Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of *Givotia moluccana* (L.) Sreem.

A thesis submitted for the award of the degree of
DOCTOR OF PHILOSOPHY
in
PLANT SCIENCES

By
NYANTHANGLO WOCH
Reg. No. 14LPPH12

हैदराबाद विश्वविद्यालय
University of Hyderabad

प्रतिष्ठित संस्थान
INSTITUTION OF EMINENCE
राष्ट्रीय अपेक्षाएँ, वैश्विक मानक
National Needs, Global Standards

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500046
INDIA

February 2024

हैदराबाद विश्वविद्यालय
University of Hyderabad

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500046, Telangana, India

प्रतिष्ठित संस्थान
INSTITUTION OF EMINENCE
राष्ट्रीय अपेक्षाएँ, वैश्विक मानक
National Needs, Global Standards

CERTIFICATE

This is to certify that **Mr. Nyanthanglo Woch** has carried out the research work embodied in this thesis under the supervision and guidance of Prof. G. Padmaja, for a full period prescribed under the Ph.D. ordinances of this University. We recommend his thesis entitled **"Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of *Givotia moluccana* (L.) Sreem."** for submission for the degree of Doctor of Philosophy of the University.

Head 28/2/2024
Department of Plant Sciences

HEAD
Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046, T.S. INDIA.

26/02/2024
Prof. G. Padmaja
Supervisor

Dr. G. PADMAJA, Ph.D.
Professor
Department of Plant Sciences
School of Life Sciences
UNIVERSITY OF HYDERABAD
Hyderabad-500 046. Telangana

हैदराबाद विश्वविद्यालय
University of Hyderabad

प्रतिष्ठित संस्थान
INSTITUTION OF EMINENCE
राष्ट्रीय अपेक्षाएँ, वैश्विक मानक
National Needs, Global Standards

DECLARATION

I hereby declare that the work embodied in this thesis entitled **"Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of *Givotia moluccana* (L.) Sreem."** has been carried out by me under the supervision and guidance of Prof. G. Padmaja, Department of Plant Sciences, School of Life Sciences. The work presented in this thesis is a bonafide research work and has not been submitted for any degree or diploma in any other University or Institute. A report on plagiarism statistics from the University Librarian has been enclosed.

26/02/2024

Prof. G. Padmaja
(Supervisor)

Dr. G. PADMAJA, Ph.D.
Professor
Department of Plant Sciences
School of Life Sciences
UNIVERSITY OF HYDERABAD
Hyderabad-500 046. Telangana

Nyanthanglo Woch

हैदराबाद विश्वविद्यालय
University of Hyderabad

Department of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500046, Telangana, India

प्रतिष्ठित संस्थान
INSTITUTION OF EMINENCE
राष्ट्रीय अपेक्षाएँ, वैश्विक मानक
National Needs, Global Standards

CERTIFICATE

This is to certify that this thesis entitled **"Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of *Givotia moluccana* (L.) Sreem."** is a record of bonafide work carried out by **Mr. Nyanthanglo Woch**, a research scholar for Ph.D. program at Department of Plant Sciences, School of Life Sciences, University of Hyderabad under my guidance and supervision. This thesis is free from plagiarism and has not been submitted in part or in full for any degree or diploma in this or any other University or Institution.

Parts of this thesis have been:

Published in the following journal:

1) **Woch N,** Laha S and Padmaja G (2023) Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. *In Vitro Cellular & Developmental Biology – Plant.* 59: 227-248. https://doi.org/10.1007/s11627-023-10335-7

Presented in the following conferences:

1) **Woch N**, Laha S and Padmaja G (2019) Enhanced accumulation of phenolics and flavonoids and antioxidant activities of callus cultures of *Givotia moluccana* (L.) Sreem. Poster presentation at 40th meeting of Plant Tissue Culture Association -India (PTCAI) & International Conference on Trends in Plant Sciences and Agro-biotechnology, 2019 at IIT Guwahati, Assam, India.
2) **Woch N**, Laha S, Muthamilarasan M, Padmaja G (2022) *De novo* transcriptome analysis provides insights into differentially expressed genes in jasmonic acid elicited callus

cultures of *Givotia molucanna* (L.) Sreem. Oral presentation at International Conference on Frontiers in Nutrition, Medical Genomics, and Drug Discovery (Inbix'22), 2022 at Vignan's Foundation for Science, Technology & Research, Guntur, Andhra Pradesh, India.

Further, the student has passed the following courses towards the fulfillment of the course work requirement for Ph.D. degree.

Sl. No.	**Course Code**	**Name**	**Credits**	**Result**
1.	PL801	Analytical Techniques	4	Pass
2.	PL802	Research Ethics, Data Analysis & Biostatistics	3	Pass
3.	PL803	Lab Work & Seminar	5	Pass

26/02/2024
Supervisor

Dr. G. PADMAJA, Ph.D.
Professor
Department of Plant Sciences
School of Life Sciences
UNIVERSITY OF HYDERABAD
Hyderabad-500 046. Telangana

26/2/2024
Head

HEAD
Dept. of Plant Sciences
School of Life Sciences
University of Hyderabad
Hyderabad-500 046, T.S. INDIA.

28/2/24
Dean

संकाय अध्यक्ष / Dean
जीव विज्ञान संकाय / School of Life Sciences
हैदराबाद विश्वविद्यालय / University of Hyderabad
हैदराबाद / Hyderabad-500 046.

Acknowledgements

As I embarked this journey in research many people have supported and helped me reach this stage. Although it would be impossible to mention each and every individual here, I would like to express my sincere gratitude to certain individuals who have made the journey smooth and easier.

Foremost, I thank the God, almighty for giving me the knowledge, strength, and courage to pursue this research and complete my thesis.

I am truly and profoundly indebted to my supervisor and mentor, Prof. G. Padmaja, for providing me guidance and support throughout my doctoral research. I am incredibly appreciative of her mentorship, which allowed me to feel completely liberated, expand my scientific perspective, and build a positive mindset. Her endurance, understanding, indefinite support, and precious recommendations have been of great assistance. Her altruistic input, innovative assistance, and knowledgeable guidance made it possible to finish my research successfully. Most of all, I am eternally grateful for her affection and backing during challenging times while I was working on my doctoral study.

My utmost gratitude to my doctoral committee members, Prof. S. Rajagopal and Prof. Ch. Venkata Ramana for their time and valuable suggestions for my research ensuring the successful completion of my thesis. I am thankful to them for allowing me to use their laboratory facilities.

My profuse thanks to the Dean of School of Life Sciences, Prof. Anand K. Kondapi, , and former Deans, Prof. N. Siva Kumar, Prof. S. Dayananda, Prof. K. V. A. Ramaiah, Prof. M. N. V. Prasad, Prof. A. S. Raghavendra and Prof. P. Reddanna. Head of Plant Sciences Department, Prof. Rajagopal and former Heads, Prof. G. Padmaja, Prof. Ch. Venkata Ramana and Prof. Attipalli R. Reddy and all the faculty of Department of Plant Sciences.

I am very grateful to Prof. A. S Raghavendra, Prof. Appa Rao Podile, Prof. P. Reddanna, and Prof. Santosh Kumar Padhi for enabling me to conduct some of my experiments in their laboratories.

I would like to thank State Silviculturist of Forest Research Station, Mulugu, Telangana and State Silviculturist, Forest Research Station, Rajahmundry for allowing us to collect the seeds from the trees of Givotia during the entire course of the study.

To my Lab mates and Colleagues- Dr. Durgeshwar Pullaiahgari, Dr. Rajesh Kumar Meena, Dr. Madhavi Bramhanapalli, Mr. Supriya Laha, Ms. Priyanka Saxena, Mr. Litu Malik, Ms. Deepika Dake and Lab Assistant Mr. Praveen Kumar, who have been an incredible source of support, both academically and emotionally. I cannot thank them enough for their help, candor and for their co-operation and camaraderie atmosphere in the lab.

To my friends who have given me tremendous support and I am immensely grateful to them for their love and encouragement. In particular, I would like to thank Mr. Lanuakum Longchar, Ms. Shanngam Kasom, Ms. Sari Luikham, Dr. Amihe Swu, Dr. Boniface Kamei, Mr. Saptarshi Dey, Dr. Limakumba Walling, Dr. Rhelo Kenye, Mr. Thano Rengma, , Mr. Imliakum Pongen, Mr. Merenmeso Pongen, Mr. Mavahrii Kemas, Mr. Shang Neyeshiu, Ms. Ctei Chhakchhuak, Ms. Ching Ngaih, Dr. Nukshimenla Lemtur, Ms. Thejasenuo Belho, Mr. Nagahoto Chophy, Dr. Vekhepu, Dr. Neikesonuo Rame, Ms. Vidila Jing, Ms. Alung Longvah, Dr. Jichule Seb and my Impact 4: 12 buddies-Mr. Hinumo Thong, Mr. Alichu Kath, Mr. Senilo Thong, Mr. Dithanglo Magh, Mr. Philemon Tep, Mr. Kedalo Semy and Chentimo Woch. Thank you all for your friendship, support, encouragement throughout my doctoral studies.

To my parents, brothers and sister, I am forever indebted for their unconditional support and prayers, endless patience, love and encouragement throughout, without which I would not have reached this far.

I am ever thankful to Department of Biotechnology, Government of India for providing me a fellowship, without which I would not have been able to pursue my doctoral studies. Infrastructural support provided by UGC-SAP, UGC-UoH-IoE, DBT-CREBB, DST-FIST and DBT-BUILDER to Department of Plant Sciences/School of Life Sciences are duly acknowledged.

Lastly, I would like to thank everyone in University of Hyderabad, Non-teaching Staff, Research Scholars, and friends, who have helped me in many ways to complete my thesis and for making my stay at University of Hyderabad a memorable one.

NYANTHANGLO WOCH

Dedicated to my late mother

Akhilu Woch

Contents

Abbreviations and Symbols

2,4-D	:	2,4-Dichlorophenoxy acid
µM	:	Micro Molar
µg	:	Micro Gram
AAE	:	Ascorbic Acid Equivalents
ABTS	:	2,2’ azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
AChE	:	Acetylcholinesterase
$AlCl_3$	:	Aluminum Chloride
APX	:	Ascorbate Peroxidase
AsA	:	Ascorbate
AsA-GSH	:	Ascorbate-Glutathione
BAP	:	6-Benzylaminopurine
BSA	:	Bovine Serum Albumin
BzSA	:	Benzoylsalicylic Acid
CAT	:	Catalase
CCl_4	:	Carbon Tetrachloride
cDNA	:	Complementary Deoxyribonucleic acid
$CuCl_2$	:	Copper Chloride
CUPRAC	:	Cupric Reducing Antioxidant Capacity
COX	:	Cyclooxygenase
DCM	:	Dichloromethane
DEG	:	Differentially Expressed Genes
DHA	:	Dehydroascorbate
DHAR	:	Dehydroascorbate reductase
DNA	:	Deoxyribonucleic acid
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DTNB	:	5,5-dithio-bis-(2-nitrobenzoic acid)
DTT	:	Dithiothreitol
DW	:	Dry Weight
EDTA	:	Ethylenediaminetetraacetic Acid
ELISA	:	Enzyme Linked Immunosorbent Assay

FDR	:	False Discovery Rate
FRAP	:	Ferric Reducing Antioxidant Power
FW	:	Fresh Weight
GAE	:	Gallic Acid Equivalents
GC-MS	:	Gas Chromatography-Mass Spectrometry
GR	:	Glutathione Reductase
GSH	:	Reduced Glutathione
GSSG	:	Oxidized Glutathione
GO	:	Gene Ontology
HCl	:	Hydrochloric Acid
H_2O_2	:	Hydrogen Peroxide
HPLC	:	High Performance Liquid Chromatography
IBA	:	Indole 3-Butyric acid
IDT	:	Integrated DNA Technologies
JA	:	Jasmonic Acid
KCl	:	Potassium chloride
KEGG	:	Kyoto Encyclopedia of Genes and Genomes
KIN	:	Kinetin
KOBAS-I	:	KEGG Orthology Based Annotation System-Intelligent.
LC-MS	:	Liquid Chromatography-Mass Spectrometry
LOX	:	Lipoxygenase
MDHAR	:	Monodehydroascorbate reductase
MDA	:	Malondialdehyde
$MgCl_2$	:	Magnesium Chloride
MS	:	Murashige and Skoog
m/z	:	Mass-to-Charge
NAA	:	1-Naphthaleneacetic acid
NADPH	:	Nicotinamide adenine dinucleotide phosphate
Na_2CO_3	:	Sodium Carbonate
NaOH	:	Sodium Hydroxide
NBT	:	Nitro Blue Tetrazolium Chloride
NCBI	:	National Centre for Biotechnology Information

NDGA	:	Nordihydroguaiaretic acid
nmol	:	Nano moles
NSG	:	Next Generation Sequencing
·OH	:	Hydroxyl Radical
$^1O_2$	:	Singlet Oxygen
O_2^-	:	Superoxide
OD	:	Optical Density
PG	:	Prostaglandin
PlantTFDB	:	Plant Transcription Factor Database
PM	:	Phosphomolybdenum method
PMSF	:	Phenylmethylsulfonyl Fluoride
PPFD	:	Photosynthetic Photon Flux Density
POD	:	Peroxidase
PVP	:	Polyvinylpyrrolidone
RE	:	Rutin Equivalents
ROS	:	Reactive Oxygen Species
qRT-PCR	:	Quantitative Real-Time Polymerase Chain Reaction
SA	:	Salicylic Acid
SAR	:	Systematic Acquired Resistance
SD	:	Standard Deviation
SDS	:	Sodium Dodecyl Sulphate
$SnCl_2$	:	Stannous Chloride
SNP	:	Single Nucleotide Polymorphism
SOD	:	Superoxide Dismutase
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic Acid
TGSH	:	Total Glutathione
Tm	:	Melting Temperature
TPM/TMM	:	Transcripts Per Million Transcripts
TPC	:	Total Phenolic Acid content
TPTZ	:	2,4,6-Tris (1-pyridyl)-S-Triazine
Tris-HCl	:	Tris Hydrochloride

List of Figures

List of Tables

Abstract

Givotia molucanna L. Sreem. Syn. *Givotia rottleriformis* Griff. ex Wight (White catamaran tree) is an economically important softwood tree species used in traditional medicine for treating inflammation related diseases such as rheumatism, psoriasis and dandruff. There are very limited reports, which validated the medicinal properties of this species. The study mainly focused on analysis of secondary metabolite profiles in different plant parts and determining the antioxidant and anti-inflammatory activities of plant parts using *in vitro* based assays. Studies were carried to establish *in vitro* callus cultures and increase the secondary metabolites by elicitation of callus cultures with salicylic acid and jasmonic acid. The effect of elicitation on total phenolic and flavonoid accumulation, and antioxidant changes were investigated. Elicitation induced changes in whole metabolome was assessed using LC-MS/MS and *de novo* transcriptome analysis was performed to identify the differentially expressed genes in response to elicitation of callus cultures with salicylic acid and jasmonic acid.

The first objective of this research work was focused on comparative assessment of phenolics and flavonoids, antioxidant and anti-inflammatory activities along with metabolites profiling using gas chromatography mass spectroscopy (GC-MS), liquid chromatography tandem mass spectroscopy (LC-MS/MS) of the different parts *viz.*, bark, leaves and pericarp of *G. moluccana*. The extracts of plant parts showed high phenolic and flavonoid contents and exhibited good antioxidant and anti-inflammatory potential when assayed by different *in vitro* methods. The methanolic extracts of bark displayed the highest content of total phenolic (TP) (62.07 mg GAE/g DW), total flavonoid (TF) (41.72 mg RE/g DW), and antioxidant activities as evaluated by phosphomolybdenum (PM) assay (70.94 mg AAE/g DW), ferric reducing antioxidant power (FRAP) assay (156.38 mg AAE/g DW), cupric ion reducing antioxidant capacity (CUPRAC) assay (246.32 mg AAE/g DW), DPPH assay (IC_{50} 48.41 µg/ml) and metal chelating ability (78.58%). The bark extracts exhibited relatively higher protein denaturation (43.46%) and higher inhibition activity against 5-LOX (41.6%) as compared to pericarp and leaves. The bark and pericarp extracts inhibited both COX-2 and 5-LOX enzyme activities, and thus can be explored for developing compounds with dual inhibition and superior anti-inflammatory agents. The metabolite profiling of bark, leaves and pericarp revealed the distribution of primary metabolites *viz.*, carboxylic acids, carbohydrates, amino acids and fatty acids. LC-MS/MS analysis revealed that the secondary metabolites predominantly comprised of flavonoids, terpenoids and terpenes, alkaloids and phenolic acids, whose distribution varied in bark, leaves and pericarp. Several putative bioactive compounds which are known to possess anti-psoriasis, anti-rheumatic, antioxidant, anti-inflammatory, and anti-cancer activities were identified. The information generated in this study could serve as a base for isolation and characterization of new bioactive compounds for treatment against inflammatory-related ailments. The study further quantified three flavonoids *viz*., myrcetin, quercetin and rutin in bark, leaves and pericarp.

In vitro callus cultures offer a promising source for secondary metabolite production with the possibility of their enhancement using elicitors. The study aimed at development of methods for callus induction from leaves, cotyledons and endosperm as well as establish plant regeneration *via* direct shoot organogenesis from zygotic embryonic axes of *G. moluccana*. The best callus induction and high proliferation was achieved from the explants on medium containing 2 mg/l 2,4-D and 1 mg/l BAP. The study was successful in establishing method for multiple shoot induction from zygotic embryo axes by initially culturing on medium with 3 mg/l BAP followed by subcultures on medium with reduced concentrations of BAP. *In vitro* root induction was achieved on medium with 0.5 mg/l NAA or 0.5 mg/l IBA and the regenerated plants were acclimatized and established in soil with 72.4% success rate.

The effects of SA and JA on production of secondary metabolites from leaves-derived callus cultures was studied for enhancing the secondary metabolite production. A marked increase in total phenolic acids and flavonoids were achieved in callus cultures treated with 50 μM SA for 5 days and 150 μM JA for 20 days as compared to control (untreated) cultures. The study also demonstrated an increase in the activity of antioxidant enzymes and the involvement of ascorbate-glutathione pathway in curbing the free radicals that were produced during elicitation treatment. Analysis of the gene expression pattern of antioxidant related enzymes genes such as *SOD, CAT, POD, APX,* and *PAL* through quantitative Real-Time Polymerase Chain Reaction (qRT-PCR), showed correlation with their enzyme activities. The LC-MS/MS analysis resulted in putative identification of 170, 218 and 203 secondary metabolites in leaves-derived callus cultures, SA and JA-treated callus cultures, respectively. These secondary metabolites belonged to different classes of secondary metabolites such as flavonoids, terpenes and terpenoids, alkaloids, phenolic acids, glycosides, sterols and steroids, vitamins and co-factors, lignans, anthroquinone, stilbenes and betacynin.

Finally, *de novo* transcriptome analysis of leaves, callus, and callus cultures treated with SA, and JA helped in unravelling the transcriptome changes associated with elicitation. The genes that were differentially expressed in callus cultures as compared to leaves, and callus cultures treated individually with 50 μM SA and 150 μM JA for 20 days were identified. It was observed that 6488 genes were differentially expressed between callus cultures and leaves, of which 2469 were upregulated and 4109 were downregulated. The differentially expressed genes were primarily involved in cell division, plant hormone signaling pathways, plants stress response, plant-pathogen interactions and photosynthesis A total of 714 transcripts encoding transcription factors were discovered in the comparison between leaves and leaves-derived callus, and these were categorized into 49 families. A total of 1623 genes were differentially expressed, with 886 being upregulated and 737 downregulated in salicylic acid (SA)-treated callus cultures compared to the control callus. On the other hand, jasmonic acid (JA)-treated callus cultures showed higher number (3504) of DEGs, with 1719 upregulated genes and 1785 downregulated genes as compared to control callus cultures. The differentially expressed genes in both SA and JA treatments indicates significant changes in gene expression related to biosynthetic pathways of secondary metabolites, plant hormone signaling, plant stress responses, and plant-pathogen interaction. A total of 1062 transcripts encoding transcription factors (TFs) were identified in control callus cultures, salicylic acid (SA)-treated, and jasmonic acid (JA)-treated callus which were categorized into 50 families. The TFs with the highest transcript levels were associated with families such as *ERF*, *bHLH*, *NAC*, *WRKY*, *GRAS*, *C3H*, *C2H2*, *MYB*, *MYB*-related, and *B3*. Several putative genes associated with biosynthesis of phenylpropanoid, flavonoids, terpenoids and lignins were identified, with their expression being enhanced in both SA and JA treatments. The expression pattern of few highly differentially expressed genes was validated using qRT-PCR.

1. Introduction

Phytochemicals are bioactive compounds naturally synthesized by plants via metabolic pathways originating from primary metabolites like carbohydrates, proteins and lipids. Plants synthesize a wide range of phytochemical compounds *viz.* terpenoids, flavonoids, alkaloids, phenolic acids, glycosides, steroids, tannins and saponins. These compounds play significant roles in the interaction between plants and their environment, contributing to tolerance against biotic and abiotic stresses (Sirikantaramas *et al.*, 2008). Secondary metabolites manifactured by plants hold economic significance, finding applications in pharmaceuticals, flavorings, food additives, fragrances, pigments and dyes and more. Medicinal plants are a valuable reservior of bioactive compounds with therapeutic properties often serving as a basis for novel drug discoveries (Atanasov *et al*., 2015). The advancement of the modern technologies has accelerated the discovery of plant based natural products with significant therapeutic properties. Plant secondary metabolites are progressively used in the treatment of different ailments such as anti-diabetic (Guerrero-Analco *et al.*, 2007; Farag *et al*., 2015; Huang *et al*., 2022; Kiem *et al*., 2020), anti-inflammatory (Chen *et al*., 2022; Cho *et al*., 2022; Youn *et al*., 2022; Yu *et al.*, 2022), anti-cancer (Chassagne *et al.*, 2018; Abreu *et al*., 2020; AlQathama *et al*., 2022), anti-microbial activities (Gonelimali *et al*., 2018; Mostafa *et al*., 2018; Akhtar *et al*., 2018) etc.

Chemical nature and composition of the secondary metabolites varies among plant species. The research on plant secondary metabolites has garnered significant attention due to their diverse array of biological activities. The antioxidant compounds obtained from plant sources play important roles in preventing oxidative damage by scavenging free radicals and modulating cell signaling pathways thus preventing chronic diseases (Valko *et al*., 2007). Intense efforts are made by the researchers to identify antioxidants from natural sources with fewer side effects. Numerous investigations have demonstrated that phenolic compounds derived from plants possess antioxidant, anti-inflammatory, and antiproliferative properties, making them highly promising in pharmaceuticals, food, cosmetics, and chemical industries (Yahfoufil *et al*., 2018; Fraga *et al*., 2019). The accumulation and concentration of polyphenolic compounds varies in different parts of plants and organs and is related to the growth phase and modulated by various environmental factors (Wegiera *et al*., 2011; Cheynier *et al*., 2013). The impact of different solvents on the composition and quantity of secondary metabolites and/or their antioxidant activities has been documented in several studies (Rebey *et al*., 2012; Kchaou *et al*., 2013; Ngo *et al*., 2017). Thus, there is a need to study the phenolic contents in plant extracts and their impending antioxidant activities as they would offer useful sources of natural antioxidants.

Inflammation serves as the body's innate defense machinery against detrimental stimuli such as toxins, pathogens, and celluar damage, and commences the healing process. However, when inflammation is not properly regulated, it can lead to a wide spectrum of disorders like allergies, cancers, cardiovascular disorders, autoimmune diseases, and metabolic ailments (Libby, 2007). Several anti-inflammatory drugs (steroidal and non-steroidal) are available for suppressing inflammatory crisis but are often associated with severe adverse effects (Wehling, 2014). Thus, the need for natural anti-inflammatory factors with high therapeutic response and low unwanted adverse effect is of prime importance and plant secondary metabolites serve as excellent prototype molecules for such drug development.

The inflammatory response is the coordinated activation of many pathways/factors that act to regulate the level of inflammatory mediators in resident cells, and recruit inflammatory cells from the blood (Lawrence, 2009). It encompasses a cascade of processes wherein the metabolism of arachidonic acid *via* LOX (lipoxygenase) and COX (cyclooxygenase) pathways into eicosanoids holds significant importance. The COX pathway involves two isoforms of cyclooxygenases enzyme namely, COX-1 (constitutive) and COX-2 (inducible) that catalyze the formation of prostaglandins, postacyclins, and thromboxanes from arachidonic acids (Rainsford, 2007). In the lipoxygenase (LOX) pathway, metabolism of arachidonic acid is fueled by different isoforms of LOX such as 15-lipoxygenase (15-LOX), 12-lipoxygenase (12-LOX), 8-lipoxygenase (8-LOX) and, 5-lipoxygenase (5-LOX) resulting in the production of pro-inflammatory leukotrienes and lipoxins (Yedgar *et al.*, 2007). Both cyclooxygenases and lipoxygenases are crucial enzymes involved in inflammation. COX-1 is a constitutively expressed under normal physiological conditions maintaining the housekeeping functions in several mammalian cells while COX-2 isofom can be induced that generates pro-inflammatory cytokines and pain signaling effectors in response to inflammation (Fiorucci *et al*., 2001; Martel-Pelletier *et al*., 2003). LOX enzymes are associated with inflammatory and allergic response due to their roles in the formation of leukotrienes (Serhan, 2007; Radmark *et al*., 2015). An elevated level of leukotrienes is expressed during inflammatory response in case of asthma, allergies, rheumatism, and psoriasis. Inhibition of LOX and COX enzymes can inhibit the production of leukotrienes and prostaglandins. The simultaneous inhibition of leukotrienes and prostaglandins has emerged as a novel and promising strategy for developing dual inhibitors targeting 5-LOX and COX-2. Such compounds hold potential as effective anti-inflammatory agents, offering the advantage of avoiding the side effects linked with COX-2 inhibitors, such as cardiovascular issues and gastrointestinal damage (Fiorucci *et al*., 2001; Davies *et al*., 2013). Bioactive compounds from medicinal plants are considered to be a promising source for generating new and effective anti-inflammatory medications (Chakraborti *et al*., 2010). Exploring natural inhibitors of lipoxygenases and cyclooxygenases could pave the way for designing biologically and pharmacologically targeted therapeutic approaches. These strategies hold promise for effectively managing inflammatory diseases.

Metabolomics is very powerful tool for characterizing the metabolites and metabolic pathways and is complementary to other omics technologies such as proteomics, genomics and transcriptomics (Heyman and Dubery, 2016). It offers a glimpse of the biochemical conditions of a biological sample. Metabolomics has become an indispensable tool in drug discovery and development by identification and profiling of secondary metabolites in medicinal plants. Metabolomics based research on medicinal plants is crucial as plants manufacture a wide arrays of metabolites which might have significant therapeutic values, including primary as well as secondary metabolites. In this context, metabolomics can be used as an effective platform to understand the phytochemical basis of such therapeutically active phyto-constituents. Some plant secondary metabolites are scarcely identified, under several instances during drug analysis because of their low abundance resulting in a limited therapeutic efficacy. However, with mecicinal plants and their combinations, biological effects can be enhanced synergistically due to the presence of multiple constituents. Under such instances, metabolomics presents a valuable platform for comprehending the phytochemical foundation of these therapeutically bioactive phyto-constituents (Mukherjee *et al*., 2016). There are two approaches for metabolomics analysis *i.e.* targeted and untargeted analysis. Targeted metabolomics is focused on a few metabolites while untargeted metabolomics is intended to analyze the whole metabolome of an organism (Waris *et al*., 2022). Various analytical techniques are employed for analysis of different metabolites of medicinal plant extracts. Mass spectrometry stands as the predominant technology in metabolomics due to its ability to swiftly, sensitively, and selectively perform qualitative and quantitative analyses of metabolites. The process involves ion formation, followed by the separation of ions based on their mass-to-charge (m/z) ratio, and ultimately the detection of these separated ions. Metabolomics approach is dependent mainly on four techniques which are liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-

MS), nuclear magnetic resonance (NMR), and spectroscopy capillary electrophoresis–mass spectrometry (CE-MS) (Salem *et al.*, 2020). GC-MS is the favored method for separating low molecular weight metabolites, particularly primary metabolites like amino acids, amines, sugars, organic acids, long-chain alcohols, and sterols, which are either volatile or can be converted into volatile and thermally stable compounds through chemical derivatization before analysis, as highlighted by Hall (2006) and Hill and Roessner (2013). Conversely, liquid chromatography-mass spectrometry (LC-MS) analysis is preferred for detecting a wider array of metabolites, encompassing secondary metabolites like alkaloids, terpenoids, flavonoids, and phenylpropanoids, as indicated by Verhoeven *et al.* (2006), De Vos *et al*. (2007), and Marr *et al*. (2021). LC-MS and GC-MS analysis have been generally used in complementary to medicinal plant metabolomics research (Zeki *et al*., 2020).

The production of secondary metabolites is often dependent on environmental factors, physiological and developmental stages of the plants and hence can be limiting (Rao and Ravishankar, 2002; Oksman-Caldentey and Inze, 2004). Plant-derived products might exhibit inconsistency in bioactivity and chemical constituents due to highly inducible variables and transitory nature of the secondary metabolites metabolism (Poulev *et al.,* 2003). The difficulties in cultivation of the source plants, low productivity, phyto-geographical and tissue/organ-specific production are some of the limiting factors in utilizing plants for secondary metabolites (Halder *et al.*, 2019). Further, excessive exploitation of medicinal plants has resulted in their depletion in natural habitats. The chemical synthesis of pharmaceutically important plant secondary metabolites is often difficult due to their diverse nature. Plant cell, organ, and tissue culture technology has gained recognition in recent years as a possible option for rapid bulk cultivation, metabolite accrual, and synthesis of precise metabolites for pharmaceutical uses without detrimental effect on the plants' natural habitats (Espinosa-Leal *et al*., 2018; Niazian, 2019). However, only a limited number of cell cultures are capable of synthesizing secondary metabolites comparable to the abundance found in intact plants (Namedo, 2007). *In vitro* techniques offer the potential of enhancing the production of high value compounds which are otherwise produced in meagre amounts.

Elicitation is an efficient strategy for enhancing the biosynthesis of secondary metabolites because of its practical feasibility (Poulev *et al*., 2003). It exploits the plants defense mechanism against stress, pathogens or external stimuli for production of secondary metabolites. Elicitors activate cascades of events such as reaction oxygen species (ROS) production, expression of defense-related genes and accumulation of secondary metabolites (Zhao *et al*., 2005). Abiotic elicitors like jasmonic acid (JA) and salicylic acid (SA) are frequently used for enhancing the production of secondary metabolites. SA, synthesized either by the phenylalanine or isochorismate pathways, plays a major role in systematic acquired resistance (SAR) during pathogen attack in plants (Klessig *et al*., 2018). JA modulates octadecanoid signaling pathway and plays a vital role in plant's tolerance to stress by modulating the defense responses (Sembdner and Parthier, 1993). Both JA and SA, when provided exogenously, trigger responses similar to pathogen exposure or other external stimuli (Giri and Zaheer, 2016). They initiate signal transduction pathways that lead to transcription of various genes thereby triggering the accumulation of molecules such as polyphenols, alkaloids, terpenoids, steroids etc. involved in defense and resistance of plants (Pieterse and van Loon, 1999; Hayat *et al*., 2010; Giri and Zaheer, 2016). These secondary metabolites are known to have a wide range of pharmacological activities such as anticancer, antidiabetic, antiasthma, antimalarial, antimicrobial, antiviral etc. They have been used in medicine either directly or as the starting points for drug discovery (Awuchi and Amagwula, 2020).

Various environmental stresses including the exogenous application of elicitors like JA and SA trigger the generation of ROS that include singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) that cause oxidative damage and ultimately cell death. ROS not only have deleterious effects, but also function as important signaling molecules that regulate normal plant growth and responses to stresses (Huang *et al*., 2019). The level of ROS is maintained by an intricate and flexible enzymatic and non-enzymatic antioxidant system to balance their production and elimination, required for normal cellular homeostasis. In plants, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) along with ascorbate-glutathione (AsA-GSH) cycle play a vital role in the elimination of ROS such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl free radicals (·OH) generated during stress conditions (Alscher *et al*., 1997; Mittler *et al*., 2004). Several non-enzymatic compounds such as phenolics, flavonoids, ascorbic acid, glutathione, carotenoids and α-tocopherol also protect the cells from cytotoxic effects of ROS. The bioactive roles of the phenolics and flavonoids are often associated with the antioxidant potency (Ghasemzadeh *et al*., 2010).

The advancement in the field of transcriptomics in the recent years has provided an unprecedented insight in understanding the gene structure, regulation and expression associated with biological processes. The inception of novel high-throughput next-generation sequencing (NGS) technology has opened avenues for quantifying and mapping transcriptomes on a larger scale with enhanced accuracy and sensitivity. The high-throughput capabilities of NGS have facilitated RNA analysis through the sequencing of complementary DNA, as outlined by Wang *et al*. (2009). The RNA sequencing is one of the key applications of NGS which has revolutionized and transformed the understanding of the genome. It combines the high throughput sequencing along with computational methods to apprehend and analyze the transcripts in the RNA (Morozova *et al*., 2009). RNA-Seq aids in identifying gense within a genome or genes active at particular point of time (Lowe *et al*., 2017) and offers comprehensive and quantitative view of gene expression (Kukurba and Montgomery, 2011). It enables the detection of alternative splicing isoforms, as demonstrated by Li *et al.* (2020), as well as marker discovery for biologically significant single nucleotide polymorphisms (SNPs), as illustrated by Dubey *et al.* (2011) and Henry *et al.* (2012). Additionally, it unveils biosynthetic pathways for secondary metabolites, as shown by Li *et al.* (2013), Senthil *et al.* (2015), and Pandey *et al.* (2016). Achieving effective phytochemical production and enhancing selective metabolites necessitates a comprehensive understanding of biosynthetic pathways, regulatory mechanisms, and metabolic alterations.

1.1. **Importance of *Givotia moluccana* (L.) Sreem. and background information**

Givotia moluccana Syn. *Givotia rottleriformis* Griff. ex Wight (White catamaran tree) is a medium-sized, dioecious tree species belonging to Euphorbiaceae family. It is distributed mostly in West Bengal, Andhra Pradesh, Telangana, Karnataka and Tamil Naidu states of India and in certain parts of Sri Lanka. It is well known for its soft and light wood which is used for crafting of toys and decors, catamarans, light boxes for packaging etc. It is an economically important tree species especially for the people of Nirmal (Telangana) and Kondapalli (Andhra Pradesh), where the artisans derive their livelihood from this tree for carving elegant and ingenious toys. The tree species is also well known for its indigenous medicinal practice in treating inflammatory diseases such as rheumatism, skin diseases and dandruff (Thammanna and Narayana Rao, 1990). The extracts of different parts of this plant species have been reported to possess various biological properties including antipsoriatic, hepatoprotective, antimicrobial and antioxidant activities (Vijayalakshmi *et al*., 2017). However, there has been a scarcity of reports validating its indigenous medicinal claim in the treatment of various inflammatory-related ailments. Vijayalakshmi *et al.* (2014) isolated flavonoid compounds such as rutin, luteolin an kaempferol from the bark, which conferred anti-psoriatic activity. Further, the inhibitory effects of bark extract on cyclooxygenase (COX) enzyme which play an important role during inflammation has been reported (Vijayalakshmi *et al*., 2016). Hepatoprotectant property of the leaves of *G. moluccana* on hepatotoxicity in rats caused by carbon tetrachloride (CCl_4) was reported by Sateesh Babu *et al*. (2014). The anti-proliferative effects of gallic acid and methyl gallate isolated from the seed coats of *G. moluccana* on human epidermoid carcinoma was demonstrated (Samuel *et al*., 2015). In the subsequent studies, Samuel *et al.* (2016) isolated and characterized benzoylsalicylic acid (BzSA) from seed coats of *G. moluccana* that proved to be an efficient inducer of systemic acquired resistance (SAR) against tobacco mosaic virus in tobacco and *Arabidopsis*. The significance of this tree species is evident through its medicinal attributes, which stem from the presence of bioactive compounds known for their therapeutic properties. Secondary metabolites produced in plants serve as the material basis for the curative effects of medicinal plants (Zhang *et al*., 2021).

1.2. Rationale of the study and Objectives

Studies on secondary metabolites constituent in different plant parts of *G. moluccana* are lacking despite being a highly valuable medicinally important tree species. Investigations on phenolic acid and flavonoid contents and the antioxidant activities of plant parts extracted in different solvents are necessary to evaluate the pharmaceutical values of the extracts. Furthermore, the production of secondary metabolites from *in vitro* cultures remain unexplored in this species. *In vitro* cultures are a promising source for investigations on the production of secondary metabolites as well as understanding their metabolism and regulation. Elicitation of the callus culture represents a promising method for augmenting the production of secondary metabolites. The integration of metabolomics with *de novo* transcriptome analysis of callus cultures exhibiting elevated levels of specific secondary metabolites offers an opportunity to identify the pivotal genes engaged in their biosynthesis and to gain insights into the mechanisms underlying secondary metabolite synthesis. Such studies would provide scope in large scale production of secondary metabolites for different applications. Building upon this background, the study was undetaken with the following objective.

i. Comparative assessment of the total phenolic acids, total flavonoids contents, *in vitro* antioxidant and anti-inflammatory activities, and metabolite profiling of different plant parts of *Givotia moluccana.*

ii. Development of methods for callus formation and plant regeneration, and enhancing the production of secondary metabolites in callus cultures by elicitation.
iii. Analysis of secondary metabolites and antioxidant metabolism in callus cultures elicited with salicylic acid (SA) and jasmonic acid (JA).
iv. *De novo* transcriptome analysis to understand the differentially expressed genes, transcription factors and biosynthesis of phenolics, flavonoids, terpenoids and lignins in elicited callus cultures.

2. Review of Literature

Plants generate a wide variety of intricate low molecular weight compounds known as secondary metabolites which are not directly involved in the growth and development of the plants. However, they play a crucial role in plant interactions with biotic and abiotic stress factors, and signal transduction processes (Dixon, 2001). These compounds have remarkable bioactivities and offer a rich source for developing pharmaceutical formulations, novel drugs, flavors, fragrances, food additives, pigments, nutraceuticals and functional foods (Guerriero *et al.*, 2018). Based on their biosynthesis, these compounds can be categorized into three main classes: phenolic compounds (including phenylpropanoids and flavonoids), terpenes (or isoprenes), and nitrogen-containing compounds such as alkaloids, glucosinolates, and cyanogenic glycosides (Harborne, 1999). Phenolic compounds are among the most prevalent secondary metabolites found in plants, characterized by the presence of one or more aromatic rings with one or more hydroxyl groups (Bravo, 1998; Dai and Mumper, 2010). The remarkable antioxidant capacity of these compounds is due to their ability to scavenge free radicals, redox property which helps in donating hydrogen atoms or act as reducing agents (Pratt *et al.*, 1992) or chelate metals (Afanas'ev *et al.*, 1989) or regulating gene expression (Krinsky, 1992). The structure-activity relationship is the key determinant of their radical scavenging ability or metal chelating ability (Balasundram *et al.*, 2006). The antioxidant activities of phenolic acids are dependent on the positions and numbers of hydroxyl groups on these molecules. Hydroxycinnamic acids exhibit higher antioxidant activity compared to hydroxybenzoic acids due to their greater capacity for hydrogen donation and radical stabilization of CH=CH-COOH group of hydroxycinnamic acids than their counterpart –COOH group of hydroxybenzoic acid (Rice-Evans *et al.*, 1996). The structure-activity relationships in flavonoids are intricate, particularly concerning the catechol group. The activity is influenced by factors such as the degree of hydroxylation and the position of the hydroxyl group in the B ring, which confer varying levels of activity (van Acker *et al.*, 1996). The activity of antioxidant is enhanced by the presence of hydroxyl group at 3', 4' 5' position of B ring in pyrogallol group of flavonoids (van Acker *et al*., 1996; Seeram and Nair, 2002).

In view of the risk factor and deleterious effects associated with the use of various synthetic antioxidants in cosmetics, food industries and therapeutics, the need to switch to natural antioxidants found in medicinal plants has become a new global trend. Natural antioxidants offer a promising alternative to synthetic antioxidants owing to their affordability, compatibility, and lack of associated ramifications (Lobo *et al*., 2010). When the endogenous antioxidants becomes compromised upon exposure to excessive ROS, the body can compensate this deficit by using exogenous supply of antioxidants through food, supplements or drugs (Sharifi-Rad *et al.*, 2020). Plants serve as a rich reservoir of antioxidants, offering valuable leads for the development of potent antioxidant agents. The intake of antioxidants such as polyphenols has demonstrated to promote the health and prevent the oxidative stress induced degenerative diseases such as cancer, atherosclerosis, diabetes, cancer etc. (Steinberg *et al*., 1989; Lai *et al*., 2001).

There is ample documentation indicating significant variations in the contents of secondary metabolites among plant populations, different plant organs, and developmental stages, all of which are influenced by environmental conditions (Achakzai *et al*., 2009; Mannan *et al*., 2011; Ghasemzadeh *et al*., 2014; Verma and Shukla, 2015). Certain secondary metabolites are universally present across various plant species, while others have been observed to be specific to particular organs or tissues (Popovic *et al*., 2021). The variations in synthesis and content of secondary metabolites suggest that they play specific physiological roles in different plant tissues and organs. As a reason, it can be inferred that different tissues and organs of medicinal plants may exhibit varying medicinal properties at different developmental stages (Bartwal *et al*., 2013). Understanding the variation and distribution of secondary metabolites in different plant parts enables the effective utilization of the medicinal properties associated with a given species.

Several factors such as temperature, time, the polarity of the solvents and concentration have found to have an effect on the type and quantity of secondary metabolites extracted from medicinal plants (Ngouana *et al*., 2021). The selection of an appropriate solvent has been demonstrated to be crucial for achieving the desired pharmacological activity of plant extracts (Dirar *et al*., 2019). Solvents of different polarities including n-hexane, chloroform, ethanol, methanol and water have been used for extracting essential phytoconstituents from different plant species. The extraction efficiency of phenolic compounds was found to be affected by the polarity of extraction solvents. The presence of hydroxyl groups in phenolic compounds contributes to their antioxidant activity, with more polar extracts typically demonstrating higher antioxidant activities (Kaczorova *et al*., 2021). However, there are studies showing that increasing solvent polarity does not necessarily enhance the extraction yield, due to variations in the nature of the compounds present in plants (Jeyaraj *et al*., 2021). These conflicting findings have emphasized the need for further research to better understand the interaction between solvents and phenolic compounds in different plant sources (Duan *et al*., 2023).

Numerous researchers have directed their efforts toward medicinal plants to isolate compounds responsible for their medicinal properties. The antioxidant potential of many medicinal plants has been investigated using various *in vitro* and *in vivo* methods (Alam *et al.*, 2013). These studies have revealed that there is no universal method to measure the antioxidant capacity as different procedures differ in their assay principles (Alonso *et al*., 2002). *In vitro* antioxidant methods, such as DPPH, ABTS, CUPRAC, and metal chelating activity assays, utilized in bioactivity assessments rely on the capacity of compounds or extracts to scavenge free radicals generated in specific reactions. In several studies, radical scavenging activities of plant extracts have been evaluated using more than one method due to varied nature of compounds present in the samples under investigation (Alam *et al*., 2013).

Reactive oxygen species (ROS) are produced as the by-product from the cellular redox reactions in almost all living organisms (Yangthong *et al*., 2009). A balanced production of ROS is beneficial for cellular functions and immune response as it acts as both a signaling molecule and a mediator of inflammation (Mittal *et al*., 2014). Uncontrolled production of ROS leads to unfavorable consequences by destroying macromolecules and lipid peroxidation of cell membranes, disrupting homeostasis and contributing to numerous inflammatory-related conditions such as cancer, asthma, rheumatoid arthritis, neurogenerative diseases and inflammatory bowel diseases (Taniyama and Griendling, 2003; Pizzino *et al*., 2017; Forrester *et al*., 2018). Inflammatory associated enzymes such as 5-lipoxygenase (5-LOX) and two isoforms of cyclooxygenase (COX-1 and COX-2) catalyze the conversion of arachidonic acids to eicosanoids such as prostaglandins, thromboxanes, prostacyclins and leukotrienes. 5-LOX enzyme catalyzes the generation of leukotrienes, which mediates inflammatory response. COX-1 is constitutive in nature and expressed in most of the tissues and mediates physiological responses, while COX-2 is inducible which produces prostaglandins involved in inflammation. Several anti-inflammatory agents have been developed based on selective inhibition of COX-2, however, long term exposure to them pose severe consequences. There have been studies showing that COX-1 is a modulator for inflammation and prostagladins produced by COX-2 are involved in inflammatory reaction as well as homeostatic processes (Armstrong *et al*., 2011; Langhansova *et al*., 2017). The emerging trend of inhibiting prostaglandins and leukotrienes holds promise for augmenting anti-inflammatory effects while potentially mitigating the side effects associated with selective COX-2 inhibitors and NSAIDs (Bertolini *et al*., 2001; Charlier and Michaux, 2003). Notable inhibition of both COX and LOX enzymes has been reported upon application of efficacious bioactive fractions derived from crude plant extract (Mukhopadhyay *et al*., 2023). Thus, medicinal plants which are widely used for treating inflammatory diseases offer potential to discover dual COX-LOX inhibitors for developing novel anti-inflammatory compounds.

Metabolomics provides an opportunity for identification and characterization of plant secondary metabolites within their metabolic environments or engineered biological systems. The swift evolution of genomics in recent years has unveiled that numerous organisms have the capability to produce a much larger array of secondary metabolites than previously believed. Many of these newly discovered secondary metabolites are only anticipated through bioinformatics analysis of probable secondary metabolite gene clusters in sequenced genomes, but they are either not naturally produced or exist at levels too minimal to be detected by conventional methods (Breitling *et al*., 2013). GC-MS and LC-MS are the most commonly utilized techniques in metabolomics research due to their superior resolution and sensitivity, as highlighted by Gowda and Djukovic (2014) and Siddiqui *et al.* (2020). In GC-MS analysis, samples are derivatized first and separated in a gas chromatograph before being analyzed for volatile metabolites or those that volatilize after derivatization, as outlined by Fernie *et al.* (2004). This platform is particularly effective for detecting primary metabolites such as amino acids, sugars, and organic acids (Fernie and Tohge, 2017). LC-MS is recognized as one of the most potent analytical method, capable of detecting wide range of hydrophobic metabolites, predominantly, secondary metabolites like flavonoids, alkaloids, terpenoids, and phenylpropanoids, without the need for sample pre-treatment. The use of liquid chromatography combined with tandem mass spectrometry (LC–MS/MS) for the identification and characterization of secondary metabolites has experienced rapid growth, offering sufficient structural insights and enabling the quantification of multiple compounds. LC-MS/MS has the advantage of higher sensitivity by using multiple-reaction monitoring (MRM) scan mode, facilitating considerable noise reduction (Qiao *et al*., 2011; Wang *et al*., 2014). Additionally, the ion source polarity can be switched between positive and negative modes, enabling simultaneous analysis of different types of compound ionization within a single chromatographic run (Yang *et al*., 2010). High-resolution GC-MS and LC-MS/MS coupled with multivariate statistics are jointly adopted as they detect different kinds of metabolites thus uncovering the diversity of compouds present in diverse plants (Fernie *et al*., 2004; Zeki *et al*., 2020). These metabolomics-based strategies (both targeted and untargeted) mandate minimal sample preparation and thus provide a better understanding of metabolites composition, allowing identification of novel bioactive compounds, evaluate its bioactivity and nutraceutical potential (López-Fernández *et al*., 2020).

Different plant parts of *G. moluccana* are used in the indigenous medicinal practice against inflammatory diseases with bark being used for treatment of rheumatism, jaundice, and bone setting. The extracts of fruits are used in the treatment of psoriasis and dandruff, leaves for wound healing and endosperm for aiding digestion (Thammanna and Narayana Rao, 1990; Madhava Chetty *et al*., 2008). Despite having high medicinal value, there have been few studies that examined the chemical constituents and pharmacological activities of different parts of *G. moluccana*. Baskar Ananda Raj *et al.* (2012) conducted an initial phytochemical assessment of hydroalcoholic extract of leaf of *G. rottleriformis* and documented the presence of alkaloids, phenolic compounds, tannis, flavonoids and sterols. Similarly, Geetha and Vijayalakshmi *et al*. (2013) reported the presence of alkaloids, glycosides, tannins, phenolic compounds, flavonoids, steroids, saponins in the alocoholic extract of bark of *G. rottleriformis*. Samuel *et al*. (2015) isolated and characterized two phenolic acids *viz*., gallic acid and its methyl ester-methyl gallate from the methanolic extract of the seed coat (pericarp) of *G. moluccana*. These compounds showed a significant inhibition on both COX-1 and COX-2 enzymes, along with a significant decline in the viability of A431 skin cancer cell lines. Further, treatment with gallic acid and methyl gallate derived from the seed coat resulted in the downregulation of Bcl-2, a protein that promotes cell proliferation and inhibits apoptosis in cancer cells. Conversely, the expression of caspase-3, an apoptotic marker protein, was upregulated. Subsequently, Samuel *et al*. (2016) also isolated benzyl salicylic acid (BzSA), a novel acyl derivative of SA from the seed coat of *G. moluccana* which induced systemic acquired resistance more effectively than SA and ASA in tobacco and *Arabidopsis.* The exogenous administration of BzSA led to the upregulation of *NPR1* (Non-expressor of pathogenesis-related gene-1) and pathogenesis-related (*PR*) genes. Additionally, it enhanced the expression of hypersensitivity-related (HSR), mitogen-activated protein kinase (*MAPK*), and *WRKY* genes in tobacco plants. As a result, *Arabidopsis NahG* plants treated with BzSA exhibited enhanced resistance to tobacco mosaic virus (TMV). Nagalakshmi *et al*. (2020) evaluated the seed oil of *G. moluccana* and found that the main components were gamma linolenic acid and linoleic acid. They found that seed oil was effective against pulse beetle (*Callosobruchus chinensis* L.).

The scientific investigations on medicinal property attributes of various plant parts of *G. moluccana* have been limited. Sana and Dogipathi (2018) synthesized silver nanoparticles (AgNPs) through rapid bio-reduction of aqueous leaves extracts of *G. moluccana*. The synthesized nanoparticles of leaves extracts were found to have anti-microbial activity against both gram-negative and gram-positive bacteria which was correlated to the presence of substantial level of secondary metabolites. Vijayalakshmi *et al*. (2014) reported the anti-psoriatic properties of three flavonoids *viz*., luteolin-7-O-β-D-glucuronide, rutin, and kaempferol 3-O-[2-O-(6-O-feruloyl)-β-D-glucopyranosyl]-β-D-galactopyranoside extracted from the ethanolic extract of *G. moluccana* bark. The maximum inhibition of keratinocyte proliferation of HaCaT cell line activity and significant orthokeratosis was observed in kaempferol and luteolin, indicating a potent anti-psoriatic activity of the bark ethanol extract. In the following study, Vijayalakshmi *et al*. (2014) developed a formulation containing kaempferol and luteolin which which notable decreased epidermal thickness, retained the stratum granulosum and prevented the migration of neutrophils in UV-B prompted psoriasis, suggesting anti-psoriatic efficacy. The formulation effectively inhibited major cytokines such as IL-17 and TNF-α involved in psoriasis development. Sateesh Babu *et al*. (2014) observed that aqueous ethanolic extract of *G. moluccana* leaves displayed hepatoprotective potential in wistar rats resulting in marked decrease in marker enzymes such as alkaline phosphatase (ALP), serum bilirubin (SB), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). Vijayalakshmi *et al*. (2016) reported the inhibition of COX-1 and COX-2 activities and protection of membrane stability by the bark extract of *G. moluccana* which was explained by the abundance of several flavonoids that have the capacity to inhibit arachidonic acid pathway.

The phytochemical profile and their production in any plant species are greatly impacted by temperature, light, soil, salinity and other environmental factors. Furthermore, plants from certain habitats can be difficult to cultivate outside of their native environments, while most secondary metabolites are present in extremely low concentrations, which pose the commercial production of these bioactive compounds an enormous challenge (Bourgaud *et al*., 2001). Chemical synthesis is feasible only for compounds with simple chemical structures but they can be highly expensive (Kolewe *et al*., 2008). *In vitro* technology offers an attractive alternative to the conventional method (Namdeo, 2007). The advantages of the plant cell culture includes consistent yields of the secondary metabolites, and the possibility to increase highly valued compounds substantially for food and medicines without the constraints of geographical, climatic and environment factors (Wilson and Roberts, 2014). Efficient production of secondary metabolites in *in vitro* requires an establishment of efficient system. Callus cultures, hairy root cultures, and micropropagation procedures are some of the most effective tissue culture techniques for producing secondary metabolites in biotechnological applications. Exogenous application of auxin and cytokinin in intermediate ratio usually promoted the formation of callus in many plant species (Skoog and Miller, 1957). There are no published reports on callus induction in *G. moluccana*. The few reports available on *in vitro* culture of this species dealt with evaluation of various factors for inducing germination and seed development (Rambabu *et al*., 2006; Samuel *et al*., 2012) and micropropagation (Samuel *et al*., 2009) using zygotic embryo axes as explant source.

Although undifferentiated cells usually do not produce sufficient secondary metabolites, but exposure to elicitors such as salicylates, jasmonates, chitosan, inorganic salts, or heavy metals can increase their synthesis. Salicylic acid (SA) is a molecule which induces systemic acquired resistance to a wide range of pathogens. The rapid accumulation of SA at the infection site during the plant-pathogen interaction causes a hypersensitive reaction, which transmits signal to other regions of the plant triggering a cascade of defense responses accompanied by accumulation of several secondary metabolites (Dučaiová *et al.,* 2013). Numerous studies have been successful in enhancement of secondary metabolites adopting SA as elicitor for example, paclitaxel production in *Taxus chinensis* (Wang *et al*., 2007), taxol production in *Corylus avellana* (Rezaei *et al*., 2011), podophyllotoxin production in *Linum album* (Yousefzadi *et al*., 2010), production of withanolides in *Withania somnifera* (Sivanandhan *et al*., 2013), bilobalide and ginkgolide A and B production in *Ginkgo biloba* (Kang *et al*., 2006), phenolics and flavonoids in *Oplopanax elatus* (Yu *et al*., 2023). Jasmonates (JAs) are signaling molecules found in plants that govern a variety of physiological and developmental processes which are provoked by pathogens and injuries, which prompts defense responses, both regionally and globally (Farmer *et al*., 2003; Ramirez-Estrada *et al*., 2016). Exogenous application of jasmonates to plant cell, organ, and tissue cultures has been successful in enhancing the production of flavonoids in *Hypericum perforatum* (Wang *et al*., 2015), *Jatropha carcus* (Lucho-Constantino *et al.*, 2017); production of terpenoids in *Persicaria minor* (Sellapan *et al*., 2018), *Taxus* spp. (Onrubia *et al*., 2013); production of alkaloids in *Catharanthus roseus* (Ruiz-May *et* *al*., 2009); production of phenylpropanoids in *Hypercium perforatum* (Gadzovska *et al*., (2007), *Celastrus paniculatus* (Anusha *et al*., 2016), *Thevetia peruviana* (Mendoza *et al*., 2018), *Ajuga bracteosa* (Saeed *et al*., 2017); production of stilbenes and trans-resveratrol in *Vitis vinifera* (Belchí-Navarro *et al*., 2012). Secondary metabolites production using elicitors and callus cultures for improving its production remains unexplored in *G. moluccana*. Hence, it is worthwhile to establish callus cultures and explore their potential for enhanced production in this species.

Oxidative burst is commonly observed plants response to elicitor treatments that causes an upsurge in plant anti-oxidative enzyme activities to scavenge the ROS (Dong *et al*., 2010). Antioxidant enzymes namely, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione reductase (GR), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) work together as a part of defense system to curb the accumulation of ROS. SOD enzyme known to catalyzes the conversion of super oxide radical ($O_2^{\cdot}$) to hydrogen peroxide (H_2O_2), which is further converted to H_2O and O_2 by APX enzyme and GR in the chloroplast, CAT enzyme then catalyzes the H_2O_2 that diffuses out of the chloroplast (Takahashi and Asada, 1983). Several studies have shown elevated activities of antioxidant enzymes in response to elicitors' treatment such as JA or SA. Modarres and Yazdi (2021) reported an improved phenolic content as well as POD and CAT activities in *Salvia leriifolia* cell cultures. Similar studies involving an improved production of phenolic acids such as salvianolic acid B and caffeic acid correlating with enhanced activity of SOD, POD, CAT and phenylalanine ammonia lyase (PAL) enzymes have been reported by Dong *et al*. (2010) in *Salvia miltiorrhiza*. Lucho-Constantino *et al*. (2017) reported an increased antioxidant response along with accumulation of flavonoids and anthocyanin in *Jatropha carcus* in response to JA treatment.

Transcriptome analysis provides information on gene structure, regulation of gene expression, function and genome dynamics by understanding genome expression at the transcript level (Dong and Chen, 2013). The emergence of next generation sequencing (NGS) technology offers a quick and efficient method for generating transcriptomic datasets in non-model species. Compared to whole-genome sequencing which is currently costly and time consuming, RNA-Seq is revolutioning the study of transcriptome as it is high throughput and cost-effective, and is specifically attractive for non-model species without genome sequences (Zhu *et al*., 2014). Transcriptomics research on medicinal plants offers valuable insights into the functional genes and regulatory mechanisms governing their medicinal properties; such studies can enhance breeding selection and cultivation techniques by providing a deeper understanding of the genetic basis underlying desirable traits. In the recent past, *de novo* transcriptome sequencing has been used in several medicinal and economically important plant species to elucidate the genome details and regulatory machineries associated in the production of novel metabolites. For instance, transcriptome analysis has been used to probe the genes associated with the production of the diterpenoid lactones andrographolide and neoandrographolide in *Andrographis paniculata* (Sun *et al*., 2019). Chen *et al*. (2018) conducted a transcriptome study in *Cinnamomum camphora*, uncovering the trends of gene expression during biosynthesis of terpenoids and accumulation of terpenoids in various chemotypes. The study of Kang *et al*. (2020) provided an insight into the anthraquinone biosynthesis in *Senna tora* through *de novo* transcriptome analysis. Zhao *et al*. (2021) identified the key genes involved in the biosynthesis of coumarins in addition to differentially expressed genes and tissue-specific genes in *Angelica dahurica*. La *et al*. (2020) identified coronatine-regulated gene associated with biosynthesis of terpenoid in *Hevea brasiliensis* by RNA-seq analysis, which deciphered the genetic basis of terpenoid production and resistance traits. Rattan and Warghat (2023) described the improved accumulation of salicydroside and rosavin along with increased expression of key biosynthetic pathway genes in cell cultures elicited with SA and JA in *Rhodiola imbricata*. To date the genome and transcriptome resources of *G. moluccana* is not available. Based on the transcriptome approach studies, it is possible to identify DEGs and transcription factors involved in biosynthesis of important biosynthetic pathways in *G. moluccana.*

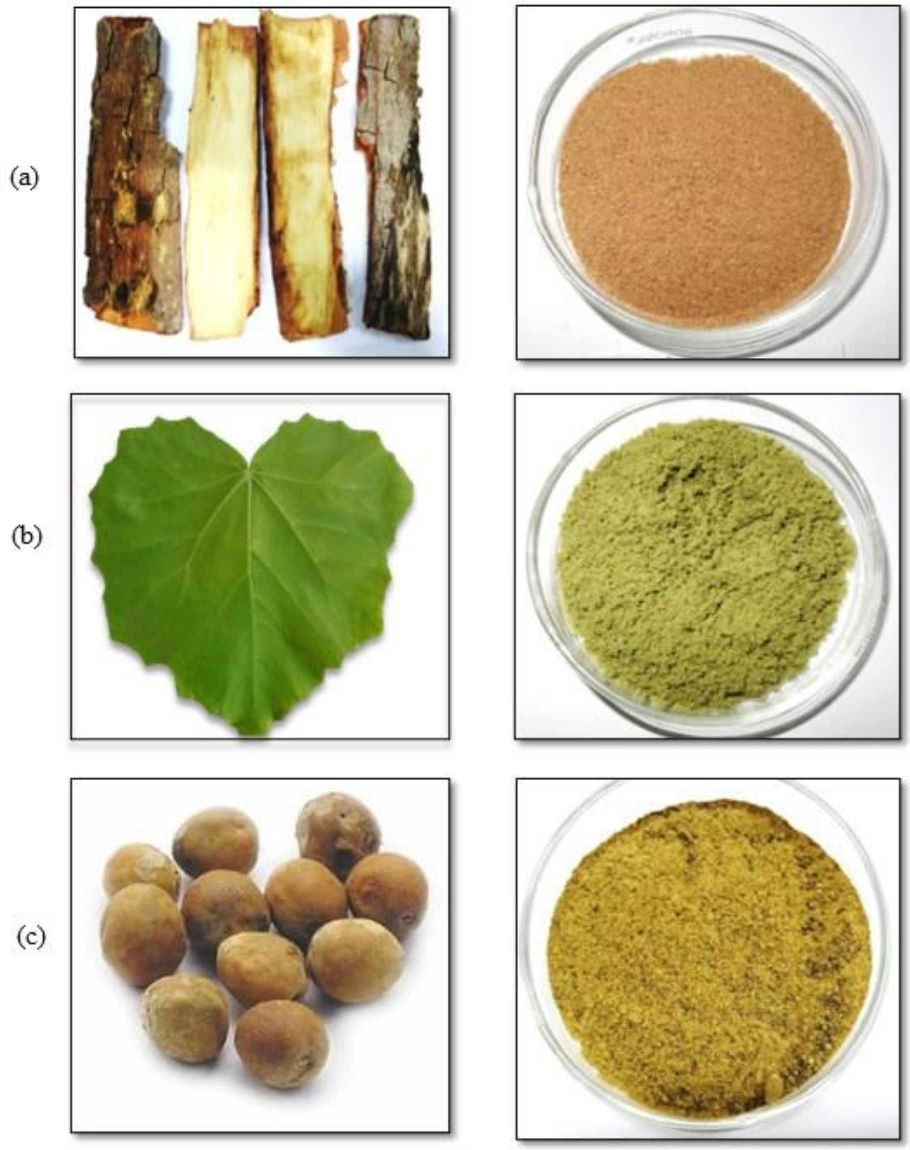
3. Materials and Methods

3.1. Plant material

The bark and mature leaves of *G. moluccana* were obtained from 15-year-old plants established at University of Hyderabad; while the matured seeds were collected from trees growing at Forest Research Station, Mulugu, Telangana (Figures 1a & b). The samples were placed in plastic covers and brought to the laboratory. They were washed thoroughly in running tap water to remove dust particles and placed in an oven at 45ºC and dried until the moisture content was completely removed. The pericarp of the mature dried seeds was removed using pruning secateurs. Subsequently, the bark, leaves and pericarp were pulverized separately in a grinder, and the powdered samples obtained were sieved. Only finely powdered samples were used for preparation of extracts (Figures 2a-c). Five grams each of the finely powdered samples were placed in a beaker comprising 100 ml each of different solvents *viz.* water, methanol, ethanol, ethyl acetate, dichloromethane (DCM), and hexane, and stirred overnight on a magnetic stirrer. The samples extracted in different solvents were filtered using Whatman filter paper (11 µm pore size), and the resultant filtrate was concentrated in a rotary evaporator (Heidolph Instruments, Schwabach, Germany). The stock of each extract (25 mg/ml) was prepared and used for performing various assays.

The seeds of *G. moluccana* were germinated *in vitro* and seedlings were produced as reported earlier by Samuel *et al*. (2009). The hard stony seeds were washed thoroughly under running tap water and placed in 0.1% Tween-20 (Sigma-Aldrich, Missouri, U.S.A.) for 10 min and rinsed with distilled water followed by 1% bavistin (BASF India Limited, Mumbai, India) treatment for another 10 min. They were surface sterilized with 70% ethanol for 3 min and subsequently with 0.1% mercuric chloride (Sisco Research Laboratories Pvt. Ltd., Mumbai, India) for 10 min in Laminar Air Flow chamber. The seeds were rinsed 3 times with sterile double distilled water after each step of surface sterilization. Then, the seeds were soaked in sterile distilled water overnight, dried using sterilized tissue paper and were cut gently using bench vice. The cut seeds with undamaged zygotic embryo axes were then placed in half-strength MS (Murashige and Skoog, 1962) medium containing 10 mg/l gibberellic acid (Sigma-Aldrich, Missouri, U.S.A.) for 3 days in the dark. The cultures were then moved to culture racks and kept under the light with 16 h photoperiod at a photosynthetic photon flux density (PPFD) of 83.6 $\mu Em^{-2}s^{-1}$ provided by white fluorescent tubes (OSRAM Lighting India Pvt. Ltd, Gurgaon,

Figures 1 (a-b). Tree of *Givotia moluccana* growing at Mulugu Research Station. (a) Tree with leaves during September, (b) Tree after leaf fall in February.



Figures 2 (a-c). Different plant parts of *G. moluccana* and their dried powders used for extract preparations. (a) Bark, (b) Leaf and (c) Dried seeds from which pericarp was removed.

Haryana, India) for germination and production of seedlings for using them as a source of explants *viz*., leaves, cotyledons and endosperm for callus formation.

3.2. Estimation of total phenolic acids (TP) and total flavonoids content (TF) in different plant parts of *G. moluccana*

Different plant parts of *G. moluccana viz.,* bark, leaves and pericarp were assayed for the total phenolic acid (TPA) and total flavonoid (TF) contents. The TPA content was estimated spectrophotometrically using Folin-Ciocalteu method as described by Singleton and Rossi (1965). Gallic acid was used as a standard. The FC reagent was obtained from a mixture of phosphotungstic acid and phosphomolybdic acid, which is reduced to blue coloration after the phenols are oxidized. The blue cloured chromophore developed correspondes to the total phenolic acids found in the samples and has a maximum absorption at 750 nm. The sample (50 µl) was taken from the extracts (25 mg of dried powder/ml of each solvent), and then 2.5 ml of 0.2 N of Folin-Ciocalteu reagent was added. After incubating for 6 min, 2 ml of 7.5% sodium carbonate solution (Na_2CO_3) was added to the sample in the tubes and the volume was made up to 5 ml with distilled water. The tubes were left in the dark at room temperature for 30 min. The absorbance of the sample in each tube was read at 750 nm wavelength. Different concentrations of gallic acid ranging from 5-50 µg were aliquoted in triplicates and the reaction was performed and the standard graph was plotted. The TPA content in each sample extract was estimated using the standard curve of gallic acid and expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW).

The total flavonoid content was estimated according to aluminum chloride ($AlCl_3$) colorimetric assay method described by Barreira *et al*. (2008) using rutin hydrate as the standard. Flavonoids chelate metal ions and form complex compounds. $AlCl_3$ forms stable complex with flavones and flavonols, and acid liable complexes with flavonoids which are measured spectrophotometrically. The sample of 50 µl was taken from the extract (25 mg of dried powder/ml of each solvent), in each tube followed by the addition of 50 µl of 5% sodium nitrite solution. After 5 min of incubation, 120 µl of 10% $AlCl_3$ solution was added. The reaction mixture was incubated for 6 min and 200 µl of 1M NaOH was added and the volume was made up to 1 ml with double distilled water. The solution was mixed well and the absorbance was read at 510 nm. A standard curve was plotted using different concentrations of rutin (5-50 µg). The TF content was estimated in sample extracts and expressed as milligrams of rutin equivalents (RE) per gram dry weight (DW) according to the standard curve.

3.3. Evaluation of antioxidant activities and free radical scavenging activities of different plant extracts of *G. moluccana* using different assays

3.3.1. Phosphomolybdenum assay

The total antioxidant capacity of bark, leaves and pericarp extracts was determined using phosphomolybdenum assay as outlined by Prieto *et al*. (1999). The basic principle of this assay is centered on the antioxidant agents reducing molybdenum (VI) to molybdenum (V) at an acidic pH, and the consequent development of a greenish phosphatemolybdenum (V) complex. Briefly, 25 µl each of the different extracts was dissolved in 75 µl double distilled water and 1 ml reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate, 0.6 M sulphuric acid) was added in each tube. The tubes were plugged and placed in water bath for 90 min at 95°C. The tubes were then allowed to cool at room temperature, and the absorbance was measured at 695 nm using blank samples consisting of the reagent solution with the carrier solvent. The antioxidant capacity was defined as milligrams of ascorbic acid equivalents (AAE) per gram dry weight (DW), with ascorbic acid as standard.

3.3.2. Reducing power assay by FRAP method

The ferric reducing antioxidant power of bark, leaves and pericarp extracts was studied following the method developed by Benzie and Strain (1996), which involves transfer of electron resulting in ferric-tripyridyltriazine $[Fe^{III}(TPTZ)]^{3+}$ being reduced to form a blue coloured ferrous complex $[Fe^{II}(TPTZ)]^{2+}$ under pH 3.6. FRAP reagent was freshly prepared and consisted of 0.3 M acetate buffer and 10 mM 2,4,6-tris (1-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ (10:1:1 v/v/v ratio) pH 3.6. TPTZ was added at the end to avoid the reduction of $FeCl_3$ by TPTZ. The sample extracts (25 µl) were added to 2 ml of FRAP reagent and the reaction mixture was left at room temperature for 30 min. The absorbance of the resulting complex was measured at 593 nm. The reducing power of the potential antioxidant extracts was expressed in milligrams of ascorbic acid equivalents per gram dry weight (AAE/g DW).

3.3.3. Cupric reducing antioxidant capacity (CUPRAC) assay

The method described by Apak *et al*. (2004) was used for CUPRAC assay. It is a colorimetric method and relies on measuring the reduction of copper (Cu), utilizing bis(neocuproine) copper(II) cation [Cu(Nc)2^2+] as a chromogenic ligand. This compound forms a complex with Cu(II), resulting in a [Cu(II)]-Nc chromogenic oxidizing reagent. This reagent further undergoes reduction in the presence of antioxidants at pH 7, yielding an orange-yellow [Cu(I)]-Nc chromophore, which displays maximum absorption at 450 nm. Briefly, one ml each of 7.5 mM neocuprine, 10 mM copper chloride ($CuCl_2$), 1 M ammonium acetate buffer (pH 7), and 0.6 ml double distilled water were homogenized in a tube. Fifty µl of the different plant extracts or standard solutions were added to the initial cocktail. A sample blank was prepared by adding 50 µl of sample solution to 1 ml cocktail mixture without $CuCl_2$. The absorbance of the mixture was evaluated at 450 nm after incubating at room temperature for 30 min. Varing concentration of ascorbic acid standards (5 to 50 µg) was used to determine CUPRAC of each sample, which was defined as ascorbic acid equivalents per gram dry weight (AAE/ g DW).

3.3.4. 2,2-dipheyl-1-picrylhydrazyl (DPPH) scavenging activity assay

The DPPH radical scavenging activity of different extracts of bark, leaves and pericarp of *G. moluccana* was assessed according to the method outlined by Dasgupta and De (2007). The extracts of different plant parts were taken in different concentrations (50-350 µg) which was made up to 100 µl with distilled water followed by addition of 900 µl of a DPPH solution (0.04 % in methanol) (Sigma Aldrich, Missouri, U.S.A.). The tube containing only distilled water (100 µl) and DPPH solution (900 µl) served as control. The tubes were placed in the dark at room temperature for a period of 30 min. The absorbance of decreasing intensity of the reaction solution due to donation of proton by different extracts was spectrophotmetrically measured at wavelength of 517 nm and compared with ascorbic acid (positive control). DPPH radical scavenging activity of each extracts was calculated using the following formula:

DPPH radical scavenging activity (%) $= \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100$, where $Abs_{control}$ is the control absorbance while, Abs_{sample} is sample absorbance.

3.3.5. Metal chelating ability assay

The chelators' iron (II) binding ability was assessed using the methods described by Dinis *et al*. (1994). Ferrozine forms a red colour complex with ferrous iron. However, the formation of this complex is interrupted in the presence of chelating agents, resulting in a reduction of the red colour. The concurrent chelator's binding capacity can be estimated by measuring the reduction of the colour intensity. Varying concentrations of the sample extracts were added to a reaction tube, which contained $FeCl_2.4H_2O$ (50 µl) and distilled water (1.7 ml). The reaction mixture was held at room temperature for a period of 1 min. To this mixture, 200 µl of ferrozine (5 mM) was added and incubated for 10 min. The final color was monitored spectrophotmetrically at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as control which was compared to plant extracts metal chelating efficiency. The inhibition percentage of the ferroxine-Fe^{2+} complex formation was calculated against a blank containing $FeCl_2$ and ferrozine using the DPPH scavenging activity calculation formula as described in **Section 3.3.4.**

3.4. *In vitro* anti-inflammatory activities of different plant parts extracs of *G. moluccana*

3.4.1. Inhibition of protein denaturation assay

The anti-inflammatory activities of bark, leaves and pericarp were studied using inhibition of albumin denaturation assay as described by Williams *et al.* (2008). The plant extracts and acetylsalicylic acid (Sigma-Aldrich, Missouri, U.S.A.) that was used as positive control were prepared at 1 mg/ml. Sample extracts or positive control and 200 µl of 1% aqueous solution of bovine serum albumin (BSA) (HiMedia, Hyderabad, India) and 0.2 M phosphate buffer (pH 7.4) made up the reaction mixture. The reaction mixture was placed at 37ºC for 15 min. Denaturation was induced by placing the reaction mixture at 70ºC water bath for a period of 10 min after which it was allowed to cool down. The absorbance was then measured at 660 nm spectrophotometrically (UV-1800, Shimadzu, Japan). The experiments were performed in triplicate. The percentage inhibition of protein denaturation was calculated as described in **Section 3.3.4.**

3.4.2. Lipoxygenase (LOX) enzyme inhibition assay

The 5-LOX enzyme was extracted from potato tubers. The enzyme was purified and assayed using the method described by Reddanna *et al*. (1990). The polarographic approach was used to assess enzyme activity using Clark's oxygen electrode on Strathkelvin Instruments (RC300, Model 782; North Lanarkshire, Scotland). Lipoxygenase enzymes are oxygen-consuming enzymes that reduce oxygen levels in the reaction mixture. The enzyme activity was measured accordingly with the rate of reduction in oxygen. The reaction mixture was made up of enzyme (100 µl), 40 mM substrate -arachidonic acid (10 µl) and 100 mM phosphate buffer (pH 6.3) was used to make up the volume upto 3 ml. The reaction was carried out at 25°C, and the highest slope obtained was used to calculate enzyme activity. The enzyme activity was represented in units/mg protein, where one unit representing one micromole of oxygen used per minute. Nordihydroguaiaretic acid (NDGA) was utilised as a positive control. The sample extract (100 µg/ml) was used in the assay to measure the enzyme inhibition activity. The lipoxygenase activity is calculated using the following equation:

$$\mathrm{VT} \times [O_2]\mathrm{s} \times \Delta\% \, [O_2]/\mathrm{ENZ\ Vol} \times 100 = \mu\mathrm{M}\ O_2 \text{ incorporated per min/ml of enzyme}$$

Where VT is the reaction mixture volume (ml); $[O_2]s$ is the O_2 concentration in air-saturated buffer at 30°C (μmol/ml); $\Delta\%$ $[O_2]$ is the maximum percentage change per minute in $[O_2]$ consumed in the course of the linear portion of the reaction (% $[O_2]$/min), and ENZ Vol is the volume of enzyme added (ml).

3.4.3. Cyclooxygenase (COX) enzyme or prostaglandin H synthase inhibition assay

The bark, leaves and pericarp extracts were tested for their ability to block the metabolism of arachidonic acid to form prostaglandin H2 (PGH2) by cyclooxygenase enzymes by following the manufacturer's protocol of COX (ovine/human) inhibitor screening assay kit (Item No. 560131; Cayman Chemical, MI, U.S.A). Enzyme linked immunosorbent assay (ELISA) was used to quantify PGF2α generated by PGH2 reduction with stannous chloride (SnCl2). The methanolic extracts of bark, leaves and pericarp (25-100 μg/ml) were used for inhibitory studies. COX reactions were first established. Briefly, for obtaining background values, background tubes were taken, cyclooxygenase enzymes were deactivated by placing it in a boiling water for 3 min; deactivated COX-1 and COX-2 enzymes (10 μl each), heme (10 μl) and reaction buffer (160 μl). Reaction buffer consisted of 0.1 M Tris-HCl (pH 8), 5 mM EDTA, 2 mM phenol. To generate tubes with 100% initial activity for the two enzymes, heme and each enzyme (10 μl each) and reaction buffer (160 μl) were added to a reaction tube. The COX inhibitor tubes consisted of extracts or positive control (Diclofenac) (10 μl each), heme and enzymes (10 μl each) and reaction buffers (160 μl), while 10 μl of inhibitor vehicle (methanol) was added to 100% initial activity and background tubes. The tubes were incubated in a water bath at 37°C for 10 min before adding arachidonic acid (10 μl) to start the reactions. After 2 minutes of incubation, saturated stannous chloride (30 μl) was added to each test tube to terminate the reactions. The tubes were vortexed and held at 37°C for 15 min before the prostaglandins were measured using ELISA.

The ELISA buffers and assay specific reagents were prepared as per the manufacturer's instructions. ELISA buffer (990 μl) was aliquoted to background samples BC1 and BC2 containing cycloxygenase enzymes (10 μl each) in the respective tubes. For obtaining COX 100% initial activity, three clean tubes were obtained and labelled as IA1, IA2 and IA3 and dilution was carried as follows. IA1 consisted of ELISA buffer (990 μl) and COX-1 or COX-2 (10 μl each) enzyme initial activity mixed thoroughly. ELISA buffer (950 μl) was added to IA2 (1:2000 dilution) containing 50 μl of IA1 and mixed properly. Subsequently, 500 μl of ELISA buffer was aliquoted to IA3 (1:4000 dilution) tube containing 500 μl of IA2. Only IA2 and IA3 were used for ELISA. Similarly, for COX inhibitors, the tubes were labelled as C1, C2 and C3 for different dilutions, however, instead of enzyme, 10 µl of the sample was used for dilution and only C2 and C3 was used for ELISA. The diluted reactions were subjected to a 96-well plate that consisted of the following: two blank wells, one total activity well (total enzyme activity of AChE-linked tracer), two non-specific binding wells (non-immunological binding of the tracer to the well), Three maximum binding well (maximum amount of the tracer that the antibody can bind in the absence of free analyte), sixteen wells for prostaglandin standards in duplicate, two background cycloxygenase (COX-1 and COX-2). The inhibitors (extracts) were placed in the remaining wells, and each reagent was added to the plate as specified in the assay kit.

The plate was developed by discarding its contents and washing them five times using wash buffer, before using Ellman's reagent (which contains AChE substrate). Five µl of tracer was introduced to the total activity well. The plate was wrapped in plastic film and incubated in Orbital Shaker (Remi Sales and Engineering Ltd., Mumbai, India) for 60 min. Finally, the plate was read at 420 nm using an ELISA plate reader. The ratio between the absorbance of the sample or reference well and that of the maximum binding well (% $B/B_{0,}$ where B represents sample or standard bound, while B_0 represents maximum bound) was calculated according to the protocol in the assay kit. The amount of prostaglandins formed was measured from the values of % B/B_0 for all the samples with the aid of standard curve obtained (% B/B_0 of the standard and raw data) and the percentage inhibition value was calculated.

In this assay, PGs and a PG-acetylcholinesterase (AChE) conjugate (PG tracer) compete with each other for a scarced antiserum. The concentration of PG in each well is determined based on the amount of PG tracer that can bind to the PG antiserum, as it is inversely proportional to the concentration of PG. The rabbit antiserum-PG (free or tracer) complex binds to a mouse monoclonal anti-rabbit antibody that is present in the well. This enzymatic reaction creates a characteristic yellow colour, whose absorbance is measured at 420 nm. The intensity of yellow color formed is proportional to the quantity of PG tracer bound to the well, which is inversely related to the amount of free PG present during the incubation. The percentage inhibition was calculated for the control and each of the samples tested.

3.5. Metabolites profiling of bark, leaves and pericarp extracts of *G. moluccana* using GC-MS/MS analysis and LC analysis

The bark and leaves were collected from 15-year-old trees of *G. moluccana* established in the campus of University of Hyderabad, while pericarp was taken from mature seeds collected from Forest Research Station, Mulugu, Telangana. Each sample was thoroughly washed under tap water followed by sterile water. The samples were parched in hot air oven to remove all the moisture. Using a grinder, each of the samples was ground to fine powder and sieved. The fine powder (5 gms) obtained was soaked overnight in 100 ml methanol under magnetic stirrer. The filterate obtained were concentrated using vacuum evaporator. The concentrated methanolic extracts of bark, leaves and pericarp (25 mg/ml) of *G. moluccana* was dissolved and filtered in membrane filter (0.22 µm) (Axiva Sichem, New Delhi, India).

3.5.1. GC-MS analysis

The GC-MS analysis was performed according to the protocol described by Weckwerth *et al*. (2004). The methanolic extracts of bark, leaves and pericarp of *G. moluccana* were subjected to derivatization prior to GS-MS analysis to increase the volatility of metabolites. Twenty five µg of each of the methanolic extracts of bark, leaves and pericarp were dissolved in methoxyamine pyridine (40 mg/ml) and placed at 55°C for 120 min. Thereafter, N-trimethylsilyl-N-methyl trifluoroacetamide (MSTFA) (80 µl) was added to the resultant mixture and vortexed, the mixture was incubated for 1 hour at 70°C for derivatization. The analysis was performed on GC/MS apparatus-Agilent 7890A mass spectrometer interfaced to MS-LECO, Pegasus® HT high throughput MS equipped with DB-1 high temperature column 29.30 m in length, 250 mm internal diameter and 0.10 µm in thickness (Agilent Technologies, J & W Scientific Products, Santa Clara, CA). One µl of the extract was injected into the instrument using helium as the carrier gas. The initial GC temperature started at 70°C which was then held at 260°C for 1 min and followed by 10 min at a rate of 4°C per min. In the MS setting, the electron impact (EI) ion source was maintained at a temperature of 260°C, with a filament bias set at -70 V. The mass range for data acquisition was set to 50–600 m/z, and the data acquisition rate was set to 5 spectra per second. The compound identification was conducted by comparing with the mass spectral available in National Institute of Standard and Technology (NIST) (Davies *et al*., 1990).

3.5.2. LC-MS/MS analysis

Ten microliter (µl) of the filtrate obtained was injected into LC-MS instrument. LC-MS/MS analysis was executed in Shimadzu Prominence-I HPLC (Shimadzu Corporation, Kyoto, Japan) coupled with a Shimadzu triple quadrupole LCMS-8045 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) equipped with C18 column 50 mm x 4.6 mm and 5 µm (Phenomenex Kinetix, California, U.S.A.). Column temperature was 30ºC, sample cooler temperature was

25ºC with mobile phases comprising of formic acid (0.1%) in water and acetonitrile (100%). Sample input and acquisition was 10 µl with flow rate of 0.8 ml/min and run time was 60 min. Electrospray ionization (ESI)–Mass spectrometer analysis was conducted in both positive and negative ion modes using the following operational parameters: drying gas flow rate of 10 liters/min, interface temperature set to 300°C, nebulizer gas flow rate of 3 litres/min, desolvation line temperature maintained at 250°C, and a heating gas flow rate of 10 litres/min. Full-scan mass spectra were obtained spanning the mass range of m/z 50–2000.

3.5.2.1. Preprocessing of raw data obtained from LC-MS/MS analysis

The preprocessing of the raw data was carried out using an open source software MZmine 2.53. (https://mzmine.github.io/). The MZML files were loaded and trimmed for a retention time of 0-60 min. The mass detection algorithm was then used to determine the masses of the samples and then create chromatograms for all the samples using chromatogram builder algorithm. Subsequently, join aligner algorithm was used to aligned peaks which aligns detected peaks for a specific sample. KEGG/Plantcyc compounds databases were used for metabolite identification. Then, the resulting data in csv (comma separated values) format were exported for further analysis. These csv files were formatted as per software requirement for statistical analysis to generate further analysis plots and data.

3.5.2.2. Processing of data and normalization

MetaboAnalyst 5.0. (https://www.metaboanalyst.ca/) was used for processing of data. The peak intensity data table containing samples in columns and features in rows, with class labels immediately following the sample IDs was uploaded in a comma separated values (.csv) file format. Data integrity check was conducted prior to data analysis, to ensure that all required information had been obtained. Filtering of data was then performed using interquantile range to remove irrelevant variables for modelling purposes to reduce noise. One or more combination of normalization processes is utized to achieve better results. In this study, the normalization procedures were carried out by following the options as (a) Row-wise procedures-normalization by the sample median. (b) Data transformation-log transformation (base 10). (c) Data scaling-pareto scaling.

3.5.2.3. Statistical data analysis

Different methods were employed to analyze the data obtained using LS-MS/MS analysis. In univariate analysis method, Fold-change analysis and Correlation analysis was performed. The Multivariate analysis method included Principal Component Analysis (PCA) and clustering analysis included heatmap.

3.6. Reverse phase high performance liquid chromatography (RP-HPLC) analysis

Quercetin, myrcetin and rutin contents in bark, leaves and pericarp were quantified using a RP-HPLC system (Agilent Technologies 1260 infinity, California, U.S.A), consisting of solvent delivery pump (Agilent 1260 infinity capillary pump) and detector (Agilent 1260 Infinity II Diode array detector WR). The separation by chromatography was accomplished on 250 x 4.6 mm; 5 µm analytical column C18 kinetex (Phenomenex, Torrance, California, U.S.A). The mobile phase consisted of methanol: 0.1% (v/v) phosphoric acid (65:35) for quercetin and rutin, and water: methanol: acetonitrile (52:42:5) (v/v) for myrcetin. The solvent used for mobile phase were all HPLC grade and degassed through ultrasonication prior to use. The delivery was carried out in isocratic mode at flow rate of 1.0 ml/min at ± 20°C with injection volume of 20 µl, and the detector was set at 370 and 575 nm. A stock of standard quercetin (5 mg/ml) was prepared by dissolving in methanol. For the preparation of calibration plot, suitable aliquots were transferred into mobile phase for obtaining concentrations of 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5 mg/ml of quercetin, rutin and myrcetin. Twenty µl of the sample was injected into the column using optimized conditions. The peak area *vs*. the concentration of standard injected was plotted to determine the linearity. The methanol extracts of bark, leaves and pericarp were dissolved and filtered through membrane filter (0.22 µm) using microsyringe/micropipette. Quercetin, myrcetin and rutin in the samples was identified and quantified by comparison with external standard. Each samples were injected in triplicate.

3.7. Evaluation of callus induction potential of different explants

The callus induction potential of leaves, cotyledons and endosperm was evaluated in the study. The cotyledons and endosperm were obtained from aseptically germinated 2-weeks-old seedlings whereas the young leaves were taken from 4-week-old *in vitro* germinated seedlings. The explants were cut into small pieces (approx. 1-1.5 cm) and inoculated in MS medium with 3% sucrose (Merck, Mumbai, India), 0.8% (w/v) agar (Sigma-Aldrich, Missouri, U.S.A.) containing plant growth regulators (Sigma-Aldrich, Missouri, U.S.A.) *viz*., either individually or in combinations for callus induction. Five different types of media consisting of 2 mg/l 2,4-dichlorophenoxyacid (2,4-D) alone or in combination with 1 mg/l 6-benzylaminopurine (BAP) or 1-naphthaleneacetic acid (NAA) or kinetin (KIN) or 2 mg/l NAA with 1 mg/l BAP were used to evaluate their effects on callus induction. The pH of the media was adjusted to 5.6 to 5.8 using

1M sodium hydroxide (NaOH) and 1N hydrochloric acid (HCl) prior to autoclaving at 120°C and 15 psi pressure for 15 min. The explants (5-6) were inoculated into each culture bottle (60 x 110 mm) containing approximately 40 ml of the media. The cultures were incubated in dark at 25 ± 2°C and examined periodically for recording the duration of callus initiation from the leaf explants. The observations on frequency of callus initiation, nature and color of callus were taken after 4 weeks of culture on the medium. For measuring the fresh weight and dry weight, approximately 1 gm of callus was placed in each culture bottle containing 40 ml of the medium augmented with different growth regulators. The calli were removed from the culture bottles and dried in an oven (45°C) for 48 h for dry weight estimation. The weight (gm) of the callus at the time of harvesting and at inoculation was determined and the difference was used for measuring the fresh weight and dry weight and expressed as grams per 100 ml medium. Each culture bottle was treated as a replicate in the analysis of the results. To determine the growth curve for leaf derived callus, fresh weight and dry weight of the callus was determined at weekly intervals for 6 weeks after sub-culture of 1 gm of callus on medium with 2 mg/l 2,4-D and 1 mg/l BAP and expressed as grams per 100 ml medium.

3.8. *In vitro* regeneration of plant through direct shoot organogenesis from zygotic embryo axes of *G. moluccana*

3.8.1. Direct shoot bud induction and multiplication

The zygotic embryo axes were carefully separated from overnight soaked sterilized seeds without damaging and used for induction of multiple shoots. Different concentrations of BAP and KN, alone or in combination were used in full-strength MS medium for inducing multiple shoots. The clump of multiple shoots was separated from the explants after 4 weeks and sub-cultured on half-strength MS medium with reduced concentrations of BAP every 3 weeks and was finally cultured into half-strength MS medium without BAP. The cultures were monitored for the frequency of multiple shoots and the average number of shoots per explant.

3.8.2. Rooting initiation and establishment in the field

After the emergence of elongated shoots from the clumps, 3-4 cm shoots were separated and cultured on half-strength MS medium containing either 0.5 mg/l of α-naphthaleneacetic acid (NAA) or indole 3-butyric acid (IBA) or without growth regulators for root induction. After 4 weeks of culture, the duration and frequency of root induction, as well as the average number of roots per shoot, were recorded. The rooted plantlets were separated from the culture bottles, which was then washed thoroughly but gently under running tapwater to remove agar stuck on the roots. The plantlets were placed in plastic pots containing soilrite and acclimatized in culture room for 2 weeks by covering with polythene covers to maintain the humidity. The polythene covers were then removed and plantlets were kept in culture room for another week, after which they were transferred to greenhouse and subsequently the plantlets were transferred to earthen pots containing soil and farmyard manure. They were subsequently transplanted in an open field and the survival frequency was determined.

3.9. Effect of salicylic acid (SA) and jasmonic acid (JA) on biomass, total phenolics, flavonoids and antioxidant activities in leaf callus cultures

Freshly harvested callus obtained after 5 weeks of subculture was used for elicitation experiments with SA and JA. The callus was inoculated in MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l BAP with different concentrations (50, 100, 150 and 200 µM) of SA and JA (Sigma-Aldrich, Missouri, U.S.A) which were dissolved in the solvent (ethanol) and added aseptically after filter sterilization with 0.22 µm membrane filter (Axiva Sichem Biotech, New Delhi, India) to the culture media. The culture medium without elicitors served as controls. The experiments were carried out by inoculating 1 gm of callus into each culture bottle containing 40 ml of medium. The effects of SA and JA on fresh weight and dry weight were determined after 5 weeks of culture on the media.

For estimating the total phenolics and flavonoids, the calli were carefully removed from the culture bottles every 5 days for the following 30 days. The calli were dried in an oven (45°C) for 48 h. The dried callus was then ground into fine power and used for preparing the extracts. The dried callus powder (5 gm each) was placed in 100 ml of methanol and stirred overnight in a magnetic stirrer. The extracts were centrifuged at 13,000 rpm for 15 min and the supernatants were recovered. The supernatants were then concentrated in rotary vacuum evaporator (Heidolph Instruments, Schwabach, Germany) until it was reduced to powder/dried form, which were stored at -20°C until use for different experiments.

Total phenolic acid (TP) and total flavonoid (TF) contents were estimated as per the protocols described in **Section 3.2.**

3.9.1. DPPH (2,2-dipheyl-1-picrylhydrazyl) and ABTS (2,2’ azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging potential of callus extracts

The free radical scavenging activities of the callus cultures treated with different concentrations (50, 100, 150 and 200 uM) of SA and JA elicitors for 20 days was assessed using

DPPH radical scavenging activity by following the protocol described in **Section 3.3.4** and ABTS decoloring assay.

ABTS decoloring assay was performed as per the specification of Re *et al.* (1999). The free radical ions were generated by mixing 20 ml each of potassium persulphate (2.45 mM) and ABTS (7 mM) (HiMedia Laboratories Pvt. Ltd, Mumbai, India). The blend was kept in the shade at ambient temperature for 15-20 h. The working stock was prepared by diluting using methanol and the absorbance was measured to achieve absorbance of 0.700 at 734 nm. Plant extracts (50-350 µg) were reacted with ABTS radical solution (200 µl) at room temperature for 30 min. The absorbance of the resulting decrease in the coloration was measured in 96-wells microplate reader at 734 nm. Ascorbic acid served as positive reference control. The percentage radical scavenging activities were calculated as shown in **Section 3.3.4.**

3.9.2. Quantification of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA)

Hydrogen peroxide levels were determined as per the method reported by Alexieva *et al.* (2001). The callus (500 mg) was homogenized in an ice-cold mortar and pestle with 5 ml of 10 % (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15000 rpm for 10 min at 4°C. Equal volume of the supernatant and 10 mM phosphate buffer (pH 7) was taken and 1 ml of 1M potassium iodide was added to it. The mixture was measured for its absorbance at 390 nm wave length spectrophotometrically. The mixture without the supernatant was used as blank. The content of the H_2O_2 in the sample was estimated as per the standard curve and expressed in micro mol per gram fresh weight (µmol g^{-1} FW).

Lipid peroxidation assay was carried out by measuring the malondialdehyde (MDA) produced during thiobarbituric acid (TBA) reaction as per the method of Kramer *et al.* (1991). The callus (100 mg) was homogenized with 10% TCA and centrifuged at 15000 rpm for 10 min at 4°C. The supernatants were collected and 0.5 ml of it was added to 1.5 ml of 0.5 % TBA (w/v) in 20% TCA. The mixture was incubated in water bath at 95°C for 25 min and the reaction was ended by incubating it on ice. The mixture was centrifuged at 15000 rpm for 5 min at 4°C. The absorbance of the solution was measured at two different wavelengths *i.e.* 532 nm and 600 nm. The OD 600 values of the non-specific absorption were subtracted from the OD 532 values of MDA-TBA complex (red color). The MDA concentration was calculated based on Beer-Lambert's law with an extinction coefficient of 155 $mM^{-1}cm^{-1}$. The results were presented in µmol g^{-1} FW.

3.9.3. Extraction and assay for antioxidant enzymes activities

3.9.3.1. Protein extraction

The protein was extracted according to the method described by Ben Amor *et al.* (2005). The fresh callus (~1 gm) were ground to fine powder using liquid nitrogen in mortar and pestle and homogenized with 100 mM tris hydrochloride (Tris-HCl buffer; pH 8) comprising of 10 mM ethylenediamine tetraaceticacid (EDTA), 20 mM magnesium chloride ($MgCl_2$), 1 mM dithiothreitol (DTT), 50 mM potassium chloride (KCl), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.1 % triton X-100 (v/v) and 10 % polyvinylpyrrolidone (PVP). The mixture was centrifuged at 14000 rpm for 30 min at 4°C. The protein concentration in the supernatant was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard. The protein was stored at -20°C and used for various antioxidant assays.

3.9.3.2. Superoxide dismutase (SOD) activity assay

Beauchamp and Fridovich (1971) method was used to determine the SOD (EC1.15.1.1) activities in the samples. To 20-50 µl of the enzyme extract, a reaction cocktail consisting of 1.5 ml of 50 mM potassium phosphate buffer (pH 7.8), 13 mM L-methionine (0.2 ml), equal volume of 75 µM nitro blue tetrazolium chloride (NBT), 4 µM riboflavin, and 1 µM EDTA was added in a final volume of 3 ml reaction. The reaction was initiated by exposing it with fluorescent light of photosynthetic photon flux of 50 µM m^{-1} s^{-1} for 15 min. The resulting blue color developed in the solution was spectrophotometrically measured at 560 nm. One unit of SOD activity was taken as 50% of the inhibition of the color developed and expressed as Umg^{-1} of protein.

3.9.3.3 Catalase (CAT) enzyme activity assay

CAT (EC 1.11.1.6) enzyme activity assay was carried out as described by Aebi (1984). The samples of 500 mg were ground in 5 ml of 50 mM buffer phosphate (pH 7) and 50 mg of PVP in a mortar and pestle that was previously cooled to 4°C. The homogenous mixture was centrifuged at 13,000 rpm for 15 min and the supernatant was collected. In order to determine the catalase enzyme activity, the reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.1 ml of 3 mM EDTA, 0.1 ml of 20 mM H_2O_2 and the enzyme extract equivalent to 25 µg protein in a final volume of 1 ml was added and incubated. The absorbance was recorded for 1 min at 240 nm. The extinction coefficient of H_2O_2 at 240 nm is 43.6 M^{-1} cm^{-1}. A unit of catalase is defined as the quantity of enzyme necessary to decompose 1 µM of H_2O_2 per min at 25°C.

3.9.3.4. Peroxidase (POD) enzyme activity assay

POD (EC 1.11.1.7) activity was determined according to the method of Polle *et al*. (1994) based on its ability to convert guaiacol to tetraguaiacol (ε =26.6 mM^{-1} cm^{-1}). The 2 ml reaction mixture comprised of 0.5 ml of 50 mM potassium-phosphate buffer (pH 7.0), 0.5 ml of 3.4 mM guaiacol, 0.5 ml of 0.9 mM H_2O_2 and 0.5 ml enzyme extract. The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm.

3.9.4. Effects of SA and JA in the regulation of AsA-GSH (ascorbate-glutathione) pathway

The influence of SA and JA on the enzymes involved in Halliwell-Asada cycle or ascorbate-glutathione (AsA-GSH) pathway in callus cultures was studied. The AsA-GsH pathway is involved in the detoxification of hydrogen peroxide using four crucial enzymes *viz*., APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase) and GR (glutathione reductase).

3.9.4.1. Analysis of APX, GR, MDHAR and DHAR enzyme activities

All enzyme assays were carried out with the crude extracts. APX (EC 1.11.1.11) was assayed according to the method of Nakano and Asada (1981). The reaction mixture of 1.5 ml for measuring APX activity contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA (0.1 ml), 0.5 mM ascorbic acid and enzyme extract equivalent to 25 µg of protein. The reaction was initiated by adding 20 mM H_2O_2 and the activity of the enzyme was recorded as decrease in the absorbance at 290 nm for 1 min. The amount of ascorbic acid oxidized was calculated from the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of the enzyme is equal to the amount of APX catalyzing the oxidation of 1 µmol ascorbate per min.

Foyer and Halliwell (1976) method was used for determining GR (EC 1.6.4.2) activity by measuring the decrease in absorbance at 340 nm wavelength and calculated from the 6.22 mM^{-1} molar extinction coefficient. The 3 ml reaction mixture contained 100 mM Tris-HCl (pH 8), 0.5 mM EDTA, 0.5 mM $MgCl_2$, 1 mM nicotinamide adenine dinucleotide phosphate (NADPH), 100 µl extract and 10 mM GSSG. The reaction was initiated by the addition of NADPH. One unit of GR activity is the reduction of 1 µmol NADPH per min.

MDHAR (EC 1.6.5.4) activity was measured by the method of Hossain (1984). The reaction mixture of 1 ml consisted of 100 mM potassium phosphate buffer (pH 7.5), 2.5 mM ascorbic acid, 0.01 mM EDTA, 0.25 mM NADH, 0.0125 % triton X-100, 50 µl extract and 0.4 units of ascorbate oxidase. The reaction was initiated by adding ascorbate oxidase. The decrease in absorbance due to NADPH oxidation was measured at 340 nm wavelength and calculated using extinction co-efficient of 6.22 mM^{-1} cm^{-1}. One unit of MDHAR activity is defined as the amount of enzyme that oxidizes 1 µmol NADH per min.

DHAR (EC 1.8.5.1) activity was assayed according to the method described by Nakano and Asada (1981). The reaction mixture of 3 ml consisted of 100 mM potassium phosphate buffer (pH 7), 20 mM GSH, 1 mM dehydroascorbate (DHA), 0.178 mM EDTA and 100 µl enzyme extract. The absorbance was measured at 265 nm and the enzyme activity was calculated from the 7.0 mM^{-1} cm^{-1} extinction co-efficient. One unit of the DHAR activity is the amount of enzyme that produces 1 µmol ascorbate per min.

3.9.4.3. Determination of ascorbate (AsA), total glutathione (TGSH), reduced glutathione (GSH) and oxidized glutathione (GSSG) contents

Ascorbate was determined using the method of Foyer *et al*. (1983). The freshly harvested callus was immersed in liquid nitrogen and ground to fine powder and homogenized with 1 ml of 4.5 M perchloric acid. The homogenate was allowed to thaw for 30 min at 4°C. The soluble fraction (100 µl) was neutralized with 1.25 M of potassium carbonate (pH 5.6) and 0.1 M sodium phosphate buffer (pH 5.6). Ascorbate oxidase (5 units) was added to the reaction mixture in a final volume of 1 ml and the decrease in the absorbance was measured at 265 nm wave length.

GSH and GSSG were assayed as per the method described by Sarker and Oba (2018). The callus samples (~1 gm) were homogenized in 10 ml of 5% sulphosalicylic acid and the homogenate was centrifuged at 15000 rpm for 30 min at 4°C. To determine the total glutathione (GSH + GSSG) in the sample, 1 ml of the supernatant was taken and then 1.5 ml of 0.5 M phosphate buffer (pH 7.5) and 50 µl of double distilled water was added. For determining GSSG in the sample, another part of supernatant (1 ml) was taken and then 1.5 ml of 0.5 M phosphate buffer (pH 7.5) and 50 µl of 2-vinylpyridine was added. The tube was mixed vigorously until an emulsion was formed and then incubated at room temperature for 1 hour. The difference between the total glutathione and GSSG is used to determine GSH content. The reaction mixture consisted of 0.2 mM NADPH, 100 mM phosphate buffer (pH 7.5), 5 mM EDTA, 0.6 mM DTNB 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB), and 3 units of GR was used for glutathione content estimation. The reaction was started by adding the sample extract of 0.1 ml to the reaction mixture and the rate of reaction was determined by measuring the change in absorbance at 412 nm for 1 min. Two standard curves were prepared based on GSSG and total glutathione. Data were represented as nmol g^{-1}FW.

3.10. Phenylalanine ammonia lyase (PAL) enzyme activity assay

The crude enzyme for PAL assay was extracted from callus (~1 gm) with borate buffer (pH 8.8). The callus was ground in the buffer with ice-cold mortar and pestle and then centrifuged at 10000 rpm and 4°C for 20 min. The supernatant obtained was used for the assay as per the method of Wu and Lin (2002). The reaction cocktail of 1 ml contained 150 mM tris-HCl (pH 8), 12 mM L-phenylalanine and 50 µg of protein followed by incubation for 30 min at 30°C. The reaction was stopped using 100 µl of 6 N HCl and the absorbance was taken at 270 nm. The extinction coefficient for PAL at 270 nm is 19.73 $\mathrm{mM}^{-1}\mathrm{cm}^{2}$.

3.11. Expression analysis of *APX*, *SOD*, *CAT*, *POD* and *PAL* genes using Real-Time PCR

3.11.1. Extraction of total RNA and cDNA synthesis

Total RNA was isolated from the callus elicited with SA and JA along with untreated controls using a modified sodium dodecyl sulphate (SDS)/TRIzol method (Wang *et al*., 2012) The quality of total RNA was analyzed by agarose gel electrophoresis. The RNA was quantified using NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, U.S.A.) at 260 nm and the purity was checked from A260/A280 ratio. First strand (cDNA) synthesis was carried out with 2 µg of total RNAs isolated from elicitor treated and control (untreated) callus cultures using the PrimeScript First Strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan) in accordance with the manufacturer's instructions.

3.11.2. Primer design and quantitative real-time Polymerase chain reaction (RT-PCR)

Primers were designed for the *APX*, *SOD*, *CAT*, *POD* and *PAL* genes and synthesized commercially for analyzing their transcript levels in elicited and control (untreated) callus cultures (**Table 1**). The coding sequence (CDS) of these genes from the related species such as *Ricinus communis*, *Manihot esculenta*, *Hevea brasiliensis* and *Jatropha curcas* belonging to the same family were downloaded from National Centre for Biotechnology Information (NCBI) database as a FASTA file and multiple alignments were carried out (T-Coffee). The position where the sequence had more homology was used for primer designing by NCBI primer design tool. The internal complementarity and formation of hairpin loops were checked by Integrated DNA technologies (IDT) tools software and the primers that did not have hairpin loops and self-complementarity was selected. First-strand cDNA samples were diluted 2.5 times and 1 µl of the diluted sample was taken as a qRT-PCR template in 10 µl of total reaction volume containing 0.4 µM gene-specific primers and 5 µl of SYBR Premix ExTaq II (TliRNase H Plus) with ROX

(Takara Bio Inc., Japan). The samples were appraised in three technical replicates. PCR analysis was carried out in a Realplex amplifier (Eppendorf, Hamburg, Germany) with the following cycle parameters: 95°C for 5 min; 40 cycles of 95°C for 20 sec, Tm (Table 1) for 20 sec, and 72°C for 20 sec; followed by a melting curve to ensure that each amplicon was a single product. The relative fold-change in RNA expression was estimated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Actin with primer sequence 5'-TGAGAGGGAAATCGTGCGTG-3' and 3'-TGCTTGCTGATCCACATCTGC-5' was used as internal control to normalize the real-time amplification data.

Table 1. Primers used for Real-Time PCR to analyze the gene expression of *APx, POD, CAT, MnSOD* and *PAL* in SA and JA-treated and control callus culture of *G. moluccana*

Gene name	Primer ID	Primer sequence (5'-3')	% GC content	Length	Tm (ºc)
Ascorbate peroxidase	*APx F*	GAACTTACGACGCGAAC	52.94	17	52.77
	APx R	GCTCAAGGAGTCTGACA	52.94	17	52.77
Peroxidase	*POD F*	TGCTTCGTCTCCATTTCC	50.00	18	53.69
	POD R	AGTTCCGATTGCTCACTG	50.00	18	53.69
Catalase	*CAT F*	CGCCCACAATTCCTCAT	52.94	17	52.77
	CAT R	GCTAGCTTCTCCACCAAA	50.00	17	53.69
Superoxide dismutase	*Mn SOD F*	CAGAGCGCCATCAAGTTCAATGGT	50.00	24	62.72
	Mn SOD R	TGGGTCCTGGTTTGCAGTTGTCT	52.17	23	62.43
Phenylalanine ammonia lyase	*PAL2 F*	TGTCGAACATGCCTTGGCGAAT	50.00	22	59.7
	PAL2 R	TCCGGTGAGCAATCCACTACCTAA	50.00	24	59.5

3.12. Metabolite profiling in callus cultures elicited with salicylic acid and jasmonic acid

The leaves-derived callus cultures were harvested after 2 weeks of subculture on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP with 3% sucrose and 0.8% agar. The callus cultures were given elicitation treatments by transferring to fresh MS medium containing 50 µM of salicylic acid and 150 µM jasmonic acid for 20 days and used for metabolite profiling. The callus cultures that were subcultured on fresh medium for the same duration (20 days) without any treatment served as controls. The metabolite profiles of callus cultures were also compared with mature leaves. The samples were dried in an oven (45ºC) until moisture content was completely removed. The dried extracts were ground to fine power, sieved, soaked in methanol and filtered. HPLC grade methanol was used to re-dissolve the concentrated methanolic extract samples (25 mg/ml) described above. The extracts were then filtered through membrane filter

(0.22 µm) (Axiva Sichem, New Delhi, India). Ten microliter (µl) of the filtrate obtained was injected into LC-MS instrument (LCMS-8045) for LC-MS/MS analysis. The details of the analysis performed are as described in **Sections 3.5.2 (3.5.2.1 to 3.5.2.3)**.

3.13. *De novo* transcriptome analysis

The leaves-derived callus cultures obtained after 2 weeks of subculture on medium with 2 mg/l 2,4-D and 1 mg/l BAP with 3% sucrose and 0.8% agar were used for treatments with SA and JA. The callus cultures treated with 50 µM of salicylic acid and 150 µM jasmonic acid for 20 days along with untreated callus cultures, and leaves of 4-week-old seedlings as described above were used for RNA extraction as well as stored at -80ºC for further validation studies.

3.13.1. RNA isolation, library creation and sequencing of the transcriptome

RNA isolation of the above mentioned samples was carried out using RNeasy plant kit (Qiagen, Hilden, Germany) following the protocol provided by manufacturer. The RNA integrity number (RIN) was verified (≥7) through TapeStation (Agilent technologies, California, U.S.A.) and quantified using Qubit fluorometer (Thermo Scientific, Massachusetts, U.S.A.). Further the library was prepared by TruSeq Stranded Total RNA with Ribo Zero Plant (Illumina INC, California, U.S.A.) and sequenced by 2 x 150 bp paired end sequencing on an Illumina HiSeq X10 platform (Illumina INC, California, U.S.A.).

3.13.2. *De novo* assembly, functional annotation and gene ontology (GO) analysis

The raw data obtained from RNA sequencing was filtered using AdapterRemoval2 to check and filter out the adapters and low quality bases (≤Q20), and rRNA were removed using bowtie2 based on SILVA database (https://www.arb-silva.de/). The cleaned reads obtained for all samples was used to generate a pooled assembly using Trinity assembler (Trinity 2.8.4) with default parameters (Grabherr *et al*., 2011). Gene expression estimation of the assembled data was carried by Kallisto 0.46.0. The assembled unigenes were annotated using Uniprot Plant Database BLASTX (2.3.0) program. The best BLASTX hits were purned out on the basis of query coverage, identity and similarity score, and gene descriptions using E-value cut off value of 10^{-3}. GO term (http://www.geneontology.org/) annotation was used to classify the function of assembled unigenes into cellular components, molecular function and biological processes through the UniProt database (Ashburner *et al*., 2000; The Gene Ontology Consortium, 2021).

3.13.3. Differential gene expression (DEGs) analysis

Trinity package (Grabherr *et al*., 2011; Haas *et al*., 2013) was used to identify the differentially expressed genes between salicylic acid and jasmonic acid treated callus cultures with untreated callus cultures (control) of *G. moluccana*. Transcript abundance and counts was estimated using kallisto and cross samples normalized TPM/TMM (Transcripts Per Million transcripts) values were obtained. The counts were used to analyze differential expression with edgeR. The differentially expressed genes with log2 fold change of ≥2 and ≤ -2 and p value of 0.05 was considered as significant. Pathway analysis was conducted by comparison of gene number of each pathway in DEG to genome background. Heat maps of top differentially expressed transcript/isoforms across samples along with expression scattered plots between pairwise samples compared were generated.

3.13.4. Pathway enrichment analysis

KOBAS-i (http://kobas.cbi.pku.edu.cn/) (Bu *et al*., 2021) was utilized to assess the statistical richness of differentially expressed genes. The DEGs involved in different biological and physiological processes were putatively annotated. The pathway enrichment analysis was based on “annotate” and “identify” programs in KOBAS-i (KEGG Orthology Based Annotation System-Intelligent) with hypergeometric test statistical method and Benjamini and Hochberg, (1995) FDR (False discovery rate) correction method.

3.13.5. Transcriptome factor identification

The transcription factors were identified from the transcript sequences of leaves, control callus, SA and JA treated callus transcripts employing plant transcription factor database PlantTFDB 5.0 (http://planttfdb.gao-lab.org/index.php) (Jin *et al*., 2017) with default parameters. In this tool, bit-score was used as domain threshold instead of e-value cutoff. ESTscan 3.0 was used to help detect CDS region from the transcripts. These CDS regions were employed to identify transcription factors of different families.

3.13.6. Identification of genes implicated in phenylpropanoids, flavonoids, terpenoids, and lignins biosynthetic pathway

The putative genes associated with phenylpropanoids, flavonoids, terpenoids, and lignins biosynthetic pathway were identified using KEGG pathway database (https://www.genome.jp/kegg/kaas/) (Moriya *et al*., 2007), and their expression pattern in SA treated, JA treated and control callus cultures were studied.

3.14. Validation of differentially expresssed genes (DEGs) by quantitative real time PCR (qRT-PCR)

To affirm the expression profile of the top differentially expressed genes, qRT-PCR was used. Briefly, the total RNA was extracted from the control callus, SA and JA treated callus using RNeasy® plant kit (Qiagen, Hilden, Germany) as per the protocol. The quality of RNA was checked by NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, U.S.A.) at 260 nm and the purity was checked from A260/A280 ratio. First strand cDNA was synthesized with 2 µg of total RNAs using the PrimeScript First Strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan) in accordance with the protocol of manufacturer. The list of primers are provided in Table 2. PCR analysis was performed as described in the Section 3.11.2. The relative gene expression was estimated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Actin was used as internal control to normalize the real-time amplification data.

3.15. Statistical analysis

The data was acquired from 3 independent experiments, with 3 replicates used per treatment in each experiment. The values are represented as mean ± standard deviation (SD). The statistical significance of the means was assessed using one-way analysis of variance followed by Duncan's multiple range tests in SigmaPlot version 13. The probability of $p \leq 0.05$ was considered as significan

Table 2. Primers used for Real-Time PCR to confirm the expression of few selected genes of different secondary metabolite pathways identified from *de novo* transcriptome analysis of SA and JA-treated and control callus cultures of *G. moluccana.*

Gene name	Primer ID	Primer Sequence	% GC content	Length	Tm (°c)
Cysteine Protease	*CP-F*	ACCTCATCATCCCTTCTC	50	18	53.69
	CP-R	CGGCCCTTCTCTTTATTC	50	18	53.69
DETOXIFICATION 41	*DXT41-F*	AAGGCCAAGAAGGGTATG	50	18	53.69
	DXT41-R	CGGTGGTTCTCTTGTAT	50	18	53.69
2-Hydroxyisoflavone dehydratase	*HID-F*	CCCACCACCATGTATGTA	50	18	53.69
	HID-R	CCAGTCTCTGCTCGTATT	50	18	53.69
Shikimate dehydrogenase	*SDH-F*	GAGTCTGTGGTGGAAGAA	50	18	53.69
	SDH-R	GGCAACTCATGGAGAAAC	50	18	53.69
Copper transporter	*Ctr*	GCCAACCTGAGAAGAGAA	50	18	53.59
	Ctr	CAACCACACCAACATGAC	50	18	53.69
Salicylate carboxymethyltransferase	*SAMT-F*	GCTTGGAGGTTTCTGAAG	50	18	53.69
	SAMT-R	CAGCTCTCATGCACTTTG	50	18	53.69
Flavonoid 3'-monoxygenase	*F3'H-F*	CGTCACCAAACACTCTTC	50	18	53.69
	F3'H-R	GGTACAACGCCAGTAAAC	50	18	53.69
Senescence-associated protein	*SAG-F*	CGCCGTCCTACCTATTTA	50	18	53.69
	SAG-R	AGTTTCCCTCAGGATAGC	50	18	53.69
Serine carboxypeptidase-like	*SCPL-F*	TCTTGTGGTGAGTCTAGC	50	18	53.69
S-adenosyl methionine	*SAMe-F*	GTGCCCTTAGAGCACTCG	61.11	18	61.11
	SAMe-R	CGACTTGGTATTACTAAT	33.33	18	46.85
	SCPL-R	TTAGCCCTACTTCTTCCC	50	18	53.69
Cytochrome P450	*Cyt-P450-F*	GACTTGGGCACTTATCCT	50	18	53.69
	Cyt-P450-R	CTGGAGTTGGGAGTTCAT	50	18	53.69
MYB transcription factors	*MYB-F*	CTGATTGGGTTACAGGGA	50	18	53.69
	MYB-R	GCAGAATGTGTGGTCTTC	50	18	53.69
Rho N domain containing protein	*RhoN-F*	GAGTCTGTGGTGGAAGAA	50	18	53.69
	RhoN-R	GGCAACTCATGGAGAAAC	50	18	53.69
Small heat shock protein	*sHsp-F*	CCTTGCGGAAACGATTAG	50	18	53.69
	sHsp-R	GCCCTCTTGGGAACAATA	50	18	53.69
Sieve elements	*SE-F*	GGTAGTGGACCGATCAAT	50	18	53.69
	SE-R	ATCGAGGAGTGAAGGATG	50	18	53.69
Vegetative storage protein	*VSP-F*	GGCTGCTAAGTCAAATGG	50	18	53.69
	VSP-R	GCACTATTGCTCTCCTCT	50	18	53.69
Tinoplast intrinsic protein 21	*TIP-F*	GGCTTGCTGGTCTTATCT	50	18	53.69
	TIP-R	GACAAACGGATCGGAAAC	50	18	53.69
Peroxisomal (S)-2-hydroxy-acid oxidase	*2-HOAX-F*	GACTAGGACAGCCAGAAA	50	18	53.69
	2-HOAX-R	AGTGAAGGGAGAGAATGG	50	18	53.69
ATP-dependent RNA helicase (ATRX)	*ATRX-F*	TGGATATGCCTTCACCTC	50	18	53.69
	ATRX-R	CATTAGCCTCCCTGTTCT	50	18	53.69
Pentatricopeptide repeat containing protein	*PPR-F*	GGTGAGGGTGAAGATGAA	50	18	53.69
	PPR-R	CTACGCTGATGACCAGAA	50	18	53.69
Peroxidase	*POD-F*	CCTCCCAACATGACAATG	50	18	53.69
	POD-R	AACCGGAAGGAAGGATAG	50	18	53.69

WALLS ARE THIN1	*WAT1-F*	TCCTCCTCCTCCTCTAAT	50	18	53.69
	WAT-R	AAGAGACTGAACCTCCTC	50	18	53.69
Pathogenesis related	*SCP-PR1-F*	TCTTGTGGTGAGTCTAGC	50	18	53.69
	SCP-PR1-R	TTAGCCCTACTTCTTCCC	50	18	53.69
Glucose/Ribitol dehydrogenase	*RDH-F*	CCCTTGATTCGGAGATGT	50	18	53.69
	RDH-F	GAGCCATCCCTTGAGAAA	50	18	53.69
Transcription factor Elongation factor	*E2F-F*	GATGGACAGCCACTTAGA	50	18	53.69
	E2F-R	CTCTTGACCCACGTAGAA	50	18	53.69
Alpha-dioxygenase	*DOX1-F*	CCGTGATTGTCTGGTTTC	50	18	53.69
	DOX1-R	TGTCTCTGACTCTCTTGC	50	18	53.69
Proteinase inhibitor	*PI-R*	GTCCGTAATCACTGCATC	50	18	53.69
	PI-R	CCGACTTCAGGTGTGATA	50	18	53.69
Kunitz trypsin inhibitor	*KTI-F*	GGAGGGTTGGTGAATTTG	50	18	53.69
	KTI-R	CAGTGGGACAGTATCTGA	50	18	53.69
Chitinase	*CHI-F*	CCCTAGCATGAACAACAC	50	18	53.69
	CHI-R	CAGAGGTCCAATCCTTCT	50	18	53.69
Patatin like protein	*PLP-F*	GTCTGCGATTCTTGCTTC	50	18	53.69
	PLP-R	TGATGGAGGTGGTGTAAG	50	18	53.69
Catalase	*CAT-F*	CCCTAGCATGAACAACAC	50	18	53.69
	CAT-R	CAGAGGTCCAATCCTTCT	50	18	53.69
Beta-glucosidase	*GBA-F*	GGATTCTGGACCACGATA	50	18	53.69
	GBA-R	GTAGCAATGGCGATGTAG	50	18	53.69
Executer	*EX-F*	CAGATGTCTAAATGTCTAAG	50	20	51.15
	EX-R	GCTATTCAACTGCTGCTCC	50	19	56.67
Lactamase domain containing protein B	*LAC-B-R*	TCTCTACTGCCACTTCAC	50	18	53.69
	LAC-B-R	TCCCAATCTCCCTTTCTC	50	18	53.69
ELMO domain-containing protein A	*ELMO-F*	TTCCCTCTTGCTCTTCTC	50	18	53.69
	ELMO-R	GACTTCTAACGGGCTCTA	50	18	53.69
GIGANTEA	*GI-F*	AGTGGGTATCCAAGTCAG	50	18	53.69
	GI-F	GCCTGACAAGATCACAAG	50	18	53.69
Aquaporin/Tonoplastic intrinsic protein	*TIP-F*	GGCTTGCTGGTCTTATCT	50	18	53.69
	TIP-R	GACAAACGGATCGGAAAC	50	18	53.69
Isoflavone 4-O-methyl transferase	*I4’OMT-F*	GCTATTCCTCCTGCTGAT	50	18	53.69
	I4’OMT-R	CCATTGCCCTGTCTACTA	50	18	53.69
SNF1 protein kinase	*SnRK-F*	TCCAACTCATCACATCCC	50	18	53.69
	SnRK-R	CCTAGAGTGTGCGGTAAA	50	18	53.69
Abscisic acid responsive elements	*ABRE-F*	GCATGTCCTAAACCAACC	50	18	53.69
	ABRE-R	CCAGGATTGAGGAGTTCA	50	18	53.69
DETOXIFICATION	*DTX-F*	CCGTGATTGTCTGGTTTC	50	18	53.69
	DXT-R	TGTCTCTGACTCTCTTGC	50	18	53.69
Phosphoenolpyruvate carboxykinase	*PEPC-F*	GCAGGAGAGAGGTTGTAA	50	18	53.69
	PEPC-R	GAAAGGAGATCCGGCTAA	50	18	53.69
Transcription factor	*bHLH144*	GCACCCATGTTCACTTTC	50	18	53.69
	bHLH144	CCATCCTTCACTCCACTT	50	18	53.69
E3 Ubiquitin protein ligase	*RtE3UT-F*	CTCCAGCTTCTTCCTCTT	50	18	53.69
	RtE3UT-R	TCCCATCTCATTCCAGTC	50	18	53.69
Sucrose synthase	*SuSy-F*	AGGGAACCTGAGTTAGTG	50	18	53.69
	SuSy-R	CCACACAACAGGAAAGTC	50	18	53.69
Ribulose-1, 5-bisphosphate	*RuBisCo-F*	TGGTGATCTGTGGTTCTC	50	18	53.69

carboxylase					
	Rubisco-R	AAGTTGCCTACCTTCTCC	50	18	53.69
Chlorophyll a-b binding protein of LHCII type-1	*CBP-LHCII-F*	CAAGCTCACGGTTCTTAG	50	18	53.69
	CBP-LHCII-R	TACTTGACTGGTGACTCC	50	18	53.69
Thioredoxin	*TRX/TXN-F*	AGTGGGAACCTCTACAAC	50	18	53.69
	TRX/TXN-R	CTGATACTGTGGCGTTTG	50	18	53.69
Lipoxygenase	*LOX-F*	AGTGTCCTTGACCCATAC	50	18	53.69
	LOX-R	GTTCATCCTGGACTACCA	50	18	53.69
Fructose-bisphosphate aldose	FBA-F	CTTCCTTCACTGCCAAAG	50	18	53.69
	FBA-R	CTCAGCTGCTTACTACCA	50	18	15.69
EGF like domain containing protein	*EGF-F*	TCCAGGTCCATTGAGAAG	50	18	15.69
	EGF-R	GTCTTCGACGAGGATGAA	50	18	15.69
Alpha amylase inhibitor domain containing protein	*AAI-F*	CCTTCCTGCTACAGACAT	50	18	15.69
	AAI-R	CTATCTGCCTTTGCACTG	50	18	15.69
Late embryogenesis abundant protein	*LEA-F*	GTTGAAGAAAGGGCTGTG	50	18	15.69
	LEA-R	GCACAATTAGCTGGTCTG	50	18	15.69
WNT family member	*WNT-F*	GTCATAGTAGCCGAAGGA	50	18	15.69
	WNT-R	ACTAGACAGAACGAGTGG	50	18	15.69
Expansin	*EXP-F*	GCCAGAATGACACCATTG	50	18	15.69
	EXP-R	CAGCATTAAGCACAGCTC	50	18	15.69
Acetylserotonin O-methyl transferase	*ASMT-F*	GGGACAGATGTTGGGTAA	50	18	15.69
	ASMT-R	CAGATGCACCTCCTAAGT	50	18	15.69
Ethylene responsive transcription factor	*ERF-F*	GCTAGTTTGTGGTGGTTG	50	18	15.69
	ERF-R	TCACTTCTCAGCCTTCTC	50	18	15.69

4. Results

4.1 Assessment on the contents of phenolics and flavonoids and *in vitro* antioxidant activities of the different plant parts extracts of *G. moluccana*

4.1.1. Total phenolics and total flavonoid contents varied in different plant parts

The total phenolic acid (TPA) contents of the bark, leaves and pericarp extracted in different solvents (hexane, dichloromethane, ethyl acetate, ethanol, methanol and water) were determined using FC assay and the findings are expressed in mg GAE/g DW (milligram gallic acid equivalents per gram dry weight). The TPA content in different extracts ranged from 1.49 mg GAE/g in pericarp to 62.07 mg GAE/g DW in bark extracts (Figure 3). Higher polar solvent like methanol and ethanol with the exception of water showed higher TPA content for all plant parts used. The methanolic extract of bark displayed the highest TPA content (62.07 mg GAE/g DW) which was followed by ethanolic extract of bark (51.66 mg GAE/g DW) and methanolic extract of leaves (46.42 mg GAE/g DW). The lowest TPA content was observed in hexane extract of pericarp (1.49 mg GAE/g DW). Overall, the bark extracts exhibited the highest TPA content as compared to leaves and pericarp extracts in all solvents used. The TPA content in leaves was higher in all solvents than pericarp except in ethyl acetate where higher TPA content was observed in pericarp (14.63 mg GAE/gDW) than leaves (8.10 mg GAE/gDW).

The total flavonoid (TF) contents of bark, leaves and pericarp in different extracts were evaluated using $AlCl_3$ method and the results are presented in mg RE/g DW (milligram rutin equivalents per gram dry weight). The TF contents ranged from 3.12 in pericarp extract to 41.72 mg RE/g DW in bark extract (Figure 4). Among the different solvents used, methanolic extract of bark showed the highest TF content (41.72 mg RE/g DW) followed by methanolic extract of pericarp (39.3 mg RE/g DW). There were no significant differences observed in TF content in ethanolic extract of bark (31.75 mg RE/g DW) and pericarp (30.15 mg RE/g DW). Polar solvents *viz*., methanol and ethanol yielded higher content of total flavonoids. Among the different plant parts, bark exhibited highest TF content followed by pericarp and leaves in polar solvents whereas the flavonoid content of bark in hexane and DCM was similar to that of leaves.

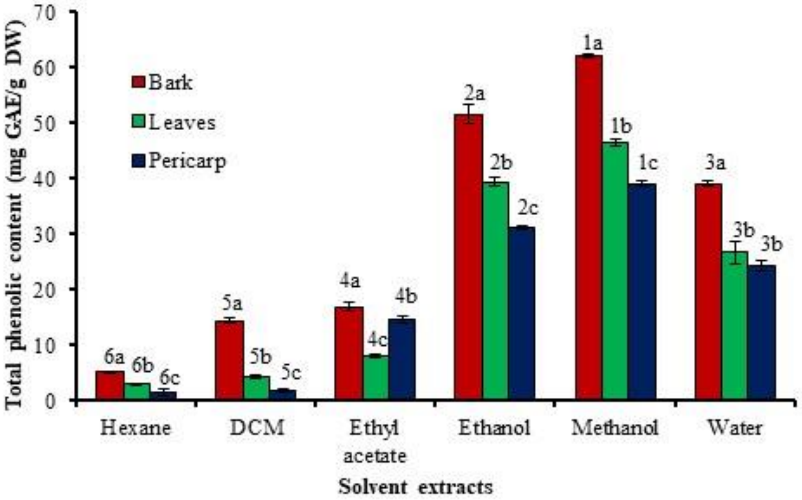


Figure 3. Total phenolic contents of bark, leaves and pericarp in different solvent extracts. Means followed by the same letter in the column are not statistically significant according to Duncan's multiple range test. Values represented are means ± standard deviation.

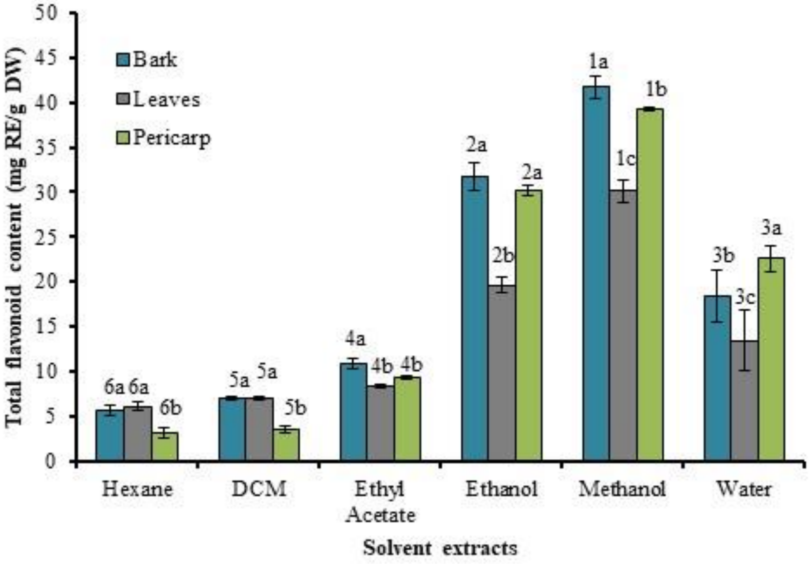


Figure 4. Total flavonoid contents of bark, leaves and pericarp in different solvent extracts. Means followed by the same letter are not statistically significant according to Duncan's multiple range test. Values represented are means ± standard deviation.

4.1.2. Bark exhibited higher *in vitro* antioxidant and free radical scavenging activities than pericarp and leaves

The antioxidant potential of the bark, leaves and pericarp extracts were appraised with several antioxidant assays as there is no single universally accepted antioxidant assay. The principle of each assay varies and depends on the type of compounds present in the extracts.

The total antioxidant capacity of plant parts extracted in different solvents was measured by phosphomolybdenum assay which relies on the capabilities of the plant extracts to reduce Mo (VI) to Mo (V) either through electron transfer or hydrogen atom transfer mechanism resulting in a greenish-blue complex. The results are expressed as milligram ascorbic acid equivalents per gram dry weight. In this study, the methanolic extracts revealed the most significant antioxidant capacity and the succeeding was ethanolic extracts in all plant parts compared to other solvents used (Figure 5). Among the plant parts, bark extract exhibited highest antioxidant activity (70.94 mg AAE /g DW) followed by leaves (32.33 mg AAE/g DW) whereas pericarp extracts exhibited the least activity in methanol (28.21 mg AAE/g DW). A similar pattern was observed for ethanolic extracts with the highest in bark (56.85 mg AAE/g DW) and the least was observed in pericarp extracts (21.74 mg AAE/g DW). The antioxidant capacity of hexane, dichloromethane, ethyl acetate and water for bark ranged from 11.83 to 17.37 AAE/g DW, leaves from 7 to 9.31 AAE/g DW and pericarp from 4.27 to 9.65 AAE/g DW. Thus, the highest antioxidant capacity exhibited by bark extracts could be due its greater ability to reduce Mo (VI) to Mo (V) as compared to leaves and pericarp.

The ferric reducing potential of bark, leaves and pericarp was estimated. The FRAP assay data reflect the concentration of electron-donating antioxidants responsible for the reduction of ferric iron (Fe3+) to ferrous iron (Fe2+). The methanolic extract of bark (156.38 mg AAE/g DW) followed by ethanolic extract of bark (137.82 mg AAE/g DW) exhibited the highest antioxidant activity (Figure 6). The ferric reducing antioxidant power of bark extracts were found to be greater than pericarp and leaves extracts in all solvents except for DCM and ethyl acetate where the values were either similar or lower than leaves. The reducing capacity of leaves in all solvents was higher than pericarp except in hexane where similar values were obtained. The FRAP values of hexane, DCM, ethyl acetate and water for bark ranged from 20.02 to 88.84 AAE/g DW, in leaves from 12.87 to 60.89 AAE/g DW whereas for pericarp it ranged from 12.87 to 44.0 AAE/g DW. The lowest reducing capacity was found in hexane extracts of different plant parts.

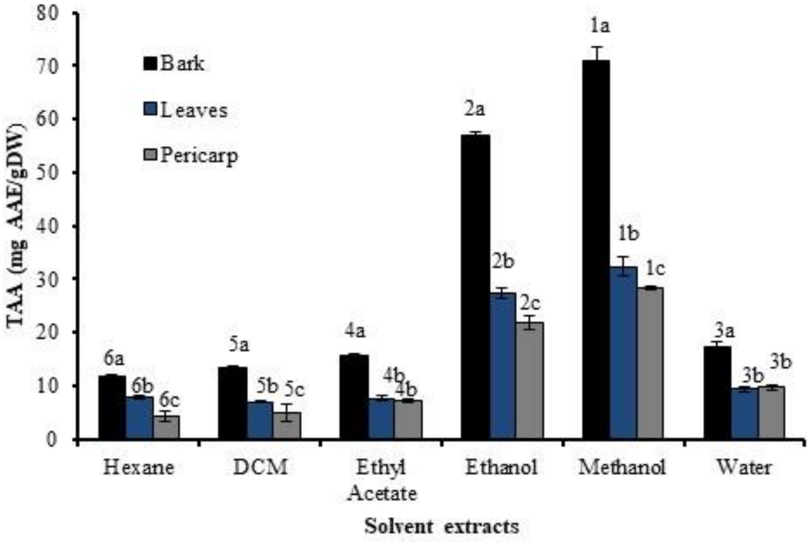


Figure 5. Total antioxidant capacity (TAC) of bark, leaves and pericarp in different solvent extracts by phosphomolydenum assay. Means followed by the same letter within the column are not statistically significant according to Duncan's multiple range test. Values represented are means ± standard deviation.

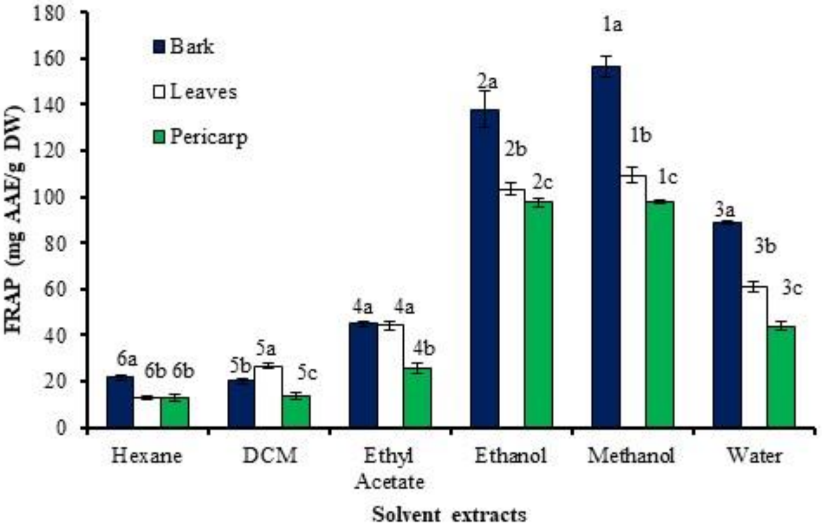


Figure 6. Ferric ion reducing antioxidant power (FRAP) of bark, leaves and pericarp in different solvent extracts. Means followed by the same letter in the column are not statistically significant according to Duncan's multiple range test. Values represented are means ± standard deviation.

Cupric antioxidant reducing capacity (CUPRAC) assay uses a chromogen known as copper (II)-neocuproine [Cu $(Nc)_2^{2+}$], a light blue complex, which is reduced by extracts to form copper (I)- neocuproine chelate [Cu $(Nc)^{2+}$], a light amber colour complex with maximum absorbance at 450 nm. The findings are presented in mg AAE/gDW (milligram ascorbic acid equivalents per gram dry weight). The methanolic extract of bark (246.32 mg AAE/g DW) followed by ethanolic extracts of bark (126.26 mg AAE/g DW) exhibited higher cupric antioxidant reducing capacity (Figure 7)**.** The CUPRAC values of leaves were higher than pericarp extract. The least values of CUPRAC were observed in hexane extract (bark-7.89 AAE/g DW; leaves- 6.64 AAE/g DW and pericarp- 3.41 AAE/g DW). CUPRAC of DCM, ethyl acetate and water extracts of bark ranged from 13.71 AAE/g DW to 64.19 AAE/g DW, leaves from 14.87 AAE/g DW to 28.83 AAE/g DW, and pericarp from 13.40 AAE/g DW to 24.50 AAE/g DW.

The total antioxidant capacities determined though different assays such as PM, FRAP and CUPRAC and total phenolic acids content were positively coorelated, similarly, it was positively coorelated with total flavonoid content as depicted with R^2 values in Table 3.

DPPH scavenging assay is based on the transfer of hydrogen atom from the antioxidant agent in the extract to the DPPH free radical which is visualized by reduction of purple colored radical to pale yellow color. The antioxidant activity of several plant extracts has been quantified by means of percentage inhibition of DPPH using ascorbic acid as positive control. The extracts showed a concentration dependent scavenging activity, with the highest scavenging activity of 94% in the methanolic extract of bark and pericarp at 300 µg/ml followed by methanolic extract of leaves (87%) in comparison to 97.6% observed in positive control at the same concentration (Figures 8-10)**.** The ethanolic extracts of different plant parts also exhibited strong scavenging activity at 300 µg/ml with the highest recorded in pericarp (89.57%) followed by bark (80.05%) and the lowest in leaves (71.6%). The lowest activity at 300 µg/ml concentration was observed in hexane extract with 29.5% for bark, 22.37% for pericarp and 21.93% for leaves. The scavenging activities of DCM, ethyl acetate and water of bark extracts at 300 µg/ml ranged from 39.2% to 48.8%, leaves from 31.6% to 43.4% while that of pericarp from 34.87% to 52.7%, which were low in comparison to ethanol, methanol and positive control (Figures 8-10)**.**

Under mild acidic conditions, the phenolics and flavonoids bind to the fractions of Fe^{2+} whilst the rest of ions interact with ferrozine to create a stable deep purple or red color complex (ferrous ion-ferrozine complex). The presence of chelators in the extract prevents the formation

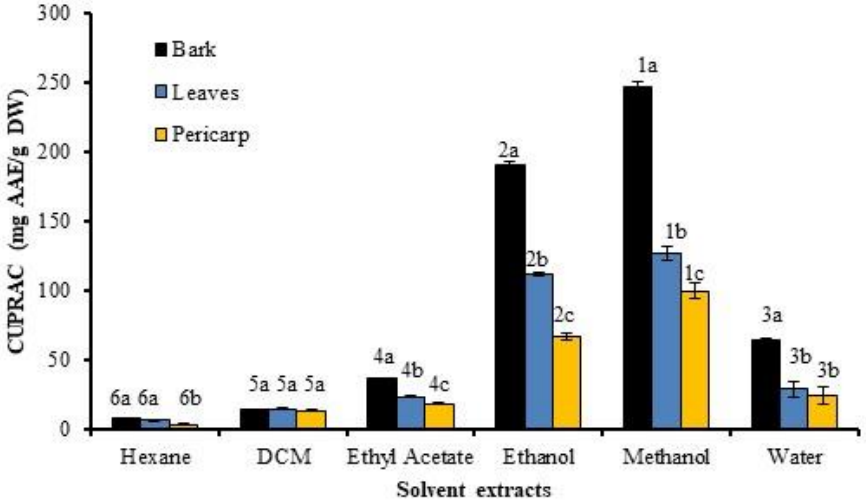


Figure 7. Cupric antioxidant reducing capacity of bark, leaves and pericarp in different solvent extracts. Means followed by the same letter are not statistically significant according to Duncan's multiple range test. Values represented are means ± standard deviation.

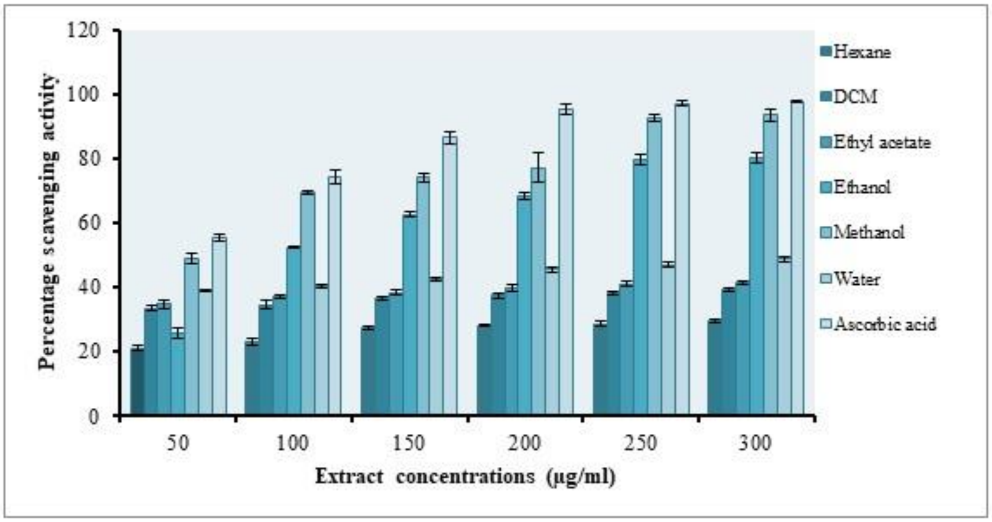


Figure 8. DPPH scavenging activity of bark extract in different solvents. Values represented are means ± standard deviation.

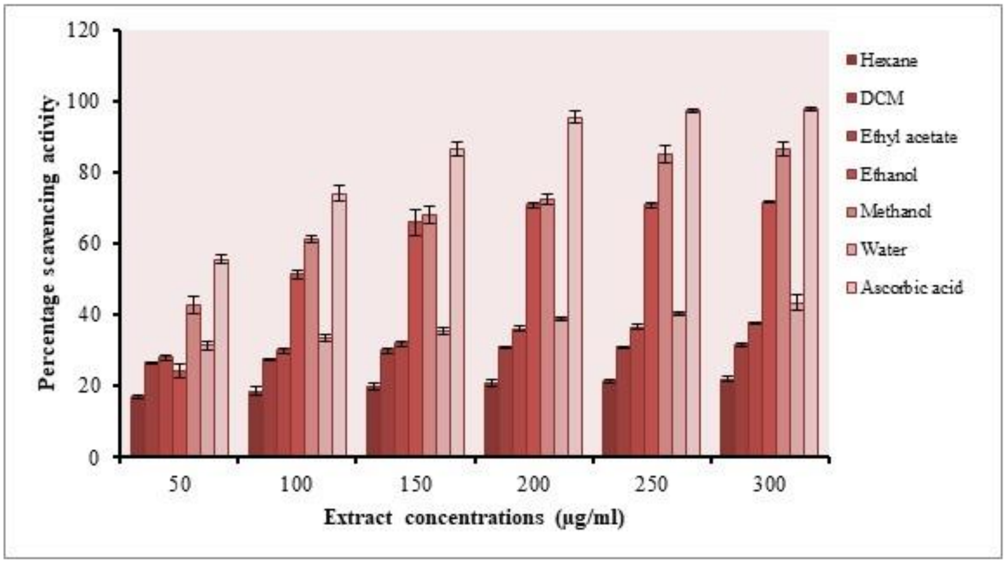


Figure 9. DPPH scavenging activity of leaves extract in different solvents. Values represented are means ± standard deviation.

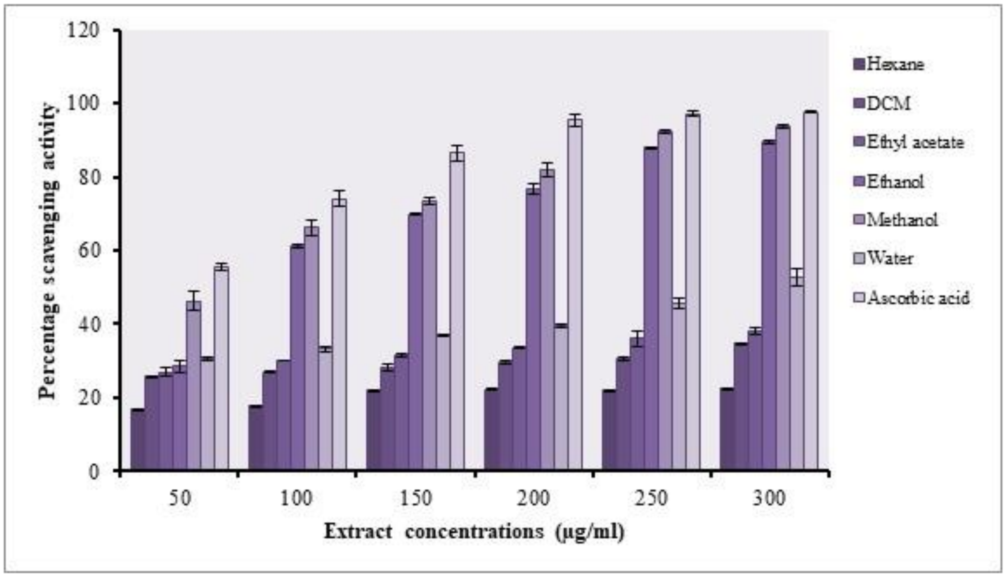


Figure 10. DPPH scavenging activity of pericarp extract in different solvents. Values represented are means ± standard deviation.

Table 3. Pearson correlations between total phenolic acids content, total flavonoids content and antioxidant potential of different plant parts of *G. moluccana*

Sample	Bark						Leaves						Pericarp					
Assay	PM assay		FRAP		CUPRAC		PM assay		FRAP		CUPRAC		PM assay		FRAP		CUPRAC	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
TPC	0.903	0.0138	0.986	0.0003	0.947	0.0041	0.919	0.002	0.977	0.0008	0.936	0.0059	0.923	0.0087	0.977	0.0008	0.908	0.0124
TFC	0.970	0.0013	0.985	0.0003	0.993	0.0000	0.949	0.004	0.936	0.0060	0.946	0.0043	0.955	0.0030	0.959	0.0025	0.940	0.0052

Note: Pairs with p-values greater than 0.050, there is no significant relationship between the two variables. The p value less than 0.05 indicates a positive relationship among the variables of this complex leading to the discoloration of the coloured complex which is measured spectrophotometrically at 562 nm. Greater the quantity of the ferrous ion-ferrozine complex, the higher the absorbance at 562 nm, indicating that the chelator has a lesser binding capacity. The Fe^{2+} metal chelating ability of the methanolic extracts of different plant parts varied with the highest metal chelating ability exhibited by bark extract for all the concentrations tested (Figure 11)**.** The metal chelating capacity of bark extract ranged from 33.36% to 78.58%, for leaves it ranged from 6.82% to 54.81% and for pericarp it ranged from 17.39% to 77.01% at 100-600 µg/ml. The metal chelating capacity of positive control (EDTA) ranged from 43.71 to 93.8% at 100-600 µg/ml.

4.1.3. *In vitro* anti-inflammatory activity of bark and pericarp was higher than leaves extracts

Denaturation of tissue protein depicts the onset of inflammatory reaction. The potential of the methanolic extracts of different parts of *G. moluccana* to inhibit the denaturation of protein induced by heat was investigated in this study. A gradual increase in the inhibition of protein denaturation was observed with increase in the concentration of the extracts which suggest a concentration dependent inhibition activity (Figure 12). The results are expressed as percentage inhibition of protein denaturation. The maximum inhibition of protein denaturation was observed in methanolic extract of bark (43.46%) at a concentration of 100 µg/ml as compared to methanolic extracts of pericarp and leaves (37.45 and 31.09%, respectively) at the same concentration (Figure 12). Acetyl salicylic acid, the positive control showed maximum inhibition activity of 74.8% at 100 µg/ml concentration. The inhibition of protein denaturation of bark extract for different concentrations of 10 to 100 µg/ml ranged from 15.9% to 43.46%, while that of leaves and pericarp ranged from 1.7% to 31.09% and 11.3% to 37.45%, respectively.

Lipoxygenase is a pro-inflammatory enzyme implicated in the progression of several inflammatory diseases. The capacity of extracts from various plant components to inhibit 5-LOX enzyme activity obtained from potato tubers was evaluated. NDGA which is the positive control eliminated 5-LOX activities up to 98.3% (IC_{50}-4.5 µM). The crude extracts of plant parts exhibited lower inhibition activity on 5-LOX than the positive control. The methanolic extract of bark showed 41.6% inhibition activity on 5-LOX whereas methanolic extract of leaves exhibited the least effect with 10.67% inhibition of LOX activity (Figure 13)**.** The methanolic extract of pericarp inhibited the activity of 5-LOX by 31.7%. Since crude extracts were used in this investigation, the inhibition activity of LOX enzyme by the extracts was lower as compared

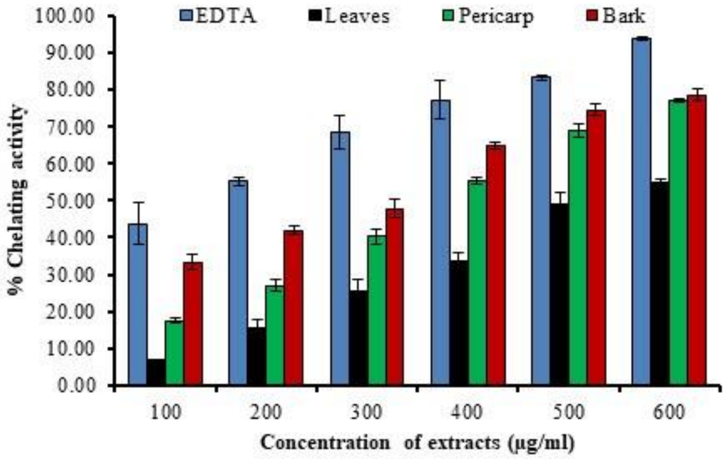


Figure 11. Ferric ions chelating activity of methanolic extract of bark, leaves, pericarp and EDTA (Positive control). Values represented are means ± standard deviation.

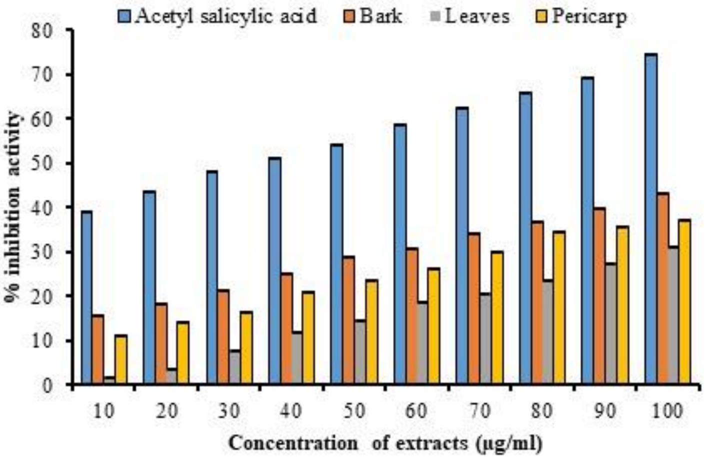


Figure 12. Inhibition of protein denaturation by methanolic extract of bark, leaves, pericarp and acetyl salicylic acid (Positive control). Values represented are means ± standard deviation.

to positive control, which is a pure compound. Thus, the results showed that bark and pericarp are the potential sources of anti-inflammatory agent. Additional investigations are essential to identify and characterize the active components of plant extracts that are responsible for inhibition of the LOX activity.

The therapeutic value of methanolic extracts of *G. moluccana* plant parts was assessed based on their potential to inhibit cyclooxygenase (COX-1 and COX-2) enzymes, which are targets of popular NSAIDs. COX-1 is a constitutive enzyme expressed in most of the tissues. The results are depicted as percentage inhibition of COX-1 at the concentrations of 25, 50 and 100 µg/ml of plant extracts. A surge in the inhibition of enzyme activity was observed with increase in the concentration of the plant extracts, although the extracts exhibited a weak inhibition on COX-1 enzyme. The methanolic extracts of leaves at 100 µg exhibited the highest inhibition (29.21%) on COX-1. The leaves extract at 50 µg and 25 µg inhibited the activities by 17.26% and 11.69%, respectively. The methanolic extract of bark showed 24.26% inhibition of COX-1 activity at 100 µg concentrations, whereas bark extract at 50 µg and 25 µg exhibited inhibition of 18.69% and 13.33%, respectively. The pericarp extract displayed the least inhibition among the extracts with enzyme activity inhibition of 13.1% at 100 µg whereas 50 µg and 25 µg exhibited 7.29% and 6.46% inhibition on COX-1 (Figure 14).

COX-2 is an inducible enzyme mediated by inflammatory stimuli. The crude methanolic extracts of different plant parts of *G. moluccana* at the concentrations (25, 50 and 100 µg) tested had the potential to inhibit COX-2 enzyme activity. The plant extracts inhibition ability of COX-2 was low at 25 µg and increased as the concentration of the extracts increased, with the highest inhibition of 86.71% for pericarp followed by bark (83.46%) and leaves (82.21%) at 100 µg (Figure 15). Similar trend was observed at 50 µg, with pericarp (62.41%) showing the highest and the least for leaves (33.24%). Overall, the extracts displayed strong inhibition on COX-2 enzyme as compared to COX-1. Diclofenac used as positive control completely inhibited the activities of both cycloxygenase enzymes.

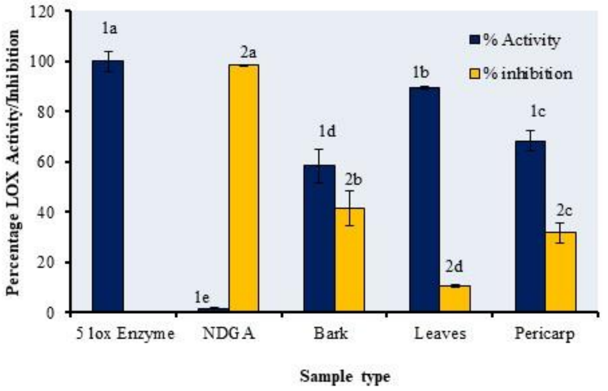


Figure 13. Percentage LOX activity and its inhibition by methanolic extracts of bark, leaves, pericarp and NDGA (Positive control). Values represented are means ± standard deviation. Means followed by the same letter are not statistically different at the 5% probability level by one-way analysis of variance (ANOVA) according to Duncan's multiple range test.

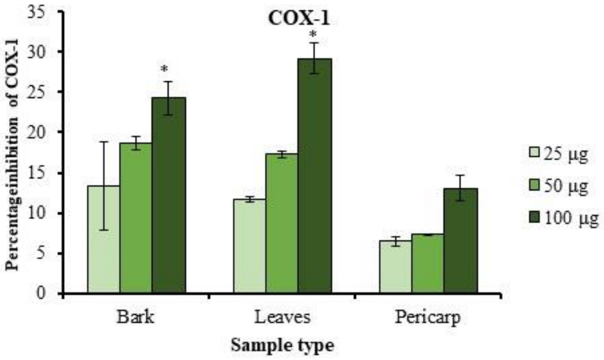


Figure 14. Percentage inhibition of COX-1 by methanolic extract of bark, leaves, pericarp and diclofenac (Positive control) which exhibited 100% inhibition (not shown). Values represented are means ± standard deviation. Asterick (*) indicates that the values are statistically significant as tested by one-way analysis of variance (ANOVA) at the 5% probability level in comparison to pericarp.

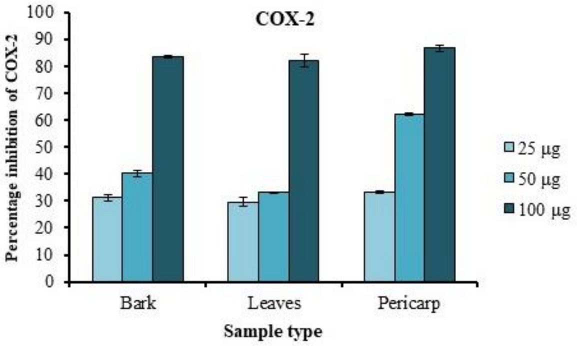


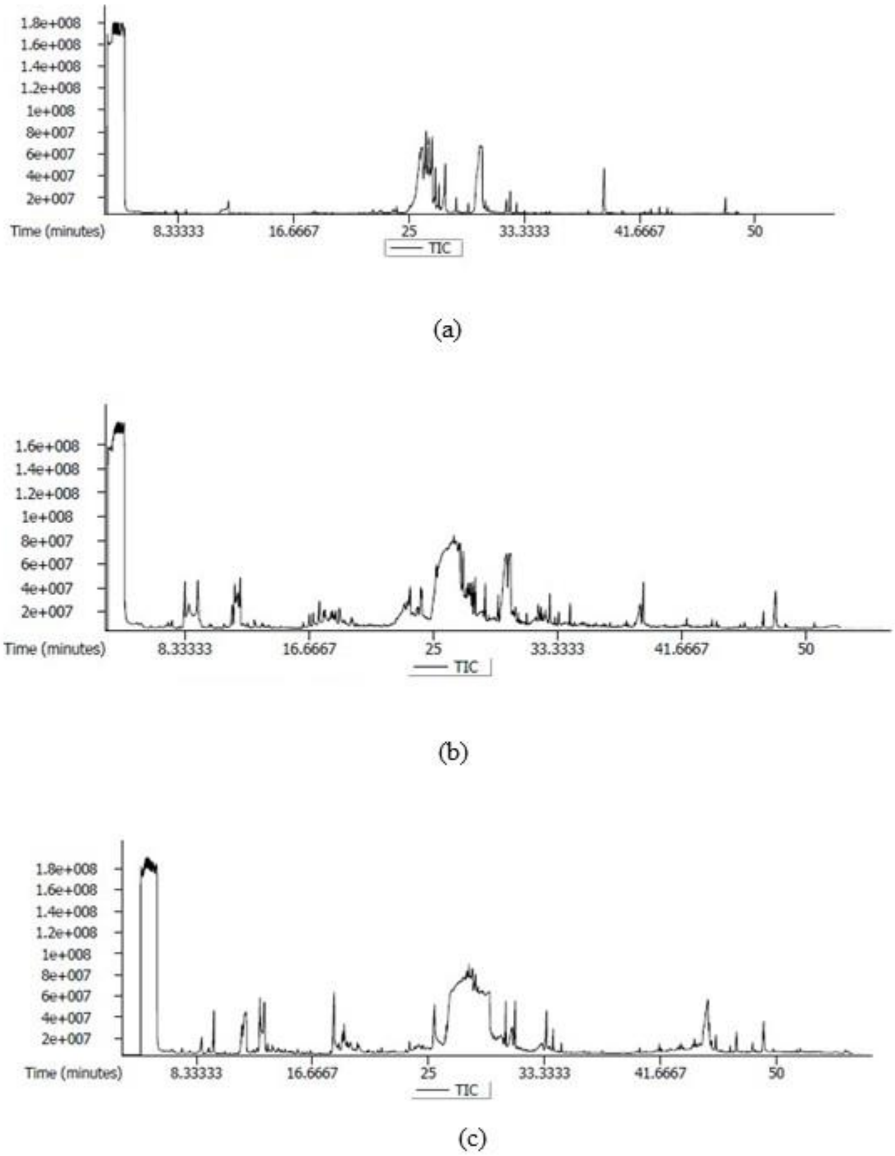
Figure 15. Percentage inhibition of COX-2 enzyme by methanolic extracts of bark, leaves, pericarp and diclofenac (Positive control) which exhibited 100% inhibition for 3 concentrations tested as that of samples (not shown). Values represented are means ± standard deviation.

4.2. Metabolite profiling in different parts of *G. moluccana*

4.2.1. GC-MS analysis revealed the profiles of primary metabolites of bark, leaves and pericarp

GC-MS identifies volatile and semi-volatile molecules of low molecular weight, usually primary metabolites. The comparative analysis of metabolites in the bark, leaves and pericarp of *G. moluccana* was carried out using GC-MS in this study. The compounds identified along with their retention time, relative abundance and molecular mass for bark, leaves and pericarp are shown in Appendix (Supplementary Tables 1-3). The polar functional groups were altered to make these diverse chemical compounds volatile and thereof suitable for GC-MS investigation. N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), the derivatization agent which is capable of reacting with a diversed array of chemical class, was utilised for the purpose. The GC-MS total ion chromatogram (TIC) of bark, leaves and pericarp revealed major, minor and trace peaks (Figures 16a-c). The TIC of each sample was distinct; however, leaves and pericarp shared more similarity as compared to bark TIC. To unveil the chemical nature of each peak, their recorded spectra were compared with accessible mass spectra libraries, such as the National Institute of Standards and Technology (NIST). The major classes of the metabolites identified were carbohydrates and carboxylic acids in all the three extracts analyzed (Figures 17a-c**).** Fatty acids, polyols (sugar alcohols), sugar acids and amino acids were identified in all the extracts but lower in number in comparison to carboxylic acids and carbohydrates. The number of metabolites that were unique to the bark, leaves and pericarp extracts were 19 (25%), 12 (15.8%) and 18 (23.7%), respectively (Figure 17d**).** Twelve metabolites (15.8%) were found to be common to all the three sample extracts.

A total of 40 compounds were identified in bark extract based on similarities of greater than 70% to mass fraction in the NIST mass spectra database. The compounds of the bark were classified into 8 chemical classes' *viz*., carbohydrates (36%), carboxylic acids (26%), fatty acids (13%), polyols (8%), terpenoids (8%), sugar acids (5%), glycols (2%), and amino acids (2%) (Figure 17a**).** The major components of the bark and pericarp extracts were found to be carbohydrates followed by carboxylic acids and fatty acids while carbohydrates, carboxylic acids and amino acids were the major classes in leaves. Thirty five compounds identified in leaves extracts were classified into 7 chemical classes' *viz*., carboxylic acids (32%), carbohydrates (29%), amino acids (21%), fatty acids (9%), polyols (6%), vitamins (3%), and sugar acids (3%) (Figure 17b). Thirty nine compounds were identified in pericarp

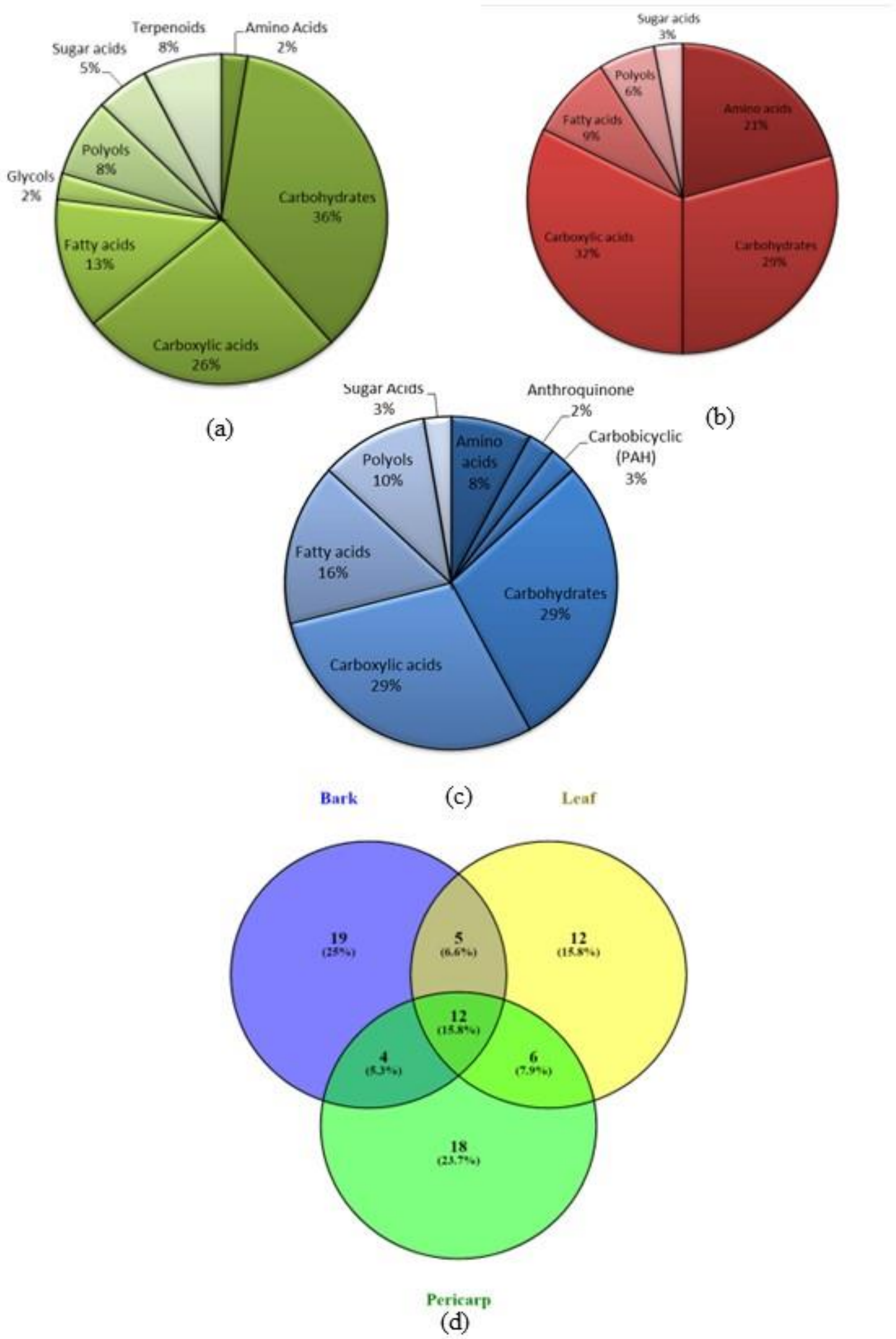


(a)

(b)

(c)

Figures 16 (a-c). GC-MS Total ion chromatogram (TIC) of bark, leaves and pericarp extracts. (a) Bark, (b) Leaves and (c) Pericarp.



Figures 17 (a-d). Distribution of different classes of metabolites in different plant parts after GC-MS analysis. (a) Bark, (b) Leaves and (c) Pericarp. (d) Venn diagram of the metabolites among the bark, leaves and pericarp.

extracts, and were classified into 8 chemical classes *viz*., carbohydrates (29%), carboxylic acids (29%), fatty acids (18%), polyols (10%), amino acids (8%), sugar acids (3%),anthroquinone (2%), and carbobicyclic (PAH) (3%) (Figure 17c).The metabolites set enrichment analysis (MSEA) was carried on each sample through MetaboAnalyst 5.0 (metaboanalyst.ca) to understand the patterns of metabolites concentration changes and most abundant metabolite group in the plant parts studied. The MSEA results provided a snapshot of the concentration of the metabolites in different tissues. Based on the enrichment ratio and p-values, metabolites classes such as monosaccharides, TCA acids, saturated fatty acids, sugar acids and medium-chain keto acids were significantly enriched in bark extracts (Figure 18; Supplementary Table 4**)**. Similarly, the enrichment ratio and p-value of metabolites in leaf extracts indicated that sugar acids, monosaccharides, saturated fatty acids, amino acids and TCA acids were significantly enriched (Figure 19; Supplimentary Table 5) whereas the enrichment ratio and p-value of pericarp demonstrated that the metabolites classes such as monosaccharides, sugar acids, TCA acids, dicarbocylic acids and saturated fatty acids were predominant (Figures 20; Supplementary Table 6).

The data from GC-MS analysis were then applied to principal component analysis (PCA) for understanding and visualization of the variation of metabolites across the different plant parts of *G. moluccana*. The PCA 2D score plot revealed clear separation of the metabolite profiles of bark, leaves and pericarp (Figure 21). The principal component 1 (PC1) elucidated 66.4% of the total variance and clearly differentiated the bark from leaves and pericarp while the principal component 2 (PC2) explained 33.6% variance and clearly distinguished the leaves from pericarp and bark. The relationships between the compounds in different plant parts of *G. moluccana* and their abundance were investigated by hierarchical clustering analysis (HCA) with correlation heat map as shown in Figure 22. The results demonstrated a distinct segregation of compounds between different plant parts as could be seen with regard to compounds such as D-fructose, lyxose, pipecolic acid, 2,3 butanediol, phytol etc. in bark than other plant parts. The compounds such as oleic acid, succinic acid, malonic acid, ribonic acid, D-xylose etc. were more abundant in pericarp whereas butanoic acid, ribitol, D-orinithine, linolenic acid, benzoic acid etc. were abundant in leaves than other plant parts.

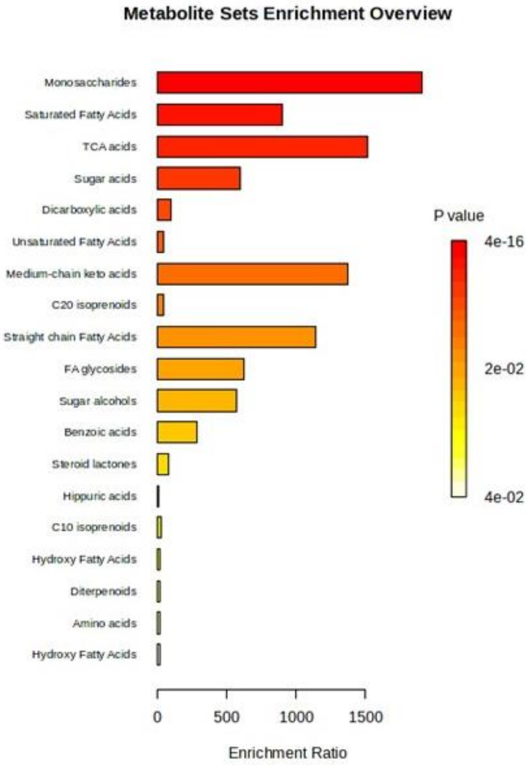


Figure 18. Summary plot of metabolite set enrichment analysis (MSEA) of bark extract.

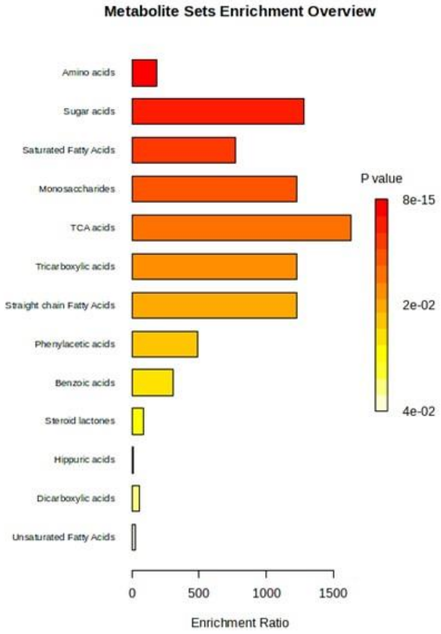


Figure 19. Summary plot of metabolite set enrichment analysis (MSEA) of leaves extract.

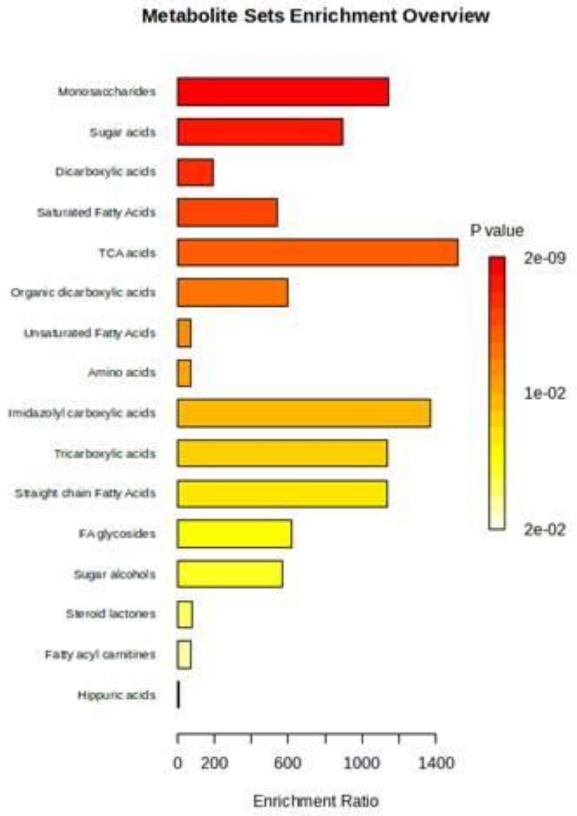


Figure 20. Summary plot of metabolite set enrichment analysis (MSEA) of pericarp extract.

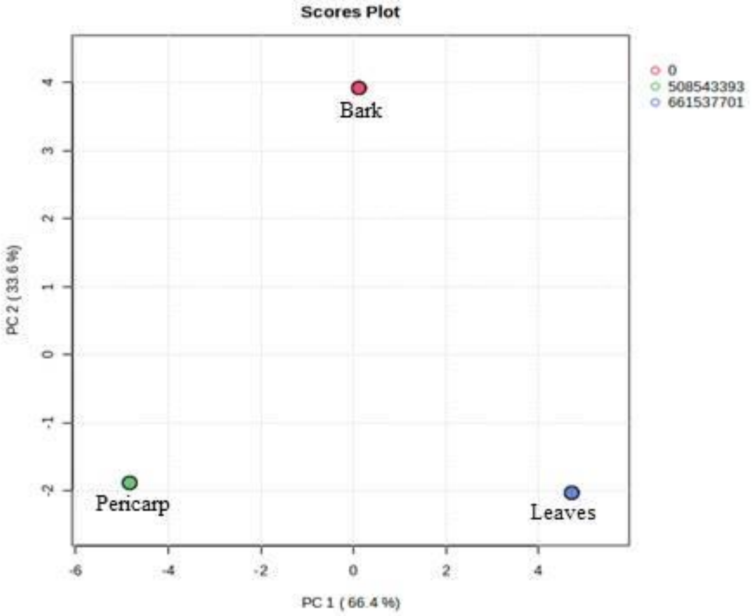


Figure 21. Principal component analysis (PCA) score plot between the selected PCs. The variances are shown in brackets.

4.2.2. LC-MS/MS analysis revealed the profiles of secondary metabolites in bark, leaves and pericarp

It would be important to compare the distribution of metabolites in different plant parts to fully exploit their medicinal and health protective properties for future research on targeted bioactive compounds. Untargeted global metabolomics platform with LC-MS/MS technique was used to investigate the secondary metabolites signatures of *G. moluccana* bark, leaves and pericarp. The total ion chromatogram (TIC) of bark was significantly different from leaves and pericarp, which shared a similar TIC (Figure 23). In this investigation, the LC-MS/MS analysis of plant parts methanolic extracts resulted in identification and annotation of 181 putative secondary metabolites in bark, 165 putative secondary metabolites in leaves and 153 putative secondary metabolites in pericarp consisting of terpenes and terpenoids, flavonoids, alkaloids, phenolic acids, glycosides, vitamins and co-factors, anthroquinone, lignans, stilbenes and betacynin (Figures 24-28). A total of 89 compounds (35%) were common to all plant parts. The number of compounds common to bark and leaves were 24 (9.4%), 22 (8.7%) for bark and pericarp, and 21 (8.3%) for leaves and pericarp. It was observed that 46 (18.1%) compounds were unique to bark extracts, 31 (12.2%) compounds were unique to leaves extracts and 21 (8.3%) compounds were unique to pericarp extracts (Figure 24).

The putatively identified secondary metabolites in bark extract comprised of 54 flavonoids, 40 terpenes and terpenoids, 36 alkaloids, 22 phenolics acids, 13 steroids and sterols, glycosides, stilbenes, vitamins, lignans, betacynin and anthroquinones (Figure 25). The flavonoids in bark extracts comprised of predominantly flavones (22%) and flavonol (20%) followed by flavonoid glycosides (17%), and other flavonoids such as flavanone, isoflavone, flava-3-ol, anthocyanin, coumestran, chalcone and dehydrochalcone were also identified (Figure 26a). Flavonol compounds such as quercetin, myricetin, kaempferide and patuletin are known to have antioxidant and anti-inflammatory effects. The biological activities of flavonoid compounds identified are listed in Supplementary Table 7. Triterpenoid (35%) and sesquiterpenoid (27%) were the predominant class of terpenoids identified in bark followed by diterpenoid (20%), tetraterpenoid (10%), monoterpenoid (5%) and norisoterpenoid (2%) (Figure 27a). The list of terpenoids identified along with their potential biological activities are listed in Supplementary Table 8. Indole alkaloid (31%) and benzylisoquinoline alkaloid (27%) were the major class of alkaloids identified in the bark extract (Figure 28a). Alkaloids belonging to other class such as benzophenanthridine alkaloid, indolizidine alkaloid, purine alkaloid, quinoline alkaloid, quinolizidine alkaloid, steroidal alkaloid, tropane alkaloid, and terpenoid alkaloids were also found in smaller number. The phenolic acids were predominated by hydroxycinnamic acid class of phenolics (59%) followed by coumarin (18%), hydroxybenzoic acid (14%) and phenylpropanes (9%) in bark extract (Figure 29a). The list of alkaloids and phenolic acids with their potential biological activities are listed in Supplementary Tables 9 & 10. The list of steroids and sterols, stilbenes, vitamins and cofactors, glycosides, anthroquinones and betacynin with their potential biological activities are listed in Supplementary Table 11.

The secondary metabolites that were putatively identified in leaves extracts consisted of 44 terpenes and terpenoids, 40 flavonoids, 29 alkoloids, 22 phenolics acids, steroids and sterols, glycosides, vitamins and co-factors, and others such as lignans, anthroquinone, betacynin stilbenes were also identified (Figure 25b). Flavones (27%) were the major class of flavonoids followed by flavonol (22%) and isoflavones (15%), and other class of flavonoids such as flavanone, flava-3-ol, anthocyanin, flavonoid glycosides, coumestan, chalones and dihydrochalcones were also identified in smaller number in leaves (Figure 26b). The terpenoids were composed mainly of sesquiterpenoids (34%) followed by diterpenoids (27%) and triterpenoids (21%) (Figure 27b). The alkaloid classes identified in the leaves were indole alkaloid (37%) and benzylisoquinoline alkaloids (37%) while other alkaloids class were meagerly represented (Figure 28b). Similar to the phenolic acids in the bark extracts, hydroxycinnamic acid (53%) were the main class of phenolic acids in the leaves extracts followed by coumarin (31%) and hydroxybenzoic acid (16%) (Figure 29b). The potential biological activities of flavonoids, terpenoids, alkaloids and phenolic acids identified in leaves extract are listed in Supplementary Tables 12-15. The other compounds such as glycosides, steroids, sterols, anthroquinones, vitamins and co-factors betacyanin were also identified in leaves extract and their potential biological activities are listed in Supplementary Tables 16-17.

The pericarp extract comprised of 42 flavonoids, 31 terpenes and terpenoids, 29 alkoloids, 22 phenolics acids, glycosides steroids and sterols, vitamins and co-factors, anthroquinone, lignans, betacyanin and stilbenes were also detected (Figure 25c). Similar to the flavonoids in leaves extract, the flavones (24%) and flavonol (24%) were the major class of flavonoids found in pericarp extract and other classes of flavonoids such as isoflavone (17%), flavanone (12%) and flavonoid glycosides (12%) were moderately present, whereas flavan-3-ol, anthocyanin, and dihydrochalone were lesser in number (Figure 26c). The terpenoids in the pericarp extract were predominated by sesquiterpenoids (32%) and diterpenoids (26%) similar to leaves extracts (Figure 27c). The remaining terpenoids

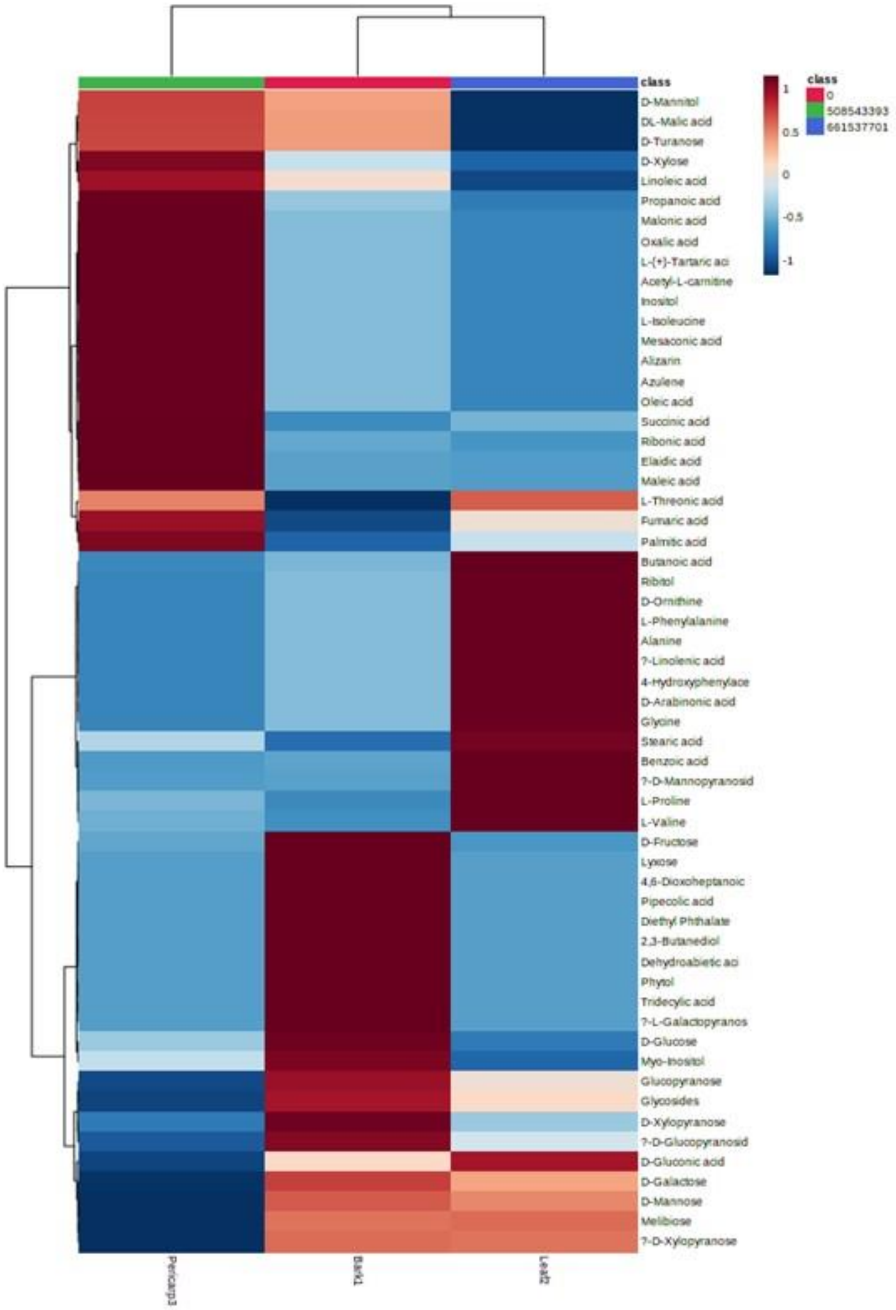
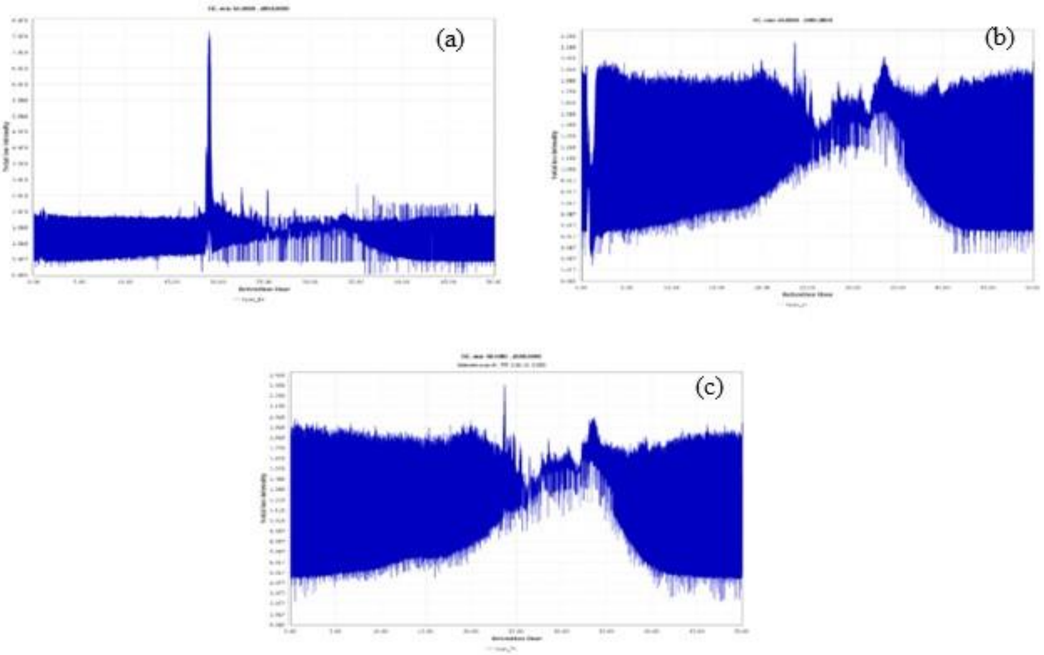


Figure 22. Hierarchical clustering results of metabolites shown as heatmap (distance measured using euclidean, and clustering algorithm using ward D).



Figures 23 (a-c). Total ion chromatogram (TIC) plot of (a) bark, (b) leaves and (c) pericarp extracts.

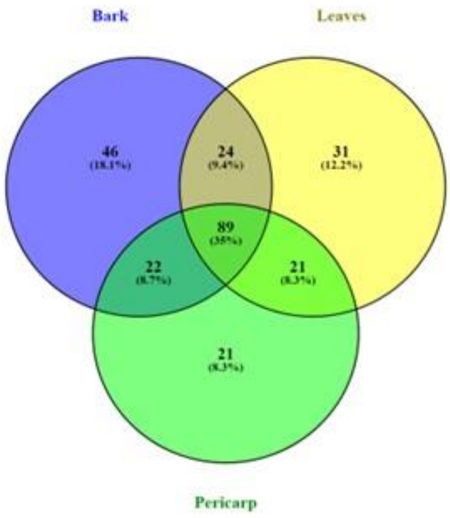
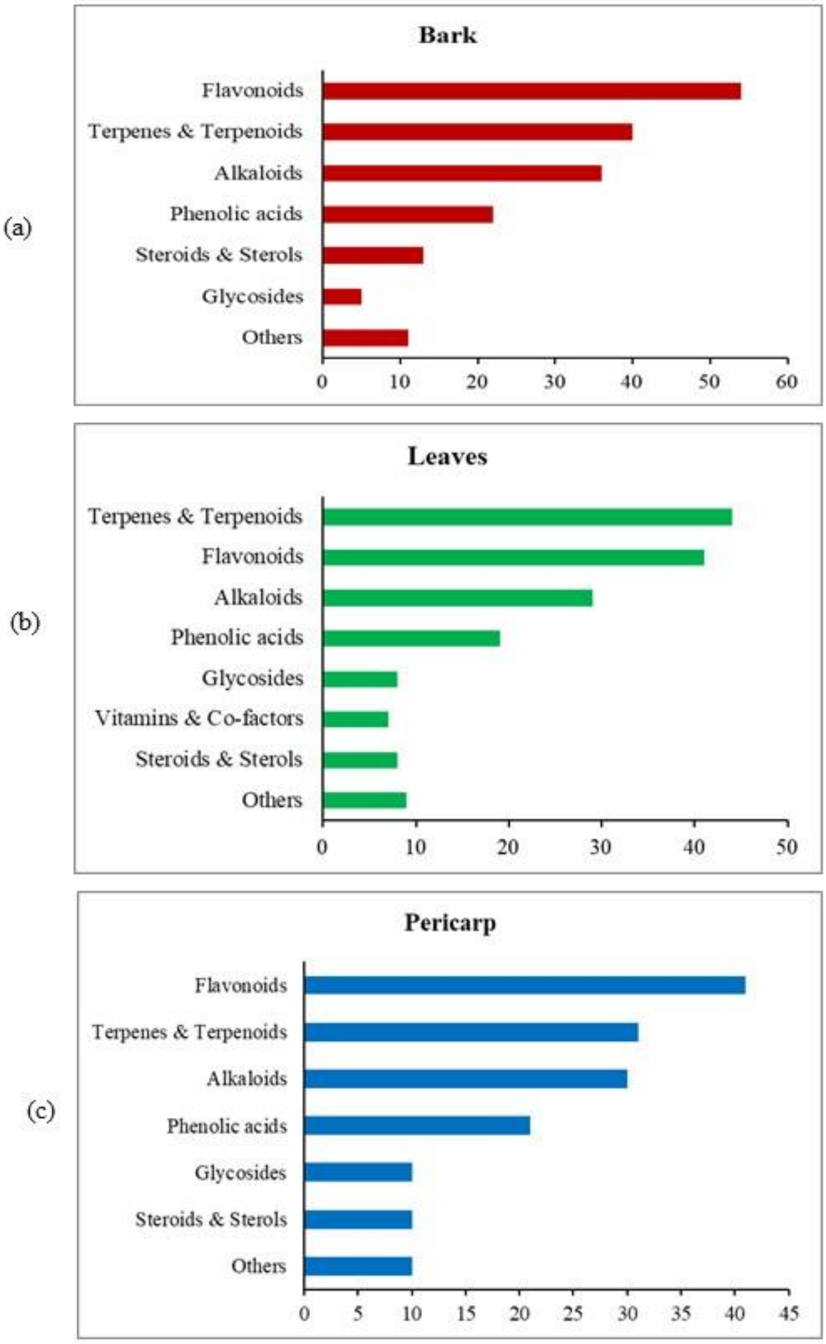
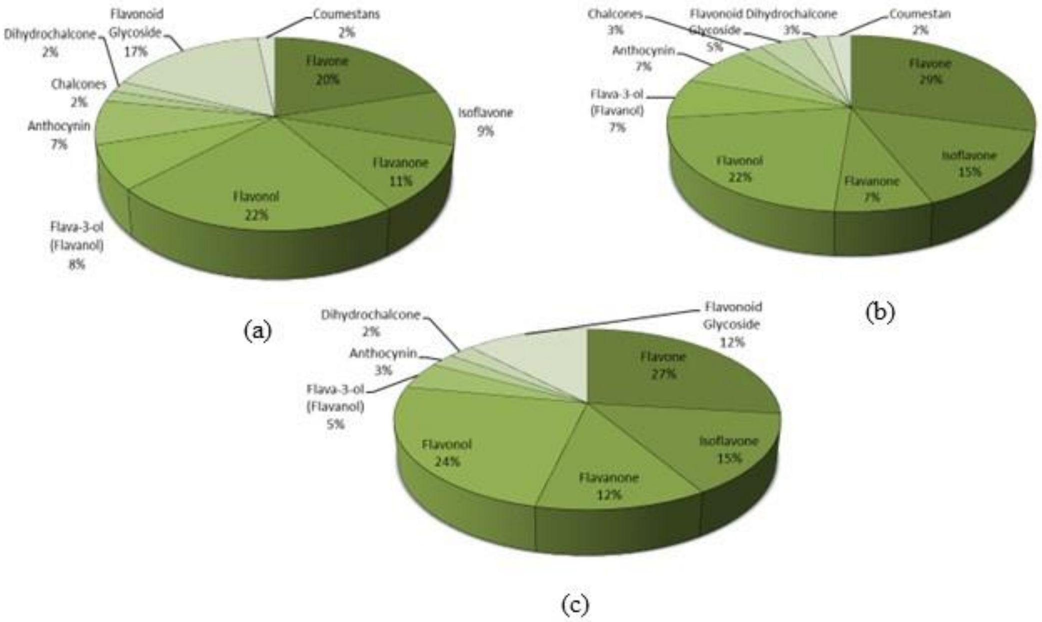


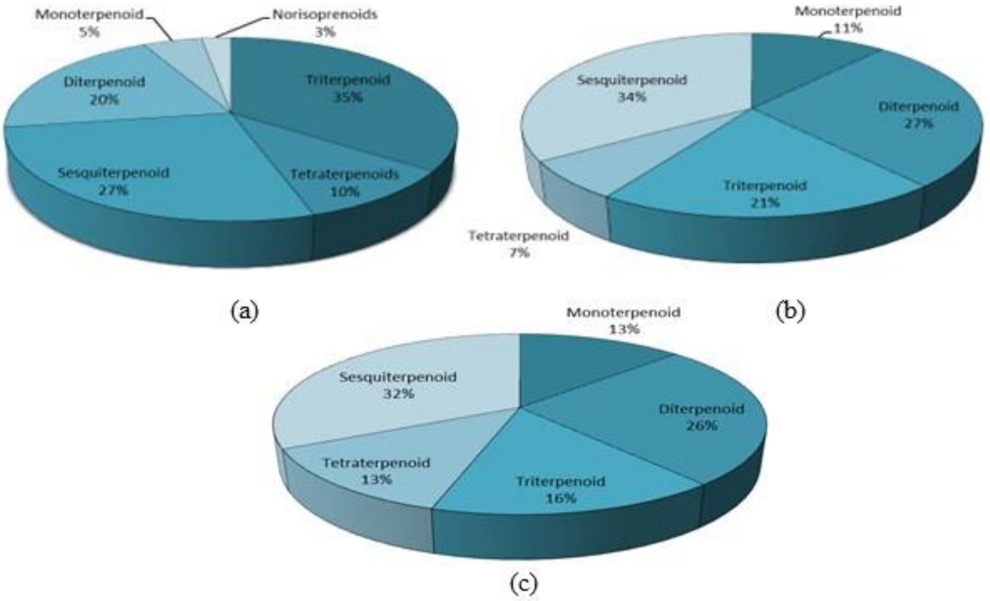
Figure 24. Distribution of secondary metabolites classes in methanolic extracts of bark, leaves and pericarp of *G. moluccana.*



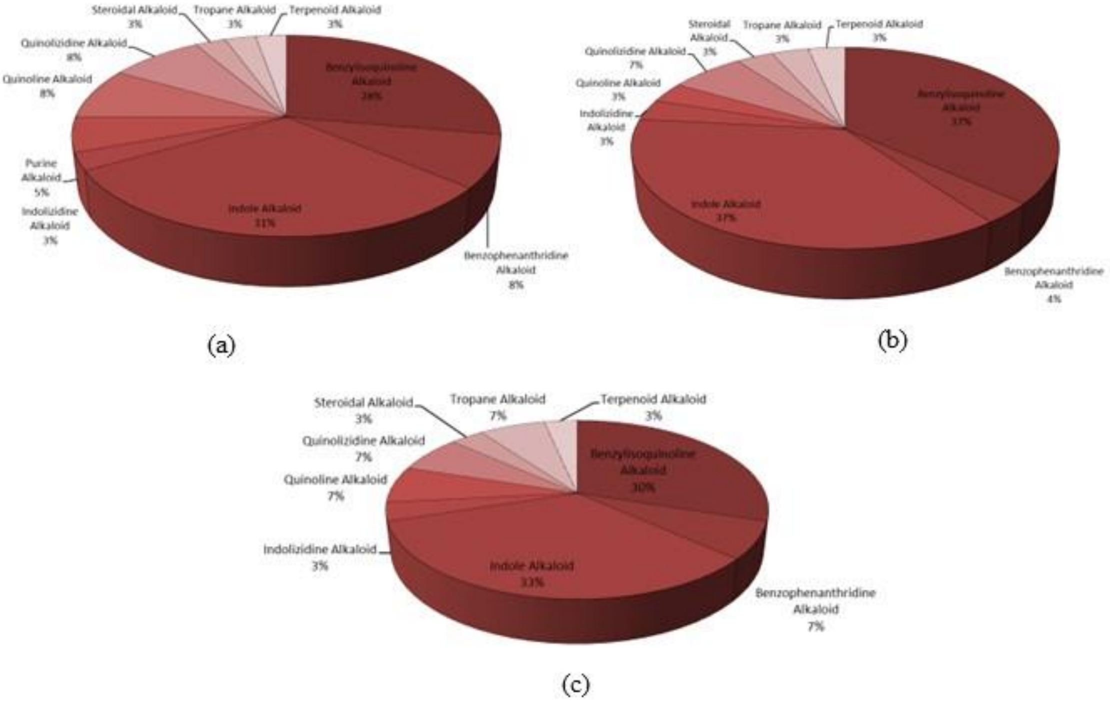
Figures 25 (a-b). Distribution of secondary metabolites classes in methanolic extracts of different plant parts. (a) Bark, (b) Leaves and (c) Pericarp.



Figures 26 (a-c). Distribution of flavonoids in methanolic extracts of different plant parts. (a) Bark, (b) Leaves and (c) Pericarp of *G. moluccana.*

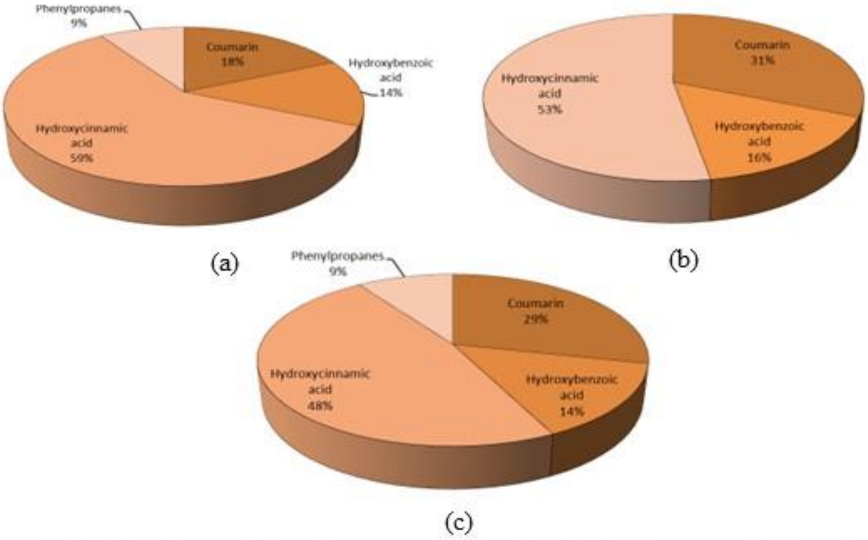


Figures 27 (a-c). Distribution of terpenoids in (a) bark, (b) leaves and (c) pericarp of *G. moluccana*



(a) (b) (c)

Figures 28 (a-c). Distribution of alkaloids in (a) bark, (b) leaves and (c) pericarp of *G. moluccana.*



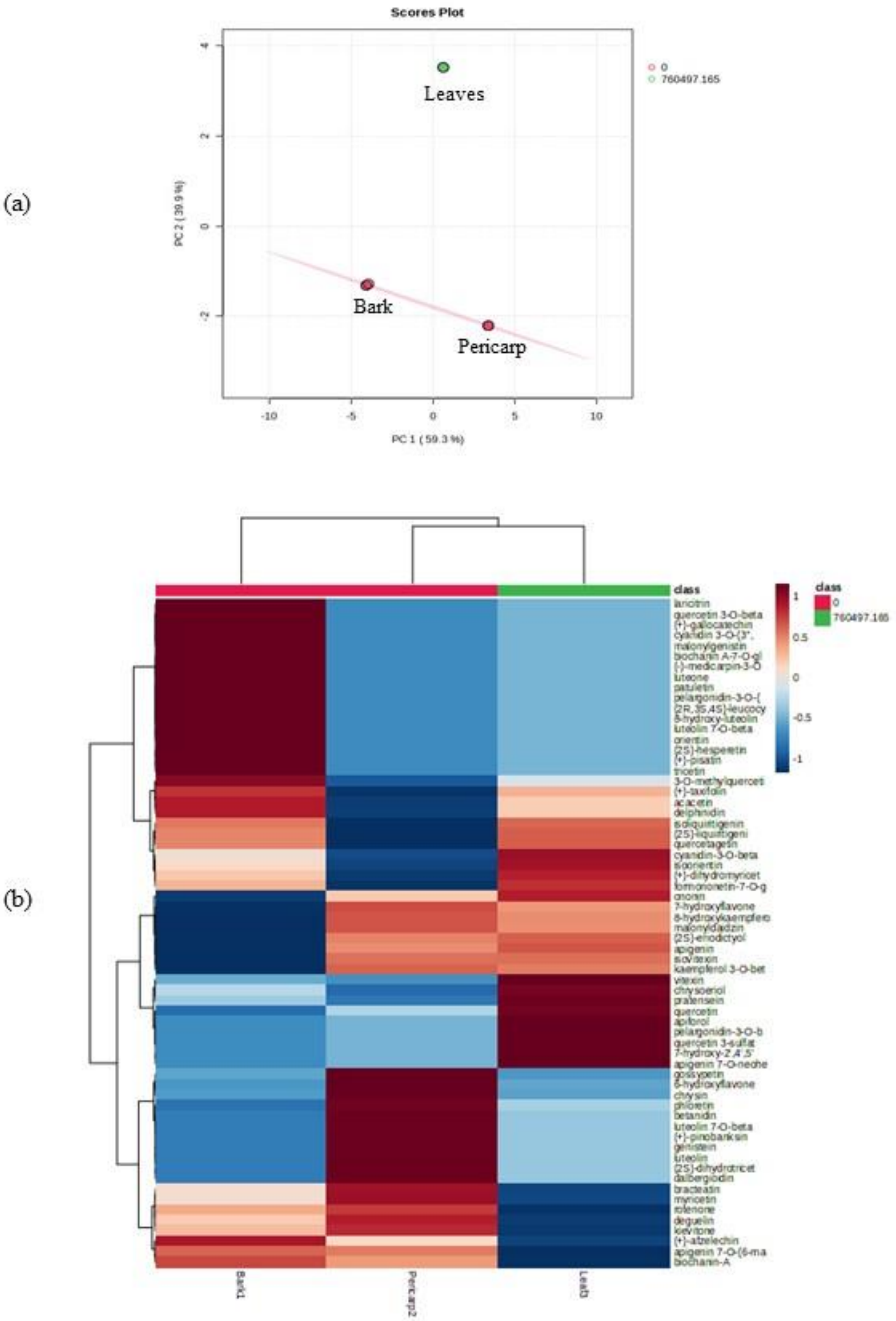
(a) (b) (c)

Figures 29 (a-c). Distribution of phenolic compounds in (a) bark, (b) leaves and (c) pericarp of *G. moluccana..*

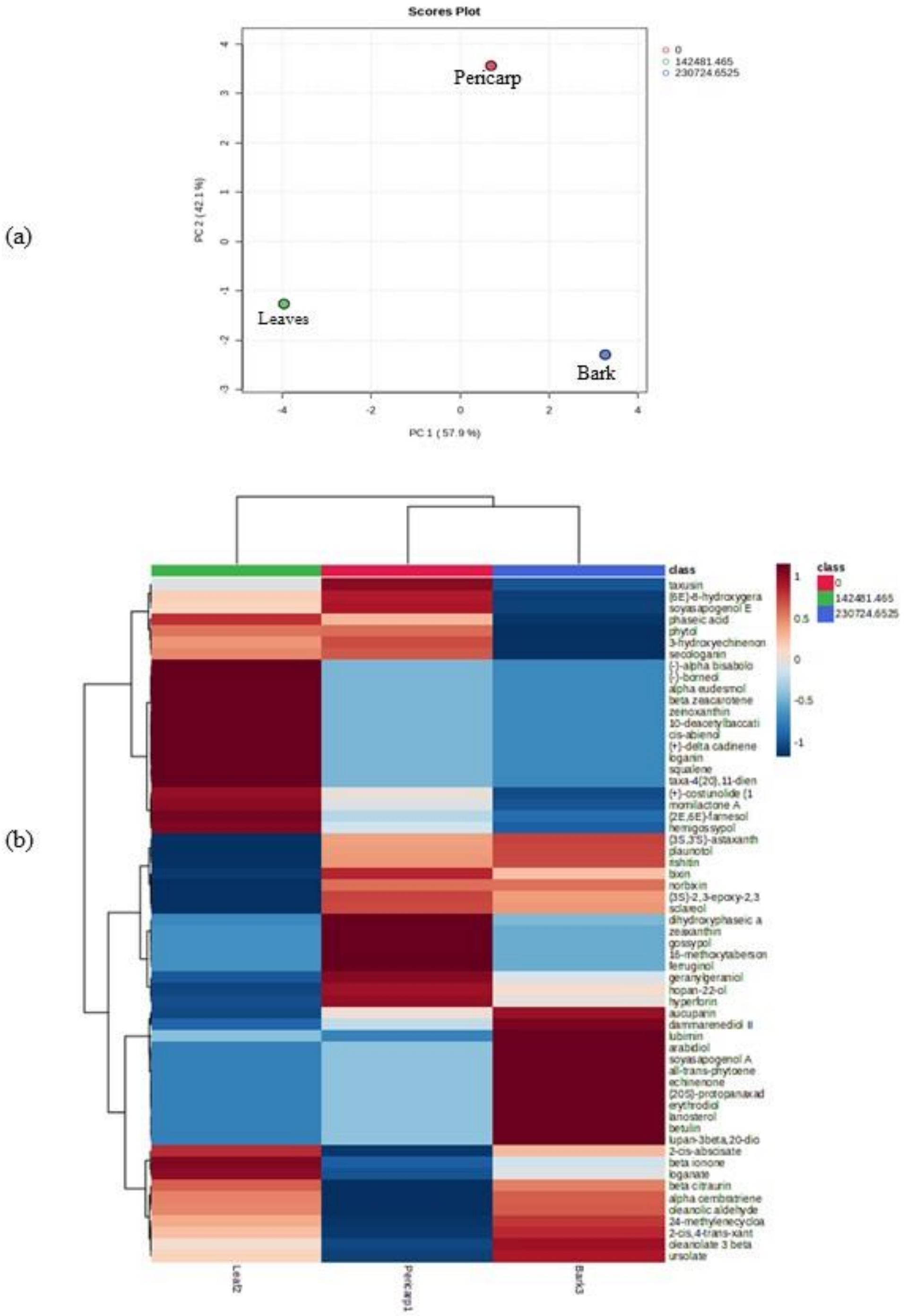
consisted of triperpenoids (16%), monoterpenoids (13%) and tetraterpenoids (13%). Similar to the alkaloids in bark and leaves extracts, indole alkaloid (33%) and benzylisoquinoline alkaloid (30%) comprised the major portion, and other class of alkaloids such as indolizidine alkaloid, quinoline alkaloid, quinolizidine alkaloid, steroidal alkaloid, tropane alkaloid, terpenoid alkaloid and benzophenanthridine alkaloids were represented in lesser proportion (Figure 28c). Hydroxycinnamic acids (48%) were the main class of phenolic acids present in pericarp followed by coumarin (29%), hydroxybenzoic acid (14%) and phenyl propanes (9%) as was observed in bark and leaves extracts (Figure 29c). The list of flavonoids, terpenoids, alkaloids, phenolics, and other compounds such as putative steroids, sterols, glycosides, anthroquinone, lignins and betacyanin are listed in Supplementary Tables 18-22.

In this study, PCA and HC were used to distinguish and group the bark, leaves and pericarp based on their flavonoids, terpenoids, alkaloids, and phenolic acids. The outcomes of this analysis are depicted in Figures 30a & b, 31a & b, 32a &b, 33a & b, respectively. The PCA score plot of the flavonoid PC1 accounted for 59.3% variance and PC1 accounted for 39.9% variance (Figure 30a). The flavonoids were clustered into two groups where the flavonoids in leaves were significantly distinct from bark and pericarp (Figure 30b). In PCA score plot of the terpenoids, the bark, leaves and pericarp were clearly separated into three clusters where the PC1 and PC2 explained 57.9% and 42.1% of total variance, respectively (Figure 31a). The clear distinction among the three tepenoid classes indicated that there were notable variations in the terpenoid profiles of *G. molucanna* in different plant parts (Figure 31b). The score plots of alkaloids were clustered into two groups, with pericarp alkaloids showing divergence from leaves and bark alkaloid contents (Figure 32a). The PC1 and PC2 of alkaloids explained 59.5% and 40.5% variance, respectively. As observed for flavonoids, the score plot of phenolic acids in bark, leaves and pericarp were clustered into two groups with dissimilarities in leaves in comparison to bark and pericarp. The PCA of phenolic acids showed 32.2% (PC2) and 67.8% (PC1).

Hierarchical Cluster Analysis (HCA) is a method used to organize and classify extensive datasets by considering qualitative or quantitative attributes from experimental data. In this study, hierarchical cluster analysis was used to group the data based on the flavonoids, terpenoids, alkaloids and phenolics in bark, leaves and pericarp of *G. moluccana*. This analysis provided insights into the inter relationships of the datasets obtained for different plant parts (Figure 30b, 31b, 32b & 33b). The flavonoids such as laricitrin, quercetin 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside, gallocatechin, cyanidin 3-o-(3'',6''-o-dimalonyl-beta glucopyranoside), malonylgenistin, biochanin A-7-O-glucoside,



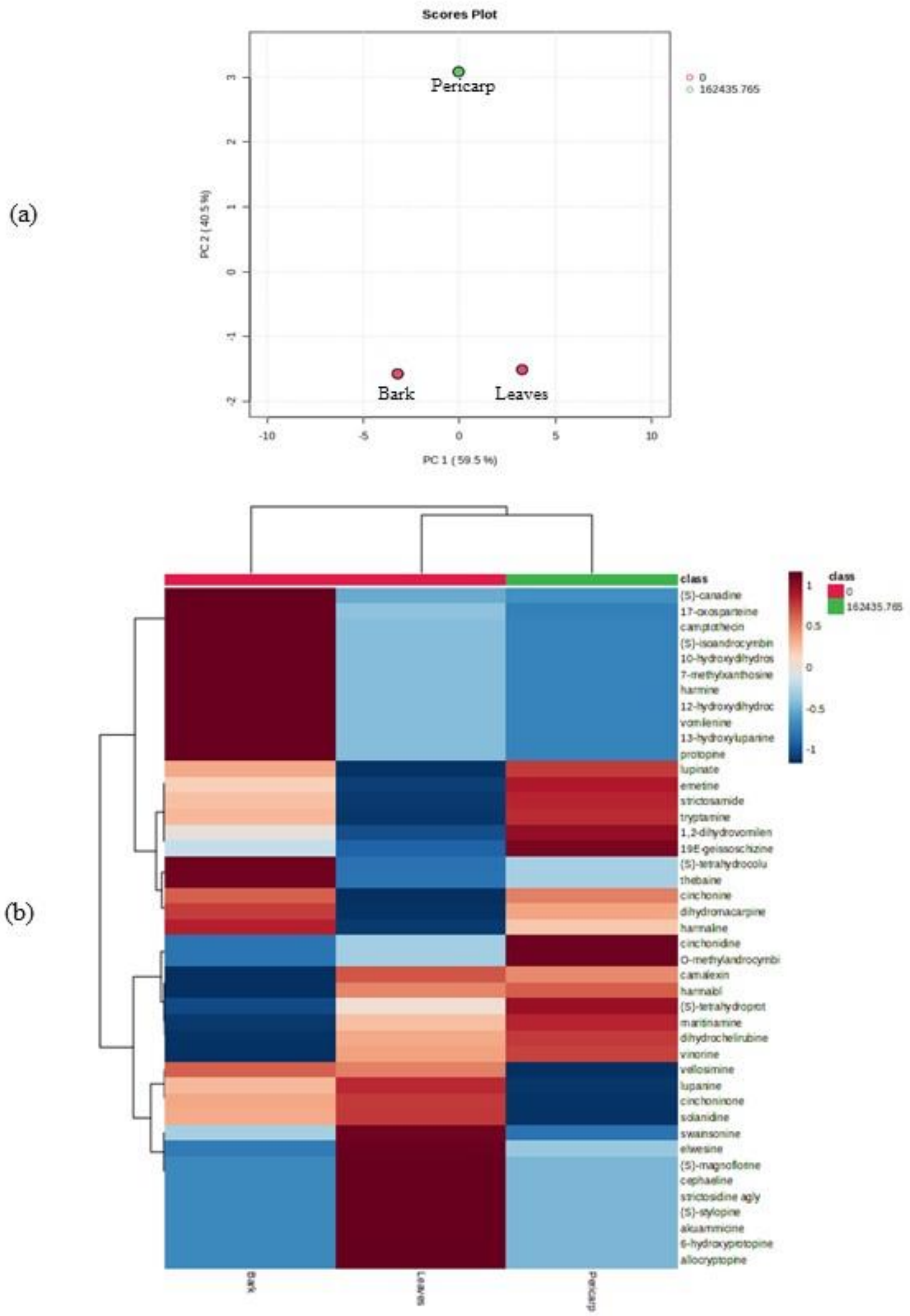
Figures 30 (a & b). (a) Principal component analysis (PCA)-scores plot of the flavonoids in bark, leaves and pericarp. The variances are shown in brackets, (b) Hierarchical clustering result of flavonoids shown as heatmap (distance measured using euclidean, and clustering algorithm using ward D).



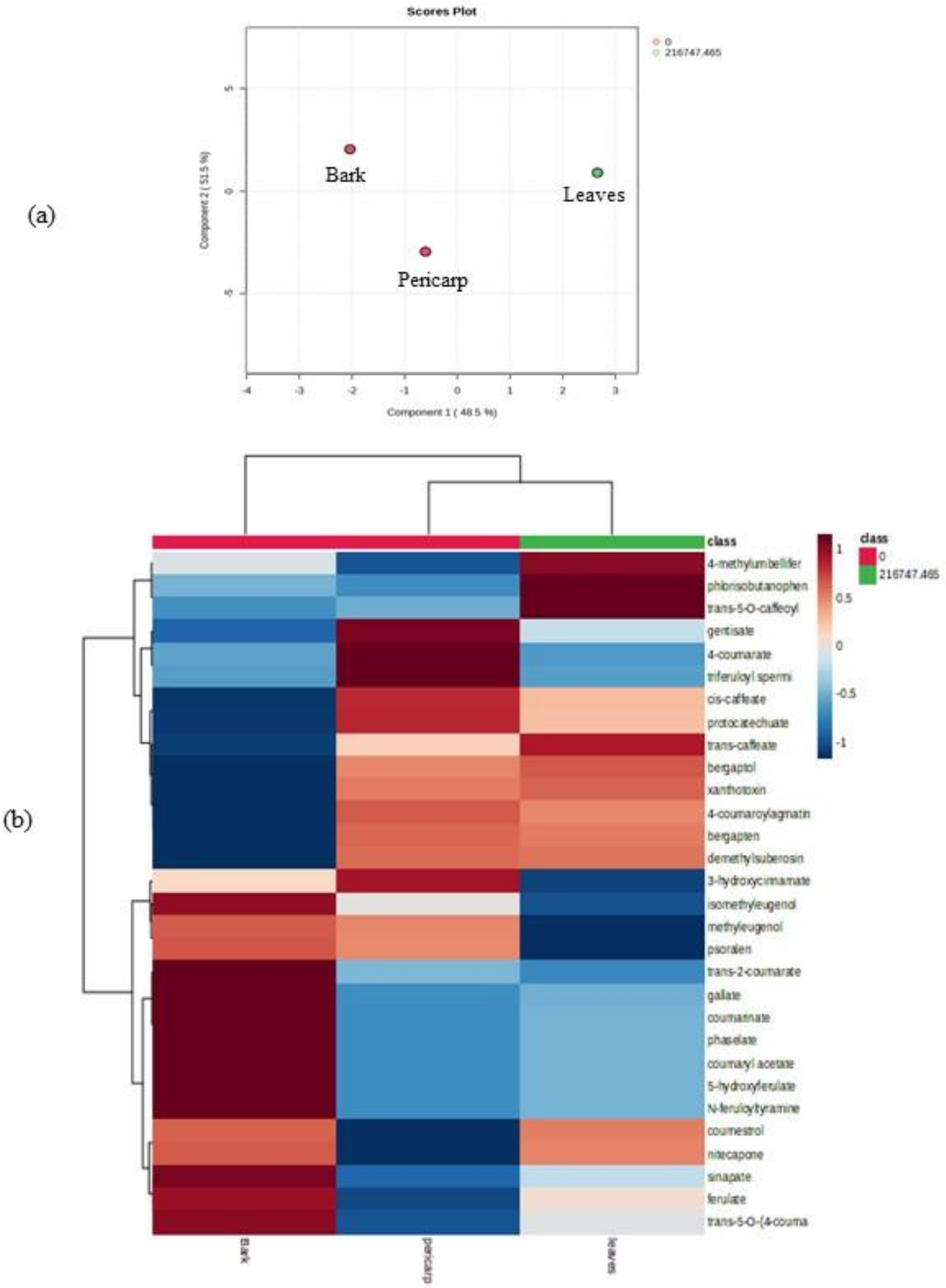
Figures 31 (a-b). (a) Principal component analysis (PCA)-Scores plot of the terpenoids in bark, leaves and pericarp. The variances are shown in brackets. (b) Hierarchical clustering result of terpenoids shown as heatmap (distance measured using euclidean, and clustering algorithm using ward. D).

medicarpin-3-o-glucoside-6"-malonate, luteone, patuletin, tricetin, pisatin, and (2s)-hesperetin were abundant in bark extracts (Figure 30b). The flavonoids that were abundant in leaves include pratensein, chrysoeriol, quercetin, vitexin, quercetin 3-sulfate and apiforol. In pericarp extracts, the most abundant flavonoids were chrysin, gossypetin, 6-hydroxyflavone, genistein, pinobanksin, luteolin, and dalbergioidin (Figure 30b).

The most abundant terpenoids in bark extracts were betulin, arabidiol, soyasapogenol a, lanosterol, lubimin, botulin, and erythrodiol (Figure 31b). The terpenoids that were most abundant in leaves were alpha bisabolo, borneol, alpha eudesmol, beta zeacarotene, zeinoxanthin, 10-deacetylbaccatin III, albienol, delta cadinene, loganin and squalene. In pericarp extracts, the most abundant terpenoids were dihydroyphaseic acid, zeaxanthin, gossypol, 16-methoxytaberson and furruginol (Figure 31b). The most abundant alkaloids in bark extracts include (s)-canadine, 17-oxosparteine, camptothecin, (s)-isoandrocymbin, 10-methylxanthosine, harmine, vomilenine, 12-hydroxydihydrochelirubine, 13-hydroxylupinine and protopine (Figure 32b). In leaves extracts, the abundant alkaloids were elwesine, s-magnoflorine, cephaeline, strictosidine, stylopine, akuammicine, 6-hydroxyprotopine and allocrytopine. On the other hand, the most abundant alkaloids in pericarp extracts were 1,2-dihydrovomilenine, 19E-geissoschizine, chinhonidine, O-methylandrocymbine, emetine, lupinate, strictosamine and tryptamine (Figure 32b). Phenolic acids such as trans-2-coumarate, gallate, coumarinate, phaselate, coumaryl acetate, 5-hydroxyferulate, sinapate and N-feruloyltyramine were the most predominant in bark extracts (Figure 33b). The most abundant phenolic acids in leaves were 4-methyllumbelliferone, trans-5-o-caffeoyl-d-quinate, trans-caffeate, bergaptol and xanthotoxin. In pericarp, the major phenolic acids were gentisate, 4-coumarate, cis-caffeate, protocatechuate, 3-hydroxycinnamate and 4-coumaroylagmatine (Figure 33b).



Figures 32 (a & b). (a) Principal component analysis (PCA) scores plot of the alkaloids content in bark, leaves and pericarp. The explained variances are shown in brackets. (b) Hierarchical clustering result of alkaloids shown as heatmap (distance measure using euclidean, and clustering algorithm using ward D).



Figures 33 (a & b). Principal component analysis (PCA)-Scores plot of the phenolic acids in bark, leaves and pericarp. The variances are shown in brackets. (b) Hierarchical clustering results of phenolic acids shown as heatmap (distance measured using euclidean, and clustering algorithm using ward D).

4.2.3. Flavonoids (quercetin, rutin, myrecetin) were quantified in pericarp, bark and leaves by RP-HPLC

The flavonoid compounds *viz.*, quercetin, rutin and myricetin which are known to have antioxidant and anti-inflammatory activities have been quantified in the methanolic extracts of bark, leaves and pericarp using RP-HPLC method. Linearity was tested by analyzing the average peak area of quercetin, rutin and myrecetin of different injection volumes. Thus, a good linearity was observed when the quercetin, rutin and myrcetin standard concentrations ranged from 0.1 to 1.5 mg/ml. The correlation co-efficient (r) obtained from the linear regression equation for quercetin, rutin and myrcetin were 0.9929, 0.9913 and 0.9955, respectively. The retention time was found to be 3.52, 3.50 and 3.55 min for quercetin (Figures 34a-c) whereas it was 3.35, 3.39 and 3.34 min for rutin in bark, leaves and pericarp, respectively (Figures 35a-c). The retention time was 3.46, 3.52 and 3.95 for myrcetin in bark, leaves and pericarp, respectively **(Figures 36a-c).** The average quercetin content in bark, leaves and pericarp was 5.71 µg/g dry weight, 9.43 µg/g dry weight, 1.44 µg/g dry weight, respectively **(Table 4)**. The rutin content in bark, leaves and pericarp was found to be 2.13 µg/g dry weight, 45.19 µg/g dry weight, and 55.39 µg/g dry weight, respectively **(Table 5)**. The myrcetin content was 0.24 µg/g dry weight, 13.22 µg/g dry weight and 8.4 µg/g dry weight, in bark, leaves and pericarp, respectively **(Table 6)**.

Table 4. Quercetin content in methanolic extracts of bark, leaves and pericarp estimated byRP-HPLC.

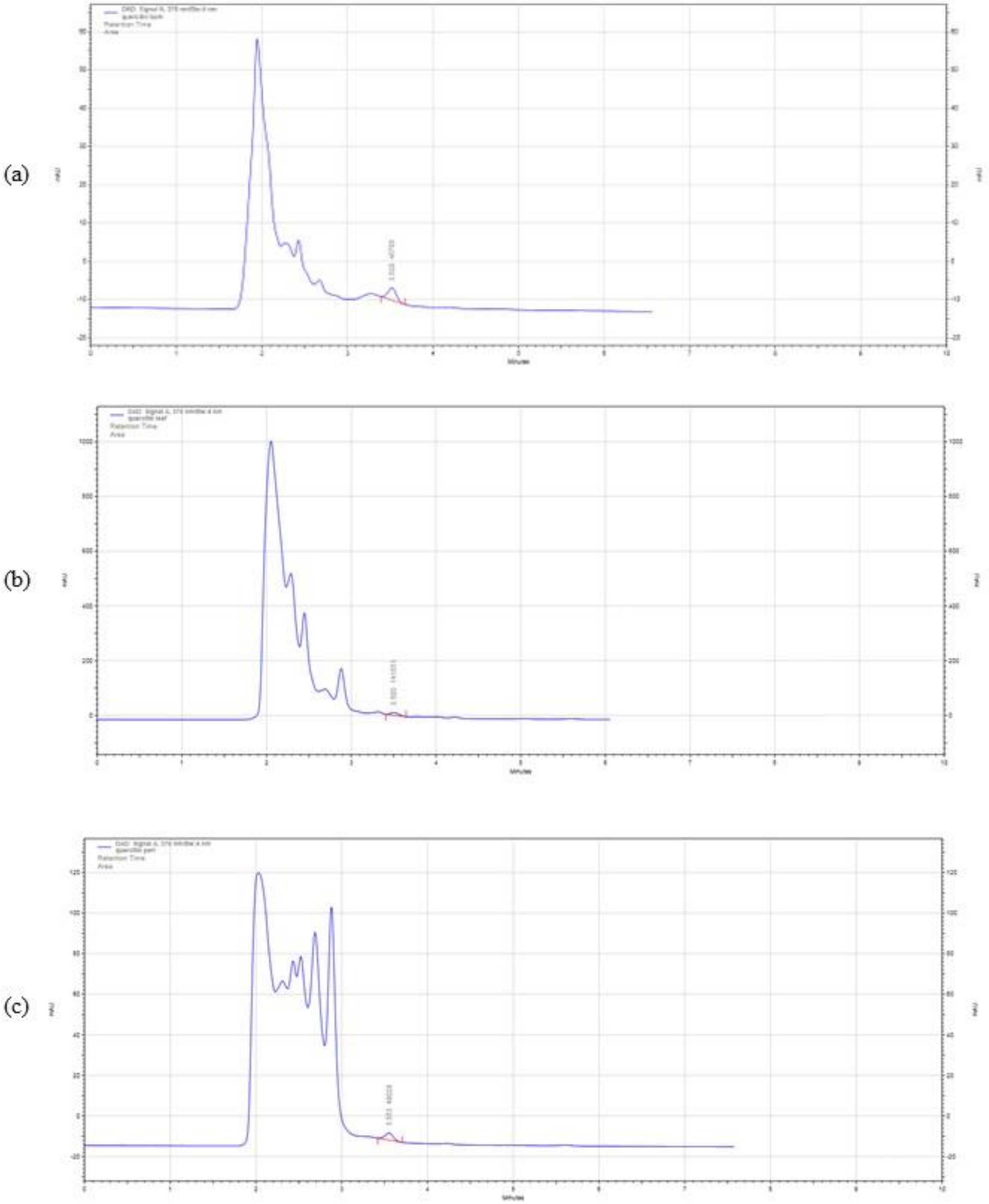
Sample	**Retention time**	**Area**	**Height**	**Quercetin content (µg/g dry weight)**
Bark	3.52	45705	6726	5.71
Leaves	3.5	141551	19691	9.43
Pericarp	3.55	49028	7467	1.44

Table 5. Rutin content in methanolic extracts of bark, leaves and pericarp estimated by RP-HPLC.

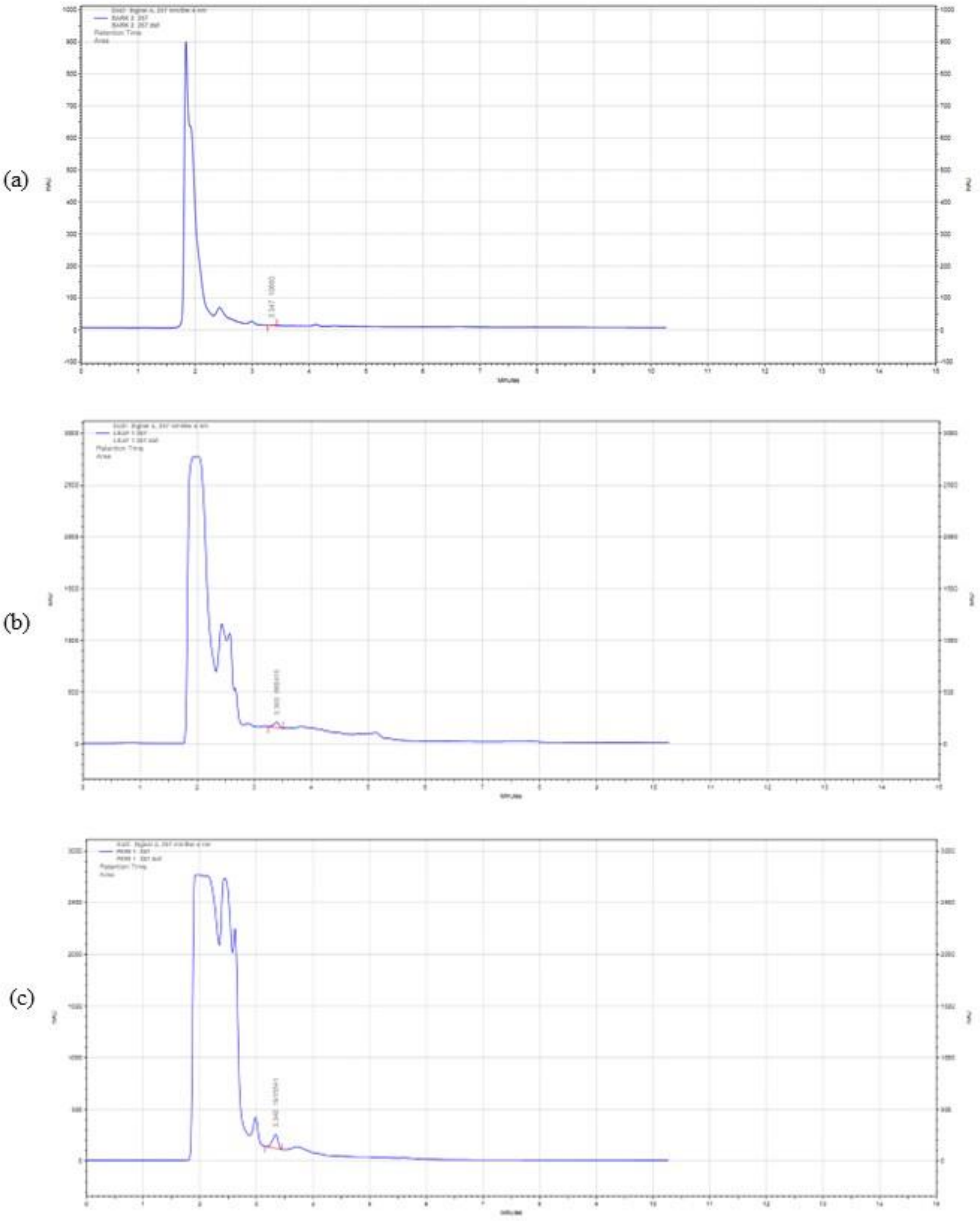
Sample	**Retention time**	**Area**	**Height**	**Rutin content (µg/g dry weight)**
Bark	3.35	9854	10680	2.13
Leaves	3.39	660415	604964	45.19
Pericarp	3.34	1915541	1951359	55.39

Table 6. Myrcetin contents in methanolic extracts of bark, leaves and pericarp estimated by RP-HPLC.

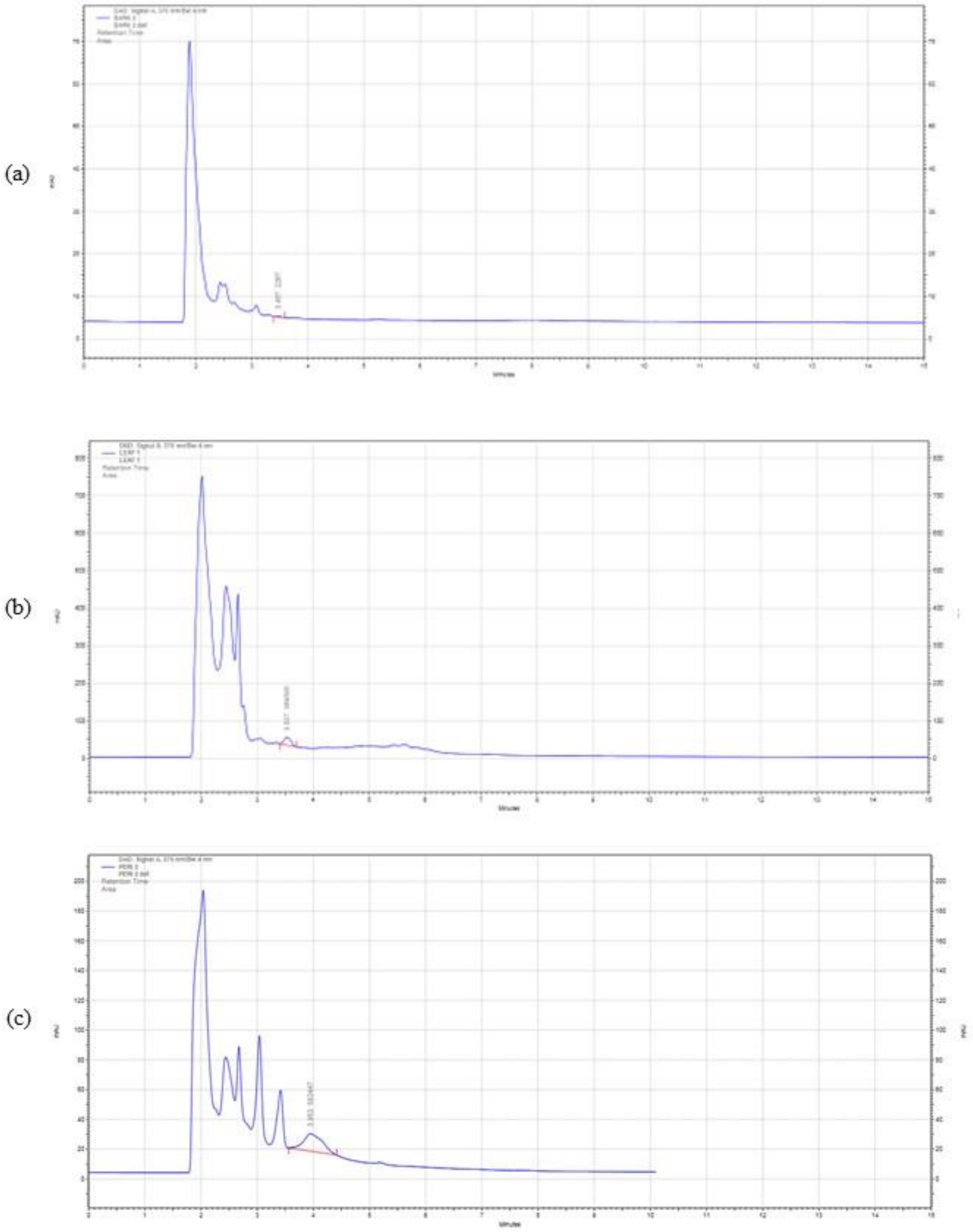
Sample	**Retention time**	**Area**	**Height**	**Myrcetin content (µg/g dry weight)**
Bark	3.46	2420	2267	0.24
Leaves	3.52	369585	370916	13.22
Pericarp	3.95	589024	583447	8.40



Figures 34 (a-c). Quantification of quercetin content in different parts using RP-HPLC. (a) Bark, (b) Leaves and (c) Pericarp.



Figures 35 (a-c). Quantification of rutin in different plant parts using RP-HPLC. (a) Bark, (b) Leaves and (c) Pericarp.



Figures 36 (a-c). Quantification of myrcetin in different plant parts using RP-HPLC: (a) Bark, (b) Leaves and (c) Pericarp.

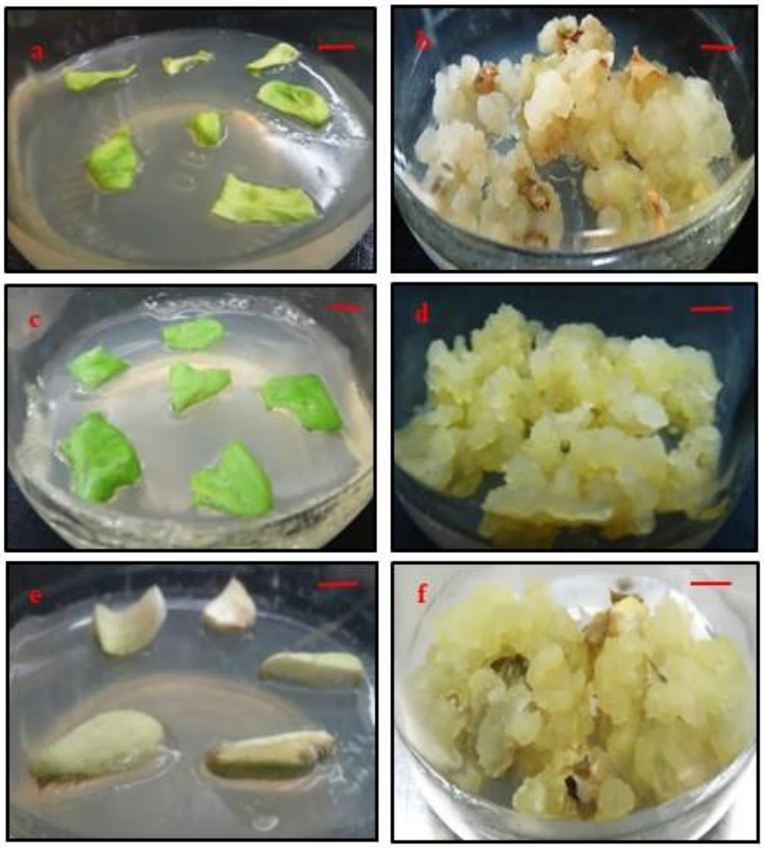
4.3. Callus induction from different explants and direct shoot organogenersis from zygotic embryo axes of *G. moluccana*

4.3.1. Callus induction varied with the explants and growth regulators

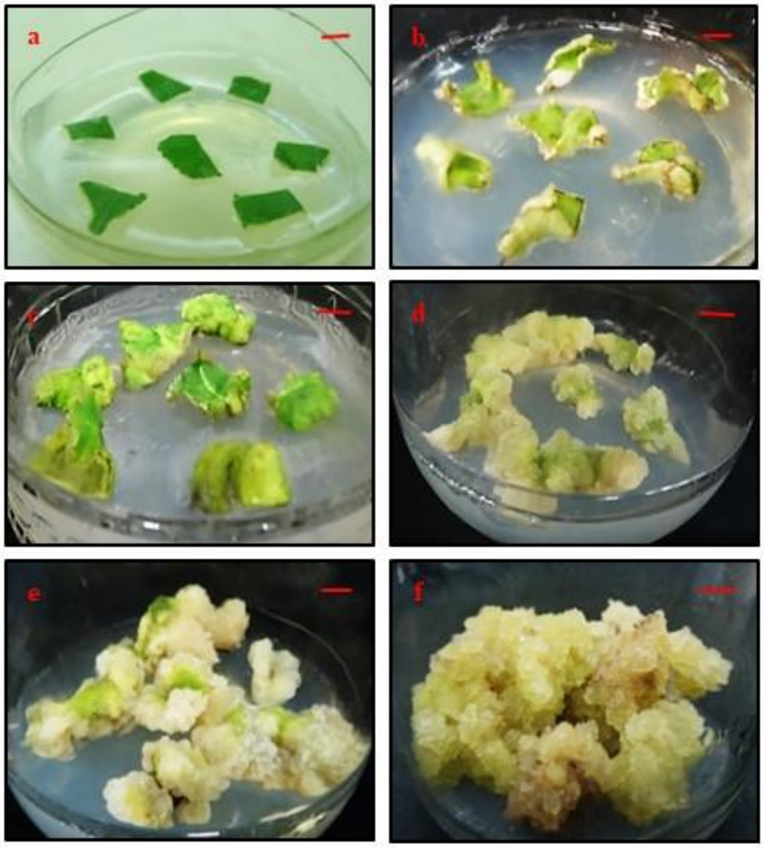
The response of different explants *viz.*, cotyledons, leaves and endosperm obtained from *in vitro* germinated seedlings for callus induction was evaluated after culture on full-strength MS medium with different growth regulators. Among the different growth regulators tested, 2 mg/l 2,4-D and 1 mg/l BAP proved to be effective in inducing high-frequency callus formation (91.3–100%) followed by 1 mg/l BAP and 2 mg/l NAA (60.3–83.9%) (Table 7). Of the different explants tested, the frequency of callus induction was found to be higher from endosperm explants (29.8%-100%) as compared to leaves (10.5-100%) and cotyledons (20.3-91.3%) on medium fortified with different growth regulators. Maximum callus induction of 100% was achieved from both leaves and endosperm on medium with 2 mg/l 2,4-D and 1 mg/l BAP. Callus was initiated from different explants after 7-16 days of culture on different media with rapid response observed from leaves and cotyledon explants in the presence of 2,4-D alone or in combination with BAP (Table 7). The morphology of the callus (color and texture) varied depending on the explant and growth regulators used. The callus induced from leaves was greenish white whereas it was mostly white or creamy white for other explants (Figures 37a-f). Callus formed from leaves in the presence of 2 mg/l 2,4-D and 1 mg/l BAP was friable and nodular (Figures 38a-f) whereas it was either hard and compact or soft in nature in the presence of other growth regulators. The callus developed from endosperm was either compact or either compact and nodular in nature while cotyledon derived callus was soft type on different media tested. The proliferation rate of the callus produced from different explants was determined after 4 weeks of subculture to fresh medium. The callus biomass in terms of fresh weight and dry weight was found to be higher on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP as compared to other growth regulators for all the explants (Figure 39). It was observed that leaves derived callus cultures showed highest biomass followed by endoperm whereas it was lowest from cotyledons in the presence of 2 mg/l 2,4-D and 1 mg/l BAP (Figure 39). Thus, the study

Table 7. Effect of plant growth regulators on callus induction from different explants of G. *moluccana* on MS full-strength medium

Growth regulator(s)	**Explant**	**Callus induction (%)**	**Days for initiation**	**Characteristics of callus**	
				Appearance	**Color**
2 mg/l 2,4-D	Leaves	48.2	7-9	Hard and compact	Greenish white
	Cotyledons	57.6	7-9	Soft and watery	White
	Endosperm	60.3	12-14	Compact	White
2 mg/l 2,4-D + 1 mg/l BAP	Leaves	100	7-9	Friable and nodular	Greenish white
	Cotyledons	91.3	7-9	Soft	White
	Endosperm	100	10-12	Compact and nodular	Creamy white
2 mg/l NAA + 1 mg/l BAP	Leaves	60.3	10-12	Soft	Greenish white
	Cotyledons	70	10-12	Hard and compact	White
	Endosperm	83.9	14-16	Compact and nodular	Creamy white
2 mg/l 2,4-D + 1 mg/l Kinetin	Leaves	44	10-12	Soft	Greenish white
	Cotyledons	49.1	10-12	Soft and watery	Greenish white
	Endosperm	58.3	12-14	Compact and nodular	White
2 mg/l 2,4-D + 1 mg/l NAA	Leaves	10.5	14-16	Soft	Greenish white
	Cotyledons	20.3	10-12	Soft	Creamy white
	Endosperm	29.8	14-16	Compact	Creamy white



Figures 37 (a-f). Callus formed from different explants after 5 weeks of subculture on MS media with 2 mg/ml 2, 4-D and 1 mg/l BAP. (a & b) Cotyledons, (c & d) Leaves and (e & f) Endosperm (Bars=1cm).



Figures 38 (a-f). Initiation and proliferation of callus from leaves explants after different durations of culture on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP. (a) Explants after inoculation, (b-f) Callus formation after 1-week, 2-weeks, 4-weeks, 5-weeks and 7 weeks of culture, respectively.

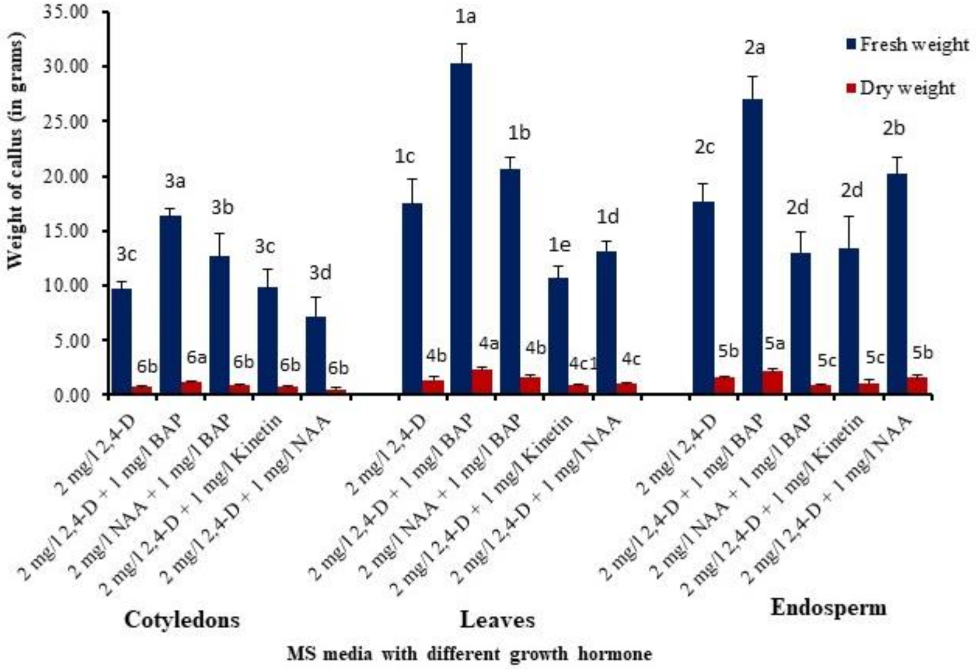


Figure 39. Effect of different growth regulators on biomass of callus derived from cotyledons, leaves and endosperm of *G. molucanna*. The fresh weight and dry weight was determined after 5 weeks of subculture of the callus on the fresh medium. Data represents Means ± SD of three independent experiments. Means followed by the same letter in the column are not statistically significant at the 5% probability level by one-way analysis of variance (ANOVA) according to Duncan's multiple range test.

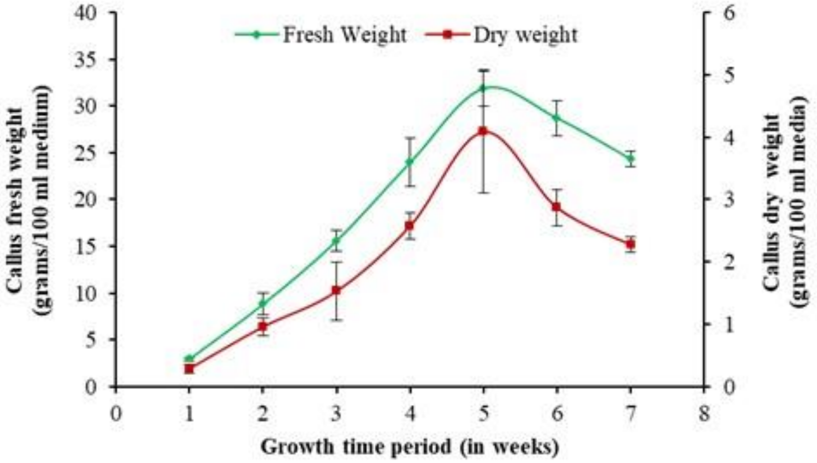
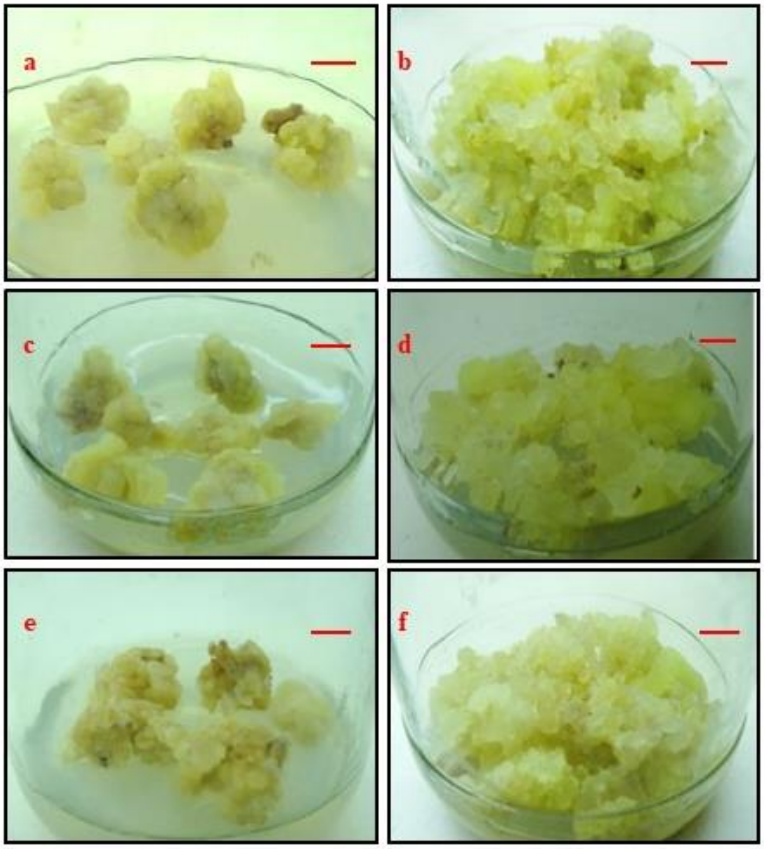


Figure 40. Growth curve of callus derived from leaves explants as determined by fresh weight and dry weight at different periods after culture on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP.



Figures 41 (a-f). Leaves derived callus cultures treated with Salicylic acid and Jasmonic acid. (a & b) Control callus (untreated), (c & d) Salicylic acid, (e & f) Jasmonic acid (after 20 days of culture (Bars=1 cm).

revealed efficient induction of callus and proliferation from leaves followed by endosperm in the presence of 2 mg/l BAP and 1 mg/l 2,4-D. The callus obtained from leaves, cotyledons and endosperm can be employed in future for studying the impact of polyploidy on secondary metabolite production in callus cultures.

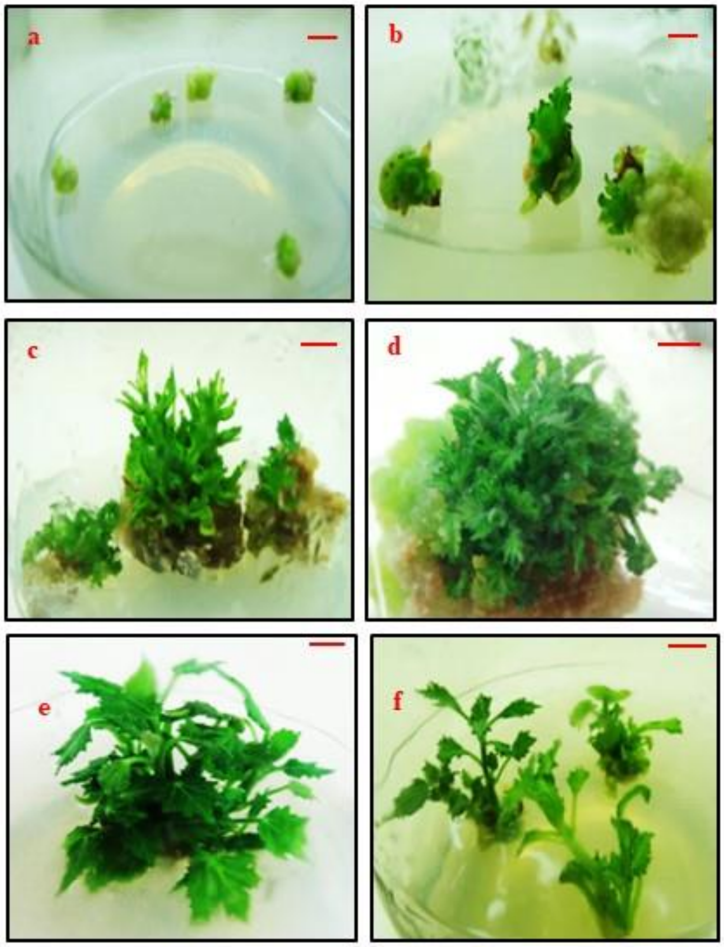
Further experiments were conducted in this study with leaves explants as they exhibited superior response and also as they are diploid with more number of explants being available for initiation. The growth rate of callus derived from leaves was further examined at different time periods which revealed highest fresh weight (31.86 gm/100 ml medium) and dry weight (4.08 gm/100 ml medium) at 5 weeks and thereafter declined (Figure 40). The leaves derived callus cultures were used in elicitation experiments by treating with SA and JA and their effects on biomass (Figure 41) and secondary metabolite production was evaluated.

4.3.2. Efficient multiple shoot induction and elongation was achieved from zygotic embryo axes

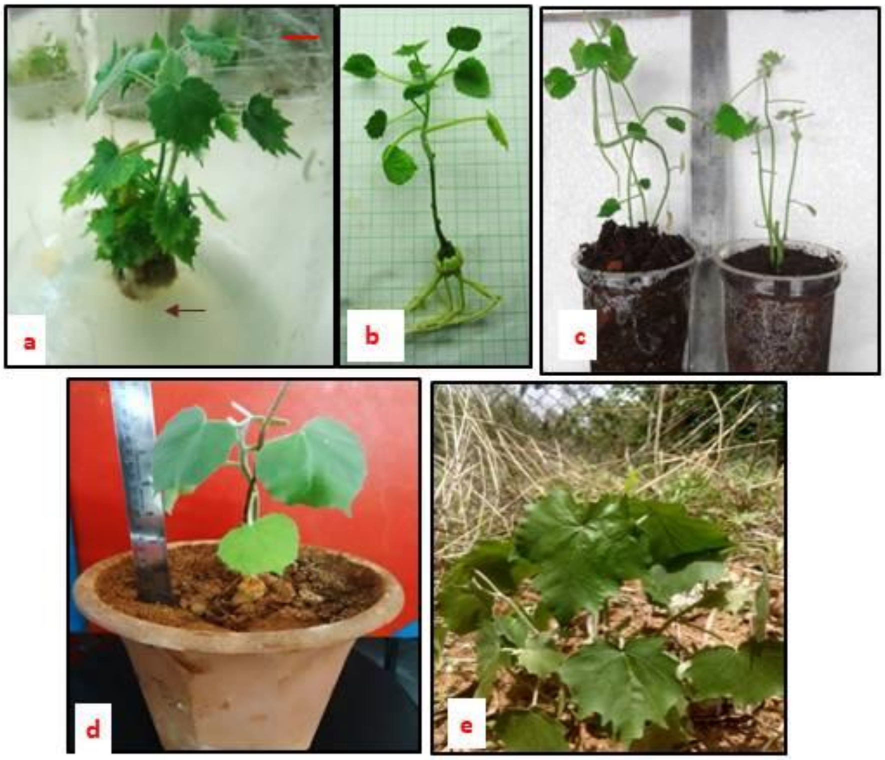
Zygotic embryo axes were cultured on medium containing different concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) of BAP and kinetin, individually or in combinations to evaluate their potential to form multiple shoots. The initial response from the explants were observed within 2 weeks of culture, however, the true sign of multiple shoot induction was observed on the 3rd week, which were induced directly from the proximal region with the emergence of several greenish structures which grew in size and formed a clump (Figures 42a-c). Among the different growth media used, 3 mg/l of BAP showed a better response by inducing multiple shoots at higher frequency (91.1%) whereas it varied (17.8-51.1%) on medium with other growth regulators. Although higher number of shoots (9-11) per explant were achieved after subculture on medium with 3 mg/l BAP, it increased the callus formation from the base of the explants. Subculture of multiple shoots clumps to half-strength medium with 2 mg/l BAP minimized callus formation and induced shoot proliferation (Figure 42e). Elongation of multiple shoots was achieved by transferring shoot clumps (3-5 shoots per clump) sequentially to half-strength medium with reduced concentrations of BAP (1 mg/l, 0.5 mg/l) followed by half-stength medium without growth regulators (Table 8; Figure 42f). Thus, efficient shoot induction and elongation was successfully achieved with 6-8 shoots per zygotic embyo axes explant at the end of last subculture on medium without growth regulators.

Table 8. Response of multiple shoots induction from zygotic embryo axes on MS media fortified with BAP and/or kinetin after 8 weeks of culture.

Growth regulators **BAP (mg/l)**	 **Kin (mg/l)**	**Shooting induction (%)**	**Average number of shoots/explant**
0	1	17.8	3.3 ± 1.0
0	2	35.5	5.6 ± 0.6
0	3	51.1	7.0 ± 1.0
1	0	17.8	5.0 ± 2.0
2	0	40.0	4.6 ± 1.5
3	0	91.1	8.3 ± 2.1
0.5	0.5	46.7	6.0 ± 0.0
1	1	48.9	6.6 ± 1.5
1	2	30.6	3.6 ± 0.6
2	1	22.0	3.6 ± 0.6



Figures 42 (a-h). Multiple shoot induction and elongation from zygotic embryo axes after culture on full-strength MS medium with 3 mg/l BAP followed by transfer to half-strength MS medium with reduced concentrations (1.0 and 0.5 mg/) of BAP (Bars=1 cm). (a-c) Multiple shoots induced on initiation medium with 3 mg/l BAP, (d) Proliferation of multiple shoots after subculture on medium with 3 mg/l BAP (e) Elongation of shoots on half-strength medium with (e) 1 mg/l BAP and (f) 0.5 mg/l BAP, respectively.



Figures 43 (a-e). Root induction, acclimatization and establishment of plants in soil. (a) Initiation of roots from shoot cultured on half-strength MS medium with 0.1 mg/l NAA, (b) Roots induced from shoots after 5 weeks of culture on 0.1 mg/l NAA (c) Acclimatization of the regenerated plantlets, (d) Regenerated plant transferred to soil and established in pots (e) Regenerated plant growing in the soil in field after 6 months of transplantation.

The elongated multiple shoots were separated and placed on half-strength MS medium containing 0.5 mg/l IBA or 0.5 mg/ NAA or without growth regulators for root induction (Figure 43a). Root induction was successfully achieved at a frequency of 71.5% and 72.8% with IBA or NAA whereas no rooting was achieved in the absence of growth regulators. Root initiation was observed in 10-12 days on medium with either IBA or NAA. About 5-6 roots per shoot were induced in each shoot after 5 weeks of culture on medium using either IBA or NAA (Figure 43b). The regenerated plants were acclimatized for 4 weeks in culture room with a survival frequency of 72.4% (Figure 43c). The acclimatized plants were transferred to earthen pots in soil in greenhouse and succesfully transplanted in the field (Figures 43d & e). Thus, multiple shoot induction and subsequent plant regeneration was successfully achieved from zygotic embryo axes which can be exploited in future for secondary metabolites production from shoot cultures which otherwise may not produced in undifferentiated callus cultures.

4.4. Elicitation of callus cultures with JA and SA for enhanced accumulation of total phenolics and flavonoids

4.4.1. SA and JA elicitation influenced the callus biomass and accumulation of total phenolics and flavonoids

The callus fresh weight and dry weight decreased upon elicitation with SA and JA for 30 days as compared to control callus cultures (FW, 33.58 gm/100 ml medium; DW, 5.75 gm/medium) (Figures 41 & 44). SA treatment resulted in greater decrease in fresh weight (21.8 to 23.12 gm/100 ml medium) and dry weight (1.24 to 1.78 gm/100 ml medium) in comparison to JA treatment where the fresh weight and dry weight was found to be 28.47 to 25.27 gm/100 ml medium and 2.15 to 4.75 gm/100 ml medium, respectively for the different concentrations tested. Elicitation of callus cultures with SA and JA had a marked effect on the accumulation of total phenolic acids in callus cultures of *G. moluccana*. The phenolic acids content varied in callus cultures treated with SA and JA depending on the concentration and duration of treatment (Figures 45a & b). The highest content of phenolics in SA treated samples (31.4 mg GAE g^{-1}DW) was recorded in callus cultures treated with 50 μM SA for 5 days. The phenolic content decreased with increase in the concentrations of SA as well as the duration of cultures (Figure 45a). The pattern of phenolic acid accumulation varied in callus cultures treated with JA, which showed an increase in phenolic content from 50 μM to 150 μM, with the highest content (81.83 mg GAE g^{-1} DW) observed in callus cultures treated with 150 μM for 20 days (Figure 45b). A

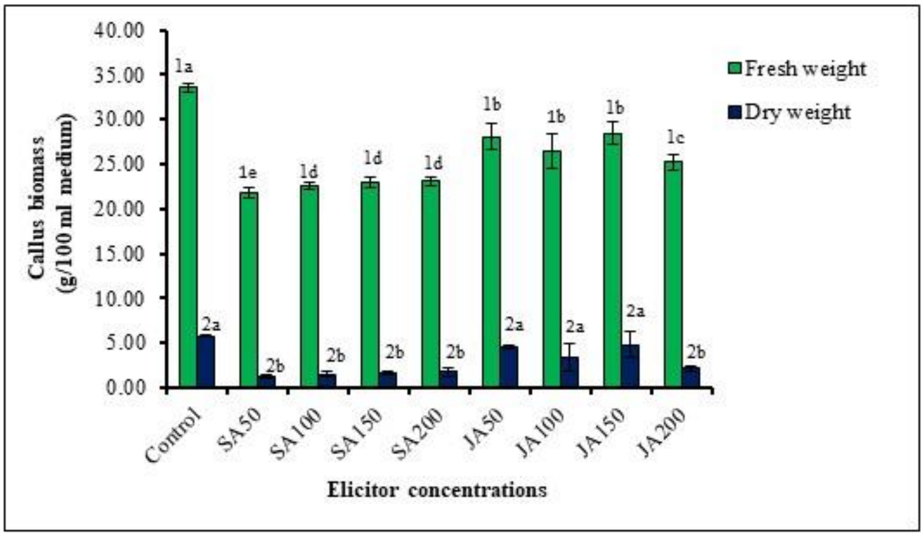
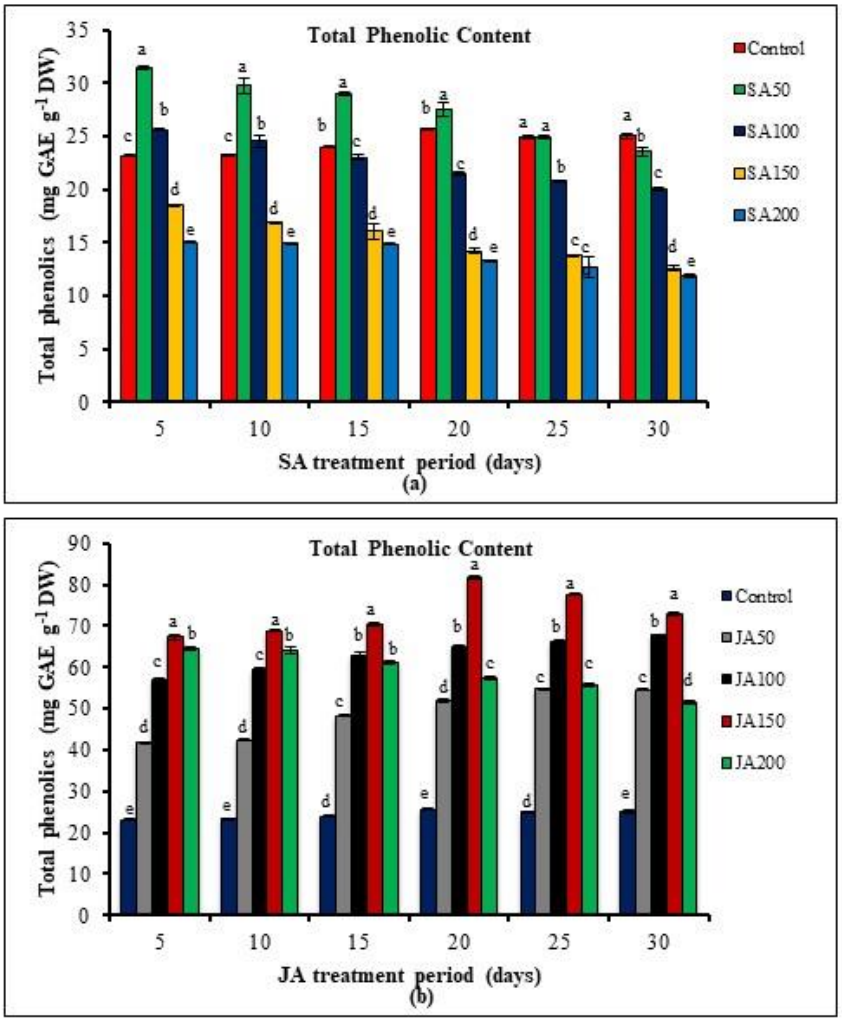
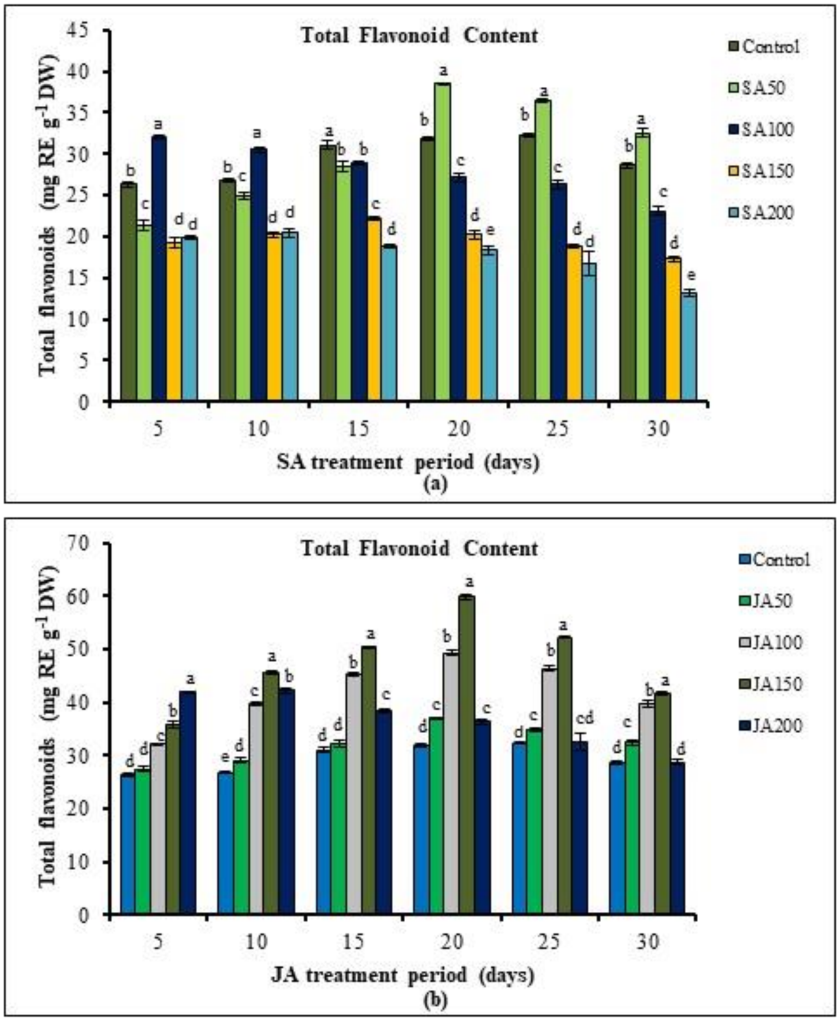


Figure 44. Effect of salicylic acid (SA) and jasmonic acid (JA) elicitation on callus biomass as determined by fresh weight and dry weight. The fresh weight and dry of the callus was recorded after 5 weeks of culture on the media. Means followed by the same letter are not statistically significant according to Duncan's multiple range test.



Figures 45 (a & b). Total phenolic acid of callus cultures after elicitation with salicylic acid and jasmonic acid along with controls. (a) Salicylic acid elicited cultures, (b) Jasmonic acid elicited cultures. Means followed by the different letter within a group are statistically significant according to Duncan's multiple range test.



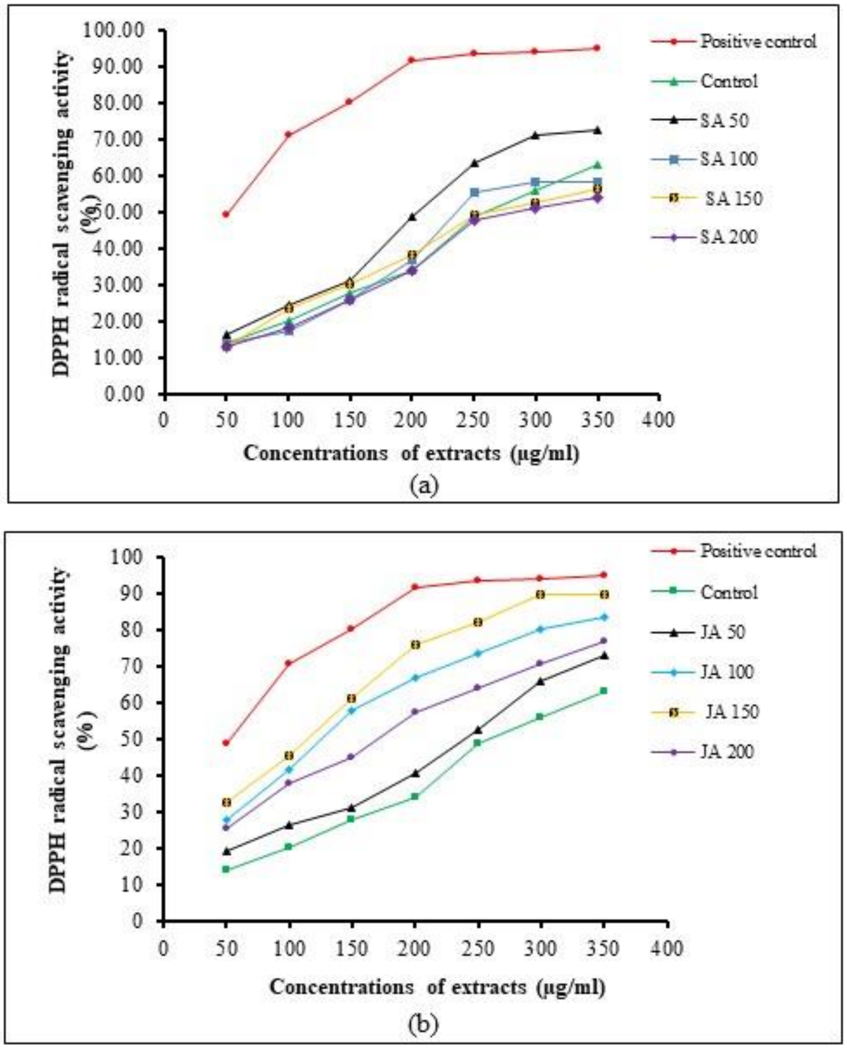
Figures 46 (a-b). Total flavonoid contents of callus cultures after elicitation with salicylic acid and jasmonic acid along with controls. (a) Salicylic acid elicited cultures, (b) Jasmonic acid elicited cultures. Means followed by the different letter within a group are statistically significant according to Duncan's multiple range test.

significant decline in phenolic content was noticed with further increase in JA concentration or duration of the cultures. The phenolic contents in the control callus cultures varied from 23.18 to 25.68 mg GAE g^{-1} DW after 5-30 days of culture.

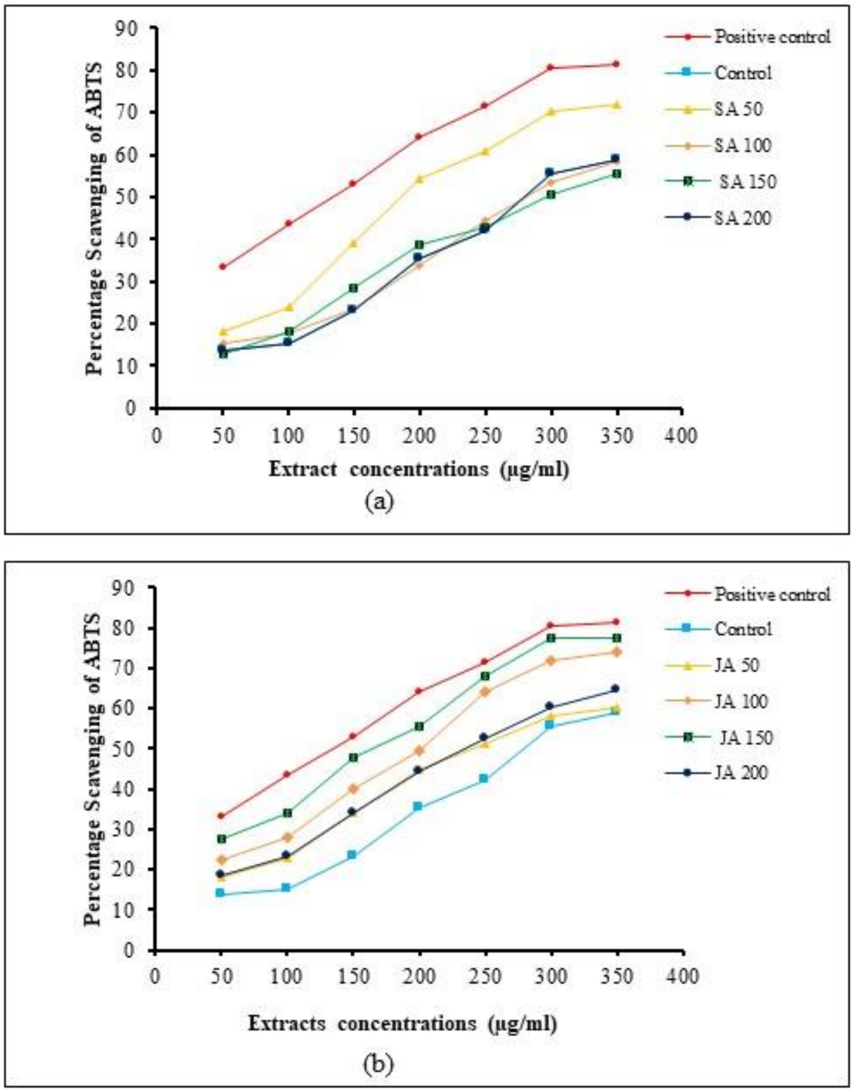
SA and JA treatments affected the accumulation of flavonoids in a concentration and time-dependent manner. The highest content of flavonoids (38.49 mg RE g^{-1} DW) in SA treated samples was recorded in callus cultures treated with 50 µM for 20 days, and thereafter declined (Figure 46a). The flavonoid content in callus cultures treated with 100 µM was higher than the controls at 5 days and 10 days, and the content was lower than controls during subsequent periods (15-30 days) of culture. The JA elicited callus cultures showed highest flavonoid content at 150 µM (60.08 mg RE g^{-1} DW) on the 20th day as compared to other treatments and controls. The flavonoid content decreased with further increase in the concentration and duration of culture (Figure 46b). Callus cultures treated with 100 µM JA also resulted in higher flavonoid content than controls at all durations of culture.

4.4.2. SA and JA treated callus cultures exhibited free radical scavenging activities

The methanolic extract of leaves-derived callus cultures of *G. moluccana* exhibited strong antioxidant activity as assayed by their ability to scavenge free radicals such as DPPH and ABTS. Callus cultures treated with SA for 20 days showed a lower percentage of radical scavenging activity in comparison to JA elicited callus cultures for the same duration but its activity was higher than controls in callus cultures treated with 50 µM SA (Figures 47a & b). The percentage DPPH radical scavenging activity varied with the concentration of salicylic acid *i.e.* SA50 (16.22–72.41%), SA100 (13.67–58.08%), SA150 (13–56.30%), and SA200 (13.05–53.95%) for different concentrations of extracts used (50–350 µg/ml). The DPPH radical scavenging activity for control callus extracts ranged between 13.87 to 62.93% while that of ascorbic acid (positive control) ranged between 48.90 to 94.95%. The DPPH radical scavenging activity decreased as the concentration of salicylic acid was increased to 200 µM and remained lower than controls at 150 to 200 µM (Figure 47a). The DPPH scavenging activity for JA-treated callus cultures was higher than the control cultures for all the concentrations tested (Figure 47b). The percentage of DPPH radical scavenging activity for different concentrations of JA-treated callus cultures were JA50 (19.17–73.07%), JA100 (27.69–83.53%), JA150 (32.59–89.85%), and JA200 (25.50–76.80 %). The most significant scavenging activity was observed in callus extracts containing 150 µM of JA and showed a decrease at 200 µM. The higher radical



Figures 47 (a & b). DPPH radical scavenging activity of extracts of callus cultures elicited with different concentrations of salicylic acid (SA) and jasmonic acid (JA) as compared with control (without elicitor treatment) and positive control (ascorbic acid), (a) Salicylic acid elicited cultures, (b) Jasmonic acid elicited cultures.



Figures 48 (a-b). ABTS radical scavenging activity of callus cultures elicited with salicyclic acid and jasmonic acid in comparison with controls (ascorbic acid was used as positive control) and cultures with any treatment are normal controls. (a) SA elicited cultures and (b) JA elicited cultures.

scavenging activity exhibited by JA and SA treated callus cultures could be due to the enriched phenolics and flavonoids as compared to control callus cultures.

Similar pattern of radical scavenging activity was observed, when the methanolic extracts of callus cultures treated with SA and JA for 20 days was assayed for ABTS. The percentage of ABTS radical scavenging activities of 50 µM SA treated callus cultures ranged from 18.36–71.98%, 100 µM SA ranged from 15.28–58.17%, 150 µM SA ranged from 13–55.36% and 200 µM SA ranged from 13.67–58.98%, while that of control callus cultures and positive controls ranged from 13.67 to 58.98%, and 33.3 to 81.5%, respectively (Figure 48a). The percentage ABTS radical scavenging activities for JA treatments varied with the concentrations *i.e.* JA50 (17.96–60.32%), JA100 (22.52–73.99%), JA150 (27.61–77.34%), and JA200 (18.49–64.74%) (Figure 48b). The highest ABTS radical scavenging activity was observed in SA treatment with 50 µM whereas for JA treatment, the highest scavenging activity was observed at 150 µM. In both SA and JA treatments, the ABTS radical scavenging activity was higher as compared to control callus cultures.

4.4.3. SA and JA elicitation increased the production of H_2O_2 and MDA levels

The accumulation of H_2O_2 in both the control and elicited callus cultures were assessed as it is one of the earliest indications of elicitor recognition by the plants. An increase in the accumulation of H_2O_2 was observed in the callus treated with SA and JA as compared to controls (0.86 µmol g^{-1} FW) (Figure 49). The levels of H_2O_2 elevated as the concentration of SA increased up to 150 µM (3.23 µmolg^{-1} FW) and did not change significantly at 200 µM (3.35 µmolg^{-1} FW). Similarly in JA elicited callus cultures, the highest level of H_2O_2 was observed with 150 µM JA (3.6 µmol g^{-1} FW) which did not differ significantly from 200 µM JA (3.7 µmol g^{-1} FW). However, at lower concentrations of 50 µM (1.61 µmolg^{-1} FW) and 100 µM (1.86 µmolg^{-1} FW), the H_2O_2 levels were found to be higher in SA-treated callus cultures as compared to JA at 50 µM (1.46 µmolg^{-1} FW) and 100 µM (1.69 µmolg^{-1} FW).

The control and elicitor-treated callus were analyzed for the MDA content which is an indicator of lipid peroxidation. All the treatments resulted in higher MDA accumulation as compared to controls (0.50 µmol g^{-1} FW) (Figure 50). Under the influence of the SA as elicitor, the content of MDA remained in the range of 0.77 to 1.45 µmol g^{-1} FW. Treatment with JA induced maximum MDA accumulation, with the highest recorded with 200 µM JA (2.27 µmol g^{-1} FW) followed by 150 µM JA (1.68 µmol g^{-1} FW).

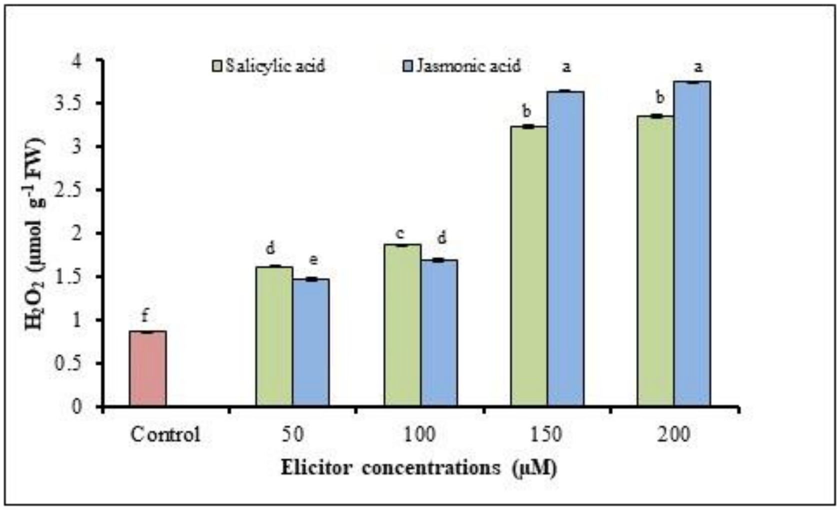


Figure 49. Effect of different concentrations of salicylic acid and jasmonic acid on generation of H_2O_2 in callus cultures of *G. moluccana*. Means followed by the same letter are not statistically significant according to Duncan's multiple range test.

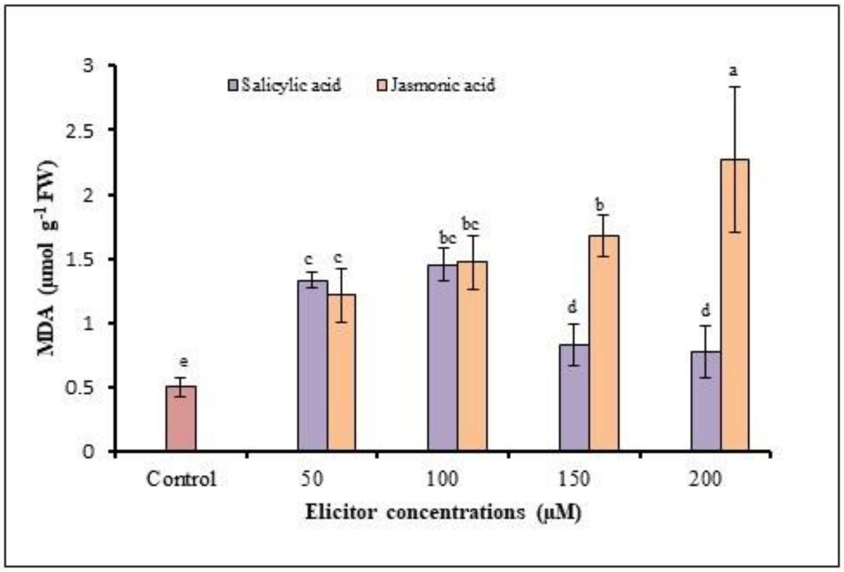


Figure 50. Malondialdehyde (MDA) levels in callus cultures elicited with salicylic acid and jasmonic acid. Means followed by the same letter are not statistically significant according to Duncan's multiple range test.

4.4.4. SA and JA elicitation of callus cultures caused alteration in antioxidant enzyme activities

For better understanding the elicitation effects of salicylic acid and jasmonic acid, the activities of several antioxidant enzymes were examined in the elicitor-treated callus cultures and compared with controls.

4.4.4.1. Superoxide dismutase (SOD) enzyme activity

The ability of SOD to reduce the superoxide into hydrogen peroxide and molecular oxygen is the basis of this assay. An elevated activity of SOD was observed in all the treatments compared to control cultures (Figure 51). Lower concentrations of SA (50 µM) stimulated higher SOD activity (20.3 U mg^{-1}) than higher concentrations (150-200 µM) where the activity did not change significantly. On the other hand, JA at 150 µM induced the maximum SOD activity (24.28 U mg^{-1}) followed by a decline at 200 µM.

4.4.4.2. Catalase (CAT) enzyme activity

Catalase is a well-known intracellular enzyme which protects against the ROS that is generated within the cell. Treatment of SA resulted in highest catalase activity at the lowest concentration of 50 µM (12.39 U mg^{-1}) followed by a decline with increase in the concentration of SA although it did not differ significantly for 150 µM and 200 µM treatments. Under the influence of JA, the catalase activity gradually increased reaching the highest (18.62 U mg^{-1}) at 150 µM, however, upon reaching 200 µM, its activity decreased to 13.06 U mg^{-1} (Figure 52).

4.4.4.3. Peroxidase (POD) enzyme activity

Peroxidases are protective enzymes of plant cells which act against a variety of stresses by regulating the concentration of O_2 and H_2O_2. Similar to CAT activity, SA at lower concentrations of 50 µM induced highest (504.25 U mg^{-1}) peroxidase activity (Figure 53). The enzyme activity decreased as the concentration of SA increased, with the activity in 150 and 200 µM treated cultures being lower than controls (338.43 U mg^{-1}). JA-elicited callus cultures showed highest activity (579.84 U mg^{-1}) at 150 µM and subsequently decreased (Figure 53). The JA induced peroxidase activity remained higher (380.13-579.84 U mg^{-1}) than controls for all the concentrations (100-200 µM) except at 50 µM where the activity was lower than controls.

4.4.4.4. SA and JA treatments regulated the AsA-GSH pathway

Significant changes in the activities of the enzymes involved in ascorbate-glutathione cycle were observed in callus cultures elicited with SA and JA (Figures 54a-d). APX is a key

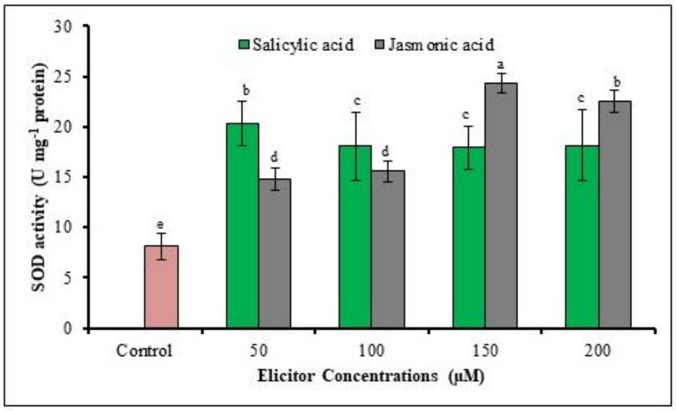


Figure 51. Effect of salicylic acid (SA) and jasmonic acid (JA) on superoxide dismutase (SOD) enzyme activity in callus cultures of *G. moluccana*.

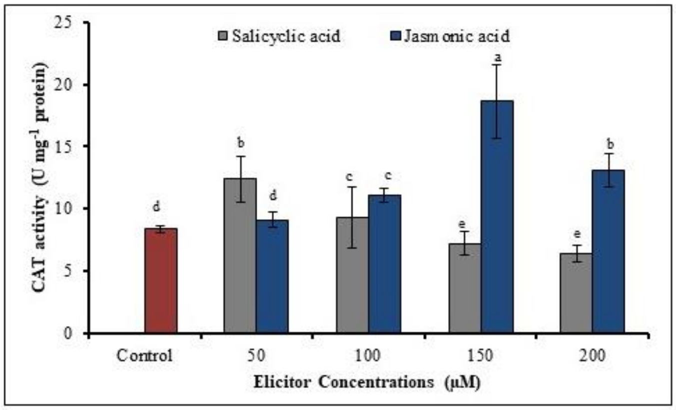


Figure 52. Effect of salicylic acid (SA) and jasmonic acid (JA) on catalase (CAT) enzyme activity in callus cultures of *G. moluccana*.

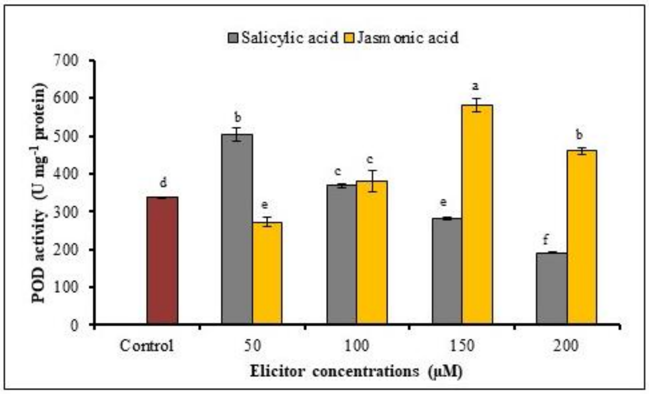
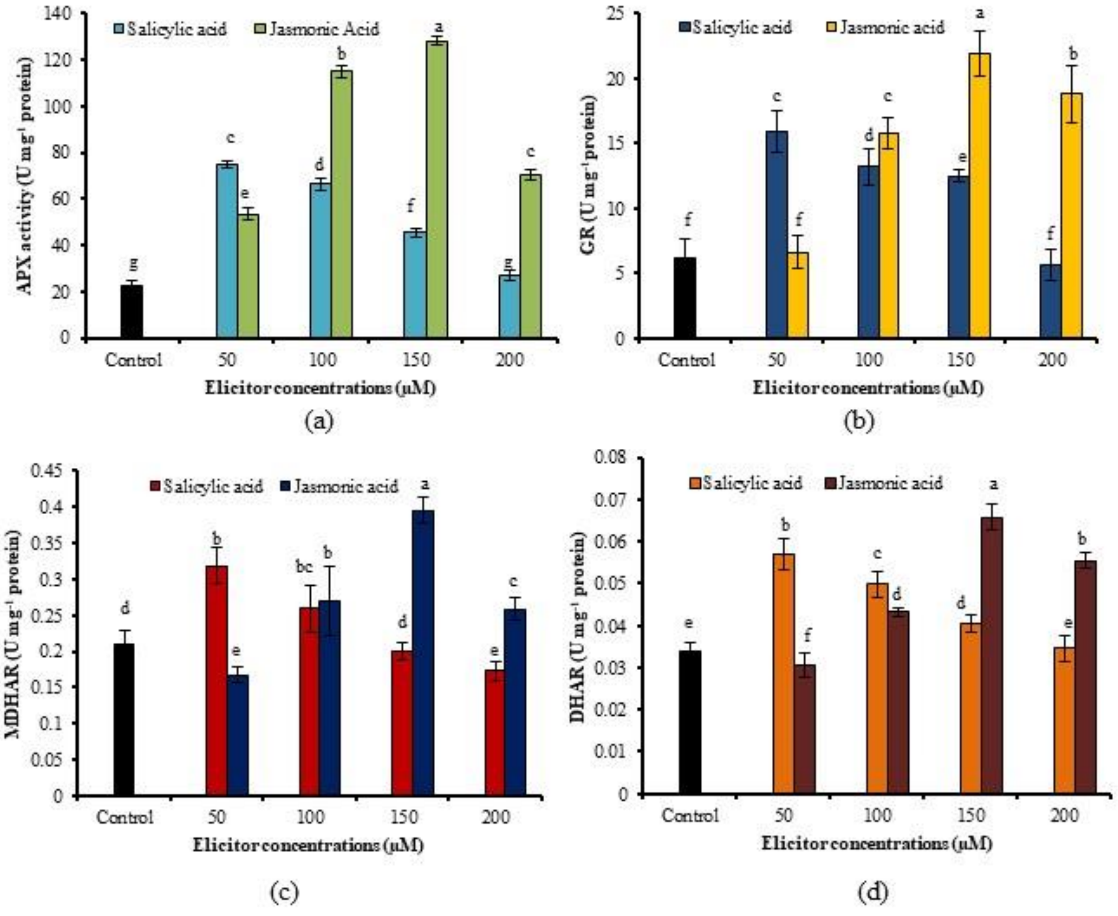


Figure 53. Effect of salicylic acid (SA) and jasmonic acid (JA) on peroxidase (POD) enzyme activity in callus cultures of *G. moluccana*.

enzyme that detoxifies H_2O_2, which utilizes ascorbic acid as its electron donor and reduces H_2O_2 to H_2O. With regard to SA treatment, the highest APX activity (74.85 U mg^{-1}) was observed at the lowest concentration of 50 µM (Figure 54a) and declined with increase in the concentration (100-200 µM). The APX activity (26.9 U mg^{-1}) of 200 µM SA-treated callus cultures did not differ significantly from the controls (22.36 U mg^{-1}). Treatment with JA resulted in a pronounced increase in APX activity being significantly higher than controls for all the concentrations, with the highest activity (127.9 U mg^{-1}) recorded at the concentration of 150 µM (Figure 54a). A similar trend was observed for GR activity where SA at 50 µM induced highest activity (15.9 U mg^{-1}) whereas it decreased to the values of controls as the concentration of SA increased to 200 µM (Figure 54b). In case of JA treated callus cultures, the activity was similar to controls at 50 µM and exhibited maximum activity (21.94 U mg^{-1}) in 150 µM treated callus cultures followed by a decrease in the activity at 200 µM (18.79 U mg^{-1}).

Salicylic acid at 50 µM triggered higher MDHAR activity (0.31 U mg^{-1}) and the values were lower than controls at higher concentrations of 200 µM (Figure 54c). In case of JA-elicited callus cultures, the MDHAR activity was lower than controls at 50 µM, and the activity raised as the concentration increased, with the maximum (0.39 U mg^{-1}) detected in 150 µM treated callus cultures. Similar pattern were observed for DHAR in response to SA treatment, with the highest activity (0.057 U mg^{-1}) observed at 50 µM and was similar to controls at 200 µM (0.034 U mg^{-1}). In JA-treated callus cultures, the DHAR activity (0.03 U mg^{-1}) at 50 µM was lower than controls whereas it elevated at higher concentrations of 100 to 200 µM (Figure 54d). The maximum activity of DHAR was observed at 150 µM JA (0.06 U mg^{-1}).

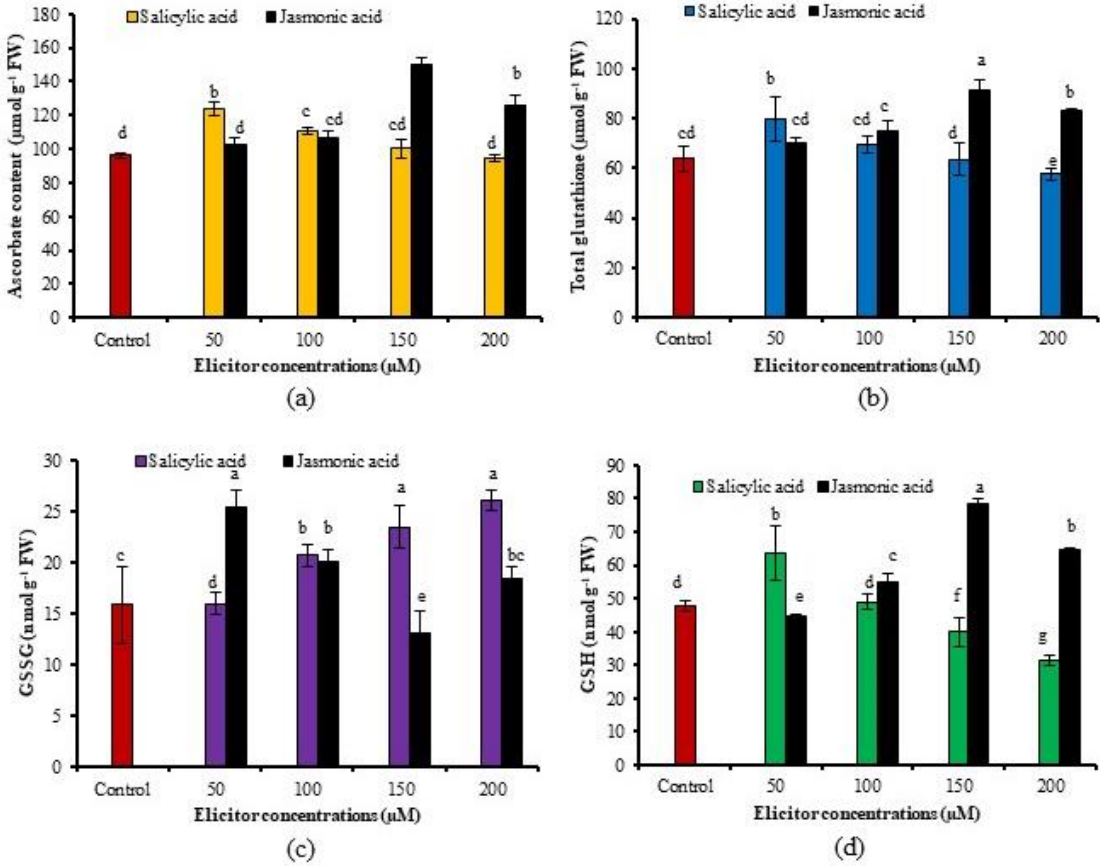
To examine the effects of SA and JA on the components of AsA-GSH in callus cultures, the contents of ascorbate, total glutathione, GSSG and GSH were determined. Ascorbate content varied in SA and JA-treated cultures in comparison to the control cultures (96.39 µmol g^{-1} FW). The highest (123.98 µmol g^{-1} FW) ascorbate content in SA-elicited callus cultures was observed in 50 µM SA and gradually decreased as the concentration increased (Figure 55a). In JA-elicited callus cultures, ascorbate content increased from 102.39 µmol g^{-1} FW to 149.68 µmol g^{-1} FW as the concentration of JA was increased from 50 to 150 µM and decreased to 125.94 µmol g^{-1} FW at 200 µM which was higher than controls. Similar trend were observed in the total glutathione content, where the highest content was observed in the cultures treated with 150 µM JA



(a) (b)

(c) (d)

Figures 54 (a-d). Changes in enzymes involved in ascorbate-glutathione pathway in callus cultures in response to elicitation with salicylic acid and jasmonic acid. (a) APX, (b) GR, (c) MDHAR and (d) DHAR. Callus cultures without elicitation are used as controls.



(a)

(b)

(c)

(d)

Figures 55 (a-d). Changes in non-enzymatic antioxidants of ascorbate-glutathione cycle in callus cultures after elicitation with salicylic acid and jasmonic acid in comparison to control callus cultures. (a) Ascorbate content, (b) Total glutathione, (c) GSSG and (d) GSH contents.

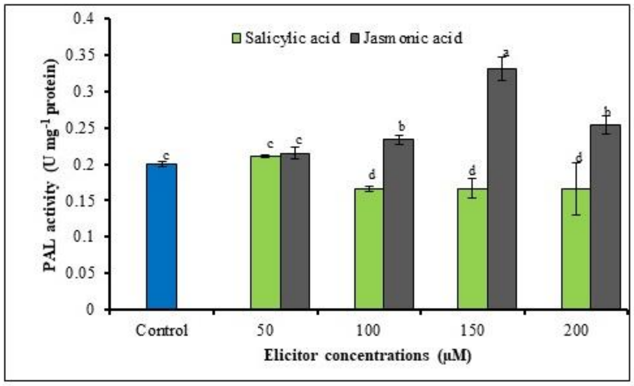


Figure 56. Effect of salicylic acid and jasmonic acid on phenylalanine ammonia lyase (PAL) enzyme activity in callus cultures in comparison to control cultures.

(91.5 µmol g^{-1} FW) followed by 200 µM JA (83.04 µmol g^{-1} FW) and 50 µM SA (79.6 µmol g^{-1} FW) as compared to other treatments (Figure 55b). The glutathione content in other treated cultures were either similar or lower than controls (63.78 µmol g^{-1} FW).

The endogenous content of GSSG and GSH were determined in both the treated and control callus cultures. Under the influence of SA, the GSSG content showed a dose-dependent increase up to 150 µM with no further significant difference observed at 200 µM (Figure 55c). On the other hand, lower concentrations of JA resulted in highest GSSG content (25.48 nmol g^{-1} FW) whereas the content decreased at higher concentrations. Overall, the highest content was detected in treatments with 200 µM SA (26.1 nmol g^{-1} FW) followed by 50 µM JA (25.48 nmol g^{-1} FW). The GSH content in callus cultures treated with 50 µM SA was significantly higher (63.57 nmol g^{-1} FW) than controls (47.84 nmol g^{-1} FW), however, it was similar to controls or showed a significant decrease at higher concentrations (Fig. 15d). Under the influence of JA, highest (78.43 nmol g^{-1} FW) content of GSH was observed at 150 µM and then declined (Figure 55d). The GSH content in 50 µM JA-treated callus cultures were significantly lower (44.62 nmol g^{-1} FW) than controls (47.84 nmol g^{-1} FW).

4.4.4.5. Phenylalanine ammonia lyase enzyme activity was differentially modulated by SA and JA treatments

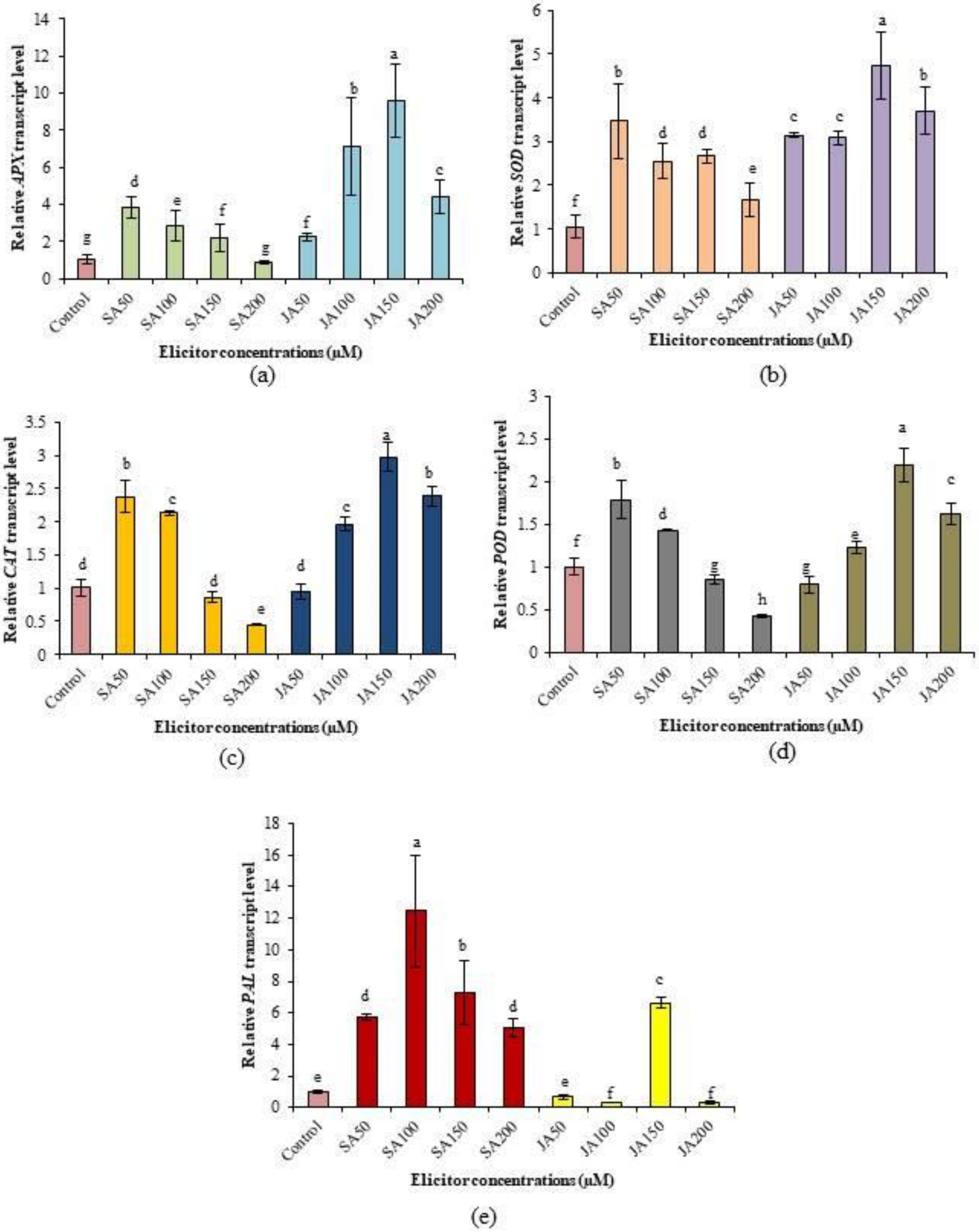
An increase in PAL activity after elicitor treatment often results in enhanced secondary metabolism in plant cells (Zhao *et al*., 2010). A significant change in PAL activity was observed in callus cultures treated with SA and JA depending on the concentration used **(Figure 56)**. The PAL activity in callus cultures treated with SA at 50 µM (0.21 U mg^{-1} protein) did not differ significantly from controls (0.20 U mg^{-1} protein), however, the activity decreased at higher concentrations. It was interesting to note that JA at 150 µM triggered highest activity (0.33 U mg^{-1} protein) as compared to 100 and 200 µM whereas the value of 50 µM did not differ from controls.

4.4.4.6. Correlation of transcript levels of genes and enzyme activities in SA- and JA-treated callus cultures showed similar or different expression patterns

Real-Time PCR analysis showing variation in transcript level of the genes encoding antioxidant enzymes *viz.*, *APX*, *SOD*, *CAT* and *POD* in callus cultures treated with SA and JA (Figures 57a-d). SA-treated callus cultures showed an increase in the transcript level of *APX* gene by 3.71-fold at 50 µM, and decreased gradually reaching the value similar to controls at

200 µM. The transcript levels of *APX* gene in JA-treated callus cultures was higher than controls for all concentrations, with the maximum fold-change of 9.24 detected at 150 µM JA (Figure 57a). Both SA and JA treatments significantly elevated the transcript levels of *SOD* gene for all concentrations used compared to control callus cultures. Among the different treatments, the maximum fold-change (4.54) in transcript levels of *SOD* gene was observed in 150 µM JA-treated callus cultures compared to controls (Figure 57b). Both *CAT* and *POD* genes showed similar expression pattern under the influence of SA and JA. The transcript levels of *CAT* and *POD* genes were found to be highest at the lowest concentration of 50 µM SA and gradually decreased being lower than controls at 200 µM (Figure 57b & c). A reverse trend was observed in JA-treated callus cultures, where the transcript levels of *CAT* and *POD* genes gradually increased reaching the maximum at 150 µM followed by a decrease at 200 µM which still remained higher than controls (Figures 57c & d). Overall, JA at 150 µM induced higher transcript levels of *APX*, *SOD*, *CAT* and *POD* genes than SA-treated cultures.

PAL is a key enzyme in phenylpropanoid pathway involved in the deamination of phenyl alanine to trans-cinnamic acid, which is the precursor for lignin and flavonoid biosynthetic pathway. SA treatment triggered higher expression of *PAL* gene at all concentrations as compared to controls, with the highest fold-change of 12.45 observed at 100 µM. Interestingly, JA-elicited callus cultures showed highest expression at 150 µM (6.6 fold-change), whereas it was similar or lower than controls at other concentrations (Figure 57e). Overall, the SA-treated callus cultures exhibited higher transcript levels than JA treatments across all concentrations. The pattern of gene expression of *PAL* detected at the transcript levels, however, did not correspond with enzyme activity for SA and JA-treated callus cultures.



Figures 57 (a-e). Relative transcript levels of genes encoding antioxidant enzymes and phenylalanine ammonia lyase in elicitor (SA and JA) treated and control (unelicited) callus cultures. (a) *APX*, (b) *SOD*, (c) *CAT*, (d) *POD* and (e) *PAL*.

4.5. LC-MS analysis for secondary metabolite profiling in leaves and leaves derived callus cultures

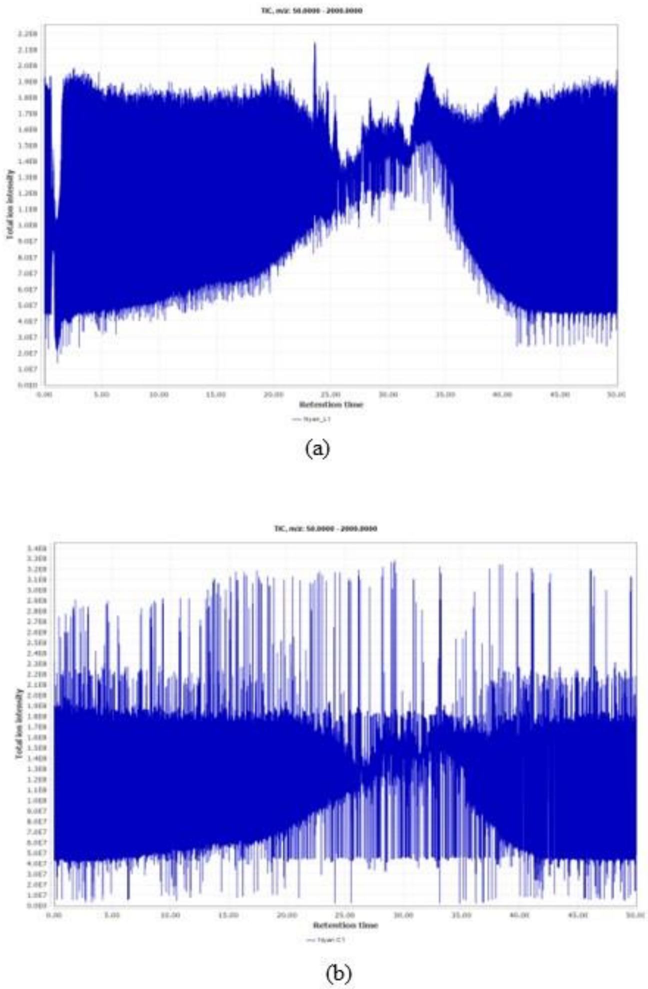
4.5.1. Metabolite analysis reveals differences in secondary metabolites in leaves derived callus as compared to leaves

The untargeted metabolite profiling of mature leaves and leaves derived callus was carried out using LC-MS/MS. The TIC of mature leaves and leaves-derived callus were distinct from each other (Figures 58a & b). A total of 165 and 170 putative secondary metabolites were detected in mature leaves and callus, respectively. Out of the total secondary metabolites detected, 55 compounds were unique to mature leaves, while 60 compounds were unique to callus and 115 compounds were common to mature leaves and callus (Figure 59). Secondary metabolites such as flavonoids, terpenes and terpenoids, alkaloids and phenolic acids were most abundant in both leaves and callus. The major distinction in the number secondary metabolites between leaves and callus was observed for terpenoids and alkaloids, where the number of alkaloids in callus were higher than leaves and the number of terpenoids were higher in leaves in comparison to callus. This was followed by flavonoids which were marginally higher in callus than leaves whereas phenolic acids were higher in leaves compared to callus. The other compounds such as sterods and sterols, lignins, glycosides, vitamins and cofactors, anthroquinones, stilbenes and betacynin did not differ between leaves and callus.

4.6. Metabolite profiling of elicited callus cultures using LC-MS/MS

4.6.1. Callus cultures elicited with SA and JA showed higher flavonoids than other secondary metabolites

The untargeted metabolite profiling was carried out through metabolomics platform with LC-MS/MS to comprehend the secondary metabolites present in the leaves derived callus cultures (untreated control), SA and JA treated callus cultures of *G. moluccana*. The total ion chromatogram (TIC) of control callus, SA-treated callus and JA-treated callus were distinct from each other (Figures 61a-c). The metabolome analysis revealed 170, 203 and 218 secondary metabolites in control callus, JA and SA treated callus, respectively (Figure 62, 63a-c, 64a-c, 65a-c, 66 a-c). Of these, 19 compounds were unique to control callus extract, 42 compounds were unique to SA treated callus extracts, while 34 compounds were unique to JA treated callus (Figure 62). Flavonoids, terpenoids, alkaloids and phenolic acids were the main constituents in each of the three samples. Other compounds such as glycosides, sterols and steroids, vitamins and co-factors, lignans, anthroquinone, stilbenes and betacynin were also identified in all the



Figures 58 (a & b). The LC-MS total ion chromatogram of methanolic extracts of mature leaf and leaf derived callus cultures, (a) mature leaf, (b) leaf-derived callus.

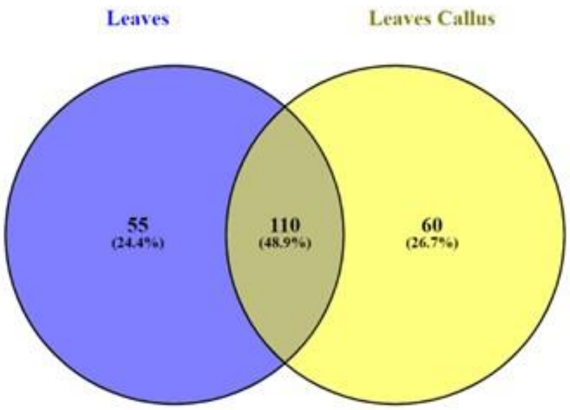


Figure 59. Venn diagram of secondary metabolites between mature leaves and leaves-derived callus.

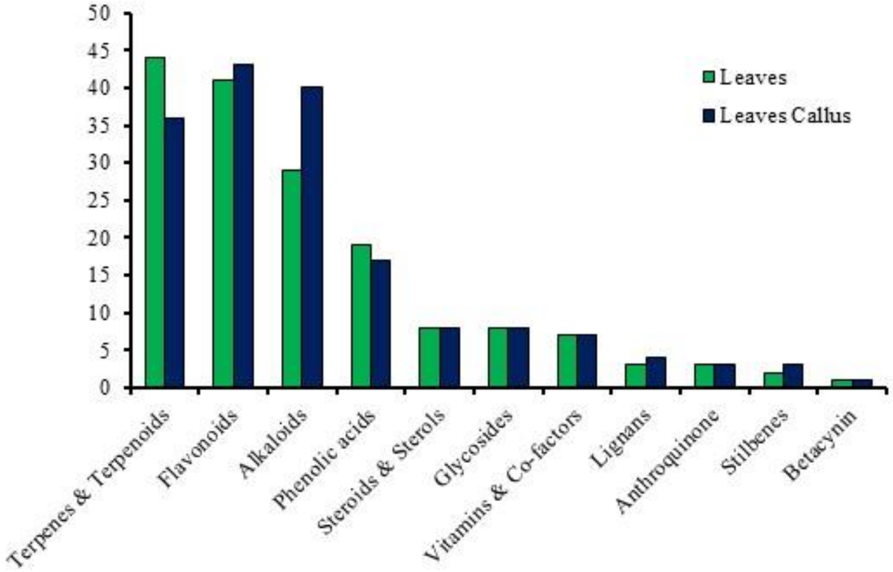
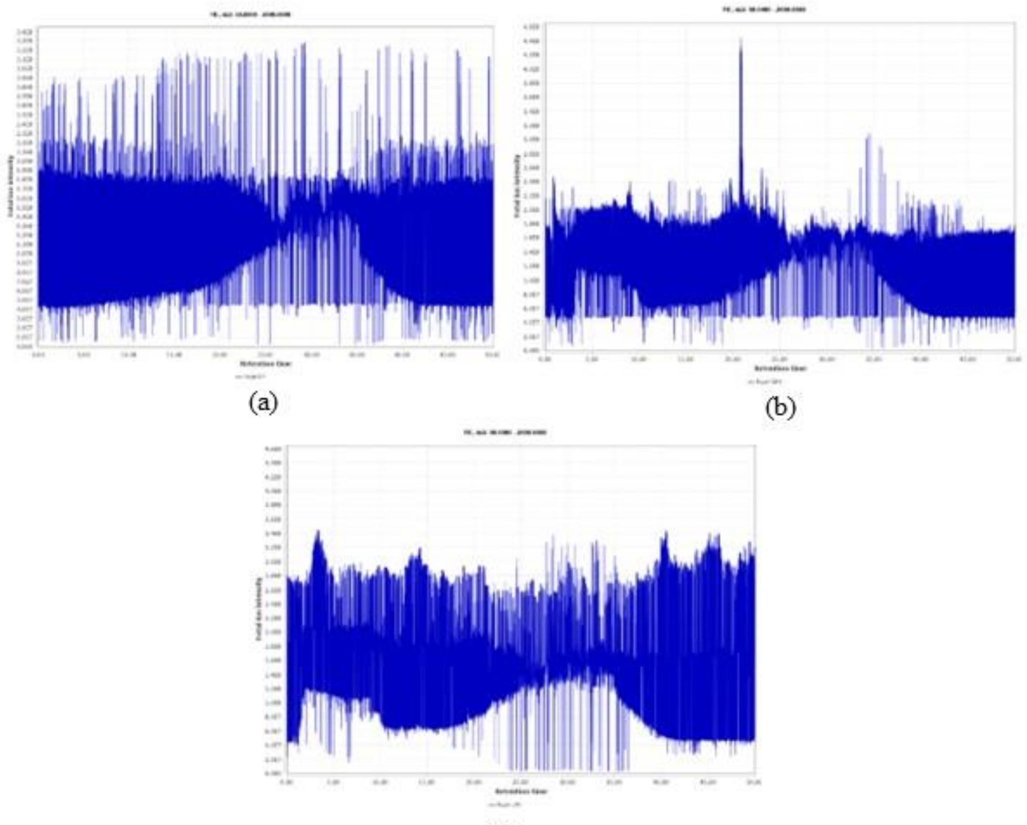


Figure 60. Comparison of different secondary metabolites in mature leaves and leaves-derived callus

samples (Figure 63a). Out of the 43 flavonoids identified in the control samples, flavone (30%) and flavonol (23%) constituted the major class of flavonoids, while other class of flavonoids such as flavonoid glycosides (14%), flavan-3-ol (9%), flavonone (7%), isoflavone (7%), anthocyanins (7%) and 3% dihydrochalcone (3%) were also found (Figure 64a).

Sesquiterpenoids (34%) were the main class of terpenoids identified followed by diterpenoids (20%), tetraterpenoids (17%), triterpenoids (17%) and monoterpenoids (12%) in control callus (Figure 65a). Benzylisoquinoline alkaloids (30%) represented the main class of alkaloids in callus cultures followed by quinoline (22%) and benzophenanthridine alkaloid (10%). Other classes of alkaloids such as tropane indole alkaloids, quinolizidine alkaloid, terpenoid alkaloid, purine alkaloid, indolizidine alkaloid were also detected (Figure 66a). Hydroxycinnamic acid (41%) and coumarins (35%) followed by hydroxybenzoic acids (18%) and phenylpropanes (6%) made up the phenolic acids types in the callus cultures (Figure 67a).

Annotation of secondary metabolites in SA treated callus cultures revealed 53 flavonoids, 52 terpenoids, 39 alkaloids, 20 phenolic acids, 18 steroids and sterols, and others such as vitamins and co-factors, anthroquinone, lignans, stilbenes and betacyanins were in lesser in number (Figure 63b). The 53 flavonoids were mostly flavones (30%) class, flavonol (23%), flavonoid glycoside (14%) and anthocyanin (13%). Other class of flavonoids such as flavanone (10%), isoflavone (9%), flavan-3-ol (4%) and dihydrochalcone (2%) formed the rest of the flavonoids (Figure 64b). The 52 terpenoids were comprised of 26% sesquiterpenoids, 24% triterpenoids, 20% tetraterpenoids, 18% diterpenoids and 12% monoterpenoids (Figure 65b). Ten different class of alkaloids were annotated from the 37 alkaloids putatively identified, with benzylisoquinoline alkaloid class dominating the group with 36% followed by indole alkaloid with 20%. The rest of the alkaloid classes such as quinoline alkaloid, benzophenanthridine alkaloid, tropane alkaloid, indole alkaloid, quinolizidine alkaloid, terpenoid alkaloid, purine alkaloid, indolizidine alkaloid and steroidal alkaloids were present in the range of 3-10% (Figure 66b). Coumarin (45%), hydroxycinnamic acids (35%), hydroxybenzoic acids (15%) and phenylpropanes (5%) were the other phenolic acids detected in SA treated callus extract (Figure 67b). The number of steroids and sterols in SA treated callus were twice as much in the control samples. Eighteen steroids and sterols with different bioactive and biological roles were putatively identified.



(a)

(b)

(c)

Figures 61 (a-c). The LC-MS total ion Chromatogram (TIC) plot of control and elicitor treated callus cultures. (a) control callus, (b) SA treated callus and (c) JA treated callus.

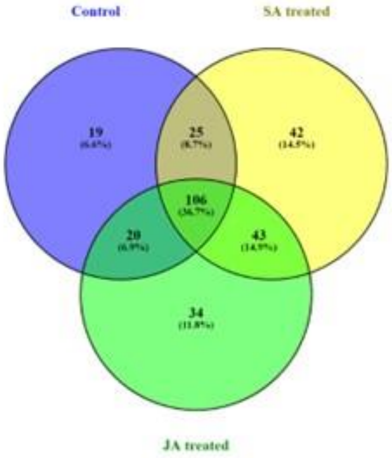
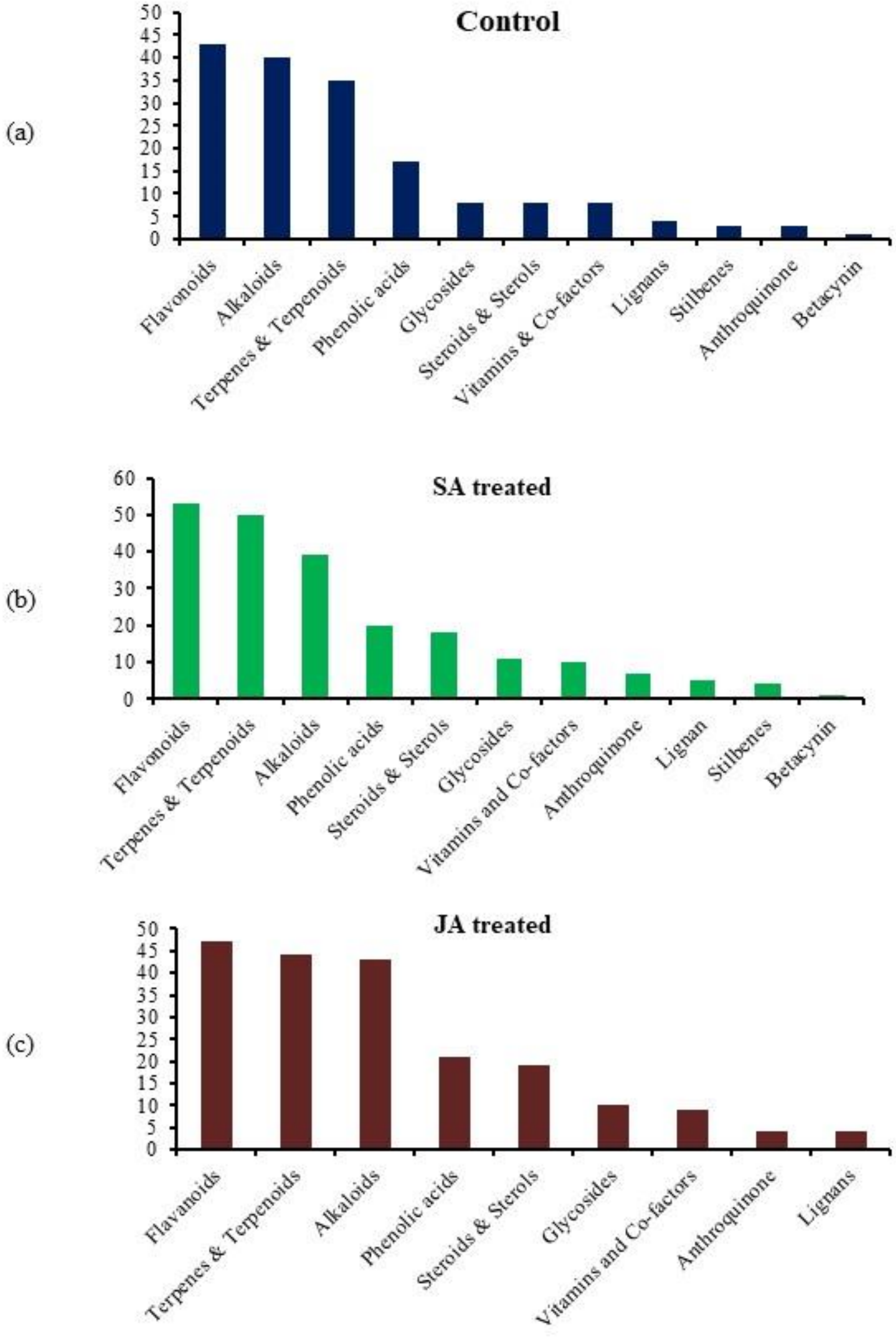
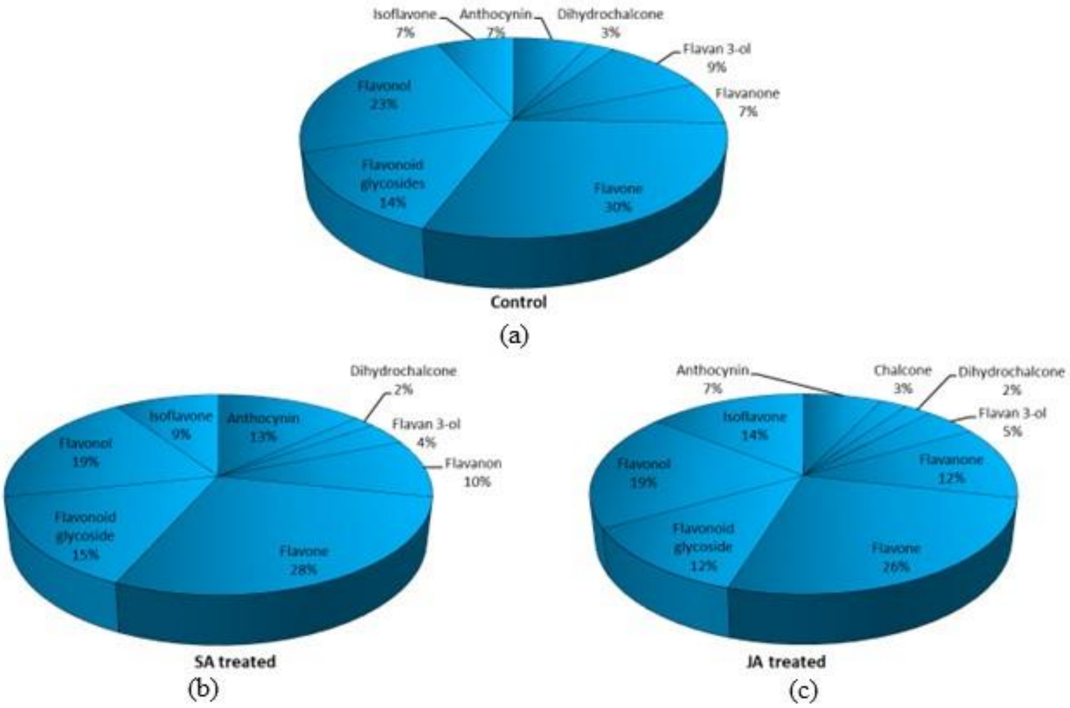


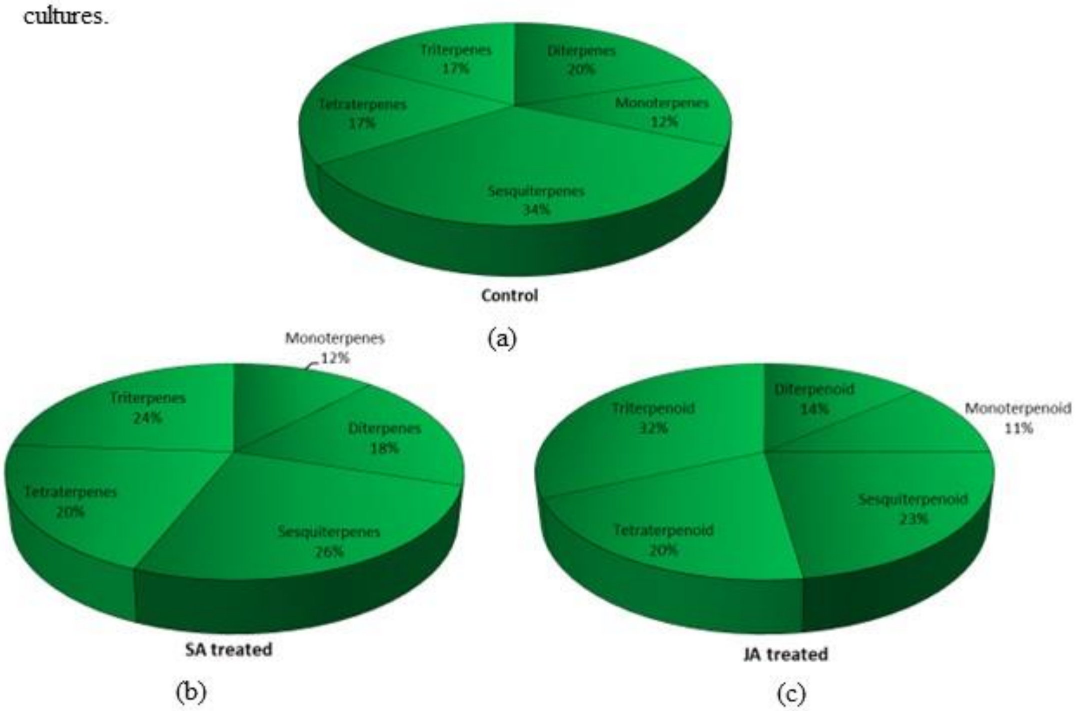
Figure 62. Venn diagram of the secondary metabolites in leaves-derived callus, SA treated and JA treated callus cultures.



Figures 63 (a-c). Distribution of secondary metabolites classes in (a) control callus, (b) SA treated and (c) JA treated callus.



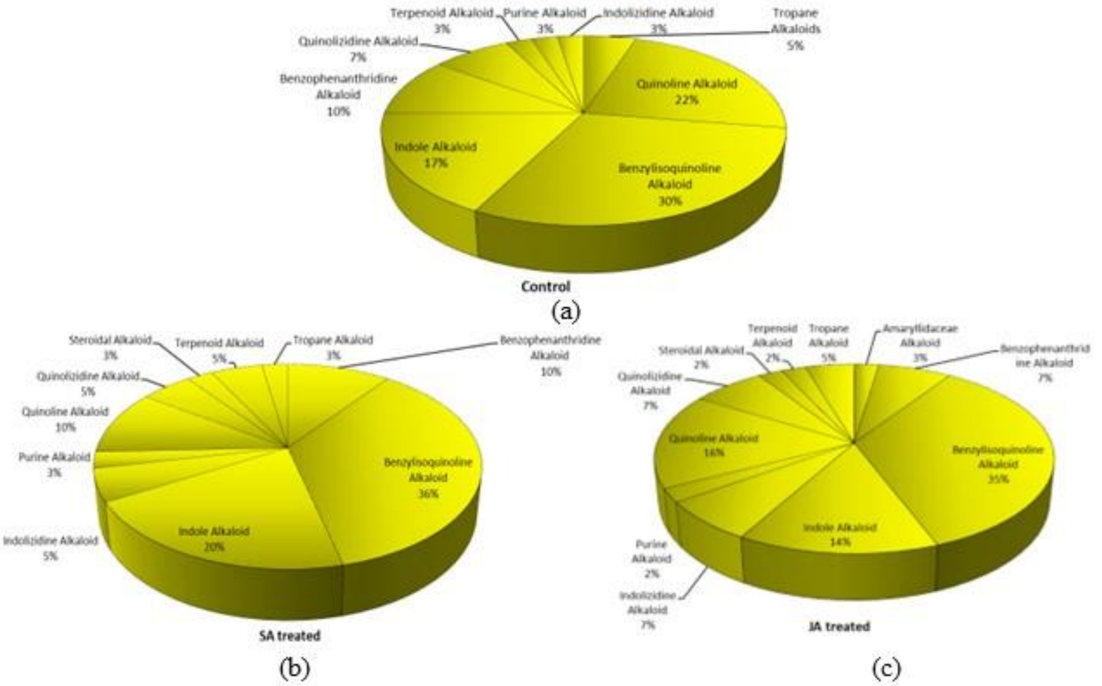
Figures 64 (a-c). Classification of flavonoids in control, SA and JA treated callus cultures.



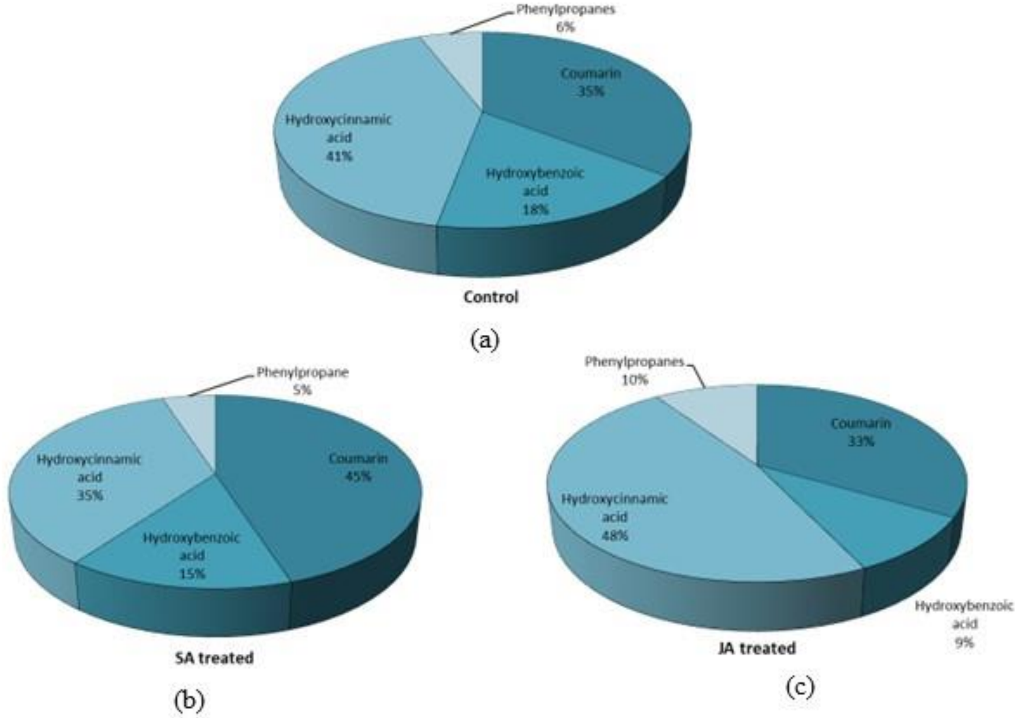
Figures 65 (a-c). Classification of terpenoids in control, SA and JA treated callus cultures.

In JA treated callus cultures, the predominant compounds were flavonoids (47), terpenoids (45), alkaloids (43), phenolic acids (21), steroids and sterols (19) (Figure 63c). The 47 flavonoids were comprised of 26% flavones, 19% flavonols, 14% isoflavone, 12% flavonoid glycosides and flavonone and others such as flavan-3-ol, anthocyanin, chalcone, dihydrochalone were in the range of 2-7% (Figure 64c). Triterpenoid (32%), sesquiterpenoid (23%), tetraterpenoid (20%), diterpenoid (14%) and monoterpenoid (11%) were the different sub-class of terpenoids identified and annotated in the JA treated callus (Figure 65c). Similar to the alkaloids in control and SA treated callus, benzylisoquinoline alkaloid (35%) was the predominant class in JA treated followed by indole alkaloid (14%). Quinoline alkaloid, benzophenanthridine alkaloid, tropane alkaloid, quinolizidine alkaloid, terpenoid alkaloid, purine alkaloid, indolizidine alkaloid, steroidal alkaloid, and amaryllidaceae alkaloid were present in the range of 2-13% (Figure 66c). Hydroxycinnamic acid accounted to 48% of the phenolic compounds annotated followed by coumarins with 33%; hydroxybenzoic acids and phenylpropanes with 9% and 10%, respectively in JA-treated callus cultures (Figure 67c).

The potential biological activities of the different classes of compounds *viz*., flavonoids, terpenoids, alkaloids, phenolic acids, and other compounds (steroids, vitamins and cofactors, anthroquinone, lignin etc.) identified in control callus cultures, SA-treated callus cultures and JA-treated callus cultures are listed in Supplementary Tables 23-39.



Figures 66 (a-c). Classification of alkaloids in (a) control, (b) SA-treated and (c) JA-treated callus cultures.

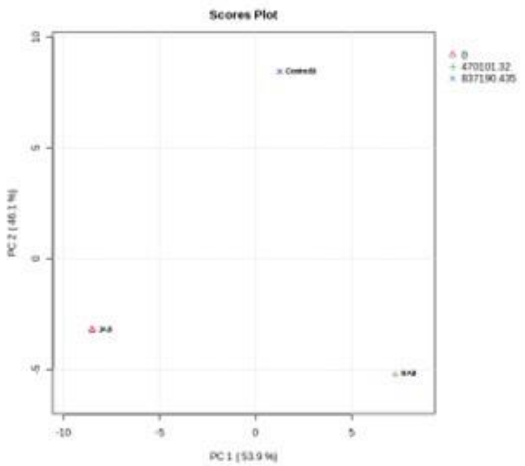


Figures 67 (a-c). Classification of phenolics in (a) control, (b) SA-treated and (c) JA-treated callus cultures.

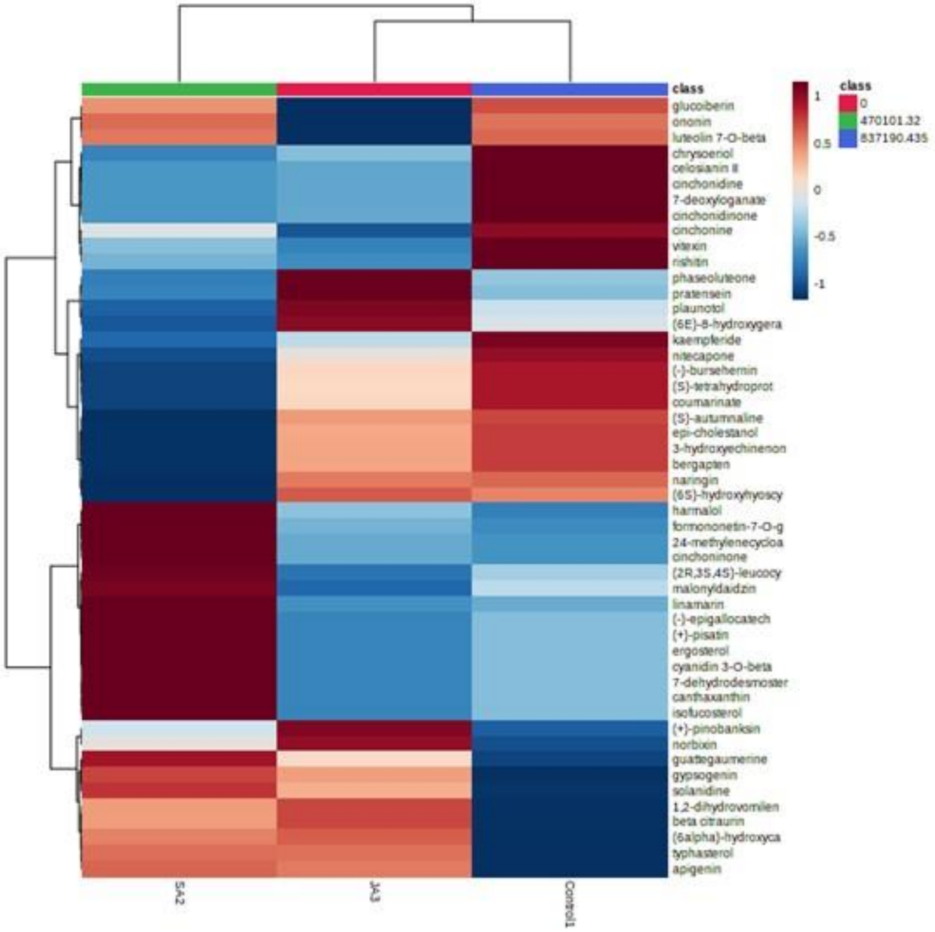
The principal component analysis (PCA) for LC-MS/MS data of the control callus, SA treated callus and JA treated callus was carried out in Metabo Analyst (Figure 68a). The PCA demonstrated that the metabolites profiles of control callus, SA treated callus and JA treated callus extracts were clearly distinct signifying the role of different elicitors in eliciting different types of metabolites. The principal component 1 (PC1) showed 53.9% variance and the principal component 2 (PC2) elucidated a further 46.1% variance. The hierarchical clustering analysis (HCA) heat map of the samples revealed a distinct pattern with regard to the abundance of the compounds of the top 50 compounds annotated in each of the extracts (Figure 68b).

The metabolites pathway analysis was performed on known metabolites by using the pathway libraries of *Arabidopsis thaliana* to associate the biological functions of identified metabolites in SA and JA treated callus cultures to different pathways (Figures 69a & b). The plots depict various metabolic pathway alterations induced by SA and JA treatments in comparison to control callus. The most significantly enriched pathways were characterized by high-log (p) value and pathway impact. In both SA and JA treated callus cultures metabolites, 21 different pathways were identified. Brassinosteroids biosynthesis, steroids biosynthesis, flavonoid biosynthesis, carotenoid biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis, and phenylpropanoid biosynthesis pathways were more affected in both SA and JA treated callus cultures. Most of the components of steroid biosynthesis and ubiquinone synthesis were found to be more enriched in SA treated callus as compared to JA treated callus cultures.

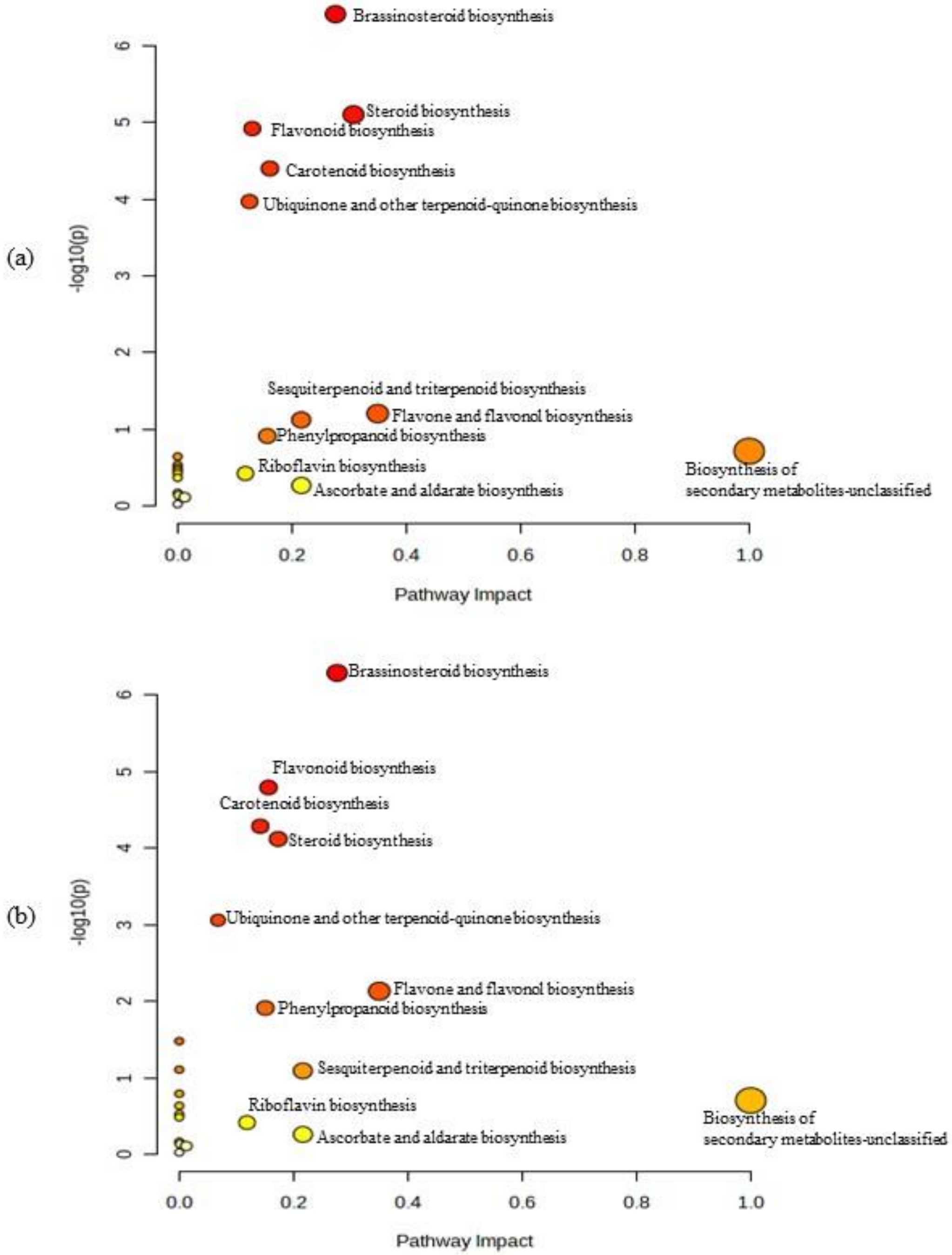
(a)



(a)



Figures 68 (a & b). Principal component analysis (PCA) and Hierarchical clustering results of secondary metabolites in treated and control callus cultures. (a) PCA-Scores plot of the secondary metabolites in leaves derived callus, SA-treated callus and JA-treated callus. The variances are shown in brackets. (b) Hierarchical clustering results of metabolites (top 50 shown as heatmap (distance measured using euclidean, and clustering algorithm using ward. D).



Figures 69 (a & b). Summary of pathway enrichment analysis of metabolites from (a) SA and (b) JA treated callus extract. The x-axis represents the pathway impact value computed from pathway topological analysis, and the y-axis is the-log of the P-value obtained from pathway enrichment analysis.

4.7. *De novo* transcriptome analysis of control and treated callus cultures

De novo transcriptome assembly is a useful approach in analyzing differentially expressed genes in non-model species where whole genome information is meagre or unavailable. As the genome of *G. moluccana* is not sequenced, RNA sequencing was executed to classify the differentially expressed transcripts in leaves, leaves derived callus (control) and leaves derived callus cultures treated with SA and JA. A total of four sequencing libraries were constructed each consisting of 5.7 Gb data. The sequences (raw data) obtained has been uploaded to National Centre for Biotechnology Information (NCBI) website with ID as PRJNA1010650. The average base quality is above Q20 for 95.23% of bases. A total of 37,379,166 raw reads were obtained for leaves, 37,885,176 raw reads were obtained for leaves-derived callus (control), 39,896,956 raw reads for SA treated callus and 38,423,972 raw reads for JA treated callus cultures (Table 9). After filtering and trimming these raw reads, 36,610,058 clean reads were obtained for leaves, 37,263,180 clean reads were obtained for leaves-derived callus (control), 30,435,498 clean reads for SA and 37,764,748 clean reads for JA (Table 9). High-quality reads from these samples were combined and provided to transcriptome assembler program Trinity 2.8.4. A total of 171,971 total trinity transcripts (isoforms) and trinity unigenes of 77,522 was generated with an average contig length of 1578.49 bp and 925.37 bp and N50 of 2,882 and 2,118 for isoform and unigenes, respectively. An overview of the assembled sequences is shown in Table 10. The assemblage of transcripts revealed distribution of the transcripts from 200 bp to >5000 bp for unigenes and isoforms. A higher number of transcripts for isoforms were distributed between 2000 bp to 5000 bp while for unigenes they were distributed between 200 to 300 bp. Large number of transcripts for both unigenes and their isoforms contained nearly 30-45% of GC content and nearly 8000 transcripts for isoforms and 3000 transcripts for unigenes contained 35-40% GC content (Figures 70a & b). The transcriptome data generated was of fine quality and found suitable for further annotation.

Gene expression estimation of the assembled data was carried by Kallisto 0.46.0. The top BLASTX hits were filtered out depending on query coverage, identity and similarity score, and gene descriptions using E-value cut off value of 10^{-3}. Out of 77,522 unigenes, 35,128 unigenes with best hit BLASTX match were generated (Table 11). The gene ontology (OG) assignment categorized the annotated unigenes to three key GO categories namely biological processes (BP: 29008 unigenes; 31.16%), molecular function (MF: 37100 unigenes; 39:85%) and cellular

Table 9. Summary of Illumina sequencing quality statistics of leaves, control and treated callus cultures

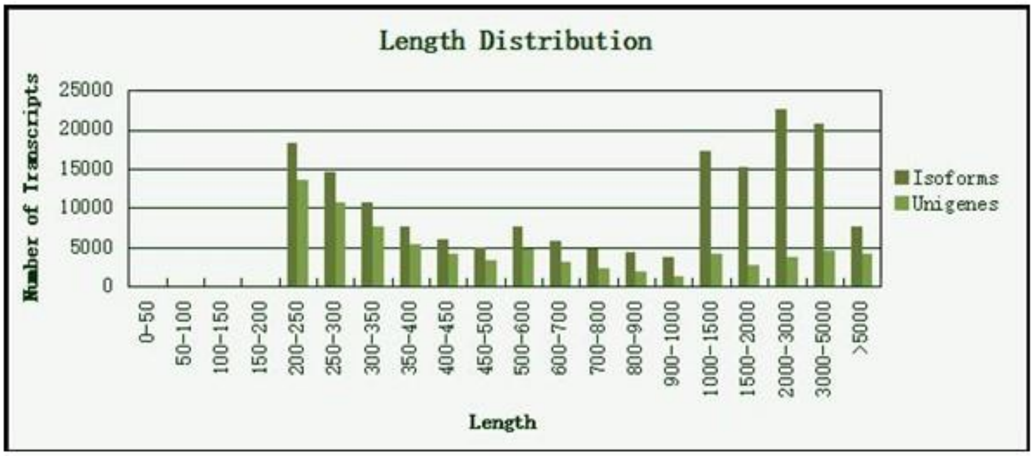
Samples used	**Raw reads**	**No. of bases (GB)**	**Clean reads**	**No. of Bases (GB)**	**GC %**	**Q30**
Leaves	37,379,166	5,607	36,610,058	5,417.01	45.17	90.9
Control	37,885,176	5,683	37,263,180	5,522.78	47.25	88.56
SA treated	39,896,956	5,985	30,435,498	4,507.31	48.01	88.32
JA treated	38,423,972	5,764	37,764,748	5,586.54	49.59	89.68

Table 10. Comprehensive *de novo* transcriptome assembled statistics of leaves, control and treated callus cultures

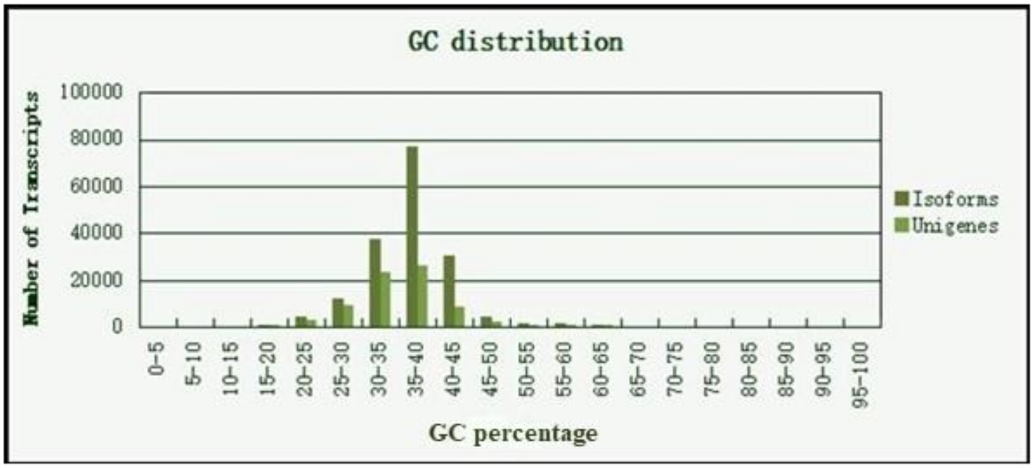
Trinity Assembly statistics	Numbers
Total trinity transcript	171,971
Total trinity unigenes	77,522
Contig N50	2,882
Contig N50 (unigene)	2,118
Median contig length	933
Median contig length (unigene)	403
Average contig length	1578.49
Average contig length (unigene)	925.37
Total assembled bases	271,454,489
Total assembled bases (unigene)	71,736,894
Mean GC% of transcripts	37.83
Mean GC% of transcripts (unigene)	36.25

Table 11. Summary of transcriptome data of leaves, control and treated callus cultures

Program used	Blastx
Database used	Ref seq Plant
Annotated transcripts	35,128
e-value cut off	<=0.001
Gene-ontology-biological processes	29008
Gene-ontology-cellular components	26957
Gene-ontology-molecular functions	37100



(a)



(b)

Figures 70 (a & b). (a) Length distribution of isoforms and unigene transcripts from leaves, leaves-derived callus, SA-treated and JA-treated callus cultures (b) GC distribution of isoforms and unigenes transcripts from leaves, leaves-derived callus, SA-treated and JA-treated callus cultures.

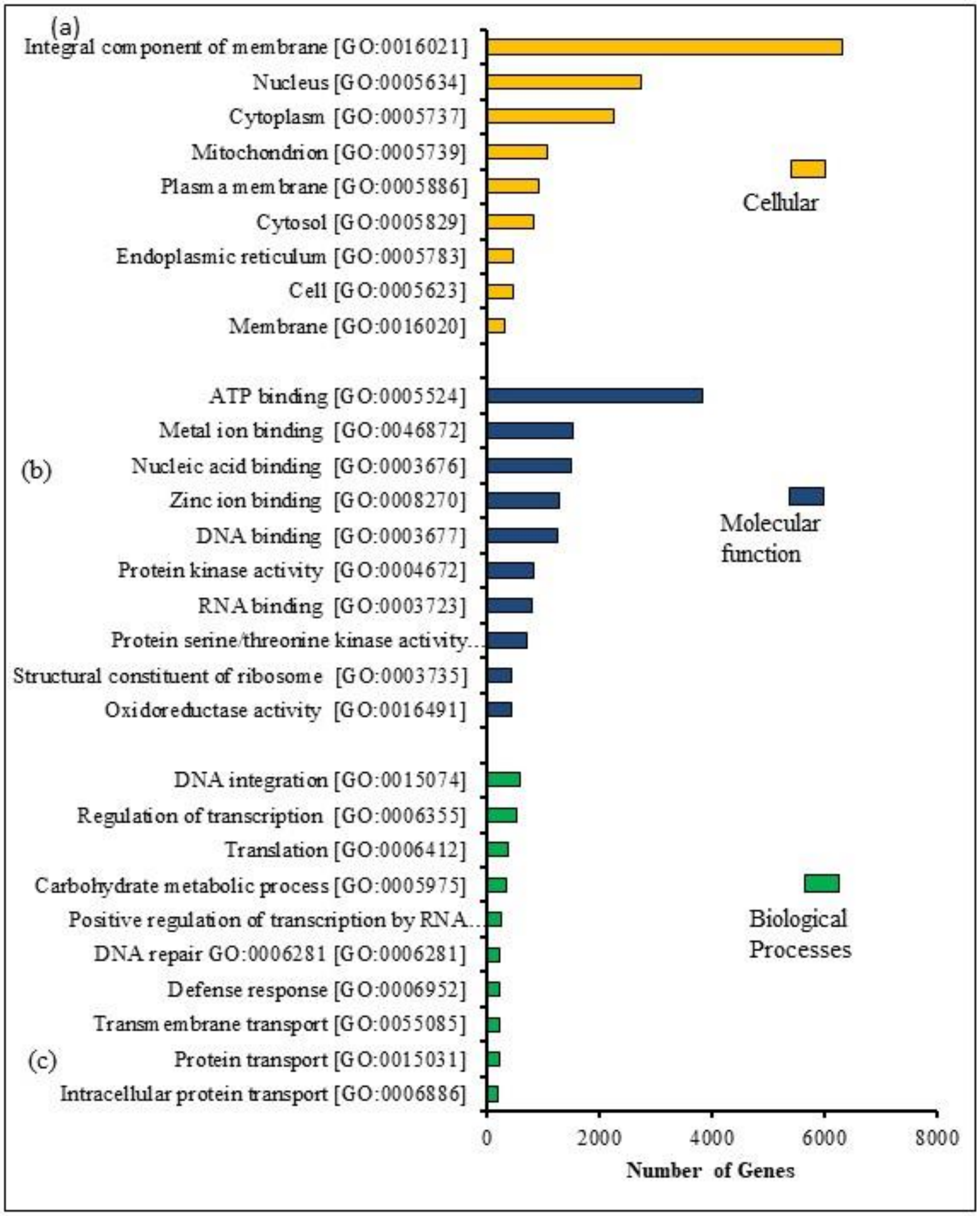
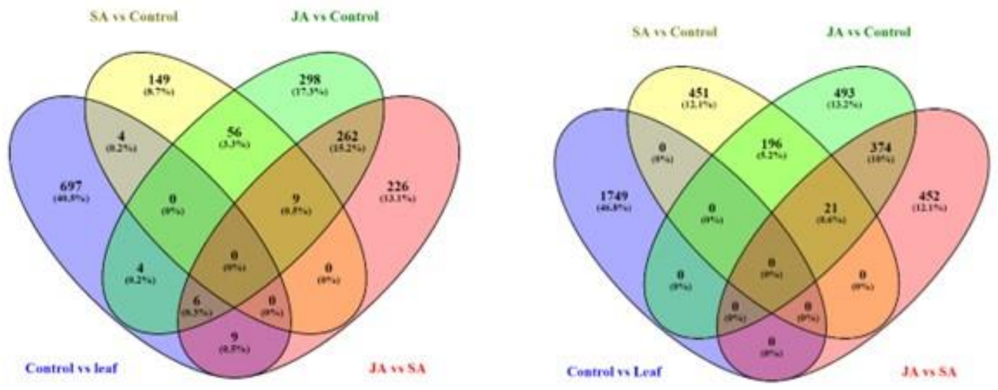
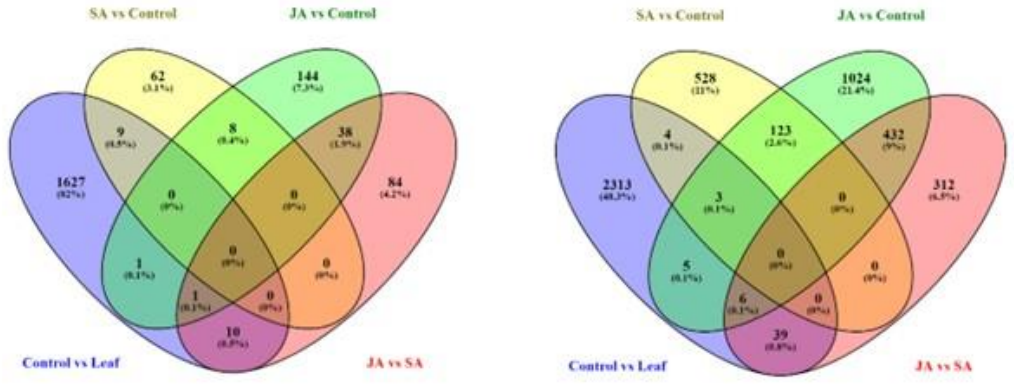


Figure 71. The gene ontology (GO) terms for unigenes (a) Biological processes, (b) Molecular function and (c) Cellular component.



(a) (b)

Figures 72 (a & b). Venn diagram of upregulated transcripts (a) unigenes (b) isoforms. in different samples analyzed.



(a) (b)

Figures 73 (a & b). Venn diagram of downregulated transcripts (a) unigenes (b) isoforms in different samples analyzed.

component (CC: 26957; 28.97%) (Figures 71a-c**).** In biological function, the major enriched functional groups were DNA integration, regulation of transcription, translation and carbohydrates metabolic processes with 592, 528, 393 and 357 unigenes, respectively. The integral components of the membrane (6315), nucleus (2753), cytoplasm (2263) and mitochondria (1079) were the dominant category in cellular component. The molecular function category consisted of ATP binding (3849), metal ion binding (1537), nucleic acid binding (1492), zinc ion binding (1278) and DNA binding (Figure 71).

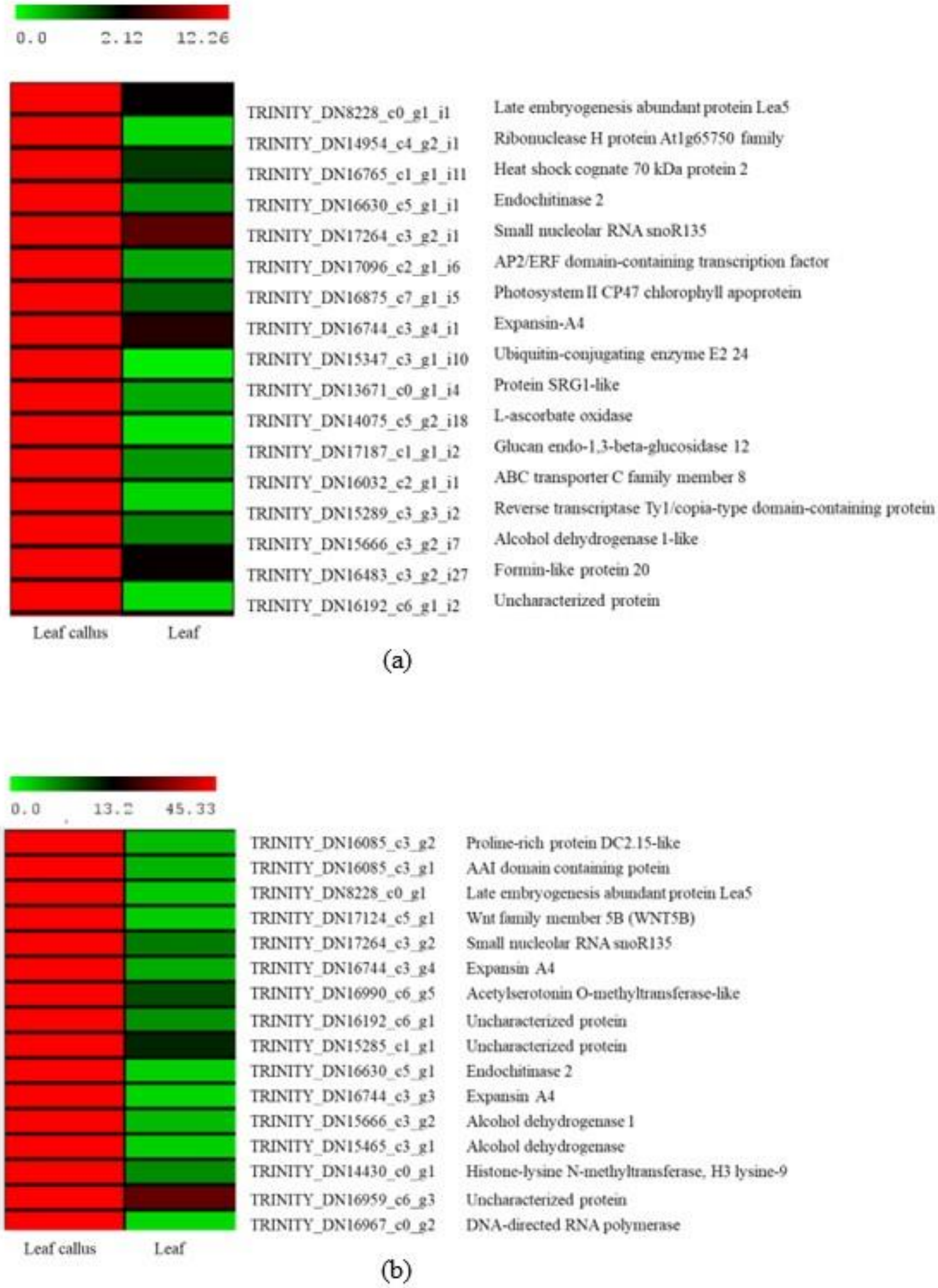
The venn diagram depicts the shared and unique transcripts and isoforms in upregulated and downregulated between the four groups namely, leaves-derived callus (control) *vs.* leaves, SA treated *vs.* callus control, JA treated control *vs.* callus, SA treated callus *vs.* JA treated callus (Figures 72 a-b & 73 a-b). The number of upregulated transcripts were found to be 5002 transcripts out of which 1040 were unigenes and 3962 were isoforms. Of these, 683 (65.7%) unigenes and 1486 (37.5%) isoforms were unique to leaves-derived callus *vs.* leaves, 67 (6.4%) unigenes and 512 (12.9%) isoforms were found only in SA treated *vs*. control callus, 131 (12.6%) unigenes and 913 (23%) isoforms were only present in JA *vs*. control callus, 85 (8.2%) unigenes and 329 (8.3%) isoforms were unique to JA *vs.* SA treated callus cultures. There were no common transcripts among the four groups. Out of 2356 unigenes and 4010 isoforms downregulated, 1333 unigenes (56.65%) and 2023 (50.4%) isoforms were unique to leaves derived callus *vs.* leaves, 176 (7.5%) unigenes and 400 (10%) isoforms downregulated were unique to JA-treated *vs.* control callus, 118 (5%) unigenes and 371 (9.3%) isoforms downregulated were unique to SA treated *vs*. control callus, while 199 (8.4%) unigenes and 420 (10.5%) isoforms downregulated were unique to JA *vs.* SA treated callus. Only one downregulated unigene and 4 isoforms were common to all four groups.

4.7.1. Transcriptomic changes associated with callus formation from leaves explants

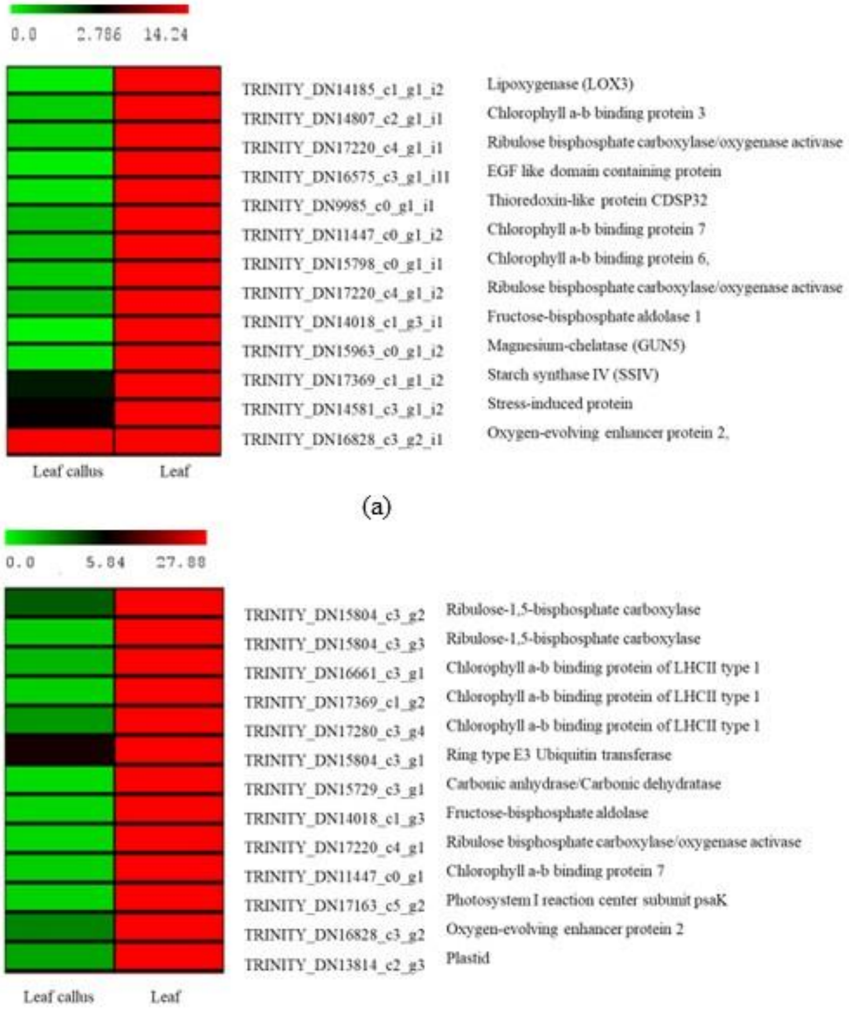
The differentially expressed unigenes/isoforms between leaves and leaves-derived callus with p-value cutoff 0.05 are mentioned in Table 11. The clustered heat maps of highly upregulated unigenes/isoforms in leaves-derived callus in comparison to leaves are shown in Figures 74a & b, 75a & b. There were 6488 differentially expressed genes between leaves and callus which were primarily involved in cell division, plant hormone signaling pathways, plants stress response and plant-pathogen interactions. Out of 6488 DEGs, 2469 were upregulated and 4019 were downregulated. The top upregulated genes in callus include stress related genes such

Table 12. Overview of differentially expressed genes (DEGs) among different samples

Sl. No.	Samples	Unregulated	Downregulated
1	SA treated *vs*. Control (Unigenes)	218	79
2	SA treated *vs*. Control (Isoforms)	668	658
3	JA treated *vs*. Control (Unigenes)	635	192
4	JA treated *vs*. Control (Isoforms)	1,084	1,593
5	JA treated *vs*. SA treated (Unigenes)	512	133
6	JA treated *vs*. SA treated (Isoforms)	847	789
7	Control *vs*. Leaves (Unigenes)	720	1,649
8	Control *vs*. Leaves (Isoforms)	1,749	2,370



Figures 74 (a & b). Heat map showing the expression pattern of highly upregulated genes in leaves-derived callus cultures in comparison to leaves. (a) Isoform and (b) Transcripts.



(a)

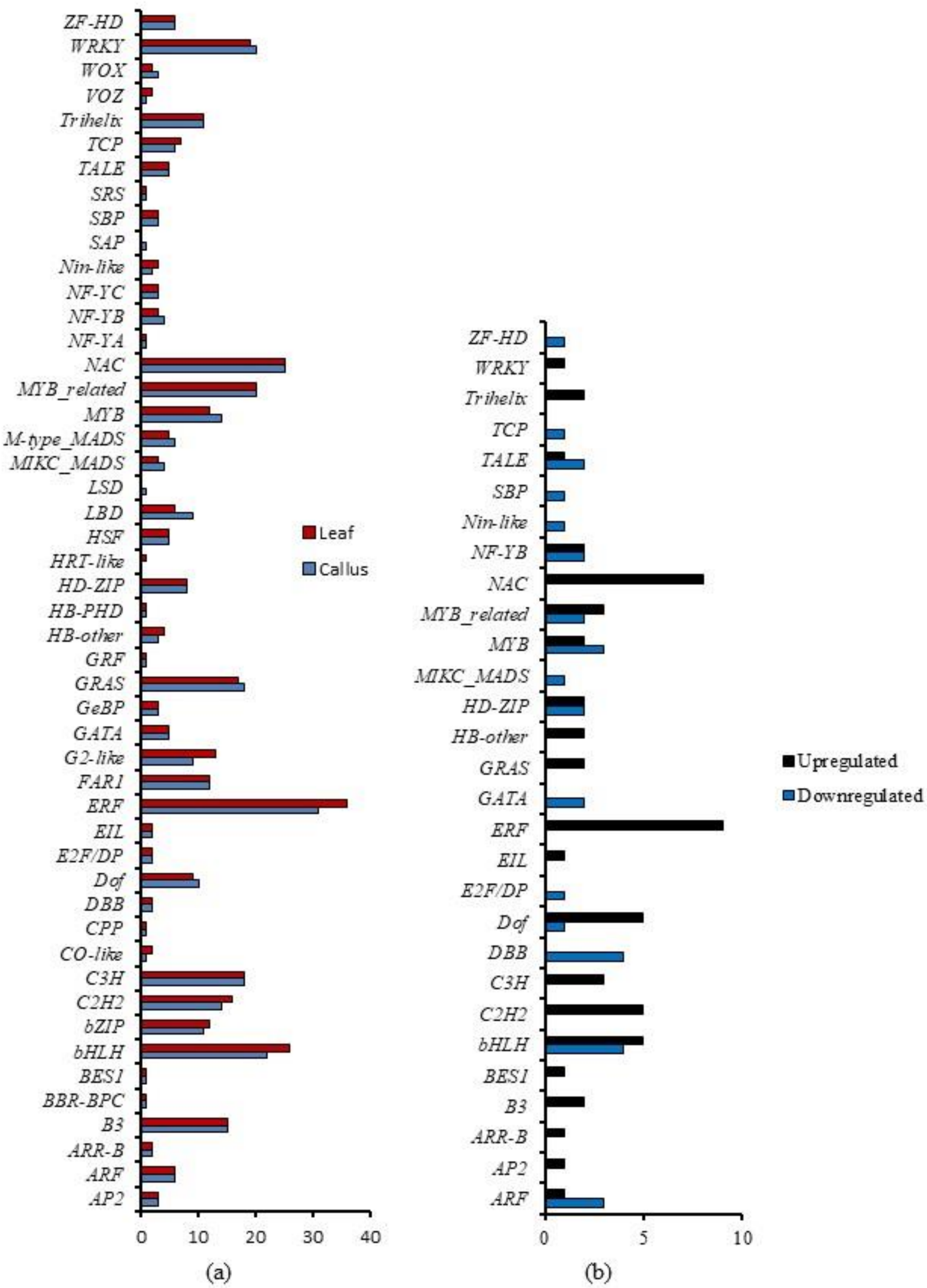
(b)

Figures 75 (a & b). Heat map showing expression pattern of highly downregulated genes in leaf-derived callus as compared to leaves. (a) Isoform and (b) Transcripts.

as proline-rich protein, alcohol dehydrogenase, and expansin (cell wall protein) which are involved in wound healing process and cold stress, AAI domain containing protein, late embryogenesis abundant protein (lea5) take part during callus formation, signaling protein like WNT family protein, acetylserotonin-o-methyl transferase which are involved in biosynthesis of important metabolites, pathogenesis-related protein such as endochitinase, heat shock protein, transcription factors AP2/EFR domain containing transcription factor and ABC transporter C family. The most downregulated genes in callus were lipoxygenase (LOX3), ribulose bisphosphate carboxylase/oxygenase activase, EGF like domain containing protein, thioredoxin-like protein CDSP32, stress-induced protein, chlorophyll a-b binding protein of LHCII type 1, ring type E3 ubiquitin transferase, carbonic anhydrase, fructose-bisphosphate aldolase, oxygen-evolving enhancer protein 2, starch synthase IV (SSIV) and magnesium-chelatase (GUN5).

The callus formation is governed by several genes some of which were identified and were upregulated in leaves derived callus such as auxin response factor (ARF), AP2/ERF domain-containing transcription factor, CYCLIN (CYC) and CYCLIN-DEPENDENT KINASES (CDK). ARF induce auxin signaling, which in turn activate the expression of two very crucial transcription factors, LATERAL ORGAN BOUNDARIES DOMAIN (LBD) and E2F TRANSCRIPTION FACTOR a (E2Fa) (Fan *et al*., 2012), AP2/ERF domain-containing transcription factor is crucial for orchestrating the cellular reprogramming that occurs in response to wounds in plants. It achieves this by augmenting ENHANCER OF SHOOT REGENERATION1 (ESR1) gene expression, which further codes for AP2/ERF transcription factor, in the model plant species *Arabidopsis thaliana* (Iwase *et al*., 2017). CYCLIN (CYC) and CYCLIN-DEPENDENT KINASES (CDK) are cell cycle regulators which trigger the process of callus development and the regeneration of organs by reactivating (Cheng *et al*., 2015.)

Several transcription factors involved in various mechanisms were identified in both leaves and callus through plant transcription factor database. Between the leaves and callus, 714 transcripts encoding transcription factors were identified, which were classified into 49 families (Figure 76a**)**. The highest number of transcripts belonged to *ERF* (Ethylene response factor), *NAC* (NAC domain protein), *bHLH* (basic/helix-loop-helix), *WRKY* (WRKY-type DNA binding protein), *GRAS* (Gibberellin-insensitive (GAI), *C3H* (Cys3His zinc finger protein), *MYB* related (myeloblastosis DNA-binding related protein). *SAP* (STERILE APETALA) and *LSD* (Lesion

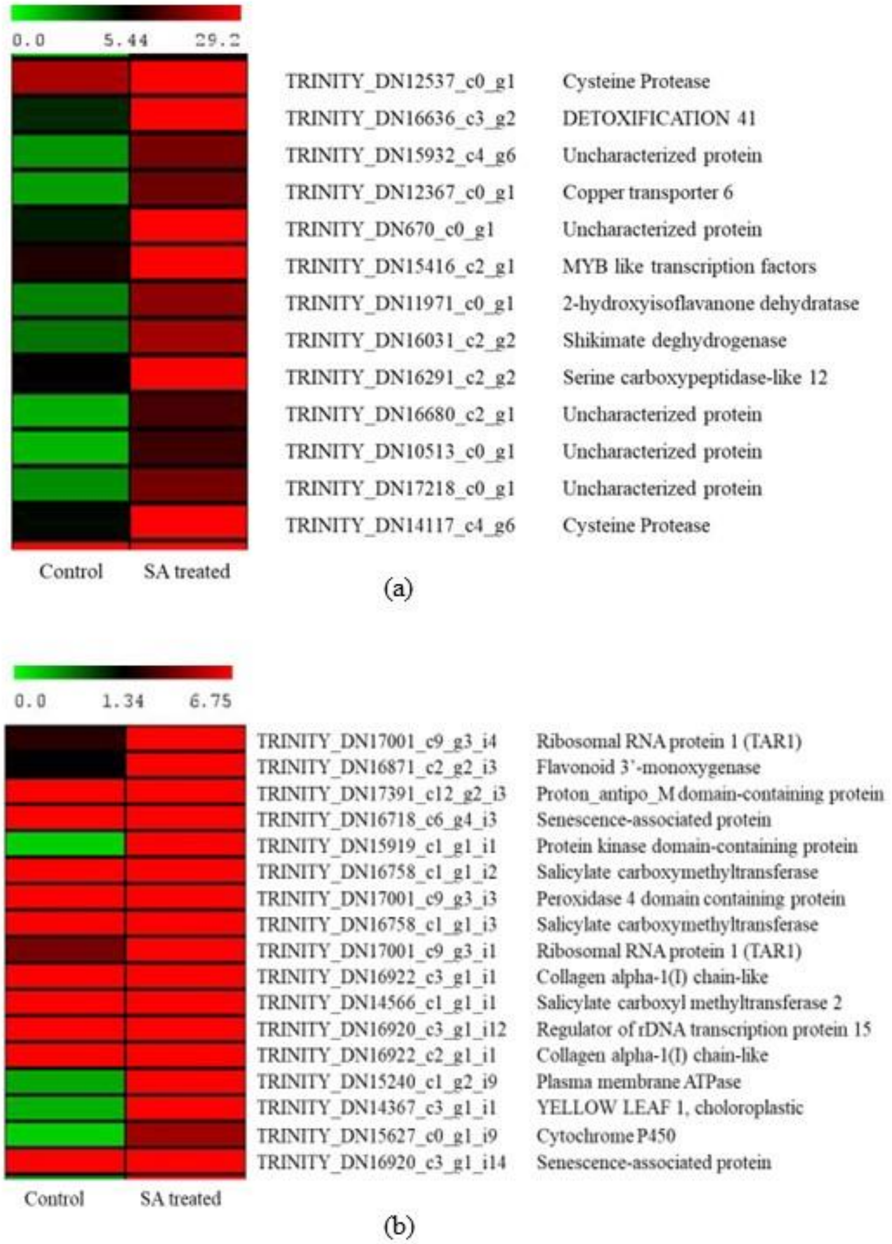


Figures 76 (a & b). Transcription factors identified and their expression pattern in leaves and leaves-derived callus. (a) Transcription factors identied in the samples and (b) Expression pattern of TF in leaves and leaves-derived callus.

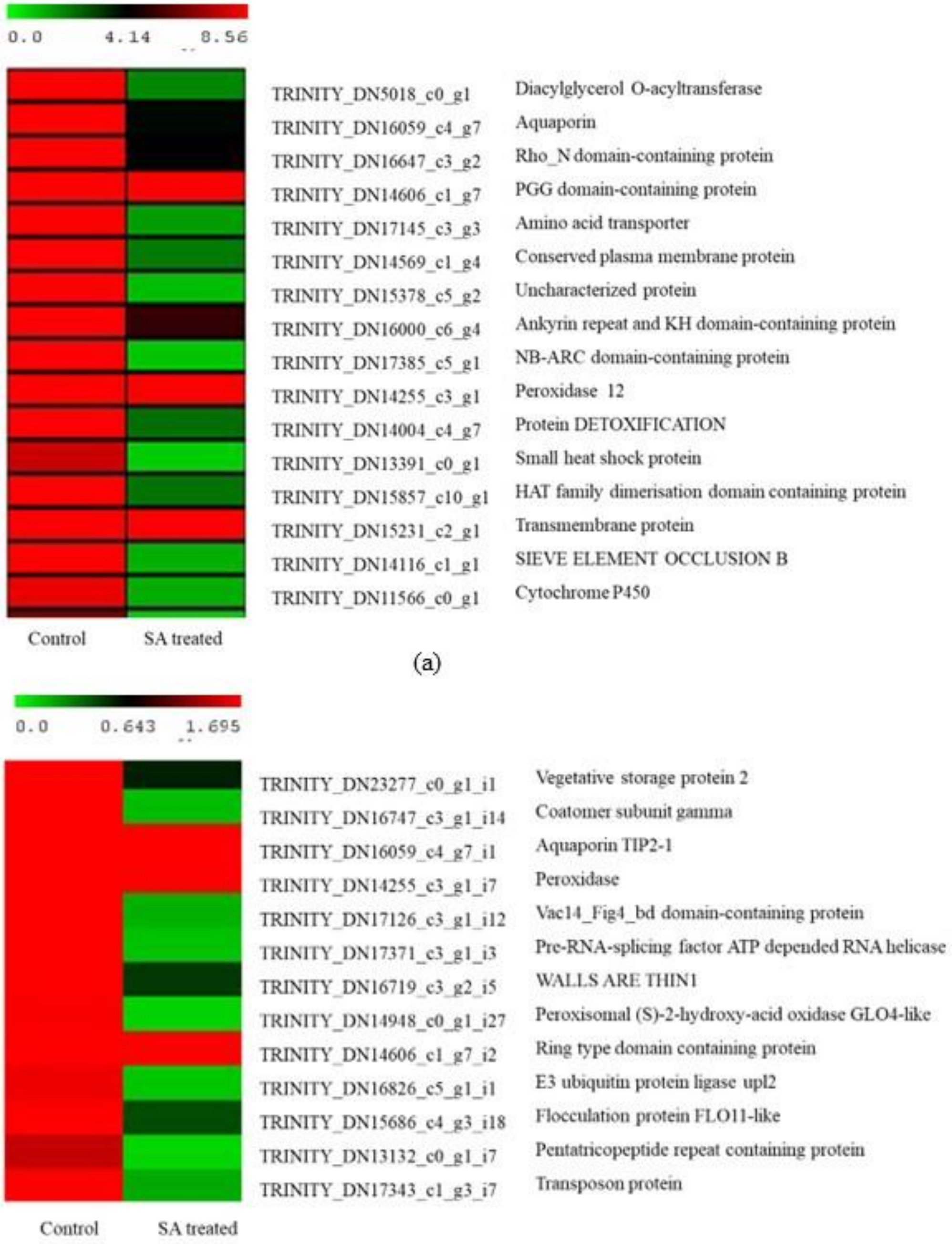
simulating disease) transcription factors were only identified in callus while *HRT*-like transcription factor was only identified in leaves. Out of the 49 familiies of TF identified, 29 were differentially expressed. The most abundant transcripts of TF that were upregulated included ERF and *NAC* in leaves-derived callus in comparison to leaves while the most abundant downregulated were *DBB* and *bHLH* transcription factors (Figure 76b).

4.7.2. Transcriptome analysis revealed differentially expressed genes in JA-treated and SA-treated callus cultures

To identify DEGs among three different samples, comparisons were made between each set of transcriptome data. Transcript abundance and counts were estimated using Kallisto and cross sample normalized TPM/TMM (Transcripts Per Million transcripts) values were obtained. The counts were used to perform differential expression with edgeR. Differentially expressed unigenes/isoforms with p-value cutoff 0.05 are mentioned in Table 12. There were 1623 differentially expressed genes between SA treated and control callus cultures which were primarily involved in biosynthetic pathways, plant hormone signaling pathways, plants stress response, plant-pathogen interactions. Out of 1623 DEGs, 886 were upregulated and 737 were downregulated in SA treated callus cultures. The clustered heat maps of top up regulated and down regulated unigenes and isoforms of SA treated callus in comparison to control callus are shown in Figures 77 a & b, 78a & b. The top upregulated genes in SA treated cultures consisted of genes associated with phenylpropanoid pathway namely 2-hydroxyisoflavanone dehydratase, flavonoid 3'-monoxygenase, and shikimate pathway such as shikimate dehydrogenase, signaling transduction proteins such as cysteine protease, serine carboxypeptidase-like 12, senescence-associated protein, salicylate carboxymethyltransferase YELLOW LEAF, stress response protein such as peroxidase 4, Cytochrome P450, MYB transcription factor, transmembrane protein such as proton_antipo_M domain-containing protein (AQP/TIP), copper transporter, plasma membrane ATPase (Figures 78a & b). The top downregulated genes in SA treatment included membrane proteins like amino acid transporter, conserved plasma membrane protein aquraporin/tonoplast intrinsic protein, transmembrane protein, SIEVE ELEMENT OCCLUSION B, stress response protein such as peroxidase 12, cytochrome P450, protein DETOXIFICATION, small heat shock protein, diacylglycerol-o-acyltransferase, signaling preotein such as Rho-N-domain containing protein, ankyrin repeat and KH domain containing



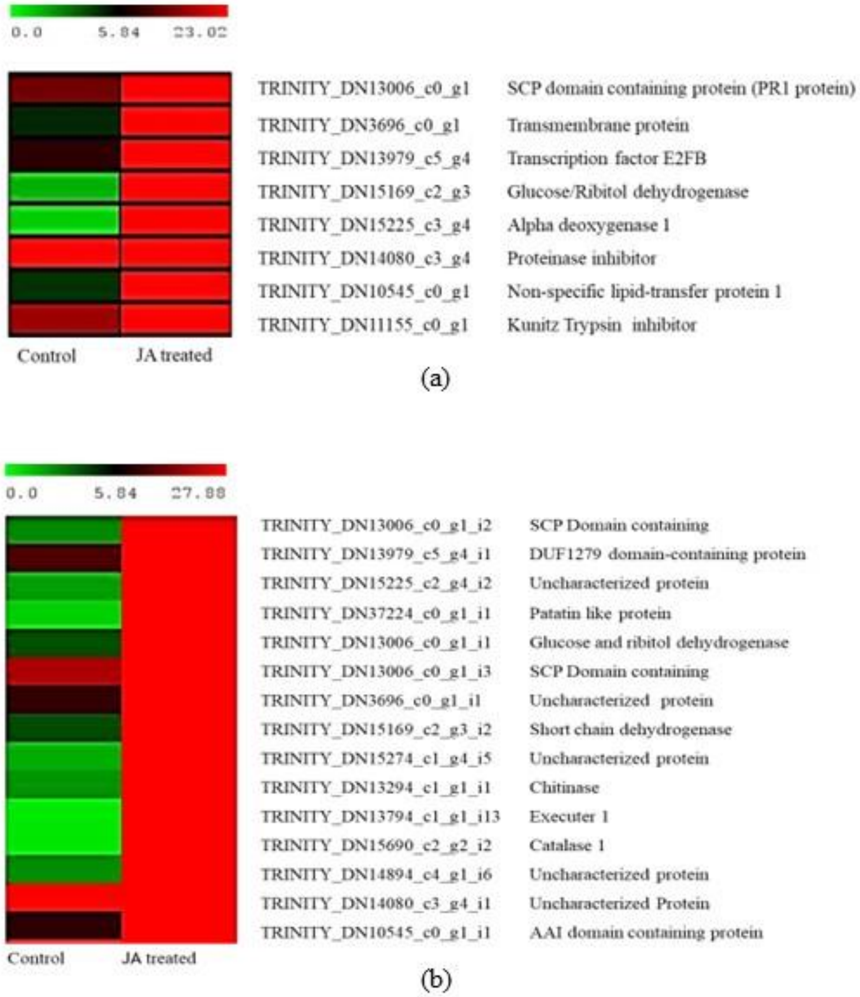
Figures 77 (a-b). Heat map showing expression pattern of highly upregulated genes in SA-treated callus in comparison to control (untreated) callus. (a) Transcript and (b) Isoform.



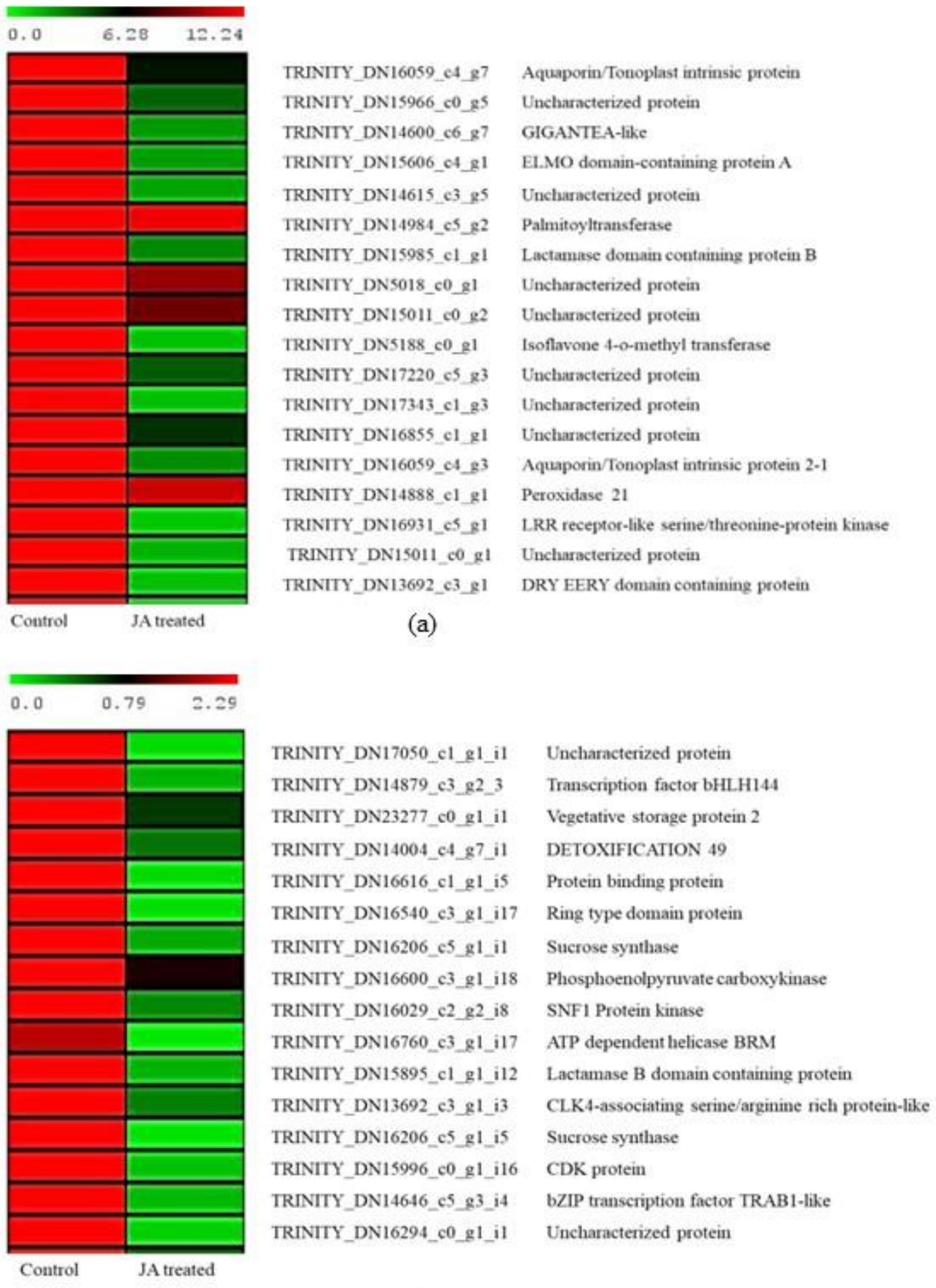
(a)

(b)

Figures 78 (a-b). Heat map showing the expression pattern of highly downregulated genes expression SA treated callus in comparison to control (untreated) callus. (a) Transcripts and (b) Isoforms.

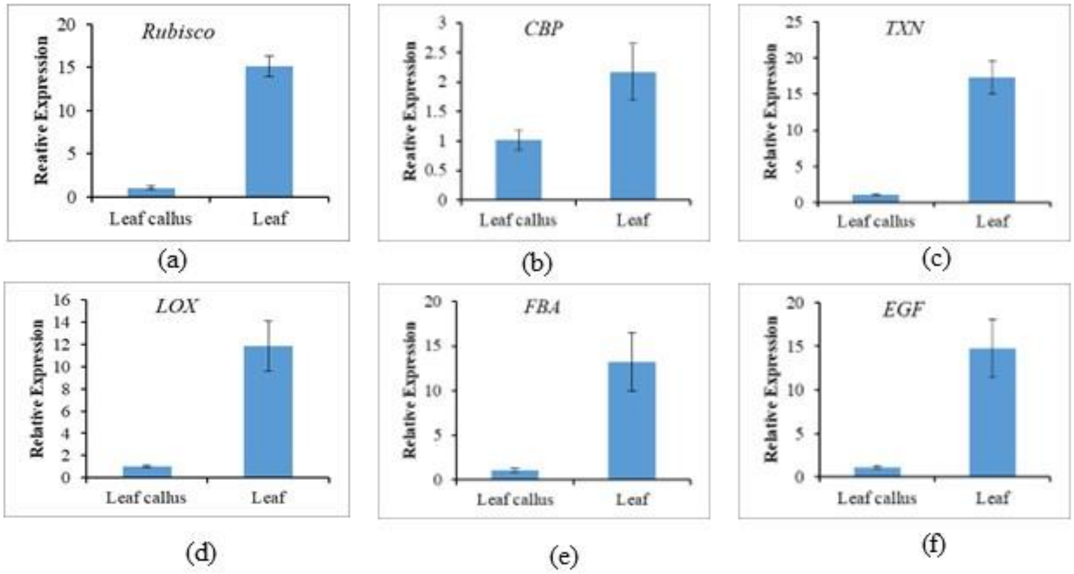


Figures 79 (a & b). Heat map showing expression pattern of upregulated genes in JA treated callus in comparison to control callus. (a) Transcripts and (b) Isoforms.

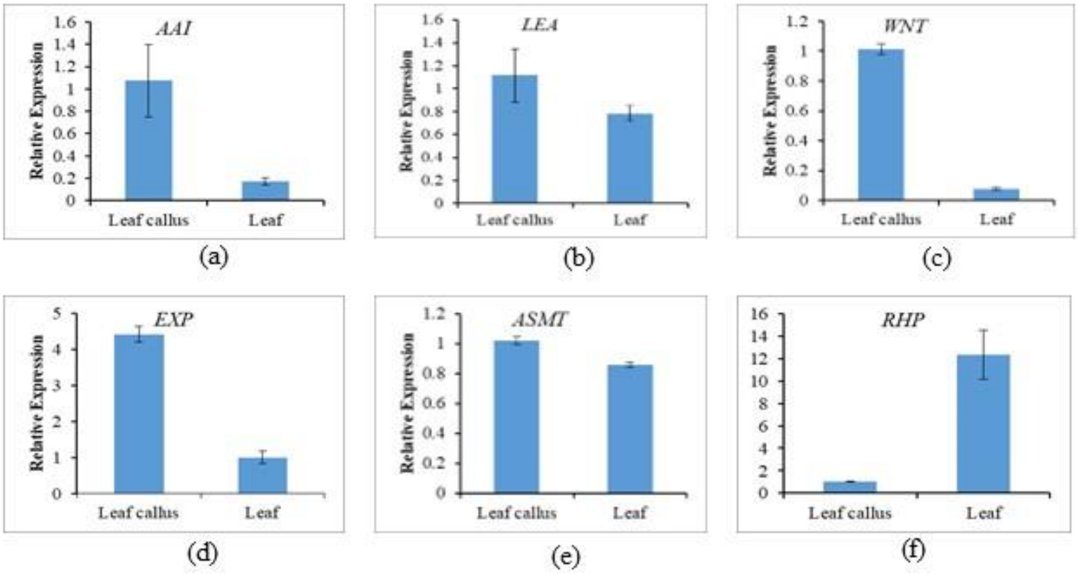


(a)

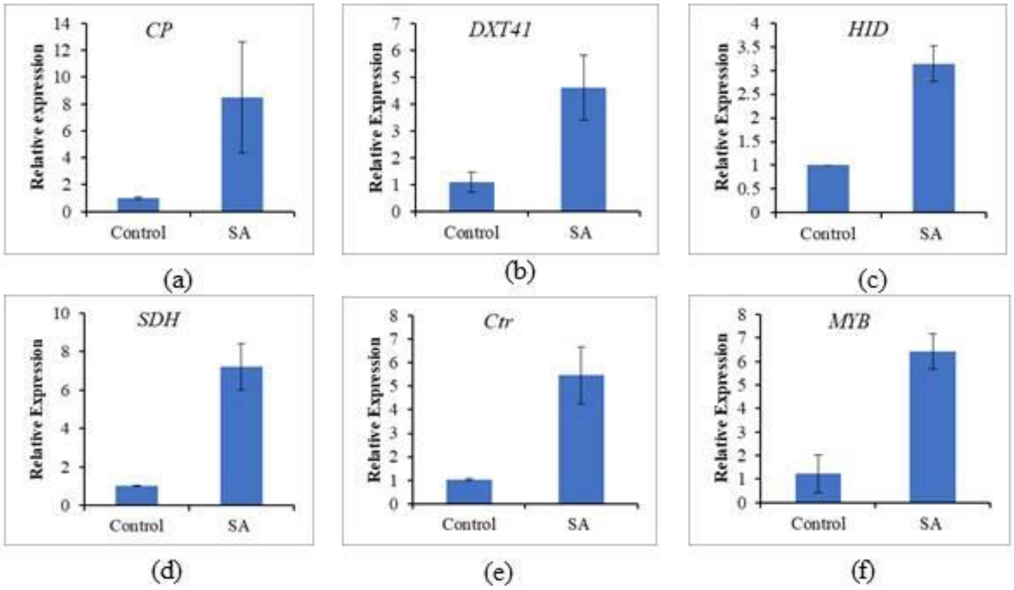
Figures 80 (a & b). Heat map showing expression pattern of downregulated genes in JA treated callus in comparison to control callus. (a) Transcripts and (b) Isoforms.



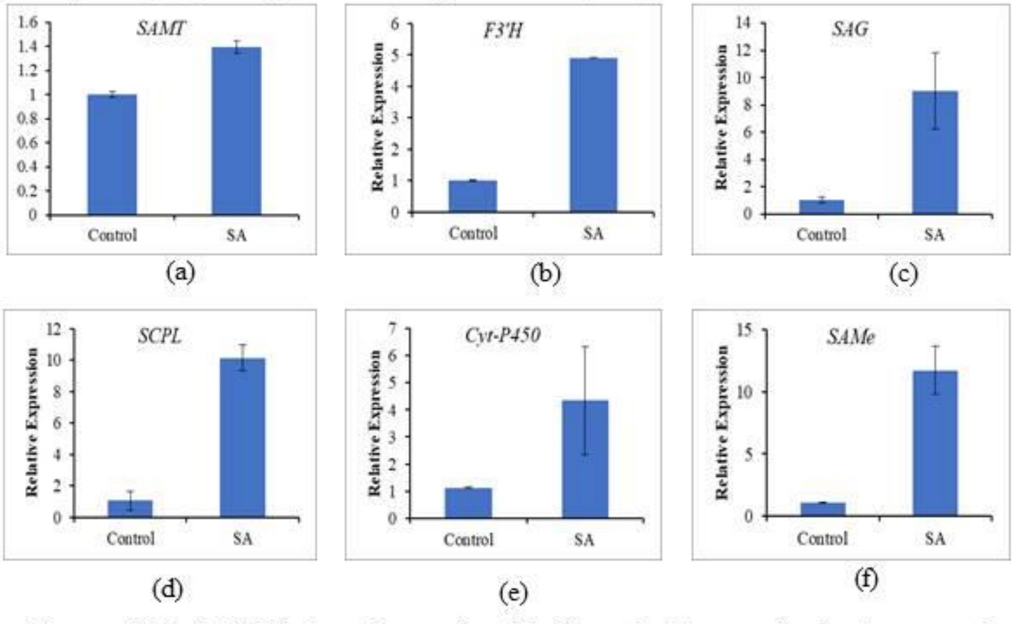
Figures 81 (a-f). Validation of top downregulated transcripts in leaves derived callus cultures in comparison to leaves (a) Ribulose-1, 5-bisphosphate carboxylase *(Rubisco)* (b) Chlorophyll a-b binding protein of LHCII type-1*(CBP-LHCII)* (c) Thioredoxin *(TRX/TXN)* (d) Lipoxygenase *(LOX)* (e) Fructose-bisphosphate aldose *(FBA)* (d) EGF like domain containing protein *(EGF)*



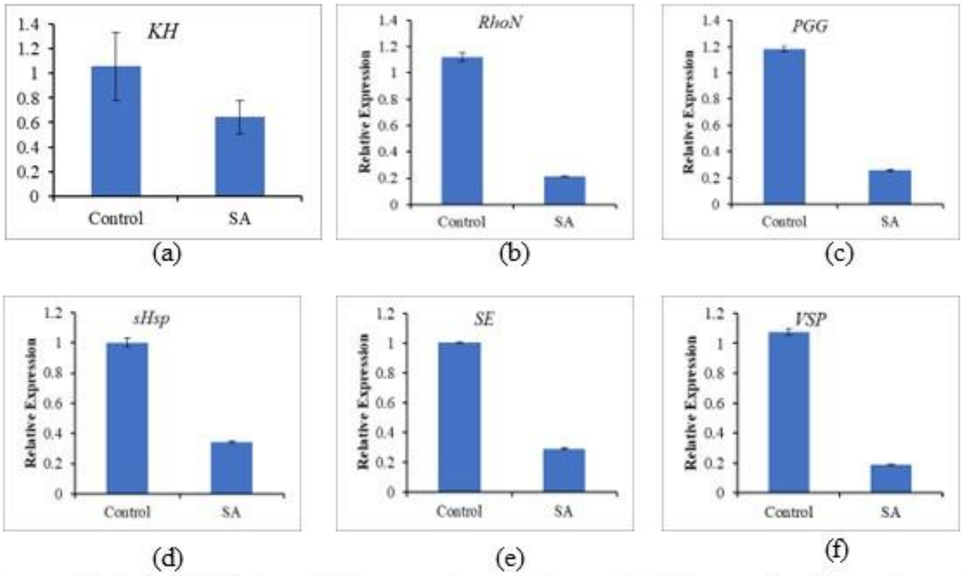
Figures 82 (a-f). Validation of top upregulated transcripts in leaves derived callus and leaves. (a) Alpha amylase inhibitor domain containing protein *(AAI),* (b) Late embryogenesis abundant protein *(LEA),* (c) WNT family member *(WNT),* (d) Expansin *(EXP),* (e) Acetylserotonin O-methyl transferase *(ASMT)* and (d) Ethylene responsive transcription factor *(ERF).*



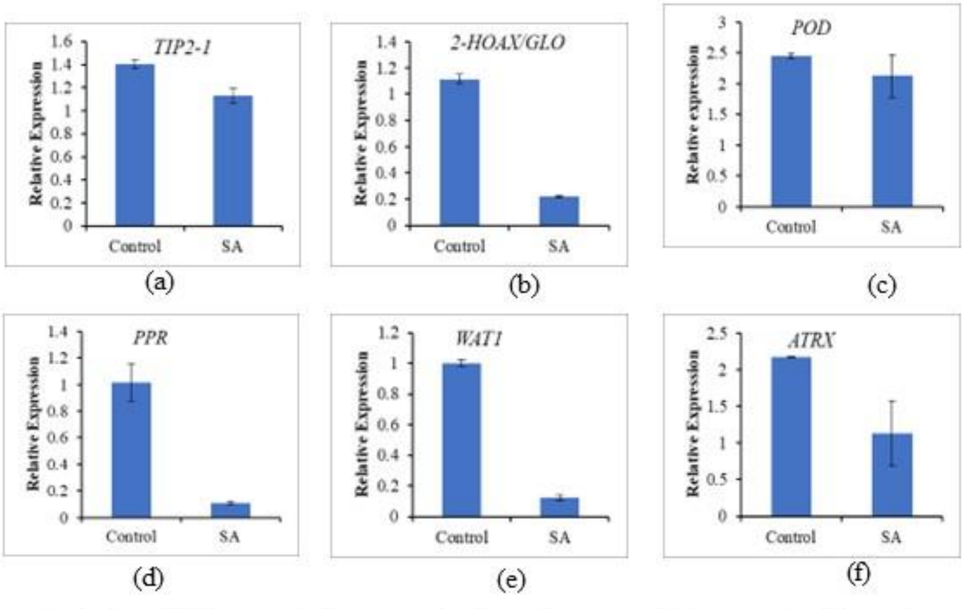
Figures 83 (a-f). Validation of upregulated unigenes in SA-treated callus in comparison to control callus. (a) Cysteine Protease *(CP)*, (b) DETOXIFICATION 41 *(DXT41)*, (c) 2-hydroxyisoflavone dehydratase *(HID)*, (d) Shikimate dehydrogenase (*SDH*), (e) Copper transporter *(Ctr)* and (f) MYB transcription factor *(MYB)*



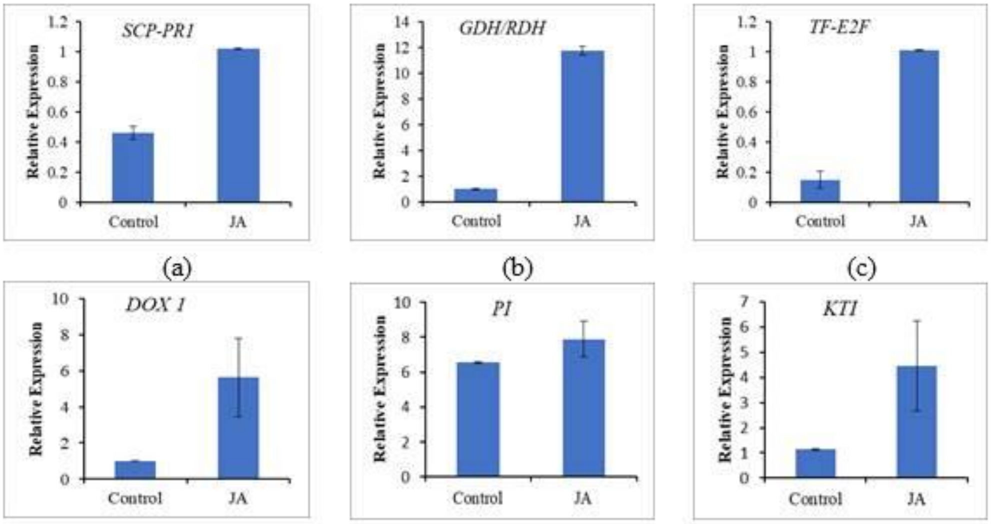
Figures 84 (a-f). Validation of upregulated isoforms in SA treated callus in comparison to control callus (a) Salicylate carboxymethyltransferase *(SAMT)*, (b) Flavonoid 3'-monoxygenase *(F3'H)*, (c) Senescence-associated protein *(SAG)*, (d) Serine carboxypeptidase-like (*SCPL*), (e) Cytochrome P450 *(Cyt-P450)* and (f) S-Adenosyl methionine *(SAMe)*.



Figures 85 (a-f). Validation of downregulated unigenes in SA treated callus cultures in comparison to control callus. (a) KH domain containing protein *(KH)*, (b) Rho N domain containing protein *(RhoN)*, (c) PGG domain containing protein, *(PGG)*, (d) Small heat shock protein *(sHsp)*, (e) Sieve elements *(SE)* and (f) Vegetative storage protein *(VSP)*.



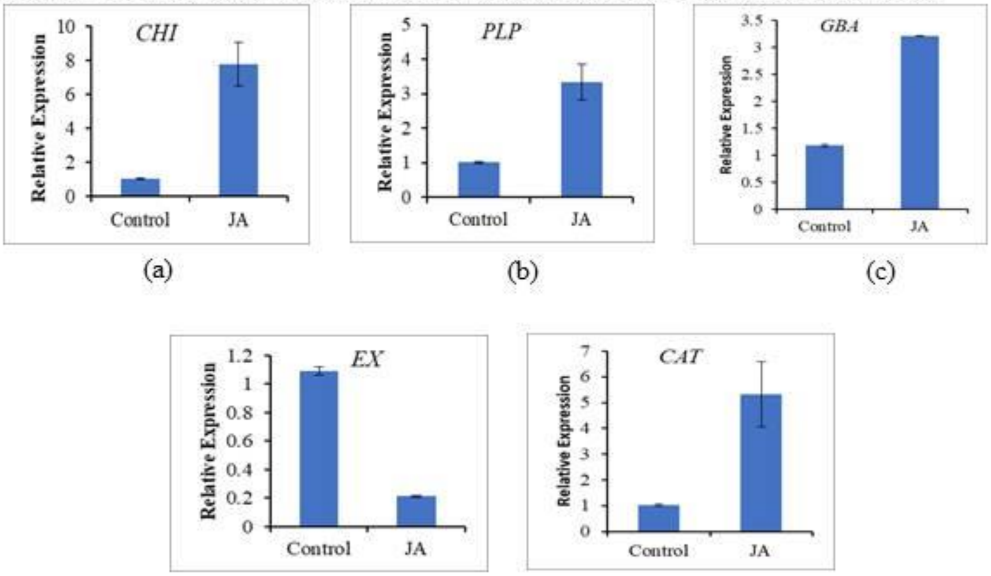
Figures 86 (a-f). Validation of downregulated isoforms in SA treated callus cultures in comparison to control callus. (a) Tonoplast intrinsic protein *(TIP2-1)*, (b) Peroxisomal (S)-2-hydroxy-acid oxidase *(2-HOAX/GLO)*, (c) Peroxidase *(POD)*, (d) Pentatricopeptide repeat containing protein (*PPR*), (e) WALLS ARE THIN1 *(WAT1)* and (f) ATP-dependent RNA Helicase *(ATRX)*.



(a) (b) (c)

(d) (e) (f)

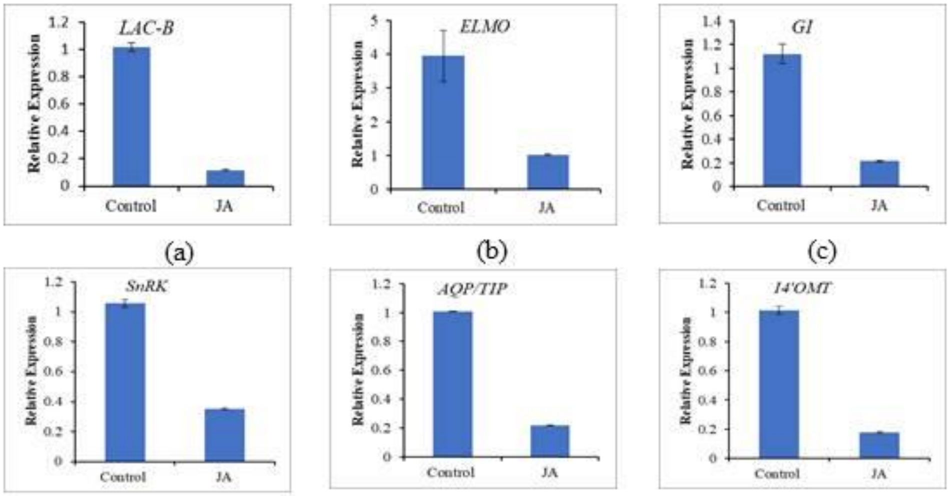
Figures 87 (a-f). Validation of upregulated unigenes in JA treated callus cultures in comparison to control callus. (a) Pathogenesis related *(SCP-PR1)*, (b) Glucose/Ribitol dehydrogenase *(GDH/RDH)*, (c) Transcription factor Elongation factor *(TF-E2F)*, (d) Alpha-dioxygenase *(DOX1)*, (e) Proteinase inhibitor *(PI)* and (f) Kunitz trypsin inhibitor *(KTI)*.



(a) (b) (c)

(d) (e)

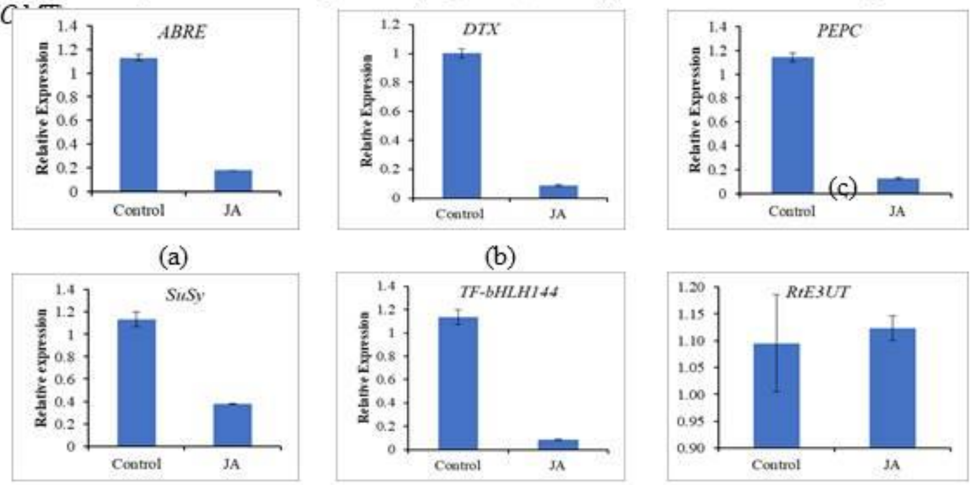
Figures 88 (a-e). Validation of upregulated isoforms in JA treated callus cultures in comparison to control callus. (a) Chitinase *(CHI)*, (b) Patatin like protein *(PLP)*, (c) beta-glucosidase *(GBA)*, (d) Executer *(EX)* and (e) Catalase *(CAT)*.



(a) (b) (c)

(d) (e) (f)

Figures 89 (a-f). Validation of downregulated genes in JA-treated callus in comparison to control callus. (a) Lactamase domain containing protein B *(LAC-B)*, (b) ELMO domain-containing protein A *(ELMO)*, (c) GIGANTEA *(GI)*, (d) SNF1 protein kinase *(SnRK)*, (e) Aquaporin/Tonoplastic intrinsic protein *(AQP/TIP)* and (f) Isoflavone 4-O-methyl transferase *(I4'C*



(a) (b)

(d) (e) (f)

Figures 90 (a-f). Validation of downregulated isoform(a) in JA-treated callus in comparison to control callus. (a) Abscisic acid responsive elements *(ABRE)*, (b) DETOXIFICATION *(DTX)*, (c) Phosphoenolpyruvate carboxykinase *(PEPC)*, (d) Sucrose synthase *(SuSy)*, (e) Transcription factor *(bHLH144)* and (f) Ring type domain containing protein/E3 Ubiquitin protein ligase*(RtE3UT)* protein, NB-ARC domain-containing protein, HAT family dimerisation domain containing protein (Figures 78a & b).

In JA treated callus cultures, 3504 differentially expressed genes were identified out of which 1719 were upregulated and 1785 were downregulated (Table 12). The top upregulated genes in JA treated callus cultures included SCP domain containing protein (PR1 protein), transmembrane protein, transcription factor *E2FB*, glucose/ribitol dehydrogenase, alpha deoxygenase 1, proteinase inhibitor, non-specific lipid-transfer protein 1, kunitz trypsin inhibitor, patatin like protein, short chain dehydrogenase, chitinase, executer 1, catalase 1, AAI domain containing protein that take part in signaling and plant oxidative stress response (Figures 79a & b). The downregulated genes in JA treated callus cultures consisted of aquaporin/tonoplast intrinsic protein, ELMO domain-containing protein A, palmitoyltransferase, GIGANTEA-like, lactamase domain containing protein B, S-adenosyl methionine, peroxidase 21, LRR receptor-like serine/threonine-protein kinase, DRY EERY domain containing protein, transcription factor bHLH144, vegetative storage protein 2, DETOXIFICATION 49, protein binding protein, ring type domain protein, sucrose synthase, phosphoenolpyruvate carboxykinase, SNF1 protein kinase, ATP dependent helicase BRM, CLK4-associating serine/arginine rich protein-like, CDK protein, bZIP transcription factor TRAB1-like (Figures 79a & b, 80a & b)**.**

4.7.2.1. Validation of DEGs by quantitative real time PCR (qRT-PCR)

To affirm and validate the accuracy and consistency of the transcriptome analysis data, 11-12 genes each of the top upregulated and top downregulated transcripts and isoforms from leaves, leaves derived callus, SA treated callus, and JA treated callus were selected for qRT-PCR. The result of each validated genes are illustrated in Figures 81a-f, 82a-f, 83a-f, 84a-f, 85a-f, 86a-e, 87a-f, 88-90. The relative expression of each selected genes were consistent with the result generated in transcriptome analysis. The results obtained therefore were reliable and would be expedient for future studies involving the phenylpropanoid, flavonoid, terpenoid and other secondary metabolite biosynthesis studies. The transcriptome analysis and qRT-PCR thus helped in understanding the gene expression changes induced by JA and SA and the putative functions of the differentially expressed genes in comparison to control callus cultures.

4.7.2.2. Pathway enrichment analysis

The transcript sequences of differentially expressed genes in SA and JA treated callus culture were entered into kobas server, where it carries out gene set enrichment analysis using the hypergeometric test and generates plot using *Ricinus communis* and *Jatropha curcas* as the reference organism. In SA treatment, pathway enrichment analysis resulted in 8 clusters. In SA, cluster 1(C1) comprised of three biosynthesis pathways namely, flavonoid biosynthesis pathway, biosyntheis of secondary metabolites, and metabolic pathway. Flavonoid biosynthetic pathway was significantly enriched. The cluster 2 (C2) comprised of two biosynthetic pathways namely biosynthesis of secondary metabolites (unclassified) and ubiquinone and other terpenoid-quinone biosynthesis. The cluster 3 (C3) comprised of linoleic acid metabolism and alpha linolenic acid metabolism. Cluster 4 (C4) comprised of taurine and hypotaurine metabolism, butanoate metabolism, alanine, aspartate, glutamine metabolism, and beta-alanine metabolism. Cluster 5 (C5) consisted of autophage and mRNA survellence pathway. Cluster 6 (C6) consisted of MAPK signaling, plant-pathogen interactions and plant-hormone signal transduction. Cluster 7 (C7) consisted of propanoate metabolism and valine, leucine, and isoleucine degradation. Cluster 8 (C8) included other biosynthesis and metabolism such as monoterpenoid biosynthesis, photosynthesis-antenna proteins, stilbenoid, diarylheptanoid and gingerol biosynthesis, phenylpropanoid biosynthesis, and ether lipid metabolism (Figure 91).

In JA treatment, the pathway enrichment showed 8 clusters. The cluster 1(C1) comprised of three biosynthesis pathways namely, phenylpropanoid biosynthesis pathway, biosynthesis of secondary metabolites, and metabolic pathways. The cluster 2 (C2) comprised of two biosynthetic pathway namely biosynthesis of secondary metabolites (unclassified), stilbenoid, diarylheptanoid and gingerol biosynthesis and flavonoid biosynthesis. The cluster 3 (C3) comprised of linoleic acid metabolism and alpha linolenic acid metabolism. Cluster 4 (C4) comprised of ether lipid metabolism, glycerophospholipid metabolism, and endocytosis. Cluster 5 (C5) consisted of isoquinoline alkaloid biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis, phenylalanine metabolism, tyrosine metabolism, tropane, piperidine and pyridine alkaloid biosynthesis. Cluster 6 (C6) consisted of alanine, aspartate and glutamate metabolism, glyoxylate and dicarboxylate metabolism, peroxisome, glycine, serine, threonine metabolism, carbon metabolism. Cluster 7 (C7) consisted of non-homologous end-joining and homologous recombination. Cluster 8 (C8) included flavone and flavonol biosynthesis, monoterpenoid biosynthesis, isoflavonoid biosynthesis, photosynthesis, glycosylphosphatidylinositol (GPI)-anchor biosynthesis (Figure 92).

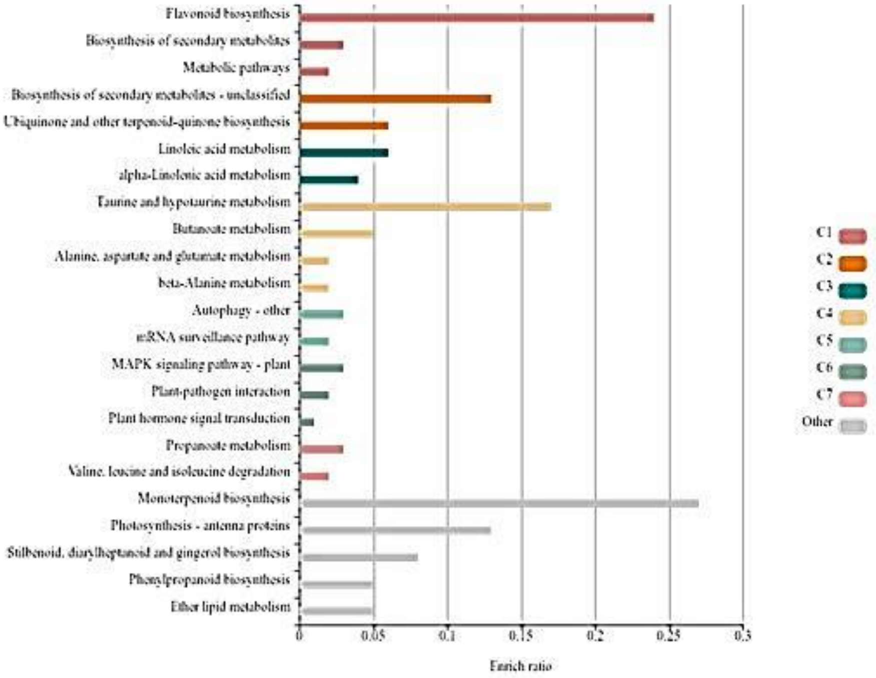


Figure 91. Pathway enrichment analysis of the differentially expressed genes in SA treated callus cultures in comparison to control callus.

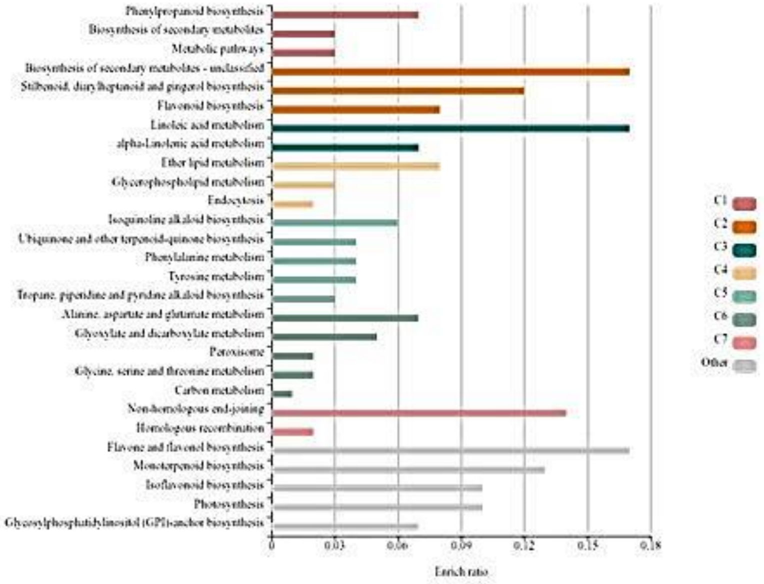


Figure 92. Pathway enrichment analysis of the differentially expressed genes in JA treated callus cultures in comparison to control callus.

4.7.3. Identification of transcription factors (TFs) and their expression pattern in JA-treated and SA-treated callus cultures

Several transcription factors involved in various mechanisms were identified in both control callus and treated callus. Herein, 1062 transcripts encoding transcription factors were identified in control, SA and JA treated callus and were classified into 50 families (Figure 93). The TFs with the highest family belonged to *ERF* (ethylene-responsive factor), *bHLH* (basic/helix-loop-helix), *NAC* (NAC domain protein), *WRKY* (WRKY-type DNA binding protein), *GRAS* (Gibberellin-insensitive (GAI), *C3H* (Cys3His zinc finger protein), *C2H2* (C2H2 zinc-finger protein), *MYB* (myeloblastosis DNA-binding protein), *MYB* related (myeloblastosis DNA-binding related protein) and *B3* (B3 DNA binding domain). *LYF* transcription factor was found specifically in JA treated callus while *SAP* was found only in control and SA treated callus. On the other hand, LSD was found only in control and JA treated callus. Of the 699 TFs, 29 belonging to 12 families were differentially expressed between SA and control callus. *WRKY*, *MYB* related and *bHLH* were found to be the largest DEGs. The most abundant upregulated transcripts in SA treated callus were *WRKY*, and *MYB* related whereas *bHLH* was the most abundant downregulated (Figure 94a). Out of 716 TFs between control and JA, 81 transcripts belonging to 31 families were differentially expressed. The upregulated TFs with the most abundant transcripts belonged to *WRKY* and *ERF*, while most abundantly downregulated TFs belong to *ERF*, *bHLH* and *B3* (Figure 94b**)**.

4.7.4. Expression profile of putative genes involved in phenylpropanoid and flavonoid biosynthesis pathway during SA and JA treatment

Phenylpropanoid pathway is very crucial for the biosynthesis of a diverse array of secondary metabolites such as flavonoids, phenolic acids, coumarins and lignins. In this study, several genes pertaining to phenylpropanoid pathway were observed to be differentially expressed such as phenylalanine ammonia lyase (PAL), trans-cinnamate 4-monooxygenase (C4H), 4-coumarate-CoA ligase (4CL), shikimate-o-hydrooxycinnamoyltransferase (HCT), 5-o-(4-coumaroyl)-D-quinate 3’-monooxygenase (C3’H), caffeoylshikimate esterase (CSE), caffeoyl- CoA o-methyltransferase (CCoAOMT), ferulate-5-hydrolate (F5H), caffeic acid 3-o-methyltransferase (COMT), cinnamyl-alcohol dehydrogenase (CAD) (Figure 95**)**. The genes such as PAL, C4H, C3’H, CCoAOMT were upregulated in both SA and JA treatments. PAL initiates the metabolism of phenylalanine to cinnamic acid, which constitutes the first step in the

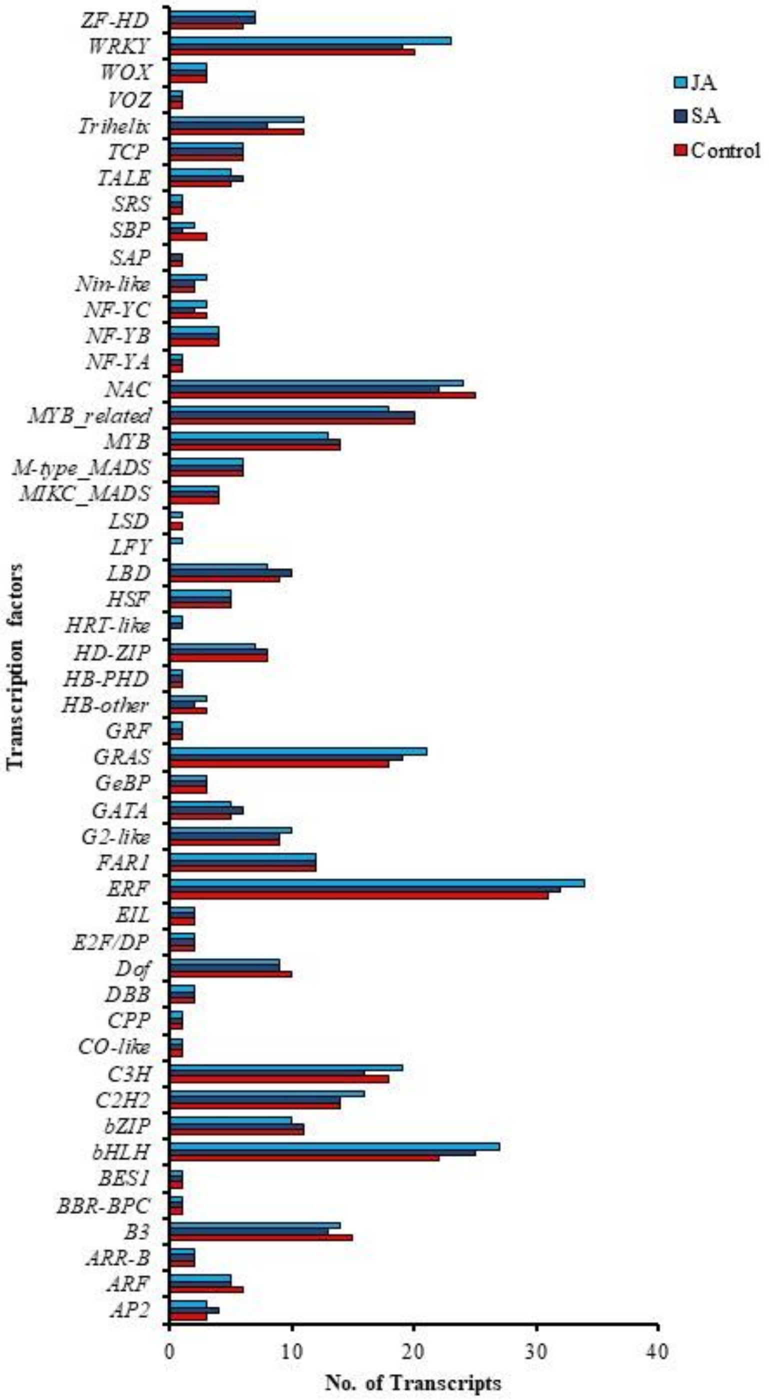
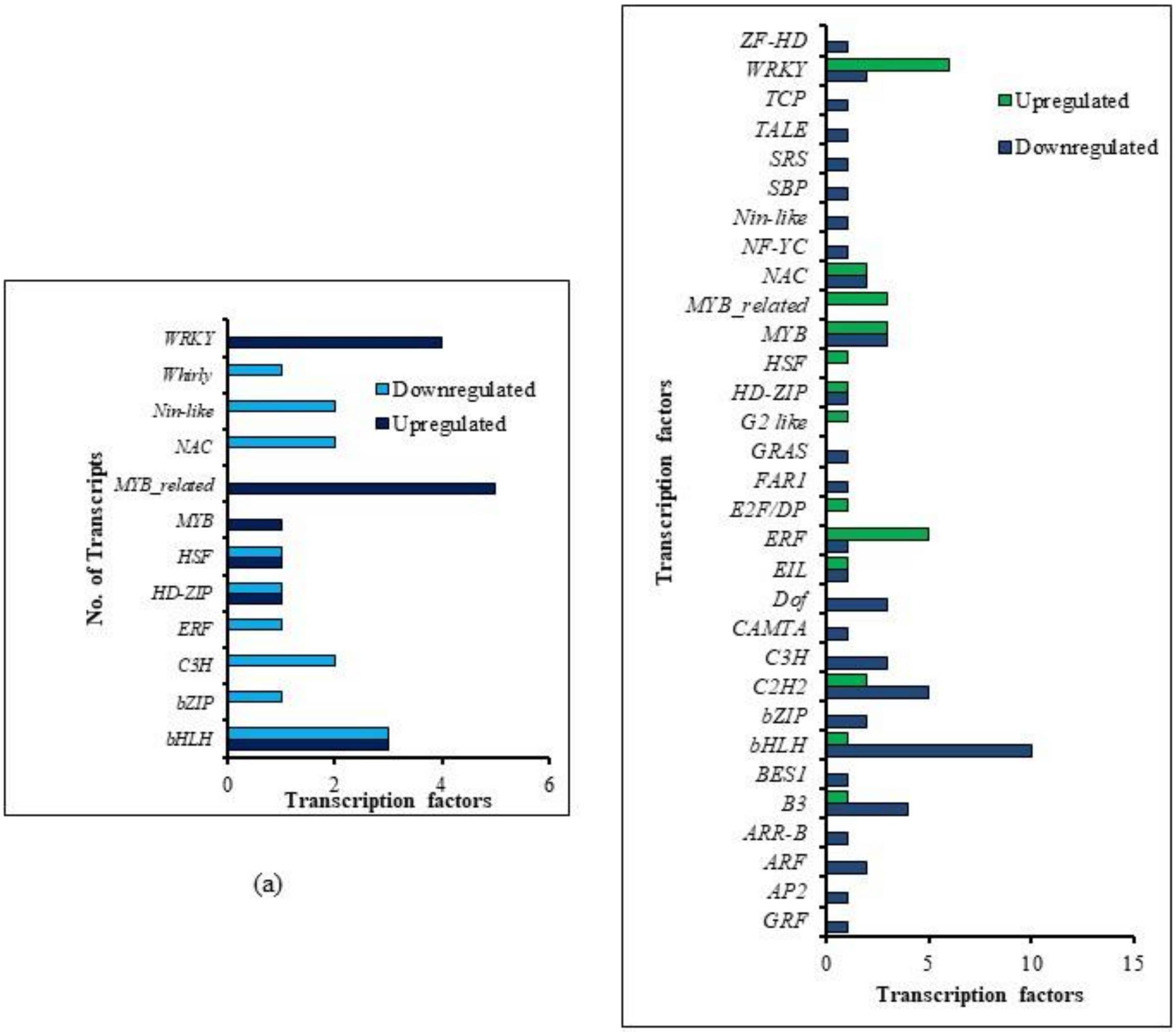


Figure 93. Various transcription factors identified in leaves-derived callus (control), SA-treated and JA-treated callus cultures.



(a)

(b)

Figures 94 (a & b). Transcription factors that were differentially expressed in SA-treated callus cultures and JA-treated callus cultures in comparison to control callus. (a) SA-treated callus cultures. (b) JA-treated callus cultures.

phenylpropanoid pathway. C4H stimulates the conversion of trans-cinnamate into p-coumarate, which is the first oxidative step of the phenylpropanoid pathway. C3’H catalyzes the conversion of 5-O-(4-coumaroyl)-D-quinate to caffeoyl-D-quinate, which is necessary for the biosynthesis of chlorogenic acids. CCoAOMT is involved in the methylation of caffeoyl-CoA to form feruloyl-CoA, an important step in the biosynthesis of lignin. CAD which is involved in biosynthesis of lignin was downregulated in JA treatment and *F5H* gene which is involved in the conversion of flavanone to dihydroflavonol was downregulated in SA treatment. Further, some of the transcripts of the genes such as *HCT*, *CSE*, *COMT*, and *4CL* which are involved in lignin biosynthesis were upregulated while some transcripts of the same genes were downregulated in both SA and JA treatments.

Ten putative genes pertaining to flavonoid biosynthesis pathway were differential expressed in SA and JA treated callus cultures (Figure 96). This includes chalcone synthase (CHS), chalcone isomerase (CHI), trans-cinnamate 4-monooxygenase (C4H), chalcone reductase (CHR), flavonoid 3’-monooxygenase (F3H), flavonone 4-reductase (FNR), flavonoid 3’,5’-hydroxylase (F3’5’H), anthocynidin synthase (ANS), anthocynidin reductase (ANR), leucoanthrocynidin reductase (LAR). CHS catalyzes the initial steps of flavonoid biosynthetic pathway, converting 4-coumaroyl-CoA and malonyl-CoA into naringenin chalcone. CHI also known as chalcone-flavanone isomerase (CFI) catalyzes the isomerization and reduction of chalcones into their corresponding flavonones. Flavonoid 3'-monooxygenase, also known as flavonoid 3'-hydroxylase (F3'H) and F3',5'H catalyzes the hydroxylation of the B-ring of flavonoids by attaching a hydroxyl (-OH) group to the carbon atom at the 3' and, 3' and 5' positions of the B-ring, respectively. This hydroxylation step is crucial for formation of different flavonoid compounds, such as quercetin and kaempferol. LAR or ANR that are known to catalyze the conversion of colored anthocyanidins to colorless leucoanthocyanidins were upregulated in SA and JA treatments. CHR, which catalyzes the reduction of chalcones into their corresponding flavonones, and ANS also known as leucoanthocyanidin dioxygenase (LDOX) which fuels the metabolism of colorless leucoanthocyanidins into colored anthocyanidins were downregulated in JA treatment. FNR enzyme is involved in the reduction of flavanones into their corresponding dihydroflavonols was downregulated in SA treatment. Four putative genes flavonoid 3’,5’-hydroxylase (F3’5’H), flavonoid 3’-monooxygenase (F3H), flavonol-3-o-glucose L-rhamnosyltransferase (F3GLRT) and anthocyanidin 3-o-glucoslyteransferase (UGT79B1),

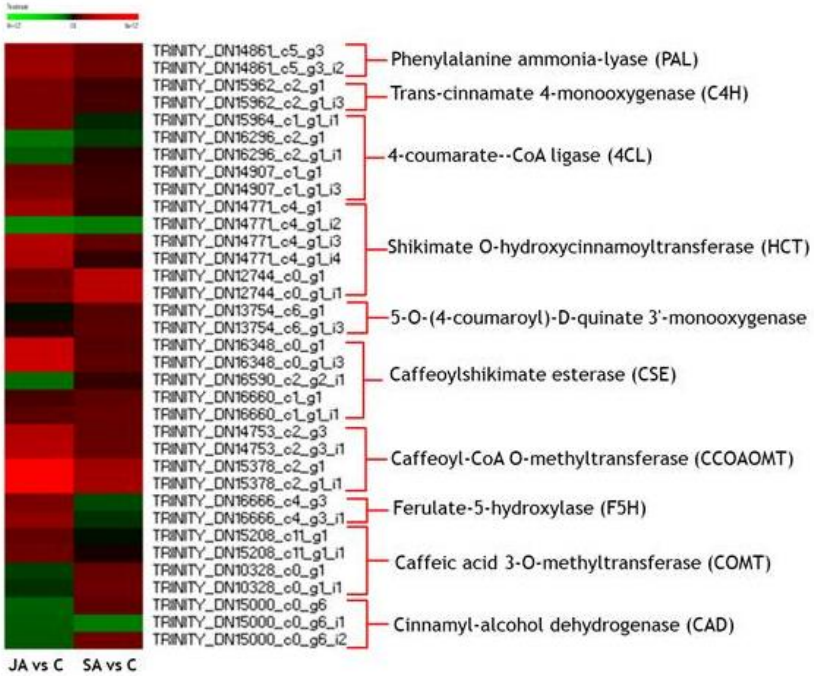


Figure 95. Heatmap diagram of expression patterns of genes involved in phenylpropanoid pathway in JA-treated, SA-treated in comparison to control callus cultures.

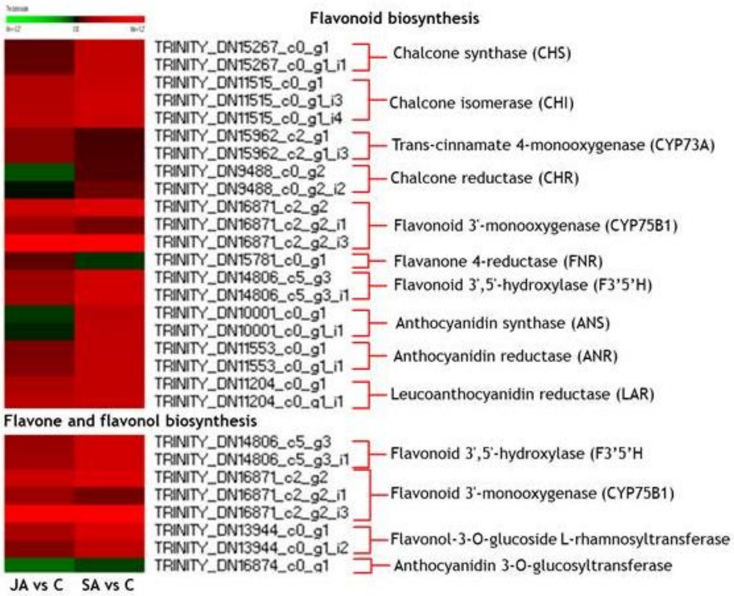


Figure 96. Heatmap diagram of expression patterns of differentially expressed genes involved in flavonoid, flavone and flavonol biosynthetic pathway in JA-treated, SA-treated in comparison to control callus.

involved in flavone and flavonol biosynthesis were identified which were also differentially expressed. All the four genes except anthocyanidin 3-o-glucoslyteransferase were upregulated in both SA and JA treatment. UGT79B1 which catalyze the transfer of glucose and L-rhamnose moieties to the 3-position of a flavonol molecule, typically quercetin, kaempferol, or myricetin were downregulated in both SA and JA treatments.

4.7.4. Expression profile of putative genes associated with terpenoid biosynthesis pathway in SA and JA treated callus cultures

Terpenoid biosynthesis pathway encompasses several enzymatic reactions and is divided into mevalonate (MVA) pathway that operates in cytosols and non-mevalonate pathway or the methylerythritol 4-phosphate (MEP) pathway which takes place in plastids. SA and JA treatments resulted in differential expression of the genes involved in both MVA and MEP pathway (Figures 97 & 98). Both the pathways contribute to the biosynthesis of wide range of terpenoids with diverse functions. Seven putative genes which are pertaining to MVA pathway were identified and differentially expressed namely acetyl-CoA C-acetyltransferase or acetoacetyl-CoA thiolase (AACT), 3-hydroxy-3-methylglutaryl coenzymeA synthase (HMGS), mevalonate kinase (MVK), phosphomevalonate kinase (PMK), diphosphomevalonate decarboxylase (MVD), isopentenyl-diphosphate delta-isomerase (IDI or IPP), farnesyl diphosphate synthase (FDPS) (Figure 97). Overall, the number of transcripts that encode enzymes involved in MVA pathway was mostly upregulated under JA treatment, while they are mostly downregulated in SA treatment. Genes such as *AACT*, *HMGS*, *MVK*, *PMK*, *IDI* and *FDPS* were upregulated while *MVD* gene which catalyzes a key step in the biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) was downregulated in JA treatment. AACT or thiolase-II catalyzes the formation of acetoacetyl-CoA by reversible condensation of two molecules of acetyl-CoA. HMGS fuels the condensation reaction between acetyl-CoA and acetoacetyl-CoA to form HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) in MVA pathway. MVK facilitates the phosphorylation of mevalonic acid to form mevalonate-5-phosphate. The primary function of PMK is to catalyze the phosphorylation of 5-phosphomevalonate, a compound derived from mevalonic acid, to form 5-diphosphomevalonate. IDI or IPP catalyze the interconversion of isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP). FDPS stimulates the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to form farnesyl diphosphate (FPP). In SA treatment, several transcripts of the genes involved in MVA pathway such as *PMK*, *MVK*, *HMGS* and *AACT* were downregulated with the exception of few transcripts which were upregulated. Only transcripts encoding *MVD* were upregulated. *IDI* and *FDPS* genes were downregulated in SA treatment.

Genes pertaining to MEP pathway such as 1-deoxy-D-xylulose-5-phosphate synthase (*DXS*), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (*DXR*), 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase (*MCT*), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (*CMK*), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (*HDS*), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (*HDR*), isopentenyl-diphosphate delta-isomerase (*IDI*), geranylgeranyl diphosphate synthase (*GGDPS*) were identified and differentially expressed in SA and JA treated callus culture as compared to control callus (Figure 99). Interestingly, majority of the transcripts encoding the genes involved in MEP pathway were upregulated in SA treatment and downregulated in JA treatment. *IDI*, *CMK* and *DXR* were the genes upregulated in both SA and JA treatments, while the transcripts of rest of the genes were both upregulated as well as downregulated.

Several genes involved in monoterpenoid, diterpenoid, triterpenoid, and sesquiterpenoid were identified (Figure 99). Neomenthol dehydrogenase (NMD) which is involved in the biosynthesis of neomenthol, a monoterpenoid was downregulated in JA treatment, while it was upregulated in SA treatment. Five genes involved in biosynthesis of diterpenoids were identified namely ent-copalyl diphosphate synthase (*CPSent*), ent-kaurene oxidase (*KO*), ent-kaurenoic acid monooxygenase (*KAO*), gibberellin-44 dioxygenase (*GA20ox*), gibberellin 2beta-dioxygenase (*GA2ox*). *CPSent*, *GA20ox*, *GA2ox* were upregulated in JA treatment, while *KO* and most transcripts of *KAO* were downregulated in JA treatment. In SA treatment, *KO* and most transcripts of *CPSent* were upregulated while *GA20ox* and *KAO* were downregulated and some transcripts of *GA2ox* were both upregulated and downregulated. Five genes involved in triterpenoid and sesquiterpenoids were identified which includes farnesyl-diphosphate farnesyltransferase (*SQS*), squalene monooxygenase (*SM*), squalene epoxidase (*SE*), germacrene D synthase (*GDS*), beta-amyrin synthase (*BAS*). *SQS* and *GDS* were upregulated in JA treatment, and *SE* was downregulated in JA treatment. *BAS* was downregulated in both SA and JA

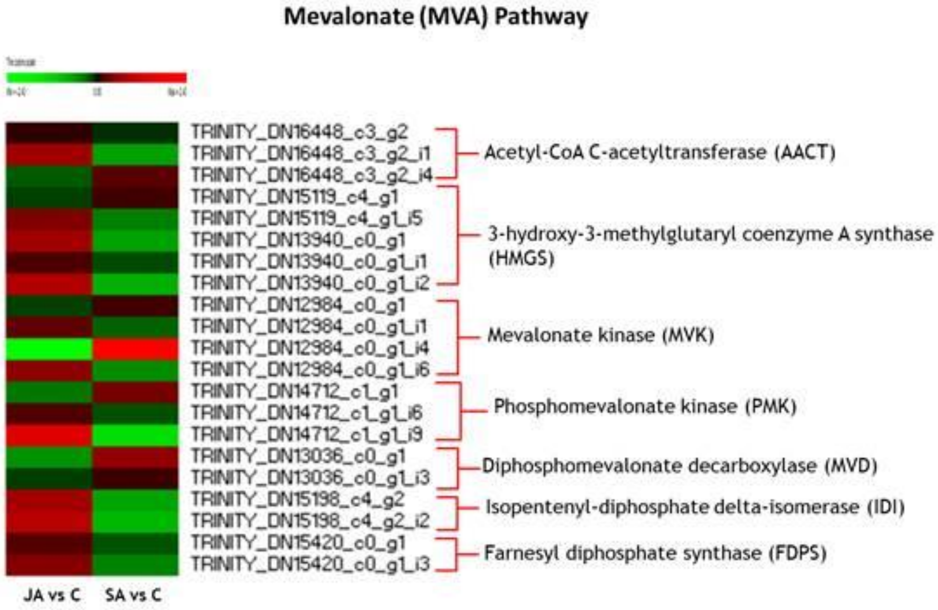


Figure 97. Heatmap diagram of expression pattern of differentially expressed genes involved in MVA pathway in JA-treated, SA-treated and control callus.

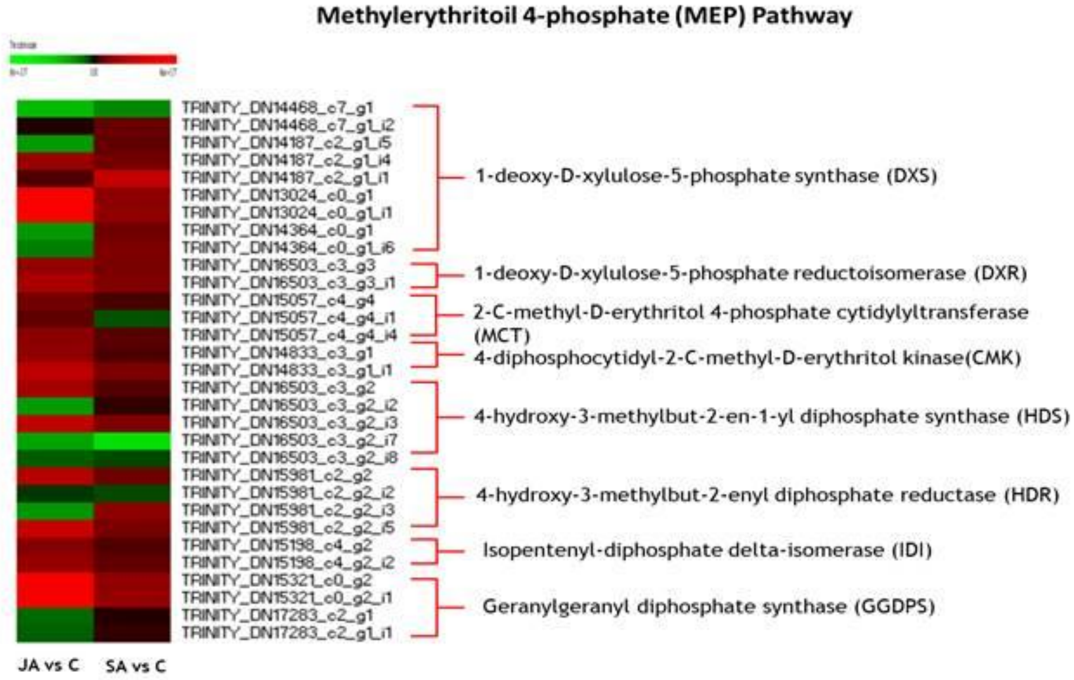


Figure 98. Heatmap diagram of expression pattern of differentially expressed genes involved in MEP pathway in JA-treated, SA-treated and control callus.

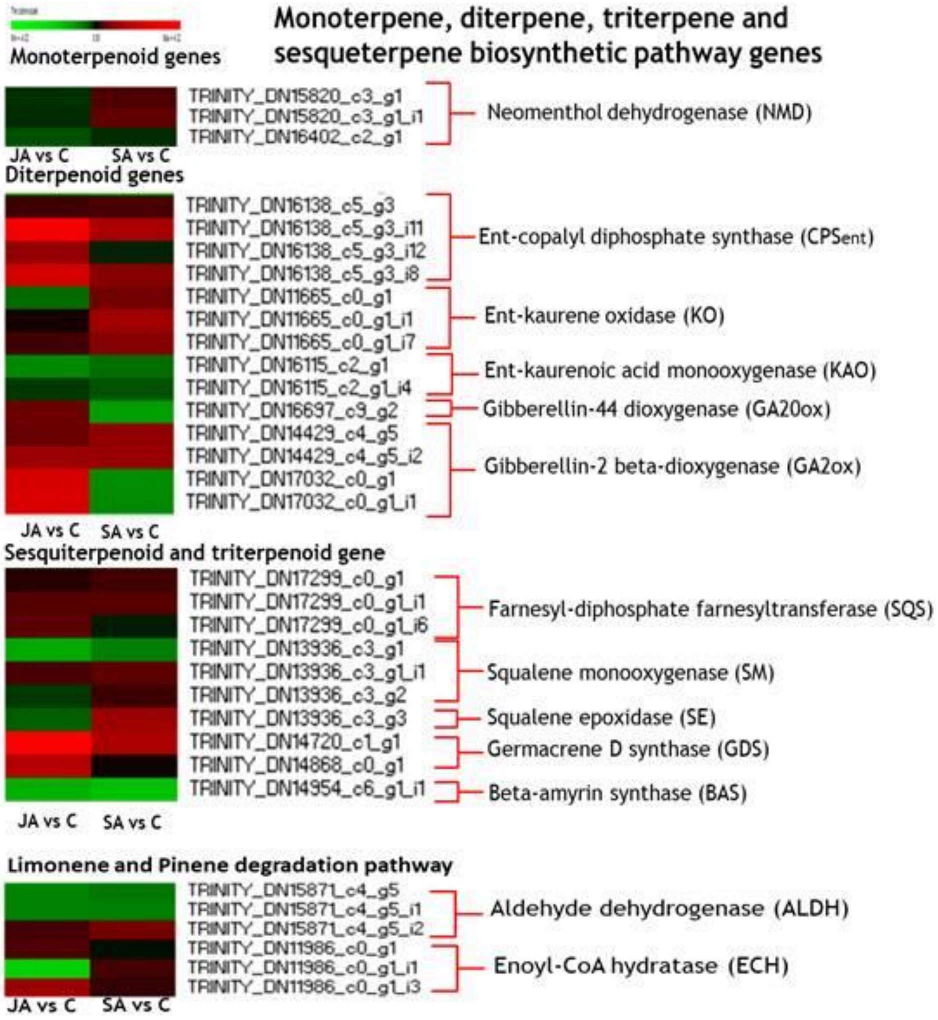


Figure 99. Heatmap diagram of expression pattern of differentially expressed genes involved in monoterpenoid, diterpenoid, triterpenoid, sesquiterpenoid, limonene and pinene degradation pathway.

treatments. *GDS* and *SE* were upregulated in SA treatment. Both *SQS* and *SM* were upregulated and downregulated in treated callus. Two genes namely aldehyde dehydrogenase (*ALDH*) and enoyl-CoA hydratase (*ECH*) related to limonene and pinene degradation pathway was identified. The trancripts of both genes were upregulated as well as downregulated.

4.7.4. Expression profile of putative genes involved in lignin biosynthesis pathway during SA and JA treatments

Lignins are intricate aromatic heteropolymers and rank among the most prevalent biopolymers following cellulose. Predominantly located in the secondary cell walls of vascular plants, lignins play pivotal roles in upholding the structural integrity of cell walls, bolstering stem strength, aiding water transport, furnishing mechanical support, and safeguarding against pathogens (You *et al.*, 2013). Lignins are synthesized through the phenylpropanoid pathway from three primary types of monolignols: p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). These monolignols are derived from the dehydrogenation of the hydroxycinnamyl alcohols p-coumaryl, coniferyl, and sinapyl (Zhao and Dixon, 2011). The expression of the enzymes involved in the biosynthetic pathway after SA and JA elicitor treatment in comparison to control were investigated. An overview of the lignin biosynthetic pathway and differentially expressed genes involved is shown in Figure 100. Phenylalanine ammonia lyase (PAL) which facilitates the conversion of phenylalanine to cinnamic acid was upregulated in both SA and JA treatments, with JA treatment resulting in higher expression than JA. Trans-cinnamate monooxygenase (C4H/CYP73A) which stimulates the conversion of cinnamic acid to p-coumaric acid were also upregulated in both SA and JA treatments. The next step in the pathway involves the formation of p-coumaroyl CoA from p-coumaric acid aided by an enzyme 4-coumarate –CoA ligase (4CL), the transcripts that encode this enzyme were both upregulated and downregulated in both SA and JA treatments. p-Coumaryl CoA is then converted to p-coumaroyl shikimate with the help of hydroxycinnamoyltransferase (HCT). The transcripts that encodes this enzyme were upregulated in both SA and JA treatments, except one transcript in each which were downregulated. The compound, p-Coumaroyl shikimate with the help of enzyme 4-coumarate 3-hydroxylase (C3H/CYP98A) and shikimic acid from chloroplast is converted into caffeoyl shikimic acid which is then catalyzed by an enzyme HCT to form caffeoyl CoA. The enzyme caffeoyl coenzyme A O-methyltransferase (CCoAOMT), catalyzes the conversion of caffeoyl CoA to

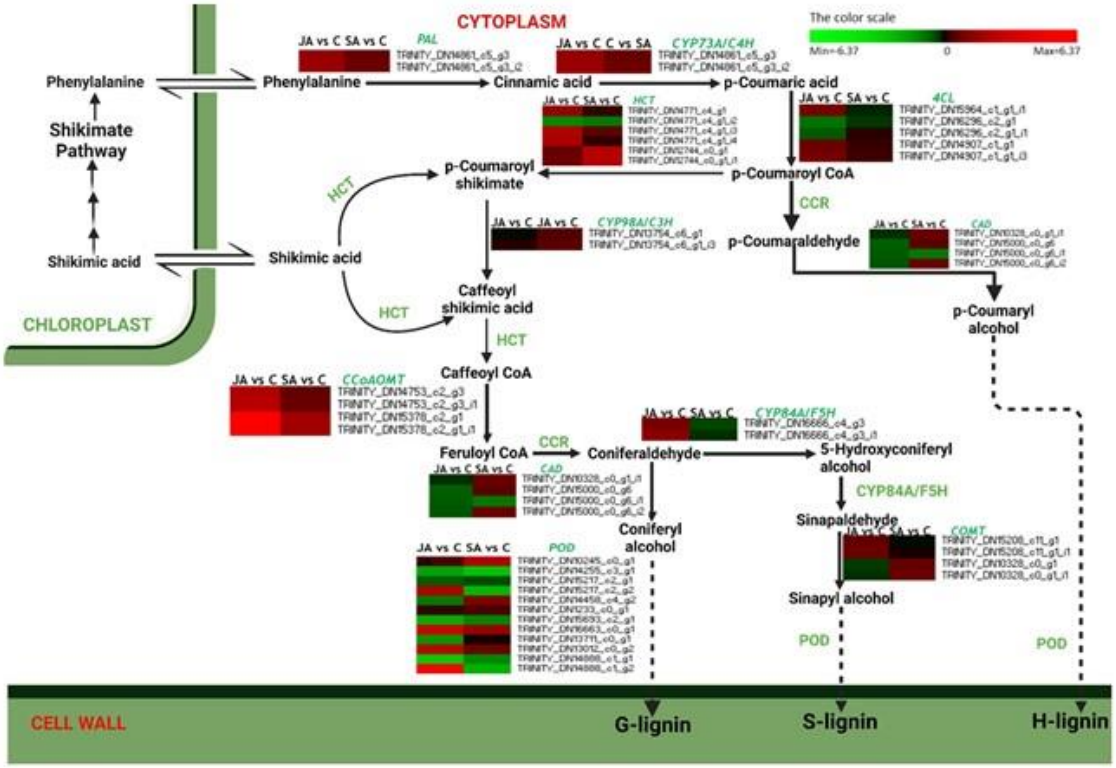


Figure 100. Overview of lignin biosynthetic pathway and differentially expressed genes involved in its biosynthetic pathway in JA-treated, SA-treated and control callus.

feruloyl CoA; the transcripts encoding CCoAOMT were upregulated in both SA and JA treatments. Feruloyl CoA is converted to coniferaldehyde catalyzed by cinnamoyl-CoA reductase (CCR). Coniferaldehyde is then converted to coniferyl alcohol with the help of cinnamyl alcohol dehydrogenase (CAD), is found to be downregulated in JA treatment and highly upregulated in SA treatment. Coniferyl alcohol in the presence of peroxidase forms G-lignins (guaiacyl) in the cell wall. Coniferaldehyde in the presence of ferulate 5-hydroxylase (F5H/CYP84A) gets converted to 5-hydroxyconiferyl alcohol. F5H/CYP84A was upregulated in JA treatment and downregulated in SA treatment. In the presence of F3H/CYP84A, 5-hydroxyconiferyl was converted to sinapaldehyde. Transcripts of catechol-O-methyltransferase (COMT) exhibited differential expression in both SA and JA treatments. COMT catalyzes the conversion of sinapaldehyde to sinapyl alcohol, which, in the presence of the peroxidase enzyme, contributes to the formation of S-lignin (syringyl). P-coumaryl CoA obtained *via* p-coumaric acid in the presence of CCR is converted to p-coumaryldehyde, which is then catalyzed by CAD to form p-coumarly alcohol, which in the presence of peroxidase is converted to H-lignin (p-hydroxyphenyl). The transcripts of peroxidase enzyme were differentially expressed in both SA and JA treatments. Thus, the transcriptome analysis of callus cultures treated with SA and JA provided insights into the differentially expressed genes involved pathways of phenylpropanoid, flavonoid, terpenoid and lignin biosynthesis including including transcripton factors.

5. Discussion

5.1. Higher phenolic and flavonoids were observed in methanolic extracts of bark than leaves and pericarp

Plant secondary metabolites exhibit remarkable diversity across different species and organs with their synthesis being highly influenced by the developmental stages and environmental factors (Sharma *et al*., 2022). Phenolics and flavonoids constitute a large group of secondary metabolites having several bio-pharmaceutical activities in addition to antioxidants capabilities. Evidence suggests that polarity and type of extracting solvents and physical characteristics of the samples influence the yield of phenolic compounds in plant extracts (Naczk and Shahidi, 2006). Solvents of different polarities selectively extract different hydrophobic or hydrophilic phenolic compounds (Ngo *et al*., 2017). Therefore, selection of a suitable solvent plays an important role for obtaining higher quantity of phenolics and flavonoids for the desired pharmaceutical activity.

The study investigated the concentrations of phenolics, flavonoids and the antioxidant activities of bark, pericarp and leaves extracted in solvents of different polarities in *Givotia moluccana*, a species that has high medicinal value. The results showed a significant variation in the concentration of phenolics and flavonoids in plant parts extracted in solvents of different polarities. Higher polar solvents *viz*., methanol and ethanol resulted in higher yields of total phenolics and flavonoids whereas their contents remained the lowest in hexane for all plant parts tested. However, the most polar solvent *i.e.* water was lesser effective than methanol and ethanol in extraction of phenolic and flavonoid compounds. It has been stated that the extraction of phenolics depend on the compatibility of the targeted constituents with the solvent system (Ahmad *et al*., 2021). Methanol and ethanol were found to be more efficient in extraction of low molecular weight phenolics and flavonoids (Ghasemzadeh *et al*., 2011; Shi *et al*., 2005; Chavan *et al*., 2013) which could be due to better solvation in polar solvents (Boeing *et al*., 2014). Further, the ethanol and methanol denatures the cell membrane more readily as compared to water, which could be another reason for higher recovery of phenolics and flavonoids in methanol and ethanol. These results are consistent with the findings of Srivani and Krishna Mohan (2022) where higher concentrations of phenolics and flavonoids were observed in methanol and the least in hexane extracts of leaves. However, the content of phenolics and flavonoids in leaves of *G. moluccana* was lower than the values reported by Srivani and Krishna

Mohan (2022). The plant source as well as the environmental and endogenous factors such as maturity stage might be the reasons for such differences observed in the same species. In this study, the highest content of total phenolics (62.07 mg GAE/g DW) and flavonoids (41.72 mg RE/g DW) were observed in methanolic extract of bark whereas the results varied concerning other plant parts tested. The phenolic content was higher in leaves (2.9 to 46.42 mg GAE/g DW) than pericarp (1.48 to 39.1 mg GAE/g DW) whereas flavonoid content was higher in pericarp (3.12 to 39.3 mg RE/g DW as compared to leaves (6.1 to 30.08 mg RE/g DW). The differences in phenolic composition in different plant parts signify the functional diversity. The phenolic content is influenced by intrinsic metabolic activity in various plant tissues as well as the molecular composition of exudate transferred through phloem channels, which generates such variations. According to Feduraev *et al.* (2019), photosynthesizing mesophilic tissue or the metabolism of a characteristic phloem exudate, sucrose, contributes significantly to the synthesis of phenolics in the stem of the sorrel species.

5.2. Antioxidant activity of methanolic extract of bark was higher than pericarp and leaves

Antioxidants confer a protective role mainly by involving single electron transfer, hydrogen atom transfer and metal chelation (Leopoldini *et al*., 2011). The antioxidant capacities of the plant extract chiefly relies on extract composition, solvent and the environments of the assessment system (Li *et al*., 2008). Several research studies have investigated the antioxidant properties of plant extracts using more than one technique to account for the multiple mechanisms of antioxidant activity (Wong *et al*., 2006). Here, the antioxidant potential of bark, leaves and pericarp extracted in different solvents was evaluated used different assays. The reducing power of the extracts of different plant parts towards phosphomolybdenum, CUPRAC, FRAP and metal chelation was investigated.

Phosphomolybenum assay evaluates the capability of samples to reduce Mo (VI) to Mo (V) either through electron transfer or hydrogen transfer mechanism. The total antioxidant activities were in the order of methanolic extracts > ethanolic extracts > water extracts > ethyl acetate extracts > dichloromethane extracts > hexane extracts for different plant parts. Overall, the methanolic extract of bark demonstrated the highest total antioxidant activity (70.94 mg AAE/ gDW) followed by ethanolic extract of bark (56.85 AAE /gDW). The antioxidant activities of methanolic and ethanolic extracts of leaves and pericarp did not differ significantly. Saeed *et al*. (2012) and Khan *et al*. (2012) highlighted that medicinal plant's phosphomolybdate scavenging action is considerably influenced by several phenylpropanoid and flavonoids. Both methanolic and ethanolic extracts of leaves and pericarp displayed a similar antioxidant capacity. Single electron transfer (SET) antioxidant assays such as FRAP and CUPRAC evaluate the capability of compounds to reduce the transition metals such as Fe (iron) and Cu (copper). In FRAP assay, the differences in antioxidant capacities were large, with the highest in bark (21.6 to 156.37 mg AAE /gDW) and the least in pericarp (12.86 to 97.72 mg AAE/gDW). Similar results were obtained in CUPRAC assay, where the values of the extracts were found to be the highest in bark extract (7.89 to 246.3 mg AAE /gDW) and the least activity being observed in pericarp (3.41 to 99.74 mg AAE /gDW).

DPPH method is widely used in the assessment of free radical scavenging activities due to its remarkable stability as free radical. In the presence of an antioxidant agent, the DPPH radical diminishes by accepting a hydrogen atom or an electron, and the degree of color shift from purple to pale color is utilized to quantify the antioxidant capacity of the sample. Phenolics and flavonoids are prominent naturally occurring antioxidants capable of inhibiting free radical-driven chemical processes (Mahdi-Pour *et al*., 2012; Sartini *et al*., 2019). The potency of phenolics and flavonoids to their antioxidant function is attributed to the benzene ring in their structure and the number and positioning of hydroxyl (OH) group (Rice-Evans *et al.,* 1996). In this study, the extracts of different plant parts exhibited dose-dependent radical scavenging activities. Our findings revealed that the methanol extract of bark showed best radical scavenging activities comparable to the positive control at higher concentrations (Figures 10a-c). The leaves and pericarp ethanolic extracts also exhibited good DPPH antioxidant activity. The IC_{50} value of a sample is the concentration at which 50% of DPPH radicals initially present in the solution were inhibited. The IC_{50} of bark in ethanol and methanol extract was 73.48 µg and 48.41 µg respectively, whereas IC_{50} of ethanol and methanol extract of leaves was 65.77 µg and 53.28 µg, respectively and IC_{50} of pericarp extract in ethanol and methanol extract was 72.15 µg and 53.74 µg, respectively. The lowest activity was observed with hexane extracts. The results are consistent with the high antioxidant activity detected for methanolic extracts of bark by phosphomolybdenum assay. Variation in compounds extracted in different solvents might have contributed to differences in antioxidant capacities of the extracts as suggested by Ngo *et al*. (2017). Previously, Srivani and Krishna Mohan (2022) appraised the antioxidant activity of leaf extracts of *G. moluccana* by DPPH and FRAP assays and observed highest activity in methanolic extracts and the least in hexane extracts. The higher antioxidant activity was ascribed to the considerable amounts of phenols and flavonoids detected in methanolic extracts as compared to other solvents. Pryzbylski *et al*. (1998) reported that the antioxidant activity of buck-wheat extracts varied with polarity of the solvent wherein methanolic extract showing comparatively higher antioxidant ability. Thus, it can be inferred that higher concentration of phenolic and flavonoid along with the presence of specific polyphenols in the plant extracts might have contributed to high scavenging activities. The correlation among total phenols, flavonoids and different antioxidant activities was performed to further augment the results. A good correlation was established between the *in vitro* antioxidant activity and the total phenolic acid content and total flavonoids content indicating that plant extracts can efficiently neutralize free radicals. These results are in conformity with several studies where positive correlations between polyphenols, flavonoids and antioxidant activities have been documented.

Although iron participates in several essential processes such as cellular metabolism, growth and death, DNA synthesis and repair, however excess accumulation can lead to generation of hydroxyl radicals, which can have damaging effects on protein, nucleic acids and lipids (Cornelissen *et al*., 2019; De Paola *et al*., 2022). Metal chelating is based on the production of a stable purple ferrous ion-ferrozine complex by Fe^{2+} ions (Dinis *et al.*, 1994). The formation of this stable colored complex is hindered by potential metal chelators in the plant extracts leading to the discoloration of the complex, which demonstrates their ability to chelate ferrous ions. The chelating abilities of the plant extracts in this study were proportional to the concentration of the extract, where with increase in the concentration of extracts, there was an increase in metal chelating ability (Figure 12). The bark extract displayed the best chelating ability (78.58%) followed by pericarp (77.01%) and leaves (54.81%).

5.3. Methanolic extract of bark and pericarp exhibited higher anti-inflammatory activtity than leaves

Denaturation of protein is reported to occur during inflammatory reaction, which leads to various maladies like rheumatoid arthritis, cancer, asthma, neurodegenerative disorders, cardiovascular disorders and autoimmune diseases (Rawdin *et al*., 2013). Protein denaturation implicates the disruption of electrostatic, disulphide, hydrophobic or hydrogen bonds as a result of which they lose their secondary and tertiary structures and thus biological functions due to exposure to external factors and stresses (Angel *et al*., 2013). Plant extract’s capacity to suppress protein denaturation is an indicator of the presence of a potential anti-inflammatory agent in the extracts as several anti-inflammatory drugs are well known to inhibit the protein denaturation. In this study, bark exhibited maximum inhibition of protein denaturation (43.46%) at 100 μg/ml concentration followed by leaves (37.45%) and pericarp (31.09%) (Figure 11). Truong *et al*. (2019) reported that methanolic extract of *Severinia buxifolia* effectively inhibited protein denaturation (IC_{50} = 28.86 µg/ml). Osman *et al*. (2016) observed greatest anti-inflammatory activity in inflorescence extract of *Barringtonia racemosa* as compared to other plant parts although its phenolic content was lower than leaves extract. It was suggested that the anti-inflammatory activity was not solely due to the phenolic content but could be due to other factors such as fatty acid composition (Osman *et al*., 2016). Sen *et al*. (2015) revealed that methanol and butanol extracts of leaves of *Amaranthus caudatus* inhibited protein denaturation by 54.1% and 49.33%. It was opined that anti-inflammatory agents in the extracts might have contributed to alteration of the synthesis of antigens related to type-III hypersensitivity reaction which are expressed when protein is denatured by heat.

The link between oxidative stress and inflammation has been well established in literature. Various studies have shown that arachidonic acid metabolism enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX) are involved in several inflammatory diseases (Hussain *et al*., 2016). Inhibitions of these enzymes suppress the synthesis of prostaglandins and leukotrienes which are primary regulators of inflammation, and hence are considered in the therapeutic aspect for the treatment of several inflammatory diseases (Mehta *et al*., 2006). This study elucidated the potential of *G. moluccana* bark, leaves and pericarp extracts on three crucial therapeutic targets of inflammatory events *viz*., LOX-5, COX-1 and COX-2 by measuring their inhibition on these enzyme activities. The methanolic crude extract of bark demonstrated LOX enzyme inhibition activity of 41.6% whereas for pericarp extract it was 31.7%. The leaves extract showed the least LOX inhibition activities (10.67%) among the three extracts used. Lipoxygenase enzymes are sensitive to antioxidants that inhibit peroxidation because they hinder the production of peroxides by scavenging lipidoxy- or lipidperoxy-radicals. Consequently, this prevents the availability of lipid hydrogen peroxide substrate necessary for LOX reaction (Rackova *et al*., 2007). Another assumption proposed was that inhibition by antioxidants could be achieved through chelation of its non-heme coupled iron (Lin *et al*., 2001) or through the reduction of its ferric form (Gutierrez-Lugo *et al.*, 2004) suggesting a competitive kind of inhibition. It can be speculated that LOX inhibition could be due to the presence of polyphenolic compound(s) and the antioxidant properties of the methanolic extracts. There have been studies showing that polyphenols are responsible for lipoxygenase inhibition (Wangensteen *et al*., 2004; Frum and Viljoen, 2006; Kamatou *et al*., 2010). According to Schneider and Bucar (2005), several LOX inhibitors may operate as nonselective antioxidants by lowering the active site ferric ion, hence limiting the enzyme's catalytic activity. Weerasena *et al.* (2016) proposed another mechanism of LOX inhibition through scavenging radical intermediates formed during arachidonate-LOX catalytic function, which is verified by a significant positive relationship between anti-arachidonate-LOX activities and DPPH free radical scavenging activities.

LOX inhibition and consequent substrate accumulation may activate the cyclooxygenase (COX) pathway, exacerbating chronic inflammatory diseases (Yang *et al*., 2007). Hence, dual LOX/COX inhibition has been recognized as a promising method to produce innovative anti-inflammatory agent (Langhansova *et al*., 2017; Mukhopadhyay *et al*., 2023; Rudrapal *et al*., 2023). COX-1 and COX-2 were shown to have independent functions. COX-2 is primarily responsible for inflammatory reactions, whereas COX-1 is associated with hemostatic activities (Hsi *et al.*, 1999), and hence COX-2 inhibitors are beneficial for the treatment of inflammation as well as cancer (Raman *et al*., 2008). The methanolic extracts of *G. moluccana* showed better inhibition activity on COX-2 (inhibition %: 86.71 %-pericarp, 83.46%-bark, 82.21%-leaves) in comparison to COX-1 (29.21%-leaves-, 24.26%-bark, 13.1%-pericarp). This selectivity for COX-2 is usually considered desirable as it can reduce the side effects associated with non-selective COX inhibitors, which often inhibit both COX-1 and COX-2. The perceptible inhibitory activity of the extracts, particularly at 100 µg concentration, indicates its potential as a potent COX-2 inhibitor. Various studies have shown that natural products such as flavonoids, terpenoids, alkaloids, glycosides, phenolics, resins etc. effectively suppress lipoxygenase and cyclooxygenase enzymes (Mukhopadhyay *et al*., 2023). The varied chemicals are known for their ability to target multiple cellular or molecular points and manifest in many ways because of their diversity which can act individually as well as synergistically. Thus, it can be inferred that higher phenolic and flavonoid contents together with specific compounds and their synergistic interactions could have resulted in bark extract showing higher inhibition activity against LOX as compared to other plant parts.

5.4. GC-MS and LC-MS/MS revealed the distribution of different metabolites in bark, pericarp and leaves

The metabolite distribution in various parts of plants serves as indicators of the overall phytochemicals distribution among different organs of plants within the same plant species (Hans *et al*., 2015). Understanding the distribution of metabolites in different plant parts would aid in identifying the most beneficial plant parts for future targeted bioactive compound studies.

The non-targeted metabolomics based metabolite profiling was carried out for the first time in bark, leaves and pericarp of *G. moluccana* using two prominent analytical methods; gas chromatography-mass spectrometry (GC-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) for comprehensive analysis of primary and secondary metabolites. GC-MS is more inclined towards detecting smaller metabolites such as alcohols, hydroxyl acids, amino acids, sugars, fatty acids, sterols, catecholamines, etc., and commonly utilizes derivatization process to render these compounds volatile for gas chromatography assessment (Fiehn, 2016). LC-MS/MS is used for identification of varied spectra of biological molecules such as phenolics and flavonoids with high accuracy (Saravanakumar *et al*., 2021).

In this investigation, the GC-MS study elaborated that the major constituents in methanolic bark and pericarp extracts were carbohydrates, carboxylic acids and fatty acids, while the major constituents in leaves extract were carboxylic acids, carbohydrates and amino acids (Figures 17a-c). Carbohydrates constitute the predominant form of carbon that is assimilated through photosynthesis in the biosphere and exhibit a wide range of functional groups tailored to their various biological functions within living organisms. Beyond their nutritional significance, carbohydrates also act as structural materials, integral components of membranes, and play a crucial role in cellular recognition (Ruiz-Matute *et al*., 2011). Several carboxylic acids were detected namely, fumaric acid, malic acid, succinic acid and maleic acid involved in tricarboxylic acid (TCA) of Kreb cycle, an iconic energy pathway for oxidation of amino acids, fatty acids and carbohydrates. Numerous studies have elucidated the significant role of the tricarboxylic acid (TCA) cycle in the development of plants. The presence of TCA cycle intermediates in plants is recognized as vital for a wide range of functions within and beyond cellular metabolism in all plant species (Zhang and Fernie, 2018). However, the extent to which these various organic acids accumulate varies significantly among species, stages of development, and types of plant tissues, indicating stringent regulation over the enzymes responsible for interconverting these metabolic intermediates (Fernie and Martinoia, 2009). Furthermore, the sequestration of organic acids into vacuoles and their release into the rhizosphere also serves as crucial mechanisms for controlling the abundance of these intermediates (Meyer *et al*. 2010). Fatty acids such as linoleic acid, linolenic acids, oleic acids, elaidic acids, palmitic acid, stearic acid, tridecylic acids, and acetyl-L-carnitine were detected among the three plant parts. The bark and pericarp were found to have a number of fatty acids whereas leaves contained 3 important fatty acids namely, palmitic acid, stearic acid and linolenic acid. Oleic acid, linoleic acid, and linolenic acids are the major unsaturated fatty acids in most plants (Harwood, 1998). These compounds are multifunctional with critical roles in cellular structure, energy storage, and response to stress. They are also important traits of commercial oil yielding plants. Their ability to act as antioxidants and precursors for bioactive molecules adds to their importance in maintaining cell health and facilitating various biological processes (He *et al.*, 2020). Palmitic acid and stearic acid are saturated fatty acids commonly observed in plants with several applications in cosmetics, food, and pharmaceuticals industries.

In the realm of plant research, LC-MS/MS methods are anticipated to play a crucial role due to the intricate and diverse biochemistry of plants. This biochemistry encompasses numerous semi-polar compounds, including significant secondary metabolite categories, which are most effectively separated and detected using LC-MS/MS techniques. In this study, the secondary metabolites in bark, leaves and pericarp were tentatively identified and classified into different classes of secondary metabolites such as flavonoids, terpenoids, alkaloids, phenolic acids, glycosides, vitamins, steroids and sterols, anthroquinones, lignins and stilbenes. The predominant classes in all the three plant parts were flavonoids, terpenoids, alkaloids and phenolic acids (Figures 25a-c). *G. moluccana* is well known for its traditional uses in medicine particularly for treating inflammatory diseases such as psoriasis, rheumatism and dandruff condition. There have been studies that have reported the presence of psoralen and furocoumarin compounds used in the treatment of psoriasis in different plant parts of *G. moluccana* (Sivanesan *et al*., 2009; Richard, 2020). Our study also revealed the presence of psoralen in all the plant parts of *G. moluccana*, substantiating its use in the treatment of psoriasis by further implicating the role of psoralen.

The study showed the presence of apigenin, kaempferol, genistein, vitexin and apiforol in plant parts of *G. moluccana* and these compounds have been demonstrated to have various medicinal activities. Apigenin is a potent inhibitor of NF-κB activation in autoimmune cells, and is known to lower the levels of cytokines such as IL-6 and IL-12 (Mirzoeva *et al*., 2018). Kaempferol attenuated the inflammatory psoriasis in mice by suppressing T helper 17 cells (Th17) and inhibiting the phosphorylation of NF-κB, a common pro-inflammatory signaling pathway in psoriasis (Liu *et al*., 2019). Wang *et al*. (2019) showed that genistein reduced the expression of cytokines such as IL-1β, IL-6, TNF-α, CCL2, IL-17, and IL-23 in mice. Vitexin showed several pharmacological effects including antioxidant (Li *et al*., 2012; Ugusman *et al*., 2012), anti-inflammatory (Borghi *et al*., 2013; Li et al., 2020), anticancer (He *et al*., 2016;

Ninfali *et al*., 2017), anticonvulsant and anxiolytic-like effects (De Oliveira *et al*., 2020) and neuroprotective effects (Yang *et al*., 2014; Min *et al*., 2015). Apiforol isolated from *Musa balbisiana* has been reported to have anti-diabetic effect by Gopalan *et al*. (2018).

Gallic acid, delphinine, and costunolide were detected in all plant parts of *G. molucanna.* Gallic acid has been reported to inhibit pro-inflammatory transcription factors which contribute to the development of psoriasis such as STAT3, RORγt, and NF-κB, or cytokines as IL-1β and TNF (Hsiang *et al*., 2013; Fan *et al*., 2019; Yang *et al*., 2021). It also decreased the frequency of IL-17 and IFN-γ producing cells, a crucial cytokine and interferon, in the pathogenesis of psoriasis (Tsiogkas *et al*., 2023). Delphinidin is a plant pigment which significantly reduced the expression of Ki-67 and PCNA which are proliferation markers in tumour cells. Furthermore, delphinidin significantly suppressed the expression of inflammatory markers, such as iNOS (inducible nitric oxide synthase), S100A7 (psoriasin), and S100A15 (koebnerisin), commonly found in psoriatic skin lesions (Chamcheu *et al.,* 2013; Chamcheu *et al*., 2015). Koo *et al*. (2001) identified costunolide, a sesquiterpene that refuted the activation of transcription factor nuclear factor-kappaB (NF-κB). Scarponi *et al*. (2014) reported that costunolide reduced the phosphorylation of STAT1 and STAT3 in IL-22 or IFN-γ-induced human keratinocytes.

Flavonoids are well recognized for their broad range of biological effects. The most abundant flavonoids in bark were laricitrin, quercetin 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside, gallocatechin, cyanidin 3-o-(3'',6''-o-dimalonyl-beta glucopyranoside), malonylgenistin, biochanin A-7-O-glucoside, medicarpin-3-o-glucoside-6''-malonate, luteone, patuletin, tricetin, pisatin, and (2s)-hesperetin (Figure 30b). Laricitrin demonstrated significant potential in alleviating the suppressive impact of lung cancer on the differentiation, maturation, and function of dendritic cells (DCs). In human lung cancer A549 and CL1-5 cells, laricitrin effectively counteracts the alterations in DC phenotype, characterized by elevated levels of IL-10 expression and a skewing of T cell priming towards an immune suppression type-2 response (Th2) (Chang *et al*., 2016); larictrin also has been reported to inhibit resistance protein (BCRP/ABCG2) of the breast cancer (Tan *et al*., 2013). Gallocatechin also has the potential for treatment of breast cancer as it has been reported to inhibit human epidermal growth factor receptor 2 (HER2), which promote cancer cell growth (Ebenyi *et al*., 2022). Gallocatechin has been described to have an efficient preventive effect on osteoclastgenesis rendering its potential for potential use as a chemoprophylaxis agent against osteoporosis (Ko *et al*., 2009).

The bark of *G. moluccana* is also used in bone setting in indigenous medicinal practices. Luteone is stated to have anti-fungal and antiviral effects (Harborne, 1976; Ahmed *et al*., 2020). Patuletin has been reported to have several bioactivities such as anti-inflammatory, cytotoxic, genotoxic, hepatoprotective, antiproliferative, and antioxidant activity (Patel *et al*., 2023); the potential of patuletin to inhibit fatty acid synthase (FASN), which plays an important role in breast cancer has been reported by Zhu *et al*. (2017). Its potential for use in the treatment of rheumatoid arthritis has been illustrated by Razzak *et al*. (2023), where patuletin effectively diminish proinflammatory cytokines, including interleukin (IL-1β and IL-6), tumor necrosis factor (TNF-α), and mitogen-activated protein kinase p38α with additional significant reduction in the genes and protein expression of matrix metalloproteinases (MMP-2 and MMP-9) while preserving the knee bone architecture.

The plant parts of *G. moluccana* have also been used in traditional medicinal practice for treatment against rheumatism. Tricetin has been reported to have potential treatment against inflammation, neurodegenerative diseases, atherosclerosis, and diabetes (Patel, 2022). Hesperetin has been described to have anti-diabetic, antioxidant and anti-inflammatory effects (Khan *et al*., 2020; Yang *et al*., 2022). The most abundant flavonoids found in leaves extract were pratensein, chrysoeriol, quercetin, vitexin, quercetin 3-sulfate and apiforol (Figure 30b). Pratensein is reported to possess strong antioxidant and anti-inflammatory activities (Chen *et al*., 2008; Liang *et al*., 2015). Pratensein also demonstrated a preventive effect on myocardial ischemia-reperfusion (I/R) injury-induced ferroptosis in cardiomyocytes, which showed its potential for therapeutic treatment against myocardial ischemia (Wang *et al*., 2022). Chrysoeriol's is reported to have several bioactivities in different studies (Wei *et al*., 2019; Min *et al*., 2020; Zingue *et al*., 2020), predominantly against tumor cell lines (Aboulaghras *et al*., 2022).

The most abundant flavonoids in pericarp extract of *G. moluccana* were compounds such as chrysin, gossypetin, 6-hydroxyflavone, genistein, pinobanksin, luteolin, and dalbergioidin (Figure 30b) which are well known for their potent bioactivities. Chrysin is a potent anti-cancer (Li *et al*., 2011; Mehdi *et al*., 2019), antidiabetic (Samarghandian *et al*., 2016), and anti-asthmatic (Wadibhasme *et al*., 2011) compound found in different plant species. Gossypetin act as a modulator of inflammatory cytokine production and acts as a suppressor of osteosarcoma cell growth (Proença *et al*., 2023). It is also reported to ameliorate oxidative stress and DNA damage by virtue of its ability to scavenge free radicals (Khan *et al*., 2013), further its anticancer role has been demonstrated by inhibition of PBK/TOPK protein kinase that modulates the signaling cascades of p38 mitogen-activated protein kinase (p38 MAPK) in cutaneous basal cell carcinoma (BCC) (Wang *et al*., 2019). 6-hydroxyflavone exhibited anti-inflammatory activities against kidney mesangial cells demonstrating its potential in treatment and prevention against nephritis (Wang *et al*., 2014). Genistein exhibited a myriad of biological activities like anti-inflammation (Frączek *et al*., 2015; Ganai *et al*., 2015), anticancer (Bolca *et al*., 2010; Löhr *et al*., 2016; Paul et al., 2017; Naeem et al., 2023, antidiabetic (Catmull *et al*., 2016; Dkhar *et al*., 2017; Schacht *et al*., 2017) and anti-osteoporotic activities. Genistein demonstrates a unique mechanism in its anti-osteoporotic effects by enhancing bone cell metabolism, leading to a shift in bone turnover that favors bone formation. In particular, genistein boosts osteoblast activity while reducing osteoclast function, mainly through modulation of the osteoprotegerin-sRANKL system (Bitto *et al*., 2010). Miao *et al*. (2012) highlighted the potential of genistein in the prevention and treatment of post-menopausal osteoporosis with undesirable side effects.

Pinobanksin is one of the bioactive compounds found in honey renowned for its antioxidant and anticancer activities (Zheng *et al*., 2018; Chen *et al*., 2023). Antiangiogenic agent potential of pinobanksin was reported by Bang and Ahn (2021), which demonstrated an antiangiogenic effect by inducing apoptosis in endothelial cells. This compound was also reported to act as apoptosis inducer by Alday *et al*. (2015). Luteolin is a natural flavone found in many plants and has been used for treatment of many inflammatory related ailments. Aziz *et al*. (2018) has reported the antioxidant activities of luteolin. The antidiabetic potential of luteolin was elucidated by Choi *et al*. (2014), where they showed the impact of C-glycosylation at various sites of luteolin on the potency and modification of luteolin's antioxidant, anti-diabetic, and anti-inflammatory properties. Dalbergioidin is an anthocyanin found in many plants, which has been reported to ameliorate renal fibrosis induced by doxorubicin which has a broad-spectrum antitumor activity, by suppression of TGF-β signal pathway (Ren *et al*., 2016).

Terpenes and terpenoids among the most extensive and varied class of secondary metabolites and are biologically active with numerous pharmacological applications. Several terpenoids were identified in bark, leaves and pericarp of *G. moluccana*. The most abundant terpenoids in bark includes betulin, arabidiol, soyasapogenol a, lanosterol, lubimin, botulin, and erythrodiol (Figure 31b). Betulin isolated from *Acacia nilotica* bark exhibited a potent antioxidant, anti-inflammatory and anticancer activity in an investigation carried out by Kaur *et al*. (2022). Several studies have shown the potential of betulin against cancer (Li *et al*., 2016; Zhou *et al*., 2018; So *et al*., 2018), while, its anti-inflammatory potential has been demonstrated in a studies of Ci *et al*. (2017) and Wu *et al*. (2014). Soyasapogenol-A is a potent anti-cancer agent which has shown to be effective against hepatocarcoma, HepG2 cells (Zhang and Popovich, 2008; Omar *et al*., 2020). Lanosterol is an important intermediate of cholesterol and Zhao *et al*. (2015) illustrated that lanosterol is capable of reversing protein aggregation which is vital for the treatment of cataracts. Lubimin and aucuparin are phytoalexins found in plants, which have anti-microbial activities. Lee *et al*. (2021) reported that aucuprin exhibited anti-inflammatory activity thus showing its potential to be a therapeutic drug against idiopathic pulmonary fibrosis (IPF).

Erythrodiol is a triterpenoid recognized for its role in anti-inflammatory and anticancer activities. Erythrodiol exhibited antioxidant, anti-proliferative and pro-apoptotic activities against MCF-7 human breast cancer cells (Allouche *et al*., 2011), skin chronic inflammation (Máñez *et al*., 1997), HT-29 human adenocarcinoma cells (Juan *et al*., 2008) and hepatocarcinoma (HepG2) cells (Peñas-Fuente *et al*., 2022). In leaves of *G. moluccana*, the most abundant terpenoids included alpha bisabolol, borneol, alpha eudesmol, beta zeacarotene, zeinoxanthin, 10-deacetylbaccatin III, albienol, delta cadinene, loganin, and squalene (Figure 31b). Bisabolol is a neuroprotectant, antioxidant, anti-inflammatory and anti-apoptotic agent as reported by Javed *et al*. (2020) and Ramazani *et al*. (2022). Borneol display an array of biopharmacological activities like antioxidant, antimicrobial, anti-inflammatory, anticancer and anti-diabetic activities (Rajput *et al*., 2023). Alpha eudesmol is a component of many essential oils in plants, and this has been reported to inhibit calcium channels suggesting a potential treatment for migraine (Asakura *et al*., 2000; Russo and Marcu, 2017). Beta zeacarotene and zeinoxanthin are carotenoids which are used as coloring agents. 10-deacetylbaccatin III is a diterpene which is an intermediate in taxol biosynthesis, has been shown to be an anti-cancer compound (Han *et al*., 2014). Delta-cadinene is a commonly occurring plant sesquiterpenoid which has an antimicrobial activity towards *Streptococcus pneumoniae* (Pérez-López *et al*., 2011). Loganin protects against MPP+-induced apoptotic cell death, neurite damage, and oxidative stress by enhancing neurotrophic signaling, activating IGF-1R/GLP-1R, and inhibiting the RhoA/ROCK pathway, supporting the claim that loganin has neuroprotective properties (Tseng *et al*., 2018). Loganin exhibited anti-inflammatory and antioxidant effects by effectively inhibiting apoptosis and extracellular matrix (ECM) catabolism induced by IL-1β in rat chondrocytes (Yang *et al*., 2019) and reduced burns-induced during intestinal inflammation and oxidative stress by regulating the TLR4/NF-κB signaling pathway (Wen *et al*., 2020). Yang *et al*. (2019) also confirmed in their *in vivo* studies that loganin mitigated cartilage degeneration suggesting that loganin holds promise as a potentially beneficial agent for treating osteoarthritis. Squalene is an intermediate compound found during cholesterol biosynthesis and exhibits several bioactivities such as anti-oxidant (Senthilkumar *et al.*, 2006; Warleta *et al.*, 2010), anti-tumor (Rao *et al.*, 1998; Smith *et al.*, 2000), and in cosmetics (Blasco *et al.*, 2006; Rissmann *et al.*, 2008).

Dihydroyphaseic acid, zeaxanthin, gossypol, 16-methoxytaberson, and furruginol compounds were the most abundant terpenoids distributed in pericarp extracts (Figure 31b). Dihydroyphaseic acid is an apocarotenoid, while zeaxanthin is a dihydroxy derivative of β-carotenoid with numerous health benefits such as neuroprotective effect through antioxidant and anti-inflammatory and anti-apoptotic mechanisms, and anti-cancer activity. Several studies have reported the potential of zeaxanthin in alleviating ocular complications and mitigated alterations in the expression of genes associated with inflammation which could potentially lower the menace of age-related macular degeneration (AMD) (Nakajima *et al.*, 2009; Bian *et al.*, 2012; Manikandan *et al.*, 2016). Zeaxanthan induced apoptosis in human choroidal melanoma by suppressing Bcl-X and Bcl-2, which are antiapoptic proteins and enhanced the expression proapoptotic proteins such as Bax and Bak along with cytochrome C (cyto C) release, which prompt the activation of caspases (Bi *et al.*, 2013). Similar observations were made in gastric cancer cells of humans by Sheng *et al.* (2020). Gossypol has a potential as a contraceptive, as it hinder sperm production and motility. Its contraceptive effects stems from the inhibition of energy metabolism enzymes within spermatogenic cells, as highlighted in various studies by Coutinho (2002), Wang *et al.* (2009), Keshmiri-Neghab and Goliaei (2014). The antitumor potential of gossypol against several cancer cell such as breast cancer (Liu *et al.*, 2002), human prostrate cancer (Jiang *et al.*, 2004; Xu *et al.*, 2005; Meng *et al.*, 2008), leukemia (Balakrishnan *et al.*, 2008; Moon *et al.*, 2008), head and neck squamous cell carcinoma (Oliver *et al.*, 2004) has been reported. Dodou *et al.* (2005) proposed the potential of gossypol for the treatment of psoriasis based on its anti-proliferative and antioxidant activities. Furruginol is a natural diterpenoid which has been reported to have inhibitory effect on proliferation of human thyroid cancer cells by triggering mitochondrial-reliant apoptosis, ROS production, compromise mitochondrial membrane, and suppressing the signaling pathways of mitogen-activated protein kinase (MAPK) and PI3K/ATK (Luo *et al.*, 2019); they are reported to have apoptotic effect on human ovarian cancer cells (Xiong *et al.*, 2017). Ferruginol has also been suggested to have a neuroprotective function against neurodegeneration induced by α-synuclein oligomers thus positioning as a promising candidate for treating Parkinson's disease (PD) and other neurodegenerative conditions (Wang *et al*., 2022).

Alkaloids are a class of plant secondary metabolites which play important roles in plant's defense against pathogens and are known to have several bioactivities with wide therapeutic applications such as anaesthetics, cardioprotectants, and anti-inflammatory agents (Heinrich *et al.,* 2021). In *G. moluccana,* alkaloids were the third most abundant class of compounds which were putatively identified in bark, leaves and pericarp. The main alkaloids putatively identified in bark extract were (s)-canadine, 17-oxosparteine, camptothecin, (s)-isoandrocymbin, 10-methylxanthosine, harmine, vomilenine, 12-hydroxydihydrochelirubine, 13-hydroxylupinine, and protopine (Figure 32b). Canadine is the intermediate in the biosynthesis of berberine, which which has anti-diabetic and antihypertensive properties. The potential of canadine as a novel antioxidant agent has been reported by Correché *et al*. (2008). 17-oxosparteine has been confirmed to have neuroprotective effects as it activates nicotinic acetylcholine receptor (nAChR), which could sever as a possible approach for treatment against Alzheimer disease (Gavilan *et al*., 2019). Camptothecin is a proven anti-cancer compound (Kepler *et al*., 1969; Pullaiah and Raveendran, 2020). Harmine has been attributed to have several pharmacological effects as it interacts with several important molecular targets such as cyclin-dependent kinases (CDKs), monoamine oxidase A (MAO A), serotonin receptors (5-HT2A), and imidazoline receptors at I1 and I2 sites accrediting its cancer prevention, anxiolytic, and anti-inflammatory properties (Patel *et al*., 2012). Protopine has been reported to have several bioactivities such as anti-thrombotic (Saeed *et al*., 1997), hepatoprotectant (Rathi *et al*., 1998), anti-inflammatory (Kim *et al*., 2022), antispasmodic (Hiller *et al*., 1998), and anti-cancer activities (Nie *et al*., 2021; He and Gao, 2014).

The most abundant alkaloids in leaves were elwesine, (s)-magnoflorine, cephaeline, strictosidine, stylopine, akuammicine, 6-hydroxyprotopine, and allocrytopine (Figure 32b). Magnoflorine exhibits various pharmacological effects such as antidiabetic, anti-inflammatory, neuroprotective effects. Magnoflorine demonstrates a therapeutic impact on inflammation and fibrosis in rats experiencing diabetic nephropathy by regulating the stability of lysine-specific demethylase 3A (Chang *et al*., 2020); further, Patel and Mishra (2012) reported the preventive activity of magnoflorine on α-glucosidase enzymes. Magnoflorine has been revealed to have anticancer activities against lung cancer, breast cancer, glioma and rhabdomyosarcoma (Okon *et al*., 2020). Wang *et al*. (2023) reported the potential anti-arthritic and anti-inflammatory effects of magnoflorine whereby signaling pathways of NF-κB and MAPK were inhibited; magnoflorine effect on inflammatory osteolysis has been reported by Sun *et al*. (2020). Cephaeline is an isoquinoline alkaloid, which possess a potential therapeutic strategy to manage mucoepidermoid carcinomas (MEC) of the salivary glands (Silva *et al*., 2022). The pharmacological potential of cephaeline against ebola and zika virus has been outlined by Yang *et al*. (2018), through inhibition of polymerase activity and disruption of lysosomal function of zika virus and inhibition of entry for ebola virus. Strictosidine is an intermediate in monoterpene indole alkaloid biosynthetic pathway, which is regarded as a core scaffold from which several other monoterpene indole alkaloids are derived (Brown *et al*., 2015). Stylopine exhibited a potent anti-tumor effect on human MG-63 osteosarcoma cells by regulating vascular endothelial growth factor 2 (VEGFR-2) signaling pathway (Velayutham *et al*., 2023). Akuammicine has been reported to enhanced uptake of glucose in fully differentiated 3T3-L1 adipocytes, which would serve as an important strategy for treatment for diabetes (Shittu *et al*., 2010). Allocrytopine is an isoquinoline alkaloid with neuroprotective effects (Dolanbay *et al*., 2021; Yang *et al*., 2023) and antiarrhythmic effects (Lin *et al*., 2013; Li *et al*., 2019). In pericarp, the most abundant alkaloids were 1,2-dihydrovomilenine, 19E-geissoschizine, cinchonidine, O-methylandrocymbine, emetine, lupinate, strictosamide, and tryptamine (Figure 32b). 1,2-dihydrovomilenine, 19E-geissoschizine, and O-methylandrocymbine are intermediate products in the biosynthesis pathway of isoquinoline alkaloid, monoterpene indole alkaloid, and colchicine, respectively. Cinchonidine is a quinidine alkaloids, which is well-known to be an antimalarial agent along with other quinine. Strictosamide has been documented to have anti-inflammatory and analgesic effects by Jia *et al*. (2021) and Li *et al*. (2014). Yüce *et al*. (2019) reported that strictosamide is a potent free-radical scavenger and α-glucosidase inhibitor rendering its antidiabetic potential. Tryptamine in plants serves as the starting point for metabolic pathways that leads to synthesis of some notable pharmacologically important indole alkaloids such as vincristine and vinblastine (Negri *et al*., 2021). There are several evidences, which suggest that tryptamine psychedelics exert significant neuromodulatory effects on crucial brain regions associated with mental imagery, theory of mind, and emotional regulation, indicating its potential therapeutic uses (Castelhano *et al*., 2021).

Phenolic acids are derived from benzoic and cinnamic acids, and are among the most abundant phenolic compounds available in plants. They occur in many forms, including free, conjugated-soluble, and insoluble-bound forms (Chandrasekara, 2019); they exhibit high antioxidant activities and are beneficial to human health (Kamath *et al*., 2004). Several important phenolic acids were also putatively identified in all the plant parts examined. In bark extracts, the main phenolic acids found were trans-2-coumarate, gallate, coumarinate, phaselate, coumaryl acetate, 5-hydroxyferulate, sinapate and N-feruloyltyramine (Figure 33b). Gallate is a well-known natural phenolic compound which possess several health promoting effects like antioxidant, anti-inflammatory, antineoplastic, antimicrobial and several cardio and neuroprotectant effects (Badhani *et al*., 2015; Kahkeshani *et al*., 2019; Bhuia *et al*., 2023). Trans-2-coumarate and coumarinate are intermediates in the biosynthesis of coumarin and cinnamic acids. Coumaryl acetate serves as substrates for the production of bioactive phenylpropanoid compounds such as eugenol and chavicol (Anand *et al*., 2016). Phaselate or phaselic acid has been reported to possess antirheumatoid arthritis activities (Wang *et al*., 2012). 5-hydroxyferulate is a hydroxycinnamic acid and an intermediate product in phenylpropanoid pathway, which serves as a precursor in the biosynthesis of sinapic acid (Maury *et al*., 1999). Sinapic acid is a hydroxycinnamic acid possessing several pharmacological effects like antioxidant (Kikuzaki *et al*., 2002; Galano *et al*., 2011), anti-inflammatory (Yun *et al*., 2008; Zhang *et al*., 2016; Lee., 2018; Lee *et al*., 2021), anticancer (Eroğlu *et al*., 2018; Huang *et al*., 2022; Pandi and Kalappan, 2022; Taştemur *et al*., 2023), antiglycemic (Kanchana et al., 2011; Cherng *et al*., 2013; Altındağ *et al*., 2021), neuroprotective (Lee *et al*., 2012; Zare *et al*., 2015; Verma *et al*., 2020), and anti-anxiety activities (Yoon *et al*., 2007). N-feruloyltyramine is a potent inhibitor of COX enzyme (Park, 2009; Jiang *et al*., 2015), while N-feruloyltyramine has anti-oxidative and anti-apoptotic properties against H_2O_2 induced cytotoxicity (Soi-ampornkul *et al*., 2022). The neuroprotective and neurogenesis effect of N-feruloyltyramine has been reported by Khan *et al*. (2021).

The most abundant phenolic acids in leaves were 4-methylumbelliferone, trans-5-o-caffeoyl-D-quinate, trans-caffeate, bergaptol, and xanthotoxin. 4-methylumbelliferone has been effectively used against tumor and cancer cells including inflammation and autoimmunity (Nagy *et al*., 2015). 4-methylumbelliferone treatment effectively inhibited hyaluronan, a conspicuous component of the extracellular matrix at several places during chronic inflammation. Bergaptol also known as 5-hydroxypsoralen is a furocoumarin compound with several anti-inflammatory, antimicrobial, anticancer, antiosteoporosis, and used for treatment for vitiligo and hypoglycemic and hypolypemic conditions (Quetglas-Llabrés *et al*., 2022). Xanthotoxin also known as 8-methoxypsoralen is another furocoumarin demonstrated considerable efficacy in addressing diverse health conditions, encompassing neuroprotection, skin repair, osteoprotection, organ protection, anticancer properties, anti-inflammatory effects, antioxidative stress mechanisms, and antibacterial activities as reported by Wu *et al*. (2022). Trans-caffeate is recognized for its antioxidant and anti-inflammatory activities, anti-tumor and immunomodulatory activities as reported by Zhang *et al*. (2014). In pericarp extracts, the most abundant phenolic acids were gentisate, 4-coumarate, cis-caffeate, protocatechuate, 3-hydroxycinnamate, and 4-coumaroylagmatine. Gentisate is a dihydroxybenzoic acid with broad spectrum pharmacological properties such as antioxidant, anti-inflammatory, anti-carcinogenesis, antimutagenic, antimicrobial, hepatoprotective, and neuroprotective as reported by Gutiérrez-Grijalva (2016) and Abedi *et al*. (2019). 4-coumarate is hydroxyl derivative of cinnamic acid and has been reported to have numerous biological activities (Boz, 2015; Aldaba-Muruato *et al*., 2021; Kaur and Kaur. 2022). Lee *et al*. (2018) reported the potential of 4-coumarate to contribute to bone growth, increased height and a viable alternative to growth hormone (GH) therapy as it enhances the chondrocyte proliferation and longitudinal bone growth by upregulating the activity of IGF-1. Neog *et al*. (2017) and Zhu *et al*. (2018) also reported the promising and therapeutic potential of 4-coumarate for the treatment of rheumatic arthritis, as it exerts its anti-inflammatory effects on rheumatic arthritis. Protocatechuate is a dihydroxybenzoic acid that has been shown in several studies to have antioxidant, antibacterial, anticancer, antiulcer, antidiabetic, antiaging, antifibrotic, antiviral, anti-inflammatory, analgesic, antiatherosclerotic, cardiac, hepatoprotective, neurological and nephroprotective uses (Kakkar and Bias, 2014; Khan *et al*., 2015; Krzysztoforska *et al*., 2017). 3-hydroxycinnamate had been reported to induce apoptosis through pathways involving p53 and caspase-3 indicating the anticancer role of 3-hydroxycinnamate (Chuang *et al*., 2005).

5.5. Quercetin, rutin and myricetin were quantified in pericarp, bark and leaves by RP-HPLC

RP-HPLC is a valuable method for evaluating crude plant extracts. It has also been successfully used in the past to study flavonoid in plants for instance; it was utilized to quantitatively assess flavonoid variation for differentiating species by Harborne *et al*. (1985) and quantitative study of flavonoid aglycones by Hertog *et al*. (1992). Flavonoids are attributed to have several pharmacological activities such as anticarcinogenic, antioxidant, antimicrobial, anti-inflammatory and anti-allergic effects (Garcia-Closas *et al*., 1999; Hollman *et al*., 1996; Vaquero *et al*., 2007; Pan *et al*., 2010). In this study, three important flavonoids namely quercetin, rutin, and myricetin belonging to flavonol class which modulates inflammation and other important bioactivities were quantified from the bark, leaves and pericarp extracts of *G. moluccana*. Quercetin is a well-known potent antioxidant flavonoid (Xu *et al*., 2019), and its ability to inhibit inflammatory enzymes such as COX-2 (Xiao *et al*., 2011) and LOX (Takahama, 1985; Sadik *et al*, 2003; Borbulevych *et al*. 2004) has been demonstrated. Quercetin administered to imiquimod (IMQ)-induced psoriasis mice model, the levels of TNF-α, IL-6, and IL-17 were significantly decreased (Chen *et al*., 2017). The content of quercetin was highest in leaves (9.43 μg/g DW), followed by bark (5.71 μg/g DW) and pericarp (1.44 μg/g DW). Quercetin also acts as anti-cancer agent by regulating cell cycle, interaction with type II estrogen binding sites, and inhibition of tyrosine kinase (Lamson and Brignall, 2000). The content of rutin in leaves was 45.19 μg/g DW. Rutin is another highly potent anti-inflammatory agent with several other bioactivities such as anti-tumor (Satari *et al*., 2021), neuroprotective (Javed *et al*., 2012), cardio protective (Trumbeckaite *et al*., 2006), hepatoprotectant (Janbaz *et al*., 2002) and so on. The content of rutin was highest in pericarp (55.39 μg/g DW), while it was least in bark (2.13 μg/g DW). Myricetin exhibits wide range of bioactivities such as anti-inflammatory (Winekenstädde *et al*., 2015; Hou *et al*., 2018), anti-cancer (Kim *et al*., 2014; Jiang *et al*., 2019), antidiabetic (Meng *et al*., 2016; Li *et al*., 2017) activities. Myricetin content was relatively high in leaves (13.22 μg/g DW) and least in bark (0.24 μg/g DW). Its content in pericarp was 8.40 μg/g DW. The inhibitory activity of quercetin and myricetin on LOX-5 and COX-1 enzymes has been reported by different research groups (Lee and Kim, 2010; Kutil *et al*., 2014; Winekenstädde *et al*., 2015). The presence of highly potent antioxidant agents such as quercetin, rutin, and myricetin could have contributed to high antioxidant and anti-inflammation activities of the *G. moluccana* plant parts extracts. This also justifies the traditional use of the various plant parts of *G. moluccana* for treatment of various inflammatory related ailments.

5.6. Leaf and endosperm exhibited high callus induction and proliferation

The increasing demand of plant derived secondary metabolites has driven the research towards using plant cell, tissue and organ cultures as a major platform for secondary metabolite production. Callus cultures or suspension cultures offer immense potential for sustainable and large scale production of secondary metabolites. It is essential to establish efficient methods for callus formation for utilization in studies on secondary metabolites in *G. moluccana* where such reports are lacking. The explant type and growth regulators are known to have a determining effect on callus induction in any plant species. In the present study, the effects of different growth regulators was evaluated on callus formation from seedling-derived explants *viz*., leaves, cotyledons that are diploid, and endosperm that is triploid in nature. The combination of 2 mg/l 2,4-D and 1 mg/l BAP was found to be superior as it resulted in high frequency (91-100%) of callus induction in all explants tested as compared to medium with 2 mg/l 2,4-D alone or other growth regulators used in full-strength MS medium (Table 7). Further, the explants differed in their ability for callus induction with relatively higher frequency of callus induction observed from endosperm than leaves and cotyledons except for the medium with 2 mg/l 2,4-D and 1 mg/l BAP where both endosperm and leaves explants formed callus with 100% frequency. Variation in the callus induction response noticed from different explants could be due to differential uptake of growth regulators applied or due to differences in the endogenous hormones in the source tissue as suggested by Preece and Imel (1991). The morphological appearance and growth of the callus induced from different explants in this study differed depending on the growth regulators used. Leaves derived callus cultures obtained on medium with 2,4-D and BAP were friable and nodular, and exhibited relatively higher growth as evidenced by increased biomass after subculture to fresh medium. Similar to our findings Bong *et al*. (2021) reported that the combination of 2,4-D and BAP was effective for callus induction and proliferation from leaf explants of Sabah Snake grass *(Clinacanthus nutans)*. Dalila *et al*. (2013) observed that 2,4-D was essential for inducing callus induction from leaf and endosperm and addition of KN promoted callus growth in endosperms of *Barringtonia racemosa*. According to the report of Ikeuchi *et al*. (2013) both auxin and cytokinin are interconnected in the hormone signaling pathways which activate cell cycle for callus initiation.

5.7. Efficient plant regeneration *via* direct shoot organogenesis was achieved from zygotic embryo axes of *G. moluccana*

Differentiated organ cultures like shoots or roots or hairy roots produced *in vitro* represent a metabolite profile similar to native plants and can overcome the limitations of low yield of secondary metabolites from cell cultures as a consequence of lack of differentiated structures (Verpoorte *et al*., 2002; Karuppusamy, 2009). Thus, *in vitro* propagation methods are needed as they could be used as a means for achieving improved production of phytocompounds (Isah *et al*., 2018). Zygotic embryo axes have been successfully used for obtaining multiple shoot formation in different plant species like castor (Sujatha and Reddy, 1998; Morre *et al*., 1998), wild species of groundnut (Gagliardi *et al*., 2000) and sunflower (Charriere *et al*., 1999). Previously, *in vitro* propagation was achieved from shoot tips of *G. moluccana* by culturing initially on 0.5 µM TDZ followed by transfer to secondary medium with 1.2 µM KN which induced shoot elongation (Samuel *et al*., 2009). Here, zygotic embryo axes were used to assess their potential to form multiple shoots in the presence of KN and/or BAP. The best results in terms of shoot proliferation and elongation was observed when 3 mg/l BAP was used initially in full-strength medium followed by transfer of shoot clumps to half-strength medium containing reduced concentrations of BAP (2.0 mg/l, 1.0 mg/l and 0.5 mg/l) and then without BAP which led to 7-8 shoots per explant. The shoot tips of primary cultures of multiple shoots obtained in this study can be subjected to repeated shoot multiplication using the procedure described earlier for this species (Samuel *et al*., 2009). In tissue culture studies using seedling derived explants of castor, multiple shoots could be proliferated when BAP was used singly at 0.5-2.0 mg/l (Athma and Reddy, 1982; Reddy *et al*., 1987) while there are reports where higher concentrations of BAP (4.0 mg/l) was used for multi-shoot formation (Khumsub, 1988; Ahn *et al*., 2007). As per the study of Morre *et al*. (1998), higher and lower concentrations of BAP yielded few shoots whereas 3 mg/l BAP was reported to be optimal resulting in induction of 3.4 shoots per embryonic axes of cotton. Based on the results, it was suggested that BAP was responsible for re-programming of the embryonic apical meristem axes of cotton towards the production of multiple buds and subsequent shoot development. In the current study, both NAA and IBA when used singly at 0.1 mg/l induced rooting at frequencies of 72.8% and 71.5%, whereas in castor (Ahn *et al*., 2007) IBA exhibited better effect on root induction than NAA. The efficient regeneration system established in this study can be exploited for generating sufficient plant material for secondary metabolites production without the need to rely on wild plants for such purposes.

Thus, the study demonstrated luxuriant growth of callus from both leaves and endosperm explants on medium augmented with 2,4-D and BAP. As endosperm cells are known to be triploid, it would be useful for future investigations on determining the impact of ploidy on primary and secondary metabolism as well as their response to elicitation. In addition, the *in vitro* produced multiple shoots offer prospect in producing secondary metabolites that are not guaranteed in callus or cell suspension cultures.

For further studies on elicitation, callus cultures were established from leaves explants as they are diploid with more number of explants being available and also as they exhibited higher proliferation than other explants.

5.8. Elicitation of callus cultures with SA and JA enhanced the phenolic and flavonoid contents

The cultured plant cells represent a potential source of biochemicals but relatively few cultures synthesize secondary metabolites comparable to those found in the whole plants. (DiCosmo and Misawa, 1985). Various approaches are used to overcome the limitations of the production of secondary metabolites from the cultured cells required for different applications. Elicitors like JA and SA have been commonly used to improve the productivity of secondary metabolites in plant species. They have been reported to have positive or negative effects on callus growth depending on the concentrations used in the cultures. It is important to determine the optimal concentration of elicitors to prevent hypersensitivity that leads to cell death (Naik and Al-Khayri, 2016). In this research, both SA and JA at the concentrations tested caused a decrease in callus biomass as compared to the control callus cultures (33.58 g FW/100 ml medium) although the biomass of SA-treated callus was lower (21.80–23.12 g FW/100 ml medium) than JA (25.27–28.47 g FW/100 ml medium) treatment (Figure 44). Cai *et al*. (2017) reported that higher concentrations of elicitors including MeJA and SA suppressed cell growth of the cell suspension cultures of *Changium smyrnioides*. A similar decrease in biomass in response to SA elicitation has been reported in the cell cultures of *S. miltiorrhiza* (Dong *et al*., 2010). Nonetheless, low concentrations of JA and SA have been shown to improve the growth of callus in normal or stressful conditions (Al-Qatrani *et al.*, 2021; Galal, 2012).

The concentration of the elicitor and the duration of its exposure are important factors to induce the optimum level of secondary metabolite production. Here, the elicitation of callus cultures with SA and JA proved to be effective in enhancing the total phenolics and flavonoid contents although it depended on the concentration and duration of treatments. The maximum accumulation of total phenolic acids (81.93 mg GAE g^{-1}DW) and flavonoids (60.88 mg RE g^{-1}DW) was observed in callus cultures when elicited with 150 µM JA for 20 days (Figures 46a-b). However, for salicylic acid elicited callus cultures, 50 µM for 5 days resulted in the highest phenolic content (31.43 mg GAE g^{-1} DW) (Figures 45a-b) while 50 µM for 20 days was optimal for flavonoid content (38.49 mg RE g^{-1} DW). Similarly, Mendoza *et al*. (2018) reported that specific concentrations of SA (300 µM) and MeJA (3 µM) increased the content of phenolics and flavonoids in cell suspension cultures of *Thevetia peruviana* suggesting an inducer effect of these elicitors in the phenylpropanoid metabolic pathway. Phenolic acids and flavonoids, which act as antioxidants, provide cellular protection by counteracting the damaging effects of excessive free radical production and oxidative stress. Increased levels of free radicals can lead to cell death through the oxidation of DNA, proteins, and lipids. Maintaining a balance between free radicals and antioxidants is essential for proper physiological functioning. The study of Stanly *et al*. (2011) demonstrated that extracts from callus or cell suspension cultures of various plant species possess the ability to scavenge free radicals. This scavenging potential can be further enhanced by treating these cultures with chemical elicitors like salicylic acid (SA) and methyl jasmonate (JA).

Interestingly, the study demonstrated that elicitation of callus cultures with 50 µM of JA for 5 days caused 1.3 and 1.45-fold increase in phenolic acid and flavonoid contents, respectively in comparison to the bark extracts which exhibited the highest phenolic acids and flavonoid content in different plant parts of *G. moluccana* studied.

5.9. Elicited callus cultures exhibited high radical scavenging activities than untreated control callus cultures

In our study, we observed that extracts from callus cultures treated with SA and JA exhibited improved free radical scavenging activities against DPPH and ABTS radicals compared to untreated callus cultures, as shown in Figures 47a-b and 48a-b. Notably, callus cultures elicited with JA demonstrated a higher capacity for free radical scavenging than those elicited with SA across various concentrations. Specifically, the extract from callus cultures treated with 150 µM JA showed the highest free radical scavenging activity, with a significant inhibition percentage against DPPH (89.85%) and ABTS (77.34%) radicals compared to other JA concentrations. This heightened free radical scavenging activity in JA-elicited cultures corresponded with higher total phenolic and flavonoid content in these cultures. Our findings align with the results reported by Manivannan *et al*. (2016), where methyl jasmonate (MeJA) stimulated the accumulation of bioactive secondary metabolites and enhanced the free radical scavenging potential of cell extracts from *Scriphularia kokudensis* Franch.

5.10. Elicitation enhanced the ROS and improved the antioxidant metabolism in callus cultures at specific concentration

The oxidative burst of H_2O_2 in many plant species is found to be necessary for the production of several secondary metabolites (Kuzniak and Urbanek, 2000). Antioxidants like phenolic acids and flavonoids confer protection to the cells by coping with the oxidative stress caused due to excess production of free radicals. The harmful effects of increased levels of free radicals resulting in cell death have been ascribed to the oxidation of DNA, proteins, and lipids.

A balance between free radicals and antioxidants is necessary for proper physiological function. The administration of exogenous chemical elicitors imitates the reaction of pathogen attacks or wound signals which activates a defense response in plants by inducing oxidative burst (Khanpour-Ardestani *et al.,* 2015; Manivannan *et al.,* 2016). Consequently, the endogenous reactive oxygen species are accumulated in the cells (Lamb and Dixon, 1999) that lead to a cascade of antioxidant metabolic changes in plants. In this study, the exogenous application of SA and JA to the callus cultures at all the concentrations stimulated higher production of H_2O_2 (1.46–3.74 µmol g^{-1} FW) compared to the control cultures (0.86 µmol g^{-1}FW). The maximum H_2O_2 levels were observed in callus cultures exposed to JA (3.74 µmol g^{-1} FW) than SA (3.30 µmol g^{-1} FW) at the highest concentration (200 µM) used in the study (Figure 49). Similarly, H_2O_2 facilitated several elicitor-induced accumulations of specific secondary metabolites such as indole alkaloids in *Catharanthus roseus* (Zhao *et al.,* 2001), saponin in Ginseng (Hu *et al.,* 2003), and tcibulin in *Alium cepa* (Kravchuk *et al.,* 2003).

Several studies have suggested that elicitor treatment to cell cultures stimulates the generation of ROS leading to increased accumulation of malondialdehyde (MDA), which is an end product of lipid peroxidation (Chong *et al.,* 2005; Ghanati and Dehaghi 2011). It is known from the literature that high levels of MDA are a reflection of an oxidative imbalance. In our study, exposure of callus cultures of *G. molucanna* to SA and JA at all the concentrations showed a significant increase in the accumulation of MDA (Figure 50). The remarkable increase of MDA level (1.68–2.27 µmol g^{-1} FW) observed in callus cultures treated with 150 and 200 µM JA might be due to jasmonic-induced enhanced expression of lipoxygenase (Kohlmann *et al.,* 1999), which catalyzes the oxygenation of polyunsaturated fatty acids (Chamulitrat and Mason 1989). In this study, the marked reduction of MDA content observed in 150 and 200 µM SA-treated callus cultures as compared to lower concentrations of 50 and 100 µM is pointing towards the inhibitor effect of SA on lipid peroxidation at higher concentrations possibly due to diminished lipoxygenase activity or altered cellular metabolism. Effect of salicylic acid inhibitory effect on lipoxygenase-dependent lipid peroxidation has been previously reported (Lapenna *et al.,* 2009). Although 150 and 200 µM JA-elicited callus cultures showed higher H_2O_2 levels, the MDA levels of 200 µM JA (2.27 µmol g^{-1} FW) were strikingly higher than that of 150 µM JA (1.68 µmol g^{-1} FW) which could due to impaired antioxidant capacity of the cells to detoxify the excess produced ROS.

Previous investigations have reported a correlation between phenolics, flavonoids, and antioxidant activities (Wong *et al.*, 2006; Zayova *et al.*, 2012). The present study revealed significant changes in the antioxidant enzyme activities of SOD, CAT, and POD in response to SA and JA elicitation of callus cultures. Varied trends were observed in this study indicating different activity levels with different concentrations of elicitors. Enzymatic antioxidant systems are usually activated during stress conditions. The first line of defense involves the SOD enzyme which catalyzes the conversion of superoxide radical (O_2^-) into H_2O_2 and O_2. The control of the steady-state O_2^- levels by SOD is known to be an important factor in protecting the cells against oxidative damage. In this study, enhanced SOD activities were observed at all the concentrations of the elicitors used indicating the protective action of elicitors to scavenge O_2^- radicals produced in the callus cultures (Figure 51). The H_2O_2 generated during the breakdown of O_2^- is further scavenged subsequently by other enzymes such as CAT, POD, and APX. CAT enzyme functions in peroxisomes and involves in the conversion of H_2O_2 to O_2 and H_2O in an energy-efficient manner (Das and Roychoudhury, 2014), while POD eliminates H_2O_2 both during stress and normal conditions (Asada, 1999). In this study, the CAT (18.62 U mg^{-1} protein) and POD (579.84 U mg^{-1}) activities markedly increased in callus cultures under the influence of JA at 100 and 200 μM indicating efficient H_2O_2 detoxification system (Figures 52 & 53). The enhanced activities of CAT and POD enzymes in response to SA and JA treatments have been reported in the cell suspension cultures of *Scrophularia kakudensis* (Manivannan *et al.*, 2016) and *Cucumis melo* (Nafie *et al.*, 2011). In contrast, Chong *et al.* (2005) reported a reduction in CAT and ascorbate peroxidase in cell cultures of *Morinda elliptica* treated with JA, indicative of the antioxidant roles played prominently by other antioxidants that were all enhanced in elicited cultures. It is observed that SA at 50 μM was effective in enhancing the CAT and POD activities while a higher concentration of 200 μM SA reduced the activity which shows the cells' inability to detoxify the H_2O_2 at higher concentrations resulting in ROS imbalance and impaired functions in the cells. Ali *et al.* (2006) observed a strong decrease in the activity of CAT while guaiacol peroxidase (G-POD) was induced in both MJ- and SA-treated roots of *Panax ginseng*. Thus, it was suggested that CAT functions were taken over by G-POD enzymes in the treated roots.

Another antioxidant mechanism for maintaining redox balance in plants is through the ascorbate–glutathione pathway consisting of four enzymes *viz.*, APX, GR, MDHAR, and DHAR. The H_2O_2 generated by ROS after elicitor treatment is converted to H_2O by APX enzyme through oxidation of AsA to monodehydroascorbate, which is converted to AsA with the help of enzyme

MDHAR. The monodehydroascorbate which escapes this cycle is further converted to dehydroascorbate (DHA) by releasing one more electron. The DHA is converted into AsA to maintain the ascorbate pool by the enzyme DHAR. GSH regenerated from its oxidized form (GSSG) *via* GR is utilized by DHAR (Murshed *et al.,* 2008). Several studies have reported an enhancement in the activities of APX, GR, MDHAR, and DHAR during stress conditions (Ali *et al.,* 2006; Jiang *et al.,* 2016; Gaafar and Seyam, 2018; Hasanuzzaman *et al.,* 2019). In this study, the activities of enzymes involved in the AsA-GSH cycle were significantly increased in JA 150 μM elicited callus cultures which corroborated with the highest contents of ascorbate and glutathione indicating better ROS regulation at this concentration (Figures 54a-d, 55a-d). Additionally, improved GSH (reduced form) and diminished GSSG (oxidized form) along with enhanced GR activity maintains the higher GSH/GSSG ratio suggesting the effectiveness of 150 μM JA in maintaining the AsA-GSH pool. These results are in agreement with the report of Li *et al.* (2017) where exogenous JA increased the activities of key enzymes in the AsA-GSH cycle, decreased the oxidized glutathione (GSSG), and increased the GSH/GSSG ratio which resulted in lower MDA and H_2O_2 contents in low root-zone temperature in roots of *Malus baccata* Borkh. Moreover, a gradual decrease in APX, MDHAR, DHAR, and GR activities with declined GSH and raised GSSG contents in callus cultures under the influence of SA at higher concentrations of 100 to 200 μM is pointing towards the concentration-dependent inhibition of the AsA-GSH cycle by SA in callus cultures in our study (Figures 54a-d). Durner and Klessig (1995) have previously demonstrated the ability of SA to inhibit APX activity supporting the hypothesis that SA-induced defense responses are mediated, in part, through H_2O_2 levels or coupled perturbations of the cellular redox state.

The PAL enzyme is a key enzyme of the phenylpropanoid pathway that is involved in the biosynthesis of several secondary metabolites. There are numerous reports indicating positive relationships between PAL activity and the accumulation of phenolics, but on the other hand, there are puzzling data concerning this correlation (Bauer *et al.,* 2011). In this study, the highest PAL activity (0.33 U mg^{-1} protein) was recorded in the callus cultures treated with 150 μM JA for 20 days, which could explain the higher accumulation of phenolics and flavonoids in these cultures (Figure 56). It can be presumed that jasmonic acid has a positive regulatory role in activating phenylpropanoid pathway although the mechanism by which specific concentration regulates higher biosynthesis is unknown. Interestingly, in our study, the PAL activity in 50 μM SA (0.21 U mg^{-1} protein) treated callus cultures did not differ significantly from control callus cultures (0.19 U mg^{-1} protein) although there was a significant increase in total phenolics and flavonoid contents in response to SA treatment. The reduction in PAL activity at higher concentrations (100–200 μM) of SA with a concomitant decrease in total phenolics and flavonoids is pointing towards the inhibitory effects of higher concentrations. In agreement with our results, Kovacik *et al.* (2009) found that PAL activity decreased at higher SA doses in *Martricaria chamomilla* plants which could be caused by a decrease in the phenylalanine pool.

The transcript levels of *SOD*, *CAT*, *POD*, and *APX* genes, as well as *PAL* which is involved in the phenylpropanoid pathway, were analyzed for ascertaining the correspondence between transcript levels and enzyme activities. The relative transcript levels of *SOD*, *CAT*, *POD*, and *APX* genes were significantly upregulated in callus cultures treated with 100 to 200 μM JA, whereas at lower concentration of 50 μM, the transcript levels of *CAT* and *POD* genes were either downregulated or equivalent to controls. On the contrary, the highest transcript levels of antioxidant genes were observed at 50 μM SA which showed a decrease with an increase in the concentration (100–200 μM). The enhanced transcript level of these genes is consistent with the activities of these enzymes, justifying similar regulation at both the transcriptional and protein levels in addition to indicating the role of these enzymes in the process of detoxification of ROS during elicitor treatment. The study of Mejia-Ieniente *et al.* (2013) revealed the inducing effect of SA on *CAT* and *PAL* gene expression which correlated with their enzyme activities in *Capsicum annuum* L. Surprisingly, we observed a lack of correlation between *PAL* transcript level and PAL activity in SA- and JA-treated callus cultures as *PAL* transcript levels were found to be higher in SA-treated callus cultures as compared to JA-treated callus cultures. Moreover, the PAL activity in SA 50 μM treated callus cultures was similar to control callus cultures despite a significant increase in total phenolic content recorded in response to SA treatment. This discrepancy between transcript levels and enzyme activity of PAL can be due to posttranscriptional modifications as stated by Ballester *et al.* (2006). Ejtahed *et al.* (2015) reported that despite the increase in the transcript rate of the *PAL* gene at the higher concentration of SA, lower amounts of rosmarinic acid were accumulated in shoots of *Salvia officinalis*. Thus, it was suggested that some other unknown factors participate in the synthesis of phenolics.

5.11. Elicitation showed an increase in number of secondary metabolite classes as compared to untreated controls

Elicitation represents a compelling strategy for enhancing the biosynthesis of secondary metabolites that are typically produced at low levels under normal conditions (Ramirez-Estrada *et al*., 2016). In this study, metabolomics strategies equipped with LC-MS/MS was employed for putative detection of secondary metabolites in leaves derived callus cultures (control) as well as SA treated and JA treated callus cultures of *G. moluccana,* resulting in detection of numerous secondary metabolites of different types such as flavonoids, terpenoids, alkaloids, phenolics, and steroids and sterols being the prominent and abundant ones. The classifications of different class of secondary metabolites in control and under SA and JA treatments are demonstrated in Figures 63a-c. SA, JA and its derivatives has been successfully used for production of various types' secondary metabolites in different plants systems (Naik and Al-Kharyi, 2016). The use of SA for the enhanced production of different class of secondary is well documented for example enhanced production of phenolic compounds in *Crocus sativus* (Tajik *et al*., 2019), *Salvia miltiorrhiza* (Guo *et al*., 2015; Li *et al*., 2016), *Ginkgo biloba* (Ni *et al*., 2018; Ye *et al*., 2020), *Vitis vinifera* (Xu *et al*., 2015), *Orostachys cartilaginous* (Wen *et al*., 2019) and several other plant species.

Enhanced production of terpenoids by SA has been reported in *Centella asiatica* (Buraphaka and Putalun, 2020), *Artemisia annua* (Aftab *et al*., 2010), *Aquilaria sinesis* (Lv *et al*., 2019), *Stevia rebaudiana* (Moharramnejad *et al*., 2019). Similarly, SA treated increased the production of alkaloids in *Catharanthus roseus* (Idrees *et al*., 2011), *Arthrospira platensis* (Hadizadeh *et al*., 2019), *Stemona curtisii* (Chotikadachanarong *et al*., 2011), *Ricinus communis* (Zavala-Gómez *et al*., 2021; Withania *somnifera* (Sivanandhan *et al*., 2013). SA enhanced the production of secondary metabolites by modulating the expression of genes related to enzymes involved in the biosynthesis of secondary metabolites (Ali, 2021). Enhanced production of phenolic compounds such as flavonoids, phenolic acids, anthocyanins and stilbenes under the treatment of JA has been reported in many plant systems such as *Vitis vinifera* (Curtin *et al*., 2003; Belhadj *et al*., 2006); *Salvia miltiorrhiza* (Xiao et al., (2009); *Lycopersicon esculentum* (Chen *et al*., 2006), *Ophiorrhiza mungos* (Deepthi and Satheeshkumar, 2007); *Glycyrrhiza glabra* (Shabani *et al*., 2009); *Calendula officinalis* (Wiktorowska *et al*., 2010); *Mentha piperita* (Krzyzanowska *et al*., 2012); *Artemisia absinthium* (Ali *et al*., 2015). Singh *et al*. (2020) reported an increase in the production of terpenoids and alkaloids in *Withania somnifera*. The mechanism that is employed by plants cells upon encountering with elicitors such as SA and JA involves a series of events. The signal from the elicitors is detected by the plant cells, which activates a series of intricate responses such as membrane depolarization, reversible phosphorylation of plasma membrane proteins, Cl and K+ efflux/H+ influx, and changes in cytosolic [Ca^{2+}] concentration, ion fluxes increased, NADPH oxidase activation, mitogen-activated protein kinase (MAPK) activation, and ROS and reactive nitrogen species (RNS) production, which results in activation of transcription and translation of defense-responsive genes/enzymes leading to production of secondary metabolites (Jeyasri *et al*., 2023).

5.12. *De novo* transcriptome analysis revealed differentially expressed genes in elicited callus cultures

De novo transcriptome analysis serves as a valuable tool not only for discovering new genes and developing molecular markers but also for establishing gene expression networks across different tissues and organs in animal and plants. It is employed in non-model species that lack comprehensive genome information (Li *et al*., 2016). The significant progress in RNA sequencing in the past decade has made our understanding of the genomics easier, cost-effective and high throughput resulting in generation of extensive transcriptome data from diverse organisms and tissue types, particularly in cases where there is no pre-existing reference genome available (Shah *et al*., 2020). This study provided the first insight on transcriptome and differentially expressed genes of *G. moluccana* leaves, leaves derived callus and callus treated with SA and JA in *G. moluccana.*

A comprehensive transcriptome analysis was carried out through Illumina Hiseq based paired end sequencing to generate four sets of *de novo* transcriptome data which were assembled, annotated and analyzed. The Illumina, a next-generation sequencing platform utilized for transcriptome assembly, produces relatively shorter sequencing reads. However, it offers significantly broader transcriptome coverage at a lower cost compared to other sequencing platforms (Deng *et al*., 2019). The Trinity assembly strategy was employed as a comprehensive approach for constructing the transcriptome as there was no available reference genome. After filtering and trimming the raw files, clean reads of 36,610,058, 37,263,180, 30,435,498, and 37,764,748 were generated for leaves, leaves derived callus (control), SA treated callus and JA treated callus, respectively. These high quality clean reads were *de novo* assembled into 77,522 unigenes and 171,971 isoforms with 37.83% GC content and N50 of 2882 bp indicating a high quality assembly. The N50 is a critical metric for evaluating assembly quality, with a longer N50 length indicating a higher quality assembly (Hou *et al.*, 2018). The N50 length of unigenes of 2882 bp in our study demonstrated that the sequence assembly was of high quality and well-suited for in-depth research. Among the 77,522 unigenes generated, 35,128 (45.31%) of the unigenes were effectively matched and annotated from public database such as nr, Pfam, Swiss-Prot, GO, COG, and KEGG using BlastX. The unannotated data could be as a result from the inadequacy of sequences available in public databases for closely related species in terms of phylogenetic relationships or poorly conserved regions (Hou *et al.*, 2011). Additionally, the presence of non-coding genes, untranslated regions (UTRs), random transcriptional noise, or incomplete spliced introns that do not match the sequences available in public databases, and further some genes might not have been expressed at the time of RNA extraction or were expressed at very low levels which contributes to challenges while annotation (Kang *et al.*, 2017; Shah *et al.*, 2020). Gene ontology (GO) emphasized the functional diversity within the transcriptome dataset, with the highest transcript abundance associated with molecular functions (39.85%) followed by biological processes (31.16%) and cellular processes (28.97 %). GO is a globally accepted and standardized system for classifying gene functions. Rastogi *et al*. (2014) highlighted that GO provides a contemporary and well-established framework for an in-depth and extensive overview of the features and activities of genes and their products across each organism. The annotation and sequence information derived from gene ontology results would serve as valuable gene resources for future prospective molecular-level investigations in *G. moluccana* and its related species.

Callus formation involves reprogramming of gene networks highlighting the importance of comprehending the molecular mechanisms behind this process. Such understanding is pivotal not only for achieving rapid plant propagation, but also for secondary metabolite production for incorporating specific genetic traits in both research and commercial setting. Over the past decade, numerous studies have shed light on the intricate regulatory mechanisms behind callus formation, which involves complex processes, with the reacquisition of cell proliferative ability being the main aspect (Ikeuchi *et al.*, 2013). It has been observed that terminally differentiated plant cells usually suppress the mitotic cell cycle, making the restoration of proliferative competence a key step in callus formation (Ikeuchi *et al.*, 2013). However, triggering callus formation typically requires more than the activation of cyclins (CYCs) or cyclin-dependent kinases (CDKs), cell cycle regulating factors. For instance, solely activating these factors does not usually lead to callus induction (Dewitte *et al.*, 2003), instead, most described callus induction mechanisms rely on transcriptional or posttranscriptional regulators that orchestrate widespread alterations in gene expression or protein translation (Ikeuchi *et al*., 2013). This study provided an insight on the transcriptome changes during callus formation from leaves of *G. moluccana*. A total of 6488 DEGs were identified between leaves and leaves derived callus. Several genes involved in cell division, plant hormone signaling pathways, plants stress response, plant-pathogen interactions were among the top upregulated including alpha-amylase inhibitor domain containing protein (*AAI*) proline rich proteins (*PRP*), late embryogenesis abundant protein (*LEA5*), WNT protein family, expansins (*EXP*), acetylserotonin-o-methyl transferase (*ASMT*), alcohol dehydrogenase (*ADH*), APETALA2/ETHYLENE RESPONSIVE FACTOR (*AP2/ERF*) domain containing transcription factors and ascorbate oxidase (*AO*).

AAI proteins are plant's natural defense system and are known to preferentially express in flower tissues and in response to phytohormones such as, methyl jasmonate, salicylic acid, indole 3-acetic acid, gibberellic acid and brassinolide. *PRP* gene plays a significant role in plant growth and development as well as in cell death processes in several plant species under normal conditions (Gujjar *et al*., 2019) and during abiotic and biotic stress conditions. Reports on *PRP* have shown that it can be either upregulated as well as downregulated during different stress conditions. Peng *et al*. (2015) and Gujjar *et al*. (2018) reported the downregulation of *PRP* in *Poncirus trifoliate* and *Solanum lycopersicum* during osmotic stress, while upregulation of *PRP* were also reported by Qin *et al*. (2013) in *Gossypium hirsutum* and in *Glycine soja* (Liu *et al*., 2015). The upregulation of *AAI* and *PRP* genes in leaves derived callus in comparison to leaves may be as a result of hormones like 2,4-D and BAP in the media or as a result of wound induced in the leaves in the process of callus induction. Gyögyey *et al*. (1997) underlined that the expression of *PRP* in dedifferentiated callus cultures of alfalfa was induced by 2,4-D and not expressed or weakly expressed in other differentiated tissues.

LEA proteins are known to play a significant role in different stress conditions such as cold stress in *Zea mays* (Liu *et al*., 2018), drought and salinity in *Oryza sativa* (Huang *et al*., 2018) and *Jatropha curcas* (Liang *et al*., 2013) and during seed desiccation (Leprince *et al*., 2017). The involvement of LEA protein during the process of callus induction was demonstrated by He *et al*. (2021) in *Arabidopsis*. The elevated levels of LEA protein in callus cultures in this study might be due to wounding of the tissue, which was similarly observed in protocorm-like body (PLB), a callus mass of *Dendrobium officinale* (He *et al*., 2021). *WNT* gene encodes highly conserved signaling molecules and plays a crucial role in cellular development and developmental processes (Das and Kumar, 2016). The role of WNT family protein during callusing and somatic embryogenesis has been reported in cotton (Aydin *et al*., 2006). Expansins are also known as cell wall-loosening protein (Choi *et al*., 2006). They have a broad range of roles in plant growth and development including vegetative growth (Cho and Cosgrove, 2000), fruit development (Kitagawa *et al*., 2005; Trivedi and Nath, 2004), cell elongation (Gookin *et al*., 2003; Harmer *et al*., 2002) and environmental stress (Rochange *et al*., 2001; Balestrini *et al*., 2005; Lee and Kende, 2001). Acetylserotonin-O-methyltransferase (ASMT) is pivotal in facilitating the last stage of melatonin production in plants, which plays important role in growth and development of plants and its response to adverse condition has been reported (Ma *et al*., 2022).

Alcohol dehydrogenases are derived from multi-gene family and are paramount to various aspects of plant growth and development including adaptation to environment, fruit maturation and generation of aromatic compounds (Jin *et al*., 2016). A substantial rise in the activity of ADH during the process of callus formation with 2-4-D in the media (van der Plas and van der Pal, 1983; Dolferus *et al*., 1985; Torquato and Machado, 1995) and slicing of potato tuber has been reported. In another study, incubation of the sliced tuber of potato in carbon such as sucrose triggered high ADH activities, and this increase was contingent on the synthesis of cytoplasmic proteins (van der Plas and van der Pal, 1983). The role of *AP2/ERF* transcription factor in the process of cell dedifferentiation and callus formation at the site of wounding has been well established in *Arabidopsis* (Iwase *et al*., 2011; Asahina *et al*., 2011; Ye *et al*., 2020) and tobacco (Marsch-Martinez *et al*, 2006; Srinivasan *et al*., 2007).

Ascorbate oxidase gene is involved in the regulation of cell division and growth of tissues (Alós, *et al*., 2013; Minutolo *et al*., 2020) and signaling between cells and its environment (Stevens *et al*., 2018). The expression of *AO* gene is triggered by wounding, hormones and other forms of stress (Stevens *et al*., 2018). Esaka *et al*. (1988) and Sekiya *et al*. (1989) also reported a marked increase in its expression and activity during callus formation in pumpkin. The majority of the top downregulated genes in callus cultures were found to be associated with photosynthesis such as ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), Rubisco activase, chlorophyll a-b binding protein, elements of light-harvesting complex-II type 1 (LHC-II type-1), magnesium chelatase and oxygen evolving enhancer protein, plastids, photosystem-I reaction center subunit PsaK and thioredoxin like protein. This could be inferred as a result of the callus cells switching to heterotroph mode of nutrition intake resulting in repression of genes associated with photosynthesis as substantiated by Bilgin *et al*. (2010) and Chen *et al*. (2020). Suppression of photosynthetic processes results in conservation of energy stimulating a defense response, paving way for accumulation of secondary metabolites (Rojas *et al*., 2014). Fructose bisphosphate aldolase (FBA), a bifunctional glycolytic enzyme which is usually highly expressed in leaves tissues (Lv *et al*., 2017) is also known to improve the photosynthesis process (Zeng *et al*., 2015). The downregulation of gene encoding the photosynthesis related proteins and FBA may also be attributed to dedifferentiation of the leaves tissue to callus formation. Other top downregulated were starch synthase, EGF like domain containing protein and carbonic anhydrase involved in several metabolism and pathways.

During callus formation, the gene expression of the explants undergo profound changes as reported in several plants species such *Arabidopsis* (Che *et al*., 2006; Okushima *et al*., 2007; Fan *et al*., 2012), *Populus trichocarpa* (Tuskan *et al*., 2018), *Glycine max* (Park *et al*., 2023), *Nicotiana tabacum* (Kosugi and Ohashi, 2003) and several other plants. The process of callus formation is governed by a complex regulatory mechanism involving several transcription factors. The TF that were identified in leaves derived callus and are known to contribute in the callus formation were *LATERAL ORGAN BOUNDARIES DOMAIN (LBD)* or *ASYMMETRIC LEAVES2-LIKE*, which is a key regulators that mediate auxin signals and directs callus formation (Fan *et al*., 2012). *AUXIN RESPONSE FACTOR (ARF)* which is intertwined with other TF such as *BRASSINOSTEROID-INSENSITIVE (BIN2)* and *LBD*, that regulate callus formation (Lee and Seo, 2017), *APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF)*, is involved in monitoring cell dedifferentiation in response to wounding (Iwase *et al*., 2011), *B3* transcription factor is involved in the callus formation and somatic embryogenesis in *Citrus sinensis* (Ge *et al*., 2012; Liu *et al*., 2020). *WRKY* and *bHLH*, act as transcriptional activator and repressor during auxin induced callus pluripotency (Zhang *et al*., 2022; Xu *et al*., 2024). *WUSCHEL-RELATED HOMEOBOX (WOX)*, promotes the callus formation in leaf petioles and hypocotyl (Ikeuchi *et al*., 2022), *SBP* transcription factors were reported in rice callus suggesting their role in cell dedifferentiation (Yang *et al*., 2008). *NIN-like* transcription factors participates in the regulation of callus formation enhancing the genes expression linked to biosynthesis of auxin and transport in the process of callus formation (Tong *et al*., 2023), *Nuclear factor-Ys-NF-YA* were reported to increase the weight of callus upon overexpression, *NF-YB* were highly expressed in embryonic callus while *NF-YC* were highly expressed during somatic embryogenesis in *Medicago truncatula* (Potsenkovskaia *et al*., 2022). *NAC* transcription factors-certain *NAC* TF are tissue specific and found only in callus tissue of rice (Fang *et al*., 2008). *MYB* and *MYB related* transcription have been reported to participate in promoting embryonic callus formation in maize (Ge *et al*., 2016) as well as suppression of callus formation in *Arabidopsis* (Dai *et al*., 2020). *MADS-box* genes *(MCM1-AGAMOUS-DEFICIENCY-SRF)* are known for their roles in floral organ identity determination, although there are reports which suggested their roles in embryonic callus formation in cotton (Yang *et al*., 2014), soyabean (Perry *et al*., 2016) and *Arabidopsis* (Xu *et al*., 2017). Heat Stress Transcription Factors *(HSFs)* has been reported to be instrumental in plant response to stress condition (Guo *et al*., 2016) as well as enhance the callus formation (Ogawa *et al*., 2007). *GROWTH REGULATING FACTOR* (*GRF*) transcription factor enhances the callus growth in canola (Kong *et al*., 2020). *GOLDEN2 (G2)* transcription factor have been reported to improve callus regeneration in rice and maize (Luo *et al*., 2023). Basic region/leucine zipper (*bZIP*) transcription factor have been reported to form a complex with *LBDs* to regulate auxin-induced callus formation in *Arabidopsis* (Xu *et al*., 2018). *BES1* (BRI1-EMS-suppressor 1) transcription factor which is associated with brassinosteroid signaling has been reported to be highly expressed during callus formation (Park *et al*., 2023).

The top upregulated genes in SA treated callus cultures are known for their roles in several metabolism and pathways. 2-hydroxyisoflavanone dehydratase vital for determining the productivity of isoflavones (Shimamura *et al*., 2007) while flavonoid 3'-monoxygenase (F3'M) is responsible for catalyzing the hydroxylation of naringenin and dihydrokampferol, which ultimately leads to the production of anthocyanins based on cyanidin in the flavonoid pathway (Jia *et al*., 2019) were among the highly upregulated genes. The upregulation of flavonoid pathways genes such as F3’M with SA treatment has been reported in *Camellia sinensis* var. *asssamica* (Jiang *et al*., 2022) and *Osmanthus fragrans* (Han *et al*., 2019). Ali *et al*. (2007) reported the increased activities of shikimate dehydrogenase when treated with SA in *Panax ginseng*. Shikimate dehydrogenase is an enzyme that assists the fourth step reactions in the shikimate pathway, which is an indispensable route for the biosynthesis of aromatic compounds in plants and microorganisms. Several signaling and transduction proteins such as cysteine protease (CP), serine carboxypeptidase-like 12 (SCPL), senescence-associated protein, salicylate carboxy methyltransferase (SAMT), and YELLOW LEAF were also among the top upregulated genes in SA treatment. Cysteine proteases (CP) are proteolytic enzymes which have been implicated in various physiological and development stages of the plants including germination, senescence, and plant immunity (Niño *et al*., 2014). CP is known to be induced and upregulated by salicylic acid (Kovács *et al*., 2016). SCPL gene family has been associated with biotic and abiotic stress (Xu *et al*., 2021), and they have been reported to exhibit significant overexpression with SA treatment in rice (Liu *et al*., 2008).

Senescence-associated proteins are associated with various plant processes such as the degradation of primary metabolites, nutrient recycling and transport, sugar metabolism, removal of oxidative by-products, and development of stress resilience. They are responsive to various environmental signals, such as abiotic and biotic stresses, and access to nutrients, implying substantial signaling crosstalk between the senescence process and stress responses (Lim *et al*., 2007; Seo *et al*., 2011). Salicylate carboxymethyltransferase is a regulator of salicylic pathway which facilitates the methylation of SA with S-adenosyl-L-methionine to form methyl salicylate (Wang *et al*., 2019). They participate in plant defense mechanism and are reported to be induced by SA in rice which confers disease resistance (Xu *et al*., 2006). Stress response proteins such as peroxidase 4, Cytochrome P450, *MYB* transcription factor along with transmembrane protein such as proton_antipo_M domain-containing protein, copper transporter, plasma membrane ATPase were some of the genes among the highly top regulated in SA treatment of *G. moluccanna.* Several reports have elucidated the role of salicylic acid in combating with different stress conditions through the involvement of stress response protein, transcription factors and transmembrane protein and antioxidant enzymes (Khanom *et al*., 2019; Brouwer *et al*., 2020; Tiwari *et al*., 2020; Khalvandi *et al*., 2021). SA has been reported to inhibit diacylglycerol O-acyltranferase (DGAT) in *Arabidopsis* (Chen *et al*., 2015). Aquaporins are transmembrane water channel protein that facilitates water movement across the cell membranes; the downregulatory role of SA on aquaporins has also been reported in *Arabidopsis* by Boursiac *et al*. (2008) and in rice by Liang *et al*. (2013) similar to our study. Peroxidase 12 is specifically expressed only in leaves and roots; the downregulation of peroxidase gene by SA has also been reported in previous studies (Durner and Klessig, 1995; Liu *et al*., 2018) as observed in this study.

The genes that were top upregulated in JA treated callus cultures mostly include signaling and plant oxidative stress response genes such as SCP domain containing protein (PR1 protein), transcription factor E2FB, glucose/ribitol dehydrogenase, alpha deoxygenase 1, proteinase inhibitor, non-specific lipid-transfer protein 1, kunitz trypsin inhibitor, patatin like protein, chitinase, executer 1, catalase 1 and AAI domain containing protein. SCP domain containing proteins (PR1 protein) are plant defense proteins which act as molecular markers in system acquired resistance (SAR) (Van Loon and Van Strien, 1999) and its expression is induced by SA and JA. The transcription factor, *E2FB*, is JA-responsive gene which is associated with cell cycle, stress and plant defense response (Vincent *et al*., 2022). Stress associated protein such as glucose/ribitol dehydrogenase, alpha deoxygenase, executer1, proteinase inhibitor, kunitz trypsin inhibitor, patatin like protein expression are induced by jasmonic acid (Farmer *et al*., 1992; Wagner *et al*., 2004; Koeduka *et al*., 2005; Gomès *et al*., 2007; Li *et al*., 2008; Lu *et al*., 2020; Zhu *et al*., 2021). Chitinase is another pathogenesis-related protein, which is induced by jasmonic acid (Rakwal *et al*., 2004). Catalase, an important antioxidant enzyme often associated with elevated activities and gene express under the influence of JA (Kumari *et al*., 2006; Woch *et al*., 2023). The downregulated genes in JA treated callus cultures comprised of aquaporin/tonoplast intrinsic protein, ELMO domain-containing protein A, palmitoyltransferase, GIGANTEA-like (GI), lactamase domain containing protein B, S-adenosyl methionine, peroxidase 21, LRR receptor-like serine/threonine-protein kinase, DRY EERY domain containing protein, transcription factor *bHLH144*, vegetative storage protein 2, DETOXIFICATION 49, protein binding protein, ring type domain protein, sucrose synthase, phosphoenolpyruvate carboxykinase, SNF1 protein kinase, ATP dependent helicase BRM, CLK4-associating serine/arginine rich protein-like, CDK protein, *bZIP* transcription factor TRAB1-like. GI is well known for its role in circadian rhythm, flowering time, environmental stress responses, plant growth and development. The down regulation of *GI* like genes have been reported to enhance plant growth and tolerance against stresses (Ke *et al*., 2016; Acharya *et al.*, 2022).

5.13. Transcription factors involved in secondary metabolites were identified in elicited callus cultures

Transcription factors act as a significant regulators of secondary metabolites, cell growth and development and plant response to various environment factors (Wang *et al*., 2016). In this study, 1062 transcripts encoding transcription factors belonging to 50 different families were identified between leaves derived calluses (control), SA treated callus and JA treated callus culture. The TF family with high number of transcripts belonged to *ERF*, *bHLH*, *NAC*, *WRKY*, *GRAS*, *C3H*, *C2H2*, *MYB*, *MYB* related and *B3*. Several members of the transcription factors such as *WRKY, MYB, NAC, AP2/ERF, bHLH, DOF* and so on are well known for their roles in regulation of secondary metabolites in plants. *ERF* transcription factor makes up one of the largest transcription families and they play essential roles in plant growth and development, hormone regulation and signaling and respond to various adverse conditions like drought, pathogens, salinity and cold; *ERF* also regulate the production of various metabolites such as proline, malondialdehyde, and flavonoids. Functioning as antioxidants, flavonoids possess the ability to neutralize reactive oxygen species (ROS) generated in plants under abiotic stress conditions (Wu *et al*., 2022). The *bHLH* transcription factors play a crucial role in various cellular activities associated with plant growth and development such as regulation of seed germination, flowering, fruit ripening, trichome formation, and root hair development. These *bHLH* transcription factors also contribute to plant reaction to abiotic stress like drought, salt, cold; additionally play an important roles in plant secondary metabolism and terpenoid biosynthesis (Liang *et al*., 2022; Yang *et al*., 2022). *MYB* transcription factors (TFs) are also among the most prominent groups of genes in plants and hold a significant regulatory role across multiple biological and physiological functions like plant growth and development, cellular structure, primary and secondary product metabolism, environmental stress response, and biosynthesis of secondary metabolites such as flavonoids, phenolic acids, and anthocyanins (Cao *et al*., 2020). *WRKY* transcription factors represent one of the most expansive families of transcriptional regulators, uniquely present in plants. These factors exhibit diverse biological functions encompassing plant disease resistance, responses to abiotic stress, nutrient deprivation, senescence, seed and trichome development, embryogenesis, and biosynthesis of flavonoids and terpenoids (Vom Endt *et al*., 2002; Bakshi and Oelmüller, 2014).

NAC transcription factors family have been demonstrated to regulate a diverse array of developmental processes which includes seeds and embryo development, shoot apical meristem formation, fiber development, leaf senescence, and cell division (Nuruzzaman *et al*., 2013). The emerging role of NAC in regulating plant secondary metabolites such as alkaloids, terpenoids in the recent times has also been highlighted in several plants (Kumar *et al*., 2021). *C3H* and *C2H2* are zinc finger protein *(ZFP)* which are known as the master regulators of many downstream genes involved in plant growth and development, morphogenesis, signal transduction, stress responses (Liu *et al*., 2020; Han *et al*., 2020). *B3* transcription factors are DNA binding proteins with diverse roles in plants including flowering (Ruan *et al*., 2021). Transcription factors respond to phytohormones by creating dynamic regulatory networks which finely adjust the timing, intensity, and tissue-specific expression of genes in metabolic pathways, influencing the subsequent accumulation of secondary metabolites. Transcriptional regulations in plants are orchestrated massively by plant hormones such a SA and JA, which directs the signaling networks of induction or repression (Caarls *et al*., 2015). Fifteen transcripts belonging to 6 transcription factor families namely *bHLH*, *WRKY*, *MYB* and *MYB* related, *HSF* and *HD-ZIP* were upregulated in SA treatment while 14 transcripts belonging to 9 transcription factor families namely *bHLH*, *bZIP*, *C3H*, *ERF*, *HD-ZIP*, *HSF*, *NAC*, *Nin-like* and *Whirly* were downregulated in SA treatment. In JA treatment, 28 transcripts belonging to 13 transcriptions factor families were upregulated including *B3*, *bHLH*, *C2H2*, *EIL*, *ERF*, *E2F/DP*, *G2-like*, *HD-ZIP*, *HSF*, *MYB*, *MYB related*, *NAC* and *WRKY*. While 53 transcripts belonging to 27 transcriptions factor families namely *GRF*, *AP2*, *ARF*, *ARR-B*, *B3*, *BES1*, *bHLH*, *bZIP*, *C2H2*, *C3H*, *CAMTA*, *Dof*, *EIL*, *ERF*, *FAR1*, *GRAS*, *HD-ZIP*, *MYB*, *NAC*, *NF-YC*, *Nin-like*, *SBP*, *SRS*, *TALE*, *TCP*, *WRKY*, and *ZF-HD* were downregulated in JA treatment.

5.14. Elicitation by SA and JA caused changes in expression of genes involved in synthesis of phenylpropanoids

Next-Generation Sequencing (NGS) techniques have enabled the identification of genes that are associated with pathways responsible for secondary metabolites synthesis. Exogenous application of SA and JA for improved biosynthesis of different secondary metabolites has been well reported in different plant species. In this study, the treatment of callus cultures with SA and JA resulted in increased accumulation of phenolic acids, flavonoids, and other class of secondary metabolites along with improved antioxidant machineries. Plants upon exposure to SA and JA treatments, exhibit a broad spectrum of intricate molecular responses such as alterations in gene expression, modifications in transcriptional regulation, and changes in signal transduction processes linked to the production of secondary metabolites (Soltani *et al*., 2022).

Here, ten differentially expressed putative genes corresponding to phenylpropanoid pathway were identified in both SA and JA treated callus cultures. Phenylpropanoids are class of plant-based compounds which are derived from deamination of aromatic amino acid phenylalanine. They partake in plant growth and development, and defense mechanisms, as these secondary metabolites responses to environmental stress, interacts with pathogens, and maintain plant cells structural integrity (Agar and Cankaya, 2020). Phenylalanine ammonia lyase (PAL) is the crucial enzyme which catalyzes the initial phenylpropanoid pathway that paves way to the regulation of several phenylpropanoid compounds. In this study, the transcripts of four key enzymes of phenylpropanoid pathway namely phenylalanine ammonia lyase, cinnamate 4-

hydroxylase (C4H), 4-coumarate 3-hydroxylase (C3'H) and Caffeoyl-CoA 3-O-methyltransferase (CCoAOMT) were upregulated with the treatments of SA and JA. Several studies has showcased that SA and JA treatments have led to enhanced expression of enzymes such as PAL (Gondor *et al*., 2016; Li *et al*., 2019), C3'H (Choi *et al*., 2012) C4H (Rostami *et al*., 2022) and CCoAOMT (Zheng *et al*., 2019). Previous studies have demonstrated that SA and JA trigger the expression of these genes which encodes the enzymes participating in lignin biosynthesis pathway (Salzman *et al*., 2005; Yaqoob *et al*., 2012). SA and JA share a similar signaling pathway with pathogen responses and wound healing such as strengthening cell wall (Maleck and Dietrich., 1999; Desender *et al*., 2007). The involvement of SA and JA in generating intracellular signaling molecules which facilitate the activation of gene expression in response to various stimuli such as wounding, elicitors, and pathogen infection has been highlighted by Sembdner and Parthier (1993) and Farmer (1994). CAD which catalyzes the final step in the biosynthesis of monolignols was found to be downregulated in JA treatment in this study. Contrastingly, Kim *et al*. (2010) reported that treatment with JA and SA induced the *CAD* gene in sweet potatoes. The exogenously applied MeJA or SA in *Camellia sinensis* changed the expression levels of CADs, reacting to different hormones differently, implying that these genes are at least partially transcriptionally regulated by hormone signaling (Deng *et al*., 2013). Liu *et al*. (2021) reported the accumulation of hydroxycinnamaldehyde, a monolignol precursor in CAD down-regulation plants. The genes that encode enzyme ferulate 5-hydrolase was downregulated in SA treatment, Kim *et al*. (2006) also highlighted the inhibitory effect of SA on ferulate 5-hydrolase gene (*F5H*) in *Camptotheca acuminate*, where F5H was induced by wounding. Some of the transcripts of the genes encoding enzymes such HCT, CSE, COMT, and 4CL were upregulated while some were downregulated which is as a result of isoforms of those genes. There are similar reports where SA and JA treatment have enhanced the regulation of the genes involved in phenylpropanoid pathway (Suzuki *et al*., 2005; Zhou *et al*., 2018; Niu *et al*., 2023).

Flavonoids are also synthesized through phenylpropanoid pathway and involve several enzymes (Winkel-shirley, 2002). Several factors can affect the biosynthesis of flavonoids like temperature, nutrient light, and water deficiency; however, phytohormones remain the most important regulators of flavonoid biosynthesis such as jasmonates, abscisic acids, auxins, cytokinins, and ethylene (Premathilake *et al*., 2020). In this study, 29 transcripts of genes that encode different enzymes involved in flavonoid pathways were annotated and identified. Most of the genes identified were upregulated in SA and JA treatments except for the gene that encode chalcone reductase (CHR), anthocynidin synthase (ANS), flavanone 4-reductase (FNR), and anthocyanidin 3-o-glucoslyteransferase, which were downregulated. *FNR* gene was downregulated in SA treatment, while *CHR* and *ANS* genes were downregulated in JA treatment, and anthocyanidin 3-o-glucoslyteransferase was downregulated in both SA and JA treatments. Salicylic acid, jasmonic acid and its methyl esters derivatives have shown to induce flavonoid biosynthesis in different plants such as *Mentha piperita* (Figueroa-Pérez *et al*. 2018), *Pyrus communis* (Premathilake *et al*., 2020), *Brassica napus* (Luo *et al*., 2021), *Citrullus lanatus* (Liu *et al*., 2023).

Two recognized terpenoids biosynthetic routes namely mevalonate (MVA) pathway which takes place in cytoplasm and 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway that takes place in plastid are well established in higher plants, which produces isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These function as precursors for sesquiterpenes, triterpenes, sterols, and polyterpenes in the cytoplasm, while in the plastid pool, they contribute to the synthesis of monoterpenes, diterpenes, and tetraterpenes (Liu *et al*., 2015). In this study, 21 transcripts which encode the enzymes that participate in MVA pathway and 31 transcripts encoding the enzymes involved in MEP pathway were annotated and identified. Overall, the JA treatment resulted in upregulation of larger number of transcripts involved in MVA pathway whereas SA treatment caused upregulation of larger number of transcripts upregulated in MEP pathway. Only diphosphomevalonate decarboxylase (MVD) gene was upregulated by SA treatment but downregulated in JA treatment in MVA pathway. Several reports have shown that SA and JA treatments resulted in enhanced expression of genes involved in MVA and MEP pathways.

Furthermore, we identified various transcripts encoding the genes of enzymes that participate in lignin biosynthesis pathway, which is also a part of phenylpropanoid pathway. Majority of the transcripts involved in lignin pathway were upregulated with the treatment of SA and JA including phenylalanine ammonia lyase (PAL), cinnamate 4-monooxygenase (C4H), 4-coumarate-coA ligase (4CL), hydroxycinnamoyl transferase (HCT), 4-Coumarate 3-hydroxylase (C3H) and CoA O-methyltransferase (CCoAOMT). Cinnamyl alcohol dehydrogenase (CAD) was downregulated in JA but upregulated in SA treatment, while ferulate 5-hydrolase (F5H) gene was upregulated in JA but downregulated in SA treatment. Meanwhile, the transcripts of peroxidase and catechol-O-methyltransferase (COMT) were differentially expressed in both SA and JA treatments.

The outcomes of this investigation demonstrates that the application of SA and JA in callus cultures of *G. moluccana* significantly impacted various crucial biological processes, including photosynthesis response and defense system, signaling and transport, and various secondary metabolic pathways. Additionally, the transcriptome profiles highlighted gene expressions linked to responses to abiotic stress and the biosynthesis of phenylpropanoids, flavonoid and terpenoids. These findings are consistent with the observed enhancement of secondary metabolites in the callus cultures of *G. moluccana*. As this is the first report of the transcriptomic data of leaves, callus and callus treated with SA and JA in *G. moluccana*, these data would serve as a valuable resource, contributing to an enhanced understanding of molecular mechanism during callus formation as well as salicylic acid and jasmonic acid response network and the molecular mechanisms governing the influence of this hormones on defense and stress responses at the transcriptional level.

6. Summary and Conclusions

G. moluccana is a tree species of economic importance valued for its soft wood and medicinal properties, especially for treatment of inflammatory diseases. It is considered to be a rich source of phenolic compounds, however, the information on secondary metabolite composition in various plant parts and their antioxidant and anti-inflammatory activities is limited. Research on production of secondary metabolite production from *in vitro* cultures remains unexplored in this species. The study focussed on assessing the phenolic and flavonoid contents, antioxidant potential of bark, pericarp and leaves extracted in different solvents and also the anti-inflammatory activity of methonolic extracts was determined. An effort was made to establish *in vitro* callus cultures and study the effect of elicition on secondary metabolite production from callus cultures. *De novo* transcriptome analysis was carried out to identify the differentially expressed genes in leaves derived callus cultures in response to elicitation with SA and JA. The transcription factors and the genes involved in different biosynthetic pathways were annotated and their expression pattern in elicited callus cultures was analyzed. The research findings of the work are summarized below:

6.1. Methanolic extract of bark showed higher phenolics, flavonoids, antioxidant activities than pericarp and leaves

Methanol and ethanol were the most effective solvents for extracting phenolic acids and flavonoids as compared to other solvents used. This could possibly be due to improved solvation of phenolic and flavonoid compounds in the extracts and their interaction with the polar solvent. Of the different plant parts, bark methanolic extract exhibited the highest contents of total phenolic acid (TP) (62.07 mg GAE/g DW), total flavonoid (TF) (41.72 mg RE/g DW). The antioxidant activities of bark methanolic extract was also higher than other plant parts, as determined by phosphomolybdenum (PM) assay (70.94 mg AAE /g DW), ferric reducing antioxidant power (FRAP) assay (156.38 mg AAE/g DW), cupric ion reducing antioxidant capacity (CUPRAC) assay (246.32 mg AAE/g DW), DPPH assay (IC_{50} 48.41 µg/ml) and superior metal chelating ability (78.58%). However, the results varied for leaves and pericarp extracts. The variation in phenolic and flavonoid contents in different parts might be due to differences in metabolic activities, which could have influenced the biosynthesis of secondary metabolites. The antioxidant efficacy of methanolic plant extracts correlated with higher phenolic and flavonoids in these plant parts.

6.2. Methanolic extract of bark and pericarp exhibited higher inhibition activity against 5-LOX and COX-2 than leaves

The methanolic extract of bark exhibited higher protein denaturation (43.46%) and high activity of 5-LOX (41.6%). The pericarp extract exhibited notable inhibition on COX-2 enzyme activity (86.71%) followed by bark, suggesting their potential for use in the treatment of inflammatory diseases. The methanolic extracts displayed moderate inhibition of the COX-1 enzyme in comparison to COX-2 enzyme activities, though the precise underlying mechanism remains to be elucidated. The bark and pericarp extracts of *G. moluccana* have demonstrated a potential as dual inhibitors of COX-2 and 5-LOX, which is a new approach for developing compounds with superior anti-inflammatory effects and mitigated cardiovascular and gastrointestinal side effects.

6.3. LC-MS/MS analysis revealed the presence of several medicinally important compounds in different plant parts

The metabolite profiling through GC-MS analysis showed that the major constituents of methanolic, bark, and pericarp extract were carbohydrates, carboxylic acids and fatty acids, while major constituents in leaves extract were carboxylic acids, carbohydrates, and amino acids. The secondary metabolites profiling by LC-MS/MS analysis in different plant parts of *G. moluccana* showed that flavonoids were the prominent class of secondary metabolites in bark and pericarp, followed by terpene and terpenoids, while in case of leaves it was *vice-versa*. Several compounds with various bioactivities such as antioxidant, anti-inflammatory, antidiabetic, anti-cancer, neuroprotectant and hepatoprotectant effects were putatively identified. Interestingly, the study led to identification of compounds with putative anti-psoriatic, anti-rheumatism and anti-osteoporotic activities providing scientific basis for its treatment in psoriasis, rheumatism and bone-setting. Quantification of flavonoids by HPLC showed that the content of quercetin (9.43 μg/g DW) and myrcetin (13.22 μg/g DW) was higher in leaves, while higher content of rutin was observed in pericarp extracts (55.39 μg/g DW).

6.4. Efficient callus proliferation and direct shoot multiplication was achieved in *G. moluccana*

Callus cultures provide an opportunity for studying secondary metabolite production, as well as gaining insights into their metabolism and regulation. In this study, leaves and endosperm explants displayed a high rate of callus induction when placed on MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l BAP. The callus generated from both leaves and endosperm exhibited vigorous proliferation, evident from the abundant callus formation after 5 weeks of subculture on fresh medium. This prolific callus formation from leaves and endosperm which differ in their ploidy has potential applications in secondary metabolite synthesis and provides an opportunity to investigate the influence of ploidy on metabolite biosynthesis.

Successful induction of multiple shoots was achieved from zygotic embryo axes by culturing them on MS medium supplemented with 3 mg/l of BAP. Subsequent subcultures on medium with lower concentrations of BAP facilitated the elongation of shoots. The rooting of shoots occurred with a frequency of 72.8% on MS medium containing 0.5 mg/l NAA. The rooted plantlets were then acclimatized and successfully established in soil, achieving a success rate of 72.4%. This method could be used for rapid production of shoots/plantlets for producing compounds associated with differentiated structures, and their enhancement by elicitation.

6.5. Elicitation of callus cultures with JA and SA resulted in enhanced phenolic and flavonoid content

The elicitation of callus cultures emerges as a promising method to enhance the production of secondary metabolites. Treating callus cultures with salicylic acid (SA) and jasmonic acid (JA) led to a reduction in biomass; however, it resulted in a significant increase in total phenolic (50 µM SA treatment, 5 days: 31.4 mg GAE g^{-1}DW; 150 µM JA treatments, 20 days: 81.83 mg GAE g–1 DW) and flavonoid contents (50 µM SA treatment, 20 days; 38.49 mg RE g-1 DW: 150 µM JA treatment, 20 days; 60.08 mg RE g–1 DW) compared to control callus cultures. Callus cultures treated with JA displayed elevated scavenging activity compared to both SA treated and control callus cultures, which is in accordance with the observed increase in phenolic compounds. This study, for the first time, highlights the high proliferation capability of callus cultures and the potential to enhance phenolic compounds through treatments with JA or SA. This finding opens avenues for genetic engineering of biosynthetic pathways to optimize the production of targeted bioactive compounds.

The exogenous application of SA and JA to callus cultures induced the production of hydrogen peroxide (H_2O_2) and resulted in a significant elevation in malondialdehyde (MDA) accumulation compared to control cultures. The callus cultures exposed to JA, particularly at the highest concentration 200 µM JA (3.7 µmol g-1 FW), exhibited the maximum levels of H_2O_2. The substantial increase in MDA levels in callus cultures treated with 150 µM JA (1.68 µmol g-1 FW) and 200 µM JA (2.27 µmol g-1 FW) is likely attributed to the jasmonic acid-induced upregulation of lipoxygenase, an enzyme catalyzing the oxygenation of polyunsaturated fatty acids. Conversely, the noticeable reduction in MDA content observed in SA treated callus cultures suggests an inhibitory effect of SA on lipid peroxidation, especially at higher concentrations. This effect could be due to the suppression of lipoxygenase activity or alterations in cellular metabolism induced by salicylic acid.

6.6. Enhancement of phenolic and flavonoid contents was associated with improved antioxidant metablosm in callus cultures

The antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), crucial in mitigating reactive oxygen species (ROS), experienced a substantial enhancement in callus cultures treated with specific concentrations of jasmonic acid (JA) and salicylic acid (SA). However, higher concentrations (200 µM) of these elicitors led to a reduction in enzyme activities, causing an imbalance in ROS levels. Concomitantly, the activities of enzymes associated with the ascorbate-glutathione (AsA-GSH) cycle were significantly increased in the elicited callus cultures. This elevation correlated with the highest contents of ascorbate and glutathione, indicating a more effective regulation of ROS. The observed improvement in reduced glutathione (GSH) levels, along with a decrease in oxidized glutathione (GSSG), contributed to an enhanced GSH/GSSG ratio. This suggests the efficacy of the elicitors in maintaining the AsA-GSH pool, further emphasizing their role in ROS regulation.

The activity of the PAL (phenylalanine ammonia-lyase) enzyme reached its peak in callus cultures treated with 150 µM JA and 50 µM SA. However, the transcript levels of PAL genes did not align consistently with the enzyme activity. Despite higher PAL enzyme activity in the 150 µM JA-treated callus (0.33 U mg-1 protein) and 50 µM SA-treated callus (0.21 U mg-1 protein) cultures, the transcript level of *PAL* gene was lower in comparison, suggesting a potential influence of post-transcriptional modifications.

The increased transcript levels of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) were in agreement with the activities of these enzymes suggesting a coordinated regulation at both the transcriptional and protein levels. These findings support the notion that these enzymes play a significant role in the detoxification of reactive oxygen species (ROS) during the treatment with elicitors.

6.7. Untargeted metabolite analysis using LC-MS/MS revealed an increase in several classes of secondary metabolites in SA and JA treated callus cultures

The LC-MS/MS analysis putatively identified 170, 203 and 218 secondary metabolites in leaves derived callus, JA, and SA treated callus cultures, respectively which belonged to different class of secondary metabolites such as flavonoids, terpenoids, alkaloids, phenolic acids, glycosides, sterols and steroids, vitamins and co-factors, lignans, anthroquinone, stilbenes and betacynin. Brassinosteroids biosynthesis, steroids biosynthesis, flavonoid biosynthesis, carotenoid biosynthesis, ubiquinone and other terpenoid-quinone biosynthesis, phenylpropanoid biosynthesis pathway were significantly enriched in both SA and JA treated callus cultures.

6.8. *De novo* transcriptome analysis revealed differentially expressed genes in leaves *vs*. callus cultures and SA and JA treated callus cultures

A total of 77,522 Trinity unigenes and 171,971 transcript isoforms were assembled, with 35,128 unigenes showing the best hit BLASTx match. The study unveiled 6488 differentially expressed genes between leaves derived callus and leaves, which were primarily involved in cell division, plant hormone signaling pathways, plants stress response, plant-pathogen interactions. Out of 6488 DEGs, 2469 were upregulated and 4018 were downregulated. A total of 714 transcripts encoding transcription factors were discovered in the comparison between leaves and leaves derived callus, and these were categorized into 49 families. The predominant families among these transcripts were *ERF*, *NAC*, *bHLH*, *WRKY*, *GRAS*, *C3H*, and *MYB*-related. The most upregulated TFs were *ERF* and *NAC* in leaves derived callus in comparison to leaves, while the most abundant downregulated were *DBB* and *bHLH* transcription factors

The study also unveiled 1623 differentially expressed genes (DEGs) in salicylic acid (SA) treated callus cultures compared to the control callus, which were primarily associated with biosynthetic pathways of secondary metabolites, plant hormone signaling, plant stress responses, and plant-pathogen interactions. Among these DEGs, 886 were upregulated, and 737 were downregulated in SA treated callus cultures. In case of jasmonic acid (JA) treated callus cultures, 3504 DEGs were identified, wherein 1719 genes showed upregulation and 1785 exhibited downregulation. The differentially expressed genes in both SA and JA treatments indicate significant changes in gene expression related to various biological processes and pathways.

A total of 1062 transcripts encoding transcription factors (TFs) were identified in control, salicylic acid (SA) treated, and jasmonic acid (JA) treated callus, categorized into 50 families. The TFs with the highest transcript levels were associated with families such as *ERF, bHLH, NAC, WRKY, GRAS, C3H, C2H2, MYB, MYB-related*, and *B3*. In SA treated callus, the most abundant upregulated transcripts were from the *WRKY* and *MYB-related* families, while *bHLH* transcripts were the most abundant downregulated. In JA treated callus, upregulated transcripts with the highest abundance belonged to the *WRKY* and *ERF* families, while downregulated transcripts were most abundant in the *ERF, bHLH,* and *B3* families. Numerous genes associated with the biosynthetic pathways of terpenoids, flavonoids, phenylpropanoid, and lignin demonstrated increased expression in callus cultures following treatments with salicylic acid (SA) and jasmonic acid (JA). However, the expression levels remained higher in JA treated cultures compared to SA. This study represents the first transcriptomic approach in *Givotia moluccana,* and the genetic resources generated are anticipated to be immensely valuable for future research endeavors, particularly in deciphering the biosynthetic pathways of secondary metabolites and facilitating metabolic engineering studies.

Major findings:

- Polar solvents *viz*., methanol and ethanol resulted in higher yields of phenolics and flavonoids from all plant parts as compared to non-polar solvents.
- The highest content of TP, TF and antioxidant activities was found in methanolic extracts of bark while varied results were observed in leaves and pericarp extracts.
- The antioxidant activities of the different plant extracts were consistent with the content of phenolics and flavonoids.
- Methanolic extract of bark showed higher inhibitiory activity against 5-LOX, while pericarp extract showed better inhibition activity on COX-2 which supports its use in treatment of inflammatory diseases.
- LC-MS/MS analysis in different plant parts of *G. moluccana* suggested that flavonoids were the prominent class of secondary metabolites in bark and pericarp, followed by terpene and terpenoids, while in case of leaves it was *vice-versa*.
- Leaves and endosperm showed potential for callus induction and proliferation on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP.
- Treatment of callus cultures with SA and JA caused a decrease in the biomass, however, resulted in a marked increased in total phenolic and flavonoid contents as compared to control callus cultures.
- Exogenous application of SA and JA to the callus cultures at all the concentrations triggered the production of H_2O_2 as well as caused a significant increase in the accumulation of MDA compared to the control cultures.

- Antioxidant enzymes such as SOD, CAT and POD involved in curbing ROS were enhanced significantly in callus cultures treated with specific concentrations of JA and SA whereas higher concentrations reduced the activities resulting in ROS imbalance.
- PAL enzyme activity was highest in 150 µM JA treated callus and 50 µM SA treated callus cultures, however, the transcript level of *PAL* genes was not consistent with the activity of PAL enzyme. *PAL* gene transcript was lower in comparison to SA treated which could be due to post-transcriptional modification.
- A total of 77,522 trinity unigenes and 171,971 total trinity transcript isoforms were assembled. 35,128 unigenes with best hit BLASTx match were generated.
- Out of 6488 DEGs identified between leaves and leaves derived callus, 2469 were upregulated and 4019 were downregulated. A toal of 714 transcripts belonging to 49 TF family were identified between leaves and leaves derived callus.
- Study revealed that 1623 genes were differentially expressed in SA treated and control callus cultures which were primarily involved in biosynthetic pathways of secondary metabolites, plant hormone signaling pathways, plants stress response and plant-pathogen interactions.
- Out of 1623 DEGs, 886 were upregulated and 737 were downregulated in SA treated callus cultures. In JA treated callus cultures, 3504 differentially expressed genes were identified, out of which the expression of 1719 genes was upregulated while 1785 were downregulated.
- Transcripts encoding transcription factors (TFs) identified (1062) in control, SA and JA treated callus were classified into 50 families. The TFs with the highest transcripts belonged to *ERF*, *bHLH*, *NAC*, *WRKY*, *GRAS*, *C3H*, *C2H2*, *MYB*, *MYB*-related and B3 family.
- The most abundant upregulated transcripts in SA treated callus were *WRKY* and *MYB* related whereas *bHLH* was the most abundant downregulated. Analysis of the upregulated TFs in SA treated callus cultures showed that the most abundant transcripts belonged to *WRKY* and *ERF*, while most downregulated TFs belonged to *ERF*, *bHLH* and *B3*.

- Several genes involved in biosynthetic pathways of terpenoids, flavonoids, phenylpropanoid and lignin exhibited higher expression in callus cultures in response to SA and JA treatments although the expression of few genes remained higher in JA as compared to SA.

Overall, the study revealed higher contents of total phenolics and flavonoids in the methanolic extract of bark along with the significant antioxidant activities. The crude extracts of bark and pericarp showed significant inhibition activities against COX-2 and 5-LOX enzymes thus demonstrating their anti-inflammatory potential. The study also provided an insight into the metabolites constituents of bark, leaves, and pericarp; wherein several compounds with bioactivities such as antioxidant, anti-inflammatory, anti-rheumatic, anti-psoriasis, anti-diabetic, anti-cancer, anti-osteoporosis, hepatoprotectant and neuroprotectant effects were putatively identified thus providing scientific clues regarding the medicinal properties. The *in vitro* studies revealed high proliferation ability of callus cultures from different explants and a significant enhancement of total phenolics and flavonoids in callus cultures upon treatment with JA or SA. Further, the increased phenolics and flavonoid contents in SA and JA-treated callus cultures correlated with improved enzymatic and non-enzymatic antioxidants showing better ROS regulation during elicitation. The study also revealed an enrichment of secondary metabolites of brassinosteroid, steroid, flavonoid, and carotenoid biosynthetic pathways in response to SA and JA treatments of callus cultures. *De novo* transcriptome analysis helped in understanding the transcriptomic changes during callus formation and in response to treatments with SA and JA, which is reported for the first time in this species. The information generated in this study could be of immense help for future research related to bioactive compound(s) isolation and their possible enhancement using elicitation. The transcriptome sequence information generated in the study could offer valuable molecular resource which can be exploited in future for deducing the biosynthetic pathways of targeted secondary metabolites using omic approaches and their increased production by metabolic engineering.

8. References

Abedi F, Razavi BM, Hosseinzadeh H (2020) A review on gentisic acid as a plant derived phenolic acid and metabolite of aspirin: Comprehensive pharmacology, toxicology, and some pharmaceutical aspects. *Phytotherapy Research* 34: 729–741. https://doi.org/10.1002/ptr.6573.

Aboulaghras S, Sahib N, Bakrim S, Benali T, Charfi S, Guaouguaou FE, Omari NE, Gallo M, Montesano D, Zengin G, Taghzouti K, Bouyahya A (2022) Health benefits and pharmacological aspects of chrysoeriol. *Pharmaceuticals* (Basel) 15:973. https://doi.org/10.3390/ph15080973.

Abreu LS, do Nascimento YM, do Espirito-Santo RF, Meira CS, Santos IP, Brandão RB, Souto AL, Guedes MLS, Soares MBP, Villarreal CF, da Silva MS, Velozo ES, Tavares JF (2020) Phenylpropanoids from *Croton velutinus* with cytotoxic, trypanocidal and anti-inflammatory activities. *Fitoterapia* 145: 104632. https://doi.org/10.1016/j.fitote.2020.104632

Achakzai AKK, Achakzai P, Masood A, Kayani SA, Tareen RB (2009) Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. *Pakistan Journal of Botany* 41: 2129–2135.

Acharya BR, Sandhu D, Dueñas C, Ferreira JF, Grover KK (2022) Deciphering molecular mechanisms involved in salinity tolerance in guar *(Cyamopsis tetragonoloba* (L.) Taub.) using transcriptome analyses. *Plants*11: 291. https://doi.org/10.3390/plants11030291

Aebi H (1984) Catalase *in vitro*. *Methods in Enzymology* 105: 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Afanas'ev IB, Dcrozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI (1989) Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochemical Pharmacology* 38: 1763–1769. https://doi.org/10.1016/0006-2952(89)90410-3.

Aftab T, Masroor M, Khan A, Idrees M, Naeem M, Moinuddin (2010) Salicylic acid acts as potent enhancer of growth, photosynthesis and artemisinin production in *Artemisia annua* L. *Journal of Crop Science and Biotechnology* 13: 183–188. https://doi.org/10.1007/s12892-010-0040-3.

Agar OT, Cankaya IIT (2020) Analysis of phenylethanoids and their glycosidic derivatives. *Recent Advances in Natural Products Analysis* 221-254. https://doi.org/10.1016/B978-0-12-816455-6.00005-6

Ahn YJ, Vang L, Mckeon TA, Chen GQ (2007) High-frequency plant regeneration through adventitious shoot formation in castor (*Ricinus communis* L.). *In Vitro Cellular and Developmental Biology – Plant* 43: 9–15. https://doi.org/10.1007/s11627-006-9009-2.

Akhtar N, Mirza B (2018) Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. *Arabian Journal of Chemistry* 11:1223–1235.

Athma P, Reddy TP (1983) Efficiency of callus initiation and direct regeneration from different explants of castor (*Ricinus communis* L.). *Current Science* 52: 256–257.

Ahmad R, Ahmad N, Aljamea A, Abuthayn S, Aqeel M (2021) Evaluation of solvent and temperature effect on green accelerated solvent extraction (ASE) and UHPLC quantification of phenolics in fresh olive fruit *(Olea europaea)*. *Food Chemistry* 342:128248. https://doi.org/10.1016/j.foodchem.2020.128248

Ahmed SR, Banik A, Anni SM, Chowdhury MMH (2020) Plant derived bioactive compounds as potential inhibitors of ZIKA virus: an *in silico* investigation. *bioRxiv*. https://doi.org/10.1101/2020.11.11.37808.

Alam MN, Bristi NJ, Rafiquzzaman M (2013) Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal* 21: 143–152. https://doi.org/10.1016/j.jsps.2012.05.002.

Alara OR, Abdurahmana NH, Ukaegbu CI (2021) Extraction of phenolic compounds: A review. *Current Research in Food Science* 4: 200–214. https://doi.org/10.1016/j.crfs.2021.03.011.

Aldaba-Muruato LR, Ventura-Juárez J, Perez-Hernandez AM, Hernández-Morales A, Muñoz-Ortega MH, Martínez-Hernández SL, Alvarado-Sánchez B, Macías-Pérez JR (2021) Therapeutic perspectives of p-coumaric acid: Anti-necrotic, anti-cholestatic and anti-amoebic activities. *World Academy of Sciences Journal* 3: 1–8. https://doi.org/10.3892/wasj.2021.118.

Alday E, Valencia D, Carreño AL, Picerno P, Piccinelli AL, Rastrelli L, Robles-Zepeda R, Hernandez J, Velazquez C (2015) Apoptotic induction by pinobanksin and some of its ester derivatives from *Sonoran propolis* in a B-cell lymphoma cell line. *Chemico-Biological Interactions* 242: 35–44. https://doi.org/10.1016/j.cbi.2015.09.013.

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment* 24: 1337–1344. https://doi.org/10.1046/j.1365-3040.2001.00778.x.

Allouche Y, Warleta F, Campos M, Sánchez-Quesada C, Uceda M, Beltrán G, Gaforio JJ (2011) Antioxidant, antiproliferative, and pro-apoptotic capacities of pentacyclic triterpenes found in the skin of olives on MCF-7 human breast cancer cells and their effects on DNA damage. *Journal of Agriculture and Food Chemistry* 59: 121–130. https://doi.org/10.1021/jf102319y.

Ali B (2021) Salicylic acid: An efficient elicitor of secondary metabolite production in plants. *Biocatalysis and Agricultural Biotechnology* 31: 101884. https://doi.org/10.1016/j.bcab.2020.101884.

Ali MB, Hahn EJ, Paek KY (2007) Methyl jasmonate and salicylic acid induced oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. *Molecules* 12: 607–621. https://doi.org/10.3390/12030607.

Ali MB, Yu KW, Hahn E-J, Paek K-Y (2006) Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. *Plant Cell Reports* 25: 613–620. https://doi.org/10.1007/s00299-005-0065-6.

Ali M, Abbasi BH, Ali GS (2015) Elicitation of antioxidant secondary metabolites with jasmonates and gibberellic acid in cell suspension cultures of *Artemisia absinthium* L. *Plant Cell, Tissue and Organ Culture* 120: 1099-1106. https://doi.org/10.1007/s11240-014-0666-2.

Alonso ÁM, Domínguez C, Guillén DA, Barroso CG (2002) Determination of antioxidant power of red and white wines by a new electrochemical method and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry* 50: 3112–3115. https://doi.org/10.1021/jf0116101.

Alós E, Rodrigo MJ, Zacarías L (2013) Transcriptomic analysis of genes involved in the biosynthesis, recycling and degradation of L-ascorbic acid in pepper fruits (*Capsicum annuum* L.). *Plant Science* 207: 2–11. https://doi.org/10.1016/j.plantsci.2013.02.007.

Alscher RG, Donahue JL, Cramer LL (1997) Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum* 100: 224–233. https://doi.org/10.1111/j.1399-3054.1997.tb04778.x.

Altındağ F, Rağbetli MÇ, Özdek U, Koyun N, Alhalboosi JK, Elasan S (2021) Combined treatment of sinapic acid and ellagic acid attenuates hyperglycemia in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology* 156: 112443. https://doi.org/10.1016/j.fct.2021.112443.

AlQathama A, Bader A, Al-Rehaily A, Gibbons S, Prieto JM (2022) *In vitro* cytotoxic activities of selected Saudi medicinal plants against human malignant melanoma cells (A375) and the isolation of their active principles. *European Journal of Integrative Medicine* 49: 102083. https://doi.org/10.1016/j.eujim.2021.102083

Al-Qatrani MKJ, Al Khalifa AAS, Obaid NA (2021) Effect of jasmonic acid on stimulating the growth and development of date palm callus (*Phoenix dactylifera* L) cultivar Shukar *in vitro* under salt stress conditions. *Earth Environmental Science* 923: 1–3. https://doi.org/10.1088/1755-1315/923/1/012017.

Anand A, Jayaramaiah RH, Beedkar SD, Singh PA, Joshi RS, Mulani FA, Dholakia BB, Punekar SA, Gade WN, Thulasiram HV, Giri AP (2016) Comparative functional characterization of eugenol synthase from four different Ocimum species: Implications on eugenol accumulation. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*. 1864:1539–1547. https://doi.org/10.1016/j.bbapap.2016.08.004

Angel GR, Vimala B, Nambisan B (2013) Antioxidant and anti-inflammatory activities of proteins isolated from eight Curcuma species. *Phytopharmacology* 4:96–105.

Anusha TS, Joseph MV, Elyas KK (2016) Callus induction and elicitation of total phenolics in callus cell suspension culture of *Celastrus paniculatus* - Willd, an endangered medicinal plant in India. *Pharmacognosy Journal* 8: 471–475. https://doi.org/10.5530/pj.2016.5.10.

Apak R, Güçlü K, Özyürek M, Karademir SE (2004) Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry* 52, 7970–7981. https://doi.org/10.1021/jf048741x.

Armstrong PC, Kirkby NS, Zain ZN, Emerson M, Mitchell JA, Warner TD (2011) Thrombosis is reduced by inhibition of COX-1, but unaffected by inhibition of COX-2, in an acute model of platelet activation in the mouse. *PloS One* 6: 5–10. https://doi.org/10.1371/journal.pone.0020062.

Asada K (1999) The water-water cycle in chloroplast: scavenging of active oxygen and dissipation of excess photons. *Annual Review of Plant Physiology and Molecular Biology* 50: 601–639. https://doi.org/10.1146/annurev.arplant.50.1.601.

Asahina M, Azuma K, Pitaksaringkarn W, Yamazaki T, Mitsuda N, Ohme-Takagi M, Yamaguchi S, Kamiya Y, Okada K, Nishimura T, Koshiba T (2011) Spatially selective hormonal control of RAP2. 6L and ANAC071 transcription factors involved in tissue reunion in *Arabidopsis. Proceedings of the National Academy of Sciences* 108: 16128–16132. https://doi.org/10.1073/pnas.1110443108.

Asakura K, Kanemasa T, Minagawa K, Kagawa K, Yagami T, Nakajima M, Ninomiya M (2000) α-Eudesmol, a P/Q-type (Ca2+) channel blocker, inhibits neurogenic vasodilation and extravasation following electrical stimulation of trigeminal ganglion. *Brain Research* 873: 94–101. https://doi.org/10.1016/s0006-8993(00)02527-0.

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. *The Gene Ontology Consortium. Nature Genetics* 25: 25–29. https://doi.org/10.1038/75556.

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H (2015) Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances* 33: 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001.

Aydin Y, Talas-Ogras T, Ipekçi-Altas Z, Gözükirmizi N (2006) Effects of brassinosteroid on cotton regeneration via somatic embryogenesis. *Biologia* 61: 289–293. https://doi.org/10.2478/s11756-006-0053-5.

Awuchi CG, Amagwula IO (2020) The biochemistry, toxicology, and uses of the pharmacologically active phytochemicals: Alkaloids, terpenes, polyphenols and glycosides. *Merit Research Journal of Food Science and Technology* 51: 6–21. https://doi.org/10.5281/zenodo.3967809.

Aziz N, Kim MY, Cho JY (2018) Anti-inflammatory effects of luteolin: A review of *in vitro, in vivo,* and *in silico* studies. *Journal of Ethnopharmacology* 225: 342–358. https://doi.org/10.1016/j.jep.2018.05.019.

Badhani B, Sharma N, Kakkar R (2015) Gallic acid: A versatile antioxidant with promising therapeutic and industrial applications. *Royal Society of Chemistry Advances* 5: 27540–27557. https://doi.org/10.1039/C5RA01911G.

Bakshi M, Oelmüller R (2014) WRKY transcription factors: Jack of many trades in plants. *Plant Signaling and Behavior* 9: e27700. https://doi.org/10.4161/psb.27700.

Balakrishnan K, Wierda WG, Keating MJ, Gandhi V (2008) Gossypol, a BH3 mimetic, induces apoptosis in chronic lymphocytic leukemia cells. *Blood, The Journal of the American Society of Hematology* 112: 1971–1980. https://doi.org/10.1182/blood-2007-12-126946.

Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry* 99: 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042.

Balestrini R, Cosgrove DJ, Bonfante P (2005) Differential location of α-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus. *Planta* 220: 889–899. https://doi.org/10.1007/s00425-004-1431-2

Ballester AR, Lafuente MT, Ganzalez-Candelas L (2006) Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia lyase in the citrus fruit-*Penicillium digitatum* interaction. *Postharvest Biology and Technology* 39: 115–124. https://doi.org/10.1016/j.postharvbio.2005.10.002.

Bang H-J, Ahn M-R (2021) Antiangiogenic effect of pinobanksin on human umbilical vein endothelial cells. *Journal of Functional Foods* 79: 104408. https://doi.org/10.1016/j.jff.2021.104408.

Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA (2008) Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chemistry* 107: 1106–1113. https://doi.org/10.1016/j.foodchem.2007.09.030.

Bartwal A, Mall R, Lohani P, Guru SK, Arora S (2013) Role of secondary metabolites and brassinosteroids in plant defense against environmental stresses. *Journal of Plant Growth Regulation* 32: 216–232. https://doi.org/10.1007/s00344-012-9272-x.

Baskar V, Murugamani V, Mounika P, Madhuri B (2011) Preliminary phytochemical investigation of *Givotia moluccana* stem. *International Journal of Research in Pharmaceutical and Biomedical Sciences* 2: 1307–1313.

Bauer N, Fulgosi H, Jelaska S (2011) Overexpression phenylalanine ammonia-lyase in transgenic roots of *Coleus blumei* alters growth and rosmarinic acid synthesis. *Food Technology and Biotechnology* 49: 24–31.

Beauchamp CO, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* 44: 276–287. https://doi.org/10.1016/0003-2697(71)90370-8.

Belchí-Navarro S, Almagro L, Lijavetzky D, Bru R, Pedreño MA (2012) Enhanced extracellular production of trans-resveratrol in *Vitis vinifera* suspension cultured cells by using cyclodextrins and methyljasmonate. *Plant Cell Reports* 31: 81–89. https://doi.org/10.1007/s00299-011-1141-8.

Belhadj A, Saigne C, Telef N, Cluzet S, Bouscaut J, Corio-Costet MF, Mérillon JM (2006) Methyl jasmonate induces defense responses in grapevine and triggers protection against *Erysiphe necator*. *Journal of Agricultural and Food Chemistry* 54: 9119–9125. https://doi.org/10.1021/jf0618022.

Ben Amor N, Ben Hamed K, Debez A, Grignon C, Abdelly C (2005) Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. *Plant Science* 168: 889–899. https://doi.org/10.1016/j.plantsci.2004.11.002.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate : A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society* 57: 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP assay. *Analytical Biochemistry* 239: 70–76. https://doi.org/10.1006/abio.1996.0292.

Bertolini A, Ottani A, Sandrini M (2001) Dual acting anti-inflammatory drugs: A reappraisal. *Pharmacological Research* 44: 437–450. https://doi.org/10.1006/phrs.2001.0872.

Bhuia MS, Rahaman MM, Islam T, Bappi MH, Sikder MI, Hossain KN, Akter F, Al Shamsh Prottay A, Rokonuzzman M, Gürer ES, Calina D, Islam MT, Sharifi-Rad J (2023) Neurobiological effects of gallic acid: current perspectives. *Chinese Medicine* 18: 27. https://doi.org/10.1186/s13020-023-00735-7.

Bian Q, Gao S, Zhou J, Qin J, Taylor A, Johnson EJ, Tang G, Sparrow JR, Gierhart D, Shang F. (2012) Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. *Free Radical Biology and Medicine* 53: 1298–1307. https://doi.org/10.1016/j.freeradbiomed.2012.06.024.

Bilgin DD, Zavala JA, Zhu J, Clough SJ, Ort DR, Delucia EH (2010) Biotic stress globally downregulates photosynthesis genes. *Plant, Cell and Environment* 33: 1597-1613. https://doi.org/10.1111/j.1365-3040.2010.02167.x

Bi MC, Rosen R, Zha RY, McCormick SA, Song E, Hu DN (2013) Zeaxanthin induces apoptosis in human uveal melanoma cells through Bcl-2 family proteins and intrinsic apoptosis pathway. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2013/205082

Bitto A, Polito F, Squadrito F, Marini H, D'Anna R, Irrera N, Minutoli L, Granese R, Altavilla D (2010) Genistein aglycone: a dual mode of action anti-osteoporotic soy isoflavone rebalancing bone turnover towards bone formation. *Current Medicinal Chemistry* 17: 3007–3018. https://doi.org/10.2174/092986710791959738.

Blasco L, Duracher L, Forestier JP, Vian L, Marti-Mestres G (2006) Skin constituents as cosmetic ingredients. Part I: A study of bio-mimetic monoglycerides behavior at the squalene-water interface by the “Pendant drop” method in a static mode. *Journal of Dispersion Science and Technology* 27: 799–810. https://doi.org/10.1080/01932690500482137.

Boeing JS, Barizão ÉO, de Silva BC, Montanher PF, Almeida VC, Visentainer JV (2014) Evaluation of solvent effect on the extraction of phenolic compounds and antioxidant capacities from the berries: application of principal component analysis. *Chemistry Central Journal* 8: 48. https://doi.org/10.1186/s13065-014-0048-1.

Bolca S, Urpi-Sarda M, Blondeel P, Nathalie R, Lynn V, Sam P, Nawaf A-M, Nigel B, Denis DK, Marc B, Arne H, Claudine M, Herman D (2010) Disposition of soy isoflavones in normal human breast tissue. *The American Journal of Clinical Nutrition* 91: 976–984. https://doi.org/10.3945/ajcn.2009.28854.

Bong FJ, Chear NJY, Ramanathan S, Kumaran NM, Subramaniam S, Chew BL (2021) The development of callus and cell suspension cultures of Sabah Snake Grass (*Clinacanthus nutans*). *Biocatalysis and Agricultural Biotechnology* 33: 1–13. https://doi.org/10.1016/j.bcab.2021.101977.

Borbulevych OY, Jankun J, Selman SH, Skrzypczak-Jankun E (2004) Lipoxygenase interactions with natural flavonoid, quercetin, reveal a complex with protocatechuic acid in its X-ray structure at 2.1 A resolution. *Proteins* 54:13–19. https://doi.org/10.1002/prot.10579

Borghi SM, Carvalho TT, Staurengo-Ferrari L, Hohmann MS, Pinge-Filho P, Casagrande R, Verri Jr WA (2013) Vitexin inhibits inflammatory pain in mice by targeting TRPV1, oxidative stress, and cytokines. *Journal of Natural Products* 76: 1141–1149. https://doi.org/10.1021/np400222v.

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: A historical perspective. *Plant Science* 161: 839–851. https://doi.org/10.1016/S0168-9452(01)00490-3.

Boursiac Y, Boudet J, Postaire O, Luu DT, Tournaire-Roux C, Maurel C (2008) Stimulus-induced downregulation of root water transport involves reactive oxygen species-activated cell signalling and plasma membrane intrinsic protein internalization. *The Plant Journal* 56: 207–218. https://doi.org/10.1111/j.1365-313X.2008.03594.x

Boz H (2015) p-Coumaric acid in cereals: presence, antioxidant and antimicrobial effects. *International Journal of Food Science and Technology* 50: 2323–2328. https://doi.org/10.1111/ijfs.12898.

Bravo L (1998) Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* 56: 317–333. https://doi.org/10.1111/j.1753-4887.1998.tb01670.x.

Breitling R, Ceniceros A, Jankevics A, Takano E (2013) Metabolomics for secondary metabolite research. *Metabolites* 3: 1076–1083. https://doi.org/10.3390/metabo3041076.

Brouwer SM, Odilbekov F, Burra DD, Lenman M, Hedley PE, Grenville-Briggs L, Alexandersson E, Liljeroth E, Andreasson E (2020) Intact salicylic acid signalling is required for potato defence against the necrotrophic fungus *Alternaria solani*. *Plant Molecular Biology* 104: 1–9. https://doi.org/10.1007/s11103-020-01019-6.

Brown S, Clastre M, Courdavault V, O'Connor SE (2015) *De novo* production of the plant-derived alkaloid strictosidine in yeast. *Proceedings of the National Academy of Sciences* 112: 3205–3210. https://doi.org/10.1073/pnas.1423555112.

Bu D, Luo H, Huo P, Wang Z, Zhang S, He Z, Wu Y, Zhao L, Liu J, Guo J, Fang S, Cao W, Yi L, Zhao Y, Kong L (2021) KOBAS-i: Intelligent prioritization and exploratory visualization of biological functions for gene enrichment analysis. *Nucleic Acids Research* 49: W317–W325. https://doi.org/10.1093/nar/gkab447.

Buraphaka H, Putalun W (2020) Stimulation of health-promoting triterpenoids accumulation in *Centella asiatica* (L.) Urban leaves triggered by postharvest application of methyl jasmonate and salicylic acid elicitors. *Industrial Crops and Products* 146: 112171. https://doi.org/10.1016/j.indcrop.2020.112171.

Caarls L, Pieterse CM, Van Wees SC (2015) How salicylic acid takes transcriptional control over jasmonic acid signaling. *Frontiers in Plant Science* 6: 170. https://doi.org/10.3389/fpls.2015.00170.

Cai J, Ma Y, Hu P, Zhang Y, Chen J, Li X (2017) Elicitation of furanocoumarins in *Changium smyrnioides* suspension cells. *Plant Cell Tissue Organ Culture* 130: 1–12. https://doi.org/10.1007/s11240-017-1199-2.

Cao Y, Li K, Li Y, Zhao X, Wang L (2020) MYB transcription factors as regulators of secondary metabolism in plants. *Biology* 9: 61. https://doi.org/10.3390/biology9030061

Castelhano J, Lima G, Teixeira M, Soares C, Pais M, Castelo-Branco M (2021) The effects of tryptamine psychedelics in the brain: A meta-analysis of functional and review of molecular imaging studies. *Frontiers in Pharmacology* 12: 739053. https://doi.org/10.3389/fphar.2021.739053.

Catmull S, Masood F, Schacht S, Dolan R, Stegman D, Leung L, Al-Nakkash L (2016) Dietary genistein rescues reduced basal chloride secretion in diabetic jejunum via sex-dependent mechanisms. *Cellular Physiology and Biochemistry* 40: 335–346. https://doi.org/10.1159/000452549.

Chakraborti AK, Garg SK, Kumar R, Motiwala HF, Jadhavar PS (2010) Progress in COX-2 inhibitors: A journey so far. *Current Medicinal Chemistry* 17: 1563–1593. https://doi.org/10.2174/092986710790979980.

Chamcheu JC, Afaq F, Syed DN, Siddiqui IA, Adhami VM, Khan N, Singh S, Boylan BT, Wood GS, Mukhtar H (2013) Delphinidin, a dietary antioxidant, induces human epidermal keratinocyte differentiation but not apoptosis: studies in submerged and three-dimensional epidermal equivalent models. *Experimental Dermatology* 22: 342–348. https://doi.org/10.1111/exd.12140.

Chamcheu JC, Pal HC, Siddiqui IA, Adhami VM, Ayehunie S, Boylan BT, Noubissi FK, Khan N, Syed DN, Elmets CA, Wood GS, Afaq F, Mukhtar H (2015) Prodifferentiation, anti-inflammatory and antiproliferative effects of delphinidin, a dietary anthocyanidin, in a full-thickness three-dimensional reconstituted human skin model of psoriasis. *Skin Pharmacology and Physiology* 28: 177–188. https://doi.org/10.1159/000368445.

Chamulitrat W, Mason RP (1989) Lipid peroxyl radical intermediates in the peroxidation of polyunsaturated fatty acids by lipoxygenase. Direct electron spin resonance investigations. *Journal Biological Chemistry* 15: 20968–20973.

Chandrasekara A (2019) Phenolic acids. In: *Encyclopedia of Food Chemistry*, Melton L, Shahidi F, Varelis P (Eds.) 3: 535–545. https://doi.org/10.1016/B978-0-08-100596-5.22395-0

Chang L, Wang Q, Ju J, Li Y, Cai Q, Hao L, Zhou Y (2020) Magnoflorine ameliorates inflammation and fibrosis in rats with diabetic nephropathy by mediating the stability of lysine-specific demethylase 3A. *Frontiers in Physiology* 22: 11. https://doi.org/10.3389/fphys.2020.580406.

Chang W-A, Hung J-Y, Jian S-F, Lin Y-S, Wu C-Y, Hsu Y-L, Kuo P-L (2016) Laricitrin ameliorates lung cancer-mediated dendritic cell suppression by inhibiting signal transducer and activator of transcription 3. *Oncotarget* 7: 85220–85234. https://doi.org/10.18632/oncotarget.13240.

Charlier C, Michaux C (2003) Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *European Journal of Medicinal Chemistry* 38: 645–659. https://doi.org/10.1016/S0223-5234(03)00115-6.

Charriere F, Sotto B, Miginiac E, Hahne G (1999) Induction of adventitious shoots or somatic embryos on *in vitro* cultured zygotic embryos of *Helianthus annuus*: Variation of endogenous hormone levels. *Plant Physiology and Biochemistry* 37: 751–757. https://doi.org/10.1016/S0981-9428(00)86688-7.

Chassagnea F, Haddada M, Amiela A, Phakeovilaya C, Manithip C, Bourdya G, Deharoa E, Martia G (2018) A metabolomic approach to identify anti-hepatocarcinogenic compounds from plants used traditionally in the treatment of liver diseases. *Fitoterapia* 127: 226–236. https://doi.org/10.1016/j.fitote.2018.02.021.

Chavan JJ, Gaikwad NB, Kshirsagar PR, Dixit GB (2013) Total phenolics, flavonoids and antioxidant properties of three *Ceropegia* species from Western Ghats of India. *South African Journal of Botany* 88:273–277. https://doi.org/10.1016/j.sajb.2013.08.007

Chen C, Zheng Y, Zhong Y, Wu Y, Li Z, Xu LA, Xu M (2018) Transcriptome analysis and identification of genes related to terpenoid biosynthesis in *Cinnamomum camphora*. *BMC Genomics* 19: 1–15. https://doi.org/10.1186/s12864-018-4941-1.

Cheng Y, Liu H, Cao L, Wang S, Li Y, Zhang Y, Jiang W, Zhou Y, Wang H (2015) Down-regulation of multiple CDK inhibitor ICK/KRP genes promotes cell proliferation, callus induction and plant regeneration in *Arabidopsis*. *Frontiers in Plant Science* 6:825. https://doi.org/10.3389/fpls.2015.00825

Chen H, Jones AD, Howe GA (2006) Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. *FEBS Letters* 580: 2540–2546. https://doi.org/10.1016/j.febslet.2006.03.070.

Chen H, Lin J, Zhu S, Zeng K, Tu P, Jiang Y (2022) Anti-inflammatory constituents from the stems and leaves of *Glycosmis ovoidea* Pierre. *Phytochemistry* 203: 113369. https://doi.org/10.1016/j.phytochem.2022.113369.

Chen H, Lu C, Liu H, Wang M, Zhao H, Yan Y, Han L (2017) Quercetin ameliorates imiquimod-induced psoriasis-like skin inflammation in mice via the NF-κB pathway. *International Immunopharmacology* 48:110–117 https://doi.org/10.1016/j.intimp.2017.04.022.

Chen HQ, Wang XJ, Jin ZY, Xu XM, Zhao JW, Xie ZJ (2008) Protective effect of isoflavones from *Trifolium pratense* on dopaminergic neurons. *Neuroscience Research* 62: 123–130. https://doi.org/10.1016/j.neures.2008.07.001.

Chen QF, Xu L, Tan WJ, Chen L, Qi H, Xie LJ, Chen MX, Liu BY, Yu LJ, Yao N, Zhang JH, Shu W, Xiao S (2015) Disruption of the *Arabidopsis* defense regulator genes *SAG101*, *EDS1*, and *PAD4* confers enhanced freezing tolerance. *Molecular Plant*. 8: 1536–1549. https://doi.org/10.1016/j.molp.2015.06.009.

Chen W-T, Zhang Y-Y, Qiang Q, Zou L-L, Zou P, Xu Y (2023) Pinobanksin from peony seed husk: A flavonoid with the potential to inhibit the proliferation of SH-SY5Y. *Food Science and Nutrition* 1–15. https://doi.org/10.1002/fsn3.3786.

Chen Y, Zhang M, Jin X, Tao H, Wang Y, Peng B, Fu C, Yu L (2020) Transcriptional reprogramming strategies and miRNA-mediated regulation networks of *Taxus media* induced into callus cells from tissues. *BMC Genomics* 21: 168. https://doi.org/10.1186/s12864-020-6576-2.

Che P, Lall S, Nettleton D, Howell SH (2006) Gene expression programs during shoot, root, and callus development in *Arabidopsis* tissue culture. *Plant Physiology* 141: 620–637. https://doi.org/10.1104/pp.106.081240.

Cherng YG, Tsai CC, Chung HH, Lai YW, Kuo SC, Cheng JT (2013) Antihyperglycemic action of sinapic acid in diabetic rats. *Journal of Agriculture and Food Chemistry* 61: 12053–12059. https://doi.org/10.1021/jf403092b.

Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S (2013) Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry* 72: 1–20. https://doi.org/10.1016/j.plaphy.2013.05.009.

Cho HM, Park EJ, Park YJ, Ponce-Zea J, Mai VH, Doan TP, Ryu B, Chin YW, Oh WK (2022) Sesquiterpene lactone and its unique proaporphine hybrids from *Magnolia grandiflora* L. and their anti-inflammatory activity. *Phytochemistry* 200: 113211. https://doi.org/10.1016/j.phytochem.2022.113211.

Cho HT, Cosgrove DJ (2000) Altered expression of expansin modulates leaf growth and pedicel abscission in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences* 97: 9783–9788. https://doi.org/10.1073/pnas.160276997

Choi B, Kang SY, Bae HJ, Lim HS, Bang WS, Bae H (2012) Transcriptional analysis of the ρ-coumarate 3-hydroxylase (C3H) gene from *Hibiscus cannabinus* L. during developmental stages in various tissues and in response to abiotic stresses. *Research Journal of BioTechnology* 7: 23–33.

Choi D, Cho H-T, Lee Y (2006) Expansins: expanding importance in plant growth and development. *Physiologia Plantarum* 126: 511–518. https://doi.org/10.1111/j.1399-3054.2006.00612.x

Choi JS, Islam MN, Ali MY, Kim YM, Park HJ, Sohn HS, Jung HA (2014) The effects of C-glycosylation of luteolin on its antioxidant, anti-Alzheimer's disease, anti-diabetic, and anti-inflammatory activities. *Archives of Pharmacal Research* 37: 1354–1363. https://doi.org/10.1007/s12272-014-0351-3.

Chong TM, Abdullah, MA, Fadzillah NM, Lai OM (2005) Jasmonic acid elicitation of anthraquinones with some associated enzymatic and non-enzymatic antioxidant responses in *Morinda elliptica*. *Enzyme and Microbial Technology* 36: 469–477. https://doi.org/10.1016/j.enzmictec.2004.11.002.

Chotikadachanarong K, Dheeranupattana S, Jatisatienr A, Wangkarn S, Mungkornasawakul P, Pyne SG, Ung AT Sastraruji T (2011) Influence of salicylic acid on alkaloid production by root cultures of *Stemona curtisii* Hook. F. *Current Research Journal of Biological Science* 3: 322–325. https://ro.uow.edu.au/scipapers/1142

Chuang JY, Tsai YY, Chen SC, Hsieh TJ, Chung JG (2005) Induction of G_0/G_1 arrest and apoptosis by 3-hydroxycinnamic acid in human cervix epithelial carcinoma (HeLa) cells. *In Vivo* 19: 683–688.

Ci X, Zhou J, Lv H, Yu Q, Peng L, Hua S (2017) Betulin exhibits anti-inflammatory activity in LPS-stimulated macrophages and endotoxin-shocked mice through an AMPK/AKT/Nrf2-dependent mechanism. *Cell Death and Disease* 8. https://doi.org/10.1038/cddis.2017.39

Cornelissen A, Guo L, Sakamoto A, Virmani R, Finn AV (2019) New insights into the role of iron in inflammation and atherosclerosis. *eBiomedicine* 47: 598–606. https://doi.org/10.1016/j.ebiom.2019.08.014.

Correché ER, Andujar SA, Kurdelas RR, Lechón MJG, Freile ML, Enriz RD (2008) Antioxidant and cytotoxic activities of canadine: Biological effects and structural aspects. *Bioorganic and Medicinal Chemistry* 16: 3641–3651. https://doi.org/10.1016/j.bmc.2008.02.015

Coutinho EM (2002) Gossypol: a contraceptive for men. *Contraception* 65: 259–263. https://doi.org/10.1016/s0010-7824(02)00294-9.

Curtin C, Zhang W, Franco C (2003) Manipulating anthocyanin composition in *Vitis vinifera* suspension cultures by elicitation with jasmonic acid and light irradiation. *Biotechnology Letters* 25: 1131–1135. https://doi.org/10.1023/a:1024556825544.

Dai J, Mumper RJ (2010) Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15: 7313–7352. https://doi.org/10.3390/molecules15107313

Dai X, Liu N, Wang L, Li J, Zheng X, Xiang F, Liu Z (2020) MYB94 and MYB96 additively inhibit callus formation via directly repressing LBD29 expression in *Arabidopsis thaliana. Plant Science* 293: 110323. https://doi.org/10.1016/j.plantsci.2019.110323

Dalila DZ, Jaafar H, Abdul Manaf A (2013) Effect of 2,4-D and kinetin on callus induction of *Barringtonia racemosa* leaf and endosperm explants in different types of basal media. *Asian Journal of Plant Sciences* 12: 21–27. https://doi.org/10.3923/ajps.2013.21.27.

Das J, Kumar R (2016) Plant Wnt: deciphering a novel signalling pathway in plants. *Current Science* 111: 1319-1324. https://www.jstor.org/stable/24908753

Das K, Roychoudhury A (2014) A reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Sciences* 2: 1–53. https://doi.org/10.3389/fenvs.2014.00053.

Dasgupta N, De B (2007) Antioxidant activity of some leafy vegetables of India: A comparative study. *Food Chemistry* 101: 471–474. https://doi.org/10.1016/j.foodchem.2006.02.003.

Davies NM, Smith GD, Windmeijer F, Martin RM (2013) COX-2 selective nonsteroidal anti-inflammatory drugs and risk of gastrointestinal tract complications and myocardial infarction: An instrumental variable analysis. *Epidemiology* 24: 352–362. https://doi.org/10.1097/EDE.0b013e318289e024.

Davies NW (1990) Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. *Journal of Chromatography* 503: 1–24. https://doi.org/10.1016/S0021-9673(01)81487-4.

Deepthi S, Satheeshkumar K (2017) Cell line selection combined with jasmonic acid elicitation enhance camptothecin production in cell suspension cultures of *Ophiorrhiza mungos* L. *Applied Microbiology and Biotechnology* 101: 545–558. https://doi.org/10.1007/s00253-016-7808-x.

Deng S, Ma J, Zhang L, Chen F, Sang Z, Jia Z, Ma L (2019) *De novo* transcriptome sequencing and gene expression profiling of *Magnolia wufengensis* in response to cold stress. *BMC Plant Biology* 19: 1–23. https://doi.org/10.1186/s12870-019-1933-5.

Deng WW, Zhang M, Wu JQ, Jiang ZZ, Tang L, Li YY, Wei CL, Jiang CJ, Wan XC (2013) Molecular cloning, functional analysis of three cinnamyl alcohol dehydrogenase (CAD) genes in the leaves of tea plant, *Camellia sinensis*. *Journal of Plant Physiology* 170: 272–282. https://doi.org/10.1016/j.jplph.2012.10.010.

De Oliveira DD, da Silva CP, Iglesias BB, Beleboni RO (2020) Vitexin possesses anticonvulsant and anxiolytic-like effects in murine animal models. *Frontiers in Pharmacology* 11: 1181. https://doi.org/10.3389/fphar.2020.01181.

Desender S, Andrivon D, Val F (2007) Activation of defence reactions in Solanaceae: where is the specificity? *Cell Microbiology* 9: 21–30. https://doi.org/10.1111/j.1462-5822.2006.00831.x.

De Vos RCH, Moco S, Lommen A, Keurentjes JJB, Bino RJ, Hall RD (2007) Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nature Protocol 2: 778–791. https://doi.org/10.1038/nprot.2007.95.

Dewitte W, Riou-Khamlichi C, Scofield S, Healy JM, Jacqmard A, Kilby NJ, Murray JA (2003) Altered cell cycle distribution, hyperplasia, and inhibited differentiation in *Arabidopsis* caused by the D-type cyclin CYCD3. *Plant Cell* 15: 79–92. https://doi.org/10.1105/tpc.004838.

DiCosmo F, Misawa M (1985) Eliciting secondary metabolism in plant cell cultures. *Trends in Biotechnology* 3: 318–322. https://doi.org/10.1016/0167-7799(85)90036-8.

Dinis TCP, Madeira VMC, Almeida LM (1994) Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics* 315: 161–169. https://doi.org/10.1006/abbi.1994.1485.

Dirar AI, Alsaadi DHM, Wada M, Mohamed MA, Watanabe T, Devkota HP (2019) Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany* 120: 261–267. https://doi.org/10.1016/j.sajb.2018.07.003.

Dixon RA (2001) Natural products and plant disease resistance. *Nature* 411: 843–847.

Dkhar B, Khongsti K, Thabah D, Syiem D, Satyamoorthy K, Das B (2017) Genistein represses PEPCK-C expression in an insulin-independent manner in HepG2 cells and in alloxan-induced diabetic mice. *Journal of Cellular Biochemistry* 119: 1953–1970. https://doi.org/10.1002/jcb.26356.

Dodou K, Anderson RJ, Lough WJ, Small DA, Shelley MD, Groundwater PW (2005) Synthesis of gossypol atropisomers and derivatives and evaluation of their anti-proliferative and antioxidant activity. *Bioorganic and Medicinal Chemistry* 13: 4228–4237. https://doi.org/10.1016/j.bmc.2005.04.026.

Dolanbay SN, Kocanci FG, Aslim B (2021) Neuroprotective effects of allocryptopine-rich alkaloid extracts against oxidative stress-induced neuronal damage. *Biomedicine & Pharmacotherapy* 140: 111690. https://doi.org/10.1016/j.biopha.2021.111690.

Dolferus R, Marbaix G, Jacobs M (1985) Alcohol dehydrogenase in *Arabidopsis*: analysis of the induction phenomenon in plants and tissue cultures. *Molecular and General Genetics* 199: 256–264. https://doi.org/10.1007/BF00330267.

Dong J, Liu YB, Liang ZS, Wang WL (2010) Investigation on ultrasound-assisted extraction of salvianolic acid B from *Salvia miltiorrhiza* root. *Ultrasonics Sonochemistry* 17: 61–65. https://doi.org/10.1016/j.ultsonch.2009.05.006.

Dong J, Wan G, Liang Z (2010) Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. *Journal of Biotechnology* 148: 99–104. https://doi.org/10.1016/j.jbiotec.2010.05.009.

Dong ZC, Chen Y (2013) Transcriptomics: Advances and approaches. *Science China Life Sciences* 56: 960–967. https://doi.org/10.1007/s11427-013-4557-2.

Duan X, Subbiah V, Xie C, Agar OT, Barrow CJ, Dunshea FR, Suleria HAR (2023) Evaluation of the antioxidant potential of brown seaweeds extracted by different solvents and characterization of their phenolic compounds by LC–ESI-QTOF–MS/MS. *Journal of Food Science* 3737–3757. https://doi.org/10.1111/1750-3841.16720.

Dubey A, Farmer A, Schlueter J, Cannon SB, Abernathy B, Tuteja R, Woodward J, Shah T, Mulasmanovic B, Kudapa H, Raju NL, Gothalwal R, Pande S, Xiao Y, Town CD, Singh NK, May GD, Jackson S, Varshney RK (2011) Defining the transcriptome assembly and its use for genome dynamics and transcriptome profiling studies in pigeon pea *(Cajanus cajan* L.*)*. *DNA Research* 18: 153–164. https://doi.org/10.1093/dnares/dsr007.

Dučaiová Z, Petruľová V, Repčák M (2013) Salicylic acid regulates secondary metabolites content in leaves of *Matricaria chamomilla*. *Biologia* (Poland) 68: 904–909. https://doi.org/10.2478/s11756-013-0217-z.

Durner J, Klessig DF (1995) Inhibition of ascorbate-peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proceedings of National Academy of Science* 89: 11312–11316. https://doi.org/10.1073/pnas.92.24.11312.

Ebenyi LN, Anyanwu CB, Halilu EM, Obialor A, Johnson TO, Adegboyega AE (2022) Identification of some bioactive compounds from *Camelia sinensis* as possible inhibitors of HER2: A structure-based drug design for breast cancer treatment. *Social Science Research Network*. http://dx.doi.org/10.2139/ssrn.4078794.

Ejtahed RS, Radjabian T, Tafreshi SAH (2015) Expression analysis of phenylalanine ammonia lyase gene and rosmarinic acid production in *Salvia officinalis* and *Salvia virgata* shoots under salicylic acid elicitation. *Applied Biochemical and Biotechnology* 176: 1846–1858. https://doi.org/10.1007/s12010-015-1682-3.

Eroğlu C, Avcı E, Vural H, Kurar E (2018) Anticancer mechanism of sinapic acid in PC-3 and LNCaP human prostate cancer cell lines. *Gene* 671: 127–134. https://doi.org/10.1016/j.gene.2018.05.049.

Esaka M, Imagi J, Suzuki K, Kubota K (1988) Formation of ascorbate oxidase in cultured pumpkin cells. *Plant and Cell Physiology* 29: 231–235. https://doi.org/10.1093/oxfordjournals.pcp.a077485.

Espinosa-Leal CA, Puente-Garza CA, García-Lara S (2018) *In vitro* plant tissue culture: Means for production of biological active compounds. *Planta* 248: 1–18. https://doi.org/10.1007/s00425-018-2910-1.

Fan M, Xu C, Xu K, Hu Y (2012) LATERAL ORGAN BOUNDARIES DOMAIN transcription factors direct callus formation in *Arabidopsis* regeneration. *Cell Research* 22: 1169–1180 https://doi.org/10.1038/cr.2012.63.

Fan Y, Piao CH, Hyeon E, Jung SY, Eom JE, Shin HS, Song CH, Chai OH (2019) Gallic acid alleviates nasal inflammation via activation of Th1 and inhibition of Th2 and Th17 in a mouse model of allergic rhinitis. *International Immunopharmacology* 70: 512–519. https://doi.org/10.1016/j.intimp.2019.02.025.

Fang Y, You J, Xie K, Xie W, Xiong L (2008) Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. *Molecular Genetics and Genomics* 280: 547–563. https://doi.org/10.1007/s00438-008-0386-6.

Farag MA, Sakna ST, El-Fiky NM, Shabana MM, Wessjohann LA (2015) Phytochemical, antioxidant and antidiabetic evaluation of eight *Bauhinia* L. species from Egypt using UHPLC-PDA-qTOF-MS and chemometrics. *Phytochemistry* 119: 41–50. https://doi.org/10.1016/j.phytochem.2015.09.004.

Farmer EE, Alméras E, Krishnamurthy V (2003) Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Current Opinion in Plant Biology* 6: 372–378. https://doi.org/10.1016/S1369-5266(03)00045-1.

Farmer EE, Johnson RR, Ryan CA (1992) Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. *Plant Physiology* 98: 995–1002. https://doi.org/10.1104/pp.98.3.995.

Farmer EE (1994) Fatty acid signalling in plants and their associated microorganisms. *Signals and Signal Transduction Pathways in Plants* 26: 187–201. https://doi.org/10.1007/978-94-011-0239-1_11.

Feduraev P, Chupakhina G, Maslennikov P, Tacenko N, Skrypnik L (2019) Variation in phenolic compounds content and antioxidant activity of different plant organs from *Rumex crispus* L. and *Rumex obtusifolius* L. at different growth stages. *Antioxidants* (Basel) 8:237. https://doi.org/10.3390/antiox8070237.

Fernie, AR, Martinoia E (2009) Malate. Jack of all trades or master of a few? *Phytochemistry* 70: 828–832. https://doi.org/10.1016/j.phytochem.2009.04.023.

Fernie AR, Tohge T (2017) The genetics of plant metabolism. *Annual Review of Genetics* 51: 287–310. https://doi.org/10.1146/annurev-genet-120116-024640.

Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L (2004) Metabolite profiling: From diagnostics to systems biology. *Nature Reviews Molecular Cell Biology* 5: 763–769. https://doi.org/10.1038/nrm1451.

Fiehn O (2016) Metabolomics by gas chromatography-mass spectrometry: Combined targeted and untargeted profiling. *Current Protocol on Molecular Biology* 114. https://doi.org/10.1002/0471142727.mb3004s114.

Figueroa-Pérez MG, Reynoso-Camacho R, Garcia-Ortega LF, Guevara-González RG (2019) Transcriptome profiling of peppermint *(Mentha piperita)* with improved antioxidant properties in response to salicylic acid elicitation. *Journal of Plant Biochemistry and Biotechnology* 28: 301–311. https://doi.org/10.1007/s13562-018-0482-6.

Fiorucci S, Meli R, Bucci M, Cirino G (2001) Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy?. *Biochemical Pharmacology* 62: 1433–1438. https://doi.org/10.1016/s0006-2952(01)00747-x.

Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK (2015) Reactive oxygen species in metabolic and inflammatory signaling. *Circulation Research* 2: 1–17. https://doi.org/10.1161/CIRCRESAHA.117.311401.

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. *Planta* 133: 21–25. https://doi.org/10.1007/BF00386001.

Foyer C, Rowell J, Walker D (1983) Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. *Planta* 157: 239–244. https://doi.org/10.1007/BF00405188.

Frączek M, Kuśmierz D, Rostkowska-Nadolska B, Kręcicki T, Latocha MT (2015) Impact of genistein and phytic acid on the viability and proliferation activity of nasal polyps' cells in an *in vitro* model. *Acta Poloniae Pharmaceutica* 72: 719–725.

Fraga CG, Croft KD, Kennedy DO, Tomás-Barberán FA (2019) The effects of polyphenols and other bioactives on human health. *Food and Function* 10: 514–528. https://doi.org/10.1039/c8fo01997e.

Frum Y, Viljoen AM (2006) *In vitro* 5-lipoxygenase and anti-oxidant activities of South African medicinal plants commonly used topically for skin diseases. *Skin Pharmacology and Physiolog* 19:329–335. https://doi.org/10.1159/000095253

Gaafar RM, Seyam MM (2018) Ascorbate-glutathione cycle confers salt tolerance in Egyptian lentil cultivars. *Physiology of Molecular Biology of Plants* 24: 1083–1092. https://doi.org/10.1007/s12298-018-0594-4.

Gadzovska S, Maury S, Delaunay A, Spasenoski M, Joseph C, Hagège D (2007) Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphtodianthrones. *Plant Cell, Tissue and Organ Culture* 89: 1–13. https://doi.org/10.1007/s11240-007-9203-x.

Gagliardi RR, Pacheco GP, Coculilo SP, Valls JFM, Mansur E (2000) *In vitro* plant regeneration from seed explants of wild groundnut species (Genus Arachis, Section Extranervosae). *Biodiversity and Conservation* 9: 943–951. https://doi.org/10.1023/A:1008960608543.

Galal A (2012) Improving effect of salicylic acid on the multipurpose tree *Ziziphur spina* – Christi (L.) Willd tissue culture. *American Journal of Plant Sciences* 3: 947–952. https://doi.org/10.4236/ajps.2012.37112.

Galano A, Francisco-Márqueza M, Alvarez-Idaboy JR (2011) Mechanism and kinetics studies on the antioxidant activity of sinapinic acid. *Physical Chemistry Chemical Physics* 13, 11199–11205. https://doi.org/10.1039/C1CP20722A.

Ganai AA, Khan AA, Malik ZA, Farooqi H (2015) Genistein modulates the expression of NF-κB and MAPK (p-38 and ERK1/2), thereby attenuating d-Galactosamine induced fulminant hepatic failure in Wistar rats. *Toxicology and Applied Pharmacology* 283: 39–146. https://doi.org/10.1016/j.taap.2015.01.012.

Garcia-Closas R, Gonzalez CA, Agudo A, Riboli E (1999) Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. *Cancer Causes and Control* 10: 71–75. https://doi.org/10.1023/A:1008867108960.

Gavilan J, Mennickent D, Ramirez-Molina O, Triviño S, Perez C, Silva-Grecchi T, Godoy PA, Becerra J, Aguayo LG, Moraga-Cid G, Martin VS, Yevenes GE, Castro PA, Guzman L, Fuentealba J (2019) 17 Oxosparteine and lupanine, obtained from *Cytisus scoparius*, exert a neuroprotection against soluble oligomers of amyloid-β toxicity by nicotinic acetylcholine receptors. *Journal of Alzheimers Disease* 67: 343–356. https://doi.org/10.3233/JAD-180945.

Geetha M, Vijayalakshmi A (2013) Pharmacognostic and phytochemical investigation of *Givotia rottleriformis* Griff . Ex Wight bark. *Journal of Pharmacognosy and Phytochemistry* 2: 130–136.

Ge F, Luo X, Huang X, Zhang Y, He X, Liu M, Lin H, Peng H, Li L, Zhang Z, Pan G (2016) Genome-wide analysis of transcription factors involved in maize embryonic callus formation. *Physiologia Plantarum* 158: 452–462. https://doi.org/10.1111/ppl.12470.

Ge X-X, Chai L-J, Liu Z, Wu X-M, Deng X-X, Guo W-W (2012) Transcriptional profiling of genes involved in embryogenic, non-embryogenic calluses and somatic embryogenesis of Valencia sweet orange by SSH-based microarray. *Planta* 236: 1107–1124 https://www.jstor.org/stable/43565307.

Ghanati F, Dehaghi MA (2011) Stimulation of taxol production by combined salicylic acid elicitation and sonication in *Taxus baccata* cell culture. *International Proceedings of Chemical, Biological and Environmental Engineering* 3: 193–197.

Ghasemzadeh A, Jaafer HZ, Rahmat A (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger *(Zingiber officinale)* Roscoe. *Molecules* 15: 4324–4333. https://doi.org/10.3390/molecules15064324.

Ghasemzadeh A, Jaafar HZ, Rahmat A (2011) Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. *Journal of Medicinal Plants Research* 5: 1147–1154. http://www.academicjournals.org/JMPR.

Ghasemzadeh A, Nasiri A, Jaafar HZE, Baghdadi A, Ahmad I (2014) Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass

(*Clinacanthus nutans* L.) in relation to plant age. *Molecules* 19: 17632–17648. https://doi.org/10.3390/molecules191117632.

Giri CC, Zaheer M (2016) Chemical elicitors versus secondary metabolite production *in vitro* using plant cell, tissue and organ cultures: Recent trends and a sky eye view appraisal. *Plant Cell Tissue Organ Culture* 126: 1–18. https://doi.org/10.1007/s11240-016-0985-6.

Gondor OK, Janda T, Soós V, Pál M, Majláth I, Adak MK, Balázs E, Szalai G (2016) Salicylic acid induction of flavonoid biosynthesis pathways in wheat varies by treatment. *Frontiers in Plant Science* 7: 1447. https://doi.org/10.3389/fpls.2016.01447.

Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR (2018) Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology* 9: 1639. https://doi.org/10.3389/fmicb.2018.01639.

Gookin TE, Hunter DA, Reid MS (2003) Temporal analysis of alpha and beta-expansin expression during floral opening and senescence. *Plant Science* 164: 769–781. https://doi.org/10.1016/S0168-9452(03)00063-3.

Gopalan G, Prabha B, Joe A, Reshmitha TR, Sherin DR, Abraham B, Sabu M, Manojkumar TK, Radhakrishnan KV, Nisha P (2018) Screening of *Musa balbisiana* Colla. seeds for antidiabetic properties and isolation of apiforol, a potential lead, with antidiabetic activity. *Journal of the Science of Food and Agriculture* 99: 2521–2529. https://doi.org/10.1002/jsfa.9462.

Gowda GA, Djukovic D (2014) Overview of mass spectrometry-based metabolomics: Opportunities and challenges. *Methods in Molecular Biology* 1198: 3–12. https://doi.org/10.1007/978-1-4939-1258-2_1.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* 29: 644–652. https://doi.org/10.1038/nbt.1883.

Guerriero G, Berni R, Muñoz-Sanchez JA, Apone F, Abdel-Salam EM, Qahtan AA, Alatar AA, Cantini C, Cai G, Hausman J-F, Siddiqui KS, Hernández-Sotomayor SMT, Faisal M (2018) Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. *Genes* 9. https://doi.org/10.3390/genes9060309.

Guerrero-Analco J, Medina-Campos O, Brindis F, Bye R, Pedraza-Chaverri J, Navarrete A, Mata R (2007) Antidiabetic properties of selected Mexican copalchis of the Rubiaceae family. *Phytochemistry* 68: 2087–2095. https://doi.org/10.1016/j.phytochem.2007.05.006.

Gujjar RS, Karkute SG, Rai A, Singh M, Singh B (2018) Proline-rich proteins may regulate free cellular proline levels during drought stress in tomato. *Current Science* 114: 915–920. https://doi.org/10.18520/cs/v114/i04/915-920.

Gujjar RS, Pathak AD, Karkute SG, Supaibulwatana K (2019) Multifunctional proline rich proteins and their role in regulating cellular proline content in plants under stress. *Biologia Plantarum* 63: 448–454. https://doi.org/10.32615/bp.2019.078.

Guo H, Zhu N, Deyholos MK, Liu J, Zhang X, Dong J (2015) Calcium mobilization in salicylic acid-induced *Salvia miltiorrhiza* cell cultures and its effect on the accumulation of rosmarinic acid. *Applied Biochemistry and Biotechnology* 175: 2689–2702. https://doi.org/10.1007/s12010-014-1459-0.

Guo M, Liu J, Ma X, Luo D, Gong Z, Lu M (2016) The plant heat stress transcription factors (HSFs): Structure, regulation, and function in response to abiotic stresses. *Frontiers in Plant Science* 7: 180954. https://doi.org/10.3389/fpls.2016.00114.

GutiErrez-Grijalva EP, Ambriz-Pere DL, Leyva-Lopez N, Castillo-Lopez RI, Heiedia JB (2016) Review: Dietary phenolic compounds, health benefits and bioaccessibility. *Archivos Latinoamericanos de Nutricion* 66: 87–100.

Gutierrez-Lugo MT, Deschamps JD, Holman TR, Suarez E, Timmermann BN (2004) Lipoxygenase inhibition by anadanthoflavone, a new flavonoid from the aerial parts of *Anadenanthera colubrina*. *Planta Medica* 70: 263–265. https://doi.org/10.1055/s-2004-818920.

Györgyey J, Németh K, Magyar Z, Kelemen Z, Alliotte T, Inzé D, Dudits D (1997) Expression of a novel-type small proline-rich protein gene of alfalfa is induced by 2,4-dichlorophenoxiacetic acid in dedifferentiated callus cells. *Plant Molecular Biology* 34: 593–602. https://doi.org/10.1023/A:1005845412667.

Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel R, LeDuc RD, Friedman N, Regev A (2013) *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols* 8: 1494–1512. https://doi.org/10.1038/nprot.2013.084.

Hadizadeh M, Ofoghi H, Kianirad M, Amidi Z (2019) Elicitation of pharmaceutical alkaloids biosynthesis by salicylic acid in marine microalgae *Arthrospira platensis*. *Algal Research* 42: 101597. https://doi.org/10.1016/j.algal.2019.101597.

Halder M, Sarkar S, Jha S (2019) Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. *Engineering in Life Sciences* 19:880–895. https://doi.org/10.1002/elsc.201900058.

Hall RD (2006) Plant metabolomics: from holistic hope, to hype, to hot topic. *New Phytologist*. 169: 453–468. https://doi.org/10.1111/j.1469-8137.2005.01632.x.

Han F, Kang LZ, Zeng XL, Ye ZW, Guo LQ, Lin JF (2014) Bioproduction of baccatin III, an advanced precursor of paclitaxol, with transgenic *Flammulina velutipes* expressing the 10-deacetylbaccatin III-10-O-acetyl transferase gene. *Journal of Science in Food Agriculture* 94: 2376–2383. https://doi.org/10.1002/jsfa.6562.

Han G, Lu C, Guo J, Qiao Z, Sui N, Qiu N, Wang B (2020) C2H2 zinc finger proteins: master regulators of abiotic stress responses in plants. *Frontiers in Plant Science* 11: 115. https://doi.org/10.3389/fpls.2020.00115.

Hans JS, Lee S, Kim HY, Lee CH (2015) MS-based metabolite profiling of aboveground and root components of *Zingiber mioga* and *officinale*. *Molecules* 20: 16170–16185. https://doi.org/10.3390/molecules200916170.

Han YJ, Dong MF, Wang HY, Wang XD, Li K, Shang FD (2019) Transcriptome sequencing flower petals reveals insights into regulation of flavonoid biosynthesis in *Osmanthus fragrans*. *Biologia Plantarum* 63: 765–775. https://doi.org/10.32615/bp.2019.146.

Harborne JB, Boardley M, Peter Lindert H (1985) Variations in flavonoid patterns within the genus *Chondropetalum* (restionaceae). *Phytochemistry* 24: 273–278. https://doi.org/10.1016/S0031-9422(00)83536-0.

Harborne JB Ingham JL, King L Payne M (1976) The isopentenyl isoflavone luteone as a pre-infectional antifungal agent in the genus *Lupinus*. *Phytochemistry* 15: 1485–1487. https://doi.org/10.1016/S0031-9422(00)88921-9.

Harborne B (1999) Classes and functions of secondary products from plants. In: Walton, J.N. and Brown, D.E., Eds., *Chemicals from Plants—Perspectives on Plant Secondary Products*, Imperial College Press, London, UK, 1-25. https://doi.org/10.1142/9789812817273_0001.

Harmer S, Orford S, Timmis J (2002) Characterisation of six α-expansin genes in *Gossypium hirsutum* (upland cotton). *Molecular Genetics and Genomics* 268: 1–9. https://doi.org/10.1007/s00438-002-0721-2.

Harwood JL (1988) Fatty acid metabolism. *Annual Review of Plant Physiolology and Plant Moecular Biology* 39: 101–138. https://doi.org/10.1146/annurev.pp.39.060188.000533.

Hasanuzzaman M, Bhuyan MHMB, Anee TI, Parvin, K, Nahar K, Mahmud JA, Fujita M (2019) Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. *Antioxidants* 8: 384. https://doi.org/10.3390/antiox8090384.

Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: a review. *Environmental and Experimental Botany* 68: 14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005.

Heinrich M, Mah J, Amirkia V (2021) Alkaloids used as medicines: Structural phytochemistry meets biodiversity—An update and forward look. *Molecules* 26: 1836. https://doi.org/10.3390/molecules26071836.

He C, Liu X, da Silva JA, Wang H, Peng T, Zhang M, Si C, Yu Z, Tan J, Zhang J, Luo J (2021) Characterization of LEA genes in *Dendrobium officinale* and one Gene in induction of callus. *Journal of Plant Physiology* 258: 153356. https://doi.org/10.1016/j.jplph.2020.153356.

He K, Gao JL (2014) Protopine inhibits heterotypic cell adhesion in MDA-MB-231 cells through down-regulation of multi-adhesive factors. *African Journal Traditional, Complement and Alternative Medicine* 11: 415–424. https://doi.org/10.4314/ajtcam.v11i2.28.

He M, Min JW, Kong WL, He XH, Li JX, Peng BW (2016) A review on the pharmacological effects of vitexin and isovitexin. *Fitoterapia* 115: 74–85. https://doi.org/10.1016/j.fitote.2016.09.011.

He M, Qin C-X, Wang X, Ding N-Z (2020) Plant unsaturated fatty acids: Biosynthesis and regulation. *Frontiers in Plant Sciences* 11: 390. https://doi.org/10.3389/fpls.2020.00390.

Henry RJ, Edwards M, Waters DLE, GopalaKrishnan S, Bundock P, Sexton TR, Masouleh AK, Nock CJ, Pattemore J (2012) Application of large-scale sequencing to marker discovery in plants. *Biosciences Journal* 37: 829–841. https://doi.org/10.18805/ag.R-1549.

Hertog MGL, Hollman PCH, Katan MB (1992) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry* 40: 2379–2383. https://doi.org/10.1021/jf00024a011.

Heyman HM, Dubery IA (2016) The potential of mass spectrometry imaging in plant metabolomics: A review. *Phytochemical Reviews* 15: 297–316. https://doi.org/10.1007/s11101-015-9416-2.

Hill CB, Roessner U (2013) Metabolic profiling of plants by GC–MS. In the handbook of plant metabolomics. Weckwerth W, Kahl G, Eds. Wiley-VCH: Weinheim, Germany. pp. 1–23. https://doi.org/10.1002/9783527669882.ch1.

Hiller KO, Ghorbani M, Schilcher H (1998) Antispasmodic and relaxant activity of chelidonine, protopine, coptisine, and *Chelidonium majus* extracts on isolated guinea-pig ileum. *Planta Medica* 64: 758–760. https://doi.org/10.1055/s-2006-957576.

Hollman PCH, Hertog MGL, Katan MB 1996) Analysis and health effects of flavonoids. *Food Chemistry* 57: 43–46. https://doi.org/10.1016/0308-8146(96)00065-9.

Hossain MA, Nakano Y, Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. *Plant and Cell Physiology* 25: 385–395. https://doi.org/10.1093/oxfordjournals.pcp.a076726.

Hou D-Y, Shi L-C, Yang M-M, Li J, Zhou S, Zhang H-X, Xu H-W (2018) *De novo* transcriptomic analysis of leaf and fruit tissue of *Cornus officinalis* using Illumina platform. *PloS One* 13: e0192610. https://doi.org/10.1371/journal.pone.0192610.

Hou R, Bao Z, Wang S, Su H, Li Y, Du H, Hu J, Wang S, Hu X (2011) Transcriptome sequencing and *de novo* analysis for yesso scallop *(Patinopecten yessoensis)* using 454 GS FLX. *PloS One* 6: e21560. https://doi.org/10.1371/journal.pone.0021560.

Hou W, Hu S, Su Z, Wang Q, Meng G, Guo T, Zhang J, Gao, P (2018) Myricetin attenuates LPS-induced inflammation in RAW 264.7 macrophages and mouse models. *Future Medicinal Chemistry* 10: 2253–2264. https://doi.org/10.4155/fmc-2018-0172.

Hsi LC, Angerman-Stewart J, Eling TE (1999) Introduction of full-length APC modulates cyclooxygenase-2 expression in HT-29 human colorectal carcinoma cells at the translational level. *Carcinogenesis* 20: 2045–2049. https://doi.org/10.1093/carcin/20.11.2045.

Hsiang CY, Hseu, YC, Chang S, Ho TY, Yang HL (2013) *Toona sinensis* and its major bioactive compound gallic acid inhibit LPS-induced inflammation in nuclear factor-κB transgenic mice as evaluated by *in vivo* bioluminescence imaging. *Food Chemistry* 15: 136: 426–434. https://doi.org/10.1016/j.foodchem.2012.08.009.

Huang H, Ullah F, Zhou D, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. *Frontiers in Plant Science* 10: 800 1–10. https://doi.org/10.3389/fpls.2019.00800.

Huang L, Zhang MY, Jia J, Zhao X, Huang X, Ji E, Ni L, Jiang M (2018) An atypical late embryogenesis abundant protein OsLEA5 plays a positive role in ABA-induced antioxidant defense in *Oryza sativa* L. *Plant and Cell Physiology* 59: 916–929, https://doi.org/10.1093/pcp/pcy035.

Huang Q, Pan Y, Wu SL, Huang XY, Hu J, Maa YB, Zhang HM, Geng CA (2022) Antidiabetic triterpenoids from the leaves of *Paeonia suffruticosa* and *Paeonia delavayi*. *Phytochemistry Letters* 48: 87–93. https://doi.org/10.1016/j.phytol.2022.02.009

Huang Z, Chen H, Tan P, Huang M, Shi H, Sun B, Cheng Y, Li T, Mou Z, Li Q, Fu W (2022) Sinapic acid inhibits pancreatic cancer proliferation, migration, and invasion via downregulation of the AKT/Gsk-3β signal pathway. *Drug Development Research* 83: 721–734. https://doi.org/10.1002/ddr.21904.

Hu X, Neill S, Cai W, Tang Z (2003) Hydrogen peroxide and jasmonic acid mediate oligogalacturonic acid-induced saponin accumulation in suspension-cultured cells of *Panax ginseng. Physiology of Plant* 118: 414–421. https://doi.org/10.1034/j.1399-3054.2003.00124.x.

Idrees M, Khan MM, Naeem M, Aftab T, Hashmi N, Alam M, Moinuddin (2011) Modulation of defence responses by improving photosynthetic activity, antioxidative metabolism, and vincristine and vinblastine accumulation in *Catharanthus roseus* (L.) G. Don through salicylic acid under water stress. *Russian Agricultural Sciences* 37: 474–482. https://doi.org/10.3103/S1068367411060127.

Ikeuchi M, Iwase A, Ito T, Tanaka H, Favero DS, Kawamura A, Sakamoto S, Wakazaki M, Tameshige T, Fujii H, Hashimoto N (2022) Wound-inducible WUSCHEL-RELATED HOMEOBOX 13 is required for callus growth and organ reconnection. *Plant Physiology* 188: 425–441. https://doi.org/10.1093/plphys/kiab510.

Ikeuchi M, Sugimoto K, Iwase A (2013) Plant callus: Mechanisms of induction and repression. *Plant Cell* 25: 3159–3173. https://doi.org/10.1105/tpc.113.116053.

Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, Fruksh A (2018) Secondary metabolism of pharmaceuticals in the plant in vitro cultures: Strategies, approaches, and limitations in achieving higher yield. *Plant Cell, Tissue and Organ Culture* 132: 239–265. https://doi.org/10.1007/s11240-017-1332-2.

Iwase A, Mitsuda N, Koyama T, Hiratsu K, Kojima M, Arai T, Inoue Y, Seki M, Sakakibara H, Sugimoto K, Ohme-Takagi M (2011) The AP2/ERF transcription factor WIND1 controls cell dedifferentiation in *Arabidopsis*. *Current Biology* 21: 508–514. https://doi.org/10.1016/j.cub.2011.02.020.

Janbaz KH, Saeed SA, Gilani AH (2002) Protective effect of rutin on paracetamol- and CCl_4-induced hepatotoxicity in rodents. *Fitoterapia* 73: 557–563. https://doi.org/10.1016/S0367-326X(02)00217-4.

Javed H, Meeran MFN, Azimullah S, Bader Eddin L, Dwivedi VD, Jha NK, Ojha S (2020) α-Bisabolol, a dietary bioactive phytochemical attenuates dopaminergic neurodegeneration through modulation of oxidative stress, neuroinflammation and apoptosis in rotenone-induced rat model of parkinson’s disease. *Biomolecules* 10: 1421. https://doi.org/10.3390/biom10101421.

Javed H, Khan MM, Ahmad A, Vaibhav K, Ahmad ME, Khan A, Ashafaq M, Islam F, Siddiqui MS, Safhi MM, Islam, F (2012) Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. *Neuroscience* 210: 340–352. https://doi.org/10.1016/j.neuroscience.2012.02.046.

Jeyaraj EJ, Lim YY, Choo WS (2021) Effect of organic solvents and water extraction on the phytochemical profile and antioxidant activity of *Clitoria ternatea* flowers. *ACS Food Science and Technology* 1: 1567–1577. https://doi.org/10.1021/acsfoodscitech.1c00168.

Jeyasri R, Muthuramalingam P, Karthick K, Shin H, Choi SH, Ramesh M (2023 Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: an updated review. *Plant Cell Tissue Organ Culture* 153: 447–458. https://doi.org/10.1007/s11240-023-02485-8.

Jiang B, Chen L, Duan S, Nian B, Liu K, Ma Y, Zhao M (2022) Transcriptomic analysis identifies differentially expressed genes in purple tender shoots and green mature leaves of Zijuan tea. *Czech Journal of Food Sciences* 40: 210–220. https://doi.org/10.17221/158/2020-CJFS.

Jiang J, Sugimoto Y, Liu S, Chang HL, Park KY, Kulp SK, Lin YC (2004) The inhibitory effects of gossypol on human prostate cancer cells-PC3 are associated with transforming growth factor beta1 (TGFβ1) signal transduction pathway. *Anticancer Research* 24: 91–100.

Jiang M, Xu F, Peng M, Huang F, Meng F (2016) Methyl jasmonate regulated diploid and tetraploid black locust (*Robinia pseudoacacia* L.) tolerance to salt stress. *Acta Physiologiae Plantarum* 38: 106. https://doi.org/10.1007/s11738-016-2120-z.

Jiang M, Zhu M, Wang L, Yu S (2019) Anti-tumor effects and associated molecular mechanisms of myricetin. *Biomedicine and Pharmacotherapy* 120: 109506. https://doi.org/10.1016/j.biopha.2019.109506.

Jiang Y, Yu L, Wang MH (2015) N-trans-feruloyltyramine inhibits LPS-induced NO and PGE2 production in RAW 264.7 macrophages: Involvement of AP-1 and MAP kinase signalling pathways. *Chemical Biology Interaction* 235: 56–62. https://doi.org/10.1016/j.cbi.2015.03.029.

Jia Q, Zhang H, Su Y, Liu X, Bai J, Lang W, Shi Q, Feng M (2021) Strictosamide alleviates the inflammation in an acute ulcerative colitis (UC) model. *Journal of Physiology and Biochemistry* 77: 283–294. https://doi.org/10.1007/s13105-021-00796-y.

Jia Y, Li B, Zhang Y, Zhang X, Xu Y, Li C (2019) Evolutionary dynamic analyses on monocot flavonoid 3′-hydroxylase gene family reveal evidence of plant-environment interaction. *BMC Plant Biology* 19: 347. https://doi.org/10.1186/s12870-019-1947-z.

Jin J, Tian F, Yang DC, Meng YQ, Kong L, Luo J, Gao G (2017) PlantTFDB 4.0: Toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Research* 45: D1040–D1045. https://doi.org/10.1093/nar/gkw982.

Jin Y, Zhang C, Liu W, Tang Y, Qi H, Chen H, Cao S (2016) The alcohol dehydrogenase gene family in melon (*Cucumis melo* L.): bioinformatic analysis and expression patterns. *Frontiers in Plant Science* 7: 670. https://doi.org/10.3389/fpls.2016.00670.

Juan ME, Wenzel U, Daniel H, Planas JM (2008) Erythrodiol, a natural triterpenoid from olives, has antiproliferative and apoptotic activity in HT-29 human adenocarcinoma cells. *Molecular Nutrition and Food Research* 52: 595–599. https://doi.org/10.1002/mnfr.200700300.

Kaczorová D, Karalija E, Dahija S, Bešta-Gajević R, Parić A, Ćavar Zeljković S (2021) Influence of extraction solvent on the phenolic profile and bioactivity of two *Achillea* species. *Molecules* 26: 1–15. https://doi.org/10.3390/molecules26061601.

Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei MH, Bishayee (2019) A. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iranian Journal of Basic Medical Sciences* 22: 225. https://doi.org/10.22038/ijbms.2019.32806.7897.

Kakkar S, Bais S (2014) A review on protocatechuic acid and its pharmacological potential. *ISRN Pharmacology* 952943. https://doi.org/10.1155/2014/952943.

Kamatham S, Kumar N, Gudipalli P (2015) Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rottleriformis* Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells. *Toxicology Reports* 2: 520–529. https://doi.org/10.1016/j.toxrep.2015.03.001.

Kamatham S, Neela KB, Pasupulati AK, Pallu R, Singh SS, Gudipalli P (2016) Benzoylsalicylic acid isolated from seed coats of *Givotia rottleriformis* induces systemic acquired resistance in tobacco and Arabidopsis. *Phytochemistry* 126: 11–22. https://doi.org/10.1016/j.phytochem.2016.03.002.

Kamatham S, Yarra R, Gudipalli P (2012) Temperature pre-treatment of seeds for overcoming the zygotic embryo dormancy of *Givotia rottleriformis* Griff . under *in vitro* conditions. *The Asian and Australasian Journal of Plant Science and Biotechnology* 6: 19–23.

Kamath VG, Chandrashekar A, Rajini PS (2004) Antiradical properties of sorghum (*Sorghum bicolor* L. Moench) flour extracts. *Journal of Cereal Science* 40: 283–288. https://doi.org/10.1016/j.jcs.2004.08.004.

Kamatou GP, Viljoen AM, Steenkamp P (2010) Antioxidant, antiinflammatory activities and HPLC analysis of South African Salvia species. *Food Chemistry* 119:684–688. https://doi.org/10.1016/j.foodchem.2009.07.010

Kanchana G, Shyni WJ, Rajadurai M, Periasamy R (2011) Evaluation of antihyperglycemic effect of sinapic acid in normal and streptozotocin-induced diabetes in albino rat. *Global Journal of Pharmacology* 5: 33–39.

Kang SH, Lee WH, Lee CM, Sim JS, Won SY, Han SR, Kwon SJ, Kim JS, Kim CK, Oh TJ (2020) *De novo* transcriptome sequence of *Senna tora* provides insights into anthraquinone biosynthesis. *PloS One* 15: 1–20. https://doi.org/10.1371/journal.pone.0225564.

Kang SM, Min JY, Kim YD, Kang YM, Park DJ, Jung HN, Kim S-W, Choi MS (2006) Effects of methyl jasmonate and salicylic acid on the production of bilobalide and ginkgolides in cell cultures of *Ginkgo biloba*. *In Vitro Cellular and Developmental Biology - Plant* 42: 44–49. https://doi.org/10.1079/IVP2005719.

Kang SW, Patnaik BB, Hwang HJ, Park SY, Chung JM, Song DK, Patnaik HH, Lee JB, Kim C, Kim S, Park HS (2017) Sequencing and *de novo* assembly of visceral mass transcriptome of the critically endangered land snail *Satsuma myomphala*: annotation and SSR discovery. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics* 21: 77–89. https://doi.org/10.1016/j.cbd.2016.10.004.

Karuppusamy S (2009) A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. *Journal of Medicinal Plant Research* 3: 1222–1239. https://doi.org/10.5897/JMPR.9000026.

Kaur J, Kaur R (2022) p-Coumaric Acid: A naturally occurring chemical with potential therapeutic applications. *Current Organic Chemistry* 26: 1333–1349. https://doi.org/10.2174/1385272826666221012145959.

Kaur P, Arora S, Singh R (2022) Isolation, characterization and biological activities of betulin from *Acacia nilotica* bark. *Scientific Reports* 12: 9370. https://doi.org/10.1038/s41598-022-13338-3.

Kchaou W, Abbès F, Blecker C, Attia H, Besbes S (2013) Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties *(Phoenix dactylifera* L.*). Industrial Crops and Products* 45:262–269. https://doi.org/10.1016/j.indcrop.2012.12.028.

Kepler JA, Wani MC, McNaull JN, Wall ME, Levine SG (1969) Plant antitumor agents. IV. An approach toward the synthesis of camptothecin. *The Journal of Organic Chemistry* 34: 3853–3858. https://doi.org/10.1021/jo01264a026.

Ke Q, Kim HS, Wang Z, Ji CY, Jeong JC, Lee HS, Choi YI, Xu B, Deng X, Yun DJ, Kwak SS (2017) Down-regulation of GIGANTEA-like genes increases plant growth and salt stress tolerance in poplar. *Plant Biotechnology Journal* 15: 331–343. https://doi.org/10.1111/pbi.12628

Keshmiri-Neghab H, Goliaei B (2014) Therapeutic potential of gossypol: an overview. *Pharmaceutical Biology* 52: 124–128. https://doi.org/10.3109/13880209.2013.832776.

Khalvandi M, Siosemardeh A, Roohi E, Keramati S (2021) Salicylic acid alleviated the effect of drought stress on photosynthetic characteristics and leaf protein pattern in winter wheat. *Heliyon* 7: e05908. https://doi.org/10.1016/j.heliyon.2021.e05908.

Khan A, Ikram M, Hahm JR, Kim MO (2020) Antioxidant and anti-inflammatory effects of citrus flavonoid hesperetin: Special focus on neurological disorders. *Antioxidants* 9: 609. https://doi.org/10.3390/antiox9070609.

Khan AK, Rashid R, Fatima N, Mahmood S, Mir S, Khan S, Jabeen N, Murtaza G (2015) Pharmacological activities of protocatechuic acid. *Acta Poloniae Pharmaceutica* 72: 643–650.

Khan A, Manna K, Bose C, Sinha M, Das DK, Kesh SB, Chakrabarty A, Banerji A, Dey S (2013) Gossypetin, a naturally occurring hexahydroxy flavone, ameliorates gamma radiation-mediated DNA damage. *International Journal Radiation Biology* 89: 965–975. https://doi.org/10.3109/09553002.2013.811310.

Khan RA, Khan MR, Sahreen S, Ahmed M (2012) Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill. *Chemistry Central Journal* 6:1-7. https://doi.org/10.1186/1752-153X-6-12.

Khanom S, Jang J, Lee OR (2019) Overexpression of ginseng cytochrome P450 CYP736A12 alters plant growth and confers phenylurea herbicide tolerance in *Arabidopsis*. *Journal of Ginseng Research* 43: 645–653. https://doi.org/10.1016/j.jgr.2019.04.005.

Khan Z, Hong S-M, Lee J-W, Moon E-Y, Huh J, Chang K-A, Kim SY (2021) Potential of N-trans feruloyl tyramine from *Lycium barbarum* fruit extract on neurogenesis and neurotrophins; targeting TrkA/ERK/CREB signaling pathway. *Journal of Functional Foods* 80: 104432. https://doi.org/10.1016/j.jff.2021.104432.

Khanpour-Ardestani N, Sharifi M, Behmanesh M (2015) Establishment of callus and cell suspension culture of *Scrophularia striata* Boiss.: An *in vitro* approach for acteoside production. *Cytotechnology* 67: 475–485. https://doi.org/10.1007/s10616-014-9705-4.

Khumsub S (1988) Tissue culture of castor bean (*Ricinus communis* L.) Dissertation, Kasetsart University, Bangkok. Pp.1–61.

Kiem PV, Yen DTH, Hung NV, Nhiem NX, Tai BH, Trang DT, Yen PB, Manh HD, Minh CV, Park SJ, Lee JH, Kim SY, Kim SH (2020) The anti-glycative potentials of pregnane glycosides from *Gymnema sylvestre. Phytochemistry Letter* 38: 19–24. https://doi.org/10.1016/j.phytol.2020.04.012.

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H (2002) Antioxidant properties of ferulic acid and its related compounds. *Journal of Agricultural and Food Chemistry*. 50: 2161–2168. https://doi.org/10.1021/jf011348w.

Kim ME, Ha TK, Yoon JH, Lee JS (2014) Myricetin induces cell death of human colon cancer cells via BAX/BCL2-dependent pathway. *Anticancer Research* 34: 701–706.

KimM, Kim H, Kim H (2022) Anti-inflammatory effect of protopine through MAPK and NF-κB signaling regulation in HepG2 cell. *Molecules* 27: 4601. https://doi.org/10.3390/molecules27144601.

Kim YH, Bae JM, Huh GH (2010) Transcriptional regulation of the cinnamyl alcohol dehydrogenase gene from sweetpotato in response to plant developmental stage and environmental stress. *Plant Cell Reports* 29: 779–791. https://doi.org/10.1007/s00299-010-0864-2.

Kim YJ, Kim DG, Lee SH, Lee I (2006) Wound-induced expression of the ferulate 5-hydroxylase gene in *Camptotheca acuminata*. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1760: 182–190. https://doi.org/10.1016/j.bbagen.2005.08.015.

Kitagawa M, Ito H, Shiina T, Nakamura N, Inakuma T, Kasumi T, Ishiguro Y, Yabe K, Ito Y (2005) Characterization of tomato fruit ripening and analysis of gene expression in F_1 hybrids of the ripening inhibitor (rin) mutant. *Physiologia Plantarum* 123: 331–338. https://doi.org/10.1111/j.1399-3054.2005.00460.x.

Klessig DF, Choi HW, Dempsey DA (2018) Systemic acquired resistance and salicylic acid: Past, present and future. *Molecular Plant-Microbe Interactions* 31: 871–888. https://doi.org/10.1094/MPMI-03-18-0067-CR.

Ko CH, Lau KM, Choy WY, Leung PC (2009) Effects of tea catechins, epigallocatechin, gallocatechin, and gallocatechin gallate, on bone metabolism. *Journal of Agriculture and Food Chemistry* 57: 7293–7297. https://doi.org/10.1021/jf901545u.

Koeduka T, Matsui K, Hasegawa M, Akakabe Y, Kajiwara T (2005) Rice fatty acid α-dioxygenase is induced by pathogen attack and heavy metal stress: activation through jasmonate signaling. *Journal of Plant Physiology* 162: 912–920. https://doi.org/10.1016/j.jplph.2004.11.003.

Kohlman M, Bachmann A, Weichert H, Kolbe A, Balkenhohl T, Wasternack C, Feussner I (1999) Formation of lipoxygenase-pathway-derived aldehydes in barley leaves upon methyl jasmonate treatment. *European Journal of Biochemistry* 260: 885–895. https://doi.org/10.1046/j.1432-1327.1999.00231.x.

Kolewe ME, Gaurav V, Roberts SC (2008) Pharmaceutically active natural product synthesis and supply via plant cell culture technology. *Molecular Pharmaceutics* 5: 243–256. https://doi.org/10.1021/mp7001494.

Kong J, Martin-Ortigosa S, Finer J, Orchard N, Gunadi A, Batts LA, Thakare D, Rush B, Schmitz O, Stuiver M, Olhoft P (2020) Overexpression of the transcription factor GROWTH-REGULATING FACTOR5 improves transformation of dicot and monocot species. *Frontiers in Plant Science* 11: 1389. https://doi.org/10.3389/fpls.2020.572319.

Koo TH, Lee J-H, Park YJ, Hong Y-S, Kim HS, Kim K-W, Lee JJ (2001) A sesquiterpene lactone, costunolide, from *Magnolia grandiflora* inhibits NF-κB by targeting IκB phosphorylation *Planta Medica* 67: 103–107. https://doi.org.10.1055/s-2001-11503.

Kosugi S, Ohashi Y (2003) Constitutive E2F expression in tobacco plants exhibits altered cell cycle control and morphological change in a cell type-specific manner. *Plant Physiology* 132: 2012–2022. https://doi.org/10.1104/pp.103.025080.

Kovács J, Poór P, Szepesi Á, Tari I (2016) Salicylic acid induced cysteine protease activity during programmed cell death in tomato plants. *Acta Biologica Hungarica* 67: 148–158. https://doi.org/10.1556/018.67.2016.2.3.

Kovacik J, Gruz J, Backor M, Strand M, Repcak M (2009) Salicylic acid induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. *Plant Cell Reports* 28: 135–143. https://doi.org/10.1556/018.67.2016.2.3.

Kramer GF, Norman HA, Krizek DT, Mirecki RM (1991) Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry* 30: 2101–2108. https://doi.org/10.1016/0031-9422(91)83595-C.

Kravchuk ZM, Perkovs’ka H, Dmytriiev OP (2003) Role of active forms of oxygen in the induction of phytoalexin synthesis in *Allium cepa* cells. *TSitologiia i genetika* 37: 30–35

Krinsky NI (1992) Mechanism of action of biological antioxidants. *Proceedings of the Society for Experimental Biology and Medicine* 200: 248–254. https://doi.org/10.3181/00379727-200-43429.

Krzyzanowska J, Czubacka A, Pecio L, Przybys M, Doroszewska T, Stochmal A, Oleszek W (2012) The effects of jasmonic acid and methyl jasmonate on rosmarinic acid production in *Mentha ×piperita* cell suspension cultures. *Plant Cell, Tissue, and Organ Culture* 108: 73–81. https://doi.org/10.1007/s11240-011-0014-8.

Krzysztoforska K, Mirowska-Guzel D, Widy-Tyszkiewicz E (2017) Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of *in vitro* and *in vivo* studies in rodents and humans. *Nutritional Neuroscience*. 22: 72-82. http://dx.doi.org/10.1080/1028415X.2017.1354543.

Kukurba KR, Montgomery SB (2015) RNA sequencing and analysis. *Cold Spring Harbor Protocols* 11: 951–969. https://doi.org/10.1101/pdb.top084970.

Kumari GJ, Reddy AM, Naik ST, Kumar SG, Prasanthi J, Sriranganayakulu G, Reddy PC, Sudhakar C (2006) Jasmonic acid induced changes in protein pattern, antioxidative enzyme activities and peroxidase isozymes in peanut seedlings. *Biologia Plantarum*. 50: 219–226. https://doi.org/10.1007/s10535-006-0010-8.

Kumar R, Das S, Mishra M, Choudhury DR, Sharma K, Kumari A, Singh R (2021) Emerging roles of NAC transcription factor in medicinal plants: progress and prospects. *3 Biotech* 11: 1–4. https://doi.org/10.1007/s13205-021-02970-x.

Kutchan TM, Ayabe S, Krueger RJ, Coscia EM, Coscia CJ (1983) Cytodifferentiation and alkaloid accumulation in cultured cells of *Papaver bracteatum*. *Plant Cell Reports* 2: 281–284. https://doi.org/10.1007/BF00270181.

Kutil Z, Temml V, Maghradze D, Pribylova M, Dvorakova M, Schuster D, Vanek T, Landa P (2014) Impact of wines and wine constituents on cyclooxygenase-1, cyclooxygenase-2, and 5-lipoxygenase catalytic activity. *Mediators of Inflammation* 178931: 1–8. https://doi.org/10.1155/2014/178931.

Kuzniak E, Urbanek H (2000) The involvement of hydrogen peroxide in plant response to stresses. *Acta Physiologiae Plantarum* 22: 195–203. https://doi.org/10.1007/s11738-000-0076-4

La VNT, Tran HTD, Nguyen CH, Nguyen TTH (2021) RNA-seq derived identification of coronatine-regulated genes putatively involved in terpenoid biosynthetic pathway in the rubber tree *Hevea brasiliensis*. *IOP Conf. Series: Earth and Environmental Science* 749: 012033. https://doi.org/10.1088/1755-1315/749/1/012033.

Lai L, Chou S, Chao W (2001) Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hemsl) leaf gum. *Journal of Agricultural and Food Chemistry* 49: 963–968. https://doi.org/10.1021/jf001146k.

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 251–275. https://doi.org/10.1146/annurev.arplant.48.1.251.

Lamson DW, Brignall MS (2000) Antioxidants and cancer III: Quercetin. *Alternative Medicine Review* 5: 196–208.

Langhansova L, Landa P, Kutil Z, Tauchen J, Marsik P, Rezek J, Lou JD, Yun ZL,Vanek, T (2017) *Myrica rubra* leaves as a potential source of a dual 5-LOX/COX inhibitor. *Food and Agricultural Immunology* 28: 343–353. https://doi.org/10.1080/09540105.2016.1272554.

Lapenna D, Ciofani G, Pierdomenico SD, Neri M, Cuccurullo C, Giamberardino MA, Cuccurullo F (2009) Inhibitory activity of salicylic acid on lipoxygenase-dependent lipid peroxidation. *Biochimica et Biophysica Acta* 1790: 25–30. https://doi.org/10.1016/j.bbagen.2008.09.007.

Lawrence, T. (2009) The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology* 1: a001651. https://doi.org/10.1101/cshperspect.a001651.

Lee EH, Shin JH, Kim SS, Seo SR (2021) Sinapic acid controls inflammation by suppressing NLRP3 inflammasome activation. *Cells* 10: 2327. https://doi.org/10.3390/cells10092327.

Lee HE, Kim DH, Park SJ, Kim JM, Lee YW, Jung JM, Lee CH, Hong JG, Liu X, Cai M, Park KJ (2012) Neuroprotective effect of sinapic acid in a mouse model of amyloid β1–42 protein-induced Alzheimer's disease. *Pharmacology Biochemistry and Behavior* 103: 260–266. https://doi.org/10.1016/j.pbb.2012.08.015.

Lee JH, Kim GH (2010) Evaluation of antioxidant and inhibitory activities for different subclasses flavonoids on enzymes for rheumatoid arthritis. *Journal of Food Science* 75: H212-H217. https://doi.org/10.1111/j.1750-3841.2010.01755.x.

Lee JY (2018) Anti-inflammatory effects of sinapic acid on 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice. *Archives of Pharmacal Research.* 41: 243–250. https://doi.org/10.1007/s12272-018-1006-6.

Lee JH, Chung YH, Kim HH, Bang JS, Jung TW, Park T, Park J, Kim U, Lee SH, Jeong JH (2018) p-Coumaric acid stimulates longitudinal bone growth through increasing the serum production and expression levels of insulin-like growth factor 1 in rats. *Biochemical and Biophysics Research Communications* 505: 1103–1106. https://doi.org/10.1016/j.bbrc.2018.10.046.

Lee K, Seo PJ (2017) High-temperature promotion of callus formation requires the BIN2-ARF-LBD axis in *Arabidopsis. Planta* 246: 797–802. https://doi.org/10.1007/s00425-017-2747-z.

Lee SY, Park SY, Lee GE, Kim H, Kwon JH, Kim MJ, Yoon HG (2021) Aucuparin suppresses bleomycin-induced pulmonary fibrosis via anti-inflammatory activity. *Journal of Medicinal Food* 24: 151–160. https://doi.org/10.1089/jmf.2020.4861.

Lee Y, Kende H (2001) Expression of β-expansins is correlated with internodal elongation in deepwater rice. *Plant Physiology* 127: 645–654. https://doi.org/10.1016/S1369-5266(00)00211-9.

Leopoldini M, Russo N, Toscano M (2011) The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food chemistry* 125:288–306. https://doi.org/10.1016/j.foodchem.2010.08.012

Leprince O, Pellizzaro A, Berriri S, Buitink J (2017) Late seed maturation: drying without dying. *Journal of Experimental Botany* 68: 827–841. https://doi.org/10.1093/jxb/erw363.

Liang B, Wan S, Ma Q, Yang L, Hu W, Kuang L, Xie J, Huang Y, Liu D, Liu Y (2022) A novel bHLH transcription factor PtrbHLH66 from trifoliate orange positively regulates plant drought tolerance by mediating root growth and ROS scavenging. *International Journal of Molecular Sciences* 23: 15053. https://doi.org/10.3390/ijms232315053.

Liang C, Tan S, Huang Q, Lin J, Lu Z, Lin X (2015) Pratensein ameliorates beta-amyloid-induced cognitive impairment in rats via reducing oxidative damage and restoring synapse and BDNF levels. *Neuroscience Letters* 592: 48–53. https://doi.org/10.1016/j.neulet.2015.03.003.

Liang J, Zhou M, Zhou X, Jin Y, Xu M, Lin J (2013) JcLEA, a novel LEA-like protein from *Jatropha curcas*, confers a high level of tolerance to dehydration and salinity in *Arabidopsis thaliana*. *PloS One* 8: e83056. https://doi.org/10.1371/journal.pone.0083056.

Liang WH, Li L, Zhang F, Liu YX, Li MM, Shi HH, Li H, Shang F, Lou C, Lin QT, Li JJ (2013) Effects of abiotic stress, light, phytochromes and phytohormones on the expression of OsAQP, a rice aquaporin gene. *Plant Growth Regulation* 69: 21-27. https://doi.org/10.1007/s10725-012-9743-x.

Liang Y, Xie L, Liu K, Cao Y, Dai X, Wang X, Lu J, Zhang X, Li X (2021) Bergapten: A review of its pharmacology, pharmacokinetics, and toxicity. *Phytotherapy Research* 35: 6131–6147. https://.doi.org/10.1002/ptr.7221.

Libby P, Shi GP (2007) Mast cells as mediators and modulators of atherogenesis. *Circulation* 115: 2471–2473. https://doi.org/10.1161/CIRCULATIONAHA.107.698480

Li C, Chen Y, Yuan X, He L, Li X, Huang S, Hou S, Liang J (2020) Vitexin ameliorates chronic stress plub high fat diet-induced nonalcoholic fatty liver disease by inhibiting inflammation. *European Journal of Pharmacology* 882: 173264. https://doi.org/10.1016/j.ejphar.2020.173264.

Li C, Zhu Y, Guo X, Sun G, Luo H, Song J, Li Y, Wang L, Qian J, Chen S (2013) Transcriptome analysis reveals ginsenosides biosynthetic genes, microRNAs and simple sequence repeats in *Panax ginseng* C.A. Mayer. *BMC Genomics* 14: 245. https://doi.org/10.1186/1471-2164-14-245.

Li G, Wang H, Cheng X, Su X, Zhao Y, Jiang T, Jin Q, Lin Y, Cai Y (2019) Comparative genomic analysis of the PAL genes in five Rosaceae species and functional identification of Chinese white pear. *PeerJ-Life and Environment* 7: e8064. https://doi.org.10.7717/peerj.8064.

Li HB, Wong CC, Cheng KW, Chen F (2008) Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Science and Technology*. 41:385–390. https://doi.org/10.1016/j.lwt.2007.03.011

Li H, Cao D, Yi J, Cao J, Jian W (2012) Identification of the flavonoids in mungbean (*Phaseolus radiatus* L.) soup and their antioxidant activities. *Food Chemistry* 135: 2942–2946. https://doi.org/10.1016/j.foodchem.2012.07.048.

Li J, Brader G, Palva ET (2008) Kunitz trypsin inhibitor: an antagonist of cell death triggered by phytopathogens and fumonisin b1 in *Arabidopsis*. *Molecular Plant* 1: 482–495. https://doi.org/10.1093/mp/ssn013.

Li J, Li B, Huang H, Han T, Li Y (2019) Allocryptopine: A review of its properties and mechanism of antiarrhythmic effect. *Current Protein and Peptide Science* 20: 996–1003. https://doi.org/10.2174/1389203720666190807123609.

Li L, Lu X, Ma H, Lyu D (2017) Jasmonic acid regulates the ascorbateglutathione cycle in *Malus baccata* Borkh. roots under low rootzone temperature. *Acta Physiologiae Plantarum* 39: 1–11. https://doi.org/10.1007/s11738-017-2469-7.

Lim PO, Nam HG (2007) Aging and senescence of the leaf organ. *Journal of Plant Biology* 50: 291–300. https://doi.org/10.1007/BF03030657.

Li N, Cao L, Cheng Y, Meng ZQ, Tang ZH, Liu WJ, Wang ZZ, Ding G, Xiao W (2014) *In vivo* anti-inflammatory and analgesic activities of strictosamide from *Nauclea officinalis*. *Pharmaceutical Biology* 52: 1445-1450. https://doi.org/10.3109/13880209.2014.895910.

Lin JK, Tsai SH, Lin-Shiau SY (2001) Anti-inflammatory and antitumor effects of flavonoids and flavanoids. *Drugs of the Future* 26: 145–157. https://doi.org/10.1358/dof.2001.026.02.858703.

Lin K, Liu Yq, Xu B, Gao J-L, Fu Y-C, Chen Y, Xue Q, Li Y (2012) Allocryptopine and benzyltetrahydropalmatine block hERG potassium channels expressed in HEK293 cells. *Acta Pharmacologica Sinica* 34: 847–858. https://doi.org/10.1038/aps.2012.176.

Li S, Yu X, Cheng Z, Zeng C, Li W, Zhang L, Peng M (2020) Large-scale analysis of the cassava transcriptome reveals the impact of cold stress on alternative splicing. *Journal of Experimental Botany* 71:422–434. https://doi.org/10.1093/jxb/erz444.

Liu A, Yu Y, Li R, Duan X, Zhu D, Sun X, Duanmu H, Zhu Y (2015) A novel hybrid proline-rich type gene *GsEARLI17* from *Glycine soja* participated in leaf cuticle synthesis and plant tolerance to salt and alkali stresses. *Plant Cell, Tissue and Organ Culture* 121: 633–646. https://doi.org/10.1007/s11240-015-0734-2.

Liu C, Liu H, Lu C, Deng J, Yan Y, Chen H, Wang Y, Liang C-L, Wei J, Han L, Dai Z (2019) Kaempferol attenuates imiquimod-induced psoriatic skin inflammation in a mouse model. *Clinical and Experimental Immunology* 198: 403–415. https://doi.org/10.1111/cei.13363.

Liu C, Xu X, Kan J, ming Cheng Z, Chang Y, Lin J, Li H (2020) Genome-wide analysis of the C3H zinc finger family reveals its functions in salt stress responses of *Pyrus betulaefolia*. *Peer J* 8: e9328. https://doi.org/10.7717/peerj.9328.

Liu F, Huang N, Wang L, Ling H, Sun T, Ahmad W, Muhammad K, Guo J, Xu L, Gao S, Que Y, Su Y (2018) A novel L-ascorbate peroxidase 6 gene, ScAPX6, plays an important role in the regulation of response to biotic and abiotic stresses in sugarcane. *Frontiers in Plant Sciences* 8:2262. https://doi.org/10.3389/fpls.2017.02262

Liu H, Wang X, Zhang H, Yang Y, Ge X, Song F (2008) A rice serine carboxypeptidase-like gene *OsBISCPL1* is involved in regulation of defense responses against biotic and oxidative stress. *Gene* 420: 57–65. https://doi.org/10.1016/j.gene.2008.05.006.

Liu M, Kang B, Wu H, Aranda MA, Peng B, Liu L, Fei Z, Hong N, Gu Q (2023) Transcriptomic and metabolic profiling of watermelon uncovers the role of salicylic acid and flavonoids in the resistance to cucumber green mottle mosaic virus. *Journal of Experimental Botany* 74: 5218–5235. https://doi.org/10.1093/jxb/erad197.

Liu S, Kulp SK, Sugimoto Y, Jiang J, Chang HL, Dowd MK, Wan P, Lin YC (2002) The (-)-enantiomer of gossypol possesses higher anticancer potency than racemic gossypol in human breast cancer. *Anticancer Research* 22: 33–38.

.

Liu X, Van Acker R, Voorend W, Pallidis A, Goeminne G, Pollier J, Morreel K, Kim H, Muylle H, Bosio M, Ralph J (2021) Rewired phenolic metabolism and improved saccharification efficiency of a *Zea mays* cinnamyl alcohol dehydrogenase 2 (zmcad2) mutant. *The Plant Journal* 105:1240–1257. https://doi.org/10.1111/tpj.15108.

Liu Y, Wang L, Xing X, Sun L, Pan J, Kong X, Zhang M, Li D (2013) ZmLEA3, a multifunctional group 3 LEA protein from maize (*Zea mays* L.), is involved in biotic and abiotic stresses. *Plant Cell Physiology* 54: 944–959. https://doi.org/10.1093/pcp/pct047.

Liu Z, Ge XX, Wu XM, Xu Q, Atkinson RG, Guo WW (2020) Genome-wide analysis of the citrus B3 superfamily and their association with somatic embryogenesis. *BMC Genomics* 21: 1–5. https://doi.org/10.1186/s12864-020-6715-9.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25: 402–408. https://doi.org/10.1006/meth.2001.1262

Li X, Guo H, Qi Y, Qi Y, Liu H, Zhang X, Ma P, Liang Z, Dong J (2016) Salicylic acid-induced cytosolic acidification increases the accumulation of phenolic acids in *Salvia miltiorrhiza* cells. *Plant Cell Tissue Organ Culture* 126: 333–341 https://doi.org/10.1007/s11240-016-1001-x.

Li X, Kong Y, Zhao QY, Li YY, Hao P (2016) *De novo* assembly of transcriptome from next-generation sequencing data. *Quantitative Biology* 4: 94–105. https://doi.org/10.1007/s40484-016-0069-y.

Li X, Wang J-N, Huang J-M, Xiong X-K, Chen M-F, Ong C-N, Shen H-M, Yang X-F (2011) Chrysin promotes tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) induced apoptosis in human cancer cell lines. *Toxicology In Vitro* 25: 630–635. https://doi.org/10.1016/j.tiv.2010.12.013.

Li Y, Liu X, Jiang D, Lin Y, Wang Y, Li Q, Liu L, Jin Y-H (2016) Betulin induces reactive oxygen species-dependent apoptosis in human gastric cancer SGC7901 cells. *Archives Pharmacal Research* 39: 1257–1265. https://doi.org/10.1007/s12272-016-0761-5.

Li Y, Zheng X, Yi X, Liu C, Kong D, Zhang J, Gong M (2017) Myricetin: A potent approach for the treatment of type 2 diabetes as a natural class B GPCR agonist. *The FASEB Journal* 31: 2603–2611. https://doi.org/10.1096/fj.201601339R.

Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 4: 118–126. https://doi.org/10.4103/0973-7847.70902.

Löhr J-M, Karimi, M, Omazic B, Kartalis N, Verbeke CS, Berkenstam A, Frödin, J-E (2016) A phase I dose escalation trial of AXP107-11, a novel multi-component crystalline form of genistein, in combination with gemcitabine in chemotherapy-naïve patients with unresectable pancreatic cancer. *Pancreatology* 16: 640–645. http://dx.doi.org/10.1016/j.pan.2016.05.002.

López-Fernández O, Domínguez R, Pateiro M, Munekata PES, Rocchetti G, Lorenzo JM (2020) Determination of polyphenols using liquid chromatography–tandem mass spectrometry technique (LC–MS/MS): A review. *Antioxidants* 9: 1–27. https://doi.org/10.3390/antiox9060479.

Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (2017) Transcriptomics technologies. *PLoS Computational Biology* 13: 1–23. https://doi.org/10.1371/journal.pcbi.1005457.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* 193: 265–275. https://doi.org/10.1016/s0021-9258(19)52451-6.

Lucho-Constantino GG, Zaragoza-Martínez F, Ponce-Noyola T, Cerda-García-Rojas CM, Trejo-Tapia G, Esparza-García F, Ramos-Valdivia AC (2017) Antioxidant responses under jasmonic acid elicitation comprise enhanced production of flavonoids and anthocyanins in *Jatropha curcas* leaves. *Acta Physiologiae Plantarum* 39: 1–10. https://doi.org/10.1007/s11738-017-2461-2.

Luo G, Zhou J, Li G, Hu N, Xia X, Zhou H (2019) Ferruginol diterpenoid selectively inhibits human thyroid cancer growth by inducing mitochondrial dependent apoptosis, endogenous reactive oxygen species (ROS) production, mitochondrial membrane potential loss and suppression of mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling pathways. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* 25: 2935. https://doi.org.10.12659/MSM.914348.

Luo W, Tan J, Li T, Feng Z, Ding Z, Xie X, Chen Y, Chen L, Liu YG, Zhu Q, Guo J (2023) Overexpression of maize GOLDEN2 in rice and maize calli improves regeneration by activating chloroplast development. *Science China Life Sciences* 66: 340–349. https://doi.org/10.1007/s11427-022-2149-2.

Luo Y, Teng S, Yin H, Zhang S, Tuo X, Tran LS (2021) Transcriptome analysis reveals roles of anthocyanin-and jasmonic acid-biosynthetic pathways in rapeseed in response to high light stress. *International Journal of Molecular Sciences* 22:13027. https://doi.org/10.3390/ijms222313027

Lu Y, Wu J, Wang R, Yan Y (2020) Identification of stress defensive proteins in common wheat-Thinopyron intermedium translocation line YW642 developing grains via comparative proteome analysis. *Breeding Science* 70: 517–529. https://doi.org/10.1270/jsbbs.19133.

Lv F, Li S, Feng J, Liu P, Gao Z, Yang Y, Xu Y, Wei J (2019) Hydrogen peroxide burst triggers accumulation of jasmonates and salicylic acid inducing sesquiterpene biosynthesis in wounded *Aquilaria sinesis*. *Journal of Plant Physiology* 234: 167–175. https://doi.org/10.1016/j.jplph.2019.02.006.

Lv G, Guo X, Xie L, Xie C, Zhang X, Yang Y, Xiao L, Tang Y, Pan X, Guo A, Xu H (2017) Molecular characterization, gene evolution, and expression analysis of the fructose-1, 6-bisphosphate aldolase (FBA) gene family in wheat (*Triticum aestivum* L.). *Frontiers in Plant Science* 8: 263430. https://doi.org/10.3389/fpls.2017.01030.

Madhava Chetty K, Sivaji K, Tulasi Rao K (2008) Flowering plants of Chittoor district, Andhra pradesh, India. Published by Students Offset Printers, Tirupati. Pp. 61.

Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S (2012) Antioxidant activity of methanol extracts of different parts of *Lantana camara*. *Asian Pacific Journal of Tropical Biomedicine* 2:960–965. https://doi.org/10.1016/S2221-1691(13)60007-6

Ma K, Xu R, Zhao Y, Han L, Xu Y, Li L, Wang J, Li N (2022) Walnut N-acetylserotonin methyltransferase gene family genome-wide identification and diverse functions characterization during flower bud development. *Frontiers in Plant Science* 13: 861043. https://doi.org/10.3389/fpls.2022.861043.

Maleck K, Dietrich RA (1999) Defense on multiple fronts: how do plants cope with diverse enemies? *Trends in Plant Science* 4: 215–219. https://doi.org/10.1016/s13601385(99)01415-6.

Máñez S, Recio MC, Giner RM, Rios JL (1997) Effect of selected triterpenoids on chronic dermal inflammation. *European Journal of Pharmacology* 334: 103–105. https://doi.org/10.1016/S0014-2999(97)01187-4.

Manikandan R, Thiagarajan R, Goutham G, Arumugam M, Beulaja M, Rastrelli L, Skalicka-Woźniak K, Habtemariam S, Orhan IE, Nabavi SF, Nabavi SM (2016) Zeaxanthin and ocular health, from bench to bedside. *Fitoterapia* 109: 58–66. https://doi.org/10.1016/j.fitote.2015.12.009.

Manivannan A, Soundararajan P, Park YG, Jeong BR (2016) Chemical elicitor-induced modulation of antioxidant metabolism and enhancement of secondary metabolite accumulation in cell suspension cultures of *Scrophularia kakudensis* Franch. *International Journal of Molecular Sciences* 17: 1–13. https://doi.org/10.3390/ijms17030399.

Martel-Pelletier J, Pelletier J, Fahmi H (2003) Cyclooxygenase-2 and prostaglandins in articular tissues. *Seminars in Arthritis and Rheumatism* 33: 155–167. https://doi.org/10.1016/S0049-0172(03)00134-3.

Maury S, Geoffroy P, Legrand M (1999) Tobacco o-methyltransferases involved in phenylpropanoid metabolism. The different caffeoyl-coenzyme A/5-hydroxyferuloyl-coenzyme A 3/5-o-methyltransferase and caffeic acid/5-hydroxyferulic acid 3/5-o-methyltransferase classes have distinct substrate specificities and expression patterns. *Plant Physiology* 121: 215–224. https://doi.org/10.1104/pp.121.1.215.

Mannan A, Ahmed I, Arshad W, Hussain I, Mirza B (2011) Effects of vegetative and flowering stages on the biosynthesis of artemisinin in *Artemisia* species. *Archives of Pharmacal Research* 34: 1657–1661. https://doi.org/10.1007/s12272-011-1010-6.

Marr S, Hageman JA, Wehrens R, van Dam NM, Bruelheide H, Neumann S (2021) LC-MS based plant metabolic profiles of thirteen grassland species grown in diverse neighbourhoods. *Scientific Data* 8:52. https://doi.org/10.1038/s41597-021-00836-8

Marsch-Martinez N, Greco R, Becker JD, Dixit S, Bergervoet JH, Karaba A, de Folter S, Pereira A. (2006) BOLITA, an Arabidopsis AP2/ERF-like transcription factor that affects cell expansion and proliferation/differentiation pathways. *Plant Molecular Biology* 62: 825–843. https://doi.org/10.1007/s11103-006-9059-1

Mehdi SY, Zafaryab M, Nafees S, Khan A, Ahmad I, Hafeez ZB, Rizvi MA (2019) Chrysin sensitizes human lung cancer cells to tumour necrosis factor related apoptosis-inducing ligand (TRAIL) mediated apoptosis. *Asian Pacific Journal of Cancer Biology* 4: 27–33. https://doi.org/10.31557/APJCB.2019.4.2.27-33.

Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G (2006) Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. *Cardiovascular Research* 69:36–45. https://doi.org/10.1016/j.cardiores.2005.09.006

Mejia-Ieniente L, Duran-Flores FDD, Chapa-Oliver AM, Torres-Pacheco I, Cruz-Hernandex A, Gonzalez-Chavira MM, Ocampo-Velazquez RV, Guevara-Gonzalex RG (2013) Oxidative and molecular responses to *Capsicum annuum* L. after hydrogen peroxide, salicylic acid and chitosan-foliar applications. *International Journal of Molecular Science* 14: 10178–10196. https://doi.org/10.3390/ijms140510178.

Mendoza D, Cuaspad O, Arias JP, Ruiz O, Arias M (2018) Effect of phenolic compounds in plant cell suspension cultures of *Thevetia peruviana*. *Biotechnology Reports* 19: 1–9. https://doi.org/10.1016/j.btre.2018.e00273.

Meng Y-L, Jia J-W, Liu C-J, Liang W-Q, Heinstein P, Chen X-Y (1999) Coordinated accumulation of (+)-δ-cadinene synthase mRNAs and gossypol in seeds of *Gossypium hirsutum* and a new member of the *cad*1 family from *G. arboretum*. *Journal of Natural Products* 62: 248–252. https://doi.org/10.1021/np980314o.

Meng Y, Su A, Yuan S, Zhao H, Tan S, Hu C, Deng H, Guo, Y (2016) Evaluation of total flavonoids, myricetin, and quercetin from *Hovenia dulcis* Thunb. as inhibitors of α-amylase and α-glucosidase. *Plant Foods for Human Nutrition* 71: 444–449. https://doi.org/10.1007/s11130-016-0581-2.

Meyer S, De Angeli A, Fernie AR, Martinoia E (2010) Intra- and extra-cellular excretion of carboxylates. *Trends in Plant Sciences,* 15: 40–47. https://doi.org/10.1016/j.tplants.2009.10.002.

Miao Q, Li L-G, Miao S, Hu N, Zhang J, Zhang S, Xie Y-H, Wang J-B, Wang S-W (2012) The bone-protective effect of genistein in the animal model of bilateral ovariectomy: Roles of phytoestrogens and PTH/PTHR1 against post-menopausal osteoporosis. *International Journal of Molecular Sciences*13: 56–70. https://doi.org/10.3390/ijms13010056

Min DY, Jung E, Ahn SS, Lee YH, Lim Y, Shin SY (2020) Chrysoeriol prevents TNFα-induced CYP19 gene expression via EGR-1 downregulation in MCF7 breast cancer cells. *International Journal of Molecular Sciences* 21: 7523. https://doi.org/10.3390/ijms21207523.

Min JW, Hu JJ, He M, Sanchez RM, Huang WX, Liu YQ, Bsoul NB, Han S, Yin J, Liu WH, He XH, Peng BW (2015) Vitexin reduces hypoxia-ischemia neonatal brain injury by the inhibition of HIF-1alpha in a rat pup model. *Neuropharmacology* 99: 38–50. https://doi.org/10.1016/j.neuropharm.2015.07.007.

Minutolo M, Chiaiese P, Di Matteo A, Errico A, Corrado G (2020) Accumulation of ascorbic acid in tomato cell culture: Influence of the genotype, source explant and time of *in vitro* cultivation. *Antioxidants* 9: 222. https://doi.org/10.3390/antiox9030222.

Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB (2014) Reactive oxygen species in inflammation and tissue injury. *Antioxidants and Redox Signaling* 20: 1126–1167. https://doi.org/10.1089/ars.2012.5149.

Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends in Plant Sciences* 9: 490–498. https://doi.org/10.1016/j.tplants.2004.08.009.

Mirzoeva S, Tong X, Bridgeman BB, Plebanek MP, Volpert OV (2018) Apigenin inhibits UV-B-induced skin carcinogenesis: The role of thrombospondin-1 as an anti-inflammatory factor. *Neoplasia* 20: 930–942. https://doi.org/10.1016/j.neo.2018.07.005.

Modarres M, Taghavizadeh Yazdi ME (2021) Elicitation improves phenolic acid content and antioxidant enzymes activity in *Salvia leriifolia* cell cultures. *Iranian Journal of Science and Technology, Transaction A: Science* 45: 849–855. https://doi.org/10.1007/s40995-021-01070-y.

Moharramnejad S, Azam AT, Panahandeh J, Dehghanian Z, Ashraf M (2019) Effect of methyl jasmonate and salicylic acid on *in vitro* growth, stevioside production, and oxidative defense system in *Stevia rebaudiana*. *Sugar Tech* 21: 1031–1038. https://doi.org/10.1007/s12355-019-00727-8.

Moon DO, Kim MO, Choi YH, Lee HG, Kim ND, Kim GY (2008) Gossypol suppresses telomerase activity in human leukemia cells via regulating hTERT. *FEBS Letters* 582: 3367–3373. https://doi.org/10.1016/j.febslet.2008.08.029.

Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M (2007) KAAS: An automatic genome annotation and pathway reconstruction server. *Nucleic Acids Research* 35: 182–185. https://doi.org/10.1093/nar/gkm321.

Morozova O, Hirst M, Marra MA (2009) Applications of new sequencing technologies for transcriptome analysis. *Annual Review of Genomics and Human Genetics* 10: 35–51. https://doi.org/10.1146/annurev-genom-082908-145957.

Morre JL, Permingeat HR, Romagnoli MV, Heisterborg CM, Vallejos RH (1998) Multiple shoot induction and plant regeneration from embryonic axes of cotton. *Plant Cell, Tissue and Organ Culture* 54: 131–136. https://doi.org/10.1023/A:1006170529397.

Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN, Bakri MM (2018) Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences* 25: 361–366. https://doi.org/10.1016/j.sjbs.2017.02.004.

Mukherjee PK, Harwansh RK, Bahadur S, Biswas S, Kuchibhatla LN, Tetali SD, Raghavendra AS (2016) Metabolomics of medicinal plants–a versatile tool for standardization of herbal products and quality evaluation of ayurvedic formulations. *Current Science* 25: 1624–1630. https://www.jstor.org/stable/24909402.

Mukhopadhyay N, Shukla A, Makhal PN, Kaki VR (2023) Natural product-driven dual COX-LOX inhibitors: Overview of recent studies on the development of novel anti-inflammatory agents. *Heliyon* 9: e14569. https://doi.org/10.1016/j.heliyon.2023.e14569.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum* 15: 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Murshed R, Lopez F, Huguette S (2008) Microplate quantification of enzymes of the plant ascorbate-glutathione cycle. *Analytical Biochemistry* 383: 320–322. https://doi.org/10.1016/j.ab.2008.07.020.

Naczk M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis* 41: 1523–1542. https://doi.org/10.1016/j.jpba.2006.04.002.

Naeem H, Momal U, Imran M, Shahbaz M, Hussain M, Alsagaby SA, Abdulmonem WA, Umar M, Mujtaba A, El-Ghorab AE, Ghoneim MM, Shaker ME, Abdelgawad MA, JBawi EA (2023) Anticancer perspectives of genistein: a comprehensive review, *International Journal of Food Properties* 26: 3305-3341. https://doi.org/10.1080/10942912.2023.2281257

Nafie E, Hathout TA, Al Mokadem AS (2011) Jasmonic acid elicits oxidative defense and detoxification system in *Curcumis melo* L. cells. *Brazilian Journal of Plant Physiology* 23: 161–174. https://doi.org/10.1590/S1677-04202011000200008.

Nagalakshmi MAH, Rajesh KK, Prasanna KT, Raghavendra A, Bakthavatsalam N (2020) Evaluation of seed oil of *Givotia moluccana* against *Callosobruchus chinensis* L. *Indian Journal of Entomology* 82: 493–497. https://doi.org/10.5958/0974-8172.2020.00124.8.

Nagy N, Kuipers HF, Frymoyer AR, Ishak HD, Bollyky JB, Wight TN, Bollyky PL (2015) 4-methylumbelliferone treatment and hyaluronan inhibition as a therapeutic strategy in inflammation, autoimmunity, and cancer. *Frontiers in Immunology* 6: 123. https://doi.org/10.3389/fimmu.2015.00123.

Naik PM, Al-Khayri J (2016) Abiotic and biotic elicitors–role in secondary metabolites production through in vitro culture of medicinal plants. In: (Ed.) Shanker AK, Shanker C). *Abiotic and biotic stress in plants – Recent advances and future perspectives*. Chapter 10: 247–277. https://doi.org/10.5772/61442.

Nakajima Y, Shimazawa M, Otsubo K, Ishibashi T, Hara H (2009) Zeaxanthin, a retinal carotenoid, protects retinal cells against oxidative stress. *Current Eye Research* 34: 311–318. https://doi.org/10.1080/02713680902745408.

Nakano Y, Asada K (1981) Hydrogen peroxide isscavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* 22: 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Namdeo AG (2007) Plant cell elicitation for production of secondary metabolites : A review. *Pharmacognosy Reviews* 1: 69–79.

Negri S, Commisso M, Avesani L, Guzzo F (2021) The case of tryptamine and serotonin in plants: a mysterious precursor for an illustrious metabolite. *Journal of Experiment Botany*: 72: 5336–5355. https://doi.org/10.1093/jxb/erab220.

Neog MK, Joshua Pragasam S, Krishnan M, Rasool M (2017) p-Coumaric acid, a dietary polyphenol ameliorates inflammation and curtails cartilage and bone erosion in the rheumatoid arthritis rat model. *BioFactors* 43: 698–717. https://doi.org/10.1002/biof.1377.

Ngo TV, Scarlett CJ, Bowyer MC, Ngo PD, Vuong QV (2017) Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality* 9305047. https://doi.org/10.1155/2017/9305047.

Ngouana V, Zeuko'O Menkem E, Youmbi DY, Yimgang LV, Toghueo RMK, Boyom FF (2021) Serial exhaustive extraction revealed antimicrobial and antioxidant properties of *Platycerium stemaria* (Beauv) Desv. *BioMed Research International* 1584141: 1-9. https://doi.org/10.1155/2021/1584141.

Niazian M (2019) Application of genetics and biotechnology for improving medicinal plants. *Planta* 249: 953–973. https://doi.org/10.1007/s00425-019-03099-1.

Nie C, Wang B, Wang B, Lv N, Yu R, Zhang E (2021) Protopine triggers apoptosis via the intrinsic pathway and regulation of ROS/PI3K/Akt signalling pathway in liver carcinoma. *Cancer Cell International* 21: 396. https://doi.org/10.1186/s12935-021-02105-5.

Ni J, Dong L, Jiang Z, Yang X, Sun Z, Li J, Wu Y, Xu M (2018) Salicylic acid-induced flavonoid accumulation in *Ginkgo biloba* leaves is dependent on red and far-red light. *Industrial Crops and Products* 118: 102–110. https://doi.org/10.1016/j.indcrop.2018.03.044.

Ninfali P, Antonini E, Frati A, Scarpa ES (2017) C-Glycosyl flavonoids from *Beta vulgaris* Cicla and betalains from *Beta vulgaris* Rubra: Antioxidant, anticancer and antiinflammatory activities – A review. *Phytotherapy Research* 31: 871–884. https://doi.org.10.1002/ptr.5819.

Niño M, Kim J, Lee HJ, Abdula SE, Nou IS, Cho YG (2014) Key roles of cysteine protease in different plant pathosystem. *Plant Breeding and Biotechnology* 2: 97–109. https://doi.org/10.9787/PBB.2014.2.2.097.

Niu Y, Ye L, Wang Y, Shi Y, Liu Y, Luo A (2023) Transcriptome analysis reveals salicylic acid treatment mitigates chilling injury in kiwifruit by enhancing phenolic synthesis and regulating phytohormone signaling pathways. *Postharvest Biology and Technology* 205: 112483. https://doi.org/10.1016/j.postharvbio.2023.112483.

Nuruzzaman M, Sharoni AM, Kikuchi S (2013) Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Frontiers in Microbiology* 4: 248. https://doi.org/10.3389/fmicb.2013.00248.

Ogawa D, Yamaguchi K, Nishiuchi T (2007) High-level overexpression of the *Arabidopsis HsfA2* gene confers not only increased themotolerance but also salt/osmotic stress tolerance and enhanced callus growth. *Journal of Experimental Botany* 58: 3373–3383. https://doi.org/10.1093/jxb/erm184.

Okon E, Kukula-Koch W, Halasa M, Jarzab A, Baran M, Dmoszynska-Graniczka M, Angelis A, Kalpoutzakis E, Guz M, Stepulak A, Wawruszak A (2020) Magnoflorine—Isolation and the anticancer potential against NCI-H1299 lung, MDA-MB-468 breast, T98G glioma, and TE671 rhabdomyosarcoma cancer cells. *Biomolecules* 10: 1532. https://doi.org/10.3390/biom10111532.

Oksman-Caldentey KM, Inzé D (2004) Plant cell factories in the post-genomic era: New ways to produce designer secondary metabolites. *Trends in Plant Sciences* 9: 433-440. https://doi.org/10.1016/j.tplants.2004.07.006.

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis. *The Plant Cell* 19: 118–130. https://doi.org/10.1105/tpc.106.047761.

Oliver CL, Bauer JA, Wolter KG, Ubell ML, Narayan A, O'Connell KM, Fisher SG, Wang S, Wu X, Ji M, Carey TE, Bradford CR (2004) *In vitro* effects of the BH3 mimetic, (-)-gossypol, on head and neck squamous cell carcinoma cells. *Clinical Cancer Research* 10: 7757–7763. https://doi.org/10.1158/1078-0432.CCR-04-0551.

Omar A, Kalra RS, Putri J, Elwakeel A, Kaul SC, Wadhwa R (2020) Soyasapogenol-A targets CARF and results in suppression of tumor growth and metastasis in p53 compromised cancer cells. *Scientific Reports* 10: 6323. https://doi.org/10.1038/s41598-020-62953-5.

Onrubia M, Moyano E, Bonfill M, Cusidó RM, Goossens A, Palazón J (2013) Coronatine, a more powerful elicitor for inducing taxane biosynthesis in *Taxus* media cell cultures than methyl jasmonate. *Journal of Plant Physiology* 170: 211–219. https://doi.org/10.1016/j.jplph.2012.09.004.

Pandey A, Swarnkar V, Pandey T, Srivastava P, Kanojiya S, Mishra DK, Tripathi V (2016) Transcriptome and metabolite analysis reveal candidate genes of the cardiac glycoside biosynthetic pathway from *Calotropis procera. Scientific Reports*: 6: 34464. https://doi.org/10.1038/srep34464.

Pandi A, Kalappan VM (2022) Mechanisms involved in the anticancer effects of sinapic acid. *Bulletin of the National Research Centre* 46: 1–9. https://doi.org/10.1186/s42269-022-00943-5.

Pan MH, Lai CS, Ho CT (2010) Anti-inflammatory activity of natural dietary flavonoids. *Food and Function* 1: 15–31. https://doi.org/10.1039/c0fo00103a.

Park CH, Park YJ, Youn JH, Roh J, Kim SK (2023) Brassinosteroids and salicylic acid mutually enhance endogenous content and signaling to show a synergistic effect on pathogen resistance in *Arabidopsis thaliana*. *Journal of Plant Biology* 66:181–192. https://doi.org/10.1007/s12374-023-09390-9

Park JB (2009) Isolation and characterization of N-feruloyltyramine as the P-selectin expression suppressor from garlic *(Allium sativum)*. *Journal of Agriculture and Food Chemistry* 57: 8868–8872. https://doi.org/10.1021/jf9018382.

Park JS, Choi Y, Jeong MG, Jeong YI, Han JH, Choi HK (2023) Uncovering transcriptional reprogramming during callus development in soybean: insights and implications. *Frontiers in Plant Science* 14. https://doi.org/10.3389/fpls.2023.1239917.

Patel DK (2022) Potential benefits of tricetin in medicine for the treatment of cancers and other health-related disorders: medicinal importance and therapeutic benefit. *The Natural Products Journal* 13: https://doi.org/10.2174/2210315512666211221113117.

Patel DK, Kumar Singh G, Husain GM, Prasad SK (2023) Ethnomedicinal importance of patuletin in medicine: Pharmacological activities and analytical aspects. *Endocrine Metabolic & Immune Disorders Drug Targets*. https://doi.org/10.2174/1871530323666230816141740.

Patel K, Gadewar M, Tripathi R, Prasad SK, Patel DK (2012) A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid

"Harmine". *Asian Pacific Journal of Tropical Biomedicine* 2: 660–664. https://doi.org/10.1016/S2221-1691(12)60116-6.

Patel MB, Mishra SM (2012) Magnoflorine from *Tinospora cordifolia* stem inhibits α-glucosidase and is antiglycemic in rats. *Journal of Functional Foods* 4: 79–86. https://doi.org/10.1016/j.jff.2011.08.002.

Paul B, Royston KJ, Li Y, Stoll ML, Skibola CF, Wilson LS, Barnes S, Morrow CD,Trygve O. Tollefsbol TO (2017) Impact of genistein on the gut microbiome of humanized mice and its role in breast tumor inhibition. *PloS One* 12: e0189756. https://doi.org/10.1371/journal.pone.0189756.

Peñas-Fuentes JL, Siles E, Rufino-Palomares EE, Pérez-Jiménez A, Reyes-Zurita FJ, Lupiáñez JA, Fuentes-Almagro C, Peragón-Sánchez J (2021) Effects of erythrodiol on the antioxidant response and proteome of HepG2 cells. *Antioxidants (Basel)* 11: 73. https://doi.org/10.3390/antiox11010073.

Peng T, Jia MM, Liu JH (2015) RNAi-based functional elucidation of PtrPRP, a gene encoding a hybrid proline rich protein, in cold tolerance of *Poncirus trifoliata*. *Frontiers in Plant Science* 6: 808. https://doi.org/10.3389/fpls.2015.00808.

Pérez-López AA, Cirio A, Rivas-Galindo V, Salazar-Aranda R, Torres N (2011) Activity against *Streptococcus pneumoniae* of the essential oil and delta-cadinene isolated from *Schinus molle* Fruit. *Journal of Essential Oil Research* 23: 25–28. https://doi.org/10.1080/10412905.2011.9700477.

Perry SE, Zheng Q, Zheng Y (2016) Transcriptome analysis indicates that GmAGAMOUS-Like 15 may enhance somatic embryogenesis by promoting a dedifferentiated state. *Plant Signaling and Behavior* 11: e1197463. https://doi.org/10.1080/15592324.2016.1197463.

Pieterse CMJ, van Loon LC (1999) Salicylic acid-independent plant defence pathways. *Trends in Plant Sciences* 4: 52–58. https://doi.org/10.1016/s1360-1385(98)01364-8.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A (2017) Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity* 8416763: 1-13. https://doi.org/10.1155/2017/8416763.

Polle A, Otter T, Seifert F (1994) Apoplastic peroxidases and lignification in needles of Norway spruce (*Picea abies* L.) *Plant Physiology* 106: 53–60. https://doi.org/10.1104/pp.106.1.53.

Popović Z, Milošević DK, Stefanović M, Vidaković V, Matić R, Janković J, Bojović S (2020) Variability of six secondary metabolites in plant parts and developmental stages in natural populations of rare *Gentiana pneumonanthe*. *Plant Biosystems* 155: 1–7. https://doi.org/10.1080/11263504.2020.1785966.

Potsenkovskaia E, Tvorogova V, Yakovleva D, Zlydneva N, Lutova L (2022) Novel *NF-Y* genes expressed during somatic embryogenesis in *Medicago truncatula*. *Plant Gene* 31: 100364. https://doi.org/10.1016/j.plgene.2022.100364.

Poulev A, O'Neal JM, Logendra S, Pouleva RB, Timeva V, Garvey KS, Gleba D, Jenkins IS, Halpem BT, Kneer R, Cragg GM, Raskin I (2003) Elicitation, a new window into plant chemodiversity and phytochemical drug discovery. *Journal of medical Chemistry* 46: 2542–2547. https://doi.org/10.1021/jm020359t.

Pratt DE (1992) Natural antioxidants from plant material. Phenolic compounds in food and their effects on health II, *ACS Symposium Series*, Chapter 5, pp. 54-71 https://doi.org/10.1021/bk-1992-0507.ch005.

Preece JE, Imel MR (1991) Plant regeneration from leaf explants of *Rhododendron* 'P.J.M. Hybrids'. *Scientia Horticulturae* 48: 159–170. https://doi.org/10.1016/0304-4238(91)90163-S.

Premathilake AT, Ni J, Shen J, Bai S, Teng Y (2020) Transcriptome analysis provides new insights into the transcriptional regulation of methyl jasmonate-induced flavonoid biosynthesis in pear calli. *BMC Plant Biology* 20: 1–4. https://doi.org/10.1186/s12870-020-02606-x.

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* 269: 337–341. https://doi.org/10.1006/abio.1999.4019.

Proença C, Rufino AT, Santos I, Albuquerque HMT, Silva AMS, Fernandes E, de Oliveira JMPF (2023) Gossypetin is a novel modulator of inflammatory cytokine production and a suppressor of osteosarcoma cell growth. *Antioxidants (Basel)* 12: 1744. https://doi.org/10.3390/antiox12091744.

Przybylskia R, Lee YC, Eskin NAM (1998) Antioxidant and radical-scavenging activities of buckwheat seed component. *Journal of American Oil Chemists' Society* 75: 1595–1601. https://doi.org/10.1007/s11746-998-0099-3.

Pulido P, Perello C, Rodriguez-Concepcion M (2012) New insights into plant isoprenoid metabolism. *Molecular Plant* 5: 964–967. https://doi.org/10.1093/mp/sss088.

Qiao S, Shi R, Liu M, Zhang C, Yang W, Shi X, Jiang X, Wang C, Wang Q (2011) Simultaneous quantification of flavonoids and phenolic acids in *Herba Scutellariae barbatae* and its confused plants by high performance liquid chromatography-tandem mass spectrometry. *Food Chemistry* 129: 1297–1304. https://doi.org/10.1016/j.foodchem.2011.05.064.

Qin LX, Zhang DJ, Huang GQ, Li L, Li J, Gong SY, Li XB, Xu WL (2013)_Cotton *GhHyPRP3* encoding a hybrid proline-rich protein is stress inducible and its overexpression in *Arabidopsis* enhances germination under cold temperature and high salinity stress conditions. *Acta physiologiae plantarum* 35: 1531–1542. https://doi.org/10.1007/s11738-012-1194-5.

Quetglas-Llabrés MM, Quispe C, Herrera-Bravo J, Catarino MD, Pereira OR, Cardoso SM, Dua K, Chellappan DK, Pabreja K, Satija S, Mehta M, Sureda A, Martorell M, Satmbekova D, Yeskaliyeva B, Sharifi-Rad J, Rasool N, Butnariu M, Bagiu IC, Bagiu RV, Calina D, Cho WC (2022) Pharmacological properties of bergapten: Mechanistic and therapeutic aspects. *Oxidative Medicine and Cellular Longevity* 8615242: 1–10. https://doi.org/10.1155/2022/8615242.

Rackova L, Oblozinsky M, Kostalova D, Kettmann V, Bezakova L (2007) Free radical scavenging activity and lipoxygenase inhibition of *Mahonia aquifolium* extract and isoquinoline alkaloids. *Journal of Inflammation* 4: 15. https://doi.org/10.1186/1476-9255-4-15.

Radmark O, Werz O, Steinhilber D, Samuelsson B (2015) 5-Lipoxygenase, a key enzyme for leudotriene biosynthesis in health and disease. *Biochimica et Biophysica Acta* 1851: 331-339. https://doi.org/10.1016/j.bbalip.2014.08.012.

Raghavendra PB (2020) Cancer biology and other disorders: beneficial role of camptothecin and novel derivatives. In: *Camptothecin and Camptothecin Producing Plants*, Chapter-5, Pullaiah T, Raghavendra PB, Karuppusamy S, Raveendran V, Anuradha M (Eds.). Academic Press, pp. 1–46. https://doi.org/10.1016/B978-0-12-820499-3.00001-X.

Rainsford KD (2007) Anti-inflammatory drugs in the 21st century. *Sub-cellular Biochemistry* 42: 3–27. https://doi.org/10.1007/1-4020-5688-5_1.

Rajput A, Aditi K, Shruti T, Madhur K (2023) Borneol: A plant-sourced terpene with a variety of promising pharmacological effects. *The Natural Products Journal* 13: 13–28. https://doi.org/10.2174/2210315512666211221115143.

Rakwal R, Yang G, Komatsu S (2004) Chitinase induced by jasmonic acid, methyl jasmonate, ethylene and protein phosphatase inhibitors in rice. *Molecular Biology Reports* 31: 113–119. https://doi.org/10.1023/b:mole.0000031407.18708.95.

Raman P, DeWitt DL, Nair MG (2008) Lipid peroxidation and cyclooxygenase enzyme inhibitory activities of acidic aqueous extracts of some dietary supplements. *Phytotherapy Research* 22: 204–212. https://doi.org/10.1002/ptr.2287.

Ramazani E, Akaberi M, Emami SA, Tayarani-Najaran Z (2022) Pharmacological and biological effects of alpha-bisabolol: An updated review of the molecular mechanisms. *Life Science*s 304: 120728. https://doi.org/10.1016/j.lfs.2022.120728.

Rambabu M, Upender M, Ujjwala D, Ugandhar T, Praveen M, Swamy NR (2006) *In vitro* zygotic embryo culture of an endangered forest tree *Givotia rottleriformis* and factors affecting its germination and seedling growth. *In Vitro Cellular and Developmental Biology - Plant* 42: 418–421. https://doi.org/10.1079/IVP2006804.

Ramirez-Estrada K, Vidal-Limon H, Hidalgo D, Moyano E, Golenioswki M, Cusidó RM, Palazon J (2016) Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. *Molecules* 21: 182. https://doi.org/10.3390/molecules21020182.

Rao CV, Newmark HL, Reddy BS (1998) Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* 19: 287–290. https://doi.org/10.1093/carcin/19.2.287.

Rao RS, Ravishankar GA (2002) Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances* 20: 101–153. https://doi.org/10.1016/s0734-9750(02)00007-1.

Rastogi S, Meena S, Bhattacharya A, Ghosh S, Shukla RK, Sangwan NS, Lal RK, Gupta MM, Lavania UC, Gupta V, Nagegowda DA (2014) *De novo* sequencing and comparative analysis of holy and sweet basil transcriptomes. *BMC Genomics* 15: 1–8. https://doi.org/10.1186/1471-2164-15-588.

Rathi A, Srivastava AK, Shirwaikar A, Singh Rawat AK, Mehrotra S (2008) Hepatoprotective potential of *Fumaria indica* Pugsley whole plant extracts, fractions and an isolated alkaloid protopine. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 15: 470–477. https://doi.org/10.1016/j.phymed.2007.11.010.

Rattan S, Warghat AR (2023) Comparative analysis of salidroside and rosavin accumulation and expression analysis of biosynthetic genes in salicylic acid and methyl jasmonate elicited cell suspension culture of *Rhodiola imbricata* (Edgew.) *Industrial Crops and Products* 198: 116667. https://doi.org/10.1016/j.indcrop.2023.116667.

Rawdin BJ, Mellon SH, Dhabhar FS, Epel ES, Puterman E, Su Y, Burke HM, Reus VI, Rosser R, Hamilton SP, Nelson JC (2013) Dysregulated relationship of inflammation and oxidative stress in major depression. *Brain, Behavior, and Immunity* 31:143–152. https://doi.org/10.1016/j.bbi.2012.11.011

Razzak ZA, Afzal ST, Najumuddin, Saifullah S, Maharjan R, Bano S, Faizi S, Shah MR, Simje SU (2023) Patuletin from *Tagetes patula*: an inhibitor of MMP-2 and MMP-9 in collagen-induced arthritis rat model and virtual screening analysis. *Revista Brasileira de Farmacognosia* 33: 1207–1222. https://doi.org/10.1007/s43450-023-00446-y

Rebey IB, Bourgou S, Debez IBS, Karoui IJ, Sellami IH, Msaada K, Limam F, Marzouk B (2012) Effects of extraction solvents and provenances on phenolic contents and antioxidant activities of cumin *(Cuminum cyminum* L.*)* seeds. *Food and Bioprocess Technology* 5: 2827–2836. https://doi.org/10.1007/s11947-011-0625-4

Reddanna P (1990) Purification of archidonate 5-lipoxygenase from potato tubers. *Methods in Enzymology* 187: 268–277. https://doi.org/10.1016/0076-6879(90)87031-w.

Reddy KR, Rao GP, Bahadur B (1987) *In vitro* morphogenesis from seedling explants and callus cultures of castor (*Ricinus communis* L.). *Phytomorphology* 37: 337–340.

Ren X, Bo Y, Fan J, Chen M, Xu D, Dong Y, He H, Ren X, Qu R, Jin Y, Zhao W, Xu C, (2016) Dalbergioidin ameliorates doxorubicin-induced renal fibrosis by suppressing the TGF-β signal pathway. *Mediators of Inflammation* 1–10. https://doi.org/10.1155/2016/5147571.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine* 26: 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3.

Rezaei A, Ghanati F, Behmanesh M, Mokhtari-Dizaji M (2011) Ultrasound-potentiated salicylic acid-induced physiological effects and production of taxol in hazelnut (*Corylus avellana* L.) cell culture. *Ultrasound in Medicine and Biology* 37: 1938–1947. https://doi.org/10.1016/j.ultrasmedbio.2011.06.013.

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science* 2: 152–159. https://doi.org/10.1016/S1360-1385(97)01018-2.

Richard EG (2020) The science and (lost) art of psoralen plus UVA. *Phototherapy* 38: 11–23. https://doi.org/10.1016/j.det.2019.08.002.

Rissmann R, Oudshoorn MH, Kocks E, Hennink WE, Ponec M, Bouwstra JA (2008) Lanolin-derived lipid mixtures mimic closely the lipid composition and organization of vernix caseosa lipids. *Biochimica et Biophysica Acta* 1778: 2350–2360. https://doi.org/10.1016/j.bbamem.2008.06.017.

Rochange SF, Wenzel CL, McQueen-Mason SJ (2001) Impaired growth in transgenic plants over-expressing an expansin isoform. *Plant Molecular Biology* 46:581–489.

Rojas CM, Senthil-Kumar M, Tzin V, Mysore KS (2014) Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. *Frontiers in Plant Sciences* 5:17. https://doi.org/10.3389/fpls.2014.00017.Roschek B, Fink RC, Li D, McMichael M, Tower CM, Smith RD, Albert RS (2009) Pro-inflammatory enzymes, cyclooxygenase 1, cyclooxygenase 2, and 5-lipooxygenase, inhibited by stabilized rice bran extracts. *Journal of Medicinal Food* 12: 615–623. https://doi.org/10.1089/jmf.2008.0133.

Rostami Z, Fazeli A, Hojati Z (2022) The isolation and expression analysis of cinnamate 4-hydroxylase and chalcone synthase genes of *Scrophularia striata* under different abiotic elicitors. *Scientific Reports* 12: 8128. https://doi.org/10.1038/s41598-022-12361-8.

Ruan CC, Chen Z, Hu FC, Fan W, Wang XH, Guo LJ, Fan HY, Luo ZW, Zhang ZL (2021) Genome-wide characterization and expression profiling of B3 superfamily during ethylene-induced flowering in pineapple (*Ananas comosus* L.). *BMC Genomics* 22: 1–2. https://doi.org/10.1186/s12864-021-07854-1.

Rudrapal, M, Eltayeb WA, Rakshit G, El-Arabey AA, Khan J, Aldosari SM, Alshehr B, Abdalla M (2023) Dual synergistic inhibition of COX and LOX by potential chemicals from Indian daily spices investigated through detailed computational studies. *Scientific Reports* 13: 1–27. https://doi.org/10.1038/s41598-023-35161-0.

Ruiz-Matute AI, Hernández-Hernández O, Rodríguez-Sánchez S, Sanz ML, Martínez-Castro I (2011) Derivatization of carbohydrates for GC and GC-MS analyses. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences* 879: 1226–1240. https://doi.org/10.1016/j.jchromb.2010.11.013.

Ruiz-May E, Galaz-Ávalos RM, Loyola-Vargas VM (2009) Differential secretion and accumulation of terpene indole alkaloids in hairy roots of *Catharanthus roseus* treated with methyl jasmonate. *Molecular Biotechnology* 41: 278–285. https://doi.org/10.1007/s12033-008-9111-2.

Russo EB, Marcu J. (2017) Cannabis pharmacology: The usual suspects and a few promising leads. *Advances in Pharmacology* 80:67–134. https://doi.org/10.1016/bs.apha.2017.03.004.

Sadik CD, Sies H, Schewe T (2003) Inhibition of 15-lipoxygenases by flavonoids: Structure-activity relations and mode of action. *Biochemical Pharmacology* 65: 773–781. https://doi.org/10.1016/S0006-2952(02)01621-0.

Saeed N, Khan MR, Shabbir M (2012) Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine* 12:1–2. https://doi.org/10.1186/1472-6882-12-221

Saeed S, Ali H, Khan T, Kayani W, Khan MA (2017) Impacts of methyl jasmonate and phenyl acetic acid on biomass accumulation and antioxidant potential in adventitious roots of *Ajuga* *bracteosa* Wall ex Benth., a high valued endangered medicinal plant. *Physiology and Molecular Biology of Plants* 23: 229–237. https://doi.org/10.1007/s12298-016-0406-7.

Saeed SA, Gilani AH, Majoo RU, Shah BH (1997) Anti-thrombotic and anti-inflammatory activities of protopine. *Pharmacological Research* 36: 1–7. https://doi.org/10.1006/phrs.1997.0195.

Salem MA, Perez de Souza L, Serag A, Fernie AR, Farag MA, Ezzat SM, Alseekh S (2020) Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. *Metabolites* 10:37. https://doi.org/10.3390/metabo10010037.

Salzman RA, Brady JA, Finlayson SA, Buchanan CD, Summer EJ, Sun F, Klein PE, Klein RR, Pratt LH, Cordonnier-Pratt MM, Mullet JE (2005) Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. *Plant Physiology* 138: 352–368. https://doi.org/10.1104/pp.104.058206.

Samarghandian S, Azimi-Nezhad M, Borji A, Hasanzadeh M, Jabbari F, Farkhondeh T, Samini M (2016) Inhibitory and cytotoxic activities of chrysin on human breast adenocarcinoma cells by induction of apoptosis. *Pharmacognosy Magazine* 12: S436–S440. https://doi.org/10.4103/0973-1296.191453.

Samuel K, Debashish D, Madhumita B, Padmaja G, Prasad SR, Murthy VBR, Rao PS (2009) *In vitro* germination and micropropagation of *Givotia rottleriformis* Griff. *In Vitro Cellular and Developmental Biology - Plant* 45: 466–473. https://doi.org/10.1007/s11627-008-9181-7.

Sana SS, Dogiparthi LK (2018) Green synthesis of silver nanoparticles using *Givotia moluccana* leaf extract and evaluation of their antimicrobial activity. *Materials Letters* 226: 47–51. https://doi.org/10.1016/j.matlet.2018.05.009.

Saravanakumar K, Park S, Sathiyaseelan A, Kim K-N, Cho S-H, Mariadoss AVA, Wang M-H (2021) Metabolite profiling of methanolic extract of *Gardenia jaminoides* by LC-MS/MS and GC-MS and its anti-diabetic and anti-oxidant activities. *Pharmaceuticals* 14: 1-19. https://doi.org/10.3390/ph14020102.

Sarker U, Oba S (2018) Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. *Scientific Reports* 8: 1–12. https://doi.org/10.1038/s41598-018-34944-0.

Sartini S, Djide MN, Alam G, Arjuna A, Djide NJ (2020) *In vitro* antioxidant and anti-mycobacterial activities of Roselle (*Hibiscus sabdariffa* L.) calyx extract against clinical isolate of multidrug resistant *Mycobacterium tuberculosis*. *Systematic Reviews in Pharmacy* 11.

Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z (2021) Rutin: A flavonoid as an effective sensitizer for anticancer therapy; insights into multifaceted mechanisms and applicability for combination therapy. *Evidence-Based Complementary and Alternative Medicine*. 9913179: 1-10. https://doi.org/10.1155/2021/9913179.

Sateesh Babu D, Yanadaiah JP, Lakshman Kumar D, Siva Sankar Prasad K, Siva Sankar S (2014) Hepatoprotective activity of aqueous ethanolic extract of aerial parts of *Givotia* *moluccana* L. on CCl4-induced hepatotoxicity in rats. *Journal of Global Trends in Pharmeutical Sciences* 5: 1864–1868. http://www.jgtps.com/.../s4cj3F.pdf.

Scarponi C, Butturini E, Sestito R, Madonna S, Cavani A, Mariotto S, Albanesi C (2014) Inhibition of inflammatory and proliferative responses of human keratinocytes exposed to the sesquiterpene lactones dehydrocostuslactone and costunolide. *PloS One* 9: e107904. https://doi.org/10.1371/journal.pone.0107904.

Schacht S, Masood F, Catmull S, Dolan, R, Altabtabaee RL, Grow W, Al-Nakkash L (2017) Dietary genistein influences number of acetylcholine receptors in female diabetic jejunum. *Journal of Diabetes Research* 3568146: 1-9. https://doi.org/10.1155/2017/3568146.

Schneider I, Bucar F (2005) Lipoxygenase inhibitors from natural plant sources. Part 1: Medicinal plants with inhibitory activity on arachidonate 5-lipoxygenase and 5-lipoxygenase [sol] cyclooxygenase. Phytotherapy research: An international journal devoted to pharmacological and toxicological evaluation of natural product derivatives. 19:81–102. https://doi.org/10.1002/ptr.1603

Seeram NP, Nair MG (2002) Inhibition of lipid peroxidation and structure-activity-related studies of the dietary constituents anthocyanins, anthocyanidins, and catechins. *Journal of Agricultural and Food Chemistry* 50: 5308–5312. https://doi.org/10.1021/jf025671q.

Sekiya J, Mizuno K, Kimura O, Shimose N (1990) Ascorbate oxidase in cucumber calli and enhancement of enzyme activity by copper sulfate. *Soil Science and Plant Nutrition* 36: 43–51. https://doi.org/10.1080/00380768.1990.10415708.

Sellapan P, Rohani ER, Noor NM (2018) Sesquiterpene production in methyl jasmonate-induced *Persicaria minor* cell suspension culture. *Sains Malaysiana* 47: 3051–3059. https://doi.org/10.17576/jsm-2018-4712-15.

Sembdner G, Parthier B (1993) The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review of Plant Physiology and Plant Molecular Biology* 44: 569–589. https://doi.org/10.1146/annurev.pp.44.060193.003033.

Sen S, Chakraborty R, Maramsa N, Basak M, Deka S, Dey BK (2015) *In vitro* anti-inflammatory activity of *Amaranthus caudatus* L. leaves. *Indian Journal of Natural Product Resource* 6:326.

Senthil K, Jayakodi M, Thirugnanasambantham P, Lee SC, Duraisamy P, Purushotham PM, Rajasekaran K, Charles SN, Roy IM, Nagappan AK, Kim GS, Lee YS, Natesan S, Min T-S, Yang TJ (2015) Transcriptome analysis reveals *in vitro* cultured *Withania somnifera* leaf and root tissues as a promising source for targeted withanolide biosynthesis. *BMC Genomics* 16: 1–16. https://doi.org/10.1186/s12864-015-1214-0.

Senthilkumar S, Yogeeta SK, Subashini R, Devaki T (2006) Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. *Chemico-Biological Interactions* 160: 252–260. https://doi.org/10.1016/j.cbi.2006.02.004.

Serhan CN (2007) Resolution phase of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annual Review of Immunology* 25: 101–137. https://doi.org/10.1146/annurev.immunol.25.022106.141647.

Seo PJ, Park JM, Kang SK, Kim SG, Park CM (2011) An *Arabidopsis* senescence-associated protein SAG29 regulates cell viability under high salinity. *Planta* 233: 189–200. https://doi.org/10.1007/s00425-010-1293-8.

Shabani L, Ehsanpour AA, Asghari G, Emami J (2009) Glycyrrhizin production by in vitro cultured *Glycyrrhiza glabra* elicited by methyl jasmonate and salicylic acid. *Russian Journal of Plant Physiology* 56: 621–626. https://doi.org/10.1134/S1021443709050069.

Shah M, Alharby HF, Hakeem KR, Ali N, Rahman IU, Munawar M, Anwar Y (2020) *De novo* transcriptome analysis of *Lantana camara* L. revealed candidate genes involved in phenylpropanoid biosynthesis pathway. *Scientific Reports* 10: 13726. https://doi.org/10.1038/s41598-020-70635-5.

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A (2020) Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology* 11: 1–21. https://doi.org/10.3389/fphys.2020.00694.

Sharma A, Sharma S, Kumar A, Kumar V, Sharma AK (2022) Plant secondary metabolites: An introduction of their chemistry and biological significance with physicochemical aspect. In Plant Secondary Metabolites: Physico-Chemical Properties and Therapeutic Applications (pp. 1-45). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-16-4779-6_1

Sheng YN, Luo YH, Liu SB, Xu WT, Zhang Y, Zhang T, Xue H, Zuo WB, Li YN, Wang CY, Jin CH (2020) Zeaxanthin induces apoptosis via ROS-regulated MAPK and AKT signaling pathway in human gastric cancer cells. *OncoTargets and Therapy* 29: 10995–1006. https://doi.org.10.2147/OTT.S272514.

Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y (2005) Extraction of polyphenolics from plant material for functional foods-engineering and technology. *Food Reviews International* 21: 139–166. https://doi.org/10.1081/FRI-200040606.

Shimamura M, Akashi T, Sakurai N, Suzuki H, Saito K, Shibata D, Ayabe S, Aoki T (2007) 2-Hydroxyisoflavanone dehydratase is a critical determinant of isoflavone productivity in hairy root cultures of *Lotus japonicus*. *Plant Cell Physiology* 48: 1652–1657. https://doi.org/10.1093/pcp/pcm125.

Siddiqui MA, Pandey S (2020) Metabolomics: An emerging potential approach to decipher critical illnesses. *Biophysical Chemistry* 267: 106462. 1–14. https://doi.org/10.1016/j.bpc.2020.106462.

Sirikantaramas S, Yamazaki M, Saito K (2008) Mechanisms of resistance to self-produced toxic secondary metabolites in plants. *Phytochemistry Reviews* 7: 467–477. https://doi.org/10.1007/s11101-007-9080-2.

Silva LC, Borgato GB, Wagner VP, Martins MD, Rocha GZ, Lopes MA, Santos-Silva AR, de Castro Júnior G, Kowalski LP, Nor JE, Squarize CH, Castilho RM, Vargas PA (2022) Cephaeline is an inductor of histone H3 acetylation and inhibitor of mucoepidermoid carcinoma cancer stem cells. *Journal of Oral Pathology and Medicine* 51: 553–562. https://doi.org/10.1111/jop.13252.

Singh M, Poddar NK, Singh D, Agrawal S (2020) Foliar application of elicitors enhanced the yield of withanolide contents in *Withania somnifera* (L.) Dunal (variety, Poshita). *3 Biotech* 10: 1–8. https://doi.org/10.1007/s13205-020-2153-2.

Singleton V, Rossi J (1965) Colorimetry of total phenolic compounds with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144-158.

Shittu H, Gray A, Furman B, Young L (2010) Glucose uptake stimulatory effect of akuammicine from *Picralima nitida* (Apocynaceae). *Phytochemistry Letters* 3: 53–55. https://doi.org/10.1016/j.phytol.2009.11.003.

Sivanandhan G, Kapil Dev G, Jeyaraj M, Rajesh M, Arjunan A, Muthuselvam M, Manickavasagam M, Selvaraj N, Ganapathi A (2013) Increased production of withanolide A, withanone, and withaferin A in hairy root cultures of *Withania somnifera* (L.) Dunal elicited with methyl jasmonate and salicylic acid. *Plant Cell, Tissue and Organ Culture* 114: 121–129. https://doi.org/10.1007/s11240-013-0297-z.

Sivanesan SP, Gattu S, Hong J, Chavez-frazier A (2009) Randomized, double-blind, placebo-controlled evaluation of the efficacy of oral psoralen plus ultraviolet A for the treatment of plaque-type psoriasis using the psoriasis area severity index score (improvement of 75% or greater) at 12 weeks. *Journal of the American Academy of Dermatology* 61: 793–798. https://doi.org/10.1016/j.jaad.2009.04.053.

Skoog F, Miller CO (1957) Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symposia of the Society for Experimental Biology* 11: 118–130.

Smith TJ (2000) Squalene: Potential chemopreventive agent. *Expert Opinion on Investigational Drugs* 9: 1841–1848. https://doi.org/10.1517/13543784.9.8.1841.

So HM, Eom HJ, Lee D, Kim S, Kang KS, Lee IK, Baek K-H, Park JY, Kim KH (2018) Bioactivity evaluations of betulin identified from the bark of *Betula platyphylla* var. japonica for cancer therapy. *Archives of Pharmacal Research* 41: 815–822. https://doi.org/10.1007/s12272-018-1064-9.

Soi-ampornkul R, Myint EEP, Thangnipon W, Tantarungsee N, Mitrpant C, Tuchinda P, Nobsathian S, Vatanashevanopakorn C (2022) N-trans-feruloyltyramine protects human neuroblastoma SK-N-SH cell line against H_2O_2-induced cytotoxicity. *Nature Product Communications.* 17: 1-8. https://doi.org/10.1177/1934578X221117312.

Soltani N, Firouzabadi FN, Shafeinia A, Shirali M, Sadr AS (2022) *De novo* transcriptome assembly and differential expression analysis of *Catharanthus roseus* in response to salicylic acid. *Scientific Reports* 12: 17803. https://doi.org/10.1038/s41598-022-20314-4.

Srinivasan C, Liu Z, Heidmann I, Supena ED, Fukuoka H, Joosen R, Lambalk J, Angenent G, Scorza R, Custers JB, Boutilier K (2007) Heterologous expression of the BABY BOOM AP2/ERF transcription factor enhances the regeneration capacity of tobacco (*Nicotiana tabacum* L.). *Planta* 225: 341-351. https://doi.org/10.1007/s00425-006-0358-1.

Srivani A, Krishna Mohan G (2022) Antioxidant activity of various extracts of *Givotia moluccana* (L.) Sreem. *International Journal of Herbal Medicine* 10: 33–38. https://doi.org/10.22271/flora.2022.v10.i6a.843

Stanly C, Bhatt A, Ali HMD, Keng CL, Lim BP (2011) Evaluation of free radical scavenging activity and total phenolic content in the petiole-derived callus cultures of *Zingiber zerumbet* Smith. *Journal of Medicinal Plant Research* 5: 2210–2217. http://www.academicjournals.org/JMPR.

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL (1989) Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *The New England Journal of Medicine* 320: 915–924. https://doi.org/10.1056/NEJM198904063201407.

Stevens R, Truffault V, Baldet P, Gautier H (2017) Ascorbate oxidase in plant growth, development, and stress tolerance. In: *Ascorbic Acid in Plant Growth, Development and Stress Tolerance*, Hossain M, Munné-Bosch S, Burritt D, Diaz-Vivancos P, Fujita M, Lorence A (Eds.). Springer, Cham. https://doi.org/10.1007/978-3-319-74057-7_11.

Sujatha M, Reddy TP (1998) Differential cytokinin effects on the stimulation of in vitro shoot proliferation from meristamatic explants of castor (*Ricinus communis* L.). *Plant Cell Reports* 7: 561–566. https://doi.org/10.1007/s002990050442.

Sun W, Leng L, Yin Q, Xu MM, Huang M, Xu Z, Zhang Y, Yao H, Wang C, Xiong C, Chen S (2019) The genome of the medicinal plant *Andrographis paniculata* provides insight into the biosynthesis of the bioactive diterpenoid neoandrographolide. *Plant Journal* 97: 841–857. https://doi.org/10.1111/tpj.14162.

Sun Z, Zeng J, Wang W, Jia X, Wu Q, Yu D, Mao Y (2020) Magnoflorine suppresses MAPK and NF-κB signaling to prevent inflammatory osteolysis induced by titanium particles *in vivo* and osteoclastogenesis via RANKL *in vitro*. *Frontiers in Pharmacology* 11: 389. https://doi.org/10.3389/fphar.2020.00389.

Suzuki H, Reddy MS, Naoumkina M, Aziz N, May GD, Huhman DV, Sumner LW, Blount JW, Mendes P, Dixon RA (2005) Methyl jasmonate and yeast elicitor induce differential transcriptional and metabolic re-programming in cell suspension cultures of the model legume *Medicago truncatula*. *Planta* 220: 696–707. https://doi.org/10.1007/s00425-004-1387-2.

Tajik S, Zarinkamar F, Soltani BM, Nazari M (2019) Induction of phenolic and flavonoid compounds in leaves of saffron (*Crocus sativus* L.) by salicylic acid. *Scientia Horticulturae* 257: 108751. https://doi.org/10.1016/j.scienta.2019.108751.

Takahama U (1985) Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: Mechanism of antioxidative function. *Phytochemistry* 24: 1443–1446. https://doi.org/10.1016/S0031-9422(00)81040-7.

Takahashi MA, Asada K (1983) Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. *Archives of Biochemistry and Biophysics* 226: 558–566. https://doi.org/10.1016/0003-9861(83)90325-9.

Taniyama Y, Griendling KK (2003) Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension* 42: 1075–1081. https://doi.org/10.1161/01.HYP.0000100443.09293.4F.

Tan KW, Li Y, Paxton JW, Birch NP, Scheepens A (2012) Identification of novel dietary phytochemicals inhibiting the efflux transporter breast cancer resistance protein

(BCRP/ABCG2). *Food Chemistry* 138: 2267–2274. https://doi.org/10.1016/j.foodchem.2012.12.021.

Taştemur Ş, Hacısüleyman L, Karataş Ö, Yulak F, Ataseven H (2023) Anticancer activity of sinapic acid by inducing apoptosis in HT-29 human colon cancer cell line. *Canadian Journal of Physiology and Pharmacology* 101. https://doi.org/10.1139/cjpp-2022-0523.

Tiwari P, Indoliya Y, Chauhan AS, Singh P, Singh PK, Singh PC, Srivastava S, Pande V, Chakrabarty D (2020) Auxin-salicylic acid cross-talk ameliorates OsMYB–R1 mediated defense towards heavy metal, drought and fungal stress. *Journal of Hazardous Materials* 399: 122811. https://doi.org/10.1016/j.jhazmat.2020.122811.

Thammanna, Narayana Rao K (1990) Medicinal plants of Tirumala (1st edition) Tirumala Tirupati Devasthanams (TTD), Tirupati, Andhra Pradesh.

The Gene Ontology Consortium (2021) The Gene Ontology resource: enriching a GOld mine, *Nucleic Acids Research* 49: D325–D334, https://doi.org/10.1093/nar/gkaa1113.

Tong Li, Feng ZQ, Zhang TT, You CX, Chao ZH, Wang XF (2023) The nitrate-responsive transcription factor MdNLP7 regulates callus formation by modulating auxin response. *Journal of Integrative Agriculture* 22: 3022–3033. https://doi.org/10.1016/j.jia.2023.08.007.

Torquato EF, Prioli AJ, Machado MF (1995) Differential alcohol dehydrogenase and malate dehydrogenase isozyme expression in long-term callus tissue cultures of *Cereus peruvianus* (Cactaceae). *Biochemical Genetics* 33: 389–399. https://doi.org/10.1007/BF00554597.

Trivedi PK, Nath P (2004) MaExp1, an ethylene-induced expansin from ripening banana fruit. *Plant Science* 167: 1351–1358. https://doi.org/10.1016/j.plantsci.2004.07.005.

Trumbeckaite S, Bernatoniene J, Majiene D, Jakštas V, Savickas A, Toleikis A (2006) The effect of flavonoids on rat heart mitochondrial function. *Biomedicine and Pharmacotherapy* 60: 245–248. https://doi.org/10.1016/j.biopha.2006.04.003.

Truong DH, Nguyen DH, Ta NT, Bui AV, Do TH, Nguyen HC (2019) Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality* 1 https://doi.org/10.1155/2019/8178294.

Tseng Y-T, Chen C-S, Jong Y-J, Chang F-R, Lo Y-C (2016) Loganin possesses neuroprotective properties, restores SMN protein and activates protein synthesis positive regulator Akt/mTOR in experimental models of spinal muscular atrophy. *Pharmacological Research* 111: 58–75. https://doi.org/10.1016/j.phrs.2016.05.023.

Tsiogkas SG, Apostolopoulou K, Mavropoulos A, Grammatikopoulou MG, Dardiotis E, Zafiriou E, Bogdanos DP (2023) Gallic acid diminishes pro-inflammatory interferon-γ- and interleukin-17-producing sub-populations *in vitro* in patients with psoriasis. *Immunologic Research* 71: 475–487. https://doi.org/10.1007/s12026-023-09361-9.

Tuskan GA, Groover AT, Schmutz J, DiFazio SP, Myburg A, Grattapaglia D, Smart LB, Yin T, Aury JM, Kremer A, Leroy T (2018) Hardwood tree genomics: unlocking woody plant biology. *Frontiers in Plant Science* 9: 1799. https://doi.org/10.3389/fpls.2018.01799.

Ugusman A, Zakaria Z, Hui CK, Nordin NAMM, Mahdy ZA (2012) Flavonoids of *Piper sarmentosum* and its cytoprotective effects against oxidative stress. *Experimental and Clinical Sciences Journal* 11: 705–714.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* 39: 44–84. https://doi.org/10.1016/j.biocel.2006.07.001

van Acker SA, van den Berg DJ, Tromp MN, Griffioen DH, van Bennekom WP, van der Vijgh, WJ, Bast A (1996) Structural aspects of antioxidant activity of flavonoids. *Free Radical Biology and Medicine* 20: 331–342. https://doi.org/10.1016/0891-5849(95)02047-0.

van der Plas LH, van der Pal RH (1983) Induction of alcohol dehydrogenase in explants of potato tuber (*Solanum tuberosum* L.). *Plant Cell Reports* 2: 40–42. https://doi.org/10.1007/BF00269233.

van Loon LC, van Strien EA (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and Molecular Plant Pathology* 55: 85–97. https://doi.org/10.1006/pmpp.1999.0213.

Vaquero MJR, Alberto MR, de Nadra MCM (2007) Antibacterial effect of phenolic compounds from different wines. *Food Control* 18: 93–101. https://doi.org/10.1016/j.foodcont.2005.08.010.

Velayutham NK, Thamaraikani T, Wahab S, Khalid M, Ramachawolran G, Abullais SS, Wong LS, Sekar M, Gan SH, Ebenezer AJ, Ravikumar M, Subramaniyan V, Mat Rani NNI, Wu YS, Jeyabalan S (2023) Stylopine: A potential natural metabolite to activate vascular endothelial growth factor receptor 2 (VEGFR2) in osteosarcoma therapy. *Frontiers in Pharmacology* 14: 1150270. https://doi.org/10.3389/fphar.2023.1150270.

Verhoeven, H.A., De Vos, C.H.R., Bino, R.J, Hall, R.D. (2006) Plant metabolomics strategies based upon quadruple time of flight mass spectrometry (QTOF-MS). In: Saito K, Dixon R, Willmitzer L, eds. *Plant Metabolomics: Biotechnology in Agriculture and Forestry.* Vol. 57. Heidelberg, Germany: Springer Verlag. https://doi.org/10.1007/3-540-29782-0_3.

Verma N, Shukla S (2015) Impact of various factors responsible for fluctuation in plant secondary metabolites. *Journal of Applied Research on Medicinal and Aromatic Plants* 2: 105–113. https://doi.org/10.1016/j.jarmap.2015.09.002.

Verma V, Singh D, Kh R (2020) Sinapic acid alleviates oxidative stress and neuro-inflammatory changes in sporadic model of Alzheimer’s disease in rats. *Brain Sciences* 10: 923. https://doi.org/10.3390/brainsci10120923.

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. *Phytochemical Reviews* 1: 13–25. https://doi.org/10.2174/97816080511441120101.

Vijayalakshmi A, Geetha M, Ravichandiran V (2014) Anti-psoriatic activity of flavonoids from the bark of *Givotia rottleriformis* Griff. Ex Wight. *Iranian Journal of Pharmaceutical Sciences* 10: 81–94. https://doi.org/10.22034/ijps.2014.15254.

Vijayalakshmi A, Ravichandiran V, Masilamani K (2014) Antipsoriatic and inhibitory effects of an oral dosage form containing bioflavonoids on inflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, IL-17 and TNF-α. *Indian Journal of Pharmaceutical Education and Research* 48: 139–148. https://doi.org/10.5530/ijper.48.4s.17.

Vijayalakshmi A, Ravichandiran V, Masilamani K, Jayakumari S (2018) Inhibitory effects of flavonoids isolated from *Givotia rottleriformis* bark and *Cassia tora* leaves on the production of pro-inflammatory cytokines in LPS stimulated human whole blood. *Indian Journal of Natural Products and Resources* 9: 16–21.

Vijayalakshmi A, Ravichandiran V, Masilamani K (2016) Inhibition of cyclooxygenase activity by standardized extract of *Givotia rottleriformis* Griff. ex Wight bark. *Indian Journal of Natural Product Resources* 7: 269–276. http://nopr.niscpr.res.in/handle/123456789/39955.

Vincent SA, Kim JM, Pérez-Salamó I, To TK, Torii C, Ishida J, Tanaka M, Endo TA, Bhat P, Devlin PF, Seki M, Devoto A (2022) Jasmonates and histone deacetylase 6 activate Arabidopsis genome-wide histone acetylation and methylation during the early acute stress response. *BMC Biology* 20: 83. https://doi.org/10.1186/s12915-022-01273-8.

Vom Endt D, Kijne JW, Memelink J (2002) Transcription factors controlling plant secondary metabolism: what regulates the regulators?. *Phytochemistry* 61: 107–114. https://doi.org/10.1016/S0031-9422(02)00185-1.

Wadibhasme PG, Mahesh M, Ghaisas MM, Thakurdesai PA (2011) Anti-asthmatic potential of chrysin on ovalbumin-induced bronchoalveolar hyperresponsiveness in rats. *Pharmaceutical Biology* 49: 508–515. https://doi.org/10.3109/13880209.2010.521754.

Wagner D, Przybyla D, Op den Camp R, Kim C, Landgraf F, Lee KP, Würsch M, Laloi C, Nater M, Hideg E, Apel K (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. *Science* 306: 1183–1185. https://doi.org/10.1126/science.1103178.

Wang A, Wei J, Lu C, Chen H, Zhong X, Lu Y, Li L, Haiding Huang H, Dai Z, Han L (2019) Genistein suppresses psoriasis-related inflammation through a STAT3-NF-κb-dependent mechanism in keratinocytes. *International Immunopharmacology* 69: 270–278. https://doi.org/10.1016/j.intimp.2019.01.054.

Wang B, Ma W, Di Y (2022) Activation of the Nrf2/GPX4 signaling by pratensein from *Trifolium pretense* mitigates ferroptosis in OGD/R-insulted H9c2 cardiomyocytes. *Natural Product Communication* 17: 1–7. https://doi.org/10.1177/1934578X2211153.

Wangensteen H, Miron A, Alamgir M, Rajia S, Samuelsen AB, Malterud KE (2006) Antioxidant and 15-lipoxygenase inhibitory activity of rotenoids, isoflavones and phenolic glycosides from Sarcolobus globosus. *Fitoterapia* 77:290–295. https://doi.org/10.1016/j.fitote.2006.03.017

Wang G, Wang G, Zhang X, Wang F, Song R (2012) Isolation of high quality RNA from cereal seeds containing high levels of starch. *Phytochemical Analysis* 23: 159–163. https://doi.org/10.1002/pca.1337.

Wang G, Li Q, Wang C, Jin C, Ji J, Guan C (2019) A salicylic acid carboxyl methyltransferase-like gene LcSAMT from *Lycium chinense*, negatively regulates the drought response in transgenic tobacco. *Environmental and Experimental Botany* 167: 103833. https://doi.org/10.1016/j.envexpbot.2019.103833.

Wang H, Wang H, Shao H, Tang X (2016) Recent advances in utilizing transcription factors to improve plant abiotic stress tolerance by transgenic technology. *Frontiers in Plant Science* 7: 67. https://doi.org/10.3389/fpls.2016.00067.

Wang H, Wang W, Huang W, Xu H (2017) Effect of salicylic acid on the gene transcript and protein accumulation of flavonoid biosynthesis-related enzymes in *Vitis vinifera* cell suspension cultures. *HortScience* 52: 1772–1779. https://doi.org/10.21273/HORTSCI12515-17.

Wang J, Qian J, Yao L, Lu Y (2015) Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of *Hypericum perforatum*. *Bioresources and Bioprocessing* 2: 1-9. https://doi.org/10.1186/s40643-014-0033-5.

Wang L, Li P, Zhou Y, Gu R, Lu G, Zhang C (2023) Magnoflorine ameliorates collagen-induced arthritis by suppressing the inflammation response via the NF-κB/MAPK signaling pathways. *Journal of Inflammation Research* 31: 2271–2296. https://doi.org/10.2147/JIR.S406298.

Wang L, Zhang Z, Ge R, Zhang J, Liu W, Mou K, Lv S, Mu X (2019) Gossypetin inhibits solar-UV induced cutaneous basal cell carcinoma through direct inhibiting PBK/TOPK protein kinase. *Anti-Cancer Agents in Medicinal Chemistry* 19: 1029–1036. https://doi.org/10.2174/1871520619666190301123131.

Wang M, Li K, Nie Y, Wei Y, Li X (2012) Antirheumatoid arthritis activities and chemical compositions of phenolic compounds-rich fraction from *Urtica atrichocaulis*, an endemic plant to China. *Evidence-Based Complementary and Alternative Medicine* 818230: 1– 10. https://doi.org/10.1155/2012/818230.

Wang X, Cao G, Ding D, Li F, Zhao X, Wang J, Yang Y (2022) Ferruginol prevents degeneration of dopaminergic neurons by enhancing clearance of α-synuclein in neuronal cells. *Fitoterapia* 156: 105066. https://doi.org/10.1016/j.fitote.2021.105066.

Wang X, Howell CP, Chen F, Yin J, Jiang Y (2009) Gossypol-a polyphenolic compound from cotton plant. *Advances in Food and Nutrition Research* 58: 215–263. https://doi.org/10.1016/S1043-4526(09)58006-0.

Wang X, Wang Z, Sidhu PS, Desai UR, Zhou Q (2015) 6-Hydroxyflavone and derivatives exhibit potent anti-inflammatory activity among mono-, di- and polyhydroxylated flavones in kidney mesangial cells. *PloS One* 10: e0116409. https://doi.org/10.1371/journal.pone.0116409.

Wang YD, Wu JC, Yuan YJ (2007) Salicylic acid-induced taxol production and isopentenyl pyrophosphate biosynthesis in suspension cultures of *Taxus chinensis* var. mairei. *Cell Biology International* 31: 1179–1183. https://doi.org/10.1016/j.cellbi.2007.03.038.

Wang Y, Liu Y, Xiao C, Liu L, Hao M, Wang J, Liu X (2014) Simultaneous determination of 15 phenolic constituents of chinese black rice wine by HPLC-MS/MS with SPE. *Journal of Food Science* 79: 3–8. https://doi.org/10.1111/1750-3841.12483.

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: A revolutionary tool for transcriptomics. *Nature Reviews Genetics* 10: 57–63. https://doi.org/10.1038/nrg2484.

Waris M, Kocak E, Gonulalan EM, Demirezer LO, Kir S, Nemutlu E (2022) Metabolomics analysis insight into medicinal plant science. *Trends in Analytical Chemistry* 157: 116795. https://doi.org/10.1016/j.trac.2022.116795.

Warleta F, Campos M, Allouche Y, Sánchez-Quesada C, Ruiz-Mora J, Beltrán G, Gaforio JJ (2020) Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cancer cells. *Food and Chemical Toxicology* 48: 1092–1100. https://doi.org/10.1016/j.fct.2010.01.031.

Weckwerth W, Wenzel K, Fiehn O (2004) Process for the integrated extraction, identification and quantification of metabolites, proteins and RNA to reveal their co-regulation in biochemical networks. *Proteomics* 4: 78–83. https://doi.org/10.1002/pmic.200200500.

Wegiera M, Grabarczyk P, Baraniak B, Smolarz H (2011) Antiradical properties of extracts from roots, leaves and fruits of six Rumex L. species. *Acta Biologica Cracoviensia Series Botanica* 53: 125–131. https://doi.org/10.2478/v10182-011-0018-z.

Wehling M (2014) Non-steroidal anti-inflammatory drug use in chronic pain conditions with special emphasis on the elderly and patients with relevant comorbidities: management and mitigation of risks and adverse effects. *European Journal of Clinical Pharmacology* 70:1159–1172. https://doi.org/10.1007/s00228-014-1734-6.

Wei W, He J, Ruan H, Wang Y (2019) *In vitro* and *in vivo* cytotoxic effects of chrysoeriol in human lung carcinoma are facilitated through activation of autophagy, sub-G1/G0 cell cycle arrest, cell migration and invasion inhibition and modulation of MAPK/ERK signalling pathway. *Journal of Balkan Union of Oncology* 24: 936–942.

Wen H, Xing L, Sun K, Xiao C, Meng X, Yang J (2020) Loganin attenuates intestinal injury in severely burned rats by regulating the toll-like receptor 4/NF-κB signaling pathway. *Experimental and Therapeutic Medicine* 20: 591–598. https://doi.org/10.3892/etm.2020.8725.

Wen T, Hao YJ, An XL, Sun HD, Li YR, Chen X, Piao XC, Lian ML (2019) Improvement of bioactive compound accumulation in cell cultures of *Orostachys cartilaginous* A. Bor. through elicitation with salicylic acid and effect of cell extract on bioactive activity. *Industrial Crops and Products* 139: 111570. https://doi.org/10.1016/j.indcrop.2019.111570.

Wiktorowska E, Długosz M, Janiszowska W (2010) Significant enhancement of oleanolic acid accumulation by biotic elicitors in cell suspension cultures of *Calendula officinalis* L. *Enzyme and Microbial Technology* 46: 14–20. https://doi.org/10.1016/j.enzmictec.2009.09.002.

Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, Conrad J, Vogler B, Rosner H, Kraus W (2008) The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (Immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals. *West Indian Medical Journal* 57: 327–331.

Wilson SA, Roberts SC (2014) Metabolic engineering approaches for production of biochemicals in food and medicinal plants. *Current Opinion in Biotechnology* 26: 174–182. https://doi.org/10.1016/j.copbio.2014.01.006.

Winekenstädde D, Angelis A, Waltenberger B, Schwaiger S, Tchoumtchoua J, König S, Werz O, Aligiannis N, Skaltsounis AL, Stuppner, H (2015) Phytochemical profile of the aerial parts of *Sedum sediforme* and anti-inflammatory activity of myricitrin. *Natural Product Communications* 10: 83–88. https://doi.org/10.1177/1934578x1501000122.

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. *Current Opinion on Plant Biology* 5: 218–223. https://doi.org/10.1016/s1369-5266(02)00256-x.

Woch N, Laha S, Gudipalli P (2023) Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. *In Vitro Cellular and Developmental Biology-Plant* 2: 1–22. https://doi.org/10.1007/s11627-023-10335-7.

Wong SP, Leong LP, Koh JHW (2006) Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* 99: 775–783. https://doi.org/10.1016/j.foodchem.2005.07.058.

Wu A, Lu J, Zhong G, Lu L, Qu Y, Zhang C (2022) Xanthotoxin (8-methoxypsoralen): A review of its chemistry, pharmacology, pharmacokinetics, and toxicity. *Phytotherapy Research* 36: 3805–3832. https://doi.org/10.1002/ptr.7577.

Wu J, Lin L (2002) Ultrasound-induced stress responses of *Panax ginseng* cells: Enzymatic browning and phenolics production. *Biotechnology Progress* 18: 862–866. https://doi.org/10.1021/bp0255210.

Wu Q, Hongyu Li H, Qiu J, Feng H (2014) Betulin protects mice from bacterial pneumonia and acute lung injury. *Microbial Pathogenesis* 75: 21–28. https://doi.org/10.1016/j.micpath.2014.08.005.

Wu Y, Li X, Zhang J, Zhao H, Tan S, Xu W, Pan J, Yang F, Pi E (2022) ERF subfamily transcription factors and their function in plant responses to abiotic stresses. *Frontiers in Plant Science* 13: 1042084. https://doi.org/10.3389/fpls.2022.1042084.

Xiao X, Shi D, Liu L, Wang J, Xie X, Kang T, Deng W (2011) Quercetin suppresses cyclooxygenase-2 expression and angiogenesis through inactivation of P300 signaling. *PloS One* 6. https://doi.org/10.1371/journal.pone.0022934.

Xiao Y, Gao S, Di P, Chen J, Chen W, Zhang L (2009) Methyl jasmonate dramatically enhances the accumulation of phenolic acids in *Salvia miltiorrhiza* hairy root cultures. *Physiologia Plantarum* 137:1–9. https://doi.org/10.1111/j.1399-3054.2009.01257.x

Xiong W-D, Gong J, Xing C (2017) Ferruginol exhibits anticancer effects in OVCAR-3 human ovary cancer cells by inducing apoptosis, inhibition of cancer cell migration and G2/M phase cell cycle arrest. *Molecular Medicine Reports* 16: 7013–7017. https://doi.org/10.3892/mmr.2017.7484.

Xu C, Cao H, Zhang Q, Wang H, Xin W, Xu E, Zhang S, Yu R, Yu D, Hu Y (2018) Control of auxin-induced callus formation by bZIP59–LBD complex in *Arabidopsis* regeneration. *Nature Plants* 4: 108–115. https://doi.org/10.1038/s41477-017-0095-4.

Xu A, Zhan JC, Huang WD (2015) Effects of ultraviolet C, methyl jasmonate and salicylic acid, alone or in combination, on stilbene biosynthesis in cell suspension cultures of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Plant Cell, Tissue and Organ Culture* 122: 197–211. https://doi.org/10.1007/s11240-015-0761-z.

Xu C, Chang P, Guo S, Yang X, Liu X, Sui B, Yu D, Xin W, Hu Y (2024) Transcriptional activation by WRKY23 and derepression by removal of bHLH041 coordinately establish callus pluripotency in *Arabidopsis* regeneration. *The Plant Cell* 36: 158–173. https://doi.org/10.1093/plcell/koad255.

Xu D, Hu MJ, Wang YQ, Cui YL (2019) Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules* 24: 1-15. https://doi.org/10.3390/molecules24061123.

Xu K, Liu K, Wu J, Wang W, Zhu Y, Li C, Zhao M, Wang Y, Li C, Zhao L (2017) A MADS-box gene associated with protocorm-like body formation in *Rosa canina* alters floral organ development in Arabidopsis. *Canadian Journal of Plant Science* 98: 309–317. https://doi.org/10.1139/cjps-2017-0027.

Xu L, Yang D, Wang S, Tang W, Liu M, Davis M, Chen J, Rae JM, Lawrence T, Lippman ME (2005) (−) – Gossypol enhances response to radiation therapy and results in tumor regression of human prostate cancer. *Molecular Cancer Therapeutics* 4: 197–205.

Xu R, Song F, Zheng Z (2006) OsBISAMT1, a gene encoding S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase, is differentially expressed in rice defense responses. *Molecular Biology Reports* 33: 223–231. https://doi.org/10.1007/s11033-005-4823-x.

Xu X, Zhang L, Zhao W, Fu L, Han Y, Wang K, Yan L, Li Y, Zhang XH, Min DH (2021) Genome-wide analysis of the serine carboxypeptidase-like protein family in *Triticum aestivum* reveals TaSCPL184-6D is involved in abiotic stress response. *BMC Genomics* 22: 1–9. https://doi.org/10.1186/s12864-021-07647-6.

Yahfoufi N, Alsadi N, Jambi M, Matar C (2018) The immunomodulatory and anti-inflammatory role of polyphenols. *Nutrients* 10:1618–1640. https://doi.org/10.3390/nu10111618.

Yang H, Wang H, Xu S, Ren J, Tang L, Gong J, Lin Y, Fang H, Su D (2022) Hesperetin, a promising treatment option for diabetes and related complications: A literature review. *Journal of Agricultural and Food Chemistry* 70: 8582–8592. https://doi.org/10.1021/acs.jafc.2c03257.

Yang J, Chen Y, Gao M, Wu L, Xiong S, Wang S, Gao J, Zhao Y, Wang Y (2022) Comprehensive identification of bHLH transcription factors in *Litsea cubeba* reveals candidate gene involved in the monoterpene biosynthesis pathway. *Frontiers in Plant Science* 13: 1081335. https://doi.org/10.3389/fpls.2022.1081335.

Yang K, Ma W, Liang H, Ouyang Q, Tang C, Lai L (2007) Dynamic simulations on the arachidonic acid metabolic network, *PLoS Computational Biology* 3. https://doi.org/10.1371/journal.pcbi.0030055

Yang L, Yang Z-M, Zhang N, Tian Z, Liu, S-B, Zhao, M-G (2014) Neuroprotective effects of vitexin by inhibition of NMDA receptors in primary cultures of mouse cerebral cortical neurons. *Molecular and Cellular Biochemistry* 386: 251–258. https://doi.org/10.1007/s11010-013-1862-9.

Yang R, Li Z, Zou Y, Yang J, Li L, Xu X, Schmalzing G, Nie H, Li G, Liu S, Liang S, Xu C (2021) Gallic acid alleviates neuropathic pain behaviors in rats by inhibiting P2X7 receptor-mediated NF-κB/STAT3 signaling pathway. *Frontiers in Pharmacology* 12: 1–12. https://doi.org/10.3389/fphar.2021.680139.

Yang S, Xu M, Lee EM, Gorshkov K, Shiryaev SA, He S, Sun W, Cheng YS, Hu X, Tharappel AM, Lu B (2018) Emetine inhibits Zika and Ebola virus infections through two molecular mechanisms: inhibiting viral replication and decreasing viral entry. *Cell Discovery* 4: 31. https://doi.org/10.1038/s41421-018-0034-1.

Yang W, Feng C, Kong D, Shi X, Zheng X, Cui Y, Liu M, Zhang L, Wang Q (2010) Simultaneous determination of 15 components in *Radix glehniae* by high performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Food Chemistry* 120: 886–894. https://doi.org/10.1016/j.foodchem.2009.10.063.

Yang Y, Ding T, Xiao G, Huang J, Luo D, Yue M, Su Y, Jiang S, Zeng J, Liu Y (2023) Anti-inflammatory effects of allocryptopine via the target on the CX3CL1–CX3CR1 axis/GNB5/AKT/NF-κB/apoptosis in dextran sulfate-induced mice. *Biomedicines* 11: 464. https://doi.org/10.3390/biomedicines11020464.

Yang Y, Gu Y, Zhao H, Zhang S (2019) Loganin attenuates osteoarthritis in rats by inhibiting il-1β-induced catabolism and apoptosis in chondrocytes via regulation of phosphatidylinositol 3-kinases (PI3K)/Akt. *Medical Science Monitor* 25: 4159–4168. https://doi.org/10.12659/MSM.915064.

Yang Z, Li C, Wang Y, Zhang C, Wu Z, Zhang X, Liu C, Li F (2014) GhAGL15s, preferentially expressed during somatic embryogenesis, promote embryogenic callus formation in cotton (*Gossypium hirsutum* L.). *Molecular Genetics and Genomics* 289: 873–883. https://doi.org/10.1007/s00438-014-0856-y.

Yang Z, Wang X, Gu S, Hu Z, Xu H, Xu C (2008) Comparative study of SBP-box gene family in *Arabidopsis* and rice. *Gene* 407: 1–1. https://doi.org/10.1016/j.gene.2007.02.034.

Yangthong M, Hutadilok-Towatana N, Phromkunthong W (2009) Antioxidant activities of four edible seaweeds from the Southern coast of Thailand. *Plant Foods for Human Nutrition* 64: 218–223. https://doi.org/10.1007/s11130-009-0127-y.

Yaqoob N, Yakovlev IA, Krokene P, Kvaalen H, Solheim H, Fossdal CG (2012) Defence-related gene expression in bark and sapwood of Norway spruce in response to *Heterobasidion parviporum* and methyl jasmonate. *Physiological and Molecular Plant Pathology* 77: 10–16. https://doi.org/10.1016/j.pmpp.2011.11.001.

Ye BB, Shang GD, Pan Y, Xu ZG, Zhou CM, Mao YB, Bao N, Sun L, Xu T, Wang JW (2020) AP2/ERF transcription factors integrate age and wound signals for root regeneration. *The Plant Cell* 32: 226–241. https://doi.org/10.1105/tpc.19.00378.

Yedgar S, Krimsky M, Cohen Y, Flower RJ (2007) Treatment of inflammatory diseases by selective eicosanoid inhibition: A double edged sword? *Trends in Pharmacology Science* 28: 459–464. https://doi.org/10.1016/j.tips.2007.07.005.

Ye J, Mao D, Cheng S, Zhang X, Tan J, Zheng J, Xu F (2020) Comparative transcriptome analysis reveals the potential stimulatory mechanism of terpene trilactone biosynthesis by exogenous salicylic acid in *Ginkgo biloba*. *Industrial Crops and Products* 145: 112104. https://doi.org/10.1016/j.indcrop.2020.112104.

Yoon BH, Jung JW, Lee JJ, Cho YW, Jang CG, Jin C, Oh TH, Ryu JH (2007) Anxiolytic-like effects of sinapic acid in mice. *Life Sciences* 81: 234–240. https://doi.org/10.1016/j.lfs.2007.05.007.

Youn I, Han K-Y, Gurgula A, Wu Z, Lee H, Chea C-T (2022) Chemical constituents of *Entandrophragma angolense* and their anti-inflammatory activity. *Phytochemistry* 201:113276. https://doi.org/10.1016/j.phytochem.2022.113276.

Yousefzadi M, Sharifi M, Behmanesh M, Ghasempour A, Moyano E, Palazon J (2010) Salicylic acid improves podophyllotoxin production in cell cultures of *Linum album* by increasing the expression of genes related with its biosynthesis. *Biotechnology Letters* 32: 1739–1743. https://doi.org/10.1007/s10529-010-0343-4.

You TT, Mao JZ, Yuan TQ, Wen JL, Xu F (2013) Structural elucidation of the lignins from stems and foliage of *Arundo donax* Linn. *Journal of Agricultural and Food Chemistry* 61:5361–5370. https://doi.org/10.1021/jf401277v

Yüce I, Agnaniet H, Morlock GE (2019) New antidiabetic and free-radical scavenging potential of strictosamide in *Sarcocephalus pobeguinii* ground bark extract via effect-directed analysis. *ACS Omega* 4: 5038–5043. https://doi.org/10.1021/acsomega.8b02462.

Yu, M., Wang, S-J., Li, H., Zhang, G-J. (2022) Xanthones from the stems of *Calophyllum membranaceum* Gardn. et Champ. and their anti-inflammatory activity. *Phytochemistry* 200:113246. https://doi.org/10.1016/j.phytochem.2022.113246

Yun KJ, Koh DJ, Kim SH, Park SJ, Ryu JH, Kim DG, Lee JY, Lee KT (2008) Anti-inflammatory effects of sinapic acid through the suppression of inducible nitric oxide synthase, cyclooxygase-2, and proinflammatory cytokines expressions via nuclear factor-κB inactivation. *Journal of Agricultural and Food Chemistry* 56: 10265–10272. https://doi.org/10.1021/jf802095g.

Yu S, Wu XH, Wang M, Liu LL, Ye WQ, Jin MY, Piao XC, Lian, ML (2023) Optimizing elicitation strategy of salicylic acid for flavonoid and phenolic production of fed-batch cultured *Oplopanax elatus* adventitious roots. *Journal of Biotechnology* 368: 1–11. https://doi.org/10.1016/j.jbiotec.2023.04.004.

Zare K, Eidi A, Roghani M, Rohani AH (2015) The neuroprotective potential of sinapic acid in the 6-hydroxydopamine-induced hemi-parkinsonian rat. *Metabolic Brain Disease* 30: 205–213. https://doi.org/10.1007/s11011-014-9604-6.

Zavala-Gómez CE, Rodríguez-deLeón E, Bah MM, Feregrino-Pérez AA, Campos-Guillén J, Amaro-Reyes A, Rodríguez-Morales JA, García-Trejo JF, Flores-Macias A, Figueroa-Brito R, Ramos-López MA (2021) Effect of salicylic acid in the yield of ricinine in *Ricinus communis* under greenhouse condition. *Plants* 10: 1902. https://doi.org/10.3390/plants10091902.

Zayova E, Stancheva I, Geneva M, Petrova M, Vasilevska-Ivanova R (2012) Morphological evaluation and antioxidant activity of in vitro and in vivo derived *Echinacea purpurea* plants. *Open Life Science* 7: 698–707. https://doi.org/10.2478/s11535-012-0054-z.

Zeng Y, Tan X, Zhang L, Long H, Wang B, Li Z, Yuan Z (2015) A fructose-1,6-biphosphate aldolase gene from *Camellia oleifera*: Molecular characterization and impact on salt stress tolerance. *Molecular Breeding* 35. https://doi.org/10.1007/s11032-015-0233-5.

Zheng X, Koopmann B, von Tiedemann A (2019) Role of salicylic acid and components of the phenylpropanoid pathway in basal and cultivar-related resistance of oilseed rape *(Brassica napus)* to *Verticillium longisporum*. *Plants* 8: 491. https://doi.org/10.3390/plants8110491

Zeki ÖC, Eylem CC, Reçber T, Kır S, Nemutlu E (2020) Integration of GC–MS and LC–MS for untargeted metabolomics profiling. *Journal of Pharmaceutical and Biomedical Analysis* 190. https://doi.org/10.1016/j.jpba.2020.113509

Zhang P, Tang Y, Li N-G, Zhu Y, Duan J-A (2014) Bioactivity and chemical synthesis of caffeic acid phenethyl ester and its derivatives. *Molecules* 19: 16458–16476. https://doi.org/10.3390/molecules191016458.

Zhang S, Zhang L, Zou H, Qiu L, Zheng Y, Yang D, Wang Y (2021) Effects of light on secondary metabolite biosynthesis in medicinal plants. *Frontiers in Plant Science* 12: 781236: 1–16. https://doi.org/10.3389/fpls.2021.781236.

Zhang W, Popovich DG (2008) Effect of soyasapogenol A and soyasapogenol B concentrated extracts on HEP-G2 cell proliferation and apoptosis. *Journal of Agriculture and Food Chemistry* 56: 2603–2608. https://doi.org/10.1021/jf0731550.

Zhang Y, Du P, Xiong F, Zhang X, Song H (2022) WRKY genes improve drought tolerance in *Arachis duranensis*. *Frontiers in Plant Science* 13: 910408. https://doi.org/10.3389/fpls.2022.910408.

Zhang Y, Fernie AR (2018) On the role of the tricarboxylic acid cycle in plant productivity. *Journal of Integrative Plant Biology* 60: 1199–1216. https://doi.org/10.1111/jipb.12690.

Zhang Y, Li X, Hao Z, Xi R, Cai Y, Liao X (2016) Hydrogen peroxide-resistant CotA and YjqC of *Bacillus altitudinis* spores are a promising biocatalyst for catalyzing reduction of sinapic acid and sinapine in rapeseed meal. *PloS One* 11: e0158351. https://doi.org/10.1371/journal.pone.0158351.

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnology Advances* 23: 283–333. https://doi.org/10.1016/j.biotechadv.2005.01.003.

Zhao JL, Zhou LG, Wu JY (2010) Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. *Applied Microbiology Biotechnology* 87:137–144. https://doi.org/10.1007/s00253-010-2443-4

Zhao J, Zhu W-H, Hu Q (2001) Selection of fungal elicitors to increase indole alkaloid accumulation in *Catharanthus roseus* suspension cell culture. *Enzyme and Microbial Technology* 28: 666–672. https://doi.org/10.3390/plants10040672.

Zhao L, Chen XJ, Zhu J, Xi YB, Yang X, Hu LD, Ouyang H, Patel SH, Jin X, Lin D, Wu F, Flagg K, Cai H, Li G, Cao G, Lin Y, Chen D, Wen C, Chung C, Wang Y, Qiu A, Yeh E, Wang W, Hu X, Grob S, Abagyan R, Su Z, Tjondro HC, Zhao XJ, Luo H, Hou R, Jefferson J, Perry P, Gao W, Kozak I, Granet D, Li Y, Sun X, Wang J, Zhang L, Liu Y, Yan YB, Zhang K (2015) Lanosterol reverses protein aggregation in cataracts. *Nature* 526: 607–611. https://doi.org/10.1038/nature14650.

Zhao L, Zhang S, Shan C, Shi Y, Wu H, Wu J, Peng D (2021) *De novo* transcriptome assembly of *Angelica dahurica* and characterization of coumarin biosynthesis pathway genes. *Gene* 791: 145713. https://doi.org/10.1016/j.gene.2021.145713.

Zhao Q, Dixon RA (2011) Transcriptional networks for lignin biosynthesis: more complex than we thought?. *Trends in Plant Science* 16:227–233. https://doi.org/10.1016/j.tplants.2010.12.005

Zheng Y-Z, Deng G, Chen D-F, Liang Q, Guo R, Fu Z-M (2018) Theoretical studies on the antioxidant activity of pinobanksin and its ester derivatives: Effects of the chain length and solvent. *Food Chemistry* 240: 323–329. https://doi.org/10.1016/j.foodchem.2017.07.133.

Zhou Y, Ma J, Xie J, Deng L, Yao S, Zeng K (2018) Transcriptomic and biochemical analysis of highlighted induction of phenylpropanoid pathway metabolism of citrus fruit in response to salicylic acid, *Pichia membranaefaciens* and oligochitosan. *Postharvest Biology and Technology* 142: 81–92. https://doi.org/10.1016/j.postharvbio.2018.01.021.

Zhou Z, Zhu C, Cai Z, Zhao F, He L, Lou X, Qi X (2018) Betulin induces cytochrome-c release and apoptosis in colon cancer cells via NOXA. *Oncology Letters* 15: 7319–7327. https://doi:10.3892/ol.2018.8183.

Zhu H, Liang QH, Xiong XG, Wang Y, Zhang ZH, Sun MJ, Lu X, Wu D (2018) Anti-inflammatory effects of p-coumaric acid, a natural compound of *Oldenlandia diffusa*, on arthritis model rats. *Evidence-Based Complementary and Alternative Medicine*. 5198594: 1–9. https://doi.org/10.1155/2018/5198594.

Zhu L, Zhang Y, Guo W, Xu XJ, Wang Q (2014) *De novo* assembly and characterization of *Sophora japonica* transcriptome using RNA-seq. *BioMed Research International*. 750961: 1-9. https://doi.org/10.1155/2014/750961.

Zhu W, Lv C, Wang J, Gao Q, Zhu H, Wen H (2017) Patuletin induces apoptosis of human breast cancer SK-BR-3 cell line via inhibiting fatty acid synthase gene expression and activity. *Oncology letters* 14: 7449–7454. https://doi.org/10.3892/ol.2017.7150.

Zhu Y, Hu X, Wang P, Gao L, Pei Y, Ge Z, Ge X, Li F, Hou Y (2021) GhPLP2 positively regulates cotton resistance to Verticillium wilt by modulating fatty acid accumulation and jasmonic acid signaling pathway. *Frontiers in Plant Science* 12: 749630. https://doi.org/10.3389/fpls.2021.749630.

Zingue S, Gbaweng Yaya AJ, Michel T, Ndinteh DT, Rutz J, Auberon F, Maxeiner S, Chun FK, Tchinda AT, Njamen D, Blaheta RA (2020) Bioguided identification of daucosterol, a compound that contributes to the cytotoxicity effects of *Crateva adansonii* DC (Capparaceae) to prostate cancer cells. *Journal of Ethnopharmacology* 247: 112251. https://doi.org/10.1016/j.jep.2019.112251.

Supplementary Table 1. GC-MS spectral analysis of methanolic bark extract of *G. moluccana*

Peak No.	Putative Metabolites	Similarity	R.T. (mins)	Peak Area %	Exact Mass
	Carbohydrates				
155	α-D-Glucopyranoside	794	39.12	7.129	918.43
149	α-D-Mannopyranoside	742	36.24	0.005	482.24
118	α-L-Galactopyranoside	775	30.94	0.010	394.20
84	D-Fructose	804	26.44	7.832	569.29
93	D-Galactose	864	27.17	2.239	569.29
89	D-Glucose	932	26.92	3.076	569.29
164	D-Turanose	771	40.42	0.284	846.39
124	D-Xylopyranose	810	32.01	0.819	438.21
176	D-Xylose	819	42.35	0.022	438.21
132	Glycosides	834	32.33	1.580	482.24
187	Lyxose	815	44.48	0.017	438.21
170	Mannose	793	41.85	0.085	452.23
151	Melibiose	773	37.94	0.284	918.43
64	Ribonic acid	774	23.86	0.305	526.25
	Carboxylic acids				
37	2-Butenedioic acid (E); Fumaric acid	877	13.20	0.073	260.09
46	2-Butenedioic acid (Z); Maleic acid	708	17.39	0.008	228.14
45	2-Piperidinecarboxylic acid; Pipecolic acid	846	16.95	0.004	273.16
63	4,6-Dioxoheptanoic acid	731	23.72	0.023	374.18
7	α-Hydroxyisovaleric acid	903	4.56	0.001	118.06
75	Benzoic acid	749	24.85	0.087	370.15
34	Butanedioic acid: Succinic acid	865	12.28	0.069	262.11
47	Butanoic acid	838	18.39	0.079	319.18
48	Diethyl Phthalate	849	18.44	0.025	222.09
66	Propanoic acid	712	24.04	0.058	322.15
	Fatty Acids				
90	Tridecanoic acid:Tridecylic acids	781	27.01	0.082	228.21
128	9,12-Octadecadienoic acid: Linoleic acid	771	32.10	0.105	294.26
107	Hexadecanoic acid; Palmitic acid	898	29.29	0.796	328.28
134	Octadecanoic acid: Stearic acid	870	32.78	0.738	356.31
131	trans-9-Octadecenoic acid: Elaidic acid	736	32.23	0.054	354.30
	Polyols/Sugar Alcohols				
92	D-Mannitol	703	27.10	0.267	614.32
99	Myo-Inositol	891	27.63	6.351	612.30
68	Ribitol	708	24.12	0.546	512.27
	Sugar acids				
103	D-Gluconic acid	807	28.42	1.281	628.30
104	L-(+)-Tartaric acid	721	28.52	0.026	438.17
	Terpenoids				
140	Dehydroabietic acid	742	34.67	0.013	372.25
56	Geraniol	810	22.20	0.000	154.14
121	Phytol	748	31.54	0.017	368.35
	Amino acids				
14	l-Valine	864	7.16	0.038	189.12
	Gylcols				
60	2,3-Butanediol	711	23.15	0.016	204.12

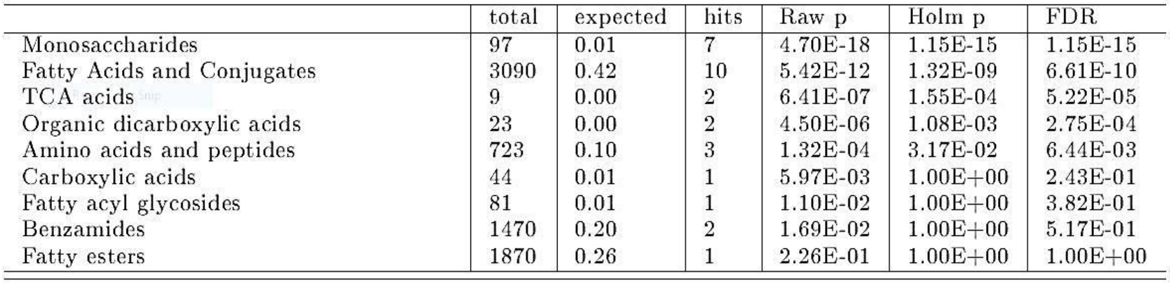
Supplementary Table 2. GC-MS spectral analysis of methanolic leaves extract of *G. moluccana*

Peak No	Putative Metabolites	Similarity	R.T. (mins)	Peak Area %	Exact Mass
	Carbohydrates				
173	α-D-Glucopyranoside	793	39.10	3.095	918.43
103	α-D-Mannopyranoside	731	25.22	0.383	482.24
144	α-D-Xylopyranose	716	32.02	0.809	438.21
122	D-Galactose	771	27.49	1.806	569.29
112	D-Glucose	880	26.80	0.942	569.29
136	D-Mannose	837	30.93	0.082	569.29
160	Glycoside	845	34.17	1.017	482.24
193	Melibiose	835	45.88	0.287	918.43
129	Ribonic acid	766	28.73	0.198	526.25
133	D-Xylopyranose	803	30.36	0.230	438.21
	Carboxylic acids				
102	1,2,3-Propanetricarboxylic acid	776	25.19	2.390	480.19
44	2-Butenedioic acid (E); Fumaric acid	815	13.48	0.157	260.09
4	4-Hydroxyphenylacetic acid	814	3.80	0.069	152.05
125	Benzoic acid	799	27.82	2.617	458.18
41	Butanedioic acid; Succinic acid	869	12.54	0.203	262.11
68	Butanoic acid	907	18.46	0.543	319.18
79	D-Arabinonic acid	764	19.82	0.225	364.16
62	DL-Malic acid	846	17.72	1.128	350.14
126	Isophthalic acid	706	27.85	0.001	274.12
71	L-Threonic acid	858	18.68	0.636	424.20
42	Propanoic acid	856	12.99	0.342	322.15
	Amino Acids				
26	Alanine	830	11.43	0.013	287.21
54	D-Ornithine	701	16.77	0.005	132.09
46	Glycine	731	13.62	0.097	175.07
59	L-Proline	883	17.36	1.645	273.12
11	L-Valine	899	7.21	0.364	189.12
143	Tryptophane	721	31.97	0.000	420.21
81	L-Phenylalanine	797	20.57	0.043	309.16
	Fatty acids				
149	α-Linolenic acid	864	32.24	0.598	350.26
132	Hexadecanoic acid; Palmitic acid	880	29.36	1.336	328.28
153	Octadecanoic acid; Stearic acid	863	32.82	1.564	356.31
	Polyols/Sugar Alcohols				
123	Myo-Inositol	889	27.65	1.433	612.30
90	Ribitol	765	23.44	2.240	512.27
	Sugar Acids				
127	D-Gluconic acid	824	28.49	2.067	628.30

Supplementary Table 3. GC-MS spectral analysis of methanolic pericarp extract of *G. moluccana*

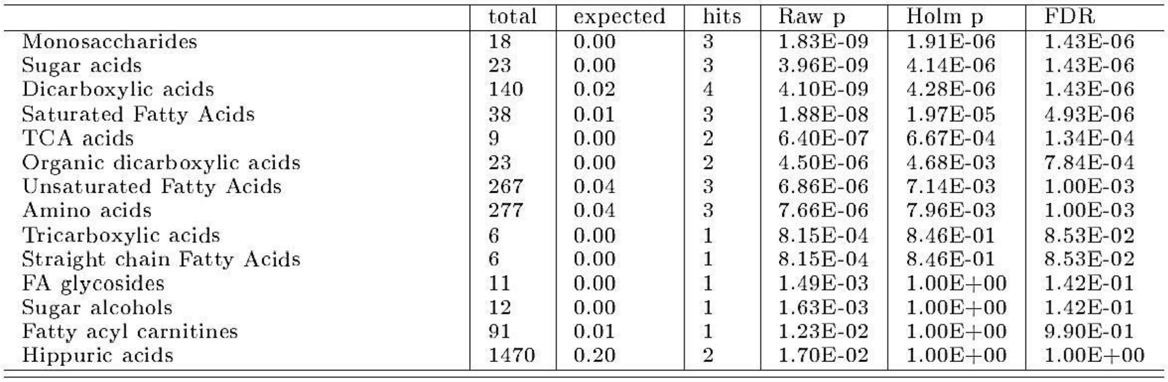
Peak No	Putative Metabolites	Similarity	R.T. (mins)	Peak Area %	Exact Mass
	Carbohydrates				
152	α-D-Glucopyranoside	780	40.18	0.269	918.43
105	D-Fructose	794	27.79	0.531	569.29
129	D-Galactose	842	31.86	0.076	569.29
109	D-Glucose	890	28.39	1.568	569.29
130	D-Mannose	819	32.00	0.058	569.29
154	D-Turanose	779	41.60	0.366	846.39
148	D-Xylopyranose	778	35.29	0.060	438.21
74	Erythro-Pentonic acid; Ribonic acid	722	18.96	2.203	438.21
155	Melibiose	765	41.72	0.134	918.43
176	D-Xylose	728	45.96	0.059	438.21
121	Glycosides	744	30.43	0.227	482.24
	Carboxylic acids				
103	1,2,3-Propanetricarboxylic acid	827	26.47	1.895	480.19
45	2-Butenedioic acid (Z): Maleic acid	895	13.46	0.452	260.09
4	4-Imidazoleacrylic acid	795	5.37	0.000	138.04
73	DL-Malic acid	873	18.95	2.310	350.14
22	Ethanedioic acid;Oxalic acid	702	9.60	3.109	234.07
80	L-Threonic acid	844	19.90	0.662	424.20
59	Mesaconic acid	800	15.95	0.034	274.11
58	Propanedioic acid; Malonic acid	712	15.62	0.358	248.09
50	Propanoic acid	849	14.22	0.268	322.15
56	2-Butenedioic acid (E); Fumaric acid	839	14.71	0.254	260.09
39	Butanedioic acid; Succinic acid	748	12.93	1.377	262.11
	Fatty Acids				
139	9,12-Octadecadienoic acid (Z,Z); Linoleic acid	846	33.51	0.196	352.28
127	9-Octadecenoic acid (Z); Oleic acid	803	31.39	0.091	296.27
112	Acetyl-L-Carnitine	702	28.70	0.058	203.12
138	Trans-9-Octadecenoic acid; Elaidic acid	905	33.49	3.207	354.30
143	Octadecanoic acid, Stearic acid	866	33.96	1.086	356.31
124	Hexadecanoic acid: Palmitic acid	845	30.57	2.465	328.28
	Amino Acids				
27	L-Isoleucine	705	10.30	0.011	203.13
68	L-Proline	874	18.56	0.329	273.12
16	L-Valine	738	8.46	0.074	189.12
	Polyols/Sugar Alcohols				
120	Inositol	700	30.22	0.334	612.30
126	Myo-Inositol	894	31.25	3.504	612.30
81	Ribitol	711	19.93	0.252	512.27
110	D-Mannitol	832	28.57	0.352	614.32
	Others				
119	D-Gluconic acid (Sugar Acids)	797	29.96	0.149	628.30
9	Azulene (Polycyclic aromatic hydrocarbon)	851	5.86	0.006	128.06
197	1,2-Dihydroxyanthraquinone; Alizarin (Anthraquinones)	705	54.22	0.094	384.12

Supplementary Table 4. Over representation analysis (ORA) of methanolic bark extract of *G. moluccana*



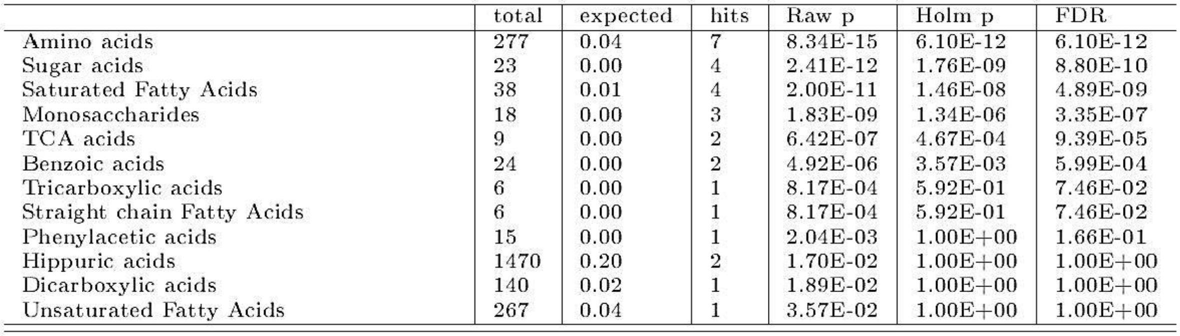
	total	expected	hits	Raw p	Holm p	FDR
Monosaccharides	97	0.01	7	4.70E-18	1.15E-15	1.15E-15
Fatty Acids and Conjugates	3090	0.42	10	5.42E-12	1.32E-09	6.61E-10
TCA acids	9	0.00	2	6.41E-07	1.55E-04	5.22E-05
Organic dicarboxylic acids	23	0.00	2	4.50E-06	1.08E-03	2.75E-04
Amino acids and peptides	723	0.10	3	1.32E-04	3.17E-02	6.44E-03
Carboxylic acids	44	0.01	1	5.97E-03	1.00E+00	2.43E-01
Fatty acyl glycosides	81	0.01	1	1.10E-02	1.00E+00	3.82E-01
Benzamides	1470	0.20	2	1.69E-02	1.00E+00	5.17E-01
Fatty esters	1870	0.26	1	2.26E-01	1.00E+00	1.00E+00

Supplementary Table 5. Over representation analysis (ORA) of methanolic leaves extract of *G. moluccana*



	total	expected	hits	Raw p	Holm p	FDR
Monosaccharides	18	0.00	3	1.83E-09	1.91E-06	1.43E-06
Sugar acids	23	0.00	3	3.96E-09	4.14E-06	1.43E-06
Dicarboxylic acids	140	0.02	4	4.10E-09	4.28E-06	1.43E-06
Saturated Fatty Acids	38	0.01	3	1.88E-08	1.97E-05	4.93E-06
TCA acids	9	0.00	2	6.40E-07	6.67E-04	1.34E-04
Organic dicarboxylic acids	23	0.00	2	4.50E-06	4.68E-03	7.84E-04
Unsaturated Fatty Acids	267	0.04	3	6.86E-06	7.14E-03	1.00E-03
Amino acids	277	0.04	3	7.66E-06	7.96E-03	1.00E-03
Tricarboxylic acids	6	0.00	1	8.15E-04	8.46E-01	8.53E-02
Straight chain Fatty Acids	6	0.00	1	8.15E-04	8.46E-01	8.53E-02
FA glycosides	11	0.00	1	1.49E-03	1.00E+00	1.42E-01
Sugar alcohols	12	0.00	1	1.63E-03	1.00E+00	1.42E-01
Fatty acyl carnitines	91	0.01	1	1.23E-02	1.00E+00	9.90E-01
Hippuric acids	1470	0.20	2	1.70E-02	1.00E+00	1.00E+00

Supplementary Table 6. Over representation analysis (ORA) of methanolic pericarp extract of *G. moluccana*



	total	expected	hits	Raw p	Holm p	FDR
Amino acids	277	0.04	7	8.34E-15	6.10E-12	6.10E-12
Sugar acids	23	0.00	4	2.41E-12	1.76E-09	8.80E-10
Saturated Fatty Acids	38	0.01	4	2.00E-11	1.46E-08	4.89E-09
Monosaccharides	18	0.00	3	1.83E-09	1.34E-06	3.35E-07
TCA acids	9	0.00	2	6.42E-07	4.67E-04	9.39E-05
Benzoic acids	24	0.00	2	4.92E-06	3.57E-03	5.99E-04
Tricarboxylic acids	6	0.00	1	8.17E-04	5.92E-01	7.46E-02
Straight chain Fatty Acids	6	0.00	1	8.17E-04	5.92E-01	7.46E-02
Phenylacetic acids	15	0.00	1	2.04E-03	1.00E+00	1.66E-01
Hippuric acids	1470	0.20	2	1.70E-02	1.00E+00	1.00E+00
Dicarboxylic acids	140	0.02	1	1.89E-02	1.00E+00	1.00E+00
Unsaturated Fatty Acids	267	0.04	1	3.57E-02	1.00E+00	1.00E+00

Supplementary Table 7. List of putative Flavonoids identified from bark extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Flavonoids)	Molecular formula	Bioactivity*
517.10	34.07	C16224	(-)-Medicarpin-3-o-glucoside-6"-malonate	C25H25O12	Unknown
256.00	46.75	C08650	Isoliquiritigenin	C15H12O4	Antioxidant; Anti-inflammtory
301.00	4.12	C00389	Quercetin	C15H9O7	Anti-inflammatory
283.00	19.95	C00814	Biochanin-A	C16H11O5	Antiproliferative
431.00	23.27	C01460	Vitexin	C21H19O10	Anti-inflammatory; Anticancer
283.10	24.72	C01470	Acacetin	C16H11O5	Anti-inflammatory; Anticancer
355.00	13.45	C01590	Kievitone	C20H19O6	Antimicrobial
303.00	23.53	C01617	(+)-Taxifolin	C15H11O7	Anticancer
446.90	19.63	C01821	Isoorientin	C21H19O11	Anti-inflammatory; Antidiabetic
301.00	14.86	C02806	8-Hydroxykaempferol	C15H9O7	Anticancer
319.10	19.53	C02906	(+)-Dihydromyricetin	C15H11O8	Antidiabetic; Anticancer
316.90	12.76	C04109	Gossypetin	C15H9O8	Antioxidant; Antibacterial
446.80	19.37	C05376	Biochanin A-7-O-Glucoside	C22H22O10	Anticancer
275.10	2.15	C09320	(+)-Afzelechin	C15H14O5	Antioxidant; Cardiopratectant
299.00	14.80	C04293	Chrysoeriol	C16H11O6	Antioxidant; Anticancer
314.90	42.32	C04443	3-O-Methylquercetin	C16H11O7	Antioxidant; Anticancer
307.00	45.55	C05906	(2R,3S,4S)-leucocyanidin	C15H14O7	Antiviral
353.00	13.73	C10486	Phaseoluteone/Licoisoflavone A	C20H17O6	Antioxidant
353.00	23.75	C10498	Luteone	C20H17O6	Antioxidant; Antimicrobial
430.90	9.90	C10509	Ononin	C22H22O9	Anti-inflammatory
314.10	19.60	C10516	(+)-Pisatin	C17H14O6	Antimicrobial
299.00	37.99	C10520	Pratensein	C16H11O6	Antioxidant
239.00	36.44	C11264	7-hydroxyflavone	C15H10O3	Anti-inflammatory; Anticancer
256.10	1.01	C09762	(2s)-Liquiritigenin	C15H12O4	Hepatoprotectant
255.00	3.37	C09827	(2S)-Pinocembrin	C15H11O4	Anti-inflammatory; Anticancer
395.00	9.64	C10417	Deguelin	C23H22O6	Anticancer
516.80	18.29	C10020	Apigenin 7-o-(6-malonyl-beta D-glucoside)	C24H21O13	Antimicrobial; Anticancer
252.90	18.89	C10028	Chrysin	C15H9O4	Anti-inflammatory
301.10	18.27	C10078	8-hydroxy-luteolin/Hypolaetin	C15H9O7	Anti-inflammatory
315.00	9.88	C10084	Isorhamnetin	C16H11O7	Antioxidant; Antidiabetic
299.00	20.21	C10098	Kaempferide	C16H11O6	Anti-inflammatory
317.00	7.72	C10107	Myricetin	C15H9O8	Anti-inflammatory
394.80	18.14	C07593	Rotenone	C23H22O6	Anticancer
447.00	18.18	C10114	Orientin	C21H19O11	Anti-inflammatory
330.90	17.52	C10118	Patuletin	C16H11O8	Anti-inflammatory
317.00	22.18	C10122	Quercetagetin	C15H9O8	Antioxidant; Antidiabetic
307.10	10.88	C12127	(+)-Gallocatechin	C15H14O7	Antioxidant
301.10	23.58	C10192	Tricetin	C15H9O7	Anticancer; Dietary
637.00	32.41	C12632	Luteolin 7-o-beta D-diglucuronide	C27H24O18	Anticancer
330.90	48.88	C12633	Laricitrin	C16H11O8	Antioxidant
772.10	21.85	C12635	Kaempferol 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside	C33H40O21	Anti-glycation
789.00	22.68	C12668	Quercetin 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside	C33H40O22	Anti-inflammatory
239.00	44.64	C14137	6-hydroxyflavone	C15H10O3	Anticancer
500.80	37.86	C16191	Malonyldaidzin	C24H21O12	Anti-inflammatory; Anticancer
516.90	33.66	C16192	Malonylgenistin	C24H21O13	Anticancer

301.20	26.45	C01709	(2s)-hesperetin	C16H13O6	Antioxidant
273.90	5.31	C00774	Phloretin	C15H14O5	Antioxidant; Anti-ageing
516.90	27.78	C12642	Pelargonidin-3-o-(6	C24H21O13	Antidiabetic
617.00	24.10	C16289	Cyanidin 3-o-(3",6"-o-dimalonyl-beta Glucopyranoside)	C27H21O17	Anticancer; Pigments
514.90	33.55	C16222	Formononetin-7-O-Glucoside-6"-Malonate	C25H23O12	Antioxidant
301.00	21.53	C05908	Delphinidin	C15H9O7	Antitumor
446.90	39.41	C08604	Cyanidin-3-o-beta D-glucoside	C21H19O11	Antioxidants; Pigments
268.70	11.72	C10205	Coumestrol	C15H8O5	Anti-inflammatory
301.00	35.03	C08577	Bracteatin	C15H9O7	Antioxidant

*As reported in literature

Supplementary Table 8. List of putative Terpenoids identified from bark extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
290.90	8.99	C20158	(13e)-labda-7,13-dien-15-ol	C20H34O	
596.90	19.69	C08580	(3s,3's)-Astaxanthin	C40H52O4	Pigments; Antioxidant
393.00	4.20	C08582	Bixin	C25H29O4	Pigments; Antioxidant
551.00	20.95	C08592	Echinenone	C40H54O	Pigments; Antioxidant
378.90	13.28	C08608	Norbixin	C24H26O4	Pigments; Antioxidant
443.00	19.19	C08618	Betulin	C30H50O2	Anticancer
444.90	19.69	C08621	Dammarenediol II	C30H52O2	Anticancer
440.80	34.90	C08830	24-methylenecycloartanol	C31H52O	Antimicrobial
306.80	19.98	C09072	Alpha Cembratriene-4,6-diol	C20H34O2	Anticancer
290.80	21.13	C09094	Geranylgeraniol	C20H34O	Anticancer
308.80	18.72	C09183	Sclareol	C20H36O2	Anticancer; Fragrant
261.00	16.11	C09680	Hemigossypol	C15H16O4	Anticancer; Anti-microbial
236.90	44.29	C09700	Lubimin	C15H24O2	Unknown
223.10	14.85	C09715	Rishitin	C14H22O2	Antimicrobial
544.90	22.59	C05413	All-trans-Phytoene	C40H64	Pigments
426.80	5.89	C01054	(3s)-2,3-epoxy-2,3-Dihydrosqualene	C30H50O	Anti-inflammatory
223.10	24.08	C01126	(2e,6e)-Farnesol	C15H26O	Antibacterial
374.90	10.65	C01512	Loganate	C16H23O10	Antioxidant
426.90	22.43	C01724	Lanosterol	C30H50O	
263.00	30.87	C06082	2-Cis-Abscisate	C15H19O4	Plant Hormone
536.00	32.03	C07608	Hyperforin	C35H52O4	Anticancer
233.00	30.08	C09382	(+)-Costunolide (12,6alpha)	C15H20O2	Anticancer
231.00	43.05	C09918	Aucuparin	C14H14O3	Anticancer
192.70	3.73	C12287	Beta Ionone	C13H20O	Antioxidant; Fragrant
306.80	45.43	C13273	Plaunotol	C20H34O2	Anticancer; Antiulcer
250.00	18.57	C13453	2-cis,4-trans-Xanthoxin	C15H22O3	Antioxidant
475.20	27.88	C17419	Soyasapogenol A	C30H50O4	Anticancer
428.90	11.92	C06309	Hopan-22-ol	C30H52O	Antimicrobial
171.10	38.64	C17621	(6E)-8-hydroxygeraniol	C10H18O2	Unknown
314.80	43.35	C18015	Momilactone A	C20H26O3	Antidiabetic; Antifungal
444.90	47.93	C19828	Arabidiol	C30H52O2	Antioomycete
445.00	27.58	C19863	Lupan-3beta,20-diol	C30H52O2	

505.00	31.10	C20152	Taxusin	C28H40O8	Anticancer
460.90	1.19	C20715	(20s)-Protopanaxadiol	C30H52O3	
432.80	16.49	C20894	Beta Citraurin	C30H40O2	
280.90	33.94	C15971	Dihydroxyphaseic Acid	C15H21O5	Plant Hormone
443.00	33.15	C20945	Erythrodiol	C30H50O2	Antioxidant
630.80	21.84	C08964	Oleanolate 3 Beta D-glucuronoside	C36H54O9	Antidiabetic
456.90	22.92	C08988	Ursolate	C30H48O3	Antidiabetic
440.70	30.53	C20946	Oleanolic Aldehyde	C30H48O2	Antioxidant

*As reported in literature

Supplementary Table 8. List of putative Alkaloids identified from bark extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloids)	Molecular formula	Bioactivity*
274.90	11.67	C21637	Elwesine	C16H21NO3	Anticancer; Antimicrobial
274.90	39.72	C21639	Maritinamine	C16H21NO3	Unknown
200.00	2.86	C21721	Camalexin	C11H8N2S	Antimicrobial
299.10	25.73	C16352	7-Methylxanthosine	C11H15N4O6	Unknown
370.90	38.84	C16708	(S)-Isoandrocymbine	C21H25NO5	Unknown
498.90	22.15	C16724	Strictosamide	C26H30N2O8	Anti-inflammatory
292.90	47.99	C16726	Cinchoninone	C19H20N2O	Antiplasmodial
292.80	8.33	C11633	16-Epivellosimine	C19H20N2O	Unknown
292.80	20.71	C11634	Vellosimine	C19H20N2O	Unknown
352.90	36.45	C11808	1,2-Dihydrovomilenine	C21H24N2O3	Anticancer
248.90	26.66	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
248.90	33.39	C10772	Lupanine	C15H25N2O	Antioxidant
172.90	7.51	C10173	Swainsonine	C8H15NO3	Anticancer
481.80	20.16	C09421	Emetine	C29H42N2O4	Antimicrobial
313.00	49.19	C06173	Thebaine	C19H22NO3	Antimicrobial
295.00	26.18	C06528	Cinchonine	C19H23N2O	Antimalarial; Antidiabetic
215.00	0.82	C06536	Harmaline	C13H14N2O	Anti-inflammatory
199.90	36.83	C06537	Harmalol	C12H12N2O	Anticancer; Antimicrobial
213.10	39.20	C06538	Harmine	C13H12N2O	Anticancer; Antimicrobial
398.80	35.78	C06543	Solanidine	C27H44NO	Unknown
378.90	34.59	C05193	12-Hydroxydihydrochelirubine	C21H17NO6	Unknown
236.90	6.29	C05204	(S)-Tetrahydroprotoberberine	C17H18N	Anti-inflammatory; Anticancer
349.10	28.76	C05247	10-Hydroxydihydrosanguinarine	C20H15NO5	Unknown
352.80	1.40	C05315	Palmatine	C21H22NO4	Antioxidant; Anti-inflammatory
353.00	34.83	C05189	Protopine	C20H19NO5	Anticancer
392.90	16.56	C05316	Dihydromacarpine	C22H19NO6	Neuroprotective
339.20	25.43	C03329	(S)-Canadine	C20H21NO4	Antioxidant; Anti-caner
341.30	26.72	C04118	(S)-Tetrahydrocolumbamine	C20H23NO4	Unknown
265.10	30.84	C02621	13-Hydroxylupanine	C15H25N2O2	Antioxidant
352.90	9.05	C02151	19E-Geissoschizine	C21H24N2O3	Unknown
348.10	18.81	C01897	Camptothecin	C20H16N2O4	Anticancer
350.10	19.04	C01761	Vomilenine	C21H22N2O3	Unknown
306.90	36.07	C01513	Lupinate	C13H18N6O3	Unknown
140.90	13.24	C00783	Tropinone	C8H14NO	Anticholinergic
161.10	2.36	C00398	Tryptamine	C10H13N2	Psychoactive

*As reported in literature

Supplementary Table 10. List of putative Phenolics identified from bark extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Phenolics)	Molecular formula	Bioactivity*
153.00	9.75	C00230	Protocatechuate	C7H5O4	Anti-inflammatory; Anticancer
312.90	42.34	C02717	N-feruloyltyramine	C18H19NO4	Anti-inflammatory
223.00	15.71	C00482	Sinapate	C11H11O5	Antioxidant; Anti-inflammatory
153.00	24.84	C00628	Gentisate	C7H5O4	Antioxidant: Anti-inflammatory
201.90	37.10	C00758	Bergaptol	C11H6O4	Anticancer
177.10	36.59	C03081	4-Methylumbelliferone	C10H8O3	Anticancer
162.90	11.06	C00811	4-Coumarate	C9H7O3	Antioxidant
185.90	26.39	C09305	Psoralen	C11H6O3	Antipsoriasis
353.00	3.36	C00852	Trans-5-o-caffeoyl-d-quinate	C16H17O9	Antioxidant
209.00	19.22	C05619	5-hydroxyferulate	C10H9O5	Antioxidant
163.00	1.85	C01772	Trans-2-coumarate	C9H7O3	Antioxidant; Anti-inflammatory
163.00	44.55	C05838	Coumarinate	C9H7O3	Antidiabetic
179.00	3.84	C01197	Trans-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
168.90	11.61	C01424	Gallate	C7H5O5	Antioxidant; Anti-cancer
193.00	1.27	C01494	Ferulate	C10H9O4	Antioxidant; Anti-aging
179.10	41.86	C10478	Isomethyleugenol	C11H14O2	Antioxidant
294.10	20.80	C10483	Phaselate	C13H10O8	Unknown
179.10	25.38	C10454	Methyleugenol	C11H14O2	Antioxidant
336.90	13.51	C12208	Trans-5-O-(4-Coumaroyl)-D-Quinate	C16H17O8	Unknown
192.70	19.51	C20465	Coumaryl Acetate	C11H12O3	Antioxidant
163.10	29.85	C12621	3-Hydroxycinnamate	C9H7O3	Anti-inflammatory
264.90	34.07	D03241	Nitecapone	C12H11NO6	Antioxidant

*As reported in literature

Supplementary Table 11. List of putative Steroids and sterol, Stilbenes, Vitamins and co-factors, Glycosides, Anthriquinone, Betacyanin identified from bark extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Steroids and Sterols)	Molecular formula	Bioactivity*
386.80	22.77	C00187	Cholesterol	C27H46O	Unknown
400.90	33.39	C01789	Campesterol	C28H48O	Anti-inflammatory; Antidiabetic
403.00	49.72	C15787	Campestanol	C28H50O	Unknown
419.30	27.68	C15788	(6alpha)-hydroxycampestanol	C28H50O2	Unknown
448.80	24.80	C15791	Teasterone	C28H48O4	Unknown
446.70	14.18	C15792	3-Dehydroteasterone	C28H46O4	Unknown
448.90	14.67	C15793	Typhasterol	C28H48O4	Unknown
382.80	30.10	C05107	7-dehydrodesmosterol	C27H42O	Unknown
412.80	1.51	C05108	14-Demethyllanosterol	C29H48O	Unknown
464.80	25.69	C15794	Castasterone	C28H48O5	Unknown
450.80	47.44	C15802	6-Deoxocastasterone	C28H50O4	Unknown
386.90	33.87	C01189	Lathosterol	C27H46O	Anti-inflammatory; Antidiabetic
466.90	18.16	C15803	6alpha Hydroxy-castasterone	C28H50O5	Unknown
			Stilbenes		
227.00	38.16	C10276	Pinosylvin Monomethylether	C15H14O2	Antioxidant
229.10	46.56	C03582	Trans-Resveratrol	C14H12O3	Anticancer
214.90	23.53	C10254	Dihydropinosylvin	C14H14O2	Antioxidant
213.10	5.21	C01745	Pinosylvin	C14H12O2	Antioxidant
			Vitamins & Co-factors		
174.90	37.56	C00072	L-Ascorbate	C6H7O6	Antioxidant
451.00	7.94	C02059	Phylloquinone	C31H46O2	Coagulation disorder
430.80	4.52	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
			Glycosides		
260.90	29.51	C08334	Lotaustralin	C11H19NO6	Unknown
297.00	16.74	C05342	Psicofuranine	C11H15N5O5	Anticancer
311.00	5.88	C01855	Taxiphyllin	C14H17NO7	Antimicrobial
422.80	13.81	C08411	Glucoiberin	C11H20NO10S3	Antimicrobial
295.00	17.74	C08481	Indican	C14H17NO6	Pigment
406.70	46.48	C08412	Glucoiberverin	C11H20NO9S3	Antimicrobial
370.80	6.05	C21183	(-)-Bursehernin	C21H22O6	Anticancer
252.90	23.94	C10628	(7s)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic
			Anthroquinone		
241.00	48.96	C01474	Alizarin	C14H8O4	Pigment
			Betacynin		
386.20	26.91	C08539	Betanidin	C18H14N2O8	Antioxidant; Pigment

*As reported in literature

Supplementary Table 12. List of putative Flavonoids identified from leaves extract through LC-MS/MS analysis

m/z value	Retention Time (mins	KEGG ID	Putative Compound (Flavonoids)	Molecular Formula	Bioactivity*
256	49.84	C08650	Isoliquiritigenin	C15H12O4	Antioxidant; Anti-inflammatory
301	15.26	C00389	Quercetin	C15H9O7	Anti-inflammatory;
380	19.41	C00616	Quercetin 3-sulfate	C15H8O10S	Antioxidant;Anti-inflammatory
274.8	38.30	C00774	Phloretin	C15H14O5	Antioxidant;
283	5.22	C00814	Biochanin-A	C16H11O5	Antiproliferative
283	13.46	C01470	Acacetin	C16H11O5	Anti-inflamatory; Anticancer
269	49.52	C01477	Apigenin	C15H9O5	Anticancer; Antidiabetic
303.1	31.42	C01617	(+)-Taxifolin	C15H11O7	Anticancer
430.9	37.72	C01714	Isovitexin	C21H19O10	Anti-inflammatory; Anticancer
446.9	22.52	C01821	Isoorientin	C21H19O11	Anti-inflammatory, Antidiabetic
301	22.55	C02806	8-Hydroxykaempferol	C15H9O7	Anticancer
319.1	20.09	C02906	(+)-Dihydromyricetin	C15H11O8	Antidiabetic; Anticancer
316.9	14.46	C04109	Gossypetin	C15H9O8	Antioxidant; Antibacterial
299	10.33	C04293	Chrysoeriol	C16H11O6	Antioxidant; Anticancer
314.9	14.32	C04443	3-o-methylquercetin	C16H11O7	Antioxidant; Anticancer
287	16.36	C05631	(2s)-Eriodictyol	C15H11O6	Anticancer; Anti-inflammatory
256.2	32.02	C09762	(2s)-Liquiritigenin	C15H12O4	Anti-inflammatory
255	9.72	C09827	(2S)-Pinocembrin	C15H11O4	Anti-inflammatory; Anticancer
253	28.65	C10028	Chrysin	C15H9O4	Anti-inflammatory
314.9	47.63	C10084	Isorhamnetin	C16H11O7	Antioxidant; Antidiabetic
299	16.93	C10098	Kaempferide	C16H11O6	Anti-inflammatory
316.9	46.85	C10107	Myricetin	C15H9O8	Anti-inflammatory
317	19.08	C10122	Quercetagetin	C15H9O8	Antioxidant; Antidiabetic
353.1	19.12	C10486	Phaseoluteone/Licoisoflavone A	C20H17O6	Antioxidant
430.8	22.03	C10509	Ononin	C22H22O9	Anti-inflammatory
275	17.87	C09320	(+)-Afzelechin	C15H14O5	Antioxidant
299	40.69	C10520	Pratensein	C16H11O6	Antioxidant
239	13.12	C11264	7-Hydroxyflavone	C15H10O3	Anti-inflammatory; Anticancer
275	48.79	C12124	Apiforol	C15H14O5	Antidiabetic
275.1	45.55	C12128	(-)-Epiafzelechin	C15H14O5	Phytoestrogen
578.8	2.87	C12627	Apigenin 7-O-Neohesperidoside	C27H30O14	Antimicrobial
772.8	33.30	C12635	Kaempferol 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside	C33H40O21	Anti-inflammatory
239	25.19	C14137	6-Hydroxyflavone	C15H10O3	Anticancer
430.9	20.16	C01460	Vitexin	C21H19O10	Anticancer
328.7	6.18	C16188	7-Hydroxy-2',4',5'-trimethoxyisoflavone	C18H16O6	Antilithiatic
500.9	35.44	C16191	Malonyldaidzin	C24H21O12	Anti-inflammatory; Anticancer
431	20.60	C12137	Pelargonidin-3-O-Beta D-Glucoside	C21H19O10	Antidiabetic
446.9	33.55	C08604	Cyanidin-3-O-Beta D-Glucoside	C21H19O11	Antioxidants/Pigments
301.1	27.70	C05908	Delphinidin	C15H9O7	Pigments
514.9	23.11	C16222	Formononetin-7-O-Glucoside-6''-Malonate	C25H23O12	Antioxidant

*As reported in literature

Supplementary Table 13. List of putative Terpenoids identified from leaves extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
410.9	36.92	C00751	Squalene	C30H50	Anticancer
223	44.90	C01126	(2e,6e)-Farnesol	C15H26O	Antibacterial
296.8	20.75	C01389	Phytol	C20H40O	Antioxidant; Antimicrobial
155.1	20.34	C01411	(-)-Borneol	C10H18O	Antimicrobial
389.1	25.59	C01852	Secologanin	C17H24O10	Neuroprotective
390.8	41.88	C01433	Loganin	C17H26O10	Anti-inflammatory
374.9	0.69	C01512	Loganate	C16H23O10	Antioxidant
538.9	31.95	C05434	Beta Zeacarotene	C40H58	Pigments
263	3.05	C06082	2-cis-Abscisate	C15H19O4	Plant Hormone
204.7	21.49	C06394	(+)-Delta Cadinene	C15H24	Antimicrobial; Fragrant
553.1	30.58	C08590	Zeinoxanthin	C40H56O	Antioxidants; Pigments
378.9	5.06	C08608	Norbixin	C24H26O4	Pigments; Antioxidant
444.9	13.86	C08621	Dammarenediol II	C30H52O2	Anticancer
441	34.61	C08830	24-Methylenecycloartanol	C31H52O	Antimicrobial
307	33.43	C09072	Alpha Cembratriene-4,6-diol	C20H34O2	Anticancer
290.6	34.90	C09094	Geranylgeraniol	C20H34O	Anticancer
308.9	16.57	C09183	Sclareol	C20H36O2	Anticancer; Fragrant
233	48.25	C09382	(+)-Costunolide (12,6alpha)	C15H20O2	Anticancer
223.1	21.30	C09621	(-)-Alpha Bisabolol	C15H26O	Anti-inflammatory
223.1	30.19	C09663	Alpha Eudesmol	C15H26O	Hepatoprotectant
261	19.24	C09680	Hemigossypol	C15H16O4	Anticancer; Anti-microbial
237	18.36	C09700	Lubimin	C15H24O2	Unknown
279	21.17	C09707	Phaseic Acid	C15H19O5	Plant hormone
223	31.85	C09715	Rishitin	C14H22O2	Antimicrobial
456.8	24.93	C08988	Ursolate	C30H48O3	Antidiabetic
544.7	24.31	C11700	10-deacetylbaccatin III	C29H36O10	Anticancer
231	13.24	C09918	Aucuparin	C14H14O3	Anticancer
330.8	43.27	C11896	Taxa-4(20),11-dien-5-alpha Yl Acetate	C22H34O2	Unknown
193	46.78	C12287	Beta Ionone	C13H20O	Antioxidant; Fragrant
428.9	44.76	C06309	Hopan-22-ol	C30H52O	Antimicrobial
250	13.06	C13453	2-cis,4-trans-Xanthoxin	C15H22O3	Antioxidant
566.8	34.54	C15966	3-Hydroxyechinenone	C40H54O2	Pigments
306.8	47.23	C13273	Plaunotol	C20H34O2	Anticancer
281	22.28	C15971	Dihydroxyphaseic Acid	C15H21O5	Plant hormone
440.8	24.37	C20946	Oleanolic Aldehyde	C30H48O2	Anti-inflammatory,
456.9	20.01	C17420	Soyasapogenol E	C30H48O3	Hepatoprotectant
432.8	13.32	C20894	Beta Citraurin	C30H40O2	Antioxidant
171.1	33.22	C17621	(6E)-8-Hydroxygeraniol	C10H18O2	Unknown
314.8	17.39	C18015	Momilactone A	C20H26O3	Antidiabetic; Antifungal
504.8	17.37	C20152	Taxusin	C28H40O8	Anticancer
290.9	33.72	C20158	(13E)-Labda-7,13-Dien-15-ol	C20H34O	Unknown
290.9	44.83	C20481	Cis-Abienol	C20H34O	Fragrant
629.9	29.23	C08964	Oleanolate 3 Beta D-glucuronoside	C36H54O9	Antidiabetic
535.9	27.60	C07608	Hyperforin	C35H52O4	Anticancer

*As reported in literature

Supplementary Table 14. List of putative Alkaloids identified from leaves extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloids)	Molecular formula	Bioactivity*
323	35.63	C09025	Akuammicine	C20H23N2O2	Anticancer
161.1	7.75	C00398	Tryptamine	C10H13N2	Psychoactive
141	14.30	C00783	Tropinone	C8H14NO	Anticholinergic
368.9	23.25	C02134	Allocryptopine	C21H23NO5	Neuroprotective
352.9	43.52	C02151	19E-Geissoschizine	C21H24N2O3	Unknown
323	29.59	C05175	(S)-Stylopine	C19H17NO4	Antimicrobial
369	35.29	C05190	6-Hydroxyprotopine	C20H19NO6	Antimicrobial
363	2.51	C05194	Dihydrochelirubine	C21H17NO5	Anti-tuberculosis
236.6	6.83	C05204	(S)-Tetrahydroprotoberberine	C17H18N	Anti-inflammatory; Anticancer
352.9	18.29	C05315	Palmatine	C21H22NO4	Antioxidant; Anti-inflammatory
368.9	32.85	C03309	Strictosidine Aglycone	C21H25N2O4	Anticancer
339.2	33.72	C03329	(S)-Canadine	C20H21NO4	Antioxidant; Anticancer
312.6	46.56	C06173	Thebaine	C19H22NO3	Antimicrobial
173	19.44	C10173	Swainsonine	C8H15NO3	Anticancer
215	8.19	C06536	Harmaline	C13H14N2O	Anti-inflammatory
199.9	12.24	C06537	Harmalol	C12H12N2O	Anticancer; Antimicrobial
398.7	11.81	C06543	Solanidine	C27H44NO	Unknown
468.7	6.20	C09390	Cephaeline	C28H40N2O4	Antioxidant
342.8	30.79	C09581	(S)-Magnoflorine	C20H24NO4	Anti-inflammatory
248.9	17.69	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
248.9	26.47	C10772	Lupanine	C15H25N2O	Antioxidant
334.8	43.93	C11807	Vinorine	C21H22N2O2	Anti-inflammatory
292.7	9.79	C11633	16-Epivellosimine	C19H20N2O	Unknown
292.7	48.59	C11634	Vellosimine	C19H20N2O	Unknown
498.8	8.28	C16724	Strictosamide	C26H30N2O8	Anti-inflammatory
274.9	19.99	C21639	Maritinamine	C16H21NO3	Unknown
292.9	38.91	C16726	Cinchoninone	C19H20N2O	Antiplasmodial
274.9	3.29	C21637	Elwesine	C16H21NO3	Anticancer; Antimicrobial
200	2.96	C21721	Camalexin	C11H8N2S	Antimicrobial

*As reported in literature

Supplementary Table 15. List of putative Phenolics identified from leaves extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Phenolics)	Molecular formula	Bioactivity*
153	43.00	C00230	Protocatechuate	C7H5O4	Anti-inflammatory; Anticancer
168.8	14.36	c01424	Gallate	C7H5O5	Antioxidant; Anticancer
223	8.14	C00482	Sinapate	C11H11O5	Antioxidant; Anti-inflammatory
153.1	20.70	C00628	Gentisate	C7H5O4	Antioxidant: Anti-inflammatory
202	1.78	C00758	Bergaptol	C11H6O4	Anticancer
162.9	28.54	C00811	4-coumarate	C9H7O3	Antioxidant
336.9	15.07	C12208	Trans-5-o-(4-coumaroyl)-d-Quinate	C16H17O8	Unknown
353	9.27	C00852	Trans-5-o-caffeoyl-d-quinate	C16H17O9	Antioxidant
386.2	26.91	C01175	1-o-Sinapoyl-beta D-glucose	C17H22O10	Antioxidant
163	28.05	C01772	Trans-2-Coumarate	C9H7O3	Antioxidant; Anti-inflammatory
179	49.50	C01197	Trans-Caffeate	C9H7O4	Anti-inflammatory
179.1	3.16	C01481	Cis-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
193	7.41	C01494	Ferulate	C10H9O4	Antioxidant; Anti-aging
217	22.60	C01557	Bergapten	C12H8O4	Anti-inflammatory; Anticancer
217	39.43	C01864	Xanthotoxin	C12H8O4	Anticancer
177.1	49.63	C03081	4-Methylumbelliferone	C10H8O3	Anticancer
276.9	45.83	C04498	4-Coumaroylagmatine	C14H21N4O2	Adrenergic receptor
264.9	31.73	D03241	Nitecapone	C12H11NO6	Antioxidant
231	49.29	C18083	Demethylsuberosin	C14H14O3	Anti-inflammatory
268.7	21.15	C10205	Coumestrol	C15H8O5	Antioxidant; Anti-inflammatory

*As reported in literature

Supplementary Table 16. List of putative glycoside and steroids and sterols identified from leaves extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Glycosides)	Molecular formula	Bioactivity*
260.9	40.69	C08334	Lotaustralin	C11H19NO6	Unknown
423.2	27.88	C08336	Neolinustatin	C17H29NO11	Unknown
406.8	18.68	C08412	Glucoiberverin	C11H20NO9S3	Antimicrobial
422.9	33.76	C08411	Glucoiberin	C11H20NO10S3	Antimicrobial
437.1	9.84	C08419	Glucoraphanin	C12H22NO10S3	Antioxidant
295	36.65	C08481	Indican	C14H17NO6	Pigment
311	36.18	C01855	Taxiphyllin	C14H17NO7	Antimicrobial
297	5.67	C05342	Psicofuranine	C11H15N5O5	Anticancer
			Steroids and Sterols		
448.8	16.64	C15791	Teasterone	C28H48O4	Unknown
446.6	49.38	C15792	3-dehydroteasterone	C28H46O4	Unknown
448.9	14.82	C15793	Typhasterol	C28H48O4	Unknown
386.8	19.38	C00187	Cholesterol	C27H46O	Unknown
384.9	24.87	C01164	7-dehydrocholesterol	C27H44O	Unknown
464.9	20.89	C15794	Castasterone	C28H48O5	Unknown
386.9	34.86	C01189	Lathosterol	C27H46O	Anti-inflammatory;Antidiabetic
414.9	13.01	C01753	Sitosterol	C29H50O	Anticancer; Anti-inflammatory

*As reported in literature

Supplementary Figure 17. List of putative Anthroquinone and Vitamins and Co-factors Betacyanin identified from leaves extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds	Molecular formula	Bioactivity*
			Anthroquinone		
241	40.80	C10314	Chrysophanol Anthrone	C15H12O3	Psosiasis
502.7	39.00	C07606	Hypericin	C30H14O8	Anticancer
241	0.69	C01474	Alizarin	C14H8O4	Pigment
			Vitamins and Co-factors		
175.1	27.68	C00072	L-Ascorbate	C6H7O6	Antioxidant
374.9	6.39	C00255	Riboflavin	C17H19N4O6	Dietary supplement
536.9	31.49	C02094	Beta Carotene	C40H56	Pigments
430.8	6.99	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
397.5	25.21	C14156	Delta Tocotrienol	C27H40O2	Anti-inflammatory
417	23.44	C02483	Gamma Tocopherol	C28H48O2	Antioxidant; Anticancer
452.7	39.19	C03313	Phylloquinol	C31H48O2	Coagulation disorder
			Betacynin		
386	26.10	C08539	Betanidin	C18H14N2O8	Antioxidant; Pigment
			Stilbenes		
214.9	37.67	C10254	Dihydropinosylvin	C14H14O2	Antioxidant
229	26.40	C03582	Trans-Resveratrol	C14H12O3	Anticancer
			Lignan		
362.9	29.27	C18167	(-)-Secoisolariciresinol	C20H26O6	Anticancer
370.8	35.92	C21183	(-)-Bursehernin	C21H22O6	Anticancer
253.1	3.54	C10628	(7s)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic

*As reported in literature

Supplementary Table 18. List of putative Flavonoids identified from pericarp extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Flavanoids)	Molecular formula	Bioactivity*
301	10.85	C00389	Quercetin	C15H9O7	Anti-inflammatory; Anticancer
283.1	27.40	C00814	Biochanin-A	C16H11O5	Antiproliferative
301.1	24.10	C08577	Bracteatin	C15H9O7	Antioxidant
285	36.47	C01514	Luteolin	C15H9O6	Anti-inflammatory; Anticancer
447.1	19.12	C01821	Isoorientin	C21H19O11	Anti-inflammatory, Antidiabetic
275	49.88	C00774	Phloretin	C15H14O5	Antioxidant; Anti-ageing
355	19.74	C01590	Kievitone	C20H19O6	Antimicrobial
302.9	13.23	C01617	(+)-Taxifolin	C15H11O7	Anticancer
430.9	3.68	C01714	Isovitexin	C21H19O10	Anti-inflammatory; Anticancer
301	21.83	C02806	8-hydroxykaempferol	C15H9O7	Anticancer
319	48.38	C02906	(+)-Dihydromyricetin	C15H11O8	Anticancer; Antidiabetic;
448	20.63	C03951	Luteolin 7-O-Beta D-Glucoside	C21H20O11	Antioxidant; Anti-inflammatory
316.9	20.52	C04109	Gossypetin	C15H9O8	Antioxidant; Antibacterial
299	1.76	C04293	Chrysoeriol	C16H11O6	Antioxidant; Anticancer
314.9	15.74	C04443	3-O-Methylquercetin	C16H11O7	Antioxidant; Anticancer
287	27.65	C05631	(2S)-Eriodictyol	C15H11O6	Anticancer; Anti-inflammatory
303	31.69	C05911	(2S)-Dihydrotricetin	C15H11O7	Anti-inflammatory
269	10.26	C06563	Genistein	C15H9O5	Phytoestrogen; Anticancer
394.9	48.96	C07593	Rotenone	C23H22O6	Anticancer
275.1	47.33	C09320	(+)-Afzelechin	C15H14O5	Antioxidant; Cardio protectant
271	39.48	C09826	(+)-Pinobanksin	C15H11O5	Antioxidant
430.8	7.74	C01460	Vitexin	C21H19O10	Antioxidant; Anticancer
254.9	34.34	C09827	(2S)-Pinocembrin	C15H11O4	Anti-inflammatory; Anticancer
517	19.88	C10020	Apigenin 7-O-(6-Malonyl-Beta D-Glucoside)	C24H21O13	Antimicrobial; Anticancer
252.9	16.96	C10028	Chrysin	C15H9O4	Anti-inflammatory
314.9	44.54	C10084	Isorhamnetin	C16H11O7	Antioxidant; Antidiabetic
299	14.84	C10098	Kaempferide	C16H11O6	Anti-inflammatory
316.9	39.15	C10107	Myricetin	C15H9O8	Anti-inflammatory
317	0.81	C10122	Quercetagetin	C15H9O8	Antioxidant; Antidiabetic
287.1	20.77	C10415	Dalbergioidin	C15H11O6	Antioxidant
395.1	26.54	C10417	Deguelin	C23H22O6	Anticancer
353	18.27	C10486	Phaseoluteone/Licoisoflavone A	C20H17O6	Antioxidant
430.7	17.23	C10509	Ononin	C22H22O9	Anti-inflammatory
299	41.51	C10520	Pratensein	C16H11O6	Antioxidant
239	3.62	C11264	7-hydroxyflavone	C15H10O3	Anti-inflammatory; Anticancer
771.8	30.79	C12635	Kaempferol 3-o-beta D-glucosyl-(1->2)-glucosyl-(1->2)-beta D-glucoside	C33H40O21	Antioxidant
239	17.64	C14137	6-hydroxyflavone	C15H10O3	Anticancer
500.9	22.62	C16191	Malonyldaidzin	C24H21O12	Anti-inflammatory; Anticancer
268.9	16.79	C01477	Apigenin	C15H9O5	Anticancer; Antidiabetic
301	35.61	C05908	Delphinidin	C15H9O7	Pigments
514.8	20.11	C16222	Formononetin-7-O-Glucoside-6"-Malonate	C25H23O12	Antioxidant

*As reported in literature

Supplementary Table 19. List of putative Terpenoids identified from pericarp extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
426.9	34.05	C01054	(3s)-2,3-epoxy-2,3-dihydrosqualene	C30H50O	Anti-inflammatory
223.1	36.21	C01126	(2e,6e)-farnesol	C15H26O	Antibacterial
296.9	22.34	C01389	Phytol	C20H40O	Antioxidant; Antimicrobial
388.8	21.88	C01852	Secologanin	C17H24O10	Neuroprotective
568.1	33.07	C06098	Zeaxanthin	C40H56O2	Pigments; Antioxidant
306.9	9.73	C13273	Plaunotol	C20H34O2	Anticancer; Antiulcer
566.8	29.77	C15966	3-Hydroxyechinenone	C40H54O2	Pigments
428.8	16.75	C06309	Hopan-22-ol	C30H52O	Antimicrobial
596.7	6.36	C08580	(3s,3's)-Astaxanthin	C40H52O4	Pigments; Antioxidant
393	5.85	C08582	Bixin	C25H29O4	Anti-oxidants; Pigments
280.9	29.86	C15971	Dihydroxyphaseic Acid	C15H21O5	Plant hormone
536.8	31.03	C07608	Hyperforin	C35H52O4	Anticancer
518.8	19.93	C07667	Gossypol	C30H30O8	Anticancer
378.8	8.86	C08608	Norbixin	C24H26O4	Pigments; Antioxidant
444.8	28.49	C08621	Dammarenediol II	C30H52O2	Anticancer
286.8	36.73	C09092	Ferruginol	C20H30O	Anti-inflammatory;
290.7	47.74	C09094	Geranylgeraniol	C20H34O	Anticancer
308.9	34.96	C09183	Sclareol	C20H36O2	Anticancer; Fragrant
192.7	23.33	C12287	Beta Ionone	C13H20O	Antioxidant; Fragrant
232.9	38.84	C09382	(+)-psounolide (12,6alpha)	C15H20O2	Anticancer
366.9	39.58	C11675	16-methoxytabersonine	C22H27N2O3	Unknown
260.9	14.14	C09680	Hemigossypol	C15H16O4	Anticancer; Anti-microbial
237	11.03	C09700	Lubimin	C15H24O2	Unknown
278.8	41.13	C09707	Phaseic Acid	C15H19O5	Plant hormone
223.1	15.59	C09715	Rishitin	C14H22O2	Antimicrobial
456.9	23.97	C17420	Soyasapogenol E	C30H48O3	Hepatoprotectant
171.1	17.45	C17621	(6E)-8-Hydroxygeraniol	C10H18O2	Unknown
504.9	35.53	C20152	Taxusin	C28H40O8	Anticancer
230.9	41.46	C09918	Aucuparin	C14H14O3	Antimicrobial; Anticancer
456.8	21.77	C08988	Ursolate	C30H48O3	Antidiabetic
314.8	17.79	C18015	Momilactone A	C20H26O3	Antidiabetic; Antifungal

*As reported in literature

Supplementary Table 20. List of putative Alkaloids identified from pericarp extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloid)	Molecular formula	Bioactivity*
161.1	47.03	C00398	Tryptamine	C10H13N2	Psychoactive
352.8	0.27	C02151	19E-Geissoschizine	C21H24N2O3	Unknown
341.1	28.40	C04118	(S)-Tetrahydrocolumbamine	C20H23NO4	Unknown
140.9	7.13	C00783	Tropinone	C8H14NO	Anticholinergic
307	46.25	C03325	(6s)-Hydroxyhyoscyamine	C17H24NO4	Anti-inflammatory; Anti-arrhythmia
339.1	26.10	C03329	(S)-Canadine	C20H21NO4	Antioxidant; Anticaner
363	11.71	C05194	Dihydrochelirubine	C21H17NO5	Anti-tuberculosis
236.9	10.94	C05204	(S)-Tetrahydroprotoberberine	C17H18N	Anti-inflammatory; Anticancer
352.7	44.83	C05315	Palmatine	C21H22NO4	Antioxidant; Anti-inflammatory
392.9	13.24	C05316	Dihydromacarpine	C22H19NO6	Neuroprotective
312.8	43.64	C06173	Thebaine	C19H22NO3	Antimicrobial
295	10.71	C06528	Cinchonine	C19H23N2O	Antimalarial; Antidiabetic
215	18.90	C06536	Harmaline	C13H14N2O	Antioxidant; Anti-inflammatory
199.9	7.38	C06537	Harmalol	C12H12N2O	Anticancer; Anti-microbial
482.8	15.75	C09421	Emetine	C29H42N2O4	Antimicrobial
172.9	38.99	C10173	Swainsonine	C8H15NO3	Anticancer
249	23.21	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
334.9	6.86	C11807	Vinorine	C21H22N2O2	Anti-inflammation
249	49.86	C10772	Lupanine	C15H25N2O	Antioxidant
295	24.11	C11379	Cinchonidine	C19H23N2O	Antimalarial
292.9	13.12	C11633	16-Epivellosimine	C19H20N2O	Unknown
293	44.81	C11634	Vellosimine	C19H20N2O	Unknown
352.9	21.48	C11808	1,2-Dihydrovomilenine	C21H24N2O3	Anticancer
385.8	36.72	C16709	O-Methylandrocymbine	C22H27NO5	Antimicrobial
498.9	26.70	C16724	Strictosamide	C26H30N2O8	Anti-inflammatory
274.9	23.25	C21637	Elwesine	C16H21NO3	Anticancer; Anti-microbial
274.9	46.71	C21639	Maritinamine	C16H21NO3	Unknown
307	2.27	C01513	Lupinate	C13H18N6O3	Unknown
200	2.12	C21721	Camalexin	C11H8N2S	Antimicrobial

*As reported in literature

Supplementary Table 21. List of putative phenolics identified from pericarp extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Phenolic acids)	Molecular formula	Bioactivity*
152.9	48.52	C00230	Protocatechuate	$C_7H_5O_4$	Anti-inflammatory ;Anticancer
202	49.69	C00758	Bergaptol	$C_{11}H_6O_4$	Anticancer
337	4.56	C12208	Trans-5-o-(4-coumaroyl)-d-quinate	$C_{16}H_{17}O_8$	Unknown
353	6.22	C00852	Trans-5-o-Caffeoyl-d-quinate (Choloringenic Acid)	$C_{16}H_{17}O_9$	Antidiabetic; Anti-inflammatory
162.9	5.88	C00811	4-Coumarate	$C_9H_7O_3$	Antioxidant
178.9	40.33	C01197	Trans-caffeate	$C_9H_7O_4$	Antioxidant; Anti-inflammatory
168.9	23.21	c01424	Gallate	$C_7H_5O_5$	Antioxidant; Anticancer
179	2.66	C01481	Cis-caffeate	$C_9H_7O_4$	Antioxidant; Anti-inflammatory
217	49.29	C01557	Bergapten	$C_{12}H_8O_4$	Anti-inflammatory; Anticancer
162.9	12.02	C01772	Trans-2-Coumarate	$C_9H_7O_3$	Antioxidant; Anti-inflammatory
217.1	22.73	C01864	Xanthotoxin	$C_{12}H_8O_4$	Anticancer
277.1	13.08	C04498	4-coumaroylagmatine	$C_{14}H_{21}N_4O_2$	Adrenergic receptor
186.1	29.13	C09305	Psoralen	$C_{11}H_6O_3$	Antipsoriasis
179.1	5.45	C10454	Methyleugenol	$C_{11}H_{14}O_2$	Antioxidant
179.1	11.70	C10478	Isomethyleugenol	$C_{11}H_{14}O_2$	Antioxidant
163	49.38	C12621	3-Hydroxycinnamate	$C_9H_7O_3$	Anti-inflammatory
673.1	27.63	C18071	Triferuloyl Spermidine	$C_{37}H_{43}N_3O_9$	
231	49.34	C18083	Demethylsuberosin	$C_{14}H_{14}O_3$	Anti-inflammatory
222.9	30.94	C00482	Sinapate	$C_{11}H_{11}O_5$	Antioxidant, Anti-inflammatory,
153	3.19	C00628	Gentisate	$C_7H_5O_4$	Antioxidant; Anti-inflammatory;
264.8	3.20	D03241	Nitecapone	$C_{12}H_{11}NO_6$	Antioxidant

*As reported in literature

Supplementary Table 22. List of putative steroids and sterols, vitamins, glycosides, anthroquinone, lignan, betacyanin identified from pericarp extract through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Steroids and sterols)	Molecular formula	Bioactivity*
386.8	34.25	C01189	Lathosterol	C27H46O	Anti-inflammatory; Antidiabetic
414.8	20.44	C01753	Sitosterol	C29H50O	Anticancer; Antidiabetic
316.8	0.04	C01953	Pregnenolone	C21H32O2	Phytohormones
384.9	30.98	C01164	7-Dehydrocholesterol	C27H44O	Unknown
386.8	19.41	C00187	Cholesterol	C27H46O	Unknown
434.8	46.82	C15799	6-Deoxoteasterone	C28H50O3	Unknown
450.9	16.56	C15802	6-Deoxocastasterone	C28H50O4	Unknown
446.7	12.20	C15792	3-Dehydroteasterone	C28H46O4	Unknown
382.9	20.72	C05107	7-Dehydrodesmosterol	C27H42O	Unknown
412.9	4.68	C05108	14-Demethyllanosterol	C29H48O	Unknown
			Vitamins and co-factors		
174.9	3.19	C00072	L-Ascorbate	C6H7O6	Antioxidant
278	35.07	C00831	Pantetheine	C11H22N2O4S	
451	11.39	C02059	Phylloquinone	C31H46O2	Coagulation disorder
431	23.04	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
452.8	9.20	C03313	Phylloquinol	C31H48O2	Coagulation disorder
			Glycosides		
260.9	6.35	C08334	Lotaustralin	C11H19NO6	Unknown
423	22.28	C08336	Neolinustatin	C17H29NO11	Unknown
423.1	20.59	C08411	Glucoiberin	C11H20NO10S3	Antimicrobial
407	21.31	C08412	Glucoiberverin	C11H20NO9S3	Antimicrobial
436.9	16.13	C08419	Glucoraphanin	C12H22NO10S3	Antioxidant
294.9	25.47	C08481	Indican	C14H17NO6	Pigment
255.2	31.31	C08574	Apigeninidin	C15H10O4	Antiproliferative; Pigments
446.9	30.93	C08604	Cyanidin-3-o-beta D-glucoside	C21H19O11	Antioxidants/Pigments
269	0.22	C05904	Pelargonidin	C15H9O5	Anti-inflammatory
297	16.46	C05342	Psicofuranine	C11H15N5O5	Anticancer
271	32.04	C08652	Luteolinidin	C15H11O5	Anti-inflammatory
			Anthroquinone		
240.9	47.95	C10314	Chrysophanol Anthrone	C15H12O3	Psoriasis
240.9	27.55	C01474	Alizarin	C14H8O4	Pigment
			Lignans		
252.9	36.52	C10628	(7s)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic
215	7.88	C10254	Dihydropinosylvin	C14H14O2	Antioxidant
			Betacynin		
386	30.89	c08539	Betanidin	C18H14N2O8	Antioxidant; Pigment

*As reported in literature

Supplementary Table 23. List of putative Flavonoids from extract of leaves derived callus cultures (control) through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Flavonoids)	Molecular formula	Bioactivity*
283	24.68	C00814	Biochanin-A	$C_{16}H_{11}O_5$	Antiproliferative
301	16.82	C00389	Quercetin	$C_{15}H_9O_7$	Anti-inflammatory; Anticancer
379.9	30.44	C00616	Quercetin 3-sulfate	$C_{15}H_8O_{10}S$	Antioxidant; Anti-inflammatory
430.8	36.80	C01460	Vitexin	$C_{21}H_{19}O_{10}$	Antioxidant; Anticancer
283.1	25.94	C01470	Acacetin	$C_{16}H_{11}O_5$	Anti-inflammatory; Anticancer
285	32.56	C01514	Luteolin	$C_{15}H_9O_6$	Anti-inflammatory; Anticancer
274.9	40.95	C00774	Phloretin	$C_{15}H_{14}O_5$	Antioxidant; Anti-ageing
430.9	14.81	C01714	Isovitexin	$C_{21}H_{19}O_{10}$	Antioxidant; Anticancer
446.9	17.51	C01821	Isoorientin	$C_{21}H_{19}O_{11}$	Anti-inflammatory; Anti-diabetic
316.9	19.97	C04109	Gossypetin	$C_{15}H_9O_8$	Antioxidant; Antibacterial
299	0.41	C04293	Chrysoeriol	$C_{16}H_{11}O_6$	Antioxidant; Anticancer
314.9	14.81	C04443	3-O-Methylquercetin	$C_{16}H_{11}O_7$	Anticancer; Antioxidant
301	34.67	C02806	8-Hydroxykaempferol	$C_{15}H_9O_7$	Anticancer
446.8	12.47	C05376	Biochanin A-7-o-Glucoside	$C_{22}H_{22}O_{10}$	Anticancer
286.9	0.32	C05631	(2S)-Eriodictyol	$C_{15}H_{11}O_6$	Anticancer; Anti-inflammatory
285.1	26.99	C05903	Kaempferol	$C_{15}H_9O_6$	Anticancer
307.1	11.83	C05906	(2R,3S,4S)-Leucocyanidin	$C_{15}H_{14}O_7$	Antiviral
395	18.86	C07593	Rotenone	$C_{23}H_{22}O_6$	Anticancer
255.2	27.16	C08574	Apigeninidin	$C_{15}H_{10}O_4$	Antiproliferative; Pigments
275	5.94	C09320	(+)-Afzelechin	$C_{15}H_{14}O_5$	Antioxidant; Cardio protectant
580.6	4.51	C09789	Naringin	$C_{27}H_{32}O_{14}$	Anti-inflammatory; Anticancer
255	16.23	C09827	(2S)-Pinocembrin	$C_{15}H_{11}O_4$	Anti-inflammatory; Anticancer
516.9	32.24	C10020	Apigenin 7-o-(6-Malonyl-Beta D-glucoside)	$C_{24}H_{21}O_{13}$	Antimicrobial; Anticancer
252.9	6.98	C10028	Chrysin	$C_{15}H_9O_4$	Anti-inflammatory
314.9	39.75	C10084	Isorhamnetin	$C_{16}H_{11}O_7$	Antioxidant; Antidiabetic
299	19.98	C10098	Kaempferide	$C_{16}H_{11}O_6$	Anti-inflammatory
317	3.66	C10107	Myricetin	$C_{15}H_9O_8$	Anti-inflammatory
331	41.43	C10118	Patuletin	$C_{16}H_{11}O_8$	Anti-inflammatory
317	22.02	C10122	Quercetagetin	$C_{15}H_9O_8$	Antioxidant; Antidiabetic
286.9	5.86	C10415	Dalbergioidin	$C_{15}H_{11}O_6$	Antioxidant
430.4	23.33	C10509	Ononin	$C_{22}H_{22}O_9$	Anti-inflammatory
299.1	13.88	C10520	Pratensein	$C_{16}H_{11}O_6$	Antioxidant
239	48.26	C11264	7-Hydroxyflavone	$C_{15}H_{10}O_3$	Anti-inflammatory; Anticancer
275	14.61	C12124	Apiforol	$C_{15}H_{14}O_5$	Antidiabetic
307.1	22.88	C12127	(+)-Gallocatechin	$C_{15}H_{14}O_7$	Antioxidant
275	49.26	C12128	(-)-Epiafzelechin	$C_{15}H_{14}O_5$	Phytoestrogen
430.9	39.35	C12137	Pelargonidin-3-o-beta D-glucoside	$C_{21}H_{19}O_{10}$	Antidiabetic
636.9	30.50	C12632	Luteolin 7-O-Beta D-Diglucuronide	$C_{27}H_{24}O_{18}$	Anticancer
239.1	18.78	C14137	6-Hydroxyflavone	$C_{15}H_{10}O_3$	Anticancer
330.9	16.02	C12633	Laricitrin	$C_{16}H_{11}O_8$	Antioxidant
500.8	32.14	C16191	Malonyldaidzin	$C_{24}H_{21}O_{12}$	Anti-inflammatory; Anticancer
514.9	19.03	C16222	Formononetin-7-O-Glucoside-6"-Malonate	$C_{25}H_{23}O_{12}$	Antioxidant
319	38.29	C02906	(+)-Dihydromyricetin	$C_{15}H_{11}O_8$	Anticancer; Antidiabetic

*As reported in literature

Supplementary Table 24. List of putative Terpenoids from extract of leaves derived callus cultures (control) through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
544.9	19.60	C05413	All-Trans-Phytoene	$C_{40}H_{64}$	Pigments
223.1	20.06	C01126	(2e,6e)-Farnesol	$C_{15}H_{26}O$	Antibacterial
296.9	48.32	C01389	Phytol	$C_{20}H_{40}O$	Antioxidant; Antimicrobial
390.7	36.61	C01433	Loganin	$C_{17}H_{26}O_{10}$	Anti-inflammatory
428.8	3.69	C06309	Hopan-22-ol	$C_{30}H_{52}O$	Anti-microbial
535.9	36.00	C07608	Hyperforin	$C_{35}H_{52}O_4$	Anticancer
518.9	34.81	C07667	Gossypol	$C_{30}H_{30}O_8$	Anticancer
393	46.02	C08582	Bixin	$C_{25}H_{29}O_4$	Pigments; Antioxidant
552.9	32.95	C08590	Zeinoxanthin	$C_{40}H_{56}O$	Antioxidant; Pigments
378.8	8.59	C08608	Norbixin	$C_{24}H_{26}O_4$	Pigments; Antioxidant
443	24.60	C08618	Betulin	$C_{30}H_{50}O_2$	Anticancer
444.8	48.59	C08621	Dammarenediol II	$C_{30}H_{52}O_2$	Anticancer
440.9	2.88	C08830	24-Methylenecycloartanol	$C_{31}H_{52}O$	Antimicrobial
456.9	32.88	C08988	Ursolate	$C_{30}H_{48}O_3$	Antidiabetic
306.7	13.54	C09072	Alpha Cembratriene-4,6-diol	$C_{20}H_{34}O_2$	Anticancer
290.8	38.00	C09094	Geranylgeraniol	$C_{20}H_{34}O$	Anticancer
308.8	7.88	C09183	Sclareol	$C_{20}H_{36}O_2$	Anticancer; Fragrant
233	9.14	C09382	(+)-Costunolide (12,6alpha)	$C_{15}H_{20}O_2$	Anticancer
223.1	46.66	C09621	(-)-Alpha Bisabolol	$C_{15}H_{26}O$	Anti-inflammatory
261	17.21	C09680	Hemigossypol	$C_{15}H_{16}O_4$	Anticancer; anti-microbial
236.9	23.02	C09700	Lubimin	$C_{15}H_{24}O_2$	Unknown
223	44.74	C09715	Rishitin	$C_{14}H_{22}O_2$	Antimicrobial
231	14.20	C09918	Aucuparin	$C_{14}H_{14}O_3$	Antimicrobial; Anticancer
359	39.53	C11636	7-Deoxyloganate	$C_{16}H_{23}O_9$	Antioxidant
193	6.37	C12287	Beta Ionone	$C_{13}H_{20}O$	Antioxidant; Fragrant
306.8	7.01	C13273	Plaunotol	$C_{20}H_{34}O_2$	Anticancer; Anticancer
250	25.05	C13453	2-Cis,4-Trans-Xanthoxin	$C_{15}H_{22}O_3$	Antioxidant
566.8	33.43	C15966	3-Hydroxyechinenone	$C_{40}H_{54}O_2$	Pigments
281	23.46	C15971	Dihydroxyphaseic Acid	$C_{15}H_{21}O_5$	Plant hormones
171	42.35	C17621	(6E)-8-Hydroxygeraniol	$C_{10}H_{18}O_2$	Unknown
444.9	18.31	C19828	Arabidiol	$C_{30}H_{52}O_2$	Anti-oomycete
504.8	34.82	C20152	Taxusin	$C_{28}H_{40}O_8$	Anticancer
290.9	35.63	C20481	Cis-Abienol	$C_{20}H_{34}O$	Fragrant
388.9	34.63	C01852	Secologanin	$C_{17}H_{24}O_{10}$	Neuroprotective
262.9	16.27	C06082	2-cis-Abscisate	$C_{15}H_{19}O_4$	Plant hormone

*As reported in literature

Supplementary Table 25. List of putative Alkaloids from extract of leaves derived callus cultures (control) through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloids)	Molecular formula	Bioactivity*
306.9	35.47	C01513	Lupinate	C13H18N6O3	Unknown
274.9	14.96	C21637	Elwesine	C16H21NO3	Anticancer; Antimicrobial
274.9	20.97	C21639	Maritinamine	C16H21NO3	Unknown
199.9	43.50	C21721	Camalexin	C11H8N2S	Antimicrobial
140.9	15.19	C00783	Tropinone	C8H14NO	Anticholinergic
330.8	41.96	C02105	(S)-Reticuline	C19H24NO4	Anti-inflammatory
352.9	8.25	C02151	19E-Geissoschizine	C21H24N2O3	Unknown
265.1	19.12	C02621	13-Hydroxylupanine	C15H25N2O2	Antioxidant
354.8	19.65	C02915	(S)-N-Methylcanadine	C21H24NO4	Antioxidant
306.9	42.23	C03325	(6s)-Hydroxyhyoscyamine	C17H24NO4	Anti-inflammatory; Anti-arrhythmia
323	14.31	C05175	(S)-Stylopine	C19H17NO4	Antimicrobial
369	30.17	C05190	6-Hydroxyprotopine	C20H19NO6	Antimicrobial
378.9	38.02	C05193	12-Hydroxydihydrochelirubine	C21H17NO6	Unknown
352.8	45.83	C05315	Palmatine	C21H22NO4	Antioxidant; Anti-inflammatory
363	20.14	C05194	Dihydrochelirubine	C21H17NO5	Anti-tuberculosis
236.9	0.95	C05204	(S)-Tetrahydroprotoberberine	C17H18N	Anti-inflammatory; Anticancer
392.9	10.03	C05316	Dihydromacarpine	C22H19NO6	Neuroprotective
393.3	27.81	C06165	Macarpine	C22H18NO6	Anti-inflammatory
313	41.50	C06173	Thebaine	C19H22NO3	Antimicrobial
295	22.50	C06528	Cinchonine	C19H23N2O	Antimalarial; Antidiabetic
215	8.40	C06536	Harmaline	C13H14N2O	Anti-inflammatory
201.1	37.25	C06537	Harmalol	C12H12N2O	Anticancer; Anti-microbial
468.7	20.79	C09390	Cephaeline	C28H40N2O4	Antioxidant
483	26.89	C09421	Emetine	C29H42N2O4	Antimicrobial
343	35.68	C09581	(S)-Magnoflorine	C20H24NO4	Anti-inflammatory
173	23.78	C10173	Swainsonine	C8H15NO3	Anticancer
248.9	18.72	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
248.9	31.41	C10772	Lupanine	C15H25N2O	Antioxidant
295.1	11.70	C11379	Cinchonidine	C19H23N2O	Antimalarial
292.9	21.90	C11633	16-Epivellosimine	C19H20N2O	Unknown
292.9	28.57	C11634	Vellosimine	C19H20N2O	Unknown
294.8	39.24	C11635	10-Deoxysarpagine	C19H22N2O	Unknown
334.8	38.38	C11807	Vinorine	C21H22N2O2	Anti-inflammatory
373	41.50	C16707	(S)-Autumnaline	C21H27NO5	Anticancer
385.8	32.93	C16709	O-Methylandrocymbine	C22H27NO5	Antimicrobial
341.1	27.92	C04118	(S)-tetrahydrocolumbamine	C20H23NO4	Unknown
292.8	42.11	C16726	Cinchoninone	C19H20N2O	Antiplasmodial
294.8	49.60	C16735	Corynantheal	C19H22N2O	Antimalarial; Analgesic
292.8	47.30	C16846	Cinchonidinone	C19H20N2O	Antimalarial
512.9	37.34	C16723	Pumiloside	C26H28N2O9	Anti-inflammatory

*As reported in literature

Supplementary Table 26. List of putative Phenolics, Vitamins and co factors and anthroquinones, Glycosides and lignan from extract of leaves derived callus cultures (control) through LC-MS/MS analysis

m/z value	**Retention time**	**KEGG ID**	**Putative Compounds (Phenolic acids)**	**Molecular formula**	**Bioactivity***
153	15.39	C00230	Protocatechuate	C7H5O4	Anti-inflammatory; Anticancer
153	37.23	C00628	Gentisate	C7H5O4	Antioxidant; Anti-inflammatory
162.8	48.65	C00811	4-Coumarate	C9H7O3	Antioxidant
202	41.01	C00758	Bergaptol	C11H6O4	Anticancer
179	6.69	C01197	Trans-caffeate	C9H7O4	Antioxidant; Anti-inflammatory
169	5.94	c01424	Gallate	C7H5O5	Antioxidant; Anticancer
179	12.40	C01481	Cis-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
162.9	7.63	C01772	Trans-2-coumarate	C9H7O3	Antioxidant; Anti-inflammatory
233	49.50	C02951	5-hydroxyxanthotoxin	C12H8O5	Anticancer
217.1	22.33	C01864	Xanthotoxin	C12H8O4	Anticancer
276.9	3.88	C04498	4-Coumaroylagmatine	C14H21N4O2	Adrenergic receptor
163	1.35	C05838	Coumarinate	C9H7O3	Antidiabetic
163	9.32	C12621	3-hydroxycinnamate	C9H7O3	Anti-inflammatory
179.1	15.29	C10454	Methyleugenol	C11H14O2	Antioxidant
231	43.27	C18083	Demethylsuberosin	C14H14O3	Anti-inflammatory
264.9	29.26	D03241	Nitecapone	C12H11NO6	Antioxidant
359	5.85	C01850	Rosmarinate	C18H15O8	Anti-inflammatory
			Vitamins and Co-factors		
452.9	38.44	C03313	Phylloquinol	C31H48O2	Coagulation disorder
375.1	11.64	C00255	Riboflavin	C17H19N4O6	Dietary supplement
450.9	22.17	C02059	Phylloquinone	C31H46O2	Coagulation disorder
536.9	30.06	C02094	Beta-Carotene	C40H56	Pigments
430.6	39.49	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
173.2	16.08	C05422	L-Dehydro-ascorbate	C6H6O6	Antioxidant
436.8	18.54	C13309	Demethylphylloquinone	C30H44O2	Unknown
			Anthroquinone		
327.1	21.42	C00113	Pyrroloquinoline Quinone	C14H3N2O8	Antioxidant
241	3.17	C01474	Alizarin	C14H8O4	Pigment
195	0.70	C00822	Dopaquinone	C9H9NO4	Unknown
			Glycosides		
260.9	25.65	C08334	Lotaustralin	C11H19NO6	Unknown
423	32.11	C08336	Neolinustatin	C17H29NO11	Unknown
422.8	21.11	C08411	Glucoiberin	C11H20NO10S3	Antimicrobial
406.9	10.96	C08412	Glucoiberverin	C11H20NO9S3	Antimicrobial
436.9	21.85	C08419	Glucoraphanin	C12H22NO10S3	Antioxidant
247.1	24.49	C01594	Linamarin	C10H17NO6	Anticancer
313.2	27.58	C01625	Vicianose	C11H20O10	Unknown
295	9.60	C08481	Indican	C14H17NO6	Pigment
			Lignan		
252.9	23.77	C10628	(7s)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic
370.8	19.20	C21183	(-)-Bursehernin	C21H22O6	Anticancer
372.9	2.27	C10545	Arctigenin	C21H24O6	Anti-inflammatory; Anticancer
380	28.07	C10559	Diphyllin	C21H16O7	Anticancer; Antiviral

*As reported in literature

Supplementary Table 27. List of putative Steroids and sterol, Stilbenes and Betacynins from extract of leaves derived callus cultures (control) through LC-MS/MS analysis

m/z value	**Retention time**	**KEGG ID**	**Putative Compounds (Steroid and sterols)**	**Molecular formula**	**Bioactivity***
388.8	33.86	C12978	Epi-Cholestanol	$C_{27}H_{48}O$	Unknown
448.8	19.18	C15791	Teasterone	$C_{28}H_{48}O_4$	Unknown
446.7	47.91	C15792	3-Dehydroteasterone	$C_{28}H_{46}O_4$	Unknown
434.7	49.00	C15799	6-Deoxoteasterone	$C_{28}H_{50}O_3$	Unknown
434.8	40.94	C15801	6-Deoxotyphasterol	$C_{28}H_{50}O_3$	Unknown
450.9	9.05	C15802	6-Deoxocastasterone	$C_{28}H_{50}O_4$	Unknown
400.7	26.68	C01789	Campesterol	$C_{28}H_{48}O$	Anti-inflammatory; Antidiabetic
466.9	19.42	C15803	6alpha Hydroxy-castasterone	$C_{28}H_{50}O_5$	Unknown
			Stilbenes		
228.9	35.70	C03582	Trans-Resveratrol	$C_{14}H_{12}O_3$	Anticancer
227	35.65	C10276	Pinosylvin Monomethylether	$C_{15}H_{14}O_2$	Antioxidant
215	0.03	C10254	Dihydropinosylvin	$C_{14}H_{14}O_2$	Antioxidant
			Betacynin		
900.7	34.04	C08542	Celosianin II	$C_{40}H_{40}N_2O_{22}$	Unknown

*As reported in literature

Supplementary Table 28. List of putative Flavonoids from extract of callus (leaves-derived) cultures treated with Salicylic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Flavonoids)	Molecular formula	Bioactivity
270.9	47.26	C01592	Licodione	$C_{15}H_{11}O_{5}$	Unknown
316.9	24.04	C04109	Gossypetin	$C_{15}H_{9}O_{8}$	Antioxidant; Antibacterial
298.9	38.75	C04293	Chrysoeriol	$C_{16}H_{11}O_{6}$	Antioxidant;Anticancer
271	11.36	C08652	Luteolinidin	$C_{15}H_{11}O_{5}$	Anti-inflammatory
255.2	30.11	C08574	Apigeninidin	$C_{15}H_{10}O_{4}$	Antiproliferative; Pigments
314.9	45.37	C04443	3-O-Methylquercetin	$C_{16}H_{11}O_{7}$	Antioxidant; Anticancer
359.9	21.15	C04552	3,6,7-Trimethylquercetagetin	$C_{18}H_{16}O_{8}$	Unknown
446.8	29.75	C05376	Biochanin A-7-O-Glucoside	$C_{22}H_{22}O_{10}$	Anticancer
286.9	31.48	C05631	(2S)-Eriodictyol	$C_{15}H_{11}O_{6}$	Anticancer; Anti-inflammatory
307	8.42	C05906	(2R,3S,4S)-Leucocyanidin	$C_{15}H_{14}O_{7}$	Antiviral
300.9	21.30	C00389	Quercetin	$C_{15}H_{9}O_{7}$	Anti-inflammatory
380	23.58	C00616	Quercetin 3-sulfate	$C_{15}H_{8}O_{10}S$	Anti-inflammatory: anticancer
283	7.86	C00814	Biochanin-A	$C_{16}H_{11}O_{5}$	Antiproliferative
431	9.42	C01460	Vitexin	$C_{21}H_{19}O_{10}$	Anti-inflammatory; Anticancer
283	14.30	C01470	Acacetin	$C_{16}H_{11}O_{5}$	Anti-inflammatory; Anticancer
269	22.09	C01477	Apigenin	$C_{15}H_{9}O_{5}$	Anticancer; Antidiabetic
303	22.94	C01617	(+)-Taxifolin	$C_{15}H_{11}O_{7}$	Anticancer
338.8	44.01	C01701	Glyceollin I	$C_{20}H_{18}O_{5}$	Anticancer
446.9	15.11	C01821	Isoorientin	$C_{21}H_{19}O_{11}$	Anti-inflammatory; Antidiabetic
300.9	37.71	C02806	8-Hydroxykaempferol	$C_{15}H_{9}O_{7}$	Anticancer
319	7.20	C02906	(+)-Dihydromyricetin	$C_{15}H_{11}O_{8}$	Anticancer; Antidiabetic
271	22.63	C09826	(+)-Pinobanksin	$C_{15}H_{11}O_{5}$	Antioxidant
255	0.35	C09827	(2S)-Pinocembrin	$C_{15}H_{11}O_{4}$	Anti-inflammatory; Anticancer
516.9	20.88	C10020	Apigenin 7-O-(6-Malonyl-Beta D-Glucoside)	$C_{24}H_{21}O_{13}$	Antimicrobial; Anticancer
252.9	25.89	C10028	Chrysin	$C_{15}H_{9}O_{4}$	Anti-inflammatory
315	40.32	C10084	Isorhamnetin	$C_{16}H_{11}O_{7}$	Antioxidant; Antidiabetic
298.9	49.36	C10098	Kaempferide	$C_{16}H_{11}O_{6}$	Anti-inflammatory
316.9	44.59	C10107	Myricetin	$C_{15}H_{9}O_{8}$	Anti-inflammatory
446.9	30.43	C10114	Orientin	$C_{21}H_{19}O_{11}$	Anti-inflammatory
331	39.89	C10118	Patuletin	$C_{16}H_{11}O_{8}$	Anti-inflammatory
317	24.14	C10122	Quercetagetin	$C_{15}H_{9}O_{8}$	Antioxidant; Antidiabetic
287	7.49	C10415	Dalbergioidin	$C_{15}H_{11}O_{6}$	Antioxidant
339.3	24.45	C10422	Glyceollin II	$C_{20}H_{18}O_{5}$	Anticancer
314	6.72	C10516	(+)-Pisatin	$C_{17}H_{14}O_{6}$	Antimicrobial
239	20.21	C11264	7-hydroxyflavone	$C_{15}H_{10}O_{3}$	Anti-inflammatory; Anticancer
307	13.71	C12127	(+)-Gallocatechin	$C_{15}H_{14}O_{7}$	Antioxidant
307	21.49	C12136	(-)-Epigallocatechin	$C_{15}H_{14}O_{7}$	Anti-inflammatory; Anticancer
578.8	19.25	C12627	Apigenin 7-O-Neohesperidoside	$C_{27}H_{30}O_{14}$	Anti-microbial
636	4.41	C12632	Luteolin 7-O-Beta D-Diglucuronide	$C_{27}H_{24}O_{18}$	Anticancer
331.1	23.15	C12633	Laricitrin	$C_{16}H_{11}O_{8}$	Antioxidant
578.8	25.63	C12644	Pelargonidin-3-o-rutinoside	$C_{27}H_{30}O_{14}$	Antidiabetic
500.8	34.16	C16191	Malonyldaidzin	$C_{24}H_{21}O_{12}$	Anti-inflammatory; Anticancer
514.9	31.45	C16222	Formononetin-7-O-Glucoside-6"-Malonate	$C_{25}H_{23}O_{12}$	Antioxidant
616.8	23.96	C16289	Cyanidin 3-o-(3",6"-o-dimalonyl-beta Glucopyranoside)	$C_{27}H_{21}O_{17}$	Anticancer; Pigments
872.9	4.14	C16303	Ternatin C5	$C_{36}H_{40}O_{25}$	Unknown
464.9	8.22	C16410	Bracteatin 6-o-glucoside	$C_{21}H_{20}O_{12}$	Unknown
578.9	16.20	C16981	Kaempferol-3,7-bis-o-alpha L-rhamnoside	$C_{27}H_{30}O_{14}$	Unknown
449	21.37	C17391	Sakuranin	$C_{22}H_{24}O_{10}$	Anticancer
579	22.07	C20490	Cyanidin 3-o-beta D-sambubioside	$C_{26}H_{27}O_{15}$	Unknown

425.1	20.37	C18053	Sophoraflavanone G	$C_{25}H_{28}O_6$	Anticancer
239	40.50	C14137	6-hydroxyflavone	$C_{15}H_{10}O_3$	Anticancer
394.9	15.51	C07593	Rotenone	$C_{23}H_{22}O_6$	Anticancer
299	21.76	C10520	Pratensein	$C_{16}H_{11}O_6$	Antioxidant

*As reported in literature

Supplementary Table 29. List of putative Terpenoids from extract of callus cultures (leaves derived) treated with Salicylic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
426.9	37.75	C01902	Cycloartenol	$C_{30}H_{50}O$	Phytosterol precursor
544.9	18.14	C05413	All-trans-Phytoene	$C_{40}H_{64}$	Pigments
542.7	19.02	C05414	All-trans Phytofluene	$C_{40}H_{62}$	Pigments
538.1	29.25	C05434	Beta Zeacarotene	$C_{40}H_{58}$	Pigments
374.8	15.70	C06071	7-Deoxyloganin	$C_{17}H_{26}O_9$	Anti-inflammatory
428.8	37.10	C06309	Hopan-22-ol	$C_{30}H_{52}O$	Antimicrobial
535.9	32.62	C07608	Hyperforin	$C_{35}H_{52}O_4$	Anticancer
518.9	32.16	C07667	Gossypol	$C_{30}H_{30}O_8$	Anticancer
411	8.34	C00751	Squalene	$C_{30}H_{50}$	Anticancer
223	48.72	C01126	(2e,6e)-Farnesol	$C_{15}H_{26}O$	Antibacterial
374.9	10.34	C01512	Loganate	$C_{16}H_{23}O_{10}$	Antioxidant
296.9	0.85	C01389	Phytol	$C_{20}H_{40}O$	Antioxidant; Antimicrobial
391	44.39	C01433	Loganin	$C_{17}H_{26}O_{10}$	Anti-inflammatory
584.9	16.18	C08579	Antheraxanthin	$C_{40}H_{56}O_3$	Pigments
597	5.25	C08580	(3s,3's)-Astaxanthin	$C_{40}H_{52}O_4$	Pigments; Antioxidant
393	12.88	C08582	Bixin	$C_{25}H_{29}O_4$	Pigments; Antioxidant
564.7	2.09	C08583	Canthaxanthin	$C_{40}H_{52}O_2$	Pigments; Antioxidant
550.7	44.93	C08592	Echinenone	$C_{40}H_{54}O$	Pigments; Antioxidant
378.8	9.67	C08608	Norbixin	$C_{24}H_{26}O_4$	Antioxidant; Pigments
442.9	7.18	C08618	Betulin	$C_{30}H_{50}O_2$	Anticancer
444.8	19.08	C08621	Dammarenediol II	$C_{30}H_{52}O_2$	Anticancer
441	7.41	C08830	24-Methylenecycloartanol	$C_{31}H_{52}O$	Antimicrobial
458.9	8.87	C08980	Soyasapogenol B	$C_{30}H_{50}O_3$	Antioxidant
457	27.78	C08988	Ursolate	$C_{30}H_{48}O_3$	Antidiabetic
307.1	5.23	C09072	Alpha Cembratriene-4,6-diol	$C_{20}H_{34}O_2$	Anticancer
290.8	37.94	C09094	Geranylgeraniol	$C_{20}H_{34}O$	Anticancer
309	8.63	C09183	Sclareol	$C_{20}H_{36}O_2$	Anticancer; Fragrant
233	13.43	C09382	(+)-Costunolide (12,6alpha)	$C_{15}H_{20}O_2$	Anticancer
223.1	10.72	C09621	(-)-Alpha Bisabolol	$C_{15}H_{26}O$	Anti-inflammatory;
223.1	24.18	C09663	Alpha Eudesmol	$C_{15}H_{26}O$	Hepatoprotectant
223.2	17.33	C09664	Beta Eudesmol	$C_{15}H_{26}O$	Hepatoprotectant
261	10.92	C09680	Hemigossypol	$C_{15}H_{16}O_4$	Anticancer; anti-microbial
236.9	18.05	C09700	Lubimin	$C_{15}H_{24}O_2$	Unknown
279	25.69	C09707	Phaseic Acid	$C_{15}H_{19}O_5$	Plant Hormone
230.8	0.68	C09918	Aucuparin	$C_{14}H_{14}O_3$	Antimicrobial; Anticancer
306.9	15.43	C13273	Plaunotol	$C_{20}H_{34}O_2$	Anticancer; Antiulcer
582.8	11.77	C15968	Adonixanthin	$C_{40}H_{54}O_3$	Pigments
280.9	21.72	C15971	Dihydroxyphaseic Acid	$C_{15}H_{21}O_5$	Plant Hormone
498.9	13.59	C16724	Strictosamide	$C_{26}H_{30}N_2O_8$	Anti-inflammatory
474.9	27.55	C17419	Soyasapogenol A	$C_{30}H_{50}O_4$	Anticancer
171	38.79	C17621	(6E)-8-Hydroxygeraniol	$C_{10}H_{18}O_2$	Unknown
314.8	48.92	C18015	Momilactone A	$C_{20}H_{26}O_3$	Antidiabetic; Antifungal
444.8	46.66	C19828	Arabidiol	$C_{30}H_{52}O_2$	Antioomycete
504.8	12.95	C20152	Taxusin	$C_{28}H_{40}O_8$	Anticancer

290.9	36.75	C20481	Cis-Abienol	C20H34O	Fragrant
477	18.20	C20716	Protopanaxatriol	C30H52O4	Anticancer
432.8	37.64	C20894	Beta Citraurin	C30H40O2	Antioxidant
587	22.78	C11900	Baccatin III	C31H38O11	Anticancer
468.9	31.43	C08950	Gypsogenin	C30H45O4	Anticancer
223	36.75	C09715	Rishitin	C14H22O2	Antimicrobial
262.9	13.01	C06082	2-Cis-Abscisate	C15H19O4	Plant Hormone

*As reported in literature

Supplementary Table 30. List of putative Alkaloids from extract of leaves derived callus cultures treated with Salicylic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloids)	Molecular formula	Bioactivity*
140.9	27.04	C00783	Tropinone	C8H14NO	Anticholinergic
306.9	35.62	C01513	Lupinate	C13H18N6O3	Unknown
327	24.11	C02106	(S)-Scoulerine	C19H21NO4	Anticancer
352.8	34.60	C02151	19E-Geissoschizine	C21H24N2O3	Unknown
339	21.30	C03329	(S)-Canadine	C20H21NO4	Antioxidant: Anti-caner
351	37.23	C03677	4,21-Dehydrogeissoschizine	C21H23N2O3	Unknown
341	22.94	C04118	(S)-Tetrahydrocolumbamine	C20H23NO4	Unknown
598.8	9.56	C05177	Berbamunine	C36H42N2O6	Unknown
369.2	29.09	C05190	6-Hydroxyprotopine	C20H19NO6	Antimicrobial
379.1	8.03	C05193	12-Hydroxydihydrochelirubine	C21H17NO6	Unknown
363	19.71	C05194	Dihydrochelirubine	C21H17NO5	Anti-tuberculosis
352.8	13.43	C05315	Palmatine	C21H22NO4	Antioxidant; Anti-inflammatory
392.9	10.96	C05316	Dihydromacarpine	C22H19NO6	Neuroprotective
372.8	18.05	C05322	7-O-Acetylsalutaridinol	C21H26NO5	Unknown
392.9	42.77	C06165	Macarpine	C22H18NO6	Anti-inflammatory
313.2	25.39	C06173	Thebaine	C19H22NO3	Antimicrobial
295	34.65	C06528	Cinchonine	C19H23N2O	Antimalarial; Antidiabetic
201	8.94	C06537	Harmalol	C12H12N2O	Anticancer; Antimicrobial
172.9	13.37	C10173	Swainsonine	C8H15NO3	Anticancer
287	22.56	C08532	Lycorine	C16H17NO4	Anticancer
467.8	5.79	C09390	Cephaeline	C28H40N2O4	Antioxidant
483	25.93	C09421	Emetine	C29H42N2O4	Antimicrobial
248.9	31.25	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
248.9	48.97	C10772	Lupanine	C15H25N2O	Antioxidant
292.9	20.98	C11633	16-Epivellosimine	C19H20N2O	Unknown
292.9	35.45	C11634	Vellosimine	C19H20N2O	Unknown
366.9	25.64	C11675	16-Methoxytabersonine	C22H27N2O3	Unknown
334.9	49.33	C11807	Vinorine	C21H22N2O2	Anti-inflammatory
352.9	18.27	C11808	1,2-Dihydrovomilenine	C21H24N2O3	Anticancer
274.9	23.91	C21637	Elwesine	C16H21NO3	Anticancer; Antimicrobial
275	49.01	C21639	Maritinamine	C16H21NO3	Unknown
201	39.54	C21721	Camalexin	C11H8N2S	Antimicrobial
385.9	28.54	C16709	O-Methylandrocymbine	C22H27NO5	Antimicrobial
292.9	48.10	C16726	Cinchoninone	C19H20N2O	Antiplasmodial
368.8	11.80	C15985	17-O-Acetylajmaline	C22H28N2O3	Unknown
339	47.48	C11818	(R)-Canadine	C20H21NO4	Antioxidant
398.8	19.75	C06543	Solanidine	C27H44NO	Unknown

598.9	3.29	C06511	Guattegaumerine	C36H42N2O6	Anticancer
512.9	20.80	C16723	Pumiloside	C26H28N2O9	Anti-inflammatory

*As reported in literature

Supplementary Table 31. List of putative phenolics and vitamins and cofactors, anthroquinones and lignans from extract of callus cultures (leaf-derived) treated with Salicylic acid

m/z value	**Retention time**	**KEGG ID**	**Putative Compounds (Phenolic acids)**	**Molecular formula**	**Bioactivity***
153	48.88	C00230	Protocatechuate	C7H5O4	Anti-inflammatory; Anticancer
223	1.96	C00482	Sinapate	C11H11O5	Antioxidant; Anti-inflammatory
153.1	13.45	C00628	Gentisate	C7H5O4	Antioxidant: Anti-inflammatory
201.9	2.62	C00758	Bergaptol	C11H6O4	Anticancer
163	1.51	C00811	4-Coumarate	C9H7O3	Antioxidant
179	12.60	C01197	Trans-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
169	15.69	c01424	Gallate	C7H5O5	Antioxidant; Anticancer
179	49.79	C01481	Cis-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
193	10.52	C01494	Ferulate	C10H9O4	Antioxidant; Anti-aging
217	25.08	C01557	Bergapten	C12H8O4	Anti-inflammatory; Anticancer
163	49.64	C01772	Trans-2-Coumarate	C9H7O3	Antioxidant; Anti-inflammatory
359	10.32	C01850	Rosmarinate	C18H15O8	Anti-inflammatory
277.1	20.74	C04498	4-Coumaroylagmatine	C14H21N4O2	Adrenergic receptor
177.1	37.67	C03081	4-Methylumbelliferone	C10H8O3	Anticancer
179.1	44.39	C10454	Methyleugenol	C11H14O2	Antioxidant
264.7	35.53	D03241	Nitecapone	C12H11NO6	Antioxidant
186	3.23	C09305	Psoralen	C11H6O3	Antipsoriasis
217	41.53	C01864	Xanthotoxin	C12H8O4	Anticancer
233.1	43.69	C02951	5-hydroxyxanthotoxin	C12H8O5	Anticancer
230.9	48.32	C18083	Demethylsuberosin	C14H14O3	Anti-inflammatory
			Vitamins And Co-factors		
175	23.61	C00072	L-Ascorbate	C6H7O6	Antioxidant
450.9	17.69	C02059	Phylloquinone	C31H46O2	Coagulation disorder
537	6.05	C02094	Beta Carotene	C40H56	Pigments
430.9	38.41	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
453	9.98	C03313	Phylloquinol	C31H48O2	Coagulation disorder
436.7	37.79	C13309	Demethylphylloquinone	C30H44O2	Unknown
425.1	4.86	C14153	Alpha Tocotrienol	C29H44O2	Anti-inflammatory
396.8	8.38	C14156	Delta Tocotrienol	C27H40O2	Anti-inflammatory
374.9	20.72	C00255	Riboflavin	C17H19N4O6	Dietary supplement
			Anthroquinones		
195	49.72	C00822	Dopaquinone	C9H9NO4	Unknown
240.8	40.93	C01474	Alizarin	C14H8O4	Pigment
502	21.15	C07606	Hypericin	C30H14O8	Anticancer
263	20.88	C18132	3"-hydroxy-geranylhydroquinone	C16H22O3	Unknown
752.9	3.33	C16695	Plastoquinol-9	C53H84O2	Unknown
327	0.95	C00113	Pyrroloquinoline Quinone	C14H3N2O8	Unknown
			Lignan		
372.8	11.39	C10545	Arctigenin	C21H24O6	Anti-inflammatory; Anticancer
380.2	24.91	C10559	Diphyllin	C21H16O7	Anticancer; Antiviral
252.9	31.13	C10628	(7s)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic
362.9	22.34	C18167	(-)-Secoisolariciresinol	C20H26O6	Anticancer
364	31.85	C10636	Justicidin B	C21H16O6	Anticancer

Supplementary Table 32. List of putative Steroids and sterols from callus cultures (leaves-derived) treated with Salicylic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Steroids and sterols)	Molecular formula	Bioactivity*
403	9.79	C15787	Campestanol	$C_{28}H_{50}O$	Unknown
419	3.48	C15788	(6alpha)-Hydroxycampestanol	$C_{28}H_{50}O_2$	Unknown
448.9	27.41	C15791	Teasterone	$C_{28}H_{48}O_4$	Unknown
446.8	20.34	C15792	3-Dehydroteasterone	$C_{28}H_{46}O_4$	Unknown
448.9	32.82	C15793	Typhasterol	$C_{28}H_{48}O_4$	Unknown
465	9.36	C15794	Castasterone	$C_{28}H_{48}O_5$	Unknown
418.9	14.47	C15798	6-Deoxocathasterone	$C_{28}H_{50}O_2$	Unknown
434.8	20.66	C15799	6-Deoxoteasterone	$C_{28}H_{50}O_3$	Unknown
435	23.87	C15801	6-Deoxotyphasterol	$C_{28}H_{50}O_3$	Unknown
413	14.59	C08821	Isofucosterol	$C_{29}H_{48}O$	Unknown
450.7	36.19	C15802	6-Deoxocastasterone	$C_{28}H_{50}O_4$	Unknown
396.9	0.66	C01694	Ergosterol	$C_{28}H_{44}O$	Unknown
426.9	8.71	C01724	Lanosterol	$C_{30}H_{50}O$	Unknown
414.8	19.33	C01753	Sitosterol	$C_{29}H_{50}O$	Anti-inflammatory; Antidiabetic
427	5.00	C01943	Obtusifoliol	$C_{30}H_{50}O$	Unknown
383	12.75	C05107	7-Dehydrodesmosterol	$C_{27}H_{42}O$	Unknown
412.2	21.36	C05108	14-Demethyllanosterol	$C_{29}H_{48}O$	Unknown
412.9	13.76	C05442	Stigmasterol	$C_{29}H_{48}O$	Unknown

*As reported in literature

Supplementary Table 33. List of Glycosides, Betacyanin from callus cultures (leaves-derived) treated with Salicylic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Glycoside)	Molecular formula	Bioactivity*
311	30.64	C01855	Taxiphyllin	$C_{14}H_{17}NO_7$	Antimicrobial
297	5.17	C05342	Psicofuranine	$C_{11}H_{15}N_5O_5$	Anticancer
254.9	18.99	C05841	Beta D-Ribosylnicotinate	$C_{11}H_{13}NO_6$	Unknown
260.9	16.09	C08334	Lotaustralin	$C_{11}H_{19}NO_6$	Unknown
423	13.23	C08336	Neolinustatin	$C_{17}H_{29}NO_{11}$	Unknown
422.9	32.40	C08411	Glucoiberin	$C_{11}H_{20}NO_{10}S_3$	Antimicrobial
406.9	8.91	C08412	Glucoiberverin	$C_{11}H_{20}NO_9S_3$	Antimicrobial
436.9	33.04	C08419	Glucoraphanin	$C_{12}H_{22}NO_{10}S_3$	Antioxidant
577	5.48	C12628	Vitexin 2''-O-Beta L-Rhamnoside	$C_{27}H_{29}O_{14}$	Anti-inflammatory; Anticancer
294.9	46.67	C08481	Indican	$C_{14}H_{17}NO_6$	Pigment
			Betacyanin		
386	27.98	C08539	Betanidin	$C_{18}H_{14}N_2O_8$	Antioxidant; Pigment

*As reported in literature

Supplementary Table 34. List of putative flavonoids from callus cultures (leaves-derived) treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Flavonoids)	Molecular formula	Bioactivity*
270.9	20.38	C01592	Licodione	C15H11O5	Unknown
301	30.55	C00389	Quercetin	C15H9O7	Anti-inflammatory
256	40.47	C08650	Isoliquiritigenin	C15H12O4	Antioxidant; Anti-inflammatory
283.1	25.86	C00814	Biochanin-A	C16H11O5	Antiproliferative
430.9	10.37	C01460	Vitexin	C21H19O10	Anti-inflammatory; Anticancer
283.2	28.32	C01470	Acacetin	C16H11O5	Anti-inflammatory; Anticancer
269.1	31.75	C01477	Apigenin	C15H9O5	Anticancer; Antidiabetic
285	39.46	C01514	Luteolin	C15H9O6	Anti-inflammatory; Anticancer
355	1.03	C01590	Kievitone	C20H19O6	Antimicrobial
339.3	26.33	C01701	Glyceollin I	C20H18O5	Anticancer
431.2	24.37	C01714	Isovitexin	C21H19O10	Anti-inflammatory; Anticancer
446.9	39.08	C01821	Isoorientin	C21H19O11	Anti-inflammatory; Antidiabetic
319	8.41	C02906	(+)-Dihydromyricetin	C15H11O8	Antioxidant; Antidiabetic
316.9	15.50	C04109	Gossypetin	C15H9O8	Antioxidant; Antibacterial
298.9	11.83	C04293	Chrysoeriol	C16H11O6	Antioxidant; Anticancer
315	14.69	C04443	3-O-Methylquercetin	C16H11O7	Antioxidant; Anticancer
446.8	21.56	C05376	Biochanin A-7-O-Glucoside	C22H22O10	Anticancer
286.9	29.56	C05631	(2S)-Eriodictyol	C15H11O6	Anticancer; Anti-inflammatory
307	12.43	C05906	(2R,3S,4S)-Leucocyanidin	C15H14O7	Antiviral
395	20.09	C07593	Rotenone	C23H22O6	Anticancer
594.9	7.46	C08064	Isovitexin 7-o-glucoside	C27H30O15	Antidiabetic
270.9	48.30	C08652	Luteolinidin	C15H11O5	Anti-inflammatory
595	2.36	C08725	Pelargonidin-3,5-Di-O-Beta D-Glucoside	C27H30O15	Pigment
458.8	48.67	C09731	(-)-Epigallocatechin-3-O-Gallate	C22H18O11	Anti-inflammatory; Anticancer
581	9.67	C09789	Naringin	C27H32O14	Anti-inflammatory; Anticancer
271	2.15	C09826	(+)-Pinobanksin	C15H11O5	Antioxidant
255.2	32.62	C09827	(2S)-Pinocembrin	C15H11O4	Anti-inflammatory; Anticancer
252.9	13.03	C10028	Chrysin	C15H9O4	Anti-inflammatory
315	35.07	C10084	Isorhamnetin	C16H11O7	Antioxidant; Antidiabetic
298.9	46.82	C10098	Kaempferide	C16H11O6	Anti-inflammatory
317	23.58	C10107	Myricetin	C15H9O8	Anti-inflammatory
317	41.09	C10122	Quercetagetin	C15H9O8	Antioxidant; Antidiabetic
287	10.28	C10415	Dalbergioidin	C15H11O6	Antioxidant
352.9	22.36	C10486	Phaseoluteone	C20H17O6	Antioxidant
352.9	42.28	C10498	Luteone	C20H17O6	Antioxidant; Antimicrobial
430.8	40.80	C10509	Ononin	C22H22O9	Anti-inflammatory
299	16.52	C10520	Pratensein	C16H11O6	Antioxidant
238.9	41.57	C11264	7-hydroxyflavone	C15H10O3	Anti-inflammatory; Anticancer
307	26.14	C12127	(+)-Gallocatechin	C15H14O7	Antioxidant
840.8	24.67	C12641	Monodemalonylsalvianin	C39H37O21	Unknown
238.9	46.64	C14137	6-Hydroxyflavone	C15H10O3	Anticancer
500.8	34.13	C16191	Malonyldaidzin	C24H21O12	Anti-inflammatory; Anticancer
514.8	15.45	C16222	Formononetin-7-O-Glucoside-6"-Malonate	C25H23O12	Antioxidant
616.9	31.14	C16289	Cyanidin 3-o-(3",6"-o-dimalonyl-beta Glucopyranoside)	C27H21O17	Anticancer; Pigments
840.8	31.80	C16299	Malonylshisonin	C39H37O21	Pigments
464.9	9.44	C16410	Bracteatin 6-O-Glucoside	C21H20O12	Unknown
596	4.97	C20491	inidin 3-O-Beta D-Sambubioside	C26H27O16	Apoptosis inducer

Supplementary Table 35. List of putative Terpenoids from extract of callus cultures (leaves-derived) treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Terpenoids)	Molecular formula	Bioactivity*
374.9	0.59	C01512	Loganate	C16H23O10	Antioxidant
223.1	24.00	C01126	(2e,6e)-farnesol	C15H26O	Antibacterial
390.9	10.37	C01433	Loganin	C17H26O10	Anti-inflammatory
469.9	1.88	C02283	Glycyrrhetinate	C30H45O4	Antioxidant
310.9	45.57	C03190	(+)-Bornyl-diphosphate	C10H17O7P2	Anti-inflammatory
544.9	22.68	C05413	All-Trans-Phytoene	C40H64	Pigments
542.9	21.17	C05414	All-trans Phytofluene	C40H62	Pigments
375	20.67	C06071	7-Deoxyloganin	C17H26O9	Anti-inflammatory
428.9	15.15	C06309	Hopan-22-ol	C30H52O	Antimicrobial
536.1	25.21	C07608	Hyperforin	C35H52O4	Anticancer
518.8	36.11	C07667	Gossypol	C30H30O8	Anticancer
597	8.29	C08580	(3s,3's)-astaxanthin	C40H52O4	Pigments; Antioxidant
393	12.87	C08582	Bixin	C25H29O4	Pigments; Antioxidant
553	7.96	C08590	Zeinoxanthin	C40H56O	Antioxidants/Pigments
550.9	23.77	C08592	Echinenone	C40H54O	Pigments; Antioxidant
378.8	38.60	C08608	Norbixin	C24H26O4	Pigments; Antioxidant
443.1	19.11	C08618	Betulin	C30H50O2	Anticancer
444.8	0.66	C08621	Dammarenediol II	C30H52O2	Anticancer
441	9.66	C08830	24-methylenecycloartanol	C31H52O	Antimicrobial
470	5.43	C08950	Gypsogenin	C30H45O4	Anticancer
458.7	26.13	C08980	Soyasapogenol B	C30H50O3	Antioxidant
456.9	10.45	C08988	Ursolate	C30H48O3	Antidiabetic
306.8	16.22	C09072	Alpha Cembratriene-4,6-diol	C20H34O2	Anticancer
290.8	23.50	C09094	Geranylgeraniol	C20H34O	Anticancer
309	24.04	C09183	Sclareol	C20H36O2	Anticancer; Fragrant
233	18.00	C09382	(+)-Costunolide (12,6alpha)	C15H20O2	Anticancer
261	37.68	C09680	Hemigossypol	C15H16O4	Anticancer; Anti-microbial
237	3.46	C09700	Lubimin	C15H24O2	Unknown
223.1	10.23	C09715	Rishitin	C14H22O2	Antimicrobial
231.1	44.63	C09918	Aucuparin	C14H14O3	Antimicrobial; Anticancer
306.8	27.38	C13273	Plaunotol	C20H34O2	Anticancer; Antiulcer
250	12.94	C13453	2-cis,4-trans-xanthoxin	C15H22O3	Antioxidant
566.9	32.78	C15966	3-hydroxyechinenone	C40H54O2	Pigments
580.8	20.45	C15967	Adonirubin	C40H52O3	Antioxidant
281.2	26.27	C15971	Dihydroxyphaseic Acid	C15H21O5	Plant Hormone
444.8	46.66	C19828	Arabidiol	C30H52O2	Anti-oomycete
504.8	45.39	C20152	Taxusin	C28H40O8	Anticancer
476.8	34.63	C20716	Protopanaxatriol	C30H52O4	Anticancer
432.9	6.93	C20894	Beta Citraurin	C30H40O2	Antioxidant
440.9	9.44	C20946	Oleanolic Aldehyde	C30H48O2	Antioxidant
475.7	18.66	C17419	Soyasapogenol A	C30H50O4	Anticancer
171.1	14.84	C17621	(6E)-8-hydroxygeraniol	C10H18O2	Unknown
314.7	39.98	C18015	Momilactone A	C20H26O3	Antidiabetic; Antifungal
410.9	8.30	C00751	Squalene	C30H50	Anticancer
262.9	20.59	C06082	2-cis-Abscisate	C15H19O4	Plant Hormone

*As reported in literature

Supplementary Table 36. List of putative Alkaloids from extract of callus (leaves-derived) treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Alkaloids)	Molecular formula	Bioactivity*
140.9	38.003	C00783	Tropinone	C8H14NO	Anticholinergic
306.9	34.162	C01513	Lupinate	C13H18N6O3	Unknown
369.1	26.123	C02134	Allocryptopine	C21H23NO5	Neuroprotective
352.7	39.231	C02151	19e-geissoschizine	C21H24N2O3	Unknown
265	26.926	C02621	13-hydroxylupanine	C15H25N2O2	Antioxidant
354.9	12.569	C02915	(S)-N-methylcanadine	C21H24NO4	Antioxidant
307	4.556	C03325	(6S)-Hydroxyhyoscyamine	C17H24NO4	Anti-inflammatory; Anti-arrhythmia
323.1	23.288	C05175	(S)-Stylopine	C19H17NO4	Antimicrobial
598.6	35.255	C05177	Berbamunine	C36H42N2O6	Anticancer
369	17.645	C05190	6-Hydroxyprotopine	C20H19NO6	Antimicrobial
378.9	28.357	C05193	12-Hydroxydihydrochelirubine	C21H17NO6	Unknown
363	7.891	C05194	Dihydrochelirubine	C21H17NO5	Anti-tuberculosis
236.9	8.897	C05204	(S)-tetrahydroprotoberberine	C17H18N	Anti-inflammatory; Anticancer
295	38.745	C06528	Cinchonine	C19H23N2O	Antimalarial; Antidiabetic
199.9	5.279	C06537	Harmalol	C12H12N2O	Anticancer; Antimicrobial
271	48.458	C08521	Caranine	C16H17NO3	Unknown
468.9	10.422	C09390	Cephaeline	C28H40N2O4	Antioxidant
248.9	28.910	C10752	17-Oxosparteine	C15H25N2O	Antimicrobial
292.8	23.288	C11633	16-Epivellosimine	C19H20N2O	Unknown
292.9	39.069	C11634	Vellosimine	C19H20N2O	Unknown
352.8	38.475	C11808	1,2-dihydrovomilenine	C21H24N2O3	Anticancer
271.1	43.477	C12162	Crinine	C16H17NO3	Unknown
373.1	0.878	C16707	(S)-Autumnaline	C21H27NO5	Anticancer
292.9	44.786	C16726	Cinchoninone	C19H20N2O	Antiplasmodial
274.9	14.931	C21637	Elwesine	C16H21NO3	Anticancer; Antimicrobial
275	45.374	C21639	Maritinamine	C16H21NO3	Unknown
200	43.686	C21721	Camalexin	C11H8N2S	Antimicrobial
322.9	49.167	C16728	Quinidinone	C20H22N2O2	Antiarrhythmic
498.9	27.925	C16724	Strictosamide	C26H30N2O8	Anti-inflammatory
370.9	47.520	C16708	(S)-Isoandrocymbine	C21H25NO5	Unknown
339.1	22.127	C11818	(R)-Canadine	C20H21NO4	Antioxidant
248.9	48.647	C10772	Lupanine	C15H25N2O	Antioxidant
173	17.793	C10173	Swainsonine	C8H15NO3	Anticancer
482.9	18.826	C09421	Emetine	C29H42N2O4	Antimicrobial
287	18.833	C08532	Lycorine	C16H17NO4	Anticancer
398.7	18.968	C06543	Solanidine	C27H44NO	Unknown
392.9	19.386	C05316	Dihydromacarpine	C22H19NO6	Neuroprotective
341	13.865	C04118	(S)-Tetrahydrocolumbamine	C20H23NO4	Unknown
338.9	23.868	C03329	(S)-Canadine	C20H21NO4	Antioxidant: Anti-caner
598.9	9.41	C06511	Guattegaumerine	C36H42N2O6	Anticancer
355	23.382	C02890	(S)-Tetrahydropalmatine	C21H25NO4	Unknown
389.2	27.68	C01852	Secologanin	C17H24O10	Neuroprotective
496.8	14.29	C16725	Deoxypumiloside	C26H28N2O8	Anti-inflammatory
512.9	13.37	C16723	Pumiloside	C26H28N2O9	Anti-inflammatory

*As reported in literature

Supplementary Table 37. List of putative phenolics from extract of callus cultures (leaves-derived) treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Phenolic acids)	Molecular formula	Bioactivity
223	12.30	C00482	Sinapate	C11H11O5	Antioxidant; Anti-inflammatory
336.8	37.80	C00796	Dicoumarol	C19H12O6	Anticoagulant
162.9	47.40	C00811	4-Coumarate	C9H7O3	Antioxidant
386.1	27.44	C01175	1-O-Sinapoyl-Beta D-Glucose	C17H22O10	Antioxidant
178.9	40.06	C01197	Trans-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
168.9	22.14	c01424	Gallate	C7H5O5	Antioxidant; Anticancer
179	2.05	C01481	Cis-Caffeate	C9H7O4	Antioxidant; Anti-inflammatory
217	49.68	C01557	Bergapten	C12H8O4	Anti-inflammatory; Anticancer
163	13.12	C01772	Trans-2-Coumarate	C9H7O3	Antioxidant; Anti-inflammatory
359.1	22.80	C01850	Rosmarinate	C18H15O8	Anti-inflammatory
217.1	24.15	C01864	Xanthotoxin	C12H8O4	Anticancer
211	35.95	C02325	Sinapyl Alcohol	C11H14O4	Unknown
313	8.36	C02717	N-Feruloyltyramine	C18H19NO4	Anti-inflammatory
233.1	0.26	C02951	5-Hydroxyxanthotoxin	C12H8O5	Anticancer
276.9	13.38	C04498	4-Coumaroylagmatine	C14H21N4O2	Adrenergic receptor
163.1	2.69	C05838	Coumarinate	C9H7O3	Antidiabetic
186	28.01	C09305	Psoralen	C11H6O3	Antipsoriasis
179.1	46.02	C10454	Methyleugenol	C11H14O2	Antioxidant
163.1	43.85	C12621	3-Hydroxycinnamate	C9H7O3	Anti-inflammatory
265.1	33.54	D03241	Nitecapone	C12H11NO6	Antioxidant
153.3	12.10	C00230	Protocatechuate	C7H5O4	Anti-inflammatory; Anticancer

*As reported in literature

Supplementary Table 38. List of putative Steroids and sterols, and glycosides from extract of callus treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Steroids and sterols)	Molecular formula	Bioactivity
400.8	8.89	C01789	Campesterol	C28H48O	Anti-inflammatory; Antidiabetic
385	34.63	C01802	Desmosterol	C27H44O	Unknown
385	39.68	C05437	Zymosterol	C27H44O	Unknown
413	22.06	C05442	Stigmasterol	C29H48O	Unknown
398.9	49.50	C08813	Brassicasterol	C28H46O	Unknown
388.8	20.77	C12978	Epi-cholestanol	C27H48O	Unknown
419.1	22.64	C15788	(6alpha)-hydroxycampestanol	C28H50O2	Unknown
448.8	22.28	C15791	Teasterone	C28H48O4	Unknown
446.8	13.86	C15792	3-dehydroteasterone	C28H46O4	Unknown
448.9	23.49	C15793	Typhasterol	C28H48O4	Unknown
464.8	17.81	C15794	Castasterone	C28H48O5	Unknown
419	2.86	C15798	6-deoxocathasterone	C28H50O2	Unknown
435	3.97	C15799	6-deoxoteasterone	C28H50O3	Unknown
435	11.39	C15801	6-deoxotyphasterol	C28H50O3	Unknown
450.9	10.85	C15802	6-deoxocastasterone	C28H50O4	Unknown
316.8	41.38	C01953	Pregnenolone	C21H32O2	Phytahormones
415.1	21.53	C01753	Sitosterol	C29H50O	Unknown
314.8	34.41	C00410	Progesterone	C21H30O2	Unknown
386.9	33.99	C01189	Lathosterol	C27H46O	Anti-inflammatory; Antidiabetic

*As reported in literature

Supplementary Table 39. List of putative Vitamins and Co-factors, Anthroquinones, Lignan from extract of callus treated with Jasmonic acid through LC-MS/MS analysis

m/z value	Retention time	KEGG ID	Putative Compounds (Glycosides)	Molecular formula	Bioactivity
247	19.87	C01594	Linamarin	C10H17NO6	Anticancer
311	22.13	C01855	Taxiphyllin	C14H17NO7	Antimicrobial
311	32.84	C05143	Dhurrin	C14H17NO7	Unknown
297	10.40	C05342	Psicofuranine	C11H15N5O5	Anticancer
457.1	32.17	C08325	(R)-Amygdalin	C20H27NO11	Anticancer
260.9	44.35	C08334	Lotaustralin	C11H19NO6	Unknown
422.9	33.70	C08411	Glucoiberin	C11H20NO10S3	Antimicrobial
406.9	22.21	C08412	Glucoiberverin	C11H20NO9S3	Antimicrobial
294.9	21.68	C08481	Indican	C14H17NO6	Pigment
			Stilbenes		
229	12.29	C03582	Trans-Resveratrol	C14H12O3	Anticancer
227	43.30	C10276	Pinosylvin Monomethylether	C15H14O2	Antioxidant
			Vitamins and Co-factors		
374.9	20.18	C00255	Riboflavin	C17H19N4O6	Dietary supplement
450.9	39.17	C02059	Phylloquinone	C31H46O2	Coagulation disorder
537	3.04	C02094	Beta Carotene	C40H56	Pigment
431	10.83	C02477	Alpha Tocopherol	C29H50O2	Antioxidant; Anticancer
452.8	15.07	C03313	Phylloquinol	C31H48O2	Coagulation disorder
403	2.88	C14151	Delta Tocopherol	C27H46O2	Antioxidant; Anticancer
397	31.13	C14156	Delta Tocotrienol	C27H40O2	Anti-inflammatory
175.1	2.32	C00072	L-Ascorbate	C6H7O6	Antioxidant
			Anthroquinones		
502	19.49	C07606	Hypericin	C30H14O8	Anticancer
752.9	8.77	C16695	Plastoquinol-9	C53H84O2	Unknown
240.9	47.14	C01474	Alizarin	C14H8O4	Pigment
195	3.29	C00822	Dopaquinone	C9H9NO4	Unknown
			Lignan		
362.9	29.86	C18167	(-)-Secoisolariciresinol	C20H26O6	Anticancer
252.8	26.76	C10628	(7S)-Cis-Hinokiresinol	C17H16O2	Antioxidant; Antiatherogenic
365	9.98	C10636	Justicidin B	C21H16O6	Anticancer
370.8	42.32	C21183	(-)-Bursehernin	C21H22O6	Anticancer

*As reported in literature

Supplementary Table 40. Summary of pathway enrichment analysis of metabolites in SA treated callus cultures

	Total	Expected	Hits	Raw p	-log10(p)	Holm adjust	FDR	Impact
Brassinosteroid biosynthesis	26	1.09	9	3.88E-07	6.41E+00	3.72E-05	3.72E-05	0.28
Steroid biosynthesis	45	1.88	10	7.91E-06	5.10E+00	7.52E-04	3.80E-04	0.31
Flavonoid biosynthesis	47	1.96	10	1.20E-05	4.92E+00	1.13E-03	3.85E-04	0.13
Carotenoid biosynthesis	43	1.80	9	3.99E-05	4.40E+00	3.71E-03	9.57E-04	0.16
Ubiquinone and other terpenoid-quinone biosynthesis	38	1.59	8	1.07E-04	3.97E+00	9.88E-03	2.06E-03	0.13
Flavone and flavonol biosynthesis	10	0.42	2	6.24E-02	1.21E+00	1.00E+00	9.98E-01	0.35
Sesquiterpenoid and triterpenoid biosynthesis	24	1.00	3	7.54E-02	1.12E+00	1.00E+00	1.00E+00	0.22
Phenylpropanoid biosynthesis	46	1.92	4	1.22E-01	9.13E-01	1.00E+00	1.00E+00	0.16
Biosynthesis of secondary metabolites - unclassified	5	0.21	1	1.93E-01	7.16E-01	1.00E+00	1.00E+00	1.00
Biosynthesis of secondary metabolites - other antibiotics	6	0.25	1	2.26E-01	6.45E-01	1.00E+00	1.00E+00	0.00
Tropane, piperidine and pyridine alkaloid biosynthesis	8	0.33	1	2.90E-01	5.38E-01	1.00E+00	1.00E+00	0.00
Monoterpenoid biosynthesis	9	0.38	1	3.20E-01	4.95E-01	1.00E+00	1.00E+00	0.00
Cyanoamino acid metabolism	29	1.21	2	3.44E-01	4.63E-01	1.00E+00	1.00E+00	0.00
Anthocyanin biosynthesis	11	0.46	1	3.76E-01	4.25E-01	1.00E+00	1.00E+00	0.00
Riboflavin metabolism	11	0.46	1	3.76E-01	4.25E-01	1.00E+00	1.00E+00	0.12
Nicotinate and nicotinamide metabolism	13	0.54	1	4.27E-01	3.69E-01	1.00E+00	1.00E+00	0.00
Ascorbate and aldarate metabolism	18	0.75	1	5.38E-01	2.69E-01	1.00E+00	1.00E+00	0.22
Glutathione metabolism	26	1.09	1	6.74E-01	1.72E-01	1.00E+00	1.00E+00	0.00
Terpenoid backbone biosynthesis	30	1.25	1	7.26E-01	1.39E-01	1.00E+00	1.00E+00	0.00
Arginine and proline metabolism	34	1.42	1	7.70E-01	1.14E-01	1.00E+00	1.00E+00	0.01
Glucosinolate biosynthesis	65	2.72	1	9.42E-01	2.62E-02	1.00E+00	1.00E+00	0.00

Supplementary Table 41. Summary of pathway enrichment analysis of metabolites JA treated callus cultures

	Total	Expected	Hits	Raw p	-log10(p)	Holm adjust	FDR	Impact
Brassinosteroid biosynthesis	26	1.12	9	5.15E-07	6.29E+00	4.95E-05	4.95E-05	0.28
Flavonoid biosynthesis	47	2.03	10	1.62E-05	4.79E+00	1.53E-03	7.75E-04	0.16
Carotenoid biosynthesis	43	1.86	9	5.19E-05	4.29E+00	4.87E-03	1.66E-03	0.14
Steroid biosynthesis	45	1.94	9	7.61E-05	4.12E+00	7.07E-03	1.83E-03	0.17
Ubiquinone and other terpenoid-quinone biosynthesis	38	1.64	7	8.75E-04	3.06E+00	8.05E-02	1.68E-02	0.07
Flavone and flavonol biosynthesis	10	0.43	3	7.41E-03	2.13E+00	6.74E-01	1.18E-01	0.35
Phenylpropanoid biosynthesis	46	1.99	6	1.23E-02	1.91E+00	1.00E+00	1.68E-01	0.15
Cyanoamino acid metabolism	29	1.25	4	3.33E-02	1.48E+00	1.00E+00	3.99E-01	0.00
Anthocyanin biosynthesis	11	0.47	2	7.85E-02	1.11E+00	1.00E+00	7.81E-01	0.00
Sesquiterpenoid and triterpenoid biosynthesis	24	1.04	3	8.13E-02	1.09E+00	1.00E+00	7.81E-01	0.22
Indole alkaloid biosynthesis	4	0.17	1	1.62E-01	7.91E-01	1.00E+00	1.00E+00	0.00
Biosynthesis of secondary metabolites - unclassified	5	0.22	1	1.98E-01	7.03E-01	1.00E+00	1.00E+00	1.00
Biosynthesis of secondary metabolites - other antibiotics	6	0.26	1	2.33E-01	6.33E-01	1.00E+00	1.00E+00	0.00
Tropane, piperidine and pyridine alkaloid biosynthesis	8	0.35	1	2.98E-01	5.26E-01	1.00E+00	1.00E+00	0.00
Monoterpenoid biosynthesis	9	0.39	1	3.28E-01	4.84E-01	1.00E+00	1.00E+00	0.00
Riboflavin metabolism	11	0.47	1	3.86E-01	4.14E-01	1.00E+00	1.00E+00	0.12
Ascorbate and aldarate metabolism	18	0.78	1	5.50E-01	2.60E-01	1.00E+00	1.00E+00	0.22
Glutathione metabolism	26	1.12	1	6.86E-01	1.64E-01	1.00E+00	1.00E+00	0.00
Terpenoid backbone biosynthesis	30	1.29	1	7.37E-01	1.32E-01	1.00E+00	1.00E+00	0.00
Arginine and proline metabolism	34	1.47	1	7.81E-01	1.08E-01	1.00E+00	1.00E+00	0.01
Glucosinolate biosynthesis	65	2.81	1	9.47E-01	2.38E-02	1.00E+00	1.00E+00	0.00

PUBLICATION

In Vitro Cellular & Developmental Biology - Plant (2023) 59:227–248
https://doi.org/10.1007/s11627-023-10335-7

PLANT TISSUE CULTURE

Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem

Nyanthanglo Woch[1] · **Supriya Laha**[1] · **Padmaja Gudipalli**[1]

Received: 25 August 2022 / Accepted: 20 January 2023 / Published online: 2 February 2023 / Editor: Yong Eui Choi

Abstract

Givotia moluccana (L.) Sreem. is a highly valued forest tree species known for its medicinal properties and the presence of bioactive compounds. Elicitation is an effective strategy used for the biotechnological production of secondary metabolites. The study aimed at determining the effects of jasmonic acid (JA) and salicylic acid (SA) elicitation on accumulation of total phenolics, flavonoids, and regulation of antioxidant metabolism in callus cultures of *G. moluccana* L. Callus cultures treated with 150 µM JA for 20 d resulted in highest contents of total phenolics (81.83 mg GAE g^{-1} DW) and flavonoids (60.89 mg RE g^{-1} DW) as compared to SA-treated and control cultures. SA-treated callus cultures showed relatively higher contents of total phenolics (31.43 GAE g^{-1} DW) and flavonoids (38.49 RE g^{-1} DW) when treated with 50 µM for 5 d and 20 d, respectively, compared to control callus cultures. Treatment of callus cultures with 150 µM JA for 20 d was found to be optimal as the maximum production of H_2O_2 was associated with increased enzymatic (SOD, CAT, and POD) and non-enzymatic (ascorbate, total glutathione, and GSH) antioxidants that were higher than SA-treated and control callus cultures. Real-time PCR analysis of transcript levels of antioxidant genes revealed similar pattern of expression as that of enzymatic activities in SA- and JA-treated callus cultures showing correlation between transcriptional and enzyme activity regulation. On the other hand, the transcript level of *PAL* gene in JA-treated callus cultures was lower than in SA-treated callus although elevated enzyme activity was observed in response to JA treatment which could be due to differential posttranscriptional modifications. Overall, the study revealed that JA is more effective than SA in achieving higher yields of total phenolics and flavonoids in callus cultures of *G. moluccana* as well as provided insights into antioxidant metabolism in response to the treatments.

Keywords *G. moluccana* · Leaves · Callus cultures · Elicitation · JA · SA · Flavonoids · Phenolics · Antioxidant metabolism

Introduction

Plants produce a wide array of secondary metabolites that play a major role in defense against biotic and abiotic stresses. Plant secondary metabolites have received considerable attention due to potential applications in pharmaceutical, nutraceutical, and agrochemical industries. The production of secondary metabolites is often dependent on environmental factors and physiological and developmental stages of the plants and hence can be limiting (Rao and Ravishankar 2002). Moreover, the chemical synthesis of pharmaceutically important plant secondary metabolites is often difficult due to their diverse nature. *In vitro* techniques offer a promising approach for production of secondary metabolites under controlled conditions along with the possibility of enhancing the production of high-value compounds which are otherwise produced in meager amounts.

Elicitation is an efficient strategy for enhancing the biosynthesis of secondary metabolites because of its practical feasibility (Poulev *et al*. 2003). It exploits the plants' defense mechanism against stress, pathogens, or external stimuli for production of secondary metabolites. Elicitors activate cascades of events such as reactive oxygen species (ROS)

✉ Padmaja Gudipalli
gudipallipadmaja@gmail.com

Nyanthanglo Woch
nyanthanglo@gmail.com

Supriya Laha
mesupriya97@gmail.com

[1] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, Telangana, India

production, expression of defense-related genes, and accumulation of secondary metabolites (Zhao *et al.* 2005). Abiotic elicitors like jasmonic acid (JA) and salicylic acid (SA) are frequently used for enhancing the production of secondary metabolites. SA, synthesized either by the phenylalanine or isochorismate pathways, plays a major role in systematic acquired resistance (SAR) during pathogen attack in plants (Klessig *et al.* 2018). JA modulates octadecanoid signaling pathway and plays a vital role in plant's tolerance to stress by modulating the defense responses (Sembdner and Parthier 1993). Both JA and SA, when provided exogenously, trigger responses similar to pathogen exposure or other external stimuli (Giri and Zaheer 2016). They initiate signal transduction pathways that lead to transcription of various genes thereby triggering the accumulation of molecules such as polyphenols, alkaloids, terpenoids, and steroids involved in defense and resistance of plants (Pieterse and Van Loon 1999; Hayat *et al.* 2010; Giri and Zaheer 2016). These secondary metabolites are known to have a range of pharmacological activities such as anticancer, antidiabetic, antiasthma, antimalarial, antimicrobial, and antiviral. They have been used in medicine either directly or as the starting point for drug discovery (Awuchi and Amagwula 2020).

Medicinal plants play an important role in preventing the detrimental consequences of oxidative stress. Various environmental stresses including the exogenous application of elicitors like JA and SA trigger the generation of ROS *viz.*, singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH) that cause oxidative damage and ultimately cell death. ROS not only have deleterious effects, but also function as important signaling molecules that regulate normal plant growth and responses to stresses (Huang *et al.* 2019). The level of ROS is maintained by an intricate and flexible enzymatic and non-enzymatic antioxidant system to balance their production and elimination, required for normal cellular homeostasis. In plants, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) along with ascorbate–glutathione (AsA-GSH) cycle play a vital role in the elimination of ROS such as hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl free radicals (•OH) generated during stress conditions (Bowler *et al.* 1992; Alscher *et al.* 1997; Mittler *et al.* 2004). Several non-enzymatic compounds such as phenolics, flavonoids, ascorbic acid, glutathione, carotenoids, and α-tocopherol also protect the cells from cytotoxic effects of ROS. The bioactive roles of phenolics and flavonoids are often associated with antioxidant potency (Ghasemzadeh *et al.* 2010).

Givotia moluccana (L.) Sreem. (syn. *G. rottleriformis* Griff. ex Wight) is a tree species of high economic importance due to its softwood and medicinal properties. The tree species has restricted distribution and is found mostly in hilly slopes in the forests of Tamil Nadu, Andhra Pradesh, Karnataka, and West Bengal States of India. This plant species is used in indigenous medicinal practices for treating inflammatory diseases such as rheumatism, psoriasis, and dandruff (Thammanna and Narayana Rao 1990). The extracts of different parts of this plant species have been reported to possess various biological properties including antipsoriatic, hepatoprotective, antimicrobial, and antioxidant activities (Vijayalakshmi *et al.* 2017). Secondary metabolites produced in plants serve as the material basis for the curative effects of medicinal plants (Zhang *et al.* 2021). This tree species is reported to be a rich source of phenolics and flavonoids, which are known to have antioxidant properties, justifying its use in traditional medicine. Vijayalakshmi *et al.* (2014) reported the antipsoriatic activity of flavonoids isolated from the bark of this species. Our research group has isolated phenolic compounds *viz.* gallic acid, methyl gallate, and benzoylsalicylic acid (BzSA) from seed coats of this species and reported their biological activities (Samuel *et al.* 2015, 2016). Owing to its medicinal properties and being a rich source of secondary metabolites like phenolics and flavonoids, it offers a good system for biotechnological studies. Callus cultures are a promising source for investigations on the production of secondary metabolites as well as understanding their metabolism and regulation. To the best of our knowledge, callus induction, growth, and secondary metabolite production from *in vitro* cultures in response to SA and JA elicitation have not been investigated so far in this species. Thus, the present study was undertaken to establish callus cultures from leaf explants and evaluate the effects of SA and JA elicitation on enhancement of phenolic and flavonoid contents and antioxidant metabolism including the expression of the *PAL* gene in callus cultures of *G. moluccana*.

Materials and Methods

Plant Materials The seeds of *G. moluccana* were collected from Forest Research Station, Mulugu, Telangana. The seeds were germinated *in vitro* and seedlings were produced as reported earlier (Samuel *et al.* 2009). The pericarp of the seeds was removed using pruning secateurs. The hard stony seeds were washed thoroughly under running tap water and placed in 0.1% Tween-20 (Sigma-Aldrich, St. Louis, MO) for 10 min and rinsed with distilled water followed by 1% bavistin (BASF India Limited, Mumbai, India) treatment for another 10 min. They were surface-sterilized with 70% ethanol for 3 min and subsequently with 0.1% mercuric chloride (Sisco Research Laboratories Pvt. Ltd., Mumbai, India) for 10 min in a laminar airflow chamber. The seeds were rinsed 3 times with sterile double-distilled water after each treatment. After surface sterilization, they were soaked in sterile distilled water overnight, dried using sterilized tissue paper, and cut gently using a bench vice. The cut seeds with undamaged zygotic embryo axes were then placed in half-strength MS (Murashige and Skoog 1962) medium containing 28.9 µM gibberellic acid (Sigma-Aldrich, St. Louis, MO) for 3 d in the dark. The cultures were then moved to culture racks and kept under the light with 16 h photoperiod at a photosynthetic photon flux density (PPFD) of 83.6 $\mu Em^{-2} s^{-1}$ provided by white fluorescent tubes (OSRAM Lighting India Pvt. Ltd, Gurgaon, Haryana, India) for germination and production of seedlings for using them as a source of leaf explants for callus formation.

Establishment of Callus Cultures from Leaf Explants The young leaves obtained from 30-d-old *in vitro* germinated seedlings were cut into small pieces of 1–1.5 cm and inoculated on MS medium with 3% sucrose (Merck, Mumbai, India), 0.8% (w/v) agar (Sigma-Aldrich, St. Louis, MO) containing plant growth regulators (Sigma-Aldrich, St. Louis, MO) *viz*., either individually or in combinations for callus induction. Five different types of media were used consisting of 2 mg/l 2,4-dichlorophenoxyacid (2,4-D) alone or in combination with 1 mg/l 6-benzylaminopurine (BAP) or 1-naphthaleneacetic acid (NAA) or kinetin (KIN) or 2 mg/l NAA with 1 mg/l BAP to evaluate their effects on callus induction from leaf explants. The pH of the media was adjusted to 5.6 to 5.8 using 1 M sodium hydroxide (NaOH) and 1N hydrochloric acid (HCl) prior to autoclaving at 120°C and 15 psi pressure for 15 min. The explants (6 nos) were inoculated into each culture bottle (60 × 110 mm) containing approximately 40 ml of the media. The cultures were incubated in dark at 25 ± 2°C and examined intermittently for recording the duration of callus initiation from the leaf explants. The observations on frequency of callus initiation and nature and color of callus were taken after 4 wk of culture on the medium. The effect of different media on callus biomass was determined based on fresh weight and dry weight after 5 wk of a subculture of callus on fresh medium containing the same growth regulators used for callus initiation. To determine the growth curve, the fresh weight and dry weight of the callus subcultured on medium with 2 mg/l 2,4-D and 1 mg/l BAP were measured at weekly intervals for 6 wk and expressed as grams per 100 ml medium. For measuring the fresh weight and dry weight, approximately 1 g of callus was placed in each culture bottle containing 40 ml of the medium augmented with different growth regulators. The calli were taken out from the culture bottles and dried in an oven (45°C) for 48 h for dry weight estimation. The weight (g) of the callus at the time of harvesting and at inoculation was determined and the difference is used for measuring the fresh weight and dry weight. Each culture bottle is treated as a replicate in the analysis of the results.

Effect of Salicylic Acid (SA) and Jasmonic Acid (JA) on Biomass, Total Phenolics, Total Flavonoids, and Antioxidant Activities in Leaf Callus Cultures Freshly harvested callus obtained after 5 wk of subculture was used for elicitation experiments with SA and JA. The callus was inoculated in MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l BAP with different concentrations (50 µM, 100 µM, 150 µM, and 200 µM) of SA and JA (Sigma-Aldrich, St. Louis, MO) which were dissolved in the solvent (ethanol) and added aseptically after filter sterilization with a 0.22-µm membrane filter (Axiva Sichem Biotech, New Delhi, India) to the culture media. The culture medium without any elicitor served as a control. The experiments were carried out by inoculating 1 g of callus into each culture bottle containing 40 ml of medium. The effects of SA and JA on fresh weight and dry weight were determined after 5 wk of culture on the media.

For estimating the total phenolics and flavonoids, the calli were carefully removed from the culture bottles every 5 d for the following 30 d. The calli were dried in an oven (45°C) for 48 h. The dried callus was then ground into fine powder and used for preparing the extracts. The dried callus powder (5 g each) was placed in 100 ml of methanol and stirred overnight in a magnetic stirrer. The extracts were centrifuged at 13,000 rpm for 15 min and the supernatants were recovered. The supernatants were then concentrated in a rotary vacuum evaporator (Heidolph Instruments, Schwabach, Germany) until it was reduced to powder/dried form, which were stored at − 20°C until used for different experiments.

Estimation of Total Phenolic Acid and Total Flavonoid Contents Total phenolic acid (TPA) estimation was carried out spectrophotometrically using the Folin-Ciocalteu method as described by Singleton and Rossi (1965). Gallic acid was used as a standard. The sample (50 µl) was taken from the diluted extract (25 mg of dried powder/ml of methanol), and then 2.5 ml of 0.2 N of Folin-Ciocalteu reagent was added. After incubating for 6 min, 2 ml of 7.5% sodium carbonate solution (Na_2CO_3) was added to the sample in the tubes and the volume was made up to 5 ml with distilled water. The tubes were left in dark at room temperature for 30 min. The absorbance of the sample in each tube was read at 760 nm wavelength. Different concentrations of gallic acid ranging from 5 to 50 µg were aliquoted in triplicates and reaction was performed and the standard graph was plotted. The TPA of each sample was estimated through the standard curve of gallic acid. The total phenolic acid was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight (DW).

Total flavonoid content was estimated according to aluminum chloride ($AlCl_3$) colorimetric assay method described by Barreira *et al*. (2008) using rutin hydrate as the standard. The sample of 50 µl was taken from the diluted extract (25 mg of dried powder/ml of methanol), in each tube followed by the addition of 50 µl of 5% sodium nitrite solution. After 5 min of incubation, 120 µl of 10% $AlCl_3$ solution was added. The reaction mixture was incubated for 6 min and 200 µl of 1 M NaOH was added and the volume was made up to 1 ml with distilled water. The solution was mixed well and the absorbance was read at 510 nm. A standard curve was plotted using different concentrations of rutin (5–50 µg). The flavonoid content was estimated for each extract and expressed as milligrams of rutin equivalents (RE) per gram dry weight (DW) according to the standard curve.

Quantification of Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA) Hydrogen peroxide levels were determined as per the method described by Alexieva *et al* (2001). The callus (500 mg) was homogenized in an ice-cold mortar and pestle with 5 ml of 10% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 rpm for 10 min at 4°C. Equal volume of the supernatant and 10 mM phosphate buffer (pH 7) were taken and 1 ml of 1 M potassium iodide was added to it. The mixture was measured for its absorbance at 390 nm wavelength spectrophotometrically. The mixture without the supernatant was used as blank. The content of the H_2O_2 in the sample was estimated as per the standard curve and expressed in micromol per gram fresh weight (µmol g^{-1} FW).

A lipid peroxidation assay was carried out by measuring the malondialdehyde (MDA) produced during thiobarbituric acid (TBA) reaction by following the method of Heath and Pecker (1968). The callus (100 mg) was homogenized with 10% TCA and centrifuged at 15,000 rpm for 10 min at 4°C. The supernatants were collected and 0.5 ml of it was added to 1.5 ml of 0.5% TBA (w/v) in 20% TCA. The mixture was incubated in a water bath at 95°C for 25 min and the reaction was ended by incubating it on ice. The mixture was centrifuged at 15,000 rpm for 5 min at 4°C. The absorbance of the solution was measured at two different wavelengths, *i.e.*, 532 nm and 600 nm. The OD_{532} values of the non-specific absorption were subtracted from the OD_{600} values of MDA-TBA complex (red color). The MDA concentration was calculated based on Beer-Lambert's law with an extinction coefficient of 155 mM^{-1} cm^{-1}. The results were presented in µmol g^{-1} FW.

Extraction and Assay for Antioxidant Enzyme Activities Protein Extraction

The protein was extracted according to the method described by Ben Amor *et al*. (2005). The fresh callus (~ 1 g) was ground to fine powder using liquid nitrogen in mortar and pestle and homogenized with 100 mM tris hydrochloride (Tris–HCl) buffer (pH 8) comprising of 10 mM ethylenediamine tetraaceticacid (EDTA), 20 mM magnesium chloride ($MgCl_2$), 1 mM dithiothreitol (DTT), 50 mM potassium chloride (KCl), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 0.1% Triton X-100 (v/v), and 10% polyvinylpyrrolidone (PVP). The mixture was centrifuged at 14,000 rpm for 30 min at 4°C. The protein concentration in the supernatant was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard. The protein was stored at −20°C and used for various antioxidant assays.

Superoxide Dismutase (SOD) Activity Assay Beauchamp and Fridovich (1971) method was used to determine the SOD (EC1.15.1.1) activities in the samples. To 20–50 µl of the enzyme extract, a reaction cocktail consisting of 1.5 ml of 50 mM potassium phosphate buffer (pH 7.8), 13 mM L-methionine (0.2 ml), equal volume of 75 µM nitro blue tetrazolium chloride (NBT), 4 µM riboflavin, and 1 µM EDTA was added in a final volume of 3 ml reaction. The reaction was initiated by exposing it to a fluorescent light of photosynthetic photon flux of 50 µM m^{-2} s^{-1} for 15 min. The resulting blue color developed in the solution was spectrophotometrically measured at 560 nm. One unit of SOD activity is taken as 50% of the inhibition of the color developed and expressed as U mg^{-1} of protein.

Catalase (CAT) Enzyme Activity Assay CAT (EC 1.11.1.6) enzyme activity assay was carried out as described by Aebi (1984). The samples of 500 mg were ground in 5 ml of 50 mM buffer phosphate (pH 7) and 50 mg of PVP in a mortar and pestle that was previously cooled to 4°C. The homogenous mixture was centrifuged at 13,000 rpm for 15 min and the supernatant was collected. In order to determine the catalase enzyme activity, the reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.1 ml of 3 mM EDTA, 0.1 ml of 20 mM H_2O_2, and the enzyme extract equivalent to 25 µg protein in a final volume of 1 ml was added and incubated. The absorbance was recorded for 1 min at 240 nm. The extinction coefficient of H_2O_2 at 240 nm is 43.6 M^{-1} cm^{-1}. A unit of catalase is defined as the quantity of enzyme necessary to decompose 1 µM of H_2O_2 per min at 25°C.

Peroxidase (POD) Enzyme Activity Assay POD (EC 1.11.1.7) activity was determined according to the method of Polle *et al*. (1994) based on its ability to convert guaiacol to tetraguaiacol ($\varepsilon = 26.6$ mM^{-1} cm^{-1}). The 2 ml reaction mixture consisted of 0.5 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.5 ml of 3.4 mM guaiacol, 0.5 ml of 0.9 mM H_2O_2, and 0.5 ml enzyme extract. The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm.

Effects of SA and JA in the Regulation of AsA-GSH (Ascorbate–Glutathione) Pathway The influence of SA and JA on the enzymes involved in the Halliwell-Asada cycle or ascorbate–glutathione (AsA-GSH) pathway in callus cultures was studied. The AsA-GsH pathway is involved in the detoxification of hydrogen peroxide using four crucial enzymes *viz.*, APX (ascorbate peroxidase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase), and GR (glutathione reductase).

Analysis of APX, GR, MDHAR, and DHAR Enzyme Activities All enzyme assays were carried out with crude enzyme extracts. APX (EC 1.11.1.11) was assayed according to the method of Nakano and Asada (1981). The reaction mixture of 1.5 ml for measuring APX activity contained 50 mM sodium phosphate buffer (pH 7.0), 0.2 mM EDTA (0.1 ml), 0.5 mM ascorbic acid, and enzyme extract equivalent to 25 µg of protein. The reaction was initiated by adding 20 mM H_2O_2 and the activity of the enzyme was recorded as a decrease in the absorbance at 290 nm for 1 min. The amount of ascorbic acid oxidized was calculated from the molar extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of the enzyme is equal to the amount of APX catalyzing the oxidation of 1 µmol ascorbate per min.

The Foyer and Halliwell (1976) method was used for determining GR (EC 1.6.4.2) activity by measuring the decrease in absorbance at 340 nm wavelength and calculated from the 6.22 mM^{-1} molar extinction coefficient. The 3 ml reaction mixture contained 100 mM Tris–HCl (pH 8), 0.5 mM EDTA, 0.5 mM $MgCl_2$, 1 mM NADPH, 100 µl extract, and 10 mM GSSG. The reaction was initiated by the addition of NADPH. One unit of GR activity is the reduction of 1 µmol NADPH per min.

MDHAR (EC 1.6.5.4) activity was measured by the method of Hossain *et al*. (1984). The reaction mixture of 1 ml consisted of 100 mM potassium phosphate buffer (pH 7.5), 2.5 mM ascorbic acid, 0.01 mM EDTA, 0.25 mM NADH, 0.0125% Triton X-100, 50 µl extract, and 0.4 units of ascorbate oxidase. The reaction was initiated by adding ascorbate oxidase. The decrease in absorbance due to NADPH oxidation was measured at 340 nm wavelength and calculated using an extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of MDHAR activity is defined as the amount of enzyme that oxidizes 1 µmol NADH per min.

DHAR (EC 1.8.5.1) activity was assayed according to the method described by Nakano and Asada (1981). The reaction mixture of 3 ml consisted of 100 mM potassium phosphate buffer (pH 7), 20 mM GSH, 1 mM dehydroascorbate (DHA), 0.178 mM EDTA, and 100 µl enzyme extract. The absorbance was measured at 265 nm and the enzyme activity was calculated from the 7.0 mM^{-1} cm^{-1} extinction coefficient. One unit of the DHAR activity is the amount of enzyme that produces 1 µmol ascorbate per min.

Determination of Ascorbate (AsA), Total Glutathione (TGSH), Reduced Glutathione (GSH), and Oxidized Glutathione (GSSG) Contents Ascorbate was determined using the method of Foyer *et al*. (1983). The freshly harvested callus was immersed in liquid nitrogen and ground to fine powder and homogenized with 1 ml of 4.5 M perchloric acid. The homogenate was allowed to thaw for 30 min at 4°C. The soluble fraction (100 µl) was neutralized with 1.25 M of potassium carbonate (pH 5.6) and 0.1 M sodium phosphate buffer (pH 5.6). Ascorbate oxidase (5 units) was added to the reaction mixture in a final volume of 1 ml and the decrease in the absorbance was measured at 265 nm wavelength.

GSH and GSSG were assayed as per the method described by Sarker and Oba (2018). The callus samples (~ 1 g) were homogenized in 10 ml of 5% sulphosalicylic acid and the homogenate was centrifuged at 15,000 rpm for 30 min at 4°C. To determine the total glutathione (GSH + GSSG) in the sample, 1 ml of the supernatant was taken and then 1.5 ml of 0.5 M phosphate buffer (pH 7.5) and 50 µl of double-distilled water were added. For determining GSSG in the sample, another part of the supernatant (1 ml) was taken and then 1.5 ml of 0.5 M phosphate buffer (pH 7.5) and 50 µl of 2-vinylpyridine were added. The tube was mixed vigorously until an emulsion was formed and then incubated at room temperature for 1 h. The difference between the total glutathione and GSSG is used to determine GSH content. The reaction mixture consisted of 0.2 mM NADPH, 100 mM phosphate buffer (pH 7.5), 5 mM EDTA, and 0.6 mM DTNB, and 3 units of GR was used for glutathione content estimation. The reaction was started by adding the sample extract of 0.1 ml to the reaction mixture and the rate of reaction was determined by measuring the change in absorbance at 412 nm for 1 min. Two standard curves were prepared based on GSSG and total glutathione. Data were represented as nmol g^{-1}FW.

Phenylalanine Ammonia-Lyase (PAL) Enzyme Activity Assay The crude enzyme for PAL assay was extracted from the callus (~ 1 g) with borate buffer (pH 8.8). The callus was ground in the buffer with ice-cold mortar and pestle and then centrifuged at 10,000 rpm and 4°C for 20 min. The supernatant obtained was used for the assay as per the method of Wu and Lin (2002). The reaction cocktail of 1 ml contained 150 mM tris–HCl (pH 8), 12 mM L-phenylalanine, and 50 µg of protein followed by incubation for 30 min at 30°C. The reaction was stopped using 100 µl of 6 N HCl and the absorbance was taken at 270 nm. The extinction coefficient for PAL at 270 nm is 19.73 $mM^{-1}cm^2$.

Expression Analysis of *APX*, *SOD*, *CAT*, *POD*, and *PAL* Genes Using Real-Time PCR Extraction of Total RNA and cDNA Synthesis

Total RNA was isolated from the callus elicited with SA and JA along with untreated controls using a modified sodium dodecyl sulfate (SDS)/TRIzol method (Wang *et al*. 2011). The quality of total RNA was analyzed by agarose gel electrophoresis. The RNA was quantified using a NanoDrop

ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 260 nm and the purity was checked from A260/A280 ratio. First-strand (cDNA) synthesis was carried out with 2 µg of total RNAs isolated from elicitor-treated and control (untreated) callus cultures using the PrimeScript First Strand cDNA Synthesis Kit (Takara Bio Inc., Shiga, Japan) in accordance with the manufacturer’s instructions.

Primer Design and Quantitative Real-Time Polymerase Chain Reaction (RT-PCR) Primers were designed for the *APX*, *SOD*, *CAT*, *POD*, and *PAL* genes and synthesized commercially for analyzing their transcript levels in elicited and control (untreated) callus cultures (Table 1). The coding sequence (CDS) of these genes from the related species such as *Ricinus communis*, *Manihot esculenta, Hevea brasiliensis*, and *Jatropha curcas* belonging to the same family was downloaded from National Center for Biotechnology Information (NCBI) database as a FASTA file and multiple alignments were carried out (T-Coffee). The position where the sequence had more homology was used for primer designing by NCBI primer design tool. The internal complementarity and formation of hairpin loops were checked by Integrated DNA Technologies (IDT) tools software and the primers that did not have hairpin loops and self-complementarity were selected.

First-strand cDNA samples were diluted 2.5 times and 1 µl of the diluted sample was taken as a qRT-PCR template in 10 µl of total reaction volume containing 0.4 µM gene-specific primers and 5 µl of SYBR Premix ExTaq II (TliRNase H Plus) with ROX (Takara Bio Inc., Japan). The samples were appraised in three technical replicates. PCR analysis was carried out in a Realplex amplifier (Eppendorf-Hamburg, Germany) with the following cycle parameters: 95°C for 5 min; 40 cycles of 95°C for 20 s, Tm (as given in Table 1) for 20 s, and 72°C for 20 s; followed by a melting curve to ensure that each amplicon was a single product. The relative fold-change in RNA expression was estimated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Actin with primer sequence 5’-TGAGAGGGAAATCGTGCGTG-3’ and 3’-TGCTTGCTGATCCACATCTGC-5’ was used as an internal control to normalize the real-time amplification data.

Statistical Analysis The data was acquired from three independent experiments, with 3 replicates used per treatment in each experiment. The values are represented as mean ± standard deviation (SD). The statistical significance of the means was assessed using a one-way analysis of variance followed by Duncan’s multiple range test in SigmaPlot 13. The probability of $p < 0.05$ was considered significant.

Results

Leaf Explants Exhibited High Potential for Callus Formation Callus was induced from the leaf explants with varying frequencies (10.5–100%) in MS medium containing different growth regulators. The highest callus induction frequency (100%) from leaf explants was achieved on medium with 2 mg/l 2,4-D and 1 mg/l BAP (Table 2). The callus was initiated rapidly after 7–9 d of culture in the presence of 2,4-D alone or in combination with BAP whereas it was observed after 10–16 d on other media tested. The callus produced on different media was greenish-white, soft, and friable in nature while medium with 2,4-D alone resulted in a hard and compact callus. The callus biomass in terms of fresh weight (30.36 g/100 ml medium) and dry weight (4.83 g/100 ml medium) was found to be higher on MS with 2 mg/l 2,4-D and 1 mg/l BAP than on other media tested (Figs. 1 and 2). Analysis of the growth rate of the callus at different time periods revealed the highest fresh weight (31.86 g/100 ml medium) and dry weight (4.08 g/100 ml medium) at 5 wk and thereafter declined (Fig. 3). Thus, the callus formed from leaves of this tree species exhibited luxuriant growth with high proliferation rate as evidenced by a substantial increase in biomass after subculture thus making it an ideal source for secondary metabolite production.

Table 1. Primers used for real-time PCR to analyze the gene expression of *APx*, *POD*, *CAT*, *MnSOD*, and *PAL* in SA- and JA-treated and control callus culture of *G. moluccana*

Gene name	Primer ID	Primer sequence (5’-3’)	% GC content	Length	Tm
Ascorbate peroxidase	*APx F*	GAACTTACGACGCGAAC	52.94	17	52.77
	APx R	GCTCAAGGAGTCTGACA	52.94	17	52.77
Peroxidase	*POD F*	TGCTTCGTCTCCATTTCC	50.00	18	53.69
	POD R	AGTTCCGATTGCTCACTG	50.00	18	53.69
Catalase	*CAT F*	CGCCCACAATTCCTCAT	52.94	17	52.77
	CAT R	GCTAGCTTCTCCACCAAA	50.00	17	53.69
Superoxide dismutase	*Mn SOD F*	CAGAGCGCCATCAAGTTCAATGGT	50.00	24	62.72
	Mn SOD R	TGGGTCCTGGTTTGCAGTTGTCT	52.17	23	62.43
Phenylalanine ammonia-lyase	*PAL2 F*	TGTCGAACATGCCTTGGCGAAT	50.00	22	59.7
	PAL2 R	TCCGGTGAGCAATCCACTACCTAA	50.00	24	59.5

Table 2. Effect of plant growth regulators on callus induction from leaf explants of *G. moluccana* on MS medium

Growth regulator(s)	No. of explants inoculated	Callus induction (%)	Days for initiation
2 mg/l 2,4-D	56	48.2	7–9
2 mg/l 2,4-D + 1 mg/l BAP	58	100	7–9
2 mg/l NAA + 1 mg/l BAP	58	60.3	10–12
2 mg/l 2,4-D + 1 mg/l KN	59	44.1	10–12
2 mg/l 2,4-D + 1 mg/l NAA	57	10.5	14–16

SA and JA Elicitation Influenced the Callus Biomass and Accumulation of Total Phenolics and Flavonoids The callus fresh weight and dry weight decreased upon elicitation with SA and JA for 30 d as compared to control callus cultures (FW, 33.58 g/100 ml medium; DW, 5.75 g/medium). SA treatment resulted in a greater decrease in fresh weight (21.8 to 23.12 g/100 ml medium) and dry weight (1.24 to 1.78 g/100 ml medium) in comparison to JA treatment where the fresh weight and dry weight were found to be 28.47 to 25.27 g/100 ml medium and 2.15 to 4.75 g/100 ml medium, respectively, for the different concentrations tested (Fig. 4).

Elicitation of callus cultures with SA and JA had a marked effect on the accumulation of total phenolics in callus cultures of *Givotia*. The phenolic content varied in callus cultures treated with SA and JA depending on the concentration and duration of treatments (Fig. 5*a*, *b*). The highest content of phenolics in SA-treated samples (31.4 mg GAE g^{-1} DW) was recorded in callus cultures treated with 50 μM SA for 5 d. The phenolic content decreased with an increase in the concentrations of SA as well as the duration of cultures (Fig. 5*a*). The pattern of phenolic acid accumulation varied in callus cultures treated with JA, which showed an increase in phenolic content from 50 to 150 μM, with the highest content (81.83 mg GAE g^{-1} DW) observed in callus cultures treated with 150 μM for 20 d (Fig. 5*b*). A significant decline in phenolic content was noticed with further increase in JA concentration or duration of the cultures. The phenolic contents in the control callus cultures varied from 23.18 to 25.68 mg GAE g^{-1} DW after 5–30 d of culture.

SA and JA treatments affected the accumulation of flavonoids in a concentration and time-dependent manner. The highest content of flavonoids (38.49 mg RE g^{-1} DW) in SA-treated samples was recorded in callus cultures treated with 50 μM for 20 d, and thereafter declined (Fig. 6*a*). The flavonoid content in callus cultures treated with 100 μM was higher than the controls at 5 d and 10 d, and the content was lower than controls during subsequent periods (15–30 d) of culture. The JA-elicited callus cultures showed the highest flavonoid content at 150 μM (60.88 mg RE g^{-1} DW) on the 20th day as compared to other treatments and controls. The flavonoid content decreased with a further increase in the concentration and duration of culture (Fig. 6*b*). Callus cultures treated with 100 μM JA also resulted in higher flavonoid content than controls at all durations of culture.

SA and JA Elicitation Increased the Production of H_2O_2 and MDA Levels The accumulation of H_2O_2 in both the control and elicited callus cultures was assessed as it is one of the earliest indications of elicitor recognition by the plants. An increase in the accumulation of H_2O_2 was observed in the callus treated with SA and JA as compared to controls (0.86 μmol g^{-1} FW) (Fig. 7). The levels of H_2O_2 elevated as the concentration of SA increased up to 150 μM and did not change significantly at 200 μM. Similarly in JA-elicited callus cultures, the highest level of H_2O_2 was observed with 150 μM JA (3.6 μmol g^{-1} FW) which did not differ significantly from 200 μM JA (3.7 μmol g^{-1} FW). However, at lower concentrations of 50 and 100 μM, the H_2O_2 levels were found to be higher in SA-treated callus cultures as compared to JA.

The control and elicitor-treated callus were analyzed for the MDA content which is an indicator of lipid peroxidation. All the treatments resulted in higher MDA accumulation as compared to controls (0.50 μmol g^{-1} FW) (Fig. 8). Under the influence of the SA as elicitor, the content of MDA remained in the range of 0.77 to 1.45 μmol g^{-1} FW. Treatment with JA induced maximum MDA accumulation, with the highest recorded at 200 μM JA (2.27 μmol g^{-1} FW) followed by 150 μM JA (1.68 μmol g^{-1} FW).

SA and JA Elicitation of Callus Cultures Caused Alteration in Antioxidant Enzyme Activities For a better understanding of the elicitation effects of salicylic acid and jasmonic acid, the activities of several antioxidant enzymes were examined in the elicitor-treated callus cultures and compared with controls.

Superoxide Dismutase (SOD) Enzyme Activity The ability of SOD to reduce the superoxide into hydrogen peroxide and molecular oxygen is the basis of this assay. An elevated activity of SOD was observed in all the treatments compared to control cultures (Fig. 9). Lower concentrations of SA (50 μM) stimulated higher SOD activity (20.3 U mg^{-1}) than higher concentrations (150–200 μM) where the activity did not change significantly. On the other hand, JA at 150 μM induced the maximum SOD activity (24.28 U mg^{-1}) followed by a decline at 200 μM.

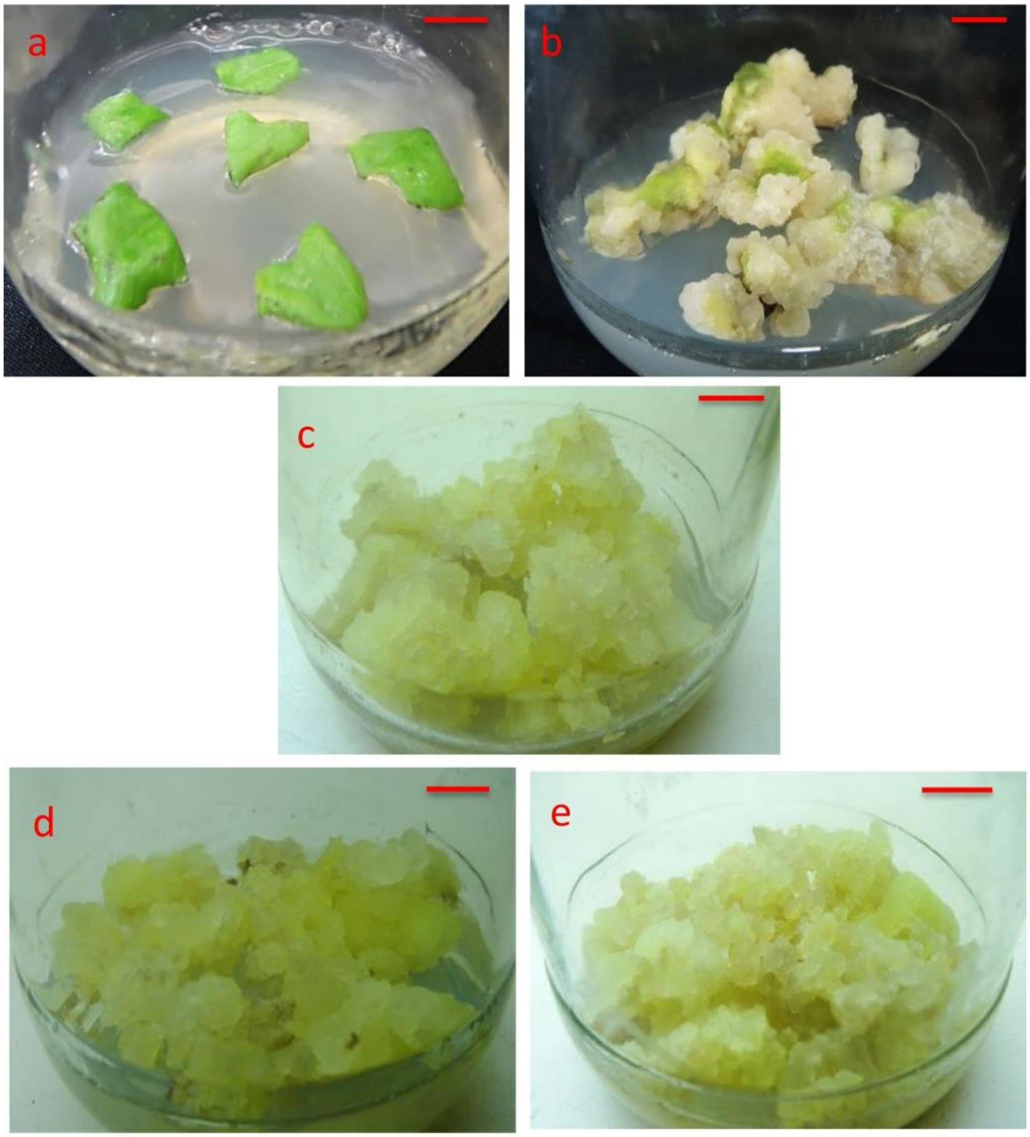


Figure 1. (*a–b*) Callus formation from leaf explants and growth of the callus of *Givotia moluccana* after elicitation with salicylic acid (SA) and jasmonic acid (JA) on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP. (*a*) Leaves cultured for callus initiation. (*b*) Callus induced from leaves after 5 wk of culture on initiation medium. (*c*) Proliferated callus after 5 wk of subculture on fresh medium. (*d*) Callus grown on medium with 50 µM SA after 5 wk of culture. (*e*) Callus grown on medium with 150 µM JA after 5 wk of culture. (*Bars* = 1 cm).

Catalase (CAT) Enzyme Activity Catalase is a well-known intracellular enzyme which protects against the ROS that is generated within the cell. Treatment of SA resulted in the highest catalase activity at the lowest concentration of 50 µM (12.39 U mg^{-1}) followed by a decline with an increase in the concentration of SA although it did not differ significantly

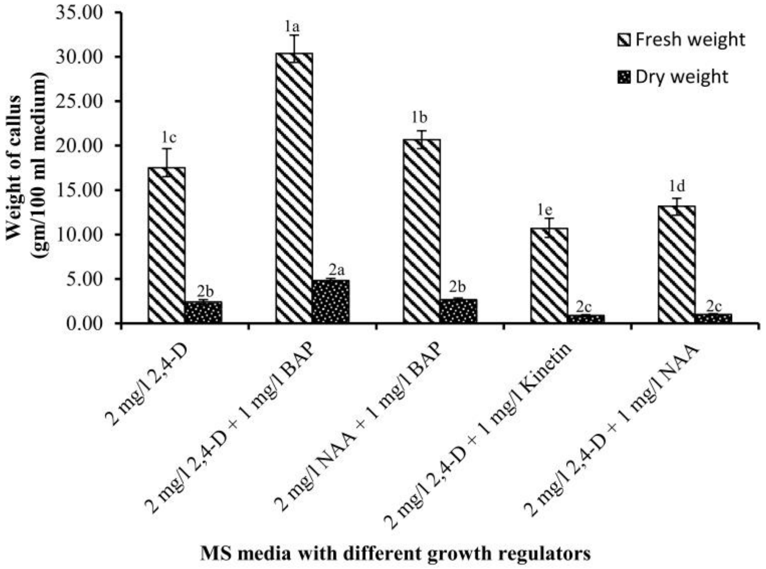


Figure 2. Effect of different growth regulators on biomass of callus derived from leaves of *G. molucanna*. The fresh weight and dry weight were determined after 5 wk of subculture of the callus on the fresh medium. Data represents means + SD of three independent experiments. Means followed by the same *letter* are not statistically significant at the 5% probability level by one-way analysis of variance (ANOVA) according to Duncan's multiple range test.

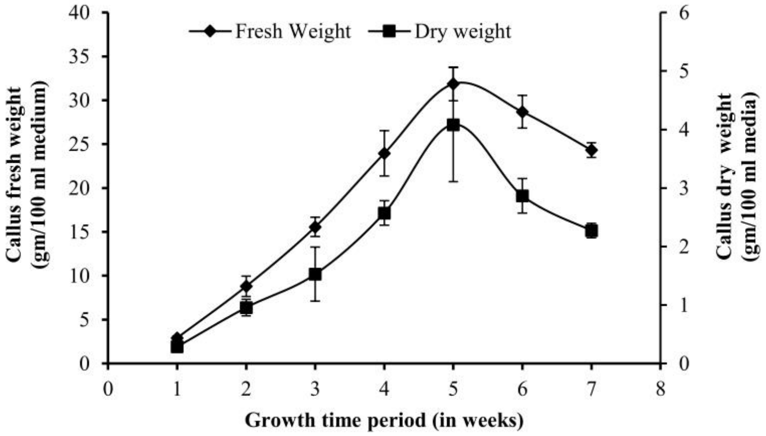


Figure 3. Growth rate of the callus as measured by fresh weight and dry weight after different time periods of culture on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP.

for 150 µM and 200 µM treatments. Under the influence of JA, the catalase activity gradually increased reaching the highest (18.62 U mg^{-1}) at 150 µM; however, upon reaching 200 µM, its activity decreased to 13.06 U mg^{-1} (Fig. 10).

Peroxidase (POD) Enzyme Activity Peroxidases are protective enzymes of plant cells which act against a variety of stresses by regulating the concentration of O_2 and H_2O_2. Similar to CAT activity, SA at lower concentrations of 50 µM induced the highest (504.25 U mg^{-1}) peroxidase activity (Fig. 11). The enzyme activity decreased as the concentration of SA increased, with the activity in 150 and 200 µM treated cultures being lower than controls (338.43 U mg^{-1}). JA-elicited callus cultures showed the highest activity (579.84 U mg^{-1}) at 150 µM and subsequently decreased. The JA-induced peroxidase activity remained higher (380.13–579.84 U mg^{-1}) than controls for all the concentrations (100–200 µM) except at 50 µM where the activity was lower than controls.

SA and JA Treatment Regulated the AsA-GSH Pathway Significant changes in the activities of the enzymes involved in ascorbate–glutathione cycle were observed in callus

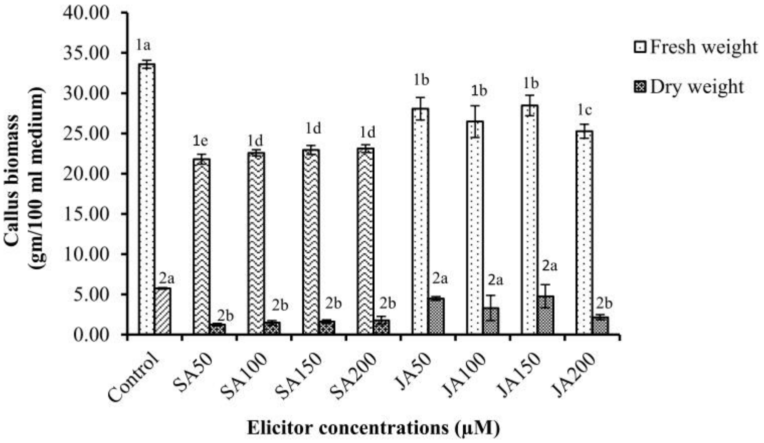


Figure 4. Effect of salicylic acid (SA) and jasmonic acid (JA) elicitation on callus biomass as determined by fresh weight and dry weight. The fresh weight and dry of the callus were recorded after 5 wk of culture on the media. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

cultures elicited with SA and JA (Fig. 12*a*–*d*). APX is a key enzyme that detoxifies H_2O_2, which utilizes ascorbic acid as its electron donor and reduces H_2O_2 to H_2O. With regard to SA treatment, the highest APX activity (74.85 U mg^{-1}) was observed at the lowest concentration of 50 μM (Fig. 12*a*) and declined with an increase in the concentration (100–200 μM). The APX activity (26.9 U mg^{-1}) of 200 μM SA-treated callus cultures did not differ significantly from the controls (22.36 U mg^{-1}). Treatment with JA resulted in a pronounced increase in APX activity being significantly higher than controls for all the concentrations, with the highest activity (127.9 U mg^{-1}) recorded at the concentration of 150 μM (Fig. 12*a*). A similar trend was observed for GR activity where SA at 50 μM induced the highest activity (15.9 U mg^{-1}) whereas it decreased to the values of controls as the concentration of SA increased to 200 μM (Fig. 12*b*). In case of JA-treated callus cultures, the activity was similar to controls at 50 μM and exhibited maximum activity (21.94 U mg^{-1}) in 150 μM treated callus cultures followed by a decrease in the activity at 200 μM (18.79 U mg^{-1}).

Salicylic acid at 50 μM triggered higher MDHAR activity (0.31 U mg^{-1}) and the values were lower than controls at higher concentrations of 200 μM (Fig. 12*c*). In the case of JA-elicited callus cultures, the MDHAR activity was lower than controls at 50 μM, and the activity raised as the concentration increased, with the maximum (0.39 U mg^{-1}) detected in 150 μM treated callus cultures. Similar patterns were observed for DHAR in response to SA treatment, with the highest activity (0.057 U mg^{-1}) observed at 50 μM and was similar to controls at 200 μM (0.034 U mg^{-1}). In JA-treated callus cultures, the DHAR activity (0.03 U mg^{-1}) at 50 μM was lower than controls whereas it elevated at higher concentrations of 100 to 200 μM (Fig. 12*d*). The maximum activity of DHAR was observed at 150 μM JA (0.06 U mg^{-1}).

To examine the effects of SA and JA on the components of AsA-GSH in callus cultures, the contents of ascorbate, total glutathione, GSSG, and GSH were determined. Ascorbate content varied in SA- and JA-treated cultures in comparison to the control cultures (96.39 μmol g^{-1} FW). The highest (123.98 μmol g^{-1} FW) ascorbate content in SA-elicited callus culture was observed in 50 μM SA and gradually decreased as the concentration increased (Fig. 13*a*). In JA-elicited callus cultures, ascorbate content increased from 102.39 to 149.68 μmol g^{-1} FW as the concentration of JA was increased from 50 to 150 μM and decreased to 125.94 μmol g^{-1} FW at 200 μM which was higher than controls. Similar trends were observed in the total glutathione content, where the highest content was observed in the cultures treated with 150 μM JA (91.5 μmol g^{-1} FW) followed by 200 μM JA (83.04 μmol g^{-1} FW) and 50 μM SA (79.6 μmol g^{-1} FW) as compared to other treatments (Fig. 13*b*). The glutathione content in other treated cultures were either similar or lower than controls (63.78 μmol g^{-1} FW).

The endogenous content of GSSG and GSH was determined in both the treated and control callus cultures. Under the influence of SA, the GSSG content showed a dose-dependent increase up to 150 μM with no further significant difference observed at 200 μM (Fig. 13*c*). On the other hand, lower concentrations of JA resulted in the highest GSSG content (25.48 nmol g^{-1} FW) whereas the content decreased at higher concentrations. Overall, the highest content was detected in treatments with 200 μM SA (26.1 nmol g^{-1} FW) followed by 50 μM JA (25.48 nmol g^{-1} FW). The GSH content in callus cultures treated with 50 μM SA was significantly higher (63.57 nmol g^{-1} FW) than controls (47.84 nmol g^{-1} FW); however, it was similar to controls or showed a significant decrease at higher concentrations (Fig. 13*d*). Under the influence of JA, the

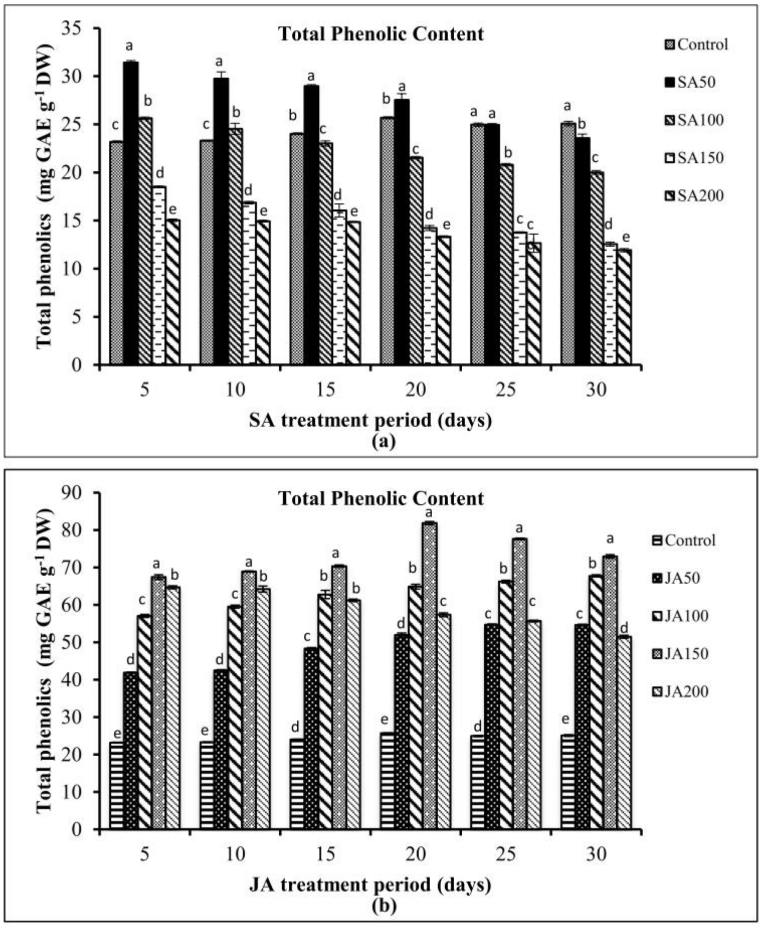


Figure 5. Total phenolic acid of callus cultures after elicitation with salicylic acid and jasmonic acid along with controls. (*a*) Salicylic acid–elicited cultures. (*b*) Jasmonic acid–elicited cultures. Means followed by the same *letter* in the *column* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

highest (78.43 nmol g^{-1} FW) content of GSH was observed at 150 μM and then declined (Fig. 13*d*). The GSH content in 50 μM JA-treated callus cultures was significantly lower (44.62 nmol g^{-1} FW) than controls (47.84 nmol g^{-1} FW).

Phenylalanine Ammonia-Lyase Enzyme Activity Was Differentially Modulated by SA and JA Treatments An increase in PAL activity after elicitor treatment often results in enhanced secondary metabolism in plant cells (Zhao *et al*. 2010). A significant change in PAL activity was observed in callus cultures treated with SA and JA depending on the concentration used (Fig. 14). The PAL activity in callus cultures treated with SA at 50 μM (0.21 U mg^{-1} protein) did not differ significantly from controls (0.20 U mg^{-1} protein); however, the activity decreased at higher concentrations. It was interesting to note that JA at 150 μM triggered the highest activity (0.33 U mg^{-1} protein) as compared to 100 and 200 μM whereas the value of 50 μM did not differ from controls.

Correlation of Transcript Levels of Genes and Enzyme Activities in SA- and JA-Treated Callus Cultures Showed Similar or Different Expression Patterns Real-time PCR analysis showing variation in transcript level of the genes encoding antioxidant enzymes *APX*, *SOD*, *CAT*, and *POD* in callus cultures treated with SA and JA (Fig. 15*a*–*d*). SA-treated callus cultures showed an increase in the transcript level of the *APX* gene by 3.71-fold at 50 μM, and decreased gradually reaching a value similar to controls at 200 μM. The transcript levels of the *APX* gene in JA-treated callus cultures were higher than controls for all concentrations, with the maximum fold-change of 9.24 detected at 150 μM JA (Fig. 15*a*). Both SA and JA treatments significantly

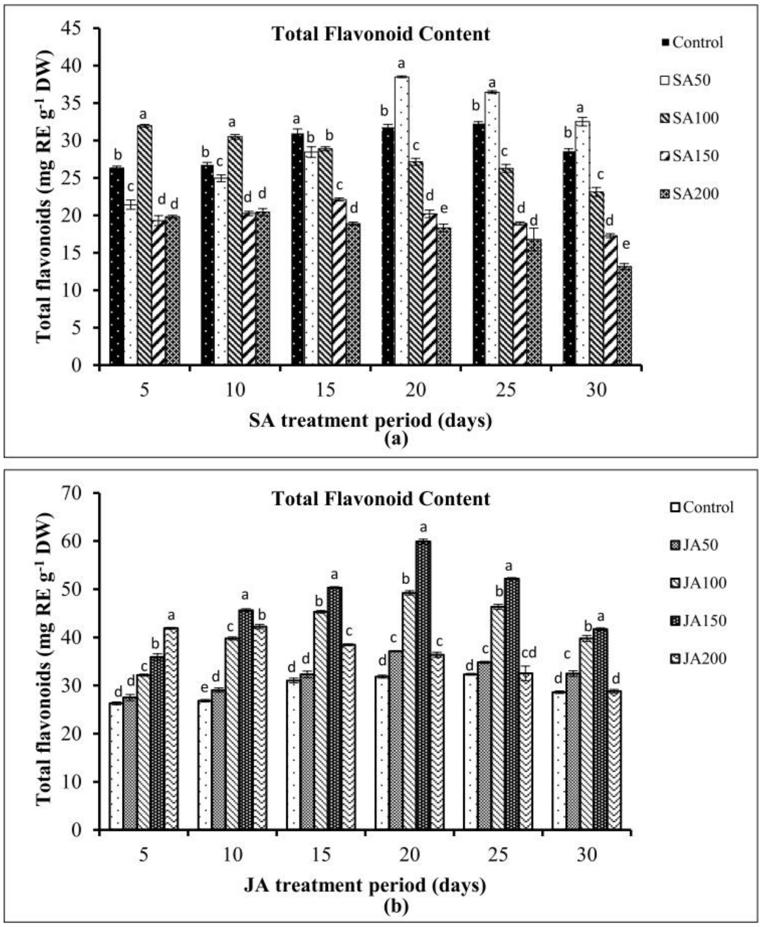


Figure 6. Total flavonoid contents of callus cultures after elicitation with salicylic acid and jasmonic acid along with controls. (*a*) Salicylic acid–elicited cultures. (*b*) Jasmonic acid–elicited cultures. Means followed by the same *letter* in the *column* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

elevated the transcript levels of the *SOD* gene for all concentrations used compared to control callus cultures. Among the different treatments, the maximum fold-change (4.54) in transcript levels of the *SOD* gene was observed in 150 μM JA-treated callus cultures compared to controls (Fig. 15*b*). Both *CAT* and *POD* genes showed similar expression patterns under the influence of SA and JA. The transcript levels of *CAT* and *POD* genes were found to be highest at the lowest concentration of SA (50 μM) and gradually decreased being lower than controls at 200 μM (Fig. 15*c*, *d*). A reverse trend was observed in JA-treated callus cultures, where the transcript levels of *CAT* and *POD* genes gradually increased reaching the maximum at 150 μM followed by a decrease at 200 μM which still remained higher than controls. Overall, JA at 150 μM induced higher transcript levels of *APX*, *SOD*, *CAT*, and *POD* genes than SA-treated cultures.

PAL is a key enzyme in the phenylpropanoid pathway involved in the deamination of phenylalanine to trans-cinnamic acid, which is the precursor for the lignin and flavonoid biosynthetic pathway. SA treatment triggered higher expression of the *PAL* gene at all concentrations as compared to the untreated control, with the highest fold-change of 12.45 observed at 100 μM. Interestingly, JA-elicited callus cultures showed the highest expression at 150 μM (6.6 fold-change), whereas it was similar to or lower than controls at other concentrations (Fig. 15*e*). Overall, the SA-treated callus cultures exhibited higher transcript levels than JA treatments across all concentrations. The pattern of gene expression of *PAL* detected at the transcript levels, however, did not correspond with enzyme activity for SA and JA-treated callus cultures.

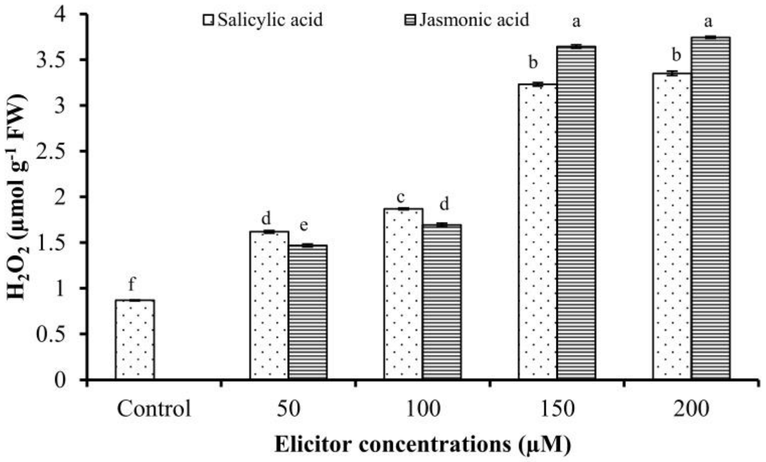


Figure 7. Effect of different concentrations of salicylic acid and jasmonic acid on generation of H_2O_2 in callus cultures of *G. moluccana*. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

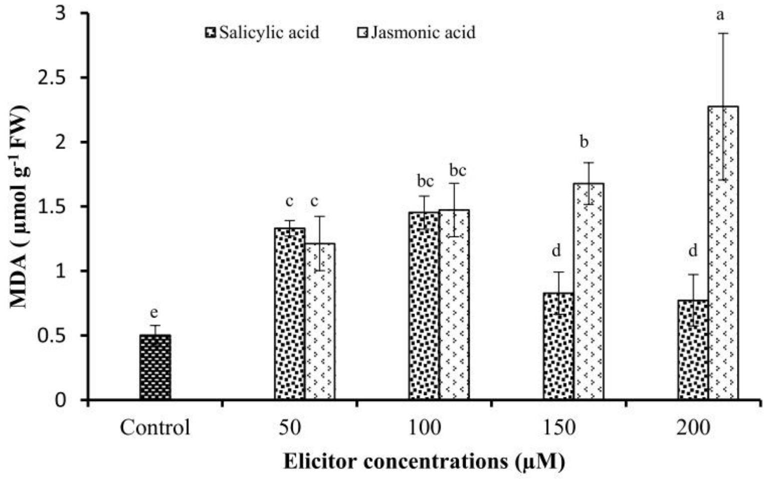


Figure 8. Malondialdehyde (MDA) levels in callus cultures elicited with salicylic acid and jasmonic acid. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

Discussion

Callus cultures offer immense potential for sustainable and large-scale production of secondary metabolites. The development of efficient methods for callus formation is a prerequisite for secondary metabolite production. The growth regulators play a determining effect on callus induction from explants. The present study showed the ability of leaf explants to form callus on MS medium with different growth regulators although the frequency and biomass varied. The combination of 2,4-D and BAP was found to be superior as it resulted in high frequency (100%) of callus induction with increased biomass as compared to medium with 2,4-D alone or other growth regulators (Table 2). The callus exhibited a high proliferation rate with a considerable increase in biomass at 5 wk of subculture on fresh medium. Similar to our finding, Bong *et al.* (2021) reported that the combination of 2,4-D and BAP was effective for callus induction and proliferation from leaf explants of Sabah Snake grass (*Clinacanthus nutans*). According to the report of Ikeuchi *et al.* (2013), both auxin and cytokinin are interconnected in the hormone signaling pathways which activate the cell cycle for callus initiation.

Figure 9. Effect of salicylic acid (SA) and jasmonic acid (JA) on superoxide dismutase (SOD) enzyme activity in callus cultures of *G. moluccana*. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

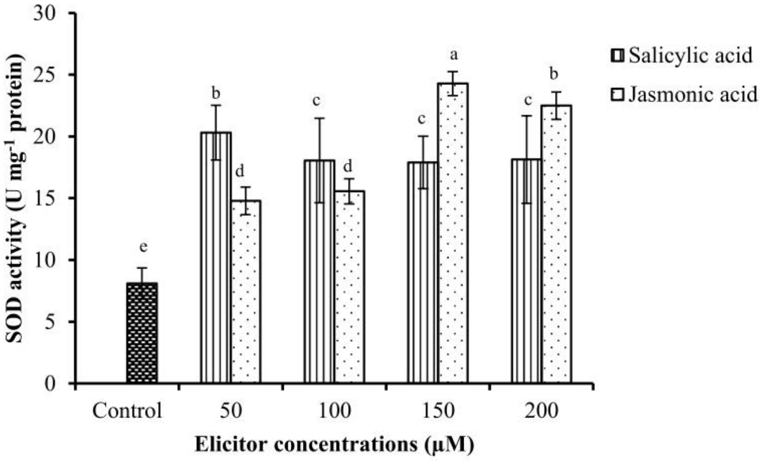
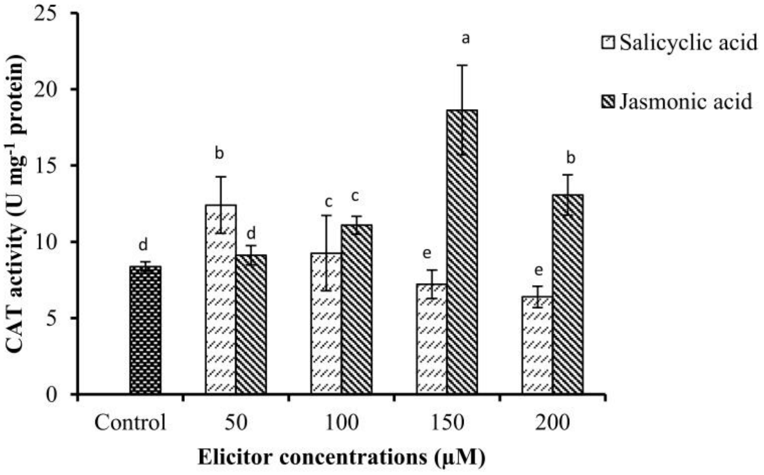


Figure 10. Effect of salicylic acid (SA) and jasmonic acid (JA) on catalase (CAT) enzyme activity in callus cultures of *G. moluccana*. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).



The cultured plant cells represent a potential source of biochemicals but relatively few cultures synthesize secondary metabolites comparable to those found in the whole plants. (DiCosmo and Misawa 1985). Various approaches are used to overcome the limitations of the production of secondary metabolites from the cultured cells required for different applications. Elicitors like JA and SA have been commonly used to improve the productivity of secondary metabolites in plant species. They have been reported to have positive or negative effects on callus growth depending on the concentrations used in the cultures. It is important to determine the optimal concentration of elicitors to prevent hypersensitivity that leads to cell death (Naik and Al-Khayri 2016). In this research, both SA and JA at the concentrations tested caused a decrease in callus biomass as compared to the control callus cultures (33.58 g FW/100 ml medium) although the biomass of SA-treated callus was lower (21.80–23.12 g FW/100 ml medium) than JA (25.27–28.47 g FW/100 ml medium) treatment (Fig. 4). Cai *et al*. (2017) reported that higher concentrations of elicitors including MeJA and SA suppressed cell growth of the cell suspension cultures of *Changium smyrnioides*. A similar decrease in biomass in response to SA elicitation has been reported in the cell cultures of *S. miltiorrhiza* (Dong *et al*. 2010). Nonetheless, low concentrations of JA and SA have been shown to improve the growth of callus in normal or stressful conditions (Al-Qatrani *et al*. 2021; Galal 2012).

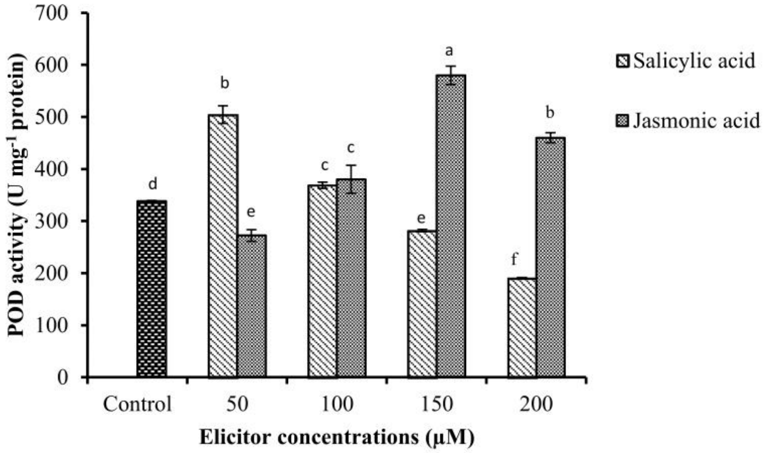
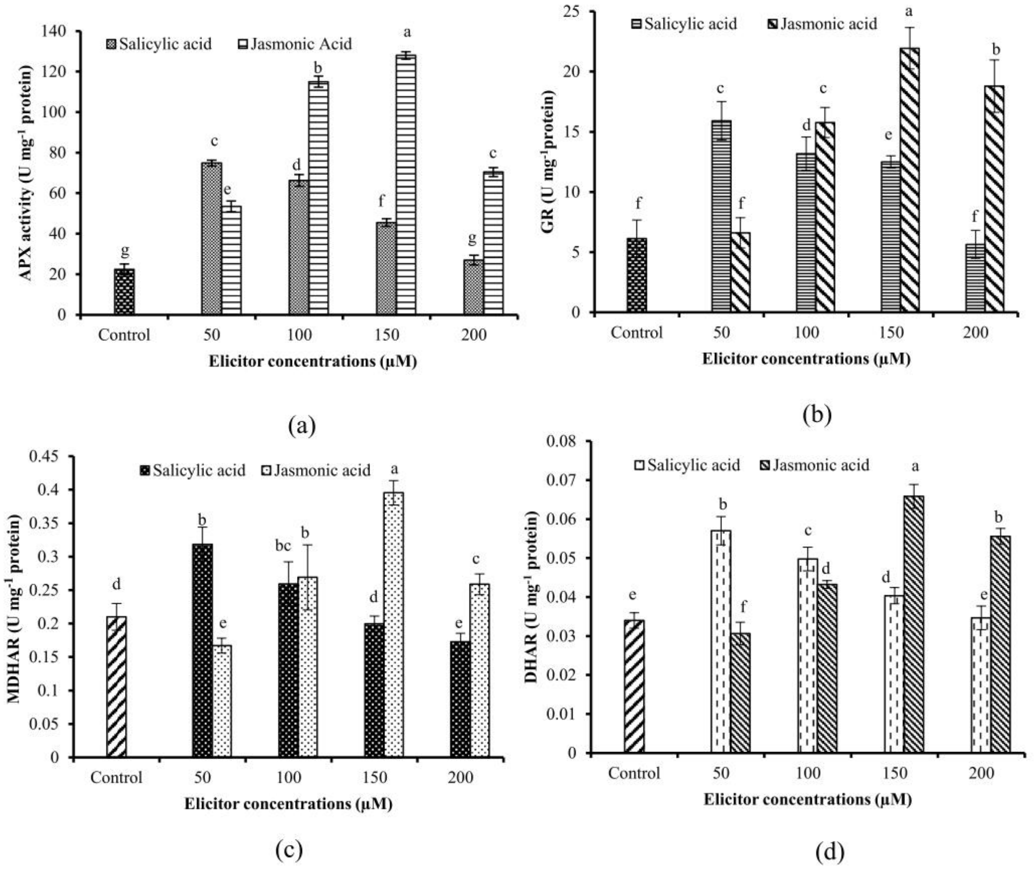


Figure 11. Effect of salicylic acid (SA) and jasmonic acid (JA) on peroxidase (POD) enzyme activity in callus cultures of *G. moluccana*. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

The concentration of the elicitor and the duration of its exposure are important factors to induce the optimum level of secondary metabolite production. In this research, the elicitation of callus cultures with SA and JA proved to be effective in enhancing the total phenolics and flavonoid contents although it depended on the concentration and duration of treatment. The maximum accumulation of total phenolics (81.93 mg GAE g^{-1}DW) and flavonoids (60.88 mg RE g^{-1}DW) was observed in callus cultures when elicited with 150 µM JA for 20 d (Figs. 5 and 6). However, for salicylic acid–elicited callus cultures, 50 µM for 5 d resulted in the highest phenolic content (31.43 mg GAE g^{-1} DW) while 50 µM for 20 d was optimal for flavonoid content (38.49 mg RE g^{-1} DW). Similarly, Mendoza *et al*. (2018) reported that specific concentrations of SA (300 µM) and MeJA (3 µM) increased the content of phenolics and flavonoids in cell suspension cultures of *Thevetia peruviana* suggesting an inducer effect of these elicitors in the phenylpropanoid metabolic pathway.

The oxidative burst of H_2O_2 in many plant species is found to be necessary for the production of several secondary metabolites (Kuzniak and Urbanek 2000). Antioxidants like phenolic acids and flavonoids confer protection to the cells by coping with the oxidative stress caused due to excess production of free radicals. The harmful effects of increased levels of free radicals resulting in cell death have been ascribed to the oxidation of DNA, proteins, and lipids. A balance between free radicals and antioxidants is necessary for proper physiological function. The administration of exogenous chemical elicitors imitates the reaction of pathogen attacks or wound signals which activates a defense response in plants by inducing oxidative burst (Khanpour-Ardestani *et al*. 2015; Manivannan *et al*. 2016). Consequently, the endogenous reactive oxygen species are accumulated in the cells (Lamb and Dixon 1997) that lead to a cascade of antioxidant metabolic changes in plants. In this study, the exogenous application of SA and JA to the callus cultures at all the concentrations stimulated higher production of H_2O_2 (1.46–3.74 µmol g^{-1} FW) compared to the control cultures (0.86 µmol g^{-1}FW). The maximum H_2O_2 levels were observed in callus cultures exposed to JA (3.74 µmol g^{-1} FW) than SA (3.30 µmol g^{-1} FW) at the highest concentration (200 µM) used in the study (Fig. 7). Similarly, H_2O_2 facilitated several elicitor-induced accumulations of specific secondary metabolites such as indole alkaloids in *Catharanthus roseus* (Zhao *et al*. 2001), saponin in *Ginseng* (Hu *et al*. 2003), and tcibulin in *Alium cepa* (Kravchuk *et al*. 2003).

Several studies have suggested that elicitor treatment to cell cultures stimulates the generation of ROS leading to increased accumulation of malondialdehyde (MDA), which is an end product of lipid peroxidation (Chong *et al*. 2005; Ghanati and Dehaghi 2011). It is known from the literature that high levels of MDA are a reflection of an oxidative imbalance. In our study, exposure of callus cultures of *G. molucanna* to SA and JA at all the concentrations showed a significant increase in the accumulation of MDA (Fig. 8). The remarkable increase of MDA level (1.68–2.27 µmol g^{-1} FW) observed in callus cultures treated with 150 and 200 µM JA might be due to jasmonic-induced enhanced expression of lipoxygenase (Kohlmann *et al*. 1999), which catalyzes the oxygenation of polyunsaturated fatty acids (Chamulitrat and Mason 1989). In this study, the marked reduction of MDA content observed in 150 and 200 µM SA-treated callus cultures as compared to lower concentrations of 50 and 100 µM is pointing towards the inhibitor effect of SA on lipid peroxidation at higher concentrations possibly due to diminished lipoxygenase activity or altered cellular metabolism. The



(a)

(b)

(c)

(d)

Figure 12. Changes in enzymes involved in ascorbate–glutathione pathway in callus cultures in response to elicitation with salicylic acid and jasmonic acid. (*a*) APX. (*b*) GR. (*c*) MDHAR. (*d*) DHAR. Callus cultures without elicitation are used as controls. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

inhibitory effect of salicylic acid on lipoxygenase-dependent lipid peroxidation has been previously reported (Lapenna *et al*. 2009). Although 150 and 200 µM JA-elicited callus cultures showed higher H_2O_2 levels, the MDA levels of 200 µM JA (2.27 µmol g^{-1} FW) were strikingly higher than that of 150 µM JA (1.68 µmol g^{-1} FW) which could due to impaired antioxidant capacity of the cells to detoxify the excess produced ROS.

Previous investigations have reported a correlation between phenolics, flavonoids, and antioxidant activities (Wong *et al*. 2006; Zayova *et al*. 2012). The present study revealed significant changes in the antioxidant enzyme activities of SOD, CAT, and POD in response to SA and JA elicitation of callus cultures. Varied trends were observed in this study indicating different activity levels with different concentrations of elicitors. Enzymatic antioxidant systems are usually activated during stress conditions. The first line of defense involves the SOD enzyme which catalyzes the conversion of superoxide radical (O_2^-) into H_2O_2 and O_2. The control of the steady-state O_2^- levels by SOD is known to be an important factor in protecting the cells against oxidative damage. In this study, enhanced SOD activities were observed at all the concentrations of the elicitors used indicating the protective action of elicitors to scavenge O_2^- radicals produced in the callus cultures (Fig. 9). The H_2O_2 generated during the breakdown of O_2^- is further scavenged subsequently by other enzymes such as CAT, POD, and APX. CAT enzyme functions in peroxisomes

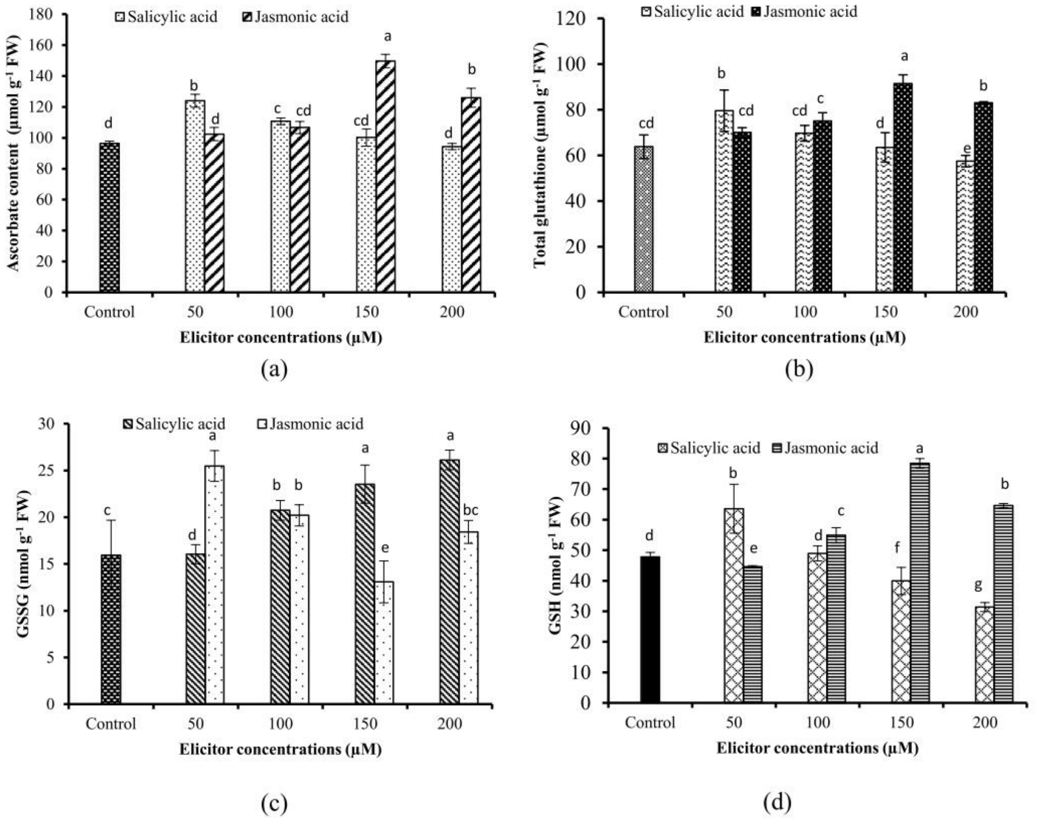


Figure 13. Changes in non-enzymatic antioxidants of ascorbate–glutathione cycle in callus cultures after elicitation with salicylic acid and jasmonic acid in comparison to control callus cultures. (*a*) Ascorbate content. (*b*) Total glutathione. (*c*) GSSG. (*d*) GSH contents. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

and involves in the conversion of H_2O_2 to O_2 and H_2O in an energy-efficient manner (Das and Roychoudhury 2014), while POD eliminates H_2O_2 both during stress and normal conditions (Asada 1999). In this study, the CAT (18.62 U mg^{-1} protein) and POD (579.84 U mg^{-1}) activities markedly increased in callus cultures under the influence of JA at 100 and 200 μM indicating efficient H_2O_2 detoxification system (Figs. 10 and 11). The enhanced activities of CAT and POD enzymes in response to SA and JA treatments have been reported in the cell suspension culture of *Scrophularia kakudensis* (Manivannan *et al.* 2016) and *Cucumis melo* (Nafie *et al.* 2011). In contrast, Chong *et al.* (2005) reported a reduction in CAT and ascorbate peroxidase in cell cultures of *Morinda elliptica* treated with JA, indicative of the antioxidant roles played prominently by other antioxidants that were all enhanced in elicited cultures. It is observed that SA at 50 μM was effective in enhancing the CAT and POD activities while a higher concentration of 200 μM SA reduced the activity which shows the cells' inability to detoxify the H_2O_2 at higher concentrations resulting in ROS imbalance and impaired functions in the cells. Ali *et al.* (2006) observed a strong decrease in the activity of CAT while guaiacol peroxidase (G-POD) was induced in both MJ- and SA-treated roots of *Panax ginseng*. Thus, it was suggested that CAT functions were taken over by G-POD enzymes in the treated roots.

Another antioxidant mechanism for maintaining redox balance in plants is through the ascorbate–glutathione pathway consisting of four enzymes *viz.*, APX, GR, MDHAR, and DHAR. The H_2O_2 generated by ROS after elicitor treatment is converted to H_2O by APX enzyme through oxidation of AsA to monodehydroascorbate, which is converted to AsA with the

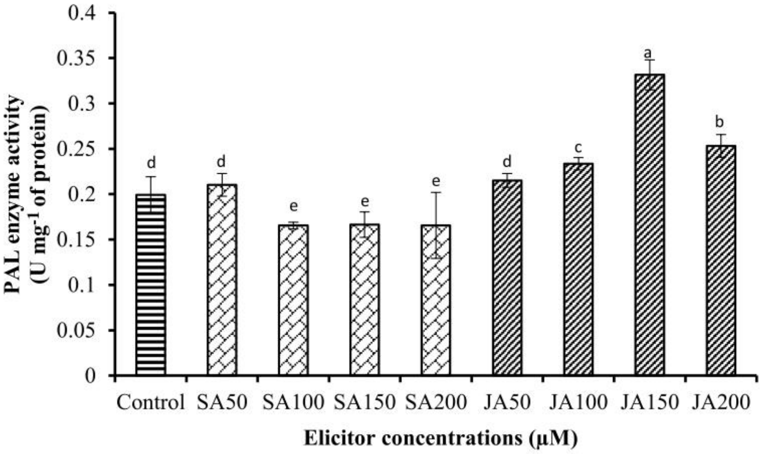


Figure 14. Effect of salicylic acid and jasmonic acid on phenylalanine ammonia-lyase (PAL) enzyme activity in callus cultures in comparison to control cultures. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

help of enzyme MDHAR. The monodehydroascorbate which escapes this cycle is further converted to dehydroascorbate (DHA) by releasing one more electron. The DHA is converted into AsA to maintain the ascorbate pool by the enzyme DHAR. GSH regenerated from its oxidized form (GSSG) *via* GR is utilized by DHAR (Murshed *et al*. 2008). Several studies have reported an enhancement in the activities of APX, GR, MDHAR, and DHAR during stress conditions (Ali *et al*. 2006; Jiang *et al*. 2016; Gaafar and Seyam 2018; Hasanuzzaman *et al*. 2019). In this study, the activities of enzymes involved in the AsA-GSH cycle were significantly increased in JA 150 µM elicited callus cultures which corroborated with the highest contents of ascorbate and glutathione indicating better ROS regulation at this concentration (Fig. 12). Additionally, improved GSH (reduced form) and diminished GSSG (oxidized form) along with enhanced GR activity maintains the higher GSH/GSSG ratio suggesting the effectiveness of 150 µM JA in maintaining the AsA-GSH pool. These results are in agreement with the report of Li *et al*. (2017) where exogenous JA increased the activities of key enzymes in the AsA-GSH cycle, decreased the oxidized glutathione (GSSG), and increased the GSH/GSSG ratio which resulted in lower MDA and H_2O_2 contents in low root-zone temperature in *Malus baccata* Borkh. roots. Moreover, a gradual decrease in APX, MDHAR, DHAR, and GR activities with declined GSH and raised GSSG contents in callus cultures under the influence of SA at higher concentrations of 100 to 200 µM is pointing towards the concentration-dependent inhibition of the AsA-GSH cycle by SA in callus cultures in our study (Figs. 12 and 13). Durner and Klessig (1995) have previously demonstrated the ability of SA to inhibit APX activity supporting the hypothesis that SA-induced defense responses are mediated, in part, through H_2O_2 levels or coupled perturbations of the cellular redox state.

The PAL enzyme is a key enzyme of the phenylpropanoid pathway that is involved in the biosynthesis of several secondary metabolites. There are numerous reports indicating positive relationships between PAL activity and the accumulation of phenolics, but on the other hand, there are puzzling data concerning this correlation (Bauer *et al*. 2011). In this study, the highest PAL activity (0.33 U mg^{-1} protein) was recorded in the callus cultures treated with 150 µM JA for 20 d, which could explain the higher accumulation of phenolics and flavonoids in these cultures (Fig. 14). It can be presumed that jasmonic acid has a positive regulatory role in activating phenylpropanoid pathway although the mechanism by which specific concentration regulates higher biosynthesis is unknown. Interestingly, in our study, the PAL activity in 50 µM SA (0.21 U mg^{-1} protein) treated callus cultures did not differ significantly from control callus cultures (0.19 U mg^{-1} protein) although there was a significant increase in total phenolics and flavonoid contents in response to SA treatment. The reduction in PAL activity at higher concentrations (100–200 µM) of SA with a concomitant decrease in total phenolics and flavonoids is pointing towards the inhibitory effects of higher concentrations. In agreement with our results, Kovacik *et al*. (2009) found that PAL activity decreased at higher SA doses in *Martricaria chamomilla* plants which could be caused by a decrease in the phenylalanine pool.

The transcript levels of *SOD*, *CAT*, *POD*, and *APX* genes, as well as *PAL* which is involved in the phenylpropanoid pathway, were analyzed for ascertaining the correspondence between transcript levels and enzyme activities. The relative transcript levels of *SOD*, *CAT*, *POD*, and *APX* genes were significantly upregulated in callus cultures treated with 100 to 200 µM JA, whereas at lower concentration of 50 µM, the transcript levels of *CAT* and *POD* genes were

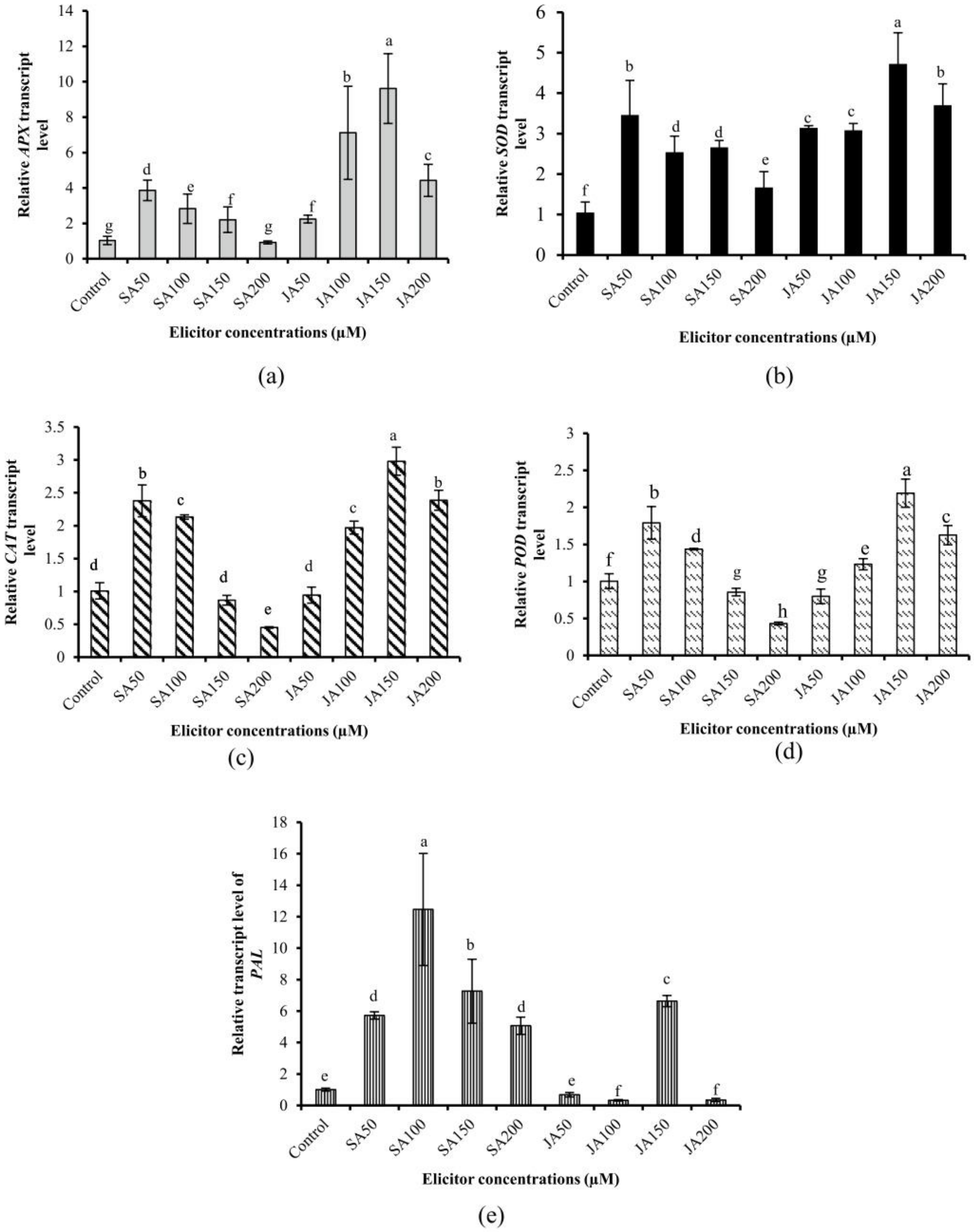


Figure 15. Relative transcript levels of genes encoding antioxidant enzymes and phenylalanine ammonia-lyase in elicitor (SA and JA) treated and control (unelicited) callus cultures. (*a*) APX. (*b*) SOD. (*c*) CAT. (*d*) POD. (*e*) PAL. Means followed by the same *letter* are not statistically significant according to Duncan's multiple range test ($p < 0.05$).

either downregulated or equivalent to controls. On the contrary, the highest transcript levels of antioxidant genes were observed at 50 µM SA which showed a decrease with an increase in the concentration (100–200 µM). The enhanced transcript level of these genes is consistent with the activities of these enzymes, justifying similar regulation at both the transcriptional and protein levels in addition to indicating the role of these enzymes in the process of detoxification of ROS during elicitor treatment. The study of Mejia-Ieniente *et al*. (2013) revealed the inducing effect of SA on *CAT* and *PAL* gene expression which correlated with their enzyme activities in *Capsicum annuum* L. Surprisingly, we observed a lack of correlation between *PAL* transcript level and PAL activity in SA- and JA-treated callus cultures as *PAL* transcript levels were found to be higher in SA-treated callus cultures as compared to JA-treated callus cultures. Moreover, the PAL activity in SA 50 µM treated callus cultures was similar to control callus cultures despite a significant increase in total phenolic content recorded in response to SA treatment. This discrepancy between transcript levels and enzyme activity of PAL can be due to posttranscriptional modifications as stated by Ballester *et al*. (2006). Ejtahed *et al*. (2015) reported that despite the increase in the transcript rate of the *PAL* gene at the higher concentration of SA, lower amounts of rosmarinic acid were accumulated in shoots of *Salvia officinalis*. Thus, it was suggested that some other unknown factors participate in the synthesis of phenolics.

Conclusions

In conclusion, the study showed efficient callus induction and proliferation from the leaf explants of *Givotia* cultured on MS medium with 2 mg/l 2,4-D and 1 mg/l BAP. SA and JA treatments resulted in a higher accumulation of total phenolic and flavonoid contents as compared to control cultures. Elicitation of callus cultures with 150 µM JA for 20 d proved to be more effective than SA as it resulted in the highest phenolic and flavonoid production for different concentrations and durations tested. The increased phenolics and flavonoid contents in elicited callus cultures correlated with enhanced enzymatic and non-enzymatic antioxidants showing better ROS regulation during elicitation. This is the first report to demonstrate higher production of secondary metabolite production in callus cultures of *G. moluccana* by elicitation with SA and JA which provides scope for enhancing the bioactive compounds and elucidate the biosynthetic pathways associated with secondary metabolites using omic approaches.

Acknowledgements The authors would like to thank the Department of Science and Technology-Promotion of University Research and Scientific Excellence (DST-PURSE), University Grants Commission-Special Assistance Programme Departmental Research Support (UGC-SAP-DRS-1), and Department of Science and Technology Fund for the improvement of S & T Infrastructure (DST-FIST Level-2 Phase-2) and the University of Hyderabad for supporting the infrastructural facilities in the Department/School that were utilized for the research work. We are highly grateful to the Officials of the Forest Research Station, Mulugu, Telangana for providing the seeds of *Givotia* used in this work.

Author contribution The study was conceived and designed by NW and GP. The materials and methods were formulated by NW. SL helped in conducting experiments on enzyme assays and assessing the transcript levels of the genes using real-time PCR and preparing the figures. The data was collected by NW, and analyzed and interpreted by all authors. The first draft of the manuscript was written by NW, and edited by all authors. The final manuscript has been checked and approved by all authors.

Funding A fellowship was provided by the Department of Biotechnology (DBT), New Delhi, to Nyanthanglo Woch (Fellow No. DBT/2014/UOH/230). Prof. Dr. G. Padmaja received a grant from the University Grants Commission University with Potential for Excellence (UGC-UPE Phase-2) to carry out the research.

Data Availability The data supporting the findings of the study are included within the article. Any further querries related to the article can be directed to the corresponding author.

References

Aebi H (1984) Catalase *in vitro*. Methods Enzymol 105:121–126

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344

Ali MB, Yu KW, Hahn E-J, Paek K-Y (2006) Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. Plant Cell Rep 25:613–620

Al-Qatrani MKJ, Al Khalifa AAS, Obaid NA (2021) Effect of jasmonic acid on stimulating the growth and development of date palm callus (*Phoenix dactylifera* L) cultivar Shukar *in vitro* under salt stress conditions. IOP Conf. Series: Earth Environ Sci 923(012017):1–3

Alscher RG, Donahue JL, Cramer LL (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224–233

Asada K (1999) The water-water cycle in chloroplast: scavenging of active oxygen and dissipation of excess photons. Annu Rev Plant Physiol Mol Biol 50:601–639

Awuchi CG, Amagwula IO (2020) The biochemistry, toxicology, and uses of the pharmacologically active phytochemicals: alkaloids, terpenes, polyphenols and glycosides. Merit Res J Food Sci Technol 51:6–21

Ballester AR, Lafuente MT, Ganzalez-Candelas L (2006) Spatial study of antioxidant enzymes, peroxidase and phenylalanine ammonia-lyase in the citrus fruit-*Penicillium digitatum* interaction. Postharvest Biol Technol 39:115–124

Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA (2008) Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. Food Chem 107:1106–1113

Bauer N, Fulgosi H, Jelaska S (2011) Overexpression of phenylalanine ammonia-lyase in transgenic roots of *Coleus blumei* alters growth and rosmarinic acid synthesis. Food Technol Biotechnol 49:24–31

Beauchamp CO, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Ben Amor N, Hamed KB, Debez A, Grignon C, Abdellva C (2005) Physiological and antioxidant responses of the perennial halophyte *Crithmum maritimum* to salinity. Plant Sci 168:889–899

Bong FJ, Chear NJY, Ramanathan S, Kumaran NM, Subramaniam S, Chew BL (2021) The development of callus and cell suspension cultures of Sabah Snake Grass (*Clinacanthus nutans*). Biocatal and Agric Biotechnol 33(101977):1–13

Bowler C, Montague MV, Inze D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 43:83–116

Cai J, Ma Y, Hu P, Zhang Y, Chen J, Li X (2017) Elicitation of furanocoumarins in *Changium smyrnioides* suspension cells. Plant Cell Tissue Organ Cult 130:1–12

Chamulitrat W, Mason RP (1989) Lipid peroxyl radical intermediates in the peroxidation of polyunsaturated fatty acids by lipoxygenase. Direct electron spin resonance investigations. J Biol Chem 15:20968–20973

Chong TM, Abdullah MA, Fadzillah NM, Lai OM (2005) Jasmonic acid elicitation of anthraquinones with some associated enzymic and non-enzymic antioxidant responses in *Morinda elliptica*. Enz Micromol Technol 36:469–477

Das K, Roychoudhury A (2014) A reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci 2:1–53

DiCosmo F, Misawa M (1985) Eliciting secondary metabolism in plant cell cultures. Trends Biotechnol 3:318–322

Dong J, Liu YB, Liang ZS, Wang WL (2010) Investigation on ultrasound-assisted extraction of salvianolic acid B from *Salvia miltiorrhiza* root. Ultrason Sonchem 17:61–65

Durner J, Klessig DF (1995) Inhibition of ascorbate-peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. Proc Natl Acad Sci 89:11312–11316

Ejtahed RS, Radjabian T, Tafreshi SAH (2015) Expression analysis of phenylalanine ammonia lyase gene and rosmarinic acid production in *Salvia officinalis* and *Salvia virgata* shoots under salicylic acid elicitation. Appl Biochem Biotechnol 176:1846–1858

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta 133:21–25

Foyer CH, Rowell J, Walker D (1983) Measurements of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. Planta 157:239–244

Gaafar RM, Seyam MM (2018) Ascorbate-glutathione cycle confers salt tolerance in Egyptian lentil cultivars. Physiol Mol Biol Plants 24:1083–1092

Galal A (2012) Improving effect of salicylic acid on the multipurpose tree *Ziziphur spina* – Christi (L.) Willd tissue culture. Amer J Plant Sci 3:947–952

Ghanati F, Dehaghi MA (2011) Stimulation of taxol production by combined salicylic acid elicitation and sonication in *Taxus baccata* cell culture. Int Proc Chem Biol Environ Eng 3:193–197

Ghasemzadeh A, Jaafer HZ, Rahmat A (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger *(Zingiber officinale)* Roscoe. Molecules 15:4324–4333

Giri CC, Zaheer M (2016) Chemical elicitors versus secondary metabolite production *in vitro* using plant cell, tissue and organ cultures: recent trends and a sky eye view appraisal. Plant Cell Tissue Organ Cult 126:1–18

Hasanuzzaman M, Bhuyan MHMB, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M (2019) Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 8:384

Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: a review. Environ Exp Bot 68:14–25

Heath RL, Pecker L (1968) Photoperoxidation in isolated chloroplast: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198

Hossain MA, Nakano Y, Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol 25:385–395

Hu X, Neill S, Cai W, Tang Z (2003) Hydrogen peroxide and jasmonic acid mediate oligogalacturonic acid-induced saponin accumulation in suspension-cultured cells of *Panax ginseng*. Physiol Plant 118:414–421

Huang H, Ullah F, Zhou D, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 10(800):1–10

Ikeuchi M, Sugimoto K, Iwase A (2013) Plant callus: mechanisms of induction and repression. Plant Cell 25:3159–3173

Jiang M, Xu F, Peng M, Huang F, Meng F (2016) Methyl jasmonate regulated diploid and tetraploid black locust (Robinia pseudoacacia L.) tolerance to salt stress. Acta Physiol Plant 38:106

Khanpour-Ardestani N, Sharifi M, Behmanesh M (2015) Establishment of callus and cell suspension culture of *Scrophularia striata* Boiss: an *in vitro* approach for acteoside production. Cytotechnol 67:475–485

Klessig DF, Choi HW, Dempsey DA (2018) Systemic acquired resistance and salicylic acid: past, present and future. Mol Plant-Microbe Inter 31:871–888

Kohlmann M, Bachmann A, Weichert H, Kolbe A, Balkenhohl T, Wasternack C, Feussner I (1999) Formation of lipoxygenase-pathway-derived aldehydes in barley leaves upon methyl jasmonate treatment. Eur J Biochem 260:885–895

Kovacik J, Gruz J, Backor M, Strand M, Repcak M (2009) Salicylic acid induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. Plant Cell Rep 28:135–143

Kravchuk ZM, Perkovs'ka H, Dmytriiev OP (2003) Role of active forms of oxygen in the induction of phytoalexin synthesis in *Allium cepa* cells. Tsitol Genet 37:30–35

Kuzniak E, Urbanek H (2000) The involvement of hydrogen peroxide in plant response to stresses. Acta Physiol Plant 22:195–203

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Ann Rev Plant Physiol Plant Mol Biol 48:251–275

Lapenna D, Ciofani G, Pierdomenico SD, Neri M, Cuccurullo C, Giamberardino MA, Cuccurullo F (2009) Inhibitory activity of salicylic acid on lipoxygenase-dependent lipid peroxidation. Biochim Biophysica Acta 1790:25–30

Li L, Lu X, Ma H, Lyu D (2017) Jasmonic acid regulates the ascorbate-glutathione cycle in *Malus baccata* Borkh. roots under low root-zone temperature. Acta Physiol Plant 39(174):1–11

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402–408

Lowry OH, Rosbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265

Manivannan A, Soundararajan P, Park YG, Jeong BR (2016) Chemical elicitor-induced modulation of antioxidant metabolism and enhancement of secondary metabolite accumulation in cell suspension cultures of *Scrophularia kakudensis* Franch. Int J Mol Sci 17(399):1–13

Mejia-Ieniente L, Duran-Flores FDD, Chapa-Oliver AM, Torres-Pacheco I, Cruz-Hernandex A, Gonzalez-Chavira MM, Ocampo-Velazquez RV, Guevara-Gonzalex RG (2013) Oxidative and molecular responses to *Capsicum annuum* L. after hydrogen peroxide, salicylic acid and chitosan-foliar applications. Int J Mol Sci 14:10178–10196

Mendoza D, Cuaspad O, Arias JP, Ruiz O, Arias M (2018) Effect of phenolic compounds in plant cell suspension cultures of *Thevetia peruviana*. Biotechnol Rep 19(273):1–9

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol 15:473–497

Murshed R, Lopez F, Huguette S (2008) Microplate quantification of enzymes of the plant ascorbate-glutathione cycle. Anal Biochem 383:320–322

Nafie E, Hathout T, Al Mokadem S (2011) Jasmonic acid elicits oxidative defense and detoxification system in *Curcumis melo* L. cells. Braz J Plant Physiol 23:161–174

Naik PM, Al-Khayri J (2016) Abiotic and biotic elicitors–role in secondary metabolites production through *in vitro* culture of medicinal plants. In: Shanker AK, Shanker C (eds) Abiotic and biotic stress in plants - Recent advances and future perspectives. Rijeka: InTech. Chapter 10, pp 247–277.

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Pieterse CMJ, van Loon LC (1999) Salicylic acid-independent plant defence pathways. Trends Plant Sci 4:52–58

Polle A, Otter T, Seifert F (1994) Apoplastic peroxidases and lignification in needles of Norway Spruce (*Picea abies* L.). Plant Physiol 106:53–60

Poulev A, O'Neal JM, Logendra S, Pouleva RB, Timeva V, Garvey KS, Gleba D, Jenkins IS, Halpem BT, Kneer R, Cragg GM, Raskin I (2003) Elicitation, a new window into plant chemodiversity and phytochemical drug discovery. J Med Chem 46:2542–2547

Rao RS, Ravishankar GA (2002) Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 20:101–153

Samuel K, Debashish D, Madhumita B, Padmaja G, Prasad SR, Bhaskara Ramana Murthy V, Rao PS (2009) *In vitro* germination and micropropagation of *Givotia rottleriformis* Griff. In Vitro Cell Dev Biol Plant 45:466–473

Samuel K, Kishore Babu N, Anil Kumar P, Reddanna P, Surya Satyanarayana S, Padmaja G (2016) Benzoylsalicylic acid isolated from seed coats of *Givotia rottleriformis* induces systemic acquired resistance in tobacco and Arabidopsis. Phytochem 126:11–22

Samuel K, Naresh K, Padmaja G (2015) Isolation and characterization of gallic acid and methyl gallate from the seed coats of *Givotia rottleriformis* Griff. and their anti-proliferative effect on human epidermoid carcinoma A431 cells. Toxicol Rep 2:520–529

Sarker U, Oba S (2018) Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. Sci Rep 8:16496

Sembdner G, Parthier B (1993) The biochemistry and the physiological and molecular actions of jasmonates. Annu Rev Plant Biol 44:569–589

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Biochim J Enol Vitic 16:144–158

Thammanna, Narayana Rao K (1990) Medicinal plants of Tirumala, 1st edn. Tirumala Tirupati Devasthanams (TTD) Press, Tirupati, Andhra Pradesh.

Vijayalakshmi A, Geetha M, Ravichandiran V (2014) Anti-psoriatic activity of flavonoids from the bark of *Givotia rottleriformis* Griff. Ex Wight Iranian J Pharma Sci 10:81–94

Vijayalakshmi A, Kinnera AG, Vishnu Prakash M (2017) *Givotia rottleriformis* Griff Ex Wight: pharmacognostical, phytochemical and pharmacological review. Int J Pharm Sci Rev Res 44:90–95

Wang G, Wang G, Zhang X, Wang F, Song R (2011) Isolation of high quality RNA from cereal seeds containing high levels of starch. Phytochem Anal 23:159–163

Wong SP, Leong LP, Koh JHW (2006) Antioxidant activities of aqueous extracts of selected plants. Food Chem 99:775–783

Wu J, Lin L (2002) Ultrasound-induced stress responses of *Panax ginseng* cells: enzymatic browning and phenolics production. Biotechnol Prog 18:862–866

Zayova E, Stancheva I, Geneva M, Petrova M, Vasilevska-Ivanova R (2012) Morphological evaluation and antioxidant activity of *in vitro* and *in vivo* derived *Echinacea purpurea* plants. Open Life Sci 7:698–707

Zhang S, Zhang L, Zou H, Qiu L, Zheng Y, Yang D, Wang Y (2021) Effects of light on secondary metabolite biosynthesis in medicinal plants. Front Plant Sci 12(781236):1–16

Zhao J, Davis LC, Verpoorte R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 23:283–333

Zhao JL, Zhou LG, Wu JY (2010) Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. Appl Microbiol Biotechnol 87:137–144

Zhao J, Zhu W-H, Hu Q (2001) Selection of fungal elicitors to increase indole alkaloid accumulation in *Catharanthus roseus* suspension cell culture. Enzyme Microb Technol 28:666–672

Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of Givotia moluccana (L.) Sreem.

by Nyanthanglo Woch

Phytochemical studies and elicitation induced changes in production of secondary metabolites in callus cultures of Givotia moluccana (L.) Sreem.

ORIGINALITY REPORT

23%	21%	23%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	link.springer.com Internet Source	16%
2	Nyanthanglo Woch, Supriya Laha, Padmaja Gudipalli. "Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of Givotia moluccana (L.) Sreem", In Vitro Cellular & Developmental Biology - Plant, 2023 Publication	4%
3	"Medicinal Plants: Biodiversity, Biotechnology and Conservation", Springer Science and Business Media LLC, 2023 Publication	<1%
4	"Anticancer Plants: Natural Products and Biotechnological Implements", Springer Science and Business Media LLC, 2018 Publication	<1%
5	www.science.gov	

26/02/

This is a part of his Ph.D. thesis work and Published by the candidate himself.

26/02/2024

Dr. G. PADMAJA, Ph.D.
Professor
Department of Plant Sciences
School of Life Sciences
UNIVERSITY OF HYDERABAD
Hyderabad-500 046. Telangana

26/02/2024

Dr. G. PADMAJA, Ph.D.
Professor
Department of Plant Sciences
School of Life Sciences
UNIVERSITY OF HYDERABAD
Hyderabad-500 046. Telangana