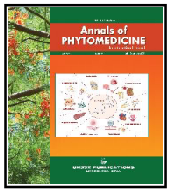
Annals of Phytomedicine 13(2): 953-962, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.98

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

Phytochemical profiling and biomedical applications of *Orthosiphon aristatus* (Blume) Miq.: *In vitro* and *in silico* approaches

M. Sabarivasan*, A. Jaya Jasmine◆, M. Maghimaa***, G. Ashokkumar**** and T. Anitha*******

**Department of Spices and Plantation Crops, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni-625604, Tamil Nadu, India*

** *Horticultural Research Station, Tamil Nadu Agricultural University, Pechiparai, Kanyakumari-629161, Tamil Nadu, India*

*** *Centre for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-602 105, Tamil Nadu, India*

**** *Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India*

***** *Department of Post-harvest Technology, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Theni-625604, Tamil Nadu, India*

Article Info

Article history
Received 16 July 2024
Revised 3 September 2024
Accepted 4 September 2024
Published Online 30 December 2024

Keywords
Orthosiphon aristatus (Blume) Miq.
Phytochemicals
Molecular docking
Pharmacological activities

Abstract

Medicinal plants have garnered considerable interest from the pharmaceutical industry because of their diverse structures and wide array of therapeutic effects. Herbal medicines have gained popularity in recent years, as they offer cost-effective solutions and are proven safe and more effective than synthetic drugs. Cat whiskers or java tea is botanically known as *Orthosiphon aristatus* (Blume) Miq., belongs to the Lamiaceae family, and is a traditional herb with medicinal properties, commonly found in Southeast Asian countries. It is used as a diuretic and can also treat urinary dysfunctions and other ailments, such as rheumatism. Java tea is shade tolerant and suitable for intercropping with rubber plantations. This study aimed to investigate the phytochemicals present in *O.aristatus* under open and intercropped conditions *via* gas chromatography-mass spectrometry (GC-MS) analysis and to explore biomedical applications by *in vitro* and *in silico* approaches. The results revealed that flavonoids, steroids, and monoterpenoids were present, and novel compounds with medicinal properties were identified when the plants were intercropped with rubber rather than when they were grown in open fields. This study paves the way for the development of herbal medicines from *O. aristatus* to treat various ailments, as well as the potential for the green synthesis of nanoparticles due to their rich array of bioactive compounds, which are recommended for intercropping with tappable rubber plantations.

1. Introduction

Plant-based remedies were a significant source of drug discovery before the development of synthetic compounds. According to the World Health Organization, approximately 80% of global population depends on herbal medicine. A significant number of commercial pharmaceuticals are utilized for the treatment of cancer (Polu *et al.,* 2015), asthma, high blood pressure, heart disease, and infectious diseases have been developed from plant extracts (Sivakumar and Jeganathan, 2018). Recently, medicinal plants have gained increased attention from researchers because of their effectiveness and potency against a variety of ailments with reduced risks compared with synthetic pharmaceuticals (Ahda *et al.,* 2023). *O. aristatus* commonly referred to as cat whiskers, is widely grown in Southeast Asia and other tropical regions. The leaves produced from java tea are utilized for the preparation of herbal tea in Southeast Asia and various tropical countries (Abinaya *et al.,* 2022). The aerial parts (dried leaves and stem tips) of this plant have medicinal properties and have been traditionally used to cure various human ailments, such as edema, gout, hypertension, and kidney disease (Gimbun *et al.,* 2019). The pharmacological activities of *O. aristatus* leaves are diverse and extensive and include antibacterial, antioxidant, anti-inflammatory, cytotoxic, antihypertensive, vasodilatory, and hepatoprotective effects (Sivakumar and Jeganathan, 2018).

Phytochemicals are nonnutritive plant compounds that possess antimicrobial and prophylactic qualities. Although, these compounds are primarily produced by plants as a self-defense mechanism, studies have shown that they can also be effective in actively treating human illnesses (Das and Gezici, 2018).

O. aristatus is a shade-tolerant crop that is suitable for intercropping with plantations. Rubber (*Hevea brasiliensis* Muell. Arg.) has emerged as an important strategic material and serves as a major economic crop in Southeast Asia and South Asia (Clermont-Dauphin *et al.,* 2018). However, the economic benefits derived from rubber plantations are limited by the vast spacing between the rows of the plantations, which also results in a waste of land resources. To maximize economic benefits while utilizing limited land resources (Du *et al.,* 2018), intercropping is a cultivation method that promotes and supplements plantation ecosystems.This strategy can enhance spatial organization and interspecies interactions among crops,

Corresponding author: Dr. A. Jaya Jasmine
Professor, Horticultural Research Station, Pechiparai (Tamil Nadu Agricultural University), Kanyakumari-629161, Tamil Nadu, India
E-mail: jayajasmine@tnau.ac.in
Tel.: +91-9751082549

optimizing resource use (Cuartero *et al.,* 2022). Additionally, it improves the spatiotemporal management of farmland soil, supports healthy crop development, and increases yield (Gou *et al.,* 2017). Intercropping systems have a substantial effect on the phytochemical and nutrient profiles of plants (Arenas-Salazar *et al.,* 2024). However, the effects of intercropping *O. aristatus* with rubber on phytochemicals that are beneficial to human health have not yet been studied. This study focused on identifying phytobionts from crude extracts of *O. aristatus* leaves from open and intercropped plants through GC-MS analysis to determine the effectiveness of *O. aristatus* leaves for their antibacterial potential through *in vitro* and *in silico* approaches. *In silico* molecular docking analysis was subsequently conducted to investigate the antibacterial capabilities of the potential phytobioactive compounds within the beta-lactamase protein.

2. Materials and Methods

The *O.aristatus* leaves were together from open fields and intercropped fields with tappable rubber plantations planted at a spacing of 30 × 30 cm during the last week of December 2023 by following the recommended package of practices at Horticultural Research Station, Pechiparai, Kanyakumari district of Tamil Nadu, India.

2.1 Extraction of essential oil

The important oil content in the leaves of *O. aristatus* was analysed (Camlica and Yaldiz, 2019). To avoid foaming, a few glass beads were placed in a flask containing 25 to 30 g of dried *O. aristatus* leaf powder. The flask was then connected to a Clevenger apparatus, which was used for essential oil extraction. Distillation was carried out using a heating mantle with a thermostat, maintaining a temperature of 90°C until boiling began, after which the temperature was adjusted to 70°C for 3 h. Once the distillate had reached room temperature, the oil layer was allowed to separate, and its volume was determined *via* the specified formula. The essential oil content was then calculated as a percentage:

Essential oil (as per the given %) (V/W)

$$= \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

2.2 Preparation of crude extract

The leaves were harvested 100 days after planting. The crude plant extracts were prepared by the leaves were dried in the shade for 2-3 days, ground into a powder, and kept in a spotless glass container for analysis (Sivalingam and Pandian, 2024). Fifty grams of the dried leaf powder was then macerated with 70% ethanol in three separate 200 ml portions. The filtrate was concentrated *via* a vacuum rotary evaporator at 125 rpm over 24 h to yield an ethanolic extract (Elavarasan *et al.,* 2024).

2.3 GC-MS analysis

The crude extracts were examined *via* GC-MS with a Thermo GC Ultra Clarus 500 system, which combines a gas chromatograph with a mass spectrometer. For the analysis, a 1 µl sample was injected at a 10:1 ratio. The relative abundance of each component was determined by comparing its average peak area to the total peak area.

2.4 Identification of phytochemicals

To analyse and interpret the mass spectra from the GC-MS data, data from the National Institute of Standards and Technology database, which includes information on over 90,000 compounds, were utilized. The reference was obtained from the NIST library.

2.5 Identification of the biological activity of phytochemicals

Predictions regarding the bioactivities of the phytocompounds were made *via* prediction of activity spectra for biologically active substances (PASS), a tool that analyses the structural formulas of the compounds. According to the PASS online database, the phytochemicals identified in *O. aristatus* leaf extract *via* GC-MS analysis, as documented in existing pharmaceutical significance.

2.6 Preparation of ligands

The major phytobioactive compounds identified *via* GC-MS from the *O. aristatus* leaf extract utilized for the study were squalene, phytol, and lanosterol. For molecular docking, the reference medication used was the beta-lactamase protein. The three-dimensional structure of the chosen phytobiocompounds was obtained from the PubChem database in the form of a structure data file (SDF), converted into a protein data bankset up and used for molecular docking analysis.

2.7 Molecular docking

Molecular docking experiments were conducted to explore the binding mechanisms of reference drug beta-lactamase proteins (Indumathy *et al.,* 2023). The coordinates of AMPc were retrieved from the PDB database (Berman and Edan, 2002). Autodock 4.0 was employed for docking simulations, utilizing a 60 × 60 × 60 Å grid with a 0.375 Å spacing to cover the entire protein structure (Murugan *et al.,* 2024).

2.8 Statistical analysis

The statistical analysis of the results was performed *via* analysis of variance (ANOVA) with the help of the statistical software KAU grapes. The data are presented as the means of three replicate measurements. A *p*-value of less than 0.05 was considered significant, indicating substantial differences across all comparisons made.

3. Results

3.1 Essential oil percentage

Shade had a significant effect on the essential oil yield (%), as shown in Table 1. The *O. aristatus* intercropped with rubber presented the highest yield, 0.52%, compared with that of the monocrop alone (0.37%).

Table 1: Effects of shade on essential oil yield (%)

Treatment	Essential oil yield (%)
Java tea alone	0.37
Java tea intercropped with rubber	0.52
SE (d)	0.007
CD (5%)	0.029**

** Significant

3.2 Phytochemical screening through GC-MS

The bioactive substances in *O. aristatus* leaves were determined *via* GC-MS. The chromatograms (Figures 1 and 2) show the different phytochemicals found in *O. aristatus* leaves under open conditions intercropped with rubber. The identified compounds are listed in Tables 2 and 3 along with their retention times (min), peak values (%), molecular formulas and molecular weights. Through GC-MS, thirty different bioactive compounds were recognised in *O. aristatus* cultivated in open media and intercropped with rubber. The major phytochemicals with high pharmaceutical value are identified in *O. aristatus* leaves intercropped with rubber plantations, which include β-elemol (32.41%), lanosterol (10.43%), 2-(4a-methyl-8-methylenedecahydro-2-naphthalenyl)-2 propanol (8.63%). Whereas, the major phytochemicals identified in *O. aristatus* under open conditions were squalene (25.61%), and phytol (9.29%).

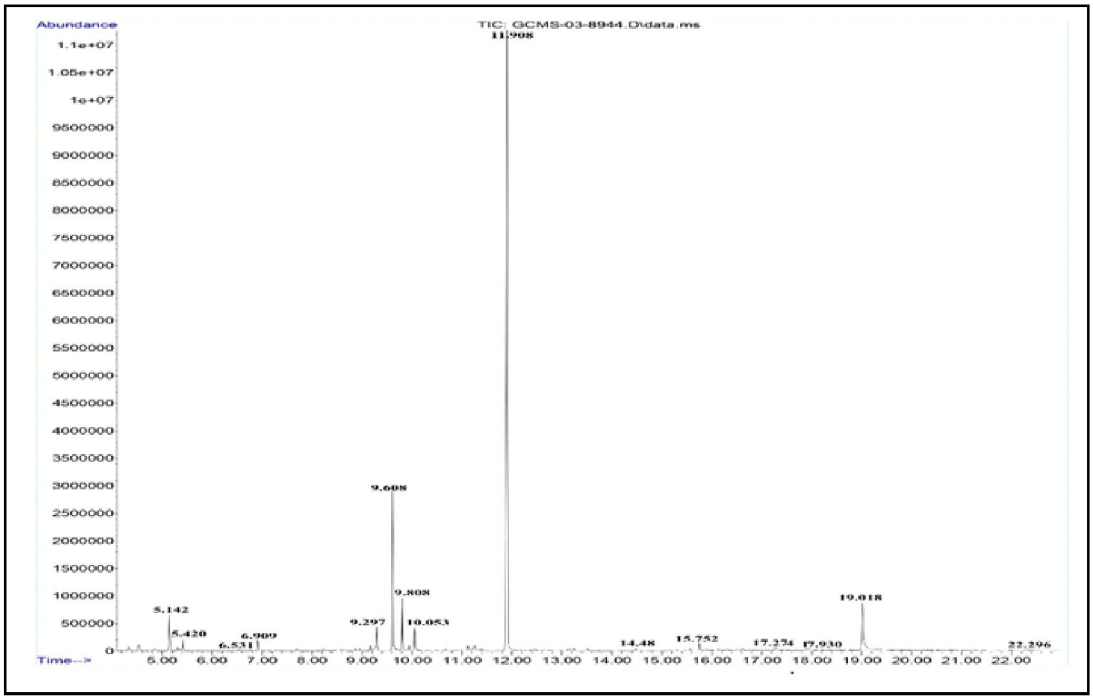


Figure 1: GC-MS chromatogram identified phytochemicals in *O. aristatus* under open condition.

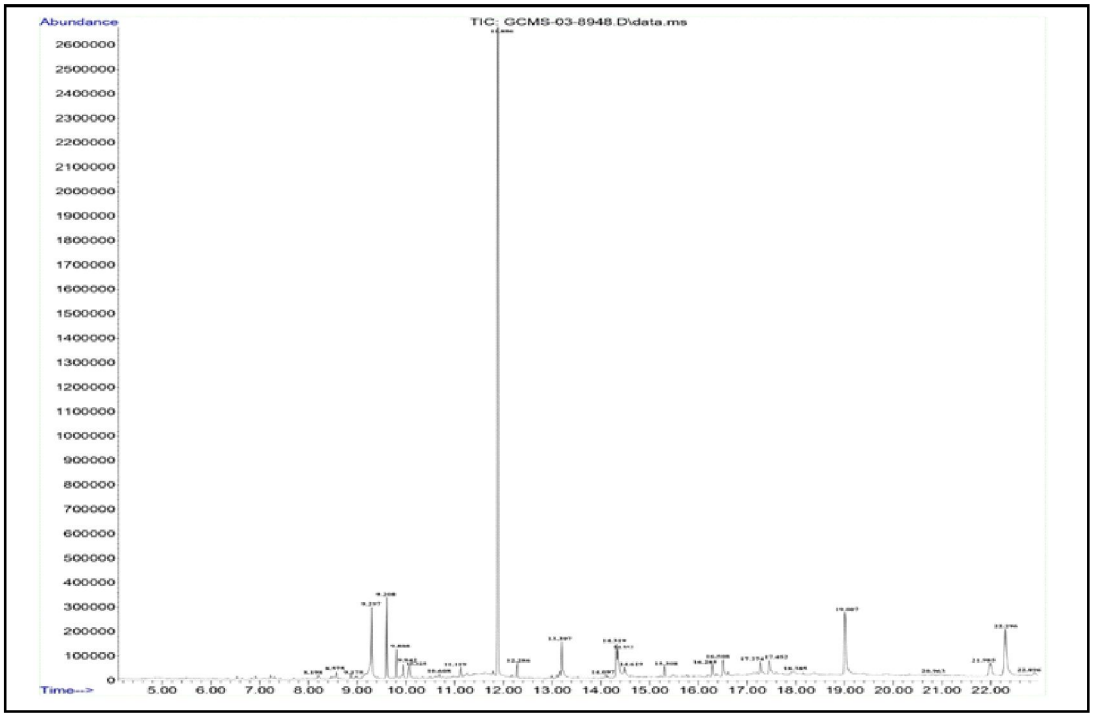


Figure 2: GC-MS chromatogram identified phytochemicals in *O. aristatus* under intercropped with rubber.

Table 2: Phytochemicals identified in Java tea under open conditions

S.No.	Bioactive compounds	Retention time (min)	Peak area (%)	Molecular formula
1	Squalene	17.885	25.61	$C_{30}H_{50}$
2	Phytol	13.997	9.29	$C_{20}H_{40}O$
3	9,12,15-Octadecatrienoic acid	14.163	6.72	$C_{18}H_{30}O_2$
4	Stigmasterol	21.718	6.65	$C_{29}H_{48}O$
5	Vitamin E	20.263	5.84	$C_{29}H_{50}O_2$
6	β-Sitosterol	22.462	5.48	$C_{29}H_{50}O$
7	n-Hexadecanoic acid	13.019	4.95	$C_{16}H_{32}O_2$
8	1H-Pyrazole-5-carboxamide, 4-amino-1-methyl-3-propyl-	4.620	4.13	$C_{18}H_{24}N_4O_3$
9	1-Propanol, 3-[(2-hydroxyethyl)thio]	4.620	4.13	$C_5H_{12}O_2S$
10	Benzoic acid, 4-ethoxy- ethyl ester	9.875	3.01	$C_{11}H_{14}O_3$
11	3-Butylindolizidine	11.942	3.01	$C_{12}H_{23}N$
12	Sinensetine	6.387	2.92	$C_6H_8O_4$
13	1-propanol,3-[(2-hydroxyethyl)thio]	20.718	2.56	$C_{19}H_{18}O_6$
14	2-Hexadecanoyl glycerol	16.308	2.50	$C_{19}H_{38}O_4$
15	Campesterol	21.440	2.06	$C_{28}H_{48}O$
16	Octadecanoic acid	14.297	1.68	$C_{18}H_{36}O_2$
17	6-Methyluracil	5.609	1.12	$C_5H_6N_2O_2$
18	Dehydroacetic acid	11.419	1.05	$C_8H_8O_4$
19	3 Oxatricyclo [4.2.0.0(2,4)] octan-7-one	18.641	0.89	$C_7H_{10}O$
20	Citronellol acetate	12.197	0.87	$C_{12}H_{22}O_2$
21	14-Tricosenyl formate	14.608	0.86	$C_{24}H_{46}O_2$
22	1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde	12.075	0.70	$C_{10}H_{16}O$
23	Metformin	6.864	0.70	$C_4H_{11}N_5$
24	Myristic acid	11.619	0.67	$C_{14}H_{28}O_2$
25	Tetrasiloxane, decamethyl	17.230	0.59	$C_{10}H_{30}O_3Si_4$
26	Silane, 1,4-phenylenebis[trimethyl	17.230	0.59	$C_{14}H_{26}Si_2$
27	α-Monostearin	17.374	0.59	$C_{21}H_{42}O_4$
28	Propyl 1-(propylthio)propyl disulfide	7.320	0.58	$C_9H_{20}S_3$
29	Mesifurane	8.809	0.55	$C_7H_{10}O_3$
30	2(3H)-Benzothiazolone	9.464	0.53	C_7H_5NOS

Table 3: Phytochemicals identified in Java tea intercropped with rubber

S.No.	Bioactive compounds	Retention time (min)	Peak area (%)	Molecular formula
1	β-Elemol	10.086	32.41	$C_{15}H_{26}O$
2	Lanosterol	21.907	10.43	$C_{30}H_{50}O$
3	2-(4a-Methyl-8-methylenedecahydro-2-naphthalenyl)-2 propanol	12.197	8.63	$C_{15}H_{26}O$
4	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	10.364	8.29	$C_{15}H_{26}O$
5	(1E)-1-Ethylidene-7a-methyloctahydro-1H-indene	10.997	4.60	$C_{12}H_20$
6	4-Hexen-1-ol, 5-methyl-2-(1-methyl ethenyl)	7.353	3.50	$C_{10}H_{18}O$
7	1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene -cyclohexane	13.274	3.21	$C_{15}H_{24}O$

8	Benzoic acid, 4-ethoxy-, ethyl ester	9.830	3.17	$C_{11}H_{14}O_3$
9	1-methyl-1-vinyl-2,4-diisopropenyl-cyclohexane	8.731	2.73	$C_{15}H_{24}$
10	Citronellol acetate	7.109	2.60	$C_{10}H_{20}O$
11	Germacrene D	9.575	1.91	$C_{15}H_{24}$
12	Glycerin	4.420	1.87	$C_3H_8O_3$
13	C-Veratroylglycol	4.420	1.87	$C_{10}H_{12}O_5$
14	Tetrasiloxane, decamethyl	22.296	1.67	$C_{10}H_{30}O_3Si_4$
15	5-methyl-2-hydroxy-2-propyl-cyclohexanol	8.264	1.14	$C_{10}H_{20}O_2$
16	Pentanamide, 4-hydroxy-4-methyl-N-phenyl	17.418	1.12	$C_{12}H_{17}NO_2$
17	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	12.563	1.09	$C_{15}H_{24}O$
18	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	10.875	1.07	$C_{15}H_{24}$
19	1-Ethyl-2-benzimidazolinone	12.741	0.95	$C_9H_{10}N_2O$
20	Squalene	11.308	0.87	$C_{30}H_{50}$
21	α-Copaen-11-ol	12.697	0.85	$C_{15}H_{24}O$
22	Silane, 1,4-phenylenebis[trimethyl	18.229	0.82	$C_{14}H_{26}Si_2$
23	O-Trifluoroacetyl-menthol	17.241	0.81	$C_{12}H_{19}F_3O_2$
24	o-Anisic acid, 2-tetradecyl ester	11.575	0.77	$C_{22}H_{36}O_3$
25	2-trimethylsiloxy-2-methyl-3-butene	9.375	0.76	$C_8H_{18}OSi$
26	6,7-Dihydro-2-cis-farnesol	13.374	0.73	$C_{15}H_{28}O$
27	Phytol	13.952	0.72	$C_{20}H_{40}O$
28	1-Chloro-4-methylcyclohexane	8.464	0.57	$C_7H_{13}Cl$
29	γ-Eudesmole	10.797	0.53	$C_{15}H_{26}O$
30	2-(Hydroxymethyl)-5-(1-hydroxy-1-methylethyl)-2-cyclohexen-1-ol	13.819	0.52	$C_{10}H_{18}O_3$

3.3 Molecular docking

The beta-lactamase protein was docked *via* AutoDock 4, and the three documented bioactive molecules were used to examine their biological activity through molecular docking with the beta-lactamase protein. The docking affinity values determined by AutoDock for lanosterol, phytol, and squalene were -7.5, -5.0, and -5.0, respectively, as depicted in Table 4, which indicated that these compounds could exhibit notable antibacterial activity. Figures 3-5 show the docking poses of various bioactive-compounds that interact with their protein drug targets. These docking poses were analysed to investigate and evaluate the amino acid residues involved in the different interactions.

Table 4: Docking score, binding energy, interaction and amino acid affinities of *O. aristatus* bioactive compounds against the beta-lactamase protein

S.No.	Name of the compound	Binding energy (kcal/mol)	Interaction	Amino acid residues
1	Lanosterol	-7.7	1. Pi-Alkyl	ALA A:32, ALA A:43, VAL A:41, TRP A:91, LEU A:209, HIS A: 234
2	Phytol	-5.0	1. Conventional hydrogen bond 2. Unfavourable Donor-Donor bond 3. Pi=Alkyl	1. HIS A:234 2. HIS A:117, HIS A:119, HIS A:172, ASP A:1213. ALA A:43, TRP A:91, LEU A:209
3	Squalene	-5.0	1. Pi- Sigma 2. Pi-Alkyl	1. HIS A:119 2. VAL A:41, ALA A:43, TRP A:91, LEU A:209, HIS A:234

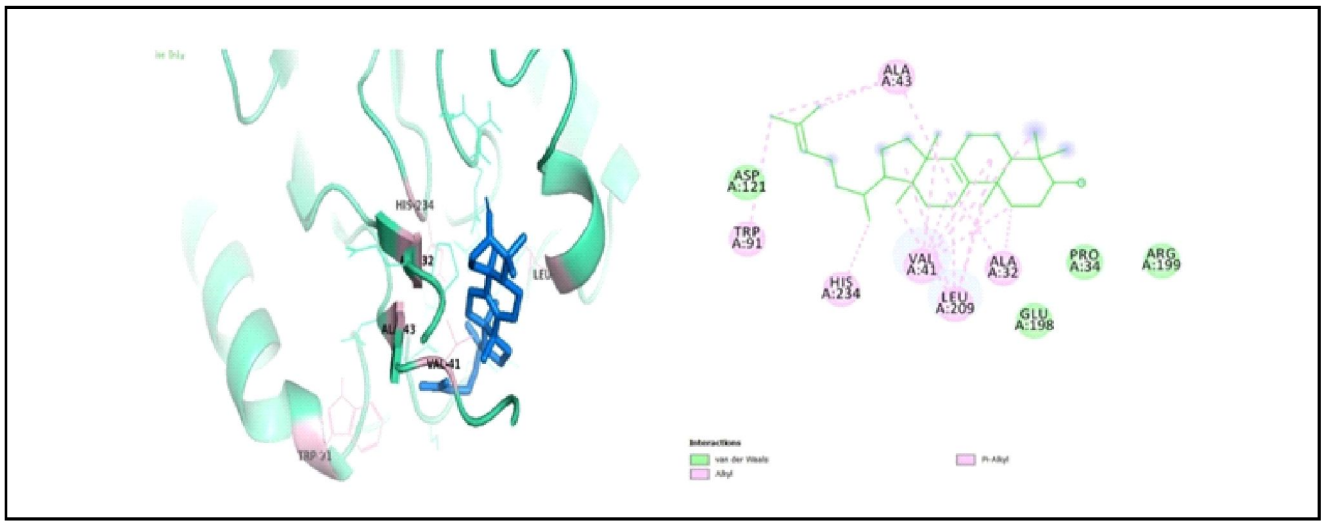


Figure 3: Lanosterol with a beta-lactamase protein docking pose and interaction effect.

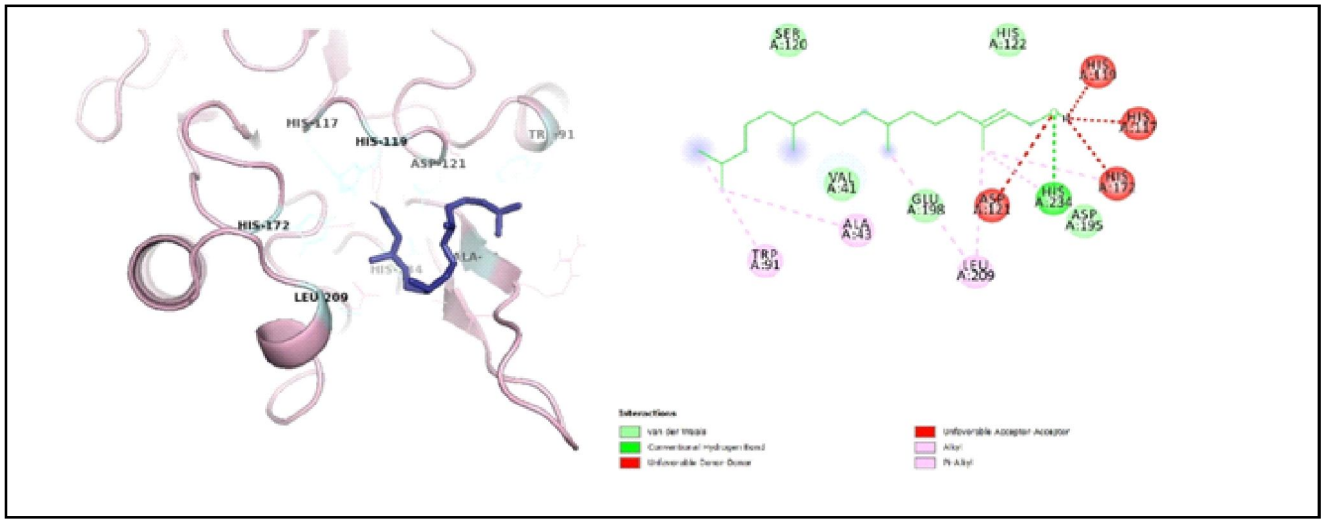


Figure 4: Phytol with a beta-lactamase protein docking pose and interaction effect.

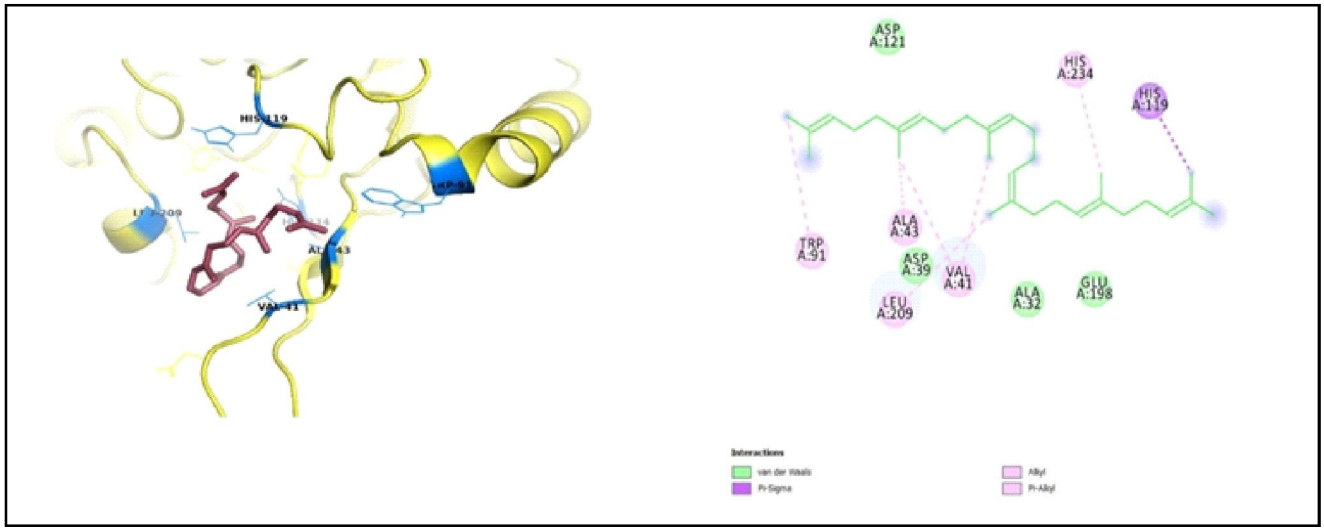


Figure 5: Squalene with a beta-lactamase protein docking pose and interaction effect.

4. Discussion

The percentage of essential oil was significantly affected by intercropping and monocropping. The essential oil yield of *O. aristatus* obtained in the present study is consistent with the range reported by (Azizan *et al.,* 2017). Agroforestry has also been reported to increase the essential oil yield of *Ocimum* sp. (Kumar *et al.,* 2015; Peker *et al*., 2023). In our study, the intercropping of *O. aristatus* with rubber resulted in a greater oil yield, although the difference was statistically significant. This is due to the microenvironment, particularly the leaf temperature, humidity, and soil fertility, and the positive effect of shade has a substantial influence on the development and composition of quality indicators. Secondary products and alkaloids are formed by breaking down and resyntizing primary products, a process that is favored by shaded conditions. The biosynthesis of secondary metabolites in plants is an adaptive mechanism that helps them cope with shaded conditions and is controlled by the ambient environment (Kumar *et al.,* 2015).

GC-MS is a powerful and accurate technique for identifying numerous metabolites, such as sugars, organic acids, polyols, and various phenolic and cyclic compounds. In our study, we detected aromatic compounds, terpenes, flavanoids, esters, steroids, ketones and alkaloids in *O. aristatus* leaves under shaded and unshaded conditions. The production of secondary metabolites in plants is thought to be an adaptation that helps them survive unfavourable environmental conditions and is influenced by various factors, including their genetic profile, ontogenetic development, external elements such as climate, pollution, diseases, pests, and edaphic characteristics (Verma and Shukla, 2015). This process involves the synthesis of intricate chemical compounds and the establishment of structural and functional stability through signalling pathways and processes (Isah, 2019).

Intercropping can lead to changes in the concentrations of specific health-related secondary metabolites. Different environmental conditions can result in variations in the concentration of a particular secondary metabolite among plants of the same species. However, shading can affect the phytochemical constituents of medicinal plants and alter their quality (Ray *et al.,* 2021).

On the basis of the results of the GC-MS analysis, we identified a total of 30 compounds in the unshaded condition and 30 compounds in the shaded condition. Only 10% of the match compounds, particularly squalene and phytol 4-hydroxy-3-[[1,3-dihydroxy-2-propoxy]methyl]-1H-pyrazole-5-carboxamide, yielded yields of 25.61%, 9.29%, and 4.13% in unshaded conditions and 0.87%, 0.72%, and 1.87% in shaded conditions. Only benzoic acid, 4-ethoxy-ethyl ester (3.17%), silane, 1,4-phenylenebis [trimethyl (0.82%), tetrasoxane, and decamethyl (1.67%) tended to increase in concentration in response to shade in the leaf extracts of *O. aristatus* under different conditions. This is because when *O. aristatus* is exposed to direct sunlight, ultraviolet radiation causes cell destruction and leads to the synthesis of cell-protective compounds in *O. aristatus* leaves, albeit at lower concentrations than those in shade-treated plants. Similar results have been reported (Ray *et al.,* 2021) in tea leaves.

The phytochemicals identified in *O. aristatus* leaf extracts *via* GC-MS analysis are known to have high pharmaceutical significance, as shown in Tables 5 and 6. In our study, we identified 24 new compounds with high pharmacological value in *O. aristatus* intercropped with rubber, particularly β-Elemol, which has antimicrobial and antioxidant activity (Arykbayev, 2023). Lanosterol is an antiviral, anticancer, and antihypertensive immunomodulatory agent (Townsend and Ebizuka, 2010). 2-(4a-Methyl-8-methylenedecahydro-2-naphthalenyl)-2 propanol has been utilized as an anticough, detoxification agent, and phlegm (Chen, 2018), and 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene has antimicrobial activity (Naido, 2014). This may be due to the interaction between the rubber roots and java tea, which produces phytochemicals as a defense mechanism. This finding also confirms the findings reported by (Ntobela *et al.,* 2022).

Table 5: Pharmacological activity of the identified compounds in Java tea leaves under open conditions

Name of the compound	Nature	Pharmacological activity	Reference
Squalene	Polyenes	Antibacterial, antifungal, antioxidant	Sundari *et al.,* 2023
Phytol	Triterpene	Detoxifier, anticancer, antioxidant,	Cheng *et al.,* 2024
9,12,15-Octadecatrienoic acid	Diterpene compound	Anticancer, antimicrobial,	De Alencar *et al.,* 2023
Vitamin E	Steroid	Anti-inflammatory, antimicrobial	Goswami *et al.,* 2023
β-Sitosterol	Vitamin E	Antioxidant, anticancer, anti-aging	Sivakumar and Jeganathan, 2018
Sinensetine	Alkaloid	Antioxidant, anti-inflammatory	Akash and Singh, 2017
Campesterol	Palmitic acid	Antibacterial, anthelmintic, anti-inflammatory, antioxidant	Thejashree and Naika, 2023
Octadecanoic acid	Steroids	Anticancer, wound healing	Uttu *et al.,* 2023
Dehydroacetic acid	Pyrimidine derivatives	Antioxidant	Shishkina *et al.,* 2023
14-Tricosenyl formate	Monoterpenoid	Antibacterial, antifungal, antioxidant	Santos *et al.,* 2019
1,3,4-Trimethyl-3-cyclo-hexenyl-1-carboxaldehyde	Aromatic compound	Antibacterial, antifungal properties	Hasan *et al.,* 2019
Silane, 1,4-phenylenebis [trimethyl	Non terpenoid	Antioxidant, anti-inflammatory	Alam and Singh, 2021

Table 6: Pharmacological activity of the identified compounds in Java tea leaves intercropped with rubber

Name of the compound	Nature	Pharmacological activity	Reference
β-Elemol	Sesquiterpenoid	Antimicrobial, antioxidant	Arykbayeva *et al.,* 2023
Lanosterol	Terpene	Antiviral, anticancer,	Al-Ansi *et al.,* 2024
(1E)-1-Ethylidene-7a-methyloctahydro-1H-indene	Polycyclic compound	Antioxidant, antimicrobial	Cours, 2020
4-Hexen-1-ol, 5-methyl-2-(1-methyl ethenyl)	Aromatic compound	Antiemetic	Li *et al.,* 2022
Citronellol acetate	Natural acyclic mono terpenoid	Antibiotic, antifungal, anticancer, antioxidant	Swantara *et al.,* 2022
Germacrene D	Sesquiterpene	Antioxidant, larvicide, antimicrobial,	Sheela *et al.,* 2021
Glycerin	Simple triol compound	Antibacterial, antiviral, anti-inflammatory,	Di Vito *et al.,* 2020
C-Veratroylglycol	Aromatic ketone	Antimicrobial	Sánchez-Hernández *et al.,* 2022
Tetrasiloxane, decamethyl	Non terpenoid	Antioxidant, antibacterial	Alam and Singh, 2021
Squalene	Triterpene	Detoxifier, anticancer, antioxidant	Kim and Karadeniz, 2012
6,7-Dihydro-2-cis-farnesol	Sesquiterpenoid	Decreases the blood cholesterol level	Sowmiya *et al.,* 2021
γ-Eudesmole	Diterpenoids	Anticough, detoxification,	Chen, 2018

Sinesetin is a major flavonoid compound usually found in *O. aristatus.* When intercropped with rubber, the sinesetin content decreased compared with that of the monocrop. Plants synthesized flavonoids and phenolic acids as defense compounds in reaction to stress induced by sun exposure (Iliæ and Fallik, 2017). The dissimilarity in the primary components of *O. aristatus* between monocultures and intercultures can be attributed to the influence of light on photosynthesis and respiration which alters the movement of metabolites and decreases the energy produced through light reactions, potentially affecting the synthesis and accumulation of the main constituents in medicinal and aromatic plants. The bio-production of phytochemicals in plants is influenced by light intensity and spectral quality (Majeed, 2017). To protect against damage from high irradiance and UV radiation, plants accumulate phenolic compounds and other antioxidants like carotenoids, flavonoids, and anthocyanins. Consequently, manipulating spectral quality and light intensity can induce morphological and physiological changes, impacting the biosynthesis, accumulation, and retention of phytochemicals (Iliæ and Fallik, 2017).

Similar findings were reported by Samson *et al.* (2022) for *Indigofera tinctoria* intercropped with cassava, Basavaraju *et al.* (2011) for kalmegh intercropped with coconut and Mohandas (2011) for medicinal plants intercropped with coconut.

Molecular docking is an effective method for identifying ligands that bind to proteins of known structure, which is crucial for structure-based drug discovery. Researchers globally utilize molecular docking software to explore and assess the binding affinity of phytobiotic compounds at protein binding sites. To carry out molecular docking analysis, it is essential to depict both the ligand and protein structures in three-dimensional form (Vakayil *et al.,* 2021). The exploration and representation of bioactive compounds derived from various phytomedicinal species have increased in importance and significance. Docking is frequently applied in structure-based drug design to predict and evaluate the molecular interactions between drug compounds and their protein targets (Baburam *et al.,* 2022; Kabeerdass *et al.,* 2022). The present study used GC-MS analysis to identify 30 bioactive compounds from java tea grown under open field conditions and 30 bioactive compounds from java tea intercropped with rubber. Among these compounds, major phytochemicals such as squalene and phytol from the open field and lanosterol from the intercropped condition were selected for molecular docking analysis. Figures 3-5 depict the docking positions of diverse compounds alongside interaction plots that illustrate the compounds docked in the enzyme's active site. The active site is the region of an enzyme where the substrate binds and the reaction is catalyzed. The compounds orient themselves in a configuration that enables them to establish several hydrogen bonds and hydrophobic interactions with the enzyme. Although hydrogen bonds are relatively weak, they are essential for stabilizing the binding of a compound to an enzyme.

The interaction plot highlights significant interactions between the compound and the enzyme, particularly emphasizing the critical hydrogen bonds formed between the carboxylate group of the compound and the amino group of the enzyme. These bonds are vital for stabilizing the compound's binding to the enzyme. Based on the docking pose and interaction plot analysis, the compound shows potential as an enzyme activity inhibitor by effectively binding to the enzyme's active site and forming numerous interactions. These interactions could hinder the enzyme's ability to bind to its substrate and carry out catalysis. Additionally, computational analysis indicated that lanosterol has greater affinity and lower binding energy than other bioactive compounds. Molecular docking also confirmed the reliable and focused binding interactions of lanosterol with the target protein, highlighting its potential therapeutic implications.

5. Conclusion

The current study revealed that *O. aristatus* is a promising intercrop for mature rubber plantations, as it increases essential oil yield (%) and produces specific secondary metabolites compared with those of the individual crops. The abundance of phytochemical components

in the aqueous leaf extract of *O. aristatus* which possesses potent pharmacological and biological properties, was determined. This finding allows researchers to conduct investigations based on the active principles present and confirm the pharmacological activity and mechanism that could support the use of the plant in traditional medicine. Ligand–protein interactions revealed that, compared with those from open field conditions, compounds from the leaves of *O. aristatus* intercropped with rubber presented greater antimicrobial properties. There are many opportunities for the large-scale production of *O. aristatus* under tappable rubber plantations to treat various kinds of diseases. The future success of the pharmaceutical industry relies on discovering novel compounds with unique activities or those aimed at specific therapeutic targets. The increasing identification and characterization of secondary metabolite gene clusters offer new genetic tools for creating novel compounds through combinatorial biosynthesis.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

Reference

Abinaya, C.; Suresh, J.; Rajamani, K.; Vennila, P. and Ganapathy, S. (2022). Studies on standardization of stage of harvest in java tea (*Orthosiphon stamineus* Benth.). Pharm. Innov., **11**(2):2253-2256.

Ahda, M.; Jaswir, I.; Khatib, A.; Ahmed, Q.U.; Mahfudh, N.; Ardini, Y.D.; Syed Mohamad, S.N.A.; Anwar, M.; Hernawan, H.and Miyashita, K. (2023). Phytochemical analysis, antioxidant, α-glucosidase inhibitory activity and toxicity evaluation of *Orthosiphon stamineus* leaf extract.Sci. Rep., **13**(1):17012.

Akash, S.S.and Singh, S.K. (2017). Phytoconstituents estimation of *Lepidium sativum* L. seed extract using GC-MS spectroscopy. World J. Pharm. Res., **7**(1):1360-1367.

Al-Ansi, Z.; Masaoud, M.; Hussein, K.; Moharram, B. and Al-Madhagi, W.M. (2024). antibacterial and antioxidant activities of triterpenoids isolated from endemic *Euphorbia arbuscula* Stem Latex. Adv. Pharmacol. Pharm. Sci., (**5**):1-9.

Alam, A. and Singh, V. (2021). Composition and pharmacological activity of essential oils from two imported *Amomum subulatum* fruit samples. Journal of Taibah University Medical Sciences., **16**(2):231-239.

Arenas-Salazar, A.P.; Schoor, M.; Parra-Pacheco, B.; García-Trejo, J.F.; Torres-Pacheco, I. and Feregrino-Pérez, A.A. (2024). Intercropping systems to modify bioactive compounds and nutrient profiles in plants: Do We have enough information to take this as a strategy to improve food quality? A review. Plants, **13**(2):194.

Arykbayeva, A.B.; Ustenova, G.O.; Sharipov, K.O.; Beissebayeva, U.T.; Kaukhova, I.E.; Myrzabayeva, A. and Gemejiyeva, N.G. (2023). Determination of chemical composition and antimicrobial activity of the CO_2 extract of *Eryngium planum* L. Int. J. Biomater., 2023(**1**):1-11.

Azizan, N.; Mohd Said, S.; Zainal Abidin, Z. and Jantan, I. (2017). Composition and antibacterial activity of the essential oils of *Orthosiphon stamineus* Benth and *Ficus deltoidea* Jack against pathogenic oral bacteria. Molecules, **22**(12):2135.

Baburam, S.; Ramasamy, S.; Shanmugam, G. and Mathanmohun, M. (2022). Quorum sensing inhibitory potential and molecular docking studies of *Phyllanthus emblica* phytochemicals against *Pseudomonas aeruginosa*. Appl. Biochem. Biotechnol., **194**:434-444.

Basavaraju, T.; Nanjappa, H.; Umesha, K.; Vasundhara, M. and Arulraj, S. (2011). Intercropping of medicinal and aromatic plants in coconut gardens. Journal of Plantation Crops, **39**(2):299-304.

Berman, S. and Edan, Y. (2002). Decentralized autonomous AGV system for material handling. Int. J. Prod. Res., **40**(15):3995-4006.

Camlica, M. and Yaldiz, G. (2019). Effect of cultural condition on seed growth and content of essential oil of two populations and one cultivar of genus Nigella. Ann. Phytomed., **8**(1):56-62.

Chen, J. (2018). GC-MS explores the health care components in the extract of *Pterocarpus pedatu*s Pierre. Saudi J. Biol. Sci.,**25**(6):1183-1188.

Chen, J.; Ni, C.; Liu, Q.; Hu, Z.; Ge, S.; Peng, W. and Ashraf, M.A. (2018). Molecular structure and functions of rosewood: *Pterocarpus Erinaceus*. Caribb. J. Sci., **51**(3):493-501.

Cheng, L.; Ji, T.; Zhang, M. and Fang, B. (2024). Recent advances in squalene: Biological activities, sources, extraction, and delivery systems. Trends Food Sci., **146**:104392.

Clermont-Dauphin, C.; Dissataporn, C.; Suvannang, N.; Pongwichian, P.; Maeght, J.l.; Hammecker, C. and Jourdan, C. (2018). Intercrops improve the drought resistance of young rubber trees. Agron. Sustain. Dev., **38**(6):56.

Cours, D. (2020). GC-MS Analysis of phytoconstituents of the essential oil from the leaves of *Melaleuca citrina* (Curtis). Advances in Zoology and Botany, **8**(3):87-98.

Cuartero, J.; Pascual, J.A.; Vivo, J.-M.; Özbolat, O.; Sánchez-Navarro, V.; Egea-Cortines, M.; Zornoza, R.; Mena, M.M.; Garcia, E. and Ros, M. (2022). A first-year melon/cowpea intercropping system improves soil nutrients and changes the soil microbial community. Agric. Ecosyst. Environ., **328**:107856.

Das, K.and Gezici, S. (2018). Secondary plant metabolites, their separation and identification, and role in human disease prevention. Ann. Phytomed., **7**(2):13-24.

De Alencar, M.V.O.B.; Islam, M.T.; Da Mata, A.M.O.F.; Dos Reis, A.C.; De Lima, R.M.T.; De Oliveira Ferreira, J.R.; De Castro E Sousa, J.M.; Ferreira, P.M.P.; De Carvalho Melo-Cavalcante, A.A. and Rauf, A. (2023). Anticancer effects of phytol against Sarcoma (S-180) and human leukemic (HL-60) cancer cells. Environ. Sci. Pollut. Res., **30**(33):80996-81007.

Di Vito, M.; Gentile, M.; Mattarelli, P.; Barbanti, L.; Micheli, L.; Mazzuca, C.; Garzoli, S.; Titubante, M.; Vitali, A. and Cacaci, M. (2020). Phytocomplex influences antimicrobial and health properties of concentrated glycerine macerates. Antibiotics, **9**(12):858.

Du, J.B.; Han, T.F.; Gai, J.Y.; Yong, T.W.; Xin, S.; Wang, X.C.; Feng, Y.; Jiang, L.; Kai, S. and Liu, W.G. (2018). Maize-soybean strip intercropping: Achieved a balance between high productivity and sustainability. J. Integr. Agric.,**17**(4):747-754.

Elavarasan, V.; Vijayakumar, S.; Aldawood, S.; Thangaswamy, S. and Prathipkumar, S. (2024). Assessment of luminescent copper nanomaterials as anti germs, anti proliferation efficiencies using green nano strategy. Lumin., **39**(7):4831.

Goswami, M.; Jaswal, S.; Gupta, G.D. and Verma, S.K. (2023). A comprehensive update on phytochemistry, analytical aspects, medicinal attributes, specifications and stability of stigmasterol. Steroids, **196**(6:109244.

Gou, F.; Yin, W.; Hong, Y.; Van Der Werf, W.; Chai, Q.; Heerink, N. and Van Ittersum, M.K. (2017). On yield gaps and yield gains in intercropping: Opportunities for increasing grain production in northwest China. Agric. Syst., **151**:96-105.

Hasan, M.M.; Al Mahmud, M.R. and Islam, M.G. (2019). GC-MS analysis of bio-active compounds in ethanol extract of *Putranjiva roxburghii* Wall. fruit peel. Pharmacognosy Journal, **11**(1):146-149.

Indumathy, P.; Kalyan, N.P.; Vishnu, K.; Kumar, R.A.; Yogeshwaran, R. and Patan, A. (2023). A review on quinazoline containing compounds: Molecular docking and pharmacological activites. Ann. Phytomed., **12**(1):220-229.

Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. Biol. Res., **52**(1):39.

Kabeerdass, N.; Thangaswamy, S.; Mohanasrinivasan, V.; Rajasekaran, C.; Sundaram, S.; Nooruddin, T. and Mathanmohun, M. (2022). Green synthesis mediated nanoparticles and their curative character against post COVID-19 skin diseases. Curr. Pharmacol. Rep., **8**(6):409-417.

Kim, S.K. and Karadeniz, F. (2012). Biological importance and applications of squalene and squalane. Adv. Food Nutr. Res., **65**:223-233.

Kumar, M.; Thakur, N. and Hegde, H. (2015). Growth, herb yield and financial flows from *Ocimum* spp. intercropped under teak (*Tectona grandis* L. f.) based silvi-medicinal system in Gujarat, India. Int. J. Innov. Hortic., **4**(2):113-118.

Iliæ, Z.S. and Fallik, E. (2017). Light quality manipulation improves vegetable quality at harvest and postharvest: A review. Environ. Exp. Bot., **139**:79-90.

Li, J.; Wang, X.; Xun, S.; Guo, Q.; Wang, Y.; Jia, Y.; Wang, W.; Wang, Y.; Li, T. and Tang, T. (2022). Study of the mechanism of antiemetic effect of *Lavandula angustifolia* Mill. Essential oil Based on Ca^{2+}/CaMKII/ERK1/2 Pathway. Drug Des. Devel. Ther., **16**:2407-2422.

Majeed, M. (2017). Evidence-based medicinal plant products for the health care of world population. Ann. Phytomed., **6**(1):1-4.

Mohandas, S. (2011). Prospects of intercropping medicinal and aromatic plants in coconut. Madras Agric. J., **98**(1-3):82-83.

Murugan, R., Selvan, S.T., Subramanian, R., Chitra, S. and Ali, S., (2024). Sustainable carbon quantum dots enhanced photoluminescence for bioimaging applications: An *in vitro* and *in silico* analysis. J. Photochem. Photobiol., **453**:115669

Naidoo, Y.; Sadashiva, C.; Kasim, N.; Nicholas, A. and Naidoo, G. (2014). Chemical composition and antimicrobial activity of the essential oil of *Ocimum obovatum* E. Mey. Ex Benth. (Lamiaceae). J. Essent. Oil-Bear. Plants., **17**(1):142-147.

Ntobela, B.; Oguntibeju, O.O.; Rautenbach, F.; Etsassala, N.G.E.R.; Akinpelu, E.A. and Nchu, F. (2022). Evaluating the effect of 40% and 0% shading levels on the secondary metabolites, antifungal and anti-insect activities of extracts of *Allium porrum* cultivated hydroponically under greenhouse conditions. J. Med. Plants Econ. Dev., **6**(1):1-9.

Polu, P.; Nayanabhirama, U. and Khan, S. (2015). Herbal medicinal plants as an anticancer agents. Ann. Phytomed., **4**(1):37-45.

Vakayil, V; Thasfeeha Abdul Nazeer and Mathanmohun, M. (2021). Evaluation of the antimicrobial activity of extracts from *Acorus calamus* rhizome against multidrug-resistant nosocomial pathogens. Res. J. Agric. Sci., **12**:1613-1617.

Ray, S.; Chatterjee, J.; Ghosh, A. and Das, S. (2021). Assessment of shade-unshade condition and subsequently pesticide treatment on first flush tea leaf metabolites through GC/MS based metabolomics approach. Cogent Food Agric., **7**(1):1930424.

Samson, D.; Sindhu, P. and Menon, M.V. (2022). Intercropping of medicinal plants with cassava (*Manihot esculenta* Crantz) under different row arrangements. J. Trop. Agric. **60**(1):118-123.

Santos, P.L.; Matos, J.P.S.; Picot, L.; Almeida, J.R.; Quintans, J.S. and Quintans Junior, L.J. (2019). Citronellol, a monoterpene alcohol with promising pharmacological activities: A systematic review. Food Chem. Toxicol., **123**:459-469.

Þeker, S.; Çakaloðullarý, U.; Bayram, E. and Tatar, Ö. (2023). Production of sage, oregano and rosemary under shading conditions and the effects of light on growth and essential oil properties. Ind. Crops Prod., **193**:116254.

Sheela, D.; Abdul Rahim, M.; Arunagirinadhan, N.; Ramya, B. and Indra, V. (2021). GC-MS profiling and antibacterial activity of *Wrightia tinctoria* and *Lantana camara* leaves extract. Intern. J. Zool. Invest. **7**(2):920-938.

Shishkina, S.V.; Shaposhnik, A.M.; Dyakonenko, V.V.; Baumer, V.M.; Rudiuk, V.V.; Yanchuk, I.B. and Levandovskiy, I.A. (2023). New polymorphic modifications of 6-Methyluracil: An experimental and quantum chemical study. ACS Omega., **8**(23):20661-20674.

Sivakumar, C. and Jeganathan, K. (2018). Phytochemical profiling of cat whisker's (*Orthosiphon stamineus*) tea leaves extract. J. Pharmacogn. Phytochem., **7**(6):1396-1402.

Sivalingam, A.M. and Pandian, A. (2024). Identification and characterization of silver nanoparticles from *Erythrina indica* and its antioxidant and uropathogenic antimicrobial properties. Microb. Pathog., **190**:106635

Sowmiya, V.; Sakthi Priyadarsini, S. and Kumar, P. (2021). GC-MS Profiling of different fractions of *Physalis minima* Linn., leaves. Nat. Vol. Essent. Oil., **8**(5):12852-12859.

Sundari, D.; Handayani, D.S. and Suryanti, V. (2023). Chemical compositions, antioxidant and antibacterial activities of kepel (*Stelechocarpus burahol)* fruit flesh and peel extracts. Biodivers. J., **24**(9):4668-4675

Swantara, M.D.; Rita, W.S.; Dira, M.A. and Agustina, K.K. (2022). Effect of the methanol extract of *Annona squamosa* Linn leaf on cervical cancer. Int. J. Vet Sci., **12**(3):295-301.

Thejashree, A.B. and Naika, R. (2023). Identification of bioactive compounds in acetone leaf and stem-bark extracts of *Psychotria dalzellii* Hook. by GC-MS analysis and evaluation of *in vitro* Anti-bacterial properties. Asian J. Biol. Sci., **12**(3):499-509

Townsend, C.A. and Ebizuka, Y. (2010). Natural products structural diversity-I: Secondary metabolites, organization and biosynthesis. Elsevier. **1**:1-1007.ISBN 978-0-08-045382-8

Uttu, A.J.; Sallau, M.S.; Ibrahim, H. and Iyun, O.R.A. (2023). Isolation, characterization, and docking studies of campesterol and β-sitosterol from *Strychnos innocua* (Delile) root bark. J. Taibah Univ. Med. Sci., **18**(3):566-578.

Vakayil, R.; Anbazhagan, M.; Shanmugam, G.; Ramasamy, S. and Mathanmohun, M. (2021). Molecular docking and *in vitro* analysis of phytoextracts from *B. serrata* for antibacterial activities. Bioinformation, **17**(7):667-672.

Verma, N. and Shukla, S. (2015). Impact of various factors responsible for fluctuation in plant secondary metabolites. J. Appl. Res. Med. Aromat. Plants, **2**(4):105-113

Yadav, K.P.; Rahman, M.A.; Nishad, S.; Maurya, S.K.; Anas, M. and Mujahid, M. (2023). Synthesis and biological activities of benzothiazole derivatives: A review. Intelligent Pharmacy, **1**(3):122-132.

Citation **M. Sabarivasan, A. Jaya Jasmine, M. Maghimaa, G. Ashok Kumar and T. Anitha (2024). Phytochemical profiling and biomedical applications of *Orthosiphon aristatus* (Blume) Miq.: *in vitro* and *in silico* approaches. Ann. Phytomed., 13(2):953-962. http://dx.doi.org/10.54085/ap.2024.13.2.98.**