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PHYTOCHEMICAL PROFILING, ANTIBACTERIAL POTENTIAL, AND GC-MS ANALYSIS OF EPIPHYTIC ORCHID *VANDA TESSELLATA* (ROXB)

P. Janaki Rao[1], J. Ramalakshmana[2*], S. B. Padal[3]

[1]Lecturer in Botany, Department of Botany, Government Degree College, Paderu-531024.

[*2]Assistant Prof. (Guest Faculty), Department of Botany, Central Tribal University, Vizianagaram-535003 (AP), India).

[3]Professor, Department of Botany, Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India.

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***Corresponding Author**

J. Ramalakshmana

Assistant Prof. (Guest Faculty), Department of Botany, Central Tribal University, Vizianagaram-535003 (AP), India).

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ABSTRACT

The present study investigates the phytochemical composition, antibacterial activity, and chemical profiling of *Vanda tessellata* (Roxb.) Hook. ex G.Don, collected from the Paderu forest region of the Eastern Ghats, Andhra Pradesh, India. Leaves were shade-dried, coarsely powdered, and sequentially extracted with methanol, acetone, benzene, and water using a Soxhlet apparatus. Preliminary phytochemical screening revealed the presence of diverse bioactive constituents, including alkaloids, flavonoids, tannins, phenols, saponins, steroids, and glycosides, with methanol and aqueous extracts showing the richest profiles. Antibacterial activity was evaluated against *Bacillus subtilis*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas fluorescens* using the agar well diffusion method. Methanol extract exhibited the highest inhibition, notably against *P. fluorescens* (22.3 ± 1.2 mm) and *S. aureus* (18.3 ± 0.57 mm), while aqueous extract showed minimal activity. GC-MS analysis of the methanol extract identified eleven compounds, with glycerin (22.96%), n-hexadecanoic acid (18.64%), and 1,2,3,5-cyclohexanetetrol (17.42%) as major constituents. The identified compounds demonstrated reported bioactivities, including antimicrobial, antioxidant, anti-inflammatory, and wound-

healing properties. These findings suggest *V. tessellata* as a promising source of phytochemicals with potential applications in antimicrobial drug development, warranting further in vivo and mechanistic studies.

KEYWORDS: Antibacterial, Methanol, GC-MS analysis, Saponins, Steroids, Flavonoids.

INTRODUCTION

The genus Vanda, comprising approximately 73 species, represents one of the most diverse and economically valuable groups of orchids distributed across the tropical and subtropical regions of Asia (Gardiner *et al*., 2013). In Nepal alone, five distinct species of *Vanda* have been recorded (Pant *et al*., 2018). The name *Vanda* is derived from the Sanskrit term "Vandak," which refers to plants with an epiphytic or sometimes parasitic nature. Members of this genus exhibit heights ranging from 20 cm to 2 m and bear brightly colored, often fragrant, and long-lasting flowers. Floral characteristics particularly structure of the labellum vary widely among species, contributing to their horticultural appeal. Among them, *Vanda tessellata* is notable for its multiple blooming seasons and highly durable flowers, placing *Vanda* among the five most valuable orchids globally (Gardiner *et al*., 2013). Beyond its ornamental importance, *Vanda* holds significant medicinal and economic value. Its aesthetic appeal has made it a dominant genus in horticultural markets across the Americas and Europe, while its traditional medicinal uses date back to the Vedic period. In Ayurvedic medicine, *Vanda tessellata* is a key ingredient in formulations such as *Rasna Panchaka Quatha*, traditionally prescribed for treating rheumatic disorders and arthritis (Mukhtar & Kalsi, 2018). Modern pharmacological studies have validated several of its therapeutic potentials, revealing antioxidant, antimicrobial, and anti-inflammatory activities in various *Vanda* species including *V. tessellata*, *V. cristata*, *V. coerulea*, and *V. spathulata* (Khan *et al*., 2019; Maharjan *et al*., 2019).

In India, ethnomedicinal surveys have documented the use of *Vanda* species by indigenous communities. For instance, Panda (2014) reported that crude extracts of *V. tessellata* were traditionally employed to treat infections, with subsequent laboratory analyses confirming antibacterial activity against multiple pathogens. Similarly, Kalaiyarasan *et al*. (2012) demonstrated that ethanol and aqueous extracts of *V. tessellata* leaves contained bioactive compounds such as phenolics, coumarins, quinine, and carbohydrates. GC–MS profiling revealed the presence of several phytoconstituents, including alkaloids, terpenoids, and esters, which contribute to its antimicrobial and antioxidant activities. Plants are vital sources of

bioactive compounds that not only sustain human nutrition but also provide therapeutic benefits for various diseases (Babu Y. R. *et al*., 2023). Traditional medicine systems, integrating plant, animal, and mineral-based remedies, have played a crucial role in global healthcare for centuries (Sailaja C. S. *et al*., 2024). The biosynthesis of primary metabolites such as sugars, amino acids, and fatty acids forms the foundation of plant survival and secondary metabolite production (Ramalakshmana J. *et al*., 2025a, 2025b).

The present study focuses on the phytochemical characterization and antibacterial potential of *Vanda tessellata*. It aims to identify bioactive compounds through GC–MS analysis and evaluate the antimicrobial activity of its leaf extracts. The study bridges traditional medicinal knowledge with modern scientific validation to highlight the therapeutic significance of this species.

MATERIALS AND METHODS

The epiphytic Orchid *Vanda tessellata* was collected from the Paderu forest region in the Alluri Sitaramaraju district of the Eastern Ghats, Andhra Pradesh. The study area lies between 16°15′–19°12′N latitude and 80°50′–84°47′E longitude, with altitudes reaching up to 1615 meters. The region features diverse soil types such as loamy, lateritic, black, and alluvial soils, and experiences a subtropical climate with summer temperatures ranging from 28 to 46°C and winter temperatures from 13 to 27°C. Annual rainfall averages around 1300 mm during the southwest monsoon (July–September), with relative humidity levels between 70 to 88%. The collected specimens were identified based on morphological characteristics, compared with standard taxonomic descriptions.

Leaves of *Vanda tessellata* were shade-dried, coarsely powdered, and subjected to sequential solvent extraction using methanol, acetone, benzene, and aqueous in a soxhlet apparatus. The extracts were concentrated under reduced pressure at low temperature to preserve thermolabile compounds. The preliminary phytochemical screening followed standard procedures to detect bioactive constituents such as alkaloids, flavonoids, carbohydrates, proteins, tannins, phenolics, saponins, steroids, and glycosides. Tests such as Benedict's, Biuret, Mayer's, Salkowski's, Keller–Killiani, Shinoda, and foam tests were employed to confirm the presence of these compounds (Chandramouli, B. et al., 2024; Rao. P. J. *et al*., 2025).

For antimicrobial analysis, the methanol, acetone, benzene, and aqueous leaf extracts of *Vanda tessellata* were evaluated using the agar well diffusion method. Bacterial strains including *Bacillus subtilis*, *Streptococcus mutans*, *Staphylococcus aureus*, and *Pseudomonas fluorescens* were procured from MTCC, IMTECH Chandigarh. The bacterial inoculum was spread onto nutrient agar plates, and 5 mm wells were loaded with 20 µL of each extract at concentrations of 125, 250, and 500 µL. Streptomycin served as the positive control and DMSO as the negative control. After incubation at 37°C for 24 hours, zones of inhibition were measured to assess antibacterial activity (Ramalakshmana. J. *et al*., 2023; Rao. P. J. *et al*., 2025). All tests were performed in triplicate.

GC-MS analysis was performed on the methanol extract of *Vanda tessellata* leaves to identify the chemical constituents present. The analysis was conducted using a GC-MS system equipped with a capillary column and a mass selective detector. Helium was used as the carrier gas, and the sample was injected in split mode. The temperature program was optimized for the separation of compounds, and peaks were identified based on their retention times and mass spectral data by comparing with NIST library databases. The GC-MS profile revealed the presence of several bioactive compounds, including phenolics, alkaloids, terpenoids, and esters, many of which are known for antimicrobial and antioxidant activities.

RESULTS AND DISCUSSION

Preliminary Qualitative Phytochemical Studies

Methanol, acetone, benzene, and distilled water extracts obtained from the leaves of *V. tessellata*, underwent comprehensive preliminary phytochemical analysis. The preliminary phytochemical screening of *V. tessellata* leaf extracts screening is aimed at identifying the presence or absence of various phytochemical constituents in the plant extract. The current investigation unveiled a diverse array of compounds within the various extracts of *V. tessellata*, as demonstrated in (Table 1). Alkaloids and flavonoids were present in nearly all extracts, except for benzene. Carbohydrates are present in aqueous extract (++), absent in other solvents. This suggests that carbohydrates are more soluble in water. Fixed Oils and Fats are present in benzene extract (+), absent in other solvents. Alkaloids are present in methanol and acetone extracts (+), absent in aqueous and benzene extracts. Solvent choice influences the extraction of alkaloids. Phenols present in highest in methanol extract (++), present in aqueous and benzene extracts, absent in acetone. Quinones are absent in all extracts. Both methanol and acetone extracts displayed a broad spectrum of secondary

metabolites, including alkaloids, phenols, tannins, terpenoids, steroids, coumarins, and flavonoids. Conversely, the aqueous extract exhibited a moderate diversity of these secondary metabolites. In contrast, benzene extract displayed the lowest levels of secondary metabolites when compared to extracts obtained with other solvents. Furthermore, both methanol and aqueous extracts contained primary metabolites such as carbohydrates, proteins, and fatty acids. Notably, the methanol extract exhibited higher levels of steroids, phenols, coumarins, and alkaloids in comparison to the aqueous extract.

Table 1: Preliminary phytochemical screening of *V. tessellate.*

Phytochemical constituents	Solvents extracts			
	Methanol	Acetone	Aqueous	Benzene
Primary metabolites				
Carbohydrates	-	-	++	--
Proteins	+	-	++	+
Fixed oils and fats	-	-	-	+
Secondary metabolites				
Alkaloids	+	+	-	-
Quinones	-	-	-	-
Phenols	++	-	+	-
Tannins	+	++	++	+
Flavonoids	+	+	++	-
Terpenoids	+	++	++	-
Glycosides	++	++	-	-
Cardiac glycosides	++	++	-	-
Saponins	++	+	-	+
Steroids	+	++	+	+
Coumarins	+	++	++	-

- Absent + Presence ++ present in high amount

The phytochemical profile of *V. tessellata* varies depending on the solvent used for extraction. Aqueous and methanol extracts seem to be rich in various secondary metabolites, while acetone extract shows a presence of certain alkaloids and glycosides. These findings are crucial for further research, guiding the selection of appropriate solvents for targeted extraction of specific phytochemicals.

Terpenoids, flavonoids, phenols, alkaloids, tannins, phalbotanin, saponins, steroids, anthocyanins, anthraquinones, cardiac glycosides, coumarins, flavonones, and glycosides all have been reported from *V. tessellata* (Bhattacharjee *et al*., 2015; Islam *et al*., 2018; Rao P. J. et al., 2025). Screened leaf, root and stem of *V. tessellata* for the detection of primary

metabolite and reported the presence of carbohydrate, protein and lipid in leaf, carbohydrate, protein, lipid and amino acid in root and carbohydrate, protein, sucrose, starch and lipid in stem.

Antibacterial activity

In this investigation, four test bacteria, encompassing one gram-negative strain (*Pseudomonas florescence*) and three gram-positive strains *(Bacillus subtilis, Streptococcus mutans and Staphylococcus aureus*), were subjected to exposure to crude extracts of Methanol, Acetone, Aqueous, and Benzene at varying concentrations (10 mg, 5 mg, and 2.5 mg for each). The table 4.2.1 presents the Zone of Inhibition (ZOI) in millimeters for different extracts at various dosage levels against four bacterial strains (*P. fluorescens*, *Streptococcus mutans*, *Bacillus subtilis*, *Staphylococcus aureus*). Additionally, a positive control (+ Ve) is included for reference.

Methanol extract demonstrated significant antibacterial activity against *P. fluorescens* at all dosages, with the highest inhibition observed at 10mg (22.3 ± 1.2 mm). Moderate inhibition was observed against *Streptococcus mutans* at 10mg and 5mg dosages respective zone of inhibitions 16.3±0.57mm and 11±1mm, while no inhibition was recorded at 2.5mg. Effective inhibition against *Bacillus subtilis* was observed at 10mg and 2.5mg dosages 20.3±0.57mm and 12±1mm respectively. *Staphylococcus aureus* exhibited notable inhibition at 10 mg and 5mg dosages, zone of inhibitions respectively 18.3 ± 0.57 and 12.6 ± 0.57 respectively. Acetone extract displayed moderate antibacterial activity against *P. fluorescens* and *Bacillus subtilis* at all dosages. Inhibition against *Streptococcus mutans* was observed at 10mg and 5mg dosages, with a reduction at 2.5mg. Remarkable no inhibition was observed against *Staphylococcus aureus*, especially at 5mg and 2.5mg dosages.

Benzene extract exhibited significant inhibition against *P. fluorescens* (ZOI 12.3 ± 0.57 mm) and *Streptococcus mutans* (16.3± 0.57mm) at 10mg dosage. Effective inhibition against *S. aureus* was noted at 10mg and 5mg dosages (10.6± 0.57 and 7 ± 1 mm respectively). *Bacillus subtilis* was completely resistant to benzene extract. Aqueous extract displayed modest inhibition against *P. fluorescens* at 10mg dosage (ZOI is 11.3± 0.57). Limited antibacterial activity was observed against *Streptococcus mutans* (ZOI is 7.3± 0.57) at 10mg dosage. *S. aureus* and *B. subtilis* are completely resistant to aqueous extracts. The positive control streptomycin demonstrated consistent and robust inhibition across all bacterial strains, serving as a reference for the effectiveness of the experimental extracts.

The methanol extract appears promising, especially against *P. fluorescens* and *Staphylococcus aureus.* Acetone extract shows versatile antibacterial activity, particularly against *Staphylococcus aureus*. Optimization of dosage and formulation may enhance its efficacy and benzene extract exhibits significant inhibition against multiple bacterial strains, indicating its potential as a broad-spectrum antibacterial agent. On the other hand aqueous extract shows limited efficacy, suggesting that water may not be an ideal solvent for extracting antibacterial compounds from the studied source. It is noteworthy that the DMSO-containing well, serving as the negative control, exhibited no discernible bioactivity. Dosage-dependent effects are evident across extracts, emphasizing the need for careful dosage selection in future studies. Mechanistic studies, isolation of active compounds, and in vivo experiments are warranted to validate and translate the observed antibacterial activity into potential therapeutic applications.

Table 2: Zone of Inhibition of different extracts (Me, Ac, Aq, and Be).

Extract	*P. fluorescens*			*Streptococcus mutans*			*Bacillus subtilis*			*Staphylococcus aureus*		
	10mg	**5 mg**	**2.5mg**	**10mg**	**5 mg**	**2.5mg**	**10mg**	**5 mg**	**2.5mg**	**10mg**	**5 mg**	**2.5mg**
Me	22.3 ± 1.2	16.6± 0.75	-	16± 0.57	11±1	9.3± 0.57	20.3± 0.57	12±1	-	18.3± 0.57	12 ± 1	9.3± 0.57
Ac	15.2 ±1.3	9 ± 1.2	7±0.57	10.6± 0.57	10.3±0.57	6.3± 0.57	10± 1	9 ±1	14 ± 0.57	7.6 ± 0.57	-	-
Be	12.6± 0.57	10 ± 0.57	9±1	16.1± 0.57	14.3± 0.75	13.3± 0.57	-	-	-	10.6± 0.57	7 ± 1	-
Aq	11.3± 0.57	-	-	7.3± 0.57	6.6 ± 0.57	-	-	-	-	-	-	-
+ Ve	25 ± 1.23 mm			28 ± 0.95			25 ± 0.57			27.6 ± 1.52		

Data showing zone of inhibition in mm. Aq: aqueous extract, Me: methanol extract, Ac: acetone extract, Be: Benzene extract. Values represent mean ± standard deviations; "-" for no zone of inhibition. A zone of inhibition with a diameter of less than 6 mm was considered inactive.

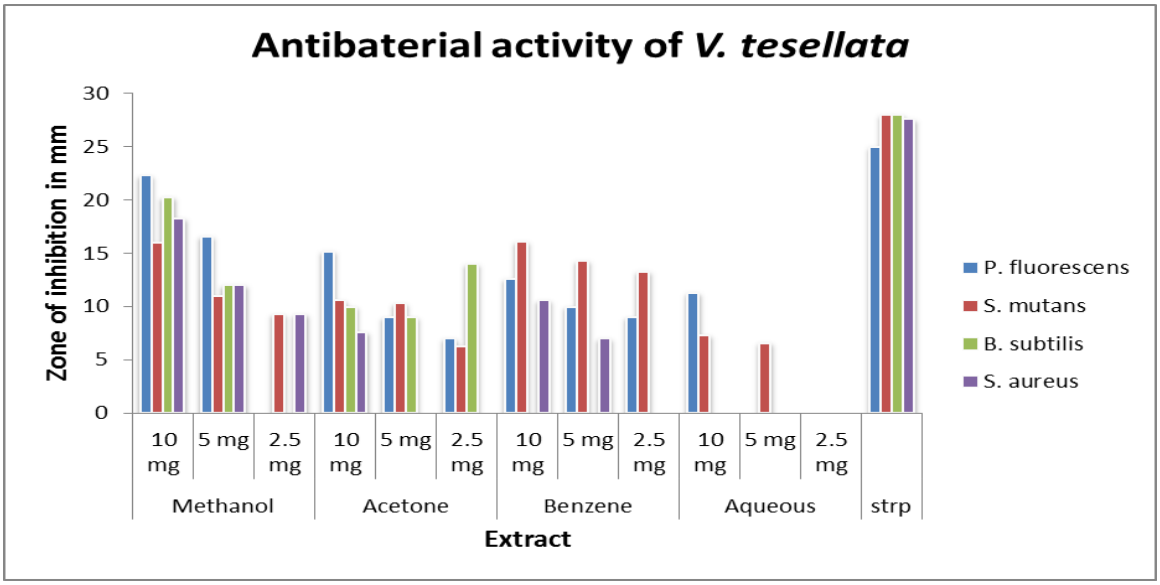

Fig 1: Antibacterial activity of *V. tesellata* (% of inhibition) with along with +'ve control.

Bacteriostatic efficacy of *V. tessellata* (NV01) against all the four bacterial strains as can be inferred from minimum inhibitory concentrations (MIC), was in accordance with the studies of earlier workers Bhattacharjee *et al*., 2015; Gupta and Ketwa, 2012). Better performance of V. tessellata (NV01) against the two gram-negative bacteria amply supports the earlier studies (Bhattacharjee *et al.*, 2015; Rao P. J. et al 2025). *Vanda* species are being examined for their antibacterial potential such as *V. spathulata*, *V. tessellata* (Ramana *et al*., 2020; against various bacterial strains and reported various degree of antibacterial activity.

GC-MS chemical profiling of *Vanda tesellata* leaf methanol extract

The experimental process described in this research resulted in the detection of several peaks in the Gas Chromatography-Mass Spectrometry (GC-MS) data obtained from the methanol extract of *V. tesellata*. These peaks indicated the presence of 11 different chemical compounds. Following this, all these compounds were meticulously recorded in (Table 3) which contains information such as percentages of peak area, molecular formulas, molecular weights, and their sequence based on retention times. Additionally, an extensive review of existing literature was conducted to investigate the biological activities associated with these compounds. The analysis unveiled that most of these compounds possess various pharmacological and therapeutic properties. Noteworthy is the wide array of bioactive effects exhibited by the compounds identified through GC-MS analysis, including anti-cancer, antimicrobial, anti-inflammatory, analgesic, antioxidant, and pain-relieving properties.

The compounds reported in the GCMS spectrum were analyzed, and their respective peak area percentages reveal the prominence of certain components. Notably, Glycerin with 22.96 % area percentage, n-hexadecanoic acid with 18.64% area percentage, and 1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β)-, with 17.42% area percentage and Isosorbide Dinitrate with 10.965% area percentage were the major compounds in this leaf methanol extract.n-Nonadecanol-1 contributing 9.39%, 13-Tetradecenal with 8.31%, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester with 5.62% and Linoelaidic acid shows the area percentage of 5.34% were also present in higher amounts. Octadecanoic acid and Decanoic acid, 2-methyl also played significant roles, with area percentages of 4.34% and 2.31%, respectively. Another noteworthy compound 2-Pentanol, 5-(2-propynyloxy)- (1.37%) is also reported.

Table 3: Compounds reported in methanol extract of *Vanda tesellata* leaf.

S. No	Compound name	Rt minute	Mol. weight g/mol	Mol. Formula	Area%
1.	Glycerin	3.329	92.09	$C_3H_8O_3$	22.96
2.	Iso sorbide Dinitrate	4.29	236.14	$C_6H_8N_2O_8$	10.965
3.	1,2,3,5-Cyclohexanetetrol, (1α,2β,3α,5β)-	17.113	148.157	$C_6H_{12}O$	17.42
4.	2-Pentanol, 5-(2-propynyloxy)-	22.625	142.2	$C_8H_{14}O_2$	1.37
5.	Decanoic acid, 2-methyl	24.822	228.37	$C_{14}H_{28}O_2$	2.31
6.	n-Hexadecanoic acid	25.762	256.42	$C_{16}H_{32}O_2$	18.64
7.	Linoelaidic acid	29.903	280.4	$C_{18}H_{32}O_2$	5.34
8.	13-Tetradecenal	30.038	210.36	$C_{14}H_{26}O$	8.31
9.	Octadecanoic acid	30.569	284.5	$C_{18}H_{36}O_2$	4.34
10.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	36.914	330.5	$C_{19}H_{38}O_4$	5.62
11.	n-Nonadecanol-1	39.838	284.5	$C_{19}H_{40}O$	9.39

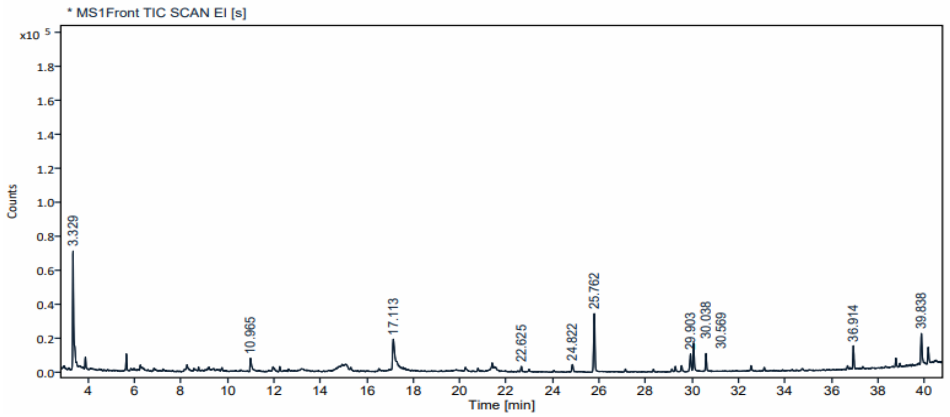

Fig 2: GC-MS chromatogram of Methanol extract of *Vanda tesellata* leaf.

Based on the existing literature, the potential bioactivities of the phytochemical compounds identified in the methanol extract of *Vanda tessellata* leaf were studied and discussed here. These compounds, as ascertained through GC-MS analysis, were categorized into various classes, including terpenes, fatty acids, flavones, phenols, steroids, and other substances. The methanol extract of *V. tessellata* leaf contains various phytochemical compounds with diverse bioactivities, including antioxidant, antimicrobial, vasodilatory, and anti-inflammatory properties. Further studies are warranted to explore the therapeutic potential of these compounds and their possible applications in medicine and healthcare. Glycerin, also known as glycerol, is a well-known compound with various applications in pharmaceuticals and cosmetics due to its moisturizing properties. It also has wound-healing effects and is used in the treatment of skin disorders like dermatitis. Additionally, it has been reported to possess anti-inflammatory and antimicrobial properties, which could contribute to its therapeutic benefits (Rawlings & Lombard, 2012).

n-Hexadecanoic acid, or palmitic acid, is a saturated fatty acid commonly found in plants and animals. It has been reported to possess anti-inflammatory properties and is involved in various biological processes. Nonetheless, consuming too much palmitic acid has been associated with negative health outcomes like insulin resistance and cardiovascular diseases (Eckel *et al*., 2014).

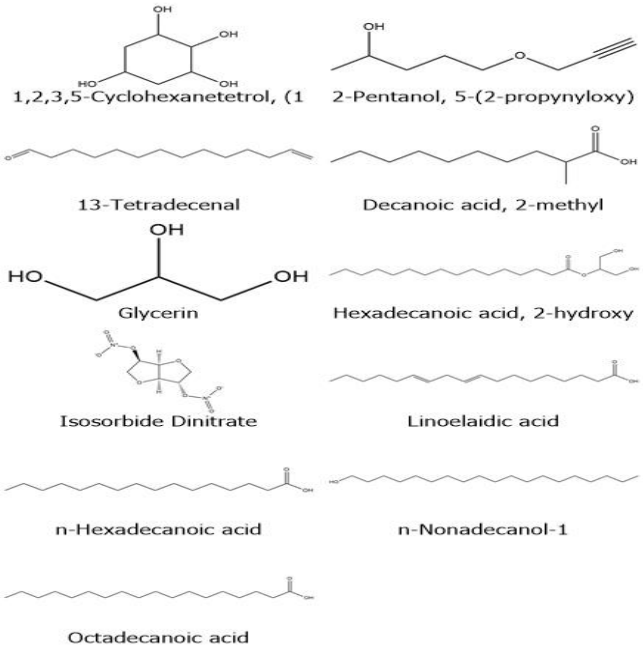

Fig 3: Chemical structures of the compounds identified from *V. tessellate* leaf methanol extract.

Further study by Promila *et al.* (2023), the leaf methanolic extracts of *Vanda cristata* revealed 54 different phytochemicals. In addition to these previous studies, current investigation reported 11 compounds from *V. tesellata* methanolic leaf extract.

CONCLUSION

The present investigation highlights *Vanda tessellata* as a valuable reservoir of bioactive compounds with significant antibacterial potential. Phytochemical screening confirmed the presence of multiple secondary metabolites, with methanol and aqueous extracts demonstrating a higher diversity of constituents. Methanol extract, in particular, showed notable antibacterial efficacy, especially against *Pseudomonas fluorescens* and *Staphylococcus aureus*, indicating its potential as a source of broad-spectrum antibacterial agents. GC-MS profiling identified eleven phytoconstituents, several of which possess documented pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, and therapeutic effects. The results underscore the importance of *V. tessellata* in ethnomedicinal contexts and its potential for pharmaceutical exploitation. Further research should focus on isolation, structural characterization, and in vivo evaluation of the bioactive compounds to develop novel plant-based therapeutics.

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