International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 11, Issue 5, pp: 50-65, 2026 (Special Issue)
https://doi.org/10.55126/ijzab.2026.v11.i05.sp006 **http://www.ijzab.com**

Research Article

PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY, TLC AND LC-MS/MS CHARACTERIZATION OF *OCIMUM GRATISSIMUM* L.

[1]Naguvanahalli Somashekar Bhavana, [2]Ballapatna Raju Swathi, [3]Sharayu shyam Deshmukh, [4]Raju Umrao Gadpayle, [5]Virendra Sangode

[1]Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore- 570006, Karnataka, India
[2] Department of Botany (PG), Teresian College, Mysore- 570011, Karnataka, India
Department of Botany science college Nagpur Maharashtra.
[4]Department of Microbiology, Yashwantrao Chavan College Lakhandur Maharashtra
[5]Department of Zoology, MB Patel College of Arts Commerce and Science Sadak Arjuni District Gondia

Article History: Received 19th July 2026; Accepted 13th September 2026; Published 31st October 2026

ABSTRACT

Ocimum gratissimum L. (Lamiaceae) is an important medicinal plant widely recognized for its diverse therapeutic properties and rich phytochemical composition. The present study aimed to evaluate the phytochemical constituents, total phenolic content, antioxidant activity, and chemical profile of *O. gratissimum* leaf extracts. Preliminary phytochemical screening of the aqueous and methanolic extracts revealed the presence of tannins, alkaloids, saponins, flavonoids, anthraquinones, steroids, and cardiac glycosides. With an IC_{50} value of 1.940 µg/mL in the DPPH radical scavenging assay, the methanolic leaf extract showed significant antioxidant activity and the highest total phenolic content (336.00 mg GAE/g dry extract). Alkaloids, flavonoids and steroids were validated by thin-layer chromatography (TLC) and 39 structurally varied bioactive metabolites, including alkaloids, flavonoids, phenolics, quinones, glycosides, and antimicrobial chemicals, were identified by LC–MS/MS profiling. On the whole, the results show that *O. gratissimum*, is a rich source of bioactive phytochemicals with strong antioxidant activity, supporting its traditional medicinal use and emphasizing its potential for pharmaceutical and nutraceutical explorations and applications.

Keywords: *Ocimum gratissimum,* Lamiaceae, Phytochemical screening, Antioxidant activity, TLC.

INTRODUCTION

Since ancient times, plants have been vital to human existence. They supply nutrients and natural medications whose therapeutic qualities are utilized to prevent and treat a wide range of illnesses. Simple herbal formulations were used in the early days of medical plant use, and information about the therapeutic qualities and application techniques of certain plants for treating a range of illnesses was passed down orally from generation to generation. This ancient wisdom has been methodically documented and conserved over time (Krstin *et al*., 2026). Over 7,000 species in 236 genera make up the Lamiaceae (mint family), which is distinguished by fragrant plants with square stems, opposite leaves, and bilabiate flowers. Species including *Ocimum*, *Mentha* and *Salvia* are widely spread in a variety of environments and are prized for their culinary, medicinal, and economic significance. The Lamiaceae family is a valuable resource for pharmaceutical and natural product research because to its abundance of bioactive secondary metabolites, such as alkaloids, terpenoids, and polyphenols, which have antioxidant, antibacterial, anti-inflammatory, antiviral and anticancer properties (Katyal *et al*., 2026). *Ocimum gratissimum* L., also known as clove basil or African basil, is a significant fragrant and medicinal plant in the Lamiaceae family that is highly prized for its pharmacological, ethnomedical and nutritional benefits. Its leaves are used as a culinary spice in many Asian and African countries, and it has historically been used to cure respiratory conditions, diabetes, diarrhoea, malaria,

*Corresponding Author: Naguvanahalli Somashekar Bhavana, Assistant Professor, Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore- 570006, Karnataka, India. Email: bhavanans.nagu@gmail.com.

inflammation, and microbiological infections. *O. gratissimum* is also a promising natural resource for pharmaceutical development and functional food applications because it is rich in essential oils, especially eugenol and thymol. The therapeutic potential of medicinal plants is greatly enhanced by phytochemicals, which are naturally occurring bioactive secondary metabolites. Numerous biological actions, including antioxidant, antibacterial, anti-inflammatory, anticancer, and antidiabetic properties, are displayed by major classes, such as polyphenols, flavonoids, alkaloids, terpenoids, tannins, and essential oils where they scavenge free radicals, modulate oxidative stress, and control important cellular signalling pathways (Maphetu *et al*., 2026). Because of their potent antioxidant qualities, phenolic compounds are among the most significant secondary metabolites found in plants. The Folin-Ciocalteu assay is frequently used to measure Total Phenolic Content (TPC), which gives an estimate of the phenolic components in plant extracts and is a crucial predictor of their potential for bioactivity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which measures these compounds capacity to scavenge free radicals by donating hydrogen atoms or electrons, is commonly used to assess their antioxidant activity. To evaluate the antioxidant activity and medicinal benefit of medicinal plants, TPC and DPPH assays are frequently used (Chedea and Pop, 2019). Thin-layer chromatography (TLC) is a crucial tool for phytochemical fingerprinting and quality control since it is a quick, easy, and affordable method that is frequently used for the initial separation, identification, and qualitative screening of phytochemicals in medicinal plants. On the other hand, liquid chromatography–mass spectrometry (LC–MS) provides great sensitivity, selectivity and accuracy for the identification and characterization of complex metabolites, allowing for thorough metabolite profiling and making it easier to find bioactive substances. For phytochemical analysis, authenticity and natural product research, the complementary use of TLC and LC-MS offers a reliable method (Siddiquee *et al*., 2023; Queiroz *et al*., 2024). Although *O. gratissimum* has been extensively recognized for its medicinal value, comprehensive studies integrating phytochemical profiling with antioxidant evaluation remain limited. Therefore, the present study was undertaken to investigate the phytochemical composition of *O. gratissimum* using TLC and LC-MS analyses to evaluate its total phenolic content and antioxidant potential through TPC and DPPH assays. The findings of this study provide scientific evidence supporting the therapeutic and pharmaceutical potential of this medicinal plant.

MATERIALS AND METHODS

Collection of plant samples

Fresh and healthy plants of *O. gratissimum* L. (Lamiaceae) were collected from the Thithimathi Forest Region of Kodagu, Karnataka, India during April 2026 (Figure 1). Thithimathi is located approximately 78 km from Madikeri, 191 km from Mangalore, and 214 km from Bangalore. The collected plant samples were placed in sterile zip-lock polythene bags and transported to the laboratory for further processing. The plant was identified using standard taxonomic keys and regional floras, including Flora of Presidency of Madras (Gamble, 1967) and Flora of Karnataka (Saldhana, 1996). The dried leaves were finely powered and 30g of resulting material was extracted with 100 mL of methanol on a rotary shaker and concentrated under reduced pressure using rotary evaporator, lyophilized and stored until further use. To compare the phytochemical profile with that of the methanolic extract, an aqueous extract was prepared from same powdered leaf material following similar procedure.

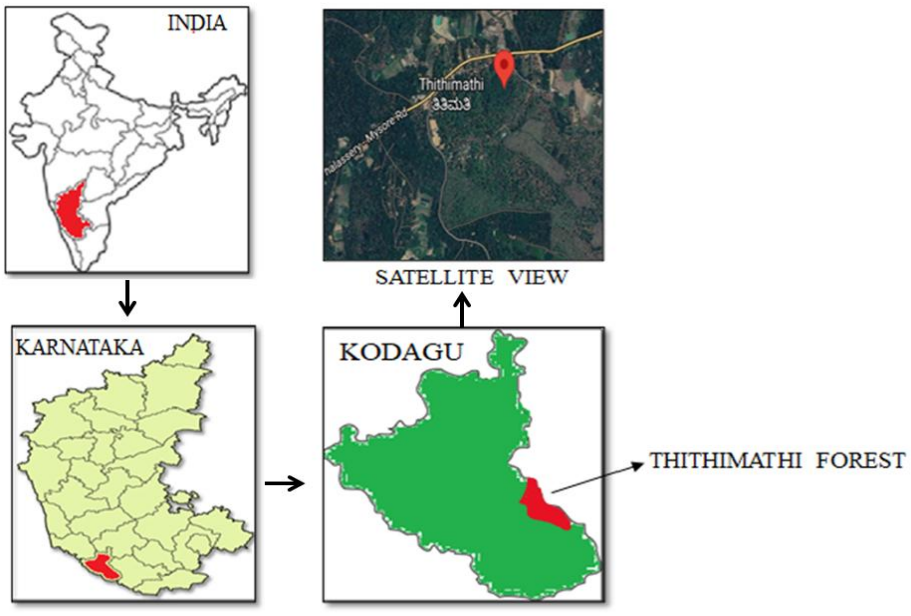


Figure 1. Map showing the location of plant sample collection.

Phytochemical screening

The aqueous and methanolic extracts were subjected to preliminary qualitative phytochemical screening for the detection of major phytochemical constituents following the standard methods of Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Determination of Total Phenolic Content

The Total Phenolic Content (TPC) of the plant extract was determined using Folin-Ciocalteu method described by Volluri *et al*., 2011, with gallic acid used as the reference standard. Briefly, the methanolic and aqueous extracts were dissolved in their respective solvents to obtain a stock concentration of 1 mg/mL, from which working solutions were prepared. An aliquot of each sample was mixed with 0.5 mL of Folin-Ciocalteu reagent followed by 1.0 mL of 20% Sodium carbonate (Na_2CO_3) solution. The reaction mixture was incubated in the dark at room temperature for 45 min and the absorbance was measured at 765 nm using a UV-Visible spectrophotometer (FM-UVS-A200). The total phenolic content was calculated from gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry extract.

DPPH Radical Scavenging Assay

The antioxidant activity of plant extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following the method of Pannangpetch *et al*. (2007). A 0.004% of DPPH solution was prepared in methanol. Different concentrations of the extracts of (20-100 µg/mL) and ascorbic acid (5-25 µg/mL) as the positive control were mixed with 2.0 mL DPPH solution and incubated in the dark room at room temperature for 20 min and absorbance was recorded at 517 nm using UV-Visible spectrophotometer (FM-UVS-A200). The percentage of DPPH decoloization was determined and the antioxidant activity was expressed as the IC_{50} value.

Thin-Layer Chromatography (TLC)

Thin-Layer Chromatography (TLC) was performed to characterize the major phytochemical constituents present in the methanolic leaf extract of *O. gratissimum*. The extract (15 mg mL^{-1} in methanol) was applied to silica gel 60 F_{254} aluminium-backed TLC plates (Merck, Germany) and developed using optimized solvent systems. Chloroform: methanol was employed for the separation of alkaloids and steroids, whereas chloroform:methanol:water was used for flavonoids. The developed plates were air-dried and visualized using iodine vapour (alkaloids and steroids) or a spray reagent containing cupric sulphate, sodium citrate and sodium bicarbonate (Flavonoids). The separated compounds were characterized by their retention factor (Rf) values.

Characterization of Phytochemical Constituents by LC-MS/MS

The phytochemical profile of the methanolic leaf extract of *O. gratissimum* L. was characterized using AB Sciex 3200 QTRAP LC–MS/MS system coupled with a PerkinElmer FX-15 HPLC (Advance Chemistry Solutions). The extract (0.1 mg mL^{-1} in methanol) was filtered through a 0.22-µm membrane filter, and 10 10 µL was injected onto an Accucore C18 column (50 × 4.6 mm, 2.6 µm; Thermo Scientific). Chromatographic separation was achieved using 0.1% formic acid in water (Mobile phase A) and acetonitrile (mobile phase B). Mass spectrometric detection was performed in positive electrospray ionization (ESI^+) mod with a capillary voltage of 2.5 kV, cone voltage of 40 V, collision energy of 30 V, source temperature of 150°C, and desolvation temperature of 450°C. Nitrogen was used as the desolvation and cone gas, while argon served as the collision gas. Phytochemical constituents were tentatively identified by comparing the m/z values and fragmentation patterns with entries in the Spectral Database for Organic Compounds (SDBS).

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the aqueous and methanolic extracts of the root, stem and leaves of *O. gratissimum* L. revealed the presence of diverse secondary metabolites which varied among different plant parts (Table 1 & Figure 2). The leaf extracts exhibited the highest diversity of phytochemicals irrespective of the extraction solvent. The aqueous extracts contained tannins in all evaluated plant parts, whereas alkaloids, saponins, flavonoids, steroids and cardiac glycosides were predominantly detected in the leaf extract. Stem extracts showed the presence of tannins, saponins, steroids and cardiac glycosides, where anthraquinines, terpenoids, phlobatannins and reducing sugar were absent in all aqueous extract. The methanolic extracts showed a comparatively distinct phytochemical profile. Tannins were detected only in the leaf extract, while alkaloids were present in the root, stem and leaf extracts. Saponins, flavonoids, were detected exclusively in methanolic leaf extract. Steroids and cardiac glycosides were present in both stem and leaf extracts. Reducing sugars were absent in all methanolic extracts. The total phenolic content of the aqueous and methanolic extracts of leaf, stem and root of *O. gratissimum* L. was determined using the Folin-Ciocalteu method. In all plant parts, the TPC increased progressively with increasing extract concentration (20-100 µg/mL). Methanolic extracts consistently exhibited higher phenolic content than the corresponding aqueous extracts. Among the plant parts, leaf methanolic extract recorded highest TPC, increasing from 42.25 to 336.00 mg GAE/g dry extract whereas the aqueous leaf extract ranged from 13.50 to 262.25 mg GAE/g dry extract (Figure 3a). The stem extract also showed a concentration-dependent increase in TPC, with methanolic extracts ranging from 14.75 to 262.25 mg GAE/g dry extract and aqueous extracts from 1.00 to 201.00 mg GAE/g dry extract (Figure 3b). Similarly, the root extracts exhibited lower phenolic content than the leaf and stem extracts, with methanolic extracts ranging from 8.50 to 164.75 mg GAE/g dry extract and aqueous extracts from 6.00 to 146.00 mg GAE/g dry extract (Figure 3c). Overall, the phenolic content followed

the order leaf>stem>root, while the extraction efficiency of solvents followed the order methanol>aqueous, signifying that the methanol was more effective in extracting phenolic contituents from *O. gratissimum* L. The highest TPC was observed in the methanolic leaf extract (336.00 mg GAE/g dry extract) at an extract concentration of 100 µg/mL.

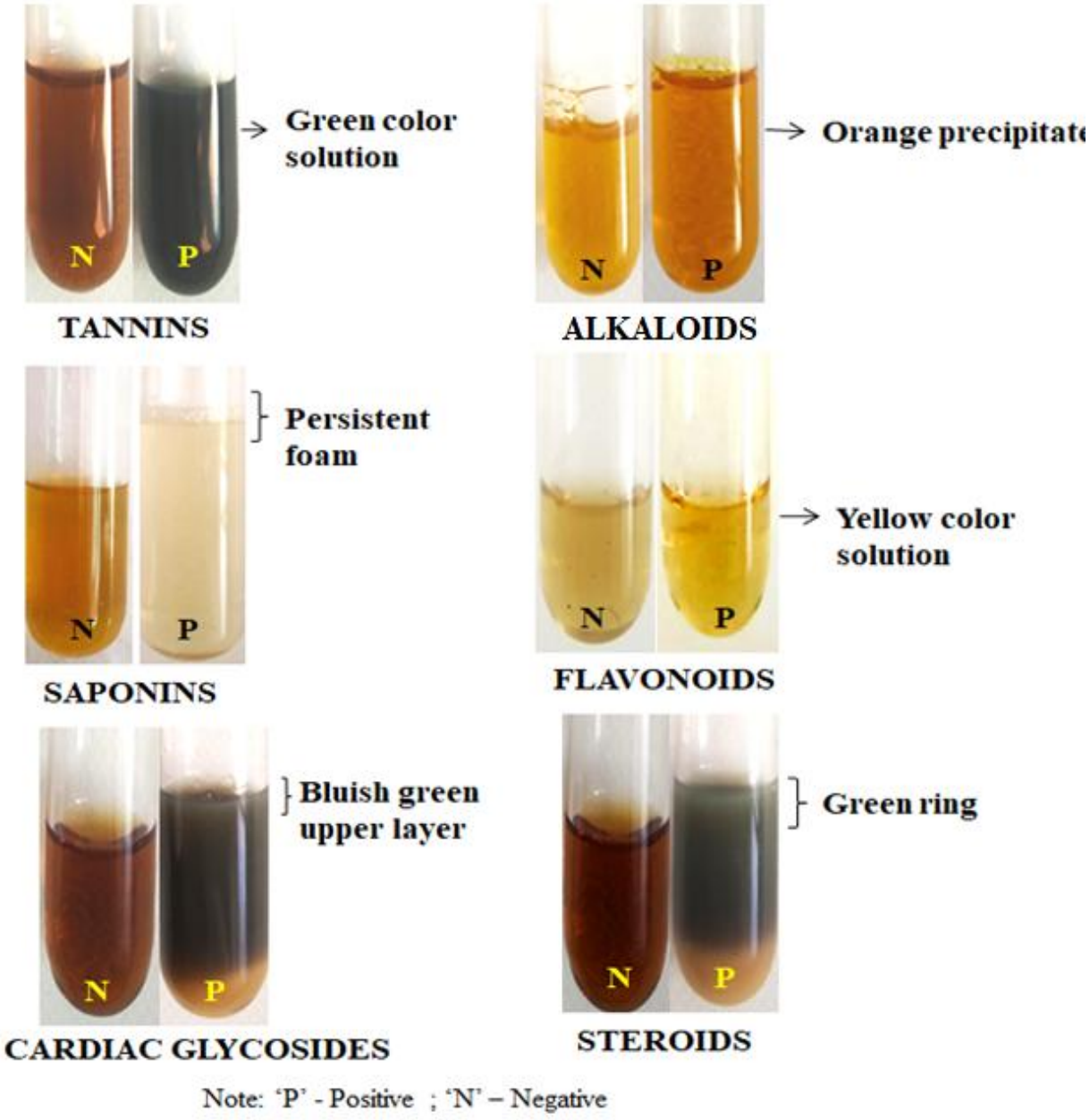


Figure 2. Qualitative detection of phytochemicals.

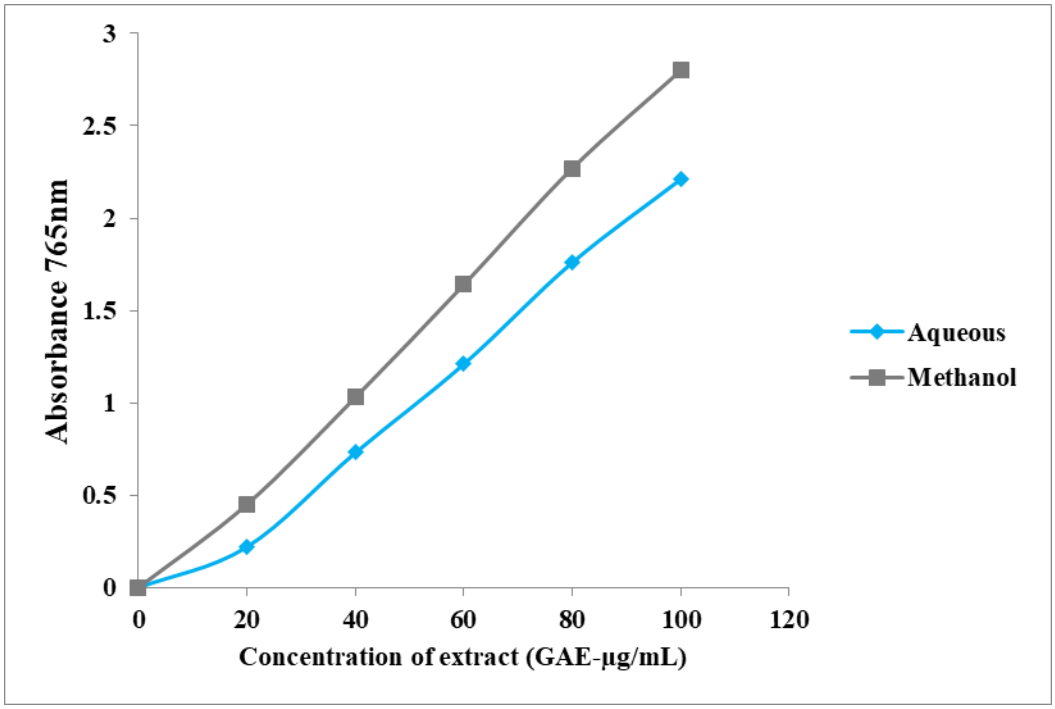


Figure 3a. Total phenolic content of leaf aqueous and methanolic extracts.

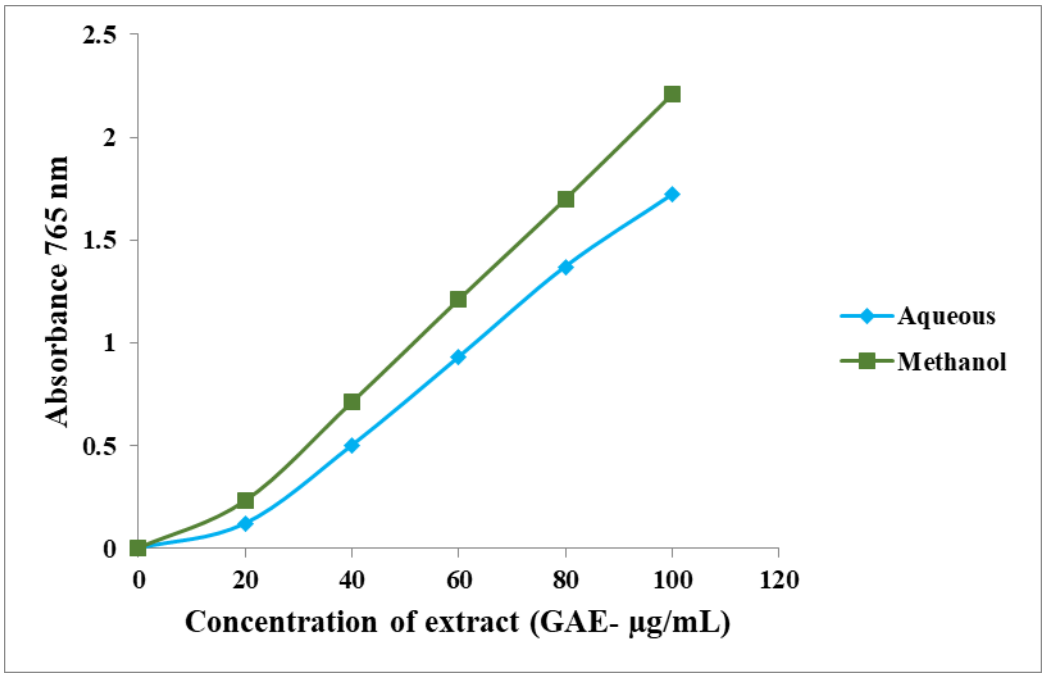


Figure 3b. Total phenolic content of stem aqueous and methanolic extracts.

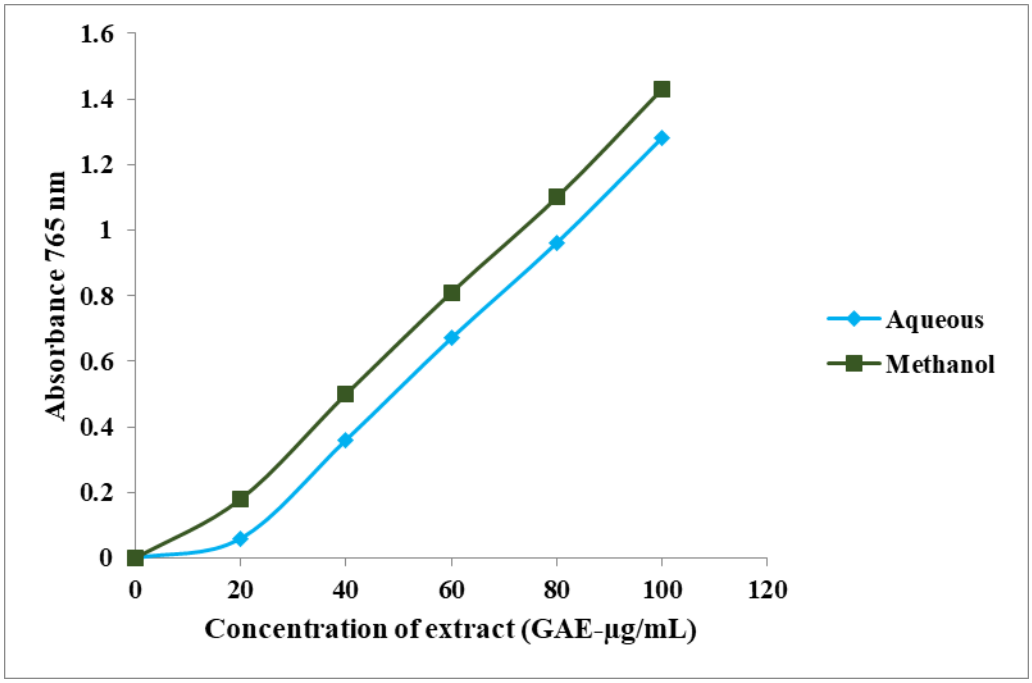


Figure 3c. Total phenolic content of root aqueous and methanolic extracts.

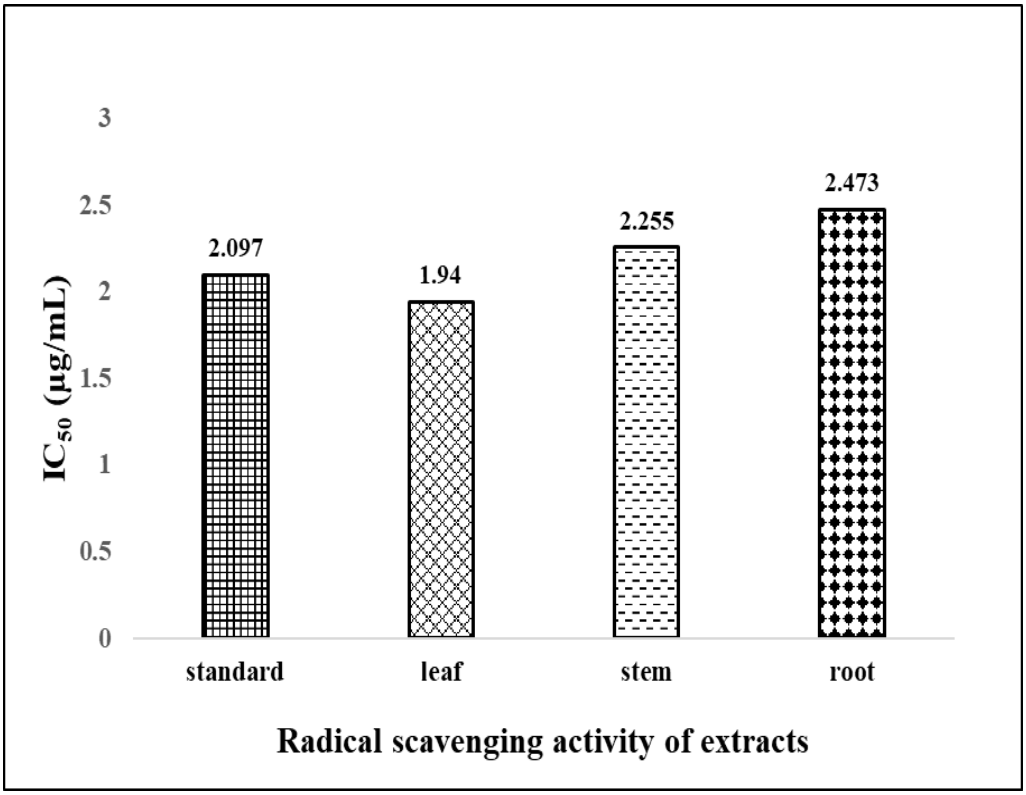


Figure 4. DPPH radical scavenging activity (IC50) of methanolic extracts of *O. gratissimum* L. leaf, stem and root compared with the standard (Ascorbic acid).

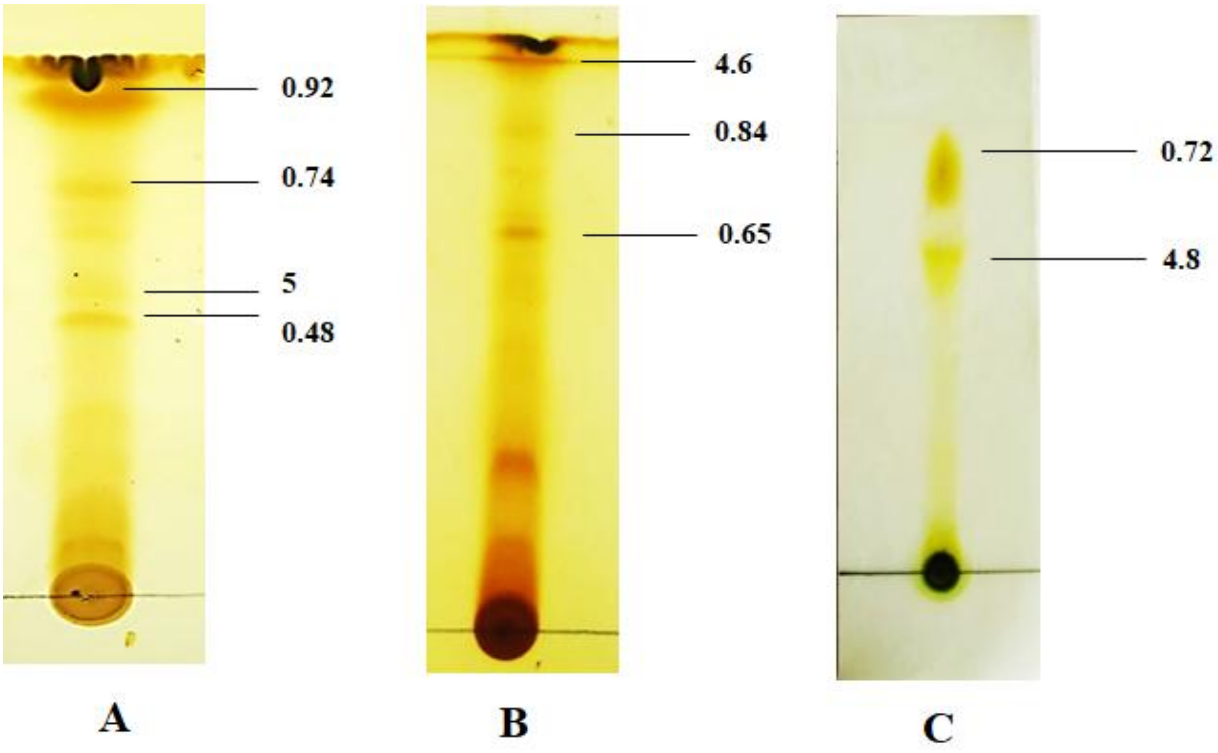


Figure 5. Thin-layer chromatographic (TLC) profiles of the methanolic leaf extract of *o. gratissimum* showing the separation of A. Alkaloids; B. Flavonoids and C. Steroids**.**

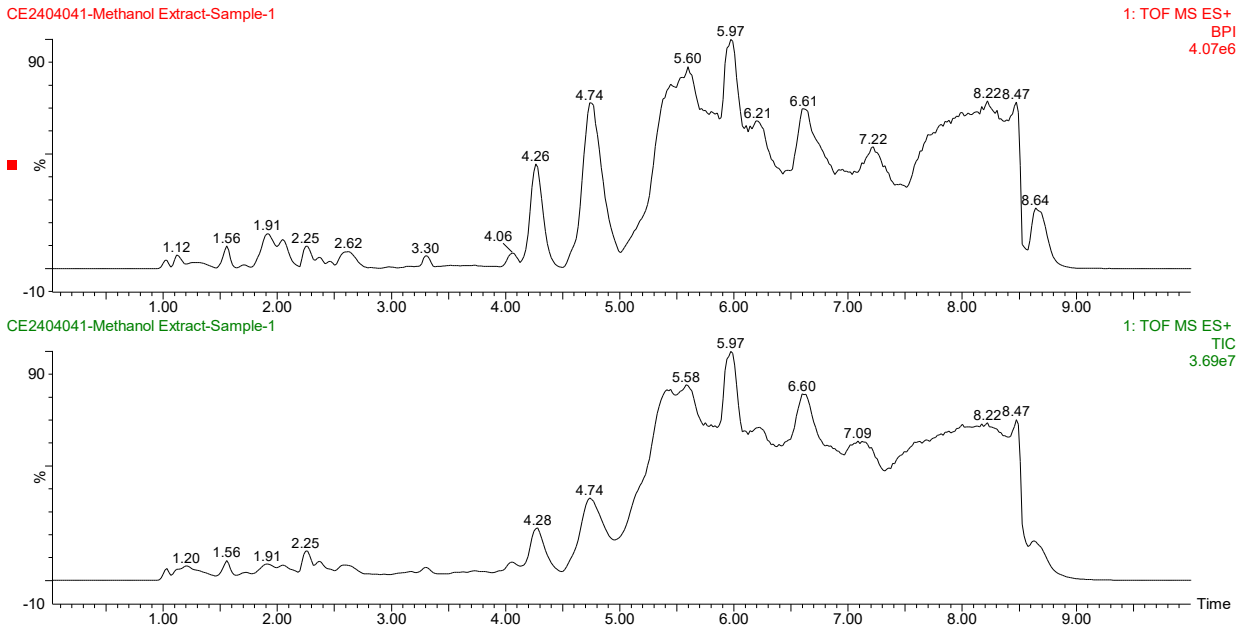


Figure 6A. Total ion chromatogram (TIC) of the methanolic leaf extract of *O. gratissimum* acquired in positive ionization mode (ESI+).

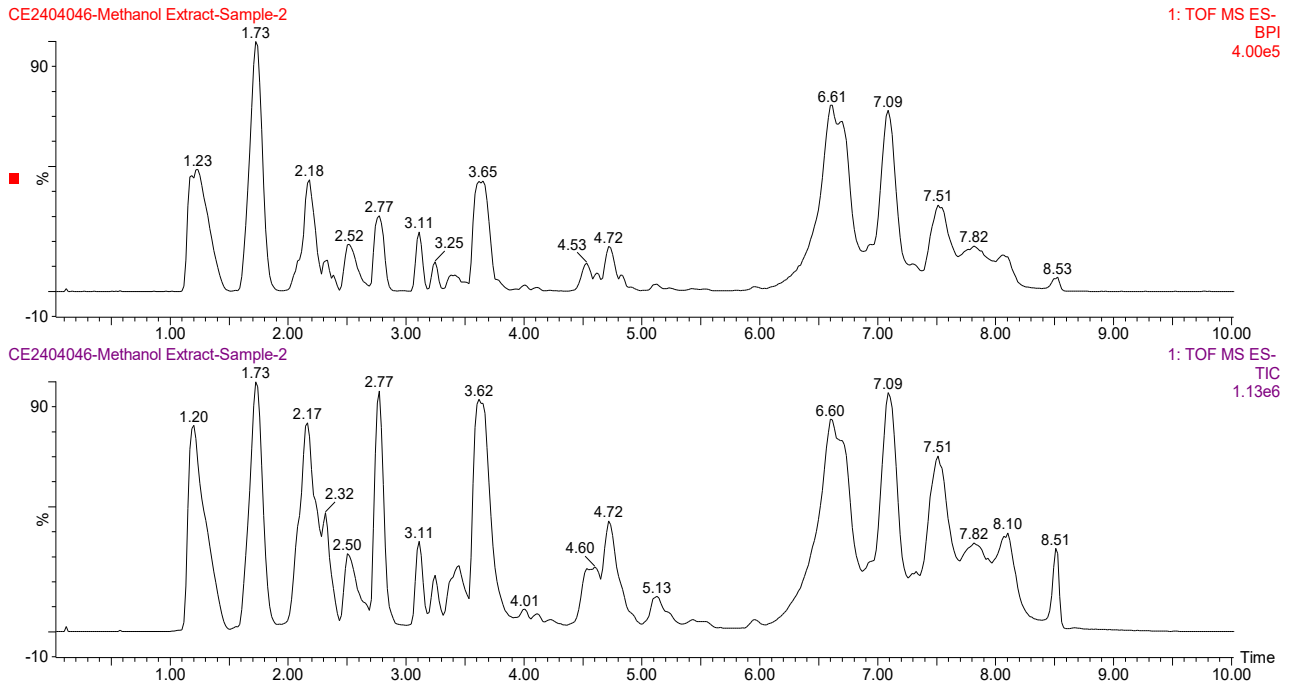


Figure 6B. Total ion chromatogram (TIC) of the methanolic leaf extract of *O. gratissimum* L. acquired in negative ionization mode (ESI-).

Table 1. Preliminary qualitative phytochemical screening of aqueous and methanolic extracts of *O. gratissimum* L. plant parts

Phytochemical tests	Aqueous extract			Methanol extract		
	Root	Stem	Leaf	Root	Stem	Leaf
Tannins	+	+	+	-	-	+
Alkaloids	-	-	+	+	+	+
Saponins	-	+	+	-	-	+
Flavonoids	-	-	+	-	-	+
Anthraquinones	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-
Steroids	-	+	+	-	+	+
Phlobatannins	-	-	-	-	-	-
Cardiac glycosides	-	+	+	-	+	+
Reducing sugars	-	-	-	-	-	-

The antioxidant activity of the methanolic extracts of *O. gratissimum* L. leaf, stem and root was evaluated using DPPH free radical scavenging assay. All methanolic extracts exhibited considerable DPPH radical scavenging activity. The percentage radical scavenging activity ranged from 87.23% to 88.05%, while the IC_{50} values ranged from 1.940 to 2.473 µg/mL. The leaf extract exhibited the strongest antioxidant activity with the lowest IC_{50} value of 1.940 µg/mL, followed by the stem extract 2.254 µg/mL and the root extract 2.473 µg/mL. The stem extract showed the highest percentage inhibition (88.05%), followed by the root (87.70%) and leaf (87.23%) (Figure 4). Thin-layer chromatography (TLC) was performed to characterize the major phytochemical constituents present in the methanolic leaf extract of *O. gratissimum*. The chromatographic profiles visualized using appropriate spraying reagents, confirmed the presence of alkaloids, steroids and flavonoids as indicated by their characteristic color, reactions and retention factor (Rf) values. The alkaloid fraction exhibited two distinct bands with Rf values of 0.92 (dark brown) and 0.74 (light brown), confirming the presence of alkaloid compounds. The steroid fraction produced two distinct bands with Rf values of 0.84 (light green) and 0.65 (brown), indicating the occurrence of

steroidal constituents. The flavonoid fraction exhibited a single yellow band with an Rf value of 0.72, confirming the presence of flavonoids in the methanolic leaf extract. The TLC chromatographic profiles of the methanolic leaf extract are presented in Figure 5. LC-MS/MS analysis performed in positive electrospray ionization mode (ESI+) (Figure 6A) resulted in the identification of 17 bioactive metabolites belonging to diverse chemical classes including alkaloids, flavonoids, steroids, phenolics, quinones, glycosides, nucleoside-peptide antibiotics, thiopeptide antibiotics, anthraquinone derivatives and diterpene quinones (Table 2A). The detected metabolites exhibited a wide range of molecular masses (m/z 146.13–1341.47) and retention times ranging from 1.01 to 8.47 min, indicating considerable chemical diversity within the extract. Alkaloids constituted one of the predominant metabolite groups, represented by 4-hydroxyquinazoline (4-quinazolinol) (RT 1.18 min; *m/z* 146.13), Rhopaladin C (RT 2.31 min; *m/z* 433.14), and Arisostatin A (RT 2.63 min; *m/z* 1341.47), all exhibiting 100% relative abundance. Among these, Aristostatin A was the highest molecular weight compound identified. Flavonoids were another major class, comprising Genistein 7-O-glucoside-6"-malonate, Cyanidin 3-(6"-dioxalylglucoside) and Malvidin 3-O-(6-O-(4-O-malonyl-α-rhamnopyranosyl)-β-glucopyranoside)-5-O-β-glucopyranoside, each detected with 100% relative abundance, suggesting their abundance in the extract.

The analysis also revealed the presence of several phenolic and quinonoid compounds. Hydroquinone, identified at RT 1.01 min (*m/z* 110.05), was the earliest eluting metabolite, whereas Obtusaquinone showed a comparatively lower relative abundance (44%), making it the last abundant compound detected among the identified metabolites. Other qionone derivatives, including 2-methoxynaphthoquinone, Neobulgarone E, Pseudohypericin, and Przewaquinone A, were also identified, with Przewaquinone A exhibiting a relative abundance of 73%. In addition to secondary metabolites, several bioactive antimicrobial compounds were identified, including the steroid Spiroxin, the glycoside Ranunculin, the nucleoside-peptide antibiotic Nikkomycin C, and the thiopeptide antibiotics Thioxamycin and Aureothricin, all of which displayed 100% relative abundance. These compounds have previously been reported to possess antibacterial, antifungal, cytotoxic and enzyme inhibitory activities. Overall, the LC-MS/MS profile demonstrated that the extract is enriched with structurally diverse metabolites possessing documented antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, antitumor, antiproliferative, kinase inhibitory, DNA-cleaving, apoptosis-inducing and chitin synthase inhibitory activities.

LC-MS/MS analysis performed in negative electrospray ionization mode (ESI-) (Figure 6B) enabled the identification of 22 bioactive metabolites distributed across nine major chemical classes, including alkaloids, flavonoids, organic acids, quinone antibiotics, nucleoside analogues, cyanogenic glycosides, azaspiro compounds, β-lactam antibiotics and aminoglycoside antibiotics (Table 2A). The identified metabolites exhibited retention times ranging from 0.118 to 8.71 min with precursor ions (*m/z*) varying from 169.03 to 1113.57, demonstrating the broad metabolite coverage achieved under negative ionization. Flavonoids represented the most abundant class, accounting for seven identified metabolites, namely Peonidin 3-rhamnoside**,** Quercetin 3-(3"-acetyl-α-L-arabinofuranoside)**,** Rhusflavone**,** [5′,5′]-Bisdihydroquercetin**,** Delphinidin 3-(2"-galloylgalactoside)**,** Catechin-(4α→8)-pelargonidin 3-O-β-glucopyranoside, and Gentiodelphin**,** all detected with 100% relative abundance. Among these Gentiodelphin possessed the highest molecular mass (*m/z* 1113.57), whereas [5′,5′]-Bisdihydroquercetin exhibited the shortest retention time (0.118 min). Alkaloids constituted another prominent metabolite group and included Norepinephrine**,** Carbazomycin F**,** Boldine**,** and Benzo[g]isoquinoline-5,10-dione, with all compounds showing 100% relative abundance except Benzo[g]isoquinoline-5,10-dione**,** which exhibited 84% relative abundance**.** Several compounds belonging to antimicrobial and pharmacologically important metabolite classes were also detected. These included the quinone antibiotic Epoxyquinomycin C**,** nucleoside antibiotics Streptozocin and Psicofuranine, the nucleoside analogue Sangivamycin B**,** the β-lactam antibiotics Valclavam and Piperacillin, and the aminoglycoside antibiotics 5-Deoxy-5-halo-gentamicin C1a and Pamamycin, all identified with 100% relative abundance**.** In addition, the cyanogenic glycoside Dhurrin, the azaspiro compound Pseurotin A**,** and the organic acid Calvatic acid were also detected.

The metabolites identified in the negative ion mode have previously been associated with diverse biological properties, including antioxidant, anti-inflammatory, antimicrobial, antifungal, antiviral, antitumor, anticancer, neuroprotective, immunomodulatory, hepatoprotective, cardioprotective, insecticidal, bactericidal and antipseudomonal activities (Table 2B). The preliminary phytochemical screening showed that *O. gratissimum* L. exhibited diverse secondary metabolites, with variation among plant parts and extraction solvents. The leaves exhibited the greatest phytochemical diversity in both aqueous and methanolic extracts, indicating them as the principal site for the accumulation of bioactive constituents.

This observation is consistent with the physiological role of leaves as the primary organs of photosynthesis and secondary metabolite biosynthesis, where phenolic compounds, flavonoids, alkaloids and glycosides are synthesized to protect the plant against oxidative stress, herbivory, microbial infection. The present findings are in agreement with the recent comprehensive review by Maphetu *et al*. (2026), which identified alkaloids, polyphenols, flavonoids, tannins and steroids as major phytochemical constituents of *O. gratissimum*. In the present study, reducing sugars were not detected in any of the methanolic extracts which is consistent with the recent

study of Olorunfemi *et al*. (2025), who also reported the absence of reducing sugars in the methanolic extract of same plant species suggesting that these metabolites may occur at concentrations below the detection limit of qualitative phytochemical assays or vary according to extraction conditions and plant material.

Table 2A. Bioactive compounds identified from the positive ion mode (ESI+) LC-MS/MS chromatogram of the methanolic leaf extract of *O. gratissimum* L.

Retention time	m/z	Bioactive compounds	Chemical class	Relative abundance	Molecular formula	Biological activity	References
1.18	146.13	4-Hydroxyquinazoline (4-quinazolinol)	Alkaloids	100%	$C_8H_6N_2O$	Anticancer, antibacterial, antifungal, anti-inflammatory, antiviral,	Tokalı, 2025; Kalakwade *et al*., 2026
2.31	433.14	Rhopaladin C	Alkaloids	100%	$BrC_{21}H_{13}N_4O_2$	Antibacterial, kinase inhibitory, anticancer.	Dvorak and Tepe, 2024
2.63	1341.47	Arisostatin A	Alkaloids	100%	$C_{69}H_{100}N_2O_{24}$	Antitumor, antibacterial and apoptosis	Furumai *et al*., 2000
4.26	518.35	Genistein 7-O-glucoside-6"-malonate	Flavonoids	100%	$C_{24}H_{22}O_{13}$	Antioxidant, anticancer, anti-inflammatory,	Shete *et al*., 2024; Gosamy *et al*., 2025
6.83	593.30	Cyanidin 3-(6"-dioxalylglucoside)	Flavonoids	100%	$C_{25}H_{21}O_{17}$	Antioxidant, anti-inflammatory, anticancer,	Kowalczyk *et al*., 2024; Bharathy *et al*., 2025
7.866	887.60	Malvidin 3-O-(6-O-(4-O-malonyl-alpha-rhamnopyranosyl)-beta-glucopyranoside)-5-O-beta-glucopyranoside	Flavonoids	100%	$C_{38}H_{47}O_{24}$	Antioxidant, anti-inflammatory,	Merecz-Sadowska *et al*., 2023
2.18	449.14	Spiroxin	Steroids	100%	$C_{20}ClH_9O_9$	Antibacterial, DNA-cleaving, cytotoxic, and antitumor activities.	Shu *et al*., 2021; Xu *et al*., 2022
1.01	110.05	Hydroquinone	Phenolics	100%	$C_6H_6O_2$	Antioxidant, anti-inflammatory, antimicrobial,	Chang *et al*., 2019; Giner *et al*., 2022
7.578	254.28	Obtusaquinone	Quinones	44%	$C_{16}H_{14}O_3$	Anticancer, induced apoptosis, anti-tumor,	Badr *et al*., 2013; Badr *et al*., 2020
1.96	188.10	2-Methoxynaphthoquinone	Quinones	100%	$C_{11}H_8O_3$	Anticancer, Antimicrobial and antiviral	Xiang *et al*., 2025
8.47	607.32	Neobulgarone E	Quinones	100%	$C_{32}C_{12}H_{24}O_8$	Antimicrobial, moderate cytotoxicity, and inhibition of appressorium formation.	Eilbert *et al*., 2000; Morehouse *et al*., 2000
1.55	276.17	Ranunculin	glycosides	100%	$C_{11}H16O_8$	Antimicrobial, anti-inflammatory, and antioxidant.	Sirak *et al*., 2025
2.48	287.08	Nikkomycin C	Nucleoside-peptide antibiotic	100%	$C_{16}H_{13}N_3O_7$	Antifungal, chitin synthase inhibitory, and	Kumar and Jha, 2023; Pumula *et*

						antimicrobial.	*al*., 2024
2.87	1265.41	Thioxamycin	Thiopeptide antibiotic	100%	$C_{52}H_{48}N_{16}O_{15}S_4$	Antibacterial	Matsumoto *et al*., 1989
3.29	242.31	Aureothricin	Thiopeptide antibiotic	100%	$C_9H_{10}N_2O_2S_2$	Antibacterial	Umezawa *et al*., 1948
4.73	520.37	Pseudohypericin	Anthraquinone derivative	100%	$C_{30}H_{16}O_9$	Anticancer	Birt *et al*., 2009
5.15	310.33	Przewaquinone A	Diterpene quinone	73%	$C_{19}H_{18}O_4$	Anticancer, Antiproliferative	Su *et al*., 2026

Table 2B: Bioactive compounds identified from the negative ion mode (ESI-) LC-MS/MS chromatogram of the methanolic leaf extract of *O. gratissimum* L.

Retention time	m/z	Bioactive compounds	Chemical class	Relative abundance	Molecular formula	Biological activity	References
1.72	169.03	Norepinephrine	Alkaloids	100%	$C_8H_{11}NO_3$	Neuroprotective, anti-inflammatory, antioxidant, and immunomodulatory.	Thoppil *et al*., 2023; Ghasemi and Mehranfard, 2024
8.52	209.04	Benzo[g]isoquinoline-5,10-dione	Alkaloids	84%	$C_{13}H_7NO_2$	Antitumor	Gomez-Monterrey *et al*., 2003
2.504	30.1.01	Carbazomycin F	Alkaloids	100%	$C_{16}H_{15}NO_5$	Antifungal, anti-inflammatory	Lin *et al*., 2024
3.113	327.23	Boldine	Alkaloids	100%	$C_{19}H_{21}NO_4$	Antioxidant, anti-inflammatory, neuroprotective, and anticancer.	Lamba *et al*., 2024
2.182	447.11	Peonidin 3-rhamnoside	Flavonoids	100%	$C_{22}H_{23}O_{10}$	Antioxidant, anti-inflammatory, anticancer, and antimicrobial.	Bharathy *et al*., 2025
4.53	476.30	Quercetin 3-(3"-acetyl-alpha-L-arabinofuranoside)	Flavonoids	100%	$C_{22}H_{20}O_{12}$	Antioxidant, hepatoprotective, and α-glucosidase inhibitory.	Moradi-Afrapoli *et al*., 2012
8.71	540.35	Rhusflavone	Flavonoids	100%	$C_{30}H_{20}O_{10}$	Anti-osteoclastogenic,	Yun *et al*., 2025
0.118	606.41	[5',5']-Bisdihydroquercetin	Flavonoids	100%	$C_{30}H_{22}O_{14}$	Antioxidant and anti-inflammatory	Filippovich *et al*., 2025
5.54	617.41	Delphinidin 3-(2"-galloylgalactoside)	Flavonoids	100%	$C_{28}H_{25}O_{16}$	Anticancer	Xu *et al*., 2026
3.99	721.39	Catechin-(4α→8)-pelargonidin 3-O-β-glucopyranoside	Flavonoids	100%	$C_{36}H_{32}O_{16}$	Antioxidant, anti-inflammatory, and cardioprotective.	Rahman *et al*., 2006; Li *et al*., 2021
3.38	1113.57	Gentiodelphin	Flavonoids	100%	$C_{51}H_{53}O_{28}$	Biological activity not specifically reported	
1.23	191.07	Calvatic acid	Organic acid	100%	$C_8H_5N_3O_3$	Carcinostatic activity	Dembitsky *et al*., 2017
4.83	291.21	Epoxyquinomycin C	Quinone antibiotic	100%	$C_{14}H_{13}NO_6$	Anti-arthritic and immunomodulatory	Matsumoto *et al*., 1997a, 1997b.

6.59	265.16	Streptozocin	Nucleoside antibiotics	100%	$C_8H_{15}N_3O_7$	Genotoxic, Antineoplastic, Broad-spectrum antibiotic	Bolzán and Bianchi, 2002
5.12	297.26	Psicofuranine	Nucleoside antibiotics	100%	$C_{11}H_{15}N_5O_5$	Biological activity not specifically reported	
7.51	309.19	Sangivamycin B (against sars cov 2)	Nucleoside analogue	100%	$C_{12}H_{15}N_5O_5$	Antitumor activity Antiviral activity Antibacterial activity	Wu *et al*., 2025
8.52	311.18	Dhurrin (against insect pest)	Cyanogenic glycoside	100%	$C_{14}H_{17}NO_7$	Herbivore deterrent, insecticidal, and plant defense metabolite.	Yadav *et al*., 2023; Wang *et al*., 2024
2.33	431.11	Pseurotin A	Azaspiro compound	100%	$C_{22}H_{25}NO_8$	Anti-inflammatory, anticancer, immunosuppressive, and antibacterial	McGehee *et al*., 2023; McGehee *et al*., 2025
3.24	329.25	Valclavam (antibiotic)	β-Lactam antibiotic	100%	$C_{14}H_{23}N_3O_6$	Bacteriostatic and antifungal.	Jensen, 2012
3.62	517.33	Piperacillin (to treat bacterial infections)	β-Lactam antibiotic	100%	$C_{23}H_{27}N_5O_7S$	Broad-spectrum antibacterial, bactericidal, and antipseudomonal	El-Kimary *et al*., 2024
4.63	559.33	5-Deoxy-5-halo-gentamicin C1a	Aminoglycoside antibiotic	100%	$C_{19}H_{38}IN_5O_6$	Biological activity not specifically reported	
4.12	649.39	Pamamycin	Aminoglycoside antibiotic	100%	$C_{38}H_{67}NO_7$	Antibacterial, antimycobacterial, anticancer	Eckert *et al*., 2023

The present study demonstrated that the phenolic content of the selected plant varied with the plant parts and extraction solvent. Among the evaluated samples, the methanolic leaf extract exhibited the highest TPC, whereas the root extracts contained comparatively lower phenolic levels. Studies conducted by Maphetu *et al*. (2026) also showed leaves were found to be rich in phenolic compounds. In addition, Achuenu *et al*., 2025 studies exhibited highest TPC and flavonoid contents in leaf extracts of *O. gratissium*, compared to stem extracts further supporting the present findings that leaves serve as the principal reservoir of phenolic metabolites. The higher phenolic content obtained with methanol compared to aqueous extracts is also in agreement with Onyebuchi and Kavaz, 2020. The methanolic leaf extract of *O. gratissimum* exhibited the highest total phenolic content (336.00 mg GAE/g dry extract), followed by the corresponding aqueous extract (262.25 mg GAE/g dry extract). These values are considerably higher than those reported by Maphetu *et al*. (2026), with the TPC of 63.05 mg GAE/g for methanolic leaf extracts, 74.59 mg GAE/g for hydromethanolic extracts and 8.47 mg GAE/g for hot-water extracts of the same plant. The methanolic extracts of the selected plant species exhibited DPPH radical scavenging activity (87.23–88.05%) and IC_{50} values (1.940–2.473 µg/mL). Among the tested plant parts, the leaf extract showed the highest activity of with an IC_{50} value of 1.940 µg/mL. Recently, Felix *et al*., 2026 reported that a nanoparticle-based herbal formulation prepared from *O. gratissimum* and *O. tenuiflorum* exhibited 90.91% DPPH radical scavenging activity at 50 µg/mL**,** Although the antioxidant activity reported was slightly higher than the percentage inhibition observed in the present study. Their investigation evaluated a nanocomposite formulation rather than crude methanolic extracts. Nanoparticle incorporation is known to enhance the stability, surface area and bioavailability of phytochemicals, thereby improving antioxidant performance.

Thin-layer chromatography (TLC) of the methanolic leaf extract in our study confirmed the presence of alkaloids, steroids and flavonoids based on their characteristic color and Rf values. These findings are in agreement with Achuenu *et al*., 2025 employing TLC analysis to detect antioxidant-active constituents in *O. gratissimum* leaves, indicating the presence of bioactive phytochemicals. Previous LC–MS investigations of *O. gratissimum* have predominantly identified phenolic acids and flavonoids, including rosmarinic acid, caffeic acid, chlorogenic acid, luteolin, apigenin, rutin, vicenin-2, nevadensin, xanthomicrol and salvigenin as the major constituents. In contrast, the present LC–MS/MS analysis identified several

additional metabolites, including 4-hydroxyquinazoline, Rhopaladin C, Arisostatin A, Genistein 7-O-glucoside-6"-malonate, Cyanidin 3-(6"-dioxalylglucoside), Malvidin glycoside, Obtusaquinone, Neobulgarone E, Pseudohypericin, Przewaquinone A, Ranunculin, Spiroxin, Nikkomycin C, Thioxamycin and Aureothricin, many of which have not been documented in previous phytochemical reports of *O. gratissimum*. These findings indicate broader metabolite diversity in the methanolic leaf extract than has been described previously. Nevertheless, because several of these metabolites are more commonly associated with other plant genera or microbial sources, their occurrence should be regarded as putative LC–MS/MS annotations pending further structural confirmation using authentic standards or complementary spectroscopic techniques (Venuprasad *et al*., 2014; Ugbogu *et al*., 2021). LC–MS/MS analysis in the negative electrospray ionization mode revealed a diverse metabolite profile comprising flavonoids, alkaloids, organic acids and other bioactive compounds. Flavonoids constituted the predominant chemical class, consistent with previous metabolomic studies of *O. gratissimum*, which reported flavonoids and phenolic acids as the major constituents responsible for its antioxidant potential (Venuprasad *et al*., 2014; Maphetu *et al*., 2025). The abundance of flavonoids detected in the present study corroborates the high total phenolic content and strong DPPH radical scavenging activity observed in the methanolic leaf extract. In addition, alkaloids, organic acids and several putatively annotated metabolites, including quinone antibiotics, nucleoside analogues, β-lactam antibiotics and aminoglycoside antibiotics, further expanded the phytochemical profile of *O. gratissimum*. Since many of these metabolites have not been previously reported in *O. gratissimum*, their identification should be considered tentative and warrants further confirmation using authentic standards and complementary spectroscopic techniques. Nevertheless, the broad spectrum of metabolites detected highlights the chemical diversity of *O. gratissimum* and provides a plausible basis for its reported antioxidant, antimicrobial, anti-inflammatory and other pharmacological activities.

CONCLUSION

Ocimum gratissimum is a rich source of bioactive phytochemicals with strong antioxidant potential, as the current study showed. The existence of a variety of secondary metabolites, including alkaloids, flavonoids, steroids, phenolics, and other pharmacologically significant chemicals, was confirmed by preliminary phytochemical screening, TLC analysis, and LC–MS/MS profiling. The methanolic leaf extract's enhanced antioxidant ability was demonstrated by its highest total phenolic content and strongest DPPH radical scavenging activity. Overall, the results highlights selected plant's potential as a promising source of natural bioactive chemicals for pharmaceutical and nutraceutical uses and offer scientific evidence in favour of the plant's traditional therapeutic use. To enable the therapeutic use of the identified metabolites, further isolation, structural characterization, and biological validation are necessary.

ACKNOWLEDGEMENT

The authors express sincere thanks to the head of the Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore, Karnataka, India for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Achuenu, C., Dashe, R., Adelakun, E. A., Edah, A. O., & Emesi, I. C. (2025). The antioxidant properties and phytochemical screening of the leaf of *Ocimum gratissimum* (scent leaf) grown in Jos, Nigeria. *Adamawa State University Journal of Scientific Research, 13*(1), 23–28.

Badr, C. E., da Hora, C. C., Kirov, A. B., Tabet, E., Amante, R., Maksoud, S., Nibbs, A. E., Fitzsimons, E., Boukhali, M., Chen, J. W., Chiu, N. H. L., Nakano, I., Haas, W., Mazitschek, R., & Tannous, B. A. (2020). Obtusaquinone: A cysteine-modifying compound that targets Keap1 for degradation. *ACS Chemical Biology, 15*(6), 1445–1454. https://doi.org/10.1021/acschembio.0c00104

Badr, C. E., Van Hoppe, S., Dumbuya, H., Tjon-Kon-Fat, L. A., & Tannous, B. A. (2013). Targeting cancer cells with the natural compound obtusaquinone. *Journal of the National Cancer Institute, 105*(9), 643–653. https://doi.org/10.1093/jnci/djt037

Bharathy, P., & Thanikachalam, P. V. (2025). Pharmacological relevance of anthocyanin derivative: A review. *Pharmacological Research - Modern Chinese Medicine, 14*, 100565.

Birt, D. F., Widrlechner, M. P., Hammer, K. D., Hillwig, M. L., Wei, J., Kraus, G. A., Murphy, P. A., McCoy, J., Wurtele, E. S., Neighbors, J. D., Wiemer, D. F., Maury, W. J., & Price, J. P. (2009). *Hypericum* in infection: Identification of anti-viral and anti-inflammatory constituents. *Pharmaceutical Biology, 47*(8), 774–782. https://doi.org/10.1080/13880200902988645

Bolzán, A. D., & Bianchi, M. S. (2002). Genotoxicity of streptozotocin. *Mutation Research/Reviews in Mutation Research, 512*(2–3), 121–134. https://doi.org/10.1016/S1383-5742(02)00007-0

Chang, M. C., Chang, B. E., Pan, Y. H., Lin, B. R., Lian, Y. C., Lee, M. S., Yeung, S. Y., Lin, L. D., & Jeng, J. H. (2019). Antiplatelet, antioxidative, and anti-inflammatory effects of hydroquinone. *Journal of Cellular Physiology, 234*(10), 18123–18130. https://doi.org/10.1002/jcp.28444

Chedea, V. S., & Pop, R. M. (2019). Total polyphenols content and antioxidant DPPH assays on biological samples. In R. R. Watson (Ed.), *Polyphenols in plants: Isolation, purification and extract preparation* (2nd ed., pp. 169–183). Elsevier. https://doi.org/10.1016/B978-0-12-813768-0.00011-6

Dembitsky, V. M., Gloriozova, T. A., & Poroikov, V. V. (2017). Pharmacological and predicted activities of natural azo compounds. *Natural Products and Bioprospecting, 7*(1), 151–169. https://doi.org/10.1007/s13659-016-0117-3

Dvorak, K. R., & Tepe, J. J. (2024). Advances in the total synthesis of bis- and tris-indole alkaloids containing N-heterocyclic linker moieties. *Natural Product Reports, 41*(8), 1264–1293. https://doi.org/10.1039/d4np00012a

Eckert, N., Rebets, Y., Horbal, L., Zapp, J., Hermann, J, Busche, T., Muller, R., Kalinowski, J. & Luzhertskyy, A. (2023). Discovery and overproduction of novel highly bioactive pamamycins through transcriptional engineering of the biosynthetic gene cluster. *Microbial Cell Factories, 22*, 233. https://doi.org/10.1186/s12934-023-02231-x

Eilbert, F., Anke, H., & Sterner, O. (2000). Neobulgarones A–F from cultures of *Neobulgaria pura*, new inhibitors of appressorium formation of *Magnaporthe grisea*. *The Journal of Antibiotics, 53*(10), 1123–1129. https://doi.org/10.7164/antibiotics.53.1123

El-Kimary, E.I., Fahmy, E.K., Esmaeel, S.E., Elkattawy, H.A., & Hegazy, W, A.H. (2024). A review on recent analytical methodologies developed for the micro-determination of piperacillin in different matrices. *Microchemical Journal*, *205*: 111203

Felix, N., Varghese, R. M., Subramanian, A. K., & Shanmugam, R. K. (2026). Comparison of free radical scavenging activity of *Ocimum gratissimum* and *Ocimum tenuiflorum* herbal formulation and its mediated nanoparticles and nanocomposite: An in vitro study. *Journal of Clinical and Diagnostic Research, 20*(1), ZC01–ZC05. https://doi.org/10.7860/JCDR/2026/75414/22181

Filippovich, S. Y., Isakova, E. P., Bachurina, G. P., & Deryabina, Y. I. (2025). Modern developing directions in the dihydroquercetin study. *Molecules, 30*(21), 4187. https://doi.org/10.3390/molecules30214187

Furumai, T., Takagi, K., Igarashi, Y., Saito, N., & Oki, T. (2000). Arisostatins A and B, new members of tetrocarcin class of antibiotics from *Micromonospora* sp. TP-A0316. I. Taxonomy, fermentation, isolation and biological properties. *The Journal of Antibiotics, 53*(3), 227–232. https://doi.org/10.7164/antibiotics.53.227

Gamble, J. S. (1967). *Flora of the Presidency of Madras* (2nd reprint ed., Vols. 1–3). Calcutta, India: Botanical Survey of India.

Ghasemi, M., & Mehranfard, N. (2024). Neuroprotective actions of norepinephrine in neurological diseases. *Pflügers Archiv – European Journal of Physiology, 476*(11), 1703–1725. https://doi.org/10.1007/s00424-024-02999-w

Giner, R. M., Ríos, J. L., & Máñez, S. (2022). Antioxidant activity of natural hydroquinones. *Antioxidants, 11*(2), 343. https://doi.org/10.3390/antiox11020343

Gomez-Monterrey, I., Campiglia, P., Grieco, P., Diurno, M. V., Bolognese, A., La Colla, P., & Novellino, E. (2003). New benzo[g]isoquinoline-5,10-diones and dihydrothieno[2,3-b] naphtho-4,9-dione derivatives: Synthesis and biological evaluation as potential antitumoral agents. *Bioorganic & Medicinal Chemistry, 11*(17), 3769–3775. https://doi.org/10.1016/s0968-0896(03)00310-9

Goswami, M., Kityania, S., Nath, R., Nath, P., Nath, D., Das, S., Sharma, B. K., & Talukdar, A. D. (2025). Genistein—A broad-spectrum bioactive compound with diverse pharmacological potential: A systematic review. *Current Molecular Medicine*. Advance online publication. https://doi.org/10.2174/0115665240377727250703130718

Harborne, J. B. (1973). *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall.

Jensen, S. E. (2012). Biosynthesis of clavam metabolites. *Journal of Industrial Microbiology & Biotechnology, 39*(10), 1407–1419. https://doi.org/10.1007/s10295-012-1191-0

Kalakwade, A., Kavalapure, R. S., Gharge, S., Alegaon, S. G., Ranade, S. D., Wong, L. S., & Ramu, R. (2025). Heterocyclic compounds with diverse biological activities: A review of quinazoline and quinazolinone derivatives. *Results in Chemistry, 19*, 102932. https://doi.org/10.1016/j.rechem.2025.102932

Katyal, R., Pandit, M. A., Gulati, S., Rawat, C. D., & Kaur, J. (2026). *Mapping global trends in Lamiaceae research*.

Plant Science Today. 13(1): 1-14. https://doi.org/10.14719/pst.11962

Kowalczyk, T., Muskała, M., Merecz-Sadowska, A., Sikora, J., Picot, L., & Sitarek, P. (2024). Anti-inflammatory and anticancer effects of anthocyanins in in vitro and in vivo studies. *Antioxidants, 13*(9), 1143. https://doi.org/10.3390/antiox13091143

Krstin, L., Katanić, Z., Turk, I., Gajski, I., & Pfeiffer, T. Ž. (2026). Current knowledge and utilization of medicinal plants and fungi in northeastern Croatia. *Plants, 15*(2), 325. https://doi.org/10.3390/plants15020325

Kumar, A., & Jha, A. (2023). Clinical status of anticandidal therapeutic agents. In A. Kumar & A. Jha (Eds.), *Anticandidal therapeutics: Discovery and development* (Chapter 8, pp. 141–160). Academic Press. https://doi.org/10.1016/C2022-0-01239-9

Lamba, D., Dwivedi, D. K., Yadav, M., & Kumar, Y. R. S. (2024). Boldine: A narrative review of the bioactive compound with versatile biological and pharmacological potential. *Journal of Complementary & Integrative Medicine, 21*(3), 269–281. https://doi.org/10.1515/jcim-2023-0224

Li, X., Sun, J., Chen, Z., Jiang, J., & Jackson, A. (2021). Characterization of carotenoids and phenolics during fruit ripening of Chinese raspberry (*Rubus chingii* Hu). *RSC Advances, 11*(18), 10804–10813. https://doi.org/10.1039/d0ra10373j

Lin, B., Zhang, D., Wang, J., Qiao, Y., Wang, J., Deng, Z., Kong, L., & You, D. (2024). *O*-methyltransferase CbzMT catalyzes iterative 3,4-dimethylations for carbazomycin biosynthesis. *Engineering Microbiology, 4*(2), 100150. https://doi.org/10.1016/j.engmic.2024.100150

Maphetu, N., Unuofin, J. O., Oladipo, A. O., & Lebelo, S. L. (2026). *Ocimum gratissimum*: Chemical composition, phytochemical properties, antioxidants, and pharmacological activities: A review. *Plants, 15*(11), 1662. https://doi.org/10.3390/plants15111662

Matsumoto, M., Kawamura, Y., Yasuda, Y., Tanimoto, T., Matsumoto, K., & Yoshida, T. (1989). Isolation and characterization of thioxamycin. *The Journal of Antibiotics, 42*(10), 1465–1469. https://doi.org/10.7164/antibiotics.42.1465

Matsumoto, N., Iinuma, H., Sawa, T., Takeuchi, T., Hirano, S., Yoshioka, T., & Ishizuka, M. (1997). Epoxyquinomicins A, B, C and D, new antibiotics from *Amycolatopsis*. II. Effect on type II collagen-induced arthritis in mice. *The Journal of Antibiotics, 50*(11), 906–911. https://doi.org/10.7164/antibiotics.50.906

Matsumoto, N., Tsuchida, T., Umekita, M., Kinoshita, N., Iinuma, H., Sawa, T., Hamada, M., & Takeuchi, T. (1997). Epoxyquinomicins A, B, C and D, new antibiotics from *Amycolatopsis*. I. Taxonomy, fermentation, isolation and antimicrobial activities. *The Journal of Antibiotics, 50*(11), 900–905. https://doi.org/10.7164/antibiotics.50.900

McGehee, O. C., Ebrahim, H. Y., Meyer, S., Ahmed, N. A., Muthumula, C. M. R., Dawud, D., King, J. A., Kaddoumi, A., & El Sayed, K. A. (2025). Safety, pharmacokinetics, translational and molecular mechanistic insights on the prostate cancer recurrence suppressor Pseurotin A. *Molecules, 30*(19), 3963. https://doi.org/10.3390/molecules30193963

McGehee, O. C., Ebrahim, H. Y., Rad, A. H., Abdelwahed, K. S., Mudhish, E. A., King, J. A., Helal, I. E., Meyer, S. A., & El Sayed, K. A. (2023). Towards developing novel prostate cancer recurrence suppressors: Acute toxicity of Pseurotin A, an orally active PCSK9 axis-targeting small molecule in Swiss albino mice. *Molecules, 28*(3), 1460. https://doi.org/10.3390/molecules28031460

Merecz-Sadowska, A., Sitarek, P., Kowalczyk, T., Zajdel, K., Jęcek, M., Nowak, P., & Zajdel, R. (2023). Food anthocyanins: Malvidin and its glycosides as promising antioxidant and anti-inflammatory agents with potential health benefits. *Nutrients, 15*(13), 3016. https://doi.org/10.3390/nu15133016

Moradi-Afrapoli, F., Asghari, B., Saeidnia, S., Ajani, Y., Mirjani, M., Malmir, M., Dolatabadi Bazaz, R., Hadjiakhoondi, A., Salehi, P., Hamburger, M., & Yassa, N. (2012). In vitro α-glucosidase inhibitory activity of phenolic constituents from aerial parts of *Polygonum hyrcanicum*. *Daru, 20*(1), 37. https://doi.org/10.1186/2008-2231-20-37

Morehouse, N. J., Flewelling, A. J., Johnson, J. A., & Gray, C. A. (2020). Halogenated bianthrones from *Penicillium roseopurpureum*: A fungal endophyte of the marine alga *Petalonia fascia*. *Natural Product Communications, 15*(2). https://doi.org/10.1177/1934578X20901405

Olorunfemi, E. S., Odelade, K. A., Adesokan, M. O., Olayiwola, R. F., Jecinta, B., & Olawuyi, B. C. (2025). Phytochemical constituents and antimicrobial activity of crude extract of scent leaf (*Ocimum gratissimum*) on fungal phytopathogens. *International Journal of Scientific Research and Technology, 2*(11), 699-708.

Onyebuchi, C., & Kavaz, D. (2020). Effect of extraction temperature and solvent type on the bioactive potential of *Ocimum gratissimum* L. extracts. *Scientific Reports, 10*, 21760. https://doi.org/10.1038/s41598-020-78847-5

Pannangpetch, P., Laupattarakasem, P., Kukongviriyapan, V., Kukongviriyapan, C., Kongyingyoes, B., & Aromdee, C. (2007). Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm. f.) Lindau. *Songklanakarin Journal of Science and Technology, 29*(1), 1–9.

Puumala, E., Fallah, S., Robbins, N., & Cowen, L. E. (2024). Advancements and challenges in antifungal therapeutic development. *Clinical Microbiology*

Reviews, 37(1), e0014223. https://doi.org/10.1128/cmr.00142-23

Queiroz, E. F., Guillarme, D., & Wolfender, J. L. (2024). Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: From metabolite profiling to pure chemical entities. *Phytochemistry Reviews, 23*, 1415–1442. https://doi.org/10.1007/s11101-024-09928-w

Rahman, M. M., Ichiyanagi, T., Komiyama, T., Hatano, Y., & Konishi, T. (2006). Superoxide radical- and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship and their synergism. *Free Radical Research, 40*(9), 993–1002. https://doi.org/10.1080/10715760600815322

Saldanha, C. J. (1996). *Flora of Karnataka* (Vol. 2). New Delhi, India: Oxford & IBH Publishing Co.

Shete, V., Mahajan, N. M., Shivhare, R., Akkewar, A., Gupta, A., & Gurav, S. (2024). Genistein: A promising phytoconstituent with reference to its bioactivities. *Phytotherapy Research, 38*(8), 3935–3953. https://doi.org/10.1002/ptr.8256

Shu, X., Chen, C. C., Yu, T., Yang, J., & Hu, X. (2021). Enantioselective total synthesis of (−)-spiroxins A, C, and D. *Angewandte Chemie International Edition, 60*(34), 18514–18518. https://doi.org/10.1002/anie.202105921

Siddiquee, Z., Parveen, R., & Ahmad, S. (2023). Effect-directed assays and biological detection approaches coupled with thin-layer chromatography as an evolving hyphenated technique: A comprehensive review. *Combinatorial Chemistry & High Throughput Screening, 26*(15), 2679–2717. https://doi.org/10.2174/1386207326666230406083555

Sirak, B., Aragaw, M., & Tadesse, S. (2025). Ranunculin, protoanemonin, and anemonin: Pharmacological and chemical perspectives. *Current Medicinal Chemistry, 32*(9), 1659–1673. https://doi.org/10.2174/0109298673294080240221115830

Sofowora, L. A. (1993). *Medicinal plants and traditional medicine in Africa* (pp. 55–71). Spectrum Books Ltd.

Su, J., Wan, T., & Tian, S. (2026). Przewaquinone A regulates cell cycle and autophagy through the Src/STAT3 signaling pathway to inhibit colorectal cancer progression. *Naunyn-Schmiedeberg's Archives of Pharmacology, 399*(7), 10223–10240. https://doi.org/10.1007/s00210-026-05042-0

Thoppil, J., Mehta, P., Bartels, B., Sharma, D., & Farrar, J. D. (2023). Impact of norepinephrine on immunity and oxidative metabolism in sepsis. *Frontiers in Immunology, 14*, 1271098. https://doi.org/10.3389/fimmu.2023.1271098

Tokalı, F. S. (2025). Recent advances in quinazolinone derivatives: Structure, design and therapeutic potential. *Future Medicinal Chemistry, 17*(9), 1071–1091. https://doi.org/10.1080/17568919.2025.2504327

Trease, G. E., & Evans, W. C. (1989). *Trease and Evans' pharmacognosy: A physician's guide to herbal medicine* (13th ed.). Baillière Tindall.

Ugbogu, O. C., Emmanuel, O., Agi, G. O., Ibe, C., Ekweogu, C. N., Ude, V. C., Uche, M. E., Nnanna, R. O., & Ugbogu, E. A. (2021). A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon, 7*(11), e08404. https://doi.org/10.1016/j.heliyon.2021.e08404

Umezawa, H., Maeda, K., & Kosaka, H. (1948). Isolation of a new antibiotic substance, aureothricin from a strain of *Streptomyces*. *Japanese Journal of Medical Science and Biology, 1*(6), 512–517.

Venuprasad, M. P., Kandikattu, H. K., Razack, S., & Khanum, F. (2014). Phytochemical analysis of *Ocimum gratissimum* by LC-ESI–MS/MS and its antioxidant and anxiolytic effects. *South African Journal of Botany, 92*, 151–158. https://doi.org/10.1016/j.sajb.2014.02.010

Volluri, S. S., Bammidi, S. R., Chippada, S. C., & Vangalapati, M. (2011). *In vitro antioxidant activity and total phenolic content of* Acalypha indica *and* Centella asiatica**.** *International Journal of Pharmaceutical Sciences Review and Research, 11*(1), 88–92.

Wang, B., Xiong, W., & Guo, Y. (2024). Dhurrin in sorghum: Biosynthesis, regulation, biological function and challenges for animal production. *Plants, 13*(16), 2291. https://doi.org/10.3390/plants13162291

Wu, Y., Wu, S., Niu, X., Yu, X., Chen, T., Liu, G., & Zhang, W. (2025). Biosynthesis and genome mining strategies for purine-derived N-nucleoside antibiotics. *Frontiers in Microbiology, 16*, 1684225. https://doi.org/10.3389/fmicb.2025.1684225

Xiang, S., Li, Y., Khan, S. N., Zhang, W., Yuan, G., & Cui, J. (2025). Exploiting the anticancer, antimicrobial and antiviral potential of naphthoquinone derivatives: Recent advances and future prospects. *Pharmaceuticals, 18*(3), 350. https://doi.org/10.3390/ph18030350

Xu, L., Ma, H., An, X., Li, Y., Zhang, Q., Liu, X., & Wang, M. (2022). Total synthesis, structure revision and cytotoxic activity of Sch 53825 and its derivatives. *RSC Advances, 12*(27), 17629–17636. https://doi.org/10.1039/d2ra02898k

Xu, Y., Jin, B., Yin, P., Cheng, K., Li, Q., & Ding, H. (2026). Targeting cancer with delphinidin: From molecular insights to therapeutic implications. *Frontiers in Pharmacology, 17*, 1778570. https://doi.org/10.3389/fphar.2026.1778570

Yadav, M., Singh, I.K., & Singh A. (2023). Dhurrin: A naturally occurring phytochemical as a weapon against insect herbivores. *Phytochemistry*, 205,113483. doi: 10.1016/j.phytochem.2022.113483.

© The Author(s) 2026. This article is published by the *International Journal of Zoology and Applied Biosciences (IJZAB)* under the terms of the **Creative Commons Attribution 4.0 International License (CC BY 4.0)**. This license permits anyone to share, copy, redistribute, adapt, remix, transform, and build upon the material in any medium or format, provided that appropriate credit is given to the original author(s), the source, and the license, and that any changes made are indicated.