

Phytochemical Profiling, Antimicrobial Activity and Characterization of Bioactive Compound from *Eucalyptus Globulus* Leaf Extracts

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ABSTRACT

Eucalyptus globulus is a medicinal plant widely employed in traditional healthcare systems for managing respiratory infections, wounds, and inflammatory disorders. Despite its ethnomedicinal relevance, the antimicrobial potential of its chemically characterized leaf fractions remains underexplored. This study was designed to screen the phytochemical constituents, isolate bioactive fractions, evaluate antimicrobial activity, and structurally characterize the most potent fractions from methanolic leaf extracts of *E. globulus*. Fresh leaves were collected, air-dried, powdered, and extracted with methanol. Qualitative phytochemical screening was conducted using standard protocols. Bioactive compounds were separated via thin-layer chromatography (TLC) and column chromatography. Antimicrobial activity was assessed against four pathogenic bacteria (*Staphylococcus* sp., *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas* sp.) using the agar well diffusion method at concentrations ranging from 1.25 to 15.00 mg/mL. The most active fractions (F1 and F2) were characterized using FT-IR spectroscopy and gas chromatography-mass spectrometry (GC-MS). Phytochemical screening revealed the presence of terpenoids, alkaloids, flavonoids, tannins, saponins, glycosides, volatile oils, and phenols. Column chromatography yielded six fractions with distinct polarity profiles. Antimicrobial testing established a clear polarity-activity relationship, with fraction F1 demonstrating the highest overall potency, particularly against *E. coli* (zone of inhibition: 30.27 mm; MIC: 2.50 mg/mL). Fraction F2 exhibited selective activity against *S. typhi* (28.63 mm; MIC: 2.50 mg/mL). FT-IR analysis confirmed C-H, C=O, C-C, C-Cl and C-O functional groups. GC-MS identified sesquiterpenes (aromadendrene, β -guaiene, alloaromadendrene) and long-chain aliphatic hydrocarbons in F2, while F1 was dominated by very-long-chain alkenes, including Z-14-nonacosane (16.90%) and 1-hexacosene (10.61%). The methanolic extract of *E. globulus* is rich in diverse bioactive phytochemicals. Non-polar fractions, particularly F1 and F2, exhibit significant antimicrobial activity attributable to sesquiterpenes and long-chain hydrocarbons. These findings validate the traditional medicinal use of *E. globulus* and highlight its potential as a source of natural antimicrobial agents for pharmaceutical development.

Keywords: Antimicrobial, chromatography, *Eucalyptus globulus*, medicinal phytochemical, sesquiterpenes.

INTRODUCTION

For centuries, medicinal plants have served as a primary source of healing, particularly in rural areas of developing countries where modern healthcare is often inaccessible. Their low cost, ease of use, and natural abundance have sustained their centrality in traditional care systems. Recent studies have confirmed that many plant-derived compounds are pharmacologically active, with demonstrated benefits against serious diseases such as cancer and Alzheimer's. These benefits arise from secondary metabolites natural chemicals plants produce for their own survival which are now being systematically studied through phytochemical screening to better understand and harness their medicinal potential (Shoaib et al., 2023).

Eucalyptus is a diverse genus of flowering trees within the family Myrtaceae, indigenous to Australia and also distributed across several Southeast Asian nations (Joy et al., 2022). The leaves of these trees are widely recognized for their extensive therapeutic applications, particularly in the management of respiratory ailments and common colds. A commercially significant product derived from the foliage is eucalyptus oil, which is valued for its antiseptic and antibacterial properties (Aleksic Sabo and Knezevic, 2019). In traditional medicinal practices, eucalyptus leaves are administered in multiple forms, including decoctions, topical preparations such as baths and lotions, and enemas, to address conditions ranging from asthma, bronchitis, and tonsillitis to urinary tract infections and hemorrhages. Inhalation of vapors from steamed or dried leaves is employed to relieve respiratory symptoms associated with asthma, cough, influenza, wheezing, and diphtheria, with finely powdered leaf material also used for this purpose. Additionally, eucalyptus leaf-based soups have been utilized in the treatment of malaria, while the leaves themselves are reported to alleviate gastrointestinal discomfort. Furthermore, resinous exudates obtained from the tree have been traditionally applied in the management of diarrhea (Siripreddy and Mandal, 2017).

It is widely acknowledged that traditional medical systems across the globe rely extensively on medicinal herbs for the treatment of both acute and chronic wounds. Consequently, a substantial number of plant species native to tropical and subtropical regions have been subjected to empirical evaluation for their wound-healing potential. In this context, plants demonstrating antimicrobial activity against multidrug-resistant (MDR) pathogens are considered particularly valuable resources (Owusu et al., 2021).

Although *Eucalyptus globulus* is widely utilized in traditional medicine, the antimicrobial potential of its leaf extracts warrants further scientific scrutiny. Additional research could pave the way for the development of potent natural antimicrobial agents. This study, therefore, aimed to screen the phytochemical constituents, isolate bioactive fractions, evaluate antimicrobial activity, and characterize the most potent fractions from methanolic leaf extracts of *E. globulus* using FT-IR and GC-MS techniques

METHODOLOGY

Sample Collection and Preparation

Fresh leaves of *Eucalyptus globulus* was collected from Modibbo Adama University, Yola, Adamawa State. The sample was identified and authenticated in plant science department where herbarium number MAU1704H was given. The sample was washed thoroughly with distilled water and dried at room temperature on clean polythene bags for two weeks. The dried leaves were then ground with a pestle and mortar, sieved, and the resulting powder was extracted using methanol as a solvent in a Soxhlet extractor.

Qualitative Phytochemical Screening

Qualitative phytochemical screening of the *E. globulus* leaf extract was conducted using the method adopted by Mustapha et al., (2016) and Onuoha et al., (2022).

Test for tannins; Approximately 5 mL of the extract solution was mixed with a small quantity of ferric chloride. A blue-green precipitate was formed, indicating the presence of tannins in the extract.

Test for Flavonoids; Five milliliters of the extract solution were mixed with two drops of ammonia. A yellow-brown color change indicates the presence of flavonoids.

Test for Saponins; The extract (5 mL) was shaken with 5 mL of distilled water and then heated to a boil. The formation of frothing indicates the presence of saponins.

Test for Glycosides; The extract solution was dissolved in glacial acetic acid with one drop of FeCl_3 . The solution was underlaid with concentrated H_2SO_4 . Absence of a brown ring at the interface between the acetic acid and H_2SO_4 layers indicate the absence of glycosides.

Test for Alkaloids; The extract solution was warmed with 1% HCl for two minutes, filtered, and then treated with a few drops of Dragendorff's reagent. A reddish-brown color and turbidity indicate the presence of alkaloids.

Test for Phenols; The extract solution was mixed with two drops of aqueous ferric chloride. A blue-black color formation indicates the presence of phenols.

Test for Volatile Oils; One gram of the extract was dissolved in 90% ethanol, and two drops of ferric chloride was added. A green coloration indicates the presence of volatile oils.

Test for Terpenoids; Salkowski test was done using 2 mL of extract 2 ml of chloroform 3 ml of concentrated sulfuric acid a reddish-brown interface suggested terpenoids.

Thin Layer Chromatography

Thin Layer Chromatography (TLC) was employed to separate, identify, and quantify components of the *E. globulus* extract, following the principles described by Poole (2003). TLC plates (10 × 20 cm) were cut to appropriate size, and a faint pencil line was drawn 1–1.5 cm from the bottom edge to mark the origin. The extract sample was dissolved in methanol, and a capillary tube was used to apply 1–5 µL of the sample as a small spot (approximately 2 mm in diameter) onto the origin line. The solvent was allowed to evaporate completely between multiple applications to concentrate the sample. The mobile phase was poured into a developing chamber to a depth of 0.5–1 cm, ensuring that the solvent level remained below the origin line. The chamber was saturated with solvent vapor by lining the walls with filter paper. The TLC plate was then placed vertically into the chamber, allowing the mobile phase to ascend by capillary action until it reached approximately 2 cm from the top of the plate. The plate was removed, the solvent front was immediately marked, and the plate was allowed to air dry. Spots were visualized under UV light at 254 nm for compounds with conjugated systems and at 365 nm for fluorescent compounds.

Column Chromatography

Column chromatography was employed to separate individual chemical compounds from the extract mixture based on differential adsorption, following the principles outlined by Kondeti et al. (2014). A glass column (5–50 mm internal diameter, 5 cm to 1 m in height) equipped with a fritted filter at the base was prepared. Silica gel (mesh size 60–120, 63–200 µm) was used as the stationary phase. It was noted that resolution is enhanced with increased column length and reduced column diameter.

Antimicrobial Activity of The Isolated Fractions

The antimicrobial activity of the isolated fractions from *Eucalyptus globulus* leaves was evaluated against four pathogenic bacterial strains and two fungal species using the agar well diffusion method (Khanam et al., 2024). The extracts were prepared in ethanol at varying concentrations: 1.25, 2.5, 5.0, 10.0, 12.5, and 15.0 mg/mL. Mueller-Hinton Agar (MHA) plates were used for the assay, and Ciprofloxacin served as the positive control. Following inoculation, the plates were incubated at 35–37°C for 24 hours. The resulting zones of inhibition were measured in millimeters, and all tests were performed in triplicate to ensure reliability.

FT-IR Spectroscopy

The functional groups of the most active isolate from *Eucalyptus globulus* were characterized using FT-IR spectroscopy on a PerkinElmer spectrometer with Spectrum software (Version 10.4.3), as described by Mamuru et al. (2015). The dried isolate was ground with KBr and pressed into a pellet, then scanned in the mid-infrared range (4000–400 cm⁻¹) at 4 cm⁻¹ resolution.

Gas chromatographic- mass spectrometry (GC/MS)

GC-MS analysis of the most active isolate was performed following the method of Schäfer et al. (2015) with slight modifications. The system comprised an Agilent 7890 GC coupled to an Agilent 5975 MSD, equipped with an HP-5ms capillary column (30 m × 250 µm, 5% phenyl methyl siloxane). The oven temperature program

began at 50°C for 1 min, then increased at 10°C/min to 300°C and held for 2 min. The injector temperature was maintained at 230°C, and helium served as the carrier gas at a flow rate of 1 mL/min. A 1 µL aliquot of the methanol-diluted extract was introduced via splitless injection. Mass spectra were acquired in electron ionization (EI) mode at 70 eV, with the ion source at 230°C, and scanned over a mass-to-charge (m/z) range starting from 6.

RESULTS AND DISCUSSION

Table 1: Percentage Yield of Crude Extract

Parameter	Amount (g)
Dry starting material	236.0
Crude extract obtained	29.6
Percentage yield (%)	12.54

Note. Percentage yield was calculated as (crude extract / dry starting material) × 100.

Phytochemical Screening of the plant

The result for the phytochemical analysis of the methanolic extract of *Eucalyptus globulus* is represented in Table 2

Table 2: Phytochemical Screening of *Eucalyptus globulus*

Phytochemicals	Results
Terpenoid	+
Alkaloids	+
Flavonoids	+
Tanins	+
Saponins	+
Glycosides	+
Volatile oils	+
Phenols	+

Key + = present

The methanolic extract of *Eucalyptus globulus* leaves is rich in phytochemicals such as tannins, flavonoids, saponins, glycosides, alkaloids, phenols, and volatile oils, which together contribute to its antimicrobial, antioxidant, and anti-inflammatory activities (Shah et al., 2016). Alkaloids, nitrogen-containing basic compounds, are linked to analgesic effects through action on the central nervous system (Shala & Gururani, 2021), while flavonoids serve as antioxidants by neutralizing reactive species and interrupting free radical chain reactions (Suman & Keshamma, 2019). The presence of alkaloids, tannins, saponins, and glycosides aligns with findings by Gurav et al. (2023), though flavonoids were reportedly absent in that study, whereas Suman and Keshamma (2019) noted the absence of alkaloids. Observed variation in phytochemical profiles across studies may be attributed to environmental factors such as soil type, climate, and collection time.

Column Chromatography

Column chromatography was used to separate individual chemical compounds from mixtures based on differential absorption using the combination of solvent from the TLC. Six different fractions (F1-F6) were obtained from the column chromatography

Antimicrobial Activity of The Isolated Fractions

Six column fractions (F1–F6) obtained were evaluated for antimicrobial activity against four bacterial strains (*Staphylococcus* sp., *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas* sp.) using the agar well diffusion method. Six concentrations (1.25–15.00 mg/mL) were tested in triplicate, and inhibition zone diameters were measured.

Table 3: Maximum Inhibition at 15.00 mg/mL (mm)

Microorganism	F1	F2	F3	F4	F5	F6	Best
<i>Staphylococcus</i> sp.	26.50	20.07	14.60	16.00	13.70	11.70	F1
<i>Escherichia coli</i>	30.27	23.30	18.43	12.90	17.10	16.83	F1
<i>Salmonella typhi</i>	19.80	28.63	14.53	11.70	11.30	10.73	F2
<i>Pseudomonas</i> sp.	27.50	21.00	19.97	19.80	15.00	15.80	F1

Table 4: Minimum Inhibitory Concentration (MIC, mg/mL)

Microorganism	F1	F2	F3	F4	F5	F6	Most potent
<i>Staphylococcus</i> sp.	5.00	10.00	10.00	12.50	12.50	12.50	F1
<i>Escherichia coli</i>	2.50	5.00	12.50	12.50	5.00	5.00	F1
<i>Salmonella typhi</i>	10.00	2.50	10.00	10.00	12.50	12.50	F2
<i>Pseudomonas</i> sp.	5.00	2.50	2.50	2.50	5.00	5.00	F2/F3/F4

Table 5: Statistical Summary (ANOVA F-values)

Microorganism	F1	F2	F3	F4	F5	F6	Strongest
<i>Staphylococcus</i> sp.	2529	5907	374	3221	8180	906	F5
<i>Escherichia coli</i>	4037	566	462	1268	409	1641	F1
<i>Salmonella typhi</i>	5394	3371	746	2926	2350	1159	F1
<i>Pseudomonas</i> sp.	3242	393	598	2393	407	828	F1

Table 6: Comparative Antimicrobial Activity of Column Fractions F1–F6

Parameter	F1	F2	F3	F4	F5	F6
Overall rank	1st	2nd	3rd	4th	5th	6th
Total inhibition (mm)	104.07	93.00	67.53	60.40	56.10	55.63
Relative potency	1.00	0.89	0.65	0.58	0.54	0.53

Fraction F1 demonstrated the highest overall potency. Maximum inhibition at 15.00 mg/mL was observed against *E. coli* (30.27 ± 0.32 mm), followed by *Pseudomonas* (27.50 ± 0.50 mm), *Staphylococcus* (26.50 ± 0.30 mm), and *S. typhi* (19.80 ± 0.20 mm). MIC values were 2.50 mg/mL for *E. coli*, 5.00 mg/mL for both *Pseudomonas* and *Staphylococcus*, and 10.00 mg/mL for *S. typhi* as shown in table 3 and table 4 respectively. The dose-response was most graded for *E. coli* (6 homogeneous subsets, Duncan's test), indicating excellent resolution between concentrations. F1 also showed low within-group variance (average 0.098) and the highest average F-value (3800), confirming exceptional experimental precision and concentration dependence as shown in table 5.

In table 3 and table 4 Fraction F2 exhibited a distinctly different spectrum. Highest activity was observed against *S. typhi* (28.63 ± 0.15 mm at 15.00 mg/mL; MIC = 2.50 mg/mL), representing a 4-fold increase in potency compared to F1 against this pathogen. *Pseudomonas* was also highly susceptible (MIC = 2.50 mg/mL). However, activity against *Staphylococcus* (20.07 ± 0.15 mm; MIC = 10.00 mg/mL) and *E. coli* (23.30 ± 0.50 mm; MIC = 5.00 mg/mL) was reduced relative to F1. Notably, F2 showed exceptional dose-response resolution for *S. typhi* (6 homogeneous subsets) and a high F-value for *Staphylococcus* (F = 5907), indicating strong concentration dependence despite lower absolute activity table 4.5. While Fractions F3–F6 showed progressively

decreasing activity with increasing polarity. F3 (medium polarity) exhibited moderate activity primarily against *Pseudomonas* (19.97 ± 1.05 mm; MIC = 2.50 mg/mL). F4 (high polarity) showed low activity, with *Pseudomonas* as the most susceptible (19.80 ± 0.20 mm; MIC = 2.50 mg/mL) but demonstrated the highest precision overall (average variance = 0.053). F5 (higher polarity) and F6 (highest polarity) showed the lowest overall activity, with total inhibitions of 56.10 mm and 55.63 mm, respectively (relative potencies of 0.54 and 0.53 compared to F1). So also, F5 showed exceptional precision for *Staphylococcus* (variance = 0.013) and the highest F-value for this bacterium (8180), indicating strong concentration dependence despite low absolute activity table 5.

When comparing all six fractions at 15.00 mg/mL, total inhibition across all four bacteria decreased monotonically from F1 (104.07 mm) to F6 (55.63 mm), establishing a clear polarity-activity relationship. Based on MIC values, the order of susceptibility varied by fraction. F1 showed *E. coli* > *Pseudomonas* = *Staphylococcus* > *S. typhi*. F2 showed *S. typhi* = *Pseudomonas* > *E. coli* > *Staphylococcus*. F3–F6 consistently showed *Pseudomonas* as the most susceptible, with variable activity against other bacteria as shown in table 6.

The clear trend in potency (F1 > F2 > F3 > F4 > F5 > F6) correlates with increasing polarity, suggesting that the most active antimicrobial compounds in this column separation are of low to medium polarity. F1 likely contains lipids, terpenes, or non-polar flavonoids; F2 contains glycosides or phenolics; while F3–F6 consist of increasingly polar compounds (tannins, salts, sugars, polar acids) with minimal antimicrobial contribution.

FTIR Analysis Of Fractions (F1 AND F2)

Fraction (F1)

The FTIR spectrum of fraction one (F1) shows the following

From table 7, it shows Aliphatic hydrocarbon chains (dominant), Weak carbonyl (ester/ketone), Strong C–O stretching (alcohol, ester, or carbohydrate) and Possible amide (minor).

Table 7 FTIR Peak Assignment for Fraction (F1)

Peak No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group	Vibration Type
1	2954.73	86.8 (weak)	CH ₃ (aliphatic)	Asymmetric stretch
2	2917.57	75.5 (medium)	CH ₂ (aliphatic)	Asymmetric stretch
3	2849.39	85.4 (weak)	CH ₂ (aliphatic)	Symmetric stretch
4	1731.57	94.2 (v. weak)	C=O (ester / ketone)	Stretch
5	1719.59	92.5 (v. weak)	C=O (saturated aldehyde/ketone)	Stretch
6	1629.91	77.6 (medium)	Amide I or C=C (aromatic)	Stretch
7	1444.23	87.9 (weak)	CH ₂	Scissor bend
8	1376.67	90.7 (v. weak)	CH ₃	Symmetric bend
9	1303.74	86.3 (weak)	C–O or Amide III	Stretch or bend
10	1177.89	73.7 (med-strong)	C–O	Stretch
11	1049.07	73.6 (med-strong)	C–O	Stretch (C–O–C)
12	647.11	95.3 (v. weak)	C–Cl / C–S or aromatic oop	Stretch or bend
13	608.02	89.1 (weak)	Unknown	–

Fraction (F2)

The FTIR spectrum of Fraction (F2) shown in table 7 it displays strong aliphatic C–H stretching absorptions at 2922 and 2853 cm⁻¹, a sharp carbonyl (C=O) peak at 1732 cm⁻¹, and an unusually intense C–O stretching band at 1052 cm⁻¹. These features are consistent with an ester-containing aliphatic compound, possibly a fatty acid ester, polyester, or lipid derivative. The fingerprint region (650–550 cm⁻¹) shows multiple sharp peaks suggesting a structured or crystalline nature.

Table 8: Peak Table with Proposed Functional Group Assignments

Peak No.	X (cm ⁻¹)	Y (%T)	Proposed Assignment
1	2954	83.77	Asymmetric CH ₃ stretch
2	2922	72.95	Asymmetric CH ₂ stretch (dominant)
3	2853	84.53	Symmetric CH ₂ / CH ₃ stretch
4	1732	100.02	C=O stretch (ester, carboxylic acid, or ketone)
5	1630	86.42	C=C (alkene) or Amide I (if protein present)
6	1458	90.46	CH ₂ bending (scissoring)
7	1376	93.38	CH ₃ bending (umbrella mode)
8	1304	94.25	Possibly CH ₂ wagging or C–O stretch
9	1052	59.25	Strong C–O stretch (alcohol, ester, ether, or carbohydrate)
10	965	78.15	=C–H out-of-plane bend (trans alkene)
11	792	85.23	Aromatic C–H bend or Si–C?
12	642	94.28	Fingerprint region – likely C–C skeletal or =C–H bend
13	620	92.90	Possibly substituted aromatic
14	604	90.50	Fingerprint
15	586	90.58	Fingerprint
16	573	87.93	Possibly C–Br, C–Cl, or aromatic substitution
17	554	85.46	Fingerprint

GC-MS Analysis of Compounds in Fractions (F1/F2)

Gas chromatography-mass spectrometry (GC-MS) was used to identify and measure the main natural product components in two samples, labeled F2 and F1. Compounds were identified by matching their mass spectra against the NIST11 and NIST14 libraries. Only those with a match quality score of at least 70 (unless stated otherwise) and a peak area percentage of 0.5% or higher were included in the detailed analysis. Phthalate esters, frequently encountered as lab contaminants, were not considered.

Fraction F2: Major Natural Product Compounds

Fraction F2 yielded a complex mixture dominated by sesquiterpenes and long-chain aliphatic hydrocarbons. Table 4.9 summarizes the six major natural product compounds identified in this sample.

Table 9: Major Natural Product Compounds Identified in Fraction F2

Peak #	Compound Name	Molecular Weight (g/mol)	RT (min)	Area %	Quality Score
4	Aromandendrene	204.35	4.975	1.53	95
4	β-Guaiene	204.35	4.975	1.53	93
1,2	Alloaromadendrene	204.35	3.979 / 4.311	0.46	74–91
14	Tricosane	324.63	8.185	4.46	92
23	Pentacosane	352.68	12.934	3.12	68
23,25,26	1-Hexacosene	64.69	12.934–15.675	7.62	78–99

Sesquiterpenes in Sample F2

The principal terpenoid compounds detected in Sample F2 were the sesquiterpenes aromadendrene, β-guaiene, and alloaromadendrene. These bicyclic sesquiterpenes are widely recognized as secondary metabolites produced by numerous higher plants, especially members of the genera *Eucalyptus*, *Juniperus*, and *Pogostemon* (patchouli) (Hafedh et al., 2025).

Aromadendrene was detected at a retention time of 4.975 min, representing 1.53% of the total peak area and showing a high spectral library match quality of 95. This sesquiterpene is commonly found in essential oils and has been associated with antimicrobial, anti-inflammatory, and cytotoxic properties (Hafedh et al., 2025).

Likewise, β -guaiene was identified at the same retention time, with its presence verified by a characteristic mass spectral fragmentation pattern and a library match quality of 93. The co-occurrence of aromadendrene and β -guaiene is a common feature of essential oils obtained from aromatic plant species.

Alloaromadendrene, a structural isomer of aromadendrene, was identified at two closely spaced retention times of 3.979 and 4.311 min, accounting for a total peak area of 0.46%. The identification was supported by a higher library match quality score of 91 at 4.311 min. Previous studies have reported the occurrence of this sesquiterpene in *Duguetiaglabriuscula*, where it has attracted interest because of its potential pharmacological activities, including antiparasitic and anticancer effects (de Siqueira *et al.*, 2003). The detection of several aromadendrane-type sesquiterpenes indicates that F2 likely originates from a plant source enriched in these biologically active secondary metabolites.

Aliphatic Hydrocarbons in Fraction F2

Besides terpenoid constituents, Sample F2 was found to contain appreciable amounts of the long-chain aliphatic hydrocarbons tricosane, pentacosane, and 1-hexacosene. These hydrocarbons are commonly recognized as major constituents of plant cuticular waxes, where they contribute to the formation of protective surface layers that help minimize water loss and provide defense against ultraviolet radiation and pathogen invasion (Buschhaus *et al.*, 2006; Jung *et al.*, 2017).

Tricosane was identified as the predominant alkane in Fraction F2, representing 4.46% of the total ion current at a retention time of 8.185 min, with a library match quality score of 92. This long-chain hydrocarbon has been reported as a major component of the epicuticular waxes of several plant species, including *Hyacinthellaleucophaea*, where it plays an important role in maintaining surface hydrophobicity and reducing water loss through the formation of a protective wax layer (Buschhaus *et al.*, 2006).

Pentacosane was detected at a retention time of 12.934 min, contributing 3.12% of the total ion current, while 1-hexacosene was identified as a major constituent with a cumulative abundance of 7.62% distributed across three peaks between 12.934 and 15.675 min. The exceptionally high library match scores obtained for 1-hexacosene, reaching up to 99, provide strong confidence in its identification. Very-long-chain alkenes such as **1-hexacosene** have been widely reported in the epicuticular waxes of plants including tomato, pea, and *Arabidopsis thaliana*, where they contribute significantly to the formation of transpiration barriers and the regulation of water loss (Vogg *et al.*, 2004).

Fraction F1: Major Natural Product Compounds

Fraction F1 exhibited a distinct chemical composition, marked by the virtual absence of sesquiterpenes and the predominance of long-chain aliphatic hydrocarbons, including both alkanes and alkenes. The six principal natural product constituents identified in this sample are presented in Table 4.10.

Table 10: Major Natural Product Compounds Identified in Sample F1

Peak	Compound Name	Molecular Weight (g/mol)	RT (min)	Area %	Quality Score	
5,6	Octadecane	254.49	5.867 / 6.660	2.61	70–95	
6	Eicosane	282.55	6.660	2.21	95	
13,23	1-Hexacosene		364.69	9.242 / 12.617	10.61	84–95
20,24	Z-14-Nonacosane		406.77	11.299 / 13.404	16.90	87–95
19	1-Nonadecene		266.50	11.002	5.94	78
26	Tetracosane		338.65	15.362	3.27	89

Alkanes in Fraction F1

In contrast to Sample F2, no detectable sesquiterpene constituents were observed in Sample F1. Instead, the chromatographic profile was dominated by long-chain saturated hydrocarbons. **Octadecane** was detected at retention times of 5.867 min and 6.660 min, contributing 0.40% and 2.21% of the total peak area, respectively, for a combined abundance of 2.61%. The identification of the compound at 6.660 min was supported by a high library match quality score of 95, indicating a reliable assignment.

Eicosane was also identified at a retention time of 6.660 min, co-eluting with octadecane and exhibiting a high library match quality score of 95, which supports its reliable identification. **Tetracosane** was detected at 15.362 min, accounting for 3.27% of the total peak area with a quality score of 89. These straight-chain alkanes are widely distributed in plant cuticular waxes, where they are produced through the fatty acid elongation pathway. As important wax constituents, they enhance the structural integrity of the cuticle and contribute to its water-repellent properties (Jung et al., 2017).

Alkenes in Sample F1

Sample F1 was distinguished by a remarkably high concentration of very-long-chain alkenes. Among these, **1-hexacosene** was detected at retention times of 9.242 min and 12.617 min, accounting for 3.25% and 7.36% of the total peak area, respectively, for a combined abundance of 10.61%. The compound was identified with high confidence, yielding library match quality scores of 95 and 84. As a terminal alkene frequently found in plant leaf and fruit waxes, 1-hexacosene has been associated with the regulation of transpiration and the maintenance of protective surface barriers (Vogg et al., 2004).

The predominant constituent of Sample F1 was **Z-14-nonacosane** a long-chain unsaturated hydrocarbon containing a double bond at the 14th carbon position. It was detected at retention times of 11.299 min and 13.404 min, contributing 4.39% and 12.51% of the total peak area, respectively, for a cumulative abundance of 16.90%. Identification of this compound was supported by strong library match quality scores of 95 and 87. Z-14-nonacosane is particularly noteworthy because it has been documented both as a constituent of plant cuticular waxes and as an insect pheromone, indicating potential ecological functions in mediating plant–insect interactions (Prasad et al., 2004). Its substantial abundance in Sample F1 may therefore suggest a specialized role in chemical signaling, defense, or other ecological processes.

CONCLUSION

This study successfully demonstrated that the methanolic extract of *Eucalyptus globulus* leaves is a rich source of diverse bioactive phytochemicals, including terpenoids, alkaloids, flavonoids, tannins, saponins, glycosides, volatile oils, and phenols. Through column chromatography, six fractions (F1–F6) were isolated, with fractions F1 and F2 exhibiting the most promising antimicrobial activity against clinically relevant bacterial pathogens. Fraction F1 showed broad-spectrum potency, particularly against *Escherichia coli* and *Pseudomonas* sp., while Fraction F2 displayed selective activity against *Salmonella typhi*.

FT-IR spectroscopy confirmed the presence of aliphatic hydrocarbons, carbonyl groups, and C–O functionalities in the active fractions. GC-MS analysis revealed that the antimicrobial activity of F2 is associated with sesquiterpenes (aromadendrene, β -guaiene, and alloaromadendrene) and long-chain aliphatic hydrocarbons, whereas F1 is predominantly composed of very-long-chain alkenes, including Z-14-nonacosane and 1-hexacosene.

The observed polarity-activity relationship (F1 > F2 > F3 > F4 > F5 > F6) suggests that the most bioactive compounds are of low to medium polarity, likely comprising lipids, terpenes, and non-polar flavonoids. These findings provide scientific validation for the traditional medicinal use of *E. globulus* and highlight its potential as a source of natural antimicrobial agent.

Conflict Of Interest

The authors declare that no competing interest exists.

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