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ORIGINAL RESEARCH ARTICLE

GC–MS Characterization and Antibacterial Activity of Terpene-Rich Extract from *Vernonia amygdalina* Leaves

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ABSTRACT

Multidrug-resistant bacterial strains represent a growing global public health concern, prompting increased interest in plant-derived antibacterial agents. *Vernonia amygdalina* is widely used in traditional medicine and has been reported to possess antimicrobial properties; however, the antibacterial potential of its volatile terpene-rich extracts has received limited investigation. Therefore, this study aimed to evaluate the antibacterial activity of a volatile terpene-rich crude extract from *V. amygdalina* leaves against selected enteric bacteria and to characterize its phytochemical constituents using gas chromatography–mass spectrometry (GC–MS). Dried powdered leaves of *V. amygdalina* were extracted by maceration using ethanol followed by aqueous reconstitution containing lead acetate solution and chloroform partitioning to obtain a volatile terpene-rich crude extract. Antibacterial activity of the extract was evaluated against clinical isolates of *Salmonella typhi* and *Escherichia coli* using agar well diffusion susceptibility tests, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Chemical profiling of the extract was performed by GC–MS analysis on an Agilent system equipped with an HP-5MS capillary column, using helium as the carrier gas. The extract exhibited concentration-dependent antibacterial activity against *S. typhi*, producing inhibition zones of 10.5 ± 0.7 mm, 6.5 ± 2.1 mm, and 5.5 ± 0.7 mm at 1000, 800, and 600 µg/ml, respectively, while *E. coli* showed no susceptibility. The MIC and MBC values against *S. typhi* were 800 µg/ml and 1000 µg/ml, respectively. GC–MS analysis identified 22 bioactive compounds, predominantly volatile terpenoids including farnesol, squalene, and lavandulol derivatives, that contributed to the observed antibacterial activity. These findings suggest that the volatile terpene-rich extract of *V. amygdalina* leaves possesses moderate antibacterial activity against clinical isolates of *S. typhi* but shows no activity against *E. coli*. This highlights the potential of these compounds as natural antibacterial agents with the possibility of optimization towards effective management of enteric bacterial infections.

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Terpene, GC-MS, *V. amygdalina, Salmonella*

INTRODUCTION

Vernonia amygdalina Del. belongs to the family Asteraceae, and it is native to Africa. In Nigeria, it is commonly called bitter leaf but known locally in Yoruba as "Ewuro", in Hausa as "Shuwaka", in Igbo as "Onugbu" and in Efik as "Etidot" (Itah and Akinjogunla, 2024; Obi *et al.* 2024; Usman *et al.* 2025). The plant is commonly consumed in Nigeria as food or medicine due to its nutritional and health benefits. In traditional medicine, it is used to treat various ailments, including intestinal worms, bloating, malaria, urinary problems, menstrual pain, and skin infections, among others, either singly or synergistically with other plant and animal parts (Degu *et al.,* 2024). Phytochemical classes such as flavonoids, terpenes, saponins, tannins, steroids, and alkaloids have been reported from the leaf section of the plant. Sesquiterpene lactones such as vanodalol, vernolide, epivernodalol, vernodalinol, flavonoids such as luteolin, cymaroside, phenolics such as quinic acid derivatives, among other compounds, have been isolated from the leaf section of the plant. Experimental studies showed that plant metabolites and isolated compounds possess anti-inflammatory, antidiabetic, antioxidant, anticancer, antiparasitic, and antidiabetic properties (Abere *et al.,* 2025).

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GC–MS is a widely used analytical technique for the identification and characterization of bioactive phytochemicals present in medicinal plants (Salisu *et al.* 2017; Zheng *et al.* 2018; Salisu and Shema 2019; Lokossou *et al.* 2022; Hamisu and Salisu 2025; Ibrahim *et al.* 2025; Musari *et al.* 2025; Said *et al.* 2025; Ado *et al.* 2026; Hassan *et al.* 2026). The technique enables the detection of volatile and semi-volatile compounds, such as terpenoids, fatty acids, alcohols, and other secondary metabolites, which may contribute to the biological activities of plant extracts (Al-Rubaye *et al.,* 2017; Njoku *et al.,* 2021). Several studies have employed GC–MS profiling to investigate the phytochemical composition of various medicinal plants and to relate the identified compounds to their antimicrobial properties. For instance, GC–MS analysis of several medicinal plant extracts revealed diverse bioactive constituents with significant antibacterial potential against pathogenic microorganisms (Nabi *et al.,* 2022; Farooq *et al.,* 2024; Endris *et al.,* 2024). These findings highlight the importance of phytochemical profiling techniques such as GC–MS in the discovery of plant-derived compounds that may serve as potential alternatives to conventional antibiotics.

The increasing spread, persistence, and prevalence of multidrug-resistant bacterial strains have emerged as a significant global public health concern largely due to the overuse of antibiotics, which has resulted in increased resistance and subsequently treatment failures, which in turn have resulted in increased healthcare costs and higher mortality, particularly in developing countries (Itah & Akinjogunla, 2024). This has prompted an increased search for novel, potent, and safe alternative sources of antibiotics with a major focus on medicinal plants due to their relative safety. Studies have shown that medicinal plant metabolites affect their antibacterial potential via mechanisms such as disruption of bacterial membrane, inhibition of protein synthesis, and disruption of nucleic acid replication (Alozie *et al.,* 2024). Volatile terpenes and terpenoids derived from medicinal plants have been reported to possess significant antimicrobial properties, primarily by altering membrane permeability, leading to the release of nucleic acids and proteins and a decrease in membrane potential (Masyita *et al.,* 2022). In vitro studies have demonstrated that specific terpenes, such as thymol, carvacrol, and linalool, exhibit significant antibacterial activity against foodborne and pathogenic bacteria, primarily by disrupting microbial cell membranes and interfering with cellular metabolic processes (Di Matteo *et al.,* 2024). Furthermore, recent reviews have highlighted the growing interest in plant-derived terpenes as potential agents to combat antimicrobial resistance, due to their diverse mechanisms of action and reduced likelihood of resistance development (Bardaji *et al.,* 2025). Thus, highlighting the potential of terpene-based compounds as promising candidates against pathogenic bacteria and antimicrobial resistance. Previous studies on the phytochemical composition and antibacterial activities of *V. amygdalina* have largely focused on conventional extracts and pure isolated compounds (Olusola-Makinde *et al.*, 2021; Tura *et al.*, 2024; Degu *et al.,* 2024). Limited attention has been given to the phytochemical composition and antibacterial activity of its volatile terpene-rich extracts. Therefore, this study aimed to evaluate the antibacterial activity of a volatile terpene-rich crude extract from *V. amygdalina* leaves against selected enteric bacteria and to characterize its phytochemical constituents using GC–MS platform.

MATERIALS AND METHOD

2.1 Collection and identification of plant material

The *Vernonia amygdalina* plant was collected from a nursery located in the Gwaram Local Government Area, Jigawa State, Nigeria. The plant material was identified by a taxonomist at the Herbarium unit of the Department of Plant Biology, Bayero University, Kano.

2.2 Preparation of plant material

Fresh leaves were collected and rinsed thoroughly under running tap water to remove any excess silt. The leaves were shade-dried at room temperature for 14 days. Dried leaves were then pulverized into a powder using a mechanical grinder, and the powdered sample was was stored in an airtight container until extraction.

2.3 Extraction of plant material

Extraction was carried out according to a previously described protocol (Watanabe *et al.*, 2005) with modifications (Fig. 1). Briefly, 50 g of powdered plant material was extracted by cool maceration with 200ml ethanol (1:4 w/v) for 72 hours. Extraction was performed twice using fresh 200ml portions of ethanol. The crude ethanol extract was reconstituted with 200 mL of distilled water to obtain an aqueous extract, which was then treated with lead acetate solution to yield a precipitate. The solution was centrifuged at 3000 rpm for 3 min, and the supernatant was extracted with chloroform (150ml x2) to obtain a chloroform extract. The extract yield obtained was 0.10 g (0.2%). The dried extract was stored in an airtight container until further analysis.

2.4 Biological activity

2.4.1 Test microorganisms and culture media

The test organisms were identified as clinical isolates of *Salmonella typhi* and *Escherichia coli* obtained from the microbiology unit of the medical laboratory department of the federal medical center, Nguru, Yobe State. The bacterial isolates were confirmed using standard microbiological identification procedures, including Gram staining and biochemical tests, before use.

Nutrient agar (Titan Biotech LTD, Delhi, India) was used for the growth of the microorganisms. The media was prepared according to manufacturer's instructions and sterilized in an autoclave at 121℃ for 15 min.

2.4.2 Susceptibility test

The agar well diffusion method was used to screen the extract as described by Sabo *et al.* (2019). Briefly, a standardized inoculum of test microorganisms was spread evenly over the surface of the medium. At the center of

each inoculated medium was a well containing 0.2 ml of extract concentrations (1000, 800, and 600 µg/ml). The inoculated plates were then incubated at 37℃ for 24 h. Thereafter, the plates were observed for growth inhibition, and the diameters of the zones of inhibition of each plate were carefully measured and recorded. The experiment was conducted using triplicate tests at each concentration.

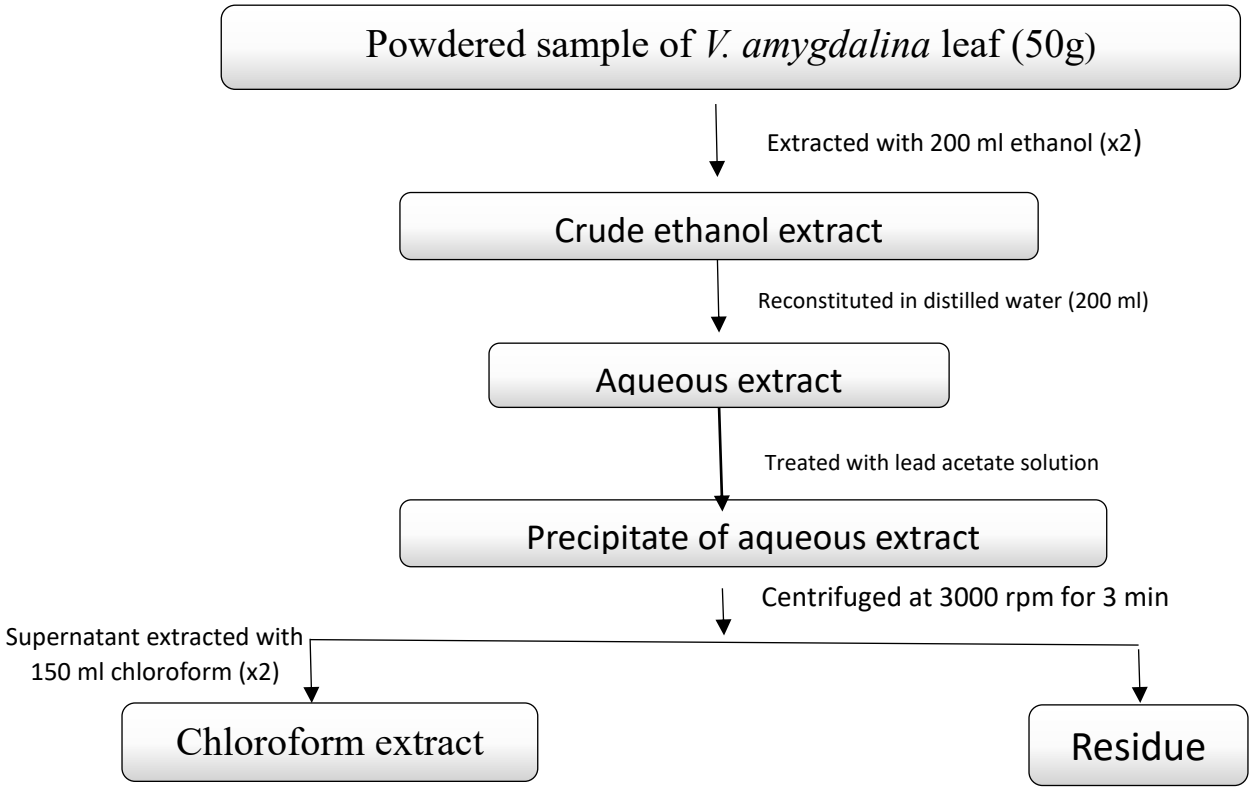

Figure 1: Extraction flow chart of powdered *Vernonia amygdalina* leaf

2.4.3 Determination of minimum inhibitory concentration (MIC)

MIC of the extracts was determined using the broth dilution method as described by Sani *et al.* (2016). Briefly, nutrient broth was prepared, sterilized (121 °C, 15 min), and dispensed into test tubes. The test microorganisms were inoculated into each tube containing different concentrations of the extract (1000, 800, and 600 µg/ml) and incubated at 37 ℃ for 24 h. The tubes were observed for turbidity (growth), and the lowest extract concentration in the broth that prevents turbidity indicates the MIC. The MIC assay was conducted in triplicate for each concentration.

2.4.4 Determination of minimum bactericidal concentration (MBC)

Here, the MIC experiment was subcultured onto a freshly prepared medium plate and incubated at 37℃ for 24 hours. Each plate was observed for colony growth, and the MBC was determined as the lowest extract concentration at which no colonies grew (Sani *et al.,* 2016). The MBC assay was conducted in triplicate for each concentration.

2.5 GC-MS analysis

GC-MS analysis was carried out on Agilent 19091S-433UI system equipped with HP-5MS ultra inert capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 0.73 mL/min. The column oven temperature was held at 50°C to 325°C at 10°C/min for 5 min. An injection volume of 2 µL was used in split mode, with a total runtime of 40 min. The mass spectrometer was operated in electron ionization mode at 70 eV, with a m/z range of 100 –600. Identification of compounds was performed by comparing the obtained mass spectra with those in the National Institute of Standards and Technology (NIST 14) library database. Only compounds with a similarity index ≥80% were considered for identification.

2.6 Data analysis

Results were presented as mean ± standard deviation (SD) where applicable. Statistical analysis was performed using One-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) for multiple comparisons. Differences among group means were considered statistically significant at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics version 29.

RESULTS AND DISCUSSION

The volatile terpene-rich crude extract exhibited concentration-dependent inhibitory activity against *Salmonella typhi*, with the highest inhibition observed at 1000 µg/ml, while *Escherichia coli* showed resistance to the extract (Table 1), which suggest *Escherichia coli* was highly resistant to both the extract and standard drug, a phenomenon most probably associated with the

formation of biofilm by the pathogen. The resistance of *E. coli* to antibiotics is quite common and has been shown in previous studies (Ali *et al.,* 2019; Onifade *et al.,* 2024). Factors such as self-medication or inappropriate administration of antibiotics due to the lack of guidelines for selecting antibiotic drugs were most probably associated with this finding, given that the *E. coli* tested in this study was a clinical isolate (Onifade *et al.,* 2024). Similar observations have been reported in previous studies, in which plant extracts exhibited lower activity against Gram-negative bacteria than against Gram-positive bacteria due to intrinsic resistance mechanisms (Nazzaro *et al.*, 2013; Bassolé & Juliani, 2012). Furthermore, the resistance of *E. coli* in this study may also be due to the specific composition and concentration of the identified compounds, as well as possible absence or low abundance of highly active constituents required to exert inhibitory effects against Gram-negative organisms. This finding highlights the selective antibacterial activity of the extract and suggests that its efficacy may be more pronounced against susceptible bacterial strains such as *S almonella typhi*.

Table 1: Shows zones of inhibition by extracts on bacterial pathogens

Extract concentrations (µg/ml)	Zones of inhibition (mm)	
	S. typhi	*E. coli*
1000	10.5 ± 0.7[a]	0.0 ± 0.0
800	6.5 ± 2.1[b]	0.0 ± 0.0
600	5.5 ± 0.7[b]	0.0 ± 0.0
5 (Ciprofloxacin)	12.5 ± 0.7[a]	0.0 ± 0.0

Values are expressed as mean ± SD of independent experiments. Means with different superscript letters within the same column are significantly different ($p < 0.05$) using one-way ANOVA followed by DMRT.

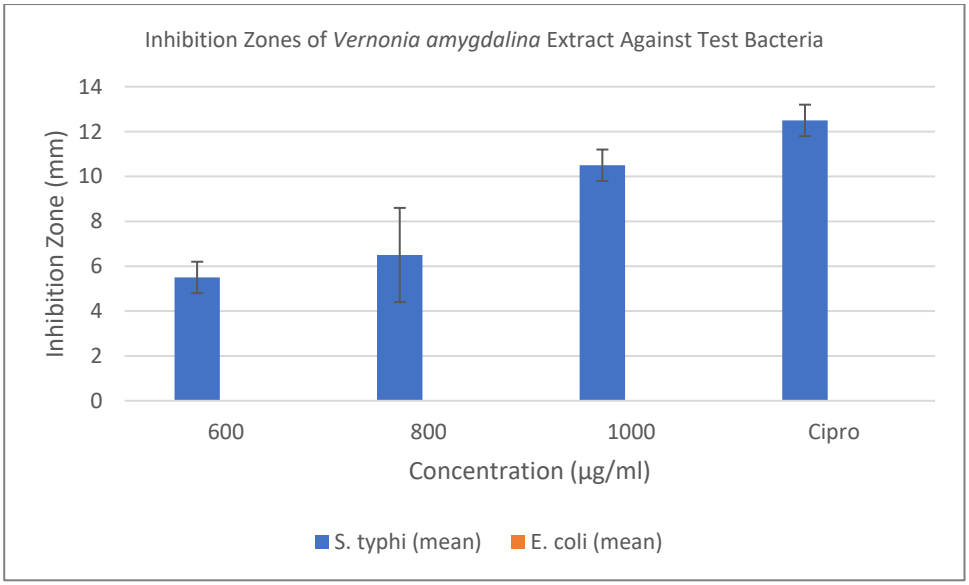

Fig. 2. Bar chart showing antibacterial activity of volatile terpene-rich crude extract of *V. amygdalina* against *S. typhi* and *E. coli*. Values represent mean inhibition zone ± SD of triplicate experiments.

Table 2: Shows the MIC and MBC of the extract against *S. typhi* at different concentrations

Test organism	Extract concentrations	MIC	MBC
S. typhi	1000	-	*
	800	*	+
	600	+	++

- = clear (no growth), * = MBC, + = moderate colony growth, ++ = heavy colony growth

The MIC and MBC values of the volatile terpene-rich crude extract against *S. typhi* are presented in Table 2. The extract exhibited inhibitory activity, with MIC and MBC values of 800 µg/mL and 1000 µg/mL, respectively (Figure 2). The higher MBC value relative to the MIC suggests that the extract was bacteriostatic at lower concentrations and bactericidal at higher concentrations against *S. typhi*. These findings indicate that the extract possesses measurable antibacterial activity against *S. typhi*.

The retention times, library identification, compound classes, molecular formulas, and molecular weights of the phytochemical constituents identified in the volatile terpene-rich extract of *Vernonia amygdalina* are presented in Figure 3 and Table 3. A total of twenty-two (22) bioactive compounds were identified through gas chromatography–mass spectrometry GC–MS analysis. The chromatogram (Figure 3) revealed several prominent peaks, indicating the presence of both major and minor constituents within the extract.

Notable compounds corresponding to prominent peaks in the chromatogram include oleic acid, squalene, farnesol (2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-), lavandulol derivative, and aspidocarpine, occurring at retention times of 38.27, 37.63, 37.48, 35.41, and 34.28 (Table 3).

Table 3: Phytoconstituents identified in volatile terpene-rich crude extract from *V. amygdalina* leaf by GC-MS

Compounds	Retention time (min)	Library/ID	Compound class	MF	MW (g/mol)
1	5.99	Octanoic acid, ethyl ester	Fatty acid acyl	$C_{10}H_{20}O_2$	172.26
2	8.91	Cyclohexasiloxane, dodecamethyl	Organosilicon	$C_{12}H_{36}O_6Si_6$	444.92
3	13.72	dihydroactinidiolide	Benzofuran (lactone)	$C_{11}H_{16}O_2$	180.24
4	17.54	3-Hydroxy-5,6-epoxy-I (2)-ionone	acyclic ketones	$C_{13}H_{20}O_3$	224.3
5	18.54	Oplopanone	Sesquiterpenoid	$C_{15}H_{26}O_2$	238.37
6	21.1	cis-Z-α-Bisabolene epoxide	Sesquiterpenoid	$C_{15}H_{24}O$	220.35
7	23.86	Palmitic acid ethyl ester	Fatty acid	$C_{18}H_{36}O_2$	284.5
8	24.12	Longifolenaldehyde	Sesquiterpenoid	$C_{15}H_{24}O$	220.35
9	24.18	2(1H)-Naphthalenone	Sesquiterpenoid	$C_{10}H_8O$	144.17
10	27.1	Elaidic acid ethyl ester	Fatty acid ester	$C_{20}H_{38}O_2$	310.5
11	27.6	Ethyl stearate	Fatty acid	$C_{20}H_{40}O_2$	312.12
12	30.64	Oleamide	Fatty amide	$C_{18}H_{35}NO$	281.5
13	34.28	Aspidocarpine	Alkaloid	$C_{22}H_{30}N_2O_3$	370.5
14	37.63	Squalene	Triterpene	$C_{30}H_{50}$	410.7
15	38.27	Oleic Acid	Fatty acid	$C_{18}H_{34}O_2$	282.5
16	15.33	Octanoic acid, ethyl ester	Fatty acid ester	$C_{10}H_{20}O_2$	172.26
17	18.41	cis-p-mentha-1(7),8-dien-2-ol	Sesquiterpenoid	$C_{10}H_{16}O$	152.23
18	26.4	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	Organosilicon	$C_{18}H_{52}O_7Si_7$	577.2
19	29.32	Cyclopentadecanone, 4-methyl-	Ketone	$C_{16}H_{30}O$	238.41
20	32.72	Ethanol, 2-(octadecyloxy)-	Alcohol	$C_{20}H_{42}O_2$	314.5
21	35.41	Trifluoroacetyl-lavandulol (S-Lavandulol)	Monoterpene alcohol	$C_{10}H_{18}O$	154.25
22	37.48	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl- (Farnesol)	Sesquiterpene alcohol	$C_{15}H_{26}O$	222.37

Key: MW = Molecular weight; MF = Molecular formular

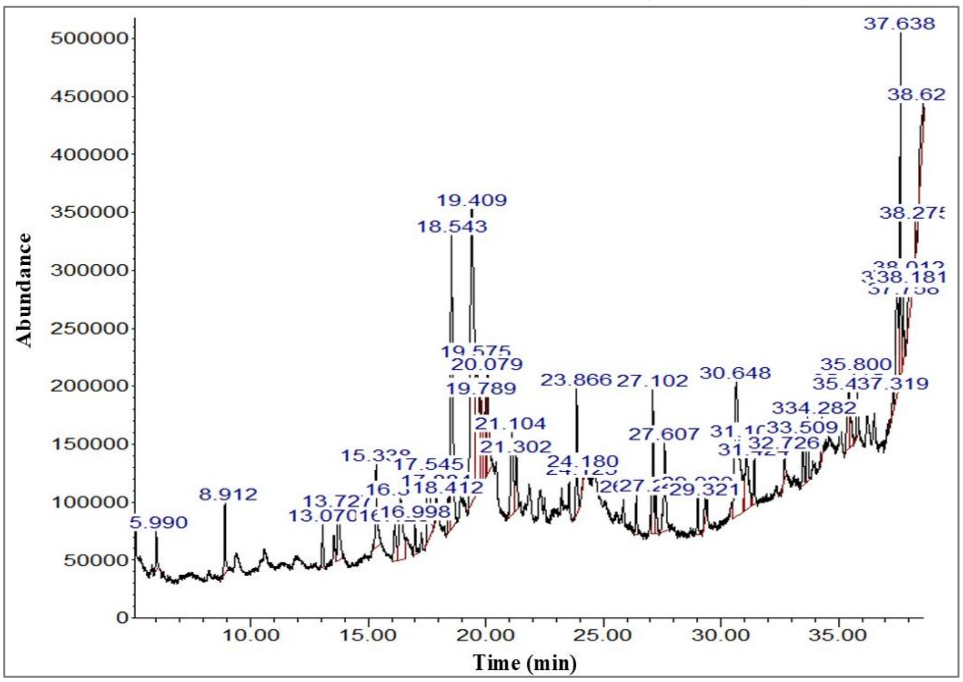

Figure 3: Total ion chromatogram showing retention time and abundance of compounds

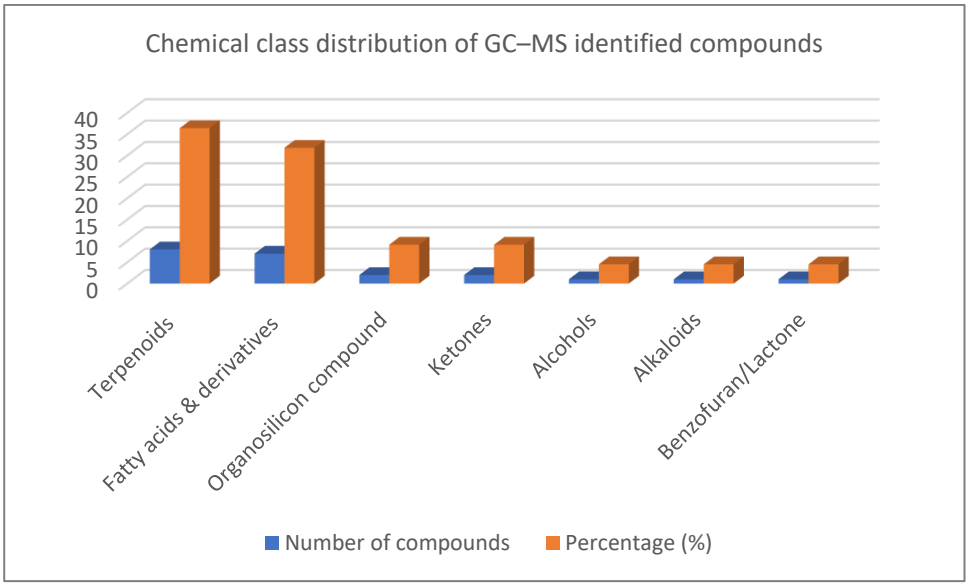

Figure 4: Bar chart showing compound classes identified in the volatile terpene-rich extract of *V. amygdalina* leaves by GC–MS analysis.

Table 4: classification of GC-MS compounds by chemical class

Chemical class	Number of compounds	Percentage (%)
Terpenoids	8	36.4
Fatty acids & derivatives	7	31.8
Organosilicon compound	2	9.1
Ketones	2	9.1
Alcohols	1	4.5
Alkaloids	1	4.5
Benzofuran/Lactone	1	4.5

The phytochemical compounds identified by GC–MS were further grouped into chemical classes, as shown in Table 4. Terpenoids constituted the largest proportion of compounds detected, followed by fatty acids and their derivatives, while other minor classes included organosilicon compounds, ketones, alcohols, and alkaloids. The distribution of these compound classes is illustrated in Figure 4.

Terpenoids are widely reported to possess significant antimicrobial, antifungal, and anti-inflammatory properties, largely due to their ability to disrupt microbial cell membranes and interfere with essential metabolic processes (Bakkali *et al.,* 2008; Guimarães *et al.,* 2019). Among the identified compounds, farnesol, an acyclic sesquiterpene alcohol, has been reported to exhibit antibacterial activity against a range of Gram-positive and

Gram-negative bacteria. Its mechanism of action involves altering membrane permeability and inhibiting biofilm formation, thereby reducing bacterial survival and virulence (Jabra-Rizk *et al.,* 2006; Gomes *et al.,* 2018).

Similarly, lavandulol, a monoterpene alcohol commonly found in essential oils, has been associated with antimicrobial activity through disruption of microbial membrane integrity and inhibition of key enzymatic systems (Burt, 2004). The presence of such compounds may contribute to the inhibitory activity observed in this study against *S. typhi*.In addition, the triterpene squalene was identified in the extract. Although primarily known for its antioxidant and pharmacological properties, squalene may also contribute to antimicrobial activity through membrane interactions and enhancement of the bioactivity of other phytochemicals (Reddy & Couvreur, 2009). The combined presence of these compounds suggests possible synergistic interactions that may enhance the extract's overall antibacterial efficacy.

The antibacterial activity observed in this study may therefore be attributed to the collective action of multiple phytoconstituents, particularly terpenoids and fatty acid derivatives. It has been reported that complex mixtures of phytochemicals often exhibit greater biological activity than isolated compounds due to synergistic effects (Bassolé & Juliani, 2012).

However, the presence of these bioactive compounds supports the antibacterial activity of the volatile terpene-rich extract of *V. amygdalina* and highlights its potential as a source of natural antimicrobial agents.

CONCLUSION

The present study demonstrated that the volatile terpene-rich extract of *V. amygdalina* exhibited antibacterial activity against *S. typhi*, with MIC and MBC values of 800 µg/ml and 1000 µg/ml, respectively. GC–MS analysis revealed the presence of 22 phytochemicals, predominantly terpenoids such as squalene, farnesol, bisabolene, and lavandulol, which may contribute to the observed antibacterial activity. These findings highlight the potential of the volatile terpene extract of *V. amygdalina* as a natural source of antibacterial agents. Further studies to isolate specific bioactive compounds and evaluate their mechanisms of action are recommended to better understand their therapeutic potential.

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