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Phytochemical Profiling, GC-MS characterization and *In-Vitro* Antibacterial Evaluation of *Boswellia dalzielii* stem Bark Extracts

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ABSTRACT

Boswellia dalzielii Hutch. (Burseraceae), is widely utilized in West African traditional medicine to manage infectious diseases, ulcers, fever, and inflammatory conditions. This study evaluated the phytochemical profile, antibacterial potency, and secondary metabolite composition of *B. dalzielii* stem bark. Successive ultrasonic-assisted extraction yielded crude extracts across non-polar to polar solvents (n-hexane, methanol, and distilled water). Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenoids, phenols, tannins, and cardiac glycosides. Antimicrobial assays using the agar well diffusion method demonstrated concentration-dependent inhibition against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Klebsiella pneumoniae*, with inhibition zone diameters ranging from 5 mm to 27 mm. Gram-positive *S. aureus* and Gram-negative *E. coli* exhibited high susceptibility to the methanolic and aqueous extracts, matching or exceeding intermediate control levels. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the bioactive methanolic extract revealed nine major phytochemical constituents, including phenolic derivatives (4-Acetoxy-3-methoxystyrene), furan derivatives (3-Methyl-2-(2-oxopropyl)furan), triazine heterocycles, quinones, and complex aromatic hydrocarbons. These identified compounds correlate directly with the observed antibacterial, antioxidant, and anti-inflammatory properties, validating the ethnopharmacological application of *B. dalzielii* bark in managing microbial infections.

Keywords: *Boswellia dalzielii*, Phytochemical screening, GC-MS analysis, Antibacterial activity, Ethnomedicine, Bioactive compounds

INTRODUCTION

Medicinal plants remain a fundamental pillar of primary healthcare systems globally, particularly within sub-Saharan Africa, where traditional phytotherapy supplies essential therapeutic remedies [1]. According to the World Health Organization, over 80% of the population in developing

nations relies on traditional plant-derived medicines for baseline therapeutic needs [2]. Secondary plant metabolites commonly termed phytochemicals function as essential natural defence mechanisms against microbial pathogens, oxidative stress, and herbivorous pests [3]. These specialized metabolites, encompassing alkaloids, flavonoids, polyphenols, terpenoids, and saponins, serve as critical structural scaffolds for modern drug discovery and semi-synthetic pharmaceutical development [4, 5].

Boswellia dalzielii Hutch. (family: Burseraceae), frequently referred to as the frankincense tree or "hararrabi" / "hano" in Hausa, is an aromatic tree widely distributed across the Sudano-Sahelian regions of West Africa [6]. Ethnobotanical surveys document extensive local rely on *B. dalzielii* bark, roots, and leaves. Decoctions of the stem bark are traditionally administered to treat peptic ulcers, gastrointestinal disorders, chronic fever, rheumatism, and skin infections, while root preparations combined with *Hibiscus sabdariffa* are employed against venereal diseases [7]. Recent pharmacological studies confirm that *Boswellia* species possess diverse bioactive properties, including anti-inflammatory, antimicrobial, hepatoprotective, and antioxidant effects, largely attributed to their rich content of pentacyclic triterpenes and volatile phenolics [8, 9].

However, the growing prevalence of multidrug-resistant (MDR) bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli,* necessitates the continuous exploration and characterization of natural plant extracts to discover novel antibacterial scaffolds [10, 11]. Although preliminary studies have explored the general bioactivity of *B. dalzielii*, detailed analytical characterization linking specific GC-MS phytochemical profiles with antimicrobial efficacy across varied solvent polarities remains limited.

Therefore, this study aimed to perform successive ultrasonic solvent extraction of *B. dalzielii* stem bark, profile its phytochemical constituents, evaluate its antibacterial efficacy against clinically relevant Gram-positive and Gram-negative pathogens, and GC-MS characterization.

MATERIALS AND METHODS

Reagents and Instrumentation

n-Hexane and methanol were purchased from BDH Chemicals Ltd, England, potassium ferricyanide, glacial acetic acid, acetic anhydride, alcoholic ferric chloride, dimethyl sulfoxide, sodium chloride, ciprofloxacin, muller Hinton agar were purchased, as designed and manufactured according to the American standard, clinical and laboratory standards institute (CLSI). All other

chemicals used are of analytical grade and purchased from BDH chemicals, England. Electronic weighing balance (Model: CS 200) was from Ohaus Corporation, USA, Whatman No 1 filter papers were from Whatman International ltd, Maidstone England, ultra-sonic cleaner (Model As3120), rotary evaporator (Model RE300), digital water bath (Model RE300DB), standard test sieve (mesh size 0.5mm), cock borer, l-shape sprider, petri-dish, micropipette, GC-MS Machine (model: Agilent technologies 7890B GC System, paired with Agilent technologies 5977A MSD) Autoclave (model DWB-280B) from Shangai Drawell Scientific Instrument Co. Ltd, China, and Vortex shaker (model:VM-20) at RS Cyclomixer in Ambala, mortar, pestle and other laboratory glass wares used were washed and sterilized.

Plant sample collection and preparation

Fresh stem bark samples of *Boswellia dalzielii* were collected from natural populations in Damaturu Local Government Area, Yobe State, Nigeria (11º 44'55" N, 11º 57'50" E). Botanical identification and authentication were conducted at the Herbarium facility of Yobe State University, Damaturu, with voucher number: YSUH111. The harvested stem bark was washed with distilled water to remove dirt, shade-dried at ambient room temperature (25 ℃) for 14 days, and pulverized into a fine powder using a mechanical wooden mortar and pestle. The powder was passed through a 0.5 mm standard mesh sieve and stored in airtight, opaque containers prior to extraction.

Successive ultra-sonic assisted extraction

About 200 g of powdered sample was weighed using an analytical weighing balance and then placed into a cleaned, dried and labelled container, in an ultrasonicator. Solvents (3), namely n-hexane, methanol and distilled water, were successively extracted in order of increasing polarity. The container, containing the sample was filled with 600 ml of n-hexane in a ratio of 3:1 and placed in an ultrasonic bath at lab temperature for 30 minutes. The extract was then filtered using Whatman No.1 filter paper, and then kept in a well-labelled container ready for recovery. The insoluble, air-dried residue was sequentially extracted using the remaining solvent namely, ethanol and distilled water. The liquid extracts were concentrated under reduced pressure at 40 ℃ using a rotary evaporator. Concentrated crude extracts were evaporated to complete dryness in a chemical fume hood, weighed to calculate extraction yields, and preserved at cool temperature.

Qualitative phytochemical screening

Phytochemical screening of stem bark of *Boswellia dalzielii* were carried out using slight modification of the standard procedures outlined by Tiwari *et al* [13] Sabri *et al* [12] and Solomons *et al.* [14].

Test for alkaloids

The extract (0.1 g) was dissolved in 2 ml of 2 N HCl and filtered. A few drops of Mayer's reagent (prepared by dissolving 1.38 g of mercury chloride in 60 ml of water and poured into a solution of 5 g of KI in 10 ml of water, made up to 100 ml with distilled water) were added. The presence of white (turbid) precipitate indicates the presence of alkaloids [12].

Test for flavonoids

The extract (0.1 g) was dissolved in 2 ml of methanol was treated with few drops of sodium hydroxide solution. The formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids [13].

Test for saponins

Foams test: The extract (0.1 g) was shaken vigorously with 2 ml of water. The formation of foams which persisted for over ten minutes indicates the presence of saponins [13].

Test for terpenoids (Solkowski's test)

About 1 ml of chloroform was added to 2 ml of the extract in methanol followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicates the presence of terpenoids [14].

Test for tannins (Braymer's test)

About 2 ml of the extract (0.2 g dissolved in 2 ml of methanol) and was treated with 10% alcoholic ferric chloride solution. Formation of blue/greenish coloration indicates the presence of tannins [14].

Test for sterols (Libermann-Burchard test): About 1 ml extract (0.2 g dissolved in 1 ml of methanol) was treated with few drops of chloroform, acetic anhydride and concentrated sulphuric acid respectively. The formation of dark pink or red colour indicates the presence of sterols [14].

Test for phenols

A fraction of the extract was treated with aqueous 5% ferric chloride solution. The formation of deep blue or black colour indicates the presence of phenols [14].

Test for cardiac glycosides (Keller- kelliani's test)

About 5 ml of each extract (0.2 g dissolved in 5ml methanol) was treated with 2 ml of glacial acetic acid in a test tube and a drop of 2% ferric chloride solution was added to it. This was carefully underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer a greenish ring may form [13].

Antibacterial sensitivity assay

Test bacterial microorganism

The antibacterial activity was evaluated against four pathogenic bacterial strains: Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) and the antibacterial sensitivity test was determined according to method described by Nostro et al. [15].

Preparation of innocula and test solution

Bacterial suspensions were standardized to a 0.5 McFarland turbidity standard (approx 1.5 X 10^8 CFU/mL). Stock solutions of crude extracts (1000 mg/mL) were prepared by dissolving 1.0 g of extract in 10 mL of 20% dimethyl sulfoxide (DMSO). Serial twofold dilutions yielded test concentrations of 300 mg/mL, 150 mg/mL, 75 mg/mL, and 37.5 mg/mL.

Agar well diffusion assay

Mueller-Hinton Agar plates were prepared and inoculated uniformly across the surface using sterile cotton swabs. Wells of 6.0 mm diameter were bored into the agar using a sterile cork borer. Aliquots (100 mL) of each extract concentration were introduced into designated wells. Ciprofloxacin (5 mg/mL) served as the positive control, while 20% DMSO served as the negative control. Assay plates were incubated at 37 ℃ for 24 h. Antibacterial potency was evaluated by measuring the clear zones of inhibition (mm) around each well using a digital caliper [15]

GC-MS analysis

The bioactive methanol extract was subjected to GC-MS analysis using an Agilent 7890B Gas Chromatograph coupled with an Agilent 5977A Mass Selective Detector (MSD).

Column: HP-5MS capillary column (30m × 0.25 mm internal diameter, 0.25 µm film thickness).

Carrier Gas: High-purity Helium at a constant flow rate of 1.0 mL/min.

Temperature Program: Initial oven temperature set at 60 ℃ (held for 2 min), ramped to 280 ℃ at 10 ℃/min, and held final for 10 min.

Injection & MS Conditions: Injector temperature 250 ℃, split ratio 10:1, electron ionization (EI) source at 70 eV, mass scan range m/z 35--500.

Identification: Phytocompounds were identified by comparing mass spectra retention times and fragmentation patterns with entries in the National Institute of Standards and Technology (NIST14) spectral library.

RESULTS AND DISCUSSION

Phytochemical screening

Qualitative screening confirmed the distribution of diverse bioactive secondary metabolites across solvent polarities (Table 1).

Table 1: Qualitative phytochemical composition of *Boswellia dalzielii* stem bark extracts.

S/N	Phytochemicals	n-Hexane	Methanol	Aqueous
1.	Alkaloids	-	+	+
2.	Flavonoids	+	+	+
3.	Saponins	-	+	+
4.	Terpenoids	+	+	+
5.	Sterols	-	-	-
6.	Tannins	+	+	-
7.	Phenols	+	+	+
8.	Cardiac glycosides	-	-	+

Key: **(+) = Present and (-) = Absent**

The universal occurrence of flavonoids, terpenoids, and phenols across all solvent fractions highlights the rich phenolic and lipophilic constitution of *B. dalzielii*. Methanol extracted the

broadest spectrum of bioactive compounds (alkaloids, flavonoids, saponins, terpenoids, tannins, phenols), consistent with findings by [16] and [17]. Phenolic compounds and flavonoids act as primary antioxidants and membrane-disrupting antibacterial agents [18, 19]. Tannins contribute to protein precipitation, wound healing, and anti-ulcer activities, providing a scientific basis for the traditional use of *B. dalzielii* bark in wound care [20, 21].

Antibacterial sensitivity

The antibacterial activities of the crude extracts against Gram-positive and Gram-negative isolates were concentration-dependent (Table 2).

Table 2: Antibacterial activity (Zone of Inhibition, mm) of *B. dalzielii* stem bark extracts.

Extracts	**Conc.mg/ml**	**Organism**	**Bacteria zone of inhibition mm/dm**		
		Escherichia coli	***Klebsiella peumoniae***	***Staphylococcus epidermis***	***Staphylococcus aureus***
n-hexane	300	15	15	17	17
	150	13	10	15	11
	75	10	NA	12	9
	37.5	10	NA	10	NA
STD Ciprofloxacin	5	30	20	20	25
Methanol	300	22	15	14	20
	150	15	10	10	15
	75	NA	NA	NA	10
	37.5	NA	NA	NA	8
STD Ciprofloxacin	5	20	30	20	25
Distilled water	300	22	17	10	27
	150	20	12	8	15
	75	15	NA	NA	13
	37.5	10	NA	NA	10
STD Ciprofloxacin	5	20	20	20	25

Key: STD= standard drug. NA = No activity

The aqueous extract (300 mg/mL) demonstrated peak inhibitory potency against Gram-positive *S. aureus* (27 mm), exceeding the benchmark inhibitory zone of the reference drug Ciprofloxacin (25 mm). Similarly, both the aqueous and methanolic extracts exhibited notable potency against Gram-negative *E. coli* (22 mm at 300 mg/mL). Gram-positive bacteria (*S. aureus*) displayed greater overall susceptibility than Gram-negative strains (*K. pneumoniae*), reflecting structural differences

in their bacterial cell walls. The outer lipopolysaccharide membrane of Gram-negative species typically restricts hydrophobic drug penetration [11, 22]. These results align with findings by Muhammad *et al* [23] regarding the broad-spectrum antibacterial activity of West African *Boswellia* species.

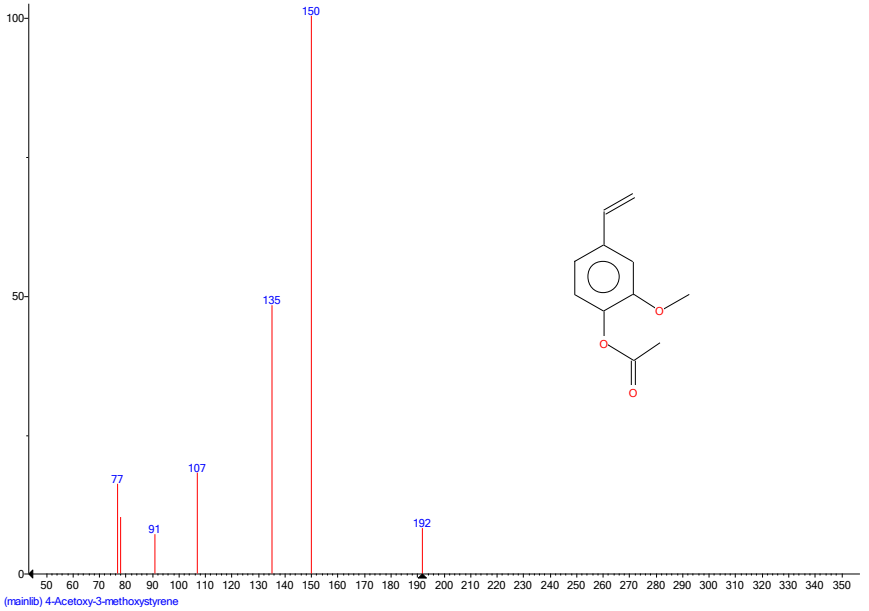
GC-MS compound identification and bioactivity profile

GC-MS analysis of the bioactive methanol extract identified nine prominent volatile phytocompounds (Table 3).

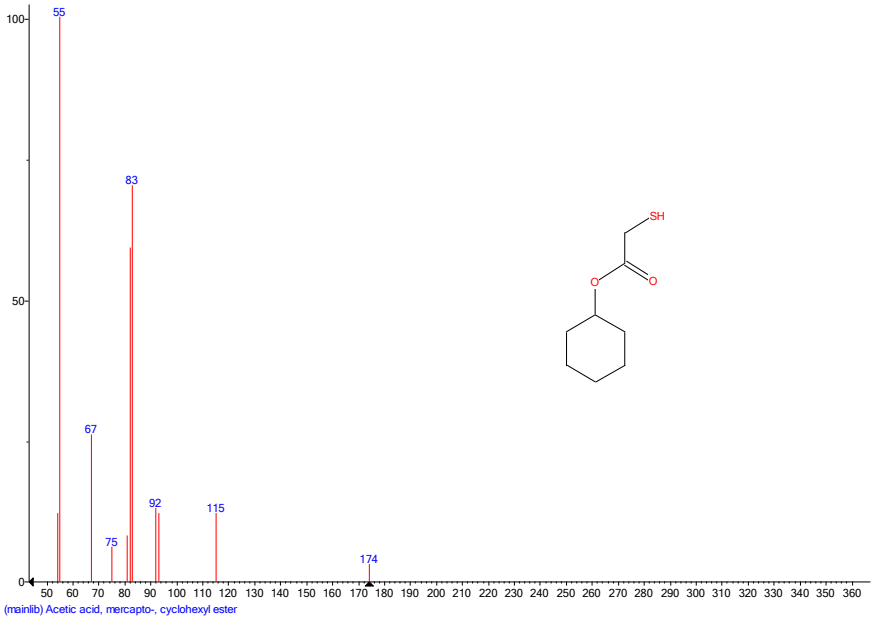
Table 3: GC-MS spectral identification of phytochemicals in the methanol extract of *B. dalzielii.*

S/N	Compound Name	Molecular Formular	Molecular Weight (g/mol)	Retention Time (min)	Reported Biological activities
1.	4-Acetoxy-3-methoxystyrene	$C_{11}H_{12}0_3$	192	5.9	Antimicrobial, Antioxidant.
2.	Acetic acid, mecarpto-cyclohexyl ester	$C_8H_{14}O_2S$	174	8.6	Antibacterial, Antifungal.
3.	3-Methyl-2-(-2-oxopropyl) furan	$C_8H_{10}O_2$	138	10.24	Antioxidant, Anti pyretic, Bactricide.
4.	2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine	$C_5H_7N_3OS$	157	10.98	Antibacterial, Antinflammatory.
5.	Culmorin	$C_{15}H_{26}O_2$	238	11.68	Fungal metabolitel/ phytomodulator
6.	3,5-Cyclohexadine-1,2-dione, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{20}O_2$	220	12.99	Antimicrobial, cytotoxic.
7.	Benzeneethanamine, 2,6-trimethyl	$C_{11}H_{17}N$	163	22.16	Vasomodulatory, Cardiovascular agent.
8.	1,2-Dibenzoyl-3-phenylcyclopropane	$C_{23}H_{18}O_2$	326	22.16	Antitumor, Antimalarial
9.	1H-Indene, 2,3-dihydro-1, 1,3-trimethyl-3-phenyl	$C_{18}H_{20}$	236	22.16	Antibiotic, Synthetic intermediate

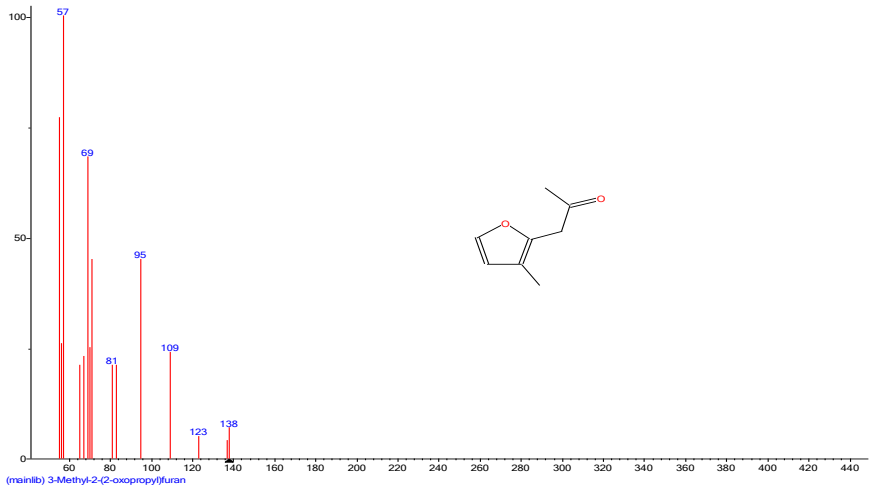
Figures 1-9 are the spectra for the identified compounds which were obtained from the GC-MS machine.



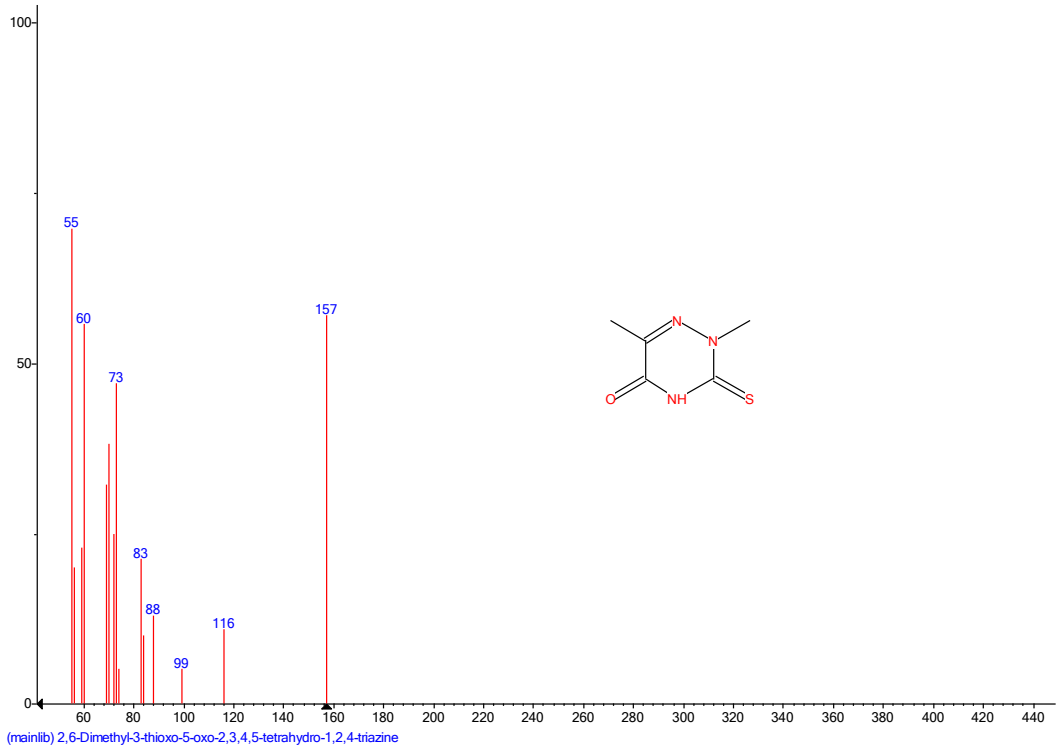
Name: 4-Acetoxy-3-methoxystyrene, Formula: $C_{11}H_{12}O_3$, MW: 192
Exact Mass: 192.078644, Retention Time: 5.9 min
Fig. 1: MS suggested spectrum of 4-Acetoxy-3-methoxystyrene



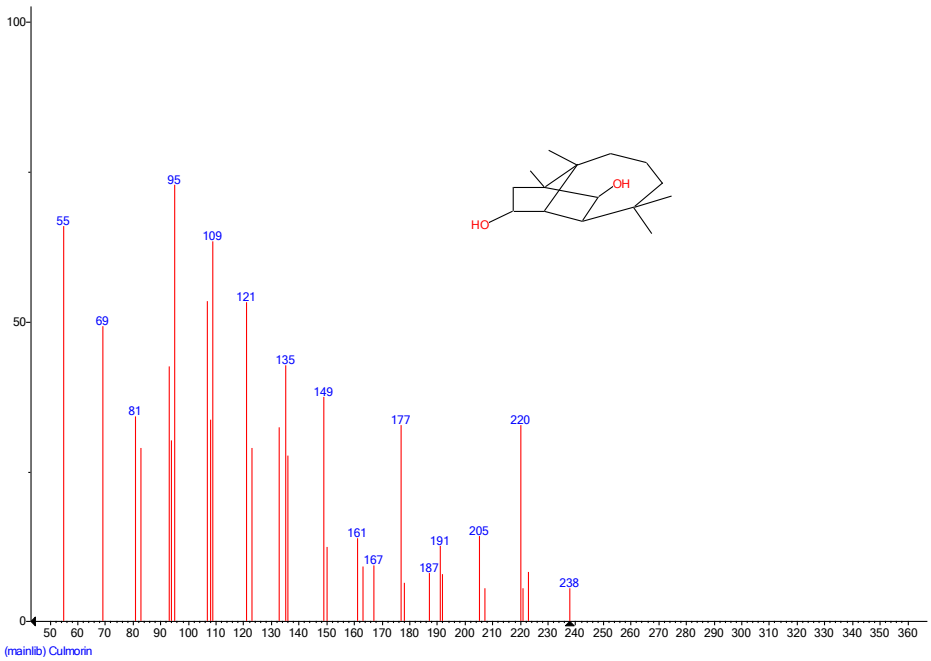
Name: Acetic acid, mercapto-, cyclohexyl ester, Formula: $C_8H_{14}O_2S$
MW: 174 Exact Mass: 174.07145, Retention Time: 8.6 min
Fig. 2: MS suggested spectrum of Acetic acid, nercapto-, cyclohexyl ester



Name: 3-Methyl-2-(2-oxopropyl)furan, Formula: $C_8H_{10}O_2$
MW: 138 Exact Mass: 138.06808, Retention Time: 10.24 min
Fig. 3: MS suggested spectrum of 3-Methyl-2-(2-oxopropyl)furan



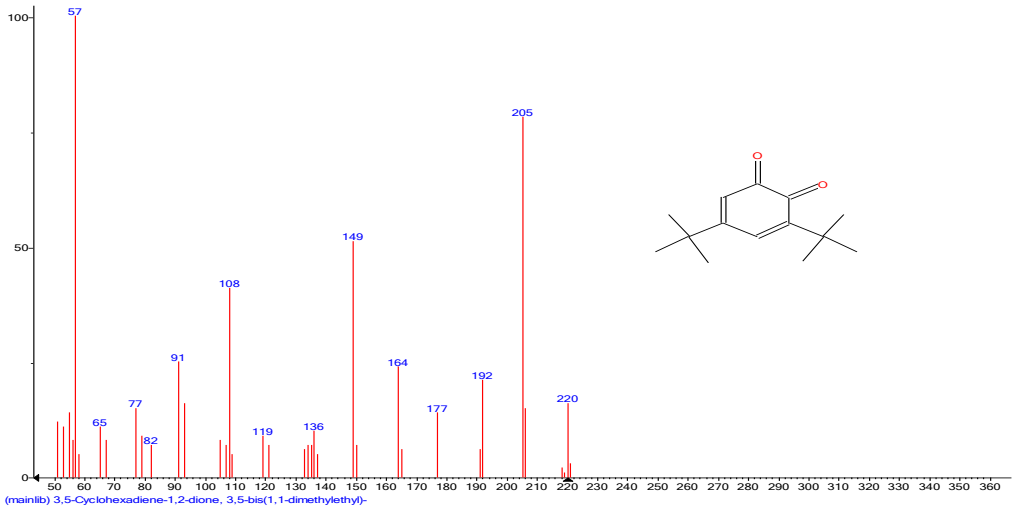
Name: 2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine, Formula: $C_5H_7N_3OS$
MW: 157 Exact Mass: 157.030983, Retention Time:10.98 min
Fig. 4: MS suggested spectrum of 2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine



Name: Culmorin, Formula: $C_{15}H_{26}O_2$, MW: 238 Exact Mass: 238.19328, Retention Time: 11.68 min

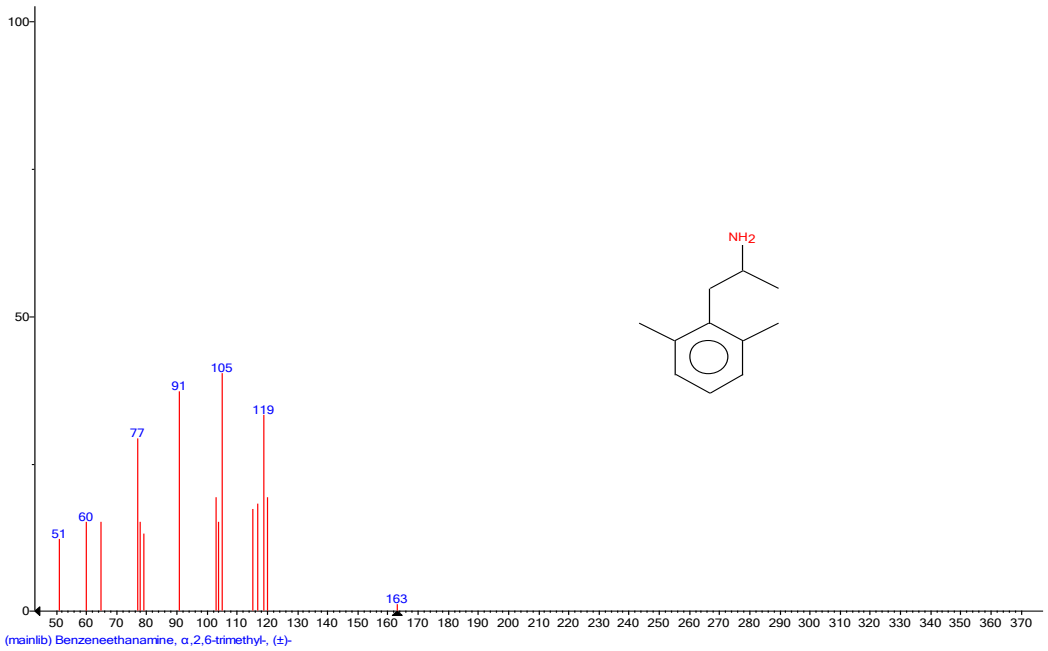
Fig. 5: MS suggested spectrum of Culmorin

12.99 min



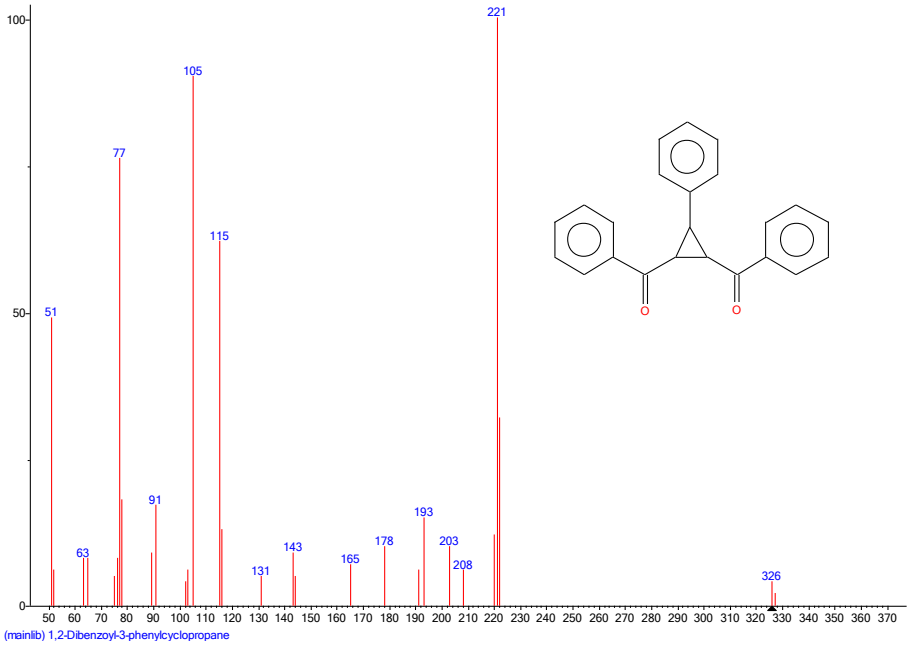
Name: 3,5-Cyclohexadiene-1,2-dione, 3,5-bis(1,1-dimethylethyl), Formula: $C_{14}H_{20}O_2$ MW: 220 Exact Mass: 220.14633, Retention Time: 12.99 min

Fig. 5: 3,5-Cyclohexadiene-1,2-dione, 3,5-bis(1,1-dimethylethyl)-



Name: Benzeneethanamine, α,2,6-trimethyl-, (±)-Formula: $C_{11}H_{17}N$MW: 163 Exact Mass: 163.1361, Retention Time: 22.16 min

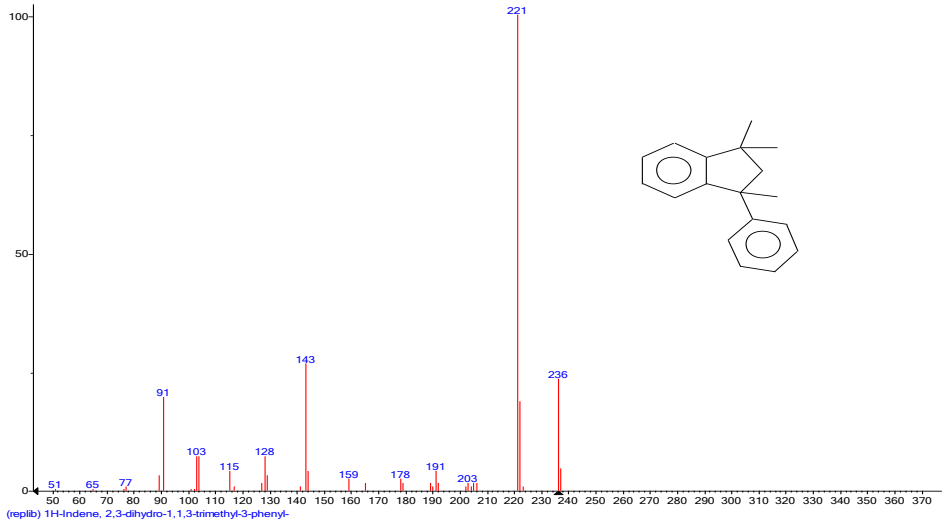
Fig. 7: MS suggested spectrum Benzeneethanamine, 2,6-trimethyl



Name: 1,2-Dibenzoyl-3-phenylcyclopropane, Formula: $C_{23}H_{18}O_2$

MW: 326 Exact Mass: 326.13068, Retention Time: 22.16 min

Fig. 8: MS suggested spectrum of 1,2-Dibenzoyl-3-phenylcyclopropane



Name: 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-, Formula: $C_{18}H_{20}$
MW: 236 Exact Mass: 236.156501, Retention Time: 22.16 min
Fig. 9: MS suggested spectrum of 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-

The identified compounds include structural classes known for therapeutic efficacy:

1. **Phenolics and Furan Derivatives:** 4-Acetoxy-3-methoxystyrene and 3-Methyl-2-(2-oxopropyl)furan disrupt microbial cell membranes, leading to cell lysis [19].
2. **Sulfur and Nitrogen Heterocycles:** Triazine derivatives (2,6-Dimethyl-3-thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine) possess documented anti-inflammatory and antibacterial properties [24].
3. **Aromatic Hydrocarbons and Amines:** Benzeneethanamine derivatives exhibit vasomodulatory activities, providing a plausible mechanism for the traditional use of *B. dalzielii* in managing palpitations and hypertension [16].

CONCLUSION

This study confirms that *Boswellia dalzielii* stem bark contains diverse bioactive secondary metabolites with broad-spectrum antibacterial activity. Methanol and aqueous extracts demonstrated inhibition against opportunistic pathogens, including *S. aureus* and *E. coli*. GC-MS profiling identified key phenolic, heterocyclic, and aromatic compounds that likely drive these pharmacological properties. Overall, these findings provide a scientific rationale for the traditional use of *B. dalzielii* in managing microbial infections, fever, and inflammatory conditions, supporting its potential as a source for natural antimicrobial drug discovery.

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