



Antimicrobial Activity of Volatile and Non-Volatile Extracts of Hyptis Suaveolens Leaves

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ABSTRACT:

Medicinal plants serve as valuable reservoirs of bioactive compounds with therapeutic potential, offering alternatives in the fight against drug-resistant infections. *Hyptis suaveolens* (L.) Poit., though widely used in traditional medicine, remains underexplored despite reports of antimicrobial, anti-inflammatory, and other pharmacological activities.

Given the global need for novel antimicrobials, this study investigated the volatile (essential oils) and non-volatile (methanolic) extracts of *H. suaveolens* leaves, aiming to assess their antimicrobial activity against selected bacterial and fungal pathogens. Fresh *Hyptis suaveolens* leaves were collected and authenticated at Adekunle Ajasin University, Nigeria. Essential oils were obtained via hydrodistillation, while methanolic extracts were prepared by maceration. Antimicrobial activity against clinical isolates (*E. coli*, *P. aeruginosa*, *B. cereus*, *K. pneumoniae*, and *C. albicans*) was assessed using agar well diffusion, with MIC and MBC determined through macro-broth dilution techniques. Hydrodistillation of fresh leaves yielded 2.7% essential oil, while methanol extraction of dried leaves produced 1.5% crude extract. Both extracts inhibited all six clinical isolates, with the methanolic extract showing greater efficacy. The highest inhibition zone was recorded against *Candida albicans* (32 mm), and the lowest against *Staphylococcus aureus* (27 mm).

Minimum inhibitory concentration (MIC) testing revealed consistent activity of the methanolic extract across all concentrations, whereas the

essential oil displayed variable inhibition. Minimum bactericidal concentration (MBC) assays confirmed bactericidal activity of both extracts, with *E. coli* and *Pseudomonas aeruginosa* eradicated at 25–50 mg/mL, while *Klebsiella pneumoniae* required the highest concentration (12.5 mg/mL). *Hyptis suaveolens* extracts exhibited broad-spectrum antimicrobial and bactericidal activity, with the methanolic extract showing superior potency, highlighting its potential as a natural alternative antimicrobial source.

KEYWORDS:

Hyptis suaveolens; antimicrobial activity; essential oil; methanolic extract; minimum inhibitory concentration.

1. Introduction

Medicinal plants, formally recognized for containing bioactive substances useful in disease treatment, have been integral to healthcare for millennia [1,2]. Derivation of therapeutic agents from plants has attracted global attention due to their perceived safety, accessibility, and cost-effectiveness. Herbal medicines are typically prepared from leaves, roots, bark, seeds, or flowers and administered via oral, inhalation, or topical routes [3]. Beyond their cultural and historical relevance, medicinal plants remain a cornerstone for drug discovery and development, serving as reservoirs of bioactive compounds with proven pharmacological potential [4].

The therapeutic activities of medicinal plants are primarily attributed to their phytochemical

constituents such as alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and essential oils. These natural compounds not only contribute to traditional healing practices but also form the structural backbone of many modern pharmaceuticals [5]. Historically, the medicinal use of plants emerged through empirical observation, where beneficial and toxic species were distinguished through trial and error. Knowledge was preserved through generations, forming the basis of traditional medicine across diverse cultures. Despite the scientific progress of synthetic chemistry, there remains a strong interest in plant-based bioactive compounds, both for their direct therapeutic use and as lead molecules for novel drug design [6].

Hyptis suaveolens (L.) Poit., commonly referred to as “mosquito plant,” represents one such species of significant ethnomedicinal value. Native to tropical and subtropical regions, *H. suaveolens* is often considered a pervasive weed but has been documented to possess diverse pharmacological properties. Its leaves are rich in pharmacologically active secondary metabolites with antispasmodic, anti-colic, anti-rheumatic, and anti-fertility effects [7]. Additional reports highlight sedative, diuretic, aromatic, anti-inflammatory, anti-pyretic, anti-catarrhal, anti-rheumatic, anti-soporific, and anti-cancer activities [8]. The essential oils of *H. suaveolens* have demonstrated antimicrobial and antifungal properties, while the roots contain ursolic acid, a triterpenoid with documented anti-retroviral potential through inhibition of retroviral integrases and proteases [9,10,11]. Phytochemical studies of the leaves have revealed alkaloids as the dominant metabolites, followed by tannins and saponins [12].

Although *H. suaveolens* is widely used in traditional medicine, its chemical diversity and antimicrobial potential remain underexplored compared to other medicinal plants of global relevance. Reports on its essential oil profile and non-volatile metabolites are still limited, particularly in relation to their activity against clinically important pathogens. This represents a significant knowledge gap, given the urgent global demand for alternative antimicrobial agents in the face of rising antimicrobial resistance.

Here, we investigate the volatile and non-volatile constituents of *H. suaveolens* leaves and evaluate their antimicrobial activities against selected pathogens. Specifically, essential

oils were extracted by hydrodistillation using a Clevenger apparatus, while methanol was employed for solvent extraction of non-volatile compounds. The antimicrobial potential of both extracts was subsequently tested to establish their comparative efficacy.

2. Materials and Methods

Plant Material

Fresh leaves of *Hyptis suaveolens* were collected from the campus of Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria. The plant was authenticated by Dr. Obembe, Department of Plant Science and Biotechnology, Adekunle Ajasin University, and a voucher specimen was deposited in the departmental herbarium.

Extraction of Plant Constituents

Essential Oil Extraction

Volatile oils were extracted from 250 g of fresh leaves by hydrodistillation using a Clevenger-type apparatus for 3 h, following standard protocols [9]. The oil was dried over anhydrous sodium sulfate and stored at 4 °C in airtight vials until use.

Methanol Extraction

Powdered air-dried leaves (50 g) were extracted with 200 mL of methanol by maceration for 72 h. The extract was filtered (Whatman No. 1) and concentrated under reduced pressure at 40 °C using a rotary evaporator (Büchi, Switzerland). The dried extract was stored at 4 °C in sterile containers.

Test Microorganisms

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Candida albicans* were obtained from the Microbiology Laboratory of Adekunle Ajasin University. The strains were subcultured on nutrient agar and maintained at 4 °C until use.

Standardization of Inoculum

The bacterial inocula were standardized to a 0.5 McFarland turbidity standard, corresponding to $\sim 1.5 \times 10^8$ CFU/mL, by adjusting the turbidity of 24 h broth cultures with sterile saline [17].

Preparation of Extract Solutions

Stock solutions (100 mg/mL) of the methanol extract were prepared in 30% dimethyl sulfoxide (DMSO) and sterile distilled water (1:3, v/v). Two-fold serial dilutions yielded working concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/mL. Essential oil dilutions were prepared similarly.

Antimicrobial Susceptibility Testing

The antimicrobial activity of extracts was evaluated using the agar well diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. Mueller–Hinton agar was seeded with standardized inocula of test organisms, and wells (5 mm diameter) were bored aseptically. Each well received 100 μ L of extract solution at different concentrations. Erythromycin (10 μ g/mL) served as the positive control, while solvent served as the negative control. Plates were incubated at 37 °C for 24 h, and the zones of inhibition were measured in millimeters.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MICs were determined by macro-broth dilution following the method of Parvekar et al. (2020) with slight modifications. Serial dilutions of extracts (50–3.125 mg/mL) were prepared in Mueller–Hinton broth. MIC was defined as the lowest concentration with no visible growth after 24 h incubation at 37 °C. To determine MBC, aliquots from MIC tubes showing no visible growth were plated onto Mueller–Hinton agar. MBC was recorded as the lowest concentration, yielding no colony growth after incubation. For *Candida albicans*, Sabouraud dextrose broth and agar were used.

3. Results and Discussion

Chemical Composition of *Hyptis suaveolens* Extracts

The chemical composition of essential oils obtained from *Hyptis suaveolens* (L.) Poit. has been reported by several studies, showing marked variation across geographic regions and even within countries. The species is widely distributed in tropical and subtropical zones of Asia, the Americas, and Africa, and at least seven major chemotypes have been identified,

including 1,8-cineole, sabinene, β -caryophyllene, eugenol, aromadendrene/alloaromadendrene, fenchone, and menthol types [13].

Comparisons among samples from Cameroon, India, and Nigeria illustrate both inter- and intra-country differences in oil profiles (Table 2). In many Indian and Nigerian collections, monoterpene hydrocarbons predominate, ranging from 32.5–64.1% and 22.9–55.5%, respectively [14]. In contrast, specific populations show divergent trends: leaves from Dehra Dun (India) contained higher oxygenated sesquiterpenes ($\approx$ 22.3%), while Cameroonian samples typically yielded 31.2–38.2% oxygenated sesquiterpenes [13].

Within Nigeria, compositional diversity is evident. Oils from Ibadan and Lagos share only a limited set of common compounds (limonene, linalool, p-cymene, α -pinene, and α -phellandrene). The Lagos specimen, as described by Tonzibo et al. (2009), may represent a distinct chemotype, with unusually high α -pinene (13.6%) and p-cymene (11.7%) levels. Similarly, Chung et al. (2020) reported that juvenile leaves contained elevated concentrations of β -caryophyllene (22.3%), α -phellandrene (10.6%), and caryophyllene oxide (10.3%), suggesting that both leaf age and local environmental conditions strongly influence oil composition. These findings highlight the chemical plasticity of *H. suaveolens*, shaped by geography, genotype, phenology, and environmental factors such as climate and soil.

In the present study, hydrodistillation of fresh leaves yielded 2.7% (w/w) essential oil, characterized as a dark green semi-viscous liquid, while methanol extraction of air-dried leaves gave 1.5% (w/w) crude extract, appearing as a green liquid (Table 1). These yields are consistent with reported values but may vary according to extraction technique, plant maturity, and origin, underscoring the importance of standardized collection and processing methods when comparing results across studies.

Table 1: Physicochemical properties and yields of *Hyptis suaveolens* leaf extracts

Extraction method	Yield (%)	Colour	Appearance
Hydrodistillation (fresh leaves)	2.7	Dark green	Semi-viscous liquid
Methanol solvent extraction (air-dried leaves)	1.5	Green	Liquid

Table 2: Representative compounds identified in the essential oils of *Hyptis suaveolens* collected from Cameroon, India, and Nigeria

Compound	Cameroon (Nga)	Cameroon (Yao)	India (Kum)	India (Luc)	Nigeria (Iba)	Nigeria (Lag)
ar-Abietadiene	–	–	–	–	–	0.3
ar-Abietatriene	9.6	–	4.4	–	–	–
ar-Abietatrienol	–	–	0.4	–	–	–
neo-Abietol	–	–	–	–	0.2	–
Acetophenone	–	–	–	–	–	0.2
allo-Aromadendrene	0.4	1.3	–	–	–	–
Aromadendrene	–	3.5	–	–	–	–
Atiserene	0.6	–	–	–	–	–
Bergamotol	–	6.2	–	–	–	–
trans- α -Bergamotene	–	–	–	–	–	5.2
trans- α -Bergamotene	–	–	–	–	–	19.8
trans- α -Bergamotol	–	–	–	0.3	–	1.9
(Z)-trans- α -Bergamotol	12.8	–	2.0	–	–	–

Nga. = Ngaoundere; Yao. = Yaoundé; Kum. = Kumaun; Luc. = Lucknow; Iba. = Ibadan; Lag. = Lagos

Antimicrobial Activity: Zone of Inhibition

Both the methanolic extract and essential oil of *Hyptis suaveolens* demonstrated inhibitory activity against all six tested clinical isolates. The methanolic extract consistently showed higher antimicrobial efficacy than the essential oil (Table 3). The strongest activity was observed against *Candida albicans* (32 mm zone of inhibition), while the weakest effect was noted against *Staphylococcus aureus* (27 mm). These results corroborate earlier findings by Pachkore et al. (2011), who also reported broad-spectrum activity of *H. suaveolens* extracts.

The superior performance of the methanolic extract relative to the essential oil likely reflects the higher solubility and wider range of bioactive phytochemicals recovered by methanol compared with volatile oil distillation. Among the tested organisms, *Pseudomonas aeruginosa* exhibited the lowest susceptibility, particularly to the essential oil, which is consistent with its known intrinsic resistance mechanisms such as biofilm formation, efflux activity, and encapsulation [21,22]. Overall, the results highlight that *H. suaveolens* contains compounds with notable antimicrobial activity, with methanolic extracts being especially potent against yeast and Gram-negative bacteria.

Table 3: Zone of inhibition (mm) of clinical isolates exposed to methanolic extract and essential oil of *Hyptis suaveolens*

Isolate	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Bacillus cereus</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>
Essential oil	24	20	23	21	20	22
Methanolic extract	28	29	32	30	30	27
Control	30	28	32	50	30	32

Sensitive (S $\geq$ 25 mm); Intermediate (I = 20–24 mm); Resistant (R $\leq$ 19 mm)

Minimum Inhibitory Concentration (MIC)

The methanolic extract of *Hyptis suaveolens* inhibited all six test organisms at every concentration tested, including the lowest level (3.125 mg/mL), indicating strong and

consistent antimicrobial potency (Table 4). In contrast, the essential oil displayed variable inhibition across the organisms (Table 5). *E. coli* and *Pseudomonas aeruginosa* were inhibited at higher concentrations ($\geq$ 12.5 mg/mL) but not at the lowest levels. *Candida albicans*

and *Staphylococcus aureus* were inhibited at 25–50 mg/mL, while *Bacillus cereus* and *Klebsiella pneumoniae* required relatively higher concentrations compared with the methanolic extract. Overall, the methanolic extract demonstrated broader and more consistent inhibitory activity than the essential oil. These findings align with earlier studies [22,23], which reported stronger antimicrobial performance of polar solvent extracts compared to essential oils. Variations in susceptibility across organisms may reflect structural differences in microbial cell walls and membranes.

Table 4: Minimum inhibitory concentration (MIC) of the methanolic extract of *Hyptis suaveolens*

Isolate	50	25	12.5	6.25	3.125
<i>E. coli</i>	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+
<i>Candida albicans</i>	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	+	+	+
<i>Klebsiella pneumoniae</i>	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+

+ = Growth observed, – = No growth observed

Table 5: Minimum inhibitory concentration (MIC) of the essential oil of *Hyptis suaveolens*

Isolate	50	25	12.5	6.25	3.125
<i>E. coli</i>	+	+	+	–	–
<i>Pseudomonas aeruginosa</i>	+	+	+	–	–
<i>Candida albicans</i>	+	+	+	–	–
<i>Bacillus cereus</i>	+	+	–	–	–
<i>Klebsiella pneumoniae</i>	+	+	–	–	–
<i>Staphylococcus aureus</i>	+	+	+	–	–

+ = Growth observed, – = No growth observed

Minimum Bactericidal Concentration (MBC)

In this study, the methanolic extract of *Hyptis suaveolens* demonstrated bactericidal activity against the tested clinical isolates. Complete eradication was observed for *E. coli* and *Pseudomonas aeruginosa* at 50 and 25 mg/L. *Candida albicans* required 12.5 mg/L

for bactericidal effect, while *Bacillus cereus* was eliminated at 50 and 25 mg/L. *Klebsiella pneumoniae* succumbed only at 12.5 mg/L, and *Staphylococcus aureus* was killed at 50 and 25 mg/L. Similarly, the essential oil showed bactericidal effects, with *E. coli* and *P. aeruginosa* eradicated at 50 and 25 mg/L. *Candida albicans* and *Staphylococcus aureus* required 12.5 mg/L, while *B. Cereus* was eliminated at 50 and 25 mg/L. *K. pneumoniae* was the least susceptible, responding only at the higher concentrations. These variations in MBC values highlight differences in microbial susceptibility, likely influenced by structural and physiological features such as cell wall composition and membrane permeability [24]. Overall, both the methanol extract and the essential oil of *H. suaveolens* exhibited promising bactericidal potential across bacterial and fungal pathogens, though activity was strain-dependent.

Table 6: Minimum bactericidal concentration (MBC) of the essential oil of *Hyptis suaveolens*

Isolate	50	25	12.5	6.25	3.125
<i>E. coli</i>	–	–	+	+	+
<i>Pseudomonas aeruginosa</i>	–	–	+	+	+
<i>Candida albicans</i>	–	–	–	+	+
<i>Bacillus cereus</i>	–	–	–	+	+
<i>Klebsiella pneumoniae</i>	–	–	+	+	+
<i>Staphylococcus aureus</i>	–	–	–	+	+

+ = Growth observed, – = No growth observed

Table 7: Minimum bactericidal concentration (MBC) of the methanolic extract of *Hyptis suaveolens*

Isolate	50	25	12.5	6.25	3.125
<i>E. coli</i>	–	–	+	+	+
<i>Pseudomonas aeruginosa</i>	–	–	+	+	+
<i>Candida albicans</i>	–	–	–	+	+
<i>Bacillus cereus</i>	–	–	–	+	+
<i>Klebsiella pneumoniae</i>	–	–	–	+	+
<i>Staphylococcus aureus</i>	–	–	–	+	+

+ = Growth observed, – = No growth observed

4. Conclusion

The present study demonstrates that *Hyptis suaveolens* contains diverse bioactive compounds with significant antimicrobial activity. Both methanolic and essential oil extracts inhibited and killed a range of bacterial

and fungal pathogens, with the methanolic extract showing superior efficacy and a broader spectrum. These findings support the potential of *H. suaveolens* as a source of natural antimicrobial agents, though activity varied by strain and warrants further pharmacological evaluation.

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