PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL EFFICACY OF *HYPTIS SUAVEOLENS* (L) POIT

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Abstract
Medicinal plants remain a prolific source of secondary metabolites and a potent source of antimicrobial agents. Researchers around the world are screening the antimicrobial compounds present in medicinal plants, as they are being used in traditional healthcare systems. The plant *Hyptis suaveolens* is an important medicinal plant that is widely distributed in different parts of the world including Nigeria and other African countries. In this study the phytochemical screening and antimicrobial activities of the methanolic leaf extract of *Hyptis suaveolens* was investigated. The quantitative and qualitative phytochemical screening was determined using standard methods, the antimicrobial activities of the methanol leaf extract of *H. suaveolens* was evaluated using the standard *in-vitro* methods against six microorganisms; *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, A. niger* and *A. flavus*. The result of the phytochemical screening revealed the presence of secondary metabolites (alkaloids, flavonoids, tannins, phenols and terpenoids) with the quantitative estimation showing phenols as the highest yield (14.74±0.08). The result showed a clear zone of inhibition both on the test microorganisms. The sensitivity result showed that the methanol leaf extract had the highest activity on *Salmonella typhi* with mean zones of inhibition in millimeter of 28.67±4.61and for the fungi *A. niger* recorded a wider zone of inhibition 28.33± 3.21 than *A. flavus*. There was a dose-dependent increase in antimicrobial activity across all the test organisms. The minimum inhibitory concentration (MIC) ranged from 6.25- 25 mg/ml. This research has shown that that the methanol leaf extract of *Hyptis suaveolens* possess bioactive compounds, which are responsible for the antimicrobial activity thus justifies its use in traditional medicine.
Keywords: antimicrobial, phytochemical, inhibition, medicinal, bioactive compounds

1.0 Introduction

Medicinal plants used in traditional healing practice have increasingly drawn the interest of Researchers and the pharmaceutical industries. Such plants have been shown to possess various activities including but not limited to antibacterial, antifungal, anti-viral, antioxidant and anti-inflammatory activities (Shikwambi *et al*., 2021). This activity has been attributed to the plant's ability to produce secondary metabolites.

The antimicrobial potential of herbal remedies is attributed to their rich content of bioactive constituents such as alkaloids, steroids, flavonoids, phenols, phlobatannins, tannins andsaponins which elicit a variety of physiological and pharmacological effects in the body (Kibe *et al.,* 2017). Naturally occurring plant compounds (phytochemicals) play a significant role in managing severe health conditions and neurodegenerative disorders. These bioactive substances are identified through the extraction of plant molecules and evaluation of their therapeutic properties (Mishra et al., 2021). There have been significant increase in antimicrobial resistance as a result of diverse mechanisms of action such as membrane disruption, enzyme inhibition, metal chelation and DNA interactions (Kim *et al*., 2025). Exploring new sources of antimicrobial agents, such as those found in *Hyptis suaveolens,* is essential for addressing this challenge and improving public health.

Hyptis suaveolens (L.) Poit. (Lamiaceae) is a widely used traditional medicinal plant, originally native to tropical America but now recognized as a common weed across many regions. It is a rapidly growing, aromatic perennial herb that reaches 0.4–2 m in height, characterized by a hairy, four-angled stem. The leaves are ovate to obovate, typically 3–5 cm long and 2–4 cm wide, with serrated edges and long petioles that may extend up to 3 cm. The plant begins flowering early, usually within 2–3 months, producing abundant blue blossoms arranged in small cymes near the branch tips, where the leaves become smaller. These flowers attract a wide range of pollinators, resulting in high seed production (Sharma *et al.,* 2013; Priya, 2015). The plant is known for its strong minty smell when crushed, which is a characteristic of the Lamiaceae family.

Various parts of this plant are incorporated into traditional medicinal preparations to manage a wide range of conditions, including respiratory and gastrointestinal infections, indigestion, colic, stomachache, colds, fever, burns, wounds, cramps, and several skin disorders. It is also employed as an antirheumatic remedy and in antisudorific baths and as a laxative (Oliveria *et al.,* 2005). *Hyptis suaveolens L.* is a medical and food plant that is commonly used to treat various microbial infections in humans in many countries of the world. (Akaraiyi *et al.,* 2023). The extract from the roots of *H. suaveolens* is good for cleansing blood in women (Sharma *et al.,* 2013). This research was conducted to determine the phytochemical composition and antimicrobial efficacy of the methanol leaf extract of *Hyptis suaveolens* on selected Microorganisms.

2.0 Material and Methods

2.1. Collection and identification of the plant

Fresh leaves of *Hyptis suavoelens* were collected from the surroundings of Federal University of Technology Minna. The plant was identified by a curator at the herbarium unit of the Department of Plant Biology, Federal University of Technology Minna, Niger State Nigeria.

2.2 Plant Preparation

The leaves were carefully washed under running water, air-dried at room temperature and then milled into fine powder. Using a weighing balance, 100 g of the powdered leaves of *H. suavoelens* was macerated with 500 ml methanol for 72 hours. The resulting mixture was filtered using a muslin cloth and subsequently concentrated using a rotary evaporator.

The extract was weighed, placed in sterile sample bottles and stored in a refrigerator until required for use (Tiwari *et al*. 2011).

2.3 Reconstitution of Plant extract

Using an analytical weighing balance one gram (1 g) of extract was dissolved in 5 ml of 50% dimethylsulphoxide (DMSO) to make 200 mg/ml stock solution from which was serially diluted to give concentrations of 100 mg/ml, 50 mg/ml and 25 mg/ml.

2.3. Phytochemical screening

The plant extracts were screened for the presence of phytochemicals, qualitative detection and quantitative estimation of some secondary metabolites such as terpenoids, tannins, alkaloids, flavonoids, phenols, and saponins were carried out using standard procedures described by Edeoga *et al*. (2005) and Tiwari *et al.* (2011)

2.4. Sensitivity Test

2.4.1 Test organisms

Pure cultures of all experimental bacteria and fungi; *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, A. niger* and *A. flavus* were obtained from the Department of Microbiology Federal University of Technology Minna. Niger State. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on the Sabouraud dextrose agar (SDA) medium. Each bacterial and fungal culture was further maintained by subculturing regularly on the same medium and stored at 4^{o} C before use in the experiments.

2.4.2 Standardization of the inoculum

The test organisms used in the study were sub-cultured on nutrient agar plates and Sabouraud dextrose agar plates for bacteria and fungi respectively and then incubated overnight. Few colonies from this overnight culture of the test organisms were taken with the aid of a sterilized wire loop and transferred into a tube containing 5mL of normal saline and vortexed until the turbidity of the suspension matched with the turbidity of the prepared 0.5 McFarland standard, this was confirmed by comparing the turbidity of the diluted inoculum with that of the standard turbidity solution against the background of the of the printed white paper, where the inoculum is more turbid, a little more normal saline was added until its turbidity matched with 0.5 Macfarland standard to give a mean of 1.5 x 10^6 CFU/mL microbial population density (Clinical and laboratory standard institute (CLSI), 2016).

2.4.3 Antimicrobial susceptibility test

Antibacterial activity test was carried out using well diffusion method as per standard protocol (Bashir *et al.,* 2019; CLSI, 2016).Using a sterile cotton swab, inoculum from the standardized bacterial cultures was evenly spread onto the freshly prepared solidified agar plates and left to stand for about 15 minutes, this was followed by making wells into the inoculated plates using 6mm cork borer and different extract concentrations (100µl) were introduced into the wells, DMSO was used as negative control while Amoxicillin (250mg) and Ketoconazole (200mg) was used as positive control. The plates were allowed for a pre diffusion time of 15 minutes The Nutrient agar plates with bacteria were incubated at 37°C for 24 h while the Sabouraud dextrose agar plates were incubated at 28°C for 48 hours. The zones of inhibition were then measured at the end of the incubation period.

2.4.4 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was carried out by tube dilution assay as described by Abdulsalami *et al.* (2014) where a twofold dilution of the plant extract was made by mixing the extract concentrations with Nutrient broth for bacteria and Sabouraud dextrose broth for fungi to give a concentration of 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/mL followed by the addition of the standardized test organism (100µl), a control tube was also set which contain the media and extracts only. The tubes were incubated at temperature of 37°C for 24 hours for the bacteria and at room temperature for 72 hours for the fungi, after incubation the tube with the least concentration of the extract with no detectable growth was checked visually for turbidity and was recorded as the MIC

2.4.5 Determination of minimum bactericidal concentration (MBC)/ minimum fungicidal concentration (MFC)

A loop full from the positive MIC tubes were streaked out on a Nutrient agar plate and Sabouraud dextrose agar plate for 24 hours and 72 hours respectively. After incubation, the lowest concentration of the extract showing a bacterial/fungicidal effect was recorded as the MBC/MFC (Abdulsalami *et al.,* 2014).

2.6. Data analysis

The statistical analyses were carried out using statistical package for social sciences (SPSS-computer package). Data from three independent replicates on the antimicrobial activities of *Hyptis suaveolens* were subjected to one-way analysis of variance (ANOVA) at $p<0.05$ level of significance for comparison of the extract activities.

3.0. Results and Discussion

3.1 Physical characteristics of Methanol Leaf Extract of *Hyptis suaveolens*

The percentage yield, colour of extract and texture of the Methanol Leaf Extract of *Hyptis suaveolens* following the extraction protocol is presented in Table 3.1.

Table 3.1: Physical characteristics of Methanol Extract of *H. suaveolens*

Sample (g)	Extract yield (g)	Percentage yield (%)	Colour of Extract	Texture
100g of powdered leaf	11.23g	11.23%	Dark green	Gummy mass

3.2 Phytochemical Composition of the Methanol Leaf extract of *H. suaveolens*

The qualitative phytochemical screening of *Hyptis suavoelens* showed the presence of alkaloid, flavonoids, phenols, tannins, and terpenoids and the absence of saponin, while the quantitative estimation showed that Phenols (14.74 ± 0.08) had the highest value as seen in Table 3.2.

Table 3.2: Phytochemical Composition of the Methanol Leaf extract of *H. suaveolens*

Phytochemicals	Observation	Value (%)
Flavonoids	+	1.36 ± 0.01
Alkaloids	+	1.78 ± 0.01
Phenols	+	14.74 ± 0.08
Terpenoids	+	10.51 ± 0.01
Tannins	+	4.34 ± 0.01
Saponins	-	0.00 ± 0.00

Key: - not present, + present

3.3 Antimicrobial activity of *H. suaveolens* against the test organisms

The antimicrobial screening of the Methanol leaf extract of *H. suaveolens* showed zone of inhibition across the concentrations (25mg/ml, 50mg/ml and 100mg/ml) on both the bacteria and fungi. The sensitivity result showed that the methanol leaf extract had the highest activity on *Salmonella typhi* with mean zones of inhibition in millimeter of 28.67± 4.61. For the Fungi, *A. niger* recorded a higher zone of inhibition 28.33± 3.21 than for *A. flavus* Table 3.3.

Table 3.3: Antimicrobial activity of *H. suaveolens* against the test organisms

Organisms		**Concentrations**		
Bacteria	100mg/ml	50 mg/ml	25 mg/ml	Control
S. typhi	28.67 ± 4.61	26.67 ± 3.05	22.33 ± 1.15	27.00 ± 3.60
E. coli	23.00 ± 3.60	23.00 ± 3.60	21.00 ± 1.00	29.33 ± 1.52
K. pneumonia	23.00 ± 1.73	17.67 ± 0.58	14.67 ± 4.04	27.67 ± 3.22
P. aeruginosa	25.33 ± 4.51	20.67 ± 3.22	18.33 ± 2.08	32.33 ± 2.52
Fungi				
A. niger	28.33 ± 3.21	26.33 ± 1.52	24.33 ± 2.88	23.67 ± 5.68
A. flavus	24.67 ± 5.03	22.33 ± 1.15	19.33 ± 1.15	20.67 ± 2.51

3.4 The minimum Inhibitory Concentration (MIC) and MBC/MFC of the Methanol leaf extract of *Hyptis suaveolens*

The Minimum Inhibitory Concentration (MIC) ranged from 6.25- 25 mg/ml for the test organisms. *P. aeruginosa* had the lowest minimum bactericidal concentration. *Aspergillus niger* had a lower minimum fungicidal concentration than *A. flavus* Table 3.4

Table 3.4: The minimum Inhibitory Concentration (MIC) and MBC/MFC of the Methanol leaf extract of *Hyptis suaveolens*

Test Organisms	MIC (mg/ml)	MBC/MFC (mg/ml)
S. typhi	25	50
E. coli	25	50
K. pneumonia	25	50
P. aeruginosa	6.25	12.5
A. niger	12.5	25
A. flavus	25	50

Discussion

Plants serve as rich sources of diverse secondary metabolites with significant pharmacognostic and pharmacological importance, many of which hold promise for development into future therapeutic agents.

The natural (*in-vivo*) synthesis of these compounds is shaped by various biotic and abiotic stress factors, leading to the continuous formation of a wide range of phytochemicals and their derivatives that can support the discovery and development of new drugs (Li, *et al.,* 2020; Mishra, *et al.,* 2021). In this research, the evaluation of the phytochemical compound of the methanol leaf extract of *Hyptis suavoelens* showed the presence of alkaloids, flavonoids, tannins, terpenoids and phenol and the absence of saponin. Several researchers have linked the presence of phytochemicals to excellent anti-bacterial and anti-fungal activities (Akharaiyi *et al*., 2023). Polyphenolics present in *H. suaveolens* is known to a have well established strong and effective antioxidative, hepatoprotective, cytoprotective, neuroprotective, and antimicrobial activity (Ghaffari, *et al.,* 2012; Oscar, *et al.,* 2020). Infections caused by micro-organisms have been the cause of mortality and morbidity in human population. Bacteria such as *E. coli* and *S.typhi* are known to be associated with a variety of clinical diseases including the urinary tract infections, gastroenteritis, and diarrhoeal diseases (Shikwambi *et al*., 2021). The methanol leaf extract of *H. suaveolens* showed both anti-bacterial and anti-fungal activity against the test organisms at the different concentrations, There was a dose-dependent increase in antimicrobial activity across all the test organisms. Similar results have also been reported by some Researchers. Mishra *et al*. (2021) and Bashir *et al*. (2019) had also reported the antimicrobial activity of the *H. suaveolens* extract on some pathogenic organisms. The Minimum Inhibitory Concentration (MIC) refers to the lowest concentration or greatest dilution of a substance required to prevent visible microbial growth. Determining the MIC is crucial in diagnostic laboratories, as it helps verify microbial resistance to antimicrobial agents and serves as a tool for evaluating the effectiveness of newly developed antimicrobials. (Sen and Batra, 2012). The MIC of the methanol leaf extract of *H. suaveolens* ranged from 6.25-25mg/ml while the MBC ranged from 12.5-50 mg/ml, implying that a different concentration is required to inhibit growth of micro-organism and a different concentration which is usually higher is required to completely kill the inoculum at a given time. The low MBC and MFC recorded showed that *H. suaveolens* is a good antimicrobial agent. Many authors have reported that most of the antimicrobial properties in different plant part extractions showed that MBC value is almost two fold higher than there corresponding MICs (Sen and Batra, 2012; Bashir *et al*., 2019).

Conclusion

The methanol leaf extract of *H. suaveolens* contains some phytocomponents with antimicrobial potentials. The broad spectrum antimicrobial activity indicates that *H. suaveolens* may be of great use for the development of drugs in the pharmaceutical industries as a therapy against various diseases caused by the test organisms. Further research on this plant is recommended to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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