

PHYTOCHEMICAL PROFILE, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *POLYALTHIA LONGIFOLIA* AND *HYPTIS SUAVEOLENS* LEAF EXTRACTS AGAINST SELECTED BACTERIAL PATHOGENS

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ABSTRACT

The increasing prevalence of antimicrobial resistance and the limitations associated with some conventional antimicrobial agents have intensified the search for bioactive compounds from medicinal plants. This study evaluated the phytochemical composition, antioxidant activity and antibacterial activity of aqueous and methanolic leaf extracts of *Polyalthia longifolia* and *Hyptis suaveolens* against selected bacterial pathogens. Dried leaf samples were extracted separately with distilled water and methanol by maceration. The extracts were screened qualitatively and quantitatively for selected phytochemicals. Antioxidant activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and ferric-reducing antioxidant power (FRAP) assays. In contrast, antibacterial activity was determined by agar-well diffusion against *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The extraction yields of methanolic and aqueous extracts of *P. longifolia* were 15.80% and 13.24%, respectively, while those of *H. suaveolens* were 18.89% and 49.63%, respectively. Tannins, phenols and terpenoids were detected in several extracts, while the distribution of other phytochemicals varied by plant species and extraction solvent. At 2 mg/mL, *P. longifolia* and *H. suaveolens* extracts exhibited DPPH radical-scavenging activities of 65.5% and 56.0%, respectively, compared with 40.1% for ascorbic acid. Their FRAP values were 399.50 and 373.00 $\mu\text{mol Fe(II)/g}$, respectively, compared with 300.25 $\mu\text{mol Fe(II)/g}$ for ascorbic acid. Both plants demonstrated concentration-dependent antibacterial activity, with the greatest inhibition generally observed at 20 mg/mL. *P. longifolia* produced the largest inhibition zone (25.7 ± 1.52 mm) against *E. coli*, while *H. suaveolens* produced a maximum zone of 23.7 ± 0.58 mm against the same organism. However, the inhibition zones produced by the plant extracts were generally lower than those of ciprofloxacin. These findings show that *P. longifolia* and *H. suaveolens* leaves contain diverse phytochemicals and exhibit appreciable antioxidant and antibacterial activities, supporting their potential as sources of bioactive compounds for further investigation.

Keywords: *Hyptis suaveolens*, *Polyalthia longifolia*, phytochemicals, antioxidant activity, antibacterial activity.

INTRODUCTION

The increasing burden of antimicrobial resistance (AMR) has intensified the search for new antimicrobial agents and alternative therapeutic resources. Bacterial infections caused by drug-resistant pathogens constitute a major global public-health challenge, with bacterial AMR estimated to have directly caused approximately 1.27 million deaths globally in 2019 and been associated with nearly 4.95 million deaths in the same year (Antimicrobial Resistance Collaborators, 2022). The continued emergence and spread of resistant bacterial strains, along with the limitations of existing antimicrobial therapies, has increased interest in discovering bioactive compounds from natural sources. Medicinal plants represent an important reservoir of structurally diverse secondary metabolites that may possess antimicrobial, antioxidant and other pharmacological activities (Newman & Cragg, 2020; Sasidharan *et al.*, 2011).

Plant-derived bioactive compounds, including phenolics, flavonoids, tannins, alkaloids, terpenoids and saponins, have been associated with a range of biological activities. These phytochemical constituents may exert antibacterial effects through different mechanisms, including disruption of bacterial cell membranes, interference with essential enzymes and proteins, alteration of cellular permeability, and inhibition of other processes required for bacterial survival (Cushnie & Lamb, 2011). Phenolic compounds and flavonoids are also recognized for their ability to scavenge reactive oxygen species and contribute to the antioxidant capacity of plant extracts. Therefore, simultaneous evaluation of phytochemical composition, antioxidant activity and antibacterial activity can provide useful preliminary evidence regarding the biological potential of medicinal plants.

Hyptis suaveolens (L.) Poit. (Lamiaceae), commonly known as pignut, is a widely distributed herbaceous plant occurring in tropical and subtropical regions. The plant has a history of traditional medicinal use and has been investigated for several pharmacological properties. Phytochemical investigations have identified diverse secondary metabolites in *H. suaveolens*, including phenolic compounds, flavonoids, alkaloids, terpenoids, saponins and other constituents, which may contribute to its reported biological activities (Jesus *et al.*, 2013; Mishra *et al.*, 2021). Its reported phytochemical diversity and ethnomedicinal importance make the plant a potential source of compounds with antioxidant and antimicrobial properties.

Polyalthia longifolia (Sonn.) Thwaites (Annonaceae) is an evergreen tree widely cultivated as an ornamental and windbreak plant in tropical regions, including Nigeria. Previous investigations have shown diverse phytochemicals in *P. longifolia*, and extracts and isolated constituents have exhibited antioxidant and antimicrobial activities (Ghosh *et al.*, 2008; Chen *et al.*, 2021; Savu *et al.*, 2022; Ranjutha *et al.*, 2023). These findings suggest that *P. longifolia* may also represent a useful source of bioactive compounds for further pharmacological investigation. The biological activity of plant extracts can vary substantially according to plant species, plant part, extraction solvent and extraction conditions. Solvent polarity influences the types and quantities of phytochemicals recovered from plant material, and differences in extract composition may consequently affect antioxidant and antibacterial activities. Comparative evaluation using solvents with different extraction characteristics, such as water and methanol, is therefore useful for determining the relative phytochemical yield and biological activity of plant extracts.

Although both *P. longifolia* and *H. suaveolens* have been investigated independently for various biological properties, comparative information on their aqueous and methanolic leaf extracts, particularly with respect to phytochemical composition, antioxidant activity and antibacterial activity against clinically relevant bacterial isolates, remains limited. A combined assessment of these properties may provide a clearer basis for comparing the biological potential of the two plants and identifying extracts that warrant further investigation. Therefore, this study evaluated the phytochemical composition, antioxidant activity and antibacterial activity of aqueous and methanolic leaf extracts of *P. longifolia* and *H. suaveolens*. Specifically, the study aimed to: (i) determine the qualitative and quantitative phytochemical constituents of the extracts; (ii) evaluate their antioxidant activities using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and ferric-reducing antioxidant power (FRAP) assays; and (iii) determine and compare the antibacterial activities of the extracts against clinically relevant isolates of *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*.

MATERIALS AND METHODS

Study Area

The study was conducted in the Department of Biological Sciences, Nigerian Defence Academy (NDA), Afaka, Kaduna State, Nigeria, located at approximately 10°36'33.55" N and 7°25'46.21" E. The study area has a mean monthly temperature ranging from 26 to 34°C. The area experiences a rainy season from March to October, with peak rainfall generally in July and August, followed by a dry, dusty Harmattan period from November to February.

Plant Collection and Authentication

Fresh leaves of *Polyalthia longifolia* and *Hyptis suaveolens* were collected within the Nigerian Defence Academy environment and transported to the laboratory in sterile polythene bags. The plant specimens were identified and authenticated, and voucher specimens were deposited in the departmental herbarium under voucher numbers NDA/BIO H202102 and NDA/BIO H202103, respectively.

Preparation of Plant Extracts

The collected leaves were washed where necessary to remove visible contaminants, chopped into smaller pieces, and air-dried at room temperature for two weeks. The dried materials were milled separately into a fine powder. For extraction, 100 g of powdered *P. longifolia* and 50 g of powdered *H. suaveolens* were separately macerated in 500 mL each of distilled water and methanol for 24 h. The resulting mixtures were filtered through cheesecloth, and the filtrates were concentrated under reduced pressure at 40–50°C using a rotary evaporator (Gallenkamp, UK). The resulting extracts were stored at 4°C until required for analysis. Extraction yield was calculated as the percentage ratio of the weight of the dried extract to the weight of the initial powdered plant material:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dried plant material (g)}} \times 100 \quad (1)$$

Qualitative Phytochemical Screening

The aqueous and methanolic extracts were screened qualitatively for selected phytochemical constituents, including saponins, tannins, flavonoids, alkaloids, glycosides, steroids, terpenoids, carotenoids, phenols and reducing sugars. The analyses were based on standard colour-development and precipitation reactions as described by Sofowora (1993).

Quantitative Phytochemical Determination

Quantitative determination of tannins, saponins, flavonoids, phenols and alkaloids was performed gravimetrically following established procedures described by Sofowora (1993). After extracting and separating the respective phytochemical fractions, the residues were dried to constant weight and quantified. The results were expressed as milligrams per gram (mg/g) of extract.

Determination of Antioxidant Activity

DPPH Radical-Scavenging Assay

The free-radical-scavenging activity of the plant extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to Brand-Williams *et al.* (1995). Extract solutions and ascorbic acid, used as the reference antioxidant, were prepared at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/mL. Each test solution was mixed with 0.5 mL of 1 mmol/L DPPH solution prepared in methanol and incubated in the dark for 15 min at room temperature. Absorbance was measured at 517 nm. Determinations were performed in triplicate. The percentage DPPH radical-scavenging activity was calculated as:

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

Where A_0 is the absorbance of the blank (DPPH radical + methanol), and A_1 is the absorbance of DPPH radical + sample or standard.

Ferric-Reducing Antioxidant Power (FRAP) Assay

Ferric-reducing antioxidant power was determined according to Benzie and Strain (1996). The assay is based on the reduction of the ferric-tripyridyltriazine complex to its ferrous form by antioxidant compounds present in the samples. Absorbance was measured at 593 nm, and antioxidant capacity was expressed as $\mu\text{mol Fe(II)}$ equivalents per gram of extract.

Bacterial Test Organisms and Antibacterial Susceptibility Testing

Five clinically relevant bacterial isolates, namely *Staphylococcus*

aureus, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*, were used for the antibacterial assay. The isolates were obtained from the Nigerian Defence Academy (NDA) Medical Centre, Kaduna and were previously identified clinical isolates. They were used as reference test organisms to evaluate the antibacterial activity of the plant extracts.

The antibacterial activity of the aqueous and methanolic plant extracts was evaluated using the agar-well diffusion method, following the procedure described by Savu *et al.* (2022). Bacterial suspensions were standardized to a turbidity equivalent to the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, as described by Babatunde *et al.* (2023). The agar-well diffusion method was adopted in line with recommended approaches for evaluating the antimicrobial activity of plant extracts (Eloff, 2019; Gonzalez-Pastor *et al.*, 2023). The standardized bacterial suspensions were evenly inoculated onto Mueller–Hinton agar plates. Wells measuring 6 mm in diameter were aseptically bored into the agar and filled with the respective plant extracts at concentrations of 1.0, 2.5, 5.0, 10.0 and 20.0 mg/mL. The extracts were prepared in 10% dimethyl sulfoxide (DMSO). Ciprofloxacin (0.5% w/v) was used as the positive control, while 10% DMSO served as the negative control. The inoculated plates were incubated at 37°C for 16–18 hours. Following incubation, the diameters of the zones of inhibition surrounding the wells were measured in millimetres.

Statistical Analysis

All experimental determinations were performed in triplicate, and results were expressed as mean $\pm$ standard error (SE). For each bacterial isolate, one-way analysis of variance (ANOVA) was used to determine whether significant differences existed among the mean inhibition-zone diameters produced by the different concentrations of each plant extract. Where a significant difference was detected, Duncan's multiple range test (DMRT) was used for post-hoc comparison of means. Statistical significance was set at $p < 0.05$.

RESULTS

Extraction Yield

Extraction yield varied by plant species and extraction solvent (Figure 1). For *Polyalthia longifolia*, methanol produced a higher extraction yield (15.80%) than water (13.24%). *Hyptis suaveolens* produced a substantially higher yield with aqueous extraction (49.63%) than with methanol (18.89%).

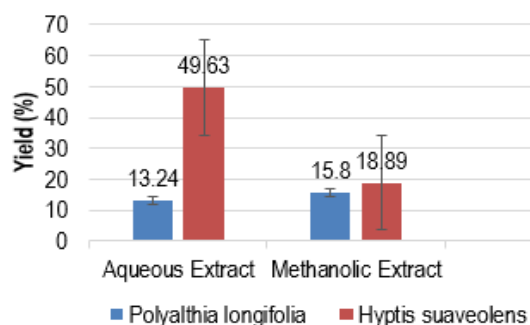


Figure 1. Extraction yield of *Polyalthia longifolia* and *Hyptis suaveolens* leaf extracts.

Qualitative Phytochemical Constituents

The qualitative phytochemical screening revealed differences in the distribution of phytochemical classes among the extracts (Table 2). Tannins and phenols were detected in all four extracts, while terpenoids were detected in the aqueous extracts of both plants but only in the methanolic extract of *H. suaveolens*. Saponins were detected in the aqueous extract of *P. longifolia* and in both extracts of *H. suaveolens*, but were not detected in the methanolic extract of *P. longifolia*. Alkaloids were detected in the aqueous extracts of both plants and in the methanolic extract of *H. suaveolens*, but were not detected in the methanolic extract of *P. longifolia*. Glycosides were detected in both extracts of *H. suaveolens* and in the methanolic extract of *P. longifolia*, but were absent from the aqueous extract of *P. longifolia*. Steroids were detected in the aqueous extracts of both plants and were absent from both methanolic extracts. Flavonoids were detected only in the methanolic extract of *H. suaveolens*. Carotenoids were detected only in the methanolic extract of *P. longifolia*, whereas reducing sugars were detected only in its aqueous extract.

Table 2. Qualitative phytochemical constituents of *Polyalthia longifolia* and *Hyptis suaveolens* leaf extracts.

Phytochemical	<i>P. longifolia</i> (Aq.)	<i>P. longifolia</i> (MeOH)	<i>H. suaveolens</i> (Aq.)	<i>H. suaveolens</i> (MeOH)
Saponins	+	–	+	+
Tannins	+	+	+	+
Flavonoids	–	–	–	+
Alkaloids	+	–	+	+
Glycosides	–	+	+	+
Steroids	+	–	+	–
Phenols	+	+	+	+
Terpenoids	+	–	+	+
Carotenoids	–	+	–	–
Reducing sugars	+	–	–	–

+ = present; – = absent; Aq. = aqueous; MeOH = methanolic.

Quantitative Phytochemical Constituents

Quantitative analysis showed variation in the concentrations of the five phytochemical groups assessed among the extracts (Table 3). The aqueous extract of *P. longifolia* had the highest tannin content (1.677 mg/g), followed by the methanolic extract of *P. longifolia* and the aqueous extract of *H. suaveolens* (0.954 mg/g each). In comparison, the methanolic extract of *H. suaveolens* had the lowest value (0.497 mg/g). The highest saponin content was recorded in the aqueous extract of *P. longifolia* (1.233 mg/g), followed by the aqueous (0.672 mg/g) and methanolic (0.667 mg/g) extracts of *H. suaveolens*. The methanolic extract of *P. longifolia* had the lowest saponin value (0.024 mg/g). For flavonoids, the methanolic extract of *H. suaveolens* had the highest value (0.732), followed by its aqueous extract (0.512 mg/g). In contrast, lower values were recorded for the aqueous (0.998 mg/g) and methanolic (0.031 mg/g) extracts of *P. longifolia*. Phenol content was highest in the aqueous extract of *P. longifolia* (0.978 mg/g), followed by the aqueous extract of *H. suaveolens* (0.554 mg/g), methanolic extract of *H. suaveolens* (0.342 mg/g), and methanolic extract of *P.*

longifolia (0.065 mg/g). Alkaloid values ranged from 0.030 to 0.079 mg/g, with the highest value recorded in the aqueous extract of *P. longifolia* (0.079 mg/g).

Table 3. Quantitative phytochemical constituents of *Polyalthia longifolia* and *Hyptis suaveolens* leaf extracts.

Constituent	<i>P. longifolia</i> (Aq.), mg/g	<i>P. longifolia</i> (MeOH), mg/g	<i>H. suaveolens</i> (Aq.), mg/g	<i>H. suaveolens</i> (MeOH), mg/g
Tannins	1.677	0.954	0.954	0.497
Saponin	1.233	0.024	0.672	0.667
Flavonoid	0.998	0.031	0.512	0.732
Phenol	0.978	0.065	0.554	0.342
Alkaloids	0.079	0.030	0.030	0.061

Antioxidant Activity

Both plant extracts demonstrated concentration-dependent DPPH radical-scavenging activity across the concentrations tested (0.5–2.0 mg/mL) (Table 4). For *P. longifolia*, DPPH inhibition increased from 23.5% at 0.5 mg/mL to 65.5% at 2.0 mg/mL. Similarly, *H. suaveolens* increased from 17.0% to 56.0% over the same concentration range. Ascorbic acid showed an increase in inhibition from 10.8% at 0.5 mg/mL to 40.1% at 2.0 mg/mL. At 2.0 mg/mL, *P. longifolia* recorded the highest DPPH inhibition (65.5%), followed by *H. suaveolens* (56.0%) and ascorbic acid (40.1%).

Table 4. DPPH radical-scavenging activity (% inhibition) of *Polyalthia longifolia* and *Hyptis suaveolens* extracts.

Sample	Concentration of Plant Extracts			
	0.5 mg/mL	1.0 mg/mL	1.5 mg/mL	2.0 mg/mL
Ascorbic acid (standard)	10.8%	15.0%	22.7%	40.1%
<i>P. longifolia</i>	23.5%	41.1%	51.4%	65.5%
<i>H. suaveolens</i>	17.0%	44.4%	50.7%	56.0%

The FRAP assay also showed higher ferric-reducing antioxidant

values for both plant extracts than for the ascorbic acid standard (Table 5). *P. longifolia* recorded a FRAP value of 399.50 $\mu\text{mol Fe(II)/g}$, while *H. suaveolens* recorded 373.00 $\mu\text{mol Fe(II)/g}$. Ascorbic acid had a value of 300.25 $\mu\text{mol Fe(II)/g}$.

Table 5. Ferric-reducing antioxidant power (FRAP) of *Polyalthia longifolia* and *Hyptis suaveolens* extracts.

Sample	FRAP value ($\mu\text{mol Fe(II)/g}$)
Ascorbic acid (standard)	300.25
<i>Polyalthia longifolia</i>	399.50
<i>Hyptis suaveolens</i>	373.00

Antibacterial Activity

Both aqueous and methanolic extracts of *Polyalthia longifolia* and *Hyptis suaveolens* exhibited antibacterial activity against the tested bacterial isolates, with inhibition generally increasing with increasing extract concentration (Tables 6 and 7). The highest inhibition zones were generally observed at 20.0 mg/mL, whereas some isolates showed little or no inhibition at 1.0 mg/mL. The 10% DMSO negative control produced no zone of inhibition. For *P. longifolia*, the aqueous extract produced inhibition zones ranging from 8.33 ± 0.58 to 23.0 ± 1.00 mm, with the largest zone recorded against *E. coli* at 20.0 mg/mL (23.0 ± 1.00 mm). The methanolic extract produced zones ranging from 8.33 ± 0.57 to 25.7 ± 1.52 mm, with the highest activity also recorded against *E. coli* at 20.0 mg/mL (25.7 ± 1.52 mm). For *H. suaveolens*, the aqueous extract produced inhibition zones ranging from 8.33 ± 0.58 to 21.7 ± 3.06 mm, with the largest zone recorded against *S. aureus* at 20.0 mg/mL (21.7 ± 3.06 mm), while the methanolic extract produced zones ranging from 8.33 ± 0.58 to 23.7 ± 0.58 mm, with the highest activity observed against *E. coli* at 20.0 mg/mL (23.7 ± 0.58 mm). Ciprofloxacin (positive control) produced inhibition zones ranging from 21.3 ± 0.96 to 30.3 ± 1.52 mm across the five bacterial isolates. Statistical analysis showed significant differences ($p < 0.05$) among the mean inhibition-zone diameters produced by the different concentrations of each extract against the respective bacterial isolates. Although the extracts generally showed a concentration-dependent increase in antibacterial activity, the significance of differences between concentrations varied by bacterial isolate and extraction solvent.

Table 6. Antibacterial activity of *Polyalthia longifolia* extracts

Aqueous extract conc. (mg/mL)	Zone of Inhibition (mm)				
	<i>S. aureus</i>	<i>H. influenzae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. pyogenes</i>
20	22.3 ± 0.57^e	19.3 ± 0.57^d	23.0 ± 1.00^e	18.7 ± 1.53^c	20.3 ± 1.16^e
10	19.3 ± 1.16^d	15.7 ± 1.15^{cd}	19.7 ± 0.58^d	13.7 ± 1.53^b	15.6 ± 1.53^d
5.0	16.0 ± 1.00^c	12.0 ± 1.00^c	15.7 ± 1.53^c	9.33 ± 1.52^b	11.6 ± 0.58^c
2.5	13.0 ± 1.00^b	8.00 ± 0.00^b	12.3 ± 1.16^b	0.00 ± 0.00^a	8.33 ± 0.58^b
1.0	8.33 ± 0.58^a	0.00 ± 0.00^a	8.67 ± 0.58^a	0.00 ± 0.00^a	0.00 ± 0.00^a
Methanolic extract conc. (mg/mL)					
20	23.0 ± 1.00^e	19.7 ± 1.52^d	25.7 ± 1.52^e	20.3 ± 1.15^d	19.0 ± 1.00^d
10	18.0 ± 1.00^d	16.7 ± 2.52^d	21.3 ± 0.57^d	16.7 ± 0.57^c	14.6 ± 0.58^c
5.0	15.0 ± 1.00^c	12.3 ± 2.08^c	18.3 ± 0.57^c	12.0 ± 1.00^b	11.3 ± 0.57^b

2.5	11.3±1.16 ^b	9.00±1.00 ^b	13.0±1.00 ^b	10.0±1.41 ^b	10.0±1.41 ^b
1.0	8.33±0.57 ^a	0.00±0.00 ^a	8.67±0.58 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Ciprofloxacin (0.5% w/v)	27.3±0.57	21.3±0.96	30.3±1.52	28.3±1.52	29.3±0.51

No zone of inhibition was recorded for the 10% DMSO negative control. Within each column, mean values bearing different superscript letters differ significantly ($p < 0.05$), whereas means sharing the same superscript letter do not differ significantly.

Table 7. Antibacterial activity of *Hyptis suaveolens* extracts

Aqueous extract conc. (mg/mL)	Zone of Inhibition (mm)				
	<i>S. aureus</i>	<i>H. influenzae</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. pyogenes</i>
20	21.7±3.06 ^d	18.3±2.08 ^d	21.3±0.58 ^e	17.3±1.53 ^d	17.0±1.00 ^c
10	18.3±3.06 ^{cd}	15.3±2.08 ^{cd}	19.3±0.57 ^d	14.0±2.00 ^{cd}	12.7±1.53 ^b
5.0	15.0±2.00 ^{bc}	10.7±2.08 ^c	16.0±1.00 ^c	10.3±1.16 ^c	9.00±1.73 ^b
2.5	12.0±1.00 ^{ab}	0.00±0.00 ^a	12.3±1.16 ^b	8.50±0.71 ^b	0.00±0.00 ^a
1.0	8.33±0.58 ^a	0.00±0.00 ^a	8.33±0.58 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Methanolic extract conc. (mg/mL)					
20	22.3±1.53 ^f	14.3±1.16 ^c	23.7±0.58 ^e	17.3±2.08 ^d	19.0±1.00 ^e
10	17.3±1.52 ^d	11.7±1.53 ^c	19.3±1.16 ^d	13.3±2.89 ^c	16.0±1.00 ^d
5.0	14.0±1.00 ^c	9.67±1.53 ^{bc}	16.7±1.53 ^c	10.0±2.00 ^{bc}	12.6±0.58 ^c
2.5	11.3±1.16 ^b	8.50±0.71 ^b	13.7±1.16 ^b	9.00±0.00 ^b	9.67±0.58 ^b
1.0	8.33±0.58 ^a	0.00±0.00 ^a	9.67±0.57 ^a	0.00±0.00 ^a	0.00±0.00 ^a
Ciprofloxacin (0.5% w/v)	27.3±0.57	21.3±0.96	30.3±1.52	28.3±1.52	29.3±0.51

No zone of inhibition was recorded for the 10% DMSO negative control. Within each column, mean values bearing different superscript letters differ significantly ($p < 0.05$), whereas means sharing the same superscript letter do not differ significantly.

DISCUSSION

This study showed that aqueous and methanolic leaf extracts of *Polyalthia longifolia* and *Hyptis suaveolens* contained several classes of phytochemicals and exhibited measurable antioxidant and antibacterial activities. The observed differences between plants and extraction solvents indicate that both plant species and solvent type influenced the quantity and profile of compounds recovered. This aligns with the established principle that extraction efficiency depends substantially on solvent polarity and physicochemical properties, as different solvents preferentially extract different classes of bioactive compounds (Sasidharan *et al.*, 2011). The extraction yield varied considerably between the two plants and solvents. Methanol produced a higher yield from *P. longifolia* leaves (15.80%) than water (13.24%), whereas water produced a markedly higher yield from *H. suaveolens* (49.63%) than methanol (18.89%). The substantially higher aqueous yield obtained from *H. suaveolens* suggests that this plant contains a relatively large proportion of water-soluble constituents. Conversely, the slightly higher methanolic yield from *P. longifolia* suggests that methanol was more effective at recovering some of its methanol-soluble constituents. Such solvent-dependent variation is expected because plant matrices contain compounds with different polarities and solubilities (Sasidharan *et al.*, 2011). The presence of several phytochemicals agrees with previous studies on the chemical composition of the two plants. *H. suaveolens* has been reported to contain alkaloids, flavonoids,

phenolic compounds, saponins, terpenes and sterols, among other constituents (Mishra *et al.*, 2021), with comparable phytochemical and antioxidant profiles reported for its leaf extracts elsewhere (Sánchez-Aguirre *et al.*, 2020). Similarly, phytochemical investigations of *P. longifolia* have documented several biologically active constituents and have associated its leaves and other tissues with antimicrobial and other pharmacological activities (Ghosh *et al.*, 2008; Chen *et al.*, 2021). The detection of flavonoids only in the methanolic extract of *H. suaveolens* may reflect the ability of methanol to extract certain moderately polar phenolic compounds more effectively than water. Flavonoids are structurally diverse polyphenolic compounds with recognized antioxidant and antibacterial properties (Cushnie & Lamb, 2011). The quantitative results further showed that *P. longifolia* aqueous extract contained the highest recorded tannin value. In contrast, the methanolic extract of *H. suaveolens* contained the highest flavonoid value among the extracts. These differences may partly explain the variation in antioxidant and antibacterial activities observed among the extracts. Tannins and phenolic compounds are of particular interest because their hydroxyl groups enable them to participate in redox reactions and interact with biological macromolecules. Similarly, alkaloids, terpenoids, saponins and flavonoids have been associated with diverse biological effects, including antimicrobial activity (Cushnie & Lamb, 2011). However, the qualitative and gravimetric phytochemical assays used in this study provide evidence of the presence and approximate abundance of

compound classes rather than identification of individual molecules.

Both plant extracts demonstrated concentration-dependent DPPH radical-scavenging activity. At 2.0 mg/mL, *P. longifolia* and *H. suaveolens* recorded DPPH inhibition values of 65.5% and 56.0%, respectively, compared with 40.1% for ascorbic acid. The increase in radical-scavenging activity with increasing extract concentration indicates a concentration-dependent antioxidant response. The comparatively high DPPH activity of *P. longifolia* may be related to its phenolic, tannin and other antioxidant-associated constituents. Phenolic compounds are capable of donating hydrogen or electrons to reactive species and may therefore contribute substantially to the radical-scavenging capacity of plant extracts (Sasidharan *et al.*, 2011). Previous studies have also identified diverse phytochemicals in *Polyalthia* species with potential antioxidant properties (Chen *et al.*, 2021). The FRAP results provided additional evidence of antioxidant capacity. The FRAP assay measures the reducing ability of an antioxidant system by assessing its capacity to reduce ferric ions to ferrous ions (Benzie & Strain, 1996; Halliwell & Gutteridge). The higher FRAP values observed for both plant extracts therefore indicate appreciable reducing capacity. The agreement between the DPPH and FRAP findings strengthens the evidence that the extracts possess antioxidant potential. However, DPPH and FRAP measure different aspects of antioxidant behaviour and do not directly reflect clinical antioxidant efficacy. The observed antioxidant activity reflects chemical antioxidant capacity under the experimental conditions and does not, by itself, demonstrate antioxidant effects in humans or animals.

Both aqueous and methanolic extracts of the two plants exhibited concentration-dependent antibacterial activity against the five clinical isolates tested. In general, inhibition zones increased with extract concentration, with the largest zones at 20 mg/mL. The 10% DMSO negative control showed no inhibition, indicating that the observed antibacterial effects were not attributable to the solvent under the conditions used. The concentration-dependent response is consistent with Babatunde *et al.* (2023) on antibacterial activity from plant extracts. It supports the possibility that higher concentrations of active phytochemicals increase their interaction with bacterial cells. For *P. longifolia*, the methanolic extract showed its greatest activity against *E. coli*, producing an inhibition zone of 25.7 ± 1.52 mm at 20 mg/mL. In comparison, the aqueous extract produced its greatest activity against *S. aureus* (22.3 ± 0.57 mm). Similarly, the methanolic extract of *H. suaveolens* showed its highest activity against *E. coli* (23.7 ± 0.58 mm). These findings are consistent with studies describing antibacterial activity of *P. longifolia* leaf extracts against *S. aureus* and *E. coli* (Babatunde *et al.*, 2023; Umar *et al.*, 2024). The antibacterial activity recorded for *H. suaveolens* extracts, particularly against *S. aureus* and *E. coli*, is likewise consistent with earlier reports of antibacterial activity in leaf extracts of this species (Erinle *et al.*, 2021). Other studies have also reported antibacterial activity of *P. longifolia* and its potential against clinically important bacteria (Ghosh *et al.*, 2008; Savu *et al.*, 2022; Ranjutha *et al.*, 2023).

Susceptibility also differed among the bacterial isolates. *E. coli* and *S. aureus* generally showed stronger responses to several extracts, whereas *H. influenzae* and *K. pneumoniae* tended to show comparatively lower inhibition. Variation in susceptibility

among bacterial species may arise from differences in cell-envelope structure, membrane permeability, efflux systems and other intrinsic or acquired resistance mechanisms. Gram-negative bacteria have an outer membrane that can act as an additional permeability barrier to many antimicrobial compounds, although susceptibility ultimately depends on the specific organism and compound involved. The antibacterial response of *P. longifolia* extracts against *E. coli* and *S. aureus* is consistent with previously reported activity against these organisms (Babatunde *et al.*, 2023; Umar *et al.*, 2024).

The antibacterial activity observed in this study may result from the combined action of several phytochemical classes rather than from a single compound. Tannins may interact with bacterial proteins and cellular structures, flavonoids may affect membrane integrity and essential bacterial processes, while saponins can interact with membrane components. Alkaloids and phenolic compounds may also interfere with important cellular functions (Cushnie & Lamb, 2011). However, these mechanisms remain proposed explanations because the present study did not isolate individual compounds or investigate the molecular mechanism responsible for bacterial inhibition. The findings suggest a possible relationship between phytochemical composition and the biological activities of the extracts. Extracts containing appreciable amounts of phenols, tannins, flavonoids and other secondary metabolites generally showed antioxidant and antibacterial activity. The relatively strong antioxidant activity of *P. longifolia* corresponds with the presence of phenols, tannins and other phytochemical constituents known to possess redox-active properties. Similarly, flavonoids and other secondary metabolites in *H. suaveolens* may contribute to its antioxidant and antibacterial effects (Chen *et al.*, 2021; Mishra *et al.*, 2021).

Conclusion

This study showed that the leaves of *Polyalthia longifolia* and *Hyptis suaveolens* contain diverse phytochemical constituents, including tannins, phenols, saponins, alkaloids, flavonoids, glycosides, steroids and terpenoids, with their occurrence varying by plant species and extraction solvent. Both plants also exhibited appreciable antioxidant activity in the DPPH and FRAP assays, with *P. longifolia* generally showing higher antioxidant activity than *H. suaveolens* under the conditions tested. The aqueous and methanolic extracts of both plants showed concentration-dependent antibacterial activity against the tested clinical isolates, although the magnitude of activity varied by plant, solvent and bacterial species. The extracts showed lower antibacterial activity than ciprofloxacin, but some produced substantial inhibition at the highest concentration tested. These findings provide preliminary in vitro evidence that *P. longifolia* and *H. suaveolens* are potential sources of biologically active phytochemicals with antioxidant and antibacterial properties. Further studies involving detailed chemical characterization, determination of minimum inhibitory and bactericidal concentrations, toxicity evaluation, elucidation of antibacterial mechanisms, and in vivo assessment are required to identify the active constituents and establish the safety and therapeutic relevance of these plant extracts.

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