

Antibacterial and antifungal activities of 10 Kenyan *Plectranthus* species in the *Coleus* clade

Fredrick M. Musila^{1*}, Joseph M. Nguta², Catherine W. Lukhoba³, Saifuddin F. Dossaji³

ABSTRACT

Background Information: *Plectranthus* L'Hér. is an economically important genus with horticultural, medicinal and food uses. Most *Plectranthus* species are used in traditional medicine and have attracted the interest of researchers who have studied them in attempt to explore the bioactivities of their phytoconstituents. **Materials and Methods:** The current study investigated the antimicrobial activities of 10 Kenyan *Plectranthus* species through disc diffusion and broth dilution method. **Results:** Results indicated that, dichloromethane/methanol (1:1) total leaf extracts from *Plectranthus barbatus* displayed the highest antimicrobial activity compared to the other nine *Plectranthus* species with minimum inhibitory concentration (MIC) values of 25, 40, 100, 50, and 100 mg/ml against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*, respectively. At a concentration of 200 mg/ml, the antibacterial activity of total leaf extracts of *P. barbatus* (MIC value = 25 mg/ml) and *Plectranthus lanuginosus* (MIC value = 40 mg/ml) against MRSA was not significantly different from positive control drug; amoxicillin. Similarity, at a concentration of 200 mg/ml, total leaf extracts from *Plectranthus ornatus* (MIC value= 50 mg/ml) and *P. barbatus* (MIC value = 50 mg/ml) exhibited antifungal activity against *C. albicans* which was not significantly different from that of the positive control; ketoconazole. **Conclusion:** The study reports for the first time, the antimicrobial activity of *Plectranthus pseudomarruboides*, *Plectranthus edulis*, *Plectranthus aegyptiacus*, *Plectranthus Otostegioides*, and *Plectranthus lanuginosus*. The study has demonstrated broad bacteriostatic activity of *P. barbatus* and thus recommends further studies on this plant aimed at discovery of novel antimicrobial agents.

KEY WORDS: Antimicrobial activity, Bioguidance, Minimum inhibitory concentration, *Plectranthus*

INTRODUCTION

Plectranthus species are extensively used in various parts of the world as ornamentals, source of food and have numerous medicinal uses.^[1] About 85% of all species of *Plectranthus* known to man have been reported to have medicinal value.^[2] Majority of species within *Plectranthus* are used in traditional medicine by various communities to treat a variety of diseases,^[3] and the potential medicinal and economic uses of *Plectranthus* are of great interest.^[4] Stems, leaves, and tubers of different *Plectranthus* species are used to treat a variety of diseases which are classified into 13 categories as described in Economic Botany Data Collection Standard.^[5] For instance, *Plectranthus barbatus* is one

of the most important species in the genus widely cited because of its use in the treatment of many diseases.^[1] Besides their medicinal value, most *Plectranthus* species have low toxicity in both man and animals.^[1]

In addition to being used in ethnomedicine, species like *P. barbatus* are also used in ethnoveterinary medicine in the management of livestock diseases; for instance, *P. barbatus* is used to manage east coast fever by the Maasai people of Kenya.^[6] Various bioactive phytoconstituents including saponins, monoterpenes, sesquiterpenes, terpenes, and phenolic compounds have been isolated from *Plectranthus* species.^[7,8] *Plectranthus* has a wealth of ethnomedicinal species which forms a basis for natural product research. Furthermore, the wide variety of ailments treated by species within *Plectranthus* is an indication of the medicinal richness of the genus, and hence the scope of drug development from this genus is endless.^[1]

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Two clades have been identified within *Plectranthus* genus: *Coleus* and *Plectranthus* clade.^[9] *Coleus* clade is well represented in Kenya, and majority of the species in the clade are used as medicinal plants.^[2] For example, *P. barbatus* and *Plectranthus amboinicus* are used in the management of toothache, stomach ache, vomiting as well as in the management of mouth, and throat infections.^[10] *Plectranthus aegyptiacus* is also used to treat stomachache.^[11] *P. barbatus* and *P. amboinicus* have been used in the treatment of burns, sores, allergies, wounds, and insect bites.^[12] *P. barbatus* is reported to treat wounds and ringworms.^[13] *P. barbatus* has been used to manage common cold, respiratory complications^[14] and malaria.^[15] With respect to respiratory diseases, *P. amboinicus* has been reported to be frequently used in the management of chronic coughs, sore throat, asthma, and bronchitis the Caribbean and India.^[2,16] Similarly, *P. aegyptiacus* has been used in the treatment of laryngitis and sore throats in Saudi Arabia.^[17]

Elsewhere, it was reported that *P. montanus* is used in the treatment of sore throats^[18] while a concoction from the roots of *Plectranthus caninus* has been used in the management of coughs in Kenya.^[19] *P. barbatus* has been reported to treat syphilis in Central Africa,^[20] and similarly, *P. amboinicus* is often used in the management of urinary tract infections in India and by the Amazon tribes.^[21] The Giriama people of Kenya use *P. aegyptiacus* to treat sexually transmitted diseases.^[11] Leaves of *P. amboinicus* and *P. barbatus* can be rubbed onto eyes to manage conjunctivitis and other forms of eyes inflammation.^[13] Majority of *Plectranthus* species have medicinal properties and in particular *P. barbatus* and *P. amboinicus*.

Members of *Plectranthus* genus have been used widely in traditional medicine in the treatment of various diseases.^[7] Various researchers have identified phytoconstituents in various species of *Plectranthus* with various bioactivities. Although most members within the *Coleus* clade are of ethnomedical importance, the majority of the species have not been investigated for their chemical composition and bioactivities. Closely related species may possess similar phytoconstituents and consequently may display similar bioactivities.^[22] As a result, the current study sought to determine antibacterial and antifungal activity of the Kenyan *Plectranthus* species, and this will allow researchers to identify potential therapeutic applications of *Plectranthus* even though they may have no documented activity.

MATERIALS AND METHODS

Collection of *Plectranthus* Species

Leaves of 10 *Plectranthus* species grouped under the *Coleus* clade were collected from various geographical

regions of Kenya. Following information available in the flora of tropical East Africa on *Plectranthus*, it was possible to identify the specific locality of the species and collect them. The following species were collected in 2014; voucher numbers are in parenthesis: *P. barbatus* Andrews (FM2014/01), *Plectranthus edulis* (Vatke) Agnew (FM2014/02), *Plectranthus ornatus* Codd. (FM2014/03), *P. caninus* Roth. (FM2014/04), *Plectranthus pseudomarrubioides* Willemse (FM2014/05), *Plectranthus otostegioides* (Gürke) Ryding (FM2014/06), *P. amboinicus* (Lour.) Spreng (FM2014/07), *P. aegyptiacus* (Forssk) C. Chr (FM2014/08), *Plectranthus montanus* Benth. (FM2014/09), and *Plectranthus lanuginosus* (Hochst. ex Benth.) Agnew (FM2014/10). Voucher specimens were deposited in Nairobi University Herbarium (NU).

Preparation of Crude Extracts of *Plectranthus* Species

Leaves from the 10 *Plectranthus* species under study were dried under shade after which they were ground into fine powder using a blender. 500 g of air dried ground material of leaves from each of the 10 *Plectranthus* species were extracted by cold maceration with 1 L of dichloromethane/methanol (DCM:MeOH [1:1]) in 2.5 L conical flasks well covered with aluminum foil for 1 week at room temperature. Filtrates were concentrated in a rotary evaporator and then left to dry in a fume chamber to ensure the total extract was free from the extraction solvents.^[23]

Preparation of Test Microbes

Nutrient agar and broth were prepared as follows, 6.5 g of nutrient agar and 6.5 g of nutrient broth were measured and dissolved in 1 L of tap water simultaneously, and stirred and then autoclaved. After sterilization, the nutrient agar was poured into Petri dishes before cooling while the nutrient broth was stored at -4°C . Microbes used for determination of the antimicrobial activity of the crude extracts were: Methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (ATCC25922), *Bacillus cereus* (ATCC11778), *Aspergillus niger* (ATCC16888), and *Candida albicans* (ATCC10231). The microbes already stored at -4°C were revived by subculturing them in agar plates and incubating at 37°C them for 24 h for MRSA, *B. cereus* and *E. coli* and 72 h for *A. niger* and *C. albicans*.

Preparation of Test Extracts

Crude extract from each species was dissolved in dimethyl sulfoxide (DMSO) followed by subsequent serial dilution with distilled water to get 200, 100, and 50 mg/ml.^[24] The percentage of DMSO in prepared extracts was below 1% to avoid carry over

effects. It is reported that a DMSO concentration of <1% in bioassay preparations has no apparent effect on microbial growth.^[25] Paper discs (5 mm in diameter) were incubated with each of the three concentrations and allowed to dry under laminar flow hood. Ketoconazole and amoxicillin were used as the positive controls for the antifungal and antibacterial activity, respectively, while 1% DMSO was used as the negative control.

Disc Diffusion Technique

Pure culture inoculum of each of the test microbes was prepared by transferring a loopful of the revived microbes into 10 ml of distilled water in a test tube followed to shaking to ensure even mixing. Using a micropipette, 1 ml inoculum of pure culture from each of the five microbes was transferred to nutrient agar on Petri dishes aseptically and spread with an L-shaped glass rod. The discs prior incubated with the crude extracts at different concentrations were then placed evenly on the nutrient agar, and the Petri dishes were placed in inverted position and then placed in an incubator set at 37°C. For the bacteria, zone of inhibition was determined after 24 h while for the fungi, zone of inhibition was determined after 72 h.

Broth Dilution Technique

Minimum inhibitory concentration (MIC) was determined by broth dilution in sterile standard test tubes. A 24-h plate culture of the test microbes was adjusted to 0.5 McFarland turbidity standard using a spectrophotometer. This 0.5 absorbance level is assumed to contain $1-2 \times 10^8$ colony forming units/mL.^[26] Organic crude extracts of each of the *Plectranthus* species were first dissolved in DMSO followed by serial dilution with nutrient broth from 200 to 25 mg/ml using micropipettes. The percentage of DMSO in prepared dilutions was below 1% to avoid carry over effects. The dilutions were then inoculated with 500 μ l of test microbes (MRSA, *B. cereus*, *E. coli*, *C. albicans*, and *A. niger*) and incubated at 37°C for 24 h for bacteria and 30°C for 72 h for fungi. The lowest concentration with no visible bacterial or fungal growth was assumed to be the MIC for the respective microbe.

Partition Liquid Chromatography

P. barbatus was found to have the highest antimicrobial activity compared to the other species, and hence the crude extract of *P. barbatus* was subjected to further study. This involved partitioning the crude extract in solvents of increasing polarity and performing bioassay of the partitions. Leaves of *P. barbatus* were air dried and ground into powder using a blender. 1000 g of the ground material was subjected to extraction using 80% ethanol for 1 week. Ethanol was then vaporized and remaining crude extract

was suspended in distilled water and then subjected to successive partitioning with organic solvents of increasing polarity. Solvents used were petroleum ether, n-hexane, dichloromethane (DCM), chloroform, and last ethyl acetate. This resulted into five fractions; petroleum ether partition, n-hexane partition, DCM partition, chloroform partition, and ethyl acetate partition. The partitions were concentrated in a rotary evaporator and then left to dry in a fume chamber to ensure the total extract was free from the extraction solvents.^[23] The resulting partitions were subjected antimicrobial activity through disc diffusion and broth dilution.

Data Analysis

Analysis of data was performed using SPSS. Using the software; descriptive statistics such as means, standard errors of the mean, variance, range, and confidence interval of the mean of inhibition zones were computed. In addition, one-way ANOVA was performed to determine whether there were significant differences in terms of antimicrobial activity among the *Plectranthus* species/partitions investigated. Further, ANOVA (*post hoc* ANOVA) was also performed using Dunnett *t*-test to compare the activity of the crude extracts of *Plectranthus* species/partitions with the antimicrobial activity of the positive controls (amoxicillin and ketoconazole) to determine where there was any significant difference. Dunnett *t*-test is recommended when we have a control group in an experiment.^[27] The level of significance/probability level used in the analysis was ≤ 0.05 .

RESULTS

Antimicrobial Activity of *Plectranthus* Species

Disc diffusion results

DCM:MeOH (1:1) crude organic extracts from each of the 10 species were subjected to antimicrobial activity through disc diffusion, and the results are represented below.

It was observed that DCM:MeOH crude extracts from all the 10 species investigated inhibited the multiplication of MRSA. Comparing all the species, highest inhibition zones were observed in 200 and 100 mg/ml of *P. lanuginosus* which were 15 and 12.3 mm, respectively, and in 200 and 100 mg/ml of *P. barbatus* which were 13 and 10.3, respectively. MRSA growth inhibition zones for the positive control (amoxicillin [50 mg/ml]) and negative control (1% DMSO) were 14 and 0 mm, respectively (Figure 1).

All the species investigated inhibited the growth of *B. cereus*. Among the 10 species, highest growth inhibitions of *B. cereus* were observed in 200 and 100 mg/ml of *P. otostegioides* which were 11.7 and 9.7 mm, respectively, 200 and 100 mg/ml of

P. lanuginosus which were 11.7 and 10.7 mm, respectively, and 200 and 100 mg/ml of *P. barbatus* which were 12.3 and 8.7 mm, respectively, *B. cereus* growth inhibition zones for the positive control (amoxicillin [50 mg/ml]) and 1% DMSO were 15 and 0 mm, respectively (Figure 2).

It was observed that only the DCM:MeOH crude extracts from *P. barbatus* and *P. edulis* were able to inhibit the multiplication of *E. coli*. 200 and 100 mg/ml of *P. barbatus* exhibited average inhibition zones of 9 and 7.7 mm, respectively, while 200 and 100 mg/ml of *P. edulis* exhibited average inhibition zones of 8.3 and 6.3 mm, respectively, against *E. coli*. The other species did not inhibit the growth of *E. coli*. *E. coli* growth inhibition zones for the positive control (amoxicillin [50 mg/ml]) and negative control (1% DMSO) were 25 and 0 mm, respectively (Figure 3).

All the species except *P. caninus* inhibited the multiplication of *C. albicans*. Comparing the 10 species, highest growth inhibitions against *C. albicans* were observed in 200 mg/ml of *P. otostegioides*, *P. ornatus*, *P. aegyptiacus*, *P. barbatus*, and *P. amboinicus* which was 10.3, 12, 10.3, 13, and 10.3 mm, respectively. *C. albicans*

growth inhibition zones for the positive control (ketoconazole [40 mg/ml]) and 1% DMSO were 13 and 0 mm, respectively (Figure 4).

P. montanus, *P. otostegioides*, *P. aegyptiacus*, *P. lanuginosus*, *P. barbatus*, *P. amboinicus*, and *P. edulis* inhibited the multiplication of *A. niger* while *P. pseudomarrubioides*, *P. ornatus*, and *P. caninus* did not inhibit the growth of *A. niger*. *P. lanuginosus* and *P. barbatus* exhibited higher inhibition zones against *A. niger* compared to the rest of the species. Average inhibition zones for 200 mg/ml *P. lanuginosus* and *P. barbatus* were 13.3 and 13.7 mm, respectively. Both ketoconazole (40 mg/ml) and 1% DMSO did not inhibit the growth of *A. niger* (Figure 5).

MIC values of total extracts from studied *Plectranthus* species

DCM:MeOH (1:1) crude organic extract from each of the 10 species were subjected to antimicrobial activity through broth dilution, and the results are represented in Table 1.

From the preliminary antimicrobial activity screening of DCM:MeOH crude extracts from the 10 species we find that all the species inhibited the growth of MRSA

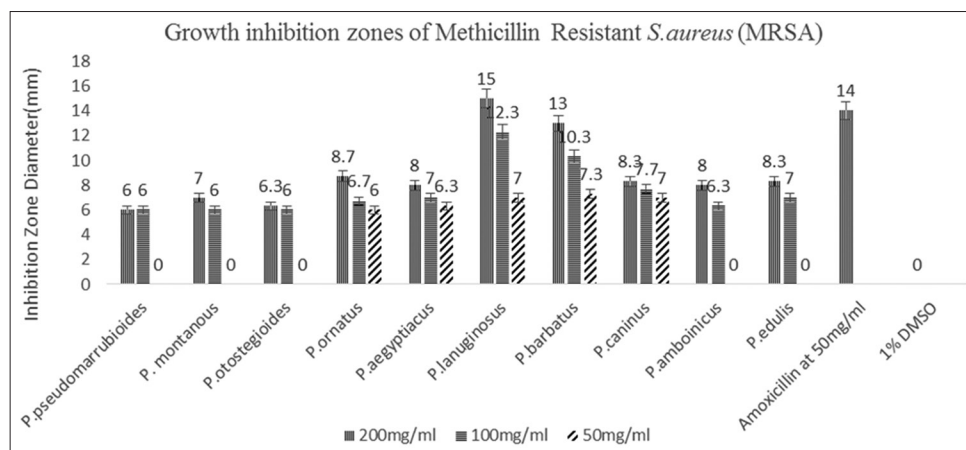


Figure 1: Antibacterial activity of *Plectranthus* species against methicillin resistant *Staphylococcus aureus*

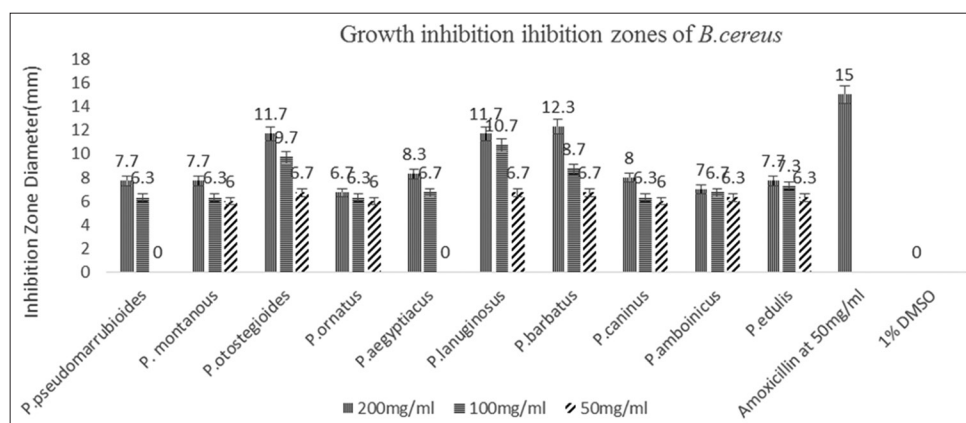


Figure 2: Antibacterial activity of *Plectranthus* species against *Bacillus cereus*

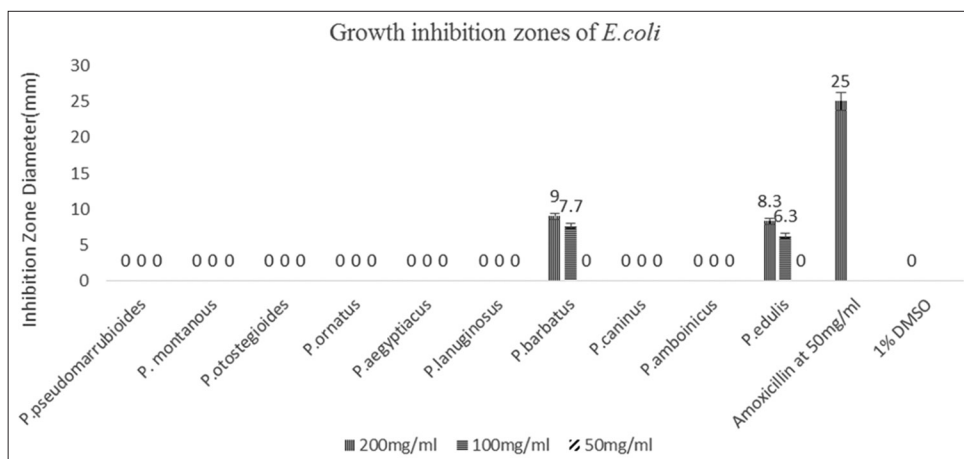


Figure 3: Antibacterial activity of *Plectranthus* species against *Escherichia coli*

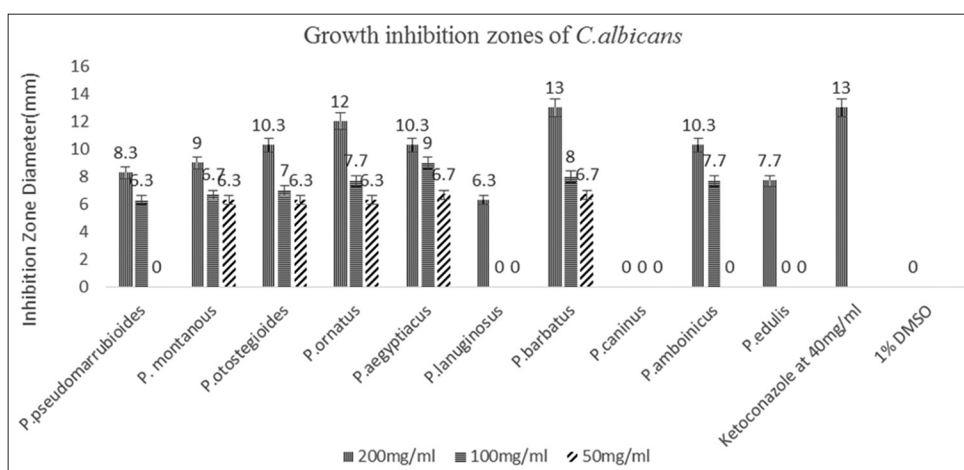


Figure 4: Antifungal activity of *Plectranthus* species against *Candida albicans*

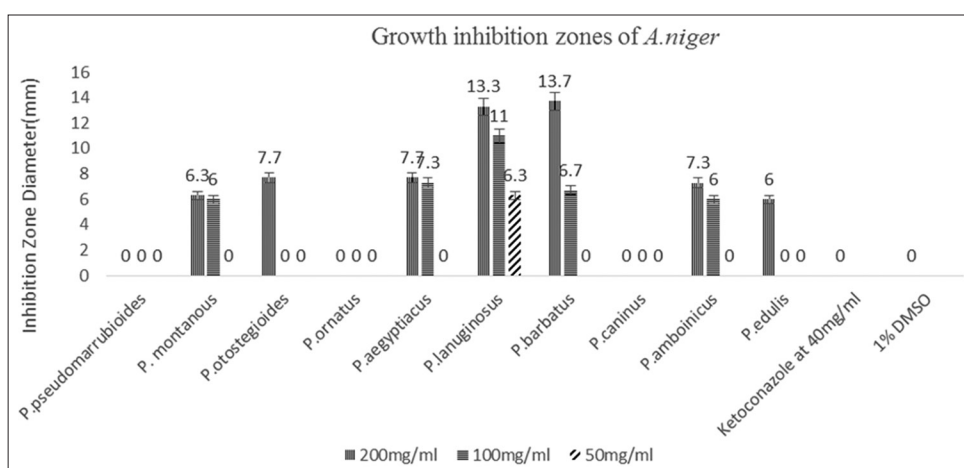


Figure 5: Antifungal activity of *Plectranthus* species against *Aspergillus niger*

and *B. cereus*. Lowest MIC values for MRSA were observed in *P. aegyptiacus* (40 mg/ml), *P. lanuginosus* (40 mg/ml), and in *P. barbatus* (25 mg/ml) while lowest MIC values for *B. cereus* of 40 mg/ml were observed in *P. otostegioides*, *P. barbatus*, and in *P. edulis*. For *E. coli*, all MIC values were above 200 mg/ml except for *P. barbatus* and *P. edulis* which both had

a MIC value of 100 mg/ml against *E. coli*. On the other hand, *P. montanus*, *P. otostegioides*, *P. ornatus*, *P. aegyptiacus*, and *P. barbatus* crude extracts had the lowest MIC value of 50 mg/ml against *C. albicans*. Finally, comparing the 10 species investigated, it was observed that crude extracts of *P. lanuginosus* had the lowest MIC value of 50 mg/ml against *A. niger*.

Table 1: MIC of *Plectranthus* species

<i>Plectranthus</i> species	MICs				
	MRSA (mg/ml)	<i>Bacillus cereus</i> (mg/ml)	<i>Escherichia coli</i> (mg/ml)	<i>Candida albicans</i> (mg/ml)	<i>Aspergillus niger</i> (mg/ml)
<i>Plectranthus pseudomarruboides</i>	100	100	No inhibition/>200	100	No inhibition/>200
<i>Plectranthus montanus</i>	75	50	No inhibition/>200	50	200
<i>Plectranthus otostegioides</i>	100	40	No inhibition/>200	50	150
<i>Plectranthus ornatus</i>	50	50	No inhibition/>200	50	No inhibition/>200
<i>Plectranthus aegyptiacus</i>	40	100	No inhibition/>200	50	100
<i>Plectranthus lanuginosus</i>	40	50	No inhibition/>200	150	50
<i>Plectranthus barbatus</i>	25	40	100	50	100
<i>Plectranthus caninus</i>	50	50	No inhibition/>200	No inhibition/>200	No inhibition/>200
<i>Plectranthus amboinicus</i>	100	50	No inhibition/>200	75	200
<i>Plectranthus edulis</i>	75	40	100	150	200

MIC: Minimum inhibitory concentrations, MRSA: Methicillin resistant *Staphylococcus aureus*

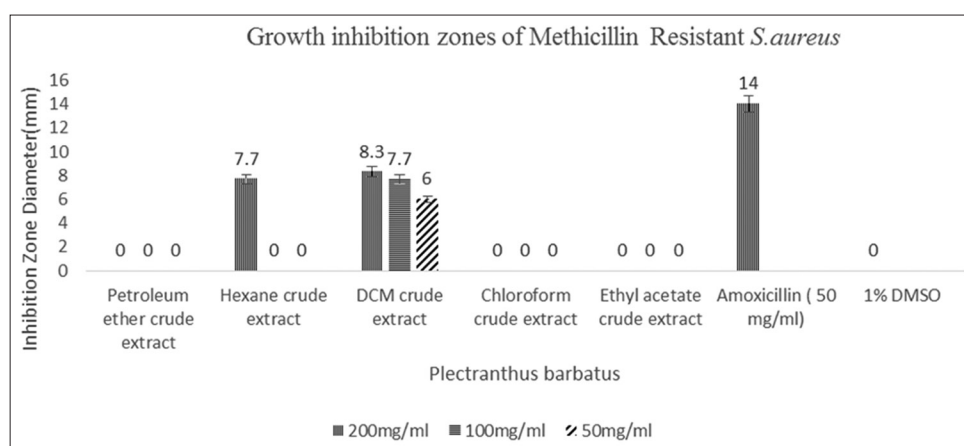


Figure 6: Antibacterial activity of *Plectranthus barbatus* fractionated crude extracts against methicillin resistant *Staphylococcus aureus*

In general, broth dilution results indicated that crude extracts of *P. barbatus* had the lowest MIC values against the test microbes compared to the other *Plectranthus* species and hence were selected for further phytochemical analysis and bioassay.

Antimicrobial Activity of Fractionated Extracts of *P. barbatus*

Disc diffusion results

Crude ethanol extract of *P. barbatus* was sequentially partitioned to give five partitions. These were petroleum ether, n-hexane, DCM, chloroform, and EtoAC. All the partitions were likewise investigated for their antimicrobial activity using disc diffusion and broth dilution technique. The results are represented.

Only hexane and DCM fractions of *P. barbatus* inhibited the growth of MRSA. Average growth inhibition diameter for 200 mg/ml of hexane fraction was 7.7 mm while average growth inhibition diameters for 200, 100, and 50 mg/ml of the DCM fraction were 8.3, 7.7, and 6 mm, respectively. Average growth inhibition zones of MRSA by amoxicillin (50 mg/ml)

and 1% DMSO were 14 and 0 mm, respectively (Figure 6).

All the generated fractions of *P. barbatus* inhibited the multiplication of *B. cereus* in a dose-dependent manner except the hexane fraction. 200 and 100 mg/ml of petroleum ether fraction exhibited growth inhibition zone of 9.3 and 8 mm, respectively, 200 mg/ml of the DCM fraction exhibited a growth inhibition zone of 9 mm. Growth inhibition zones of *B. cereus* by 200 and 100 mg/ml of the chloroform fraction were 12 and 8.3 mm, respectively, while *B. cereus* growth inhibition zones by both 200 and 100 mg/ml of the ethyl acetate fraction were 11.3 and 9.3 mm, respectively. *B. cereus* growth inhibition zone by amoxicillin (50 mg/ml) and 1% DMSO was 15 and 0 mm, respectively (Figure 7).

All generated fractions inhibited the growth of *E. coli* with highest growth inhibition zones being observed in the DCM and chloroform crude extracts. 200, 100, and 50 mg/ml of the DCM fraction exhibited growth inhibition diameters of 13.3, 10.7, and 10 mm, respectively, while 200, 100, and 50 mg/ml of the chloroform fraction exhibited growth inhibition

diameters of 11, 10.3, and 8.3 mm, respectively. Average growth inhibition zones of *E. coli* by amoxicillin (50 mg/ml) and 1% DMSO were 24 and 0 mm, respectively (Figure 8).

All generated fractions of *P. barbatus* inhibited the growth of *C. albicans*. Among the five fractions investigated, highest growth inhibitions were observed in the DCM and chloroform fractions of *P. barbatus*. 200, 100, and 50 mg/ml of the DCM fraction exhibited growth inhibition diameters of 11.7, 10.7, and 8.3 mm, respectively, while 200, 100, and 50 mg/ml of the chloroform fraction exhibited growth inhibition diameters of 10, 9.3, and 7.7 mm, respectively. Average growth inhibition zones of *C. albicans* by ketoconazole (40 mg/ml) and 1% DMSO were 14 and 0 mm, respectively (Figure 9).

Only the hexane and DCM fractions of *P. barbatus* inhibited the multiplication of *A. niger*. The other fractions of *P. barbatus* together with the control did

not inhibit the growth of *A. niger*. Average growth inhibition diameters of the 200 mg/ml of the hexane fraction, 200 and 100 mg/ml of the DCM fraction were 8.7, 9.7, and 6.7 mm, respectively (Figure 10).

MICs of *P. barbatus* fractionated crude extracts

From the broth dilution results lowest MIC values were observed in DCM fraction (40 mg/ml) against MRSA, EtoAC (60 mg/ml) fraction against *B. cereus*, 25 mg/ml of both the DCM and chloroform fraction against both *E. coli* and *C. albicans* and last in DCM (75 mg/ml) against *A. niger*. Broth dilution results indicated that *P. barbatus* generally had the lowest MIC values against all the microbes tested (Table 2).

DISCUSSION

Majority of *Plectranthus* species have been used in ethnomedicine for the management of various disease conditions. Bioassay studies have identified

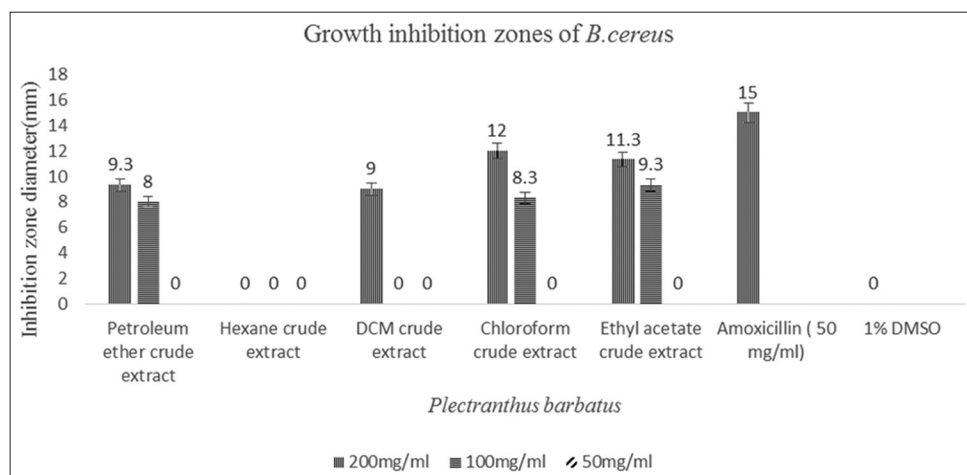


Figure 7: Antibacterial activity of *Plectranthus barbatus* fractionated crude extracts against *Bacillus cereus*

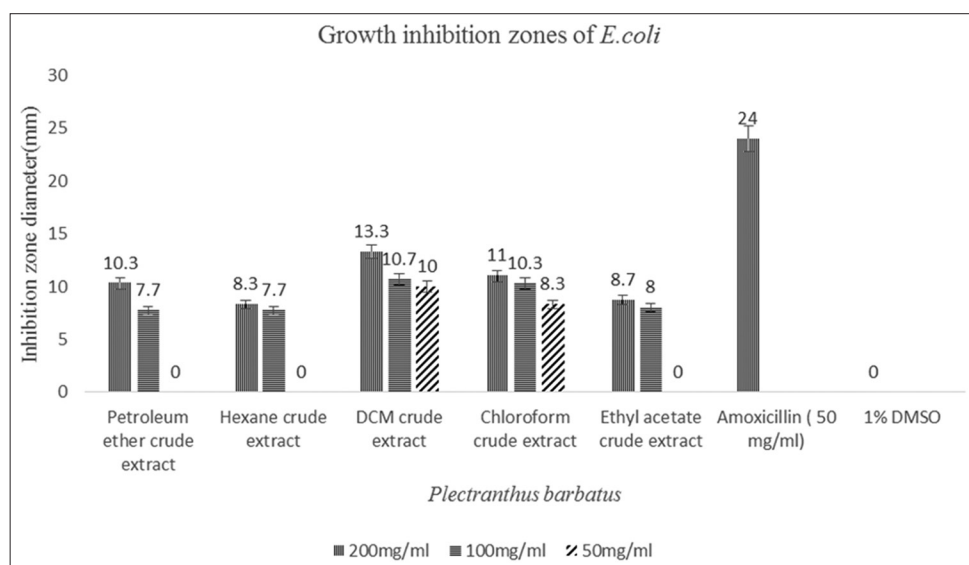


Figure 8: Antibacterial activity of *Plectranthus barbatus* fractionated extracts against *Escherichia coli*

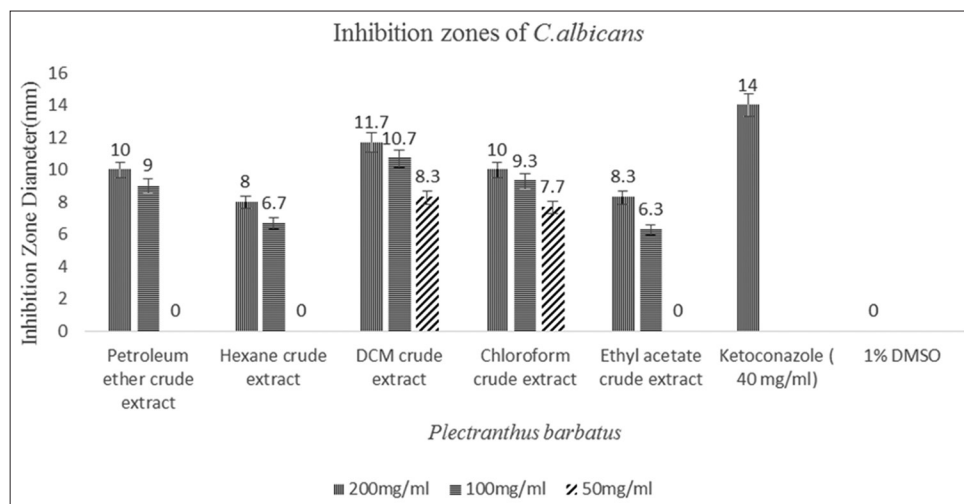


Figure 9: Antifungal activity of *Plectranthus barbatus* fractionated crude extracts against *Candida albicans*

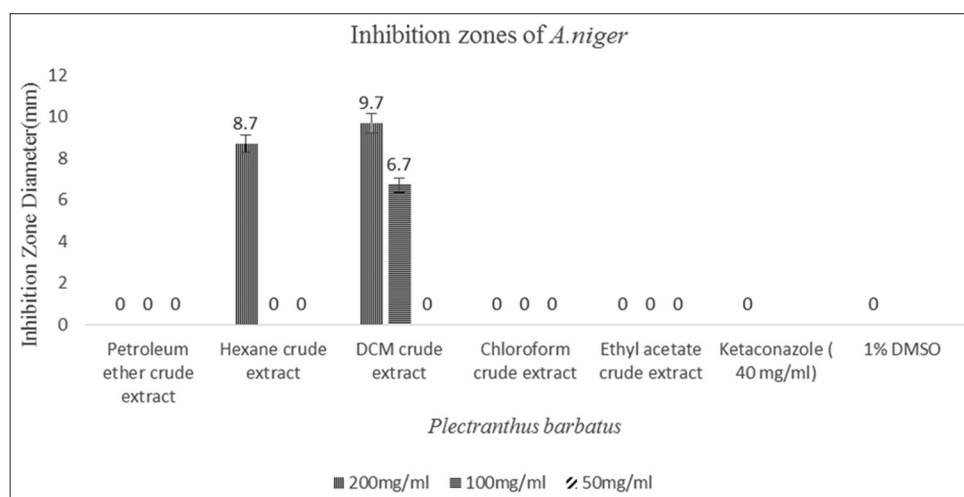


Figure 10: Antifungal activity of *Plectranthus barbatus* fractionated crude extracts against *Aspergillus niger*

Table 2: MIC of *Plectranthus barbatus* crude extracts

<i>Plectranthus barbatus</i> fraction	MICs				
	MRSA	<i>Bacillus cereus</i> (mg/ml)	<i>Escherichia coli</i> (mg/ml)	<i>Candida albicans</i> (mg/ml)	<i>Aspergillus niger</i> (mg/ml)
Petroleum ether	No inhibition/>200	75	75	60	No inhibition/>200
n-hexane	150	No inhibition/>200	75	75	150
DCM	40	150	25	25	75
Chloroform	No inhibition/>200	75	25	25	No inhibition/>200
Ethylacetate	No inhibition/>200	60	75	75	No inhibition/>200

MIC: Minimum inhibitory concentrations, MRSA: Methicillin resistant *Staphylococcus aureus*, DCM: Dichloromethane

several members from the genus with promising bioactivities while phytochemical analysis has led to the isolation of important secondary metabolites from *Plectranthus* species.^[28] In this study, crude organic extracts from all the 10 species were tested against three bacteria (MRSA, *B. cereus*, and *E. coli*) and two fungi (*C. albicans* and *A. niger*). DCM:MeOH (1:1) leaf crude organic extracts from most of the species were found to possess antibacterial activity against the microbes investigated. *P. barbatus* was found to be the most active species compared to the other

species investigated and was subsequently subjected to partitioning resulting to five fractions which were likewise subjected to antimicrobial activity.

To begin with, all the crude organic extracts from the 10 *Plectranthus* species were found to inhibit the multiplication of MRSA with *P. lanuginosus* and *P. barbatus* leaf crude extracts inhibiting the growth of MRSA most compared to the other *Plectranthus* species. MIC values of *P. barbatus* and *P. lanuginosus* leaf crude extracts against MRSA were

25 and 40 mg/ml, respectively. When the crude organic extracts were tested against *B. cereus*, highest growth inhibition was observed in *P. otostegioides* (MIC value = 40 mg/ml), *P. lanuginosus* (MIC value = 50 mg/ml), and *P. barbatus* (MIC value = 40 mg/ml), while only crude extracts of *P. barbatus* (MIC value = 100 mg/ml) and *P. edulis* (MIC value = 100 mg/ml) inhibited the multiplication of *E. coli*. The other species did not inhibit the growth of *E. coli*. On the other hand, when the crude organic extracts were subjected to *C. albicans* to access their antifungal activity, *P. otostegioides*, *P. ornatus*, *P. aegyptiacus*, *P. barbatus*, and *P. amboinicus* were found to inhibit the growth of *C. albicans*, highest growth inhibition of *C. albicans* was observed under *P. ornatus* (MIC value = 50 mg/ml) and *P. barbatus* (MIC value = 50 mg/ml). Finally, growth inhibition of *A. niger* was observed in the crude organic extracts of *P. lanuginosus* (MIC value = 50 mg/ml) and *P. barbatus* (MIC value = 100 mg/ml).

Multiple comparisons were carried out using Dunnett *t*-test whereby various concentrations of the DCM:MeOH (1:1) crude leaf extracts of the *Plectranthus* species were compared with the positive controls. Only the comparison of the growth inhibitions of 200 mg/ml of both *P. barbatus* and *P. lanuginosus* leaf crude extracts against MRSA with growth inhibitions of 50 mg/ml of amoxicillin against MRSA gave a significance level of ≥ 0.05 , i.e., 0.451 and 0.439, respectively. Hence, it was concluded that growth inhibition of MRSA due to 200 mg/ml of both *P. barbatus* and *P. lanuginosus* leaf crude extracts was not significantly different from the growth inhibition of MRSA due to amoxicillin at significance level (*P*) of ≤ 0.05 . With respect to antifungal activity, various concentrations of the DCM: MeOH leaf crude extracts of *Plectranthus* species were likewise compared with ketoconazole. Only the comparison of growth inhibition of 200 mg/ml of both leaf crude extracts of *P. ornatus* and *P. barbatus* against *C. albicans* with growth inhibition of 40 mg/ml of ketoconazole against *C. albicans* gave a significance level of ≥ 0.05 , i.e., 0.291 and 1.00, respectively. This implied that *C. albicans* growth inhibition by 200 mg/ml of *P. ornatus* and *P. barbatus* leaf crude extracts was not significantly different from *C. albicans* growth inhibition by 40 mg/ml of ketoconazole at $P \leq 0.05$.

From the disc diffusion results of DCM:MeOH leaf crude extracts from the 10 *Plectranthus* species, *P. barbatus* displayed the highest growth inhibition zones against the test microbes compared to the other species under study. On the determination of MICs of the DCM: MeOH crude extracts from the 10 *Plectranthus* species using broth dilution, it was also observed that *P. barbatus* displayed the lowest MIC values against the microbes. MIC values of the leaf crude extracts of *P. barbatus* against MRSA, *B. cereus*,

E. coli, *C. Albicans*, and *A. niger* were as follows: 25, 40, 100, 50, and 100 mg/ml, respectively (Table 1). Hence, it was subjected to further study whereby leaf crude extracts of *P. barbatus* were fractionated using five solvents of increasing polarity, namely, petroleum ether, n-hexane, DCM, chloroform, and ethyl acetate. These fractionated crude extracts were tested against each of the five microbes using disc diffusion and broth dilution techniques.

From the *P. barbatus* fraction results, DCM (MIC value = 40 mg/ml) and hexane (MIC value = 150 mg/ml) leaf crude extracts of *P. barbatus* were found to have activity against MRSA while petroleum ether, chloroform, and ethyl acetate crude extracts had no activity against MRSA and their MIC values against MRSA were >200 mg/ml. Second, petroleum ether (MIC value = 75 mg/ml), DCM (MIC value = 150 mg/ml), chloroform (MIC value = 75 mg/ml), and EtoAC (MIC value = 60 mg/ml) leaf crude extracts of *P. barbatus* inhibited the multiplication of *B. cereus*. When all the crude extracts of *P. barbatus* were tested against *E. coli*, they all inhibited the growth of *E. coli* with DCM (MIC value = 25 mg/ml) and chloroform (MIC value = 25 mg/ml) crude extracts displaying the highest growth inhibition. On antifungal activity, *C. albicans* growth was likewise inhibited by all *P. barbatus* leaf crude extracts with DCM (MIC value = 25 mg/ml) and chloroform (MIC value = 25 mg/ml) extracts inhibiting the growth most. Finally, only two leaf crude extracts of *P. barbatus* inhibited the growth of *A. niger* which were the hexane (MIC value = 150 mg/ml) and DCM (MIC value = 75 mg/ml) crude extracts.

From the multiple comparisons of growth inhibitions of various concentrations of *P. barbatus* fractions with that of the respective positive control using Dunnett *t*-test, it was observed that the comparison of inhibition zone of 200 mg/ml of the DCM crude extract with ketoconazole activity gave a significance level of 0.109. This implied that the 200 mg/ml of the DCM fraction of *P. barbatus* had an antifungal activity which was not significantly different from that of ketoconazole at $P \leq 0.05$. However, all the other fractions of *P. barbatus* had antimicrobial activities against the microbes tested only that the activities were low and significantly different from the positive control activities. After subjecting the crude extracts of *P. barbatus* to broth dilution, DCM crude extract was found to have the lowest MIC values against the microbes tested. MIC values of the *P. barbatus* DCM leaf crude extracts against MRSA, *B. cereus*, *E. coli*, *C. albicans*, and *A. niger* were as follows: 40, 150, 25, 25, and 75 mg/ml, respectively (Table 2). As a result, DCM crude extracts of *P. barbatus* were assumed to possess the highest antimicrobial activity compared to the rest of the crude extracts.

Various previous studies have reported the presence of antimicrobial activity in *Plectranthus* species and corroborate the findings of this study. For example, crude ethanol extracts of *P. barbatus* have been shown to possess antimicrobial activity against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, and *E. coli*.^[29] Aqueous methanol and hexane extracts of *P. barbatus* have likewise been reported to have antimicrobial activity against *S. aureus*.^[3] Elsewhere, methanolic extracts from the roots of *P. barbatus* were also reported to possess strong anti-*Candida* activity.^[30] Aqueous extracts of *P. barbatus* have also been reported to have antibacterial activity against *Streptococcus sobrinus* and *Streptococcus mutans*.^[31] Petroleum ether, DCM, and water extracts of *P. barbatus* have also been reported to inhibit the growth of *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa* and this is in line with the current study findings.^[32]

Elsewhere, essential oil of *P. amboinicus* has been reported to possess antifungal activity against *Aspergillus flavus*, *A. niger*, *Aspergillus ochraceus*, *Aspergillus oryzae*, *Candida versatilis*, *Fusarium moniliforme*, and *Saccharomyces cerevisiae* in stored food products.^[33] Methanolic extracts of *P. barbatus* and *P. amboinicus* have also been reported to have antifungal activity against *Candida krusei*.^[34] Essential oil of *P. amboinicus* has also been reported to inhibit the growth of *C. albicans*, *Candida tropicalis*, *Candida guilliermondii*, and *C. krusei*.^[35] Another study revealed that essential oil of *P. ornatus* has antibacterial activity against *S. aureus*, *S. pyogenes*, *E. coli*, and *Salmonella typhimurium*.^[35] Essential oil of *P. montanus* is said to have antifungal activity and has been shown to inhibit the growth of *C. albicans*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Fusarium oxysporum*, *Curvularia lunata*, and *Stemphylium solani*.^[36]

Another study showed that *P. montanus* has significant antibacterial activity against *E. coli*, *Shigella* spp. and *Vibrio cholera* and antifungal activity against *C. albicans*, and *A. niger*.^[37] Finally, *P. caninus* has been reported to have antimicrobial activity.^[38] Several previous studies have reported antimicrobial activity of some of the plant species investigated in this study. However, the antimicrobial activity of *P. pseudomarruboides*, *P. edulis*, *P. aegyptiacus*, *P. otostegioides*, and *P. lanuginosus* has not been reported before although these species have been reported to possess other bioactivities. For example, aqueous extracts of *P. lanuginosus* have been reported to have antioxidant activity,^[39] *P. caninus* has shown to exhibit diuretic activity and anti-tumor activity.^[40] *P. pseudomarruboides* has been used as an insect repellent.^[41]

Plectranthus species have been reported to have low toxicity in both man and animals.^[1] For example, *P. barbatus* has been reported to generally possess low toxicity and high antibacterial and antioxidant activities.^[31] Similarly, ethyl acetate extract from the leaves of *P. barbatus* has been reported to have low toxicity on peripheral blood mononuclear cells.^[42] Aqueous extract from the leaves of *P. amboinicus* was found to be non-toxic to *Artemia salina*.^[43] Moreover, 2000 mg/kg of methanolic crude leaf extract from *P. amboinicus* was reported to be non-toxic to male and female Swiss albino mice and did not cause a change in hematological and biochemical parameters in the mice.^[44] Ethanolic crude extracts from the aerial parts of *P. ornatus* was reported to be moderately toxic to *A. salina*.^[45] It has also been reported that concoctions from *P. ornatus* and *P. amboinicus* do not present any adverse effects to people using them in the management of ailments.^[46] *P. caninus* was reported to exhibit low cytotoxic with high radical scavenging and anti-inflammatory activity.^[38,40] No previous studies have reported the toxicity of *P. lanuginosus*, *P. edulis*, *P. pseudomarruboides*, and *P. aegyptiacus*. The fact that *P. edulis* is used as food proves that this species does not have adverse effects on cells.

CONCLUSION

From this investigation, DCM:MeOH (1:1) crude extracts of *P. barbatus*, *P. lanuginosus*, and *P. ornatus* have been reported to have antimicrobial activity. 200 mg/ml of DCM:MeOH (1:1) leaf crude extracts of *P. barbatus* and *P. lanuginosus* had high antibacterial activity against MRSA, and the activity was not significantly different from the antibacterial activity of 50 mg/ml amoxicillin against MRSA at significance level (*P*) of ≤ 0.05 . Again *P. ornatus* and *P. barbatus* had antifungal activity against *C. albicans* which was not significantly different from that of 40 mg/ml of ketoconazole at $P \leq 0.05$. The study also reports for the first time, the antimicrobial activity of *P. pseudomarruboides*, *P. edulis*, *P. aegyptiacus*, *P. otostegioides*, and *P. lanuginosus*, species which have been used in ethnomedicine by various communities around the world. DCM:MeOH (1:1) crude extracts of *P. barbatus* displayed the lowest MIC levels ranging from 25 to 100 mg/ml against MRSA, *B. cereus*, *E. coli*, *C. albicans*, and *A. niger*. Further antimicrobial activity of *P. barbatus* fractions showed that the DCM partition of *P. barbatus* leaves had lowest MIC levels ranging from 40 to 150 mg/ml against the microbes investigated compared to the other fractions of *P. barbatus*.

Many species within the genus have been used in the management of many diseases, and several studies have confirmed that these species have antimicrobial activities. Studies on *Plectranthus* species have

also led to the isolation of compounds from these species and screening of the isolated compounds for bioactivities has been done. Moreover, *Plectranthus* species are rich in terpenes notably diterpenes and phenolics and more research geared toward isolation of these compounds from *Plectranthus* species coupled with bioassays should continue. This could lead to the isolation of more compounds with promising bioactivities. Wide usage of *Plectranthus* species in ethnomedicine notably *P. barbatus* and its documented high antimicrobial activities and low toxicity prove that this plant can be vital as a source of other bioactive novel products or chemical templates due to its ability to inhibit the growth of pathogenic microbes. Furthermore, research on the distribution of phytoconstituents in *Plectranthus* species can help in the classification of the genus and compliment classification based on molecular and morphological characters.

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