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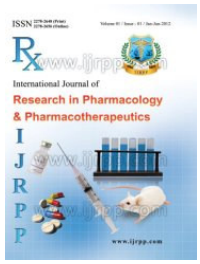

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Evaluation of antimicrobial activity of the roots of *Plectranthus Sp.*

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ABSTRACT

Introduction

Antimicrobial-resistance infection is currently one of the main threats to humanity. This study investigates the antimicrobial activity of the different extracts of the *Plectranthus coleoides roots* that belongs to the Lamiaceae family.

Methods

100 g of *P. coleoides* root dried, pulverised extracted with methanol. A further 400 g of the plant content was also macerated successively with n-hexane, ethyl acetate, and methanol. These extracts were evaluated using agar diffusion method for antimicrobial activity on clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans*, and minimal inhibitory concentration was determined. Ciprofloxacin has been used as a standard antibacterial drug, while Ketoconazole has been used as a standard antifungal.

Results

The most active extract on *E. coli* has been the ethyl acetate extract with an inhibitory minimum concentration (MIC) of 0.049 mg / mL. Also the n-hexane extract had activity on *E. Coli* with MIC 0.196 mg / mL. Only n-hexane extract had been successful against *Candida albicans* with a 6.2 5 mg / mL MIC.

Conclusion

The *n*-hexane extract was active against *C. albicans*, ethyl acetate extract was active against *E. coli*. Thus, suggesting that the *P.coleoides* could be of great value in the development of a potent antimicrobial agent with further studies.

Keywords: *P.coleoides,* Ketoconazole, *C. albicans, E. Coli, extract,* n-hexane, ethyl acetate

INTRODUCTION

Infectious diseases are still the main cause of death, accounting for a quarter of all deaths in the world [1]. Infections due to antimicrobial resistance (AMR) are increasing [2]. Antimicrobial resistance is currently one of the major threats facing mankind. The emergence and rapid spread of multi-drug resistant organisms (vancomycin, methicillin, extended-spectrum β-lactam, carbapenem, and colistin) have put the world in a dilemma The health and economic burden associated with AMR on a global scale are dreadful. Available antimicrobials have been misused and are almost ineffective while some of the drugs are associated with dangerous side effects in some individuals [3]. Therefore, there is a need to discover new antimicrobial agents to combat the growth of resistant microorganisms. [4].

Plant-derived drugs have made great contributions to human health and well-being. They provide key chemical compounds for the development of new antimicrobial drugs. In this regard, the most interesting bioactive components of plants are phenolic compounds, alkaloids, tannins and flavonoids [5]. Antibacterial agents are compounds or substances that kill or inhibit the growth of microorganisms.

Plectranthus coleoides is also known as *Coleus glabratus, Coleus paniculatus, Solenostemon paniculatus* (Lamiaceae). The main phytochemical components of are diterpenoids, essential oils and phenols. About 140 diterpenes were identified Plectranthus species. The main components are of abietanons, phyllocladanes. It has been used as in dermatitis, as antibacterial, deodorant, and cooling agent. An attempt of antimicrobial study for the roots were studied.

MATERIALS AND METHODS

Plant Identification and Collection

The plant was collected from Kanyakumari, Tamil Nadu, India in January 2016. The herbarium specimens of plants are stored in the Pharmacognosy Department. The plant was supplied Dr. K. Senthilkumar, Scientist, Epoch Ayurvedic Division.

Extraction Procedure

The collected cut roots are dried for 10-12 days in a cool place. After being completely dried, the dried root is crushed into coarse powder. 100 grams of powdered root was macerated with methanol for 3 days. The extract obtained is called crude methanol (CME). Also 400 grams of powdered root was continuously macerated with n-hexane, ethyl acetate and methanol for 3 days. The combined filtrate of each solvent was evaporated in a rotary evaporator at 40°C under vacuum and then weighed. These three extracts were named NHE, EAE and ME.

Antimicrobial Assay Method

Preparation of test extracts: Weigh 500 mg (0.5 g) of dry extracts and dissolve them in 10 ml of appropriate solvent (the extracts of n-hexane and ethyl acetate are dimethyl sulfoxide (DMSO), methanol The extract is water) to obtain a stock solution with a concentration of 50 mg/mL. A two-fold serial dilution of the 50 mg/mL stock solution was prepared.

Preparation of Standard Drugs

Ciprofloxacin is used as a standard antibacterial agent. Dissolve 500 mg of ciprofloxacin in 100 ml of water to obtain a concentration of 5 mg/mL stock solution. Ketoconazole at 5 mg/mL was also prepared as a standard antifungal agent. From the 5 mg/mL stock solution of ciprofloxacin and ketoconazole standard agents, lower concentrations (2.5, 1.25, 0.625, 0.3125 mg/mL) were also obtained by two consecutive dilutions.

Test Microorganisms

The clinical isolates of the test organisms; *Staphylococcus aureus* (Gram-positive bacterium), *Escherichia coli* (Gram-negative bacterium), *Pseudomonas aeruginosa* (Gram-negative bacterium) and *Candida albicans* (Fungus) were standardized to 0.5 Mcfarland standard.

Antimicrobial Susceptibility Test Using Agar Diffusion Method

Add 0.1 ml of each test microorganism aseptically to the prepared Muller Hinton agar [6] in the universal bottles and properly mixed. Then the mixture is poured into the corresponding sterile petri dish, and each test microorganism is labeled and solidified. Use sterile cork to make five holes on each plate. In the fifth well of the standard antibiotic center, mark the well as four (4) extracts. Carefully and aseptically add 50 mg/mL of each extract dropwise to the well. Also add 5 mg/mL of standard antibiotic (ciprofloxacin) aseptically to the fifth well. Keep the petri dish for 15 minutes to spread properly. The plate (Petri dish) was incubated at 37°C for 24h. Use a meter ruler to measure the diameter of the suppression zone formed in millimeters (mm) through the bottom of the board. Repeat this step for the fungus Candida albicans, but use Sabouraud's Dextrose Agar as the nutrient medium [7] and 5 mg/mL of ketoconazole as a standard anti-fungal agent.

Determination of Minimum Inhibitory Concentration (MIC)

Agar layers containing sensitive microorganisms from the above-mentioned extract activity test were prepared in various petri dishes and labeled. Make duplicate plates (dishes) for each organism. Five holes were made using sterile cork, and serial dilutions of various concentrations were marked. A drop of the extract and the respective concentration of the standard drug are added dropwise to the respective marked wells and kept for 15 minutes to allow the extract to diffuse into the agar layer. The plate was incubated at 37°C for 24 hours. The diameter of any resulting suppression zone is in millimeters. The MIC of the obtained extract for various microorganisms is less than or equal to the concentration of the extract, and the concentration of the extract has no inhibitory area (activity) on the microorganisms when further diluted.

RESULTS AND DISCUSSION

According to the results of the antibacterial susceptibility test shown in Table 1, the antibacterial activity of the 50mg/mL extract against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* was obtained through their growth inhibition zone. microorganism. Obviously, although the ethyl acetate extract has the highest inhibition zone of 7 mm, all the extracts show antibacterial activity against *E. coli*. The n-hexane extract is not active against *Staphylococcus aureus*, but other extracts show activity, of which methanol extract (ME) is the most active and has an inhibition zone of 13 mm. Among *Pseudomonas aeruginosa*, only the ethyl acetate extract showed an inhibitory effect on 4 mm, but not as wide as the standard drug 25 mm. *Candida albicans* is inhibited by n-hexane extract (10 mm). Therefore, it can be said that the antifungal activity of the root of *P. coleoides* lies in its n-hexane extract, although it is not as high as the standard drug (25 mm). The table also shows that the dissolving solvent does not affect the activity of the extract because it has no inhibitory effect on organisms.

Table 1: Antimicrobial susceptibility test for activity of extracts at 50 mg/mL

Microorganism	Plate	Inhibition zone diameter (mm)					
		CME	NHE	EAE	ME	Standard Drug	DMSO
S. aureus \	1	3	0	9	10	14	0
	2	2	0	9	13	10	0
E. coli	1	3	4	7	4	24	0
	2	2	5	5	4	25	0
P. aeruginosa	1	0	0	5	0	9	0
	2	0	0	5	0	25	0
C. albicans	1	0	10	0	0	25	0
	2	0	8	0	0	23	0

CME = Crude methanol extract (50 mg/mL); N-HE = n-hexane extract (50 mg/mL); EAE = Ethyl acetate extract (50 mg/mL); ME = Methanol extract (50 mg/mL); Standard drugs = Ciprofloxacin 5 mg/mL (for bacteria), Ketoconazole 5 mg/mL (for fungi)

The susceptibility of clinical isolates on commercially isolated antibiotic discs was tested on a large number of standard antibiotics to compare the activity of available antibiotics and extracts against the same organism, Table 2. At the concentration used, none of the commercial antibiotics produced activity against *Pseudomonas aeruginosa* (very strong resistance to most antibiotics), while 50 mg/mL EAE showed activity against *Pseudomonas aeruginosa*. However, *E. coli* also showed the most sensitivity to a large number of commercial antibiotics.

Table 2: The susceptibility of clinical isolates using commercially prepared antibiotic discs

Standard Antibiotics (For gram-positive organism)	Inhibition zone diameter (mm)		Standard Antibiotic (For gram-negative organisms)	Inhibition zone diameter (mm)			
	S. aureus (Clinical isolates)			*E. coli* (Clinical isolates)		*P. aeruginosa* (Clinical isolates)	
	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2
S	0	0	S	19	18	0	0
SXT	0	0	SXT	19	14	0	0
E	0	0	CH	24	23	0	0
PEF	12	13	SP	19	17	0	0
CN	0	0	CPX	26	28	0	0
APX	17	14	AM	0	0	0	0
Z	0	0	AU	0	0	0	0
AM	0	0	CN	0	0	0	0
R	0	0	PEF	20	21	0	0
CPX	12	9	OFX	0	0	0	0

S= Streptomycin (30 µg); CN= Gentamicin (10 µg); SXT= Septrin (30 µg), APX= Ampiclox (30 µg); E= Erythromycin (10 µg); Z= Zinnacef (20 µg); PEF= Perfloxacin (10 µg); SP = Sparfloxacin (10 µg); CH= Chloramphenicol (30 µg); AM= Amoxacillin (30 µg); OFX= Tarivid (10 µg); CPX= Ciprofloxacin (10 µg); AU= Augmentin (30 µg)

Determine the minimum inhibitory concentration (MIC) of the extract that produces antimicrobial activity against specific microorganisms. When diluted to 25 mg/mL, all extracts lose their antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, Table 3. However, the antibacterial activity of CME, N-HE and EAE against *E. coli* continued to a concentration of 3.125 mg/mL, as shown in Table 4 & Figure 1. When diluted to 25 mg/mL shown in Table 3, only ME loses its activity against *E. coli*.

Table 3: Determination of the minimum inhibitory concentration (MIC) of extracts

Extract	Conc mg/mL	Average Zone Inhibition (MM)			
		S. aureus	*P. aeruginosa*	*E. coli*	*C. albicans*
Crude Methanol Extract	25.00	-		4 ± 0.02	
	12.50	-		3 ± 0.03	
	6.25	-		2 ± 0.01	
	3.125	-		2 ± 0.03	
	2.50	-		30 ± 0.02	
N-Hexane Extract	25.00			6±0.54	10±0.02
	12.50			5±0.12	4.5±0.44
	6.25			5±0.12	-
	3.125			4.5± 0.23	-

	2.50			30±0.02	33±0.21
Ethyl acetate Extract	25.00	-	-	6±0.54	
	12.50	-	-	5±0.12	
	6.25	-	-	5±0.12	
	3.125	-	-	4.5± 0.23	
	2.50	-	-	30±0.02	
Methanol Extract	25.00	-			
	12.50	-			
	6.25	-			
	3.125	-			
	2.50	-			

[-] = No activity at present; [ ] = No previous activity; CME = Crude methanol extract; NHE = *n*-Hexane extract; EAE = Ethyl acetate extract; ME = Methanol extract; * Standard drugs: Ciprofloxacin (for bacteria) and Ketoconazole (for fungi).

Table 4: Continuation of the determination of Minimum Inhibitory Concentration of extracts on *Escherichia coli*

Extract	Average zone of inhibition diameter (mm) of various concentrations								
	1.563 mg/mL	0.782 mg/mL	0.391 mg/mL	0.196 mg/mL	0.098 mg/mL	0.049 mg/mL	0.025 mg/mL	0.0125 mg/mL	*0.625 mg/mL
CME	0	0	0	0	0	0	0	0	30.5 ± 0.21
N-HE	9 ± 0.65	8 ± 0.34	6 ± 0.32	5 ± 0.43	0	0	0	0	25 ± 0.33
EAE	7± 0.08	7 ± 0.56	6 ± 1.34	6 ± 0.34	4.5 ± 0.32	4 ± 0.054	3 ± 0.21	0	25 ± 0.43

*** Ciprofloxacin (0.625 mg/mL); CME = Crude methanol extract; NHE = n-Hexane extract; EAE = Ethyl acetate extract.**

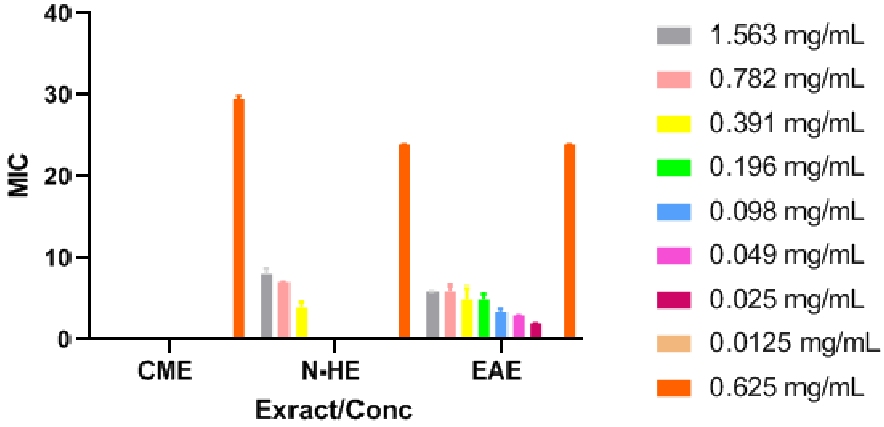

Fig:1 Minimum Inhibitory Concentration of different extracts on *Escherichia coli*

Table 5: Summary of the Minimum Inhibitory Concentration (MIC) of the extracts

Extract	**Crude methanol extract**		***n*-hexane extract**		**Ethyl acetate extract**			**Methanol extract**	
Organism	*S. aureus*	*E. coli*	*E. coli*	*C. albicans*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. coli*
MIC (mg/mL)	≤500.0	≤ 3.125	≤0.196	≤12.50	≤50.0	≤0.025	≤50.0	≤ 50.0	≤50.0

This may be because most of the effective plant components of plants will be extracted by n-hexane and ethyl acetate, which is different from the extracts in crude methanol extract, because they are polar solvents in crude plant raw materials, so they will all be present. The antifungal activity of NHE against *Candida albicans* was lost at a concentration of 6.25 mg/mL. Therefore, the MIC of the n-hexane extract on *Candida albicans* is ≤12.5 mg/mL, because the lowest concentration that produces activity is 12.5 mg/mL. Similarly, the MIC value of other extracts should be less than or equal to the lowest concentration of the last antibacterial activity recorded on the microorganism.

Therefore, although the inhibition range is not as wide as the standard drug at 0.625 mg/mL, the MICs of n-hexane and ethyl acetate extracts in *E. coli* are ≤0.196 mg/mL and ≤0.025 mg/mL, respectively. . Graphical determination of the MIC of ethyl acetate extract on *E. coli* also yielded 0.025 mg/mL [8], [9]. Therefore, the results of this study prove that it is reasonable to use *P. coleoides* roots to treat diarrhea, skin infections (such as *E. coli*), while *Candida albicans* causes diarrhea and skin infections (vaginal candidiasis), respectively of pathogenic bacteria.

CONCLUSION

The ethyl acetate extract has the highest anti-*E. coli* activity of 0.025 mg/mL against MIC, followed by the n-hexane extract, which has antifungal activity against *Candida albicans*, with the lowest inhibitory concentration of 12.5 mg/mL. Studies have shown that the root of *P.coleoides* has antibacterial and antifungal activities, which proves its rationality for the treatment of diarrhea and skin diseases in folk medicine.

REFERENCES

1. WHO Health Organization: Containing antimicrobial resistances 1999, WHO/CDS/CSR/DRS/99.2
2. Pfaller MA, Jones RN, Doern GV and Kugler K: SENTRY participants group: Bacterial pathogens isolated from patients with blood stream infection: Frequencies of occurrence and antimicrobial susceptibility patterns from SENTRY antimicrobial surveillance program (the United States and Canada). Antimicrobial Agents Chemotherapy 1998; 42: 1762-1770
3. Anyanwu MU and Rosemary CO: Antimicrobial activity of Nigerian medicinal plants. Journal of Intercultural Ethnopharmacology 2017; 6(2): 240-259.
4. Guyue C, Haihong H, Shuyu X, Xu W, Menghong D, Lingli H and Zonghui Y: Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiology 2014; 5: 217.
5. Hill AF: Economic Botany. A Text Book of Useful Plants and Plant Products. McGraw-Hill Book Company Incorporated New York, Edition 1st, 1952.
6. Ruangpan L and Tendencia EA: Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment. Southeast Asian Fisheries Development Center, Aquaculture Department 2004.
7. Nisha R: Sabouraud Dextrose Agar (SDA): Principles, composition, uses, and colony morphology. Micro-beonline.com, 2015. https://microbeonline.com/ sabouraud -dextrose-agar-sda-principle-composition-uses-colony-morphology/.
8. Lamikanra A: Essential Microbiology for Students and Practitioners of Pharmacy, Medicine and Microbiology. Amkara Books, Edition 2nd, 1999.
9. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HAB and Odugbemi TO: Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (Clove). African Journal of Microbiology Research 2008; (2): 162-166.