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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF VARIOUS EXTRACTS AND VOLATILE OIL FROM THE WHOLE PLANT OF *COLEUS VETTIVEROIDS* K.C. JACOB: AN *IN VITRO* STUDY

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ABSTRACT

Objective: The present study was aimed to prove the traditional use of the plant *Coleus vettivereoids* as antibacterial and antifungal agent.

Methods: The antibacterial and antifungal activities of Petroleum ether, Chloroform, Ethyl acetate, Ethyl alcohol and Water extracts of the dried whole plant of *Coleus vettivereoids* were evaluated. The study also covers antimicrobial studies on the volatile oil and the acetone dilution of the volatile oil from the fresh whole plant. The organisms for the studies include Bacillus *subtilis*, *Staphylococcus aureus* (Gram+ve), *Psuedomonas aeruginosa*, and *Proteus mirabilis* (Gram-ve) for evaluating antibacterial activity by well diffusion method and the Aspergillus *niger* and *Candida albicans* for antifungal study by streak method.

Result: Out of the various extracts petroleum ether extract showed greater antibacterial and antifungal activity towards the organisms under study.The pure volatile oil and its actone dilution also showed good anti bacterial and anti fungal activities

Conclusion: The study provides clear evidence of its use as antimicrobial agent.

Keywords: Antimicrobial activity, *Coleus vettiveroids*, Hrivera, Labiatae,

INTRODUCTION

Before the discovery of modern medicine, plants were the main remedy for treating various diseases. Due to the development of microbial resistance by using synthetic medicines, researchers have a great interest towards the plants having antimicrobial properties. The plant extracts show various medicinal properties including antimicrobial property. Over the past several years, intensive efforts have been made by the researchers to discover clinically useful antimicrobial plant drugs [1].

Coleus vettivereoids K .C. Jacob (*Plectranthus vettiveroids*) commonly known as Hrivera is a small profusely branched, succulent, aromatic herb belongs to the family Labiatae and the whole plant is used traditionally for treating wide range of diseases. It is cultivated in south India through vegetative cuttings. It has antibacterial, deodorant and cooling properties. It also has anti-inflammatory action. lruveli is therapeutically used in a number of Ayurvedic formulations. It is recommended for cardiac disorders, respiratory diseases, asthma and breathing troubles, cough, pneumonia, gastric irritation and anemia[2]. It is also marketed as an antiseptic herbal hand wash. The constituents are said to possess antiseptic and antimicrobial properties[3].

The preliminary phytochemical screening showed the presence of carbohydrates, steroids, proteins, amino acids, starch, phenolic compounds, tannins and alkaloids in various extracts[4].The volatile oil from the root of the plant showed significant antibacterial activity[5]. The present study was aimed to evaluate the antimicrobial activity of various extracts of the whole plant and volatile oil from the fresh whole plant of *Coleus vettiveroids.*

MATERIALS AND METHODS

Collection and authentication of plant material

The plant material for the proposed study was collected from the Botanical Garden, Poojappura, Thiruvananthapuram, Kerala, India. The species of the proposed study was identified as *Coleus vettiveroids* by Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

Preparation of extracts

Washed the plant material thoroughly to remove soil and other impurities, dried in shade, and pulverized.100g of the powdered drug was subjected to successive solvent extraction by cold maceration method using petroleum ether, chloroform, ethyl acetate, ethyl alcohol and water. Each extract was dried in a vacuum drier[6].

Antimicrobial studies on plant extracts

The antibacterial and antifungal activities of different extracts of the plant Coleus vettiveroids were evaluated. Studies were carried out in the Microbiology laboratory of the College of Pharmaceutical sciences, Medical College, Thiruvananthapuram.

Evaluation of antibacterial activity

The organisms used were *Bacillus subtilis*, *Staphylococcus aureus* (Gram+ve), *Psuedomonas aeruginosa*, and *Proteus mirabilis* (Gram-ve). They were obtained from the Department of Microbiology, Medical College, Thiruvananthapuram.

Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Nutrient Broth (NB). They were prepared and sterilized at 121^{o}C at 15 psi for 15-30 minutes in autoclave.

Plate preparation

25ml of pre autoclaved Mueller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri plates. These petri plates were allowed to solidify at room temperature.

Well diffusion method

Agar well diffusion method was performed for the determination of anti bacterial activity. The Bacterial cultures were lawn cultured on Mueller-Hinton agar (MHA) media using sterile cotton swab under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 6 mm diameter. In these well, each extracts individually was loaded. Petri plates were incubated for 24 hrs at37^{o}C in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well was measured in mm compared to the standard drug Ampicillin (10 µg/ml).The antibacterial studies were carried out in triplicate[7,8,9].

Evaluation of antifungal activity

The fungal organisms used were *Aspergillus niger* and *Candida albicans*. The organisms were collected from Department of Microbiology, Medical College, Thiruvananthapuram. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25°Cfor about 72-96 hours and then stored at 4°C as stock cultures for further antifungal activity.

Streak method

Fresh cultures were obtained by transferring a loop full of cultures into Sabouraud's dextrose broth, and then incubated at 25°C for 72 hrs. To test antifungal activity, the streak plate method was used. The sterile medium was poured into sterile test tube, plant extracts were added to the media, shaken well, kept in slanting position and allowed to settle. The organisms were streakeover the media. The test tubes were labeled for identification. These were then incubated at 27° C ±0.5° C for 48 hrs. Inhibition of growth indicates antifungal activity. Grieseofulvin (100µg/ml) was used as the standard. The antifungal studies were also carried out in triplicate[8,10,11].

Antimicrobial studies on the volatile oil

Extraction of Volatile Oil

One hundred and fifty grams of fresh plant material (fresh whole plant, leaves stem and root) were subjected to hydro distillation using Clevenger apparatus for 4 hours. The volume of volatile oil which separated out as the upper layer in the graduated tube was noted and the %v/w calculated on fresh weight basis.

Evaluation of antibacterial activity

The volatile oil collected from the fresh whole plant and its acetone dilution was used for the study. The organisms used and the method used was same as the case described above.

Evaluation of Antifungal activity

The volatile oil collected from the fresh whole plant and its acetone dilution was used for the study. The organisms used and the method used was same as the case described above.

RESULTS AND DISCUSSION

Antimicrobial studies on the extracts

Antibacterial activity

Table 1: Antibacterial activity of various extracts of *Coleus vettiveroids*.

Treatment	Conc. (µg/ml)	Zone of inhibition (mm)			
		Gram +Ve		Gram-Ve	
		S .aureus	*B.subtilis*	*P.aeruginosa*	*P .mirabilis*
Petroleum ether extract	250	-	-	-	-
	500	5	8	-	8
	1000	20	25	18	25
Chloroform Extract	250	-	-	-	-
	500	-	10	-	-
	1000	12	25	12	10
Ethyl acetate Extract	250	-	-	-	-
	500	-	-	-	-
	1000	10	15	18	12
Ethanol extract	250	-	-	-	-
	500	-	8	5	-
	1000	10	25	20	10
Water extract	250	-	-	-	-
	500	-	-	-	-
	1000	-	-	-	-
Standard (Ampicillin)	10	25	30	20	28

-indicates no zone of inhibition.Out of the various extracts petroleum ether showed greater antibacterial activity towards *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*, in the concentration of 1000 µg/ml. Water extracts showed no activity in the three concentrations towards the organisms under study. The chloroform and alcohol extracts showed activity towards *Bacillus subtilis* in the concentration of 1000 µg/ml. Results of antibacterial studies of various extracts are shown in table 1

Antifungal activity

Results of antifungal studies of various extracts are shown in Table 2. Petroleum ether extract showed good antifungal activity against *Aspergillus niger and* Candida *albicans* in the concentrations of 250 and 500 µg/ml. The activity in the concentration of 500 µg/ml is comparable to the activity produced by Greiseofulvin (100µg/ml).The ethanol and ethyl acetate extracts showed greater activity against *Aspergillus niger* in the concentration of 500 µg/ml. But the Water extracts showed no activity towards the organisms under study

Table 2: Antifungal activity of various extracts of *Coleus vettiveroids*

Treatment	Concentration (µg/ml)	Antifungal activity	
		Aspergillus niger	*Candida albicans*
Petroleum ether Extract	250	+	+
	500	++	++
Chloroform Extract	250	-	-
	500	+	+
Ethyl acetate Extract	250	-	-
	500	++	+
Ethanol Extract	250	+	-
	500	++	+
	500	-	-
	250	-	-
Greiseofulvin extract	100	++	++

++ indicates good activity, + indicates presence of activity, - indicates no activity

Antimicrobial studies on volatile oil from the whole plant

Extraction of volatile oil

The percentage yield of volatile oil from various parts of the plant is shown in table: 3. Results showed that the fresh stem of the plant yields maximum quantity of oil followed by root and leaves.

The oil collected has brown orange colour and has aromatic, characteristic odour and taste.

Anti bacterial activity

The pure volatile oil and its acetone dilution showed anti bacterial and anti fungal activity comparable to that of the standard drugs. The results are shown in table: 4 and table: 5

Table 3: Comparison of % yield of volatile oil from different parts of the plant *Coleus vettiveroids*

Plant material	%v/w
Whole plant	0.15
Stem	0.06
Leaves	0.13
Root	0.12

Table 4: Antibacterial activity of volatile oil and acetone dilution of volatile oil from the whole plant of *Coleus vettiveroids*

Treatment	Zone of inhibition (mm)			
	Gram +Ve		Gram-Ve	
	S .aureus	***B.subtilis***	***P.aeruginosa***	***P .mirabilis***
Pure volatile oil	12	17	15	13
Acetone dilution of Volatile oil (1:1)	8	13	12	8
Standard Ampicillin (10µg/ml)	15	20	20	18

Anti fungal Activity:

Results are shown in table 5

Table 5: Antifungal activity of volatile oil from the whole plant of *Coleus vettiveroids*

Treatment	Antifungal activity	
	Aspergillus niger	***Candida albicans***
Standard (grieseofulvin)	++	++
Pure volatile oil	++	++
Acetone dilution(1:1)	+	+

++ indicates good activity, + indicates presence of activity, - indicates no activity

CONCLUSION

The study covers the evaluation of antibacterial and antifungal activity of Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water extracts and the volatile oil and acetone dilution of volatile oil extracted from the fresh whole plant of *Coleus vettiveroids.* Out of the various extracts petroleum ether in the concentration of 1000 µg/ml showed comparable antibacterial activity as standard Ampicillin against the organisms *Staphylococcus aureus, Bacillus subtilis* and *Proteus mirabilis*. Petroleum ether extract in the concentration of 250 and 500 µg/ml showed good antifungal activity against *Candida albicans*. Ethyl acetate (500 µg/ml) and ethanol extract (250 and 500 µg/ml) also showed anti fungal activity against *Aspergillus niger*. The volatile oil also showed comparable anti bacterial and antifungal activity towards the organisms under study. The study proved the traditional use of the plant as antimicrobial agent.

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