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ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACTS OF LEAVES AND STEMS OF *PERISTROPHE BIVALVIS* MERRILL.

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ABSTRACT
The antimicrobial activity of ethanolic extracts of leaves and stems of *Peristrophe bivalvis* Merrill was tested by agar diffusion streak well method. The zone of Inhibition produced by the extracts was measured against doses 10 mg, 40 mg, 70 mg and 100 mg/ml and compared with standard Gentamicin 10 µgm/ml for bacteria and Clotrimazole 25 µgm/ml for fungi. Their minimum inhibitory concentrations were calculated. It was found that *Peristrophe bivalvis* Merrill is having broad spectrum of activity. Leaves showed more antimicrobial activity as compared to stems.

Keywords: *Peristrophe bivalvis* Merrill; Agar diffusion; Antimicrobial activity

1. INTRODUCTION

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. They have genuine utility and over 80% of rural population depends on it for primary health care. The World Health Organisation(WHO) has been advocating the needs for orthodox medical practitioners to interact with traditional herbal healers with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and nonmicrobial organisms (WHO 1978)[1].

Peristrophe bivalvis Merrill Nees, Family Acanthaceae It is an erect spreading and often much branched herb, grows along watercourses in forests. It is cultivated upto 1600 m altitude in India. It is a herbaceous perennial plant growing to 50-100 cm tall. It is cultivated in India in Bengal and Assam. It is a flowering plant and flowers are rose or purple. An extract of it's leaves impart magenta tone to Vietnamese food[2].

2. EXPERIMENTAL

2.1 Materials and Methods:

2.1.1 Collection of plant: *Peristrophe bivalvis* Merrill plant was collected from Naldehra(near Shimla) region. It was identified and authenticated by Dr. (Mrs.) U.S.Yadav, Dept. of Botany,Willingdon College, Sangli. The voucher specimen of the plant has been kept in the herbarium of Appasaheb Birnale College Sangli for future reference.

2.1.2 Preparation of the Extract: From the collected plant material leaves and stems were separated. They were crushed, dried separately into shade at room temperature. They were separately powdered and sieved through No.20 mesh sieve. The successive solvent extraction was carried out using Soxhlet apparatus. About 50 gm of powder was extracted with 400 ml of solvent. The extracts were dried by using rotary vacuum evaporator. Ethanolic extract of leaves (having percentage yield- 8.65%) and stems (having percentage yield -7.48%) of *Peristrophe bivalvis Merrill* were used for screening antimicrobial activity[4,5].

2.2 Antimicrobial assay.

2.2.1 Test microorganisms: Test microorganisms, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6633) *Candida Albicans* (ATCC 10231) and *Candida tropicalis* (ATCC 4563) were procured from National Chemical Laboratory (NCL), Pune, and were maintained on slants of the medium under incubation conditions and transferred weekly to fresh slants.

2.2.2 Procedure: Preparation of sample solutions: The test ethanolic extracts of stems and leaves of *Peristrophe bivalvis* Merrill 10 mg were weighed and dissolved in 1 ml of ethanol to get 10 mg/ml test solution. In the same way 40 mg, 70 mg and 100 mg extracts were weighed and each separately dissolved in 1 ml of ethanol to get 40 mg/ml, 70 mg/ml and 100 mg/ml test solutions respectively. Gentamicin and clotrimazole were used as standards for antibacterial and antifungal activity.

2.2.3 Antibacterial activity[5,6.8,10.] **:** The sterile nutrient agar medium (43^{o} to 45^{o}C) is inoculated by suspension of microorganisms and the inoculated medium is poured immediately into sterile petriplates by using an assay medium and then the microorganisms were spread on plate surface by streak method. By using a sterile cork borer, 6 mm of bores were prepared in the solidified agar medium. The sample solutions of different concentrations were added into each well. The wells were marked at the back of petriplates. Standard gentamicin solution was added. Ethanol and distilled water were used as negative controls.

The plates were left standing for 1 to 2 hours at 4^{o}C for diffusion of solution. All plates were then incubated at 37^{o}C for 18 to 24 hours. The diameters of circular zone of inhibition produced by standard and test solutions were accurately measured including the diameter(6mm) of well prepared by cork borer. An average of three independent determinations was recorded.

Ethanol and distilled water which served as negative controls produced no zones of inhibition.

2.2.4 Antifungal activity[7,8,10] **:**The sterile Saboured dextrose agar (43^{o} to 45^{o}C) was inoculated by suspension of microorganisms and the inoculated medium was poured immediately into sterile petriplates by using an assay medium and then the microorganisms were spread on plate surface by streak method. Sterile cotton tipped applicators were used for streaking. 6mm bores were prepared. The sample solutions of different concentrations were added into each well. Clotrimazole solution was used as standard. All plates were then incubated at 30-32^{o}C for 24 hours. The diameters of circular zone of inhibition produced by standard and test solutions were accurately measured.

2.2.5 Determination of minimum inhibitory concentration: Sterile capped test tubes were used which contain a double strength medium. They were labeled. In the first test tube inoculum was not added. In all other test tubes 3 to 4 drops of inoculum was added. The second tube was used as a control to check suitability of the medium for growth of microorganisms and viability of inoculums. In all test tubes serial dilutions were made from test extract to get concentrations ranging from 70, 60, 50, 40, 30, 20, and 10 mg/ ml except in the inoculated and control tube (The quantity of range of test solution is calculated by carrying out a preliminary experiment). Final volume was adjusted and incubated at 37^{o}C for 2 to 3 days. After incubation all test tubes were examined for growth in form of turbidity. Results were recorded and minimum inhibitory concentration was calculated[8].

3. RESULTS:

3.1 Table: 1.Antibacterial activity of ethanolic extracts of leaves and stems of *Peristrophe bivalvis* Merrill.

Bacteria	Type of ethanolic extract	Zone of Inhibition in mm Doses of different concentrations in mg/ml.				MIC range mg/ml	Standard Gentamicin 10 µgm/ml
		10 mg/ml	40 mg/ml	70 mg/ml	100 mg/ml		
Pseudomonas aeruginosa. ATCC 27853	leaves	00	12	18	23	20-30	22
	stems	00	09	15	20	30-40	
Escherichia coli ATCC 25922	leaves	00	13	19	24	20-30	24
	stems	00	10	16	20	20-30	
Staphylococcus aureus ATCC 25923	leaves	00	09	15	22	30-40	22
	stems	00	00	10	17	50-60	
Bacillus subtilis ATCC 6633	leaves	00	07	12	20	30-40	28
	stems	00	00	10	18	50-60	

Zone of Inhibition, including the diameter of well prepared by cork borer: - 6mm
Zone of Inhibition is average of triplicate experiments**.**
Gentamicin used as positive control.

Table -2: Antifungal activity of ethanolic extracts of leaves and stems of *Peristrophe bivalvis* Merrill.

Type of extract	Type of Fungi	Zone of Inhibition in mm Doses of different concentrations in mg/ml.				MIC mg/ml	Standard Clotrimazole 25 µgm/ml
		10 mg/ml	40 mg/ml	70 mg/ml	100 mg/ml		
Ethanolic extract of leaves.	*Candida Albicans* ATCC 10231	00	09	15	21	30-40	23
	Candida tropicalis ATCC 4563	00	10	15	20	20-30	22
Ethanolic extract of stems.	*Candida Albicans* ATCC 10231	00	07	12	16	30-40	23
	Candida tropicalis ATCC 4563	00	08	12	15	30-40	22

Zone of Inhibition, including the diameter of well prepared by cork borer: - 6mm
Zone of Inhibition is average of triplicate experiments**.**
Clotrimazole used as positive control.

4. DISCUSSION

The results were recorded in table 1 and table 2. The present study showed that *Peristrophe bivalvis* Merrill is effective against at least four microorganisms, gram+ve *Bacillus subtilis, Staphylococcus aureus and gram –ve. Escherichiat coli, Pseudomonas aeruginosa.* So it can be concluded that *Peristrophe bivalvis* Merrill is having broad spectrum of activity. Leaves extracts of *Peristrophe bivalvis* Merrill showed more antibacterial activity and more antifungal activity as compared to stem extracts.'[11]

Antibacterial effect of plant on *Staphylococcus aureus, E.Coli* and *Pseudomonas aeruginosa* showed that the plant can be used in treatment of gastrointestinal infection and Diarrhea in man. The extracts can also be used to treat boils sores and wounds since *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been implicated as causative agents of these diseases. As the extract is effective against *Candida Albicans* and *Candida tropicalis*,it can also be used to treat skin and mucosal infections.[12]

As microorganisms are developing resistance to antibiotics, this plant extract can be one of therapeutic option[13].

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