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Antimicrobial studies on *Poeciloneuron indicum* Bedd and *Suregada angustifolia* (Baill.ex Muell.Arg) Airy Shaw

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Abstract

In this study, the antimicrobial potentials of solvent extracts from bark and leaves of *Poeciloneuron indicum* and *Suregada angustifolia* were investigated. Chloroform, ethanol and aqueous extracts of leaves and bark were tested against two strains each of Gram +ve and Gram-ve bacteria and two strains of fungi by disc diffusion method. The ethanol bark extracts of *S. angustifolia* showed maximum inhibition zone against *Pseudomonas aeruginosa* with an inhibition zone of 22±0.00. Chloroform bark extracts of *P. indicum* showed a wide spectrum of activities against majority of the microbes tested. The study lends support for the use of the plants as folklore medicine for the cure of infectious diseases.

Keywords: Antimycotic activity, minimum inhibitory concentration, *Argemone mexicana* L.

Introduction

Microbial immunity to existing drugs is steadily increasing and has become a serious problem. This has necessitated in the continuous research into novel groups of antimicrobials. (Woodford 2003) [7]. New compounds which are not based on present synthetic antimicrobial agents are to be used as a method to overcome the anti biotic resistance of pathogens. (Chanda *et al.*; 2011) [1]. Studies to determine phytochemical and antimicrobial components of plants having medicinal properties have been taken up. These components are being used for treating both topical and systemic microbial infections. As, many pathogens have become resistant to chemical synthetic drugs these microbial compounds can be used as effective alternatives (Kumar *et al.*, 2011) [3]. World health organisation has listed 21,000, plants to be utilised for medicinal causes worldwide. Among these, India has 2500 species, and 150 species out of these are utilised on a relatively large scale (Seth & Sharma, 2004) [5]. An endemic tree species, seen mostly in Western Ghats, *Poeciloneuron indicum* Bedd. Comes under the family Clusiaceae. The members of this family have been reported for antimicrobial activity. *P. indicum* is a large evergreen tree with brown or brownish grey bark. The genus *Suregada* of Euphorbiaceae is represented by 40 species. These are mostly distributed in old world tropics. *Suregada angustifolia* (Baill.ex Muell.Arg) Airy Shaw is a small tree mostly found in evergreen and deciduous forests of India. The antimicrobial activity of plant extracts is because of various biochemical agents in the extract. The action of repelling harmful and drawing useful organisms is attributed to these biochemicals which are usually the secondary metabolites in the plants, these plant chemicals act as phytoprotectants and react to the habitat imbalances in plants. (Maiyo *et al.*, 2010) [4]. Hence there is an immense need to evaluate the potential of plants to manage the microbial infections and identify the secondary metabolites which could be least toxic and cost effective, with minimum side effects.

Materials and Methods

Collection of plants

Poeciloneuron indicum and *Suregada angustifolia* were collected from the natural forests of Western Ghats, Shimogga district, southern Karnataka. The plant parts such as bark and leaves were excised with sharp knife and placed in zip lock plastic pouches, labeled and brought to the laboratory for further analysis.

Extraction of phytochemicals

In this study, the given sample was extracted using three different solvent system i.e., water,

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chloroform and ethanol. Briefly, 10g of each given sample was suspended in 100 ml of respective solvents (water, chloroform and ethanol) and continuously shaken in a water bath at 40°C for 12 h. Whatman No.1 filter paper was used to filter the extracts and the extracts were stored in closed containers for further use. The remnant was re-extracted two times, once with 50 ml and again with 25ml of respective solvent for 4h each. Rotary evaporator was utilized to pool and evaporate the respective filtrates. The residue was dissolved in water, filter sterilized and used for antimicrobial activity assay.

Test organisms

Bacterial pathogens: The pathogenic cultures were *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*. Brain heart Infusion (BHI) media was used to grow these bacteria for 24 h at 37°C under constant shaking (150 rpm).

Fungal strains: *Aspergillus niger* and *Candida albicans*. Potato dextrose broth was used to grow the fungal strains for 3 days at 30°C under constant shaking (150 rpm).

Antimicrobial activity

Freshly grown bacterial pathogenic cultures were spread on BHI agar plates making use of aseptic swabs. Similarly PDA plates were used for the spread of fungal cultures. Sterile discs of filter paper measuring 4 mm in diameter was placed on the media at equidistance. Then, 10 µl of filter sterilized extract was spotted on the disc carefully and allowed to absorb. Later, incubation of plates was done at 37°C for 24–48 h. After incubation, the inhibition zone was estimated in mm and recorded. Positive control for the bacterial pathogens was antibiotic chloramphenicol (10mg/ml) and nystatin (10mg/ml) was used for fungal strains.

Results and Discussion

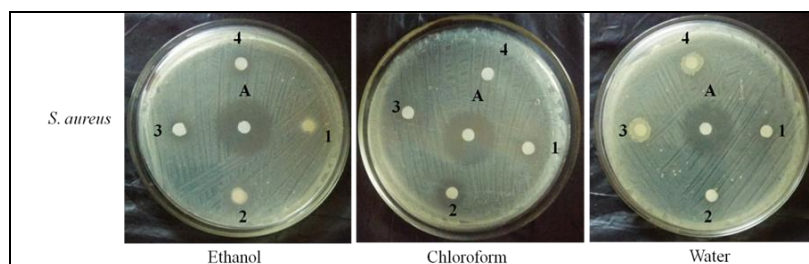
The antibacterial potential of the solvent extracts on the test organisms were evaluated in terms of diameters of inhibition zones are represented in table 1. Inhibition zones (clear zones on agar) for all the organisms tested were measured in mm. Of the solvent extracts tested, *Suregada angustifolia* aqueous leaf extracts showed an inhibition zone of 18.00±0.00 against *P. aeruginosa* like wise 8.00±0.00 against *B. subtilis*. The aqueous bark extracts showed an inhibition zone of 20.00±0.00 against *P. aeruginosa* as well as 15.00±0.00 against *B. subtilis*. Antimicrobial activity was nil in the aqueous extracts of *P.indicum*, against the pathogens tested. But the chloroform bark extract of *P. indicum* showed potent antimicrobial activity on *S. aureus* (12.00±0.00), *P. aeruginosa* (25.00±0.00), *Y. enterocolitica* (12.00±0.00), and *B. subtilis* (18.00±0.00). The chloroform leaf extract of *P. indicum* showed an inhibition zone of 7.00±0.00 against *Bacillus subtilis*. The chloroform bark extracts of *S. angustifolia* showed an inhibition zone of 18.00±0.00 against *P.aeruginosa* and 15.00±0.00 against *B. subtilis*. Similarly,

the ethanol bark extracts of *P. indicum* showed an inhibition zone of 16.00±0.00 in opposition to *P. aeruginosa* and 12.00±0.00 against *B. subtilis*. Whereas, the ethanol leaf extracts of *S. angustifolia* showed an inhibition zone of 9.00±0.00 against *S. aureus*, 20.00±0.00 against *P. aeruginosa* in addition to 12.00±0.00 against *B. subtilis*. However maximum inhibition zone was observed in ethanol bark extracts of *S. angustifolia* against *P.aeruginosa* with an inhibition zone of 22.00±0.00, An inhibition zone of 15.00±0.00 was seen against *B. subtilis* and 8.00±0.00 against *S.aureus*. Positive results were not seen in any of the fungal species tested. Antibacterial activity against *B. subtilis* was shown by most of the extracts. *B. subtilis* is an encapsulated, Gram +ve, endospore forming, obligate aerobe which causes food poisoning in immuno compromised patients and *P. aeruginosa* which is an encapsulated, Gram -ve, aerobic, rod shaped, multiple drug resistant bacteria, which causes various diseases in plants, animals and humans. Similarly, *S.aureus* is an Gram +ve, round bacteria which mostly infects the upper respiratory tracts and skin in humans. It is an important human pathogen that causes a wide spectrum of clinical infections (Tong *et al.*) [6]. *Y. enterocolitica*, a Gram -ve bacillus causing broad spectrum infections in humans listing from acute bowel disease to extra intestinal manifestations such as reactive arthritis, erythema nodosum and uveitis (Heesemann *et al.*, 1993) [2]. The present study is a result of the antimicrobial screening of 12 solvent extracts from two plant species. Both the plant species showed potent activity against both Gram +ve and Gram-ve microorganisms tested. This shows the extracts to be having potent phytoconstituents.

Table: Antimicrobial activity of plant extracts in mm

Pathogenic strains	S1/PL	S2/PB	S3/SL	S4/SB
Water				
<i>S.aureus</i>	-	-	-	-
<i>P.aeruginosa</i>	-	-	18	20
<i>Y.enterocolitica</i>	-	-	-	-
<i>Bacillus subtilis</i>	-	-	8	15
<i>Candida</i>	-	-	-	-
<i>Aspegillus</i>	-	-	-	-
Chloroform				
<i>S.aureus</i>	-	12	-	-
<i>P.aeruginosa</i>	-	25	-	18
<i>Y.enterocolitica</i>	-	12	-	-
<i>Bacillus subtilis</i>	7	18	-	15
<i>Candida</i>	-	-	-	-
<i>Aspegillus</i>	-	-	-	-
Ethanol				
<i>S.aureus</i>	-	-	9	8
<i>P.aeruginosa</i>	-	16	20	22
<i>Y.enterocolitica</i>	-	-	-	-
<i>Bacillus subtilis</i>	-	12	12	15
<i>Candida</i>	-	-	-	-
<i>Aspegillus</i>	-	-	-	-

Values are zone of inhibition (mm in diameter). Values are average of two independent experiment.



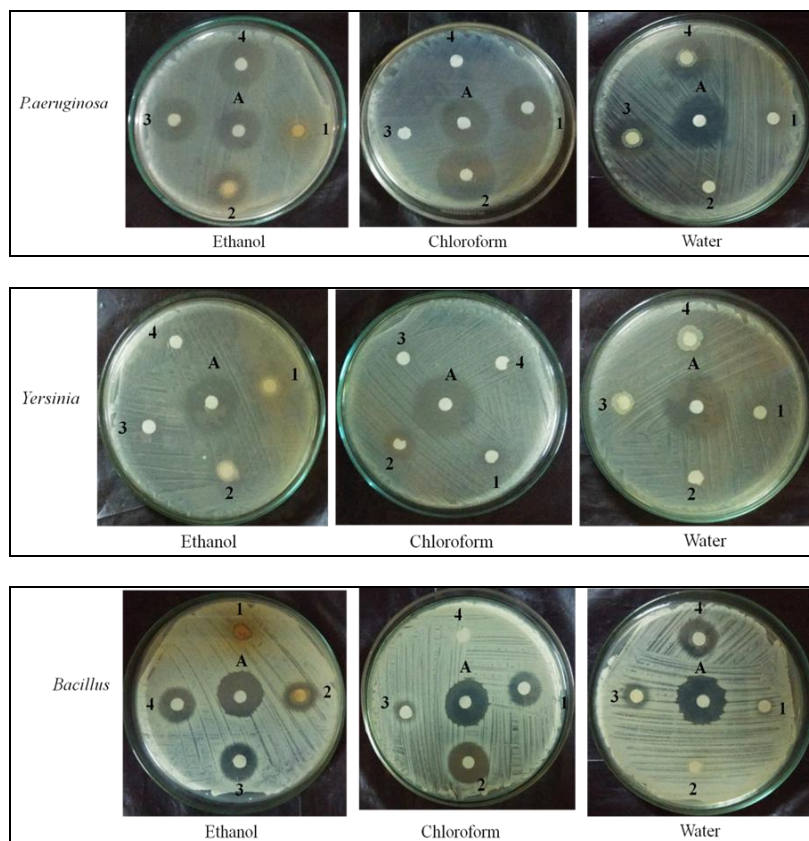


Fig: Antimicrobial activity of given plant samples (1) S1; (2) S2; (3) S3; (4) S4. "A" represents antibiotic chloramphenicol.

Conclusion

This work is based on the antimicrobial activities of various extracts of *Poeciloneuron indicum* and *Suregada angustifolia*. The ethanol bark extracts of *S. angustifolia* showed maximum inhibition zone against *P.aeruginosa* with an inhibition zone of 22.00 ± 0.00 . Chloroform bark extracts of *P. indicum* showed a broad spectrum of activity against most of the microbes tested. This may be attributed to the phytochemicals present in the plant species. Further studies on the fractionation of the extracts and characterization by spectroscopy techniques may reveal the compounds showing the antimicrobial potentials.

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