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Antimicrobial activity of the roots of *Ichnocarpus frutescens*

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Abstract
A large number of medicinal plants are claimed to be of great value in treating skin diseases in all traditional systems of medicine. The present study was carried out to investigate the antimicrobial effect of the ethanolic extract of roots of *Ichnocarpus frutescens*. The antimicrobial activity of the extract was tested against five bacterial and two fungal strains by disc diffusion method. Broad spectrum activity was seen against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium).* Among all the bacterial strains, *Bacillus subtilis* showed the lowest MIC values (62.5µg/ml). The antifungal study revealed a significant effect against *Candida albicans* and no antifungal activity against *Aspergillus niger.*

Keywords: Antimicrobial, MIC, disc diffusion

1. Introduction
Plants are a potential source of antimicrobial compounds and several researchers throughout the world are investigating the antimicrobial activity of medicinal plants, which are utilized in the traditional or alternative healthcare systems [1, 2].
Ichnocarpus frutescens (Linn) R.Br. (Family-Apocynaceae) is an evergreen plant and this plant is used in traditional Indian medicine for centuries to treat several illness. This plant is also known as Dudhi; 'Shyamalata' in Bengali, 'Black creeper' in English and 'Ananta', 'Sariva' in Sanskrit. I*chnocarpus frutescens,* is a large, much branched twining shrub, found throughout India and in the Himalayas less than 5,000 feet. *Ichnocarpus frutescens* leaf, stem and root were investigated for its physicochemical and phytochemical screening [3, 4].
Various parts of this plant are used as a cure for fever, dyspepsia, skin troubles and headache. Laboratory studies have demonstrated that extracts of the plant inhibit tumors, protect liver cells from damage in aceteminophen overdose, and correct hyperlipidemia in diabetic rats. It also has analgesic and anti-inflammatory properties, reduces fever, and lowers fasting glucose and improves glucose tolerance in diabetes [5, 6].
Decoction of the shoots is used in fevers. Leaves are boiled in oil and applied in headaches and fevers [7-9]. However, not much work has been reported on antimicrobial activities of roots of *I. frutescens.* Therefore the present study has been undertaken to evaluate the same.

2. Materials and methods
2.1 Collection of plant species
The roots of *I. frutescens* were collected from Gomantak Ayurveda Mahavidyalaya and Research Centre, Shiroda, Goa. The collected plant material was air dried under shade and dried roots were crushed into powder by the mechanical blender.

2.2 Preparation of ethanolic extract
The roots of *I. frutescens* were collected, washed and dried in shade. The dried roots were powdered (200 gm) and exhaustively extracted by maceration with ethanol (95%) for three days. After three days, ethanol layer was decanted off. The process was repeated three times. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency using rotary vacuum evaporator (25 rpm; 60 °C) and then evaporated to dryness (18.75g) [10]

2.3 Antimicrobial Susceptibility Testing
2.4 Microorganisms
In the present study, ethanolic extract of the roots of *I. frutescens* was tested for antimicrobial activity by disc diffusion method. Five bacterial strains used included two gram-positive-*Staphylococcus aureus (6538P)* and *Bacillus subtilis (6633)* and three gram-negative bacteria -

Escherichia coli (35218), Pseudomonas aeruginosa (19429) and *Salmonella typhimurium (23564)*. Two fungal strains, *Candida albicans (10231)* and *Aspergillus niger (10864)* were used. All the bacterial strains and fungal strains were maintained on Nutrient Agar and Saborauds Dextrose Agar respectively and were freshly sub cultured for 24-48 hrs at 37 ^{o}C and 25 ^{o}C respectively.

2.5 Antimicrobial Agents

Streptomycin S25 (Hi media) and tetracycline S25 (Hi media) disc were used for antibacterial studies and Fluconazole S25 (Hi media) disc was included in the study as standard reference for antifungal activity.

2.6 Antimicrobial Activity

The Mueller Hinton Agar plates and Saborauds Dextrose Agar were prepared as mentioned above. The 500 µg/ml and 1000µg/ml concentrations of extracts were loaded on sterile paper disc. The loaded disc was placed on the surface of medium using sterile forceps and the compound was allowed to diffuse for 5 minutes. After that, the plates were incubated at 37 ^{0}C for 24 hr. For each bacterial and fungal strain, pure solvent (alcohol) is used as control. Streptomycin 25 mcg/disc, tetracycline 25 mcg/disc for antibacterial studies and fluconazole 25 mcg /disc for antifungal studies were used as positive control. The diameters of inhibition zones were measured to evaluate antibacterial activity. All experiments were carried out in triplicate and the mean of the readings were recorded [3, 11].

2.7 *MIC determination*

Streptomycin and test extracts were prepared in Muller Hinton Broth at 31.25-1000 µg/mL (Two fold dilutions). 90µl of antibiotic / test extracts of different concentrations were mixed with 10µl inoculum in well plate in triplicate. Streptomycin in Muller Hinton Broth with inoculum was treated as positive control. Muller Hinton Broth with inoculum and without antibiotic or test extracts was treated as negative control. Treated bacterial cultures were incubated at 37 °C for a period of 24 hours. After incubation period, optical density was measured at 600nm. The lowest concentration of test extract or antibiotic giving 50% inhibition of OD as compared with control was taken as minimum inhibitory concentration [7].

3. Results & Discussion

The ethanolic extract of the rhizomes of *I. frutescens* was subjected to antimicrobial studies. The results indicated that the ethanolic extract showed broad spectrum antibacterial activity against both gram-positive- *Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria - *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium* which is indicated by the zone of inhibition (Refer Tables I and II).

The results also indicated that the ethanolic extract of rhizomes of *I. frutescen* showed antifungal activity against *Candida albicans* and no activity against *Aspergillus niger*. (Refer Tables I and III).

It was observed that as the concentration of the ethanolic extract of the rhizomes of *I. frutescens* increased, there was significant inhibition seen in the growth of the culture as was evident from the drastic decline in absorbance values. Decline in the absorbance indicated the decrease in the number of bacteria thus resulting in a less turbid solution.

The ethanolic extract of *I. frutescens* showed a significant decline in growth of *Bacillus subtilis* and MIC value was seen to be 62.5 µg/ml. A sharp decline in of growth with MIC value of ethanolic extract were found to 125 µg/ml against both Salmonella typhimurium, and Pseudomonas aeruginosa respectively. (Refer Tables I and IV).

In case of *Escherichia coli* and *Staphylococcus aureus* both the extract showed a gradual decline in the growth with MIC value of 250 µg/ml. (Refer Tables I and IV).

In the present study has shown that the root extract of *I. frutescens* has a potent antibacterial property against majority of organisms tested. Due to a rapid increase in the rate of infections and antibiotic resistance in microorganisms medicinal plants are gaining popularity over these drugs. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.

The results of phytochemical analysis of different crude extracts of *I. frutescens* have been reported to contain alkaloids, tannins, phenols, flavonoids, glycosides and steroids in all tested extracts [11, 12].

Most of the flavonoids are known to exhibit antibacterial and antifungal activity. Flavonoids are said to reduce the infectivity and cellular replication of various bacteria and viruses [11]. This and probable synergistic activity with other organic compounds could be probable mechanism of action of antimicrobial activity of *Ichnocarpus frutescens*. However, further investigations are required to find out which active ingredient is responsible for this antimicrobial activity.

3.1 Tables

Table 1: Antibacterial/ Antifungal activity of ethanolic extract of the roots of *Ichnocarpus frutescens*

Microorganism	Anti-Microbial / Antifungal Activity	Percent inhibition of growth at (%)	
		concentration of 500 µg/ml	concentration of 1000 µg/ml
Staphylococcus aureus	+ve	76.47	88.23
Bacillus subtilis	+ve	Total inhibition	Total inhibition
Escherichia coli	+ ve	67.24	84.48
Pseudomonas aeruginosa	+ ve	91.30	Total inhibition
Salmonella typhimrium	+ve	90.56	92.45
Aspergillus niger	-ve	-	-
Candida albicans	+ve	-	-

Table 2: Antibacterial activity of ethanolic extract of the roots of *Ichnocarpus frutescens* by disc diffusion method

Microorganism	*I.frutescens* roots ethanolic extract		Zone of inhibition in mm	
	500 µg/ml	1000 µg/ml	Streptomycin control	Tetracycline control
Staphylococcus aureus	27	35	22	22
Bacillus subtilis	19	45	27	30
Escherichia coli	15	32	10	12
Pseudomonas aeruginosa	22	40	27	32
Salmonella typhimrium	25	30	26	46

Table 3: Antifungal activity of ethanolic extract of the roots of *Ichnocarpus frutescens* by disc diffusion method

Microorganism	Zone of inhibition in mm		
	I.frutescens roots ethanolic extract		Fluconazole control
	500 µg/ml	1000 µg/ml	
Aspergillus niger	-	-	-
Candida albicans	14	30	36

Table 4: Percent inhibition in growth of cultures by ethanolic extract of the roots *Ichnocarpus frutescens* on bacterial cultures

concentration µg/ml	Percent Inhibition In Growth Of Cultures				
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***
31.25	7.62	40	4.82	4.34	11.89
62.5	11.32	77.14	15.51	21.73	22.64
125	21.76	80	31.03	60.86	69.81
250	64.70	85.71	60.34	82.60	83.01
500	76.47	TI	67.24	91.30	90.56
1000	88.23	TI	84.48	TI	92.45

*TI-Total inhibition

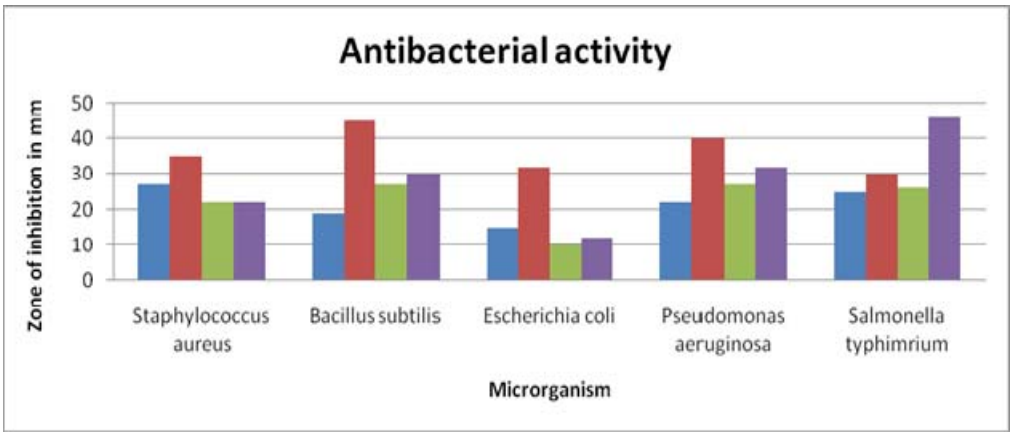

Fig 1: Antibacterial activity of ethanolic extract of the roots of *Ichnocarpus frutescens*

4. Conclusions

The ethanolic extract of the roots of *I.frutescens* was subjected to the antibacterial studies. The results indicate significant activity against both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis)* and gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhimrium).* The antifungal study revealed significant activity against *Candida albicans* and no inhibition against *Aspergillus niger.*

It has been observed that as the concentration of the ethanolic extract of the roots of *I. frutescens* increases, there is significant inhibition seen in the growth of the cultures. The above activity has been reported for the first time from the ethanolic extract roots of this plant.

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