

Antimicrobial activities of *Ichnocarpus frutescens* (L.) R.Br. and *Hemidesmus indicus* R.Br. Roots.

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Abstract: Hexane, chloroform and aqueous extracts from *Hemidesmus indicus* and *Ichnocarpus frutescens* roots were evaluated for their antimicrobial activity. The chloroform extract of both plants showed antibacterial and antifungal activities against the tested organisms. Both the plants showed highest antibacterial and antifungal activity against *Escherichia coli* and *Aspergillus flavus* respectively. With increase in concentration of the extract a corresponding increase in diameter of inhibition zone was observed. The roots of the common substitute of *H.Indicus* namely *I.frutescens* possess similar antimicrobial properties.

Key words: Antibacterial, antifungal, roots, chloroform extract, *H.indicus*, *I.frutescens*

Introduction

The interest in the study of medicinal plants as a source of pharmacologically active compounds has increased tremendously^[1, 2]. The increase in the Multiple Drug Resistant strains of pathogens and reports of new incidences of diseases due to microbial pathogens has triggered the study of new antimicrobial compounds and medicinal plants are looked upon as a source of safe and potential drugs^[3]. According to WHO (2001) herbal medicines serve the health needs of about 80% of the world's population especially for millions of people in the vast rural areas of developing countries.

A perusal through the literature revealed the absence of insufficient scientific reports on *Ichnocarpus frutescens*. Moreover *I.frutescens* is often used as a substitute for *Hemidesmus indicus* in Ayurveda and both plants are referred by the Sanskrit name 'saariva'. *H.indicus* is a laticiferous, prostrate or slender twining under shrub and the root is sweet, bitter, cooling, aphrodisiac, alterative, astringent, demulcent, antipyretic, antidiarrhoeal, antileprotic and cures skin diseases. *I.frutescens* is also laticiferous but a large evergreen twining shrub and its roots are also known for a number of traditional medicinal uses like demulcent, alterative, tonic, diuretic and diaphoretic^[4]. The aim of this paper is to investigate the antimicrobial properties of roots of *I.frutescens* and *H.indicus*.

Materials and Methods:

Plant material: Dried roots of *H.indicus* were obtained from the shop of traditional medicinal herb sellers in Pallakad, Kerala. Fresh roots of *I.frutescens* growing in the wild was collected by digging in August 2005, from Mondur, a semi urban village 14km from Palakad, Kerala. The identification of the plant specimen was confirmed at the Botanical Survey of India, Coimbatore. The roots were washed, shade dried and powdered by milling.

Preparation of crude extract: Ten grams of dried root powder was extracted sequentially with 200mL of solvents namely hexane, chloroform and distilled water. The extracts were filtered and the filtrate concentrated to dryness and re dissolved in mother solvents so as to give a final concentration of 6 mg extract per ml.

Microorganisms used: Test microorganisms (local clinical isolates of bacteria) and fungi were obtained from the laboratory stock culture. Antimicrobial activity was tested against *Bacillus pumilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Proteus* sp.,

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Aspergillus flavus, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Cladosporium oxysporum*, *Penicillium candidum*, *Penicillium pergenum* and *Trichoderma viride*. The bacteria were cultured in nutrient agar and fungi on Czapek-Dox agar media. Stock cultures were maintained at 4^o C.

Antimicrobial screening: Antibacterial and antifungal activity of the crude extracts was determined by disk diffusion assay^[5]. Nutrient agar or Czapek- Dox agar plates were smeared with a suspension of bacteria or fungal spores so as to obtain a uniform thick lawn of growth. Sterile filter paper discs (Whatman No.1) of 5mm diameter were saturated with various extracts of the medicinal plants and air dried to evaporate the solvent completely before placing the discs on seeded agar plates. An aqueous solution of chloramphenicol (30µg/disc) was used as positive control and a disc impregnated with equal volume of solvent served as negative control. The plates

were incubated at 30°C for a period of 24hrs in the case of bacteria and 72hrs in the case of fungi. The experiments were carried out in triplicates and the average diameter of inhibition zone was recorded.

Results:

Preliminary studies of hexane, chloroform, and aqueous extracts of roots of *H.indicus* and *I.frutescens* on *E.coli* and *B.pumilis* revealed antimicrobial property in chloroform extract only. Therefore chloroform extracts of *H.indicus* and *I.frutescens* roots were screened for their antimicrobial activity on all the test microorganisms. The chloroform extract of both plants inhibited all the tested microorganisms (Table1). Amongst bacteria *E.coli* followed by *B.pumilis* were most sensitive and among fungi *A.flavus* and *A.niger* were most sensitive. The solvent (negative control) did not have any inhibitory activity. Chloramphenicol (positive control for bacteria) had a stronger inhibition than the extracts (Table1).

Table: 1.
Effect of chloroform extracts of *Ichnocarpus frutescens* and *Hemidesmus indicus* roots on bacteria and fungi

Sl.No.	Name of the organism	Diameter of inhibition zone in mm		
		<i>I.frutescens</i>	<i>H.indicus</i>	Chloramphenicol
1	<i>Bacillus pumilis</i>	21	26	43
2	<i>Staphylococcus aureus</i>	18	21	47
3	<i>Eschericia coli</i>	22	27	40
4	<i>Pseudomonas aeruginosa</i>	17	21	40
5	<i>P.putida</i>	16	17	31
6	<i>Proteus sp.</i>	19	24	47
7	<i>Aspergillus flavus</i>	14	13	-
8	<i>A.fumigatus</i>	12	11	-
9	<i>A.niger</i>	13	13	-
10	<i>A.terreus</i>	08	08	-
11	<i>Cladosporium oxysporum</i>	12	11	-
12	<i>Penicillium candidum</i>	09	06	-
13	<i>P.pergenum</i>	12	11	-
14	<i>Trichoderma viride</i>	11	11	-

Fig. 1. Effect of chloroform extract of *Ichnocarpus frutescens* roots on microbes

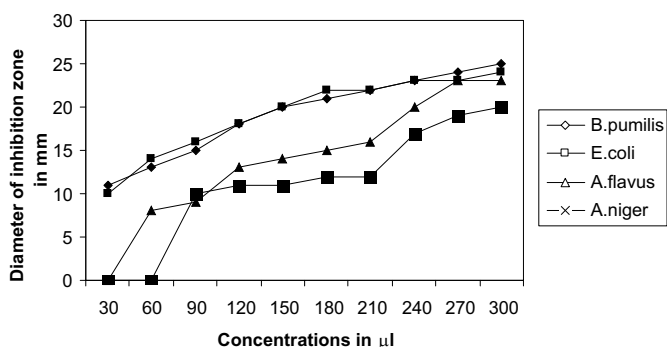
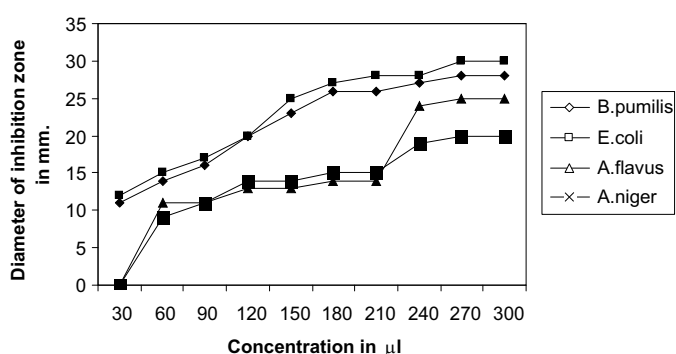


Fig. 2. Effect of chloroform extract of *Hemidesmus indicus* root on microbes.



The effect of various concentrations (30 to 300 µl) of the chloroform extracts of roots of *I. frutescens* and *H. indicus* was tested on *E. coli*, *B. pumilis*, *A. flavus* and *A. niger* by disc diffusion assay on respective media. Both *E. coli* and *B. pumilis* were inhibited even at the lowest concentration (30 µl) of the extract of both the plants but the two fungi tested were inhibited only at concentrations of 60 µl and above and the extent of inhibition increased with increase in concentration (Figs. 1 and 2) of the extract.

Discussion:

To our best knowledge this is the first report on the antimicrobial properties of *I. frutescens* roots. Aqueous extracts of roots of *H. indicus* has been reported to have antileprotic action^[6] and its essential oil to have antibacterial but not antifungal activity^[7]. Chloroform and ethanol extracts of *H. indicus* have been reported to exhibit antifungal activity against *A. niger*^[8]. In the present study also chloroform extract of both *H. indicus* and *I. frutescens* roots possessed antimicrobial activity. In addition the antimicrobial profile of *I. frutescens* and *H. indicus* is similar. Further bioassay guided screening can lead to the identification of active principle.

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