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Evaluation of antimicrobial activity of *Ichnocarpus frutescens* (L.) R.Br. against human pathogens

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ABSTRACT

Leaves of *Ichnocarpus frutescens* (L.) R.Br. were collected from the Gahamakhunti of Dhenkanal districts, Odisha, commonly called as *swamnai*. The leaves were extracted with hexane, chloroform, diethyl ether, methanol and water for the *in vitro* study of antimicrobial property. The chloroform extract was found to be more active against *Pseudomonas putida* and *Aspergillus fumigatus*. Methanol extract was found to possess maximum antimicrobial activity against most of the test pathogens but maximum effect was found against *Bacillus sphaericus* (15 mm), *Bacillus polymyxa* (14 mm), *Aspergillus niger* (25 mm) and *A. fumigatus* (22 mm) and minimum activity was found against *Bacillus circulans.* Negative response was found against *Escherichia coli* and *Pseudomonas putida*. In case of chloroform extract of *I. frutescens,* maximum inhibition (21 mm) was found against *Pseudomonas putida* whereas minimum activity (5 mm) was found against *Bacillus circulans*. The diethyl ether extract did not show any response against the test pathogens except *Bacillus sphaericus* (15 mm) and *Bacillus circulans* (11 mm). A maximum inhibition zone of 17 mm and minimum of 8 mm was found against *Bacillus polymyxa* and *Bacillus sphaericus,* respectively. The present screening result demonstrated that the methanol and chloroform extracts of leaf of the fiber yielding plant *I. frutescens* has potent antibacterial as well as antifungal activity and the studied plant may be a new source for novel antimicrobial compound discovery for treating disease causing human pathogens.

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1. Introduction

The World Health Organization reported recently that at least 75 - 95% of the world populations of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involves the use of plant extract products or their active constituents (Robinson, 2011). Traditional medicine usage is a common practice in developed countries at the primary healthcare level (Essawi and Srour, 2000). Presently thousands of plants are yielding valuable medicines of great use to man and are commonly known as medicinal and drug plants (Bradshaw, 1992). Herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost. The use of plant extracts and phytochemical, with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Behera and Mishra, 2005; Govindraj *et al.,* 2006). These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils, as well as tannins.

India is a vast repository of medicinal plants that are used in medical treatments (Bradshaw, 1992), which also forms a rich source of knowledge (Nadkarni, 1984). Thus according to Ganesan *et al.* (2013) medicinal plant constitutes a group of industrially important plants which bring appreciable income to the country by way of export. With the eventual development, the mankind and industries led to the sole dependences of the human being on manufactured medicines.

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The elemental analysis of plant parts of *Ichnocarpus frutescens* was carried out by EDX (Energy Dispersive X-Ray spectroscopy) analysis which showed the presence of Ca, Si, Mg, Cl, K, C in ethanolic extract using $CaCO_3$, SiO_2, MgO, KCl, K-MAD, Cawollastonite as the standards. The EDX analysis showed root possesses all the tested elements. The weight % of carbon was found to be maximum i.e. 51.60 % in leaf part in comparison with the root and stem. The percentage of essential element was higher in root in compared to leaf and stem *Ichnocarpus frutescens* (L.) RBr. (Apocynaceae) have been used as folk medicine and as an ingredient in Ayurvedic and Unani preparations against diseases of blood, skin, headache, snake bite and inflammation (Starlin *et al.,* 2012). *I. frutescens* is rich in polyphenols and flavonoids. Distribution of various flavonoids and phenolic acids in the leaves of *I. frutescens* has been systematically studied. The root portion of this plant was much more used in traditional as well as in modern era. It has shown the presence of phenylpropanoids, phenolic acids, coumarines, flavonoids, sterols and pentacyclic triterpenoids. Pharmacological study revealed hepatoprotective, antioxidant, anti-inflammatory, and analgesic, anti diabetic and anti-tumor activity (Mishra *et al.,* 2009). In the present study realizing the medicinal values *in vitro* screening of leaf extracts of *I. frutescens* for antimicrobial activity of the proposed plant with different organic solvents has been carried out.

2. Materials and Methods

2.1 Botany of Ichnocarpus frutescens (L.) R.Br.

Ichnocarpus frutescens (L.) R.Br. (Apocynaceae; common name-swamnai) is an evergreen plant with white, small, 7.5mm diameter flowers It is a large, evergreen, lactiferous, woody creeper with red appearance found almost throughout India, ascending up to an altitude of 4000 ft. The roots of the plants are used in the medicine as a substitute for Indian Sarsaparilla (*Hemidesmus indicus*) and are often mixed with the latter; neither their therapeutic properties nor their suitability for use as "sarsaparilla" substitute have been established.

2.2 Collection of sample

The fresh leaves of *I. frutescens* free from disease were collected from the Gahamakhunti of Dhenkanal districts, Odisha. The specimen was deposited at the herbarium, Institute of Minerals and Materials Technology (IMMT), Bhubaneswar, Orissa for authentification and identified following Flora of Orissa (Saxena and Brahmam, 1994-96). Specimen was labeled, numbered, annotated with the date of collection, the locality and medicinal uses were recorded.

2.3 Solvent extraction

The leaves were collected, washed thoroughly (2-3 times) with running tap water followed by distilled water and dried under sterile blotting paper. These were then dispersed in a tray and kept under shade for 8-10 days for air drying at room temperature. After drying the leaves were powdered with the help of electric blender and stored in a zipper polythene bag. The powdered material (15-20 g) was filled in the thimble and extracted successively with different organic solvents such as methanol, chloroform, hexane, petroleum ether and water in Soxhlet extractor for 24-48 hr (Bradshaw, 1992). The collected extracts were filtered using standard filter paper and concentrated to dryness under reduced pressure below 60^0C under a rotary flash evaporator and stored at 4^0C in an air tight bottle for further use.

2.4 Growth and maintenance of test organisms

For antimicrobial study different human pathogenic bacterial strains viz., *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas putida, Bacillus polymyxa, B. sphaericus,* and *B. circulans* from SUM hospital and fungal cultures such as *Aspergillus fumigatus* and *Aspergillus niger* obtained from OUAT, Bhubaneswar, Orissa were used for the present study. The bacteria were maintained in Nutrient agar (NA) slants at 37^0C and fungi were maintained in Potato Dextrose Agar (PDB) medium at 28^0C.

2.5 Antibacterial assay

In vitro antibacterial screening was carried out by disc diffusion method (Bauer *et al.,* 1996). The test organisms were inoculated into nutrient broth (NB) and Potato Dextrose Broth (PDB) and incubated at 37^0C for 18-24 hrs. The inoculum of individual pathogens was swabbed with the help of a sterile cotton swab on Nutrient Agar (NA) plates in duplicates. Filter paper discs loaded with extracts (20 µl) of each solvent extract were placed in the surface of growth media at equidistant points and then were incubated at 37^0C±0.5^0C for 18-24 hrs after which the diameter of inhibition zones formed around the discs were recorded. Control and standard set of experiments were also carried out with different solvents and standard antibiotics (streptomycin, gentamycin, penicillin, ampicillin and amikacin. Minimum Inhibitory Concentration (MIC) was determined by standard test procedures using disc diffusion method.

2.6 Antifungal assay

Inhibition of mycelial growth using the agar well diffusion technique (Groover and Moore, 1962; Shahi *et al.,* 1999). Potato Dextrose Agar (PDA, Dehydrated culture media) was

autoclaved and then maintained in water bath at 40 °C. The extracts or the solvent fractions were added to sterile molten PDA to obtain final concentration of 250 µg/ml. The media was poured into sterile petridishes, solidified and fungal discs with seven day old mycelium of the pathogen were placed in the centre of the plate. Plates were incubated for 2-3 days at 28-30^{0}C. The diameter of inhibition zone formed around the discs were recorded. Control and standard set of experiments were also carried out with different solvents and standard antibiotic (nystatin).

3. Results

Results obtained in the present study revealed that the tested leaf part of *I. frutescens* showed considerable antimicrobial activity against the studied microorganisms. Different extracts of the plants exhibited antibacterial and antifungal activity in a concentration dependant manner. Methanol extract was found to possess maximum antimicrobial activity against most of the test pathogens but maximum response was found against *B. sphaericus* (15 mm) and *B .polymyxa* (14 mm) and minimum (12 mm) was found against *B. circulans* whereas negative response was found against *E. coli* and *P. putida*. In case of chloroform extract of *I. frutiscens* maximum inhibition (21 mm) was found against *P. putida* whereas minimum activity (5 mm) was found against *B. circulans*. The diethyl ether extract did not show any response against the test pathogens except *B. sphaericus* (15 mm) and *B. circulans* (11 mm). A maximum activity (17 mm) and minimum activity (8 mm) was found against *B. polymyxa* and *B. sphericus* respectively whereas a positive result was also found against *B. sphaericus, B. circulans, B. polymyxa, K. pneumoniae, P. vulgaris* and *P. putida* in hexane extract (Fig.1). In case of standards penicillin showed maximum inhibition (33 mm) against *P. putida* whereas ampicillin showed maximum activity (24 mm) against *E. faecalis*. Gentamycin showed maximum activity (22 mm) against *E. coli* and *Proteus mirabilis*. Streptomycin showed maximum activity (19 mm) against *B. sphaericus* and *E. faecalis* whereas amikacin was showing maximum activity (24 mm) against *B. circulans* and *B. sphaericus* (Fig. 1).

Antifungal activity of different solvent extracts of leaves of *I. frutiscens* showed significant activity. Maximum activity i.e. 25 mm and 23 mm was observed in hexane extract whereas minimum i.e. 8 mm and 15 mm was found in chloroform extract against *Aspergillus niger* and *Aspergillus fumigatus* respectively. Aquous extract did not show any activity against both the strains. In case of standard nystatin was showing 24 mm and 27 mm against *A. niger* and *A. fumigatus,* recpectively (Fig. 2).

The MIC value of methanol extract against *B. sphaericus* and *K. pneumoniae* was found to be 30 µg/ml and 40 µg/ml whereas in case of chloroform extract MIC value was found to be 5 µg/ml against *P.putida*. In case of hexane extract it was found to be 30 µg/ml against *B.*

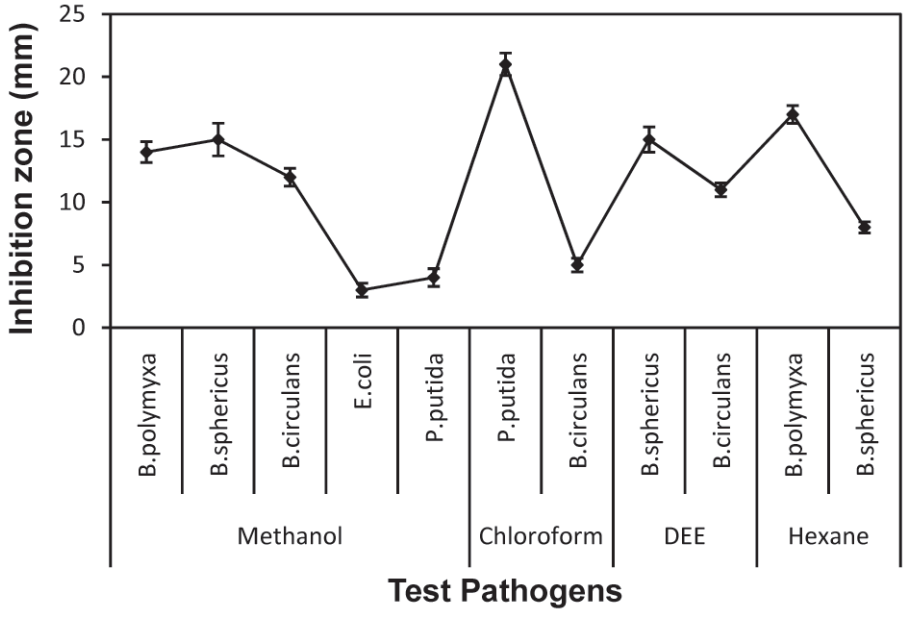

Fig.1 Antibacterial activity of leaf extracts of *Ichnocarpus frutescens*

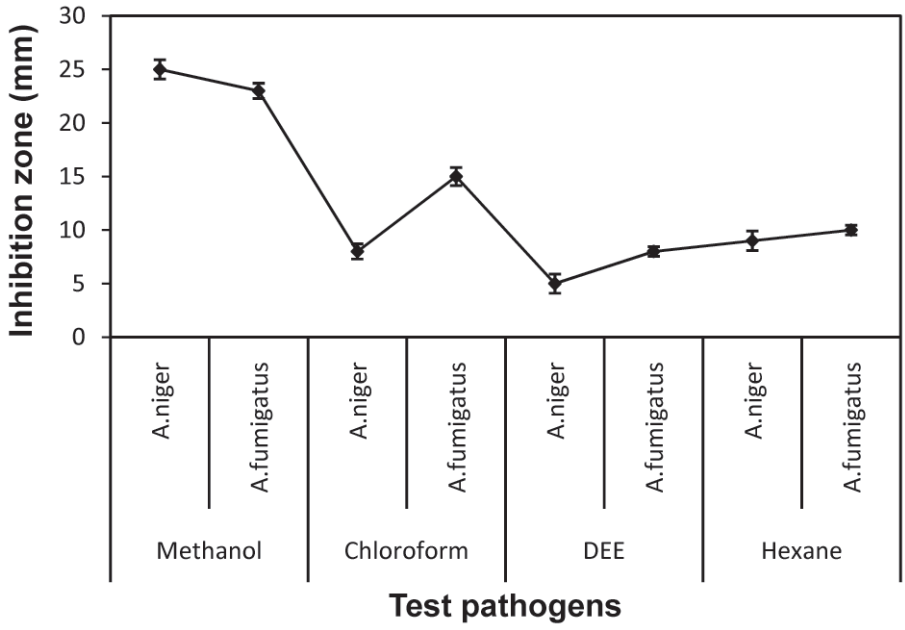

Fig.2 Antifungal activity of leaf extracts of *Ichnocarpus frutescens*

polymyxa whereas in diethyl extract it was 20 µg/ml against *B. sphaericus*. In methanol extract, the MIC was 35 µg/ml against *A. niger*.

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, as a result plants are one of the bed rocks for modern medicine to attain new principle (Evans *et al.,* 2002; Saxena and Brahmam, 1996; Bonjar, 2004; Mahesh and Satish, 2008). In the present study methanol, hexane, and chloroform extracts of the *I. frutescens* showed greater antibacterial as well as antifungal activity than the corresponding water extracts. This finding is interesting, because in the traditional method of treating a bacterial infection, decoction of the plant parts in water was employed. Whereas, according to present study preparing an extract with an organic solvent was shown to provide a better antimicrobial activity, in accordance with the results obtained by Nair *et al.* (2005). These observations may be attributed to two reasons: Firstly, the nature of biological active components whose activity can be enhanced in the presence of methanol, hexane and chloroform; secondly, the stronger extraction capacity of the above solvent could have produced greater number of active constituents responsible for antimicrobial activity. So, *I. frutiscens* plant can be used to discover bioactive natural products that will lead to the development of new pharmaceuticals. Such screening of various natural organic compounds and identifications of active agents must be considered as a fruitful approach in the search of new herbal drugs. Moreover, plant extracts are far more economical because of being freely available around in nature. This can result in control of disease at the required concentration of extracts. Needless to say that it would be great service to mankind if the scientific community can create awareness among people about the judicious use of this gift of natural resources.

The antimicrobial activity can be enhanced if the active components are purified and adequate dosage determined for proper administration. This may go a long way in preventing the administration of inappropriate concentrations, a common practice among many traditional medical practitioners.

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