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Antimicrobial effects of Aqueous Butanolic extract of *Saraca Indica* (Linn).

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ABSTRACT

Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties. Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Hence, monitoring resistance is of paramount importance. The use of traditional herbal medicines in crude or refined form may help in the treatment of microbial infections with two advantages, i.e. the cure is achieved and the chances of microbes becoming resistant are minimized. The herbal medicines have the advantage of not producing major side effects as is found in case of usual antibiotics. Therefore this study was undertaken to focus on the in vitro antimicrobial effects of two plants. Plant materials of *Saraca indica* were tested for their antibacterial activities on selected strains of bacteria namely, *Bacillus cereus, Shigella flexneri* and *Pseudomonas aeruginosa*. These activities were compared with standard antibiotic namely Broad spectrum antibiotics tetracycline. Antimicrobial activity was measured using the standard method of diffusion disc plates on agar and the MIC was calculated using dilution method. Our results clearly indicate that the plant have the antimicrobial properties.

Keywords: Antimicrobial, *Pseudomonas aeruginosa*, *Shigella flexneri,* Resistance, Antibiotics.

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INTRODUCTION

Saraca indica belongs to the family Caesalpiniaceae. It is found in India, China, Ceylon and Malaysia. It occurs almost throughout India up-to an altitude of 750 m in the central and in the eastern Himalayas and Khasi, Garo and Lushai hills, wild in Chittagong, Bihar, Orissa, Konkan, Deccan, Mysore. It has become quite scarce in several localities and is reported to be threatened in North Eastern Region of India. The initial introduction of new medicinal agents into the health care system some times, requires information beyond that is recorded in libraries relying instead, on reports available through traditions and healers within a society. Thus traditional medicinal practices, conserved over years by civilizations, can serve as an effective basis for the discovery and development of modern therapeutic drugs (Silva junior etal 1994).

Many potent drugs have been purified from medicinal plants which range from antibacterial, anti-malarial, anti-cancer and anti-diabetic (Holetz etol 2002). The present study focuses on the antibacterial properties of *Saraca indica.*

MATERIALS AND METHODS

Plant Materials

Plant material were collected from their authorized Ayurvedic store. Both the plants were identified and authenticated by reputed botanist.

Extraction from plants

The plant materials were dried in shade and powdered in a mechanical grinder. The powder of the plant materials were initially de-fatted with petroleum Benzene (60^0C-80°C), followed by extraction with 500 ml of water and 500 ml of butyl alcohol by using a Soxhlet extractor for 72 hrs at a temperature not exceeding the boiling point of the solvent .The extract was filtered using Whattman filter paper (No.1) and then was concentrated and dried at 45°C for butanol elimination, and the extracts were kept in sterile bottles, under refrigerated conditions until further use. The dry weight of the plant extracts was obtained by solvent evaporation and used to determine concentration in mg/ml. The extract thus obtained was directly used in the assay of antimicrobial activity.

Antibiotics

Broad spectrum antibiotics, Tetracycline were used as control drug.

Bacterial Strains

The strains of microorganisms (*Bacillus cereus, Shigella flexneri and Pseudomonas aeruginosa* were used.

Determination of Antimicrobial Activity

Antimicrobial activity was measured using the standard method of diffusion disc plates on agar and the MIC was calculated using dilution method (Kirby- Bauer method).

Dilution Methods

Dilution susceptibility testing methods were used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganisms. This was achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are generally tested in log2 serial dilutions (two fold).

Broth Dilution Method

The Broth Dilution Method is a simple procedure for testing a small number of isolates, even single isolates.

Preparation of microorganisms for experiment

The pure cultures of organisms (*Bacillus cereus, Pseudomonas aeruginosa and Shigella flexneri)* were sub-cultured in nutrient broth. They were inoculated, separately, into nutrient broth and kept at 37°C for 24 hours. Then, they were kept at 4°C until use.

Growth Method

At least three to five well-isolated colonies, of the same morphological type, were selected from an agar plate culture of a particular microorganism. The top of each colony was touched with a loop, and the growth was transferred into a tube, containing 4 to 5 ml of Nutrient broth medium. The broth culture was incubated at 35°C for 8 hours. After the incubation period broth culture became turbid.

Disc Diffusion Method

Mueller-Hinton Agar Medium

Mueller-Hinton Agar is considered to be the best for routine susceptibility testing of non-fastidious bacteria for the following reasons; It shows acceptable batch-to-batch reproducibility for susceptibility testing. Medium is transparent, so that the inhibition zone can be visualized clearly. It gives satisfactory growth of most non fastidious pathogens. A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Preparation of Mueller-Hinton Agar

Mueller-Hinton Agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in a 45 to 50°C water bath. The freshly prepared and cooled medium was poured into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm. The agar medium was allowed to cool to room temperature and unless the plate is used the same day, stored in a refrigerator. Plates were used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar. A representative sample of each batch of plates was examined for sterility by incubating at 30 to 35°C for 24 hrs or longer.

Preparation of antibiotic stock solutions

Powders of the two antibiotics (Tetracycline) were brought from authorized medical shop. They were accurately weighted and dissolved in sterile distilled water in appropriate dilutions to yield the required concentrations. The stocks were kept in aliquots of 5 ml volumes and frozen at -20°

Preparation of plant extract solutions for the experimen:

The dried plant extracts were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations (1.0mg/ ml, 1.5mg/ ml, 2.0 mg/ ml, and 2.5 mg/ ml). They are kept under refrigeration.

Preparation of dried filter paper discs

Whattman filter paper (No.1) was used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven. After the sterilization, the discs were poured into the different concentration of broad spectrum antibiotics and into the prepared plant extract solutions and again kept under refrigeration for 24 hrs.

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RESULTS AND DISCUSSION

Reading of Minimum Inhibition Concentration Minimum inhibition concentration was expressed as the lowest dilution which inhibited growth judged by lack of turbidity in the tube, because very faint turbidity might be given by the inoculums itself. The inoculate tube was kept in the refrigerator overnight and was used as the standard for the determination of complete inhibition. The plant extracts were found to be effective against the three selected bacterial species.

Reading Zone of Inhibition and Interpreting Results after 16 to 18 hrs of incubation each plate was examined. Once the resulting zones of inhibition came uniformly circular and in a confluent lawn of growth, the diameters of the zone of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest mm using a ruler, which was held on the back of the inverted Petri plate.

Table 1: Minimum inhibitory concentration (MIC)

Microoraganisms	Tetracycline	*Saraca indica*
Bacillus cereus	**0.260**	**0.300**
Pseudomonas aeruginosa	**0.400**	**0.480**
Shigella flexneri	**0.220**	**0.260**

Clear inhibition zones indicated the presence of antimicrobial activity.

Table: 2

Microorganisms	Zone of Inhibition		
	Concentrated drug (mg/ ml)	Group I	Group II
B. cereus	1.0	12.0	15.0
	1.5	16.0	17.2
	2.0	18.0	18.2
	2.5	20.0	22.0
P. aeruginosa	1.0	8.0	10.0
	1.5	12.0	12.8
	2.0	14.0	15.6
	2.5	16.4	18.0
S. flexneri	1.0	10.0	11.2
	1.5	12.0	13.4
	2.0	14.0	15.0
	2.5	17.4	19.0

Group II, III and IV are compared with Group I.
Group I, III and IV are compared with Group II.

Natural drugs are obtained from the plant, animal or mineral kingdoms. The plant kingdom is the store house of the organic compounds. *Saraca indica* (Roxb) de wild (Family-Caesalpinaceae) is commonly known as Asoka, Sita Asoka and Haempushpam. It is an evergreen tree which is 9 m in height. The flowers are orange yellow in colour and arranged in dense corymbs. It occurs throughout India up to an altitude of 750m in central and eastern Himalayas. Useful parts of the plant are barks, leaves, flowers and seed.

The Asoka tree is considered sacred throughout India. This tree has many folklorical, religious and literary associations in the religions. Due to its high value and handsome appearance this tree is found close to the temples throughout India. The use of medicinal plants in the world and especially in India, contribute significantly to primary health care. The antimicrobial medicinal plants are well documented (Buckles, 1995). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agent even against some antibiotic resistant strains (Daxenbichler etal 1971).

In the present study the results show that the extract from *Saraca indica* possess antimicrobial activities against *Bacillus cereus, Shigella flexneri* and *Pseudomonas aeruginosa.* The extract compared favorably with the standard antibiotics Tetracycline. The plant extract showed more activity that broad spectrum antibiotic activities. The MIC of *Saraca indica* were shown in table-2. The standard Tetracycline had MIC values varying between 0.220 mg/ml and 0.260 mg/ml. The results indicated that the extract of Saraca indica has stronger activity than that of standard antibiotics (Table-1). Since ancient times, herbs and /or their essential oils have been known for their varying degrees of antimicrobial activities (Bell etal 1971, Carson etal 1995, Lin etal 1999, Valero etal 2003).

More recently medicinal plant extracts were developed and proposed for use in food as natural antimicrobials (kone etal 2004, shelef, 1983). Antimicrobials are powerful but controversial tools. Food animals are often exposed to antimicrobial compounds to treat or prevent infectious diseases and/or to promote growth. The early history of supplementing animals feeds with antimicrobials parallels the isolation, identification and characterization of Vitamin B (Agbafor etal 2011). in 1948.

CONCLUTION

Ashoka is the most ancient tree of India, generally known as a “ashok briksh”, botanist known as a *Saraca asoca* (Roxb.), De.wild or *Saraca indica* belonging family *Caesalpinaceae*. Medicinal herbs are moving from fringe to mainstream use with a great number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Saraca asoca is reported to contain glycoside, flavanoids, tannins and saponins. It is used as spasmogenic, oxytocic, uterotonic, anti-bacterial, anti-implantation, anti-tumour. Therefore it is of interest to investigate the anti-bacterial effect of aqueous butyl extract of *Saraca indica* againt the selected pathogens light Bacillus cereus, Pseudomonas aeruginosa and *Shigella flexneri*. Our results clearly indicate that the medicinal plant saraca indica linn processes the anti-microbial effect against Bacillus cereus, Pseudomonas aeruginosa and Shigella Flexner.

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