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Comparative Screening of Selected Mangrove Plant Methanolic Extracts against Clinical and Plant Pathogens

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ABSTRACT

Antimicrobial effect of the crude methanol extracts of mangrove plants *Tamarix aphylla*, *Sesuvium portulacastrum* and *Xylocarpus granatum*. The antimicrobial activity was determined in methanol extracts using agar well diffusion method by measuring the diameter of the zone of inhibition in term of millimeter. Screening revealed that the crude methanol extract of the studied mangrove extracts possess antimicrobial activity against most of the test organisms depending upon the nature of their active ingredients in the extract and capacity of diffusion into the agar medium. Among the test organisms, the extract showed significant antimicrobial activity against *Acremonium strictum Asperigillus niger, Candida albicans Erwinia carotovara, Fusarium oxysporum, Pseudomaonas marginales*, *Ustilago maydis*, and no activity against *Pseudomaonas marginales* with *S. portulacastrum.*

Keywords: Antibacterial activity, Agar well diffusion, *Sesuvium portulacastrum*, Zone of inhibition.

INTRODUCTION

Life and diseases go together where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through their uses as food, clothing and shelter, but also plants have been used to control diseases, therefore, the use of plants as medicines is an ancient and reliable practice (Arshad and Rao, 2001). Plants are serving several purposes whether health, nutrition, beauty or medicinal. With the development in techniques and recent researches, it has been proved that certain nutritive and non-nutritive chemicals in plants which are of very much importance to dietary and healing properties. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al*., 1995). The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity should be tested against appropriate microbial models to confirm the activity and to ascertain the parameters associated with it. Interest in large number of traditional natural products has been increasing (Taylor *et al*., 1996) now a day.

Mangroves have long been a source of astonishment for the layman and of interest for scientist. Mangroves are biochemically unique, producing a wide array of novel natural products. Substances in mangroves have long been used in folk medicine to treat diseases (Bandaranayake, 1998). *Tamarix aphylla* belongs to a family Tamaricaceae is an evergreen tree that can grow to 18 m tall. They usually grow on saline soils, tolerating up to 15,000 ppm soluble salt and can also tolerate alkali conditions. The bark of young branches is smooth and reddish-brown. The leaves are scale-like, 1-2 mm long, and overlap each other along the stem. The bark is used as astringent and bitter (Chopra *et al.*, 1986). *Sesuvium portulacastrum* belongs to a family Aizoaceae commonly known as shoreline purslane or (ambiguously) "sea purslane", is a sprawling perennial herb that grows in coastal areas throughout much of the world. Grows as sprawling perennial herb up to thirty cm hight with thick and smooth stems up to one meter long. It has smooth, fleshy, glossy green leaves that are linear or lanceolate, from 10–70 long and 2–15 mm wide. Flowers are pink or purple.

Xylocarpus granatum belongs to a family Meliaceae trees is 3-8 m tall, buttresses long and snaking laterally; bark light brown, yellowish or greenish, smooth, flaking off. The bark is used as astringent, dysentery, Diarrhea, and other abdominal troubles and as febrifuge. (Ghani, 1998) The seed ash mixed with sulphur and coconut oil is applied as ointment for itch. (Ghani, 1998) Fruit is used as a cure for swelling of the breast and elephantiasis. The family is distinguished by the occurrence of characteristic substances called limonoids (Ambrozin *et al*., 2006). These substances have wide spectrum of biological activities, particularly insecticidal action (Ambrozin *et al*., 2006). Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Okeke *et al*., 2001; Ebi and Ofoefule *et al*., 1997). *X. granatum* also possesses alkaloidal substances which also have biological activities (Chou *et al.*, 1977).

Keeping in view the importance of diverse medicinal flora research work was conducted to investigate ethno botanical uses and create awareness about the mangrove plant species enlisted for the welfare of human communities throughout the world. In the present

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work three mangrove plants were selected and screened for potential antimicrobial activity.

Table 1. Screening of mangrove plants for potential antibacterial activity

Sno	Botanical name	Family	Vernacular name	Parts used
1	*Tamarix aphylla*	Tamaricaceae	Palisa	Leaf and bark
2	*Sesuvium portulacastrum*	Aizoaceae	Thikkakura	Bark
3	*Xylocarpus granatum*	Meliaceae	Chenuga	Seed and bark

MATERIALS AND METHODS

Plants and extraction:

The material was taxonomically identified and the voucher specimen is stored. The plant parts were collected from Kakinada-Godavari mangrove forest, Andhra Pradesh, India. The plant material were dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to soxhlet extraction with solvent methanol.

Test microorganisms:

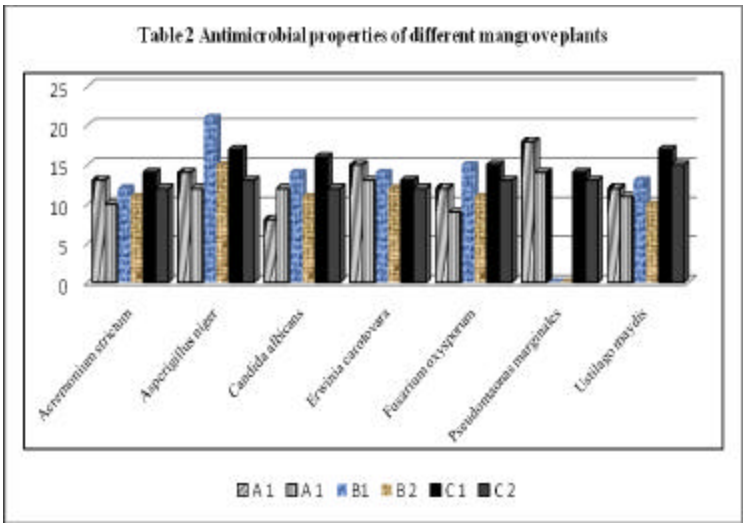
The microorganisms (including fungi and bacteria) selected were *Acremonium strictum* (MTCC 3072), *Asperigillus niger* (MTCC 2723), *Candida albicans* (MTCC 3017), *Erwinia carotovara* (MTCC 3609), *Fusarium oxysporum* (MTCC 1755), *Pseudomaonas marginales* (MTCC 2758) and *Ustilago maydis* (MTCC 1474) obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. Active cultures were generated by inoculating a loopful of culture in separate 100mL nutrient broths and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5 x 10^5cfu/mL.

Determination of antibacterial activity:

The crude methanol extracts of the different mangrove species were subjected to antimicrobial assay using the agar well diffusion method of Murray *et al.*, (1995) modified by Olurinola, (1996). 20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µl of the extract concentration of 100mg/ml and allow diffusing for forty five minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°c for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°c for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates. The extracts and the phytochemicals that showed antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for each bacterial and fungal sample.

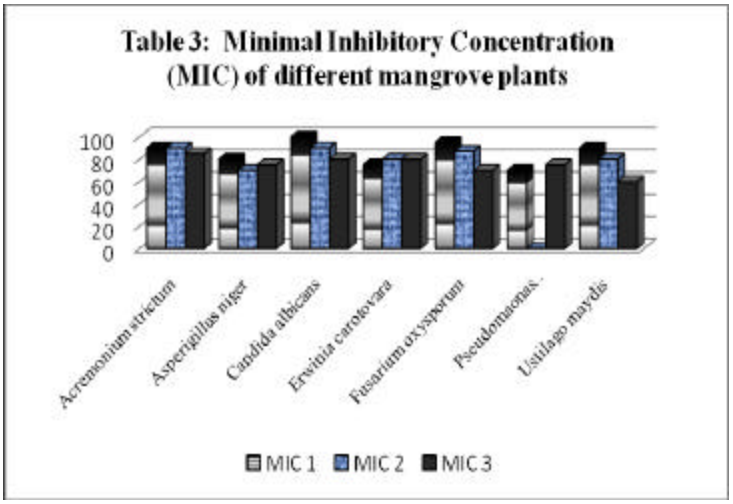
RESULTS AND DISCUSSION

Table 1 represents the ethno botanical data including botanical name, vernacular name, and part used of selected plant species. Table 2 summarizes the antimicrobial activities of the various crude extracts. The antimicrobial activity of *T. aphylla* leaf and bark methanol extract showed significant inhibition against *P. marginales* and *E. carotovara*. No inhibitory effect was observed with *S. portulacastrum* on *P. marginales* but exhibited good inhibition against *A. niger* where as *X. granalum* produced overall outstanding antimicrobial effect than *T. aphylla* and *S. portulacastrum*.



A1 (250mg/ml) and A2 (100mg/ml) T. aphylla methanol extract concentration; B1 (250mg/ml) and B2 (100mg/ml) S. portalucastrum methanol extract concentration; C1 (250mg/ml) and C2 (100mg/ml) X. granalum methanol extract concentration; Borer size used:6mm, 0-25 Zone of inhibition in mm.

The lowest MIC (60 mg/ml) was recorded against *Ustilago maydis* with *X. granalum* and highest MIC (100 mg/ml) with *T. aphylla* against *Candida albicans*. It appears that best antimicrobial activity was shown by *X. granalum* methanolic crude extracts and hence it can be selected for further studies. The discovery of a potent remedy from plant origin will be a great advancement in fungal and bacterial infection therapies. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from Kakinada coringa mangrove forest and Godavari region. The variation of antimicrobial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial action of the plant extract.



MIC 1= T. aphylla methanol extract concentration; MIC 2= S. portalucastrum methanol extract concentration; MIC 3= X. granalum methanol extract concentration; Borer size used: 6mm, 0-100 Drug concentration in mg/ml.

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