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In vitro antimicrobial screening of mangrove plant *Avicennia officinalis*

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ABSTRACT

In India *Avicennia officinalis* is commonly used for herbal preparations in the treatment of small pox sores, scabies, as a contraceptive, boils and tumors. This has, therefore, led to the investigation of the antimicrobial activities of methanolic extract of *A. officinalis*. Eighteen different bacterial and fungal belonging to clinical and plant pathogenic microorganisms were used. The results show that *A. officinalis* extracts exhibited antimicrobial activities at a concentration of 20 mg/mL. Antibacterial activity of mature leaves and bark extracts of *A. officinalis* was evaluated using soxhelt extraction method. Hexane, chloroform and methanol were used as solvents in order to get the plant extracts. The antibacterial activity was screened by using agar well diffusion technique against human and plant pathogenic bacteria and fungi. The length of inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone. Mature leaf extracts of *A. officinalis* in methanol exhibited promising antimicrobial activity than other solvent extracts. Phytochemical screening revealed that mature leaf of *A. officinalis* contained alkaloids, steroids, triterpenoids and flavonoids.

Key words: Antimicrobial activity, *Avicennia officinalis.*

INTRODUCTION

A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Farnsworth 1994, Srivastava *et al*. 1996). Biologically active compounds present in the medicinal plants have always been of great interest to scientists working in this field. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing (Clark and Hufford 1993). Further acquaintance with different ethnic groups has contributed to the development of research on natural products to the increase in knowledge about the close relationship between the chemical structure of a certain compound and its biological properties, and to the understanding of the animal/ insect-plant interrelation that act as new anti-infectious agents. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Bacterial resistance to almost all antibacterial agents has been reported (Truiti *et al*., 2003), This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used in treatment of infectious diseases. Apart from resistance, some antibiotics have serious undesirable side effects which limit their applications, so there is urgent need to develop new antimicrobial agents that are very effective with minimal unwanted side effects, and higher plants represent a potential

source of novel antibiotic prototypes. *Avicennia officinalis* (Indian mangrove) is used as a folk remedy for boils and tumors (Duke and Wain 1981; Kirtikar and Basu 1975). The main objective of this study is to screen for potential antimicrobial activity of selective mangrove plant *A. officinalis.* is belongs to family Avicenniaceae it vernacular name is nallamada and it is growing as tree/shrub along seaward fringe and inter tidal areas like coringa Kakinada-Godavari, Andhra Pradesh.

MATERIAL AND METHODS

Plant material

Plant parts were collected randomly from mangrove forest, coringa near to Kakinada - Godavari dist, Andhrapradesh, India. The samples mangrove plant parts were shade dried, cut into small pieces and powdered in a mixer grinder the residues (crude extracts) obtained were finally dried under vacuum.

Extraction of Plant Material

The extraction method employed here is a known amount of coarsely powdered plant materials of different plant species were successively extracted with organic solvents like chloroform, methanol basing on order of their polarity using soxhlet apparatus. The different extracts obtained were subsequently concentrated under reduced pressure to get their corresponding residues. The extracts were screened for antimicrobial activity using the method described under the section.

Hundred grams of each of the air-dried and coarsely powdered plant material was exhaustively extracted for 2 hours with organic solvents like hexane, chloroform and methanol basing on order of their polarity using soxhlet (60-80°C) apparatus. The solvent extract was filtered and evaporated under reduced pressure using rotavapor to get their corresponding residues. The extracts were dissolved in dimethy lsulphoxide (DMSO) to make the final concentrations which kept in refrigerator till used.

Organisms used in this study

The antimicrobial activity of *A. officinalis* extracts was tested against *Asperigellus flavus* (MTCC 1884), *Asperigellus niger* (MTCC 2723), *Acremonium strictum* (MTCC 3072), *Candida albicans* (MTCC 3017), *Cladosporium herbarum* (MTCC 2143), *Erwina caratovara* (MTCC 3609), *Fusarium oxysporaum* (MTCC 7229) *Lactobacillus acidophilus* (MTCC 447), *Pencillium expansum* (MTCC 2006), *Pseudomonas syringe* (MTCC), *Rhizoconia solani* (MTCC 4633), *Streptococcus gordonii* (MTCC 2695), *Streptococcus mutans* (MTCC 890), *Streptococcus salivarious* (MTCC 1938), *Tiarosporella phaseolina* (MTCC 2165), *Ustilago maydis* (MTCC 1474) and *Xanthomonas compestris* (MTCC 2286). These microorganisms were procured from The Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology Sector39-A, Chandigarh, India. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

Preparation of the tested organisms

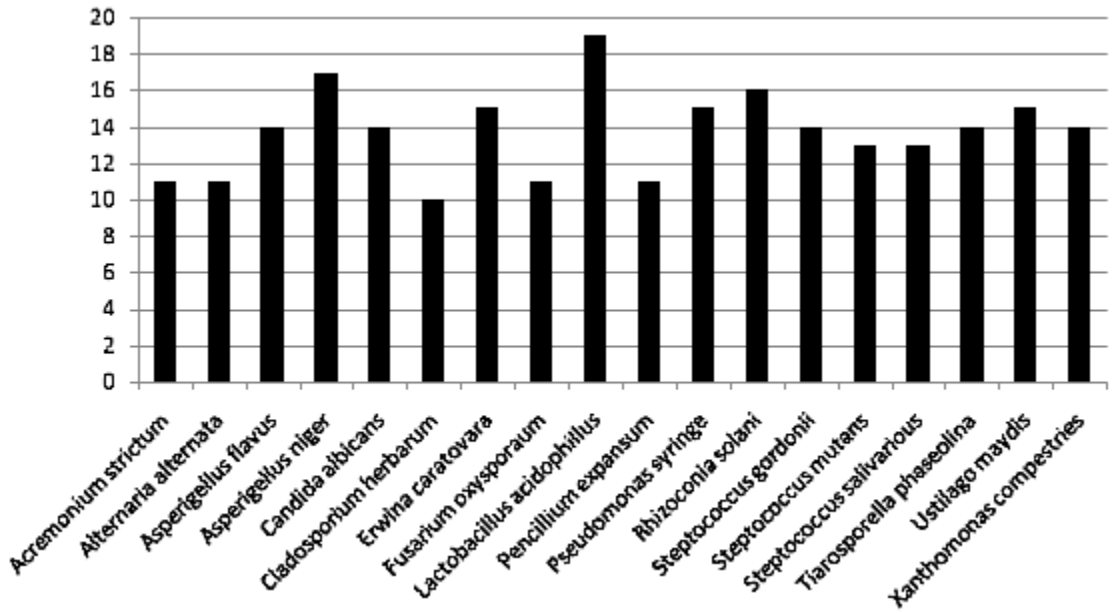
The average number of viable bacterial organisms per ml of the stock suspensions was determined by means of the surface viable counting technique (Miles and Misra, 1938). About (10^8-10^9) colony-forming units per ml was used. Each time, a fresh stock suspension was prepared; the experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained. The fungal growth was harvested and washed with sterile normal saline and finally suspended in (100ml) of sterile normal saline and the suspension was stored in refrigerator till used. The fungal cultures were maintained on Potato Dextrose Agar (PDA), incubated at 25ºC for 4days. Testing for antibacterial activity:

The Cup-plate agar diffusion method was adopted according to Kavanagh, (1972) to assess the antibacterial activity of the prepared extracts. 0.6 ml of standardized bacterial stock suspensions (10^8-10^9) colony- forming units per ml was thoroughly mixed with 60ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agars was left to set and in each of these plates 4 cups, 6 mm in diameter, were cut using a sterile cork borer No. 3 and the agar discs were removed. Alternate cups were filled with 0.1ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then

incubated in the upright position at 37ºC for 18 hours. Two replicates were carried out for each extract against each of the test organism. Simultaneously addition of the respective solvents instead of extracts was carried out as controls. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated. The same method as for bacteria was adopted. Instead of nutrient agar, yeast and mould extract agar was used. The inoculated medium was incubated at 25ºC for two days for the *Candida albicans* and three days for *Aspergillus niger*.

RESULTS AND DISCUSSION

The antimicrobial activity of areal parts of *Avicennia officinalis* methanolic extracts (Table 1) against some common clinical and plant micro organisms are presented here. Apart from the studies bacterial strains of *Lactobacillus acidophillus* showed maximum antibacterial activity followed by *Pseudomonas syringe* while coming to antifungal activity *Asperigellus niger* and *Rhizoconia solani* showed the highest activity. All the micro flora were sensitive to *A. officinalis* methanolic extracts to some extent. So this plant can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. Comparisons with pertinent data from literature indicate that, according to the methodology adopted in studies on antimicrobial activity, the most diverse results can be obtained. Plant extracts have great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganisms. Further phytochemical studies are required to determine the type of compounds responsible for the antimicrobial effects of this medicinal plant.



(0-20) Zone of inhibition in mm; 6mm cup borer size used

Graph 1: Antimicrobial activity of methanol extracts *Avicennia officinalis* of Arial parts

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