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EXCOECARIA AGALLOCHA L. ANTIMICROBIAL PROPERTIES AGAINST IMPORTANT PATHOGENIC MICROORGANISMS

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ABSTRACT: *Excoecaria agallocha* L. leaves were extracted by various extracting procedures, using different solvents for testing the antimicrobial activities against important microorganisms using agar well diffusion method. Chloroform and methanolic extracts were found to be effective against these organisms, whereas hexane extracts were inactive. The purpose of this study was to find preliminary data for the development of alternative treatments to chemical microbicides for the control of plant diseases from natural plant extracts.
Keywords: *Excoecaria agallocha*, Agar well diffusion method; Antimicrobial activity.

INTRODUCTION

Medical plants have been used for years in daily life to treat disease all over the world. It is well known that some plants containing active compounds are able to inhibit the microbial growth. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. Secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity. These plant compounds have different structures and actions when compared with conventional fungicides used to control the microbial growth and survival.

Excoecaria agallocha L. (Euphorbiaceae) is a small mangrove tree found extensively in the tidal forests and swamps of the Krishna-Godavari area. This plant is also well-distributed in a number of other countries of temperate and tropical Asia. The bark oil has been found effective against rheumatism, leprosy and paralysis. This plant also has been traditionally used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic. However, the milky sap of this tree can cause temporary blindness if it enters the eyes. The sap can also cause skin blisters and irritation. Clinical trials carried out on this plant have shown its potential anti-HIV, anticancer, antibacterial and antiviral properties. Previous phytochemical investigations on this species revealed the presence of diterpenoids[1, 2, 3, 4] triterpenoids[5] flavonoids[6] and phorbol esters[7]. As part of our on-going phytochemical and bioactivity studies on mangrove plants from Kakinada and Godavari, we now report assessment of *in vitro* antimicrobial activity including pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

E. agallocha L. commonly known as milky mangrove and its vernacular name is Tilla and this species of mangrove tree classified in the plant family Euphorbiaceae and widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines. It has been reported to tolerate extreme weather conditions, high winds. The material was taxonomically identified and the voucher specimen is stored. The plant parts were collected from Coringa Mangrove Wetland, 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 successive soxhlet extraction with the organic solvents with increasing order of polarity respectively.

The antibacterial activity of the extracts was assessed against microbial strains of clinical, plant and aquatic origin i.e. *Acremonium strictum* (MTCC 2599), *Aspergillus flavus* (MTCC 4633), *Asperigillus niger* (MTCC 2723), *Candida albicans* (MTCC 3017), *Curvularia lunata* (MTCC 2030), *Fusarium oxysporum* (MTCC 1755), *Lactobacillus fermentum* (MTCC 903),

Macrophomina phaseolina (MTCC 2165), *Penicellium expansum* (MTCC 2006), *Rhizoctonia solani* (MTCC 4633), *Staphylococcus aureus* (MTCC 96), *Ustilago maydis* (MTCC 1474) and *Xanthomonas compestris* (MTCC 2286) including fungi and bacteria were obtained from Microbial Type Culture Collection (MTCC), Chandigarh were used as test organisms. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

Determination of antibacterial activity:

The crude extracts of *E. agallocha* leaf extract were subjected to antimicrobial assay using the agar well diffusion method of Murray[8] modified by Olurinola[9]. 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.

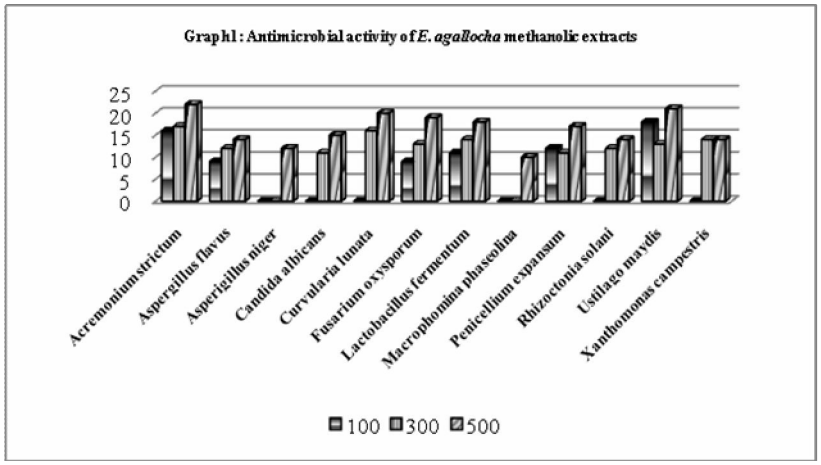
The cups/wells were filled with 50µℓ of the extract concentration of 100mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°c for 24 hours for bacteria. The above procedure is allowed for fungal assays but 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

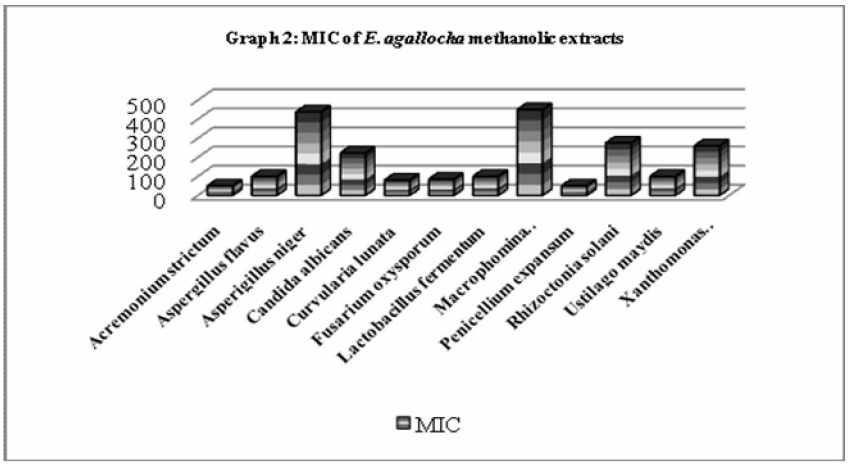
The antibacterial activity of *Excoecaria agallocha* extracts (hexane, chloroform and methanol) against twelve microorganisms from different sources is shown in Graph 1 and 2. The methanol extract showed considerably more activity than hexane and chloroform extracts. Maximum antibacterial activity was shown against *A. strictum*, followed by *P. expansum*. Neither of the extracts hexane and chloroform was able to inhibit any of the tested microbial strains. The methanolic extract showed more activity than the hexane and chloroform extracts. Hence only methanolic extracts results were analyzed.

Methanol extracts of *E. agallocha* inhibited most bacterial and fungal growth, but their effectiveness varied. The above results concluded that this plant extracts have greater potential compounds against microorganisms and that they can be used as novel antimicrobial agents. Our study undoubtedly confirms that the leaves of *E. agallocha* contain higher relative percentage of the above mentioned crude organic extracts has potential antibacterial and antifungal principle for chemotherapeutic application. According to McGaw[10] and Seidel[11] Lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic acids are known to have potential antibacterial and antifungal agents.

From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Excoecaria agallocha* showed maximum antibacterial activity and 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 important lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development.



Borer size used: 6mm; 100, 300 and 500 mg/ml concentration; 0-25 Zone of inhibition in mm;



Volume per well: 50µl, Borer size used: 6mm, 'x' axis = Medicinal plants, 'y' axis =0-500 = Extract concentration in mg/ml

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