In vitro antimicrobial activity of leaf extracts of certain mangrove plants collected from Godavari estuarine of Konaseema delta, India

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Abstract: Several varieties of Mangrove plants were collected from Godavari estuarine of Konaseema delta which is an extended forest of Coringa. In the present study selected mangrove plants leaf extracts were screened for their antimicrobial activity against bacteria and fungi. The mangroves, Avicennia marina (Forsk) (Avicenniaceae), Excoecaria agallocha (Linn) (Euphorbiaceae), Lumintzera racemosa (Combretaceae), Derris trifoliata (Fabaceae), Brugiera gymnorrhiza (Rhizophoraceae), Ceriops decandra (Rhizophoraceae) and Acanthus ilicifolius (Acanthaceae) were selected for this study. The crude extracts from dried leaves of the mangroves were extracted using soxhlet extraction method and obtained extracts were concentrated by using rotary evaporator. Four solvents, Chloroform, Petroleum ether, Methanol and Ethanol were used to extract the leaf material. The crude extract obtained was evaluated for antimicrobial activity against bacteria & fungi by agar well diffusion method and the zone of inhibition diameters were calculated. The extracts at a concentration of 1500 µg/ml exhibited antibacterial and antifungal activity against test microorganism with degree of variation. Out of the screened seven mangrove plants D. trifoliata extracts of four solvents showed promising antimicrobial activity with inhibition zone diameter ranging from 22-14mm. B. gymnorrhiza and C. decandra extracts showed moderate antibacterial and antifungal activity (20-14mm). Among seven mangroves plant extracts screened for antibacterial and antifungal activity, extracts of A. marina, E. agallocha, L. racemosa and A. ilicifolius exhibited least activity.

Keywords: Derris trifoliata; antimicrobial activity; Godavari estuarine; Mangroves; zone of inhibition.

Introduction

Mangrove forests are the rich source for Biodiversity. Mangrove forests are widely distributed throughout coastal areas of India, especially estuaries. In Andhra Pradesh mangroves are widely distributed at Godavari & Krishna estuarine where it associates with Bay of Bengal. In East Godavari, Konaseema is popularly known as rice bowl of Andhra Pradesh. Coringa mangrove forest is rich in biodiversity of mangrove plants where it is located near to Kakinada and extended upto Konaseema deltaic zone throughout the Godavari estuarine. Mangrove plants and their products have been extensively used in traditional medicine. These plants are well known to have diverse natural products with great pharmaceutical importance and also exhibiting antimicrobial, anti larval, anti viral and anti insecticidal activity (Premnathan et al. 1992; Premnathan et al. 1996; Kokpal et al. 1990). There is a need of development of new drugs because of the resistance developed to existing antibiotics by pathogens. Hence there is a need to search and design new alternative drugs from natural plant products to control microbial infections. Mangrove plants are the best choice to isolate bioactive natural products active against bacteria and fungi. Antibacterial activity of mangroves against fish pathogens has already been studied by many authors. Extracts of Avicennia species showed a board spectrum of antimicrobial activity against Candida albicans, Mycobacterium vaccae, Mycobacterium aurum, Mycobacterium smegmatis, Mycobacterium fortuitum, and Staphylococcus aureus (Han et al. 2007; Jun et al. 2008). The leaf and stem extracts of E. agallocha collected from Bhitar Kanika mangrove forest (India) showed antibac-
terial activity against human pathogenic microorganisms. It showed antibacterial activity against both gram positive and gram negative bacteria (Konishi et al. 1998, 2000; Masuda et al. 1999; Patra et al. 2009a). The fatty acid methyl ester extract from the leaf of *E. agallocha* showed strong antimicrobial and antifungal activity (Agoramoorthy et al. 2007). *Acanthus ilicifolius*, a mangrove plant, which is useful in the treatment of paralysis, asthma, rheumatic pains and possess analgesic, anti-inflammatory, antileukemic activity and Leishmanicidal activities. *Clerodendron inerme*, exhibits larvicidal, antiviral and uterine stimulant activity (Sampson et al. 2000). The present study is focused on the exploitation of mangrove plant leaf extracts for their antibacterial and antifungal activities.

Materials and methods

**Collection of Sample**

The leaves of *Avicennia marina* (Forsk) (Avicenniaceae), *Excoecaria agallocha* Linn (Euphorbiaceae), *Lumnitzera racemosa* (Combretaceae), *Derris trifoliata* (Fabaceae), *Brugiera gymnorrhiza* (Rhizophoraceae), *Ceriops decandra* (Rhizophoraceae) and *Acanthus ilicifolius* (Acanthaceae) were collected from Coringa Mangrove wetland forest which is extended to Konaseema deltaic zone located at 16° 33’ 25.32” N and 82° 12’ 28.49” E (Figure 1). The plant materials were taxonomically identified and stored as specimens (Voucher no: ANU BT-01) in Biotechnology Department, Acharya Nagarjuna University, Guntur.

**Preparation of plant powders & extracts**

The collected mangrove plants leaves were dried under shade and then powdered with mechanical grinder. The obtained plant powder was used to prepare crude extracts by using Soxhlet extraction method with the organic solvents. The crude obtained was connected with rotary evaporator. Four different solvents such as chloroform, petroleum ether, methanol and ethanol were employed for extraction of each plant species.

**Antimicrobial activity**

**Microbial strains**

Bacterial and fungal cultures used in this study to evaluate the antimicrobial activity of the various mangrove plant extracts are *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermides* (MTCC 435), *Escherichia coli* (MTCC 443), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 39), *Aspergillus niger* (MTCC 1344), *Rhizopus oryzae* (MTCC 262), *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). These cultures were procured from microbial type culture collection centre (MTCC), IMTECH, Chandigarh. Cultures were maintained on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) medium for bacteria and fungi respectively.

**Determination of Antimicrobial activity**

The crude extracts of different mangrove plants leaves were used to determine the antimicrobial activity by agar cup plate method or agar well diffusion method described Murray et al. (1995) later modified by Olurinola (1996). The four organic solvents of all plants leaves were used in this method. Sterile nutrient agar and potato dextrose agar medium were prepared as per the composition and 0.2 ml of bacterial & fungal culture was spreader on each sterile petriplates. Approximately 6mm diameter wells were punched over the agar plates using sterile borer. The crude extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain specific concentration. The cups were filled with 100 µl of leaf extract at a concentration of 1500 µg/ml was allowed to diffuse over the medium for 30-45 min. The plates were incubated at 37°C for 24 hrs for bacteria. The above procedure was allowed for fungal assays except the media PDA instead of NA and incubated at 25°C for 48 hrs. The zone of clearance around each well after the incubation period, confirms the antimicrobial activity of each respective leaf extract. Similar procedure was followed to the remaining plant extracts to determine the anti microbial activity. Each experiment was carried out in triplicates. DMSO is used as control. The clear zones around the well were measured and average diameter of the inhibition zone was expressed in mm.
Results and Discussion

The mangrove plants leaves powder (1 kg) was obtained by mechanical grinding of dried leaves of seven plant species. The amount of crude leaf extract obtained by Soxhlet extraction using chloroform, petroleum ether, methanol and ethanol was summarized in Table-1.

The antimicrobial activity of the mangrove leaf crude extracts (chloroform, petroluim ether, methanol and ethanol) of Avicennia marina (Forsk), Excoecaria agallocha Linn, Lumintzera racemosa, Derris trifoliata, Brugiera gymnorrhiza, Ceriops decandra and Acanthus ilicifolius were assayed in vitro by agar well diffusion method against bacterial and fungal strains. The zone inhibition diameter (IZD) measured was summarized in Table-2.
Chloroform and methanolic extracts of E. agallocha showed antibacterial and antifungal activity with a zone of inhibition range of 14-18 mm. The chloroform extract of E. agallocha was active against S. epidermide, B. subtilis, A. niger, R. oryzae and C. albicans with a zone of inhibition diameter of 18 mm. Chloroform Extracts of A. marina showed high antimicrobial activity against C. albicans, A. niger and S. aureus with an inhibitory zone diameter of 18 mm. The extracts of L. racemosa were moderately active against test bacteria and fungi IZD with a range of 16 - 11 mm. Chloroform extracts of D. trifoliate were highly active against S. aureus and S. epidermides with a zone of inhibition of 22 mm. A. niger and S. cerevisiae were more sensitive to chloroform extract of D. trifoliate, exhibiting an IZD value of 20 mm. D. trifoliate other solvent extracts were moderately active against test bacteria and fungi. All the four solvent extracts of B. gymnorrhiza were active against S. aureus, E. coli, P. aeruginosa, A. niger and C. albicans with a zone of inhibition diameter of 20 mm. Gram negative bacteria was more sensitive to the crude extracts of B. gymnorrhiza. The solvent extracts of C. decandra were equipotent in their activity against the bacteria and fungi. The extracts are moderately active with IZD value in between 20 to 14 mm. A. illicifolius extracts were active against S. aureus, B. subtilis, P. aeruginosa, A. niger, R. oryzae, C. albicans and S. cerevisiae with zone of inhibition diameter of 18 mm. Fungal cultures were more sensitive to A. illicifolius extracts. Among the solvent extracts, Chloroform, Petroleum ether, Methanol and Ethanol extracts of D. trifoliate and B. gymnorrhiza were significantly active against the tested bacteria and fungi. The solvent extracts of C. decandra and A. illicifolius were moderately active, whereas A. marina, E. agallocha and L. racemosa extracts were less active. Further the extracts of D. trifoliate, B. gymnorrhiza, C. decandra and A. illicifolius can be explored for the study of phytochemicals present in them and to evaluate their biological activities.

Conclusion
The present study is conducted to develop new chemotherapeutic agents from the mangroves. The plant extracts showed antibacterial and antifungal activity with degrees of variation. Out of the seven plant extracts tested for biological activity, Derris trifoliata extracts exhibited higher inhibitory activity against bacteria and fungi. Further studies are needed to isolate pure compounds from these plant extracts and to establish the mode of action of the isolated compounds.

References
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