Antimicrobial Activities of Extracts of Some Species of Mangrove Plants and a New Compound Isolated Towards some Selected Strains

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ABSTRACT

The bio-materials of four marine mangrove medicinal plants viz., Aegiceras Corniculatum (AGC), Excoecaria agallocha (EA), Rhizophora mucronata (RM) and Xylocarpus granatum (XG) are extracted with methanol and hexane. These extracts are submitted to the antibacterial activity towards the strains: Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholerae adopting Agar-well diffusion method. It is found that a new Flavone Compound isolated from hexane extract of EA is effective towards Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholerae strains while RM MeOH extract is effective towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholerae strains while XG MeOH extract is effective towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholerae strains while AGC MeOH extract is found to be effective towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholerae strains. The order of effectiveness is found to be: EA Hexane > RM MeOH > XG MeOH > AG MeOH. Finally a new flavone compound is found to be more effective than the extracts.

Keywords: Mangrove plants, extracts, flavone compound, antimicrobial activity on different strains.
INTRODUCTION

The recent investigations are concentrating on the bio-screening of natural products have revived due to the paucity of safe antimicrobial drugs, anti-reverse transcriptase, anti-HIV and the perilous upsurge of new and re-emerging infectious diseases\(^1\)\(^2\)\(^3\). The antibiotics from natural sources are efficacious, biodegradable, less toxic and cost effective and therefore, it could supplement the costly synthesized antibiotic drugs\(^4\)\(^5\)\(^6\). Biopotentiality of mangrove vegetal makes them a reserved for the development of pharmaceuticals, fish and animal feed additives, agrichemicals and natural pigments\(^7\)\(^8\)\(^9\). The mangrove preparations used successfully in the hospitalization of infectious diseases and ailments are envisaged to possess antimicrobial potency\(^10\)\(^11\)\(^12\).

In the present investigation, the different biological parts of four mangrove species namely Aegiceras Corniculatum, Excoecaria agallocha, Rhizophora mucronata and Xylocarpus granatum have been extracted with different solvents like hexane and methanol. These extracts have been screened for antimicrobial activity towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniforms, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigellaflexneri, Sphingomonaspaucimobilis, Escherichia coli and Vibrio cholera and found to be results are encouraging hence they are presented comprehensively in this article.

MATERIALS and METHODS

Collection of Mangrove Medicinal plants

The different species of mangrove plants viz., Excoecaria agallocha and Xylocarpus granatum, were collected from corangi Mangrove forest near Bhiravapalem in Godavary Estuary (Latitude 16°15'N and Longitude 82°15'E) and further, Aegiceras Corniculatum and Rhizophora mucronata (Latitude 80°99'N and Longitude 76°87'E) were collected from Kollam mangrove forest near Krishnapatnam Port, Nellore.

Plant preparation and extraction

The fresh plants were washed under running tap water and dried in a warm room for 2 to 6 d. The samples were grinded into fine powder and extracted with n-hexane and methanol successively to get n-hexane and methanol extracts. Then, all the crude extracts were kept at -20°C until further use. The flavone compound getting By using column chromatography over a column of silica gel (Acme brand, 100-200 mesh, and 450 g) using solvents of increasing polarity from n-hexane through EtoAc. In all 200 Fractions (500 ml) were collected. The fractions displaying similar spots in TLC were combined and the residues from therein were subjected to re-chromatography over silica gel column to yield one pure compound Fig.I in the form of an off-white solid.

preparation of a sample

A sample of 100 mg from each extract and compound was dissolved in 1 mL DMSO. The extract and compound was then sterilized by filtration through sterile syringe filter (0.2 µm pore). Finally the filtered extract and compound was stored as aliquots until it was used.

Microbial strains

Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigellaflexneri, Sphingomonaspaucimobilis, Escherichia coli and Vibrio cholera.

Agar Ditch diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the extracts according to Qaralleh et al\(^14\)some modification. Briefly, inoculum containing 120°(15 lb/in2) was spread on Nutrient agar Medium with the respective bacterial strains of bacteria and medium potato dextrose agar for fungus strains. Testing sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (1 or 1.5 mg),
standard antibiotics (30 µg of chloramphenicol or 100 µg of amphotericin B) or negative control (DMSO) were laid down on the coverage of inoculated agar plate. The plates were incubated at 37 ±2°C for 24 h for the bacteria and at room temperature 28±2°C for 12 h for yeasts strains. Each sample was tested in duplicate and the zone of inhibition was measured as 50 micro liters diameter.

Screening for Antimicrobial Activity

The antimicrobial activity was carried out by the employing 24h young cultures with the given compounds by using Agar-well diffusion method. The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 20 ml of the medium (Nutrient Agar Medium) with the respective bacterial strains of bacteria and medium (Potato Dextrose Agar) for Fungal strains were transferred aseptically into each sterilized petri Plate. The plates were left at room temperature for solidification. Each plate is made 5 wells with equal distance with 6mm sterile borer. The test compounds were freshly reconstituted with suitable solvents (DMSO) and tested at various concentrations. The samples and the control along with standard (Ciprofloxacin) were places in 6-mm diameter well. In Antimicrobial assays plates were incubated at 28±2°C for fungi about 24h and 37±2°C for bacteria 12h. Standard with 5µg/ml used as a positive control for antibacterial activity. Activity diameter of the zone of inhibition was measured using Himedia antibiotic zone scale. Observations and results were represented in Table 2.

RESULTS and DISCUSSION

The Agar well diffusion method which belongs to Gram positive & Gram negative Bacteria’s of different plant extracts and flavone compound towards different strains have been presented in Table 2. The following observations are significant: of all the extracts and compound tested, AGC, EA, RM, XG have shown some remarkable antimicrobial behaviour.

With AGC extract, the antimicrobial activity for strains

*Bacillus puvvilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.*

The Gram Positive Bacteria’s are *Bacillus subtilis* with the values 12 and 10 respectively, *Bacillus coagulans* with the values 13, 11 and 10 respectively. And *Staphylococcus aureus* with the value 7 respectively. These strains have no activity against the Gram Negative Bacteria’s.

With EA flavone compound, the antimicrobial activity for strains

*Bacillus puvvilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.*

The Gram Positive Bacteria’s

Table 1: Abbreviation of Mangrove Medicinal Plant Extracts & Compound

<table>
<thead>
<tr>
<th>Name of the Plant Species</th>
<th>Parts used</th>
<th>Extraction of solvent</th>
<th>Abbreviation</th>
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<tr>
<td>AegicerasCorniculatum</td>
<td>Fruits</td>
<td>Methanol</td>
<td>AGC</td>
</tr>
<tr>
<td>Excoecariaagallocha (compound)</td>
<td>Roots</td>
<td>Hexane</td>
<td>EA</td>
</tr>
<tr>
<td>Rhizophoramucronata</td>
<td>Fruits</td>
<td>Methanol</td>
<td>RM</td>
</tr>
<tr>
<td>XylocarpusGranatum</td>
<td>Roots</td>
<td>Methanol</td>
<td>XG</td>
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Table 2: Results of Antimicrobial Assay mangrove medicinal plants

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<th>250mg/ml</th>
<th>100mg/ml</th>
<th>Standard</th>
<th>Control</th>
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Diameter of the well = 0.6mm
Volume of the Compound = 50 Micro liters.
With XG extract, the antimicrobial activity for strains

*Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.* The Gram Positive Bacteria's are *Bacillus puvuilis & Corynebacterium diphtheria* these strains are no activity. And *Bacillus subtilis* with the values 12 and 10 respectively, *Bacillus coagulans* with the values 15 and 11 respectively, *Staphylococcus aureus* with the values 14, 13 and 12 etc. And *Bacillus licheniformis* with the values 11 and 10 respectively. The Gram Negative Bacteria's are *Escherichia coli* with the values 12 and 11 respectively. Remaining in all Negative Strains has no activity.

Finally the order of effectiveness is found to be: EA Hexane > RM MeOH > XG MeOH > AG MeOH. Finally a new flavone compound is found that more effective than the extracts.

The order of Activity is: EA Hexane (4) > RM MeOH (1) > XG MeOH (2) > AG MeOH (3).

**CONCLUSION**

The extracts and new flavone compound of parts of different species of mangrove plants have been tested for their antimicrobial activity towards the strains *Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.* The Gram Positive Bacteria's are *Bacillus puvuilis & Bacillus coagulans* with the values 12 & 15, 13, 11 respectively. These strains have no activity was found against *Bacillus subtilis, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.*

The Gram Negative Bacteria's *Klebsiella pneumonia* with the values 20, 13 and 10 respectively. And *Pseudomonas aeruginosa* with the value 15 respectively, *Shigella flexneri* with the values 16 and 12 respectively, *Sphingomonas paucimobilis* with the values 19, 13 and 11 respectively, *Escherichia coli* with the values 16 and 12 respectively. And *Vibrio cholera* with the values 19, 13 and 10 respectively.

With RM extract, the antimicrobial activity for strains

*Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.* The Gram Positive Bacteria's are *Bacillus puvuilis & Bacillus coagulans* with the values 19, 18 and 17 respectively. These strains are no activity was found against *Bacillus subtilis and Bacillus coagulans, Staphylococcus aureus* with the values 13, 12 and 11 respectively. *Bacillus licheniformis* with the values 14, 12 and 11 respectively. *Corynebacterium diphtheria* with the values 11 and 10 respectively. The Gram Positive Bacteria's are *Bacillus subtilis and Bacillus coagulans* with the values 11 and 10 respectively. The Gram Negative Bacteria's are *Escherichia coli* with the values 12 and 11 respectively. Remaining in all Negative Strains has no activity.

Finally the order of effectiveness is found to be: EA Hexane > RM MeOH > XG MeOH > AG MeOH. Finally a new flavone compound is found that more effective than the extracts.

The order of Activity is: EA Hexane (4) > RM MeOH (1) > XG MeOH (2) > AG MeOH (3).

**CONCLUSION**

The extracts and new flavone compound of parts of different species of mangrove plants have been tested for their antimicrobial activity towards the strains *Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera.* The Gram Positive Bacteria's are *Bacillus puvuilis & Bacillus coagulans* with the values 12 & 15, 13, 11 respectively. These strains have no activity. And *Bacillus subtilis* with the values 12 and 10 respectively, *Bacillus coagulans* with the values 15 and 11 respectively, *Staphylococcus aureus* with the values 14, 13 and 12 etc. And *Bacillus licheniformis* with the values 11 and 10 respectively. The Gram Negative Bacteria's are *Escherichia coli* with the values 12 and 11 respectively. Remaining in all Negative Strains has no activity.

The order of Activity is: EA Hexane (4) > RM MeOH (1) > XG MeOH (2) > AG MeOH (3).
aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera. The XG MeOH extract is found to be effective towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Sphingomonas paucimobilis, Escherichia coli and Vibrio cholera strains while AGC MeOH extract is found to be effective towards the strains Bacillus puvuilis, Bacillus subtilis, Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis, Corynebacterium diphtheria, Klebsiella pneumonia, Pseudomonas aeruginosa.

The order of effectiveness is found to be: EA Hexane > RM MeOH > XG MeOH > AG MeOH. Finally a new flavone compound is found that more effective than the extracts.

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