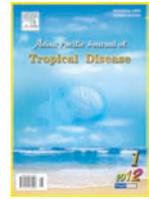


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In vitro antimicrobial activity of plant extracts of *Avicennia alba* against some important pathogens

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ABSTRACT

Objective: In this present study antimicrobial activity of aerial parts of *Avicennia alba* were evaluated against the resistant pathogens belong to aquatic, human and plant origin. **Methods:** Soxhlet extraction method was used to get the corresponding extracts of hexane, chloroform and methanol. The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria and fungi investigated using agar well diffusion technique. The length of inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone. Methanol and chloroform extracts exhibited promising antimicrobial activity than hexane extracts. **Results:** The zone of inhibition of chloroform varies from (9 to 17 mm) where as with methanol (11 to 28 mm) at 100 mg/ml concentration. Among all microorganisms studied *Erwinia carotovora* and *Pseudomonas syringae* showed the considerable growth inhibition with chloroform and methanolic extracts. **Conclusions:** *A. alba* can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms. Further studies are being carried out in order to separate the individual components that are present in plant extracts of *A. alba* using column chromatography.

1. Introduction

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Medicinal plants represent a rich source of antimicrobial agents [1–20]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines [21]. Mangrove *Avicennia alba* Linn. Classified in the plant family Avicenniaceae This plant is mainly found in the salty regions [22] it is native and common throughout much of India, Burma and Malacca and dry areas of Ceylon and is often grown in Southern Asia to Southeast Asia, Australia and Oceania. *A. alba* bark and seeds are used as a fish poison and resin used in birth control. It provides a rich source of steroids, triterpenes,

saponins, flavonoids, alkaloids and tannins. Recently three new naphthoquinones and their analogues, named avicequinone–A (1), –B (2), –C (3), and avicenol–A(4), –B (5), –C (6), respectively, were isolated from the stem bark of *A. alba* [23]. The present study was to screen the antimicrobial activities of *A. alba* and search for new compounds from mangrove plants against the resistant pathogens belong to aquatic, human and plant origin

2. Materials and methods

2.1 Plant material and extraction

Avicennia alba was taxonomically identified and the Voucher specimen is stored in the department of botany, Andhra University, Visakhapatnam, INDIA. The aerial plant parts were collected from Coringa Mangrove Wetland, Kakinada, 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

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successive soxhlet extraction with organic solvents with increasing order of polarity i.e. Hexane, Chloroform and Methanol respectively.

2.2 Test microorganisms

Microbial strains of clinical, plant and aquatic origin i.e. *Aeromonas hydrophyla* (MTCC 646), *Alternaria alternate* (MTCC 1362), *Ustilago maydis* (MTCC 1474), *Asperigellus niger* (MTCC 2723), *Acremonium strictum* (MTCC 3072), *Pencillium expansum* (MTCC 2006), *Fusarium oxysporum* (MTCC 1755), *Xanthomonas compestries*(MTCC 2286), *Erwinia caratovara* (MTCC 3609), *Lactobacillus acidophilus* (MTCC 447), *Pseudomonas marginalis* (MTCC), *Pseudomonas syringae* (MTCC 1604), *Pseudomonas aeruginosa* (MTCC 1688), *Streptococcus mutans* (MTCC 890), *Streptococcus salivarius* (MTCC 1938) and *Staphylococcus aureus* (MTCC 96) including both fungi and bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Active cultures were generated by inoculating a loopful of culture in separate 100 mL nutrient/potato dextrose 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 5×10^5 cfu/mL.

2.3 Determination of antimicrobial activity

The crude extracts of the different plant parts of different species were subjected to antimicrobial assay using the agar well diffusion method of [24] modified by [25]. 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 50 μ l 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 $^{\circ}$ 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 $^{\circ}$ C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

3. Results

Table 1: Antimicrobial activity of chloroform and methanol extracts *A. alba*.

In the present study, chloroform and methanol extract exhibited different degree of growth inhibition against tested bacterial and fungal strains. According to Table 1, methanolic extracts of *A. alba* exhibited considerable antimicrobial activity against tested microbial strains. (Table 1) summarizes the antimicrobial activities zone of inhibition of chloroform varies from (9 to 17 mm) where as with methanol (11 to 28 mm) at 100 mg/ml concentration. 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. Among all microorganisms studied *Erwinia caratovara* and *Pseudomonas syringae* showed the considerable growth inhibition with chloroform and methanolic extracts.

Table 1

Antimicrobial activity of chloroform and methanol extracts *A. alba*.

| Pathogen name | Extract concentration 100 mg/ ml DSMO | |
|----------------------------------|---------------------------------------|----------|
| | Chloroform | Methanol |
| <i>Aeromonas hydrophyla</i> | 16 | 20 |
| <i>Alternaria alternate</i> | 13 | 14 |
| <i>Asperigellus niger</i> | 11 | 12 |
| <i>Acremonium strictum</i> | 9 | 11 |
| <i>Erwinia caratovara</i> | 17 | 25 |
| <i>Fusarium oxysporum</i> | 14 | 21 |
| <i>Lactobacillus acidophilus</i> | 10 | 12 |
| <i>Pencillium expansum</i> | 10 | 12 |
| <i>Pseudomonas marginalis</i> | 12 | 13 |
| <i>Pseudomonas syringae</i> | 16 | 28 |
| <i>Pseudomona aeruginosa</i> | 12 | 14 |
| <i>Streptococcus mutans</i> | 10 | 11 |
| <i>Streptococcus salivarius</i> | 13 | 15 |
| <i>Stephylococcus aureus</i> | 10 | 11 |
| <i>Ustilago maydis</i> | 13 | 15 |
| <i>Xanthomonas compestries</i> | 14 | 16 |

Volume per well: 50 μ l, Borer size used: 6mm, Zone of inhibition in mm

4. Discussion

Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Plants are employed as important source of medication in many traditional medications [26]. Continued further exploration of plant-derived antimicrobials is needed today. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytochemicals present in the plants [27–41]. The results of the present study clearly showed that mangrove plant *A. alba* extracts showed antimicrobial activity against tested pathogenic strains including antibiotic resistant strains. *A. alba* extract is harmless and nonphytotoxic; it has been proved that extracts inhibitory effects on germination and on the viability of fungal spores as well. I showed moderate activity against *A. niger* as it is a saprophyte in soil causes black mould of onion, garlic and shallot; stem rot of *Dracaena*; root stalk rot of *Sansevieria*; and boll rot of cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune. The effectiveness of the active compounds present in plant extracts cause the production of growth inhibition zones that appear as clear are as surrounding the wells. However, plant extract was unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation [42] or the concentration of the compound used may not be sufficient. Lowest activity was observed against *A. strictum* with Chloroform and *A. strictum*, *S. mutans* and *S. salivarius* with methanolic extracts respectively. No inhibition was observed with controls, which proves that solvents could not act as antibacterial agents. In almost all tests, crude methanolic extracts showed better inhibition against all tested bacterial and fungal strains, indicating that active ingredients in plant materials could be extracted into methanol. However, highest antibacterial activity was observed due the presence of secondary metabolites such as alkaloids, flavonoids and steroids against *P. syringae* (28 mm) which is a rod shaped, Gram-negative bacterium with polar flagella. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques. Further studies are being carried out in order to separate the individual components that are present in plant extracts of *A. alba* using column chromatography to develop Biopesticide which is alternative to synthetic agents.

Conflict of interest statement

We declare that we have no conflict of interest

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