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RESEARCH PAPER

Study on antibacterial activity of root extract from mangrove plants

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ABSTRACT

The antibacterial activity of the roots of mangrove plants, *Avicennia marina, Bruguiera cylindrica, Exoecaria agallocha* and *Salavadora persica* was evaluated against Gram positive and Gram negative pathogenic bacteria including Gram positive nonpathogenic spore bearer *Bacillus* species. Soxhlet extracts of petroleum ether, methanol, chloroform and water were prepared and assessed for the antibacterial activity using agar diffusion method. All of the plant extracts showed promising antibacterial activity against Gram positive pathogenic bacteria and spore bearer non pathogenic *Bacillus* species too. All methanol extracts showed inhibition against Gram positive bacteria while some extracts of petroleum ether gave inhibition against gram positive organisms except *Bruguiera cylindrica* and *Salavadora persica.* However, only the chloroform root extract of *E. agallocha* exhibited antibacterial activity against both Gram negative and gram positive bacteria. None of the aqueous extracts exhibited inhibition against any of the bacterium tested. The root extract of *Exoecaria agallocha* in chloroform was characterized using TLC which revealed the presence of alkaloids and saponins.

Key Words : Antibacterial activity, Zone of inhibition, Mangroves, Soxhlet extraction

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Mangroves are the characteristic intertidal plants distributed in tropical and subtropical coastlines which create a unique ecological environment that is composed of rich assemblages of species being a valuable source of useful metabolites for medicinal usage (Chapman, 1976; Lin, 1984; Tomlinson, 1986). Mangroves produce from their trunks aerial roots that become embedded in the mud and form a tangled network, this serves both as a prop for the tree and as a means of aerating the root system. Such roots also form a base for the deposit of silt and other material carried by the tides, and thus land is built up which is gradually invaded by other vegetation. The mangrove forests also can protect inland coastal areas by absorbing the effects of storm and some tsunami waves. Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins (Agoramoorthy *et al*., 2007; Bandaranayake, 1995, 2002).

Extracts from *Rhizophora* and *Avicennia species* have been used for making tonics, wines and fruit drinks. The leaves, fruits and seeds of *Avicennia marina* have been used as vegetables. Bark of red mangrove trees have been used in folk remedy for a wide array of diseases (Duke and Wain, 1981; Morton, 1981). Headaches, snakebites, wounds, boils and many more conditions are traditionally treated with mangrove plants *e.g. A. illicifolius*. Skin disorders and sores, including leprosy, may be treated with ashes or bark infusions of certain species e.g. *Lumnitzera racemosa.* Root, leaf and stem extracts of Rhizophora trees have inhibitory properties, affecting the growth of various human pathogenic organisms. Among these are bacteria, fungi and viruses (Hernandez *et al.,* 1978). They have also antihelmintic, antifeedant, molluscicidal, and pesticidal properties (Kokpol *et al*., 1984; Chandrasekaran *et al*., 2009). Extracts from *Avicennia marina*

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are believed to be able to cure rheumatism, smallpox and ulcers. Extracts from *Rhizophora mucronata* are believed to help remedy or cure diarrhoea, elephantiasis, haematoma and hepatitis (Bandaranayke, 1998). A physician in Cali, Colombia, reported to cure throat cancer, with gargles of mangrove bark (Garcia-Barriga, 1975). Premanathan *et al.* (1999) reported that a polysaccharide extracted from the leaf of *Rhizophora apiculata* (designated as RAP) inhibited HIV-1 or HIV-2 or SIV strains in various cell cultures and assay systems. Alarcon-Aguilara *et al*. (1998) reported that extracts of *Rhizophora mangle* had anti-diabetic and anti-hyperglycemic property. Roots of *Derris trifoliata,* known colloquially as "dynamite" because of its extensive use as fish poison, has also yielded rotenone, used as an insecticide (Molyneux, 1972).

The rise of antibiotic resistant microorganisms is one of the severe problems in health care systems of the world (Nascimento *et al*., 2000). Therefore, new drugs have to be found, in order to combat such diseases and it is essential to find new compounds that have antimicrobial properties. Concerning the above facts, it is worthwhile to screen plant species which have the above properties to synthesize new drugs. The antibacterial activity of the leaves and bark of mangrove plants was evaluated against antibiotic resistant pathogenic bacteria, *Staphylococcus aureus* and *Proteus* species (Abeysinghe *et al.,* 2010).

The aim of this study was to evaluate the antibacterial activity of root extracts of medicinal mangrove plants using different solvents: chloroform, methanol, petroleum ether and sterilized water in order to get maximum compounds from the mangrove roots and screening them *in vitro* for antibacterial activity.

RESEARCH METHODOLOGY

Plant materials and bacterial strains:

Fresh roots of *Avicennia marina, Bruguiera cylindrica, Exoecaria agallocha* and *Salavadora persica* were collected from the Western bank of the Thane Creek, which is the single largest mangrove belt in Mumbai (A substantial tract of mangrove land is adjoining the Godrej and Boyce township, Pirojshanagar, in Vikhroli a suburb of Mumbai).

Laboratory Gram positive pathogenic cultures like *Staphylococcus aureus*, *Streptococcus pyogenes, Corynebacterium diphtheriae* and Gram negative cultures like *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used as test bacterial species. In addition non pathogenic gram positive spore bearer *Bacillus cereus* was also used. All the bacterial cultures were maintained in Brain Heart infusion agar slants.

Extraction by Soxhlet extractor:

Roots of plant materials were washed, air dried and used immediately for extraction. Ten grams of the roots of each plant materials *of Avicennia marina, Bruguiera cylindrica, Exoecaria agallocha* and *Salavadora persica* were separately crushed to a powder form using sterilized mortar and pestle. These crushed materials were extracted sequentially into 100 ml of petroleum ether, chloroform, methanol and sterilized distilled water. Resulting extracts in different solvents were tested for antibacterial activity against bacterial strains by agar diffusion technique.

Testing of antimicrobial activity for plant extracts:

Antibacterial activity was determined by Agar cup method. A loopful of the given test strain was inoculated in 10 ml of Brain Heart infusion broth and incubated at 37^0 C for 24 hours in order to activate the bacterial strain activity. Sterile 20 ml of Luria Bertani agar was melted and cooled and 0.2 ml test strain (0.1 O.D. at 530nm) was seeded and poured into a 9cm diameter Petri plate. After solidification of the medium agar cups were punched in the plate with the help of a sterile 8mm cup borer. The cups were then filled with 70ml of different concentration of the test sample solution. The plates were incubated at 37^0C for 24 hours. The inhibitory zone formed by these compounds against the particular test bacterial strain determined the antibacterial activity of the root extracts of different mangrove plants (zone size measured in mm). The mean value obtained for three individual replicates was used to calculate the zone size of inhibition of each plate. Controls were run (for each bacterial strain and solvent) where pure solvent was added into the cup.

Separation of active components of extracts by thin layer chromatography (TLC):

Alumina coated silica gel size (5cm x 10cm) plates were air dried at room temperature for one hour and dried at 80^0C in an oven for 30 min. Extract of roots *of Exoecaria agallocha* in chloroform was separated using thin layer chromatography (TLC). A small amount of each extract (10 µl) was spotted at the bottom of the plate. Then plates were placed in a 50 ml beaker containing a combination of toluene, ethyl acetate and diethylamine (7:2:1) and ethyl acetate, methanol, water and ammonia (6.5:2.5:0.8:0.2) were used as mobile phase. Beakers were covered with watch glasses. When the mobile phase reached the top of the plate, plates were removed from the developing chamber and dried at room temperature. Plates were developed using Dragendorff's reagent and anisaldehyde sulphuric acid spray (Bandaranayke, 1998) and were visualized under ultra violet light. HPTLC of the chloroform extract from roots of *Exoecaria agallocha* was performed by Anchrome enterprises, Mulund (E), Mumbai.

RESEARCH AND REMONSTRATION FINDINGS

Root extracts of *Avicennia marina, Bruguiera*

cylindrica, Exoecaria agallocha and Salavadora persica in petroleum ether, chloroform, methanol and sterile distilled water were used to test the growth of *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium diphtheriae* and Gram negative cultures like *Escherichia coli, Proteus vulgaris, Pseudomonas aerugeniosa* and *Klebsiella pneumoniae*. In addition non pathogenic Gram positive spore bearer *Bacillus cereus* was also tested. Antibacterial assays for plant extracts of mangrove plants and the controls were also carried out. Four different extracts (petroleum ether, chloroform, methanol and sterilized distilled water) exhibited different degree of growth inhibition against bacterial strains.

Only chloroform extract of *Exoecaria agallocha* inhibited and showed the highest antibacterial activity against Gram positive, Gram negative pathogenic bacteria and the non pathogenic spore bearing *Bacillus cereus* (Table 1). Root extracts of *Avicennia marina* and *Exoecaria agallocha* in methanol, petroleum ether and chloroform exhibited inhibition for gram positive pathogenic bacteria, while only methanol and chloroform extracts of *Bruguiera cylindrica* and methanol extract *Salavadora persica* exhibited inhibition for Gram positive bacteria (Table 1). None of the water extracts exhibited inhibition zones against both Gram positive and Gram negative bacteria.

It has been recorded that, a number of mangrove plant extracts in methanol, ethyl acetate, ethanol and aqueous showed antibacterial activity against pathogenic isolates (Chandrasekaran *et al*., 2009). Abeysinghe (2010) showed almost all mangrove extracts of leaves and barks in ethyl acetate showed the highest inhibition towards antibiotic resistant *S. aureus* than *Proteus* species compared to the extracts obtained with petroleum ether, chloroform, ethanol and water. In present studies chloroform extracts of all mangrove plants showed highest inhibition against Gram positive pathogens including non pathogenic *Bacillus cereus* which is a spore bearer and also heat resistant. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components of the outer layer of Gram-negative bacteria. So the outer lipopolysaccharides layer may hinder access of antibacterial compounds to the peptidoglycan layer of the cell wall. This may be the reason that Gram negative bacteria were resistant to most of the extracts. Water extract may contain a low concentration of antibacterial compounds or may not extract antibacterial compounds. All antibacterial compounds may have been extracted by other solvents during sequential Soxhlet extraction by methanol, petroleum ether and chloroform. No inhibitions were observed for controls.

Amongst all the mangrove plants tested, root extract of *E. agallocha* in chloroform exhibited maximum antibacterial activity. Therefore, the root extract of the above mangrove plant species in chloroform can be used as a source which could yield drugs to improve the treatment of infection caused by these bacteria *e.g*. wound infections, skin infections, ear and eye infections, urinary tract infections, sore throat and diphtheria. Reports are available on *E. agallocha* which has been used to treat haematuria, conjunctivitis, dermatitis, leprosy, toothache and also as an herbicide and purgative (Bandaranayke, 1998).

When TLC of the chloroform extract of *Excoecaria agallocha* root sample was carried out, it showed the presence of brown spots after derivatisation with Dragendroff's reagent signifying the presence of alkaloids while appearance of blue spots after derivatisation with anisaldehyde sulfuric acid signified presence of saponins. The HPTLC of the same was performed and were further analyzed to determine the type of alkaloids and saponins present in the extract. Several peaks were obtained on performing the analysis. One of the peaks obtained was at R_f 0.67 which was close to 0.64, the value obtained from the literature (Conine and Paul, 1972). On comparing the results obtained with the standard R_f values from the literature it could be speculated that the alkaloids

Table 1: Antibacterial activity of root extracts of different mangrove plants in 3 different solvents

Name of Mangrove spps	*Avicennia marina*			*Exocecaria agallocha*			*Bruguiera cylindrica*			*Salavadora persica*		
	Inhibition zone size in mm			Inhibition zone size in mm			Inhibition zone size in mm			Inhibition zone size in mm		
Solvents	ME	PE	CH	ME	PE	CH	ME	PE	CH	ME	PE	CH
Bacterial strains												
Bacillus cereus	14	13	15	13	13	18	17	--	10	12	--	--
C.d.	14	13	14	17	15	18	16	--	10	11	--	--
Strepto. pyo.	11	11	11	14	11	16	13	--	10	10	--	--
S. aureus	14	14	15	11	12	18	13	--	10	10	--	--
Pseudo.	--	--	--	--	--	14	--	--	--	--	--	--
E.coli	--	--	--	--	--	12	--	--	--	--	--	--
K.p.	--	--	--	--	--	10	--	--	--	--	--	--
P.v.	--	--	--	--	--	12	--	--	--	--	--	--

Key -- = no zone of inhibition obtained

ME- Methonal; PE- Petroleum ether; CH- Chloroform;*C.d. - Corynebacterium diphtheria*e; *Strepto.pyo - Streptococcus pyogenes; S.aureus - Staphylococcus aureus*; *Pseudo - Pseudomonas aerugeniosa; E.coli - Escherichia coli; K.p. - Klebsiella pneumoniae; P.v. - Proteus vulgaris*

present may be caffeine. It was reported that leaves, bark and stem extracts of *Excoecaria agallocha* contain triterpenes, saponins, tannins, alkaloids and flavonoids (Bandaranayke, 1998). These phytochemicals are present in other plants as well (Barnabas and Nagarajan, 1988). Mangroves contain these secondary metabolites which may show antibacterial activity. Therefore, the beneficial medicinal effects of mangrove plant may result from the combinations of secondary products present in the plant. Secondary products play a role in a plant's defense through cytotoxicity towards microbial pathogens. This could prove the usefulness of these as antimicrobial medicines for humans.

Conclusion:

The mangroves have always been considered to be an important facet of the environment. Present study has helped to emphasize the medicinal value of the root extracts, giving mangroves a new unexplored dimension. Further studies could be focused upon the isolation, extraction and identification of the antibacterial compounds and their activity *in vivo,* which could help in discovery of new drugs.

REFERENCES

Abeysinghe, P.D., Vithanawasam, M., Pathirana, R.N. and Abeysinghe, S. (2000). Preliminary *in vitro* screening of some mangrove plant extracts for antibacterial compounds against clinical bacterial isolates from different sources. 1st Science Symposium proceeding, **1**:22-25.

Abeysinghe, P.D. (2010). Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. *Indian J. Pharm. Sci*., **72** : 167-172.

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V. and Hsu, M.J. (2007). Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your eye mangrove from India. *Braz. J. Microbiol*., **38**:739-742.

Alarcon-Aguilara, F.J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C.C. and Flores-Saenz, J.L. (1998). Study of the anti-hyperglycemic effect of plants used as antidiabetics. *J. Ethnopharmacol*., **61**:101-110.

Barnabas, C.G. and Nagarajan, S. (1988). Antibacterial activity of flavonoids of some medicinal plants. *Fitoterapia*, **3**:508-510.

Bandaranayake, W.M. (1995). Survey of mangrove plants from northern Australia for phytochemical constituents and uv-absorping compounds. *Curr. Topics Phytochem*., **14**:60-72.

Bandaranayake, W.M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology & Management*, **10**:421-452.

Chandrasekaran, M., Kannathasan, K., Venkatesalu, V. and Prabhakar, K. (2009). Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant *Staphylococcus aureus. World J. Microbiol Biotechnol*., **2**:155-160.

Chapman, V.J. (1976). Mangrove *vegetation*. Cramer, Vaduz.

Conine, F. and Pau, I.J. (1972). A Thin-Layer Chromatographic Procedure for the Separation of Aspirin, Cocaine, Caffeine, Codeine, Heroin, 6-Monoacetyl Morphine and Morphine. *Mikrochimica Acta., [Wien]* 443-448.

Duke, J.A. and Wain, K.K. (1981). *Medicinal plants of the world*. Computer index with more than 85,000 entries. 3 vols.

Garcia-Barriga, H. (1975). Flora medicinal de Colombia. Botanica Medica. *Talleres Editoriales de la Imprenta Nacional*. BOGOTA.

Hernandez, Rojas N.M. and Perez, Coto O. (1978). Antimicrobial properties of extracts from Rhizophora mangle L. *Rev. Cubana Med. Trop*., **30**:181-187.

Kokpol, U., Chittawong, V. and Mills, H.D. (1984). Chemical constituents of the roots of *Acanthus illicifolius*. *J. Nat. Prod*., **49**:355-356.

Lin, P. (1984). Mangrove Vegetation. Beijing, China: Ocean Press.

Molyneux, F. (1972). Derris - a natural pesticide. *Australian Chemical Processing and Engineering,* 25: 9-12

Morton, J.F. (1981). Atlas of medicinal plants of middle America. Bahamas to Yucatan. C.C. Thomas, Springfield, IL.

Nascimento, GGF. Locatelli, J., Freitas, P C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbiol*., **31** : 247-256.

Premanathan, M., Arakaki, R.,Izumi, H., Kathiresan, K., Nakano, M., Yamamoto, N. and Nakashima, H. (1999). *Antiviral properties of a mangrove plant, Rhizophora apiculata blume, against human immunodeficiency virus. Antiviral Res*., **44**:113-122.

Tomlinson, P.B. (1986). *The Botany of Mangroves*. New York: Cambridge University Press. 433.

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