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RESEARCH ARTICLE

SCREENING OF THE ANTIBACTERIAL PROPERTIES OF AVICENNIA MARINA FROM PICHAVARAM MANGROVE

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ABSTRACT

Avicennia marina were extracted from different solvents like Acetone, methanol, diethylether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts against 12 bacterial strains. The present study exhibit maximum antibacterial activity was recorded from chloroform extracts against *Enterococci* sp. and minimum activity was noted from ethyl acetate extract against *Klebsiella pneumoniae, Enterococci* sp., *Salmonella* sp. and *Shewanella* sp.

INTRODUCTION

The recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties (Dahanukar et al., 2000). Life and diseases go together where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through their uses as food, clothing and shelter, but also plants have been used to control diseases, therefore, the use of plants as medicines is an ancient and reliable practice (Arshad and Rao, 1998). Plants are serving several purposes whether health, nutrition, beauty or medicinal. With the development in techniques and recent researches, it has been proved that certain nutritive and non-nutritive chemicals in plants which are of very much importance to dietary and healing properties. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker *et al.*, 1995).

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The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity should be tested against appropriate microbial models to confirm the activity and to ascertain the parameters associated with it. Interest in large number of traditional natural products has been increasing (Taylor *et al.*, 1996) now a day. Mangroves have long been a source of astonishment for the layman and of interest for scientist. Mangroves are biochemically unique, producing a wide array of novel natural products. Substances in mangroves have long been used in folk medicine to treat diseases (Bandaranayake, 1998). Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds (Okeke, et al., 2001). They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins. Therefore, it is worth to screen mangrove plants for the presence of new antibacterial compounds to combat the pathogenic bacteria *A. marina* (Forssk.) is commonly known as gray mangrove tree classified in the plant family *Avicenniaceae*, and is commonly used for ulcers. So the main objectives of this study was to screen antibacterial activity of the organic solvent extracts

of *A.marina* mangrove plant species against pathogenic and antibiotic resistant bacterial strains and also to isolate and characterization of chemical components which are responsible for the claimed activity.

MATERIALS AND METHODS

Antimicrobial effect of the crude organic extract of *Avicennia maraina* leaves was studied in the Department of Plant Biology and Biotechnoogy, A.A. Government Arts College, Villupuram, Thiruvalluvar University, Tamilnadu, India, during the period from October to December 2009.

Plants and extraction

Plant material and extraction

A. marina is commonly known as gray mangrove and vernacular name is Tella mada belongs to the family Aviceniaceae, grow as a shrub or tree to a height of three to ten metres, or up to 14 meters in 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 aerial plant parts were collected from Pichavaram Mangrove Wetland, Tamilnadu, 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 organic solvents with increasing order of polarity i.e. acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water respectively.

Preparation of the discs

The agar disc diffusion techniques involved placing sterile paper discs (Whitman No. 1 filter paper) of 5 mm diameter impregnated with different crude extracts and dried in a hot air oven at 60°C on agar plates seeded with the test organism. Three types of discs were used for antimicrobial screening; sample discs, standard discs and blank discs. Then the sample disc was prepared by applying sample solution of the desired concentration on the sterile filter paper discs (5 mm in diameter) with the help of a micropipette in an aseptic condition. Similarly blank discs and other discs were prepared to serve as negative control and test sample respectively. In this investigation kanamycin (30 µg/disc) standard disc was used as reference and methanol was used as blank. These discs were left for few minutes in aseptic condition under UV light for complete sterilization.

Determination of antibacterial activity

The crude methanol extracts of the different mangrove species were subjected to antimicrobial assay using the agar well diffusion method of Murray *et al.,* (1995) modified by Olurinola, (1996). 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 forty five 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 sample.

Table 1: Dry extracts of mangrove *Avicennia marina* obtained from Pichavaram mangroves against bacterial pathogens Solvent used (Inhibition zone diameter in mm)

Sl. No	Bacterial pathogens	Aqueous	Acetone	Methanol	Ethyl acetate	Chloro -form	Ethanol	Diethyl ether	Petroleum ether	Control
1	*Klebsiella pneumoniae*	12	14	2	2	3	NS	NS	2	NS
2	*Escherichia coli*	2	7	NS	8	11	4	4	13	1
3	*Staphylococcus* sp	NS	15	23	16	4	2	12	NS	NS
4	*Enterococci* sp	7	9	3	2	29	3	2	NS	NS
5	*Proteus* sp	3	11	22	4	6	18	NS	NS	NS
6	*Streptococcus* sp.	5	6	NS	NS	4	NS	NS	3	1
7	*Pseudomonas aeruginosa*	NS	NS	4	NS	3	NS	NS	NS	NS
8	*Vibrio parahaemolyticus*	7	9	NS	NS	3	NS	NS	NS	NS
9	*Salmonella* sp.	NS	2	4	2	3	NS	10	9	1
10	*Shewanella* sp.	NS	NS	3	2	6	3	4	2	NS
11	*Vibrio flurialis*	2	3	2	5	4	3	NS	NS	NS
12	*Vibrio splendidus*	6	2	4	NS	NS	NS	2	NS	NS

Test microorganisms

The microorganisms obtained residues were kept in a freezer at-80°C until testing like antibacterial activity against some disease causing bacteria (*Klebsiella pneumoniae, Escherichia coli, Staphylococcus* sp, *Enterococci* sp *Proteus* sp, *Streptococcus* sp, *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp, *Shewanella* sp *Vibrio flurialis, Vibrio splendidus*).

RESULT

The antibacterial activity of acetone, methanol diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts of the *Avicennia marina* against the tested microorganisms was estimated and the basis of the presence or absence of inhibitory zones, in the results were exhibited in (Table 1). The aqueous extract showed no activity against *Pseudomonas aeruginosa, Staphylococcus* sp*., Salmonella* sp. and *Shewanella* sp. The trace activity was recorded against *Escherichia coli, Vibrio flurialis* and *Proteus* sp.

The minimum activity was noted against *Streptococcus* sp. (5 mm) followed by *Vibrio splendidus* (6 mm), *Enterococci* sp. (7 mm) and *Vibrio parahaemolyticus* (7 mm). The maximum activity was found against *Klebsiella pneumoniae* (12 mm). The acetone extract showed no activity against *Pseudomonas aeruginosa* and *Shewanella* sp. The trace activity was observed against *Salmonella* sp. (2 mm), *Vibrio splendidus* (2 mm) and *Streptococcus* sp. (3 mm), *Vibrio flurialis* (3 mm). The minimum activity was scrutinized against *E.coli*, (7 mm), *Enterococci* sp. (9mm), *Vibrio parahaemolyticus* (9mm) and *Proteus* sp. (11 mm). The maximum activity was observed against *Staphylococcus* sp. (15 mm).

The extracts by way of methanol observed no activity *E.coli, Streptococcus* sp. and *Vibrio parahaemolyticus.* The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Vibrio flurialis* (2 mm) and *Enterococci* sp. (3 mm), *Shewanella* sp. (3 mm). The minimum activity was recorded against *Pseudomonas aeruginosa* (4 mm), *Salmonella* sp. (4 mm), *Vibrio splendidus* (4 mm). The maximum activity was observed against *Staphylococcus* sp. (23 mm) and *Proteus* sp. *(*22 mm). Ethyl acetate extract showed no activity against *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio splendidus.* The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Enterococci* sp. (2 mm), *Salmonella* sp. (2 mm), *Shewanella* sp. (2 mm) and *Proteus* sp. *(*4 mm). The minimum activity was recorded against *Vibrio flurialis* (5mm) and *E.coli* (8 mm). The maximum activity was observed against *Staphylococcus* sp. (16 mm).

The chloroform extract showed no activity against *Vibrio splendidus.* The trace activity was observed against *E.coli*, *Pseudomonas aeruginosa, Vibrio parahaemolyticus* (3mm) and *Staphylococcus* sp., *Streptococcus* sp., *Vibrio Splendidus* (4 mm). The minimum activity was recorded against *Proteus* sp., *Shewanella* sp. (6 mm). The maximum activity was observed against *Enterococci* sp. (29 mm). The ethanol extract showed no activity against *Klebsiella pneumoniae, Streptococcus* sp. and *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Salmonella* sp. The trace activity was observed against *Staphylococcus* sp*.,* (2 mm) and *Enterococci* sp., *Shewanella* sp. and *Vibrio flurialis* (3 mm). The minimum activity was recorded against *E.coli* (4 mm). The maximum activity was observed against *Proteus* sp. (18 mm). The diethyl ether extract showed no activity against *Klebsiella pneumoniae, Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio flurialis*. The trace activity was observed against *Enterococci* sp*., Vibrio Splendidus,* (3mm) and *Staphylococcus* sp*.,* (2mm). The minimum activity was recorded against *Salmonella* sp. (10mm). The maximum activity was observed against *Staphylococcus* sp., (12mm). The petroleum ether extract showed no activity against *Staphylococcus* sp., *Enterococci* sp*., Proteus* sp*., Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio flurialis* and *Vibrio splendidus.* The trace activity was observed against *Klebsiella pneumoniae, Shewanella* sp. (2 mm) and *Streptococcus* sp.,(3 mm). The minimum activity was recorded against *Salmonella* sp. (9mm). The maximum activity was observed against *E.coli* (13mm).

DISCUSSION

The mangroves provide food and wide variety of traditional products and artifacts for the mangrove dwellers. Extracts and chemicals from mangroves are used mainly in folkloric medicine (e.g. bush medicine), as insecticides and pesticides and these practices continue to this day. However the extraction of novel natural chemical compounds from mangroves, in addition to those already known to the pharmacopoeia of the people is in its infancy. A knowledge of the biological activities and/or chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents, but because such information may be of value in disclosing new sources of already known biologically active compounds. *Xylocarpus granatum* belongs to the family Meliaceae includes many plants that are sources of valuable timber and many that have wide ranging uses in ethno medicine (Ambrozin *et al.*, 2006).

The family is distinguished by the occurrence of characteristic substances called limonoids (Ambrozin *et al.*, 2006). These substances have wide spectrum of biological activities, particularly insecticidal action (Ambrozin *et al.*, 2006). Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Okeke *et al*., 2001; Ebi and Ofoefule *et al*., 1997). *Xylocarpus granatum* also possess alkaloidal substances which also have biological activities (Chou *et al.,* 1977). In our study, some of the bacterial strains did not respond to crude extracts, whereas the fractions showed broad-spectrum activity against multiple strains. This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract or synergism by the presence of some compounds or factors in the extract. The variation of antibacterial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract.

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