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Screening of Antibacterial Activity of Mangrove Leaf Bioactive Compounds Against Antibiotic Resistant Clinical Isolates

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Abstract: Microorganisms have potential to cause human diseases. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, screening of antibacterial activity of mangrove plants is very important since vast number of mangrove plants have been used for centuries as remedies for human diseases. The present investigations mainly focused to screen the antibacterial activity of chloroform bioactive crude and column fractionated compounds from leaves of the plant *Exoecaria agallocha* against five species of antibiotic resistant pathogens *viz., Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Pseudomonas aeruginosa* and *Klebsiella* sp. using disc diffusion method. Results showed that crude chloroform extracts have maximum activity (10mm) against *Pseudomonas aeruginosa* and Column purified fraction 3rd showed maximum activity (12mm) against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa.* No activity was recorded for crude and all the fractions of mangrove leaf extract against the *klebsiella* sp. Extracts showing good antibacterial activity are undergoing further analysis to identify the active compounds.

Key words: Antibacterial activity · Bioactive compounds · Mangrove leaf and Clinical isolates

INTRODUCTION

Mangroves have been a source of several bioactive compounds. Mangrove plants have been used in folklore medicine and extracts from mangrove species have proven activity against human, animal and plant pathogens. Secondary metabolites like alkaloids phenolics, steroids and terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance [1, 2]. The earliest references to the uses of mangroves are date back to the year 1230 [3]. Reference was made to the use of *Rhizophora* seedlings as food in times of famine, to cure sore mouth, to produce fuel, tannin and dye and wine having an aphrodisiac effect when ingested and of their use as philters in Arabia. A fundamental function of all forests has been to supply timber for cooking, heating and constructing dwellings and mangrove forests are no exception [4, 5].

Microorganisms have potential to cause human diseases. Most of the time viruses, bacteria and fungi act as major pathogenic organisms. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Therefore, screening of antibacterial activity of medicinal plants is very important since vast number of medicinal plants have been used for centuries as remedies for human diseases. Among them extracts from different parts of mangroves and mangrove associates are widely used throughout the world. For instance, stem of *Avicennia marina* is used for ulcers and bark of *Bruguiera sexangula* is used for antitumors [6]. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds [6]. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins [7]. Scanty literature is available on the antibacterial activity of mangroves. However, studies of other biological activities in general are available. The study of Premnathan [8, 9] revealed that

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the mangroves were found highly effective for antiviral activity as compared to seaweeds and sea grasses. Kokpal [2] had also reported the bioactive compounds from mangrove plants. Some mangroves had shown insecticidal activity [10, 11]. Wu [12] reported the cytotoxic and antiplatelet aggregation activity of methanol extract of *Aglaia elliptifolia*.

Many bioactive and pharmacologically active substances have been isolated from algae. For instance, extracts of marine algae were reported to exhibit antibacterial activity [13-15]. Many authors had found antibacterial activities of microalgae due to fatty acids [16-19]. Changyi [20] opined that the fatty acids (PUFA) in litter fall of mangroves might have positive role on the growth of fishes and shrimps. The objective of present investigation was mainly focused on to screen the antibacterial activity of mangrove plant *Exoecaria agallocha* bioactive compounds against antibiotic resistant bacterial pathogens.

MATERIALS AND METHODS

Sample Collection: Fresh elder leaves from mangrove specie *E. agallocha* was collected from Pichavaram Mangrove forest (Lat. 11° 27' N, Long. 79° 47' E) Southeast Coast of India and washed thrice in tap water to remove the adhering soil particles and salts and once with sterile distilled water.

Extraction of Bioactive Compounds: The chopped air-dried leaves of *E. agallocha* (1kg) were taken separately in an air tight glass jars and required quantity of ethanol and water mixture (3:1 ratio) was added and kept under dark (percolation method). After 7 days, the contents were stirred well and then filtered by using muslin cloth. The plant extracts were concentrated to two third of the volume by distillation. The colloidal form of the plant extract from *E. agallocha* (207g) was stored in a sterile glass container for further use. The percentage of extraction was calculated using the following formula:

$$\text{Percentage of extraction (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

Column Chromatography Fractionation of Bioactive Compounds: The colloidal form of extract obtained through percolation were separately suspended in water and defatted with diethyl ether [21]. Individual aqueous layer of extracts were subjected to column chromatography packed with 500g of silica gel (230-400 mesh) (MERCK) with the maximum height of 50cm and eluted successively with 30ml of chloroform. MERCK AR grade solvent was used throughout the study. The obtained fractions were labeled and stored at -80°C (SANYO-JAPAN) for further use.

Antibacterial Assay [22]: Disc diffusion method was used for screening the medicinal plant extract against chosen antibiotic resistant bacterial pathogens *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella* sp., *Streptococcus pneumoniae* and *Pseudomonas aeruginosa.* Whatmann No. 1 filter paper disc (5mm diameter) impregnated with different fractions ($mg.disc^{-1}$) were placed on Muller Hinton Agar (HIMEDIA, MUMBAI) which was previously inoculated with test organisms. Control disc was maintained without the fractions. All the plates were incubated overnight at 37°C under static conditions. After 24 hrs, the zone of inhibition appearing around the discs were measured and recorded in millimeter in diameter. Triplicate samples were maintained for each bacterial strain.

RESULTS

The percentage of bioactive compound extraction from the *E. agallocha* is 20.7%. The six chloroform fractions of *E. agallocha* leaf extracts were collected and tested against the antibiotic resistant pathogens.

The crude chloroform extracts from *E. agallocha* leaf was showed maximum activity (10mm) against *Pseudomonas aeruginosa* bacterial strains. *Staphylococus epidermidis* showed (9mm) and minimum activity (7mm) was recorded in two bacterial strains *Staphyloccus aureus* and *Streptococcus pneumoniae*. No activity was observed in *klebsiella* sp. (Table 1).

The 3rd fraction of chloroform from *E. agallocha* leaf extracts showed maximum activity against four (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Pseudomonas aeruginosa*) of the five antibiotic resistant pathogens. This fraction showed maximum activity (12mm) against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa.* This fraction also showed activity (9mm and 7mm) against *Staphylococcus aureus* and *Streptococcus pneumoniae* respectively. The detailed result was shown in Table 1 and Figure 1.

Table 1: Antibacterial sensitivity of crude and column chromatographic fractions (1-6) of *E. agallocha* bioactive compounds against chosen antibiotic resistances pathogens

	Zone of inhibition in diameter (mm) and Chloroform ($CHCl_3$) fractions						
Name of the antibiotic resistances pathogens	Crude	I	II	III	IV	V	VI
Klebsiella sp.	-	-	-	-	-	-	-
Staphylococus epidermidis	9	7	-	12	-	10	-
Staphyloccus aureus	7	-	-	9	-	-	-
Pseudomonas aeruginosa	10	-	-	12	7	9	-
Streptococcus pneumonaiae	7	-	-	7	-	7	-

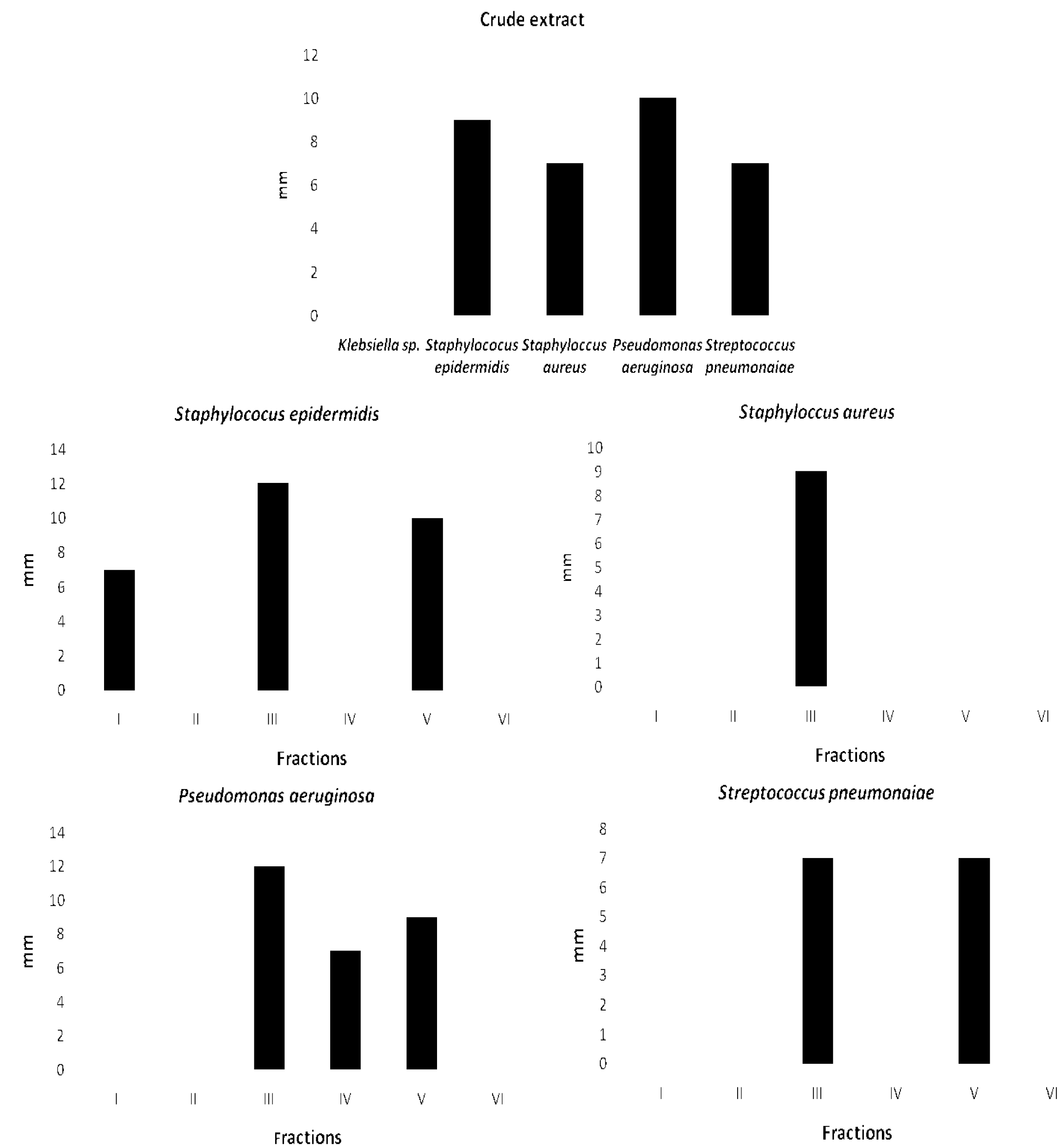

Fig. 1: Pattern of average zone of inhibition by chloroform crude and chromatographic fractions against antibiotic resistant pathogens

The 5th fraction of chloroform from *E. agallocha* leaf extracts showed activity against three (*Staphylococcus epidermidis, Streptococcus pneumoniae* and *Pseudomonas aeruginosa*) of the five antibiotic resistant pathogens. This fraction showed maximum activity (12mm) against *Staphylococcus epidermidis*. This fraction also showed activity (9mm and 7mm) aganist *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* respectively.

The 1st and 4th fractions of chloroform from *E. agallocha* leaf extracts showed activity against only one pathogen. The 1st fraction of chloroform from *E. agallocha* leaf extract showed maximum activity (7mm) against *Staphylococcus epidermidis* and the 4th fraction of chloroform from *E. agallocha* leaf extract showed maximum activity (7mm) against *Pseudomonas aeruginosa*. There is no activity was recorded in crude and all the fractions of mangrove leaf extract against the *klebsiella sp.*

DISCUSSION

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 and the family Meliaceae includes many plants that are sources of valuable timber and many that have wide ranging uses in ethno medicine. The family is distinguished by the occurrence of characteristic substances called limonoids. These substances have wide spectrum of biological activities, particularly insecticidal action [23]. The *E. agallocha* is well known to contain skin irritants. In traditional Thai medicine, the bark and wood of the plant is used to treat flatulence [24]. In Srilanka, the smoke of the burning wood is used in the treatment of leprosy and the root, when pounded with ginger, as an embrocating for swollen hands and feet [25]. The milky latex exuded from the bark may cause Blindness or blistering of the skin [24]. This latex has been used as a poison for fish by adding it to water and to poison arrowheads. Some triterpenoids isolated from this plant have been found to posses anti-tumor promoting activity [26].

Padmakumar and Ayyakkannu [27] reported toluene-methanol (1:3) extracts of species belonging to Rhodophyceae exhibited broad-spectrum activity when compared to Chlorophyceae and Phaeophyceae. Vidyavathi and Sridhar [28] reported chloroform-methanol extract of fully grown *G. corticata* showed maximum activity against *S. aureus* compared to medium and young stages of growth. Srinivasa Rao and Parekh [29] analysed *Enteromorpha intestinalis* and *G. corticata* collected from Gujarat coast of India for antibacterial activity and found that the algae were active throughout the year with a peak during the winter season. Acetone and ethanol extracts of marine algae *Cladophora fascicularis, Caulerpa taxifolia, Chaetomorpha antennina, Ulva lactuca* and *G. corticata* collected from south-west coast of India in three seasons showed good inhibitory activity against *Bacillus subtilis*. The results differ from the findings of Crasta *et al.* [30] who had recorded significantly different inhibitory activity from season to season.

Some of the preliminary studies have been demonstrated that the mangrove plant extracts have antibacterial activity against pathogenic bacterial strains; *Staphylococcus sp.* and *E. coli* and *Pseudomonas sp.* [31] and antibiotic resistant bacterial strains; *Staphylococcus. sp* and *Proteus sp.* [32]. Sastry and Rao [33] found the benzene extract of *G. corticata* showed antibacterial activity only against *Salmonella typhi* and *E. coli* whereas the methanol and chloroform extracts had activity against *P. aeruginosa*. In our studies, fractions of methanol (Me) extract of *G. corticata* had shown good activity against *P. aeruginosa*.

Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity [34. 35]. *Xylocarpus granatum* also possess alkaloidal substances which also have biological activities [36].

Mangroves have enormous ecological value. They protect and stabilize coastline, enrich coastal water, yield commercial forest products and support coastal fisheries. Mangroves forests are among the world's most productive ecosystems, producing organic carbon well in excess of the ecosystem requirements and contributing significantly to the global carbon cycle. Extracts from mangroves and mangrove-dependent species have proven activity against human, animal and plant pathogens. Mangroves may be further developed as sources of high-value commercial products and fishery resources and as sites for a burgeoning ecotourism industry. Their unique features also make them ideal sites for experimental studies of biodiversity and ecosystem function [37].

In our studies results of crude chloroform extracts showed maximum activity in 10mm against *Pseudomonas aeruginosa* and minimum activity (7 mm) was recorded for two bacterial strains; *Staphyloccus aureus* and *Streptococcus pneumoniae*. The 3rd fraction showed maximum activity (12mm) against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The 5th fraction showed maximum activity (12mm) against *Staphylococcus epidermidis*. The 1st and 4th fractions showed maximum activity (7mm) against *Staphylococcus epidermidis* and the 4th fraction of chloroform from *E. agallocha* leaf extract showed maximum activity (7mm) against *Pseudomonas aeruginosa*. No activity was recorded for crude and all the fractions of mangrove leaf extract against the *klebsiella* sp. The non polar extracts of mangroves have shown antibacterial activity against fish pathogens earlier [38].

In conclusion the present investigation showed that the potential mangrove bioactive compounds could be used for the development of anti-pathogenic agents against antibiotic resistant pathogens.

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