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Evaluation of Antibacterial Properties of Mangrove Plant *Sonneratiaapetala* Buch. Ham Leaf

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Abstract: Mangroves are collection of plants growing in saline coastal habitats around the world and are a rich source of secondary metabolites of various types. Secondary metabolites from various plant species are known to possess interesting bioactive properties especially with respect to their antimicrobial activity. The aim of this study thus was to evaluate the antibacterial potential of a mangrove plant *Sonneratia apetala*. Acetone extract was prepared from the leaves and was tested against various bacterial pathogens. For this purpose, both gram-negative as well as gram-positive bacterial strains were tested in this study. Antibacterial potency of the extract was tested by standard growth inhibitory assay methods. The tested extract showed to varying degrees of antibacterial potential against tested gram-negative as well as gram-positive bacteria. These promising findings suggest antibacterial activity of the plant material indicating presence of bioactive compounds against bacterial pathogens and exhibiting an alternative source of antimicrobial compounds against diseases caused by these micro-organisms.

Key words: *Sonneratia apetala* · Mangrove · Acetone extract · Antibacterial principles · Bioactive principles

INTRODUCTION

Sonneratia apetala is a mangrove plant belonging tothe Sonneratiaceae family and it grows as tree or shrub along seaward fringes and intertidal areas. Mangrove plants are rich source of secondary metabolites like steroids, triterpenes, Saponins, Flavonoids, alkaloids and Tannins which play an important role in suppression of deleterious microorganisms [1]. Extracts from different mangrove plants are reported to possess diverse medicinal properties [2].

Leaves of this plant have been used traditionally to treat hepatitis [3]. Report also exists where *in vitro* study of this plant using hexane, chloroform and methanol as extractant and involving aerial parts were used randomly to prepare extracts for bioassay. These extracts showed species specific activity in inhibiting the growth of both bacteria as well as fungi [4]. However, very little is known about the chemical constituents specifically present in *S. apetala* leaf apart from presence of Gibberelin A_{25}, a growth promoter [5].

Most of the groups working on the chemistry of secondary constituents tend to use dried materials because there are fewer problems associated with the large scale extraction of dried plant material as compared with fresh material. It is difficult to work with fresh material because differences in water content may affect the solubility or subsequent separation by liquid-liquid extraction. The secondary metabolite should be relatively stable especially if it is to be used as an antimicrobial agent and lastly many, if not most plants are used in the dried form (or as aqueous extract) by traditional healers [6]. Hence dried leaf powder extract has properties which make it a convenient choice for most of the studies including the present one.

One of the major challenges in drug development with plants is the choice of extracts one should use to isolate compound(s) [7, 8]. The choice of the solvent depends on what is intended with the extract. If extraction is to screen plants for antimicrobial components, the effect of the extractant on subsequent separation procedure is not important but the extractant should not inhibit the bioassay procedure. Acetone is a convenient

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solvent to use as evident in multiple studies. Its biggest advantages are volatility, miscibility with polar and non-polar solvents and its relatively low toxicity for the test microorganisms. Considering all these facts, acetone was selected as solvent to prepare extract for the present study.

Two gram-positive bacteria like *Bacillussubtilis* and *Staphylococcusaureus*and four gram-negative bacteria like *Escherchiacoli, Klebsiellapneumoniae, Pseudomonasauregenosa* and *Salmonella typhi*were selected for the study. *B. subtilis*, bacterium commonly found in soil. *B. subtilis* spores can survive the extreme heat during cooking and are responsible for causing ropiness, a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides [9]. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections [10].

E. coli is rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans. The harmless strains are part of the normal flora of the gut and can benefit their hosts by producing vitamin K2 and by preventing the establishment of pathogenic bacteria within the intestine. Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis. In rarer cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicemia and gram-negative pneumonia [11].

Klebsiella is a genus of non-motile, rod-shaped bacteria with a prominent polysaccharide-based capsule. Klebsiella organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, ankylosing spondylitis and soft tissue infections [12].

An opportunistic, nosocomial pathogen of immune-compromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections [13]. *S. typhi*causes illnesses like typhoid fever, paratyphoid fever and the foodborne illness [14].

This study is therefore focused specifically to test anti bacterial constituents present in acetone extract of *S. apetala* leaf against the above selected human pathogens.

MATERIALS AND METHODS

Plant Collection: *S. apetala* leaves were collected from Ghodbunder Road, Thane (Maharashtra), India. Identity of the plant material was confirmed by an expert taxonomist of University of Mumbai, Mumbai.

Extraction: Leaves were dried in the shade at room temperature and dried leaves were ground to a fine powder in a Jankel and Kunkel model A10 mill. 10 grams of powder material was extracted with 200 ml of acetone and extract was evaporated to dryness on water bath.

Microbial Analysis

Microorganisms: For microbial analysis, bacterial species used included clinical isolates of *Escherichia coli*, *Staphylococcus aureus, Pseudomonas auriginosa, Streptococcus pyogens, Bacillus subtilis, Salmonella typhi* and *Klebsillaaerogens* which were procured from Haffkines Laboratory, Mumbai (India). They were maintained on nutrient agar (Himedia Lab Ltd., India) slants at 4°C prior to use for antimicrobial susceptibility test.

Susceptibility Test: Susceptibility tests were carried out by Agar cup method [15]. 24 hrs mature bacterial culture (0.4 ml) was added to 20 ml of sterile medium. Poured in to each plate and slowly shaken the medium to allow the culture to mix methodically. Thereafter plates were set aside on a plain surface and allowed for proper solidification. After solidification, 4 wells were made with the help of cork borer (8 mm) as per requirement. In each well, 80 µl of sample was loaded using micropipette. Among these wells one was used only for pure solvent served as control. Later plates were kept for diffusion at 40°C temperature in refrigerator for 45 minutes and incubated at 37°C for 24 hrs and 48 hrs respectively.

Determination of Inhibitory Zone Diameter (IZD): All the tests were carried out in triplicate and the average values for zones of inhibition in millimeters (mm) were taken after 24 hrs and 48 hrs of incubation. Final results were expressed as the arithmetic average of triplicate experiments.

Determination of Minimal Inhibitory Concentration (MIC): The crude extract shown zone of inhibition above 16 mm was selected for MIC. The extracts were evaporated to dry residues and by using fresh solvents different concentrations (0.5, 1, 5 and 10 mg/ml) were prepared for the MIC study. The bioassay of all the concentrations was carried out by Agar cup method.

RESULTS

Susceptibility Test: Bioassay results shown by acetone extract of *S. apetala* leaves against bacterial pathogens are listed in Table 1. Acetone extract showed inhibition against all the bacterial species except *Kl. aerogens,* which was found to be resistant against this extract.

S. aureus, S. typhi and *E. coli* exhibited 21 mm size zones of inhibition. *S. pyogen* showed zone of inhibition of 20 mm. 19 mm and 16 mm size zone of inhibition was shown by *Ps. auregenosa* and *B. subtilis* respectively.

Minimum Inhibition Concentration (MIC): The bioassay of acetone leaf extract showed positive results against all the bacteria except *Kl. aerogens* and hence the MIC of extract was carried against the bacteria. Zones of inhibition were noted against *S. typhi, B. subtilis*, *Ps.* auregenosa and *E. coli*. Among these *S. typhi, B. subtilis* and *E. coli* microorganisms were inhibited at the concentration 0.5 mg/ml. While 5 mg/ml concentration shunted the growth of *Ps. auregenosa*. In contrast, *S. aureus* and *S. pyogen* showed negative results, even up-to the concentration of 10 mg/ml (Table 2).

Table 1: Zones of inhibition in Bioassay Experiment

Microorganisms	Zones of inhibition (mm)
Kl. pneumoniae	--
S. aureus	21
S. typhi	21
E. coli	21
S. pyogen	20
Ps. auregenosa	19
B. subtilis	16

Table 2: Zones of inhibition for Minimum Inhibition Concentration (MIC)

Microorganisms	10 mg/ml	5mg/ml	1mg/ml	0.5mg/ml
S. aureus	--	--	--	--
S. typhi	16	15	12	10
E. coli	15	13	11	10
S. pyogen	--	--	--	--
Ps. auregenosa	15	13	--	--
B. subtilis	15	14	12	10

DISCUSSION

Infectious diseases are the world's leading cause of premature deaths. Therefore, the importance of identifying new effective antimicrobial agents cannot be overemphasized. The use of plant extracts or phytochemicals with known antimicrobial properties can be of great significance for therapeutic treatments.

Results of our findings revealed that the tested extract of *S. apetala* leaves exhibited growth inhibitory activity against the bacterial strains evaluated which are similar to the findings of Varaprasad *et al*., 2009.

Varaprasad *et al*., 2009 studied antifungal and antibacterial activity of aerial parts of *S. apetala* using chloroform, methanol and hexane extracts. The microorganisms used for study were, *Asperigellusflavus, Asperigellusniger, Acremoniumstrictum, Candida albicans, Cladosporiumherbarum, Curvularialunata, Erwinacaratovara, Lactobacillus acidophilus, Pseudomonas aeruginosa, Pencilliumexpansum, Rhizoconiasolani, Streptococcus gordonii, Streptococcus mutans, Streptococcus salivarious, Tiarosporellaphaseolina, Ustilagomaydis* and *Xanthomonascompestris.*

The chloroform and methanolic extracts showed considerably more activity than the hexane extract. Maximum antimicrobial activity was shown against *Asperigellusflavus, Streptococcus mutans* with chloroform. The greatest activity of the methanolic extract was against *Asperigellusflavus* followed by *Candida albicans*and *Cladosporiumherbarum*. The hexane extract appears to have less antibacterial and antifungal activity than the chloroform and methanolic extracts.

In the present study as well the study by Varaprasad *et al.,* the results obtained are significant with respect to solvent specific bioactivity. The reason behind this is not clear right now but it may be because of the greater solubility of bioactive compounds responsible for growth inhibition of specific bacterial test strains in respective solvents. If we compare the polarity of hexane, acetone, chloroform and methanol, it is evident that hexane is non-polar solvent. Methanol is more polaras compared to acetone and water is highly polar if compared with methanol and acetone. This shows the possibility of presence of polar solvent soluble bioactive compounds in *S. apetala* plant.

The result further revealed that antibacterial potency of the bioactive compounds was not affected when extracted in hot conditions indicating that

the plant material contains thermo-stable bioactive compounds. The present findings are hence encouraging in recognizing a plant showing interesting antibacterial activity.

Thus *S. apetala* leaves has strong antibacterial activity against both the gram-negative and gram-positive bacteria and the compounds responsible for this activity are thermo-stable, suggesting the importance of ethno-medical approach as a potential source of bioactive compounds. Leaves of *S. apetala* can be helpful against diseases caused by these microorganisms and further studies both on the extract and/or its chemical constituents are needed to elucidate the chemical nature of the bioactive compounds. Thisreport may serve as a footstep in this aspect.

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