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Evaluation of anti-infective potential of fruits of common mangrove tree *Sonneratia apetala* against some selected pathogenic fungi and bacteria

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Abstract
The present investigations mainly focused to evaluate the antimicrobial activity of aqueous and acetone extract of fruits of the mangroves plants *Sonneratia apetala* against six pathogen (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Aspergillus flavus and Candida albicans)* using disc diffusion method. The aqueous extracts are more effective rather acetone and the aqueous extract(s) of fruit were found to higher antifungal activity than bacteria. Result showed that aqueous extract has maximum activity against *A. flavus* and *C.albicans* as 25mm, 17mm respectively. The aqueous extract (s) was found to inhibit the isolates of *A. flavus* and *C.albicans* at an MIC of 1.75 mg/ml and 0.1 mg/ml, respectively. But, antibiotics fluconazole found to possess the MIC value 0.75 mg/ml against *C. albicans* and 2.0 mg/ml against *A. flavus.* Therefore, the results suggested that these plants could be exploited in the management of various infectious diseases and their fruit extracts might have roles as pharmaceuticals and preservatives. Phytochemical analysis of fruit extract revealed the presence of carbohydrate, protein, flavonoid and phenolic compounds.

Keywords: Antimicrobial activity, Mangroves, MIC, Fruit extract, *Sonneratia apetala*

1. Introduction
Mangrove forest are the rich source for Biodiversity and it have been rich source of medicine because they produce a host of bimolecules most of which probably involved as chemical against predation or infection [1]. The use of traditional medicine is widespread and plants still present a large source of novel active biological compounds (glycoside, alkaloids, terpenes, essential oils, steroids, hormones, vitamins, enzymes, plant acids, sugars, starches, fats, waxes, oleoresins, oleo gum –resins, balsams etc) with different activities, including anti-inflammatory, anticancer, antiviral, antibacterial and cardio protective activities. Antioxidants may play a role in these health promoting activities [1]. Scientific studies of plants used in ethno medicine led to the discovery of many valuable drugs, including taxol, comptothecin, vincristine and vinblastine [2].

In west Bengal, mangroves are widely distributed at Sundarban. Mangrove and their products have been used in traditional medicine. These plants are well known to have diverse natural products with great pharmaceutical importance and also exhibiting antimicrobial, anti Larval, anti fungi and anti insecticidal activity. There is a need of development of new drugs because of the resistance developed to existing antibiotics by pathogen. Hence there is a need to search and design new alternative drugs from natural plant product to control microbial infection. Mangrove plants are the best choice to isolate bioactive natural product active against bacteria and fungi [3].

Because of their medicinal values, different parts of these plants have been used for ages by the local people for curing many diseases. Examples are; stem of *Avicennia marina* has been used for the treatment of rheumatism, small pox, ulcers and fodder for livestock; resin of *Avicennia alba* plant extract has been used for a long time as folk medicine for the treatment of antifertility, skin diseases, tumors, ulcers, etc.; bark, leaf and stem of *Aegiceras corniculatum* are used for the treatment of asthma, diabetes, rheumatism and as a fish poison [4]. Mangrove plant *Clerodendrum inerme* is used for antibacterial activity against some human pathogenic bacteria and as antifungal against some human and plant pathogenic fungi [5-6]. Some scattered studies have also been carried out earlier by different authors on these plants for the purpose of identifying various activities related to human health and other

industrial uses. For example, Roome *et al*., [7] have studied antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *A. corniculatum* (stem) extracts. Agoramoorthy *et al*., [8] have also reported antioxidant activity in *A. corniculatum*. Moreover, some researchers have studied the antimicrobial activity of *A. corniculatum* at very preliminary levels [9- 10].

Sonneratia apetala is a common mangrove tree abundantly found in the western sector of Indian Sundarbans [11], where salinity is relatively low (average water salinity is ~12 psu) compared to the central sector (average value is ~18 psu). The fruit of this species is sour in taste with greenish-yellow pulp and appear in plenty during the months of August and September, the period characterized by heavy rainfall, low salinity and relatively low pH of the aquatic phase. The fruits are mainly preferred by deer population of Sundarbans, but a large fraction of island dwellers consume the fruit after processing and cooking. Recently, Pramanick *et al*. [12] have found that the fruit products of *S. apetala* were compared with the jelly prepared from other edible fruits to evaluate the nutritional status of the present product. Vitamin C of the fruit pulp and jelly were significantly higher than other citrus fruits. The major and trace elements of the jelly were well within the range of accepted level for human consumption. But, no report on the antimicrobial property of the fruit is yet available from this part of sub-continent. The present observation is therefore a baseline study of anti-infective potential of fruits of common mangrove tree *Sonneratia apetala* against some selected pathogenic fungi and bacteria and has the potential of opening an alternative livelihood for the people of the deltaic region, if proper backup nursery of the species can be created in the islands involving the local people.

2. Materials and methods

2.1. Collection and preparation of plant materials

The western sector of the Indian Sundarbans, adjacent to Hooghly estuary sustains a large population of *S. apetala*, from where the ripe fruits (Figure-1) were collected during August, 2014. The fruits parts of the plant were shade dried for 15 days and then pulverized into fine powder using pestle and mortar.

Fig 1: *Sonneratia apetala* fruit

2.2. Aqueous extract by soxhlet methods

25 gm of powdered plant materials (fruits) were packed in a thimble and placed in a soxhlet extractor. It was extracted with 250ml d.H2O and temperature was controlled at 60 ˚C for 6hrs [13]. After complete extraction the extract was collected into conical and stored at 4 ˚C.

2.3. Acetone extract

10gm of powdered plant materials (fruits) were extracted with 100ml of acetone and incubated in a rotary shaker for 24 hrs. at 37 ˚C. Thereafter, it was filtered with the help of Whatman No.1 filter paper and centrifuged at 5000 rpm for 15 min [13]. The supernatant was collected and tested against microorganisms and was stored at 4 ˚C for further use.

2.4. Phytochemical tests

The aqueous extracts were subjected to phytochemical group tests including flavonoid, phenolic compound [14], carbohydrate (Benedict's and Fehling's tests), and protein (Lowery test).

2.5. Determination of antimicrobial activity of the extract

Antimicrobial sensitivity was tested by the agar well diffusion method using Mueller-Hilton and PD agar media [15]. Petri dishes (100 mm) containing 25 ml of Mueller–Hinton Agar and potato-Dextrose agar seeded with 100 µl inoculum of bacterial strain and fungal strain (Inoculum size was adjusted so as to deliver a final inoculum of approximately 10^6 CFU/ml). Media was allowed to solidify and then individual Petri dishes were marked for the bacteria and fungi inoculated. Wells were cut into solidified agar media with the help of sterilized cup-borer. 100 µl of each extract was poured in the respective wells and the plates were incubated at 37 ºC (for bacteria overnight) and 28 ºC (fungi for 96 hours). Sterilized distilled water and acetone was used as negative control. The experiment was performed in triplicate under strict aseptic conditions and the antibacterial and antifungal activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by the respective fruit extract [16].

2.6. Determination of minimum inhibitory concentration (MIC) of the aqueous extract

Minimum inhibitory concentration (MIC) was determined using Inhibitory Concentration in Diffusion (ICD) method [17]. It was done by carrying out the disc diffusion tests with 4 to 6 discs spotted with serially diluted concentrations of mangrove plant extracts. For making these dilutions, the dried crude extracts (lyophilized) were dissolved at a concentration of 2 mg/ml and serially diluted (1:1) in distilled water to obtain crude extract concentrations of 2, 1.75, 1.5, 1.2, 1, 0.75, 0.5, 0.2, 0.1 and 0.05 mg/ml respectively. 100 µl of aqueous extract of different concentration was poured in the respective wells and the plates were incubated at 28 ˚C (fungi for 96 hours). Sterilized distilled water was used as control. The experiment was performed in triplicate under strict aseptic conditions.

2.7. Determination of MIC value of selected antibiotics

For this present study we used fluconazole antibiotic (fungi). Agar diffusion method was applied to determine the MIC [17]. Petri dishes (100 mm) containing 25 ml of potato-Dextrose agar seeded with 100 µl inoculum of fungal strain (Inoculum size was adjusted so as to deliver a final inoculum of approximately 10^6 CFU/ml). Media was allowed to solidify and then wells were cut into solidified agar media with the help of sterilized cup-borer. 100 µl of each antibiotic (30 to 0.5 mg/ml for fungi) was poured in the respective wells and the plates were incubated at 28 °C (fungi for 96 hours). Sterilized distilled water was used as negative. The experiment was performed in triplicate under strict aseptic conditions and the antifungal activity of different concentration of antibiotics was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by the antibiotics at the end of incubation period.

2.8. Statistical analysis

All the data was analyzed statistically using SPSS-10.0. Each sample was analyzed and data were represented as mean ±SD

3. Result

Table 1: Antimicrobial activity of aqueous and acetone extract of fruits of *Sonneratia apetala* against all the six pathogens

plant extract	Bacteria				Fungi	
	***E.coli* (mm) mean±SD**	***S.aureus* (mm) mean±SD**	***B.subtilis* (mm) mean±SD**	***P. aeruginosa* (mm) mean±SD**	***A.flavus* (mm) mean±SD**	***C.albicans* (mm) mean±SD**
Aqueous	9.0±0.1	9±0.1	10±0.1	15±0.2	25±1.1	17±1.0
Control	6.0	6.0	6.0	6.0	6.0	6.0
Acetone	10±0.2	11±0.1	13±0.2	11±0.1	22±0.9	16±0.7
Control	9.0	9.0	9.0	9.0	9.0	9.0

Table 2: MIC value of lyophilised crude extract against *Candida albicans*

Organism	Lyophilized crude extract (mg/ml)	Zone of inhibition (mm) mean±SD
Candida albicans	0.05 mg/ml	-
	0.1 mg/ml	16±0.2
	0.2 mg/ml	18±0.4
	0.5 mg/ml	19±0.3
	0.75 mg/ml	19±0.2
	1 mg/ml	20±0.3

Table 3: MIC value of lyophilised crude extract against *Aspergillus flavus*

Organism	Lyophilized crude extract (mg/ml)	Zone of inhibition (mm) mean±SD
Aspergillus flavus	1 mg/ml	-
	1.2 mg/ml	-
	1.5 mg/ml	-
	1.75 mg/ml	10±0.1
	2 mg/ml	11±0.1
	2.5 mg/ml	15±0.3

Table 4: Antimicrobial assay of antibiotic (Fluconazole) against fungi

Name of fungi	Concentration	Antimicrobial zone(mm)
Candida albicans	1mg/ml	11±0.1
	0.75 mg/ml	10±0.1
	0.5 mg/ml	-
	0.2 mg/ml	-
Aspergillus flavus	2 mg/ml	8±0.1
	1.75 mg/ml	-
	1.5 mg/ml	-
	1 mg/ml	-

Table 5: Phytochemical analysis of aqueous extract

Substance	Result
Carbohydrate	Present
Protein	Present
Flavonoids	Present
Quinone	Absent
Phenolic compound	Present

4. Discussion

This work presents a comparative survey of aqueous and acetone extracts of fruits of mangrove plants *Sonneratia apatala* collected from Sundarban estuary with respect to their antimicrobial activities against microbial models *B. subtilis*, *E. coli, S. aureus, C. albicans, Aspergillus sp*.

It has been found that the aqueous extracts are more effective rather acetone and the aqueous extract(s) of fruit were found to higher antifungal activity *(A.flavus* and *C.albicans)* than bacteria (Tables 1- 3). Similarly, many researcher found that plants are rich in a wide variety of phytochemicals like flavonoids, antimicrobial sugar, etc., that have been found to have antifungal activities than bacteria [18-20].

The antibiotic, fluconazole has been used to test the sensitivity against fungi because generally all people used antifungal drug to cure the skin disease and allergy [21]. Present result shows that the effectiveness of fruits of *Sonneratia apatala* has much higher antifungal activity than commonly used antibiotics as fluconazole (Tables 4- 6). The aqueous extract (s) was found to inhibit the isolates of *A. flavus* and *C.albicans* at an MIC of 1.75 mg/ml and 0.1 mg/ml, respectively. But, antibiotics fluconazole found to possess the MIC value 0.75 mg/ml against *C. albicans* and 2.0 mg/ml against *A. flavus.* Therefore, it may be a promising natural substance as clinically useful antifungal drug for treatment of skin disease and allergy. From the phytochemical analysis, it was observed that the effective compound may be carbohydrate or phenolic compound. Therefore, further purification is required to find the exact component present in plant extract which have higher antifungal activity.

5. Conclusion

Further researches are needed to characterize various phytochemicals of these plants and isolate the active compound/s responsible for the antimicrobial activities that we have observed in the fruit extracts of these mangrove plants. Along with this, the fruit extract can be tested against other pathogens in higher animals like mice and if it is not having any adverse effect on the animal body, then it can be taken as medicine after clinical trials.

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