J. Mycopathol. Res. ***59(2)*** *: 165-169, 2021;* (ISSN 0971-3719)

Antimicrobial and antioxidant properties of common mangrove plants of Sundarban, Patharpratima, West Bengal

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Received : 11.02.2021 Accepted : 17.05.2021 Published : 28.06.2021

The aqueous, 95% ethanolic and 80% acetone extracts of leaves of *Herittiera fomes, Aegialitis rotundifolia, Avicennia alba, Avicennia marina, Avicennia officinales, Rhizophora mucronata, Ceriops decandra, Ceriops tagal, Bruguiera gymnorrhiza and Aegiceras corniculatum* were tested for their antimicrobial activities on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. The free radical scavenging activity or antioxidant effects of the leaf extracts were also checked. *Aegialitis rotundifolia, Rhizophora mucronata, Herittiera fomes, Aegiceras corniculatum, Avicennia alba, Avicennia officinales, Ceriops decandra* and *Bruguiera gymnorrhiza* showed antibacterial activity against *Bacillus subtilis* in different solvents. In growth of *Staphyllococcus, Aegialitis, Avicennia marina, Rhizophora mucronata* and *Aegiceras corniculatum* showed hindrance. In case of *E. coli*, *Aegialitis, Rhizophora* and *Aegiceras* were effectives. *Aegialitis, Herittiera, Avicennia* and *Rhizophora* showed resistance in growth of *Klebsiella*. They can be used to develop natural drugs in lieu of commonly used strong allopathic drugs.

Key words: Antimicrobial property, antioxidant property, flavonoid, mangrove leaves

INTRODUCTION

It is known for a long time that extracts of different parts of the mangrove plants are widely used for different pathophysiological conditions worldwide. The stem of Avicennia marina is used for ulcers and bark of Bruguiera sexangula is used for antitumor. There are compounds present in mangrove leaves that have biologically active antiviral, antibacterial and antifungal compounds. Mangrove plants provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, and tannins . Marine environmental conditions are extremely different fromterrestrial ones; so, mangrove plants have different characteristics from those of terrestrial plants, and therefore, might produce different types of bioactive compounds (Ravikumar and Gnanadesigan. 2011). Among the different mangrove plants, Avicennia marina is previously proved to have bactericidal activities and it is proved to have high content of secondary metabolites, like, polyphenols, flavonoids, alkaloids and tannins (Ravikumar et al. 2010).

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At the same time, as microorganisms are getting resistant to antibiotics because of increasing use and misuse, it is high time that we to screen the endogenous and medicinal plants for the presence of new antibacterial compounds. In the present investigation attempts have been made to detect antibacterial and antioxidant properties of ten common mangrove plants of Sundarbans forest of West Bengal which are used by the local people.

MATERIALS AND METHODS

Sample collection and preparation

Fresh twigs of ten different mangrove plants, *Herittiera fomes, Aegialitis rotundifolia, Avicennia alba, Avicennia marina, Avicennia officinales, Rhizophora mucronata, Ceriops decandra, Ceriops tagal, Bruguiera gymnorrhiza* and *Aegiceras corniculatum* were collected from Sundarban area, West Bengal (Fig 1) with prior permission from Forest department in the month of March. Twigs of each sample were preserved for herbarium sample and identified and authenticated by experts from Indian Botanical Garden, Shibpur, Kolkata. Leaves from

twigs of each sample were collected, washed, sun-dried and kept in clean sterilised glass container in powdered form in 25^{0}C. List of these plant samples with their chromosome numbers have been presented in Table 1.

Preparation of crude extract

The crude extracts of all the samples for each experiment was obtained by using powdered sample of same dry weight with different solvents in same volumes. For protein extraction 0.02 (M) Tris-HCL (pH=6.8) was used as solvent. For flavonoid assay and antioxidant study the solvent used wasmethyl alcohol. For antimicrobial study three different solvents were selected, those were, de ionized double distilled cold water, 50% ethyl alcohol and 80% acetone. In each case 5 mg of dried leaf powder was soaked in 10 ml of solvent overnight, then centrifuged with 20000 rpm and supernatant was filtered with the help of Whatman no. 1 filter paper. The filtrate in each case was used as crude sample extraction (Saravanan *et al*. 2016).

Microorganisms

Pure cultures of two Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, and two Gram negative bacteria, *Escherichia coli* and *Klebsiella pneumonia* were obtained from MTCC, India.

Protein assay

Bradford assay was performed to detect protein amount in each sample. 0.5 ml of reagent was added to 0.5ml of aqueous extract of each sample and the mixtures were incubated for 10 minutes at room temperature. Absorbance was measured at 595 nm and the amount of protein in each sample was compared with standard BSA concentrations (Bradford,1976).

Flavonoid assay

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content in the samples.0.5 ml of each extract was mixed with 0.5ml of 2% aluminium chloride-ethanol, mixed and incubated for 60min at room temperature. The absorbance of the end products was measured against blank at 420nm. Quercetin was used to make the standard calibration curve and the concentration of total flavonoid content in all the samples was calculated from the calibration plot (Y=0.0162x + 0.0044, R^2 = 0.999)and expressed as mg quercetin equivalent (QE)/g of dried plant material. (Asha et al, 2012)

Free radical scavenging activity determination

The free radical scavenging activity of ethanolic extracts of all the plants were determined using a stable radical DPPH(1,1-diphenyl-2-picrylhydrazyl). 2.5 mg of DPPH was dissolved in100 ml of 95% ethanol and 3.9 ml of freshly prepared DPPH was mixed with 100 ul of plant extract and incubated for 30 mins in room temperature. Absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated, using the following equation:

$$\text{DPPH scavenged (\%)} = \{(Ac - At) / Ac\} \times 100$$

where Ac is the absorbance of the control reaction and at is the absorbance of the sample reactions. An antioxidant value of 100% indicates the strongest antioxidant activity and 95% ethanol as blank and the DPPH – ethanol mixture as control was used (Ravikumar and Gnanadesigan 2011).

Qualitative estimation of bactericidal activity

Cup plate method was used to evaluate the antimicrobial property of all the ten selected mangrove plants. This method is one of the official methods for measurement of inhibition property, where the test samples diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms is restricted entirely to a circular area or zone around the cavity containing the solution of a test substance. Following proper sterilization procedure, selected microorganisms were mixed with molten agar and poured on Petri plates and solidified. 5 mm diameter cups were made with sterile borer in the agar medium on each Petri dish.The centre cup was used for control.All the plates were kept at room temperature after addition of plant extracts for effective diffusion.Later, they were incubated at 37±1°C for 24 h.The presence of clear zones around any cup is an indication of antibacterial activity. The zone of inhibition were measured by their diameter.All the experiments were conducted thrice and presented as Mean ± standard deviation. (Walsh , 2003)

RESULTS AND DISCUSSION

Protein content was found to be relatively constant in all the ten plant extracts in the range between 4~5 mg/ml whereas flavonoid contents varied

Table 1: Common and scientific families and chromosome numbers of selected mangrove plants.

Sample	Common Name in Bengali	Scientific Name	Family	Chromosome number
S1	SUNDARI	*Herittiera fomes*	Sterculiaceae	38
S2	TORA	*Aegialitis rotund ifolia*	Plumbaginaceae	34
S3	KALI BAANI	*Avicennia marina*	Avicenniaceae	62
S4	PEYARA BAANI	*Avicennia alba*	Avicenniaceae	66
S5	JAT BAANI	*Avicennia officinales*	Avicenniaceae	64
S6	GORJON	*Rhizophoramucronata*	Rhizophoraceae	36
S7	MAATH GORAAN	*Ceriopsde candra*	Rhizophoraceae	36
S8	CHAAND GORAAN	*Ceriopstagal*	Rhizophoraceae	36
S9	KAANKRAA	*Bruguiera gymnorrhiza*	Rhizophoraceae	36+
S10	KHOLSHI	*Aegiceras corniculatum*	Primulaceae	46

Table 2: Flavonoid and Protein contents of selected mangrove plants

No.	Sample	Flavonoid(mg/ml)	Protein(mg/ml)
S1	*Herittierafomes*	80	4
S2	*Aegialitis rotundifolia*	80	4
S3	*Avicennia marina*	150	5
S4	*Avicennia alba*	80	4
S5	*Avicenniaofficinales,*	90	5
S6	*Rhizophora mucronata*	150	5
S7	*Ceriopsdecandra*	120	5
S8	*Ceriops tagal*	60	5
S9	*Bruguiera gymnorrhiza*	120	4.5
S10	*Aegiceras corniculatum*	170	4.4

Table 3: DPPH inhibition (percentage) of plant samples

No.	Sample	Anti - oxidant Properties (%)
S1	*Herittierafomes*	99
S2	*Aegialitisrotundifolia*	98
S3	*Avicennia marina*	32
S4	*Avicennia alba*	20
S5	*Avicennia officinales,*	98
S6	*Rhizophoramucronata*	98
S7	*Ceriopsdecandra*	33
S8	*Ceriops tagal*	53
S9	*Bruguiera gymnorrhiza*	98
S10	*Aegiceras corniculatum*	96

greatly among the extracts ranging from 60 to 170 mg/ml. *Aegiceras corniculatum* showed maximum flavonoid content of 170 mg/ml while *Ceriops tagal* was found to have minimum flavonoids (60 mg/ml). The flavonoid contents of the other plant extracts were in between these two values. *Herittiera fomes*, *Aegialitis rotundifolia* and *Avicennia alba* are found to have flavonoid content of 80mg/ml. *Avicennia officinales* has flavonoid content of 90 mg/ml. *Ceriops decandra*, *Bruguiera gymnorrhiza* have flavonoid content of 120 mg/ml and *Avicennia marina, Rhizophora mucronata* have flavonoids of 150 mg/ml.(Table 2)

The ethanolic extracts of all ten mangrove plant leaves showed antioxidant property. *Herittiera fomes* showed 99% antioxidant property while *Aegialitis rotundifolia*, *Avicennia officinales*, *Rhizophora mucronata*, *Bruguiera gymnorrhiza* showed 98% activity. *Aegiceras corniculatum* showed 96% activity. Four plant extracts ie, *Avicennia alba*, *Avicennia marina*, *Ceriops decandra* and *Ceriops tagal* did not show considerable antioxidant activity. (Table 3)

The water, alcohol and acetone (all three) extracts of *Aegialitis rotundifolia* and *Rhizophora mucronata* showed antibacterial activity against *Bacillus*, in acetone extract *Herittiera fomes*, *Avicennia alba*, *Avicennia officinales* and *Bruguiera gymnorrhiza* also showed antibacterial effect against it. *Aegiceras corniculatum* showed antibacterial property against *Bacillus* in both water and acetone, whereas *Ceriops decandra* showed this property against *Bacillus* only in water extract (Fig: 4). In growth of *Staphyllococcus, Aegialitis* showed hindrance in water and acetone extract whereas, *Avicennia marina, Rhizophora mucronata* and *Aegiceras corniculatum* resist the growth of *Staphyllococcus* only in acetone extract. In case of *Escherichia coli* only water extract of *Aegialitis, Rhizophora* and *Aegiceras* were effectives. Both alcohol and acetone extract of *Aegialitis* and *Rhizophora* showed resistance in growth of *Klebsiella* and alcohol extract of *Herittiera* and water extract of *Avicennia* showed antibacterial effect against *Klebsiella.* From the result it can be concluded that, growth of *Bacillus subtilis* was inhibited by most of the plant extracts while growth of *Escherichia coli* was minimally inhibited. Among the samples, *Agialitis rotundifolia* (Tora),*Rhizophora mucronata* (Gorjon) and *Agicerus corniculatum* (kholsi) showed highest inhibition while *Ceriops tagal* (Goran) and *Avicennia marina* (Bani) did not show any inhibition. The acetone extract of the plant leaves exhibited the best result and the concentration of 500 mg/ml had been proven to be the best performing dose overall. (Table 4). Inhibition zones have been identified by arrow sign on Fig 4.

Mangrove plant leaves are rich in polyphenols and

Table 4: Calculated mean values of diameter of inhibition zones with standard deviations for all those responding species of mangrove plant leaves.

SAMPLES	Inhibition against *Bacillus subtilis* (cm)			Inhibition against *Staphyllococcus aurius* (cm)			Inhibition against *Escherichia coli* (cm)			Inhibition against *Klebsiella pneumoni* (cm)		
	Water	Alcohol	Acetone	Water	Alcohol	Acetone	Water	Alcohol	Acetone	Water	Alcohol	Acetone
Herittiera fomes			1.4 ±0.11								1 ±0.09	
Aegialitis rotundifolia	1.91 ±0.13	1.8 ±0.14	1.83 ±0.125	1.72 ±0.11		1.84 ±0.15	2.06 ±0.12				2 .1 ±0.1	1.51 ±0.12
Avicennia marina	No inhibition											
Avicennia alba			1.3 ±0.11			1.3 ±0.08				1.72 ±0.12		
Avicennia officinales			1.3 ±0.13									
Rhizophoram ucronate	1.62 ±0.12	1.4 ±0.1	1.72 ±0.2			1.4 ±0.1	1.82 ±0.13				1.6 ±0.12	1.22 ±0.15
Ceriop s decandra	1.4 ±0.11											
Ceriops tagal	No inhibition											
Bruguiera gymnorrhiza			1.54 ±0.2									
Aegiceras corniculatum	1.5 ±0.1		1.6 ±0.09			1.2 ±0.1	1.68 ±0.2					

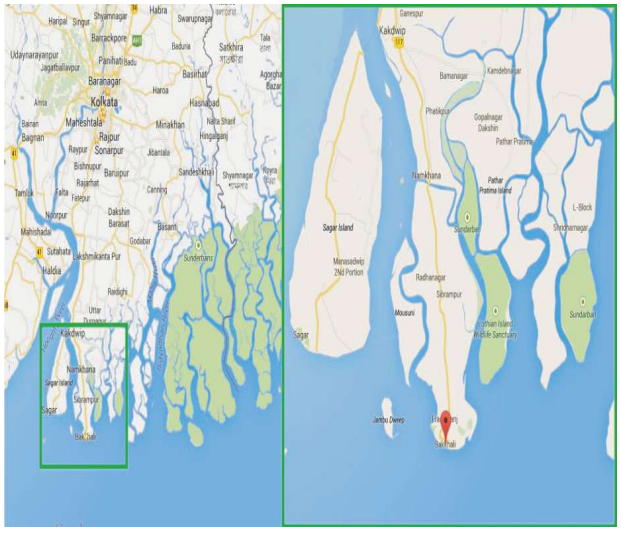

Fig. 1 : Map of the work field of present investigation (Photo Source: Google Map)

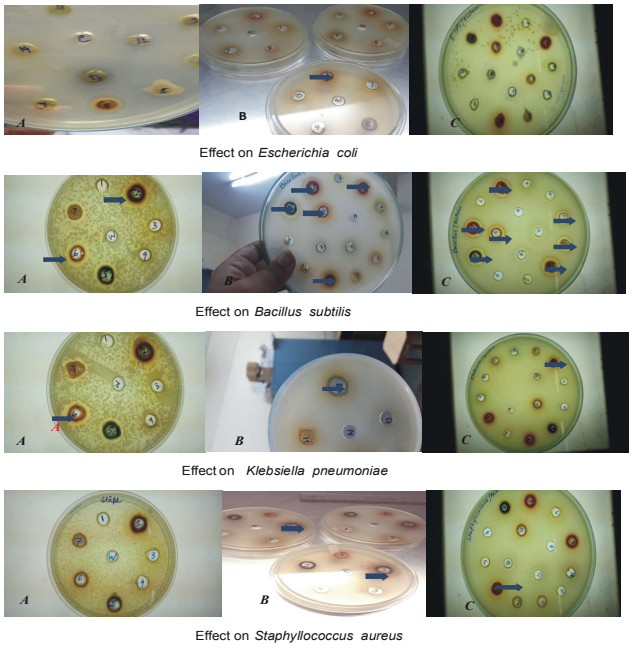

Fig.2 : Inhibition effects of plant extract against different target organisms Solvents used for extraction (A= alcohol, B= water, C= acetone)

flavonoids but have less protein contents. They have high quantity of secondary metabolites and thus act as good antioxidant as well as antimicrobial agents. The water extracts of some leaves showed considerable bactericidal property, which is very promising as they can be consumed as crude extracts and surely will add some feathers on mangrove plants in their ethnobotanical uses. Ethanol and acetone extract of some leaves also showed considerable antimicrobial activity and further studies on their properties and isolation of the active compound(s) will be helpful in the formulation of proper drugs. The results obtained on the antioxidant properties of some leaves are very promising and taking together both antimicrobial and antioxidant properties mangrove plants or leaves can be used in future as supplements along with prescribed drugs.

ACKNOWLEDGEMENTS

The authors are grateful to the authority of St. Xavier's College, Kolkata, and Bhairab Ganguly College, Belghoria for their kind collaboration and

willingness to be supportive whenever and wherever required.

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