Biosciences, Biotechnology Research Asia Vol. **4**(2), 633-638 (2007)

Antimicrobial screening and phytochemical analysis of mangrove species *Acanthus ilicifolius*

PRIYANKA A. KASATKAR and B.L. JADHAV*

Department of Life Sciences, University of Mumbai
Vidyanagari campus, Santacruz (E), Mumbai - 400 098 (India).

(Received: October 17, 2007; Accepted: November 06, 2007)

ABSTRACT

Antimicrobial properties and phytochemical analysis of leaf and stem of *Acanthus ilicifolius* were studied. The cold and hot extracts of these parts were made in petroleum-ether, chloroform, acetone, ethanol, methanol and water and tested against microorganisms. Overall higher activity was observed in hot extracts than cold. Hot extracts of acetone, ethanol and methanol of both the parts showed mild to moderate activity against *Staphylococcus aureus* and *Bacillus subtilis*, while *Escherichia coli* was inhibited by chloroform, ethanol and methanol hot extracts. HPTLC analysis and various confirmatory tests for bioactive principles have revealed the presence of alkaloids, anthraglycosides, arbutins, bitter drugs, cardiac glycosides, coumarins, essential oils, flavonoids, saponins and valeportriates.

Key words: Mangroves, *Acanthus ilicifolius, Antimic*robial property, HPTLC, Phytochemical analysis.

INTRODUCTION

The term 'mangrove' refers to the highly adapted plants found in tropical intertidal forest communities or the ecosystem itself. Mangroves (halophytes) are highly productive ecosystem with various important economic and environmental functions. Few medicines have been derived from mangroves that can cure abdominal troubles, elephantiasis, skin diseases, AIDS, various types of cancer etc. (Bandaranayke M., 1998). Mangroves and mangal associates produce novel metabolites unique to the environment. Hence, they are of interest to the "curious" chemist and the exploration of nature as source of novel active agents.

The Indian mangroves consist of approximately 65 species belonging to 31 families (Banergee and Ghosh, 1998) out of 77 species throughout the world (Santhakumar *et al*., 1998). Mumbai coast has 21 species belonging to 20 genera. The most dominating genera include *Acanthus, Avicennia, Rhizophora* and *Sonneratia* (Deshmukh and Karmarkar, 1991). Among these *A. alba, A. marina (*Mangaokar and Jadhav, 2001), *A. officinalis* L, *E. agallocha* (Suraiya and Jadhav, 2000) and *S. apetela* (Maurya, 2006) are found to be potent.

The mangrove plant *A ilicifolius* L. (family Acanthaceae) an erect spiny shrub commonly known as sea holly is found along the sea coasts of India, Sri Lanka, Philippines, Malaysia, Australia and South Africa. Most of the parts of the *A. ilicifolius* are found to contain various medicinal properties from anticancerous (Graham *et.al.,* 2000), HIV-integrase inhibitory activities (Tewtrakul *et.al*., 2006), anti-tumour activity (Babu *et.al*., 2002) to various comman ailments like rheumatism, kidney stones, neuralgia, snake bites, poison arrow wounds, colds, skin allergies, antiseptic, asthma, dyspepsia, acidity, hepatitis, swelling-enlargement of the liver, lymph nodes and spleen, analgesic, leismanicidal activity, anti-inflammatory and antioxidant properties. Mostly

the medicinal properties of the plants mainly antimicrobial are due to the presence of secondary metabolites like alkaloids, anthraglycosides, arbutin, bitter drugs, cardiac glycosides, coumarins, essential oils, flavonoids, saponins and valeportraites (Cowan, 1999). In view of this a few mangroves species like *Sonnertia apetela* (Maurya, 2006) and *Rhizophora mucronata* (Patil, 2006) are studied for quality and quantity of these bioactive classes. However there are no comprehensive studies on the phytochemical composition of *A ilicifolius.* Therefore, present paper deals with the scientific screening of antimicrobial properties and phytochemicals analysis of this species.

MATERIAL AND METHODS

Plant collection

The plant was collected from Mumbai coast in the month of July-August. Leaves and stem of the plant were brought to the laboratory and identified by an expert taxonomist, separated into individual parts and washed thoroughly under running tap water to free them from dust and other contaminant particles.

Test microorganisms and media

Two gram positive *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative miroorganisms, *Escherichia coli and Salmonella typhi* were used. Nutrient agar was the media used for culturing the microorganisms.

Methods of extraction

All cold and hot extracts were prepared 50%w/v in Petroleum-ether, chloroform, acetone, ethanol, methanol and water.

Cold extract preparation

The fresh plant parts were used to prepare cold extracts. 10gms of leaves and stem were homogenized individually in a mortar and pestle using different solvents and kept aside for 45 min for extraction. It was then filtered through Whatmans filter paper no.1.To the residue again 6-8 ml of solvent was added and filtered similarly after 15 minutes. The final volume of extract was made to 20ml and stored in an airtight borosil glass bottles at 4°C.

Hot extract preparation

The individual parts were oven dried at a constant temperature of 40°C, powdered and sieved through the muslin cloth. These powders were used to prepare extracts by Soxhlet method, using 10 gm of fine powder placed in thimble. To prepare the hot extract 200 ml of solvent was taken into the round bottom flask and temperature of the heating mantle was adjusted just above the boiling point of the solvent. The vapours of the solvent pass through the extraction tube to the condenser and percolates back into the thimble. This process continues, the level at which it flows back into the flask through the capillary attached to the side of extraction tube, this completes one cycle. This cycle continues till the solvent overflowing from the tube becomes colourless. The extract was then evaporated on water bath to reduce the volume to 20 ml (50%w/v) and stored in air tight bottles and kept at 4°C.

Bioassay (*In-vitro*)

Primary screening was carried by Agar cup method (Spooner and Skyes, 1972). On each plate four wells were made with the help of cork borer (8mm diameter). In one of the wells 80 µl of solvent was added as negative control while similar quantity of standard antibiotic Ampicillin (10µg/ml) was added as positive control. In the other two wells 80 µl of leaves and stem extracts were loaded with help of micropipette and kept for diffusion in the refrigerator at 4°C for 20min. The incubation temperature for *E. coli, S. typhi, S. aureus, and B. subtilis* was 37°C. Results were observed after 24 hrs as zone of inhibition measured in millimeter (mm).

Extract preparation for phytochemical analysis

The individual plant parts were oven dried at a constant temperature of 40°C and ground thoroughly with help of a mortar and pestle. The resultant powder was individually sieved through the muslin cloth and used to prepare various extracts for the phytochemicals study (Wagner, 1996).

Phytochemical analysis

Phytochemical analysis of leaf and stem was carried out for the presence alkaloids, anthraglycosides, arbutins, bitter drugs, cardiac glycosides, coumarins, essential oils, flavonoids,

saponins and valeportriates using High Performance Thin Layer Chromatography (CAMAG) at Anchrome R & D (TLC/HPTLC specialist), Mulund. The extracts were loaded on a ready made fluorescent pre-coated silica gel-G aluminum plate (supplied by MERCK) and developed using appropriate solvent systems and detected with appropriate reagent for each of the bioactive classes as mentioned (Table 1).

Table 1: HPTLC analysis of the ten major Bioactive principles.

Bioactiveprinciples	Solvent system	Detecting agent
Alkaloids	Toluene : Ethyl acetate : Diethylamine(7: 2 : 1)	At 254nm
Anthraglycosides	Ethyl acetate : Methanol : Water(10 : 1.35 : 1)	Alcoholic kOH
Arbutin	Ethyl acetate : Methanol : Water(10 : 1.35 : 1)	Berlin Blue
Bitterdrugs	Ethyl acetate : Methanol : Water(7.7 :1.5 : 0.8)	Vannilin Sulphuric Acid
Cardiac glycosides	Ethyl acetate : Methanol : Water(10 : 1.5 : 1)	Anisaldehyde sulphuric Acid
Coumarins	Toluene : Ethyl acetate(6 : 4)	Alcoholic kOH
Essential oils	Toluene : Ethyl acetate(9.3 : 0.7)	Vannilin Sulphuric Acid
Flavonoids	Ethyl acetate : formic acid : Glacial acetic acid : Water(10 : 0.5: 0.5: 2.6)	at 254nm
Saponins	Chloroform : Glacial Acetic acid : Methanol : Water(6.4:3.2 : 1.2: 0.8)	Anisaldehyde sulphuric acid
Valeportriates	Toluene : Ethyl acetate(9.3 : 0.7)	Anisaldehyde sulphuric acid

RESULTS

Antimicrobial assay

Cold extract

Amongst all the cold extracts only chloroform extracts of leaf and stem showed moderate activity against *E. coli.* The zones of inhibition for both the extracts were found to be 14mm. None of the remaining cold extracts showed any activity against any test microorganisms.

Hot extract

All the hot extracts of leaf and stem showed activity against test microorganisms except petroleum ether (Table-2). Overall, stem showed better results than leaf extracts. All the hot extracts of stem showed higher activity against *E. coli* which ranged between 18-22mm. Rest of the test microorganisms exhibited mild activity for these extracts. Similarly hot leaf extracts showed mild to moderate activity against most the test microorganisms. In this, the highest activity was registered by methanol and ethanol extracts against *E. coli* and *S. typhi*. The other microorganisms exhibited mild zones of inhibition.

Phytochemical analysis

After development all the chromatograms were scanned, Rf values noted and number of compounds in each of the class were recorded (Table-3).

Alkaloids

Scanning of chromatogram at 254nm and 366nm showed 12 peaks in leaves with Rf value ranging from 0.04 to 0.80 while and stem showed 17 peaks with Rf values ranging from 0.06 to 0.92. Amongst these, two bands in both leaves and stem showed same Rf values.

Anthraglycoside

Anthraglycoside fraction was observed at 254nm and 580nm. The chromatogram showed 17 peaks in leaves and 11 peaks in stem with Rf values ranging from 0.06 to 0.93. Six bands of same Rf values were seen in leaves and stem.

Arbutin

The chromatogram showed presence of 25 peaks in leaves and 20 in stem with Rf values ranging from 0.05 to 0.93, of which 12 bands showed matching Rf values in parts of the plants.

Bitter principles

Scanning of chromatogram at 254nm showed 19 peaks in leaves and 14 in stem with Rf values ranging from 0.04 to 0.93. Among these three bands of leaves and stem have shown similar Rf values.

Cardiacglycoside

The HPTLC chromatogram for the detection of cardiac glycoside observed at 254nm showed four peaks in leaves and two in stem with Rf values ranging from 0.38 to 0.68 and 0.43 to 0.89 respectively.

Table 2: **Effects of hot extract on the test microorganisms.**

		Zone of inhibition (mm)			
	Microorganisms	***E.coli***	***S.typhi***	***S.aureus***	***B.subtilis***
Plant parts	**Solvents**	***(mm)***	***(mm)***	***(mm)***	***(mm)***
Leaves	Petroleum ether	-	-	-	-
	Chloroform	13	-	-	-
	Acetone	-	-	14	12
	Ethanol	17	-	13	15
	Methanol	17	16	14	14
Stem	Petroleum ether	-	-	-	-
	Chloroform	22	-	-	-
	Acetone	-	-	14	13
	Ethanol	18	-	17	14
	Methanol	19	18	13	12

+: Inhibition. - : No Inhibition mm: Zone in millimeter

Table 3: Number of bioactive principles in *Acanthus ilicifolius*.

Bioactive principle	**Leaves Wavelength in nm**			**Total**	**Stem Wavelength in nm**			**Total**	**Total (Leaves &stem)**
	254	**366**	**Vis.**		**254**	**366**	**Vis.**		
Alkaloids	8	7	-	12	10	8	-	17	27
Anthraglycoside	11	-	9	17	7	-	4	11	22
Arbutin	11	-	17	25	7	-	13	20	33
Bitter drugs	10	-	9	19	7	-	5	14	30
Cardiac glycosides	4	-	-	4	2	-	-	2	6
Coumarins	12	11	-	22	11	10	-	19	35
Essential oils	10	-	9	13	9	-	6	15	25
Flavonoids	8	-	-	8	5	-	-	5	10
Saponins	-	-	9	9	-	-	10	10	18
Valeportriates	-	-	11	11	-	-	11	11	20

Coumarins

Chromatogram scanned at 254nm and 366nm showed 22 peaks in leaves and 19 in stem with Rf values ranging from 0.05 to 0.91. Six bands in leaves and stem recorded same Rf values.

Essential oil

Scanning at visible wavelength showed 13 peaks in leaves and 15 in stem with Rf values ranging from 0.03 to 0.83. Among these three bands gave same Rf value in these parts of the plant.

Flavonoid

Scanning of chromatogram developed for flavonoid at 254 nm showed 8 peaks in leaves with Rf value ranging from 0.05 to 0.93 and 5 in stem with Rf value ranging from 0.05 to 0.47. Of these three peaks of flavonoids found in stem have similar Rf value to that found in leaves.

Saponins

Analysis of saponin fraction was only done at visible range. It showed the presence of 9 peaks in leaves and 10 in stem with Rf values ranging from 0.03 to 0.90, of which only one band showed similarRfvalue.

Valeportriate

Scanning of chromatogram of leaves and stem for the valeportriate showed the presence of 11 peaks in both the parts of the plant at visible wavelength with Rf values ranging from 0.08 to 0.83. Except two all bands were different from each other.

DISCUSSION

Antimicrobial assay

Overall hot extracts gave better zones of inhibition against test microorganisms. In this, polar extracts showed better activity than non polar solvents. Among the tissues, stem showed higher potency than leaf for the antimicrobial properties. The most susceptible organism was found to be *E. coli* followed by *S. typhi*. This result shows that *A. ilicifolius* contains mild to moderate antimicrobial principles. Further, stem was found to be more potent than leaf indicating it contains potent antimicrobials. Similar results were found in other mangrove spp. occurring along Mumbai coast (Maurya, 2006), indicating that mangrove plants are potential source of antimicrobials. Present results also indicates that *A. ilicifolius* contains thermostable antimicrobial principles of wide spectrum activity.

Phytochemical Analysis

The antimicrobial principles of plant belong to the ten classes of secondary metabolites (Cowan, 1989). The quality and quantity of these ten bioactive classes of secondary metabolites together are responsible for the medicinal properties of the plant. The ongoing results on the phytochemical analysis of *A. ilicifolius* showed presence of all ten bioactive principles indicating potent medicinal nature of the plant. Scanning of the chromatogram and comparing the Rf value of the plant parts under study, about 35 different types of coumarins, 33 arbutin, 30 bitter principles, 27 alkaloids, 25 essential oils, 22 anthraglycoside, 20 valeportriates and 18 saponins were observed in *A. ilicifolius*. These results indicate that this species is a rich source of these secondary metabolites. Besides this few numbers of flavonoids (10) and cardiac glycosides (6) adds to the medicinal potency of this plant. Therefore it is suggested that *A. ilicifolius* can serve as best source for these phytoconstituents. Further attempts may be made to study this plant for its medicinal property against several diseases which may lead to novel drug discovery.

REFERENCES

1. Bandaranayke W. M. Traditional and medicinal uses of mangroves. *Mangroves and salt marshes*, Springer Netherland, **2**: 133 (1998).
2. Banergee L. K. and Ghosh D. Species diversity distribution of mangroves in India. *An anthology of Indian mangroves*, Ed. Prof. T. Kannupandi: 20 (1998).
3. Santhanakumar G., Velmoni A., Selvaraj A. and Nagarajan T. A pilot project of Mangrove

afforestation. *An Anthology of Indian Mangroves*. Environmental information system centre, Annamalai Univ. Ministry of Environment and Forest, Govt. of India, New Delhi: 49 (1998).

4. Deshmukh S.V. and Karmarkar. Plantation of mangroves in disturbed habitat of Maharashtra. *Proceeding of the symposium on the significance of mangroves.* Maharashtra association of cultivation of science research Institute, Pune: 24 (1991).
5. Mangaonkar M. K. and Jadhav B. L. Screening of antimicrobial properties of *Avicennia marina, Avicennia alba* and *Sesuvium portulacastrum* along the Mumbai coast. *Journal of University of Mumbai*, **56**: 99 (2001)
6. Suraiya N. N. and Jadhav B. L., Screening of the antimicrobial properties of *A ilicifolius* and *Excoecaria agallocha* species of the Mumbai coast. *Trends in Life Sciences* (India), **15**(2): 85 (2000).
7. Maurya B. R., "Studies on the bioactive components of alcoholic stem extract of *Sonneratia apetela*." *Ph.D. Thesis* (Life Sciences). University of Mumbai, Mumbai-98 (2006).
8. Graham J. G., Quinin M. L. and Fabricant D. S. Plants used against Cancer. *Journal of Ethnopharmacology*, **73:** 347 (2000).
9. Babu B.H., Shylesh B.S. and Padikkala J. Tumour reducing and anti-carcinogenic activity of *Acanthus ilicifolius* in mice. *Journal of Ethnopharmacology*, **79**: 27 (2002).
10. Tewtrakul S., Subhadhirasakul S. and Kummee S. Anti-HIV activity of medicinal plants used as self medication by AIDS patients, *Songklanakarin J. Sci. Technology.*, **28**(4): 785 (2006).
11. Cowan M. M. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**: 564 (1999).
12. Patil Amitkumar. Phytochemical analysis for the presence of bioactive principles in *Rhizophora mucronata.* M.Sc Thesis, University of Mumbai, Mumbai 98 (2006).
13. Spooner D. F. and Skyes G. Laboratory assessment of antimicrobial activity. *Methods of Microbiology*. (Eds. Norris J. R. and Ribbons D. W.) Academic press, London **7**B: 217 (1972).
14. Wagner H. and Bladt S. Plant Drug Analysis: A thin layer chromatography, Atlas, published by Springer-Verlag Berlin, Heidelberg, Newyork, Tokyo, 349 (1998).