Journal of the Andaman Science Association Vol. 18(2):147-151 (2013)
ISSN 0970-4183, Printed in India

www.asapb.org

IN VITRO ANTIBACTERIAL ACTIVITY OF MANGROVE AND SEAWEEDS OF ANDAMAN ISLAND, INDIA

Baskaran R[1], Mohan P.M[1], Vijayakumar S[2]

[1]Department of Ocean Studies and Marine Biology, Pondicherry University, Port Blair-744112, Andaman and Nicobar Island-India
[2]PG and Research Department of Botany and Microbiology, AVVM Sri Pushpam College (Autonomous) Poondi 613503, Thanjavure, Tamil Nadu, India
Corresponding Author E-mail*: sivan.thamilan@gmail.com*

ABSTRACT

Aim of this study was to evaluate antibacterial activity of commonly occurring algae *Halimeda* sp. and *Gracillaria* sp. (Seaweed) and leaves extract of *Acanthus ilicifolius* (Mangrove). In the current study three different solvents namely ethyl acetate, methanol and aqueous were used for extraction. Of these solvents, ethyl acetate extract of *A. ilicifolius*, *Halimeda* sp. and *Gracillaria* sp. were showed maximum activity against most of the tested Gram positive and Gram negative bacterial strain than methanol and aqueous extracts. The study concluded that ethyl acetate would be suitable solvent for extraction of antibacterial compound from mangrove and marine macroalgae and also these have high potential antibacterial compound. However, further research is necessary to confirm the activity of the crude extracts to determine the chemical structure of the active compounds.

Keywords: *Acanthus ilicifolius*; *Halimeda* sp.; *Gracillaria* sp.; antibacterial activity.

INTRODUCTION

Nature has been found a source of medicinal agents for thousands of years and an impressive number of modern drugs were isolated from natural source, because of their use on traditional medicine. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives (1).The urge of find new bioactive compounds for treating bacterial infection is becoming a pressing issue on a worldwide scale due to the progressive increase in antimicrobial resistance in enteric pathogens in developing countries is becoming a decisive area of concern (2). *Acanthus ilicifolius* L. (Acanthaceae) is a medicinal plant used against paralysis, asthma, snake bite, analgesic, anti-inflammatory, antioxidant, heptoprotective and also against smallpox and ulcer (3, 4). Phytochemical screening indicates the presence of flavonoids and terpenes (5). Recent research evident that Indian mangrove contained antibacterial properties (6).

The aptitude of seaweeds to produce secondary metabolites of promising interest has been extensively documented. There are several reports of compounds resultant from macroalgae with a wide range of biological activities such as antivirals and antibacterials (7,8). The chemical compounds responsible for the antibacterial activity in algae have been variously identified as bromophenols, carbonyls, halogenated aliphatic compounds, terpenes, isoprenylated and brominated hydroquinone, as well as phlorotannins (9). The capacity of marine macroalgae to produce inhibitory substances against bacteria has been reported, but not widely exploited as sources of new drugs (10). The present study describes the antibacterial activity of *A. ilicifolius*, *Halimeda* sp. and *Gracillaria* sp. from Andaman Island, India.

MATERIALS AND METHODS

Algal and plant material

The leaves of mangrove were collected from the mangrove forests of Carbyns Cove and algae were collected by hand picking from the Barmanallah, South Andaman Island (Lang. 11°30’1" N - 11°44’56"E and 92°34’56"E – 92°47’18"E), India (Figure 1). *A. ilicifolius* was identified using the Manual of Indian Mangrove and seaweeds were identified by seaweed specialist. The work

was carried out at Department of Ocean Studies and Marine Biology, Pondicherry University, Andaman during April to July 2012.

Preparation of extracts

Plant leaves and algal samples were washed in sterile water to remove salts, sand and other extraneous matters and then dried at room temperature. The dried algal and mangrove materials were cut into small piece and powdered with a grinding machine. 10g of all sample powder was transferred separately into 80ml of the solvents (ethyl acetate, methanol and aqueous) left for 48 hours in rotary shaker. Extracts were filtered through Whatman No1 filter paper then the residues were dried up to concentrate volume. The collected materials were used for antibacterial assay (11).

Bacterial strains

The following bacterial strains were used as test microbes *Klebsiella pneumoniae* subs- MTCC-3040, *Salmonella infantis* - MTCC- 1167, *Staphylococcus aureus* - MTCC- 3160, *Loctococcus lactis* sup sp .1-440, *Eschericia coli* (Electro aggregative) ICMR, *Vibrio cholerae* 01 Inaba- ICMR, *Pseudomonas* sp. (KU), *Proteuse* sp. MTCC- 425, *Citrobacter diserus* (KU), *Bacillus* sp. MTCC-3133.

Antibacterial assay

Antibacterial activities of extracts were evaluated by agar well diffusion method against gram positive and gram negative bacteria. The bacterial inocula was grown in nutrient broth overnight and spread over in Muller Hinden Agar medium. 100µl extract was placed in well for testing the crude extracts. The plates were incubated at 37°c and zones of inhibition were measured after the 24 hours incubation (12).

RESULTS AND DISCUSSION

Acanthus ilicifolius extracts have been used in various folk medicines as remedies against neuralgia, pison arrow wounds, coughs, asthma and bacterial infection (13). The literatures suggest that use of organic solvents provides a higher efficiency in extracting antimicrobial activities (14). In this study, three solvents were used for *A. ilicifolius* extraction. Among them, ethyl acetate extract showed highest activity against seven bacteria such as *L. lactis*, *E. coli*, *Pseudomonas* sp, *Proteuse* sp. *V. cholerae*, *S. aureus* and *Bacillus* sp. As well as methanol extract showed highest activity against four bacteria such as *Proteuse* sp., *E. coli*, *S. aureus*, and *Pseudomonas* sp. however, aqueous extract showed activity against *Proteuse* sp. only (Table 1 and Figure 2A).

The present study revealed that ethyl acetate extract showed clear zone of inhibition against most of the used bacterial strains than methanol and aqueous.

Table 1: Antibacterial activity of *Acanthus ilicifolius*

Strains	Ethyl acetate (mm)	Cont. (mm)	Methanol (mm)	Cont. (mm)	Aqueous (mm)	Cont. (mm)
K. pneumoniae	-	6	-	7	-	0
S.infantis	-	4	-	5	-	0
S. aureus	14.33±2.51	3	13.33±1.52	4	-	0
L. lactis	17.33±1.52	4	-	7	-	0
E. coli	17±1	5	14.66±1.52	3	-	0
V. cholerae	14±1	3	-	4	-	0
Pseudomonas sp.	16.33±1.52	4	13.33±1.52	4	-	0
Proteuse sp.	16.66±1.52	4	16.66±1.52	3	13.33±1.52	0
C. diserus	-	2	-	6	-	0
Bacillus sp.	13.33±1.52	3	-	2	-	0

Zone of inhibition (mm) - (-) no activity, Cont. (Control) Zone of inhibition (mm) (-)-no activity

Similarly, reported that ethyl acetate extract of whole plant showed significant anticancer activity (15). Thirunavukarasu *et al*. reported that chloroform and aqueous crude leaves extract of *A. ilicifolius* showed clear inhibition zone against *V. cholerae* and *Pseudomonas* sp. Data generated from this assay suggested that *A. ilicifolius* extracts are strongly active to gram positive and gram negative bacteria also ethyl acetate extract would be a

suitable solvent for extraction of antibacterial compound alone.

In the present study ethyl acetate extract obtained from *Halimeda* sp. showed maximum activity against six bacteria out of ten such as *L. lactis, E. coli, Pseudomonas* sp., *Proteuse* sp., *V. cholerae*, and *Bacillus* sp. did not show any activity against *S. aureus, K. pneumoniae, S. infantis* and *C. diserus* and methanol extract showed activity against only three tested strains, such as *K. pneumoniae, Proteuse* sp. and *C. diserus*. However, aqueous extract showed activity against only *Bacillus* sp. (Table 2 and Figure 2B).

Table 2: Antibacterial activity of Algae

Strains	*Halimeda* sp.			*Gracillaria* sp.		
	Ethyl acetate	Methanol	Aqueous	Ethyl acetate	Methanol	Aqueous
K.pneumoniae	-	12.00±2.00	-	-	-	-
S. infantis	-	-	-	-	-	-
S. aureus	-	-	-	-	14.66±2.08	-
L. lactis	20.33±1.15	-	-	14.00±2.00	-	-
E. coli	12.66±1.15	-	-	13.33±1.52	15.33±1.52	-
V. cholerae	15.66±1.52	-	-	-	18.33±1.52	-
Pseudomonas sp.	13.33±1.52	-	-	16.66±2.51	15.00±1.00	-
Proteuse sp	14.33±2.51	12.66±2.08	-	13.33±1.52	16.66±1.52	-
C. diserus	-	12.66±2.08	-	11.66±1.52	16.66±2.08	-
Bacillus sp.	16.33±1.52	-	15.66±2.08	16.33±1.15	12.33±1.52	18.66±2.08

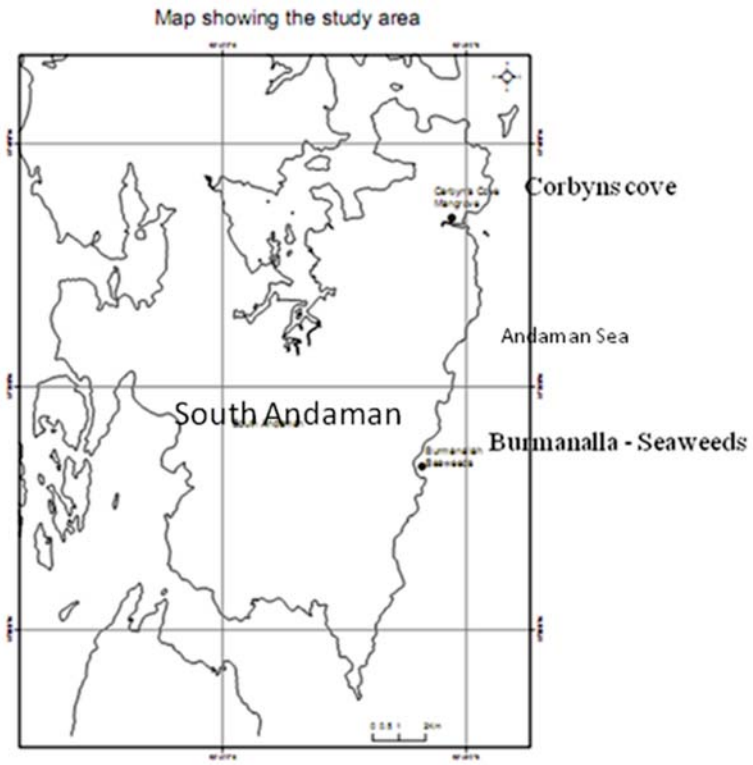

Fig. 1: Showing the sampling site

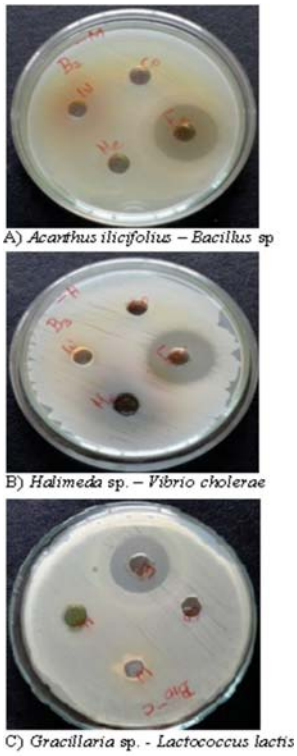

Fig. 2: Showing the zone formation of *Acanthus ilicifolius* and seaweeds

Methanol extracts of *Gracillaria* sp. showed activity against seven pathogens such as *E. coli*, *Pseudomonas* sp., *Proteuse* sp., *V. cholerae*, *S. aureus*, *C. diserus* and *Bacillus* sp., but ethyl acetate extract showed activity against only six pathogens like *L. lactis*, *E. coli*, *Pseudomonas* sp., *Proteuse* sp., *C. diserus* and *Bacillus* sp. The aqueous extract proved activity against *Bacillus* sp. (Table 2 and Figure 2C). The result from the present investigation revealed that the strongest antibacterial activity was exhibited by the ethyl acetate and methanol extract of *Halimeda* sp. and *Gracillaria* sp. against gram positive and gram negative bacterial pathogens than aqueous extract. Literatures suggested that maximum activity was noted in ethanol extract of seaweed against *Staphylococcus* sp. (13mm) and ethyl acetate showed least activity against bacterial pathogens (17) and ethanol extract of *Gracillaria ferusonii* was found to be active against *P. aeroginosa* and *B. subtilis* (10).

This result revealed that *Gracillaria* sp. has also showed antibacterial activity. However, methanol extract of *Gracillaria* sp. showed maximum activity against used pathogens followed by ethyl acetate and aqueous whereas ethyl acetate extract of *Halimeda* sp. and *Gracillaria* sp. showed activity against most of the tested organisms than methanol and water extract. It may be influenced by some factors such as the habit and water nutrient, season of algal collection, different growth stage of plant, experimental method etc. The inhibitory property may be due to the compounds such as glycerides, hydrocarbons, steroids, or phenolic substances present in the marine algal extracts (18, 19).

CONCLUSION

This study supporting that ethyl acetate would be suitable solvent for extraction of antimicrobial compound from marine macroalgae and would be expected to have potential utility. However, further research is necessary to confirm the activity of the crude extracts to determine the chemical structure of the active compounds.

ACKNOWLEDGMENT

We are thankful to the authorities who provided facility, to carry out this work in Pondicherry University. This work was accomplished with the help of University Grants Commission, Government of India's Research Fellowship in Sciences to Meritorious Students (RFSMS) granted to the first author.

REFERENCE

Allen, M. B. (1965). Antibiotic properties of marine algal. Proceeding of the seminar on sea salt and plant. CSMCRI, pp. 366-369.

Audu, J.A., Kela, A.Z. & Vnom, V.V. (2000). Antimicrobial activity of some medicinal plants. *J. Eco. Bot*., 24 : 641- 649.

Ballesteros, E., Martin, D. & Uri, M.J. (1992). Biological activity of extracts from some Mediterranean Macrophytes. *Bot. Mar.*, 35 : 481-485.

Baskaran, R. & Mohan, P.M. (2012). *In Vitro* antibacterial activity of leaf extracts of *Rhizophora mucronata* L. against multi drug resistant *Vibrio* spp. isolated from labstar larvae hatcheries. *Indian J. Geo-Marine Sciences*, 41 (3) : 218-222.

Glombitza, L.W. (1979). Antibiotics from algae. In: marine algae in pharmaceutical science. Walter de Gruyter, Berlin, pp. 303-342.

Harborne, J.B. (1973). *In phytochemical methods*, Chapman and Hall (1st ed). London and Hall, London, P.288.

Kannadasan, K. & Chandrasekaran, M. (2009). Antibacterial activity of some salt marsh halophytes and mangrove plants against methicilin resistant *Staphylococcus aureus*. *World. J. Microbi. & biotech*., 25 :155-160.

Kapil, A., Sharma, S. & Wahidulla, S. (1994). Leishrnanicidal activity of 2-benzoxazolinone from. *Acanthus ilicifolius in vitro. Planta medica.*, 60: 187-188.

Karthikaidevi, G., Manivannan, K., Thirumaran, G., Anandaraman, P. & Balasubramanian T. (2009). Antibacterial properties of selected green seaweeds from Vedalai Coastal water; Gulf of Mannar marine Bioshere Reserve. *Global J. pharm.*, 3 (2) : 107-112.

Masteller, M. (1997). Mangroves: the forgotten forest and phytochemical analysis of *Rubus imperialis*. *Z Naturalforsh*. 57 : 272-276.

Pradeep, V., Khajure, J. & Rathod, L. (2011). Potential anticancer activity of *Acanthus ilicifolius* extracted from the mangrove forest of Karwar, west coast of India. *World J. Sci. tech*. 1(1) : 01-06.

Renuka Bai, N. (2010). Evaluation of *Gracillaria frgusonii* for phytochemical analysis and antibacterial activity. *Plant Archives* : 711-713.

Rosell, K.G. & Srivastava, I.M. (1987). Fatty acids as antimicrobial substance in brown algae. *Hydrobiologia*, 151 : 471-475.

Smith, P., Hiney, M.P. & Samuelsen, O.B. (1994). Bacterial resistance to antimicrobial agents used in fish farming. *Annu. Rev. Fish Dis*., 4: 273-313.

Srinivasa, Padmini & Roa, P. (1991). Biological investigation of Indian marine algal and screening of some green, red and brown seaweed for their antimicrobial activity, *Seaweed Res.Utiln*., 14 (1) : 37-43.

Subudhi, H.N., Choudhary, B.P. & Acharya, B.C. (1992). Potential medicinal plants from Mahanadi delta in the state of Orissa. *J. Econ. Tax. Bot.*, 16 (2) : 479-487.

Thirunavukarasu, P., Ramanathan, T. & Ramkumar, L. (2011). Hemolytic and Anti Microbial Effect in the Leaves of *Acanthus ilicifolius*. *J. Pharm. Toxi*., 6 (2) : 196-200.

Tjaniadi, P., Lesmana, M. & Subekti, D. (2003). Antimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. *Am. J. Trop. Med. Hyg*., 68: 666-670.

Vlachos, V.A., Critchley, T. & Von Holy, A. (1997). Antimicrobial activity of extracts from selected south east African marine macroalgae. *S. Afr. J. Sci*., 93 : 328-332.