Advances in Biological Research 5 (1): 38-44, 2011
ISSN 1992-0067

Antibacterial Potential of Six Seaweeds Collected from Gulf of Mannar of Southeast Coast of India

R. Lavanya and N. Veerappan

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences,
Annamalai University, Parangipettai-608 502 Tamil Nadu, India

Abstract: This work reported on the *in vitro* antibacterial activity of six selected marine algae (seaweeds) which have been selected and their extracts have been tested as an alternative to commonly used antibiotics. Extracts of six seaweed samples namely *Codium decorticatum, Caulerpa scalpelliformis, Gracilaria crassa, Acanthophora spicifera, Sargassum wightii* and *Turbinaria conoides* were collected from Gulf of Mannar, southeast cost region, Mandapam, Tamil nadu, India. were selected for antibacterial activity extract solvents viz., Acetone, Methanol, Chloroform, Diethyl ether, Ethyl acetate, Hexane and aqueous were listed against selected human pathogens such as species *Vibrio parahaemolyticus*, *Salmonella* sp, *Shewanella* sp, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. All the seaweeds extracts have shown moderate antibacterial activity <10mm of zone of inhibition, out of which only Methanolic extract has shown significant activity. Results showed higher antibacterial activity were found in *Acanthophora spicifera* and minimum found in *Codium decorticatum*. From these further studies on phytochemical analysis of seaweeds is necessitated to confirm them as a better source for antimicrobial properties.

Key words: Seaweeds-antibacterial activity-extracts of different solvents-human pathogens

INTRODUCTION

Bacterial infection causes high rate of mortality in human population and aquaculture organisms. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems [1]. Nowadays the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alteration [2, 3]. Approximately 2500 new metabolites were reported from a variety of marine organisms during the years from 1977 to 1987 [4]. The Antibacterial activity of the seaweed *Gracilaria edulis* associated epiphytic bacteria against human bacterial pathogens from Indian waters [5] and also [6] from west coast of India. From SriLankan waters [7] have also screened some marine algal for their antibacterial properties.

Numerous substances were identified as antimicrobial agents from algae such as Chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocylic compounds, Phenolic inhibitors etc. Nowadays there is an increasing demand for biodiversity in the screening programmes for selecting therapeutic drugs from natural products, the marine organisms; especially seaweeds are of with immense interest, since they are having a broad range of biological activities such as antibacterial, antifungal, antiviral, antitumorals, anti-inflammatory and antioxidants. Seaweeds have been recognized as potential sources of antibiotic substances. The production of antimicrobial activities was considered to be an indicator of the seaweeds to synthesize bioactive secondary metabolites [8-10].

So in this context, the present experimental study has been made to reveal the biological and medical properties of the following marine flora such as Chlorophyceae (*Codium decorticatum, Caulerpa scalpelliformis*); Rhodophyceae (*Gracilaria crassa, Acanthophora spicifera*) and Phacophyceae (*Sargassum wightii, Turbinaria conoides*).

Corresponding Author: R. Lavanya, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502 Tamil Nadu, India. E-mail: lavanmarine@gmail.com.

MATERIALS AND METHODS

Fresh seaweeds, *Codium decorticatum, Caulerpa scalpelliformis, Gracilaria crassa, Acanthophora spicifera, Sargassum wightii* and *Turbinaria conoides* were collected from the intertidal regions of the Mandapam coast (Lat. 09° 17.417'N; Long. 079° 08.558'E) of Gulf of Mannar during the period 2008-2010 and was immediately brought to the laboratory in plastic bags containing water in order to prevent evaporation. Then these algae were washed thoroughly with tap water to remove extraneous materials. The samples were shade-dried until constant weight obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

Seaweed powder were soaked in the organic solvents with the increasing order of polarity *viz*., Acetone, Methanol, Chloroform, Diethylether, Ethyl acetate, Hexane and aqueous (1:4 w/v) and kept for two weeks at room temperature and the extracts were collected and concentrated. The concentrates were reconstituted with their respective extracts (5 mg mL^{-1}) [11-13].

Pathogens Used for the Assay: The bacterial species *Vibrio parahaemolyticus*, *Salmonella* sp, *Shewanella* sp, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa, Proteus mirabilis* were obtained from Department of Microbiology, Raja Muthiah Medical College and Hospital, Annamalai University, Annamalainagar. The pathogens were maintained on Nutrient Agar (NA) (Hi Media, India).

Antibacterial Assay: Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes [14]. Briefly, sterile filter paper discs, 6 mm in diameter (Whatman # 1), were loaded with different extracts and air-dried. Discs containing solvents alone were used as controls. The discs were placed on Muller Hinton agar (Hi Media, India) plates inoculated with each of the previously mentioned microorganisms. Plates were incubated in triplicate for each treatment for 24 h at 37°C. Zone of inhibition was recorded in millimeters.

RESULTS

The antibacterial activity of seaweeds (*C. decorticatum, C. scalpelliformis, G. crassa, A. spicifera, S. wightii* and *T. conoides)* using seven different solvents were tested against 10 human bacterial pathogen viz. *V. parahaemolyticus*, *Salmonella* sp, *Shewanella* sp, *E. coli*, *K. pneumoniae*, *S. pyogenes*, *S. aureus*, *E. faecalis*, *P. aeruginosa* and *P. mirabilis* were presented in Table 1 and Fig. 1-6.

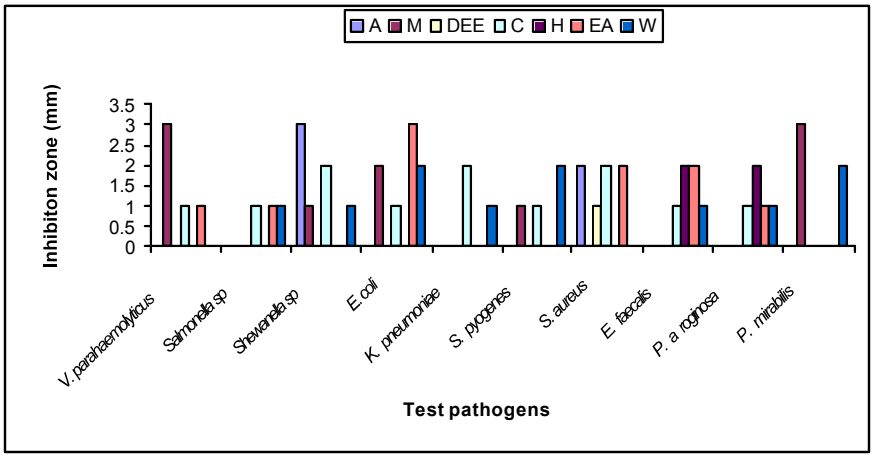
Table 1: Antibacterial activity of Seaweeds against Bacterial pathogens

Seaweed	*C. decorticatum*							*C. scalpelliformis*							*G. crassa*						
Solvent	A	M	DEE	C	H	EA	W	A	M	DEE	C	H	EA	W	A	M	DEE	C	H	EA	W
Vibrio sp	-	3	-	1	-	1	-	-	-	-	1	2	1	1	-	3	-	1	-	2	1
Salmonella sp	-		-	1	-	1	1	-	-	-	1	-	1	2	-	-	-	1	-	2	2
Shewanella sp	3	1	-	2	-	-	1	-	2	-	3	-	-	2	-	4	-	1	-	-	3
E. coli	-	2	-	1	-	3	2	-	4	2	2	-	2	-	-	2	1	3	-	2	3
Klebsiella sp	-		-	2	-	-	1	-	-	-	1	-	2	1	-	-	-	1	-	2	1
Streptococcus sp	-	1	-	1	-	-	2	-	1	-	2	-	2	1	-	1	-	1	--	2	1
Staphylococcus sp	2	-	1	2	-	2	-	3	5	-	-	-	2	2	4	5	2	2	-	2	1
Enterococci sp	-	-	-	1	2	2	1	2	2	-	-	2	2	1	5	6	4	-	3	2	2
Psedomonas aeroginosa	-	-	-	1	2	2	1	-	1	1	4	-	2	2	-	-	-	2	-	2	1
Proteus sp	-	3	-		-	-	2	-	3	-	2	-	-	1	-	6	-	1	2	-	3

A-Acetone, M-Methanol, DEE-Diethyl Ether,C-Chloroform, H-Hexane, EA-Ethyl Acetate and W-Water

Table 1: Cont.

Seaweed	*A. spicifera*							*S. wightii*							*T. conoides*						
Solvent	A	M	DEE	C	H	EA	W	A	M	DEE	C	H	EA	W	A	M	DEE	C	H	EA	W
Vibrio sp	-	-	1	1	-	2	2	-	-	-	-	3	1	2	-	-	-	1	-	2	2
Salmonella sp	-	-	-	2	-	-	1	-	1	-	1	3	-	2	-	-	-	-	-	4	-
Shewanella sp	-	2	-	1	-	1	4	-	-	-	-	1	2	2	-	3	-	-	-	2	1
E.coli	-	1	1	-	-	1	2	-	-	-	-	2	2	3	-	4	1	1	-	-	1
Klebsiella sp	-	-	-	1	-	3	3	-	-	-	2	-	4	4	-	-	-	1	-	-	2
Streptococcus sp	-	-	1	1	-	-	2	-	1	1	1	-	-	1	-	2	-	1	1	-	2
Staphylococcus sp	-	10	1	1	-	2	1	-	5	5	2	3	2	1	6	7	1	5	1	3	2
Enterococci sp	5	8	3	-	-	3	4	-	3	4	3	2	3	3	4	3	3	4	2	-	2
Psedomonas aeroginosa	-	-	-	4	-	4	3	--	-	-	1	-	1	2	-	-	-	-	1	1	2
Proteus sp	-	-	-	2	-	1	2	-	4	-	2	-	1	2	-	2	-	1	-	-	-



Acetone, M - Methanol, DEE- Diethylether, C- Chloroform, H- Hexane, EA - Ethyl Acetate and W - Water.

Fig. 1: Antibacterial activity of C. *decorticatum* against bacterial pathogens

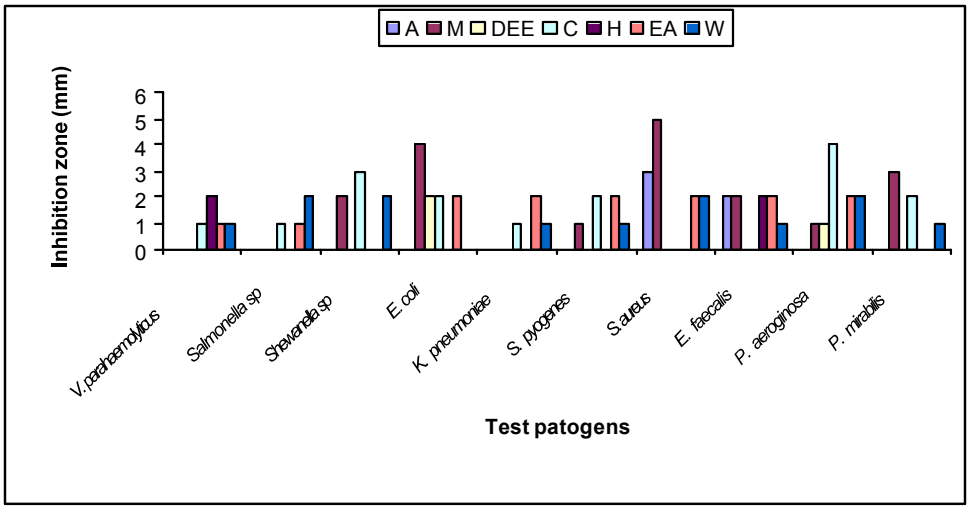


Fig. 2: Antibacterial activity of *Caulerpa scalpelliformis* against bacterial pathogens

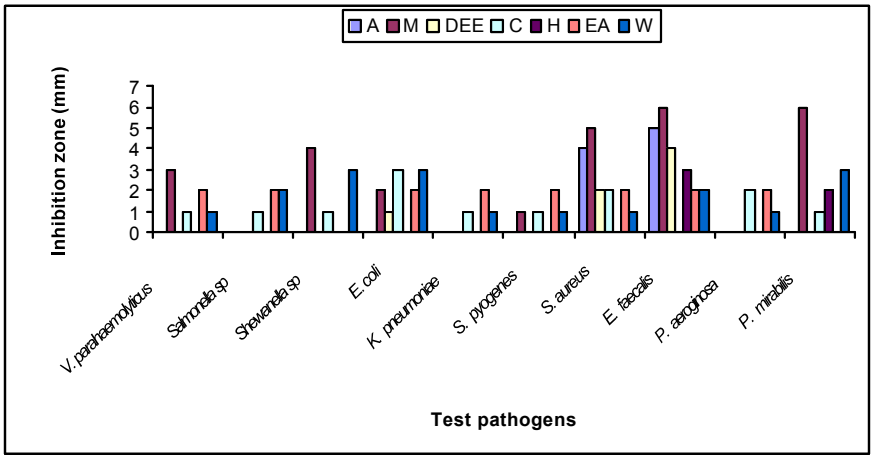


Fig. 3: Antibacterial activity of *G. crassa* against bacterial pathogens

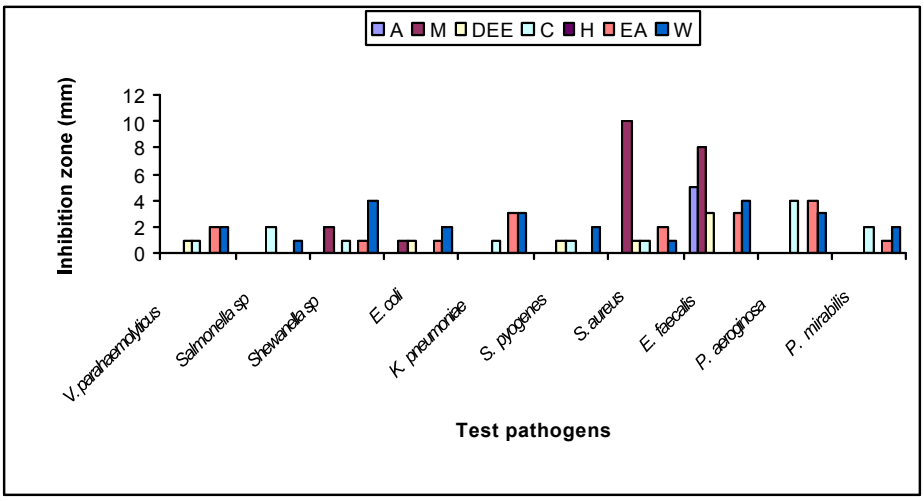


Fig. 4: Antibacterial activity of *A. spicifera* against bacterial pathogens

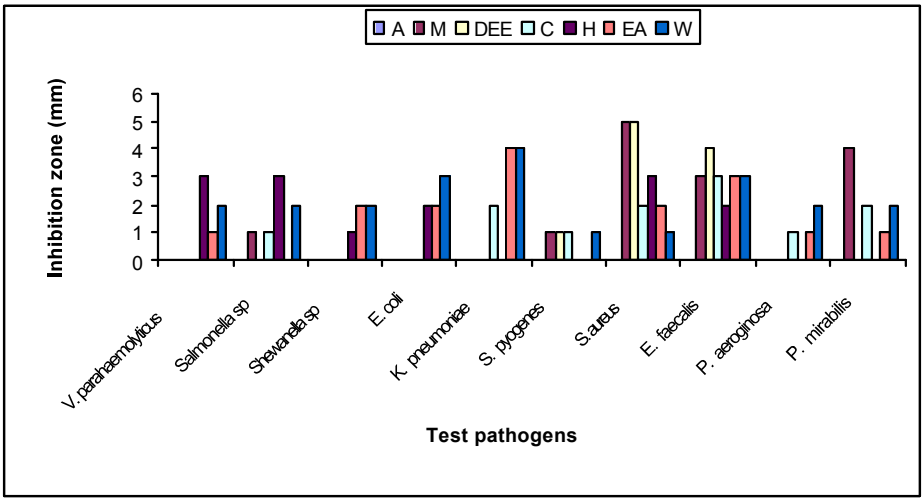


Fig. 5: Antibacterial activity of *S. wightii* against bacterial pathogens

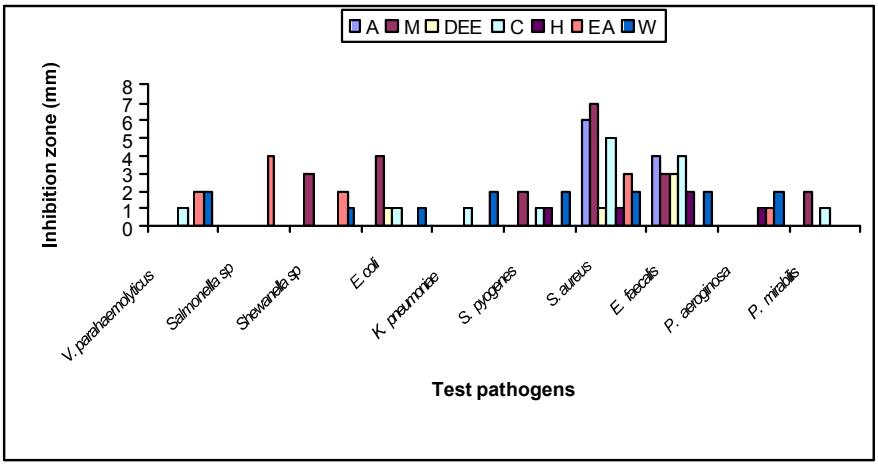


Fig. 6: Antibacterial activity of *T. conoides* against bacterial pathogens

Codium Decorticatum: The maximum inhibition zone (Fig. 1) was recorded in Methanol extracts against *V. parahaemolyticus* sp and *P. mirabilis* (3 mm) followed by Chloroform, Ethyl acetate and Aqueous extract showed slight activity against some pathogens. Acetone, Diethyl ether and Hexane extracts are inactive against most of the test pathogens.

Caulerpa Scalpelliformis: The highest inhibition zone (Fig. 2) was recorded in Methanol extracts against *S. aureus* (5mm) followed by Chloroform, Ethyl acetate and Aqueous extract also showed some moderate activity against the most of the tested pathogens. Acetone, Diethyl ether and Hexane were active only against two pathogens each.

Gracilaria Crassa: The maximum inhibition zone (Fig. 3) was obtained from Methanol extracts against *E. faecalis* and *P. mirabilis* (6mm). Aqueous, Ethyl acetate and Chloroform also showed some moderate activity against the most of the tested pathogens and Acetone and Hexane was active only against two pathogens.

Acanthophora Spicifera: The highest activity (Fig. 4) was observed in Methanol extracts against *S. aureus* (10) followed by Aqueous, Ethyl acetate and Chloroform showed moderate activity against most of the pathogens, whereas acetone is active only against *E. faecalis* and Hexane is inactive against all the tested pathogens.

Sargassum Wightii: The maximum activity (Fig. 5) was recorded from methanol and Diethyl ether extracts against *S. aureus* (5mm). Aqueous, Ethyl acetate, Chloroform, Hexane, Methanol and Diethyl ether showed moderate activity against most of the pathogens and Acetone was no activity against all the pathogens.

Turbinaria Conoides: The highest activity (Fig. 6) was obtained from methanol extracts against *S. aureus* (7mm) and acetone extracts of same pathogen (6mm). All the extracts showed moderate activity followed by Aqueous, Chloroform, Methanol, Ethyl acetate, Hexane and Acetone against all the pathogens.

DISCUSSION

Seaweeds are great potential production of secondary metabolites, which are not found in terrestrial environment. Thus, marine algae are among the richest sources of known novel bioactive compounds [15, 16].

Seaweeds extracts are considered to be a rich source of phenolic compounds [17]. The large majority of these terpenes, but fatty acids are also common with nitrogenous compounds.

The seaweeds tested in the present study for their antibacterial property includes green, brown and red algae. There are a number of reports regarding the medicinal importance of seaweeds belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae from the corners of the world [1, 8, 10, 18-27]. All the studies were made to detect the antibacterial activity from seaweeds.

In the present investigation, red and brown algae were inhibited the growth higher than the green algae. The earlier investigations [21, 28-33] found higher antibacterial activity in the extracts of brown algae than the red algae extracts, while Majin and Tan Wel [34] obtained positive results from red and brown algae. Caccamese *et al*. [29, 30] has reported that the brown algal extracts showed higher activity than the extracts of red algae. Antibacterial activity of nine species of seaweeds belonging to brown, red and green algae revealed that red and brown seaweeds had greater antibacterial activity than the green algae [35]. Similarly Selvi and Selvaraj [36] noted higher activity in brown algae and also in earlier work had [1] also, they stated that the Chlorophyceae members showed higher antibacterial activity than the Phaeophyceae and Rhodophyceae members.

In the present investigation, methanol, chloroform, ethyl acetate and aqueous extracts of the tested algae were more active than the acetone, diethyl ether and hexane extracts against the bacterial pathogens. Several earlier workers have used different solvent systems to extract bioactive principles from seaweeds and arrived at varying conclusions. Martinez - Nadal [37] mentioned that benzene and diethyl ether were the suitable solvents for extracting of antibiotic principles. Hornsey and Hide [38] used acetone as a solvent for extracting antimicrobial compounds from British marine algae. The mixture of toluene and methanol has been used by Padmakumar and Ayyakkannu [39, 40] to extract the antibacterial substances from *Enteromorpha intestinalis,* while Rao and Karmarkar [41] confirmed diethyl ether as a suitable solvent to extract the active compounds from various species of *Sargassum* but in the present investigation also revealed the methanol were found as a suitable solvent in extracting majority of the algae.

Gonzalez *et al*. [8] reported Methanol extract of *Codium decorticatum* showed no activity against Gram positive and Gram negative bacteria. But, in the Present

observation it was noticed that the extract of *Codium decorticatum* inhibiting the growth of all pathogenic bacteria except *Proteus mirabilis.* Margret *et al*. [27] reported that methanol extract of *Acanthophora spicifera* was active against Gram negative bacterial pathogen *Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli.* But, in the present study, the same extract is inactive against the same pathogens, while chloroform, ethyl acetate and aqueous extract inhibited the growth. Chiheb *et al*. [9] reported methanol extract of *Sargassum vulgare* did not show antibacterial activity against *Escherichia coli* and *Staphylococcus aereus*. Similarly in the present investigation also the methanol extract of *Sargassum wightii* was inactive against *Escherichia coli* but the same extract was inhibit the growth of *Staphylococcus aereus.* Kandhasamy and Arunachalam [1] had studied the Chlorophyceae members and it was showed with high antibacterial activity than other members. In the present study inferred with Rhodophyceae members showed higher antibacterial activity than Phaeophyceae and Chlorophyceae.

In conclusion, differences between the results of the present investigation and the results of other studies may be due to the production of bioactive compounds related to the organic solvents used for the extraction. Methanol was found to be the best solvent for majority of the algae from the collection. Rhodophycea members showed a higher antibacterial activity against the human pathogens used. They are the potential sources for bioactive compounds and it should be thoroughly be investigated for natural antibiotic properties. But, further studies should be made to identify and evaluate the actual substances responsible for the antibacterial property.

REFERENCES

1. Kandhasamy, M. and K.D. Arunachalam, 2008. Evaluation of in *vitro* antibacterial property of seaweeds of southeast coast of India. African J. Biotech., 7(12): 1958-1961.
2. Smith, P., M.P. Hiney and O.B. Samuelsen, 1994. Bacterial resistance to antimicrobial agents used in fish farming Annu. Rev. Fish. Dis., 4: 273-313.
3. Ireland, C.M., D.M. Roll, T.F. Molinsk, T.C. Mckee, T.M. Zarbriske and J.C. Swersey, 1988. Uniqueness of the marine environment: categories of marine natural product from invertebrates In: D.G. Fautin, (ed.) *Biomedical importance of marin e organisms*. California Academy of Sciences, San Francisco, pp: 41-58.
4. Arul Senthil, K.R., P. Rajesh and A. Murugan, 2008. Antibacterial activity of the crude extracts of the Seaweed *Padina boergesenii*. Seaweed Res. Utiln., 30: 177-182.
5. Emmanuel, S., J. Jebasingh, P. Raja and A. Murugan, 2008. Antibacterial activity of the seaweed *Gracilaria edulis* associated epiphytic bacteria against human bacterial pathogens. Seaweed Res. Utiln., 30: 183-189.
6. Naqvi, S.W, A. Solimabi, S.Y. Kamat, L. Fernandes, C.V.G. Reddy, D.S. Bhakuni and B.N. Dhawan, 1981. Screening of some marine plants from the India coast for biological activity. Bot. Mar., 24: 51-55.
7. Sachithananthan, K. and A. Sivapalan, 1975. Antibacterial properties of some marine algae of Sri Lanka. Bulletin of Fisheries Research Station, Sri Lanka, 26: 5-9.
8. Gonzalez Del Val, A., G. Platas, A. Basilio, J. Gorrochategui, I. Suay, F. Vicente, E. Portillo, M. Jimenez Del Rio, R.G. Garcia and F. Pelaez, 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. Microbial., 4: 35-40.
9. Chiheb, I., R. Hassane, Martinez- L. José, D.S.J. Francisco, G.V.J. Antonio, B. Hassan and K. Mohamed, 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr. J. Biotechnol., 8(7): 1258-1262.
10. Srivastava, N., K. Saurav, V. Mohanasrinivasan, K. Kannabiran and M. Singh, 2010 Antibacterial Potential of Macroalgae Collected from the Mandapam Coast, India British J. Pharmacol. and Toxicol., 1(2): 72-76.
11. Evans, W.C., 1997. An index of medicinal plants A text Book of Pharmacognosy 14th edn. Bailliere Tindall Ltd., London, 7(5): 12-14.
12. Omulokoli, E.B. Khan and S. Chahabra, 1997. Antiplasmodial activity of four Kenyan medicinal plants. J. Ethano Pharmacol., 56: 133-137.
13. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.
14. Norris, J.N. and W.H. Fenical, 1985. Natural products chemistry: uses in ecology and systematics. In: M.M. Littler and D.S. Littler, eds, Handbook of phycological methods. Ecological field methods: macroalgae. Cambridge University Press, Cambridge, pp: 121-147.
15. Faulkner, D.J., 2002. Marine natural products. Nat. Products Rep., 19: 1-48.

16. Blunt, J.W., B.R. Copp, M.H.G. Munro, P.T. Northcote and M.R. Prinsep, 2006. Marine natural products. Natur. Prod. Report, 23: 26-78.
17. Heo, S.J., E.J. Park, K.W. Lee and Y.J. Jeon, 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour. Technol., 96: 1613-1623.
18. Rao, P. and K.S. Parekh, 1981a. Antibacterial activity of Indian seaweeds. Phykos, 23: 216-221.
19. Rao, P.S. and K.S. Parekh, 1981b. Antibacterial activity of Indian seaweed extracts. Bot. Mar., 24: 577-582.
20. Rao, O.S., K.S. Parekh, H.H. Parekh, H.M. Mody, S.B Trivedi and B.H. Dave, 1982. In Marine algae in pharmaceutical science vol.2. Ec. Hoppe and levering walter de Gruyter and co., Berlin.
21. Reichelt, J.L. and M.A. Borowitzka, 1984. Antimicrobial activity from marine algae: Results of a large-scale screening programme. Hydrobiologia, 116/117: 158-168.
22. Zheng, Yi, Chen Yin-shan and L.U. Hai-sheng, 2001. Screening for antibacterial and antifungal activities in some marine algae from the Fujian coast of China with three different solvents. Chinese J. Oceanol. and Limnol., 19(4): 327-331.
23. Tuney, I., B.H. Cadirci, D. Unal and A. Sukatar, 2006. Antimicrobial activities of the extracts of marine algae from the coast of Urla (izmir, Turkey). Turk. J. Biol., 30: 171-175.
24. Selvaraj, R., M. Ayyappan, R. Shiva and S. Kabilan, 2006. Screening for antifungal activity of macro algae. Seaweed Res. Utiln., 28(1): 89-98.
25. Taskin, E., M. Ozturk, E. Taskin and O. Kurt, 2007. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. Biotechnol., 6(24): 2746-2751.
26. Salvador, N., A. Gómez Garreta, L. Lavelli and L. Ribera, 2007. Antimicrobial activity of Iberian macroalgae. Sci. Mar., 71: 101-113.
27. Margret, R.J, S. Kumaresan and G. Indra jasmine, 2008. Antimicrobial activities of some macro algae from the coast of Tuticorin, Tamilnadu. Seweed Res. Utiln., 30: 149-15.
28. Burkholder, R., L.M. Burkholder and L.R. Almodovar, 1960. Antibiotic activity of some marine algae of Puerto Rico. Bot. Mar., 2: 149-156.
29. Caccamese, S., R. Azzolina, G. Furnari, M. Cormaci and S. Grasso, 1980. Antimicrobial and antiviral activities of extracts from Mediterranean algae. Bot. Mar., 23: 285-288.
30. Caccamese, S., R.M. Toscana, G. Funari and M. Cormaci, 1985. Antimicrobial and antiviral activities of some marine algae from southern Italy coast. Bot. Mar., 24: 505-507.
31. Takaki, D.C., M.L. Koenong and G.C. Perira, 1988. Screening of marine algae fromn Brazilian northeastern coast for antimicrobial activity. Bot. Mar., 31: 375-377.
32. Pesando, D., 1989. Antibacterial and Antifungal activities of marine algae. In: Introduction to applied Phycology, I. Akatsuka, (Ed.), Acad. Publishing Co., The Hague., pp: 359-372.
33. Pesando, D. and B. Caram, 1984. Screening of marine algae from the French Mediterranean coast of antibacterial and antifungal activity. Bot. Mar., 27: 381-386.
34. Majin and Tan WeI-Ci, 1984. Screening for antimicrobial activities in marine algae from the Quingdao Coast, China. Hydrobiologia, 22: 517-520.
35. Rao, P.P.S., 1995. Biological investigation of Indian Phaeophyceae 12. Antimicribial activities of frozen samples of genus *Sargassum* collected from Okha, west coast of India. Seaweed Res. Utlin., 17: 105-109.
36. Selvi, M. and R. Selvaraj, 2000. Antibacterial activity of Indian seaweeds. J. Seaweed Res. Utiln., 22: 161-166.
37. Martinez-Nadal, N.G., C. Casillas, J.R. Rodriquez Perazza and L. Torreera, 1966. Antibiotic properties of marine algae. 3 *Cymoplia barbata*. Botanica Marina., 9: 21-26.
38. Hornsey, I.S. and D. Hide, 1974. The production of antimicrobial compounds by British marine algae. I. Antibiotic producing marine algae. Br. Phycol. J., 9: 353-361.
39. Padmakumar, K. and K. Ayyakkannu, 1986. Antimicrobial activity of some Marine Algae of PortoNovo and Pondicherry Waters, East Coast of India. Indian J. Marine Sci., 15: 187-188.
40. Padmakumar, K. and K. Ayyakkannu, 1997. Seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from southern coasts of India. Bot. Mar., 40: 507-515.
41. Rao, P.S. and S.M. Karmarkar, 1986. Biological investigations of the genus *Sargassum* I. Antifungal activity of fractions of different species of Sargassum. Phykos., 25: 6-11.