

RESEARCH ARTICLE

Evaluation of antibacterial properties of selected red seaweeds from Rameshwaram, Tamil Nadu, India

Krishnapriya Madhu Varier¹, Muthu Chinnadurai John Milton², Chinnasamy Arulvasu^{1*} and Babu Gajendran¹

¹Dept. of Zoology, University of Madras, Guindy Campus, Chennai-600025

²PG and Research Dept. of Advanced Zoology and Biotechnology, Loyola College, Chennai-600034, India
carulvasu@gmail.com ; +91-44-22202836; 91-44-22301003

Abstract

Crude extracts of the red seaweeds, *Gellidella acerosa*, *Gracillaria verrucosa* and *Hypnea musciformis* were analyzed for their antimicrobial activity against gram positive *Salmonella paratyphi*, *Enterococcus aerogenes*, *Staphylococcus epidermidis* and gram negative *Salmonella typhi* and *Shigella flexneri*. Methanol, ethanol, chloroform and aqueous solvents were used for extraction of seaweeds in their absolute forms. Disc diffusion assay was performed for crude extracts and shade-dried powdered samples of seaweeds. Chloroform extract of *Gracillaria verrucosa* showed highest activity of 21±1.0 mm inhibition zone than its control with 15±1.0 against *Salmonella paratyphi*. *Gellidella acerosa* showed the lowest activity against *Salmonella typhi* (7±1.0 mm) than its control with 15±1.0. None of the aqueous extracts showed antibacterial activity. This study intends at spotting out the efficiency of the selected seaweeds as a potential antibacterial agent against common human pathogenic bacteria.

Keywords: Red seaweeds, methanol, ethanol, chloroform, disc diffusion assay, *Gracillaria verrucosa*.

Introduction

Seaweeds are potential renewable resource in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes like green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae. Most of the compounds of marine algae show anti-bacterial activities (Vlachos *et al.*, 1996; Vairappan *et al.*, 2001). Many metabolites isolated from marine algae possess bioactive principles (Yang *et al.*, 2006; Venkateswarlu *et al.*, 2007; Oh *et al.*, 2008). Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Lindequist and Schweder, 2001; Newman *et al.*, 2003). Seaweed *Ulva fasciata* have showed antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are commonly found in human infections (Selvin and Lipton, 2004). Usage of organic solvents provides a higher efficacy in extracting compounds always for antimicrobial activity assay (Manivannan *et al.*, 2011). Several extractable compounds such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and they are responsible for the antibiotic activity of seaweeds. The extraction of antimicrobials from different species of seaweeds was solvent dependent. Methanol was a good solvent for extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species (Cox *et al.*, 2010). Different parts of the thalli are also known to differ in their antimicrobial potential. Extracts prepared from fresh seaweed samples are reported to show negligible antimicrobial activity as compared to that obtained with dried seaweeds.

Seasonal and geographical variation also contributes in the antimicrobial activity levels of marine algae. However, information is lacking on the seasonal and geographic variations in the specific metabolites of marine algae of antimicrobial potential, especially for the marine algae of South India (Rajasulochana *et al.*, 2009). Sreenivasa Rao and Parekh (1981) showed that crude extracts of Indian seaweeds are active only against gram positive bacteria. Antimicrobial activities against bacteria and fungi were reported by Hellio *et al.* (2000). The bactericidal agents found in algae include aminoacids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids. As a consequence of an increasing demand for biodiversity in the screening programmes seeking therapeutic agents from natural products and there is now a greater interest in marine organisms (Manilal *et al.*, 2009). Microorganisms have developed adaptation mechanisms against the action of antimicrobial drugs (Al-Haj *et al.*, 2009). This problem is one of the main reasons for continued research into antimicrobial compounds, including molecules from marine algae (Kim *et al.*, 2007). Hence, this study was aimed to screen and evaluate the efficiency of different solvent extracts of selected red seaweeds as antibacterial agents and to find out the most active seaweed species against the common pathogenic bacteria.

Materials and methods

Chemicals: Chemicals were purchased from Sigma (St. Louis, USA). Mueller–Hinton broth, agar, and sterile discs were purchased from Hi Media, Mumbai, India.

The mother broth cultures were obtained from Dept. of plant biology and plant biotechnology, Loyola College, Chennai.

Seaweed collection: Seaweeds were collected from Rameshwaram, TN, India (Latitude: 9° 16' 48" N; Longitude: 79° 18' 0" E) (Fig. 1-3). The collected samples are sorted and brought to laboratory and identified by a field botanist at University of Madras, Chennai, India. Seaweeds are shade-dried and powdered in a blender.

Extraction of seaweeds: The weighed powdered seaweed samples are mixed with solvents in the ratio 1:5 (g/mL) and kept soaked for 4 d with mechanical shaking at regular intervals. Extracts are filtered by squeezing using a muslin cloth, followed by filtration using whatman no.1 filter paper. Then, the solvents are allowed to evaporate in hot plate. The dried extracts are used for assay (Raman, 2006).

Disc diffusion assay: The antibacterial activity of the seaweed extracts was carried out by agar disc diffusion assay (Kumar *et al.*, 2001). The Muller Hinton agar (MHA) medium was used for this study. The strains were inoculated by swabbing the respective broth cultures of bacterial pathogens (diluted to 0.5 McFarland standard) using sterile swabs. Sterile filter paper discs were impregnated with the seaweed extracts (10 mg/mL) and used for the assay. The sterile discs not impregnated with the seaweed extracts serve as negative control and the commercial antibiotic discs (ciprofloxacin) serves as positive control. These discs were placed over the culture plates and incubated at 37°C overnight. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms and expressed in millimetres (Gulluce *et al.*, 2003).

Statistical analysis: Test samples were carried out independently in triplicates, data was expressed as the mean \pm standard deviation (SD) and the results were processed using Excel 2003 (Microsoft, Redmond, WA, USA).

Results

Antibacterial activity was tested with shade-dried powdered seaweed samples, diluted in both saline and water (10 mg/mL). None of these showed activity. Among the solvent extracts, methanol extract showed best results for both positive and negative strains. Methanol and ethanol extracts of *Hypnea musciformis* showed an inhibition zone of 19 \pm 1.0 and 16 \pm 1.0 mm that of control (15 \pm 1.0 mm) against *Salmonella typhi*. Chloroform extract of *G. Verrucosa* gave the highest zone of inhibition measuring 21 \pm 1.0 mm. Ethanol extract of *G. acerosa* also showed a zone of inhibition of 12 \pm 1.0 mm. Methanol extract of *G. acerosa* alone gave a zone of 9 \pm 1.0 mm compared to control with 19 \pm 1.0 mm against *Enterococcus aerogenes*.

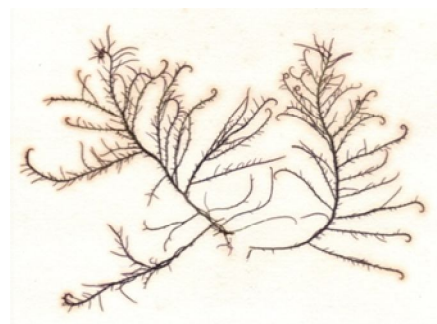
Fig. 1. *Gellidella acerosa*.



Fig. 2. *Gracillaria verrucosa*.



Fig. 3. *Hypnea musciformis*.



Ethanol and chloroform extracts of *G. verrucosa* gave clearly distinct zone of inhibition measuring 8 \pm 1.0 and 9 \pm 1.0 mm, with respect to control (25 \pm 1.0 mm) against *Staphylococcus epidermidis*. Chloroform extract of *G. verrucosa* showed similar zone of inhibition while its methanol extract showed 7 \pm 1.0 mm against *Salmonella typhi*. Methanol extract of *H. musciformis* and chloroform extract of *G. verrucosa* showed zone of inhibition around 9 \pm 1.0 and 10 \pm 1.0 mm against *Shigella flexneri*. None of the aqueous extracts showed antibacterial activity in any of the bacterial cultures (Table 1).

Table 1. Antibacterial activity of seaweed extracts against different bacterial strains (Inhibition zone in mm).

Bacteria	Seaweeds	Control	Methanol	Ethanol	Chloroform
Gram positive					
<i>Salmonella paratyphi</i>	<i>Gellidella acerosa</i>		-	12±1	-
	<i>Gracillaria verrucosa</i>	15±1	-	-	21±1
	<i>Hypnea musciformis</i>		19±1	16±1	-
<i>Enterococcus aerogenes</i>	<i>Gellidella acerosa</i>		9±1	-	-
	<i>Gracillaria verrucosa</i>	19±1	-	-	-
	<i>Hypnea musciformis</i>		-	-	-
<i>Staphylococcus epidermidis</i>	<i>Gellidella acerosa</i>		-	-	-
	<i>Gracillaria verrucosa</i>	25±1	-	8±1	9±1
	<i>Hypnea musciformis</i>		-	-	-
Gram negative					
<i>Salmonella typhi</i>	<i>Gellidella acerosa</i>		7±1	-	-
	<i>Gracillaria verrucosa</i>	15±1	-	-	15±1
	<i>Hypnea musciformis</i>		-	-	-
<i>Shigella flexneri</i>	<i>Gellidella acerosa</i>		-	-	-
	<i>Gracillaria verrucosa</i>	20±1	-	-	10±1
	<i>Hypnea musciformis</i>		9±1	-	-

Discussion

Infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem nowadays. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and emerging infectious diseases (Adaikalaraj *et al.*, 2012). It was obvious that the dried powdered samples failed to exhibit antibacterial properties. Even, reports have suggested that the samples of powdered fresh or dried seaweeds show less antibacterial activity than from extracts of powdered samples. It was also proved that, different parts of the thalli of seaweeds show antibacterial activity (Hellio *et al.*, 2000). Crude extracts showed antibacterial activities for both gram positive and negative bacteria. However, literature suggests that crude extracts of Indian seaweeds are active only against gram positive bacteria. The better results for gram positives, in general, and moderate activity of gram negative bacterial strains throw a light in this aspect. Within gram negative strains, extracts of low polar solvents showed better activity. While, in relation to gram positives, high polar solvents gave better zone of inhibition. *Hypnea* extracts showed best results among the various families of Rhodophyta (Manilai *et al.*, 2009). In this study, *H. musciformis* extracts showed best results in both methanol and ethanol extracts; while *G. verrucosa*, showed best results in chloroform extract against gram positive bacterial strains. In aqueous extracts, none of them showed activity. Comparing gram negative *Salmonella typhi* and *Shigella flexneri*, a considerable zone was revealed by *G. verrucosa* against *Salmonella typhi*, which exhibits the same activity as that of ciprofloxacin control measuring 15±1.0 mm. The resistance of gram negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules.

The membrane is also associated with the enzymes in the periplasmic space which are capable of breaking down the molecules introduced from outside (Shan *et al.*, 2007). However, the gram positive bacteria do not possess such outer membrane and cell wall structures (Kalamba and Kanicka, 2003). In case of gram negative bacterial strains, thin layer of peptidoglycan in the cell may fail to provide an active site for the binding of the bioactive compound leading to reduced inhibitory effect. The concentration at which the extracts have been loaded in the discs may also contribute to its inhibitory action. The importance of defensins in innate immunity of humans is underscored by the observation that certain disorders characterized by recurrent infections associated with a lack of defensins in blood phagocytes (Ganz and Lehrer, 1988). Moreover, transposon mutants of a pathogenic *Salmonella* strain is known to infect and grow inside phagocytes simultaneously lost their resistance to defensins (and other antimicrobial peptides) and their virulence (Groisman *et al.*, 1992). A novel class of plant peptides whose structural and functional properties resemble those of insect and mammalian defensins and hence, we termed this family of peptides 'plant defensins' (Terras *et al.*, 1995). Most defensins function by binding to the microbial cell membrane and once embedded, forming pore-like membrane defects that allow efflux of essential ions and nutrients from the bacteria. Such proteins are found among the seaweed *Ulva Lactuca* (Dang *et al.*, 2011). Results can be interpreted that these seaweeds may also confer the presence of the proteins or peptides responsible for the antibacterial activity. Moreover, such proteins or peptides become more competent in the crude solvent extraction, as it confers its availability in secondary metabolites.

Conclusion

From the study, it can be concluded that the antibacterial activity of extracts was found to be high against gram positive than gram negative strains.

The activity also varies according to the seaweeds species and the type of solvents used for extraction. The polarity of the solvents also may result in extraction of various bioactive compounds. Different solvents with different polarity may result in extraction of different types of biologically active compound from seaweeds. These bioactive compounds may go and bind to the cell wall of the microbes leading to inhibition of its growth.

Acknowledgements

Authors are thankful to UGC-SAP, New Delhi, India for financial assistance.

References

1. Adaikalaraj, G., Patric Raja, D., Johnson, M., Janakiraman, N. and Babu, A. 2012. Antibacterial potential of selected red seaweeds from Manapad coastal areas, Thoothukudi, Tamil Nadu, India. *Asian Pacific J. Trop. Med.* S1077-1080.
2. Al-Haj, N.A., Mashan N.I., Shamsudin M.N., Mohamad, H., Vairappan, C.S. and Sekawi, Z. 2009. Antibacterial activity in marine algae *Euचेuma denticulatum* against *Staphylococcus aureus* and *Streptococcus pyogenes*. *Res. J. Biol. Sci.* 4: 519-524.
3. Cox, S., Abu-Ghannam, N. and Gupta, S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Int. Food Res J.* 17: 205-220.
4. Dang, T., Yan, L., Peter, S. and Kirsten, B. 2011. Effects of micro and macroalgal diet supplementations on growth and immunity of greenlip abalone, *Haliotis laevigata*. *Aquaculture*. 320: 91-98.
5. Ganz, T., Metcalf, J.A., Gallin, J.I., Boxer, L.A. and Lehrer, R.I. 1988. Microbicidal or cytotoxic proteins of neutrophils are deficient in two disorders: Chediak-Higashi syndrome and 'specific' granule deficiency. *J. Clin. Invest.* 82: 552-556.
6. Groisman, E.A., Parra-Lopez, C., Salcedo, M., Lipps, C.J. and Heffron, F. 1992. Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. *Proc Natl. Acad. Sci.* 89(11): 939-943.
7. Gulluce, M., Sokmen, M., Deferera, D., Agar, G., Ozkan, H., Kartal, N., Polissiou, M., Sokmen, A. and Sahin, F. 2003. In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. *J. Agric. Food Chem.* 51(39): 58-65.
8. Hellio, G.B., Bremer, G., Pons, Y., Cottenceau Le Gal. and Borgougmon. 2000. Antibacterial and antifungal activities of extracts of marine algae from Brittany France. Use as antifouling agents. *Appl. Microbiol. Biotech.* 54: 543-549.
9. Kalamba, D. and Kanicka, A. 2003 Antibacterial and antifungal properties of essential oils. *Curr. Med. Chem.* 10: 813-829.
10. Kim, I.H., Lee, S.H., Ha, J.M., Ha, B.J., Kim, S.K. and Lee, J.H. 2007. Antibacterial activity of *Ulva lactuca* against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Biotechnol. Bioproc. Engg.* 12: 579-582.
11. Kumar, S., Narain, U., Tripathi, S. and Misra, K. 2001. Syntheses of curcumin bioconjugates and study of their antibacterial activities against β -lactamase-producing microorganisms. *Bio Conj. Chem.* 12: 464-469.
12. Lindequist, U. and Schweder, T. 2001. Marine biotechnology. In: Rehm, H.J Reed, G. (Eds.). *Biotechnol.* 10: 441-484.
13. Manilal, A., Sujith, S., Selvin, J., Shakir, C. and Seghal, K.G. 2009. *Int. J. Ex. Botany.* 78: 161-166.
14. Manivannan, K., Karthikaidevi, G., Anantharaman, P. and Balasubramanian, T. 2011. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. *Asian Pac. J. Trop. Biomed.* 1(2): 114-120.
15. Newman, D.J., Cragg, G.M. and Snader, K.M. 2003. Natural products as source of new drugs over the period 1981-2002. *J. Nat. Prod.* 66: 1022-1037.
16. Oh, K.B., Lee, J.H., Chung, S.C., Shin, J., Shin, H.J. and Kim, H.K. 2008. Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives. *Bioorg. Med. Chem. Lett.* 18: 104-108.
17. Rajasulochana, P., Dharmotharan, R., Krishnamoorthy, P. and Murugesan, S. 2009. Antibacterial activity of the extracts of marine red and brown algae. *J. Amer. Sci.* 5(3): 20-25.
18. Raman, N. 2006. Phytochemical analysis, New India Publications, p.10.
19. Selvin, J. and Lipton, A.P. 2004. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the Peninsular Coast of India. *J. Mar. Sci. Technol.* 12: 1-6.
20. Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. 2007. The in-vitro antibacterial activity of dietary spice and medicinal herb extract. *Int. J. Food Microbiol.* 117: 112-119.
21. Sreenivasa Rao, P. and Parekh, K.S. 1981. Antibacterial activity of Indian seaweed extracts. *Bot Mar.* 24: 577-582.
22. Terras, F.R.G., Eggermont, K., Kovaleva, V., Raikhel, N.V., Osborn, R.W., Kester, A., Rees, S.B., Torrekens, S., Van Leuven, F., Vanderleyden, J., Cammue, B.P.A. and Broekaert, W.F. 1995. Small cysteine-rich antifungal proteins from radish (*Xaphanus sativus* L.). Their role in host defense. *Plant Cell.* 7: 573-588.
23. Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M. 2001. Antibacterial halogenated metabolites from the Malaysian Laurencia species. *Phytochem.* 58: 291-297.
24. Venkateswarlu, S., Gopala, K.P., Aditya, L., Gottumukkala, V. and Subbaraju. 2007. Synthesis, structural revision, and biological activities of 4'-chloroaurone, a metabolite of marine brown alga *Spatoglossum variabile*. *Tetrahedron.* 63(29): 6909-6914.
25. Vlachos, V., Critchley, A.T. and Von Holy, A. 1999. Differential anti-bacterial activity of extras from selected southern African macro algal thalli. *Bot. Mar.* 42: 165-173.
26. Yang, R.Y., Li, C.Y., Lin, Y.C., Peng, G.T., She, Z.G. and Zhou, S.N. 2006. Lactones from a brown alga endophytic fungus (No. ZZ36) from the South China Sea and their antimicrobial activities. *Bio. Org. Med. Chem.* 45: 46-49.