Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60364-5 ©2012 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Antibacterial potential of selected red seaweeds from Manapad coastal areas, Thoothukudi, Tamil Nadu, India

Adaikalaraj G[1], Patric Raja D[1], Johnson M[1*], Janakiraman N[2], Babu A[1]

[1] *Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India*

[2] *Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli – 620 002, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 18 June 2012
Received in revised from 5 July 2012
Accepted 7 August 2012
Available online 28 August 2012

Keywords:
Antibacterial activity
Seaweeds
Methanol extract
Aqueous extract

ABSTRACT

Objective: To evaluate the antibacterial activity of *Gracilaria verrucosa* (*G. verrucosa*) (Hudson), *Hypnea musciformis* (*H. musciformis*) (Wulf) Lamour, *Enatiocladia prolifera* (*E. prolifera*) (Grev.) falk, *Gracilaria ferugosoni* (*G. ferugosoni*), *Gelidium* species and *G. verrucosa* var. against the selected bacterial pathogens. **Methods:** The antibacterial activities of methanol and aqueous hot extracts were tested against various organisms by using disc diffusion method. **Results:** The highest antibacterial activity (13 mm) was shown by the aqueous extract of *G. verrucosa* var. against *Pseudomonas aeruginosa* (*P. aeruginosa*) and the lowest activity (6 mm) was observed in the methanol extract of *E. prolifera* against *Escherichia coli* (*E. coli*). However in most of the seaweeds, methanol extract was found to be more effective. The microbial strains *Salmonella typhi* (*S. typhi*), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and *Candida albicans* (*C. albicans*) were resistant to the aqueous extracts of all seaweeds. **Conclusion:** Further work is needed to identify the principle compound which is responsible for antibacterial activity against pathogenic bacteria especially those causing the human diseases.

1. Introduction

Seaweeds are macroscopic algae found attached to the bottom in relatively shallow coastal waters. They grow in the intertidal and deep sea areas up to 180 meter depth and also in estuaries and back waters on the solid substrate such as rocks, dead corals, pebbles, shells and other plant materials. They form one of the important living resources grouped under three divisions *viz*. Chlorophyceae, Phaeophyceae and Rhodophyceae[1]. Seaweeds are the renewable living resources which are used as food, feed and fertilizer in many parts of the world. They are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals, proteins, polysaccharides and dietary fibres. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities with antiviral, antibacterial and antifungal activities[2-3]. Most of the bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides[4].

Recently, infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem. 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 reemerging infectious diseases. The new therapeutic agents should be effective and have a novel mode of action that renders them impervious to existing resistance mechanisms[5]. The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produced. These limitations demand for improved pharmacokinetic properties which necessitate the continued research for new antimicrobial compounds for the development of drugs[6]. There have been a number of

*Corresponding author: Johnson M, Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai – 627 002, Tamil Nadu, India.
Tel: +91 97869 24334;
Fax: + 91 462 2561765
E-mail: ptcjohnson@gmail.com

reports on antimicrobial activity of marine seaweeds against several pathogens[7-13].

Using organic solvents for antimicrobial activity always provides a higher efficacy in extracting compounds[14]. 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 the 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[15]. Screening of organic extracts from marine algae and other marine organisms is a common approach to identify compounds of biomedical importance. Hence, the present work was aimed to screen and evaluate the efficiency of methanol and aqueous hot extracts as antibacterial agents from selected marine seaweeds of Rhodophyceae and to select the most active species against the common pathogenic bacteria.

2. Materials and methods

2.1. Collection and preparation of seaweeds

Seaweeds belongs to the family Rhodophyceae *viz.* *Gracilaria verrucosa* (*G. verrucosa*) (Hudson), *Hypnea musciformis* (*H. musciformis*) (Wulf) Lamour, *Enatiocladia prolifera* (*E. prolifera*) (Grev.) falk, *Gracilaria ferugosoni* (*G. ferugosoni*), *Gelidium species* and *G. verrucosa* var. were collected by handpicking from the rocky shores of Manapad, Thoothukudi, Tamil Nadu, India during December 2010 to March 2011. The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, necrotic parts, pebbles and shells and brought to the laboratory in sterile polythene bags. The samples were then thoroughly washed with tap water followed by sterile distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were cut into small pieces and powdered in a mixer grinder. The powdered samples were then stored in refrigerator for further use.

2.2. Extract preparation

For Soxhlet extraction, the powdered materials (5 g) were extracted successively with 250 mL of methanol and distilled water using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman No.1 filter paper and then concentrated in vacuum at 40 ℃ using Rotary evaporator. The residues obtained were stored in a freezer −20 ℃ until further tests.

Table 1
Antibacterial activity of methanol and aqueous extracts of selected seaweeds of Rhodophyceae.

Name of the pathogen	Extract	Zone of inhibition in mm					
		G. verrucosa	*H. musciformis*	*E. prolifera*	*G. ferugosoni*	*Gelidium* sps.	*G. verrucosa* var.
Pseudomonas aeruginosa	Methanol	8	7	7	12	–	7
	Aqueous	10	10	10	11	10	13
Escherichia coli	Methanol	8	7	6	8	8	7
	Aqueous	8	10	8	8	–	7
Salmonella typhi	Methanol	8	8	–	7	7	8
	Aqueous	–	–	–	–	–	–
Streptococcus aureus	Methanol	7	11	7	7	8	11
	Aqueous	8	–	–	7	–	8
Staphylococcus aureus	Methanol	8	7	–	8	11	7
	Aqueous	–	–	–	–	–	–
Bacillus subtilis	Methanol	7	8	7	8	10	8
	Aqueous	–	–	–	–	–	–
Candida albicans	Methanol	8	10	–	12	10	10
	Aqueous	–	–	–	–	–	–

2.3. Antibacterial assay

Antibacterial activity was carried out using paper disc diffusion method. Seven bacterial strains were used *viz*. *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*), *Salmonella typhi* (*S. typhi*), *Streptococcus aureus* (*S. aureus*), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) and *Candida albicans* (*C. albicans*). Paper disc of 6 mm in diameter was prepared from Whatman No. 1 filter paper. The antibacterial assay using Gram +ve and Gram −ve bacteria was carried out using the agar plate method The bacterial strains preserved in Muller-Hinton agar medium at 4 ℃ were revived in Muller-Hinton broth (liquid medium) and incubated at (37 ± 1) ℃ for overnight. The diluted bacterial culture was placed on Muller-Hinton agar medium and spread throughout the plate using sterile glass 'L' rod. The sterile filter paper disc of 6 mm diameter soaked with the fixed volume of plant extract was inoculated placed into 10 mL aliquot of the medium and incubated at 37 ℃ for 24 h. Antibacterial activity was recorded by measuring the diameter of zone of inhibition. Chloramphenicol was used as positive reference. Each and every test was performed in triplicates.

3. Results

The antibacterial efficacy of the methanol and aqueous extracts of selected seaweeds using agar disc diffusion method is tabulated in Table 1. The results revealed that the plant extracts showed significant antibacterial activity with varying magnitudes. Most of the algal extracts exhibited antibacterial activity against all the tested bacterial species. Among the Gram +ve bacteria, *S. aureus* and Gram −ve bacteria, *P. aeruginosa* was the most sensitive to all the seaweed extracts. The highest antibacterial activity (13 mm) was shown by the aqueous extract of *G. verrucosa* var. against *P. aeruginosa* and the lowest activity (6 mm) was observed in the methanol extract of *E. prolifera* against *E. coli*. However in most of the seaweeds, methanol extract was found to be more effective. The microbial strains *S. typhi*, *S. aureus*, *B. subtilis* and *C. albicans* were resistant to the aqueous extracts of all seaweeds.

The highest antibacterial activity of *G. verrucosa* was seen in the aqueous extract (10 mm) of *P. aeruginosa* and the lowest activity was found in the methanol extract (7 mm) against *Streptococcus aureus* and *B. subtilis*. In *H. musciformis*, the highest antibacterial activity was observed in the methanol extract (11 mm) of *Streptococcus aureus* and the lowest activity was noticed in the methanol extract (7 mm) against *P. aeruginosa*, *E. coli* and *S. aureus*. The highest antibacterial activity of *E. prolifera* was seen in the aqueous extract (10 mm) of *P. aeruginosa* and the lowest activity was detected in the methanol extract (6 mm) against *E. coli*. In *G. ferugosoni*, the highest antibacterial activity was examined in the methanol extract (12 mm) of *P. aeruginosa* and *C. albicans*. The lowest activity was studied in the methanol extract (7 mm) against *S. typhi* and *S. aureus*. The highest antibacterial activity of *Gelidium* species was found in the methanol extract (11 mm) of *S. aureus* and the lowest activity was observed in the methanol extract (7 mm) against *S. typhi*. The uppermost antibacterial activity of *G. verrucosa* var. was scrutinized in the aqueous extract (13 mm) of *P. aeruginosa* and the lowest activity was seen in the methanol extract (7 mm) against *P. aeruginosa*, *E. coli* and *S. aureus*.

4. Discussion

Antibacterial assay of red, brown and green algae against the Gram +ve and Gram −ve bacteria has been established by several scientists[4]. The antibacterial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods etc. But variation in antibacterial activity may be due to the method of extraction and solvent used in extraction[16]. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kind of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antibacterial activity of seaweeds and made comparisons[17-19]. Kolanjinathan and Stella[17] indicated that acetone was the best solution for extracting the effective antimicrobial compounds. Cordeiro *et al*[18] showed successive extraction with acetone, methanol-toluene, ether and chloroform-methanol. Kim and Lee[19] used methanolic extract to observe strong antibacterial activities. Our results also correlate with the previous observations showing better antibacterial activity in methanol extract.

In the disc diffusion antibacterial assay, the selected seaweed extracts were most effective against Gram −ve strains (*P. aeruginosa* and *E. coli*) compared to Gram +ve strains (*B. subtilis* and *S. aureus*). These results are in agreement with observations on antibacterial activities of different medicinal plants as reported by previous workers[20, 21]. The resistance of Gram −ve 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[22]. However, the Gram positive bacteria do not possess such outer membrane and cell wall structures[23].

Bacterial infection causes high rate of mortality in human population and aquaculture organisms[16]. For example, *B. cereus* is responsible for causing food borne diseases[24]. *E. coli*, *S. aureus* and *P. aeruginosa* cause diseases like

mastitis, abortion and upper respiratory complications, while *S. typhi* causes diarrhoea and typhoid fever. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness[16]. This research finding gives further scope to screen the chemical constituents of the extracts which will be very useful to combat the various diseases caused by pathogenic bacteria.

Taking all the previous results together, we conclude that both the hot extracts of seaweeds showed promising activity against the tested pathogens. Among the marine algal extracts tested, some appeared to be specific in their activity against several test bacteria. This point may be important for the development of specific antibiotics and further work is needed to identify the principle compound which is responsible for antibacterial activity against pathogenic bacteria especially those causing the human diseases. Finally we recommend that, seaweeds from the coastal areas are potential sources of bioactive compounds and should be investigated for natural antibiotics.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Anantharaman P. Manual on identification of seaweed. All india coordinate project on survey and inventorization of coastal and marine biodiversity. *J Mar Bio Ass Indian* 2002; **29**: 1-9.

[2] Del Val AG, Platas G, Basilio A, Gorrochategui J, Suai I, Vicente F, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001; **4**: 35-40.

[3] Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT- Food Sci Technol* 2008; **41**: 1067-1072.

[4] Kolanjinathan K, Ganesh P, Govindarajan M. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. *Eur Rev Med Pharmacol Sci* 2009; **13**: 173-177.

[5] Westh H, Zinn CS, Rosdahl VT, Sarisa. An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. *Microbial Drug Resistance* 2004; **10**: 169-176.

[6] Al- Haj NA, Mashan NI, Shamudin MN. Antibacterial activity in marine algae *Eucheuma denticulatum* against *Staphylococcus aureus* and *Streptococcus pyogenes*. *Res J Biol Sci* 2009; **4**(4): 519-524.

[7] Taskin E, Ozturk M, Kurt O. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). *Afr Biotechnol* 2007; **6**: 2746-2751.

[8] Kolanjinathan K, Stella D. Antibacterial activity of marine macro algae against human pathogens. *Recent Res Sci Technol* 2009; **1**(1): 20-22.

[9] Rajasulochana P, Dhamotharan R, Krishnamoorthy P, Murugesan S. Antibacterial activity of the extracts of marine red and brown algae. *J Am Sci* 2009; **5**(3): 20-25.

[10] Rangaiah SG, Lakshmi P, Manjula E. Antimicrobial activity of seaweeds *Gracillaria*, *Padina* and *Sargassum* sps. on clinical and phytopathogens. *Int J Chem Anal Sci* 2010; **1**(6): 114-117.

[11] AL-Haj NA, Mashan NI, Mariana N Shamsudin, MN, Mohamad H, Vairappan CS, Sekawi Z. Antibacterial activity of marine source extracts against multidrug resistance organisms. *Am J Pharmacol Toxicol* 2010; **5**(2): 95-102.

[12] Osman MEH, Abushady AM, Elshobary ME. *In vitro* screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egypt. *Afr J Biotechnol* 2010; **9**(12): 7203-7208.

[13] Aruna P, Mansuya P, Sridhar S, Kumar JS, Babu S. Pharmacognostical and antifungal activity of selected seaweeds from Gulf of Mannar region. *Recent Res Sci Technol* 2010; **2**(1): 115-119.

[14] Manivannan K, Karthikai devi G, Anantharaman P, Balasubramanian T. Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar. *Asian Pac J Trop Biomed* 2011; **1**(2): 114-120.

[15] Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *Int Food Res J* 2010; **17**: 205-220.

[16] Kandhasamy M, Arunachalam KD. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *Afr J Biotechnol* 2008; **7**(12): 1958-1961.

[17] Kolanjinathan K, Stella D. Antibacterial activity of marine macro algae against human pathogens. *Recent Res Sci Technol* 2009; **1**(1): 20-22.

[18] Cordeiro RA, Gomes VM, Carvalho AFU, Melo VMM. Effect of proteins from the red seaweed *Hypnea musciformis* (Wulfen). Lamouroux on the growth of human pathogen yeasts. *Brazilian Arch Boil Technol* 2006; **49**(6): 915-921.

[19] Kim IH, Lee JH. Antimicrobial activities against methicillin-resistant *Staphylococcus aureus* from macroalgae. *J Indian Eng Chem* 2008; **14**: 568-572.

[20] Gurinder JK, Daljit SA. *In vitro* antibacterial activity of three plants belonging to the family Umbelliferae. *Int J Antimicrob Agent* 2008; **31**: 380-399.

[21] Salem WM, Galal H, Nasr El-deen F. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). *Afr J Microbiol Res* 2011; **5**(15): 2160-2167.

[22] Shan B, Cai Y-Z, Brooks JD, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extract. *Int J Food Microbiol* 2007; **117**: 112-119.

[23] Kalamba D, Kanicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem* 2003; **10**: 813-829.

[24] Wijnands LM. *Bacillus cereus* associated food borne disease: quantitative aspects of exposure assessment and hazard characterization. PhD Thesis, Wageningen University, Wageningen, Netherlands, 2008, p. 1-175.