International Journal of ChemTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.1, pp 01-05, Jan-March 2014

An Antibacterial activity of the Green Seaweed Caulerpha Sertularioides using Five Different Solvents

A. Pushparaj*, R.S. Raubbin and T. Balasankar

Department of Zoology, TDMNS College, T. Kallikulam – 627113.Tamil Nadu, India.

***Corres. Author: aquapush@yahoo.com**

Abstract: The green seaweed *Caulerpha sertularioides* was treated with five different types of solvents such as Acetone, Chloroform, Ethanol, Ethyl acetate and methanol. The extracts of the selected seaweeds was extracted by using soxhlet apparatus. The antibacterial activities were carried out by the agar well diffusion method. In this present study showed the significant antibacterial activity against six bacterial pathogens (*Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, E.coli., and Proteus mirabilis*) and the best activities were recorded in the 100µg/ml concentration. In low concentration (40µg/ml), the highest activities were seen in ethanol extract against *B. subtilis, E. coli and Proteus mirabilis* like 6mm, 6mm and 7mm respectively. The extracts of ethanol and ethyl acetate were showed considerable activity against all the organisms but at the same time there were no activity in the chloroform extract.
Key words: Antibacterial activity, *Caulerpha sertularioides*, agar well diffusion method.

Introduction

Seaweeds serve as an important source of bioactive natural substances. Seaweeds are commonly classified into three main groups based on their pigmentation. Phaeophyta or brown seaweeds are predominantly brown due to the presence of the carotenoid fucoxanthin and the primary polysaccharides present include alginates, laminarins, fucans and cellulose[1, 2]. Chlorophyta, or green seaweeds, are dominated by chlorophyll a and b, with ulvan being the major polysaccharide component[3]. Seaweeds are considered as a source of bioactive compounds with cystostatic, antiviral, antihelminthic, antifungal and antibacterial activities. They have also been used to treat some diseases like cancer, arthritis etc. Seaweeds are naturally renewable sources which are also used as food, feed and fertilizer in many parts of the world. They have been screened extensively to isolate life saving drugs or biologically active substances all over the world[4]. The revolutionized therapy of infectious diseases by the use of antibacterial 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 antibacterial compounds for the development of drugs[5, 6]. The extraction of major compounds from the different species of seaweeds was solvent dependent. There are a lot of reports from around the world related to that seaweed species were extracted using organic solvents[7-12]. In the present study we have focused our vision to investigate the potential ability of the green seaweed *Caulerpha sertularioides* extracted using five different solvents against six bacterial pathogens.

Material and Methods

Sample collection

The seaweed *Padina tetrastromatia* was collected from collected from Gulf Of Mannar Sea shore Thoothukudi, India. The collected sample was thoroughly washed with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and sample was brought to the laboratory in plastic bag. Then again the sample was cleaned with the fresh water and distilled water, then shade dried and powdered using mixture grander. The powdered sample stored in freezer until the extraction.

Preparation of seaweed extract

The powdered sample (10g) was extracted in soxhlet apparatus using acetone, chloroform, ethanol, ethyl acetate and methanol (250 ml) as solvents for 8h at 60^0C. The extracts were filtered using Whatman No.1 filter paper and kept it under Hot air oven (40^0 C) for the solvent evaporation. The residues obtained were stored in a freezer at -20^0 C.

Test pathogens

The identified organisms were obtained from the Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India. Extracts were tested against gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and *Lactobacillus acidophilus*) and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis*).

Antibacterial assay

The antibacterial activities carried out by agar well diffusion method[13]. The acetone, chloroform, ethanol, ethyl acetate and methanol of collected the test sample extracts were tested against the pathogens in four different concentration levels of 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively. The bacterial satins were inoculated in nutrient broth and incubated for 18 hrs at 27^0C. The Mueller Hinton agar (Hi-Media) medium prepared was using a sterile swab with 18 hours old cultures of the above mentioned test organisms, 5 wells of 6mm diameter were made in each plate with the help of a sterile cork borer. The different concentrations of the extract (40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml) were placed in the wells separately using sterile pipettes. In each plate one well was used for control (solvent alone). The Petri dishes thus prepared were incubated for 18-24hrs at 37°C. Each experimental result was measuring the zones of inhibition around the well and determined by the average of triplicates.

Result and Discussion

The seaweed *Caulerpha sertularioides* was extracted by five solvents and tested for their antibacterial activity against six strains of Gram positive and Gram negative bacteria. The antibacterial activity results were tabulated in the Table 1 to 6. In the study results showed that all the test pathogens were resistance to the extract obtained from chloroform, similarly Parekh reported that the Seaweed extract prepared in acetone, ethyl alcohol and diethyl ether showed higher antibacterial activity than the samples extracted with chloroform[14].

The maximum activity was recorded in ethanol and ethyl acetate extracts, both of these extracts were exhibit reasonable activity against all the tested pathogens. The highest activity of ethanol and ethyl acetate extracts (12mm and 18mm) were recorded against *Proteus mirabilis* at 100µg/ml. In the low concentration (40µg/ml) of ethanol extracts were exhibit the activities like 6mm, 6mm, 7mm against *B. subtilis*, *E.* coli., and *P. mirabilis* respectively and the minimum activity was also recorded by using the ethyl acetate extracts such as 6mm, 2mm, 4mm respectively against *S. aureus*, *B. subtilis* and *L. acidophilus*. This may be due to active components which are present in the seaweed extracted by the solvents.

There were no inhibitory effect from acetone extracts against *L. acidophilus* and *P. aeruginosa*, as well as methanol extract against *B. subtilis*. As suggested by Schwarz and Noble, these bacterial strains may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the compound used may not be sufficient[15]. Sastry and

Rao carried out a successive activity extraction using benzene, chloroform and methanol reported the chloroform extract exhibited the strongest activity[16], but the present study we conclude that acetone, ethanol, ethyl acetate and methanol were better than chloroform extract.

Karthikaidevi *et al*., also studied that the extraction of green seaweeds using seven solvents and were reported that antibacterial activity of marine macro alga *Chaetomorpha aerea*, the maximum antibacterial potential was recorded from the ethanol extract against *P. aeruginosa,* and the minimum was noted in methanol extracts against *Micrococcus* sp. and *S. typhii*[17]. Rajasulochana *et al*., performed antibacterial activity studies invitro with three extracts namely acetone, methanol and ethanol. They observed that *Ulva fasciata* in selective media produced good results against *E.coli*[18]. Subba Rangaiah *et al*., carried out the antibacterial activity of Chlorophycean algae *Ulva lactuca, Caulerpa taxifolia* and *Spongomorpha indica* extracted using three solvent against Gram positive, Gram negative bacterial and Fungal organisms and reported that bacterial strains were more sensitive to the seaweed extracts when compared to the fungal organisms[19]. However, seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. Most of the secondary metabolites produced by seaweeds have bacteriocidal or the antibacterial compounds derived from seaweeds consist of diverse groups of bacteriostatic properties terpenols, sterols, polysaccharides, dibutenolides peptides and proteins metabolites. Compounds with antibacterial activity have been detected in green, brown and red algae[20]. So the present study was concluded that the microorganism is the important component to create an infectious disease in human beings. So it is the right time to discover the new drugs to improve the immune power as well as the control of diseases against the human pathogens also concluded that the acetone, ethanol, ethyl acetate and methanol were better than chloroform extract.

Table No: 1 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Staphylococcus aureus* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extracts	–	–	6	7
Chloroform Extracts	–	–	–	–
Ethanol Extracts	–	7	8	11
Ethyl acetate Extracts	6	9	11	13
Methanol Extracts	–	–	–	8

Table No: 2 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Bacillus subtilis* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extracts	–	–	–	7
Chloroform Extracts	–	–	–	–
Ethanol Extracts	6	6	7	9
Ethyl acetate Extracts	2	4	7	9
Methanol Extracts	–	–	–	–

Table No: 3 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Lactobacillus acidophilus* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extracts	–	–	–	–
Chloroform Extracts	–	–	–	–
Ethanol Extracts	–	–	6	7
Ethyl acetate Extracts	4	9	11	14
Methanol Extracts	–	7	8	10

Table No: 4 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Pseudomonas aeruginosa* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extract	–	–	–	–
Chloroform Extracts	–	–	–	–
Ethanol Extract	–	–	7	9
Ethyl acetate Extract	–	7	9	11
Methanol Extract	–	–	–	7

Table No: 5 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Escherichia coli* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extract	–	–	–	6
Chloroform Extract	–	–	–	–
Ethanol Extract	6	7	8	9
Ethyl acetate Extract	–	6	9	10
Methanol Extract	–	–	–	6

Table No: 6 Antibacterial activity of the *Caulerpha sertularioides* extracts against *Proteus mirabilis* - Zone of inhibition in diameter (mm)

Test samples	Concentrations (µg/ml)			
	40	**60**	**80**	**100**
Acetone Extract	–	–	–	6
Chloroform Extract	–	–	–	–
Ethanol Extract	7	10	11	12
Ethyl acetate Extract	2	5	13	18
Methanol Extract	–	7	9	9

References

1. Goni I, Valdivieso L. and Gudiel-Urbano M., Capacity of edible seaweeds to modify in vitro starch digestibility of wheat bread. Nahrung. 2002, 46, 18–20.
2. Haugan J.A. and Liaaenjensen S., Algal Carotenoids 54. Carotenoids of Brown-Algae (Phaeophyceae). Biochem. Syst. Ecol., 1994, 22, 31–41.
3. Robic A, Gaillard C, Sassi J.F, Lerat Y, Lahaye M., Ultrastructure of Ulvan: A Polysaccharide from Green Seaweeds. Biopolymers, 2009, 91, 652–664.
4. Hellio G.B, Brrener A.M, Pons G, Cotlenceau Y. and Le Gal Borgongman., Antibacterial and antifungal activities of extracts of marine algae from Breittany. France. Use as antifouling agents. Applied microbiology and Biotechnology, 2002, 54, 543 – 549.
5. Tuney I, Cadirci B.H, Unal D. and Sukatar A., Antibacterial activities of the Extracts of marine algae from the coast of Urla (Izmir, Turkey). Turkish Journal of Biology, 2006, 30, 171-175.
6. Patra J.K, Rath S.K, Jena K, Rathod V.K. and Thatoi H.N., Evaluation of antioxidant and antibacterial activity of seaweed (Sargassum sp.) extract: A study on inhibition of Glutathione-Stransferase activity. Turkish Journal of Biology, 2008, 32, 119-125.
7. Adaikalaraj G, Patric Raja D, Johnson M, Janakiraman N. and Babu A., Antibacterial potential of selected red seaweeds from Manapad coastal areas, Thoothukudi, Tamil Nadu, India. Asian Pacific J Trop Med, 2012, S1077-1080.
8. Cox S, Abu-Ghannam N. and Gupta S., An assessment of the antioxidant and antibacterial activity of six species of edible Irish seaweeds. Int Food Res J., 2010, 17, 205-220.
9. Rizvi M.A and Shameel M., Pharmaceutical Biology of seaweeds from the Karachi Coast of Pakistan. Pharmaceut. Biol.,2005, 43(20), 97-107.
10. Vlachos V, Critchley A.T. and von Holy A., Antibacterial activity of extracts from selected southern African marine macroalgae. South Afr. J. Sci., 1997, 93(7), 382-332.
11. Caccamese S, Toscana R.M, Funari G. and Cormaci M., Antibacterial and antiviral activities of some marine algae from southern Italy coast. Bot. Mar., 1985, 24, 505-507.
12. Lima-Filho J.V.M, Carvalho A.F.F.U and Freitas S.M., Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian Coast. Braz J Microbiol., 2002, 33, 311-313.
13. Holder I.A. and Boyce S.T., Agar well diffusion assay testing of bacterial susceptibility to various antibacterials in concentrations nontoxic for human cells in culture, Burns, 1994, 20, 426- 429.
14. Parekh K.S., Antibacterially and pharmaceutically active substances from marine sources, *Ph.D Thesis*, Saurashtra Univercity, Rajkot, India, 1978.
15. Schwarz S. and Noble W.C., Aspects of bacterial resistance to antibacterials used in veterinary dermatological practice. Veterinary Dermatol., 1999, 10, 163-176.
16. Sastry V.M.V.S and Rao G.R.K., Antibacterial substances from marine algae: Successive extraction using benzene, chloroform and methanol. Botanica Marina, 1994, 37, 357-360.
17. Karthikaidevi G, Manivannan K, Thirumaran G, Anantharaman P. and Balasubaramanian T., Antibacterial properties of selected green seaweeds from Vedalai coastal waters; Gulf of Mannar marine biosphere reserve. Global J Pharmacol., 2009, 3, 107-112.
18. Rajasulochana P, Dhamotharan R, Krishnamoorthy P. and Murugesan S., Antibacterial activity of the extracts of marine red and brown algae. J Am Sci, 2009, 5(3)**,** 20-25.
19. Subba Rangaiah G, Lakshmi P. and Sruthikeerthi K., Antibacterial activity of the crude extracts of Chlorophycean seaweeds *Ulva, Caulerpa* and *Spongomorpha* sps. against clinical and phytopathogens. Drug Invention Today, 2010, 2(6), 311-314.
20. Kayalvizhi K, Vasuki S, Anantharaman P. and Kathiresan K., Antibacterial activity of seaweeds from the Gulf of Mannar. Int J Pharm Appl., 2012, 3(2), 306-314.

*****