ejpmr, 2015,2(4), 900-907

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ANTIBACTERIAL ACTIVITIES OF *CAULERPA SCALPELLIFORMIS* (R. BROWN EX TURNER) C.AGARDH FROM THE GULF OF MANNAR SOUTH EAST COAST OF INDIA

Kotteswari, M., Shanthi, N., Elamvaluthi, M and Murugesan, S*

*Unit of Algal Biotechnology and Bionanaotechnology, PG and Research Dept of Botany, Pachaiyappa's College, Chennai-600 030.

Article Received on 02/06/2015 **Article Revised on 23/06/2015** **Article Accepted on 14/07/2015**

***Correspondence for Author**

Dr. Murugesan, S

Unit ofAlgal Biotechnology and Bionanaotechnology, PG and Research Dept of Botany, Pachaiyappa's College, Chennai. 600 030.

ABSTRACT

The antibacterial activities of methanolic extract of marine alga *Caulerpa scalpelliformis* (R. Brown ex Turner) C.Agardh from Mandapam, Ramnad District, South East Coast of Tamilnadu were tested against Gram positive strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* and Gram-negative strains *Klebsiella pneumonia*, *Escharichia coli* and *Proteus vulgaris*. The results of the present study demonstrated that the methanolic extract of *Caulerpa scalpelliformis* exhibited the antibacterial activity against both Gram-positive and Gram-negative bacteria. It was observed that the extract of *Caulerpa scalpelliformis* recorded the maximum activity against *Staphylococcus aureus*.

KEYWORDS: *Caulerpa scalpelliformis*, antimicrobial activity, Disc diffusion method.

INTRODUCTION

Marine macro algae is a rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents.[1] In recent years, there are several reports of macro algae have a significant attraction as the natural source of bioactive molecules with a broad range of biological activities. There are a number of reports regarding the medicinal importance of seaweeds belonging to Chlorophyceae, Phaeophyceae, and Rhodophyceae from all over the world.[2-3] Several studies were made earlier on the antimicrobial activities of marine algae.[4-7] Marine macro algae are the most interesting group because of their broad spectrum of biological activities such as antimicrobial[8],

antiviral[9-11], antifungal[12], anti-allergic[13], anti-coagulant[14], anti-cancer [11], anti-fouling[15] and antioxidant activities.[16] They produce a wide variety of chemically active metabolites in their surroundings as an aid to protect themselves against other settling organisms.[15]

Antibacterial activity has been detected in extracts from a number of macroscopic algae from almost all groups and in different geographic areas. The secondary metabolites with antibacterial properties in algae may function as active defense mechanisms against epiphyton in the marine environment, and to maintain the capacity to recover and regenerate rapidly after predator or abrasion damage.[17] It is well known that biotic factors, such as reproductive state [18] and different thallus portions of algae [19], together with abiotic factors such as seasonality and geographic location[18, 20-21] can influence the bioactivity of algal extracts.

Infectious diseases are a major cause of morbidity and mortality worldwide.[22] Synthetic drugs are not only expensive but are also often with adulterations and side effects. Therefore, there is a need to search for new strategies to control microbial infections.[23] Pharmaceutical industries increasingly recognizing the importance of compounds derived from soil, plants and other sources such as marine organisms [24] Selective utilization of marine algae as a source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bioactive components which inhibit the growth of some of the Gram positive and Gram negative bacterial pathogens. Hence, the objective of the present study was to evaluate the antibacterial activities of the methanol extract of marine green alga *Caulerpa scalpelliformis* collected from the Mandapam, Ramnad District the South East Coast of Tamilnadu, India.

MATERIALS AND METHODS

Algae Sample collection

The marine green alga *Caulerpa scalpelliformis* (R.Brown ex Turner) C.Agardh was collected from intertidal regions of Mandapam, Ramnad District, the South East Coast of Tamilnadu, India. Details regarding the ecology of the Ramnad shore type (latitude 8°14'23.10" N, longitude 77°20'04.02"E), exposure of the collection have been described earlier [25] Seaweeds were immediately washed with seawater to remove the foreign particles, sand and epiphytes. The washed samples were transported to an ice box containing slush ice to the laboratory. In the laboratory the samples were washed thoroughly in running tap water (to remove salt) and were a shade dried.

Preparation of algal extract

Collected seaweeds were immediately washed with seawater to remove the foreign particles and shade dried. Dried seaweed was powdered and then soaked in methanol (1:20, w/v) overnight and filtered and then concentrated to obtain the crude methanolic extract residue.

Bacterial cultures

Six bacterial cultures were used in the bioassay namely *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Escherichia coli* and *Proteus vulgaris.* The pure strains were obtained from the American type culture collection (ATCC), and maintained on agar slopes at 4^0C and sub cultured for 24 hrs before use.

Inoculum preparation

The strains were maintained on nutrient agar slants at 4^0C. A loopful of each bacterial strain was added to a 50 mL sterile nutrient broth in a 100 mL conical flask. The flasks were then incubated on a rotary shaker for 24 hours to activate the strains.

Antibacterial assay

The antibacterial activity of the algal extract was evaluated by the disc diffusion method [26] A sterile cotton swab was dipped into the bacterial suspension and then was evenly streaked over the entire surface of a sterile Muller Hinton agar plate to obtain uniform inoculums. Sterile disc (6 mm dia) loaded with various of algal extract (250 to 750 µg/mL) were impregnated onto the plates and incubated overnight at 37^oC. The antibacterial activity was determined by measuring the diameter of the zone of inhibition (mm).

RESULTS AND DISCUSSION

In the past, many of our important antimicrobial compounds have been derived from terrestrial plants and microorganisms. However, with the increase in the rate of emerging resistance among the pathogenic bacteria population, the earth's oceans will have the potential to provide a novel source of structurally unique bioactive agents. Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals.[27] The cell extracts

and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria.[28]

The methanol extract of green alga *C. scalpelliformis* was investigated for *in vitro* antimicrobial activity (Table.1). The concentration of 100 µg/mL of methanolic extract of *C. scalpelliformis* inhibited the growth of all microorganisms. Some studies concerning the effectiveness of extraction methods highlight that methanol extraction yields the highest antimicrobial activity than hexane and ethyl acetate, whereas, others report that chloroform extraction is better than methanol and benzene [29] According to the present experimental results, methanol extract caused better for all strains. The seaweed extracts are responsible for its activity against Gram (+) bacteria, especially *Bacillus subtilis* and *Staphylococcus aureus*. The previous reports showed that the algal extracts were generally more effective against Gram (+) than Gram (–) bacteria, probably due to the more complex structure of the cell wall of Gram (–) bacteria.[30]

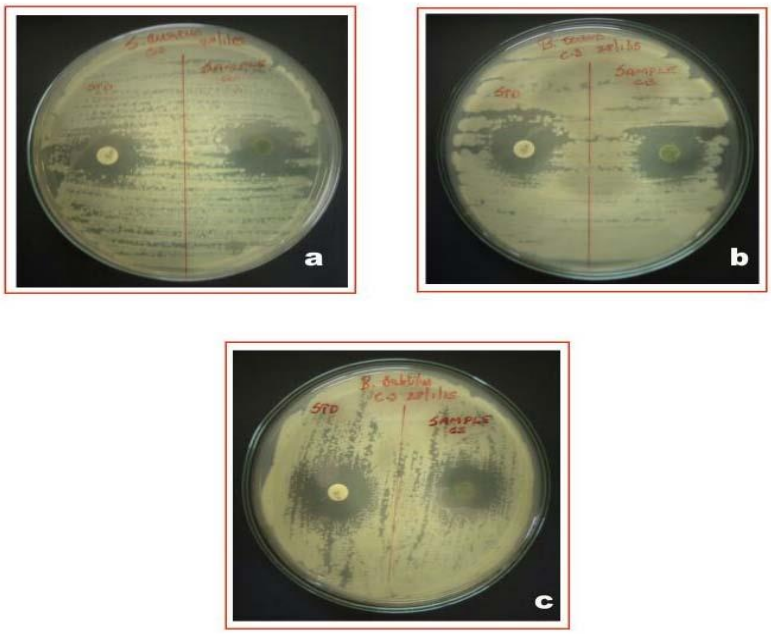
In the present study, the methanolic extract of *C. scalpelliformis* exhibited a broad spectrum antibacterial activity (Table.1; Plates.I-II). This extract inhibited both Gram-positive and Gram-negative bacteria. It is clear that organic solvents always provide a higher efficiency in extracting compounds for antimicrobial activities.[31] In the present study, there are remarkable differences in the activity of algal extract against the tested pathogenic species. It is amazing that, mostly, the antimicrobial activity toward Gram-positive species is lower than that detected against Gram-negative species. Kandhasamy and Arunachalam [32] revealed that Gram-positive organisms were more susceptible to the crude extracts of algae used.[33-34] Taskin *et al*., [35], also made similar observations, indicating that the more susceptibility of Gram-positive bacteria to the algal extract was due to the differences in their cell wall structure and their composition.[36] In Gram-negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics.[37] The presence of a thick murine layer in the cell wall also prevents the entry of the inhibitors.[38]

The result shows that the methanol extract possesses a strong antimicrobial activity against both Gram positive as well as Gram negative bacteria when compared with streptomycin as standard. However, the exact mechanism and the compound responsible for the antimicrobial activities are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and characterization of the compound. The present investigation has also proved that seaweed *C. scalpelliformis* possess antimicrobial activity to achieve

excellent results by inhibiting the growth of maximum human pathogenic bacteria. This may be due to the masking of antibacterial activity by the presence of some inhibitory compounds in the crude extracts.[39]

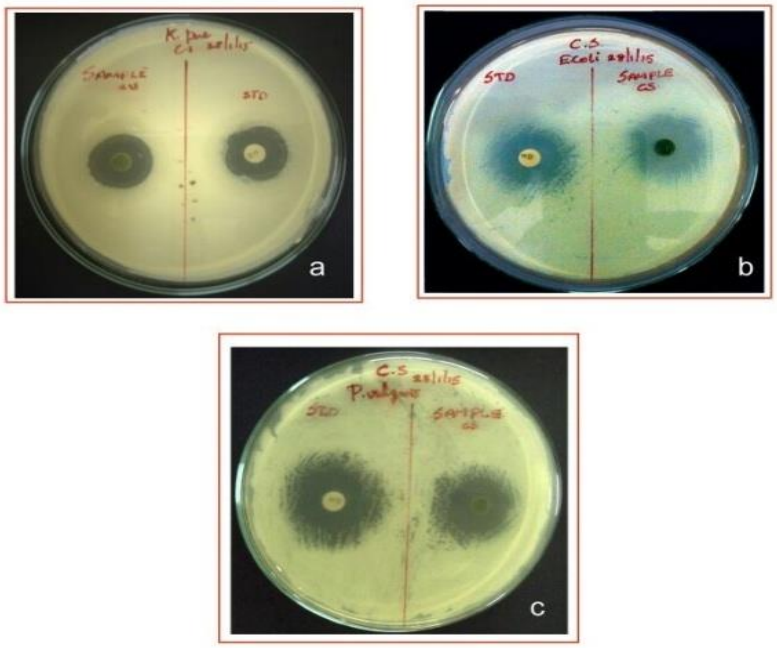
Table 1: Antimicrobial activity of methanolic extract of *C. scalpelliformis*

S.No	Microorganisms	Standard (Streptomycin) (100 µg/mL)	Methanol extract (100 µg/mL)
Gram positive bacteria			
1	*Staphylococcus aureus*	19 ± 0.001	16 ± 0.0002
2	*Bacillus subtilis*	17 ± 0.002	14 ± 0.004
3	*Bacillus cereus*	17 ± 0.001	14 ± 0.0001
Gram negative bacteria			
4	*Klebsiella pneumonia*	18 ± 0.005	12 ± 0.0002
5	*Escherichia coli*	18 ± 0.002	17 ± 0.0001
6	*Proteus vulgaris*	15 ± 0.001	13 ± 0.003



a. *Staphylococcus aureus*, b. *Bacillus subtilis*, c. *Bacillus cereus*

Plate. I Antibacterial activity of the methanolic extracts of *C. scalpelliformis* against negative microorganisms



a. *Klebsiella pneumonia* b. *Eschercia coli* c. *Proteus vulgaris*

Plate.II Antibacterial activity of the methanolic extracts of *C. scalpelliformis* against positive microorganisms

CONCLUSION

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. The marine green alga *C. scalpelliformis* is a potential sources of bioactive compounds and should be investigated for natural antibiotics.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article is reported.

REFERENCES

1. Ara, J.V, Sultana, S, Ehteshamul-Haque, R, Qasim, V.U and Ahmad. Phototherapy Res. 2002; 13: 304-307.
2. Kolanjinathan, K, Ganesh, P, Govindarajan, M. Eur.Rev. Med.Pharmacol.Sci. 2009; 13: 173-177.
3. Rajasulochana, P, Dhamotharan, R, Krishnamoorthy, P, Murugesan, S. J.Am.Sci. 2009; 3(4): 399-401.
4. Veeragurunathan, V and T. Geetha. Seaweed Res.Utili. 2009; 31(1&2): 151-155.

5. Veeragurunathan, V., R. Vellaiyan, S.Siva Subramanian, G.Sadannantham and R. Bahkyaraj. Seaweed Res.Utilin. 2008; 30(1&2): 147-152.
6. Battu, G.R, Ethadi, S, Prayaga Murthy, P, Praneeth, V.S, Rao, M. India. Int. J. Pharm. Sci. 2011; 3(4): 399-401.
7. Selvi, M.R. Selvaraj and Anandhi Chidambaram. Seaweed Res.Utilin. 2001; 23(1&2): 59-63.
8. Chiheb, I. Riadi, H. Martine –Lopez, J. Dominguez-Seglar, J.F. Gomez-Vidal, J.A. Bouziane, H. Kadiri, M.In African Journal Of Biotechnology, 2009; 8: 1258-1562.
9. Bouhlal, R., Riadi, H. Bourgougnon, N. In African Journal of Biotecnology. 2010; 9: 7968-7975.
10. Bouhlal, R. Haslin, C, Chermann, J.C Colliec Jouault, S. Sinquin, C, Simon, G. Cerantola, S, Riadi, H,Bourgougnon, N. In Marine Drugs. 2011; 9: 1187-1209.
11. Kim, S.K, Thomas, N.V. – LI, X. Advanced Food and Nutrition Research, 2011; 64: 213-224.
12. De Felicio, R, De Albuquerque, S. Young, M.C.M. Yokoya, N.S, Debonsi, H.M. In Journal Of Pharmaceutical And Biomedical Analysis, 2010; 52: 763-769.
13. Na, H.J. Moon, P.D., Lee, H.J.Kim, H.R,Chae, H.J.Shin,T,Seo,Y.Hong,S.H,Kim, H.M. In Journal of Ethnopharmocology, 2005; 101: 43-48.
14. Dayong, S.Jing, L, Shuju, G.Lijun, H. In Journal of Biotechnology, 2008; 136: 763-769.
15. Bhadury, P. Wright, C.P. In Planta, 2004; 219: 561-578.
16. Devi, G.K.Manivannan, K. Thirumaran, G. Rajathi, F.A.A. Anantharaman, P.In Asian Pacific Journal Of Tropical Medicine, 2011; 4: 205-211.
17. Vlachos, V., A.T. Critchley and A. von Holy. S. Afr. J. Sci. 1997; 93: 328–332.
18. Robles-Centeno, P.O., D.L. Ballantine and W.H. Gerwick. Hydrobiologia.1996; 326/327: 457–462.
19. Vlacos, V.A.T and Critchley Von Holy A. Botanica Marina. 1999; 42: 165-173.
20. Sreenivasa Rao, P. P. Seaweed Research and Utlization.1995; 17: 105-109.
21. Moreau, J., D. Pesando and B. Caram. Hydrobiologia.1984; 116/117: 521–524.
22. WHO (World Health Organization) Geneva, Switzerland, 2004; 120-121.
23. Sieradzki K.W.S.W. & Tomasz A. Micro. Drug. Resist. 1999; 5: 253-257.
24. McGee P. Drug Disc. Develop. 2006; 9: 18–26.
25. Krishnamurthy, V and A. Blasundaram. Seawwed Res. Utiln.1990; 12(1 &2): 1-22.
26. Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M., American Journal Clinical Pathology. 1996; 45: 493-496.

27. Freitas, A.M. Brazilian J. Microbiol.2002; 33: 311-313.
28. Ely, R., T. Supriya and C.G. Naik, J.Exp.Mar.Biol.Ecol. 2004; 309: 121 – 127.
29. Tuney, I, Cadirci, B.H, Unal, D, Sukatar A. Fres Environ Bull. 2007; 16: 428-434.
30. Stirk, W. A. Reinecke, D. L. Staden, J. V. J. Appl. Phycol. 2007; 19: 271.
31. Ortega, P.C. and F.R.M. Gonzales, Benthos.1990; 6: 31-35.
32. Kandhasamy, M. and K.D. Arunachalam African J.Biotechnol. 2008; (12): 1958-1961.
33. Tuney, I., B.H. Cadirci, D.Unal and A. Sukatar.Turk.J.Biol. 2006; 30: 171-175.
34. Salvador N, Gomez-Garreta A, Lavelli L, Ribera L. Sci. Mar. 2007; 71: 101-113.
35. Taskin E, Ozturk M, Taskin E and Kurt O. African Journal of Biotechnology. 2007; 6(24): 2746-2751.
36. Paz EA, Lacy RN, Bakhtian M. Hodder strong, London: 1995; 324.
37. Tortora GJ, Funke BR Case CL. Benjamin Cummings. San Francisco, 2001. p. 88.
38. Martin GJ. Champan and Hall, London. 1995.
39. Sastry and Rao. Botanica Marina, 1994; 37: 335-360.