Evaluation of the Antimicrobial and Antioxidant Activity of Two Chlorophyceae and Two Rhodophyceae Seaweeds from Porbandar Coast

Abhishek Dhanki¹, Smit Sindhav¹ and B. A. Jadeja¹*

¹Department of Botany, M. D. Science College, Porbandar, Gujarat, India.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors AD and SS wrote the protocol, wrote the first draft of the manuscript and managed the analysis of study. Author BAJ drafted and designed the study and organized the data of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2020/v31i1630328

Editors:
(1) Dr. Naseem A. Qureshi, National Center of Complementary and Alternative Medicine, Saudi Arabia.
(2) Prof. Marcello Iriti, University of Milan, Italy.

Reviewers:
(1) João Andrade da Silva, Universidade Federal da Paraíba, Brasil.
(2) Saravanan Gengan, K. L. Deemed to be University, India.

Complete Peer review History: http://www.sdiarticle4.com/review-history/62353

Received 17 August 2020
Accepted 22 October 2020
Published 25 November 2020

ABSTRACT

Seaweeds are significant marine sources of bioactive compounds with prospective use in inefficient foods and nutraceutical products. Four marine macroalgae species from the western coasts of Gujarat were evaluated for their antimicrobial and antioxidant activity. The antimicrobial activity of extracts of red seaweed Hypnea valentiae and Spyridia filamentosa and green seaweed Enteromorpha compressa and Caulerpa racemosa using cold percolation extraction in an in-vitro method and testing against six pathogenic bacteria and one fungi. Results showed effective inhibition zone. The antioxidant activity of the seaweeds methanol extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for measuring potential scavenger of free radical. Both activities were shown promising result of all four seaweeds. This study suggested that seaweeds could have shown a potential role in the future remedy and nutrition which can be used as drug or food additive.
1. INTRODUCTION

Marine macro-algae, commonly known as seaweeds compose one of the commercially consequential marine algae they are primordial plants without true root, stem and leaves and Seaweeds are good source of medicine. They are one of the living renewable resources of the oceans with potential vituals applications and Consumption of seaweeds as sea vegetables in human diets has been the mundane practice in several Asian countries [1]. All seaweeds produced have secondary metabolites and functions of these secondary metabolites are bulwark against herbivores, fouling organisms, and pathogens. More research can explore the possibility of optimizing the engendering of biologically active compounds and obtain bioactive metabolites with pharmaceutical activities [2] such as antimicrobial, anti-viral [3], anti-mutagenic [4], anti-inflammatory [5], anti-coagulant and antithrombotic actions [6].

A variety of in-vitro studies have demonstrated antimicrobial and antioxidant activity of algal extract and derived polyphenols and flavonoids exhibit Phytochemicals are generally safer than synthetic chemical [7]. Antioxidants in biological systems have various utility, including resistance against oxidative damage and participating in the most important signaling pathways of cells. Oxidative stress may play a key role in the expansion of cancers and cardiovascular disease and in recent times, there is a growing awareness of the finding of natural antioxidants from seaweeds, essentially there is epidemiological and clinical evidence suggesting that consumption of seaweeds reduces the risk of developing chronic disease [8].

There are immense diversity existing in the terrestrial environment, diversity prevalent in the oceans is extraordinarily rich offering an infinite resource for novel compounds as in Chlorophyceae and Rhodophyceae are equally important for biological activity because of various kinds of secondary metabolites present in various amount. Selective utilization of marine algae is important for many aspects like antibacterial and anti-fungal which inhibit the growth of some Gram-positive bacteria, Gram negative bacterial and fungal pathogens. The algal extracts were used as a curative and defensive against various diseases [9].

2. MATERIALS AND METHODS

2.1 Algae Collection and Extract Preparation

The seaweed was collected from the Porbandar and kuchhdi region in the Gujarat west coast of India. Hypnea valentiae (Rhodophyceae), Spyridia filamentosa (Rhodophyceae), Enteromorpha compressa (Chlorophyceae) and Caulerpa racemosa (Chlorophyceae) was thoroughly washed with ambient seawater to remove the epiphytes and extraneous matter than the samples were thoroughly washed with sterile distilled water and ground in to fine powder. The coarse powder of H. valentiae S. filamentosa, E. compressa and C. racemosa was subjected to cold percolation extraction method [10]. In this method Ten grams of dried H. valentiae S. filamentosa, E. compressa and C. racemosa powder was placed in 100 ml of methanol in a conical flask, plugged with cotton wool, and then kept on a rotary shaker at 220 rpm for 24 h. Then the extract was filtered with 8 layers of muslin cloth. Then filtrate was centrifuged for 10 min, the supernatant was collected, and the solvent was evaporated. The dried Extracts were stored in a refrigerator in air tight containers. Crude extracts were analyzed for preliminary antimicrobial and antioxidant activity.

2.2 Antimicrobial assay

In vitro antimicrobial activity of different four seaweed extracts was studied against 6 Pathogenic bacterial strains collected from HiMedia such as Bacillus spizizenii (ATCC 6633), Enterococcus faecalis (ATCC 19433), Staphylococcus aureus (ATCC 29737), Salmonella abony (NTCC 6017), Proteas mirabilis (ATCC 12453), Euterobacter aerogenes (ATCC 13048) and 1 pathogenic fungal strain is Candida albicans (ATCC 10231) by the agar well diffusion method. Muller hinton agar/sabouraud dextrose agar was used for antimicrobial and antifungal susceptibility test respectively. The extracts were prepared in 100% DMSO at concentration of 20 mg/ml. the microbial activity was evaluated at a concentration 2.0 mg/Well. The muller hinton agar/ sabouraud dextrose agar was melted to 50°C and wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well. The plates were incubated over night at 37°C for bacteria and

Keywords: Seaweed; seaweed extract; antimicrobial; antioxidant.
28°C for fungi. DMSO was used as negative control and the antimicrobial spectrum of the extract was determined in term of zone sizes around well.

2.3 DPPH Radical Scavenging Activity

The scavenging effects of crude methanolic extract and fractions were determined by the method of [11]. Briefly, 1 ml of 0.1 mM DPPH solution (in methanol) was added to the test tube containing 2.5 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all four sample solutions was measured at 518 nm. The scavenging effect was calculated by using % DPPH radical scavenging = [(B – A)/B] x 100 where B is the absorbance of the blank (DPPH + methanol) and absorbance of the sample is A. Sample blank and control samples were performed according to the method.

3. RESULTS AND DISCUSSION

Antimicrobial activity of various algal extracts of *H. valentiae* (Rhodophyceae), *S. filamentosa* (Rhodophyceae), *E. compressa* (Chlorophyceae) and *C. racemosa* (Chlorophyceae) against 7 different human pathogens are presented in Fig. 1(A) and 1(B). Maximum activities was recorded in the red marine algae *S. filamentosa* against *E. aerogenes* (14.23 mm) and lower inhibitory effects for *P. mirabilis* organisms were recorded in the *E. compressa* (10.16 mm).

As observed, Fig. 1(A) represent gram positive bacteria *B. spizizenii* against algal extract of *H. valentiae* showed maximum zone of inhibition (13.36 mm) and minimum zone of inhibition *E. compressa* (12.03 mm). *H. valentiae* extract displayed maximum activity against *E. faecalis* (13.46 mm) and minimum activity against *E. faecalis* showed by *C. racemosa* (12.9 mm). *S. filamentosa* had the maximum activity against *S. aureus* (12.83 mm) and minimum activity *H. valentiae* (12.83 mm). As shown in Fig. 1(B) gram negative bacteria *S. abony* against maximum activity was recorded in *S. filamentosa* (12.76 mm) and minimum activity in *E. compressa* (12.16 mm). *H. valentiae* had maximum activity against *P. mirabilis* (11.96 mm) and minimum activity in *C. racemosa* (10.07 mm). Gram negative bacteria *E. aerogenes* against maximum zone of inhibition showed by *S. filamentosa* (14.23 mm) and minimum zone of inhibition of *C. racemose* (12.53 mm). Fig. 2 shown antifungal activity showed by *C. racemosa* (13.06 mm) followed by *E. compressa* (12.07 mm), *S. filamentosa* (12.33 mm) and *H. valentiae* (11.76 mm) against *C. albicans*.

Many of the studies have reported that red algae have higher antimicrobial activity compared to other two groups and extracts prepared with methanol showed the best activity [12]. The high percentage of phenolics might be the reason for the high antimicrobial activity as high antimicrobial activity can always be directly linked to higher phenolic content suggested that phlorotannins, eckol and eckol related-compounds that have strong bactericidal activity and gave the strong antibacterial activities from brown seaweed may be due to the compounds. Agar diffusion methods using various test bacteria are significant tools for describing the antibacterial effects of aqueous and methanol extracts [13,14].

![ Gram Positive Bacteria ](image)

**Fig. 1(A).** Antibacterial activity against gram positive bacteria
DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) scavenging assay. The ability of a compound to scavenge DPPH radicals is dependent on their ability to couple with the unpaired electron of a radical reduction of antioxidant property and its absorption reduce is due to the creation of its non-radical form and this process has been attained in the presence of hydrogen donating atom [15].

In the present study observed crude extract and its fractions from red and green seaweeds. In Rhodophyceae shown in Fig. 3(A) H. valentiae at 1000 mg/ml showed higher DPPH radical scavenging activity (46.65%) compared to S. filamentosa (35.01%). Fig. 3(B) shown Chlorophyceae seaweed C. racemosa at 1000 mg/ml showed higher DPPH radical scavenging activity (32.57%) compared to E. compressa (25.72%). Fig. 3 (A) and (B) shown the radical scavenging activity of the seaweed extract increased with the increasing concentration of extract. At different concentration capacity of % inhibition of H. valentiae 23.21% to 46.65%, S. filamentosa 21.23% to 35.01, E. compressa 20.32% to 25.72% and C. racemosa 26.78% to 32.57%. DPPH scavenging activity compared with standard ascorbic acid 93.60 to 96.02 at 200 to 1000 µg/ml showed highest scavenging activity. The scavenging activity increased with increasing concentration as reported by Nagavani V and Raghava Rao [16]. The major antioxidant elements in seaweeds are thought to be polyphenols. Research had confirmed the correlation between Polyphenol content and radical scavenging activity Kuda [15].
4. CONCLUSION

*In-vitro* antimicrobial and antioxidant properties of seaweeds, 2 red seaweeds (*Hypnea valentiae* & *S. filamentosa*) and 2 green seaweeds (*Enteromorpha compressa* & *Caulerpa racemosa*) were evaluated. The antibacterial activity of extracts of *S. filamentosa* seaweeds showed very good results and showed the maximum zone of inhibition against *E. aerogenes*. Also Methanol extract of *H. valentiae*, *E. compressa* and *C. racemosa* gave positive result against bacteria as well as fungi. DPPH radical scavenging activity showed moderate results as compared to standard ascorbic acid but radical scavenging activity of the seaweed extract increased with the increasing concentration of extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors thank to Food Testing Laboratory, Department of Biotechnology, Junagadh Agricultural University, Junagadh (Gujarat) India.
for providing necessary research facilities and timely supports in order to complete research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Norziah MH, Ching CY. Nutritional composition of edible seaweed Gracilaria changgi. Food Chemistry. 2000;68(1):69-76.
2. Khalid S, Abbas M, Saeed F, Bader-Ul-Ain H, Suleria HA. Therapeutic potential of seaweed bioactive compounds. In Seaweed Biomaterials. Intech Open. 2018;5:7.
3. Chan YS, Ong CW, Chuah BL, Khoo KS, Chye FY, Sit NW. Antimicrobial, antiviral and cytotoxic activities of selected marine organisms collected from the coastal areas of Malaysia. J. Mar. Sci. Technol. 2018;26:128-136.
4. Mansour HA, Mahfouz H, Maher N. Antimitogenic potential of algal extracts on chromosomal aberrations in Allium cepa L. Acta Biologica Hungarica. 2017;68(2):137-149.
5. Khan MN, Yoon SJ, Choi JS, Park NG, Lee HH, Cho JY, Hong YK. Anti-edema effects of brown seaweed (Undaria pinnatifida) extract on phorbol 12-myristate 13-acetate-induced mouse ear inflammation. The American Journal of Chinese Medicine. 2009;37(02):373-381.
6. Kim DW, Sapkota K, Choi JH, KimYS, Kim S, Kim SJ. Direct acting anti-thrombotic serine protease from brown seaweed Costaria costata. Process Biochemistry. 2013;48(2):340-50.
7. Domettila C, Joselin J, Jeeva S. Phytochemical analysis on some South Indian seaweeds. Journal of Chemical and Pharmaceutical Research. 2013;5(4):275-278.
8. Ermakova S, Sokolova R, Kim SM, Um BH, Isakov V, Zvyagintseva T. Fucoids from brown seaweeds Sargassum hornery, Ecklonia cava, Costaria costata: Structural characteristics and anticancer activity. Applied Biochemistry and Biotechnology. 2011;164(6):841-850.
9. Lu H, Xie H, Gong Y, Wang Q, Yang Y. Secondary metabolites from the seaweed Gracilaria lemaneiformis and their allelopathic effects on Skeletonema costatum. Biochemical Systematics and Ecology. 2011;39(4-6):397-400.
10. Wiart C, Hannah A, Yassim M, Hamimah H, Sulaiman M. Antimicrobial activity of Acalypha siamensis Oliv. ex Gage. Journal of Ethnopharmacology. 2004;95(2-3):285-286.
11. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry. 2007;103(3):1003-1008.
12. Salvador N, Garretta GA, Lavelli L, Ribera L. Antimicrobial activity of Iberian macroalgae. Sci. 2007;71:101-113.
13. Ravikumar S, Ramanathan G, Subhakaran M, Inbaneson SJ. Antimicrobial compounds from marine halophytes for silkworm disease treatment. International Journal of Medicine and Medical Sciences. 2009;1(5):184-91.
14. Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T. Bactericidal activity of phlorotannins from the brown alga Ecklonia kurome. Journal of Antimicrobial Chemotherapy. 2002;50(6):889-93.
15. Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. Journal of Food Composition and Analysis. 2005;18(7):625-633.
16. Nagavani V, Raghava Rao T. Evaluation of antioxidant potential and identification of polyphenols by RP-HPLC in Michelia champaca flowers. Adv Biol Res. 2010;4(3):159-168.