Phytochemical and Antimicrobial Analysis of *Portieria Hornemannii*, A Marine Red Macro Algae

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Abstract

The present study was designed to evaluate the phytochemical activity of *Portieria hornemannii*. The primary metabolites from *Portieria hornemannii* were obtained by soxhlet extraction using various solvent like acetone, chloroform, ethyl acetate and methanol. The phytochemical analysis determined the presence of flavonoids, terpeniods Saponins, Phenol and Cardiac Glyciosides. The extracts of ethyl acetate exhibited a higher phenolic content of **764.413 ± 22.11 mg/GAE.** The antibacterial activity determined that the extracts of ethyl acetate exhibited a good zone of inhibition of 19mm and 14mm at 20µg against *Klebsiella pneumonia* and *Staphylococcus aureus.* and in the case of antifungal activity no zone of inhibition was obtained in any of the extracts.

Key words: *Portieria hornemannii, Seaweed, Phytochemical Analysis, Red algae, Antibacterial activity, anti-fungal activity.*

Introduction

Seaweeds are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities and because of these properties they are considered to be the most predominant source for bioactive compounds. Seaweeds during metabolic process, infrequently suffer serious photodynamic damage even though they grow in a harsh environment. This fact suggests that seaweed cells possess some protective compounds and mechanisms.[1] Marine algae, like other photosynthesizing plants, are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents. However, the absence of oxidative damage in the structural components of macro algae (i.e., polyunsaturated fatty acids) and their stability to oxidation during storage suggest that their cells have protective anti-oxidative defence systems which are similar to vascular plants[1,2].

In developing countries diseases are the major cause of death and accounts to 50% of it. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. But pathogens with resistance develop and spread, because of which the effect of those antibiotic drugs is reduced. This kind of resistance by bacterial species to the antimicrobial agents invoke a serious threat worldwide[3,4]. Bacterial resistance to antibiotics increases mortality likelihood of hospitalization and also increases the period of hospitalization.[5] Hence, there occurs an urge of antimicrobials with alternate strategies.[6,7] It has been well established by several scientific teams that seaweeds belonging to all three major pigments exhibit inhibitory action against both Gram negative and Gram positive bacteria. Antibacterial activity of nine species of seaweeds belonging to all major pigmentations revealed that brown and red seaweeds had greater antibacterial activity than the green and brown algae.[8] This study reveals the antibacterial and the phytochemical aspects exhibited by the marine red algae. *Portieria hornemannii* is a small red marine algal species which is widely distributed in tropical and subtropical water bodies of the Pacific and Indian Ocean.[9] Portieria belongs to the family Rhizophyllidaceae. The family Rhizophyllidaceae includes 4 genera Contarinia, Ochtodes, Nesophila and Portieria. The geographical distribution of the species belonging to the genera is interesting and exclusive.[10–12]with an evaluation of the ordinal classification of

the Florideophyceae (Rhodophyta The present study was designed to investigate the presence of major phytochemical compounds present in *Portieria hornemannii*.

Materials and Method

The crude metabolites from the sample were extracted using Soxhlet extraction method using solvents like acetone, chloroform, ethyl acetate and methanol. The extracts were subjected to phytochemical analysis to detect the presence of following biomolecules using the standard qualitative and quantitative procedures as described by Trease and Evans[13] and Total phenolic assay was determined by using Folin-Ciocalteu assay.[14]

The screening of anti-bacterial and antifungal activity against fastidious pathogens was performed with extracts of *Portieria hornemannii* by determining the zone of inhibition using disc diffusion method.[15–17]

Result and Discussion

The phytochemical characters of *Portieria hornemannii* were determined for all the crude extracts derived from the solvents after condensation in rotary vacuum evaporator.[13] From the present study, it was observed that Flavonoids, Terpenoids, Phenol and Cardiac glycosides were present in all the four crude extracts derived from acetone, Ethyl Acetate, Chloroform and methanol. However, Saponins were present only in methanol and ethyl acetate extracts (Table 1).

Table 1: Qualitative analysis of Phytochemicals from*Portieria hornemannii.*

Phytochemicals	Solvents			
	Acetone	Methanol	Ethyl Actetate	Chloroform
Tannins	-	-	-	-
Saponins	-	+	+	-
Flavanoids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	-
Cardiac Glycosides	+	+	+	+
Phenol	+	+	+	+

The seaweeds are known for their secondary active metabolites which are used in several medical and pharmaceutical industries. Metabolites like Tannins, Saponins, Flavanoids, Terpenoids, Alkaloids and phenolic compounds have a great medicinal value and are extensively used for the manufacturing of new drugs.[13] Saponins are widely used in the treatment of hypercholesterolaemia and hyperglycaemia. It is also used as a mild detergent. Apart from this saponins also possess several medical properties like anti-microbial, cholesterol lowering, anti-oxidant, anti-cancer, anti-carcinogenic, and immune modulatory activities. It also helps in the treatment of congestive heart failure by inhibiting Na^+ and Ca^{2+}antipotal by producing cytosolic Ca^{2+} which reduces congestive heart failure by strengthening heart muscles.[18]

Tannins possess antibacterial anti-cancerous and anti-viral activities, is also used for the inhibition of HIV replication.[19] Flavonoids also possess similar activities like tannins like antioxidant, anti-inflammatory, anti-cancer, antimicrobial and anti-allergic activity.[20]

Determination of Total phenols

Earlier reports have stated that the polyphenols obtained from marine algae possess a good anti-oxidant property [21,22]. Total phenolic content of the extracts was calculated from the regression equation of calibration

curve (Y = 0.001+ 0.25x; R2 = 0.966) and expressed as mg gallic acid equivalents (GE) per gram of sample in dry weight (Figure 1) The Results of the phenolic content of *Portieria hornemannii* determined the ethyl acetate extract has high phenolic content followed by methanol extract comparing to other extracts (Table 2; Figure 2). The differences between the content of phenolic compound between each solvents and extraction methods were statistically significant ($p < 0.05$) (Table 3). The presence of high phenolic compound could be useful for the prevention of oxidative activities of the extract.

Table 2. Mean ± SD of Total Phenolic Content of *Portieria hornemannii*

Solvent	Total Phenolic Content (mgGAE/g)
	Mean ± Std. Deviation
Acetone	486.206 ± 4.84
Methanol	696.146 ± 3.85
Ethyl Acetate	764.413 ± 22.11
Chloroform	449.026 ± 14.43

Table 3. Two way ANOVA Total Phenolic Content of *Portieria hornemannii*

	Sum of Squares	**Degrees of freedom**	**Mean Square**	**F-Value**	**Sig.**
Between Groups	216040.115	3	72013.372	391.723	0.000
Within Groups	1470.699	8	183.837		
Total	217510.814	11			

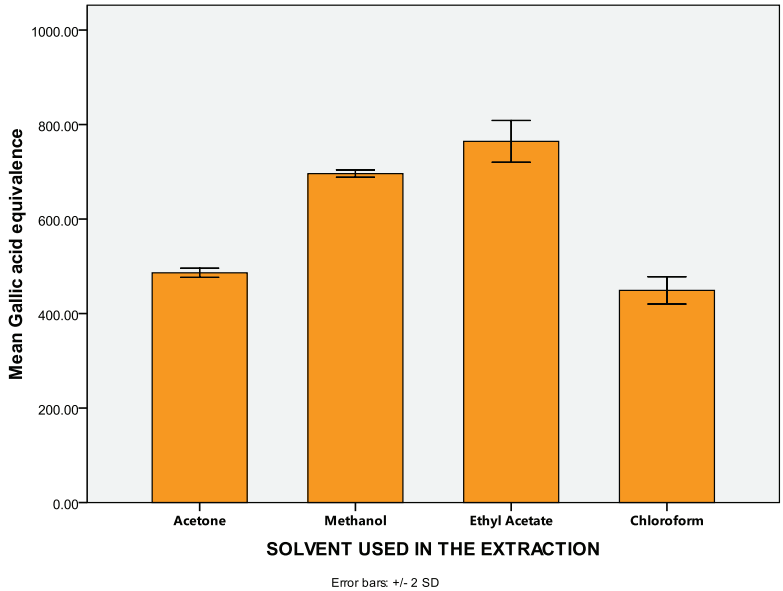

Figure 1. Mean ± SD of Total Phenolic Content of *Portieria hornemannii.*

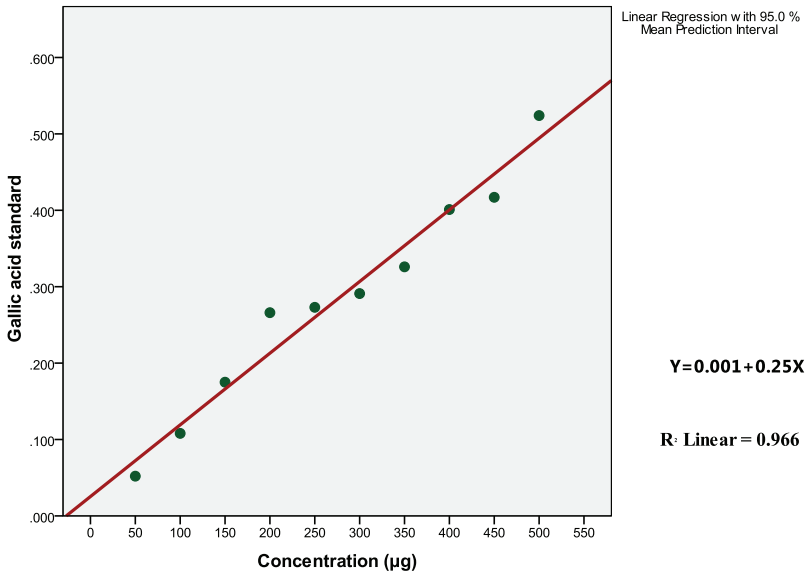

Figure 2. Gallic Acid Equivalence

Phenols are the most significant constituent in seaweed as of for their scavenging activity due to presence of the hydroxyl groups [23]. Pedersen reported that the increase in the phenolic content depends upon two major factors i.e. it increases with the aging of the tissue and also the salinity concentration of its habitat. [24] The bioactive compounds of macro algae is determined by certain aspects like environment, salinity, maturity of the algae and climatic conditions.[25] A lower phenolic content range of 1.5 to 4.1 mg GAE/g, with crude methanolic extracts of red seaweeds which is comparatively very lower with the range obtained with present study [26]. The Phenolic compounds derived from the marine algae play a vital role against the abiotic and biotic stress conditions through cell defense mechanism.[27]8 Phaeophyta and 23 Rhodophyta. In general, the biological activities of the seaweed are reflected by the phenolic compounds present in the seaweed.

Antimicrobial Activity

Antibacterial activity

In this present study the screening of the antibacterial activity was performed against five different bacterial strains *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* under various concentration of each crude extracts ranging from 2, 5, 10, and 20µg. From the study, the zone of inhibition of *Bacillus subtilis* was obtained at a concentration range of 20µg only in chloroform extracts (10 mm). Whereas, in *Klebsiella pneumonia*, the zone of inhibition (10 mm and 19 mm) was obtained at a conc. range of 20µg in both the acetone and ethyl acetate extracts. Also, the zone of inhibition against, *Escherichia coli* was observed at a conc. range of 20µg in the extracts of ethyl acetate (11 mm), methanol (10 mm) and chloroform (12 mm). Apparently, the zone of inhibition against *Staphylococcus aureus* was observed at a conc. range of both 10µg and 20µg in the extracts of acetone (10 mm, 11 mm), and ethyl acetate (10 mm, 14 mm). Finally, the zone of inhibition against *Pseudomonas aeruginosa* was observed at a conc. range of 20µg only in the extracts of chloroform (10 mm) (Table 4). Therefore the highest zone of inhibition was seen only in the ethyl acetate extracts comparing with other extracts and also the ethyl acetate extracts inhibited the growth of three bacterial strains followed by acetone and chloroformic extracts which inhibited two bacterial strains and finally methanolic extracts inhibited only a single bacterial strain (Table 4).

Table 4. Antibacterial activity of *Portieria hornemanii*

Bacterial Species	Acetone				Ethyl Acetate				Methanol				Chloroform			
	Concentration in µg															
	2	5	10	20	2	5	10	20	2	5	10	20	2	5	10	20
Bacillus subtilis	-	-	--	--	-	-	--	--	-	-	--	--	-	-	--	10
Klebsiella pneumonia	-	-	--	10	-	-	--	19	-	-	--	--	-	-	--	--
Escherichia coli	-	-	--	--	-	-	--	11	-	-	--	10	-	-	--	12
Staphylococcus aureus	-	-	10	11	--	--	10	14	-	-	--	--	-	-	--	--
Pseudomonas aeruginosa	-	-	--	--	-	-	--	--	-	-	--	--	-	-	--	10

The acetone extract of Caulerpa scalpelliformis showed broad spectrum antibacterial activity when compared to other seaweed extracts.[28,29] But in the present study the maximum zone of inhibition was obtained from the extracts of Ethyl acetate. The maximum antibacterial activity was observed in the methanol extract of Caulerpa scalpelliformis against Salmonella typhi, Micrococcus sp., and Shigella bodii. [30]. Ely *et al*., studied with the methanolic extracts of *Chadophorea profileria* exhibited a moderate antibacterial activity against *Staphylococcus aureus* and *Vibrio cholera* [31].

Anti-fungal activity

In the present study the screening of the antifungal activity was performed by agar disc diffusion method against five different fungal strains *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatius, Fusarium solanum, Exoserohium species* under various concentration of each crude extracts ranging from 2, 5, 10, and 20µg. From the study, the zone of inhibition was not observed in any of the concentrations.

In contradiction to this, the methanolic extracts of *Aspergillus taxiformis* showed inhibitory activity against fungal species like *Fusarium solanum, Aspergillus flavus,* and *trichoderma species*. The organic extracts obtained from *Aspergillus taxiformis* showed low inhibitory zone against *Aspergillus fumigatius* [32]. From the reports of El-Baroty *et al*., it was observed that the hexane and ethyl acetate extracts of *Aspergillus taxiformis* showed a good anti-fungal activity against *Fusarium oxysporees* [33] .

Conclusion

The phytochemical (Flavanoids, Terpenoids, Saponins, Phenols and Cardiac Glycosides) studies were determined for *Portieria hornemannii*. Ethyl acetic extracts showed a significant amount of phenolic content on comparing with other crude extracts. The antibacterial activity for different solvents extracts was measured in range of 10 mm to 19 mm. The extracts of ethyl acetate showed a maximum zone of inhibition against *Staphylococcus aureus, Klebsiella pneumonia, E. coli* at 10µg and 20µg/ml. It was followed by chloroform and acetone extracts. *Portieria hornemannii* was also analyzed for antifungal activity against five ocular pathogens. But, there was no significant zone of inhibition in any of the extracts at any concentration. This algal species possesses a good antibacterial activity which can be used for the drug developmental against various fastidious bacterial strains.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Clearance: Animal study was not involved in this research.

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References

1. Matsukawa, R., Dubinsky, Z., Kishimoto, E., et al., A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol.*, 1997, **9**, 29.
2. Stein, E. M., Colepicolo, P., Afonso, F. A. K., and Fujii, M. T., Screening for antifungal activities of extracts of the Brazilian seaweed genus Laurencia (Ceramiales, Rhodophyta). *Brazilian J. Pharmacogn.*, 2011, pp. 290–295.
3. Levy, S. B. and Marshall, B., Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.*, 2004, **10**, S122.
4. Mandal, S. and Mallick, N., Microalga Scenedesmus obliquus as a potential source for biodiesel production. *Appl. Microbiol. Biotechnol.*, 2009, **84**, 281–291.
5. Winstanley, T. G., Limb, D. I., Eggington, R., and Hancock, F., A 10 year survey of the antimicrobial susceptibility of urinary tract isolates in the UK: the Microbe Base project. *J. Antimicrob. Chemother.*, 1997, **40**, 591–594.
6. Basualdo, C., Sgroy, V., Finola, M. S., and Marioli, J. M., Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet. Microbiol.*, 2007, **124**, 375–381.
7. Mandal, S., DebMandal, M., Pal, N. K., and Saha, K., Synergistic anti--Staphylococcus aureus activity of amoxicillin in combination with Emblica officinalis and Nymphae odorata extracts. *Asian Pac. J. Trop. Med.*, 2010, **3**, 711–714.
8. Kamat, S. Y., Wahidulla, S., D'Souza, L., et al., Bioactivity of marine organisms. VI. Antiviral evaluation of marine algal extracts from the Indian coast. *Bot. Mar.*, 1992, **35**, 161–164.
9. Guiry, M. D., AlgaeBase. World-wide electronic publication, National university of ireland, Galway. *http//www. algaebase. org/*, 2010.
10. Saunders, G. W., Chiovitti, A., and Kraft, G. T., Small-subunit rDNA sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 3. Delineating the Gigartinales sensu stricto. *Can. J. Bot.*, 2004, **82**, 43–74.
11. Krayesky, D. M., Norris, J. N., Gabrielson, P. W., Gabriel, D., and Fredericq, S., A new order of red algae based on the Peyssonneliaceae, with an evaluation of the ordinal classification of the Florideophyceae (Rhodophyta). *Proc. Biol. Soc. Washingt.*, 2009, **122**, 364–391.
12. Verbruggen, H., Maggs, C. A., Saunders, G. W., Le Gall, L., Yoon, H. S., and De Clerck, O., Data mining approach identifies research priorities and data requirements for resolving the red algal tree of life. *BMC Evol. Biol.*, 2010, **10**, 16.
13. Trease, G. E. and Evans, W. C., Pharmacognosy, Saunders. *Elsevier, Amsterdam, the Netherlands*, 2002, **36**, 51.
14. Sadasivam, S. and Manickam, A., *Biochemical methods for agricultural sciences* Wiley eastern limited, 1992.
15. Kumar, G. and Sahoo, D., Effect of seaweed liquid extract on growth and yield of Triticum aestivum var. Pusa Gold. *J. Appl. Phycol.*, 2011, **23**, 251–255.
16. Patra, J. K., Rath, S. K., Jena, K., Rathod, V. K., and Thatoi, H., Evaluation of antioxidant and antimicrobial activity of seaweed (Sargassum sp.) extract: A study on inhibition of glutathione-S-transferase activity. *Turkish J. Biol.*, 2008, **32**, 119–125.
17. Bayer, E. A., Zalis, M. G., and Wilchek, M., 3-(N-Maleimido-propionyl) biocytin: a versatile thiol-specific biotinylating reagent. *Anal. Biochem.*, 1985, **149**, 529–536.
18. Oloyede, O. I., Chemical profile of unripe pulp of Carica papaya. *Pakistan J. Nutr.*, 2005, **4**, 379–381.
19. Haslem, E., Plant Polyphenols—Vegetable Tannins. Cambridge University Press, UK, 1989.
20. Pietra, F., Secondary metabolites from marine microorganisms: bacteria, protozoa, algae and fungi. Achievements and prospects. *Nat. Prod.* Rep., 1997, 14, 453–464.
21. Xu, N., Fan, X., Yan, X., Li, X., Niu, R., and Tseng, C. K., Antibacterial bromophenols from the marine red alga Rhodomela confervoides. Phytochemistry, 2003, 62, 1221–1224.
22. Kuda, T., Tsunekawa, M., Hishi, T., and Araki, Y., Antioxidant properties of driedkayamo-nori', a brown alga Scytosiphon lomentaria (Scytosiphonales, Phaeophyceae). Food Chem., 2005, 89, 617–622.
23. Wang, T., Jonsdottir, R., and Ólafsdóttir, G., Total phenolic compounds, radical scavenging and metal

chelation of extracts from Icelandic seaweeds. *Food Chem.*, 2009, **116**, 240–248.

24. Pedersen, A., Studies on phenol content and heavy metal uptake in fucoids. In *Eleventh International Seaweed Symposium* 1984, pp. 498–504.
25. Kumar, M., Kumari, P., Trivedi, N., et al., Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *J. Appl. Phycol.*, 2011, **23**, 797–810.
26. Ganesan, P., Kumar, C. S., and Bhaskar, N., Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour. Technol.*, 2008, **99**, 2717–2723.
27. Zubia, M., Robledo, D., and Freile-Pelegrin, Y., Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *J. Appl. Phycol.*, 2007, **19**, 449–458.
28. Thillairajasekar, K., Duraipandiyan, V., and Perumal, P., Antimicrobial activity of Trichodesmium erythraeum (Ehr) (microalga) from South East coast of Tamil Nadu , India Plant material Antifungal activity and minimum Gas chromatography-mass spectrometry Antimicrobial assay. *Int. J. Integr. Biol.*, 2009, **5**, 167–170.
29. Liao, W. R., Lin, J. Y., Shieh, W. Y., Jeng, W. L., and Huang, R., Antibiotic activity of lectins from marine algae against marine vibrios. *J. Ind. Microbiol. Biotechnol.*, 2003, **30**, 433–439.
30. TÜney, \.Inci, Cadirci, B. H., Ünal, D., and Sukatar, A., Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turkish J. Biol.*, 2006, **30**, 171–175.
31. Ely, R., Supriya, T., and Naik, C. G., Antimicrobial activity of marine organisms collected off the coast of South East India. *J. Exp. Mar. Bio. Ecol.*, 2004, **309**, 121–127.
32. Mtolera, M. S. P. and Semesi, A., Antimicrobial Activity of Extracts from Six GreenAlgae from Tanzania. 1996.
33. El-Baroty, G. S., Moussa, M. Y., Shallan, M. A., Ali, M. A., Sabh, A. Z., and Shalaby, E. A., Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: Asparagopsis taxiformis (Delile) Trevisan. *J. Appl. Sci. Res*, 2007, **3**, 1825–1834.