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ANTIOXIDANT ACTIVITY OF MARINE RED ALGAE – *PORTIERIA HORNEMANNII*

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Abstract

An *in vitro* evaluation study was conducted to assess the antioxidant capacity of the red algae *Portieria hornemannii* by using DPPH, Reducing power, ABTS radical scavenging activity and Superoxide scavenging activity.

Crude extracts (acetone, ethyl acetate, methanol and chloroform) was prepared by Soxhlet extraction and tested for the presence of active antioxidants using DPPH assay, Reducing power, ABTS assay and Superoxide scavenging activity and was statistically analysed with Tukey-HSD Homogenous subsets.

From the results the following inference can be obtained, in DPPH assay the ethyl acetate extract and methanol extract shows highest inhibition absorbance of 0.472 ± 0.002 and 0.484± 0.001 respectively. In reducing power assay, among the four extracts the chloroform extract shows absorbance of 0.198 ± 0.002 at 2.5 µg, 0.297 ± 0.001 at 5 µg, 0.305 ± 0.00 at 7.5 µg and 0.333 ± 0.002 at 10 µg respectively. In ABTS assay, the Ethyl acetate extract and chloroform extract shows highest inhibition absorbance of 0.435 ± 0.0010 and 0.488 ± 0.0025. In Superoxide scavenging activity, the acetonic and methanolic extract shows 0.128 ± 0.001 and 0.138 ± 0.009. The one-way ANOVA results of all the four assays showed a significance value of $p < 0.01$ which makes them highly significant.

The results prove the red algae *Portieria hornemannii* is a potential source as antioxidants against the reactive oxygen species which obstructs the cell metabolism and physiological activities.

***Keywords*:** *Portieria hornemannii*, Anti-oxidant activity, Macro algae, Seaweed.

Introduction

Bioactive molecules from natural sources are attaining thrust in the field of natural product discovery. The terrestrial plants are being widely used while marine plants such as seaweeds are less exploited for the bioactive molecule's discovery. Hudson *et al.* (1998). In contrast to terrestrial vegetation, the macro algae produce bioactive metabolites in response to ecological pressures such as competition for space and ability to reproduce constitute valuable sources for drug development. De Vries & Beart (1995). In the past decade, the search for natural antioxidant compounds has gained considerable attention and the number of publications on antioxidants and oxidative stress has nearly quadrupled Huang, Ou & Prior (2005). Antioxidant compounds play an important role against various diseases like chronic inflammation, atherosclerosis, cancer and cardiovascular disorders. Kohen & Nyska (2002), which explains their considerable commercial potential in medicine, food production and the cosmetic industry. Moreover, interest in employing antioxidants from natural sources is considerably enhanced by consumer preference for natural products and concern about the potential toxic effects of synthetic antioxidants. Safer & Al-Nughamish (1999). 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 antioxidative defence systems. Fujimoto (1990); Matsukawa *et al.* (1997). In fact, algae have protective enzymes (superoxide dismutase, peroxidase, glutathione reductase, catalase) and antioxidative molecules (phlorotannins, ascorbic acid, tocopherols, carotenoids, phospholipids, chlorophyll related compounds, bromophenols, catechins, mycosporine-like amino acids, polysaccharides, etc.) which are similar to those of vascular plants. Fujimoto (1990); Le Tutour *et al.* (1998); Rupérez, Ahrazem & Leal (2002); Yuan & Song (2005).

This study reveals the antioxidant 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. Guiry (2010) 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. Saunders, Chiovitti & Kraft (2004); Krayesky *et al.* (2009); Verbruggen *et al.* (2010). The present study was designed to investigate the antioxidant activity of *Portieria hornemannii*.

Materials and Methods

Collection of sample

Healthy and matured seaweeds were handpicked from the rocky intertidal region at the coastal region of Mandapam 9º28"31"0ºN and 79º15"7"11ºE, processed and stored at the laboratory. Y\ild\ir\im *et al.* (2000) The crude metabolites from the sample were extracted using Soxhlet extraction method using solvents like acetone, chloroform, ethyl acetate and methanol; the crude metabolites were exhibited for the antioxidant activity of *Portieria hornemannii.*

Anti-Oxidant Activity

DPPH Radical Scavenging Activity : DPPH (2, 2-diphenyl-2-picryl hydrazyl hydrate) is a free radical and it is relatively stable and a widely accepted a widely accepted radical for the estimation of radical scavenging activity. The reaction of reducing agents reducing agents with the free DPPH radical depends upon the electron taken up and the stoichiometric

degradation of the color solution. Sala *et al.* (2002). DPPH Radical Scavenging Activity was carried determined according to the method of Molyneux and others, (2004).

Reducing Power : Reducing power of the extracts was evaluated according to the method of Oyaizu (1986) and Yen & Chen (1995) Potassium Ferro cyanide reacts with Fe^{3+} ions in the presence of an antioxidant agent and form a Prussian blue color which leads to the conversion of blue color to yellow color in the presence of antioxidant agent and hence the greater the reducing power of the compound greater the absorbance at 700nm. Zou *et al.* (2008)

ABTS Radical Scavenging Activity : The antioxidant effect of the extracts was studied by ABTS (2, 2, –azino-bis-3-ethyl benzothiazoline -6-sulphonic acid) radical cationide colorization assay according to the method of Shirwaikar, Prabhu & Punitha (2006).

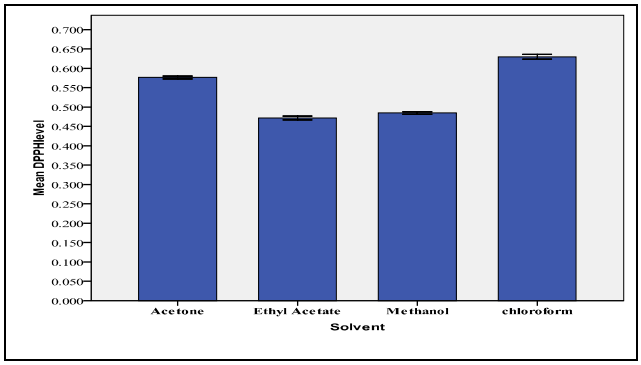
Superoxide Scavenging Activity : The superoxide scavenging ability of the extracts was assessed by the method of Winterbourn *et al.* (1975).

Statistical Analysis : The data collected were analyzed through statistical package for social studies version 17 (SPSS 17.0 ver.). The data were expressed as mean ± SD statistical significance of variance was evaluated with one-way ANOVA, Two-way ANOVA with Tukey Multiple comparison. If the significance of variance i.e. P value (given under the head sign.is less than 0.05 ($p < 0.05$) the difference between the experimental conditions was considered significant. Regression and descriptive analysis were also carried out. The results obtained was also represented graphically.

Results and Discussion

DPPH Radical Scavenging Activity

These radical comprises of highly reactive molecules or atoms which are unstable due to the presence of single electron or the presence of unbalanced electrons. Even though due to the presence of unbalanced electrons these radicals can be used for the normal cellular function under the physiological concentration, but the interruption in the function or the damage in the cellular metabolites such as lipids proteins and also in cellular components like nucleotide can occur due to the presence of excessive free radicals Zheng & Wang (2001). DPPH (2, 2-diphenyl-2-picryl hydrazyl hydrate) Radical Scavenging Activity of the extracts (acetone, ethyl acetate, methanol and chloroform) was determined according to the method of Molyneux & others (2004). The mean ± SD value were 0.576 ± 0.001, 0.472 ± 0.002, 0.484 ± 0.001, 0.629 ± 0.002 (Graph 1). Higher level of DPPH was observed in the chloroformic extracts followed by the acetonic, methanolic and finally ethyl acetate extracts. From the results it was observed that chloroform extracts have high DPPH Radical Scavenging Activity compared with other extracts. The one-way analysis of variance showed that there is a significant ($P<0.05$) difference between the extract's treatments (Table 1). The results of Tukey-HSD Homogenous subsets formation showed that there are four subsets for the DPPH Radical Scavenging Activity (Table 2). The free radical scavenging activity of these antioxidants can protect from serious molecular and cellular damage and inhibit the process of several chronic disease and also lipid peroxidation in the food. Han *et al.* (2010) studied that the extracts of seaweeds pose anti-oxidant activity through DPPH radical scavenging activity and observed that the brown seaweed *Cendaria pinnatifida* expressed a good antioxidant activity through radical scavenging activity. Yan *et al.* (1999); Wang, Jonsdottir & Ólafsdóttir (2009) reported that higher amounts of polyphenols through DPPH radical scavenging activity and confined that brown algae exhibit more antioxidant activity than red and green algae. But in contrast to that Chandini, Ganesan & Bhaskar (2008) described in her studies that brown algae possess lower levels of DPPH radical scavenging activity ranging from17.79 to 23.16% at an extract concentration of 1000 µg/ml Duan *et al.* (2006) also discussed the crude extract of red alga, *Polysiphonia urceolata* exhibited a good DPPH radical scavenging activity. Hence DPPH a free radical can be extensively used as a free radical to evaluate the free radical scavenging activities of compounds.



Graph 1: Mean DPPH level of *Portieria hornemannii*

Table 1: One-way ANOVA – DPPH of *Portieria hornemannii*

		Sum of Squares	Df	Mean Square	F	Sig.
DPPH level	Between Groups	0.051	3	0.017	4869.778	0.000
	Within Groups	0.000	8	0.000		
	Total	0.051	11			

Table 2: Multiple Comparison – DPPH of *Portieria hornemannii*

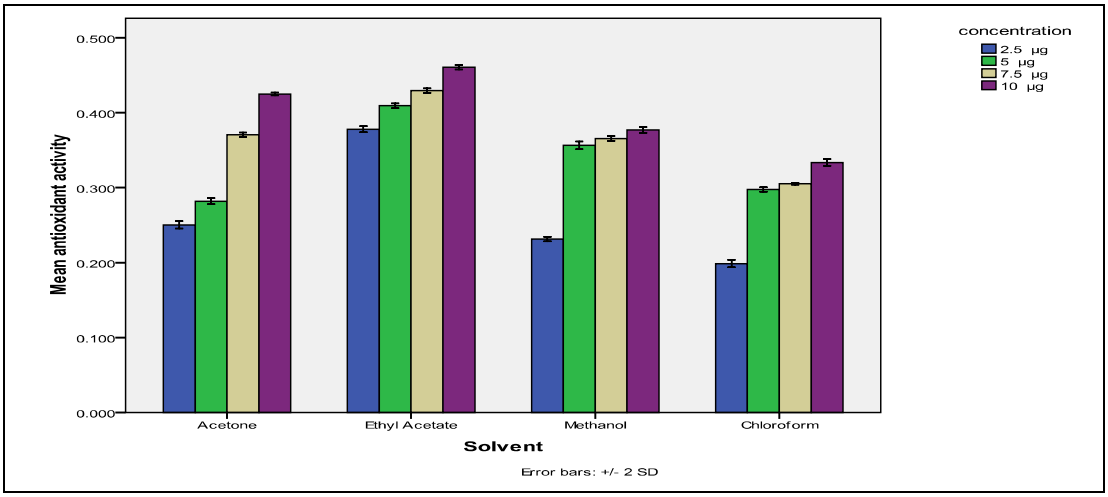
	Solvent	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Ethyl Acetate	3	.47200			
	Methanol	3		.48467		
	Acetone	3			.57633	
	chloroform	3				.62967
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Reducing Power

The anti-oxidant potential activity of the compound can be indicated by the presence of reducing power of the compound. Lin *et al.* (2009) Reducing power of the extracts was evaluated according to the method of Oyaizu (1986); Yen & Chen (1995). The mean ± SD value of the reducing power of the extracts (acetone, ethyl acetate, methanol and chloroform) at the concentration of 2.5 µg were 0.250, 0.378, 0.231, 0.198 and at the concentration of 5 µg were 0.282, 0.409, 0.356, 0.297 and at the concentration of 7.5 µg were 0.370, 0.429, 0.365, 0.305 and at the concentration of 10 µg were 0.425, 0.460, 0.377, 0.333. From the results it was observed that ethyl acetate extracts have high reducing power compared with other extracts (Graph 2). The two-way analysis of variance showed that there is a significant (P<0.05) difference between the extract's treatments (Table 3). The multiple comparison test Tukey-HSD (Homogenous subsets) also showed that there is a significant (P<0.05) difference between the extracts treatments (Table 4). Jiménez-Escrig *et al.* (2001) observed that red algae showed higher reducing power than brown algae due to the presence of polyphenols which is higher in red seaweeds comparing with green and brown seaweeds. Reducing power is exhibited in Seaweeds is due to the presence of flavonoids and phenols. Hence the seaweeds possess antioxidant, anti-allergic, anti-inflammatory, anti-microbial, anti-cancer activity. The antioxidant compounds during the reducing power assay donate electrons and also immediately reduce the lipid peroxidation process through reduced oxidation by which it can act has primary and secondary antioxidants. Yen & Chen (1995)

Table 3 : Two-way ANOVA – Reducing power of *Portieria hornemannii*

Tests of Between-Subjects Effects					
Dependent Variable: antioxidant activity					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Solvent	.115	3	.038	11334.887	.000
concentration	.119	3	.040	11793.043	.000
solvent * concentration	.022	9	.002	733.323	.000
Error	.000	32	3.37		
Total	5.870	48			
Corrected Total	.257	47			



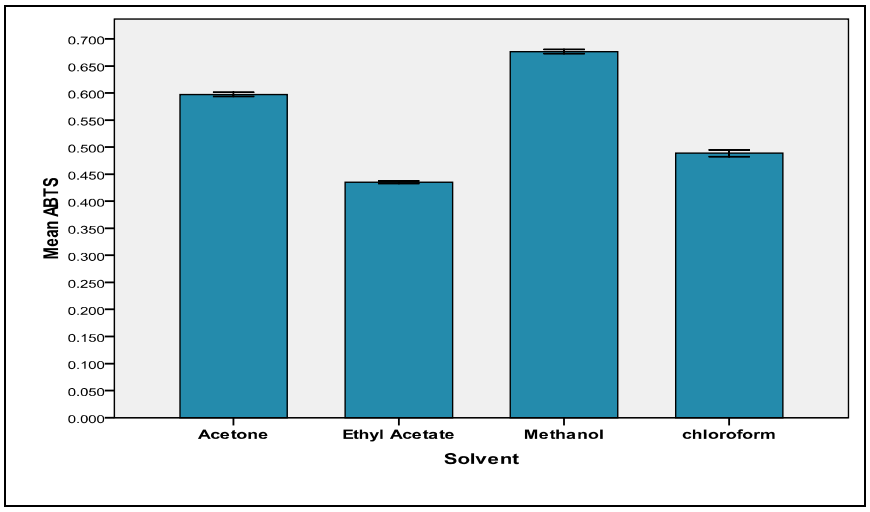
Graph 2: Mean ± SD – Reducing power of *Portieria hornemannii*

Table 4: Multiple Comparison – Reducing power of *Portieria hornemannii*

Multiple Comparisons of reducing power Tukey HSD						
(I) solvent	**(J) solvent**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Acetone Vs	Ethyl Acetate	-.08742*	.000750	.000	-.08945	-.08538
	Methanol	-.00067	.000750	.811	-.00270	.00137
	Chloroform	.04817*	.000750	.000	.04613	.05020
Ethyl Acetate Vs	Acetone	.08742*	.000750	.000	.08538	.08945
	Methanol	.08675*	.000750	.000	.08472	.08878
	Chloroform	.13558*	.000750	.000	.13355	.13762
Methanol Vs	Acetone	.00067	.000750	.811	-.00137	.00270
	Ethyl Acetate	-.08675*	.000750	.000	-.08878	-.08472
	Chloroform	.04883*	.000750	.000	.04680	.05087
Chloroform Vs	Acetone	-.04817*	.000750	.000	-.05020	-.04613
	Ethyl Acetate	-.13558*	.000750	.000	-.13762	-.13355
	Methanol	-.04883*	.000750	.000	-.05087	-.04680
Based on observed means. The error term is Mean Square (Error) = 3.38E-006.						
*. The mean difference is significant at the .05 level.						

ABTS Radical Scavenging Activity

ABTS radical scavenging activity is an indirect method for the determination of natural antioxidants these ABTS radical like the DPPH radical is also a free and rather stable ion in the absence of phenolic compound but it reacts energetically and gets converted into a non-colored form of ABTS with the help of H-atom donor like phenolic compound. Rauchová *et al.* (1995) The antioxidant effect for the extracts (acetone, ethyl acetate, methanol and chloroform) was studied by ABTS (2, 2, –azino-bis-3-ethyl benzothiazoline -6-sulphonic acid) radical cationide colorization assay according to the method of Shirwaikar *et al.* (2006). The mean ± SD value were 0.597±0.0015, 0.435±0.001, 0.76±0.0015, 0.488±0.0025. From the results it was observed that methanol extracts have high ABTS Radical Scavenging Activity compared with other extracts (Graph 3). The one-way analysis of variance showed that there is a significant (P<0.05) difference between the extracts treatments (Table 5). The results of Tukey-HSD Homogenous subsets formation showed that there are four subsets for the ABTS Radical Scavenging Activity (Table 6). Sachindra *et al.* (2007) reported that the extracts obtained from the brown alga *Turbinaria conoides* and *Padina tetrastomatica* exhibited a higher ABTS radical scavenging activity compared to other four red seaweeds in its crude as well as fractionate extract derived from various solvents.. Bonnet, Camares & Veisseire (2000) observed 98% inhibition of the ABTS molecule from the brown algae *Padina minor.* The results of the present study indicate that the methanolic extracts from *Portieria hornemannii* seaweed exhibited higher ABTS radical activity compared with other solvent extracts. However, the ABTS assay also possess certain limitations such as the competence of the sample to reacts with ABTS radical instead of inhibiting the oxidative process and decrease the reaction of many phenolics. Rauchová *et al.* (1995). Marinova & Yanishlieva (1997)., reported that the antioxidant activity is directly proportional to the types of solvent used for the extraction because the compounds obtained by the extraction depends upon the polarity difference and exhibit antioxidant potential at differing rates. Senthil Nathan, Kalaivani & Sehoon, (2006) reported that the ethanolic extract exhibited the maximum antioxidant activity which revealed that the maximum antioxidant activity was obtained from the polar solvents like ethanol than with extracts obtained from non-polar solvents.. Wang *et al.* (2009) reported that 70% aqueous acetone is more proficient to derive polyphenolic compounds from seaweeds rather than using water for extraction because the solubility of the Phenolic compounds is high in polar organic solvents than water. Gakunju *et al.* (1995) recommended that 70% aqueous acetone (v/v) was found to be the most efficient solvent and for the effective extraction of metabolites for the antioxidant activity the aqueous mixtures of methanol, ethanol and acetone are highly recommended.



Graph 3 : Mean ABTS level of *Portieria hornemannii*

Table 5: One-way ANOVA – ABTS of *Portieria hornemannii*

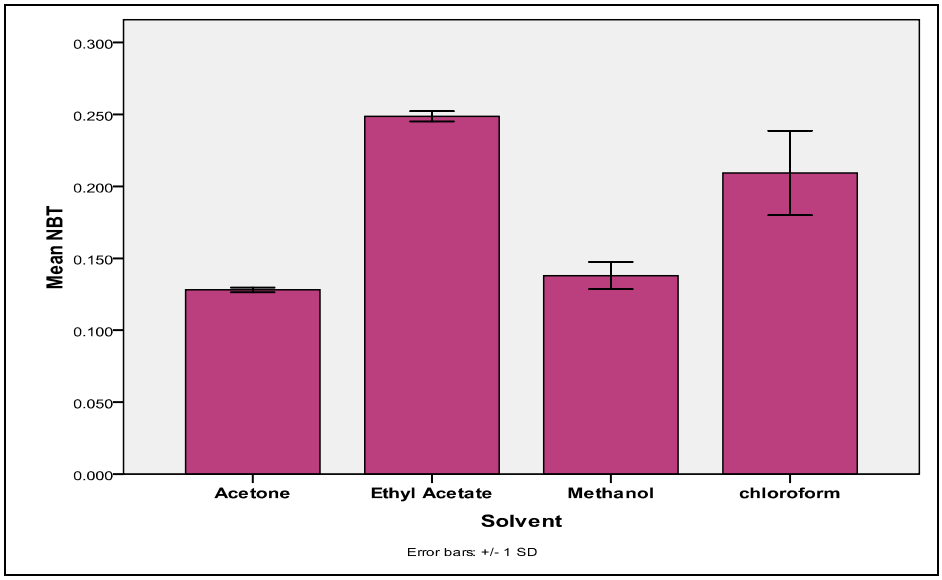
		Sum of Squares	Df	Mean Square	F	Sig.
ABTS	Between Groups	0.106	3	0.035	11756.769	0.000
	Within Groups	0.000	8	0.000		
	Total	0.106	11			

Table 6: Multiple Comparison – ABTS of *Portieria hornemannii*

	Solvent	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	Ethyl Acetate	3	.43500			
	chloroform	3		.48867		
	Acetone	3			.59733	
	Methanol	3				.67667
	Sig.		1.000	1.000	1.000	1.000
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Superoxide Scavenging Activity

The superoxide scavenging ability for the extracts (acetone, ethyl acetate, methanol and chloroform) was assessed by the method of Winterbourn *et al.* (1975). The mean ± SD value were 0.128±0.001, 0.248±0.003, 0.138±0.009, 0.209±0.0029. From the results it was observed that ethyl acetate extracts have high superoxide Scavenging Activity compared with other extracts (Graph 4). The one-way analysis of variance showed that there is a significant ($P<0.05$) difference between the extracts treatments (Table 7). The results of Tukey-HSD Homogenous subsets showed that there are two subsets formation for the superoxide Scavenging Activity. One subset for the acetone and methanol extracts and another subset for chloroform and ethyl acetate are tabulated (Table 8). Super oxide is one of the most important and a very effective free radical and it is first radical to be generated in the cellular oxidation reaction and it acts as a precursor in the species utilizing oxygen to prevent the cellular damage by inhibiting the pathological activity of many diseases. Siriwardhana *et al.* (2003). obtained high values with *Loboohora variegata* and with some other species of Phaeophyta in the super oxide anion radical scavenging activity.. Athukorala *et al.* (2007) studied that the marine algae *Grateloupia filficina* obtained a higher super oxide anion radical scavenging activity and it was observed that the concentration range of 1mg/ml. *Copernicia baileyana* induced the production of superoxide anion radical instead of inhibiting it, in this context Zhang & Omaye (2001) stated that certain antioxidant like ascorbic acid and α tocopherol can act as pure oxidant depending upon the dose of the experimental conditions.



Graph 4: Mean Superoxide Scavenging Activity level of *Portieria hornemannii*

Table 7: One-way ANOVA- Superoxide Scavenging Activity level of *Portieria hornemannii*

		Sum of Squares	Df	Mean Square	F	Sig.
NBT	Between Groups	0.030	3	0.010	41.372	0.000
	Within Groups	0.002	8	0.000		
	Total	0.032	11			

Table 8: Multiple Comparison - Superoxide Scavenging Activity level of *Portieria hornemannii*

	Solvent	N	Subset for alpha = 0.05			
			1			
Tukey HSD[a]	Acetone	3	.12800			
	Methanol	3	.13800			
	Chloroform	3		.20933		
	Ethyl Acetate	3		.24867		
	Sig.		.859	.058		
Means for groups in homogeneous subsets are displayed.						
a. Uses Harmonic Mean Sample Size = 3.000.						

Conclusion

Herbal medicines are chief source of drug discovery as they are widely used for the control of pathogens and human related diseases. In the present investigation, it is proved the red algae *Portieria hornemannii possess* antioxidant activity and paves way in elucidating the key metabolite responsible for the activity against the reactive oxygen species. Further studies can be conducted on red algae *Portieria hornemannii,* marking them a significant source of metabolites for the cure of many fatal diseases like cancer, Alzheimer's disease.

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