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EXPLORING NEW SOURCES OF ANTIOXIDANTS AND PHENOLIC CONTENTS FROM A MARINE RED ALGA *AGARDHIELLA ROBUSTA* (GREVI.) BORG. COLLECTED FROM KARACHI COAST

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ABSTRACT

The antioxidant potential and total phenolic contents of a red alga *Agardhiella robusta* (Grevi.) Borg belonging to Rhodophycota collected from coastal areas of Karachi, Pakistan was investigated through various in vitro protocols. Antioxidant potential measured through total antioxidant determination, total phenolic contents, ferric reducing antioxidant power (FRAP) and DPPH analysis. The antioxidant components were initially extracted in methanol and were further fractionated in solvents of different polarity. The results showed that the petroleum ether fraction exhibited highest DPPH radical scavenging activity, i.e. (79.5±1.9%) at a concentration of 1000ug/ml with IC_{50} 286.13±0.34 µg/mL relative to the BHT. While Methanol extract showed highest phenolic contents i.e. 1542.5 ±1.2 GAEmg/mL, highest FRAP values i.e. 18.95± TEuM/mL as well as least IC_{50} value of 25.81 ± 0.65 µg/mL which indicated its potential for scavenging the free radicals. On the basis of these results obtained here *Agardhiella robusta* (Grevi.) Borg considered as a rich source of antioxidant.

Key words: Antioxidants, *Agardhiella robusta*, total phenolic contents, ferric reducing antioxidant power (FRAP) and DPPH.

INTRODUCTION

Antioxidant properties of the marine algal compounds have been reported to possess tremendous functions in all biological systems. The marine environment is a unique reservoir of bioactive natural compounds, many of which show chemical and structural features not reported in terrestrial plants (Vijayavel and Martinez, 2010). Among marine organisms, marine macroalgae (more commonly known as seaweeds) have emerged as rich sources of natural products/chemicals with considerable biological activities. In accordance with this approach, Ziconotide, isolated from a tropical cone snail, was the first marine-derived compound for the treatment of pain and trabectedin later followed as first marine anticancer drug thus displaying tremendous potential of natural products for treatment of various diseases (Maeda *et al*., 2008; Kim *et al*., 2008). Seaweeds are the best source of vitamins (A, B, B12, C, D & E), riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na and K. Dietary fibers in seaweeds are used as antioxidant, anti-mutagenic, anticoagulant, antitumor, et (Dela *et al*., 2012;Vadlapudi and Naidu, 2010). Many Clinical studies of chronic disorders e.g cancer, cardiac and degenerative brain diseases revealed that such disorders entails oxidative disorder to cellular components (Murgan and Iyer, 2012). Confluence of information suggested that chemically unstable free radicals induce damage in cells. The destructive effects of ROS cause lipid peroxidation and vehemence of tissue proteins in membrane, destruction of DNA and enzymes (Umayaparvathi *et al*., 2012). Antioxidants are considered to be crucial in the cure of enduring diseases and health issues by terminating radical-mediated oxidative reactions and nonstop AOS attacks (Ganesan *et al*., 2011). In seaweeds it is being realized that phenolics, proanthocyanidins and flavonoids, are the major sources of antioxidants (Kuda *et al*., 2005). Coastal areas of Pakistan are diversified with the rich algal flora. (Chandini *et al*., 2008). Several studies have been reported on the phytochemistry of seaweeds during last decade (Ananthi *et al*., 2011).

Based on this background, the main objective of this research is to find out naturally occurring antioxidants which can be suggested as nutraceuticals because now a day there is an increasing collaboration between marine chemist and pharmacologists that will lead to the discovery of such natural defensive molecules. This study aims to investigate possible correlation between total phenolic contents (TPC) and antioxidant potential of these seaweeds.

MATERIALS AND METHODS

Collection of Seaweed and description of study area: *Agardhiella robusta* (Greiv.) Borg. (red seaweed) was collected from the intertidal and subtidal habitat of the Hawsbay, Paradise , Sandspit and Buleji located between longitude 66º 59º E and Latitude 24º 48º N located on the coast of Karachi, Pakistan. Collection was performed

during the April 2012 to 2014, when algal diversity remains dominant and luxuriant growth of the seaweed was observed. The specimen was identified by late Phycologist Mustafe Shameel, Department of Botany, University of Karachi in April 2012 and preserved in Kashyap Botany Museum GCU Lahore for voucher specimen. Seaweed was collected as drift material and after washing with running water to remove epiphytes, animal castings, sand matters. Cleaned seaweed was placed in shade for drying to prevent photolysis, thermal degradation of biocompounds.

Chemicals and instrumentation: All solvents used for sample preparation were of analytical grade Methanol, n-Hexane, Chloroform, Ethyl acetate, DPPH, Potassium persulfate, Gallic acid, Ethanol, Anhydrous sodium carbonate, Folin- Ciocalteu's reagent, Acetate buffer, Ferric chloride, Ferrous sulphate , Ferrozine, Sodium nitrite, Aluminum chloride, Sodium hydroxide, Tripyridyltriazine (TPTZ), Phosphate buffer, etc. were purchased from Sigma-Aldrich Chemical Co. Inc.

Preparation of Extract: The shade-dried powdered seaweed (300 g) was soaked in methanol (2.5 L) at room temperature for 25 days. Whatman filter paper No. 1 was used for filtration and clarified filtrates (1.25 L), was later evaporated (60°C) using rotary evaporator. After Evaporation a dark green viscous mass (40 mL) of methanolic fraction was obtained. This mass (40 mL) was subjected to fractionation mixed with an equal volume of distilled water and partitioned successively with *n*-hexane (200 mL), Petroleum Ether (200 mL), Chloroform (200 mL), Methanol (200mL) to furnish all fractions respectively. Each fraction was then stored in glass vials for antioxidant evaluation. Following antioxidant assays were performed on all the fractions.

DPPH radical scavenging activity: BHT as standard was used to study the DPPH radical scavenging activity of seaweed according to (Prieto *et al*., 1999). Different concentrations of the samples (1000µg/ml, 500µg/ml, 250µg/ml and 125µg/ml) were dissolved with 3ml of methanolic solution of DPPH (0.1mM). The extent of DPPH discoloration of the extracts was calculated according to following formula:

$$\text{Antiradical activity (\%)} = _(A_{control} - A_{sample})/A_{control} \times 100$$

Total antioxidant activity by phosphomolybdenum method: The total antioxidant activities of different fractions of red algae was evaluated by phosphomolybdenum complex formation method as described by (Benzie and strain, 1996).

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay followed with slightly modification as described by Benzie and Strain (1996). The values were expressed in (TE) per ml of the sample solution. Standard curve was plotted against different concentrations of trolox. Results were expressed in TE µM/ml.

Total Phenolic contents: Total phenolic contents of different fractions of seaweed were determined by the standard method (Gupta and Abu-Ghannam, 2011). Total phenols were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. Results were expressed in GAE mg/ml.

Statistical analysis: All the readings were recorded in triplicate and excel sheet was used to statistically analyze data. All the data were expressed as Mean ± S.E.M.

RESULTS AND DISCUSSION

Physical properties of extract of *Agardhiella robusta* (Grevi.) Borg: The *Agardhiella robusta* (Grevi.) Borg extract was prepared in non-polar and polar solvents for the investigation of antioxidant potential. The physical properties of extracts were observed on the basis of color, appearance and texture (Table 1).

Table 1. Physical properties of extract of *Agardhiella robusta* (Grevi.) Borg.

Sr. No.	Extract	Color	Appearance	Texture
1	Petroleum Ether	Brown	Sticky	semi-Granular
2	Chloroform	Reddish Green	Sticky	Smooth
3	Methanol	Rust	Non-Sticky	Granular
4	Water	Dark Brown	Non-Sticky	Granular
5	Hexane	Greenish Brown	Greesy	Smooth oily

% Extraction Yield of *Agardhiella robusta* (Grevi.) Borg: Methanol extract of *Agardhiella robusta* (Grevi.) Borg. exhibited maximum percentage yield, i.e. 2.6% which is due to the tendency of the solubility of compounds in non polar solvent whereas water extract displayed 1.9%. Minimum yield was shown by petroleum ether extract (1.3%) shown in (Table 2).

DPPH radical scavenging activity: % DPPH free radical scavenging activity of various fractions **of** *n*-hexane, P.E, Chloroform, Methanol and aqueous extract of *Agardhiella robusta* (Grevi.) Borg. was evaluated (Table 3). The results obtained directly compared with the standard BHT. The petroleum ether extract of the *Agardhiella robusta* (Grevi.) Borg. had displayed maximum value (79.646±1.1) at a concentration of 1000**µl**. The DPPH potential of the different fractions of

the extracts at various concentrations was found to be between the range of 79.646±1.1 to 24.012±3.3.

Table 2. % Extraction Yield of *Agardhiella robusta* (Grevi.) Borg.

Sr. No.	Extracts	% Yield
1	*n*-hexane	1.5
2	Petroleum ether	1.3
3	Chloroform	1.7
4	Methanol	2.6
5	Water	1.9

The methanol extract showed the least IC_{50} with a value 25.81±0.65 showing its strong potential of good scavenger for free radicals. When compared with n-hexane having 423.124±0.15 showed the least effectiveness of radical scavenging (Table 4).

Total antioxidant activity by Phosphomolybdenum method: Total Antioxidant activity of different fraction of *Agardhiella robusta* (Grevi.) Borg. was investigated by Phosphomolybdenum assay (Table 5). The maximum antioxidant potency exhibited by n-Hexane extract i.e. 1.038±.230 at a concentration of 250 µl while the minimum effectiveness was showed by aqueous extract i.e. 0.218±.004 at a concentarion of 250 µl. The results indicated that the total antioxidant activity of seaweed dependant on the concentration gradient.

Ferric reducing antioxidant power (FRAP) assay: The FRAP investigation was carried out to estimate the reduction potential of the different solvents at various concentrations. It is basically the sum of antioxidant with the reducing capacity of the sample. The assessment was executed and the results were expressed in TEµM/mL. The standard curve of trolox was drawn (appendix 2) to express the results in accordance. The FRAP assay revealed a maximum antioxidant activity in methanolic extract (16.1±.635) at a concentration of 500 µL. The minimum antioxidant activity in n-Hexane extract (0.9±.115) at a concentration of 125 µL (table 5).

Total phenolic contents: The total Phenolic Content of *Agardhiella robusta* (Grevi.) Borg were found maximum in methanol fraction 1632±0.12 as shown in figure 2 while the lowest were shown by chloroform extract i.e. 343±0.03. The results were calculated in comparison with Gallic acid standard and expressed in terms of GAEmg/mL

Table 3. DPPH free radical scavenging activity of various fractions of *n*-hexane, P.E, Chloroform, Methanol and aqueous extract of *Agardhiella robusta* (Grevi.) Borg.

Sr. No.	Extracts	Conc. (µg/mL)	% scavenging
1.	*n*-hexane	1000	63.646±0.8
		500	57.497±0.3
		250	52.642±1.3
		125	41.963±0.2
2.	P.E	1000	79.646±1.1
		500	75.497±2.3
		250	55.642±0.7
		125	45.963±1.3
3.	Chloroform	500	45.928±1.2
		250	33.829±0.6
		125	25.199±3.3
		60	36.785±2.3
4.	Methanol	500	58.317±1.1
		250	49.687±2.4
		125	32.998±2.0
		60	24.012±3.3
5.	Water	500	44.584±0.3
		250	31.423±0.8
		125	38.263±1.3

Table 4. IC_{50} values of different extracts of *Agardhiella robusta* (Grevi.) Borg.

Sr. No.	Extracts	IC_{50} values (µg/mL)
1.	*n*-hexane	423.124±0.15
2.	Petroleum ether	286.139±0.34
3.	Chloroform	337.27±0.2
4.	Methanol	25.81±0.65
5.	Water	318.27±0.1
	Standard BHT	**12**±0.5

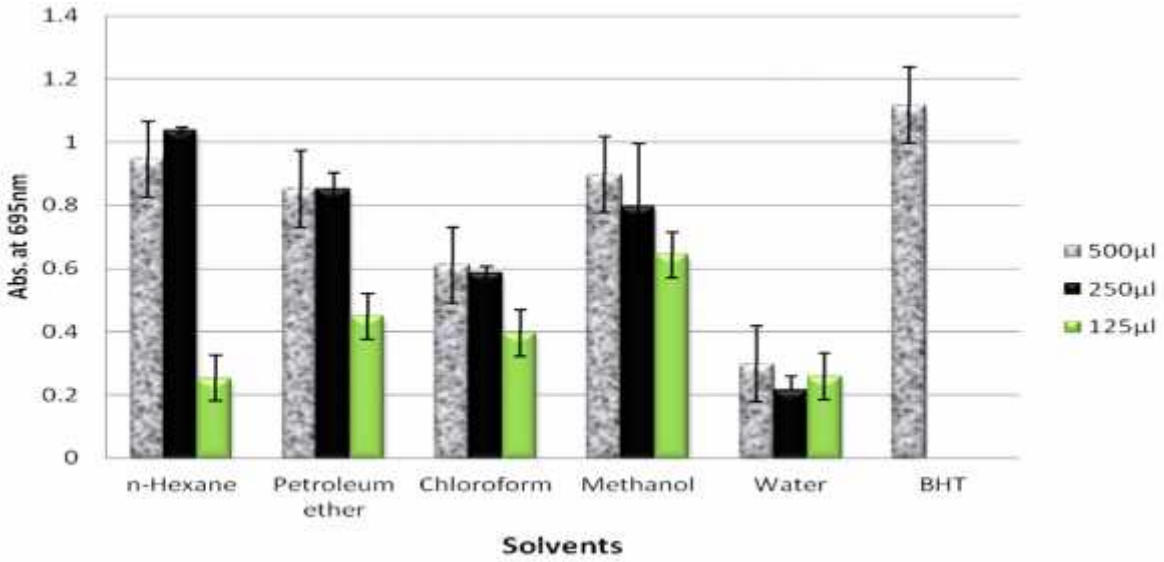

Figure 1. Antioxidant activity of *Agardhiella robusta* (Grevi.) Borg. **by Phosphomolybdenum assay.**

Table 5. FRAP Assay of *Agardhiella robusta* (Grevi.) Borg.

	FRAP values(TEµM/mL)		
Concentration	500	250	125
***n*-hexane**	1.8±0.76	1.4±0.56	0.9±0.45
P.E	1.72±0.53	1.09±0.63	1.2±0.45
Chloroform	2.8±0.98	2.5±0.87	2.1±0.88
Methanol	16.1±0.06	15.2±0.04	12.3±0.08
Water	3.45±0.96	3.21±0.93	2.98±0.89

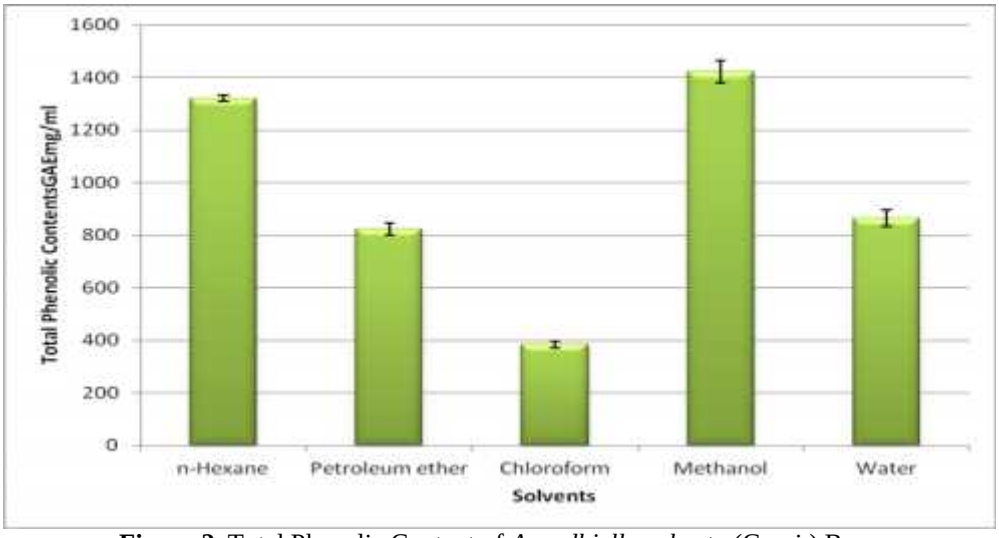

Figure 2. Total Phenolic Content of *Agardhiella robusta* (Grevi.) Borg

DISCUSSION

Many studies have been reported for the radical scavenging activities of the macroalgae. This was due to the presence of secondary metabolites in seaweeds such as fucoxanthin and astaxanthin, chlorophyll related pigments, flavonoids, bromopbhenols and sugars (Zubia *et al*., 2009; Vijayabaskar and Shiyamala, 2012). The IC_{50} values are used to compare the scavenging activity. Lower IC_{50} value indicated efficient DPPH radical scavenging activity. The crude methanolic extract of *Agardhiella robusta* (Grevi.) Borg. provided the IC_{50}

value of 25.81 ± 0.65 µg/ml than the non-polar fraction (n-hexane) with IC_{50} of 423.124±0.15 as shown in (Table-4). Similar work was also done on the methanolic extract of red alga *G. corticata* showed similar percentage inhibition as of 67.9% against DPPH radical (Vadlapudi and Naidu, 2010). Our results are also in accordance with Ananthi *et al*. (2010) in *Gracillaria edulis* who stated that %age inhibition is affected by the type of solvent because the solubility of the secondary metabolites directly associated with the polarity of the solvents because polar compounds have the tendency to dissolve in polar solvents and vice versa A high activity of DPPH has also been reported by Ganesan *et al*., (2011)

When the total antioxidant ability of the fractions were compared with the available standard antioxidant BHT there was a remarkable difference in all the fractions. *n*- hexane which is a non-polar solvent exhibited the highest total antioxidant activity. It was noteworthy that aqueous fraction showed the least antioxidant activity as in. The same results were also reported by (Farasat *et al*., 2014; Chakraborty *et al*., 2013) who stated different extracts of seaweeds exhibited different potential and in case of n-hexane showed maximum activity observed.

The FRAP assay measures the total antioxidant levels in a sample by taking into consideration the reducing power of ferrous to ferric showing the oxidation reduction potential of the plant (Nair *et al*., 2012; Heo *et al*., 2005) Among all fractions of *Agardhiella robusta* (Grevi.) Borg, the crude methanolic fraction showed the highest FRAP value and then the other fractions which followed the order water extract > chloroform > n-Hexane while the petroleum ether soluble fraction revealed the lowest FRAP. Similar kind of results were also reported by Vijayabaskar and Shiyamala (2012) who stated that *T. ornate* methanolic fraction had a good potential of scavenging broad range of synthetic and natural free radicals and *T. ornata can be utilized as a good source of natural antioxidants*. The higher total phenolic contents resulted in the higher antioxidant potential. Radical scavenging properties of Phenolic compounds are currently being studied and phenolic contents have a strong correlation with total antioxidant activity also concluded by (Farasat *et al*., 2014). The same findings were also described by Chakraborty *et al*., (2013). As *n*-hexane fraction of *Agardhiella robusta* (Grevi.) Borg indicated higher percentage of TPC also described by (Murgan and Iyer, 2014) that red algae posses higher antioxidant capacity. The screening of medicinally important natural products from seaweeds for the commercial production of antioxidant is very safe. Because these are the best source of dietary supplements, as ingredients in pharmaceutics, as cosmetic agents, nutraceuticals with the objective of improvement in the consumer health, and mitigating the effect of various chronic diseases.

Conclusion: Present study concluded that the antioxidant activities of *Agardhiella robusta* (Grevi.) Borg. exhibited high phenolics and also, high antioxidant activity with a low IC_{50}. Strong positive and significant correlations between DPPH radical scavenging, phenolics and Phenolic contents showed that, these are the main contributors of antioxidant activity in this alga. However, to the best of our knowledge, this is the initial investigation on the antioxidant capacity and total phenolics of *Agardhiella robusta* (Grevi.) Borg species from Pakistan. Further work is under way in Phycology laboratory GC University, Lahore which are aimed at investigation of antioxidant capacity of the other seaweeds of Karachi Coast.

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REFERENCES

Ananthi, S., H. Rao, B. Raghavendran, A.G. Sunil, V. Gayathri, and G. Ramakrishnan (2010). In vitro antioxidant and in vivo anti-inflammatory potential of crude polysaccharide from *Turbinaria ornata* (Marine Brown Alga). Food. Chem. Toxicol., 48:187–192.

Benzie, I. F., and J.J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Bio., 239(1): 70-76.

Chakraborty, K., and R. Paulraj (2010). Sesquiterpenoids with free radical scavenging properties from marine macroalga *Ulva fasciata* Delile. Food. Chem., 122:31–41.

Chakraborty, K., Praveen, N. K., K Vijayan, and G. Syda Rao (2013). Evaluation of Phenolic contents and antioxidant activities of Brown Seaweed beloing to Turbinaria spp. (Pheaophyta, Sargassaceae) collected from the gulf of Mannar. Asia. Pac. J. Trop. Biomed., 3(1):6–18.

Chandini, S. K., P. Ganesan, and N. Bhaskar (2008). In vitro antioxidant activities of three selected brown seaweeds of India. Food. Chem., 107:707–713

De la, C. F., J. Aguilera, F.L. Figueroa, M.V. DeGalvez and E. Herrera. (2008). Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. J. Appl. Phycol., 21:161–169.

Ebrahimzadeh, M. A., S. M. Nabavi, S. F. Nabavi, F. Bahramian, and A. R. Bekhradnia.(2010).

Antioxidant and free radical scavenging activity of *H. officinalis* L. var. angustifolius, *V. odorata*, *B. hyrcana* and *C. speciosum*. Pak .J. Pharm. Sci., 23(1): 29-34.

Farasat, M., R. A. Khavari-nejad, S.M.B. Nabavi, and F. Namjooya. (2014). Antioxidant Activity, Total Phenolics and Flavonoid and Contents of Some Edible Seaweeds from Northern Coasts of Persian Gulf. Iran. J. Pharm. Res., 13(1):163-170

Ganesan, K., S. K. Kumar, and P.V. Subba Rao. (2011). Comparative assessment of antioxidant activity in three edible species of green seaweed, *Enteromorpha* from Okha, Northwest coast of India. Innov. Food. Sci. Emerg. Technol., 12:73–78.

Gupta, S., and N. Abu-Ghannam (2011). Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innov. Food. Sci. Emerg. Technol., 12:600–609.

Heo, S. J., E. J. Park, K. W. Lee, Y. J. Jeon (2005). Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresource Technol., 96:1613–1623.

Kim, Y. A., C. S. Kong, Y. R. Um, J. I. Lee, T. J. Nam, and Y. Seo (2008). Antioxidant efficacy of extracts from a variety of seaweeds in a cellular system. Ocean. Sci. J., 43:31–37.

Kuda, T., M. Tsunekawaa, H. Goto and Y. Araki (2005). Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J .Food. Comp. Anal., 18: 625–633.

Liu, L., M. Heinrich, S. Myers, and S.A. Dworjanyn (2012). Towards a better understanding of medicinal uses of the brown seaweed *Sargassum* in traditional Chinese medicine: A phytochemical and pharmacological review. J. Ethnopharmacol., 142:591–619.

Maeda, H., T. Tsukui, T. Sashima, M. Hosokawa, and K. Miyashita (2008). Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. Asia. Pac. J. Clin. Nutr., 17: 196–199.

Makkar, H. P., M. Blummel, N.K. Borowy, and K. Becker (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agri., 61(2); 161-165.

Murgan, K., and V.V. Iyer (2012). Antioxidant and antiproliferative activities of Seaweeds from Chenni Coast. Int. J. Cancer. Res., 8: 15-26

Murgan, K., and V.V. Iyer (2014). Antioxidant and antiproliferative activities of extracts of selected red and brown Seaweeds from the mandapam Coast of Tamil nadu. J. Food. Biochem., 38: 92-26

Nair, V. D., R. Paneerselvam, and R. Gopi. (2012). Studies on methanolic extract of *Rauvolfia* species from Southern Western Ghats of India - In vitro antioxidant properties, characterisation of nutrients and phytochemicals. Ind. Crop. Prod., 39:17–25.

Prieto, P., M. Pineda, and M. Aguilar. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Bio., 269(2): 337-341.

Umayaparvathi, S., M. Arumugam, T. Balasubramanian, and S. Meenakshi. (2012). In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. Asian. Pac. J. Trop. Biomed., 1(Suppl 1): 66-70.

Vadlapudi, V., and K.C. Naidu (2010). In vitro bioevaluation of antioxidant activities of seaweeds. J. Pharm. Res., 3(2): 329-331

Vijayabaskar, P., and K. Shiyamala (2012). Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh. Asian. Pac. J. Trop. Biomed., 1(Suppl 1):S90–S98.

Vijayavel, K., and J.A. Martinez 2010. In vitro antioxidant and antimicrobial activities of two Hawaiian marine *limu*: *Ulva fasciata* (Chlorophyta) and *Gracilaria salicornia* (Rhodophyta). J. Med. Food., 13: 1494–1499.

Zubia, M., M. S. Fabre, V. Kerjean, K.L. Lann, V. Stiger-Pouvreau, and M. Fauchon (2009). Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. Food Chem., 116: 693–701.

Zubia, M., Payri C, Deslandes E. and J. Guezennec (2003). Chemical composition of attached and drifted brown algae, *Sargassum mangarevense* and *Turbinaria ornata*, from Tahiti (French Polynesia). Bot. Mar., 46: 562–571.