Complementary Therapies in Medicine 46 (2019) 19–23

Contents lists available at ScienceDirect

Complementary Therapies in Medicine

journal homepage: www.elsevier.com/locate/ctim

Short Communication

The antioxidative effects of bioactive products from *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh against inflammation and other pathological issues

M. Johnson[a], S. Asha Kanimozhi[a], T. Renisheya Joy Jeba Malar[a], T. Shibila[a], P.R. Freitas[b], S.R. Tintino[b], I.R.A. Menezes[b], J.G.M. da Costa[b], H.D.M. Coutinho[b,*]

[a] Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai, 627 002, Tamil Nadu, India
[b] Department of Biological Chemistry, Centre of Biological Science and Health, Regional University of Cariri – URCA, 63105-000, Crato, CE, Brazil

ARTICLE INFO

Keywords:
Antioxidant
DPPH
Scavening activities
Total phenol
Total flavonoids

ABSTRACT

The present study was aimed to determine the phenol, total flavonoids and antioxidant potentials of *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh from south east coast of Tamil Nadu and India using DPPH, phophomolybdenum and hydrogen peroxide scavenging activity. The total phenols, total flavonoids and antioxidant activities of *S. polycystum* and *S. duplicatum* were determined. Highest phenols (33.49 and 149.52 mg GAE/g) were observed in chloroform extracts of *S. polycystum* and methanolic extracts of *S. duplicatum*. The acetone extracts of *S. polycystum*and *S. duplicatum* demonstrated maximum amount of flavonoids compared to other studied extracts. Maximum amount of phosphomolybdenum reduction was observed in acetone extracts of *S. polycystum* and methanolic extracts of *S. duplicatum*. The DPPH radical scavenging activity of different extracts of *S. polycystum* and *S. duplicatum* were as follows acetone > chloroform > Petroleum ether > methanol and acetone > chloroform > methanol > Petroleum ether respectively. The H_2O_2 scavenging activity of *S. polycystum* extracts were as follows Petroleum ether extracts of *S. polycystum* (67.9%) > acetone (67.3%) > chloroform (58.6%) > methanol (51.78%). Acetone extracts of *S. duplicatum* showed maximum inhibition (90.39%) followed by petroleum ether, chloroform and methanolic extracts (75.11, 72.37 and 54.59%) respectively. The present study results confirmed the antioxidant properties of the two selected brown seaweeds viz., *S. polycytum* and *S. duplicatum*. The total phenols, flavonoids and alkaloids may be responsible for the antioxidant activities.

1. Introduction

The genus Sargassum belongs to the family Sargassaceae, order Fucales, subclass Cyclosporeae, class Phaeophyceae and generally recognized as gulf-weed or sea holly. The genus *Sargassum* contains approximately 400 species distributed in the tropical and subtropical areas of the world. Phytochemical analysis confirmed the occurrence of terpenoids, polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides etc. Due to their therapeutic and nutritional values they are considered as a medicinal food of the twenty-first century. Among the various natural antioxidants, marine seaweeds are now being considered to be a rich source of antioxidants. Various methods viz., 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, 2,2′-azinobis-3-ethylbenzo thizoline-6-sulfonate (ABTS) radical scavenging, NO scavenging, lipid peroxide inhibition, superoxide and hydroxyl radical scavenging assays are adopted to examine the antioxidant activities of Sargassum. A number of studies were carried out on the antioxidant potentials of the *Sargassum* viz., viz[1], *Sargassum boveanum*,[2] *Sargassum fulvellum*,[3] *Sargassum tenerrimum*,[4,5] *Sargassum siliquastrum*,[6] *Sargassum polycystum*,[7] *Sargassum vulgare* and *Sargassum angustrifolum*,[8] *Sargassum vulgare*,[9] *Sargassum micracannthum*,[10] *Sargassum myriocystum, S. plagiophyllum* and *S. ilicifolium*,[11] *Sargassum ringgoldianum* subsp. subsp,[12] *Sargassum vulgare*,[13] *Sargassum baccularia*,[14] *Sargassum baccularia* and *Sargassum binderi*,[15] *Sargassum coreanum*,[16] *Sargassum dentifolium*,[17] *Sargassum filipendula, S. crassifolium* and *S. binderi*,[18] *Sargassum graminifolium*,[19] *S. horneri*,[20] *Sargassum plagiophyllum*,[21] *Sargassum siliquastrum*,[22] *Sargassum swartzii* and *S. variegatum*[23] and *Sargassum wightii*.[24,25,11,26] The cold ethyl acetate extracts of *S. echinocarpum, S. duplicatum*and *S. polycystum*from Java Island,[27] *S. duplicatum* from Java Tengah also showed antioxidant

* Corresponding author at: Universidade Regional do Cariri, URCA, Rua Cel. AntonioLuis 1161, Pimenta, 63105-000, Brazil.
E-mail address: hdmcoutinho@urca.br (H.D.M. Coutinho).

https://doi.org/10.1016/j.ctim.2019.06.014
Received 30 April 2019; Received in revised form 13 June 2019; Accepted 23 June 2019
Available online 10 July 2019
0965-2299/

properties using DPPH.[27] Revealed the phytochemical composition of *S. polycytum*and *S. duplicatum*.[28] But there is no report on the total phenol, total flavonoids and antioxidant activities of *Sargassum polycyctum* and *Sargassum duplicatum* from Manapad / Rasthkadu / Hare Island of Tamil Nadu and India. Hence, the present study was aimed to determine the phenol, total flavonoids and antioxidant potentials of *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh from Manapad / Rasthkadu / Hare Island of Tamil Nadu and India using DPPH, phophomolybdenum and hydrogen peroxide scavenging activity.

2. Materials and methods

2.1. Collection of plant materials

Seaweeds *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh were collected from the South East Coast of Tamil Nadu, India by handpicking method.The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. It was then thoroughly washed with tap water followed by distilled water to remove the unwanted debris. Seaweeds were blotted on the blotting paper and spread out at room temperature in shade for 20 days. The shade dried samples were grounded to fine powder using tissue blender. The powdered samples were then stored in refrigerator for further analysis.

2.2. Preparation of extracts

The dried and powdered materials (10 g) of *S. polycystum* and *S. duplicatum* were extracted with 60 ml of solvents viz., petroleum ether, chloroform, acetone, methanol and aqueous. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

2.3. Determination of total phenolics

The total phenolic content was determined according to the method described.[30] 100–200 µL aliquots were taken in the test tubes and made up to the volume of 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min, and the absorbance was recorded at 725 nm against blank. The analysis was performed in triplicates and the results were expressed as GAE.

2.4. Estimation of total flavonoids

The flavonoid contents of all the extracts were quantified as it acts as a major antioxidant in plants, reducing oxidative stress, estimated as per described.[31] Initially, 500 µL of extracts was taken in different test tubes. To each extract, 2 mL of distilled water was added. Then 150 µL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 µL of $AlCl_3$ (10%) was added to all the test tubes including the blank. All the test tubes were incubated for 6 min at room temperature. Then 2 mL of 8% NaOH was added, which was made up to 5 mL using distilled water. The contents in all the test tubes were vortexed well and they were allowed to stand for 15 min at room temperature. The pink color developed was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated in GAE.

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating of radical scavenging ability using the stable radical DPPH, according to the method.[32] Sample extracts at various concentrations were taken and the volume was adjusted to 10 µL with methanol. About 5 ml of 0.1 mM methanolic solution of DPPH was added to the aliquots of samples and standards (BHA, BHT, Rutin and Vitamin E) and shaken vigorously. Negative control was prepared by adding 100 µL of methanol in 5 ml of 0.1 mMmethanolic solution DPPH. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 514 nm against the blank (Methanol). Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH concentration.

2.5.2. Phosphomolybdenum assay

The antioxidant activity was evaluated by the green phosphomolybdenum complex formation according to the method described.[33] An aliquot of 100 µL of sample solution (in 1 mM dimethyl sulfoxide) was combined with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 mL vial. The vials were capped and incubated in a water bath at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results are mean values expressed as g of ascorbic acid (AA) equivalents/100 g extract.

2.5.3. Scavenging of hydrogen peroxide

The ability of extracts to scavenge hydrogen peroxide was determined according to the method.[34] A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined from absorption spectra at 230 nm with molar absorbtivity 81 (mol/L)/cm. Extracts (100–200 mg/mL) were added to hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated using the formula

$$\% \text{ scavenging activity} = [(\text{Control OD} - \text{Sample OD}) / \text{Control OD}] \times 100$$

3. Results

In the present study, the total phenols obtained from *S. polycystum* and *S. duplicatum* using different solvent systems were represented in Table 1. The maximum extractable value of total phenols (33.49 mg GAE/g) was recorded in chloroform extracts followed by acetone and methanolic extracts in *S. polycystum*. The petroleum ether extracts of *S. polycystum* showed the lowest level of total phenolics (17.46 mg GAE/g). In *S. duplicatum* the higher amount of phenols was observed in methanolic extracts (149.52 mg GAE/g) whereas acetone and chloroform extracts demonstrated moderate level of phenolics. Petroleum ether extracts of *S. duplicatum*showed the minimum amount of phenols (8.41 mg GAE/g).

The flavonoid content of different extracts of *S. polycystum* varied considerably from 865.55 to 340 mg GAE/g extract. Highest amount of flavonoids was present in acetone extracts (865.55 mg GAE/g) followed by methanolic and petroleum ether extracts. Chloroform extracts gave the lowest level of total flavonoids (340 mg GAE/g) compared to other extracts. In *S. duplicatum* the highest amount of total flavonoids was observed in methanolic extracts (149.52 mg GAE/g) whereas the other

Table 1
Total flavonoids and phenols of various extracts of *S. polycystum* and *S. duplicatum*.

Extracts	Phenols in mg GAE/g extract		Flavonoids in mg GAE/g extract	
	S. polycystum	*S. duplicatum*	*S. polycystum*	*S. duplicatum*
Pet.Ether	17.46 ± 0.99	511.11 ± 19.24	429.0 ± 13.33	8.41 ± 0.99
Chloroform	33.49 ± 4.42	340 ± 3.33	455.5 ± 7.69	18.41 ± 3.09
Acetone	18.09 ± 3.33	865.55 ± 19.24	953.33 ± 11.54	28.57 ± 3.77
Methanol	18.88 ± 3.34	700 ± 33.33	691.11 ± 10.18	149.52 ± 2.07

Table 2
Phosphomolybdenum reduction of different extracts of *S. polycystum*and *S. duplicatum*.

Extracts	TAC-mg AA/g extract	
	S. polycystum	*S. duplicatum*
Petroleum Ether	126.21 ± 1.41	41.57 ± 0.56
Chloroform	98.68 ± 1.16	75.84 ± 30.24
Acetone	240.26 ± 36.41	165.91 ± 2.12
Methanol	127.90 ± 0.85	688.98 ± 8.33

extracts showed moderate level of flavonoids (Table 1).

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate / Mo (V) complex with the absorption at 695 nm. The results of phosphomolybdenum assay of different extracts of *S. polycystum* and *S. duplicatum* were illustrated in Table 2. Among the various extracts of *S. polycystum* acetone extracts showed the strongest phosphomolybdenum reduction (240.26 g AA/100) followed by methanolic extracts (127.90 g AA/100 g). Petroleum ether and chloroform extracts showed the lowest level of phosphomolybdenum reduction in *S. polycystum*. In *S. duplicatum*the methanolic extracts had highest phosphomolybdenum reduction (688.98 g AA/100 g) activity followed by moderate reduction was noticed in acetone extract (165.91 g AA/100 g). Petroleum ether and chloroform extracts demonstrated the lowest level of phosphomolybdenum reduction.

The DPPH is a stable free radical which is commonly used for assessing antioxidant activity in plant samples. The DPPH assays were expressed in IC_{50} values. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined. Therefore, the lower value of IC_{50} indicates a higher antioxidant activity.[35] The best DPPH activity was exerted by acetone extracts of *S. polycystum* (IC_{50} 183.82 µg/ml) and acetone extracts of *S. duplicatum* (IC_{50} 225.22 µg/ml). The DPPH radical scavenging activity of different extracts of *S. polycystum* and *S. duplicatum* were as follows acetone (183.82 µg/ml) > chloroform (192.3 µg/ml) > Petroleum ether (211.86 µg/ml) > methanol (214.59 µg/ml) and acetone (225.22 µg/ml) > chloroform (231.48 µg/ml) > methanol (251.25 µg/ml) > Petroleum ether (287.35 µg/ml) respectively.

The extracts of *S. polycystum* and *S. duplicatum* showed an efficient H_2O_2 scavenging activity in concentration-dependent manner. The H_2O_2 scavenging activity of *S. polycystum* extracts were as follows Petroleum ether extracts of *S. polycystum* (67.9%) > acetone (67.3%) > chloroform (58.6%) > methanol (51.78%). Acetone extracts of *S. duplicatum* showed maximum inhibition (90.39%) followed by petroleum ether, chloroform and methanolic extracts (75.11, 72.37 and 54.59%) respectively.

4. Discussion

Biopotency of seaweeds were proven by number of biological and pharamcological studies. The seaweeds possess various biological activities viz., antibacterial, antiviral, antitumour, antifungal, antiprotozoal, antioxidant and mosquito and larva control.[36–39] Among the seaweeds, *Sargassum* possesses various threapeutic potentials. Listed out the therapeutic potential and health benefits of *Sargassum* species.[40] To strengthen the therapeutic value of *Sargassum*, in the present study the antioxidant potentials of *S. polycystum* and *S. duplicatum* are documented.

The maximum extractable value of total phenols (33.49 mg GAE/g) was recorded in chloroform extracts of *S. polycystum*. In *S. duplicatum* the higher amount of phenols was observed in methanolic extracts (149.52 mg GAE/g). Highest amount of flavonoids was present in acetone extracts (865.55 mg GAE/g) of *S. polycystum* and *S. duplicatum* (953.33 mg GAE/g).

The best DPPH activity was exerted by acetone extracts of *S. polycystum* (IC_{50} 183.82 µg/ml) followed by acetone extract of *S. duplicatum* (IC_{50} 225.22 µg/ml). The petroleum ether extracts of *S. polycystum* showed an efficient H_2O_2 scavenging activity. Among the various extracts of *S. polycystum* acetone extracts showed the strongest phosphomolybdenum reduction (240.26 g AA/100).

Flavonoids, the largest groups of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties.[41] Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids.[42] The phosphomylpdenum method of activity is directly correlated with flavonoids concentrations of *S. duplicatum*. The concentration of flavonoids and antixodiant activity of *S. duplicatum* were as follows methanol > acetone > chloroform > petroleum ether. The observed results explained the significance of the solvent polarity and solubility for the extraction. Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation.[43–45] Many marine plants, including seaweeds, often carry significantly less macro and microepibionts on their thalli compared to co-occurring biofilms on inanimate substrata.[46,47] Therefore it has been assumed that seaweeds defend themselves against bacterial fouling by production of secondary metabolites that prevent attachment and growth of bacterial colonizers.[48] Recently, consumers are demanding foods which are fresh, natural and minimally processed along with the requirement for enhanced safety and quality. This perspective has put pressure on the food industry for progressive removal of chemical preservatives, and has fuelled research into alternative natural antimicrobials. Plant products with antimicrobial properties have obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth.[49] In the present study, the presence of flavonoid was observed in acetone and methanolic extracts of *Sargassum* species. In *S. duplicatum*, flavonoid showed its presence in all the extracts except in petroleum ether and acetone extract.Highest amount of flavonoids was present in acetone extracts (865.55 mg GAE/g) followed by methanolic and petroleum ether extracts. Chloroform extracts gave the lowest level of total flavonoids (340 mg GAE/g) compared to other extracts. In *S. duplicatum*the highest amount of total flavonoids was observed in acetone extracts (953.33 mg GAE/g).

Seaweeds are of applications in several industries due to the presence of polyphenols such as phenolic acids, flavonoids, anthocyanidins, lignin, tannins, catechin, epicatechin, epigallocatechin and gallic acid.[50,51] The polyphenolic compounds have much health benefits such

as antioxidant, anticancer, antiviral, anti-inflammatory and an ability to inhibit human platelet aggregation.[52] However, polyphenol from brown seaweeds and their relationship with antioxidant activities are poorly validated. Have revealed that polyphenolic compounds are one of the most effective antioxidants in brown algae.[53] the total antioxidant activity in the methanolic extract of *Sargassam muticum* are estimated previousuly.[54] Revealed the phytochemical composition of *S. polycytum* and *S. duplicatum*.[29] In the present study also the total phenolics of *S. polycytum* and *S. duplicatum* is quantified. The existence of phenol quantity and antioxidant activities of *S. polycytum* and *S. duplicatum* are coincided. The total phenolics was maximum in acetone and metahnolic extrcats of *S. polycytum* and *S. duplicatum*, both extracts showed maximum antixodant activity. The observed results of metabolites quantification and antioxidant activity clearly explained the relationship between the metabolites existence and activity. The acetone and methanolic extracts of *S. polycytum* and *S. duplicatum* showed higher activity than the other tested extracts (chloroform and petroleum ether). The *t*-test between phenol concentration and antioxidant activity showed the significance with $t = 0.011$. The correlation coeeficient $r = 0.867$ was observed between the flavonoids concentration of *S. polycystum* and antioxidant activity (phosphomolybdenum assay). The correlation coeeficient $r = 0.800$ was observed between the flavonoids concentration of *S. duplicatum* and antioxidant activity (phosphomolybdenum assay). The preliminary phytochemical analysis confirmed the existence of steroids, flavonoids, phenols, cardiac glycosides, saponoins, alkaloids from *S. polycytum* and *S. duplicatum* etc.

5. Conclusion

The present study results confirmed the antioxidant properties of the two selected brown seaweeds viz., *S. polycytum* and *S. duplicatum* and noted that the highest antioxidant activity was expressed in methanolic extract of the selected *Sargassum* species. The total phenols, flavonoids, alkaloids may be responsible for the antioxidant activities. The results of the present study provided a clue for the antioxidant properties of *S. polycytum* and *S. duplicatum.* The studied magroalgae *S. polycytum* and *S. duplicatum* extracts showed good antioxidant properties, showed the potential health ingredient for human nutrition. Further research is required to isolate active fractions may bring out a natural antioxidant with nutritive value.

References

1. Hwang PA, Wu CH, Gau SY, Chien SY, Hwang DF. Antioxidant and immune stimulating activities of hot water extract from seaweed Sargassumhemiphyllum. *J Mar Sci Technol.* 2010;18:41–46.
2. Zahra R, Mehrnaz M, Farzaneh V, Kohzad S. Antioxidant activity of extract from a brown alga, *Sargassumboveanum*. *Afr J Biotechnol.* 2007;6:2740–2745.
3. Kim SH, Choi DS, Yasantha A, Jeon YJ, Mahinda S, Rha CK. Antioxidant activity of sulfated polysaccharides isolated from *Sargassumfulvellum*. *J Food Sci Nutr.* 2007;12:1–10.
4. Marudhupandi T, Ajithkumar TT, Lakshmana Senthil S, Nanthini Devi K. In vitro antioxidant properties of Fucoidan fractions from Sargassumtenerrimum. *Pak J Biol Sci.* 2014;17:402–407.
5. Patra S, Muthuraman MS, Prabhu AR, Priyadharshini RR, Parthiban S. Evaluation of antitumor and antioxidant activity of *Sargassumtenerrimum*against Ehrlich ascites carcinoma in mice. *Asian Pac J Cancer Prev.* 2015;16:915–921.
6. Lee JI, Seo Y. Chromanols from Sargassumsiliquastrum and their antioxidant activity in HT 1080 cells. *Chem Pharm Bull (Tokyo).* 2011;59:757–761.
7. Anggadiredja J, Ria A, Hayati Muawanah. Antioxidant activity of *Sargassumpolycystum* (Phaeophyta) and *Laurenciaobtusa* (Rhodophyta) from Seribu Islands. *J Appl Phycol.* 1997;9:477–479.
8. Rastian Z, Mehranian M, Vahabzadeh F, Sartavi K. Antioxidant activity of brown algae *Sargassum vulgar* and *sargassumangustrifolum*. *J Aquat Food Prod Technol.* 2007;16:17–26.
9. Sarojini Y, Sujatha B, Lakshminarayanana K. Total phenol content and antioxidant activities of ethanolic extracts of two marine brown macroalgae. *Int J Curr Sci.* 2013;8:43–49.
10. Ham MY, Kim KN, Lee WJ, Lee NH, Hyun CG. Chemical constituents from Sargassummicrantham and antioxidant activity. *Int J Pharmacol.* 2010;6:145–151.
11. Kokilam G, Vasuki S, Sajotha N. Biochemical composition, alginic acid yield and antioxidant activity of brown seaweeds from Mandapam region, Gulf of Mannar. *J Appl Pharm Sci.* 2013;3:99–104.
12. Yang XD, Kang MC, Lee KW, Kang SM, Lee WW, Jeon YJ. Antioxidant activity and cell protective effect of loliolide isolated from *Sargassumringgoldianum* subsp. *Coreanum*. *Algae.* 2011;26:201–208.
13. Khaled N, Hiba M, Asma C. Antioxidant and antifungal activities of PadinaPavonica and Sargassum vulgare from the lebanese Mediterranean Coast. *Adv Environ Biol.* 2012;6:42–48.
14. Sheikh TZB, Yong CL, Lian MS. *In vitro*antioxidant activity of the hexane and methanolic extracts of *Sargassum baccularia* and *Cladophora patentiramea*. *J Appl Sci.* 2009;9:2490–2493.
15. Sarini AW, Nor'Aishah H, MohdZaini N. Determination of antioxidant activity for seven types of macroalgae. *5Th International Conference on Food Engineering and Biotechnology.* 2014. https://doi.org/10.7763/IPCBEE 11.
16. Ko SC, Kang SM, Ahn G, Yang HP, Kim KN, Jeon YJ. Antioxidant activity of Enzymatic extracts from Sargassumcoreanum. *Korean Soc Food Sci Nutr.* 2010;39:491–499.
17. Shanab MMS. Antioxidant and Antibiotic activities of some seaweeds (Egyptian Isolates). *Int J Agric Biol.* 2007;9:220–225.
18. Bambang BS, Kumalaningsih S, Susinggih W. Polyphenol content and antioxidant activities of crude extracts from Brown algae by various solvents. *J Life Sci Biomed.* 2013;3:439–443.
19. Zhang CY, Wu WH, Wang J, Lan MB. Antioxidant properties of Polysaccharide from the Brown Seaweed *Sargassumgraminifolium*(turn.), and its effects on calcium oxalate crystallization. *Mar Drugs.* 2012;10:119–130.
20. Park PJ, Shahidi F, Jeon YJ. Antioxidant activities of enzymatic extracts from an edible seaweed *Sargassumhorneri*using ESR spectrometry. *J Food Lipids.* 2004;11:15–27.
21. Suresh V, Kumar NS, Murugan P, Palani P. Antioxidant properties of sequential extracts from brown seaweed Sargassumpalgiophyllum C. Agardh. *Asian Pac J Trop Dis.* 2012:937–939.
22. Cho SH, Cho JY, Kang SE, Hong YK, Ahn DH. Antioxidant activity of mojabanchromanol a novel chromene, isolated from brown alga Sargassumsiliquasrum. *J Environ Biol.* 2008;29:479–484.
23. Tariq A, Ara J, Sultana V, Haque SE, Athar M. Antioxidant Potential of seaweeds occurring at Karachi coast of Pakistan. *J Appl Bot Food Qual.* 2011;84:207–212.
24. Devi KP, Suganthy N, Kesika P, Karuthapandian S. Bioprotective properties of Seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Complement Altern Med.* 2008;8:38.
25. Meenakshi S, Umayaparvathi S, Arumugam M, Balasubramanian T. In vitro antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. *Asian Pac J Trop Biomed.* 2012:S66–S70.
26. Syad AN, Shunmugiah KP, Kasi PD. Antioxidant and anti-cholinesterase activity of Sargassumwightii. *Pharm Biol.* 2013;51:1401–1410.
27. Widowati I, Susanto AB, Puspita M, Stiger-Pouvreau V, Bourgougnon N. Potentiality of using spreading *Sargassum*Species from Indonesia as an interesting source of antibacterial and radical scavenging compounds: A preliminary study international. *J Mar Aquat Resour Conserv Co-existence.* 2014;1:63–67.
28. Septiana AT, Asnani DA. Antioxidant activity of *Sargassumduplicatum*seaweed extract. *J Teknologi Pertanian.* 2013;14:79–86.
29. Asha Kanimozhi S, Johnson M, Malar TRJJ. Phytochemical composition of SargassumPolycystum C. Agardh and SargassumDuplicatum J. Agardh. *Int J Pharm Pharm Sci.* 2016;7:393–397.
30. Siddhuraju P, Becker K. Studies on antioxidant activities of Mucuna seed (*Mucunapruriens* var. *utilis*) extracts and certain non-protein amino/imino acids through *in vitro*models. *J Sci Food Agri.* 2003;83:1517–1524.
31. Zishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging affects on superoxide radicals. *Food Chem.* 1999;64:555–559.
32. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I. Antioxidant principles from Bauhinia terappotensis. *J Nat Prod.* 2001;64:892–895.
33. Prieto P, Pineda M, Aguilar M. Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem.* 1999;269:337–341.
34. Ruch RJ, Cheng SJ, Klauning JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidants catechins isolated from isolated from Chinese green tea. *Carcinogenesis.* 1989;10:1003–1008.
35. Sivaraman A, Johnson M, Parimelazhagan T, Irudayaraj V. Evaluation of antioxidant potential of ethanolic extracts of selected species of Selaginella. *Indian J Nat Prod Resour.* 2013;4(3):238–244.
36. Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *J Food Compost Anal.* 2005;18:625–633.
37. Bansemir A, Blume M, Schroder S, Lindequist U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture.* 2006;252:79–84.
38. Asker MMS, Mohamed SF, Ali FM, El-Sayed OH. Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from Sargassumlatifolium. *J Appl Sci Res.* 2007;3:1178–1185.
39. Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.* 2009;116:240–248.
40. Subhash RY, Uday NH, Bhupal BC. Therapeutic potential and health benefits of Sargassum species. *Pharmacogn Rev.* 2014;8:1–7.
41. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem.* 1999;47:3954–3962.

42. Ndhlala AR, Kasiyamhuru A, Mupure C, Chitindingu K, Benhura MA, Muchuweti M. Phenolic composition of Flacourtiaindica, Opuntiamegacantha and sclerocaryabirrea. *Food Chem.* 2007;103:82–87.
43. Harbourne JB. *The flavonoids: Advances in research since.* London, UK: Chapman and Hall; 1986 1994.
44. Herrmann K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in food. *CRC Crit Rev Food Sci Nutr.* 1989;28:315–347.
45. Wallace G, Fry SC. Phenolic components of the plant cell wall. *Int Rev Cytol.* 1994;151:229–267.
46. Hellio C, De La Broise D, Dufosse L, Le Gal Y, Bourgougnon N. Inhibition of marine bacteria by extracts of macroalgae: Potential use for environmentally friendly antifouling paints. *Mar Environ Res.* 2001;52:231–247.
47. Lam C, Harder T. Marine macroalgae effect abundance and community richness of bacterioplankton in close proximity. *J Phycol.* 2007;43:874–881.
48. Maximilien R, De Nys R, et al. Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga Deliseapulchra. *Aquat Microbial Ecol.* 1998;15:233–246.
49. Lanciotti R, Gianotti A, Patrignani A, Belleti N, Guerzoni ME, Gardini F. Use of natural aroma compounds to improve shelf-life of minimally processed fruits. *Trends Food Sci Technol.* 2004;15:201–208.
50. Yoshie Y, Wang W, Hsieh YP, Suzuki T. Compositional Difference of Phenolic Compounds between Two Seaweeds, Halimeda spp. *J Tokyo Univ Fish.* 2002;88:21–24.
51. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants Wetland Ecol. *Management.* 2002;10:421–452.
52. Mohsen SM, Ammar ASM. Total phenolic contents and antioxidant activity of corn tassel extracts. *Food Chem.* 2009;112:595–598.
53. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphoniaurceolata. *Food Chem.* 2006;95:37–43.
54. Bhaskar N, Miyashita K. Lipid composition of *Padinatetrastomatica*a brown seaweed of the west coast of India. *Ind J Fish.* 2005;52:263–268.