NVEO
Natural Volatiles & Essential Oils

Phytochemical analysis and DPPH radical scavenging activity of marine red seaweed Gracilaria corticata

V. Sornalakshmi[1*], P.S. Tresina[2], K. Jeba Ananthi[3]

[1]Department of Botany, A.P.C. Mahalaxmi College for Women Thoothukudi, Tamil Nadu, India*
Email – sornalakshmiapcm@gmail.com
[2]Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Thoothukudi, Tamil Nadu, India Email – tresi.bot@voccollege.ac.in
[3]Department of Botany Sarah Tucker College (Autonomous), Tirunelveli, Tamil Nadu, India
Email - jebajeyakumar18@gmail.com

Abstract

In recent times, researchers paid more attention on marine macro algae or seaweeds owing its biological activities with their multiple applications to the humans. In the marine environment red seaweeds are renowned for their biological activities due to the presence of various bioactive substances including phenolic residues. The present investigation deals with the qualitiative and quantitative phytochemical analysis of red seaweed *Gracilaria corticata*. DPPH radical scavenging activity using different solvent extracts also performed. Preliminary phytochemical screening confirmed the existence of proteins, carbohydrates, lipids, aromatic acids, alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, saponins, coumarins, quinones, anthroquinones and catechins. *G. corticata* contains 16.7% protein, 1.0 % lipid, 7.9% carbohydrate, 3.14 mg GAE/g DW phenol and 1.05 mg GAE/g DW flavonoid. Maximum DPPH radical scavenging ability was recorded by ethanol extract (74.5%) followed by methanol (73.82%), water (73.62%), chloroform (36.9%) and petroleum ether (18.2%). No scavenging activity recorded by benzene extract.

Keywords: *Gracilaria corticata*, seaweed, phytochemicals, DPPH radical

Introduction

More than 70 % of the world covered by ocean which provide shelter for wide variety of marine planktons, plants and animals. These unique marine environmental organisms offer a rich foundation for natural products and several miracles of this location still remains unknown. Recent investigation suggested that variety of phytocompounds like polyphenolic compounds, peptides, polysaccharides, antioxidants, essential vitamins polyunsaturated fatty acids and minerals are derived from these marine organisms (Fernando et al., 2017). Abundant number of organisms and different kind of species are present in the vast marine environment. Seaweeds otherwise called as macroalgae with lot of biological properties are an important eco – friendly therapeutically important species from marine resources (Umavandhana and Jayanth, 2018).

This macrophytic marine algae growing in salt water and largely available in surface sea waters. It provides a extensive variety of healing properties. In Japan and China seaweeds are used by man for eras and used as part of staple food. Many countries are well-known for the use of marine algae as food, animal feed and fertilizer. It contains more than 60 trace elements and it is higher than the land plants. Stimulatory and antibiotic substances and other components like carbohydrates, protein, amino acids, iodine, bromine and vitamins are occur in seaweeds (Kannan, 2014).

There is increasing attention in substances that possess antioxidant properties, which are used in nutrition and pharmaceuticals. (Ekrem and Ilhami, 2008). As enormous amount of antioxidant compounds is present in seaweeds it plays a vital part against many illnesses and anti – ageing processes by preventing the cells from oxidative injury. Bioactive substances like polyphenols and phlorotannins present in seaweeds are act as prominent antioxidant compounds. Progression of numerous chronic diseases were retarded by these substances and also shelter the human body from free radicals. Due to this reason food industries and pharmaceuticals targeting antioxidants from seaweeds for the development of health promoting constituents (Dhinakaran *et al*. 2015). With this background information this work was aimed to assess phytochemical content and DPPH scavenging capacity of marine red seaweed *G.corticata.*

Materials and methods

Collection of seaweed

The commonly available red seaweed *Gracilaria corticata was* collected from the Red Gate end of Hare Island, Thoothukudi, Tamil Nadu, India. After collection it was carried to the laboratory and washed thoroughly then subjected to shade dry. Dried matter was powdered and used for further analysis.

Preliminary phytochemical analysis

The qualitative assessments to categorize the numerous chemical ingredients were carried out in different solvent extracts of red seaweed *G.corticata* using the procedures suggested by Brindha *et al* (1981).

Estimation of Biochemical constituents

Estimation of carbohydrates (Roe, 1955)

10 mg of the seaweed powder was homogenised with 4 ml of distilled water, 1ml of 10% TCA (trichloroacetic acid) and centrifuged at 3000 rpm for 15 minutes. 4 ml of anthrone reagent was added to 1 ml of the supernatant solution. It was incubated in a hot water bath for about 10 minutes. After it was measured at 620 nm with Elico SC-177 Scanning mini spectrophotometer. Standard was prepared by liquifying 10 mg glucose with 100 ml of distilled water.

Estimation of proteins

10 mg of the seaweed powder was ground with 10% (TCA) trichloroacetic acid and then it was centrifuged for 10 minutes at 3000 rpm. The supernatant trichloroacetic acid (TCA) was decanted and to the sedimented protein After 10 minutes, the supernatant was decanted and 0.1 ml of 1N Sodium hydroxide was added to the sedimented protein. This was located in hot water bath at 60 - 70° C for 10 minutes. To 0.5 ml of the above solution 4.5 ml of reagent D (Copper carbonate solution) was added. After 10 minutes 0.5 ml of folin-ciocalteau was added then mixed well. After the development of blue colour, it was measured at 640 nm. Peptone was used as standard (Lowry *et al*., 1951).

Estimation of lipids

10 mg of the seaweed powder ground with sufficient amount of chloroform. This was evaporated to dryness in a water bath. To this, 3 ml of potassium dichromate reagent (2% potassium dichromate in H_2So_4) was mixed. Then equal amount of distilled water and read at 640 nm. Coconut oil is used as standard. A blank was prepared using distilled water to equal quantity of reagent (Bragdon, 1951).

Estimation of phenols

Phenolic content in ethanol solution was measured by Folin- Ciocalteu's Reagent method (FCR). Seaweed extract was mixed with 0.4 ml by Folin- Ciocalteu's Reagent. Afterwards 4 ml of Na_2Co_3 solution was added. The ending capacity was made up to 10 ml by distilled water. After 90 minutes absorbance of sample was measured at 750 nm. A standard was run using 100 mg of catechol in 100 ml water (Chang *et al*., 2002)

Estimation of flavonoids

Aluminium chloride technique was used to determine flavonoid content. To 1 ml of ethanolic extract 4 ml of water was added. 0.3 ml of 10% aluminium chloride and 0.3 ml of 5 % sodium nitrite were added after 5 minutes. 2 ml of 1 M sodium hydroxide was added after six minutes and the end capacity was made to 10 ml by distilled water. Then the absorbance was read at 510 nm. A standard was run using 10 mg of catechin in 100 ml of water (Mervat *et al.*, 2009).

DPPH Radical Scavenging Assay (Brand-Williams et al., 1995)

For this 1ml of 0.1mM DPPH solution in methanol was allowed to mix with 1ml of different concentration of seaweed extract (50, 100, 150 and 200µg/ml). L-Ascorbic acid was used as standard. For

control methanol was used instead of seaweed sample. The reaction was carried out in triplicate and after 30 minutes in dark the decrease in absorbance was read at 517nm

Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control

As is the absorbance of the sample

Statistical Analysis

All the studies were estimated in triplicate. Standard deviation and standard error were calculated following Zar, (1974).

Results

Qualitative analysis

Preliminary phytochemical screening of 15 different chemical compounds (proteins, carbohydrates, lipids, aromatic acids, alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, saponins, coumarins, quinones, anthroquinones and catechins) were tested in four different extracts of *G.corticata*. Thus 60 (1 x 15 x 4 = 60) tests were executed for the presence or absence of the above compounds. Out of sixty tests 32 showed positive results and the remaining 28 showed negative results (Table 1).

Table 1 Preliminary phytochemical screening of different solvent extracts of *Gracilaria corticata*

Tests	Petroleum Ether	Benzene	Ethanol	Water
Carbohydrates	+	-	+	+
Proteins	+	-	+	+
Lipids	+	-	+	+
Aromatic acids	+	-	+	-
Phenols	-	+	+	+
Flavanoids	-	+	+	+
Alkaloids	+	-	+	+
Terpenoids	-	-	+	+
Steroids	-	-	+	-
Coumarins	-	-	+	-
Tannins	-	-	+	+
Saponins	-	-	+	-
Quinones	-	-	+	-
Anthroquinones	-	-	+	+
Catechins	+	-	+	-

Among the four various solvent extracts tested, ethanol showed the presence for all compounds studied. It is followed by water extract which showed presence for 9 compounds namely carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, terpenoids, tannins and anthroquinones. Petroleum ether extract showed positive result for six compounds (carbohydrates, proteins, lipids, aromatic acids, alkaloids and catechins). Benzene extract confirmed the presence of only two compounds (phenols and flavonoids).

Quantitative analysis

Table 2 Biochemical content of *G. corticata*

Constituents	(%) dry wt.
Carbohydrates	7.9 ± 0.3
Proteins	16.7 ± 0.4
Lipids	1 ± 0.01

Table 3 Phenol and flavonoid content of *G. corticata*

Constituents	mg GAE/g DW
Phenol	3.14 ± 0.26

Flavonoid	1.05 ± 0.41

The chemical composition of *G.corticata* in the existing study is shown in Table 2 and 3. It was found to contain carbohydrates 7.9%, protein 16.7%, lipids 1%, phenols 3.14 mg GAE/g DW, and flavonoids 1.05 mg GAE/g DW.

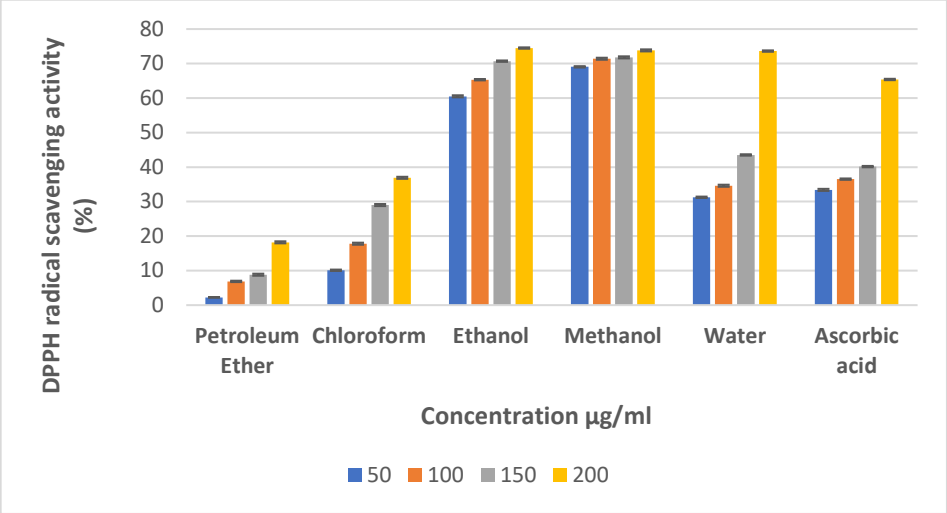

Fig. 1. DPPH radical scavenging potential of various solvent extract of *Gracilaria corticata*

DPPH radical scavenging capacity

The DPPH radical scavenging potential by different solvent extracts of the seaweed *G.corticata* was assessed at four concentrations such as 50, 100, 150 and 200 µg/ml of the extracts and the results showed in Figure 1. Among the solvent studied ethanol methanol and water extracts were found to be effective in quenching DPPH radical and there is no scavenging activity was recorded by benzene extract. Ethanol, methanol and water extracts at 200 µg/ml concentration showed 74.5%, 73.82% and 73.62% of DPPH radical scavenging activity respectively. These values were higher than the standard ascorbic acid (65.39). Minimum activity was recorded by petroleum ether extract.

Discussion

Recently seaweeds have more environmental and commercial status all over the world as they are widely used in food, medicine and agricultural sectors. This widespread application is due to the presence of variety of bioactive components with therapeutic properties. In the current study, phytochemical screening of Gracilaria showed the presence of all the phytochemicals studied such as proteins, carbohydrates, lipids, aromatic acids, alkaloids, phenols, flavonoids, tannins, terpenoids, steroids, saponins, coumarins, quinones, anthroquinones and catechins.

Carbohydrate, protein and lipids are the chief vital biochemical elements of seaweeds. The amount of protein in green and red seaweeds are generally higher (10 to 30%) compare to brown seaweeds (5 to 15%) (Burtin, 2003). In the current study, when compare to other reports *G.corticata* contains higher amount of protein (16.7%). Previous studies reported lower content of protein than the present study like 0.61% in *G. acerosa* (Syad et al., 2013), 6.68% in *G. edulis* (Sakthivel and Devi, 2015), 6.2% in *G. domingensis,* 7.1% in *G. birdiae* (Gressler et al., 2010), 9.55% in *G. Salicornia* (Mwalugha et al., 2015), 12.57% in *G. changii* (Chan and Matanjun, 2017) and 11.6% in *G.fisheri* (Benjama and Masniyom, 2012).

At the same time higher values than the present one also recorded by *G. verrucosa* (18.7%) (Nazni and Deepa, 2015) *G. cervicornis* (19.70%) (Marinho-Soriano et al., 2006) *G. tenuistipitata* (21.6) (Benjama and Masniyom, 2012), *G. corticata* (22.84%) and *G. edulis* (25.29%) (Rosemary et al., 2019). Seaweed proteins have antioxidant, anti-inflammatory, antibacterial, antithrombotic and immunostimulating activities. Subsequently, they can be used for treating diabetes, hypertension and hepatitis (Francavilla et al., 2013).

The lipid content observed in this study was 1.0%. This amount is lower than other species of *Gracilaria* such as *G. arcuate* (1.07%) and *G. salicornia* (1.47%) (Mwalugha et al., 2015) *G. salicornia* (2.00%) (Tabarsa et al., 2012) and higher than *G. gracilis* (0.19%) (Rasyid et al., 2019).

Carbohydrate is vital constituent of metabolism, chiefly in supplying the energy required for growth and other metabolic activities (Khairy and El-Sharay, 2013). In the current study, carbohydrate content was distinctly lower than that of other reports in *G. edulis* (10.2%) (Sakthivel and Devi, 2015), *G. changii* (29.44%) (Chan and Matanjun, 2017), *G. verrucosa* (33.67%) (Nazni and Deepa, 2015), *G. cornea* (36.29%) (Robledo and Freile-Pelegrin, 1997), *G. cervicornis* (63.12%) (Marinho-Soriano et al. 2006), *G. gracilis* (63.13%) (Rasyid et al., 2019).

However lower value of 1.05% in *G. acerosa* (Syad et al., 2013) and 4.7% in *G. edulis* (Rosemary et al., 2019) were reported. The extensive difference in the amount of carbohydrate detected in Rhodophycean and Phaeophycean species due to the impact of diverse features like sunlight intensity, temperature and salinity (Torres et al., 2019). Furthermore, carbohydrate content is also affected by biomass, which exposes the connection between carbohydrate content and growth (Mabeau and Fleurence 1993).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a firm radical has a higher absorbance at 517 nm that can be reduced by an antioxidant. Owing to the ease and suitability of this, it is used in the free radical-scavenging studies (Brand-Williams et al., 1995). In the current work, it exhibited dose dependency, the scavenging potential was increased with increasing concentration of the seaweed extract and the standard. There is correlation between % inhibition of free radicals and concentration of the extract (Rana et al., 2010).

Solvent used for extraction is a chief parameter for the efficacy of antioxidant substances. Ethanol, methanol and water extracts were seems to be effective in eliminating DPPH radical and no scavenging activity was recorded by benzene extract in the present work. Previous study reported high DPPH radical scavenging activity in methanol extract from red seaweed *G. corticata* (Taheri, 2016) *Euchema kappaphycus* (Ganesan et al., 2008). Viswanathan et al., (2014) and Adnan (2011) stated the effective antioxidant compound phenol yield was high in methanol extracts. Souza et al., (2011) reported ethanolic extract of *G. birdiae* and *G. cornea* revealed high DPPH radical scavenging activity.

Boonchum et al., (2011) reported that aqueous extract was a good source of antioxidants. Water extract of macroalgae contained maximum antioxidant activity (Kuda and Ikemori, 2009). Hence, it can be presumed that they have different antioxidant capability in different extraction medium. This might be due to different polarities of individual antioxidant components occur in the seaweeds (Marinova and Yanishlieva, 1997)

The red alga *G.corticata* found to contain 3.14 mg GAE/g DW of phenol and 1.05 mg GAE/g DW of flavonoid in the present work. These values were lesser than the previous study by Arulkumar et al (2018). There is high correlation was reported between DPPH radical-scavenging activity and total polyphenolics. Devi et al. (2008) reported that the antioxidant activity of macroalgal extracts could be the outcome of the presence of phenolic substances. flavonoids are the natural phenol with lot of biological potential including free radical scavenging and antioxidant properties (Kahkonen et al., 1999). A large number of diseases are prevented through the free radical scavenging activity of flavonoids (Duan et al., 2006).

From the study we found that the seaweed *G.corticata* showed potential source for bioactive compounds which could be the reason for DPPH radical scavenging activity. Further studies are needed to use this red seaweed as expected natural antioxidants that could be employed in nutraceutical, cosmetic, therapeutic and other applications.

References

1. Adnan A.: Antioxidant properties of methanolic extract from Inula graveolens L. Turk J Agri For, 2011, 35. 591-596. 10.3906/tar-1010-1268.
2. Arulkumar A. Rosemary T. Paramasivam S. and Rajendran R.B.: Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay, India. Biocatal. Agric. Biotechnol., 2018, 15 63-71. DOI: 10.1016/j.bcab.2018.05.008

3. Benjama O. Masniyom P. Songklanakarin J. Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand, Sci. Technol., 2012, 34 (2) 223-230.
4. Boonchum W. Peerapornpisal Y. Kanjanapothi D. Pekkoh J Pumas C et al. : Antioxidant activity of some seaweed from the Gulf of Thailand. Int. J. Agri.Biol, 2011 (13) 95-99.
5. Bragdon J.H. Colorimetric determination of lipids, Journal of Biology and Chemical Sciences, 1951, 190: 513.
6. Brand Williams W. Cuvelier M.E. and Berset C.: Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol., 1955, 28 (1):25-30. doi.org/10.1016/S0023-6438(95)80008-5
7. Brindha P. Sasikala B. and Purushothaman K.K. Pharmacognostic studies on Merugankilzhangu, Medico Ethno Bot. Res., 1981, 3 (1) 84-96
8. Burtin P.: Nutritional value of seaweeds. Electronic Journal of Environmental Agriculture Food Chem, 2003, 2 (4) 498-503.
9. Chan P.T. Matanjun P.: Chemical composition and physicochemical properties of tropical red seaweed, *Gracilaria changii*. Food Chem., 2017, 221: 302–310.
10. Chang C.C. Yang M.H. Wenand J.C. and Chern J.C.: Estimation of total flavonoids content in propolis by two complementary colorimetric methods, J. Food Drug Analysis, 2002, 10: 178-182.
11. Devi K. P. Suganthy N. Kesika P. and Pandian S. K.: Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. BMC Complementary Altern Med., 2008, 8: 38.
12. Dhinakaran D.I. Geetha P. and Rajalakshmi J.S.: Antioxidant activities of marine algae *Valoniopsis pachynema* and *Sargassum swartzii* from the south east coast of India. Int. J Fisheries Aquatic Studies, 2015, 3(2): 426-430.
13. Duan X.J. Zhang W.W. Li W.X.M. and Wang B.G.: Evaluation of antioxidant property of extract and fraction obtained from red alga *Polysiphon ieurcelata,* 2006, Food Chem. 95 :1022-1037.
14. Ekrem K. and Ilhami G.: Antioxidant Activity of Cauliflower (*Brassica oleracea* L.) Turk J Agri For, 2008, 32 :65-78.
15. Fernando S. P. Sanjeewa A. K. K. Kalpa W. Samarakoon. and Won Woo Lee.: FTIR characterization and antioxidant activity of water soluble crude polysaccharides of Sri Lankan marine algae, Algae, 2017, 32(1) 75-86
16. Francavilla M. Franchi M. Monteone M. and Caroppo C.: The red seaweed *Gracilaria gracilis* as a multi products source, 2013, Marine Drugs 11. 3754–3776.
17. Ganesan P. Chandini S. Kumar N. and Bhaskar.: Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresource Technol, 2008, 99 :2717–2723.
18. Gressler V. Yokoya N.S. Fujii M.T. Colepicolo P. and Filho J.M et al.: Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chem, 2010, 120 :585–590.
19. Kahkonen M. Hopia P. Vuorela Hj. Rauha H. and Kujala J.P.: Antioxidant activity of plant extract containing phenolic compounds, J Agri Food Chem, 1999, 47 :349-36.
20. Kannan S.: FT-IR and EDS analysis of the seaweeds *Sargassum wightii* (brown algae) and *Gracilaria corticata* (red algae), Int. J Curr Microbiol App Sci, 2014, 3(4): 341-351.
21. Khairy H.M. El-Syafay S.M. Seasonal variation in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt, Oceanologia, 2013, 58 (2): 435-452. doi: 10.5697/oc.55-2.435.
22. Kuda T. and Ikemori T.: Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macro algal beach casts in the Noto Peninsula, Ishikawa, Japan. Food Chem, 2009, 112: 575-581.
23. Lowry O.H. Farr A.L. Randall R.J. and Rosebrough N.J.: Proteins measurements with Folin phenols reagent, J Biol Chem Sci, 1951, 193: 265-275.
24. Mabeau S. and Fleurence J.: Seaweed in food products: Biochemical and nutritional aspects. Trends Food Sci. Technol, 1993, 4 :103–107.

25. Marinho-Soriano E, Fonseca P.C, Carneiro M.A, Moreira W.S.C.: Seasonal variation in the chemical composition of two tropical seaweeds. Bioresource Technol, 2006, 97(18): 2402-2406. doi: 10.1016/j. biortech.2005.10.014.
26. Marinova E.M. and Yanishlieva N.V.: Antioxidative activity of extracts from selected species of the family Lamiaceae in sunflower oil. Food Chem, 1997, 58 (3):245-248. https://doi.org/10.1016/S0308-8146(96)00223-3.
27. Mervat M. El Far M. Hanan A. and Taie A.: Antioxidant activities, total anthrocyanins, phenols and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol, Aus. J. Basic Appl Sci, 2009, 3: 3609-3616.
28. Mwalugha H.M. Wakibia J.G. Kenji G.M. and Mwasaru M.A.: Chemical composition of common seaweeds from the Kenya Coast. Journal of Food Research, 2015, 4: 28-36. doi: 10.5539/jfr.v4n6p28.
29. Nazni P. and Deepa S.: Evaluation of proximate and pigment composition of red seaweed in South Coastal Regions of Tamilnadu. Int. J Res Marine Sci, 2015, 4(2): 38-41.
30. Rana M. G. Katbamna R. V. Padhya A. A. Dudhrejiya A. D. and Jivani N. P. et al. In vitro antioxidant and free radical scavenging studies of alcoholic extract of *Medicago sativa* L. Rom J Biol. Plant Biol, 2010, (55): 15-22.
31. Rasyid A. Ardiansyah A. and Pangestuti R.: Nutrient Composition of Dried Seaweed *Gracilaria gracilis* Indonesian J Marine Sci, 2019, 24 (1):1-6.
32. Robledo D. and Freile Pelegrín Y.: Chemical and Mineral Composition of Six Potentially Edible Seaweed Species of Yucatan. Botanica Marina, 1997, 40 (4):301-306. DOI:10.1515/botm.1997.40.1-6.301.
33. Roe J.R.: The determination of sugar in blood and spinal fluid with anthrone reagent. J Biol Chem Sci, 1955, 20: 335-345
34. Rosemary T, Arulkumar A, Paramasivam S, Mondragon A, Portocarrero Manuel Miranda J (2019). Biochemical, Micronutrient and Physicochemical Properties of the Dried Red Seaweeds *Gracilaria edulis* and *Gracilaria corticata* Molecules 24 (12): 2225. doi:10.3390/molecules24122225.
35. Sakthivel R and Devi K.P.: Evaluation of physicochemical properties, proximate and nutritional composition of *Gracilaria edulis* collected from Palk Bay. Food Chem, 2015, 174: 68–74.
36. Syad A.N. Shunmugiah K.P. and Kasi P.D.: Seaweeds as nutritional supplements: Analysis of nutritional profile, physicochemical properties and proximate composition of *G. acerosa* and *S. wightii*. Biomed Prevent Nutri, 2013, 3. 139–144.
37. Tabarsa M. Rezaei M. Ramezanpour Z. and Robert W.J.: Chemical composition of the marine algae *Gracilaria salicornia* (Rhodophyta) and *Ulva lactuca* (Chlorophyta) as a potential food source. J Sci Food Agri, 2012, 92(12): 2500-2506.
38. Taheri A.C.: Antioxidant activity in some Iranian seaweed species from Iranian J Fisheries Sci, 2016, 15(2) 802-817.
39. Torres P. Santos J.P. Chow F. and dos Santos D.Y.: A comprehensive review of traditional uses, bioactivity potential, and chemical diversity of the genus *Gracilaria* (Gracilariales, Rhodophyta). Algal Res, 2019, 37. 288–306.
40. Umavandhana R. and Jayanth S: Phytochemical Screening and Free Radical Scavenging Activity on Some Selected Seaweeds from Gulf of Mannar, India, Res J Pharmaceu Technol, 2018, 11(8): 3385-3388.
41. Viswanathan S. Ebciba C. Santhiya R. and Nallamuthu S.: Phytochemical screening and Invitro antibacterial, antioxidant and anticancer activity of *AmphiroFragilissima* International Journal of Innovative Research in Science: Engineering Technology, 2014 3 p. 2319- 8753.
42. Zar J.M. Biostatistical Analysis. Prentice Hall, New Jersy. 1974, p260.