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# Assessment of the nutritional value and native agar content of the red alga *Gracilaria foliifera* (Forsskal) Borgesen from the Red Sea coast of Sudan

**Nahid Abdel Rahim Osman[1*] Balsam Abdo[1] and Sayadat El-Tigani Mohamed[2]**

[1]Faculty of Marine Science and Fisheries, Red Sea University, Sudan, [2]Faculty of Science, University of Khartoum, Sudan.
*corresponding author email: nahidcoast@ yahoo.com

**Abstract**
The paper aimed to investigate the proximate composition, the native agar content, selected macro-minerals and micro-minerals concentrations, amino acids profile, fatty acids mixture, and some selected phytochemical groups of compounds in *Gracilaria foliifera* collected from Sudan Red Sea coast. Based on the dry weight, the alga contained 39.33±1.2 ash, 50.0±0.0 total carbohydrates, 8.93±0.094, 8.93±0.094 crude protein, 6.03±0.2 total lipid. The native agar constituted up to 36.5±2.5% of the alga dry matter. The concentrations of the following 4 macro-minerals were as follows: K was 12.9±1.63%, Ca was 8.50±2.87%, Mg was 3.39±1.69%, and P was 0.51±0.02%. according to their concentration the micro minerals could be arranged as follows: Fe (157.54±0.4 ppm), Mn (48.72±1.02 ppm), Zn (2.99±0.08 ppm), and Cu (0.51±0.04 ppm). Eight of the 9 essential amino acids were present in *G. foliifera* protein. In total 5 fatty acids were detected as methyl esters in the alga lipids at this time. The alga was rich in flavonoids which made up to 21.11±1.11% as well as in phlorotannin (5.0±0.0%) and in alkaloids (4.44±2.22%). These findings could be taken as an indication of the good prospective of the *G. foliifera* from the Red Sea of the Sudan in food and pharmaceutical industries.

**Keywords**: *Gracilaria foliifera*, agar, red algae, red sea,

## Introduction

*Gracilaria foliifera* (Forsskal) Borgesen is a common red alga along the Sudanese Red Sea coast. It is found growing attached to solid natural as well as artificial substrata below the intertidal zone. The alga flourishes in winter from September to April when the sea water temperature is cooler compared to the hot summer. It belongs to the genus Gracilaria Greville (Gracilariaceae, Gracilariales). The genus has wide distribution in temperate and tropical regions. Most of the genus members are of economic significance as they are agar-producer (agarophytes), edible, and phytochemicaly interesting species. Accordingly, these species have been commercially cultivated and extensively studied where they naturally occur in temperate as well as tropical coasts. However, information on the Red Sea Gracilaria species is hardly available. The aim of this work was to investigate selected biochemical constituents of *G. foliifera* from the Sudanese Red Sea coast in order to assess its nutritional value, native agar content and to contribute to the knowledge on *Gracilaria* species.

## Materials and methods

The sample of *G. foliifera* was collected from Port Sudan coast at 19° 36'56 N and 37° 12' 58 E along Sudan Red Sea coast. The collected algal biomass was thoroughly washed in situ with seawater, and then 3 times under running tap water in the laborotory to remove epiphytic elements and extra salt. The sample was then air-dried in the shade to constant weight. The air-dried plant materials were ground to fine powder with mixer grinder and kept in air tight containers at room temperature until analyzed. All experiments were repeated until reproducible results were obtained or in triplicates. Values obtained were expressed as mean ± standard deviation on dry weight bases.

*Proximate and agar contents*
Determinations of moisture contents, ash, crude protein, total lipid, agar, amino acids, and fatty acids were undertaken as described below.

*Determination of Moisture content*
Two grams of the alga powder were kept in an oven at 105° C until the weight of the sample was constant. Moisture content was reported as loss in weight and its percentage content was determined gravimetrically.

*Determination of ash*
Two grams of alga were ignited in a muffle furnace at 550° C until light grey ash was obtained. Ash content was calculated gravimetrically.

*Minerals composition*
The elemental analyses were carried out following the methods described by Richards (1954). The ash was dissolve in 10 ml of hydrochloric acid (5 N) and the volume was completed to 100 ml with distilled water. This solution (thereafter stock solution) was used to determine minerals concentration. Quantitative assessments were obtained with titration against ethylene diamine tetra acetic acid (EDTA), atomic absorption spectrophotometer, flame spectrophotometer, and UV/VIS spectrophotometer.

*Measurements of macro-minerals*
Quantitative assessment of the measurable concentrations of magnesium (Mg), calcium (Ca), potassium (K), and phosphorus (P) was performed as described in Richards (1954).
Measurements of Mg and Ca concentrations were obtained by titrating diluted volume of the stock solution against 0.01 N EDTA.
For Ca Measurement Approximately about 50 mg of muroxide indicator and 7 drops of 4 N NaOH were added to 10 ml of the stock extract and titrated against 0.01 N EDTA.
Phosphorus concentration was determined by reading the absorbance of the mixture of 5 ml of stock solution and 5 ml of ammonium molybdate vandate solution at 470 nm with UV/VIS spectrophotometer (Jenway 6505). Concentration of P was calculated from a calibration curve made by plotting the absorbance of a series of standard solutions of potassium dihydrogen phosphate ($KH_2PO_4$) against their respective P concentrations.
Potassium concentration was measured with flame spectrophotometer (Jenway PFP7) after appropriate dilution of the mineral extract.

*Measurements of micro-minerals*
The concentrations of manganese (Mn), Copper (Cu), Iron (Fe) and Zinc (Zn) were measured with atomic absorption spectrometer (Shimadzu A 6800) directly from the stock extract. The elements were measured with air C2H2 flame atomizer. Mn concentration was measured at 279.5 nm, Cu at 324.8 nm, Fe at 248.3 nm, and Zn at 213.9 nm.

*Determination of total carbohydrates*
Determination of total carbohydrates in *G. foliifera* was achieved using the Anthrone Method (Hedge and Hofreiter, 1962) as described in Mazumdar and Majumder (2003).

*Measurement of crude protein contents (Nx6.25***)**
Protein in the sample was determined by Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid ($H_2SO_4$) in the presence of digestion mixture. The mixture was then made alkaline with 40% NaOH. Boric acid was used to capture the ammonium released by the mixture. The resultant mixture was titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was calculated.

*Determination of total Lipid and fatty acids mixture*
The method of Folch et al. (1957) with the modification of Christie (1993) for extraction of total lipids was performed to approximate total lipid content on dry weight basis. The dry residual product of this experiment was transesterified to determine fatty acids profile according to the method described by Christie (1990).

*Extraction of native agar*

Native agar was extracted following the guidelines provided by Santos (1990) with some modifications. One 100 ml of distilled water was added to known weight of seaweed powder and the pH of the resultant mixture was adjusted to 7.0 with 1 M HCl or KOH. The mixture was incubated at 121° C for 15 minutes. The hot solution was filtered through gauze to remove algal residue and left at room temperature to gel. The filtrate was transferred to trays and frozen at – 20° C overnight. The frozen agar gel was thawed on the next day and the water of hydration was removed to exclude as much as possible of the impurities that were extracted together with the agar. The agar was then washed twice with 100 ml 85% and 99% isopropanol respectively. The agar was dried at 50° C for overnight and the content of agar per dry weight was calculated on dry weight basis.

*Profiling of total amino acids*

Quantitative determination of total amino acids (AA) was carried out with gradient HPLC using Sykam Amino Acid Analyzer (Sykam S 433, Germany) according to the protocols recommended by the manufacturer.
The following variables were calculated from the results obtained:

i. Total amount of AA,
ii. Total amount and percent of essential AA (EAA),
iii. Total amount and percent of non-essential AA (NEAA),
iv. Ratio of EAA to NEAA.

*GC Determination of fatty acid composition*

Fatty acids were determined by gas chromatographic quantification of their fatty acids methyl esters (FAMES) obtained above. The FAMES samples were analyzed using capillary Gas Chromatography GC 2010 (Shimadzu, Japan) equipped with FID detector and capillary column (DB-1, 30m x 0.25 µm x 0.25 µm). The injection and detection temperatures were 250° C and 280° C respectively with split ratio of 10 using nitrogen as carrier gas. The running method was through temperature gradient from 150° C up to 230° C with an increase rate of 20° C/min. identification of fatty acids in the sample was performed by comparing their retention time with those of standard fatty acids mixture (Supelco Analytical, PA, USA ) injected under same conditions.

*Phytochemical screening and quantification*

The experimental work covered 3 of the most general phytochemical groups of compounds and was based on the recommendations given in Harborne (1998). Regular phytochemical procedures were followed for the qualitative and quantitative analysis. Experiments were performed in duplicates and\or triplicates.

*Extraction*

The extraction method described in Tiwari et al. (2011) was followed to extract the materials. Ten grams of *G. foliifera* dry powder were first macerated in 100 ml of ethyl acetate for 3 days at room temperature with frequent shaking. The mixture was centrifuged (10000g/10m) and filtered with Whatman No. 1 filter paper into a clean glass vial and kept at 4° C until further analysis. Then the residues were successively extracted with chloroform and methanol respectively.

*Qualitative screening of alkaloids, flavonoids, and phlorotannin*

The extracts were tested for the presence of alkaloids, flavonoids, saponins, sterols, tannins and terpenes phytochemical groups of compounds. This was based on development of colouration and precipitation upon addition of certain chemical reagents to the extracts. The presence of alkaloids in the alga extract was tested with Wagner`s reagent following the procedure described by Sabri et al. (2012). The test of flavonoids was done as described in Pamar et al. (2012) with NaOH solution. Two mL of concentrated $H_2SO_4$ were added to the extract to test the presence of sterols (Solihah et al., 2012). Equal volumes of the extract and the distilled water were added to a test tube and vigorously mixed to check the presence of saponins (Sathya et al., 2013)). Two ml chloroform and 3 ml of concentrated $H_2SO_4$ were added to 5 ml of the extract to examine the presence of terpenes (Mir et al., 2013). Phlorotannins were detected with 3% ferric chloride solution according to the methods described in Ugochuhwu et al. (2013).

*Quantitative determination of alkaloids, flavonoids, and phlorotannins*

The crude concentration of the three tested phytochemical groups of compounds was gravimetrically determined in the alga tissue on dry weigh basis. The concentration of alkaloids was determined as described in Agoreyo et al. (2012). The method described by Eleazu et al. (2012) was performed to determine the concentration of flavonoids. Phloroannins were quantified with the methods illustrated by Vetter and Barbosa (1995).

**Results and discussion**

Proximate composition and native agar content of *G*. *foliifera* (Sudan) are shown in Table (1). Only 5± 2.8% of the dry alga matter were water indicating that the alga dry mass is very high. Moisture content in *G. foliifera* is approximately comparable to that of *G*. *fisheri* (5.5%) and to that of *G*. *tenuistipitata* (3.6%) as stated in Benjama and Masniyom (2012). The ash content (39.33%) of *G*. *foliifera* is the second abundant proximal component and is comparable to the ash content of 43.18% reported for *G*. *crassa* (Baghel et al., 2014). Available studies have recorded high values of ash in Red Sea seaweeds. For instance Omer et al. (2013) recorded 40.34% of ash in *Turbinaria triquetra* from the eastern coast of the Red Sea and 53.79% of ash for *Halimeda opuntia*. Similarly, Al-Harithi and El-Deek reported that the ash content of *Sargassum dentifebium* was 47.15% to 48.31% and attributed these high values to the high salt concentration in the Red Sea. Also Khalil and El-Tawil (1982) averaged the ash content in 10 algal species of Red Sea to 33.27%. The values of ash content of analogous macroalgae from other Seas are comparatively lower than those of the Red Sea. El-Tawil and Khalil (1983) reported the ash content of some Mediterranean macroalgae to range from17.23% for *Caulerpa prolifera* to 25. 80% for *Cystoseira fimbriata*. Additionaly the ash content of *Fucus vesiculosis* from the Baltic Sea changed from 14.2% to 21.40% with no seasonal pattern (Truus et al., 2001). Therefore, it seems that high ash content may be a feature of Red Sea seaweeds due to its high elements concentration. Generally seaweeds exhibit high ash content compared to freshwater algae (Mišurcová1 et al., 2010).

**Table 1. The proximate composition and agar content of *Gracilaria Foliifera* (Sudan).**

| **Parameter** | **Content (%)*** |
|---|---|
| **Moisture.** | 5± 2.8 |
| **Ash.** | 39.33±1.2 |
| **Total carbohydrate.** | 50±0.0 |
| **Crude protein.** | 8.93±0.094 |
| **Total lipid.** | 6.03±0.2 |
| **Native agar** | 36.5±2.5 |

*** mean value of 3 replicates** ± SD.

Carbohydrates are the main structural component of all plant cell walls and they are stored in different forms as food reserve. Gracilariaceae was reported to contain high carbohydrate content suitable for bioconversion to biofuel (Bird et al., 1990). In the present alga the carbohydrates are the major constituent (50%) of the dry matter. This is in conformity with previous reports on the carbohydrates contents of Red Sea macroalgae (Khairy et al., 2013) in particular with the carbohydrates concentration of *G*. *corticata* (52.93%) from the Red Sea (Omer et al., 2013). Inverse relationship between the content of the carbohydrates and protein was observed in plants surviving under limited nitrogen availability condition (Araya et al., 2010) and in seaweeds (Lobban and Harison, 1994). The Red Sea is known as an oligotrophic sea (Figueroa et al., 2009) with comparatively low nutrients levels mainly for nitrates and nitrites. Therefore, most of the records on the proximate composition of its macroalgae have reported the carbohydrates as the most abundant component (Omer et al., 2013; Khalil and El-Tawil, 1982; El-shafay, 2014) constituting up to 90.83% of the dry matter and this was linked to the intense illumination of Red Sea water that leads to high photosynthesis. Comparatively, the seasonal variation in carbohydrates content of some macroalgae from Mandapam (India) was found to range from 13.0 to 13.3% (Kaliaperumal et al., 2002). Chakraborty and Santra (2008) reported that the carbohydrates concentration of 8 benthic algae of Sunderban region varied from 14.34 % to 35.27 %. The carbohydrates of the red alga *catenella repens* varied seasonally, however, remained in the range of 21.52 %

to 35.74 % (Banerjee et al., 2009). The seasonal variation in carbohydrates content of 3 macroalgae from the Mediterranean Sea was in the range of 34.57% to 50.96% (Khairy and El-Shafay, 2013). It could be construe from these values that the Red Sea macroalgae contain more levels of carbohydrates than macroalgae from other seas. This could be attributed to the well illumination of Red Sea water due to high transparency that makes more sun light available for photosynthesis.

Approximately 8.93% of *G*. *foliifera* (Sudan) dry matter was protein. This value is noticeably higher than the maximum protein level (5.1%) reported for *G*. *cornea* (Orduña-Rojas et al., 2002). However it concurs with the protein levels reported for some Red Sea macroalgae. For instance *Padina pavonica* contained 8.35% of protein and *Sargassum fusiforma* containd 8.85% (El-Shafay, 2014). High light intensity and nutrient availability stimulated accumulation of hydrolysable proteins (Ramlov et al., 2011). Of these two conditions the availability of nitrogen and phosphate in the Red Sea has been debated and were considered as limiting factors by some authors. These two nutrients were reported to be below detection limits in some parts of the Red Sea (Shaikh et al., 1986; Li et al., 1998). Therefore, this condition may have an effect on protein synthesis of some Red Sea seaweed. Depending on the species the values of the total protein content recorded for different green, brown, and red macroalgae from the Red sea was in the range of 5 to 25% of dry weight. This is comparable to the protein level in the Indian seaweeds that was reported to fall in the range of 10% to 30% DW (Murugaiyan et al., 2012). Interspecific and intraspecific variation in protein content was attributed to the dissimilarity in the ambient environmental parameters of salinity, water temperature, and dissolve nitrogen (Nascimento et al., 2014). An inverse relationship between the concentration of protein and carbohydrate was observed in seaweeds (Nascimento, 2014; Khalil and El-Tawil, 1982; Orduña-Rojas et al., 2002). Consequently, the protein level in Red Sea seaweeds may have been reduced due to high carbohydrates synthesis and accumulation.

Generally variable statements on lipid levels in seaweeds are encountered in the literature. While some studies stated that some seaweed contained high total lipid content above 10% of dry weight (Gosch et al., 2012) other studies reported seaweed lipid content to vary from 1 to 6% (Polat and Ozogul, 2013; Ambrozova et al., 2014). The value of the lipid content (6.03%) in the present species is analogous to the value of lipid content in *G*. *corticata* (5.98%) from the eastern Red Sea coast (Omer et al., 2013), to *Laurencia papillosa* (5.07%), and to lipid content in some brown macroalgae from the Red Sea reported in Khalil and El-Tawil (1982). In contrast to this, very low values of lipids were reported in El-Shafay (2014) for *Ceramium rubrum* (0.0082%), *Sargassum valgare* (0.0403%), *S*. *fusiforma* (0.0204%), and *P*. *pavonica* (0.006%) from the Egyptian Red Sea. It seems that lipid content of seaweeds may depend on species, the ambient environmental parameters, and treatment and extraction methods. For example the Red Sea *G*. *foliifera* species contained more lipids than the Indian species which recorded to contain 0.8% of total lipids (Bhaskar et al., 2004). This remarkable difference could possibly ascribe to the marked differences in the environmental conditions prevailing in the two regions, and to sample treatment and extraction methods.

Agar is a polysaccharide (also known as hydrocloids or phycocoloids) produced by the 3 red algal families Gracilariaceae, Gelidiaceae, and Gelidiellaceae. However, approximately 60% to 65% of world agar is produced by the members of the genus Gracilaria (Roa and Kaladharan, 2003; Niu et al., 2013). Agar polymers are widely used in pharmaceutical, cosmetics, food industry and scientific research with the agar produced by Gracilaria species commonly classified as food and sugar reactive grade agar (Marinho-Soriano, 2001; Murano, 1995). Agar yield of Gracilaria species is typically 33% ranging from 10% to 50% of the dry weight (Briggs and Funge-Smith, 1993). Although, Chung et al. (2011) reported a remarkably higher value of 82.56% for *G*. *edulis* after alkali treatment. Consequently, the present alga is rich in native agar which constituted 36.5% of it dry matter. This value is comparable to the values of agar content (29.4% to 30.7%) reported by Matsuhashi and Hayashi (1972) for *G*. *foliifera* and to agar content of 28% to 51% recorded for *G*. *corticata* (Andriamanantoanina et al., 2007). Nevertheless, lower values of native agar are reported for other gracilariacean species in Niu et al. (2013) for *G*. *lemaneiformis* which reported to contain 15.7% of agar, and in Younis et al. (2000) who report agar content in *G corticata* (17.7%) and in *G*. *crassa* (15.8%) from the Red Sea.

*Minerals content*

In total 8 elements were quantitatively determined in the present alga (Table 2). This included the 4 macro-elements magnesium (Mg), calcium (Ca), phosphorus (P), potasium (K), and the 4 micro-elements manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn). This Red Sea Alga is rich in K (12.49%), Ca (8.5%), Mg (3.39%), and P (0.51%). This pattern is in conformity with that reported in Smith et al. (2010) for some New Zealand seaweeds. In the edible

Hawaiian seaweeds a very wide range of K concentration between a minimum of 0.7% in *Caulerpa lentillifera* to a maximum of 22.1% in *G*. *coronopifollia* was recorded (Smith et al., 2010). The concentration of K in this alga is relatively similar to its concentration in *G*. *edulis* (13.49%) reported in Mageswaran and Sivasubramaniam (1984) and to its concentration (12.0%) in both *Himanthalia elongata* and *Laminaria digita* reported in (Hotchkiss, 2010). In general red and brown seaweeds were reported to accumulate K in their cell sap as a salt inclusion mechanism to withstand saline conditions (Sivakumar and Arunkumar, 2009). Further, accumulation of K in plants was associated with resistance of biotic and abiotic stresses (Cakmak, 2005; Wang et al., 2013). In this case, the high salinity of the Red Sea (40‰) may cause this seaweed to accumulate K to maintain osmotic adjustment. As an essential element for human health, K plays an important role in electrolyte regulation, nerve function, muscle control, and blood pressure. Increased K uptake reduces hypertension and consequently the risk of heart attack and stroke (WHO, 2012). Therefore, this alga may serve as an affordable source of K to mitigate the risk of hypertension. Seaweeds are one of the most important sources of calcium as it accounts up to 7% of its dry matter (Burtin, 2003). The content of Ca in *G*. *foliifera* (Sudan) is greater than the stated level. Commonly calcified seaweeds contained high level of Ca in commensuration with the degree of calcification. In *Jania natalensis*, a heavy calcified alga, the Ca content is 23.85%, while in *Padina pavonica*, a partially calcified alga, Ca content is 10.96% (Mageswaran and Sivasubramaniam, 1984). This is of a particular medicinal value because Ca functions in human are related to maintaining strong bones and teeth, transmitting nerve impulses; contractions of muscles; blood clotting; activation of some enzyme reactions and secretion of hormones (Millikan, 2012). Calcium deficiency has been identified as a major risk factor for osteoporosis although other important minerals in bone health have also been identified including magnesium, copper, manganese, selenium and zinc (Fujita, 2004). Therefore, the utilization of seaweed Ca in curing human ailment emanating from Ca deficiency has been investigated. In particular oral ingested calcium supplement derived from seaweeds is better absorbed in the intestine compared to other calcium compounds and is therefore most efficient in suppressing osteoporosis and calcium paradox disease.

**Table 2. Macro and micro minerals concentration in *Gracilaria foliifera*.**

| Macro elements | Concentration (%) | Micro elements | Concentration (ppm) |
|---|---|---|---|
| **K** | 12.49±1.63 | **Fe** | 157.54±0.4 |
| **Ca** | 8.50±2.87 | **Mn** | 48.72±1.02 |
| **Mg** | 3.39±1.69 | **Zn** | 2.99±0.08 |
| **P** | 0.51±0.02 | **Cu** | 0.51±0.04 |

Magnesium is an important component in chlorophyll and it also vital for the biochemical functioning of living cells. For instance Mg is directly involved in chlorophyll formation, in photophosphorylation, and in protein synthesis (Cakmak and Yazici, 2010). Therefore, Mg is very vital in crop production. Substantial amounts of Mg were reported from different species of edible seaweeds (Perira, 2011; Nakamura et al., 2012; Kumar et al., 2015). The content of Mg in seaweeds is commonly less than 1%; in Mageswaran and Sivasubramaniam (1984) only 4 macroalgae out of 24 tested for Mg content contain more than 1% of the element. The present alga exhibited significant content of Mg (3.39%) indicating that it could be a good candidate as a source of dietary Mg, as a fertilizer as well as a feed. The essentiality of Mg to human health was comprehensively explained (FAO/WHO, 2001; Pasternak et al., 2010; Faryadi, 2012, Castiglioni et al., 20013).

The concentration of P (0.51±0.02%) in *G*. *foliifera* from Sudan is the lowest compared to the other 3 macro-minerals. However, this value is comparable to the P content (0.54%) reported for *Centrocerus clavulatum* (Diniz et al., 2011) as well as to that reported for *G*. *salicornia* (0.4%) in Nelson et al. (2009). These values compares well with the typical content of P (0.1% to 0.5%) in plants tissue (Mullins, 2009). Virtually P is found in every living cell.
Generally the content of iron in seaweeds was reported to be higher than in land plants (Tabarsa et al., 2012) however with high inter-specific and intra-specific variation (Khristoforova and Bogdanova, 1980; Stengel and Dring , 2000; Smith et al., 2010; Murugaiyan and Narasimman, 2012). Of all the trace elements present in seaweeds the content of iron was reported to be the highest (Flores et al., 2015; Astorga-Espana et al., 2015). The iron concentration in the present *G*. *foliifera* (157.54±0.4 ppm) is significantly higher than the iron concentration in *G*.

*textorii* (89.2±24.40 ppm) and lowers than the iron concentration in *G*. *vermiculophylla* (190.3 ± 51.5 ppm) (Rodriguez et al., 2013). Surprisingly much lower iron content value were reported for some species from the eastern Red Sea which ranged from a minimum value of 0.277±0.003 ppm in *Turbinaria triquetra* to a maximium value of 0.428±0.018 ppm in *Halimedia opuntia* (Omer et al., 2013). Nevertheless, comparatively higher values of iron content were reported for *S*. *longifolium* (69. 05±3.12 ppm) in Murugaiyan and Narasimman (2012) and for Lessonia and Heterosiphonia genera (3810 mg/kg) in Astorga-Espana et al. (2015). Variation in iron content could largely be due to inter and intra-specific variation as well as to the variation in the environmental conditions of the alga habitat.

Similar to the content of iron, Manganese content in seaweed is remarkably variable based on the available literature. The present alga contained 48.72±1.02 ppm of Mn. This value is relatively similar to the Mn content (49.8±2,8 ppm) in *Hypnea musciformis* provided in Venkateshwarlu et al, (2013) and relatively similar to its value (58.4 ppm) in S. *naozhouense* (Peng et al., 2013). This micro-element is an important component of the chlorophyll in the photosynthetic plants required for the water splitting step. It is also required for amino acids formation. In humans and animals, manganese is an essential element, necessary for bone mineralization, energy and protein metabolism, regulation of cell metabolism, and protection against oxidative stress and formation of glycosaminoglycans (Menezes-Filho et al., 2009). On the other hand, excess exposure to manganese could cause neurotoxicity (Rahbar et al., 2014) that result in a progressive disorder of the extra-pyramidal system which is similar to Parkinson's disease.

The content of Zn in G. *foliifera* from the Red Sea coast of Sudan (2.99±0.08 ppm) is remarkably higher than its content in G. *foliifera* from India which was reported to be 0.036 ppm (Qari and Siddiqui, 2010). However, this value is in conformity with the values of zinc concentration reported for 3 macroalgae from the Mediterranean coast of Egypt. The content of Zn in these macroalgae ranged from 1.3±0.03 to 3.1±0.05 ppm (Khairy and El-Sheikh, 2015). Essentiality of zinc for human health was recognized only 50 years ago (Prasad, 2014). This relatively non toxic trace element is a component of more than 300 enzymes and an even greater number of other proteins, which emphasizes its indispensable role for human health (Plum et al., 2010). In addition to, optimal nucleic acid and protein metabolism, as well as cell growth, division, and function, require sufficient availability of zinc.

Among the trace elements tested in *G*. *foliifera* the concentration of Cu (0.51±0.04 ppm) was the lowest. This is in agreement with some previous records on the trace metal content of macroalgae (e.g. Saeed and Moustafa, 2013). Copper content in the present alga is lower than its content in the red alga *Geleidella acerosa* (1.04±0.024 ppm) but higher than its content (0.31±0.019 ppm) in *Turbinaria ornata* reported in Sudharsan et al. (2012). Copper is essential to human health (Squitti et al., 2014) because it is part of many proteins including vital enzymes. Therefore, copper deficiency or excess can be life threatening. Recent studies have indicated that alteration of copper metabolism is one of the pathogenetic mechanisms of Alzheimer's disease (Squitti et al., 2014). Accordingly, the WHO recommends a minimal intake of copper of about 0.9 to 1.3 mg per day.

*Amino acids profile of Gracilaria foliifera (Red Sea, Sudan)*

*Gracilaria foliifera* from the Red sea coast of Sudan contained 16 amino acids, of these 8 are EAA and 8 are NEAA and this is in consistence with the amino acids profile in Gracilariaceae (Benjama and Masniyom, 2012; Wen et al., 2008). The profile of the EAA in Sudanese Gracilariaceae is topped by structurally similar branched chain amino acids namely, valine, luecine, and isoluecine that scored the highest concentration among the group. Despite their structural similarity, they have different metabolic pathways (Letto et al. 1986). Valine converted into carbohydrate, luecine to fat, and isoluecine to both. This could explain the value obtained for the carbohydrate and fat in the alga, since carbohydrates was the major constituent of the proximate content. In addition to, these amino acids are essential for human health and nutrition particularly in muscle tissue repair, muscle metabolism, and regulation of immune system.

**Table 3. Amino acids profile of *Gracilaria foliifera* (Red Sea, Sudan).**

| Amino acids | Concentration | |
|---|---|---|
| | mg/g | % |
| Valine. | 3.63 | 12.45 |
| Luecine | 2.93 | 10.06 |
| Isoluecine | 2.90 | 9.95 |
| Phenylalanine | 2.3 | 7.8 |
| Threonine. | 1.5 | 5 |
| Lysine | 0.96 | 3.5 |
| Histidine. | 0.35 | 1.2 |
| Methionine. | 0.3 | 0.9 |
| Aspartic acid. | 1.96 | 6.7 |
| Glutamic acid. | 1.31 | 4.5 |
| Tyrosine. | 0.33 | 1.1 |
| Alanine, | 3.51 | 11.94 |
| Glysine. | 0.8 | 2.65 |
| Serine. | 1 | 3.35 |
| Cystine. | 0 | 0 |
| Proline. | 3.53 | 12 |
| Arginine | 2.5 | 8.5 |
| Total AA | 16 | |
| Total amount of AA | **29.75** | **101.34** |

In the NEAA profile proline was the prevalent in this species. In addition to be a major constituent of protein, proline also acts as osmotic protectant in plant under osmotic stress as well as under thermal stress (Chang and Lee, 1999). This may provide an assertion to the harsh environmental conditions under which the Sudanese species survives. The alga grows in the intertidal zone where the temperature of the shallow water and the light intensity would be higher during the day time. Also high temperature increase evaporation that in turn increases water salinity. For these reasons, the present alga may produce more proline to counter act the unfavourable impact of these conditions.

The second abundant NEAAs were alanine (12%) and arginine (8.5%) respectively. The two are basic amino acids and together constituted the largest amount (20.44%) of the alga AA.

The limiting amino acid in this alga was the methionine, a sulfur containing amino acids. This result concord with Lurenco et al. (2002) but in contradiction to Ortiz et al. (2008) and Qasim (1991) who denoted that some species of Gracilariaceae contained higher amount of sulfur containing amino acids compared to brown and green algae. Due to differences in analytical methods performed to quantify amino acids in algae, comparison of the present values of individual amino acids concentrations and chemical scores could not be accomplished accurately.

In principle all amino acids are important for health and nutrition of human and animals. They perform vital biological roles, for example amino acids could be used as a source of energy during fasting when the breakdown of muscle protein is a major energy source. Some amino acids act as neurotransmitters and some act as starting materials for biosynthesis of neurotransmitters, hormones, and other important biochemical compounds.

*Protein quality*

The quality of the protein of *G. foliifera* could be inferred from the summary of amino acids composition (Table 4). The percentage of the essential amino acids (EAA) in *G. foliifera* was 49.98% of its total amino acids (AA). This value is greater than the 39% considered to be adequate for ideal protein food for infants, the 26% for children, and the 11% for adults (FAO/WHO/UNU, 1985). This finding is very similar to that of Kumar and Kaladharan (2007).

**Table 4. Summary of *Gracilaria foliifera* amino acids profile**.

| Parameter | Value in *Gracilaria foliifera* |
|---|---|
| **Total AA (mg\g).** | 29.75 |
| **Total amount of EAA (mg\g).** | 14.87 |
| **Percent of EAA (%)** | 49.98% |
| **Total NEAA (mg\g).** | 14.88 |
| **Percent of NEAA (%),** | 50.02% |
| **EAA: NEAA ratio** | 0.99 |

*Fatty acids*

In total only 5 fatty acids were identified in *G*. *foliifera* from the Sudanese Red Sea coast in this analyses. The saturated fatty acids (SFA) included lauric acid or dodecanoic acid (C 12:0), myristic acid or tetradecanoic acid (C 14:0), and palmitic acid or hexadecanoic acid (C 16:0). The mono unsaturated fatty acids (MUFA) were represented by oleic acid or cis-9-octadecenoic acid (C 18:1). The poly unsaturated fatty acids (PUFA) were represented by linoliec acid or cis-9, cis-12-octadecadienoic acid (C 18:2). Based on the present fatty acids concentrations, the major fatty acid in *G. foliifera* is palmitic acid. The alga contained lesser proportion of PUFA (3.35%) compared to SFS (58.5%) and MUFA (32.18%). This pattern of fatty acids mixture corresponds with that reported for *G*. *dura* (Xu et al., 2015)

**Table 5. Fatty acids mixture of *Gracilaria foliifera* (Sudan)**

| Fatty acids | Concentration (%) |
|---|---|
| Lauric acid | 2.50 |
| Myristic acid | 3.13 |
| Palmitic acid | 52.87 |
| Oleic acid | 32.18 |
| Linoleic acid | 3.35 |
| **Total amount of SFA** | **58.5** |
| **Total amount of MUFA** | **32.18** |
| **Total amount of PUFA** | **3.35** |

The profile of fatty acids obtained in this study is in agreement with the current knowledge on fatty acids in Gracilariaceae. The available information on fatty acids profile in the family indicated that species may contain similar fatty acids profile with different relative content. According to Rajasulochana et al. (2010) major fatty acids in Rhodophyta are myristic, palmitic, oleic acids along with the PUFA 20:6ω4 and 20:5ω3 and trivial concentrations of arachidonic (2.1 to 10.9%) and linoliec acid (1.3 to 2.5%).

Fatty acids mixtures in seaweeds were reported to exhibit remarkable temporal and spatial interspecific variations (Bramarambica et al., 2014; Gosch et al. 2012). These variations were interpreted as the effect of environmental factors or as presence of genotypes. Accordingly, since the present fatty acids mixture of this species does not reflect any of these variations, it should be considered as inconclusive account.

*The content of alkaloids, flavonoids, and phlorotannin in Gracilaria foliifera (Sudan)*

The qualitative and quantitative content of alkaloids, flavonoids, and tannins in *G*. *foliifera* are shown in Table 6. Whilst alkaloids and tannins were detected in the 3 organic solvent used, flavonoids were only detected in the chloroform extract. This could be attributed to the ability of the solvent to extract certain group of these groups of compounds whose molecular weight is in proportion to the polarity of the solvent.

**Table 6. Qualitative and quantitative content of alkaloids, flavonoids, and phlorotannins in *Gracilaria foliifera* (Sudan).**

| Phytochemicals | Crude extracts of *G. foliifera* | | | Content (%) |
|---|---|---|---|---|
| | Ethyl acetate | chloroform | Methanol | |
| **Alkaloids** | **+** | **+** | **+** | **4.44±2.22** |
| **Flavonoids** | **-** | **+** | **-** | **21.11±1.11** |
| **Phlorotannin** | **+** | **+** | **+** | **5.0±0.0** |

Some members of the genus Gracilaria were reported to contain important bioactive primary and secondary metabolites (De Almeida et al., 2011; Maithili et al., 2014). The presence of alkaloids, flavonoids, and tannins in the members of the genus Gracilaria has been reported. The presence of these 3 groups of compounds is reported for *G*. *foliifera* (Leelavathi and Prasad, 2015) as well as for *G*. *corticata* (Eahamban et al. 2012; Divya et al. 2013). These groups of compounds were reported to have therapeutic effects on some human pathogens. Antibacterial and antifungal activity of the crude methanolic extract of the Sudanese *G*. *foliifera* against some selected pathogenic strains has experimentally proven (Abdalla et al., 2016 in press). These activities were attributed to the presence of phenolic compounds (Jeyanthi Rebecca et al, 2013). Therefore, the presence of these phytochemical groups of compounds in an alga is indicative to the nature of the therapeutic prospective of that alga.

Few investigations provided quantitative information on the content of phytochemicals in macroalgal species. The crude alkaloids content (4.44±2.22%) of this *G*. *Foliifera* is relatively comparable to that of *Dictyopteris membranacea* (6.11%), *Sargassum vulgare* (5.84%), *Ulva. lactuca* (5.33%) quantified by (Alghzeer et al. 2013). Nevertheless, higher values (14% to 18%) were reported for the content of crude alkaloids in 3 Gracilaria species (Leelavathi and Prasad, 2015). This could possibly be attributed to the variations within species, between species, and to the variation in environmental conditions. Also, high content of certain alkaloids such as caulerpin, an indole alkaloids isolated from members of the genus Caulerpa and other seaweeds, were recorded to constitute about 15% of *C*. *lentilifera* and 8% of *C*. *sertulorides* (Güven et al. 2010).

Flavonoids content of this alga (21.11±1.11%) is relatively conform with its content in *G*. *foliifera* from India which made about 17% of the alga dry weight (Leelavathi and Prasad, 2015). On the other hand, flavonoids constituted more than 10% of the green alga *Acetabularia ryukyuenais,* and the red algae *Chondrus verruscosus, G. textorii*, and *G. asiatica* (Yumiko et al. 2003).

The phlorotannin content of *G*. *foliifera* (5.0±0.0%) from the Red Sea of the Sudan congruent with the relative abundance (5%-30%of dry weight) of phlorotannin in algae (Heffernan et al., 2015).

**Ethical statement**

The collection of the plant materials was undertaken after the acquisition of appropriate permissions from concerned Sudanese authorities.

**Conflict of interests**

The authors have no conflicting interests.

**Conclusion**

The profile of the bioconstituents of *G*. *foliifera* from the Red Sea coast of Sudan is of nutritional and therapeutic prospective. Further biological examinations are needed to confirm the possibilities of utilizing the alga in food and pharmaceutical industries.

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