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Biochemical Studies on Red Algae *Gelidium* sp. Grown in Egypt

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Abstract The present work aims to study the potentialities of macroalgae *Gelidium sp.* collect from Mediterranean Sea as new source of bioactive substance. Thus, the chemical and biochemical components for these algae were isolated and characterized which includes antioxidant activity. The proximate chemical composition of *Gelidium* sp. were protein content (13.23 ± 1.06% DW), crude lipid (1.16 ± 0.21% DW), fiber content (5.5 ± 1.05% DW), ash content (26.45 ± 0.74%), and carbohydrate content (53.66 ± 1.21% DW). The fatty acid profiles of *Gelidium* sp. were analyzed as follow, the saturated fatty acids are caproic, caprylic, capric, lauric, myristic, pentadecanoic, palmitic, heptadecanoic, stearic and arachidic acid, and the unsaturated fatty acids are tetradecenoic, oleic, vaccinic, octadecosaenoic, linoleic, linolenic and alpha octadecatetraenoic acid. Most of the essential amino acids found in *Gelidium* sp., which accounted for 50.26% of the total amino acid**,** glutamic and aspartic acids are the most abundant amino acids in *Gelidium sp.* (24.41 % of total amino acids). Polysaccharides from *Gelidium* sp. were extracted and the yield was (15.2%), Analyses of total sugars by the method of DNS colorimetric showed high percentages of these compounds (82.9%). The monosaccharide composition of galactans obtained from *Gelidium sp* by reductive hydrolysis and quantified by gas chromatography, showed that these galactans have a high content of galactose (83.4%) and 6-O-Me-galactose (14.1%) where glucose was found in smaller quantity (2.5%). DPPH is a free-radical compound that has been widely used to determine the free-radical scavenging ability of samples; result demonstrated that the sulfated polysaccharide had a noticeable effect on inhibiting the formation of these radicals (68%). Where the result obtained for the inhibition of hydroxyl (OH^-) radical formation demonstrated that scavenging activity of *Gelidium* sp. polysaccharide was (57%).

Keywords Biochemical Studies, Red Algae, *Gelidium* sp.

Introduction

Marine algae have along been used as food and medicine in Asian contries such as Japan, china and Korea. Most of the algae intake in the daily diet is derived from undaria (wakame) and laminaria (kombu) algae species [1]. The marine macroalgal better known as seaweeds, are classified according to their pigmentation into brown (phaeophyta), red (Rhodophyta) and green (chlorophyte) seaweeds. They are widely used as food, as ingredients in cosmetics and fertilizers and in hydrocolloid production (e.g. agar and alginate) [2]. There are 250 macro algal species which have been listed as commercially utilized worldwide, among which 150 are consumed as human foods [3]. Traditionally, marine algae are often used in human or animal foods for their mineral content or for the functional properties of their polysaccharides. They are rarely promoted for the value of their proteins and pigments. However, some red seaweed can have protein contents up to 47% of dry seaweed. Nowadays the use of seaweeds as sources of pigments, proteins, or amino acids appears to be an interesting opportunity to valorize this marine resource. Numerous studies have been conducted to extract components using various methods and new, essentially

mild procedures, such as enzymatic, ultrasound, microwave, or supercritical fluid-assisted extractions have been developed to improve the extraction yield of native molecules. The protein in algae contains all essential amino acids (EAA) which available throughout the year although seasonal variations in there concentrations are known to occur for example, EAA of algae varieties namely *Hizikia* sp. and *Eisenia bicyclis* ranged from 45 to 59% [4]. The crude protein content of red algae (*Asparagopsis taxiformis*) was 9.1% and of brown algae (*Sargassum vulgare*) was 13.6% [5]. The most common fatty acids from microalgae are: Palmitic (hexadecanoic-C16:0), Stearic (octadecanoic-C18:0), Oleic (octadecenoil-C18:1), Lenoleic (octadecadienoic-C18:2) and Linolenic (octadecatrienoic - C18:3) acids [6]. *Gelidium* and *Gracilaria* are two important genera as raw materials for agar resources. The best quality of agar is extracted from *Gelidium* species. All *Gelidium* used for commercial agar extraction comes from natural resources, principally from France, the republic of Korea, Mexico, Morocco, Portugal and Spain [7]. The predominant algal polysaccharides are the alginates in brown macroalgae, and the sulfate-esterified polysaccharides of macro- and microalgae that are widespread in red, brown, and green seaweeds [8]. Polysaccharides promote antioxidant activity, which exhibited the greater of abstraction of anomaric hydrogen from the internal monosaccharide units [9-10]. Also, polysaccharide like alginic acid can absorption of toxic chemicals and free radical [11]. The brown alga and the red alga could be used as a food supplement to help meeting the recommended daily intake of some minerals, macro elements (Na, K, Ca, Mg) and trace elements (Fe, Zn, Mn, Cu) [12].

Materials and Methods

1- Materials

The red algae (seaweed) *Gelidium* sp. was obtained from Edfina Company. It was collected from Alexandria water (Mediterranean sea). These algae were identified by specialized researchers.

2- Methods

Chemical Composition of Algae

2.1. Determination of Moisture Content: An appropriate amounts of red algae was accurately weighed (ca. 10 g) and dried in an oven at 105 ° C until a constant weight was reached [13].

2.2. Determination of Crude Protein: The total nitrogen was determined using Kjeldahel method [13]. The crude proteins were calculated by multiplying the total nitrogen by a factor of 6.25.

2.3. Determination of Crude Fiber: A known weight of the dried algae sample (ca 2 g) was digested with sulfuric acid (200 ml 1.25 %), then with sodium hydroxide solution (200 ml 1.25 %) and washed several times with diethyl ether. The residue was then dried at 110 °C, then fibers at 550 °C [14].

2.4. Determination of Total Lipid: The total lipid of dried algae were determined [13] as follows: a known weight of the dried sample (ca 10 g) was placed in Soxhlet apparatus and the lipid were extracted by petroleum ether (B.P. 60/80 ° C) for 24 hr. the solvent was then removed and the percentage of total lipid was calculated.

2.5. Determination of Ash Content: Ashing process was carried out in a muffle furnace by heating at 550 °C until a constant weight [13].

2.6 Determination of Amino Acids: Performic oxidation methods: Applicable to determination of amino acids (including methionine and cystine) in feeds, not applicable to determination of tyrosine and tryptophan. Performic acid oxidation prior to hydrolysis to oxidize cystine and methionine cystic acid methionine cystic acid methionine sulfon. Hydrolysis was carried out in closed conical flask for determining all amino acid other than tryptophan. Sample equal to 10 mg of protein was weighted in the conical flask and 5.0 ml of performic acid was added, the flask was closed and placed in ice bath 16 hour sodium disulfide was added, 25.0ml HCl 6.0 N was added to the oxidized mixture, the flask was placed in an oven at 110 °C for 24 hour, the flask was then opened using rotary evaporator to reduce the volume 5–10 ml under vacuum at 60 °C. Adjust the pH to 2.20 with sodium hydroxide solution, suitable volume of sodium citrate buffer (2.20) was added to hydrolyzed sample. After all soluble material completely dissolved, the sample is ready for analysis. The system used for the analysis was high performance amino acid analyzer (Biochrom 30) [13].

2.7. Identification of Fatty acids Content of *Gelidium* sp. Saturated, unsaturated and total fatty acids were determined in the oil by using methyl esters boron triflorid methods [14]. The fatty acids were methylated with boron triflorid in methanol, extracted with heptanes and determined on a gas chromatography with FID detector (PE Auto system XL) with auto sampler and Ezchrom integration system. Carrier gas (He); ca 25 Psi–air 450 ml/min – Hydrogen 45 ml – split 100 ml/min oven temperature 200 °C. Injector and detector 250 °C. GC/MS analysis : was carried out using a HPLC (Aglint Technologies 7890 A) interfaced with a mass – selective detector (MSD , Agilent 7000) equipped with an apolar Agilent HP-5ms (5 % - phenyl methyl poly siloxane) capillary column (30 m $^{0.25}$ mm i.d and 0.25 um film thickness) the carrier gas was helium with the linear velocity of ml/min. The identification of components based on comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature.

2.8. Agar Extraction and Analysis: The dried sample of 30 g was boiled for 2 hour with 900 ml of distilled water. The extracted agar solution was filtered through muslin bad while hot extract agar was kept at room temperature until it solidified. The solidified agar was cut into strips, then frozen at -20° C for 48 hour, and thawed at in tap water to the hydrogel and dried.

2.8.1. Determination of Sulfate Content in Polysaccharides: Sulfate content in polysaccharides was determined by the barium chloride-gelatine method. A standard curve was made as a follow: 0.02: 0.04 : 0.6 : 0.08 : 0.10 : 0.12 : 0.14 : 0.16 : 0.18 : and 0.20 ml K_2SO_4 standard solution (0.6 mg ml^{-1}) were accurately put into test tube: hydrochloric acid (1M) was compensated to 0.2 ml solution, then 3.8 ml of tricholoro acitic acid (3 % v / v) and 1.0 ml of barium chloride-gelatin solution (5 g l^{-1}) were added, vortexted, and absorbance were measured at 360 nm after incubation for 15 minutes at room temperature ; 0.2 ml hydrochloric acid solution was used as a blank.

2.8.2. Polysaccharides Analysis: The monosaccharides composition of red seaweed galactans was obtained by reductive hydrolysis [15]. The polysaccharide was hydrolysed with 2M H_2SO_4 for 4 h at 100 °C in the presence of inositol as internal standard. The hydrolysate was neutralized with barium carbonate, deionized and the sugars were then reduced with an excess of $NaBH_4$ for 16 h at room temperature. Borate compounds were eliminated by MeOH/HCl 1% v/v treatment. The reduced sample was acetylated with acetic anhydride/pyridine (v/v) at 100 °C for 1h. After cooling and dilution with water, the mixture was evaporated to dryness. At end, the alditol acetate derivatives prepared were analyzed by gas chromatography equipped with a flame-ionisation detector (CPG/ FID) on a SP2380 macrobore column (0.53 mm x 30 m) in a Hewlett Packard 5890A system using various alditol acetate carbohydrates as standards. The carrier gas was high-purity nitrogen and the injector port was heated at 250 °C. For sample separation the following conditions were applied: 4 min at an initial temperature of 195 °C followed by an incremental increase (2.5 °C/min) to a final temperature of 225 °C during 2 min.

2.9. Determination of Antioxidant Activity

2.9.1. Effect of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The free–radical scavenging capacity of the sulfated polysaccharides was analyzed using a 1,1-diphenyl-2-picrylhydrazyl (DPPH). With some modification, BHT was used a reference material. Briefly, 0.2 ml of MeOH and 0.3 ml of various sample concentrations (0.1–2.0 mg ml^{-1}) dissolved in MeOH were mixed in a 10 ml test tube. DPPH (2.5 ml of 75 µM in MeOH) was then added to achieve a final volume of 3.0 ml. The solution was kept at room temperature for 30 min, and the absorbance at 517 nm (A_{517}) was measured. The DPPH scavenging effect was calculated as follows:

$$\text{Scavenging effect \%} = [\ A_0 - (\ A - A_b\ )\ /\ A_0\ ] \times 100$$

Where:

$A_0 = A_{517}$ of DPPH without sample

$A = A_{517}$ of sample and DPPH

$A_b = A_{517}$ of sample without DPPH

2.9.2. Hydroxyl Radicals Scavenging Activity

The scavenging activity of seaweed polysaccharides against the hydroxyl radical was investigated using fenton's reaction:

$$Fe^{2+} + H_2O_2 \xrightarrow{\quad Fe^{3+} \quad} + OH^- + {}^{\cdot}OH$$

The results were expressed as an inhibition rat. Hydroxyl radical exhibit a small diffusion capacity and are most reactive in the induction of injuries cellular molecules and, accordingly, deserve special attention. Hydroxyl radical were generated using a modified [16] method: in 3 ml sodium phosphate buffer (150 mM, pH 7.4), which contained 10 mM $FeSO_4.7H_2O$, 10 mM EDTA, 2 mM sodium salicylate, 30 % H_2O_2 (200 µl) and varying concentrations of polysaccharides (0.1-2.0 $mgml^{-1}$). In the control sample, sodium phosphate buffer replaced H_2O_2. The solution were incubated at 37 °C for 1 hour, and the presence of Hydroxyl radical was detected by monitoring absorbance at 510 nm .

Results and Discussion

Chemical Composition of *Gelidium sp.*

The proximate chemical composition of *Gelidium* sp. are shown in Table (1). The mean protein content of *Gelidium* sp. (13.23 ± 1.06% DW) was within the range of 10-47% for green and red seaweeds [17], the mean percentages of crude lipid (1.16 ± 0.21% DW) and fibre content (5.5 ± 1.05% DW) of *Gelidium* sp. Edible seaweeds are not considered as a good source of lipid content as they contain less than 4% of crude lipid at dry weight basis [18]. The mean percentage of crude lipid (1.16 ± 0.21% DW) obtained from this study is higher than some edible red seaweeds (*Gracilaria cervicornis* 0.43% DW, *Porphyra tenera* 0.7%) reported in previous studies [19]. The mean percentage of fibre and Carbohydrate content were 5.5 ± 1.05% DW and 53.66 ± 1.21% DW respectively. The mean percentage of ash contents (26.45 ± 0.74%) found in *Gelidium* sp. was similar with other red seaweeds. In general, high level of ash was associated with the amount of mineral elements. Previous studies reported that ash content of seaweed varies between 8 and 40% (at dry weight basis) [20].

Table 1: Proximate chemical composition of *Gelidium* sp. (dry weight basis)

Nutrient	*Gelidium sp.*
Protein %	13.23 ± 1.06
Crude lipid (%)	1.16 ± 0.21
Carbohydrate (%)	53.66 ± 1.21
Fibre (%)	5.5 ± 1.05
Ash (%)	26.45 ± 0.74
Moisture (%)	10.44 ± 0.94

Amino acids Composition of *Gelidium sp.*

Most of the essential amino acids found in *Gelidium* sp., which accounted for 50.26% of the total amino acid [Level of total EAAs % /sum of all measured amino acids% x 100%]. The amino acid profiles and the essential amino acid scores of *Gelidium* sp. are presented in Table (2). Wong and Cheung [21] observed that most of the essential amino acids in some subtropical seaweed (*H. japonica*, *H. charoides* and *U. lactuca*), which accounted for 42.1-48.4% of the total amino acids. *Gelidium sp.* seemed to be able to contribute adequate levels of total EAA for human. This study revealed that glutamic and aspartic acids are the most abundant amino acids in *Gelidium sp.* A number of studies argued that red seaweed contains higher percentages of both aspartic and glutamic acids [21-22]**.** In *Gelidium* sp., aspartic and glutamic acids constituted a substantial amount of the total amino acids (24.41% of total amino acids). Similar results were reported in various other studies previously [17, 23-24]. In general, most of the seaweeds contain relatively higher amount of free amino acids [25]. These amino acids provide different types of flavours to several edible seaweeds. Glycine and alanine give a sweet flavour to edible seaweeds [26] and aspartic and glutamic acids were responsible for the special flavour and taste of seaweeds [23]. With the increasing level of education in the developing countries, people are now more concern about nutritional value of consumable food items [27-28].

Table 2: Amino acids composition of *Gelidium sp*

Amino acid	%	Amino acid	%
Aspartic (ASP)	1.51	Leucine (LEU)	1.34

Therionine (THR)	0.11	Tyrosine (TYR)	0.92
Serine (SER)	0.11	Phenylalanine (PHE)	1.01
Glutamic (GLU)	1.72	Histidine (HIS)	0.74
Glycine (GLY)	0.65	Lysine (LYS)	1.12
Alanine (ALA)	0.8	Argnine (ARG)	0.35
Valine (VAL)	1.12	Proline (PRO)	0.71
Isoleucine (ILE)	0.69	Cystine (CYS)	0.16
Methionine	0.17		

Fatty acids Composition of *Gelidium sp.*

The fatty acid profiles of *Gelidium* sp. are presented in Table (3). Only 10 saturated fatty acids from C6 to C20 and eight unsaturated fatty acids from C14:1 to C18:4 including some highly unsaturated fatty acids such as C18:1 ω9 (oleic acid) and C18:2 ω6 (linoleic acid) which recorded 21.5% and 9.14% respectively. The fatty acids *i.e.* (PUFA) play an important role in human metabolic pathways, particularly as specific precursors for prostaglandin E1 [29].

The PUFA including the essential fatty acids namely, linoleic acid, α-linolenic acid (ALA) and γ- linolenic acid (GLA) are important in pharmaceutical industry. The γ-linolenic acid is recognized as a promising therapeutic agent for numerous health disorders acting as a precursor for prostaglandin E1, an important compound necessary for reducing inflammation and in treatment of heart disease, Parkinson disease, multiple sclerosis, plasma cholesterol levels, dermatitis, diabetes, and pre-menstrual syndrome [31]. The mono and polyunsaturated fatty acids such Palmitoleic acid (C16:1), Oleicacid (C18:1), Cis-Linoleic acid (C18:2), Linolenic acid (C18:3) and alpha octadecatetraenoic acid (C18:4) were detected from *Gelidium* sp.

Gelidium sp., had 18 fatty acids, among the 18, ten were saturated fatty acids with the highest content 30.87% in palmitic acid (C16:0) and remaining eight unsaturated fatty acids were detected with five of them monounsaturated and three of them were polyunsaturated fatty acids namely Tetradecenoic acid (C14:1), Palmitoleic acid (C16:1), Oleic acid ((C18:1 ω9), Vaccinic acid (C18:1 ω7), Octadecosaenoic acid (C18:1 ω5), Cis-Linoleic acid (C18:2), Linolenic acid (C18:3) and octadecatetraenoic acid (C18:4) respectively .ten saturated fatty acids were detected in *Gelidium sp*, among the ten, the maximum percent 30.87% of Palmitic acid (C16:0) and minimum percent 0.21% of Arachidic acid (C20:0).

Table 3: Fatty acids composition of *Gelidium sp.*

Fatty acids	**%**
Caproic acid C6:0	0.45
Caprlyic acid C8:0	1.13
Capric acid C10:0	1.51
Lauric acid C12:0	5.62
Myristic acid C14:0	8.91
Tetradecenoic acid C:14:1 ω5	0.50
Pentadecanoic acid C15:0	1.87
Palmitic acid C16:0	30.87
Palmitoleic acid C16:1 ω9	2.03
Heptadecanoic acid C17:0	1.42
Stearic acid C18:0	10.13
Oleic acid C18:1 ω9	21.50
Vaccinic acid C18:1 ω7	2.72
Octadecosaenoic acid C18:1 ω5	0.43
Linoleic acid C18:2 ω6	9.14
Linolenic acid C18:3 ω3	0.95
Alpha octadecatetraenoic acid C18:4 ω3	0.30

Arachidic acid	C20:0	0.21

Agar extraction and analysis

Agar yield, sulfate content, total sugars and monosaccharide composition were presented in table (4), the milled red seaweed was washed with water at room temperature and the residue left was exhaustively extracted with hot water (90°C). This method revealed to have a higher yield (15.2%).

As can be seen, the extraction with hot water originated levels of sulfate of 8 %, higher than those found in the literature for other tropical species, such as G. cornea (collected in Mexico), which showed variations from 4.8 to 5.5% [19]. The G. birdiae polysaccharide obtained by cold extraction has a sulfate content (6.4%) [31].

Analyses of total sugars by the method of DNS colorimetric [32], showed high percentages of these compounds (82.9%).

The monosaccharide composition of galactans obtained from *Gelidium sp* by reductive hydrolysis and quantified by gas chromatography, show that these galactans have a high content of galactose (83.4%) and 6-O-Me-galactose (14.1%) where glucose was found in smaller quantity (2.5%).

Table 4: Agar yield, sulfate content, total sugars and monosaccharide composition of *Gelidium sp*

Parameter	**Percent %**
Yield	15.2
Sulfate content	8
Total sugars	82.9
Monosaccharide composition	
Galactose	83.4
6-O-Me-galactose	14.1
Glucose	2.5

Rahelivao *et al.* [33] reported that agar from the red alga (*Gelidium* sp) was extracted with hot water and precipitated in presence of ethanol (representing 16 w % on the basis of dried crude algae) forms strong gels in aqueous solutions.

Agar yield could be stimulated by manipulating each extraction variable although exogenous factors, such as species, location and environmental parameters and stage of the life-cycle [34].

Antioxidant Activity of Sulfated Polysaccharides

DPPH is a free-radical compound that has been widely used to determine the free-radical scavenging ability of samples. This method allows determining that anti-radical activity of an antioxidant by measuring the decrease in absorbance of the DPPH radical caused by the scavenging of the hydroxyl radical through hydrogen donation. In this work, DPPH free-radical scavenging effect of the sample was measured. Result in table (5) demonstrated that the sulfated polysaccharide had a noticeable effect on inhibiting the formation of these radicals (68%). The result obtained for the inhibition of hydroxyl (OH^-) radical formation demonstrated that scavenging activity of *Gelidium* sp. polysaccharide increased was (57%).

Table 5: Antioxidant activity of sulfated polysaccharides for *Gelidium sp*

Antioxidant methods	**% Inhibition**
DPPH scavenging activity	68
Hydroxyl radicals scavenging activity	57

Concluded that the OH^- scavenging activity of different polysaccharides was related to the presence of the same structural feature in which all of the polysaccharides had one or more -OH and $-OSO_3H$ groups in the molecule. These results proved that sulfate content had a significant effect on OH^- scavenging activity. Souza *et al.* [35] isolated a SP by aqueous extraction from the red seaweed Gracilaria birdiae and observed that the slimy substance exhibits moderate antioxidant properties as measured by DPPH free-radical scavenging effect.

Several works have demonstrated that the presence of sulfate groups in seaweed polysaccharides is responsible for numerous types of biological activities, such as antioxidant activities [36].

References

1. Yubin V.S. and Guangmei Z. (1998). Pharmacological action and application of available antitumor composition of traditional Chinese medicine, Heilongijang, hinam Heilongijang sci. technec. Press.
2. Chan C., Ho C. and Phang S. (2006). Trends in seaweed research, trends in plant science 111 – 165:166.
3. Barrow C. J. (2007). Marine by-products as functional food ingredient, Food Technology International Journal 5 – 1:5.
4. Galland-lrmoul A.V., Fluerence J., Lucan M. and Rouxel C. (1999). Nutritional value of proteins from edible seaweeds, nutritional biochem. Journal 10 – 353:359.
5. Marinho- Soriano E., Fonseca P. C., Carneiro M. A. A. and Moreira W. S. C. (2006). Seasonal variation in the chemical composition of two tropical seaweeds, Bioresouces technol. 97 – 2402:2406.
6. Knothe, G. (2009). "Improving biodiesel fuel properties by modifying fatty ester composition." Energy Environ. Sci. 2(7): 759-766.
7. Mchugh G. (2003). A guide to the seaweed industry, FAO Fisheries Technical paper No. 441.
8. Popper ZA, Michel G, Hervé C, Domozych DS, Willats WGT, Tuohy MG, Kloareg B, Stengel DB (2011) Evolution and diversity of plant cell walls: from algae to flowering plants. Annu Rev Plant Biol 62: 8.1–8.24
9. Abd El-Baky, HH, El-Baz FK, El-Baroty GS (2009). Potential Biolo-Gical Properties of Sulphated Polysa-Ccharides Extracted From The Macroalgae Ulva Lactuca L. Acad J Cancer Research 2: 1-11.
10. Athukorala Y, Kim K, Jeon Y (2006) Antiproliferative and Antioxidant Properties of an Enzymatic Hydrolysate from Brown Algae Ecklonia Cava. Food Chem Toxicol 44: 1065-1074.
11. Kim IH, Lee JH (2008) Antimicrobial Activities against Methicillin-Resistant *Staphylococcus Aureus* From Macroalgae. J Indus Engin Chem 14: 568-572.
12. Ruperez P. (2002). Mineral content of edible marine seaweeds. Food Chemistry; 79:23-6.
13. A.O.A.C. (2007). Official method of analysis of Association of the Analytical chemistry. 17th ed., Published by association of the official Analytical chemistry. PO. Box 540. Benjamin Franklin Station Washington DC 20044.
14. A.O.A.C. (2012). Official method of analysis of the Association of Official Agriculture Analytical Chemistry. 17th ed., . Published by Washington, DC., USA.
15. Marie Pascaline Rahelivao, Hanta Andriamanantoanina , Alain Heyraud2 and Marguerite Rinaudo (2014). Structure and Rheological Behaviour of Agar Extracted From Madagascar Sea Coast Algae , The Open Macromolecules Journal, 2014, 7, 1-6
16. Smirnoff Cumbes N. and Cumbes Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes, Phyto Chem.. J. 28– 1057:1060.
17. Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses. Trends in Food Science and Technology 10: 25-28.
18. McDermid, K.J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. Journal of Applied Phycology 15: 513-524.
19. Marinho-Soriano, E., & Bourret, E. (2005). Polysaccharides from the red seaweed Gracilaria dura (Gracilariales, Rhodophyta). Bioresource Technology, 96, 379e382.
20. Mabeau, S. and Fleurence, J. (1993). Seaweed in food products: bio-chemical and nutritional aspects. Trends in Food Science and Technology 4: 103-107.
21. Wong, K.H. and Cheung, C.K. (2000). Nutritional evaluation of some subtropical red and green seaweeds Part I: proximate composition, amino acid profiles and some physicochemical properties. Food Chemistry 71: 475-482.

22. Lourenço, S.O., Barbarino, E., De-Paula, J.C., Pereira, L.O.S. and Marquez, U.M.L. (2002). Amino acid composition, protein content and calculation of nitrogen-to-protein conversion factors for 19 tropical seaweeds. Phycological Research 50: 233-241.
23. Mabeau, S., Cavaloc, E., Fleurence, J. and Lahaye, M. (1992). New seaweed based ingredients for the food industry. International Food Ingredients 3: 38-45.
24. Wang, J., Zhang, Q., Zhang, Z., Zhang, J., & Li, P. (2009). Synthesized phosphorylated and aminated derivatives of fucoidan and their potential antioxidant activity in vitro. International Journal of Biological Macromolecules, 44(2), 170e174.
25. Ratana-arporn, P. and Chirapart, A. (2006). Nutritional evaluation of tropical green seaweeds *Caulerpa lentillifera* and *Ulva reticulata*. Kasetsart Journal of Natural Science 40: 75-83.
26. McLachlan, J., Craigie, J.S., Chen, L.C.M. and Ogetze, E. (1972). Porphyra linearis Grev: an edible species of Nori from Nova Scotia. Proceedings of the International Seaweed Symposium 7: 473-476.
27. Siddique, M.A.M. (2012). Explaining the role of perceived risk, knowledge, price and cost in dry fish consumption within the Theory of Planned Behaviour. Journal of Global Marketing 25 (4): 181-201.
28. Siddique, M.A.M., Mojumder, P. andZamal, H. (2012). Proximate composition of three commercially available marine dry fishes (*Harpodon nehereus*, *Johnius dussumieri* and *Lepturacanthus savala*). American Journal of Food Technology 7: 429-436.
29. Mendes RL, Reis AD, Palavra AF (2006) Supercritical CO2 extraction of γ-linolenic acid and other lipids from Arthrospira (Spirulina) maxima: comparison with organic solvent extraction. Food. Chem. 99: 57-63.
30. Wainwright PE, Huang YS, Levesque S, Mutsaers L, Mccutcheon D, Balcaen P, Hammond J (1996). Effects of dietary γ-linolenic acid and prenatal ethanol on mouse brain and behaviour. Pharmacol. Biochem. Beh. 53: 843-852.
31. Maciel, J. S., Chaves, L. S., Souza, B.W. S., Teixeira, D. I. A., Freitas, A. L. P., Feitosa, P. A., et al. (2008). Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from red seaweed Gracilaria birdiae. Carbohydrate Polymers, 71, 559e565.
32. Miller, G.L., (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem., 31, 426.
33. Rahelivao, M. P.; H. Andriamanantoanina; A. Heyraud and M. Rinaudo (2014). Structure and rheological behaviour of agar extracted from Madagascar sea coast algae. Open Macromol J 7: 1-6.
34. Sousa, A. M. M., Alves, V. D., Morais, S., Delerue-Matos, C., and Goncalves, M. P. 2010. "Agar Extraction from Integrated Multitrophic Aquacultured *Gracilaria Vermiculophylla*: Evaluation of a Microwave-Assisted Process using Response Surface Methodology." *Bioresource Technology* 101 (9): 3258-67.
35. Souza BWS, Cerqueira MA, Bourbon AI, Pinheiro AC, Martins JT, Teixeira JA, Coimbra MA, Vicente AA (2012) Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed Gracilaria birdiae. Food Hydrocol 27:287–292.
36. Qi H. , Zhang T.Q., Li Z, Zhao Z ,Xing R (2005) Antioxidant Activity Of Different Molecular Weight Sulfated Polysaccharides From *Ulva Lactucapertusa Kjellm (Chlorophyta)*. J Appl Phycol 17: 527–534.