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The content of some selected metabolites of *Hypnea valentiae* (Turner) Montagne from the Red Sea coast of Sudan

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ABSTRACT

Objective: To investigate some selected metabolic constituents of *Hypnea valentiae* (Turner) Montagne collected from the Red Sea coast of Sudan to evaluate the economic potential of the alga.

Methods: Healthy thalli of the alga under investigation were collected, thoroughly cleaned, air dried and milled. Authentic analytical methods were used to determine moisture, ash, protein lipid and carrageenan contents. Fatty acids profile was revealed with gas chromatography instrumentation. Testing of phytochemical groups of compounds was based on the development of colouration and precipitation upon addition of certain chemical reagents to the extracts.

Results: The ash content of the alga [(40.3 ± 0.2)% of dry weight] was comparatively the highest among the parameters tested. The protein, lipid, and carrageenan constituted (9.30 ± 0.70)%, (6.50 ± 0.34)%, and (33.70 ± 0.01)%, respectively of the alga dry matter. *Hypnea valentiae* (Turner) Montagne from Sudan Red Sea coast composed of 12 fatty acids, 8 of which were unsaturated fatty acids and 4 were saturated fatty acids. The major unsaturated fatty acids in the alga was the trans-isomer of linoleic acid and linolelaidic acid (36.32%) followed by palmitoleic acid (13.64%). The major saturated fatty acids was the heptadecanoic acid amounting to 19.10% of the total fatty acids. Phytochemically the alga contained alkaloids, flavonoids and tannins.

Conclusions: This alga may represent a promising source of functional food and therapeutic metabolites. Further investigation and critical evaluation of the bioactivity of the phytochemical compounds is required to assure the therapeutic potential of this alga.

1. Introduction

The red algal genus *Hypnea* (Lamouroux, 1813) is one of the widest spread macroalgae on tropical and subtropical shores[1,2]. The genus, known to include 53 species, is of economic importance as a source of carrageenan. Several studies were undertaken to investigate the biochemical and bioactive constituents as well as the morphological and molecular characteristics of the species of the genus. The content and variation of carrageenan were examined for Indo-Pacific species and Atlantic species[1,3]. The studies done to investigate the biochemical constituents and the antibacterial potential of different organic solvent extracts of species of *Hypnea* have shown that species of the genus might represent a potent source of new antibacterial drugs[4-7]. Some organic solvent extracts of *Hypnea valentiae* (Turner) Montagne (*H. valentiae*) exhibited the highest and significant antibacterial activity against 7 standard pathogenic bacterial strains[8]. Additionally, Mazhar *et al.*[9] tested the toxicity, analgesic, behavioural, and anti-emetic activities of the ethanolic extract of *Hypnea pannosa* (*H. pannosa*) and concluded that the extract has shown promising bioactive activities. The antioxidant activity and the potent inhibitory effects of the organic extracts of *Hypnea* species on both Gram-positive and Gram-negative bacteria were demonstrated and correlated to the algal extract content of total phenols and free fatty acids[10,11]. Furthermore, the potent analgesic and anti-emetic effects reported for the ethanolic extract of *H. pannosa* were ascribed to the presence of phytochemicals such as sterols and sesquiterpenes[9]. The

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nutritional value of some *Hypnea* species was assessed. According to Shareef Khan *et al.*[12], *Hypnea musciformis* (*H. musciformis*) could be utilized as a functional food ingredient because the alga contains essential amino acids and fatty acids. Morphological and molecular studies on *Hypnea* species were also undertaken to reveal and/or to confirm the taxonomic status of the tested species. Morphological and molecular techniques were performed to confirm the introduction of an Indo-Pacific *Hypnea* species to the Mediterranean and to provide a comparative morphological and molecular assessment of *H. valentiae* from Indian subcontinent[13,14].

To date, only *H. valentiae* was known to occur in the Sudanese Red Sea coast. The information on the biochemicals and bioconstituents of this alga is lacking. The main objective of this paper was to investigate selected biochemical and phytochemical variables of *H. valentiae* from the Red Sea coast of Sudan in order to assess the economic potential of this alga.

2. Materials and methods

2.1. Materials

The sample of *H. valentiae* was collected from Kilo 8 Embayment at 19°35' N and 37°15' E along Sudanese Red Sea coast in mid winter season (March, 2014). In the field, the sample was washed thoroughly with seawater to remove extraneous materials. In the laboratory, the sample was washed 3 times sequentially with running freshwater and distilled water to assure the removal of foreign matters and salts. Then the excess water was removed with paper tissues and the sample was spread in the shade to dry at room temperature for 6 days. The air-dried salt free plant materials were ground to fine powder using Kinematica M20 universal Muhle grinder and kept in air-tight containers at room temperature until analyzed.

2.2. Methods

Experiments were repeated until reproducible results were obtained or in triplicates. Values obtained were reported relative to the dry weight of the seaweed unless otherwise stated.

2.3. Proximate compositions

Determinations of moisture contents, ash, crude protein, total lipid, carrageenan and fatty acids were undertaken as described below.

2.3.1. Determination of moisture content

Two grams of the alga powder were weighed in a dish and kept in an oven at 105 ˚C until constant weight was reached. The dish was covered while still in the oven and placed in a desiccator and weighed soon after reaching room temperature. The moisture content was reported as loss in weight and its percentage content was determined gravimetrically.

2.3.2. Determination of ash

An ashing dish was ignited, cooled in a desiccator to room temperature and weighed. The sample was well mixed and 2 g were placed in the dish. The contents were ignited in a muffle furnace at 550 ˚C until light grey ash was obtained. The dish was cooled in the desiccator and weighed soon after reaching room temperature and the ash percentage content was calculated gravimetrically.

2.3.3. Measurement of crude protein contents (Nx6.25)

The crude protein of the red alga was measured with Buchi instruments for protein analysis that consisted of a digester block (Buchi, K-424) and a distillation unit (Buchi, 350). The protein content of the alga was calculated by multiplying its nitrogen content obtained with micro Kjeldahl technique with a correction factor of 6.25. One gram of the sample was weighed and transferred to a digestion tube. About 0.4 g of a catalyst mixture [potassium sulphate and copper II sulphate pentahydrate (5:1, w/w)] were mixed with the sample. Ten milliliters of concentrated sulphuric acid were added to the mixture. The tube was then incubated in the digester block adjusted at 100 ˚C for 20 min. The temperature was raised gradually to 380 ˚C until clear solution was formed. Then, the tube was fitted in the distillation unit. In a 250 mL conical flask, 20 mL of boric acid was added. The flask was installed in the distillation unit. Then, 20 mL of 40% sodium hydroxide was added as a reagent to help capturing NH_4 in the boric acid under steamed condition. The tube was left in the distillation unit for 5 min until the colour of boric acid changed from red to blue. The product of the distillation was titrated against HCl (0.1 mol/L) until the colour of boric acid changed to red. The consumed volume of HCl was recorded and used to calculate the percentage of the nitrogen in the sample with the following formula:

$$\frac{1.4 \times \text{T.V.} \times \text{N}}{\text{W} - \text{MC}\%}$$

where, 1.4 referred to a constant, T.V. was titration volume, N meant HCl normality and W was sample weight.

2.3.4. Determination of total lipid content and fatty acids profile

The method of Folch *et al.*[15] with the modification of Christie[16] for the extraction of the total lipids was performed to determine the 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[17].

2.3.5. Determination of fatty acids composition

Fatty acids were determined by gas chromatographic quantification of their fatty acid methyl ester obtained above. The fatty acid methyl ester samples were analyzed using capillary gas chromatography GC-2010 (Shimadzu, Japan) equipped with capillary column (DB-1, 30 m × 0.25 μm × 0.25 μm) and flame ionization detector. The injection volume and temperature were 0.2 μL and 250 ˚C, respectively. The split ratio was 10 and the detection temperature was 280 ˚C. Nitrogen was used as a carrier gas with the column flow rate of 1 mL/min. The running temperature was set gradient from 150 ˚C up to 230 ˚C with an increase rate of 20 ˚C/min. The identification of fatty acids in the sample was performed by comparing their retention time with those of the standard fatty acids mixture (Supelco Analytical, PA, USA) injected under the same conditions.

2.3.6. Measurement of carrageenan content

A conventional method was used to determine the carrageenan content in *H. valentiae*. The dry alga tissue was soaked in NaOH solution in a water bath for 90 min. The mixture was blended and filtered through a nylon cloth. The precipitate was washed with isopropyl alcohol, dried and weighed.

2.4. Extraction

The serial exhausted extraction method[18] was followed to extract the materials. Ten grams of *H. valentiae* dry powder were first macerated in 100 mL of ether for 3 days at room temperature with frequent shaking. The mixture was centrifuged (10 000 g/10 m) 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.

2.5. Qualitative screening of phytochemicals

Three of the most general phytochemical groups of compounds was investigated based on the recommendations given by Harborne[19]. Regular phytochemical procedures were followed for the qualitative analysis. The extracts were tested for the presence of alkaloids, flavonoids, and tannins of phytochemical groups of compounds. This was based on the 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 Zohra *et al.*[20]. The test of flavonoids was done as described by Pamar *et al.*[21] with NaOH solution. Tannins were detected with ferric chloride solution according to the methods described by Ugochuhwu *et al.*[22].

3. Results

The concentrations of the tested metabolites of *H. valentiae* from the Red Sea coast of Sudan are presented below.

3.1. Proximate composition of H. valentiae

Based on dry weight, the moisture content of the alga was (9.30 ± 0.30)% and the ash content was (40.30 ± 0.20)%. The protein, lipid, and carrageenan were (9.30 ± 0.70)%, (6.50 ± 0.34)%, and (33.70 ± 0.01)%, respectively.

3.2. Fatty acids profile

Twelve fatty acids and fatty acids methyl esters were detected in the lipid of *H. valentiae* in Sudan (Table 1). Among them, 8 belonged to the unsaturated fatty acids (USFA) and 4 belonged to the saturated fatty acids (SFA). The total concentration of the SFA was 31.10% and that of USFA was 68.90% of the total fatty acids.

Table 1
Fatty acids compositions and concentrations of *H. valentiae*.

Fatty acids		Concentration (%)
SFA	Arachidic acid M.E	0.70
	Heptadecanoic acid	19.10
	Palmitic acid M.E.	7.70
	Pentadecenoic acid	3.60
	Total	31.10
USFA	cis-11-Eicosenoic acid	4.12
	Elaidic acid M.E.	5.20
	Erucic acid M.E.	2.80
	Lambda-linolenic acid	5.00
	Linolelaidic M E.	36.32
	Myristoleic M.E	1.60
	Nervonic acid M.E.	0.22
	Palmitoleic acid M.E	13.64
	Total	68.90

3.3. Phytochemical profile

The contents of crude alkaloids, flavonoids, and tannins in the 3 different organic solvent extracts of *H. valentiae* in Sudan were investigated. Alkaloids were detected in ether and chloroform extracts. Flavonoids were detected in ether and methanol extracts and the tannins were present in all the three solvent extracts.

4. Discussion

Generally, most of the findings on the biochemical constituents of the Sudanese *H. valentiae* obtained in this investigation were comparable with the findings of some previous studies on *Hypnea* species on the same parameters.

The moisture content of the alga [(9.30 ± 0.30)%] was relatively in conformity with the moisture content of *Hypnea japonica* (9.95%), *H. pannosa* (12.35%) and *H. musciformis* (11.54%)[23]. However, the variation in moisture content could be attributed to the variation in gum polymer content in different species. These substances have more hydrogen bonds and open chains resulting in high water holding capacity[24].

The ash content of *H. valentiae* from the Red Sea coast of Sudan [(40.30 ± 0.20)%] was relatively higher than the content of ash in *Hypnea* species from other geographical areas. For example, *Hypnea japonica* contained 21.10% of ash[25], *H. pannosa* contained 18.65%, and *H. musciformis* had 21.57% of ash. However, significant seasonal variation in ash content (11%–55% of dry matter) was reported for *H. musciformis* from Morocco[3]. Available records on Red Sea macroalgae indicated that they contained high ash content. For instance, the ash content in *Turbinaria triquetra* from the eastern coast of the Red Sea was 40.34% and that of *Halimeda opuntia* was 53.79%[26]. The ash content in algae may range from 3.5% to 46.0% with that of *Laminaria* typically being 33%[27]. A high inorganic content in seaweeds is very common and it is due to the extraordinary ability of seaweeds to accumulate elements present in the water where they live[28]. The average concentrations of the major elements Na, Mg, Ca, and K in the Sudanese Red Sea waters were reported to be 11 870.250, 1 449.200, 468.967, and 388.955

mg/L (or other units?), respectively[29]. These values, with exception to that of K, are higher than those of these minerals in seawater of other seas. Therefore, the ash content of seaweeds from the Red Sea may be relatively higher than that of seaweeds from other marine environments due to its high elements contents.

The protein content in *H. valentiae* from Sudanese Red Sea coast [(9.30 ± 0.07)%] of its dry weight is congruent with the reported range of protein content (10%–47%) in red macroalgae[30]. This value fairly approximates the protein content in *H. valentiae* which ranges from 11.8%–12.7%[31]. However, the present value is also relatively lower as compared to the protein contents in *H. pannosa* (16.31%) and *H. musciformis* (18.64%) recorded by Siddique *et al.*[23]. High salinity and water temperature were listed among the environmental factors that may produce negative bearing on the protein content[32]. The Red Sea experiences some of the extreme physic-chemical oceanographic conditions which occur in any marine area on earth[33]. Therefore, it was well established that Red Sea seaweeds contained less protein as compared to their counterpart species from the Mediterranean Sea[34]. Accordingly it is likely that the high salinity of the Red Sea water (39.82‰–40.00‰) besides the high water temperature may have influenced the protein content of this alga.

In contrast to protein content, the lipid content [(6.50 ± 0.34)%] of *H. valentiae* was greater than the lipid contents of other *Hypnea* species such as *H. pannosa* which contained 1.56% of lipid and *H. musciformis* that contained 1.27% of lipid. Contradicting statements on the lipid content of marine algae were present in the literature. Some studies stated that marine algae produce low lipid content that may not exceed 4% or 5% of their dry matter[30,35,36]. So in this context, the present lipid value is slightly greater than that statement. However, for *H. valentiae* from India, higher lipid content values (9.6%–11.6%) were reported[31]. Lipid composition and content of marine algae were reported to be influenced by some environmental conditions including high light intensity, high salinity, high temperature, and low nitrogen availability[37-40]. Compared to other seas, the Red Sea is well-illuminated, warm and oligotrophic sea. These conditions may have affected the lipid content of the present alga. It was proved that the growth of algae at high light intensity resulted in a 1.5-fold increase in the level of storage lipids, *i.e.* triacylglycerols and that the content of the most USFA, 20:5 nώ3, was reduced under high light intensity[37].

The carrageenan yield of the Sudanese *H. valentiae* [(33.70 ± 0.01)%] is in agreement with the yield reported in similar studies. In an investigation on the seasonal variation of carrageenan content of *H. valentiae* from Morocco, the yield fluctuated from 13.5% to 41.0% with no clear observed pattern[3]. Similar findings were also reported from tropical regions by Mtolera and Buriyo[1] for *H. musciformis* in which the carrageenan yield range was 20.56% to 35.63%. In both studies, no clear seasonal pattern was deduced in carrageenan contents and qualities. Therefore, the yield obtained from the present species is promising, and a further investigation on chemical constituents, gel strength and melting and gelling temperature is needed.

Available literature on the fatty acids composition of *Hypnea* species indicated that the relative contents of SFA of species was greater than those of the USFA with the palmitic acid consistently reported as the main constituent which in some investigations constituted more than half of the SFA[41]. In *H. valentiae*, *H. pannosa* and *H. musciformis,* palmitic acid was the major SFA and the oleic acid was the major USFA[42]. Apart from this, the three species remarkably differed in their fatty acid mixtures. Similarly in *Hypnea cornuta* palmitic acid was the most abundant SFA, palmitoleic and stearic acids were the predominant USFA[43]. In *H. musciformis*, pentadecanoic acid was recorded as the second major fatty acids followed by palmitoleic acid[12]. In this study, the USFA contain 2 *trans*-isomers of the linoleic acid and oleic acid, the linoeli adic and elaidic acids. Linolelaidic acid (36.32%) was the major USFA followed by palmitoleic acid (13.64%). Isomers of linoleic acid and other fatty acids as well as long chain fatty acids previously known to occur in animals only were reported from marine macroalgae. For instance, 8.9% of *cis*-vaccinic acid was reported for the red alga *Odonthalia floccosa* and elaidic acid was reported for *Cladostephus spongiosus*, *Sargassum granuliferum* (13.6%) and for *Gracilaria manilaensis* (3.169%)[44-46]. The long chain fish fatty acids eicosapentaenoic acids (C20:5, n-3) and docosahexaenoic acid (C22:6, n-3) were found in brown and red macroalgae[47]. Isomers of fatty acids are produced in rumens due to the action of specific enzymes that act on them to produce different conjugated and non-conjugated isomers of *cis* and *trans* configurations. However, the presence of similar enzymes was confirmed in marine macroalgae[48-50]. The biosynthesis of conjugated triene-containing fatty acids in the red alga *Ptilota filicina* was verified to be catalyzed by a novel enzyme, polyenoic fatty acid isomerase[49]. Bhaskar *et al.*[51] reported the occurrence of conjugated polyenoic fatty acids from the red alga *Acanthophora spicifera* from the Indian Ocean and suggested that the alga may contain an isomerase enzyme similar to *Ptilota filicina*. Subsequently, it is possible that this *H. valentiae* from Sudan may contain similar enzyme that produced the *trans* isomers eliadic and linolelaidic acid. A further investigation is required to confirm this assumption. On the other hand, linolelaidic acid has been reported to form 3.269% of the total fatty acids of the red macroalga *Gracilaria manilaensis*, 2.1% of the fatty acids of the marine green alga *Chaetomorpha linum* (Miller) Kutz[52], and 4.31% of the total fatty acids of *Ulva reticulata* Forsskal[53]. This is beside other natural sources of plant origin. This fatty acid was found to constitute 49.69% of the total fatty acids composition in the seed oil of *Schleichera oleosa* (Lour.) Oken[54], 14.57% in the moss *Kindbergia stokesii* (Turn.) Ochyra[55], 24.01% of the total fatty acids of medlar fruit from Iran[56], and 26.02% of the seed oil of *Hibiscus sabdariffa* Linn.[57]. The heptadecanoic acid was the major SFA amounting to 19.10% of the present alga dry weight. This odd chain fatty acid was reported from the genus *Hypnea* in varying concentrations ranging from 3.16% in the species *H. musciformis* to 5.57% in *H. valentiae* and to 13.58% in *H. pannosa*[42]. In addition, other macroalgae from the Red Sea and Mediterranean Sea were also reported to contain considerable concentrations of heptadecanoic acid. The brown alga *Hormophysa triquetra* from the Red Sea contained 9.70% of this fatty acid[58], the green macroalga *Ulva fasciata* from the Mediterranean Sea and the red macroalga *Laurencia papillosa* from the Red Sea contained 16.03% and 8.10%, respectively[59]. In addition, the concentrations of heptadecanoic acid in *Ulva reticulata* and *Kappaphycus* sp. were recorded to be

40% and 34%, respectively[53,60]. Therefore, these macroalgae could be considered as a potential source of this essential fatty acid in addition to its traditional sources of animal origin.

In the present alga the relative concentration of the USFA (68.90%) is higher than that of the SFA (31.10%) which is in conformity to the fatty acids profile of red seaweeds[44,46,51,58]. Four of the fatty acids reported here were not reported to date from Hypneaceae. However, they were reported from other species of red macroalgae. These included elaidic acid, erucic acid, linolelaidic acid and nervonic acid. Interspecific as well as intraspecific variations in fatty acids composition were largely attributed to variations in the environmental conditions prevailing in the seaweeds habitats. The fatty acids mixture of this alga seems to contain some fatty acids with beneficial and nutritional potential. For instance, linolelaidic acid, the major USFA of the Sudanese *H. valentiae* was reported to inhibit carcinogenesis[61-63]. Both linolelaidic and linoleic acids inhibited the growth of MOLT-4 T-lymphoblastic leukemia cells at 400 μmol/L[64]. The former fatty acid was usually reported from animal sources (dairy products) as a result of biohydrogenation of linoleic acid in mammalian tissue. The Sudanese *H. valentiae* may represent a natural plant source of this fatty acid. Oral administration of palmitoleic acid was reported to dramatically improve diabetic condition in mice[65]. This fatty acid was reported to enhance insulin sensitivity, reduce inflammation and minimize the destruction of insulin secreting pancreatic beta cells. Heptadecanoic acid, 16-methyl- and methyl ester exerted the most potent effect on skin cancer protein as compared to two other fatty acids isolated from two fungal strains of the *Hypocrea* species and the standard anticancer drug dyclonine[66]. Very low concentration (0.22%) of nervonic acid was detected in the fatty acid mixture of *H. valentiae* from Sudan. This very long chain fatty acid might be beneficial to neurological development and function[67]. From nutritive perspective, the fatty acids mixture of *H. valentiae* contained the linolenic acid (5%), one of the three basic essential fatty acids and the precursor of ω-3 fatty acids family[68]. The fatty acids mixture of *H. valentiae* from Sudan may also have economic and environmental propensity, as fatty acids with carbon atom number between C16–C18 are appropriate for biodiesel production[39].

This is in conformity with some studies demonstrating that some species of *Hypnea* contained these phytochemicals[69-71]. These metabolites were reported to produce a wide array of biological effects[72,73]. In particular, caulerpin isolated from macroalgae was the only indole alkaloid from marine sources which has been reported to have anti-inflammatory potentials. In addition, the anticancer activity of two alkaloids derivatives, lophocladine A and lophocladine B, which have been isolated from the red alga *Lophocladia* species has been successfully proven in various cancer cell lines. As for tannins, it has been established that the phytochemicals of seaweed extracts, especially polyphenols, have antioxidant activity[74,75]. Therefore, the antibacterial, antiviral, anticoagulant and anticancer activities of *Hypnea* species extracts were attributed to their phytochemical contents. In higher plants, secondary metabolites were reported to have a protective role against biotic and abiotic stresses such as herbivory, pathogens, high/low temperature, UV light exposure and high salinity[76-80]. Taking the harsh oceanographic conditions of the Red Sea into account, a similar role of secondary metabolites in this seaweed could be expected.

The ash, lipid, carrageenan and fatty acids contents of *H. valentiae* from the Red Sea coast of Sudan reasonably indicate that this alga may represent a promising source of functional food. In particular, the fatty acids of this alga are of nutrition as well as therapeutic potential. Similarly, the concentrations of alkaloids, flavonoids and tannins are encouraging for more investigations on the chemical characterization and bioactivity of these groups of compounds. Therefore, this alga may have an economic potential for food and pharmaceutical industries.

Conflict of interest statement

We declare that we have no conflict of interest.

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