Brown Seaweeds from Black Sea Coast as an Important Source of Bioactive Compounds of Interest for Human Health

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Abstract

For human nutrition, algae are important organisms that can offer essential compounds and new bioactive substances with pharmaceutical and medicinal value. Macroalgae contain nutritional elements such as lipids, proteins, vitamins and minerals and they are used as food supplements because they are known to be high in mineral content. There are a lot of nutritionally compunds that can be identified in algae such as polysaccharides, polyphenols, diterpenes, sterols, carbohydrates, peptides, polyunsaturated fatty acids, pigments and dietary fibers. Nutraceuticals are confirmed to be used as medicines because they are a good protector against chronic diseases and they have physiological benefits. Algae bioactive compounds could be used as an anticoagulant, antioxidant, anti-inflammatory, antibacterial, antimicrobial, antifungal, anticancer, antiviral, antidiabetic, antiobesity, antihypertensive and hypercholesterolemic nutraceuticals. Although only some of the algae have been studied properly for their chemical composition and properties, they are a considerable biological resource with ability for use as a raw material. In this paper the compounds from red and brown algae from the Romanian seaside are evaluated as sources of biologically active ingredients with benefits in human health.

Keywords: brown algae, antioxidant, Cystoseira barbata, polysaccharides, dietary fibers, fucoidan.

Introduction

One of the most popular organisms that can be found on earth are the algae. They have applications in agricultural, cosmetic and pharmaceutical industries because they have a wide variety of bioactive compounds and they have a rich chemical

composition [1]. Corresponding to many studies, a lot of bioactive compounds extracted from algae such as PUFAs, polysaccharides, peptides, pigments, carbohydrates, polyphenols, vitamins and phytosterols have implicated much interest in recent years for the significant chemical and biological diversity [2]. Anti-inflammatory, antimicrobial, immunostimulatory and immunomodulatory are some of the properties that these compounds had recorded. Both the recent studies about their benefical properties in human health and according to their nutritional composition, has highlighted the growing interest to incorporate algae into human diet [3, 4]. The Black Sea is known to be one of the richest seas with an impressive fauna containing green, red and brown seaweeds, which have not been fully studied for their properties and for their bioactive compounds. For hundreds of years the brown algae have been used by humans. In the recent studies it was highlighted that brown algae are very important sources of bioactive compounds with great nutritional value and they could be considered as functional foods with health benefits [5]. *Cystoseira* genus includes approximately 294 species [6]. *Cystoseira barbata* (Phylum: *Ochrophyta*, Class: *Phaeophyceae*, Order: *Fucales*, Family: *Sargassaceae*) is a genus of brown algae that can be found in the Romanian seacoast of the Black Sea. This brownalgae is defined by apical regions and vastly differentiated basal and catenate pneumatocysts are present too. In time the principal laterals come to be proportionally elongated, but the old plants have an elongated main axis. They have strong flattened lower parts into 'foliar-expansions' or basal leaves. Receptacles are the fertile parts which carry conceptacles and these can be normally situated at the tips of the branches. The apical and basal regions are notably differentiated. This brown seaweed can float in strong currents due to the aerocyst or air vesicles which keep the organism erect [7]. After the investigation of other members of the genus, *Cystoseira* has been discovered to include many biological activities such as: antiprotozoal, antibiotics, anti-inflammatory, antioxidant and cytotoxic [8]. An important source of polysaccharides is represented by the algae, notably the brown ones. The polysaccharides can be found commonly in nature, and these are omnipresent biopolymers. These can be determined in different types. Because of the structural differences, they can have distinct physical and chemical properties. Polysaccharides are nontoxic, biodegradable biopolymers. The sulfated polysaccharides are another essential group. The brown algae are also rich in soluble and insoluble polysaccharides, known as dietary fibres. The soluble polysaccharides that can be found in the brown algae are, fucans, alginates and laminarins. The sulfated polysaccharide that is principally composed of fucose interconnescted by β (1,3) glycoside bonds, alternating β (1,4) and β (1,3) bonds and rarely β (1,2) bonds, is the fucoidan. Its sulfate content is situated between 5% and 38 and it can contain, apart from fucose, other monosaccharides, including xylose, galactose, mannose, glucose, uronic acids and rhamnose [9]. The studies have demonstrated that fucoidan presents biological properties such as anti-inflammatory, antioxidant, liver protection [10] and antitumor [11] and it continues to be the most researched algae molecule. It has an important potential in the production of cosmeticals, functional

foods and pharmaceutical products. A small glucan obtained from the brown algae is the laminaran, which has a molecular weight of 1–10 kDa [12]. The two types that have been defined are the one with the chains terminated by D-glucose residues (G-series) and the other one with the chains that are terminated by D-mannitol residues (M-series). These laminarans have various advantages like biodegradability, low cellular toxicity and high biocompatibility [13].

Algal Material

The algal biomass, see Fig.1 that was collected manually has to be exposed to a pretreatment which is represented by repeated washings with drinking water and in the end with distilled water. On the fresh product only the macroscopic and the microscopic examinations can be performed [14]. For other types of examinations, the marine material has to be dried at temperatures between 25-35 degrees [15]. After that, the dry algal biomass has to be grounded to a powder and to use a sieve of 0.5 mm in order to obtain an uniform powder. May-november is the period that the algal flora was collected from the Black Sea coastline, from water at a distance of 5-25 m from the shore, from the areas of Constinesti, Mangalia, Eforie Sud, Eforie Nord, Navodari, Vama Veche and Constanta Casino.

Fig. 1. Brown algae in algal colony

Macroscopic and microscopic examinations

Macroscopic and microscopic examinations have to be applied on the algae selected for analysis. The first step in the research of the known or untested products was represented by the macroscopic examination [16]. In order to notice its dimension, appereance, taste, colour and smell, this part to be done through the whole plant examination (phylloid, rhizoid, celluloid) with the human eye, as well as with a magnifying glass [17]. The materials that are used in this examination are fragments of thallus from the red seaweed, a Microscope and Micros photomicroscope (10/0.25), forceps, blades, spatulate needles and Petri dishes. The microscopic exam

of the collected algae was realized directly on the fresh thallus fragments which were sliced, because the macrophytic algae species have single-cell or two-layer thallus and brought into a Petri dish with distilled water [18].

Chemical composition determination

Determination of humidity and ash - The humidity was determined in the termoregulator oven by drying the algae at 105 °C. After 12 hours was done, the calculation of the algae calcination at 550 ± 10 °C was performed. The organic substance was calculated by the difference between 100% and the sum of the values (%) of humidity and ash [19].

Determination of the sulfate content – Quantitive chemical analysis was used to determine the sulfate content. According to the national standard STAS 3069-87, the analysis for the determination of sulfates was realized spectrophotometrically [18].

Determination of protein and total nitrogen – Using the UdK DK6 digestor equipment was determined the total nitrogen and protein content of the algae, using the Kjeldahl method. In the presence of sulfuric acid under the catalytic action of selenium and mercury the mineralization was done. Then the alkalization was realized. After that, the ammonia was steam driven and captured in a boric acid solution which was titrated with hydrochloric acid. The result was expressed as a percentage [20].

Determination of lipids – The Soxhlet method was used to determine the lipids from the algae, an extraction method for 5 hours with dichloromethane as solvent. Gravimetrically the lipid content was determined and the results were expressed as a percentage [21].

Determination of carbohydrates – Using 15% acetic acid solution, the carbohydrate extraction was completed. With the Dubois method (1956), at a 190 nm maximum absorption, the carbohydrate content was determined spectrophotometrically. An Aquamate 8000 UV-VIS spectrophotometer was used, which uses spectral bandwidth of 1.8 nm and selectable wavelengths of 190-1100 nm. The results were calculated based on a standard glucose calibration curve [19].

Determination of total dietary fiber – Hipsley used the term "dietary fiber" to involve indigestible constituents of plants that compose the cell wall of plants, known to include hemicellulose, cellulose and lignin. Based on resistance and edibility to digestion in the human small intestine, the definition of dietary fiber, which has been extended by Trowell et al. became principally a physiological definition. In the definition are included indigestible polysaccharides such as modified cellulose, gums, mucilages and pectin and non-digestible oligosaccharides. The method used was the official method AOAC 985.29 "Total dietary fiber in food and the enzymatic-gravimetric method" [20]. The method was extended to allow total measurement of insoluble and soluble fiber in foods (AOAC official method 991.43) and various other modified fiber methods approved by AOAC International [20, 22].

Identification of phenol acids by HPLC

The method that was used to identify the phenol acid was HPLC-DAD. According to the method that was defined by Goupy [23], the HPLC analysis of phenolic compounds was determined in the ethanolic extract of marine brown algae. Using high performance liquid chromatography, the detection was conducted. That was equipped with auto sampler, quaternary pump, auto sampler, multi wavelength detector sett 280nm, 330nm and column compartment set at 35°C. Chem 32 integrator computed and monitored automatically the peak areas and the retention times. The retention time and standard deviations of phenolic standards chosen were: protocatechuic acid (3.130±0.008), pyrogallol acid (0.910±0.025), ferulic acid (8.865 ± 0.06), 4-amino-benzoic acid (3.455±0.005), chlorogenic acid (3.501±0.015), gallic acid (0.990±0.03), vanillic acid (6.919± 0.05), caffeic acid (8.281±0.07), benzoic acid (9.468±0.098), salicylic acid (15.952±0.051) and elagic acid (15.303±0.03), and for which a 0.05 mg/mL concentration was settled.

Extraction of sulfated polysaccharides

Using the method of Ermakova et al. with few modifications, the separation and isolation of water-soluble polysaccharides were realized. Under constant stirring (250 rpm) for 24h at 30 °C, the powdered algal biomass was treated with acetone–methanol (7:3, 500 ml), twice and chloroform (300 ml), twice. Under constant stirring (250 rpm) for 2h at 60 °C, the dried and defatted algal biomass was extracted twice with 0.1 M HCl (500 ml). Using two volumes of absolute ethanol, the supernatant obtained by centrifugation for 10 minutes at 5000 rpm was concentrated and precipitated [24]. The pellet was dissolved in distilled water after centrifugation for 10 minutes at 8000 rpm and 4 °C, dialyzed (cut-off 12–14 kDa) and lyophilized to yield the *C. barbata* sulfated polysaccharides.

Results and discussions

Macroscopic and microscopic examinations

This examination shows that *Cystoseira Barbata* is a large brown monoic alga, 1.5 – 2 m, which grows in the Black Sea on a rocky substratum. It has a thalle with various ramifications, fixed on rocks through a rhizoid as a disclike clip bolt. A lot of cilindric sticks stem from the clip bolt, on which a big number of primary and secundary branches are being developed, flat or cilindric, along which chains of air-bearing vesicles. Shaped as a conic cilinder or as a cilinder, on the top of the branches the receptacles develop. The conceptacles are situated in the receptacles, on the bottom of which various eggs are found, each with a pedicel cell and a single eggsphere. A new plant can form when the eggspheres, eliminated into sea water, merge with the anteroids. The microscopic analysis of the thalli of the brown algae taken in the study showed that *Cystoseira barbata* has a thali consisting of a single layer of cells. In *Cystoseira barbata*, the cells are elongated, arranged in longitudinal rows, they have a membrane on the outside that contains a small amount of cellulose. Inside the cell

there is a nucleus, numerous brown chromatophores with pyrenoids and numerous vacuoles.

Result for the chemical composition determination

The chemical composition of *C. barbata* seaweed is shown in products and affects stability of food materials. The moisture content of C. barbata was 14.45% and the ash content of the brown algae C. barbata was 16.3%. Comparable ash contents were reported by Frikha et al. [25] for C. barbata (14.24%) and by Marinho-Soriano et al. [26] for Sargassum vulgare (14.2%). Generally, the brown seaweed has a higher ash content than red and green algae [27]. C. barbata revealed a total protein amount of 17.9%, which was higher than other seaweeds determination [28]. C. barbata exposed a high level of dietary fibers (TDF= 59.875%), that is why algae are known as a great source of polysaccharides, which could signify a high level of soluble and insoluble dietary fibres. This percentage of dietary fiber from C. barbata was higher than the one recorded for the brown seaweeds Fucus vesiculosus (50.09%), B. bifurcata (37.42%), Undaria pinnatifida (33.58%), and H. elongata (37.14%), L. digi- S. Sellimi et al [32] [33]. The content of lipids present in this brown algae was reltively low, but Himanthalia elongata (L.), L. saccharina, Mastocarpus stellatus and Gigartina pistillata seaweeds showed significanly lower contents of lipids (0.3-0.9%) [28]. In Tabel 1 are presented all the results for this determination. The seaweeds are still an important source of health promoting PUFAs compared with plants and animals, even though they have a total content of lipids generally low.

Table 1. Chemical Composition of algae *Cystoseira barbata* from Romanian Black Sea coast

Parameters	Cystoseira barbata	Literature data values
Ash%	16.3±1.73	12.4-29.9
Moisture%	12.3±0.42	12.6-18.5
Total nitrogen%	2.765±0.34	-
Sulphates%	70.91±1.94	65.3-70.5
Lipid%	1.65±0.54	1.5-3.6
Protein%	17.9±2.11	8-17
Carbohydrate%	59.9±1.06	59.1-61.5
Total dietary fiber%	59.875±1.66	50.3-60.5
Soluble fiber%	30.3±1.33	27.2-30.5
Insoluble fiber%	28.76±1.26	24.2-32.6

HPLC analysis of phenol compounds

The phenol acids in the extracts were identified by HPLC- DAD. In Table 2 are presented the individual phenol concentrations from Cystoseira barbata, determined by HPLC – DAD and they are expressed in mg/100 g f.w. The highest quantity that

Cystoseira barbata from the Romanian Black Sea coast contains is the vanillic acid (99.5 mg/100 g f.w.), followed by benzoic acid (65.7 mg/100 g f.w.) and feluric acid (54.5 mg/100 g f.w.).

Table 2. The individual phenol concentrations determined by HPLC - DAD in brown seaweed from the Romanian Black Sea coast [8]

Type of acid	Mean Value±SD mg/100 g.f.w.	Percentage
Pyrogallol Acid	4.2±0.05	1.10
Protocatechiuc Acid	7.12±0.01	1.85
Gallic Acid	3.5±0.03	0.91
4-Amino-benzoic Acid	5.2±0.09	1.35
p-Hydroxy-benzoic Acid	26.9±0.06	6.97
Chlorogenic Acid	5.3±0.05	1.37
Caffeic Acid	21.2±0.06	5.49
Vanilic Acid	99.5±0.08	25.8
Benzoic Acid	65.7±0.06	17
Feluric Acid	54.5±0.01	14.13
Ellagic Acid	5.6±0.02	1.45
Salicylic Acid	10.5±0.03	2.72
Total	309.22	80.14

The smallest quantities were for 4-aminobenzoic acid (5.2±0.09 mg/100 g f.w.), pyrogallol acid (4.2±0.05 mg/100 g f.w.) and gallic acid (3.5±0.03 mg/100 g f.w.). From the total phenolic content identified, the phenolic acid from hydroethanolic extract of the brown algae, quantified by HPLC-DAD, was 80.14%. Salicylic acid (10.5 mg/100 b f.w.), caffeine (21.2 mg/100g f.w.) and p-hydroxybenzoic acid (26.9 mg/100 g f.w.) were another important compounds that were identified. The remainder phenol acids which were identified and quantified through HPLC-DAD, were found in smaller amounts.

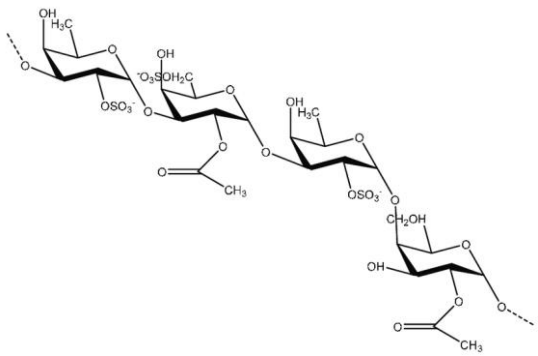


Fig. 2. The chemical structure of fucoidan

Results for the sulfate polysaccharides extraction

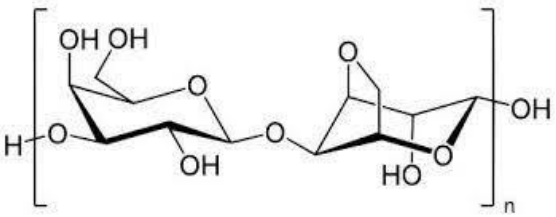
Fucoidans

Fucoidans are sulfated polysaccharides that can be determined in brown algae, see Fig.2. Even if most of them are essentialy made of sulfated α -L-fucose residues, they may also contain xylose, mannose, galactose, uronic acids and acetyl groups, sometimes in substantial amounts. In other algae or terrestrial plants such polysaccharides are absent [30]. Algal fucoidans are a virtually non-toxic bioactive polymers and they can be found in natural sources in high amounts [31]. The most interesting property is their heparin-like anticoagulant and antithrombotic activity, but many other activities, such as anti-inflammatory, antiviral, antitumor, antiadhesive, antiangiogenic, etc., are promising for new drug design [32]. Depending on the species, the harvest period and the part of the thallus, fucoidans usually constitute about 5–10% of dry seaweed biomass [33]. By extraction of algal biomass followed by oxidative decoloration and determination of this sugar with specific color reaction for 6-deoxyhexoses or by using GLC after acid hydrolysis, the fucoidan content may be evaluated by determination of L-fucose. Their biological activity is dependent on anatomical part of the algae, species, extraction procedures, growing conditions and locations and analytical methods and each of these properties is associated with a specific fucoidan [34].

Laminarans

Polysaccharides that are commonly distributed in nature are the one built up predominantly of 3-linked β -D glucopyranose residues [35]. Due to the ordered triple helical molecular conformation, high molecular weight glucans of this type are insoluble in water and therefore serve as structural components, for example, in fungal cell walls. Laminarans are known as similar glucans of lower molecular mass function as storage polysaccharides in brown algae, see Fig.3. Their content can be as high as about 35% of dry biomass in the algae and this percentage is dependent on the environmental conditions and the species. Percival and McDowell monograph reviewed the results of structural investigations of laminarans by classical chemical methods. Containing a backbone of about 20–30 (1 → 3)-linked β -D-glucopyranose residues with some single β -D-glucopyranose stubs attached to the main chain by (1 → 6)-linkages and differing slightly in the degree of branching, polysaccharides from different species were shown to be very similar. The two types that have been defined are the one with the chains terminated by D-glucose residues (G-series) and the other one with the chains that are terminated by D-mannitol residues (M-series). The biological activity can be modified or enhanced by the chemical modification of laminarans. In this way, it has been demonstrated that laminar sulfate causes several plant defense mechanisms and inhibits the proliferation of endothelial cells. Sulfated alkyl laminara-oligosaccharides have potent inhibitory effects on AIDS virus infection [36].

Fig. 3. The chemical structure of laminaran



Biodegradability, low cellular toxicity and high biocompatibility are some of the advantages of the laminarans [37]. The bioactivities that have been identified for the laminarans were anti-inflammatory, anti-apoptotic [38], antitumor, immunoregulatory, antioxidant activities and anticoagulant [39].

Alginic acid and alginates

A polysaccharide composed of two different uronic acids, glucuronic and mannuronic, are one of the principal active compounds of edible brown algae. They act like prebiotics and as potential immunomodulators, supporting the production of short chain fatty acids, because the alginates can not be digested by human enzymes [40]. The alginates have been used for the treatment of gastric reflux, stomach ulcers and heatburn and also as tablet excipients. The absorption, swelling and hemostatic properties of alginates are involved in their mode of action against such health conditions [41].

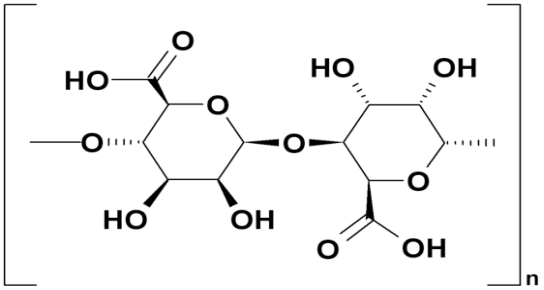


Fig. 4. The chemical structure of the alginic acid

These characteristics also support the use of alginate in wound treatment. They have been linked to many other health effects and their absorption and swelling characteristics. For example, binding of glucose and α-amylase inhibition, which reduce post-prandial glucose levels. Alginates have the property of reducing the level of cholesterol and the level of lipids, because they have the ability to absorb bile acids and lipids. Alginic acids are linear copolymers of two linked uronic acid residues (1 → 4), α-L-guluronic (G) and β-D-mannuronic (M) acids, see Fig.4. They are present as components in the intercellular matrix and cell walls in all known brown algae in the

form of salts mixed with several cations such as Na + , K + , Mg 2+ and Ca 2+ [42]. The polymer is extracted in the form of easily soluble sodium salt by treatment at high temperature with sodium carbonate solution, the algal biomass being treated beforehand with dilute acid to remove the polyvalent cations [43]. Sodium alginate can be precipitated with ethanol, insoluble calcium alginate can be obtained by adding calcium chloride and alginic acids are generally precipitated after acidification of the extract. The alginate content of algae can reach up to 40% of the dry algal biomass and depends on the part of the thallus, the harvest period and the species [44].

Conclusions

Algal biodiversity produces a vast resource of different polysaccharides. Some have been characterized by chemical and physical methods of structural analysis and have found a wide practical application, such as agar-agar and carrageenans from red algae and alginates from brown algae. Several chemical and enzymatic procedures have been developed to improve their practically valuable properties, therefore mariculture seems to be the main source of raw materials for the production of these polysaccharides in the future. This is true even for fucoidans from brown algae, which are the most intensively studied group of biologically active polysaccharides. Their biological activity depends on the composition of monosaccharides, degree of sulfation, conformation and fine structure of polysaccharides. For a high anticoagulant activity of sulfated fucans, the appropriate spatial arrangement of the sulfate groups is necessary, and also the distribution of the molecular mass has a particular importance on the biological properties of fucoidans. The results of this study indicate that Black Sea brown seaweed species *C. barbata* may be a good source of fucoidan. The data also confirmed the long known facts that brown algae cell wall PS are complex, and that their yield and chemical composition are significantly influenced by the algae species and the conditions used to extract them.

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