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BIOCHEMICAL CHARACTERIZATION AND EXPLOITATION POSSIBILITIES OF *Gongolaria Barbata* (Stackhouse) Kuntze 1891 FROM THE ROMANIAN BLACK SEA COAST

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Abstract. *Black Sea macroalgae are a marine resource with many benefits. Gongolaria barbata (Stackhouse) Kuntze 1891 (formerly known as Cystoseira barbata (Stackhouse) C. Agardh, 1820) is a brown macroalga with an essential role in biodiversity and in the marine ecosystem functioning, considered an ecologically valuable species at the Romanian Black Sea coast. Samples were collected during 2002 and 2023 years (August-September) from 6 stations of the Romanian Black Sea coast in order to characterize biochemically and highlight the presence of some biologically active compounds with therapeutic, pharmaceutical and cosmetic interest. Biochemical composition (dry matter, moisture, ash, organic matter, crude proteins, crude lipids, carbohydrates, chlorophyll a, chlorophyll b, carotenoids) and dietary fiber (CF, ADF, NDF, NDF, NDS, ADL) were evaluated in this study. The obtained results indicate some differences between the two years and these are influenced by the environmental conditions and physiological state of macroalgae. Although present on the Romanian coast, Gongolaria barbata has not been intensively studied in terms of its valorization potential, but it has recently attracted attention due to its biochemical properties that lead to some possibilities of use for economic purposes. The existence of valuable biochemical compounds in the composition of this brown macroalga qualifies it in the marine resources category of interest with biotechnological applications.*

Keywords: *Gongolaria barbata,* biochemical characterization, valorization, Romanian Black Sea coast

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INTRODUCTION

Brown algae are a diverse class of algae renowned for their color, ranging from olive green to light golden brown. This is because their chromatophores contain the golden brown xanthophyll pigment fucoxanthin; because of the large amounts of fucoxanthin and carotenoid covering the residual pigments chlorophyll *c* and *a* and other xanthophylls, it looks brownish.

The most significant marine algae, and the majority of brown algae is found in the intertidal zone (Zhang *et al.*, 2020).

Gongolaria barbata (Stackhouse) Kuntze 1891 (formerly known as *Cystroseira barbata* (Stackhouse) C. Agardh, 1820) is one of the largest algae in the Black Sea basin that belongs to the Phaeophyta (class Phaeophyceae, order Fucales, family Cystoseiraceae). It reaches a maximum length of 170 cm. The study of these seaweed associations is used for biomonitoring, to calculate indices that help determine the ecological status and the level of eutrophication of a habitat. *G. barbata* is an indicator of water purity (Manev *et al.,* 2013).

On the Romanian the Black Sea coast, this brown alga is more present in the southern part and has started to develop more and more, being an indicator of sea water quality (Marin and Spinu, 2023).

Regarding the use and valorization of this living marine resource from the Romanian coast, it has not been fully investigated, although specialized studies indicate a biochemical composition and nutritional qualities that make it suitable for use in different branches of the economy.

Brown seaweed *G. barbata* is known to possess functional nutritional properties and contain compounds with antimicrobial, antihypertensive and antioxidant properties (Abdala-Diaz *et al.,* 2006; Sellimi *et al.,* 2018). It is an edible brown alga, traditionally used as a functional food and as a source for the extraction of alginates (Trica *et al*., 2019). *G. barbata* has been scientifically proven to contain compounds that have biological activity, such as laminarin, which has antibacterial, antioxidant and healing properties (Sellimi *et al.,* 2018) fucoxanthin, which is used as an enhancer of colorants and oxidative stability of topical products, and polyphenolic protein polysaccharides (Sellimi *et al.,* 2017). Being edible algae, seaweeds are consumed as food in many coastal regions throughout the world (Mabeau and Fleurence, 1993. In many countries algae are important part of regular diet. Since algae are rich source of fiber, protein, and high levels of omega-3 fatty acids, they have excellent nutritional value (Adharini *et al.,* 2019; Burtin, 2003). Moreover, algae are rich source of many vitamins, and minerals suggesting that they are new potentiality for the food industry (Fleurence *et al.,* 2012). The significant pigments in brown algae consist of chlorophyll *a, c1, c2*, *β*-carotene, lutein, fucoxanthin, dioanthin, and violaxanthin. Also, some storage food includes laminarin, mannitol, and some oils, and the brown algae cell walls are made up of cellulose and alginic acid (Fleurence and Levine, 2016).

In the last three decades, interest has grown in seaweeds as nutraceuticals, or functional foods, which gave dietary benefits beyond their macronutrient content. In addition, seaweed has been mined for metabolites with biological activity, to produce therapeutic products (Zerrifi *et al.,* 2018). Incorporating seaweeds or their extracts into foods to improve nutritional properties is a recent practice, prompted by improved understanding of dietary sciences and the nutrient-dense nature of algae.

The incorporation of a variety of seaweeds has produced several meat products of high consumer acceptance with significantly lower levels of saturated fat and salt, no artificial additives, plus increased fibre and polyunsaturated fat content (Cofrades *et al.,* 2017). In addition, polyphenolic compounds, selenium, and vitamins A, C, and E in seaweed act as natural antioxidants and preservatives within the meat matrix. Aside from meat and grain-based foods, seaweed has been used to enhance many other products such as dairy, fish, desserts, mayonnaise, sauces and fermented products (Uchida *et al.,* 2018). Seaweeds are suitable for vegan diet. As functional foods, seaweeds offer a low-cost, alternative, sustainable source of protein without the saturated fat associated with meat.

Since the beginning of human history marine macroalgae have been viewed as amajor source of food and food products and, more recently, of bioactive compounds to be used in different areas such as pharmaceuticals and cosmetics (Faulkner, 2002).

Marine algae are among the richest sources of chemically diverse natural

products (Dias *et al.,* 2012), although their potential in drug discovery has remained largely unexplored.

MATERIAL AND METHODS

The brown algae *G. barbata* (Fig. 1) were collected from the Romanian Black Sea coast during August-September period in 2022 and 2023 years.

The algal material was collected from several stations of the Romanian Black Sea coast: Cazino-Constanța, Jupiter, Saturn, Golf Mangalia, 2 Mai, Vama Veche, at 0 - 3m depths. These sampling stations correspond to the macroalgae monitoring network established by the National Institute for Marine Research and Development "Grigore Antipa" Constanta, Romania (Fig. 1).

The global biochemical parameters analyzed are the following:

- dry weight, DW (gravimetric method, according to the Romanian Pharmacopoeia, 1993) by drying at 105°C in the Caloris EC100 oven,
- moisture content, H_2O (gravimetric method, according to the Romanian Pharmacopoeia, 1993) by drying at 105°C in the Caloris EC100 oven,
- organic substance, OS (gravimetric method, according to the Romanian Pharmacopoeia, 1993), was calculated by difference,
- ash (gravimetric method, according to the Romanian Pharmacopoeia, 1993), by calcination at 550°C in the SNOL calcination furnace,
- total nitrogen, total N (Kjeldahl method, AOAC, 1995c),
- total proteins (calculation: total nitrogen x 6.25),
- carbohydrates (Dubois method, 1956),
- total lipids (Soxhlet method).

Total nitrogen was analyzed with the DK 6 Velp Scientifica digestion unit, the UDK 149 Velp Scientifica distillation unit and the Titroline 5000 SI Analytics titrator. Carbohydrates were analyzed spectrophotometrically using a Spekord 205

UV-VIS Spectrophotometer, Analytic Jena. Total lipids were performed in the Soxhlet Behrotest extractor, with extraction in 1,2-dichloroethane solvent.

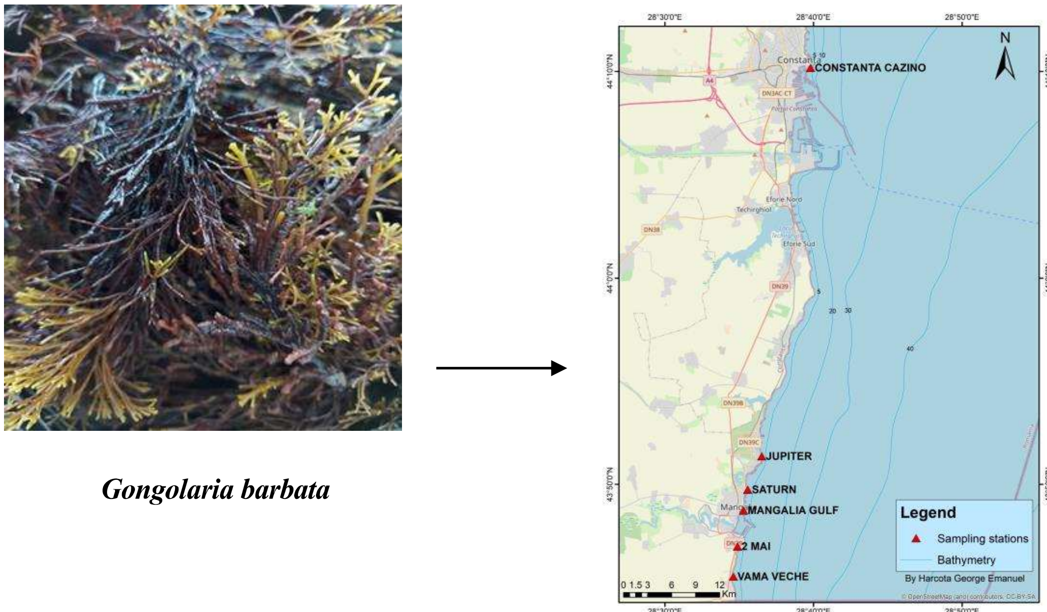


Fig. 1. *G. barbata* sampling stations along the Romanian Black Sea coast.

Chlorophyll *a*, chlorophyll *b* and carotenoid pigments were assessed according to a spectrophotometric method described by Lichtenthaler and Welburn 1983, using a Spekord 205 UV-VIS Spectrophotometer, Analytic Jena. All pigments were analyzed from fresh material (WW), extraction being carried out in 80% acetone.

The method for determination of crude fiber content, CF is based on the solubilization of non-cellulosic compounds with sulfuric acid and potassium hydroxide solutions (Weende method, AOAC, 1995a).

Determination of neutral detergent fiber, NDF is according to Van Soest, AOAC, 1995b. The method is based on the solubilization, in a neutral surfactant solution, of: a) soluble carbohydrates, including pectins, b) most proteins, c) lipids, d) soluble mineral substances, part of silicon dioxide included.

Soluble content is defined as neutral detergent soluble, NDS (according to Van Soest, AOAC, 1995b). The residue is composed by the fibrous components of plant cells: hemicellulose, cellulose, lignin, cutin, insoluble mineral substances and some cell wall proteins.

Determination of fiber content with acid detergent, ADF was accomplished according to Van Soest, AOAC, 1995b. The method is based on the acid solution solubilization of: a) soluble carbohydrates, b) proteins, c) lipids, d) hemicellulose, e) soluble mineral substances. The fibrous residue is composed of cellulose, lignin, cutin and mineral substances insoluble in an acidic medium (silica) and is defined as ADF.

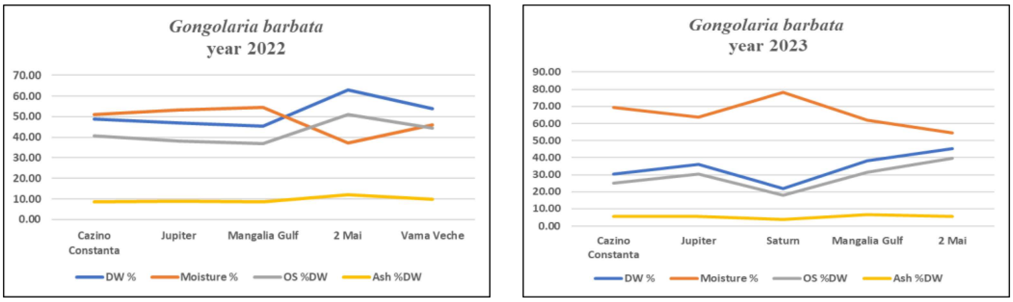
The method for determination of lignin with acid detergent, ADL (according to Van Soest, AOAC, 1995b) is based on the solubilization of cellulose with 72% sulfuric acid and provides a "crude lignin" which may also contain cutin (removed from plant cell membranes with a complex lipid structure).

The fiber content analysis was performed on dry seaweed obtained according to the protocol from the Romanian Pharmacopoeia, 1993. Fiber content analysis was performed with the COEX cold extractor, Velp Scientifica and the FIWE fiber extractor, Velp Scientifica.

RESULTS AND DISCUSSIONS

Brown algae samples of *G. barbata* harvested from the Romanian Black Sea coast were analyzed in three replicates and it was taken into account the average of the results and standard deviation.

Dry weight (DW) of macroalgae ranged in year 2022 from 45.4 ±0.35 % to 62.94±0.42 % (min. Mangalia Gulf, max. 2 Mai), Fig. 2a and in year 2023 from 21.69±0.41 % to 45.40±0.47 % (min. Saturn, max. 2 Mai), Fig. 2b.



a) **b)**

Fig. 2. Global biochemical content: a) year 2022, b) year 2023.

Dry weight (DW) represents the sum of organic substance (OS) and mineral substance (Ash), indicating the biochemical value of the analyzed material according to the purpose of its use.

The moisture (H_2O content) of algae varied in year 2022 from 37.06±0.46% to 54.55±0.38 % (min. 2 Mai, max. Mangalia Gulf), Fig. 2a and in year 2023 from 54.60±0.38 % to 78.31±0.44 % (min. 2 Mai, max. Mangalia Gulf), Fig. 2b. Water content indicates the degree of a living organism hydration and is influenced by the environmental conditions in which it developed (in this case, the marine environment) and its physiological state.

Organic matter (OS) varied in year 2022 from 37.01±0.24 %DW to 51.00±0.31 %DW (min. Mangalia Gulf, max. 2 Mai), Fig. 2a and in year 2023 from 17.84±0.39 %DW to 39.68±0.29 %DW (min. Saturn, max. 2 Mai), Fig. 2b.

Organic matter (OS) is mainly the summary of total proteins, total carbohydrates and total lipids content to which other biochemical elements are added. The OS value is given by the physiological state of the living organism, the development environment and varies seasonally. The time of harvesting algae must be chosen according to the purpose of use and the valorization of certain interest compounds.

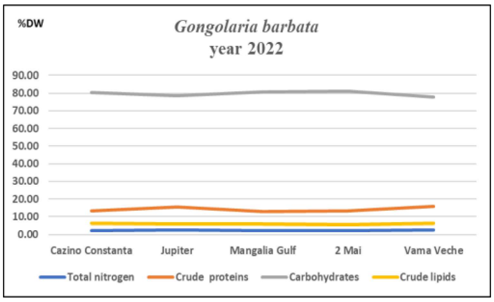
Ash content ranged in year 2022 from 8.44±0.15 %DW to 11.94±0.21 %DW (min. Cazino Constanta, max. 2 Mai), Fig. 2a and in year 2023 from 3.85±0.28 % DW to 6.53±0.17 %DW (min. Saturn, max. Mangalia Gulf), Fig. 2b.

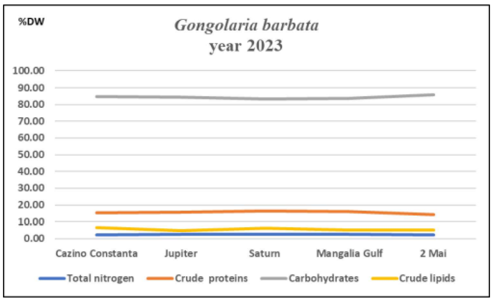
The ash content of macroalgae is generally high, especially compared to that of land plants. Ash concentration levels are known to be associated with the amount of mineral elements. Macroalgae are known as a significant source of minerals due to their ability to absorb inorganic ions from the environment, mainly iron, potassium, calcium and sodium (Misurcova *et al.,* 2011).

Total nitrogen ranged in year 2022 from 2.09±0.48 %DW to 2.54±0.54 %DW (min. Mangalia Gulf, max. Vama Veche), Fig. 3a and in year 2023 from 2.27±0.34 %DW to 2.65±0.28 %DW (min. 2 Mai, max. Saturn), Fig. 3b.

Total nitrogen content is a parameter that is analyzed in order to accurately determine the total protein content of a biological material.

Crude proteins concentration varied in year 2022 from 13.06±0.48 %DW to 15.86 ±0.54 %DW (min. Mangalia Gulf, max. Vama Veche), Fig. 3a and in year 2023 from 14.18±0.34 %DW to 16.56±0.28 %DW (min. 2 Mai, max. Saturn), Fig. 3b.





a) **b)**

Fig. 3. Biochemical constituents: a) year 2022, b) year 2023.

Macroalgae, in general, contain significant amounts of proteins (Lourenço *et al.,* 2002), which are important and essential factors determining the nutritional value of foods. In brown algae, the protein content is usually lower compared to the other categories of algae, ranging between 5 and 15% (Burtin 2003; Dawczynski *et al.,* 2007).

The representative biochemical component in macroalgae is the carbohydrates content.

Carbohydrates content varied in year 2022 from 77.94±0.41 %DW to 81.14±0.33%DW (min. Vama Veche, max. 2 Mai), Fig. 3a and in year 2023 from 83.44± 0.39 %DW (min. Saturn, max. 2 Mai), Fig. 3b.

The carbohydrate content of macroalgae is considered high, over 70%. However, the digestibility of these carbohydrates is low (Bocanegra *et al.,* 2009). The standard polysaccharides in brown algae are laminarin, cellulose, alginates, mannitol and fucoidan. Most of these polysaccharides are not digestible by the human gastrointestinal tract and can therefore be considered dietary fibers (Dawczynski *et al.,* 2007).

Crude lipids content ranged in year 2022 from 5.48±0.14 %DW (min. 2 Mai, max. Cazino Constanta), Fig. 3a and in year 2023 from 4.87±0.11 %DW to 6.50±0.16 %DW (min. Jupiter, max.Cazino Constanta), Fig. 3b.

Lipids play diverse and critical roles in metabolism. The lipid content of macroalgae is only 1–5% and thus, the contribution of this class of molecules as an energy source appears to be limited (Burtin 2003). However, Phaeophyceae such as *Gongolaria* are among the algae with higher lipid content, especially in terms of polyunsaturated fatty acids (PUFA), compared to algae belonging to other categories (Colombo *et al.,* 2006; Pereira *et al.,* 2012).

Algae pigment concentrations are highly dependent on light intensity, so the sampling period is important to highlight them. In the present case, in *G. barbata* the results of the pigment analysis are represented in Fig. 4a. The concentration values obtained are influenced by the sampling period, respectively, the beginning of autumn when the light intensity is lower.

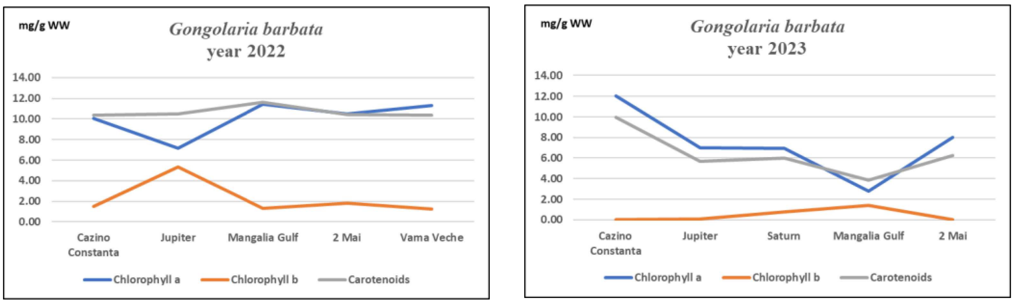
Chlorophyll *a* content ranged in year 2022 from 7.18±0.07 mg/g WW to 11.44±0.09 mg/g WW (min. Jupiter, max. Mangalia Gulf), Fig. 4a and in year 2023 from 2.82±0.04 mg/g WW to 12.00 ± 0.08 mg/g WW (min. Mangalia Gulf, max. Cazino Constanta), Fig. 4b.

Chlorophyll *b* content varied in year 2022 from 1.26±0.05 mg/g WW to 5.34±0.06 mg/g WW (min. Vama Veche, max. Jupiter), Fig. 4a and in year 2023 from 0.00 mg/g WW to 1.41±0.02 mg/g WW (min. Cazino Constanta, 2 Mai, max. Mangalia Gulf). Fig. 4b.

By comparing the results with those of other authors, it was observed that not only the location but also the extraction agent affects the chlorophyll content in brown algae (Manev and Petrova, 2021). This makes it difficult to compare chlorophyll data for the same species, even for carotenoid pigments that were analyzed using the same extractant and concentration as the chlorophylls.

Carotenoids concentration varied in year 2022 from 10.37±0.12 mg/g WW to 11.66±0.15 mg/g WW (min. Vama Veche, max. Mangalia Gulf), Fig. 4a and in year 2023 from 3.83±0.03 mg/g WW to 9.97±0.04 mg/g WW (min.Mangalia Gulf, max. Cazino Constanta), Fig. 4b.

Zucchi and Necchi (2001) reported that physical factors such as light density and quality, photoperiod and temperature can also alter pigment contents.



a) **b)**

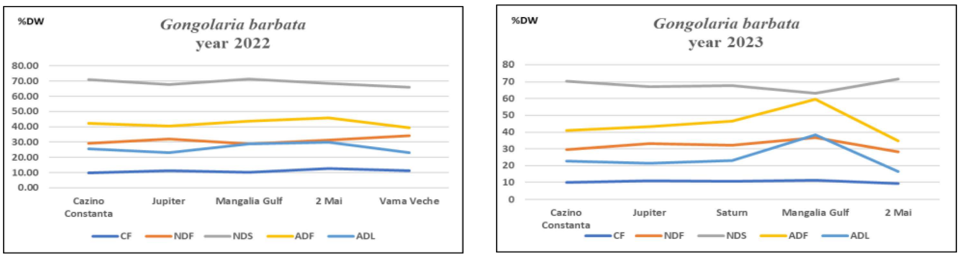
Fig. 4. Chlorophyll *a*, chlorophyll *b*, carotenoid pigments: a) year 2022, b) year 2023.

Dietary fiber content (CF, NDF, NDS, ADF, ADL) is represented in Fig.5.

Crude fiber content, CF, ranged in year 2022 from 9.7±0.24 %DW to 12.7±0.38 %DW (min. Cazino Constanta, max. 2 Mai), Fig. 5a and in year 2023 from 9.4± 0.41%DW (min. 2 Mai, max. Mangalia Gulf), Fig. 5b.

Neutral detergent fiber, NDF, ranged in year 2022 from 28.7±0.22 %DW to 34.2±0.13 %DW (min. Mangalia Gulf, max.) Fig. 5a and in year 2023 from 28.4±0.29 %DW to 36.9±0.17 %DW (min. 2 Mai, max. Mangalia Gulf) Fig. 5b.

Neutral detergent soluble fiber, NDS, varied in year 2022 from 65.80±0.11 %DW to 71.30±0.16 %DW (min. Vama Veche, max. Mangalia Gulf), Fig. 5a and in year 2023 from 63.1±0.17%DW to 71.6±0.21%DW (min. Mangalia Gulf, max. 2 Mai), Fig. 5b.



a) **b)**

Fig. 5. Dietary fiber content: CF, NDF, NDS, ADF, ADL.

Acid detergent fiber, ADF, varied in year 2022 from 39.2±0.31 %DW to 45.7±0.28 %DW (min. Vama Veche, max. 2 Mai), Fig. 5a and in year 2023 from 34.9 ±0.34%DW to 59.6±0.29 %DW (min. 2 Mai, max. Mangalia Gulf), Fig. 5b.

Lignin detergent fiber, ADL, varied in year 2022 from 23.0±0.19 %DW to 30.10±0.25 %DW (min. Vama Veche, max. 2 Mai), Fig. 5a and in year 2023 from 16.4±0.19 %DW to 38.3±0.21 %DW (min. 2 Mai, max. Mangalia Gulf), Fig. 5b.

Data in the literature generally indicate a global assessment of dietary fiber: total dietary fiber, soluble fiber, insoluble fiber (Cadar *et al.,* 2019) and not by exact components, as it was analyzed in the present study. It is often found that large compositional differences can occur between macroalgae of the same genus and species (Martínez and Rico, 2002; Dawczynski *et al.,* 2007), making comparisons difficult.

The protein content of algae varies not only between species but also between habitats, maturity and time of the year (Zucchi and Necchi, 2001; Stirk *et al.,* 2007).

Several studies have reached the same conclusion that the biochemical contents of macroalgae depend not only on season and geography (Haroon *et al.,* 2000; Stirk *et al*., 2007), but also on the nutrient content of the environment (Mohamed et al., 2012).

Table 1. Biochemical composition of *Gongolaria barbata* from Romanian Black Sea coast (Cadar *et al*., 2019).

Parameters	*Gongolaria barbata* **Black Sea coast Average September 2018-April 2019**
Moisture %	12.27 ± 0.42
Ash %	18.63 ± 1.73
Total nitrogen %	2.826 ± 0.34
Protein %	18.13 ± 2.11
Lipid %	1.63 ± 0.54
Carbohydrate %	61.95 ± 1.06
Total dietary fiber %	61.075 ± 1.66
Insoluble fiber %	30.62 ± 1.26
Soluble fiber %	30.45 ± 1.33

Biochemical parameters determined in the brown alga species *Gongolaria barbata*: indicate variations that are attributed to marine and seasonal environmental conditions, Table 1 (Cadar *et al.*, 2019).

CONCLUSIONS

The biochemical substances and complexes are of interest in terms of their biotechnological valorization from living marine resources (brown algae), with multiple economic applications in the medical field, including cosmetics and pharmaceuticals.

Chlorophyll and carotenoid pigments are sources of bioactive substances contained in autochthonous algae from the Black Sea, in this case *G. barbata*, which can be exploited in the medical, pharmaceutical and other areas of economic interest, taking into account the special properties they possess.

CF, NDF, NDS, ADF, ADL, which are part of the group called "dietary fibers", are of interest in their valorization from the macrophyte algae *G. barbata* present on the Romanian coast of the Black Sea, in the food industry and beyond.

Seaweeds from the Black Sea continue to attract attention, especially for isolation, purification, chemical characterization and experiments at the laboratory and pilot phase in order to obtain biologically active substances and new bio-nano-pharmaceutical formulas intended for innovative therapies.

Brown algae generally have a high content of organic and mineral biochemical components that confirm their high nutritional value, as well as their use in order to obtain biologically active substances with subsequent practical applications (marine biotechnology).

G. barbata by the values obtained for the biochemical parameters analyzed qualifies it as suitable for exploitation, essential being the harvesting period and the quality of the marine environment.

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