ASSESSMENT OF SOME PIGMENTS FROM ULVA RIGIDA AT THE ROMANIAN BLACK SEA COAST FOR BIOTECHNOLOGICAL PURPOSE *Daniela Mariana Rosioru*	“Cercetări Marine“ Issue no. 50 Pages 95 - 107	2020

ASSESSMENT OF SOME PIGMENTS FROM *ULVA RIGIDA* AT THE ROMANIAN BLACK SEA COAST FOR BIOTECHNOLOGICAL PURPOSE

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ABSTRACT

The aim of this paper was to evaluate the chlorophyll (*a, b*) and carotenoids pigments content of the macroalgae species with the highest frequency and abundance in the Romanian Black Sea coast, namely green alga *Ulva rigida* C. Agardh. (1823).

At the same time, it was considered to evaluate the possibilities of further exploitation of the mentioned algal species in order to capitalize the determined pigments for the biotechnological purpose on the future. The algae were harvested from 9 stations distributed along the Romanian Black Sea coast during May, 2019. The spectral analysis of the algal extracts confirmed the presence of the chlorophyll pigments, and the values of the analyzed pigment concentrations (chlorophyll *a* and *b*, carotenoids) indicate a possible recovery in the biomedical and pharmaceutical field.

The natural pigments are usually useful for food supplement to optimize body metabolic system, immunity, detoxification, minimize inflammation and hormonal system balanced.

The Black Sea algae continue to pay particular attention to isolation, purification, chemical characterization in order to obtain biologically active substances and new bio-nano-pharmaceutical formulas for innovative therapies.

Key-Words: *Ulva rigida*, Black Sea coast, pigments, biotechnological capitalization

AIMS AND BACKGROUND

The aim of this study was to analyze the pigment contents (chlorophyll *a* and *b*, carotenoids) of the green alga *Ulva rigida* C. Agardh. (1823) collected from the Romanian Black Sea coast. That gives to these green algae from the Black Sea, the great potential to be fully exploited by biotechnology and to provide health benefits.

Algal classification into brown (*Phaeophyceae*), red (*Rhodophyta*), and green algae (*Chlorophyceae*) are based on their natural pigments contents (Khan *et al*., 2010) that they are considered potential sources of nonanimal natural pigments.

Green macroalgae are included in the phylum *Chlorophyta*, and their pigmentation is identical to that of vascular plants (chlorophylls *a* and *b* and carotenoids).

Red macroalgae belong to the phylum *Rhodophyta*; they have chlorophyll *a*, phycobilins, and some carotenoids as photosynthetic pigments.

Brown macroalgae belong to the phylum *Ochrophyta*, and all of them are grouped in the class *Phaeophyceae*; their pigments are chlorophylls *a* and *c* and carotenoids (where fucoxanthin predominates, responsible for their brownish color).

Blue-green algae are included in the phylum *Cyanobacteria*; they have chlorophyll *a* (green), carotenoids (yellow), phycocyanin (blue), and, in some species, phycoerythrin (red) (both phycobilins) (Pereira, 2021).

Macro- and microalgae (including cyanobacteria) have been recognized to provide a wide diversity of metabolites including pigments for energy capture and photo-protection (Alam, 2019).

The marine algae contain three basic classes of natural pigments: chlorophylls, carotenoids and phycobiliproteins. The natural pigments are used not only as colorants, but also as antioxidant, anticancer, anti-inflammatory, anti-obesity, neuroprotective and antiangiogenic activities (Pangestuti and Kim, 2011).

The marine algae used in the industries are exploited either from natural environment or from cultivated crops. The rapid increase of industrial applications of algae and their products has escalated the aquaculture seaweed production. More than thirty-five countries across the world (cold to tropical waters) are involved in commercial algal exploitations, especially China, Japan and Korea (Bixler and Porse, 2011; FAO, 2018).

The total production of macroalgae rises every year about 5.7 % and in 2011, from the natural and farming more than eighteen million tons were produced. Of these, over 96 % was produced through aquaculture and Asian countries contributed over 99 % of the total biomass (FAO, 2014), and in 2016 production was 31 million tons (FAO, 2018).

EXPERIMENTAL

In order to determine the chlorophyll pigments (chlorophyll *a* and chlorophyll *b*) as well as the total content of carotenoid pigments (xantophylls and carotenes), samples of *U. rigida* were collected (Fig.1), during May, 2019 in the area of the Romanian Black Sea coast from the depth of 2-3 m. (Table.1, Fig. 2).

The mentioned pigments were evaluated according to a spectrophotometric method described by Lichtenthaler and Welburn (1983) and the absorption spectra in the 400-700nm range were analyzed.

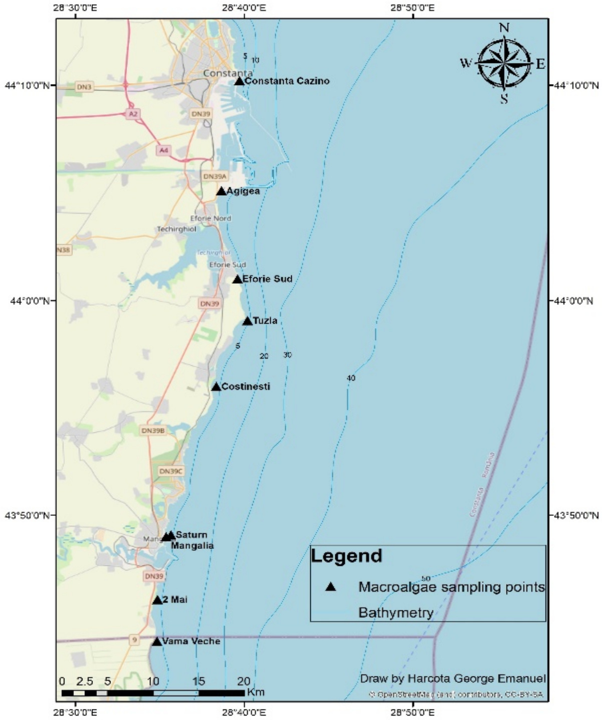

Fig. 1. *U. rigida* sampling stations on the Romanian Black Sea coast.	**Fig. 2.** *U. rigida*- thallus appearance (Marin and Timofte, 2011).

Table. 1. Geographical coordinates of *U. rigida* macroalgae sampling stations.

No	Station	Geographical coordinates	
1	Constanta Cazino	N 44°10'12.3"	E 28°39'43.2"
2	Agigea	N 44°05'58.6"	E 28°38'39.0"
3	Eforie Sud	N 44°01'42.0"	E 28°39'35.6"
4	Tuzla	N 43°59'14.4"	E 28°40'11.8"
5	Costinesti	N 43°56'18.0"	E 28°38'20.2"
6	Saturn	N 43°49'48.5"	E 28°35'38.8"
7	Mangalia	N 43°49'02.0"	E 28°35'21.7"
8	2 Mai	N 43°46'55.4"	E 28°34'51.9"
9	Vama Veche	N 43^{0}44'36.7"	E 28°34'49.6"

The collected algae were cleaned with tap water, rinsed with distilled water and dried on filter paper. Samples were stored in a freezer at -20 °C until further analysis. An amount of 100 mg fresh algae (FW) was homogenized with 10 mL acetone 80% (v/v) and maintained 24 hours at 4 °C. The extract was centrifuged to become fully transparent and further used for pigments and the absorption spectra analysis. The measurements were made with the spectrophotometer UV-VIS Specord 205, Analytik Jena, Germany.

The concentrations for chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and carotenoids (C_{x+c}) were calculated with the following equations:

Chl_a (mg/g) = [(12.21 × A663) - (2.81 × A646)] × mL acetone/mg algae

Chl_b (mg/g) = [(20.13 × A646) - (5.03 × A663)] × mL acetone/mg algae

C_{x+c} (mg/g) = [1000 A470 – 3.27Chl_a – 104 Chl_b]/229

(x+c = xantophylls and carotenes = carotenoids)

RESULTS AND DISCUSSION

U. rigida is a cosmopolitan, annual species that grows abundantly in the warm season and can, under certain conditions, dominate the hard substrate (Marin and Timofte, 2011). The quantitative analysis of the *U. rigida* samples from the last years showed the dominance of this algae along the Romanian Black Sea coast in comparison with the other opportunistic species (Marin *et al*, 2018). Evaluation of the pigments concentration: chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b) and carotenoids (Chl_{x+c}) is shown in Fig.3.

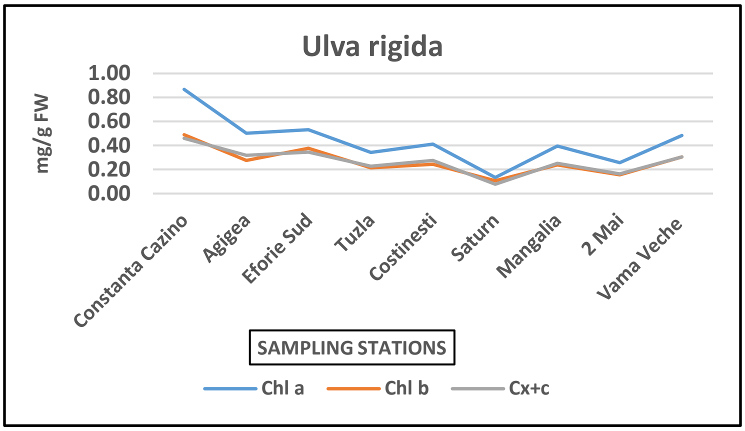

Fig. 3. The concentrations of chlorophyll (chlorophyll *a* and chlorophyll *b*) and carotenoids in *U. rigida*, from sampling stations on the Romanian Black Sea coast.

Chlorophyll *a* varied in the concentration range (0.13±0.15-0.87±0.18mg/g FW), average 0.44±0.21mg/g FW, with a minimum at Saturn and a maximum at Constanta Cazino station. Chlorophyll *b* varied in the concentration range (0.11±0.26-0.49±0.12mg/g FW), average 0.27±0.11mg/g FW, with a minimum at Saturn and a maximum at Constanta Cazino station.

The carotenoid pigments were in the concentration range (0.08±0.16-0.46±0.19mg/g FW), average 0.27±0.11mg/g FW, with a minimum at Saturn and a maximum at Constanta Cazino station. It is noticed, the same variation of the concentration of the analyzed pigments, in all the sampling stations of algae (Fig.3).

The biochemical composition of marine seaweeds is generally known to be highly influenced by geographical location and local environmental conditions (Renaud and Luong-Van, 2006; Mohamed *et al.,* 2012). A higher chlorophyll amount is usually correlated with a good health status of vegetative organs and with a better higher assimilation of nitrogen from the environment (Biris-Dorhoi *et al.*,2018).

U. rigida collected from the Marmara Sea coast of Turkey, contains excessive levels of both chlorophyll *a* (0.51mg/g FW) and chlorophyll *b* (0.43mg/g FW) and high total carotene concentration (0.97mg/g FW) (Gamze *et al.,* 2012).

In *U. rigida* collected from the marine coastal region of Chilka Lake, India, chlorophyll *a* content determined is 130mg/g FW and chlorophyll *b*, 75mg/g FW, respectively. Carotenoid was present in considerable quantities 45mg/g FW (Satpati and Pal, 2011).

Taking into account these scientific results may be considered that *U. rigida* from the Romanian Black Sea coast contains an important quantity of chlorophyll and carotenoids, being suitable for biotechnological use with different applications. There are different classes of pigments in marine algae generally occurring in bound and non-bound forms in the cells.

Chlorophylls (Fig.4) are greenish pigments which contain a porphyrin ring around which are electrons free to migrate. Because the electrons transfer freely, the porphyrin ring has the potential to gain or lose electrons easily, and thus the potential to provide energized electrons to other molecules. This is the fundamental process by which chlorophyll captures the energy of sunlight (Duka and Cullaj, 2009; İnanç, 2011).

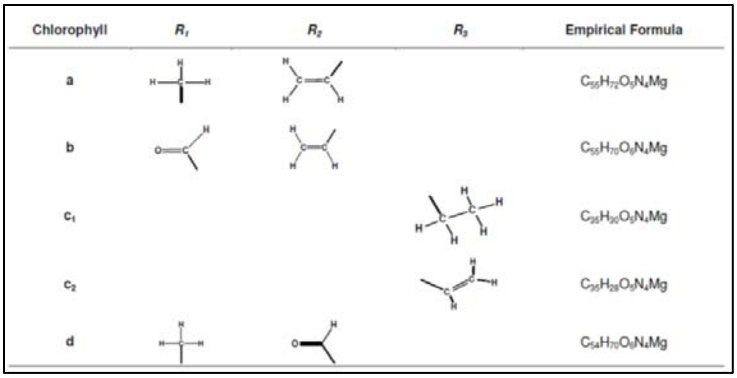

Fig. 4. Different forms of chlorophyll (Inanc, 2011).

Chlorophyll is a measure of all greenish pigments. Chlorophyll *a* is a measure of the portion of the pigment that is still active (Sava *et al.,* 2012).

Cyanobacteria typically contain chlorophyll *a* while species of green algae mostly have chlorophyll *b*. Accessory pigments in photosynthesis transfer light energy to chlorophyll *a*. One of these is chlorophyll *b*, blue-green in solution, found in higher plants and green algae with chlorophyll *a*. Chlorophyll *c* is also an accessory pigment found with chlorophyll *a* in brown algae and diatoms.

Chlorophyll *d*, together with chlorophyll *a*, there are present in some red algae (recent literature suggests that chlorophyll *d* presence in red algae is that of the associated cyanobacteria). All forms of chlorophyll are lipo-soluble (Inanc, 2011). Chlorophyll absorbs light mainly in the red (650 – 700 nm) and the blue-violet (400 – 500 nm) regions of the visible spectrum.

Green light (~550 nm) is not absorbed but reflected giving chlorophyll its characteristic color. Chlorophyll *a* possess a green-blue color, and chlorophyll *b* possess a green-yellow color. The structural difference between chlorophyll *a* and *b* molecules increases the range of sun light captured by plants during photosynthesis (Fig. 5).

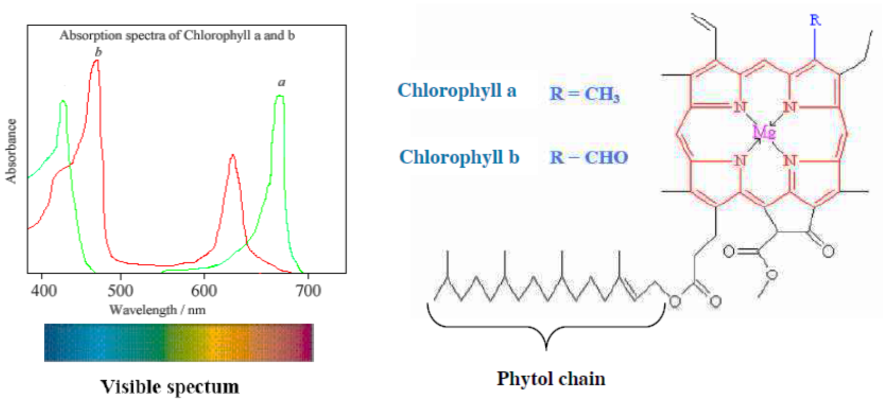

Fig. 5. Chemical structure and absorption spectrum of chlorophyll *a* and *b*. (http://www.chm.bris.ac.uk/motm/chlorophyll/chlorophyll_h.htm).

The carotenoids are natural pigments derived from five carbon isoprene units that are polymerized enzymatically to form regular highly conjugated 40-carbon structures (with up to 15 conjugated double bonds). Carotenoids are essential constituents of the photosynthetic organisms, and they are as accessory pigments for light-harvesting processes during photosynthesis, as structural stabilizers for protein assembly in photosystems, and as inhibitors of both photo and free radical oxidation provoked by excess light exposure (Karuppiah and Zhi-Yong, 2015).

The absorption spectra made in the range 400-700nm, for the chlorophyll pigments *a* and *b* confirm their presence in all *U. rigida* samples from the analyzed stations and are found in the absorption ranges 400-500nm, 650-700nm (Fig.6, Fig. 7, Fig.8, Fig.9, Fig.10, Fig.11, Fig.12, Fig.13, Fig.14).

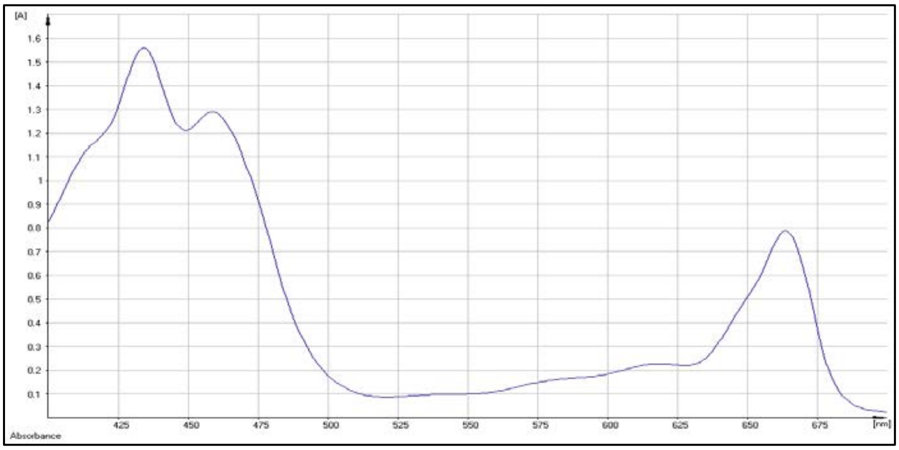

Fig. 6. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Constanța Cazino station.

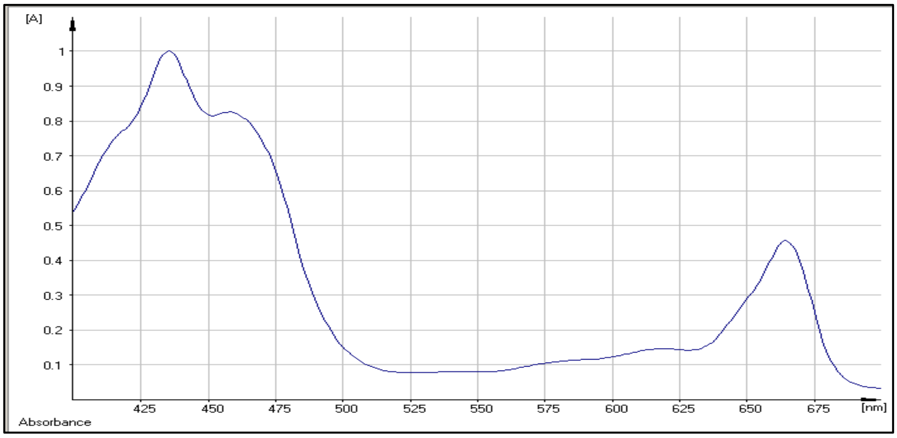

Fig. 7. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida*

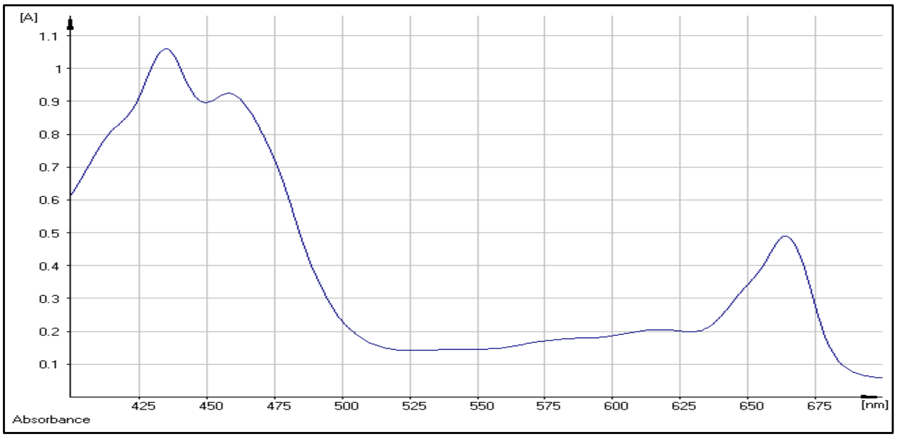

Fig. 8. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Eforie Sud station.

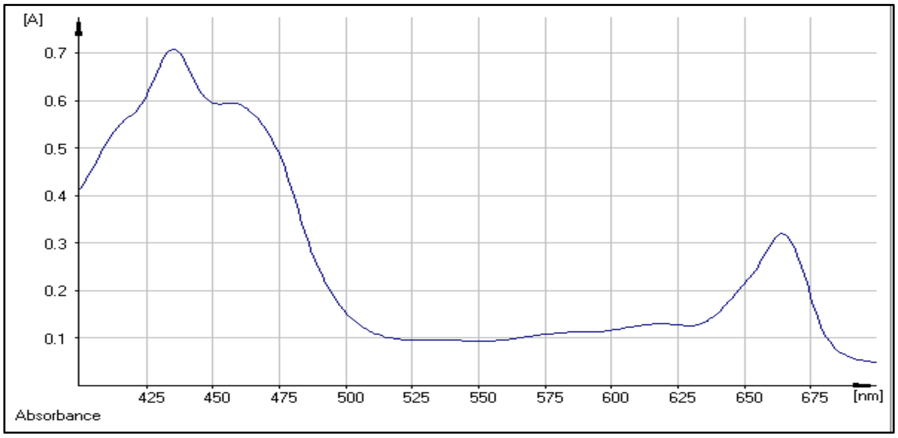

Fig. 9. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Tuzla station.

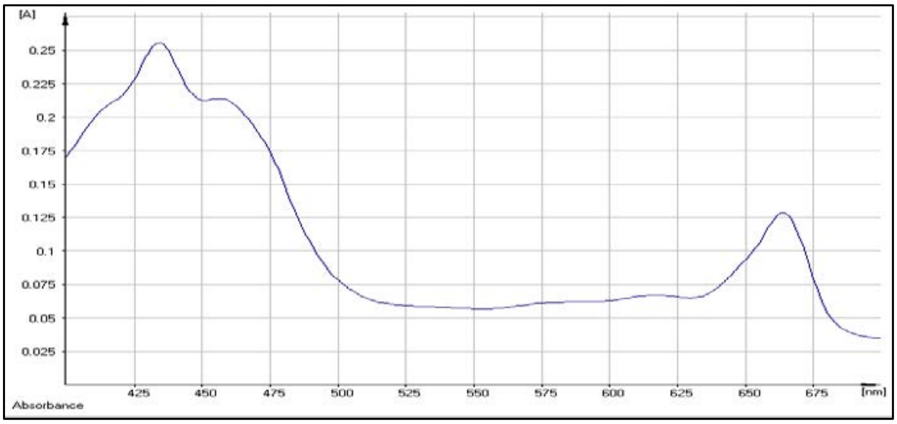

Fig. 10. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Costinesti station.

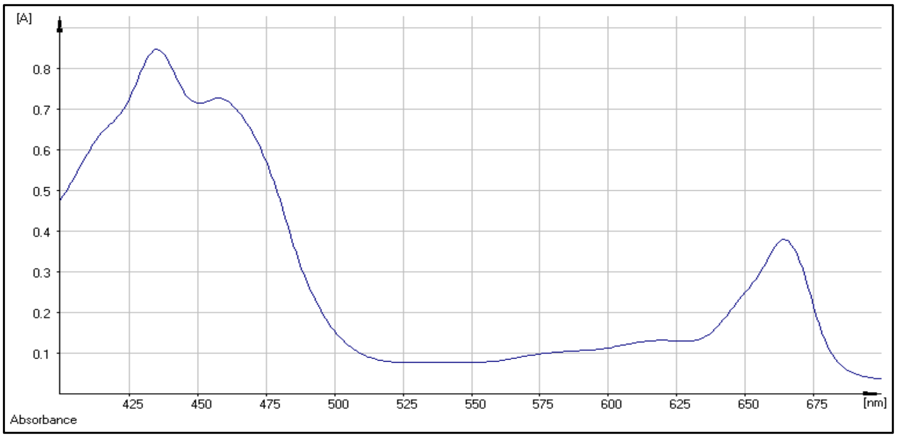

Fig. 11. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Saturn station

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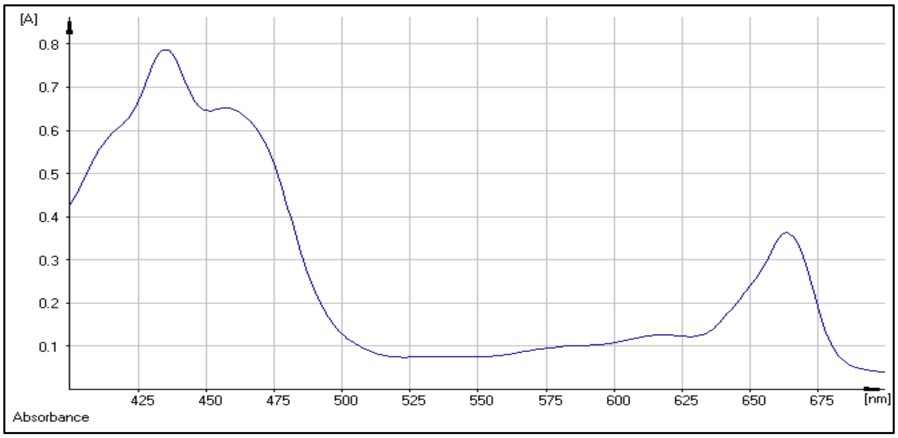

Fig. 12. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Mangalia station.

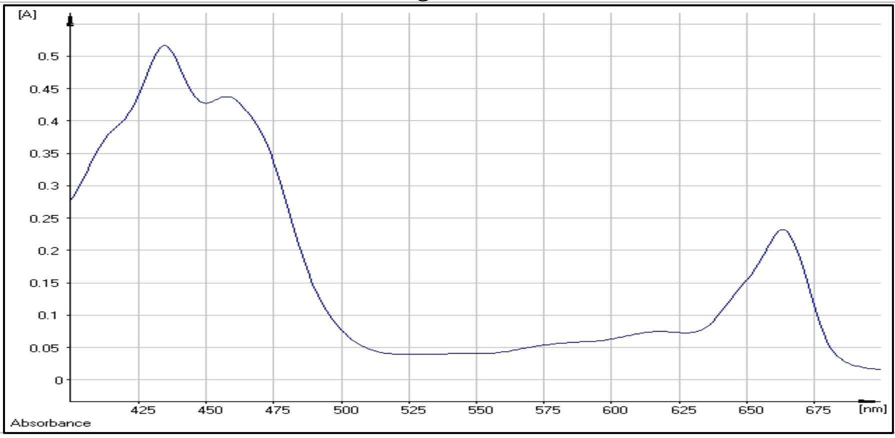

Fig. 13. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in 2 Mai station.

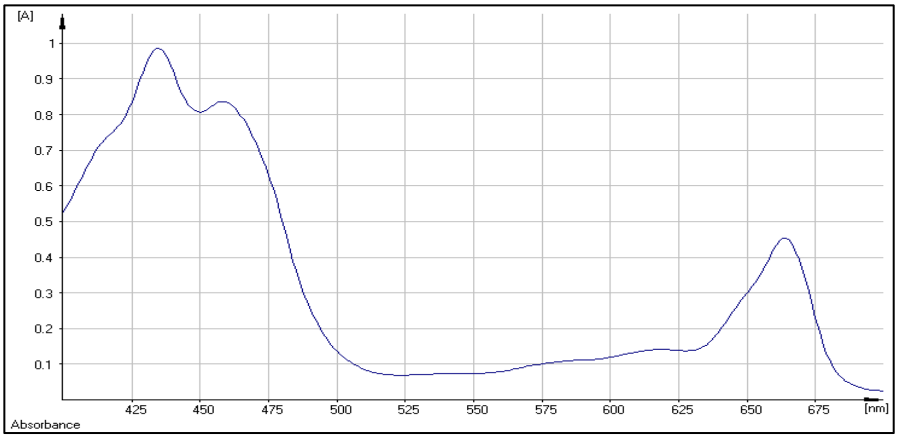

Fig. 14. Absorption spectrum of chlorophyll *a* and *b* from *U. rigida* in Vama Veche station.

The Black Sea seaweed continues to pay particular attention to isolation, purification, chemical characterization, laboratory and pilot experiments in order to obtain biologically active substances and new bio-nano-pharmaceutical formulas for innovative therapies. Chlorophyll and carotenoid pigments are such bioactive substances contained in native algae in the Black Sea and can be used in this direction taking into account the special properties they have.

Chlorophyll is a good source of antioxidant nutrients. Antioxidant nutrients such as vitamins A, C and E help to neutralize harmful molecules (free radicals) in the body that can cause damage to healthy cells. Many studies support that chlorophylls and its derivatives have antioxidant properties **(**Hoshina *et al*.,1998; Sakata *et al*., 1990; Ferruzzi *et al*., 2002; Sato *et al*., 2002) but some studies shown that chlorophyll was responsible for a pro-oxidant effect on the oxidation of oils **(**Wanasundara and Shahidi, 1998; Endo *et al*., 1985; Usuki *et al*., 1984). The pro-oxidant and antioxidant properties of chlorophylls and its derivatives depend on the presence of light.

Therapeutic properties of chlorophyll can be summarized as followings **(**Ferruzzi and Blakeslee, 2007):

- Stimulating immune system;
- Benefit against sinusitis, fluid buildup, and skin rashes;
- Ability to help combat anemia;
- Eliminating molds in the body;
- Purifying the blood and the organism, cleaning it of toxins;
- Ability to help prevent cancer and is being used in cancer therapy;
- Cleaning the intestines;
- Ability to help to rejuvenate and energize the body;
- Detoxification of the liver;
- Ability to normalize blood pressure;
- Combating bad odors, bad breath as well as body odor; due to the magnesium salts that it contains.

In human nutrition some carotenoids offer provitamin A activity and they directly provide photoprotection against UV light photooxidation in the skin. They play a key role in the prevention of several human pathological processes, such as skin UV-mediated photooxidation, inflammation, prostate and mammary carcinogenesis, ulcers due to *Helicobacter pylori* infection and age-related diseases.

The results of several investigations have confirmed that these compounds can play important roles in prevention (and even treatment) of human diseases and health conditions, e.g., cancer, cardiovascular problems, atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts and some neurological disorders.

Carotenoids are effective antioxidants, they can suppress lipid peroxidation and prevent oxidative damage. The consumption of carotenoid-rich vegetables and fruits could protect humans against cardiovascular disease, certain cancers and other degenerative diseases as evidenced from several investigations. The average concentration of carotenoids in most of the algae is only 0.1–2 %, but *Dunaliella* when grown under the right conditions of high salinity and light intensity will produce up to 14 % beta-carotene **(**Hu *et al*., 2008).

CONCLUSIONS

- ✓ The concentrations of the chlorophyll pigments and carotenoids analyzed in *U. rigida* from the Romanian Black Sea coast are quite high for the analyzed period (May, 2019).
- ✓ Spectral analysis of the extracts with chlorophyll pigments (chlorophyll *a* and chlorophyll *b*) in the absorption range 400-700nm, confirms their presence in *U. rigida* algae in high quantities in all analyzed sampling stations.
- ✓ Chlorophyll and carotenoid pigments are sources of bioactive substances contained in native algae in the Black Sea that can be exploited by biotechnology taking into account their special healing properties.

Acknowledgement. This research has been carried out with financial support from the NUCLEU Programme (INTELMAR), funded by the Ministry of Education and Research, project no. PN 19260202.
I would like to thank to my colleague George Emanuel Harcota for the map drawing.

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