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**Fund “Nauka” Project № 17018 Resume – Competition-Based Session 2017:**
“Determination of carbohydrates, proteins and phenolic compounds in Black Sea algae of *Cystoseira barbata* and *Ulva rigida*”
**Project leader:** Prof. Lubomir Evstatiev Makedonski, PhD

In the modern world, one of the main challenges is the fight against oxidative stress, respectively the fight against free radicals and the prevention of their harmful effects.

A large group of damaging particles are so-called reactive oxygen species (ROS), which are an array of metabolites produced by molecular oxygen. They have the ability to damage cellular DNA, proteins and lipids, alter biochemical compounds and destroy cell membranes. These compounds, respectively, play an important role in the development of various diseases such as malignant tumors, atherosclerosis, respiratory diseases. In addition to damaging living cells, free radicals are a major cause of food degradation through lipid oxidation or rancidity.

Oxidative degradation of lipids in raw or processed foods causes loss of nutritional value, deterioration of taste, and also contributes to carcinogenesis, atherosclerosis and acceleration of the aging process in individuals. Currently, ROS and lipid oxidation in the food industry are controlled or minimized by the addition of synthetic antioxidants. Due to serious concerns about the toxicity of synthetic antioxidants, there is a growing interest in discovering natural substances with similar properties which will be use in the food industry. Current studies have focused on characterizing the composition and antioxidant potential of uncultivated plants, including seaweed.

Seaweed is used in many countries as a food source due to its high nutritional value, vitamin and mineral content. At the same time, the low calorie capacity makes them suitable to overweight people. The intake of seaweed varied as food, in the form of spices and delicacies or food supplements. In addition to the food industry, seaweed is currently used in cosmetic, pharmaceuticals and agriculture industry. Scientific studies show the seaweed’s potential as raw materials, sources of natural antioxidants and macronutrients. These biocomponents can help and satisfy the demand from various types of industries for substitutes of synthetic antioxidants and anti-aging agents with harmful effects on the human body.

Phenolic acids and flavonoids are bioactive components with similar properties derived from marine macroalgae. Their individual subspecies diversity in marine plant species is determined by the habitat, the difference in habitat conditions and the surrounding climatic indicators. The intake of this type of compounds has a positive effect on human health and has

proven anti-inflammatory, anti-allergic and anti-viral properties, as well as the potential for prevention of certain cardiovascular diseases, hypertension, diabetes and others. Additionally natural polyphenolic compounds (including flavonoids) are also known as safe and non-toxic antioxidants. Many studies show that high dietary intake of natural phenols is strongly associated with longer life expectancy and reduce the risk of developing some chronical diseases, various types of cancer, diabetes, obesity and low blood pressure.

There is a wide biodiversity of seaweed in the waters of Bulgaria represented by 165 species of red (*Rhodophyta*), green (*Chlorophyta*) and brown (*Phaeophyta*) macroalgae but unfortunately lack in data in the scientific literature.

The aim of the project is to determine the macronutrient composition (carbohydrates, proteins, lipids, selected phenolic compounds) in Black Sea algae of the species *Cystoseira barbata* (genus Phaeophyta, brown algae) and *Ulva rigida* (genus Chlorophyta, green algae), collected from the Bulgarian waters of the Black Sea. This research will provide information for characterizing the macronutrient composition of the algae from species *Cystoseira barbata* and *Ulva rigida* grown in the Bulgarian waters of the Black Sea. It will show the potential of the selected species as a valuable source of substances with high antioxidant activity. The established chromatographic determinations of selected phenolic compounds will expand the merits of the obtained information and respectively its applicability. This will allow the inclusion of algae in various diets accompanying treatment or used in disease prevention.

The implementation of the project will lead to expanding the knowledge about the Bulgarian Black Sea flora and algology. It will raise public awareness of the benefits of Black Sea algae and their extracts for human health.

During the implementation of the project liquid chromatographic method for identification and quantification of polyphenolic acids and flavonoids in the Black Sea algae will be developed. This will allow to use this method in the study of these components in various plant matrices.

The research aims to expand scientific knowledge in the field of “The role of food in the treatment and rehabilitation of various diseases” and to increase the competence of research group members through the acquisition and implementation of the analytical methodologies.

**Achieved results:**

**Activity 1: Analysis**

Study of the literature suggested the two popular and widespread species that are the subject of analysis, namely *Cystoseira barbata* and *Ulva rigida*. *Cystoseira barbata* belongs to the family of brown algae (genus Phaeophyta), and *Ulva rigida* belongs to the species of green algae (genus Chlorophyta). They provide bioactive compounds such as vitamins, minerals,

carotenoids, proteins, essential fatty acids, dietary fiber and various functional polysaccharides.

**Activity 2: Choice of methodologies**

- Ultrasonic extraction and vortex with two types of extractants – ethanol and methanol;
- Liquid chromatographic technique with UV-detection, the separation was performed by reverse phase analytical column;
- Spectrophotometric determination of the total phenolic content (TPS) of the studied two species of Black Sea algae using the Folin – Ciocalteu reagent;
- Acid hydrolysis with concentrated sulfuric acid and 5% phenol solution to determine total carbohydrate content (TCC).

**Activity 3: Sampling**

The sampling of the selected macroalgae took place in the period April – September 2018. The collected marine plants *Cystoseira barbata* and *Ulva rigida* are wild growing near the northern Black Sea coast (Varna region). The affiliation to class and genus of algae was determined by fellow biologists from the Institute of Oceanology at the BAS.

**Activity 4: Content of individual phenolic acids and total phenolic content**

- Content of individual phenolic compounds

Both extractants extract analytes and can be used to extract polar phenolic compounds. Ethanol is many times weaker than methanol, and for 4HBC and Kv the amount of extracted phenolic compounds is insufficient for detection – below the limits of quantification. The 4HBC contents of the methanol extracts of both macroalgae are significantly higher than those of the other phenolic acids and flavonoids. Regarding the levels of all analyzed substances, it may be said that they are close in both samples, with the lowest being reported for *Ulva rigida* and for the six components for the extractant ethanol.

- Total phenolic content

The total phenolic content (TPC) of the seaweed extracts was determined spectrophotometrically using the Folin – Ciocalteu reagent.

The levels of total phenols for the two studied Black Sea species show similar results in methanol extracts. However, there is a significant difference between the two ethanol extracts – the content of total phenols in the ethanol extract of *Ulva rigida* is almost twice as high as in the ethanol extract of *Cystoseira barbata*. This is most likely due to species differences, habitat and other exogenous factors specific to the area.

From the review of the obtained data for TPC, it should be said that the detected quantities are significant and definitely complement the overall antioxidant potential of the extracts from the analyzed seaweed species.

Along with all useful components such as vitamins, minerals, fatty acids, essential elements, amino acids, polysaccharides, etc., evaluations of the antioxidant properties of natural compounds are very important due to their use in medicine, food and cosmetics. Subjected to various external influences, as well as as a result of metabolic processes, living systems generate active particles that provoke a quick and easy side effect of oxidation. The results of this type of reaction lead to harmful side effects, sometimes even death for the living cell. Such particles are free radicals and reactive oxygen species (ROS). Their increased level in living organisms damages the structure of biomolecules and changes their functions, can lead to cell dysfunction and even cell death. The cumulative effect of ROS can increase oxidative stress at systemic level and manifests itself in the form of various health problems such as cancer, age-related diseases and cardiovascular diseases. Antioxidants in the form of phenolic acids, flavonoids, carotenoids and others ensure that the concentration of these particles is within such a range that the undesired effect is minimal. For this reason, all the new data on the inhibitory ability of the relevant extracts of Black Sea algae make an additional scientific contribution by enriching the information on the antioxidant properties of marine vegetation, in particular the two species *Ulva rigida* and *Cystoseira barbata*. The results of the study of both types of algae show a close dependence on the type of the extractor. Methanol extracts at the three concentration levels showed higher inhibitory activity in both cases.

**Activity 5: Determination of total carbohydrate content**

The analysis of total carbohydrates in two species of Black Sea algae was performed by a method involving an acid hydrolysis reaction with concentrated sulfuric acid and 5% phenol and subsequent spectrophotometric determination at a wavelength of 490 nm. The content of total carbohydrates in the Black Sea algae *Cystoseira barbata* was studied by Z. MANEV and team [Z. MANEV et. al., 2013]. The established results are in the range of 2.3 – 9.6 g/ kg, which are very close and comparable to ours for *Cystoseira barbata* – 5.6 g/ kg; The data for total carbohydrates in the form of *Ulva rigida* are higher than those quoted for brown algae – 17.0 g/ kg, which is most likely due to the difference in the extractants used and the habitat of the collected samples for analysis.