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Evaluation of the biochemical composition and antioxidant activity of preparation based on pigments extracted from the remaining biomass of *Arthrospira platensis*

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Abstract

Biotechnological research is currently focused on obtaining preparations based on natural pigments due to their properties and positive impact on human and animal health. Thus, this study aimed to evaluate the biochemical composition and antioxidant activity of the preparation based on pigments obtained from the remaining biomass of *Arthrospira platensis*. The obtained results established that the preparation is characterized by a high content of β-carotene, lutein, chlorophyll pigments, and sulfated polysaccharides. Due to its composition, the preparation also possesses high antioxidant activity and the catalase and superoxide dismutase enzymes. These findings highlight the high biological value of the new preparation and the enormous potential for implementation in medicine, the animal husbandry sector, and the food and cosmetic industry.

Introduction

Currently, special attention to biotechnological research is directed toward the reuse of industrial secondary products obtained in enormous quantities following the manufacturing process.

The repurposing of microalgae *Arthrospira platensis* for deriving pigment-based formulations holds significant interest.

Owing to this, it possesses high biological activity and is commonly used as a nutritional supplement with a beneficial effect on human and animal health.[1,2] For the Republic of Moldova, the reuse of algal biomass remaining after the production of the BioR remedy is of importance. According to the specialized literature, the active part of the active part of BioR remedy preparation is composed of a combination of biologically active compounds (amino acids and oligopeptides, phospholipids, macro-, and microelements) that have been extracted from *Spirulina* biomass and purified. Clinical studies have demonstrated that the preparation exhibits antioxidant, cytoprotective, regenerating, immunomodulatory, anti-inflammatory, antiviral, hepatoprotective, and antiatherogenic activities.[3,4] However, after obtaining the preparation, the

remaining biomass can be used for extraction of carotenoids, chlorophyll pigments, and sulfated polysaccharides which are of vital importance functioning primarily as aids in the photoprotection process. Carotenoid pigments have important metabolic functions, including conversion of vitamin A, enhancement of immune response, protection against eye diseases such as cataracts and macular degeneration, and cardiovascular diseases.[5,6] The major carotenoids of *Arthrospira platensis* are *β*-carotene and lutein which also have an important role in the antioxidant defense contributing to the protection of vulnerable biomolecules in the cell from the harmful effects of environmental stress, reactive oxygen species (ROS), and other aggressive chemical species.[7-9]

Another important pigment in the structure of the microalgae *Arthrospira platensis* is chlorophyll, a powerful antioxidant with anti-inflammatory and anti-cancer properties. It is an essential additive and colorant in pharmaceutical, cosmetic, and food products.[10,11]

It was also established that the carotenoid and chlorophyll pigments obtained from *Arthrospira platensis* play an important role in zootechnics, being used to increase the productive and reproductive potential of animals. Their use contributes to the improvement of the quality of meat, eggs, and dairy products and offers several benefits for animals but also for human health.[12] According to some studies, carotenoid pigments contribute to the protection of the plasma membrane against peroxidation lipids and give membranes fluidity and flexibility that help sperm engage in the membrane fusion events associated with fertilization.[13]

Based on the above, the research aims to evaluate the biochemical composition and the antioxidant activity of the preparation based on pigments extracted from the remaining biomass of *Arthrospira platensis*.

Materials and Methods

Chemicals

The following reagents were used to determine the biochemical and antioxidant composition in the preparation based on pigments extracted from the remaining biomass of *Arthrospira platensis*: Total antioxidant activity was assessed utilizing 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), generated by reacting equal volumes of 7 mM ABTS with 2.45 mM potassium persulfate (Sigma Aldrich Reagents, Darmstadt, Germany). This mixture was incubated in darkness for 12 hours to form the ABTS radical. The reaction mixture comprised 0.3 mL of pigment-based preparation and 2.7 mL of ABTS solution.

Catalase (CAT) activity was assessed using molybdenum as a catalyst and hydrogen peroxide 3% as the substrate for the reaction (Sigma Aldrich Reagents, Darmstadt, Germany).

The determination of superoxide dismutase (SOD) activity was carried out by assessing its capacity to inhibit the reduction of nitro-blue tetrazolium by superoxide in the presence of riboflavin (Sigma Aldrich Reagents, Darmstadt, Germany).

Protein extraction was carried out using 0.1N NaOH. Subsequently, 0.1 mL of the sample was collected, followed by the addition of 0.4 mL of distilled water and 2.0 mL of a mixture containing reagent A (sodium carbonate 2% in 0.1N sodium hydroxide) and reagent B (copper sulfate 0.5% in sodium citrate 1.0%). Finally, 0.2 mL of the Folin-Ciocalteu reagent was added and vigorously stirred (Sigma Aldrich Reagents, Darmstadt, Germany).

Total carbohydrates were determined using the anthrone reagent and D-glucose as a standard (Ecochimie, Chisinau, Republic of Moldova).

Sulfated polysaccharides were assessed by mixing 50 mL of alcian blue stock solution with 150 mL of 0.1N HCl and 1.5 mL of 96% ethanol containing 0.05M $CaCl_2$. To the pectin precipitate, 2 mL alcian blue working solution and 7% CH_3COOH were added.

The pigment extraction was performed using ethanol 96% (Eladum Pharma, Cojusna, Republic of Moldova).

***Process for obtaining the remaining biomass of* Arthrospira platensis**

The BioR remedy was obtained by cultivating *Arthrospira platensis* in a nutrient medium enriched with biochemical stimulators at a temperature of 30-35ºC, under illumination of 18-24 thousand Erg/cm^2/s and pH 8.5-10.0. The algal biomass was separated through filtration, followed by the extraction, fractionation, and purification of the bioactive compounds.[14]

The biomass of *Arthrospira platensis* cyanobacteria, left over from the production of the BioR remedy, offered by the company «Ficotehfarm» LLC, Chisinau, Republic of Moldova, was used in the research.

Process for obtaining the biologically active preparation based on pigments

Initially, the remaining *Arthrospira platensis* cyanobacteria biomass after BioR remedy production was dried at a temperature of 50±5°C until constant mass. Then, the biomass was subjected to grinding in an electric grinder for 3 minutes until it was transformed into a fine powder. In the next step, the biomass was mixed with 96% ethyl alcohol in a volume of 1:10. The obtained suspension was placed in a water bath with a thermostat at a temperature of 45˚C for 30 minutes with periodic stirring. At the end of the process, the extract was separated from the biomass by centrifugation at 3500 rpm for 5 minutes. The preparation was concentrated by removing the ethyl alcohol in a rotary evaporator. Afterward, 0.1 mol/L phosphate buffer with pH 6.0 and 0.4 mmol/L EDTA were added to the preparation, and it was homogenized. The

preparation was then standardized according to the dry substance up to a concentration of 40-44 mg/mL.

Antioxidant activity

The total antioxidant activity was determined by the spectrophotometric method using the radical cation 2,2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The radical $ABTS^{+}$ is generated by the oxidation of ABTS with potassium persulfate and is reduced by the addition of hydrogen atoms.[15]

Determination of pigment content

The β-carotene and lutein content was determined spectrophotometrically (Shimadzu UV-1280, Tokyo, Japan) using 96% ethyl alcohol, at room temperature, by shaking at 200 rpm for 30 minutes, separating the extract by centrifugation and determining the absorbance of the extract at the wavelength 450 nm.[16,17]

Supplementation of the preparation with chlorophyll pigments was carried out to improve the detoxifying and purifying properties of the preparation.

The content of chlorophyll pigments was determined by the spectrophotometric method, calculating the concentration of chlorophyll a, chlorophyll b, and the sum of chlorophylls for the ethanol solvent, according to the method described by Ritchie.[18]

Determination of the biochemical composition

The protein content in the samples was determined according to the method described by Lowry *et al.*[19] The principle of the method is based on the formation of a copper complex with peptide bonds and its subsequent reduction in an alkaline medium.

The total carbohydrate content was determined spectrophotometrically at a wavelength of 620 nm using the anthrone reagent and D-glucose as standard.[20]

Determination of sulfated polysaccharide content

To determine the content of sulfated polysaccharides, a method based on the binding of anionic carboxyl groups and sulfated ester groups of algal acid polysaccharides with alcian-blue dye was used. This results in the formation of an insoluble precipitate. The optical density was measured at a wavelength of 610 nm. The difference between the absorbance of the dye in the blank sample and the absorbance of the dye remaining in the solution after precipitation was then calculated. This difference is proportional to the polysaccharide content in the sample, as described.[21]

Evaluation of the antioxidant enzymes CAT and SOD

The activity of the CAT antioxidant enzyme was determined by the method that is based on the ability of hydrogen peroxide to interact with molybdenum salts, forming a stable-colored complex. The optical density was determined at a wavelength of 410 nm, as described.[22] SOD activity was determined by the method based on the inhibition of tetrazolium-nitro blue salt reduction in the presence of TEMED and riboflavin; the optical density was determined at a wavelength of 560 nm.[23]

Statistical analysis

The statistical analysis of results was done using the statistical software kit Statistics 12. All experiments were performed in 3 replicates. Results were expressed by calculating the mean ± standard deviation and confidence interval for a mean. All differences between values were considered statistically significant for $p \leq 0.05$.

Results

The preparation based on yellowish-green pigments, with a neutral smell, was obtained from the remaining biomass of *Arthrospira platensis* with a percentage yield of 34.25±0.28% d.w.

Initially, through spectrophotometric analysis in the preparation, the total content of carotenoid pigments was determined; the results obtained are shown in Figure 1.

The content of carotenoid pigments was 14.77±3.93 mg/100g, with a β-carotene content of 14.21±0.02 mg/100g. Additionally, the extract exhibited a well-balanced content of lutein which resulted in 0.56±0.02 mg/100g.

Subsequently, the concentration of chlorophyll pigments was evaluated, and the obtained results are illustrated in Figure 1. It was found that the preparation was characterized by a content of 1.42±0.07 mg/100g of chlorophyll a and 0.16±0.004 mg/100g of chlorophyll b. The results of the biochemical composition of the preparation are presented in Table 1. According to the biochemical tests performed, it was determined that the preparation contains 30.64±0.22% d.w. proteins and 28.44±0.05% d.w. carbohydrates. Sulfated polysaccharides are an important component of the preparation. In this study yields of sulfated polysaccharides in the preparation showed a content of 44.25±0.58 mg/100g.

The result of the free radical capture efficiency of the preparation based on pigments obtained from the remaining biomass of *Arthrospira platensis* is presented in Table 1.

The reported value of 195.94±9.15% inhibition of the preparation demonstrates its strong ability to neutralize free radicals and oxidative species.

CAT and SOD are crucial antioxidant enzymes in algae, known for their role in defending against peroxidation activity and maintaining the redox state. In this study, the enzymatic activity of CAT and SOD was assessed, and the results are presented in Table 2. CAT activity in the experimental samples was 1235±30.59 mmol/min per mg of protein, while SOD activity was 618±2.6 U/mg protein.

The results demonstrate a high level of CAT enzyme's efficiency in breaking down harmful hydrogen peroxide into water and oxygen, preventing cellular damage from reactive oxygen species. The observed SOD activity indicates the enzyme's ability to convert superoxide radicals into less harmful forms, reducing oxidative damage. These findings highlight the potent antioxidant capacity of the pigment preparation obtained from the remaining biomass of *Arthrospira platensis*.

Discussion

The spectrophotometric analysis revealed the presence of various pigments in the composition of the preparation obtained from the remaining biomass of *Arthrospira platensis*.

Our data on the composition of carotenoid pigments are in line with previous scientific research, which demonstrated that extracts obtained from *Arthrospira platensis* exhibit a higher content of β-carotene compared to other carotenoids. This finding indicates that the preparation is a safe and abundant source of β-carotene, making it suitable for consumption in large quantities for various production purposes.[24]

Another essential class of pigments found in the composition of the cyanobacterium *Arthrospira platensis* is chlorophyll pigments. These pigments serve as detoxifying and purifying phytonutrients that enhance the metabolism of carbohydrates, proteins, and lipids and have important antioxidant properties.[25]

The chlorophyll a content obtained in our research is in close agreement with the findings of Romero *et al.*, who reported a content of 11.08 µg/mL. However, there is a slight difference between the two studies regarding the content of chlorophyll b which could be attributed to variations in culture conditions and quantification methods. The growth environment, such as light intensity, temperature, nutrient availability, and pH, can influence chlorophyll synthesis in *Arthrospira platensis* cyanobacteria. Additionally, different quantification methods may have been employed, and variations in analytical techniques can lead to subtle differences in reported chlorophyll content.[26]

The results regarding the biochemical composition are in concordance with other studies in which it is described that the extracts obtained from *Arthrospira platensis* register a carbohydrate content that usually varies within the limits of 10 to 27% d.w.[27,28] Additionally, the studies

presented by Abd El Baky and collaborators demonstrated that ethanol extraction from *Arthrospira platensis* gave the highest concentration of total carbohydrates.[29] The protein content cannot be compared with other studies because the previous extraction from the biomass was carried out for the production of the BioR remedy.

A significant advantage of the preparation is the presence of sulfated polysaccharides in its composition. Sulfated polysaccharides are heterogeneous complex natural polymers, featuring sugar units linked with sulfate groups with a different abundance depending on the species and extraction methods.[29]

They are of interest due to the complex group of macromolecules that give them a wide range of antioxidant, anticoagulant, antithrombotic, immunomodulatory, antiviral, and antibacterial biological properties.[30-32] They can also absorb toxic chemicals and play a major role as dietary fibers in maintaining animal and human health.[33] Similar results regarding obtaining sulfated polysaccharides from *Arthrospira platensis* have been reported in other studies, which gave high concentrations when extracted with alcohol due to the ability of alcohol to dissolve and separate the polysaccharides.[29]

The ABTS method was used in the research to determine the total antioxidant activity due to its ability to offer a comprehensive evaluation of the antioxidant power, along with its speed and stability.

Moreover, it is sensitive and can be used to measure both hydrophilic present in the preparation and lipophilic antioxidants such as carotenoid pigments.

The results of the evaluation of antioxidant activity showed the strong ability of the preparation based on pigments to neutralize free radicals and reactive oxygen species.

These results are due to the presence of pigments that significantly reduce oxidative stress that attacks and damages DNA, RNA, proteins, and lipids, leading to metabolic disturbances, tissue damage, and cell death.[34,35] It was established a positive correlation between the concentration of carotenoids in *Arthrospira platensis* biomass extracts and their radical scavenging activity, suggesting that these carotenoids are major contributors to the observed antioxidant properties.[36,37] The extracts obtained from *Arthrospira platensis* showed high antioxidant activity also in another study, in which a high content of pigments in the composition was recorded.[38] Furthermore, the antioxidative properties are determined by the presence of proteins, carbohydrates, and minerals within the composition.[39]

The high enzymatic activity is attributed to the unique biochemical composition of the extract, wherein the combination of pigments and other biochemical components present in the structure plays a key role in enhancing enzymatic activity.

Finally, we should mention that the preparation based on pigments extracted from the biomass of *Arthrospira platensis* remaining from the production of the BioR remedy may offer significant advantages from an economic and environmental point of view. It helps reduce energy consumption and eliminates the need for cultivation and the application of many high-cost stimulation factors. The method aims to obtain the biomass extract with a more efficient approach, while also saving resources and has a lower cost compared to similar products.

Conclusions

In this study, a new ecological and less expensive procedure was obtained to produce the preparation based on pigments from the remaining biomass of *Arthrospira platensis* cyanobacteria. The results demonstrate that the preparation is a good source of carotenoid pigments, chlorophylls, and sulfated polysaccharides. Due to its biochemical composition, the preparation also has high antioxidant and enzymatic activity.

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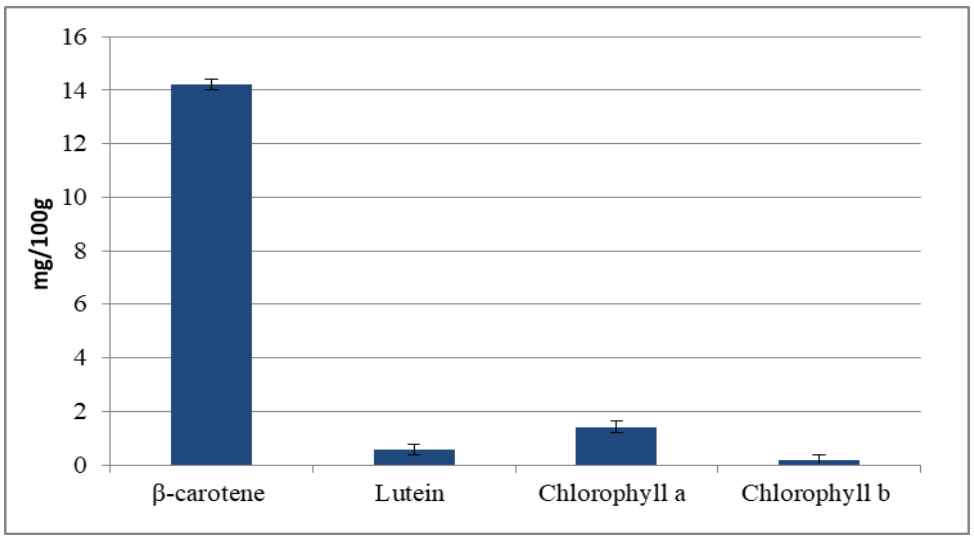

Figure 1. Composition of carotenoid and chlorophyll pigments in the preparation obtained from the remaining biomass of *Arthrospira platensis.* Values are presented as mean ±SD.

Table 1. Biochemical composition and antioxidant activity of the preparation based on pigments obtained from the remaining biomass of *Arthrospira platensis.*

Biochemical parameters	**Value (mean ± SD)**
Protein content, % dry weight	30.64±0.22
Carbohydrates content, % dry weight	28.44±0.05
Sulfated polysaccharides content, mg/100g	44.25±0.58
Antioxidant activity, % inhibition	195.94±9.15

Table 2. Enzymatic activity of the preparation based on pigments obtained from the remaining biomass of *Arthrospira platensis*.

Enzymatic activity	**Value (mean ± SD)**
CAT activity, mmol/min per mg	1235±30.59
SOD activity, U mg/protein	618±2.6