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CIANOBACTERIIE- SURSE DE COMPUȘI BIOACTIVI CU PROPRIETĂȚI ANTIOXIDANTE

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În ultimele decenii, interesul cercetătorilor față de cianobacterii și alge a crescut mult, fiind privite nu doar ca produse alimentare sau nutraceutice, ci și ca surse de substanțe bioactive naturale cu efecte benefice asupra sănătății, care ar servi drept alternativă la medicamentele de sinteză, precum și ar lărgi spectrul de remedii naturale utilizate în prevenirea și combaterea unor maladii. Această lucrare prezintă rezultatele cercetărilor mondiale recente privind identificarea compușilor bioactivi cu activitate antioxidantă produși de alge și cianobacterii, analizate metodele de determinare a activității antioxidante a diferitelor clase de compuși biochimici, precum și speciile de cianobacterii cu un continut bogat de substante cu efecte antioxidante în vederea evaluarii tulpinilor de cianobacterii de interes biotehnologic și a preparatelor obtinute pe baza acestora cu activitate antioxidantă ridicată.

***Cuvinte-cheie**: activitate antioxidantă, cianobacterii, compuși bioactivi, radicali liberi.*

CYANOBACTERIA- SOURCES OF BIOACTIVE COMPOUNDS WITH ANTIOXIDANT PROPERTIES

In recent decades, the interest of researchers towards cyanobacteria and algae has increased not only as functional foods or nutraceuticals, but also as sources of natural bioactive components with beneficial health effects, which would serve as an alternative to synthetic drugs, as well as to broaden the spectrum of natural remedies used in the prevention and combating of some diseases. This review presents the results of recent research on the world map regarding the identification of bioactive compounds with antioxidant activity produced by algae and cyanobacteria, analyzed the methods of determining the antioxidant activity of different biochemical compounds classes, as well as the species of cyanobacteria with a rich content of substances with antioxidant effects in order to evaluate the cyanobacterial strains of biotechnological interest and the preparations obtained based on them with high antioxidant activity.

***Keywords**: antioxidant activity, cyanobacteria, bioactive compounds, free radicals.*

Introduction

Oxidative stress is the main cause of many chronic diseases, including atherosclerosis, obstructive pulmonary disease, Alzheimer’s disease, cancer [1], diabetes [2], as well as of cardiovascular and prostatic diseases [3]. Antioxidant molecules are molecules with the ability to inhibit the oxidation of other molecules. In the composition of algae and cyanobacteria, there are such bioactive substances with effects similar to synthetic antioxidants, the use of which sometimes causes adverse reactions in the body. Bioactive compounds from different species of algae and cyanobacteria, including phytochemicals and pigments can prevent oxidative stress by scavenging free radicals and active oxygen, which can contribute to the prevention of cancer and other chronic diseases [4]. Algae and cyanobacteria in natural environments are often exposed to increased irradiance and high oxygen levels. As a result, these microorganisms have developed defense mechanisms through the synthesis of bioactive compounds with antioxidant action [5-7].

Cyanobacterial and algal compounds with antioxidant and other properties

Bioactive substances that act as antioxidants are compounds isolated from cyanobacteria and algae,

such as cyanovirin, polyunsaturated fatty acids (oleic acid, linolenic acid and palmitoleic acid), vitamin E, B12, β-carotene, phycocyanin, lutein and zeaxanthin, which can manifest and antimicrobial, and anti-inflammatory effects, helping to reduce or prevent disease [4, 8-11]. Green microalgae are evaluated as rich sources of chlorophylls as well as carotenoids (β-carotene and astaxanthin being quantitatively dominant). The green microalga Dunaliella salina, which is recognized as an important source of β-carotene, can produce up to 14% β-carotene from dry biomass [12, 13]. Astaxanthin is a xanthophyll carotenoid, obtained from green microalgae, which is mainly used as a food coloring. Green microalga Haematococcus sp. contains a high content of astaxanthin (about 3.4-3.5%) with an antioxidant effect and is used as a food supplement [12,14].

Pigments in cyanobacterial cells, such as chlorophyll a, carotenoids, phycobiliproteins, phenolic compounds and other substances, also exhibit antioxidant activity. Chlorophyll a and its derivatives have structural functional properties and pharmaceutical applications. Based on the structural properties of chlorophyll, which contains a heme portion similar to the hemoglobin structure, it may serve to facilitate CO2/O2 exchange. Due to this fact it is used in the treatment of ulcers, stimulation of cell growth, acceleration of tissue formation and increase of healing rate. Other chlorophyll derivatives have demonstrated antioxidant and antimutagenic properties by trapping mutagens in the gastrointestinal tract [15]. It was established that different strains of *Spirulina sp*. are rich sources of protein-pigments, such as phycobiliproteins, as well as other pigments that include chlorophyll a, B-carotene (49.6 -319.5 μg g-1), followed by xanthophyll carotenoids (myxoxanthophyll, zeaxanthin, etc.) [8]. The presence of pigments varies by species. Phycobiliproteins are important pigments in red algae and cyanobacteria soluble in water and possess high antioxidant capacity and other therapeutic properties (anti-inflammatory, anti-carcinogenic, anti-viral, and anti-neurodegenerative) [16-18]. In the species of *Spirulina sp*. phycocyanin predominates quantitatively, and allophycocyanin is in more reduced quantities.

Studies have shown that both C-phycocyanin as well as allophycocyanin from cyanobacteria acts as strong antioxidants [18-20]. Phycobiliproteins from the filamentous cyanobacteria *Oscillatoria limosa* and *Scytonema aquatilis* included phycocyanin and allophycocyanin. Phycobiliproteins *N. spongiaeforme* contain phycoerythrin, phycocyanin and allophycocyanin. As a result of some research it was shown that all these pigments have the ability to annihilate hydroxyl radicals, superoxide and alkoxyl radicals [21].

Significant antioxidant properties were also recorded for Se-phycocyanin. Thus, the study undertaken by Chen and Wong revealed that Se-phycocyanin, obtained from spirulina biomass enriched with selenium, exhibits more pronounced antioxidant effects thanks to the presence not only of phycocyanobilin, but also to the presence in the protein of selenium-containing amino acid residues [22] . Other research undertaken on the antioxidant activity of phycocyanin from the biomass of the cyanobacterium *S. platensis* demonstrated a higher antioxidant capacity in the extracts of phycobiliproteins obtained from zinc and germanium enriched biomass [19,20] .

Apart from pigments and other compounds, such as lipids from cyanobacteria also exhibit antioxidant and anti-inflammatory activity. The research carried out by E. da Costa and the co-authors allowed the identification of 162 molecular components of a lipid nature (glycolipids, glycerolipids of the betaine lipid type and phospholipids) in the lipid extracts from the cyanobacterium *Gloeothece sp*. Some of the lipid compounds identified in the mixture are known for their bioactives. The antioxidant capacity of polar lipid extracts was evaluated by neutralization tests of superoxide radicals ($O2^{\bullet -}$) and nitric oxide radical (NO•). Their anti-inflammatory capacity was also examined using the COX-2 Enzyme Activity Assay Kit [23]. In addition to pigments and lipids, other components of cyanobacterial biomass with antioxidant activity are polysaccharides. Polysaccharides from microalgae and cyanobacteria have been described in detail by Delattre et al.[24]. Algal and cyanobacterial polysaccharides represent a class of components with applications in the food industry, cosmetics, as well as biomedical applications [25, 26]. Many microalgal polysaccharides can modulate the immune system by activating macrophages, inducing different types of cytokines/chemokines. A significant antioxidant activity of polysaccharides was established in the cyanobacterium *Nostoc commune*, recording a hydroxyl radical annihilation activity of 92.71% and a reducing power of 0.445 [27]. Thus, polysaccharides possess valuable properties for be used as food additives and therapeutic agents. Sulfated polysaccharides

have also found application as antiviral and antioxidant agents, as well as potential therapeutic agents in neurodegenerative diseases [4, 28]. Some studies have shown that sulfated polysaccharides, extracted from algae and cyanobacteria, show high antiviral activity in vitro against HIV-1 and other strains of viruses. These compounds interfere with the attachment of the virus to its target cells, thereby inhibiting the fusion between viral cells and blocking the entry of the virus into the cells. Purified sulfated polysaccharide from *S. platensis* Calcium spirulan (Ca-Sp) has shown the capacities to prevent replication of several viruses and exhibit antiviral activity against human immunodeficiency virus type 1 (HIV-1), HSV-1, influenza virus, herpes simplex virus-1, cytomegalovirus human (HVMV and other viruses [29].

There are others antioxidant substances produced by cyanobacteria, such as phenol compounds, antioxidant enzymes SOD, catalase, peroxidase and some pigments which will be discussed lower.

Methods for determination of antioxidant activity. Compounds that readily release electrons or hydrogen atoms and form more stable radicals may be potential antioxidants. It is known that there are two main mechanisms by which antioxidant molecules can deactivate free radicals: hydrogen atom transfer (HAT) and single electron transfer (SET). One of them can dominate depending on the conditions and the structure of the antioxidant. For more than two decades one of the most used methods for determining the antioxidant capacity is the test called Trolox equivalent antioxidant activity (TEAC). This assay applies the radical cation ABTS•+ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as a model radical and differs from the original version of the TEAC assay by a radical initiator - potassium persulfate (PP) instead of metmyoglobin/H2O2 [30].

The method with ABTS•+ presents some advantages. The cation radical cationic ABTS•+ is soluble in both organic and aqueous media, unlike the DPPH• radical, which a soluble only in organic media. The ABTS•+ test can thus be used to estimate the antioxidant activity of both lipophilic and hydrophilic samples [31]. It can be used to determine the antioxidant capacity of numerous compounds, namely carotenoids, phenolic compounds, flavonoids, phycobiliproteins (phycocyanin.) etc. The ABTS•+ radical is stable for more than two days when stored in the dark at room temperature, compared to DPPH, which has a rather short life. The redox activity of the antioxidant compounds can also be evaluated by the ability to interact with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•). Delocalization of the radical in the aromatic rings of DPPH• ensures its stability [3]. Therefore, this method is widely used to determine the radical scavenging action of antioxidant compounds. In the DPPH assay, the reaction proceeds according to the one hydrogen atom transfer (HAT) mechanism. SET-based assays measure the reducing capacity of the antioxidant, while HAT-based assays quantify the hydrogen atom donating capacity [32].

Antioxidant activity can also be assessed by the CUPRAC assay based on the ability of the antioxidant to reduce Cu2+ complexed with 2,9-dimethyl-1,10-phenanthroline (neocuproine) or by Fe3+ reducing power (FRAP similar to CUPRAC). Trolox, the water-soluble vitamin E analogue, is used as a standard. [33].

Phenolic compounds with chemo-preventive and therapeutic values have been of scientific interest in the management of countless chronic diseases since 1990. The main contributors to the antioxidant capacity of fruit, vegetable, grain or plant samples are phenolic compounds. The Folin–Ciocalteu (F–C) test is the most commonly used to determine the total content of phenolic compounds in various plant or food samples. The Folin-Ciocalteu test is a colorimetric method based on SET reactions between F-C reagent and phenolic compounds. [34].

Another test that allows the evaluation of the antioxidant capacity is the β-carotene decolorization test based on the oxidation of linoleic acid which produces free radicals derived from hydroperoxide that attack the β-carotene chromophore, leading to the partial decolorization of the reaction emulsion, and the antioxidant activity is expressed as percent inhibition relative to control [5].

Besides of described methods, some researchers determine also the ability of cyanobacterial extracts to chelate Fe2+. The determination of unchelated Fe2+ is carried out by the reaction with ferrozine (0.25mM) [35].

There is also a known method for determining the antioxidant activity based on the nitric oxide (NO•) radical elimination test. It has been established that flavonoids can quickly neutralize NO• radicals and this test could be applied in the case of determining the antioxidant activity in samples containing not only flavonoids but also polar lipids [36].

The oxygen radical absorbance capacity (ORAC) method is also used to determine the antioxidant capacity. The (ORAC) assay is widely used to quantify the peroxyl radical scavenging capacity of pure natural and synthetic compounds, as well as to determine the antioxidant capacity of both cyanobacterial phycocyanin biopreparations and and organometallic coordinating compounds with antiproliferative action [37]. The test is based on the fluorescence method using fluorescein. The ORAC test has been one of the most commonly used tests for the determination of antioxidant activity in biomedical research and the food industry. The given method presents some disadvantages related to the need for special equipment (fluorometers), which may not be routinely available in all analytical laboratories [38, 39].

In the following, the results of research on the antioxidant potential of extracts and biopreparations obtained from various genera and species of cyanobacteria are presented

Antioxidant activity of bioactive substances from cyanobacteria.

Reactive oxygen species (ROS) are known to play an important role in a variety of cellular functions, including signal transduction and regulation of enzyme activity [40, 41]. An excessive amount of reactive oxygen species, on the other hand, can also interact with biological molecules and generate secondary products such as peroxides and aldehydes, which can cause damage to cell structure and function [42, 43]. Under normal conditions, cells have an antioxidant defense system, including enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and non-enzymatic antioxidants such as glutathione and vitamins, to combat excessive ROS [44, 45]. Cyanobacteria eliminate ROS with the help of antioxidant substances, such as carotenoids, α-tocopherol as well as with antioxidant enzymes, including peroxidases, catalases and superoxide dismutases (SOD) [13, 46, 47].

Phenolic compounds cyanobacteria also possess a high level of specific antioxidant activity. In your research Blagojević et al. evaluated the phenolic content and antioxidant activity of ethanolic extracts of ten cyanobacterial strains, including 8 nitrogen-fixing from the genera *Nostoc* (6 strains) and *Anabaena* (2 strains), grown under different nitrogen conditions, as well as 2 strains of the genus Arthrospira. The amount of detected phenolic compounds varied from 14.86 to 701.69 μg/g. HPLC-MS/MS analysis revealed the presence of the following phenolic compounds: gallic acid, chlorogenic acid, quinic acid, catechin, epicatechin, kaempferol, rutin and apiin. Only catechin, among the detected phenolic compounds, was present in all tested strains, while quinic acid was the most dominant compound in all tested *Nostoc sp*. Strains. The results also indicated that by manipulating the amount of nitrogen in the cultivation medium, an increase in the phenolic content of the cyanobacterial biomass of *Nostoc 2S7B* strain was observed, especially quinic acid (from 70.83 to 594.43 μg/g). The highest radical scavenging activity by DPPH test was recorded for the alcoholic *Nostoc LC1B* extract with an IC50 value of 0.04 ± 0.01 mg/mL, while the *Nostoc 2S3B* extract much lower [48]. Through the FRAP test, the reducing power of the alcoholic extracts was determined within the limits of 8.36 ± 0.08 - 21.01 ± 1.66 mg AAE/g, registering significantly different values, which in the case of the *Arthrospira S1* strain was approximately twice higher compared to those in nitrogen-fixing strains. This study highlights cyanobacteria of the genera *Nostoc*, *Anabae*na and *Arthrospira* as producers of antioxidants and phenolic compounds with pharmacological and health-beneficial effects, namely quinic acid and catechin, in particular [48].

In another study performed the screening and characterization of the antioxidant profile as well as the evaluation of the anticancer effect of 12 species of cyanobacteria and two species of microalgae strains isolated from soil and agricultural runoff [49]. The data demonstrated that total phenolic content was highest in *Anabaena oryzae* and *Aphanizomenon gracile* (27.39 and 26.83 mg GAE/g, respectively), followed by *Leptolyngbya fragilis* (22.96 mg GAE/g). Among the identified 14 species, the cyanobacterium *Dolichospermum flos-aquae HSSASE2 s*howed the highest antioxidant activity in terms of NO• radical scavenging activity and anti-lipid peroxidation potential (IC50 = 28.7 ± 0.1 and, respectively 11.9 ± 0.2 μg/ml) and the lowest DPPH radical scavenging activity. (467.7 μg/ml) [49].

In the study presented by Ismaiel and co-authors, the antioxidant activity of the aqueous extracts and the activity of the antioxidant enzymes SOD, catalase and peroxidase, obtained by extraction from 11 species of freshwater cyanobacteria with phosphate buffer solution (pH 7.0), were evaluated. The content of phycobiliproteins in the biomass was also determined, with the highest content of phycobiliproteins (150.68

mg/g), including phycocyanin (91.16mg/g) identified in the strain of *Spirulina platensis*. Antioxidant activities were determined by testing free radical scavenging activity, reducing power and chelating activity. The results revealed that activity and reducing power (524 and 244%, respectively, more than the control). The highest chelating activity (195%) was observed for the Nostoc linkia extract. The pigments such as chlorophyll a, carotenoids and phenolic compound content was also examined and *S. platensis* was found to have the highest chlorophyll a content (10.6 mg/g), followed by carotenoids (2.4 mg/g). Phenolic compounds content from S. platensis was 9.7 mg gallic acid equivalent /g [50].

Qi et al. mentioned that sulfated polysaccharides with low molecular mass have a potential ability to stop free radical reactions from the very beginning thus inhibiting the damage induced by the excess of free radicals [51]. The antioxidant activity may be due to the –OH and –OSO_3H groups in polysaccharide molecules that can have a significant effect on free radical scavenging. Therefore, sulfated polysaccharides in *S. platensis* exert antioxidant action by breaking the chain of free radicals by donating a hydrogen atom.

Abd El Baky et al. studied the antioxidant activity of sulfated polysaccharides from cyanobacterium *S. platensis* using 2 methods: DPPH• (BHA and BHT as standard) and ABTS•+ (Trolox standard). Polysaccharide extracts were obtained by extraction with 85% alcohol (80°C) and hot water (100°C) from biomass grown on Zarrouk medium enriched with nitrogen in concentrations: 45, 128, 293, 412 and 622 ppm. The highest scavenging activity was shown in the polysaccharide extract (300 μg) obtained by hot water extraction from *S. platensis* grown on medium containing 128 ppm nitrogen. Highest ABTS•+ radical scavenging activity. was highlighted in the case of the polysaccharide sample (400 μg) obtained by hot water extraction from the biomass of *S. platensis* grown on medium with the addition of 293 ppm nitrogen [52].

Guerreiro and co-authors evaluated the antioxidant potential of 8 distinct cyanobacterial strains, of which 6 strains were isolated from freshwater environments), a soil strain (*Nostoc (LMECYA 291*)) and a strain (*Planktothrix mougeotii* (LEGE 06224)) from wastewater. Ethanol and methanol extracts were obtained from the lyophilized biomass of in vitro cultures of these strains. The antioxidant potential was evaluated by the DPPH radical scavenging method, and the determination of total phenolic compounds and total flavonoids by the β-carotene discoloration test. The methanolic extracts from the biomass of *M. aeruginosa* (LMECYA 127), *Leptolyngbya sp*. (LMECYA 173), *Dolichospermum flos-aquae* (LMECYA 180), *Planktothrix agardhii* (LMECYA 257) and *P. mougeotii* (LEGE 06224) showed high yields of antioxidant activity by the DPPH• test (inhibition % up to 10.7%), which corresponds with 20.7 Trolox equivalent (TE) μg/mL). The (ethanolic) extracts of *Aphanizomenon gracile* (LMECYA 009) and *A. flos-aquae* (LMECYA 088) showed significantly higher values in the β-carotene bleaching test (690 .47 and 828.94 AAC, respectively) than the other tested strains. A significant content of phenolic compounds (102,3 and 123.16 mg GAE/g dry biomass), as well as flavonoids (606 and 900.60 mg QE/g dry biomass) was established also in ethanolic extracts of these strains [5].

Researchers Hossain et al. evaluated the content of total phenolic compounds (TPC), total flavonoid content (TFC), antioxidant activity, phycobiliproteins content (PBP) and other bioactive compounds in four species of cyanobacteria: *Oscillatoria sp*., *Lyngbya sp*., *Microcystis sp*. and *Spirulina sp.,* isolated from freshwater sources in Sri Lanka. In this study, *Lyngbya sp*. demonstrated the highest content of total phenols and flavonoids (5.02 ± 0.20 mg/g and 664.07 ± 19.76 mg/g, respectively) and total phycobiliproteins (127.01 mg/g). Ferric reducing antioxidant power (FRAP) was recorded the highest in *Oscillatoria sp*. (39.63 ± 7.02), while the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was reported in the extracts of Oscillatoria sp with a maximum value of 465.31 ± 25.76 and of 248.39 ± 11.97 in the case of the extract of *Lyngbya sp*.[53].

A valuable component of aqueous extracts obtained from *Spirulina sp*. with antioxidant activity are phycobiliproteins, especially phycocyanin. The antioxidant action of phycocyanin on hydroxyl and peroxyl radicals is due to the presence of conjugated bonds in phycocyanobilin as well as some amino acid residues in the polypeptide chain [55, 56]. Romey et al. 2003; and Safari et al. 2017 studied the antioxidant and antibacterial activity of C-phycocyanin (C-PC) extracted from *S. platensis* and subjected to purification using lysozyme and ammonium sulfate precipitation [57, 56]. Radical scavenging activity by DPPH•, ferric reducing antioxidant power (FRAP) and Fe2+ chelating activity were used to highlight the antioxidant ac-

tivity of C-PC. In the result of this study, it was established that the activity with DPPH• constituted 45.75% of inhibition, the activity of FRAP - 0.051 mg equivalent of Trolox /g, and 40.23% the activity of chelating Fe^{2+}[56]. The antioxidant activity of phycocyanin was also determined by the ORAC method. Benedetti et al. applied this method to investigate the ability of phycocyanin (PC) and phycocyanobilin (PCB) purified from the cyanobacterium *Aphanizomenon flos-aquae* to directly neutralize peroxyl radicals compared to well-known antioxidant molecules used as standard (Trolox, ascorbic acid, reduced glutathione). As a result, PCB was found to have an ORAC value (22.18 μmol of Trolox/μmol of compound), comparable to that of PC (20.33 μmol of Trolox/μmol of compound) demonstrating that both phycocyanobilin and phycocyanin exhibit antioxidant properties and can be used in the treatment of clinical conditions related to oxidative stress [38].

Indian researchers Ananya and Aisha Kamal evaluated the antioxidant capacity of the total polar lipid fraction obtained from the cyanobacterium *Nostoc muscurum*. Antioxidant analysis of total polar lipids was performed by the DPPH• free radical scavenging assay. The highest percentage of free radical inhibition (65%) was detected at the concentration of 500 μg/ml, which could be due to the presence of hexadecanoic acid in the extract. In the mixture of summary lipids, other bioactive compounds were also identified, especially phthalic acid and the ethyl ester of 9-octadecenoic acid, the latter predominating quantitatively and it was also proven to have antimicrobial, anti-inflammatory, anticarcinogenic, hypocholesterolemic, antiandrogenic activities etc. [57].

Thus, cyanobacteria are important sources of bioactive substances with an antioxidant effect: phycobiliproteins, pigments (carotenoids, chlorophylls, xanthophylls), sulfated polysaccharides, polar lipids, phenolic compounds (including quinic acid and catechin), flavonoids, antioxidant enzymes (SOD, catalase, peroxidase) et al., many of which are structurally bioactive metabolites that may have cytotoxic, antiviral, anticancer, antimitotic, antimicrobial, specific enzyme inhibitory, and immunosuppressive activities.

Conclusions

Cyanobacteria are producers of a wide range of bioactive substances including substances with an antioxidant effect, having a high potential for biotechnological applications. Such natural bioactive substances can find applications in the pharmaceutical, cosmetic, medicine, food industry, as an alternative to synthetic chemical compounds. Oxidative stress is the cause of many diseases, including neurodegenerative diseases, cancer, cardiovascular diseases, atherosclerosis, obstructive pulmonary diseases, etc. In order to overcome all the unwanted effects following the installation of oxidative stress, the application of natural products is welcome due to the presence in their composition of biologically active compounds with antioxidant properties, including phycobiliproteins, phenolic compounds, pigments, sulfated polysaccharides, etc. which are certainly preferable to synthetic analogues. The results obtained by some researchers highlighted the fact that cyanobacteria from the genera *Nostoc*, *Anabaena*, *Oscilatoria*, *Lyngbya*, *Phormidium*, *Dolichospermum, Leptolyngbya*, *Planktothrix Aphanizomenon*, *Arthrospira* etc. can be used as producers of antioxidants: phycobiliproteins, pigments (carotenoids, chlorophylls, xanthophylls), sulfated polysaccharides, polar lipids, phenolic compounds, including quinic acid and catechin, flavonoids, antioxidant enzymes (SOD, catalase, peroxidase), as well as other secondary metabolites with therapeutic and beneficial health effects. For the comparative analysis of the antioxidant activity, it is necessary to use the same appropriate antioxidant standard (Trolox, ascorbic acid, BHA, BHT, gallic acid, quercitin) and it is necessary to express the antioxidant activity in the same measurement units. The standard is selected depending on the biochemical composition of the analyzed samples. Many of these properties are also due to the antioxidant effects.

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