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БИОЛОГИЧЕСКИЕ НАУКИ

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SCREENING OF BIOCHEMICAL COMPOSITION AND INTEGRAL ANTIRADICAL ACTIVITY OF BEGONIACEAE REPRESENTATIVES¹

Objective of the study is to evaluate the integral antiradical activity (hereinafter referred to as IAA) and conduct biochemical screening of the content of biologically active compounds in representatives of Begoniaceae.

Materials and methods. Standard methods of biochemistry and electrochemistry used.

Results. IAA was evaluated and biochemical screening of the content of biologically active compounds (flavonols, anthocyans, catechins, ascorbic acid, hydrophilic pectin and protopectin) of eight representatives of the Begoniaceae family was performed. The prospects of using such an indicator as IAA for screening plant materials in order to expand the range of medicinal plants is shown, which indicates the need for research in the field of antiradical properties and biochemical composition of medicinal plants, herbal preparations and biologically active additives of plant origin.

Conclusion. IAA and the obtained data on the content of biologically active compounds are a comprehensive indicator of the quality of plant materials and can be successfully used to standardize it. The introduction of a test for IAA in the future may allow to: quickly and accurately assess the quality of the used plant materials of the Begoniaceae family; make multicomponent preparations with a given antioxidant activity; expand the indications for the use of drugs and determine the most selective solvent for the extraction of active substances from plant material.

Keywords: Begoniaceae, secondary metabolic substances, antiradical activity, coulometric titration, electrochemistry.

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СКРИНИНГ БИОХИМИЧЕСКОГО СОСТАВА И ИНТЕГРАЛЬНОЙ АНТИРАДИКАЛЬНОЙ АКТИВНОСТИ ПРЕДСТАВИТЕЛЕЙ *ВЕGONIACEAE*

Цель работы: дать оценку интегральной антирадикальной активности (далее – ИАА) и провести биохимический скрининг содержания биологически активных соединений у представителей Begoniaceae.

Материалы и методы. Стандартные методы биохимии и электрохимии.

Результаты. Дана оценка ИАА и проведен биохимический скрининг содержания биологически активных соединений (флавонолов, антоцианов, катехинов, аскорбиновой кислоты, гидрофильного

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пектина и протопектина) восьми представителей семейства Begoniaceae. Показана перспективность использования такого показателя как ИАА для скрининга растительного сырья с целью расширения номенклатуры лекарственных растений, что свидетельствует о необходимости исследований в области антирадикальных свойств и биохимического состава лекарственных растений, фитопрепаратов и биологически активных добавок растительного происхождения.

Заключение. ИАА и полученные данные по содержанию биологически активных соединений являются комплексным показателем качества растительного сырья и могут быть успешно использованы для его стандартизации. Введение теста на ИАА в дальнейшем может позволить: быстро и достаточно точно проводить оценку качества используемого растительного сырья семейства Веgoniaceae; составлять многокомпонентные препараты с заданной антиоксидантной активностью; расширить показания к применению лекарственных препаратов и определять наиболее селективный растворитель для извлечения действующих веществ из растительного материала.

Ключевые слова: вещества вторичного обмена, антирадикальная активность, кулонометрическое титрование, электрохимия

What this paper adds

The article presents the results of the assessment of IAA and biochemical screening of the content of biologically active compounds in representatives of Begoniaceae.

Научная новизна статьи

В статье представлены результаты оценки ИАА и биохимического скрининга содержания биологически активных соединений у представителей *Begoniaceae*.

Introduction. In the last decade, free radicals and their role in the development of diseases have become the subject of many studies. So, the activation of lipid peroxidation processes in the tissues of the body leads to the development of free radical-related pathologies, such as atherosclerosis, hypertension, coronary heart disease, oncology, cataract, etc. To manage these conditions, therapeutic and prophylactic agents are recommended, and nowadays, low-toxic herbal drugs containing a large set of bioantioxidants: vitamins, polyphenols, flavonoids, catechins and tannins, which have a mild effect on the body and relatively low toxicity, are increasingly used [1-9].

An analysis of the trend in the development of new drugs shows that over the last decade there has been an increased interest in herbal drug substances throughout the world. This dynamics is characteristic not only for countries that traditionally use medicinal herbs (India, China, Vietnam), but also for states with a highly developed chemical and pharmaceutical industry (USA, Germany), which have great potential for work in the field of synthesis of a wide range of dosage forms.

Currently, biologically active substances used in the pharmaceutical industry are isolated from plants, often belonging to rare species. In this regard, there is an active search for new sources of biologically active compounds of plant origin. An important place among them is occupied by taxons of tropical plants, in particular, the *Begoniaceae* family.

The *Begoniaceae* family has over 800 species. Representatives of the family are characterized by the presence of a wide range of secondary metabolic products – alkaloids, isoprenoids; phenolic compounds – the practical use of plants of this family in the pharmaceutical industry as the basis of drugs is largely determined [10-11].

Materials and methods. As the objects of study, we used leaves of plants of the *Begonia-ceae* family from the collection of the Central Botanical Garden of the National Academy of Sciences of Belarus, collected in the blooming period.

Electrochemical assessment of IAA [12-14]. The extraction of plant materials was carried out in accordance with the State Pharmacopoeia of the Republic of Belarus. In a 100 ml volumetric flask, 10 g of extractable plant material, ground to a particle size of 3 mm, was placed and diluted to volume with 70% ethanol. Then allowed to stand for 3 days at a temperature not exceeding 10°C. The resulting extract was filtered.

IAA was determined using coulometric titration with internal generation of bromine compounds on a P-5827M potentiostat (Ukraine). A platinum plate with an area of 1 cm^2 was used as a working electrode. The auxiliary electrode consisted of a platinum spiral. The cathode chamber, in which the auxiliary electrode was placed, was separated from the anolyte by a semipermeable partition. Coulometric titration was carried out in the galvanostatic mode (1 = 5 mA). The endpoint was set amperometrically with two polarized needle-shaped platinum electrodes (AE = 300 mV). Bromine was generated from an aqueous solution of 0.2 mol/L potassium bromide in the presence of 0.1 mol/L sulfuric acid with a current efficiency of 100%.

20 ml of background electrolyte was introduced into a cell with a capacity of 50 ml, the electrodes were lowered, and the generating circuit was switched on. Upon reaching a certain value of the indicator current, an aliquot of the test solution was introduced into the cell and at the same time the stopwatch was turned on. The titration endpoint was fixed after the initial indicator current was established, the stopwatch and the generating circuit were turned off.

The amount of electricity in coulombs spent on titration was calculated by the equation:

Q = (100 It)/V,

where

Q is the amount of electricity, C; I is the current rate, A; t is the time to reach the titration endpoint, s; V is the aliquot volume, ml. IAA units: kC/100 ml. Assay of ascorbic acid [15] 5 g of plant

Assay of ascorbic acid [15]. 5 g of plant material was ground in the presence of 2% metaphosphoric acid and diluted to 100 cm³ with the same acid. The extract was centrifuged. Three 10 cm³ samples were taken into conical flasks and 1 cm³ of a 0.025% solution of 2,6dichlorophenolindophenol was added to them. After 35 s, the optical density was measured on an Agilent 8453 UV-visible spectrophotometer (USA) at a wavelength of 530 nm in a cuvette with a working length of 10 mm vs. 2% metaphosphoric acid. In parallel, spectrophotometry of 10 cm³ of 2% metaphosphoric acid was performed with 1 cm³ of a 0.025% solution of 2,6dichlorophenolindophenol (control). To calculate the content of ascorbic acid, a calibration graph was built, and then calculated by the equation:

x = a - V/m,

where

x is the amount of ascorbic acid, mg/100 g;

a is the content of ascorbic acid, μ g/cm³ of the extract, found according to the calibration graph;

m is the mass of the sample, g;

V is the extract volume, cm³.

Assay of flavonols [15]. 2 cm³ of a 2% solution of aluminum chloride and 6 cm³ of a 5% solution of sodium acetate were added to 2 cm³ of the initial alcohol solution. 2 cm³ of distilled water were added to the control sample, instead of 2 cm³ of a 2% solution of aluminum chloride. After 2.5 hours, spectrophotometry was performed at 440 nm with a working cuvette length of 10 mm. The content of the sum of flavonols (mg/100 g in terms of rutin) was found by the equation: x = k-(D-D1)-V-p-100/m,

where

g;

x is the content of the sum of flavonols, mg/100

k is the conversion factor according to the calibration curve, built according to the rutin (0.655);

D is the optical density of the test solution;

 D_1 is the optical density of the control solution;

V is the volume of alcohol extract, cm³;

p is the degree of dilution;

m is the weight of a plant material sample, g.

Assay of anthocyans [15]. 2 g of ground plant material was poured with a mixture of 20 cm³ of ethanol with 1% content of hydrochloric acid and kept for 24 hours at 4°C. Then anthocyans were triturated and removed on a Buchner funnel. Then spectrophotometry was performed at 529 nm with a working cuvette length of 1 cm. The amount of anthocyans was found from the calibration graph. The content of anthocyans (mg/100 g) was calculated by the equation:

where

x is the content of anthocyans, mg/100 g;

k is the coefficient calculated by the calibration curve;

x = kDV-100/m,

D is the optical density of the solution;

V is the extract volume, cm³;

m is the weight of the sample, g.

Assay of catechins [15]. After removal of the hydrophilic phenolic compounds, the catechins fraction was isolated. For this, the filter residue was washed with 15 cm³ of 50% methyl alcohol, and then with 10 cm³ of 70% methyl alcohol. 1 cm³ was taken from the obtained methanol eluate, 5 cm³ of the vanillin reagent was added, and after 15 min spectrophotometry was performed in a 3 mm thick cuvette at 530 nm. A calibration curve was built using the d-catechin. The catechins content was calculated by the equation:

 $x = k - DV_2 - V_3 - 100/(mV_1),$

where

x is the amount of free catechins, mg/100 g;

D is the optical density of the test solution;

k is the conversion factor calculated by the calibration curve;

 V_3 is the volume of methanol eluate, cm³;

 V_2 is the volume of the initial extract, cm³;

 V_1 is the volume of the extract applied to the polyamide, cm³;

m is the weight of the sample, g.

Assay of hydrophilic pectin and protopectin [15]. Extraction of hydrophilic pectin. The ground sample of plant material with a weight of 10 g was poured with hot ethanol (based on the final alcohol concentration of 80-82°) and heated in a boiling water bath with a reflux column for 30 min to extract sugars, then filtered through a paper filter into a volumetric flask. The alcohol treatment of the same sample was repeated 3 times to completely remove the sugars. The filter along with the residue was dried at 50°C until ethyl alcohol was removed. Then, the residue together with the filter was placed in a flask and 50 cm³ of water heated to 45°C was added, and at this temperature hydrophilic pectin was extracted in a water bath for 1 h. The liquid was filtered into a 100 cm³ volumetric flask, washed with distilled water and, after cooling, diluted to volume.

Extraction of protopectin. The residue after extraction with water was transferred to an extraction flask, 50 cm³ of 0.3 N hydrochloric acid was poured and heated for 30 min in a boiling water bath with a reflux column. It was filtered into a 200 cm³ volumetric flask and washed 3 times with hot distilled water. Then the filter together with the residium was returned to the same extraction flask, 50 cm³ of a 1% solution of ammonium citrate was poured, and placed in a boiling water bath for 30 min. It was filtered into a volumetric flask, where the filtrate of the hydrochloric acid extract was located, washed with hot water and, after cooling, diluted to volume with water.

Conducting a demethoxylation reaction. 10 cm³ of 0.05 N potassium hydroxide solution and, after 30 minutes, 10 cm³ of a solution of 0.05 N hydrochloric acid were added to 10 cm³ of extract. 0.5 cm³ of hydrophilic pectin extract (or protopectin) was taken in 3 test tubes, cooled and 3 cm³ of a solution of borate in sulfuric acid ($p = 1.84 \text{ g/cm}^3$), cooled to a temperature of 4°C, were poured into each tube. Then they were heated for 6 min in a boiling water bath, after which they were cooled again.

Conducting a reaction with carbazole. In each 2 tubes (out of three) with pectin extract, 0.1 cm³ of a 0.2% carbazole solution was added and heated in a water bath for 10 minutes. After cooling, spectrophotometry was performed at 535 nm with a working cuvette length of 5 mm. The third test tube, in which a solution of borate

in sulfuric acid was mixed with water (3:0.5), was used as a control sample. Using a calibration curve, built according to galacturonic acid, its content (μ g) was found from the optical density of the sample. The content of hydrophilic pectin or protopectin (%) was determined by the equation:

$$x = aV_2 - V - 10^2 / (m - V_3 - V_1 - 10^6),$$

where

x is the content of hydrophilic pectin (or protopectin),%;

a is the content of galacturonic acid in the sample, found from the calibration curve, μg ; m is the weight of the sample, g;

V is the volume of the extract obtained from the sample, cm³;

 V_I is the volume taken for dilution, cm³;

 V_2 is the volume obtained after dilution, cm³;

 V_3 is the volume of the sample taken for the reaction with carbazole, cm³;

 10^2 is the conversion factor into percent;

 10^6 is the conversion factor into grams.

Statistical processing of experimental data [16]. All analyses were performed in quadruplicate, the results were processed using the "Statistica" software, the data were considered reliable at P<0.05. The discrepancy between the studied data in the sampling and the entire assembly was calculated using statistical error for the average. The range in which the studied values for the entire assembly were found with a given probability was calculated using the confidence interval for the average. Comparison of the samplings was performed according to the average value of the dispersions. When solving such problems, the probability of the zero hypothesis was calculated.

Results and discussion. For physiologically active compounds of various classes isolated from natural objects, from the point of view of the manifestation of biological activity, the most commonly used characteristic is IAA. The study of this parameter is especially relevant for galenical preparations and dietary supplements used for the treatment and prevention of pathological conditions of the human body. The most widely known preparations of this property include plant extracts.

Model systems in which electrochemical processes accompanied by redox reactions and the generation of free radicals are the IAA criterion are now widely used. One of the research directions is the study of IAA of herbal preparations in a model system based on the electrochemical generation of active bromine compounds.

As a result of electrochemical screening, IAA was revealed for eight taxons of the *Begonia*-

ceae family, namely *Begonia rex* Put-zeys. cv. Merry Chrystmas, *Begonia diadem a* Linden cv. Kupfer Koenigin, *Begonia heracleifolia* Cham, et Schlecht, *Begonia lucema* (Wettst.) hort., *Begonia bowery* Ziesenh., *Begonia* Tiger, *Begonia masoniana* Irmsch. and *Begonia x erytrophylia* Neum.

The sodium salt of poly-(para-dioxi-paraphenylene) thiosulfuric acid (trade name – Mitophen) was used as the IAA standard. Mitophen is a long-acting antihypoxanthantioxidant. Its structural analogues are coenzyme Q, cytochrome C, coenzyme Q_{10} , solcoseryl, biophen, oliphen and hypoxene.

Extract from the leaves of Begonia bowery Ziesenh. showed the greatest IAA - 38.6% of the IAA standard - the sodium salt of poly-(para-dioxi-para-phenylene) thiosulfuric acid. A slight decrease in antioxidant activity has been reported for Begonia rex Putzeys. cv. Merry Chrystmas, 33.8% from mitophen activity. For Begonia masoniana Irmsch. IAA of 29.8% is registered. Such taxons as: Begonia Tiger, Begonia lucema (Wettst.) hort, and Begonia diadema Linden cv. Kupfer Koenigin showed a relatively similar level of 21.9% and 19.9% of mitophen IAA. Begonia heracleifolia Cham. et Schlecht. и Begonia x ervtrophylla Neum. showed the lowest level of IAA - 17.4% and 13.5%, respectively.

The electrochemical oxidation of bromide anions on a platinum electrode in acidic media leads to the formation of Br_3^- , Br_2 , as well as short-lived bromine radicals. The bromine compounds formed during electrooxidation are capable of entering into radical, redox, electrophilic substitutions and additions via multiple reaction bonds, which made it possible to cover a wide range of physiologically active compounds of the studied taxons. On the basis of this it can be stated that the level of IAA is a complex indicator characterizing the total content of active substances of various classes in extracts of plants of the *Begoniaceae* family.

The next stage of the study was a biochemical screening of the content of biologically active compounds in taxons of the *Begoniaceae* family (Table).

In the course of analytical and biochemical studies, the content of the total amount was established for: anthocyans, catechins, flavonols, hydrophilic pectin, protopectin, as well as ascorbic acid. The choice of these groups of biologically active compounds as objects of biochemical research is due to their significant contribution to the formation of the therapeutic properties of plant materials.

The results of the analysis showed significant heterogeneity in the content of phenolic compounds among representatives of the *Begoniaceae family*. Anthocyans are not the main active ingredients of plant materials, but generally participate in the general pharmacological action of the studied taxons. Their structure is based on the flavilium cation, in which oxygen in the pyran ring has a free valence.

Taxon	Anthocyans	Catechins	Flavonols	Ascorbic acid	Hydrophi-lic pectin	Proto- pectin
<i>Begonia rex</i> Putzeys. cv. Merry Chrystmas	3654±136	788.6±35	2963±110	6.6±0.29	1.38±0.07	3.97±0.2
<i>Begonia diadema</i> Linden cv. Kupfer Koenigin	3939±163	376.3±18	1215±64	19.2±0.84	1.86±0.08	9.32±0.4
<i>Begonia heracleifolia</i> Cham, et Schlecht.	2148±96	394.4±18	1744±93	19.5±0.63	0.93±0.03	6.32±0.2
Begonia lucema (Wettst.) hort.	4886±210	478.7±16	1429±63	12.4±0.73	0.28±0.01	8.72±0.3
Begonia bowery Ziesenh.	5465±267	479.1±18	2696±115	9.6±0.38	0.8±0.02	12.79±0.5
Begonia Tiger	3766±163	304.6±13	2526±103	13.6±0.33	0.68±0.05	7.76±0.5
<i>Begonia masoniana</i> Irmsch.	4963±263	359.7±19	2456±183	8.6±0.54	0.91±0.05	7.94±0.4
<i>Begonia x erytrophylla</i> Neum.	1569+53	436.3±18	1759±87	7.5±0.33	0.87±0.06	2.26±0.1

Table – The content of physiologically active compounds in representatives of *family Begoniaceae*, mg/100 g

Anthocyans determine the intense red color of the leaves of plants of the *Begoniaceae* family due to the large number of methoxy groups in their composition, as well as the ability to form complexes with calcium and magnesium cations [17].

The highest content of anthocyans was found for Begonia bowery Ziesenh. - 5465±267, in terms of catechins and flavonols, the species is inferior to Begonia rex Putzeys. cv. Merry Chrystmas, accordingly 479.1±18, 2696±115 mg/100 g - Begonia bowery Ziesenh. and 788.6±35, 2963±110 mg/100 g for Begonia rex Putzeys. cv. Merry Chrystmas. A relatively equal number of anthocyans was detected in Begonia masoniana Irmsch. and Begonia lucerna (Wettst.) hort. (Table). Then there is a slight decrease in the content of the total amount of anthocyans in the plant material of Begonia rex Putzeys. cv. Merry Chrystmas, Begonia diadema Linden cv. Kupfer Koenigin, Begonia Tiger. 3654±136, 3939±163 and 3768±163 mg/100 g, respectively. The minimum content of biologically active compounds of anthocyan nature was established for Begonia heracleifolia Cham. et Schlecht. and Begonia x erytrophyila Neum. -2148±96 and 1569±53 mg/100g.

Among the studied classes of phenolic compounds, the least heterogeneity in the quantitative distribution of taxons is characteristic for catechins. These are colorless compounds based on the 2-phenylchroman system. Catechins are the most reduced flavonoid compounds. They are easily oxidized; during the oxidation they acquire color. Catechins are characterized by a pronounced ability to polymerize and are the precursors of condensed tannins. According to C. Freudenberg, condensation of catechins is accompanied by rupture of heterocyclic rings and the formation of linear polymers with a large molecular weight. Catechins exhibit Pvitamin activity, namely, strengthen the vascular wall by inhibiting the oxidation of adrenaline, which stimulates the pituitary gland, and the latter, in turn, the secretion of corticosteroids. In addition, they affect vascular permeability by acting on the hyaluronic acid - hyaluronidase system, inhibiting hyaluronidase [17, 18]. For such representatives of Begoniaceaefamily as: Begonia diadema Linden cv. Kupfer Koenigin, Begonia heracieifolia Cham, et Schlecht., Begonia lucerna (Wettst.) hort., Begonia Tiger, Begonia masoniana Irmsch. и Begonia x ervtrophylla Neum., Begonia bowery Ziesenh. the difference in the content of the total catechins varied within small limits (Table) and averaged 404.1±17 mg/100 g. The maximum amount of biologically active substances of a catechin nature is established for *Begonia rex* Putzeys. cv. Merry Chrystmas.

Flavonols are a large group of flavonoids, which, unlike flavones, have an additional hydroxyl group [19]. According to the content of flavonols, the studied taxons can be divided into two groups. In the first group, the content of the total amount of flavonols ranged from 2963±110 to 2456±183 mg/100 g. It includes Begonia rex Putzeys. cv. Merry Chrystmas, Begonia bowery Ziesenh., Begonia Tiger and Begonia masoniana Irmsch. The number of biologically active compounds of flavonol nature in the second group ranged from 1215±64 to 1759±87 mg/100 g: Begonia diadema Linden cv. Kupfer Koenigin, Begonia heracleifoiia Cham, et Schlecht., Begonia lucerna (Wettst.) hort. and Begonia x ervtrophylla Neum.

Ascorbic acid is one of the most widely distributed vitamins in nature. Due to reversible redox reactions, vitamin C is a component of the biological antioxidant system. Ascorbic acid is a cofactor of proline hydroxylation reactions in the synthesis of collagen, hydroxylation of phydroxyphenylpyruvate to homogentizinic acid, and transformations of corticosteroids and transferrin [18]. The content of ascorbic acid in all studied representatives of the *Begoniaceae* family ranged from 6.6 ± 0.29 (*Begonia rex* Putzeys. Cv. Merry Chrystmas) to 19.5 ± 0.63 mg/100g (*Begonia heracieifolia* Cham, et Schlecht.) and the average vitamin C content for all eight taxons was 12.1 ± 0.50 mg/100g.

Data on the content of hydrophilic pectin and protopectin in the leaves of some representatives of the Begoniaceae family are presented in the Table. Pectin substances are high molecular weight heteropolysaccharides, the main component of which is a-D-galacturonic acid (83-90%). Hydrophilic pectin and protopectin in vivo are in dynamic equilibrium and can be converted into each other, for example, under the influence of pectolytic enzymes. In clinical practice, pectin substances are used as hemostatic drugs, antiseptics and chelators of heavy metals, as well as anti-inflammatory and antihypertensive drugs. The total hydrophilic pectin content is lower compared to protopectin in the studied samples. Its maximum content was established for Begondiadema Linden cv. Kupfer Koenigin ia (1.86±0.08 mg/100g) and Begonia rex Putzeys. cv. Merry Chrystmas (1.86±0.08 mg/100g), for

all other representatives of begonia, the content of hydrophilic pectin is below 1 mg/100g and averaged 0.74 ± 0.04 mg/100g. The content of protopectin among the studied taxons ranged from 2.26 ± 0.1 (Begonia x erytrophylla Neum.) to 12.79 ± 0.5 mg/100 g – Begonia bowery Ziesenh.

Thus, the biochemical composition and IAA of some representatives of the Begoniaceae family were screened. The process of oxidation of extractive substances of studied taxons by active forms of bromine is one of the promising model systems for evaluating IAA preparations based on begonia plant materials. Such a system made it possible to evaluate the antioxidant activity of complex mixtures of plant origin without preliminary isolation of individual components. The study of the IAA of the presented plant objects under uniform conditions is an urgent task of pharmaceutical analysis, as this will further develop common requirements for the quality of medicinal plant raw materials and herbal preparations based on the Begoniaceae family.

IAA and the obtained data on the content of biologically active compounds are a comprehensive indicator of the quality of plant materials and can be successfully used to standardize it. The introduction of a test for IAA in the future may allow to: quickly and accurately assess the quality of the used plant materials of the *Begoniaceae* family; make multicomponent preparations with a given antioxidant activity; expand the indications for the use of drugs and determine the most selective solvent for the extraction of active substances from plant material.

Conclusion. IAA was evaluated and biochemical screening of the content of biologically active compounds (flavonols, anthocyans, catechins, ascorbic acid, hydrophilic pectin and protopectin) of eight representatives of the *Begoniaceae* family was performed. The prospects of using such an indicator as IAA for screening plant materials in order to expand the range of medicinal plants is shown, which indicates the need for research in the field of antiradical properties and biochemical composition of medicinal plants, herbal preparations and biologically active additives of plant origin.

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