PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2013, № 2, p. 23–31

Chemistry

ANTIOXIDANT ACTIVITY OF CREEPING THYME (*THYMUS SERPYLLUM* L.) IN CUMENE OXIDATION REACTION

S. A. HAYRAPETYAN, L. R. VARDANYAN, R. L. VARDANYAN *

Department of Biology and Chemistry Goris State University, Armenia

Antioxidant activity of ethyl acetat extraction of creeping thyme, growing in the regions with various altitude above the sea level, was investigated. In the paper is demonstrated that the highest amount of antioxidants (14.06%) is in ethyl acetat extraction of creeping thyme, growing at the altitude of 650 *m* a.s.l., where the temperature under the sun is above 30^0C. It was found as well, that from the studied solvents the best extractant for antioxidants of creeping thyme is the solvent with the greatest polarity (acetone).

Keywords: medical herbs, *Thymus serpyllum* L., extraction, extragent, *antioxidants*, antioxidant activity.

Nowadays, the search and investigation of promising natural products with antioxidant and antiradical activity is a very urgent problem. Disturbances in the balance of the velocity of free radical oxidation and activity of antioxidant protection of the body, caused by unfavourable factors (environmental pollution, ultraviolet and radiation emissions, decrease of bioantioxidants) play an important role in the pathogenesis of many diseases: cardiovascular, oncological, gastrointestinal, neurodegenerative etc. [1–3].

The curing effect of medical herbs is associated with the contain of pharmacologically active substances, which demonstrate their medical activity and healing properties after entering the human or animal body. These substances have various composition and belong to different classes of chemical compounds. The main acting substances refer to flavonoids, vitamins, essential oils, acids and tannins [4, 5].

Thereby, at present in the medical practice a major importance is attached to medicines with herbal origin.

It is long ago that the representatives of the genus of thyme (*Thymus*) from the dead nettle family (*Laminaceae*) are considered as one of the most active types of medical herbs, which are revealed to have anti-microbial [6], antioxidant [7–9], anti-inflammatory, spasmolytic [10] activities.

The therapeutic activity of remedies from thyme is associated with the presence of various classes of biologically active substances: tanning agents,

* E-mail: vrazmik@rambler.ru

phenol compounds, are basic components of essential oil of thyme and posses antiseptic and fungicide characteristics [11].

It is necessary to mention, that the chemical composition of essential oils from the same medical herb mainly depends on natural and geographical regions of its growth. For example, as mention authors of a recent publication [12], there is much higher content of carvacrol (21–37%) than thymol (10–17%) in essential oils extracted from thyme growing in northern latitude, on the other hand, in essential oils obtained from Indian thyme the content of thymol is 65% and the content of carvacrol is just 5% (<0.3%). Therefore, in order to prescribe extraction or essential oil from the medical herb, growing in exact geographical region, it is necessary to investigate its chemical composition or any other specific characteristic.

In this study we have investigated anti-oxidant characteristics of the extraction of creeping thyme (*Thymus serpyllum* L.) growing in Goris region of Armenia. Creeping thyme is a small odoriferous boscage with 10–15 *cm* height. The stem is brown and creeping, leaves are small and crosswise, with opposite localization to each other, with very short petioles with elongated white cilia along the border. Flowers are small, pinkish-purple, bilabiate and connected in loose cephalanthium. Creeping thyme is widespread in Goris region and in the whole territory of Armenia. It grows in dry sandy soil, on slopes and rocks of mountains, in wood clearings, in cuttings, meadows, highlands from 400–3500 *m* a.s.l.

Creeping thyme has many applications in Armenia since ancient times, both in folk and scientific medicine. It is being prescribed as expectorant and disinfectant in pulmonary diseases and as a medicine for gastrointestinal diseases, atherosclerosis, chill etc. Creeping thyme is widely used also in cookery, especially, when the digestion of heavy meal is demanded.

Materials and Methods. Considering the fact, that the chemical composition mainly depends on geographical and climatic conditions of growth, the collection of thyme is carried out on the altitude 650–2400 *m* a.s.l. during the period of flowering (from the end of April till the beginning of July). Tiny shoots of thyme (leaves and flowers) were dried in 40^0C till air-dry condition. Dried raw material was grinded in ceramic pounder till getting powder (dimensions of particles $\leq 1\ mm$). The extragent with the dilution 1:20 (20 *ml* solvent on 1 *g* ground) was added to the powder, the mixture is left to settle down in room temperature during one day, then it was filtrated through paper filter and dryed in vacuum drier till constant weight. From all specimens of creeping thyme a greenish brown solid mass was obtained.

Extragents with various polarity (e.g. benzol, diethyl ether, ethyl acetat, acetone, methanol, chloroform) were used in order to study the influence of extragent polarity on the level and capability of the extraction of antioxidants from the dry raw material of creeping thyme.

Total content of antioxidants in processed extractions and their antioxidant characteristics were investigated by kinetic methods. As a model reaction initiated cumene acidification reaction was selected azodi isobutyronitrile (AIBN). Chlorobenzene acted as a solvent in the reaction mixture. The volume of reaction mixture in all experiments made 5 *ml* and the cumene concentration made 2.87 *mol*/*l*. The experiments of oxidation were carried out on the gasometric installation with autonomic pressure adjustment [13]. The purification of AIBN, cumene, chlorobenzene and used extragents was performed according to methods described in [14]. Experiments for cumene oxidation were conducted at temperature 348 *K*.

Results and Discussion. The results of the experiments showed that clearly defined induction periods appear on the kinetic curves of oxygen absorption in the presence of studied extractions, which evidences about the presence of higly active antioxidant substances in these extractions.

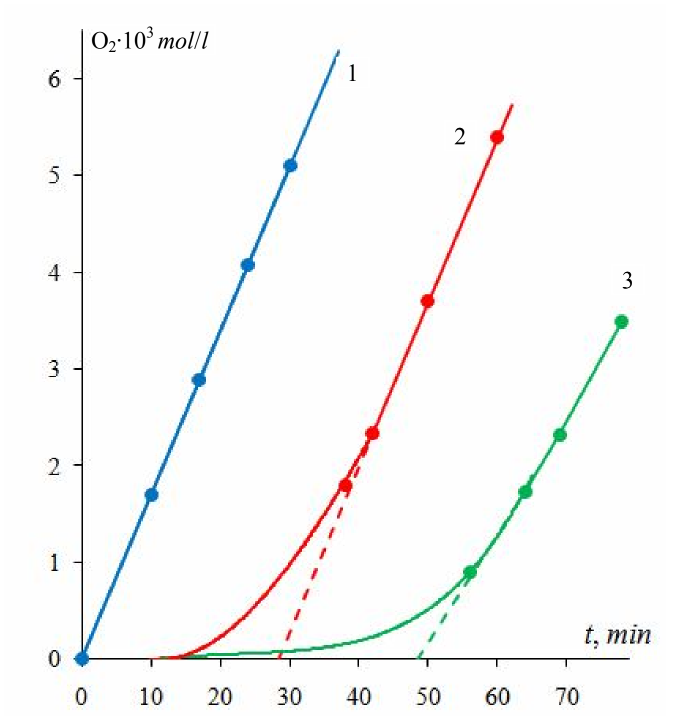

Fig. 1. Kinetic curves of oxidation of cumene in the absence (1) and in the presence of 0.1*g*/*l* (2), 0.2 *g*/*l* (3) ethyl acetat extraction of creeping thyme from Vorotan gorge $V_i = 1.25 \cdot 10^{-7}$ *mol*/*l·s*, $T = 348$ *K*.

As an illustration, the typical kinetic curves of oxygen absorption during cumene oxidation reaction in the presence and in the absence of extractions of creeping thyme (collected in Vorotan gorge, 650 *m* a.s.l.) in different concentrations (*g*/*ml*) are presented in Fig. 1. Received experimental data of induction periods are described in Fig. 2 and in equation

$$\tau = f\ [In\mathrm{H}]_0 / V_i, \qquad (1)$$

where $[In\mathrm{H}]_0$ is initial concentration of antioxidants in used extraction, f is stoichiometric coefficient of inhibition, e.g., the number of radicals terminated on one molecule of inhibitor, V_i is initiation velocity, *mol*/*l·s*. Considering the fact that in the extractions of creeping thyme the thymol (2-isopropyl-5-methyl-phenol) and carvacrol (2-methyl-5-isopropyl-phenol) dominate among antioxidant substances, calculating on the content of antioxidans in 1g of extractions f was considered to be equal to 2 as in substituted phenols [15].

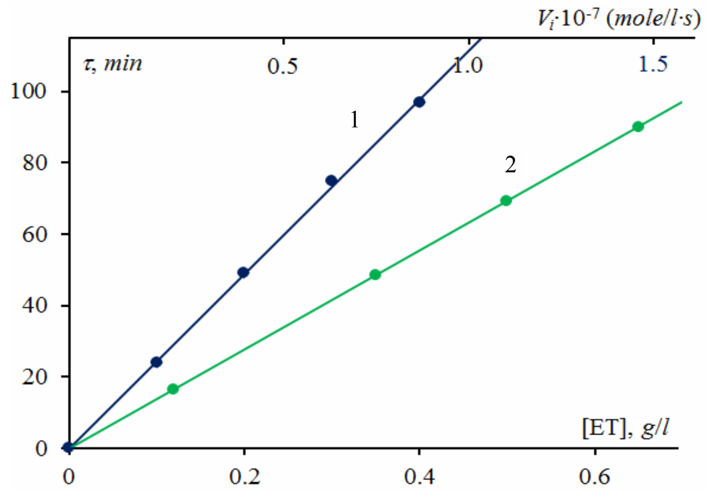

Fig. 2. The dependence of periods of induction of cumene
1. from the content of creeping thyme extraction (V_i=1.25·10^{-7} *mol/l·s*);
2. from reciprocal value of initiation velocity, T=348 *K*.

The content of antioxidants in percent was determined in induction periods. Meanwhile, we used the equation (2)

$$\omega = \frac{\vartheta\, \tau\, V_i\, Mr}{m\, f} 100\%, \qquad (2)$$

where ϑ is the volume of reaction mixture, *ml*; Mr is molar mass of antioxidants (in calculations we supposed that only thymol and carvacrol are contained in thyme extraction); m is band-and-hook hinge of extraction in the reaction mixture, *mg*.

Results of these calculations are brought in Tab. 1.

Table 1

Content of antioxidants and antioxidant activity of ethyl acetate extraction of creeping thyme collected depending on area of growth

Area of growth, height a.s.l., *m*	Date of the collection of herbal raw material	Content of antioxidants in 1 *mg* extraction		$k_7 \cdot 10^{-4}$, $l/mol \cdot s$	$k_{71} \cdot 10^{-2}$, $l/mol \cdot s$
		$[In\text{H}] \cdot 10^4$, *mol/l*	ω, %		
Vorotan gorge, 650	08.05.12	1.875	14.06	3.92	1.74
Old City Goris, 1250	12.06.12	0.294	2.21	2.70	5.26
Village Verishen, 1600	25.06.12	0.60	4.50	3.69	4.13
Gorge in village Brun, 1700	20.07.12	0.876	6.57	5.58	2.19
Gorge in village Chndzoresk, 1350	15.06.12	1.661	12.46	4.56	1.51
Gorge in village Tegh, 1200	10.06.12	0.525	3.94	4.74	3.39
Village Gorayk, 2400	25.08.12	0.378	2.84	5.20	6.83

As it is presented in Tab. 1, the biggest amount of antioxidants is found in ethyl acetate extraction of creeping thyme growing in the Vorotan Gorge (650 *m* a.s.l.), which evidently is associated with earlier dates of collection of herbal raw material and relatively high temperature. These data evidence that geographical and climatic conditions actually influence upon the biosynthesis of antioxidants in plants.

It is necessary to mention that antioxidant characteristics of extractions depend not only on quantitative content of antioxidant substances, but also on their activity. Antioxidant activity is characterized by the constant of velocity of reaction of peroxide radicals $(\mathrm{RO}_2^\bullet)$ with the inhibitor $(In\mathrm{H})$:

$$\mathrm{RO}_2^\bullet + In\mathrm{H} \xrightarrow{k_7} \mathrm{ROOH} + In^\bullet,$$

e.g., the velocity constant of the reaction of linear breakaway of chain k_7.

The values of k_7 were determined by equation (3) [15]

$$\frac{\Delta[\mathrm{O}_2]}{[\mathrm{RH}]} = -\frac{k_2}{k_7}\ln\left(1-\frac{t}{\tau}\right), \tag{3}$$

where $\Delta[\mathrm{O}_2]$ is the concentration of oxygen absorbed by cumene during $t<\tau$; $[\mathrm{RH}]$ is the cumene concentration; k_2 is the velocity constant of the reaction of chain elongation, $k_2 = 4.677\cdot 10^7 \exp(-6800/RT)$ [16]:

$$\mathrm{RO}_2^\bullet + \mathrm{RH} \xrightarrow{k_2} \mathrm{ROOH} + \mathrm{R}^\bullet.$$

The absolute values of k_7 characterizing antioxidant activity of inhibitors contained in extractions were calculated, when the experimental data on the coordinates $\frac{\Delta[\mathrm{O}_2]}{[\mathrm{RH}]}$ from $\ln\left(1-\frac{t}{\tau}\right)$ were rectified by the tangent of the angles of inclination (Fig. 3). Results of these calculations are brought in Tab. 1. As follows from extracted data, the ethyl acetate extraction of creeping thyme growing in the territory of village Brun differs by antioxidant activity from other investigated extractions.

As a result of all experiments it was found out that the velocity of cumene oxidation reaction in the presence of extractions after induction period is significantly low comparing with that in pure cumene oxidation reaction (it becomes evident, if compare tangents of angles of kinetic curves after exit from induction periods, demonstrated on Fig. 1). It evidences that the products of oxidizing transformation of initial antioxidants (QH) in extractions also possess inhibiting properties, as in case of flavonoids, investigated in [17, 18]. Besides, during free radical acidification of cumene in the presence of the products of chein termination is carried out both linear

$$\mathrm{RO}_2^\bullet + \mathrm{QH} \xrightarrow{k_{71}} \mathrm{ROOH} + \mathrm{Q}^\bullet$$

and quadratically

$$RO_2^{\bullet} + RO_2^{\bullet} \xrightarrow{k_6} \text{molecular products.}$$

In this case the length of chains of inhibited oxidation of cumene $\vartheta = V/V_i$ remains more than 5 and between cutoff velocities of oxygen absorption, and the dependence is observed between inhibited (V) and non-inhibited (V_0) oxidation [15]:

$$\frac{V_0}{V} - \frac{V}{V_0} = \frac{k_{71} f [\text{QH}]}{\sqrt{k_6 V_i}}. \tag{4}$$

The values of maximum velocities V_0 and V are subordinated to the Eq. 4 in cumene oxidation reaction in the presence of all investigated extractions (Fig. 4). It allowed to determine the relation $k_{71}/\sqrt{k_6 V_i}$. Considering, that the concentration of initial antioxidants (InH) and products (QH) of their oxidation are identical, and that $k_6 = 4.77 \cdot 10^5 \exp(-1800/RT)$ for cumene [16], the values of k_{71} were calculated, characterizing inhibiting activity of products of oxidizing transformation. As it is evident from data in Tab. 1, the extraction of creeping thyme collected in the area of Old City Goris possesses the highest antioxidant activity among all investigated extractions.

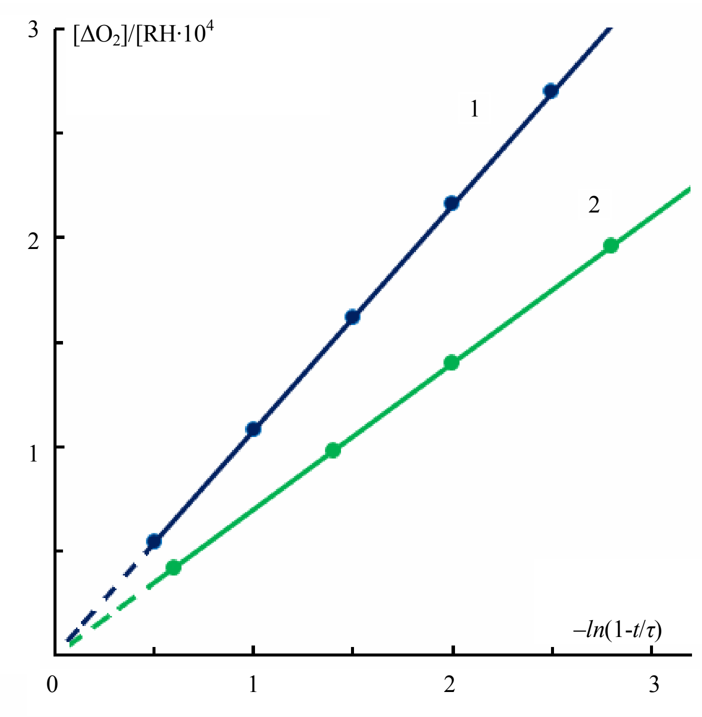

Fig. 3. Dependence from ln(1–t/τ) extraction of creeping thyme growing in: 1. Vorotan gorge; 2. Village Brun; T=348 K.

It is well known that the nature of applied organic solvent significantly influences on the completeness of extraction of antioxidant substances from the raw herbal material [19]. In order to find the best solvent capable to maximum extraction of antioxidants from the powder of creeping thyme, several solvents with different polarity were used. As a subject of investigation creeping thyme growing in the area of village Vernishen of Goris region of Armenia was chosen.

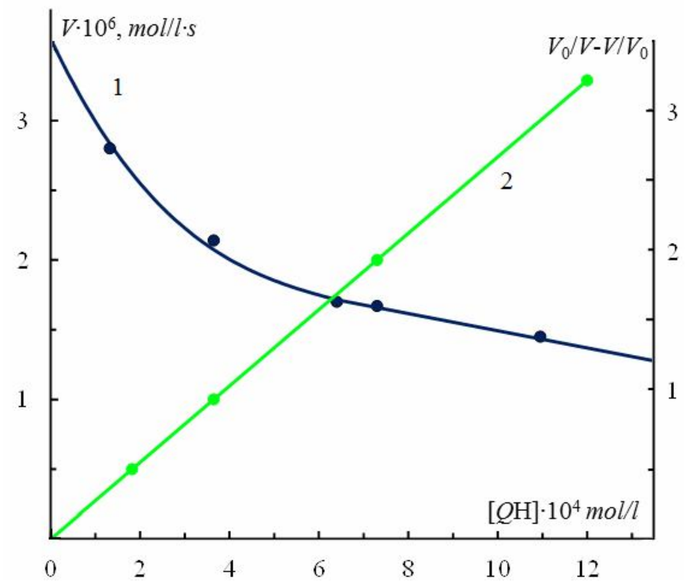

Fig. 4. 1 – The dependence from concentration *QH*; 2 – of the maximum oxygen absorption rate $V_0/V–V/V_0$ parameter. V_i =1,25·10^{-7} *mol/l·s*, *T*=348 *K*.

Content of antioxidants and their activity were measured by above mentioned methods. Experimental data are given in the Tab. 2.

Table 2

Content of antioxidants and antioxidant activity of creeping thyme extraction collected in village Vorotan depending on polarity of extragent

Extragent	Content of antioxidants in 1 *mg* extraction		Boiling temperature 645 *mm* Hg, $T_{boiling}$, *K*	$\vec{\mu}$, *Debye* [20]	$k_7 \cdot 10^{-4}$, *l/mol·s*	$k_{71} \cdot 10^{-2}$, *l/mol·s*
	$[In\mathrm{H}] \cdot 10^4$, *mol/l*	ω, %				
Ethyl acetate	0.600	4.500	72.5	1.8295	3.685	4.130
Chloroform	0.474	3.555	57.5	1.8160	5.150	3.578
Methanol	0.451	3.383	61.0	1.6770	3.540	2.380
Acetone	0.714	5.356	53.0	2.6295	1.400	2.170
Benzol	0.410	3.075	75.5	0	1.740	1.740
Ethanol (100%)	0.204	1.530	74.0	1.7700	1.960	4.760
Diethyl ether	0.502	3.765	33.5	1.1657	10.41	3.71

As follows from data, the best extragent for antioxidant compounds is the solvent with the highest polarity, e.g. acetone, and as a the best extragent with antioxidant activity diethyl ether was recognized.

Therefore, as a result of carried investigation we can conclude that in order to get the extraction of thyme with the highest content of antioxidants, it is necessary to collect thyme during its blossoming period in relatively low geographical areas, and the extraction has to be carried out by solvents with the highest polarity in volatile solution.

Received 15.02.2013

REFERENCES

1. **Miliauskas G., Venskutonis P.R., van Beek T.A.** Screening of Radical Scavenging Activity of Some Medicinal and Aromatic Plant Extracts. // Food Chemistry, 2004, v. 85, № 2, p. 231–237.
2. **Samuelsen A.B.** The Traditional Uses, Chemical Constituents and Biological Activities of Plantago Major L. // J. Ethnopharm., 2000, v. 1, p. 1–21.
3. **Silva B.A, Ferreres F., Malva J.O., Dias A.C.P.** Phytochemical and Antioxidant Characterization of *Hypericum Perforatum* Alcoholic Extracts. // Food Chemistry, 2004, v. 85, № 1, p. 157–167.
4. **Kluchnikova N.F., Golubkina N.A., Senkevich O.A., Kluchnikov P.F.** Selenium in Medicinal Plants of Khabarovskii Krai. // Bulleten Botanicheskovo Sada-Instituta DVO RAN, 2009, v. 4, p. 37–40 (in Russian).
5. **Maslennikov P.V., Chupakhina G.N., Skrypnik L.N., Maltseva E.J., Poltavskaya R.L.** The Content of Low Molecular Antioxidants in Medicinal Plants in the Kaliningrad Region. // Chemistry of Plant Raw Materials, 2012, № 3, p. 127–133.
6. **Ismaili H., Sosa S., Brkic D., Fkih-Tetouani S., Ilidrissi A., Touati D., Aquino R.P., Tubaro A.** Topical Anti-Inflammatory Activity of Extracts and Compounds from *Thymus Broussonettii*. // Journal of Pharmacy and Pharmacology, 2002, v. 54, № 8, p. 1137–1140.
7. **Jukic M., Milos M.** Catalytic Oxidation and Antioxidant Properties of Thyme Essential Oils (*Thymus Vulgarae* L.). // Croatica Chemica Acta, 2005, v. 78, № 1, p. 105–110.
8. **Nejad-Ebrahimi S., Hadian J., Mirjalili M.H., Sonboli A., Yousefzadi M.** Essential Oil Composition and Antibacterial Activity of *Thymus Caramanicus* at Different Phenological Stages Original Research Article. // Original Food Chemistry, 2008, v. 110, № 4, p. 927–931.
9. **Figueiredo A.C., Barroso J.G., Pedro L.G., Salgueiro L., Miguel M.G., Faleiro M.L.** Portuguese Thymbra and Thymus Species Volatiles: Chemical Composition and Biological Activities. // Curr. Pharm. Des., 2008, № 14, p. 3120–3140.
10. **Broucke C.O., Lemli J.A.** Spasmolytic Activity of the Flavonoids From *Thymus Vulgaris*. // Pharmacy World Science, 1983, v. 5, № 1, p. 9–14.
11. **Vasilenko Yu.K. , Oganesyan E.T., Lisevitskaya L.I.** Study and Review of Physiological Activity of Triterpene Substances Extracted From the Waste of Thyme. // Khimiko-Farmatsevticheskii Zhurnal, 1978, № 9, p. 61 (in Russian).
12. **Alexeeva L.I., Gruzdiev I.V., Bystrushkin A.G., Teteryuk L.V.** The Chemical Composition of Essential Oil of Thyme Endemic European North-East Russia and the Urals. // Bulletin of the Institute of Biology, Komi Science Center RAN, 2011, № 10, p. 9–14 (in Russian).
13. **Emanuel N.M., Denisov E.T., Maizus E.K.** Chain Reactions in the Liquid Phase Oxidation of Hydrocarbons. M.: Nauka, 1965.
14. **Gordon A., Ford R.** Satellite Chemist. M.: Mir, 1976, 514 p. (in Russian).
15. **Denisov E.T., Azatian V.V.** Inhibition of Chain Reactions. Chernogolovka, 1997, 266 p. (in Russian).

16. **Denisov E.T.** The Rate Constants of Homolytic Liquid-Phase Reactions. M.: Nauka, 1971, 711 p. (in Russian)
17. **Denisov E.T.** Handbook of Antioxidants: Bound Dissociation Energies, Rate Constants, Activation Energies and Enthalpies of Reactions. NY–Tokyo: CRC Press, Boca Ration, 1995, 174 p.
18. **Vardanyan R.L., Vardanyan L.R., Atabekyan L.V.** Antioxidant Effect of Extracts of White Mistletoe (*Viscum Album* L.) Grown in Different Trees. // Chemical Journal of Armenia, 2011, v. 64, № 3, p. 335–343.
19. **Kostiuk V.A., Potapovich A.I.** Bioradicals and Bioantioxidant. Minsk, 2004, 174 p. (in Russian).
20. **Yakupova L.R., Khairullina V.R., Baimuratova G.R., Ostroukhova L.A., Safiullin R.L., Gerchikov A.Ja., Babkin V.A.** Antioxidizing Activity of Flavonoids of a Bark of the Siberian Larch. // Bashkir Chemical Journal, 2007, v. 4, № 1, p. 51–54 (in Russian).
21. **Osipov O.A., Minkin V.I., Garnovskii A.D**. Handbook of Dipole Moments. M.: Visshaja Shkola, 1971, 414 p. (in Russian).