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THE STUDY OF ANTIOXIDANT ACTIVITY OF BIOLOGICALLY ACTIVE SUBSTANCES IN THE ALCOHOL EXTRACT OF NETTLE LEAVES

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The purpose of this research is the extraction of phenolic compounds from nettle leaves, grown in urban conditions, with different concentrations of ethanol and the study of the antioxidant properties of these extracts. Nettle (*Urtica dioica*) is a great source of bioactive compounds. The content of biologically active compounds in nettle leaves depends on the growing conditions of the plant. Analysis of the electronic spectra of the extract shows that all extracts contain aurones and coumarins, hydroxybenzoic acid and flavonoids. The antioxidant activity (AO) of polyphenols in alcoholic extracts of nettle was studied using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). It was found that the total amount of biologically active substances is greatest in a 70% ethanol solution of nettle leaves, but the antioxidant activity is higher in a 95% solution. The total amount of flavonoids in the leaves of urban nettles is higher than in the leaves of the nettle grown in the meadow. Perhaps this is connected with the process of the plant's protective mechanisms caused by air pollution in the city.

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***Keywords*:** *Urtica dioica*, antioxidant properties, plant extracts, DPPH, flavonoids.

Introduction. Oxidative stress occurs in the human body when the balance of biochemical mechanisms is disturbed. Usually, free radicals accumulate in cell membranes and begin to destroy them. As a result, the cells gradually disintegrate and die. This leads to accelerated aging, tissue damage, impaired immunity and other health problems. In living cells, there is a perfect system of antioxidant protection that regulates the formation of free radicals. But the body does not always cope without outside help. The removal of oxidative stress is achieved with the help of biologically active substances, in particular antioxidants. Antioxidants stop the rapid development of oxidative processes, form inactive radicals and remove them from the body. Flavonoids are natural antioxidants [1]. The biological activity of flavonoids

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is due to their specific structure [2]. Determination of antioxidant activity (AO) is usually carried out using stable free radicals. The study of the properties of natural compounds containing phenolic groups for antiradical and antioxidant activity makes it possible to expand the range of their use. Plants are an excellent source of natural ingredients and active compounds such as polyphenols, flavonoids, etc. [2]. The plant parts most commonly used for extraction are leaves, fruits, seeds or roots [3]. Most plant extracts are rich in phenolic compounds that provide antioxidant properties. *Urtica dioica*, commonly known as stinging nettle, is a perennial plant. Among the main components of nettle leaves are polyphenolic compounds such as flavonoids and phenolic acids (syringic, caffeic, ferulic, gallic acids) [4]. These compounds are known for their antioxidant, anti-inflammatory, and antiviral properties, among others. Flavonoids and phenolic acids exercise their AO through various mechanisms. It is believed that the predominant mechanism is the removal of radicals due to the donation of hydrogen atoms [5]. Fatty acids are also present in nettles. The predominant fatty acids are α-linolenic acid, palmitic acid and cis-9,12--linoleic acid. Fatty acids *n*-3 and *n*-6 are present in significant amounts, providing anti-inflammatory, vasodilating, antithrombotic and hypolipidemic properties [6]. It should be borne in mind that the composition of nettles varies depending on the time of collection, the soil in which it was grown, the exposure of the plant to the sun and other factors.

Experimental Part. Stinging nettle from the central part of Yerevan City (Armenia) was used for the study. Stinging nettles were collected from April to October. In the lab, the plants were cleaned and defoliated to produce healthy leaves. Nettle leaves were dried in a dehydrator at 45℃ for 12 *h* to remove moisture. After that, the leaves were crushed. The moisture content and dry matter of the samples were determined. To do this, 1 *g* of the sample was weighed and dried at a temperature of 60℃ in an oven for 18 *h*. The percentage of moisture was calculated from the difference in masses after and before drying. In all cases, 1 *g* of dry nettle powder was weighed, transferred to a screw cap tube, and diluted with 30 *mL* of solvent (50%, 70%, 95% ethanol). Extraction was carried out through a warm bath at a temperature of 65℃ for 1 *h*. Determination of AO was carried out using a stable free radical. The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from "Sigma-Aldrich". AO was determined according to the method described in [7]. A series of solutions was prepared in test tubes by adding 2 *mL* of extract and 1.5 *mL* of DPPH solution. After that, the samples were kept in the dark and at room temperature for 30 *min*. Spectrophotometric measurements were conducted on a UV 1800 PC instrument. The absorption measurements were carried out at a wavelength of 517 *nm*. The content of the sum of flavonoids in terms of rutin is calculated by the formula:

$$x = \frac{A \cdot m_0 \cdot 100 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 100(100 - W)},$$

where A and A_0 are the optical density of the test and SSS (State standard sample) rutin solutions, respectively; m and m_0 are the mass of raw materials and SSS rutin, respectively, g; W is the weight loss during drying of raw materials, % [8, 9]. The amount of carotenoids and chlorophyll in the studied plants was calculated according to the formula from [10].

Results and Discussion. Moisture of dried nettle leaves was very low ($0.8 \pm 0.04\%$). A moisture percentage of less than 12% is considered of good quality and adequate for the analysis. The extraction process of natural products can be carried out through several steps:

1) the solid matrix is penetrated by the solvent;
2) the solute obtained is dissolved in the solvent;
3) the solute is removed from the solid matrix;
4) the extracted solutes are collected.

It is known that individual groups of phenolic compounds differ in their spectral characteristics [11]. For the identification of phenolic compounds, electronic absorption spectra of nettle samples were recorded. The absorption spectra of nettle extracts are presented in Fig. 1.

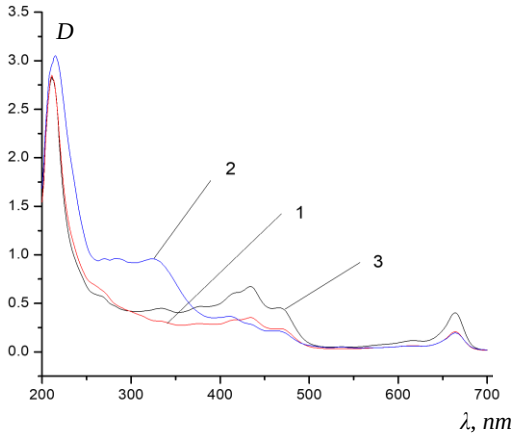

Fig. 1. Electronic absorption spectra of nettle ethanol extracts: 1 – 50%; 2 – 70%; 3 – 95%.

The phenolic composition (Fig. 1) of nettle alcoholic extracts is quite diverse. The presence of absorption bands at λ = 322, 324, 334, 406–413 *nm* in all extracts indicates the presence of 3-glycosides, coumarins and aurones. However, in addition to these named groups of phenolic compounds, the maximum at λ = 285 *nm* in a 70% alcohol extract of nettle indicates the presence of flavanone (Tab. 1).

The reaction of complex formation of flavonoids with aluminum chloride in a slightly acidic medium gives stable results, in particular, in the determination of complex plant objects.

Table 1

Absorption bands of some phenolic compounds in the spectra of nettle extracts (nm)

Ethanol, %	Coumarin, 310–350	Flavanone	Hydroxybenzoic acid, 235–270	Aurone, 390–430	Carotenoids	Chlorophyll
50	330	–	260	390	470	664
70	325	285	270	410	470	664
95	340	–	267	390, 406	470	664

For quantitative determination of the amount of flavonoids, a complex of extract from nettle grass with a 2% solution of aluminum chloride was obtained [9]. On the other hand, chlorophylls, carotenoids, and fatty acids are also important

compounds in the composition of nettle. The chlorophyll and carotenoids play a role in nettle as light-harvesting pigments. Specifically, carotenoids can suppress harmful photochemical reactions involving oxygen, therefore, antioxidant capacity is attributed to them. The amount of flavonoids, carotenoids and chlorophyll was calculated according to the method in [10, 11]. The results are shown in Tab. 2.

Table 2

The results of determining the optimal solvent for the extraction of flavonoids, carotenoids and chlorophyll from nettle leaves

Ethanol, %	Content of flavonoids, %	Chlorophyll *a+b, mg/mL*	Carotenoids, *mg/mL*
50	1.75	3.54	0.51
70	2.8	4.08	0.08
95	2.3	8.17	0.3

During the experiment, it was found that the optimal solvent is 70% ethanol. The total amount of biologically active substances in 70% extracts of meadow nettle leaves is approximately 2 times greater than the amount of biologically active substances in the leaves of nettle grown in Yerevan City [12]. To study the antioxidant activity, a decrease in the optical density of the radical was observed after its addition to the nettle extract at the beginning of the reaction at a fixed wavelength of 517 *nm*. Nettle extract, DPPH solution, and acetic acid solution were introduced into the cuvette of the spectrophotometer. The acid slows down the reaction rate, making it easier to conduct kinetic studies. When interacting with antioxidants, DPPH passes into a non-radical form, which is accompanied by the disappearance of the absorption band maximum at a wavelength λ_{max} = 517 *nm*.

An analysis of the kinetic curves shows that most of the DPPH molecules are reduced in the first 5 *min* of the reaction. The reaction of DPPH with antiradical antioxidants proceeds according to a series-parallel mechanism. At the first stage, the antioxidant molecule donates the most mobile hydrogen atom to the radical. Its structural formula and mechanism of action are given below (Fig. 2).

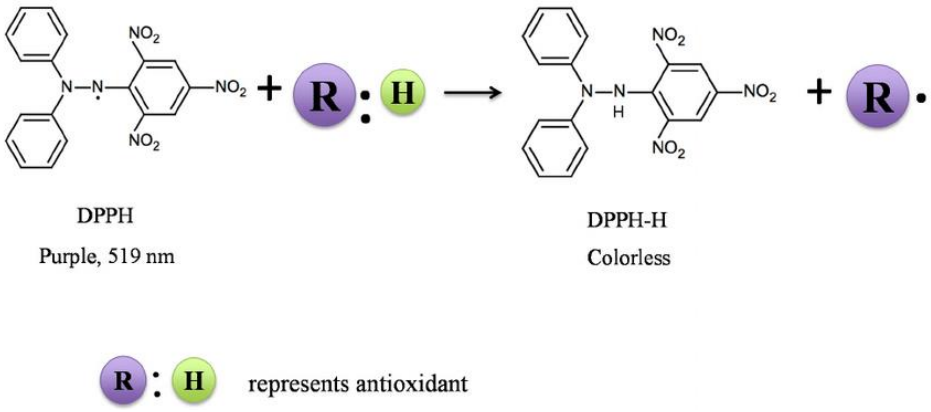

Fig. 2. Reaction mechanism of DPPH with antioxidant. R : H – antioxidant radical scavenger; R – antioxidant radical [5].

Antiradical activity (% inhibition of DPPH) was determined by the formula:

$$\% \text{ inhibition} = (D–D_x)/D \cdot 100.$$

The results are shown in Tab. 3.

Table 3

Antiradical activity of nettle extracts

Ethanol, %	Antioxidant activity, %
50	37
70	57
95	65

The extract of 95% ethanol has the highest antioxidant properties. Although the total amount of flavonoids is higher in 70% ethanol solution. But the amount of carotenoids is higher in 95% ethanol solution. Carotenoids also have antioxidant properties, which led to an increase in the AO of the 95% alcohol extract.

Conclusion. It is shown that the total amount of biologically active substances is the largest in 70% ethanol solution of nettle leaves, and AO is higher in 95% ethanol solution. This may be due to carotenoids passing through the 95% ethanol solution. Carotenoids also have antioxidant activity. The total amount of flavonoids in the leaves of nettles grown in urban areas is lower than in the leaves of nettles grown in the meadow.

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ԵՂԻՆՋԻ ՏԵՐԵՎՆԵՐԻ ՍՊԻՐՏԱՅԻՆ ԷՔՍՏՐԱԿՏՈՒՄ ԿԵՆՍԱԲԱՆԱԿԱՆ ԱԿՏԻՎ ՆՅՈՒԹԵՐԻ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Հետազոտության նպատակն է քաղաքային պայմաններում աճած եղինջի տերևներից ֆենոլային միացությունների լուծահանումը էթանոլի տարբեր կոնցենտրացիաների դեպքում և այդ էքստրակտների հակաօքսիդանտային հատկությունների ուսումնասիրությունը: Եղինջը (*Urtica dioica*) կենսաբանական ակտիվ միացությունների մեծ աղբյուր է: Եղինջի տերևներում կենսաբանական ակտիվ միացությունների պարունակությունը կախված է բույսի աճման պայմաններից: Եղինջի տերևների էթանոլային էքստրակտների էլեկտրոնային սպեկտրների վերլուծությունը ցույց է տվել, որ բոլոր էքստրակտները պարունակում են առուրոններ, կումարիններ, հիդրօքսիբենզոյաթթու և ֆլավանոիդներ: Այս էքստրակտների հակաօքսիդանտային ակտիվությունը ուսումնասիրվել է կայուն ազատ ռադիկալ 2,2-դիֆենիլ-1-պիկրիլհիդրազիլի օգնությամբ: Ցույց է տրվել, որ եղինջի տերևների 70%-ոց էթանոլային լուծույթում կենսաբանական ակտիվ նյութերի գումարային քանակը ամենաբարձրն է, իսկ հակաօքսիդանտային ակտիվությունը բարձր է 95%-ոց էթանոլային լուծույթում: Քաղաքային պայմաններում աճած եղինջի տերևներում ֆլավոնոիդների գումարային քանակը ավելի ցածր է, քան մարգագետնում աճած եղինջի տերևներում: Դա կարող է կապված լինել քավաքում օդի աղտոտվածությամբ պայմանավորված բույսի պաշտպանական մեխանիզների գործընթացով:

С. А. АПОЯН, С. М. ВАРДАПЕТЯН, Г. Ф. МКРТЧЯН, А. М. ОГАНЕСЯН

ИЗУЧЕНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ В СПИРТОВОМ ЭКСТРАКТЕ ЛИСТЬЕВ КРАПИВЫ

Цель исследования – извлечение фенольных соединений из листьев крапивы, выращенной в городских условиях, при различных концентрациях этанола, и изучение их антиоксидантных свойств. Крапива (*Urtica dioica*) является отличным источником биологически активных веществ. Содержание биологически активных соединений в листьях крапивы зависит от условий роста растения. Анализ электронных спектров экстрактов показал, что все они содержат ауроны и кумарины, гидроксибензойную кислоту и флаваноиды. Антиоксидантную активность (АО) полифенолов спиртовых экстрактов крапивы изучали с использованием стабильного радикала 2,2-дифенил-1--пикрилгидразила (ДФПГ). Показано, что общее количество биологически активных веществ наибольшее в 70%-ом этанольном растворе листьев крапивы, а антиоксидантная активность выше в 95%-ом растворе. Общее количество флавоноидов в листьях выращенной в городских условиях крапивы ниже, чем в листьях крапивы, выращенной на лугу. Возможно, это связано с процессом срабатывания защитных механизмов растения, вызванным загрязнением воздуха в городе.