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PREPARATION AND STUDY OF JUNIPER BERRY AND SEED EXTRACTS

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The study of antioxidants in plants is one of the modern scientific and practical problems. The aim of this work is to obtain the extracts of pharmacy sale juniper berries and seeds, and to study their antioxidant properties by the method of competitive reactions. In this work, a number of extraction methods were used, and the investigation of the obtained extracts was performed by spectrometric and chromatographic methods.

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Introduction. It is known that various herbs such as juniper, possess a number of beneficial and healing properties. These properties are largely due to the biologically active, antioxidant, and antiradical substances present in them [1]. The main function of antioxidants is that they bind free radicals and remove them from the body. The antioxidant properties of extracts obtained from plants are advantageous as they contain antioxidant substances with different functional groups, and therefore with different nature and mechanisms of action. The latter makes them more effective and universal. This is also the reason for the wide use of plants in modern medicine and drug production [2].

The purpose of this work is to study the antioxidant properties of extracts obtained from juniper berries and seeds, and to determine the quantitative content of some biologically active substances in the extracts. Quinones, phosphoric acid, citric acid, catalase enzyme are widely used in medicine [3]. The juniper (*Juniperus communis*), which is the subject of research in this work, is widely used in medicine. Berries and seeds of juniper (*Fructus Juniperi communis*, *Baccae Juniperi*), collected in autumn and dried at a temperature of up to 30°C, are used as medicinal raw materials [4]. Juniper berries yield a terpene oil or "juniper resin" that is recommended as an external analgesic. Juniper berries contain essential oil up to 2%, which is rich in terpenes, organic acids – acetic acid, malic acid, formic acid, invert sugar – up to 40%, and many other biologically active substances [5].

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Materials and Methods. The extraction of juniper berries and seeds was carried out using several methods taken from the literature: cold and hot maceration, steam distillation, and Soxhlet extraction [6]. Distilled water, as well as water–alcohol 30/70 and 60/40 (w/w) ratio mixtures were used as extraction solvents. Dry berries of juniper plant species with equal weight were used for each extraction process. The berries were taken from the same production lot. Detailed information about the extraction methods, conditions, and solvents used in this work are presented in Tab. 1.

Table 1

Serial number of obtained extracts, mass of dry berries, used solvent, method and conditions, volume of standardized extracts

Extracts	Mass of berries, *g*	Solvent used	Method used, duration, temperature	Volume of standardized extract, *mL*
1	10	70% ethanol	hot maceration, 2 *h*, 70°C	250
2	10	40% ethanol	hot maceration, 2 *h*, 70°C	250
3	10	40% ethanol	hot maceration, 24 *h*, 20°C	250
4	10	70% ethanol	hot maceration, 24 *h*, 20°C	250
5	10	70% ethanol	Soxhlet extraction, 4 *h*, 83°C	250
6	10	40% ethanol	Soxhlet extraction, 4 *h*, 87°C	250
7	10	distilled water	steam distillation, 4 *h*, 96°C	250

Method of Competitive Reactions for the Study of Antioxidant Properties. In the p-nitrosodimethylaniline (PNDMA) assay, the assessment of antioxidant properties involves the study of the kinetics of competitive reaction between hydroxyl radicals and PNDMA. Under the influence of UV light at 313 *nm*, hydrogen peroxide forms hydroxyl radicals. These radicals react with PNDMA, resulting in dye decolorization. The rate of the reaction between hydroxyl radicals and PNDMA is determined by measuring the absorption at 440 *nm* using a spectrophotometric technique. The addition of a sample affects this reaction due to the competitive reaction between the antioxidants present in the extract and hydroxyl radicals, resulting in slower decolorization of radical target PNDMA. By the determination of the rate of dye decolorization the rate constant of the reaction between antioxidants derived from the extracts and hydroxyl radicals may be determined according to the following equation [7, 8]:

$$k_{\mathrm{OH+polyphenol}} = 1.25 \cdot 10^{10} \frac{[\mathrm{PNDMA}]}{[\mathrm{polyphenol}]} \cdot \left(\frac{W_1}{W_2} - 1\right),$$

The factor $1.25 \cdot 10^{10}$ represents the rate constant of the hydroxyl radical and PNDMA reaction, $mol^{-1} \cdot L \cdot s^{-1}$. Meanwhile, W_1 and W_2 denote the slopes of the plots illustrating the absorption of PNDMA over time during hydrogen peroxide radiation in the absence and presence of infusions, respectively. A similar approach was employed to determine the rate constant of the hydroxyl radical and PNDMA reaction in the presence of the well-known antioxidant ascorbic acid, the value of which is $9.45 \cdot 10^9\ M^{-1} \cdot s^{-1}$ [8].

Instrumentation. UV-Vis absorption spectra were recorded using PG-instruments T60 spectrophotometer. In this work, measurements were carried out using "Waters Separation module e2695" (USA) liquid chromatograph. An Alltima C18 250×4.6 *mm*, 5 μm column was used. The eluent was a mixture of phosphoric acid,

methanol, acetonitrile, and water in the following ratio: 0.5% methanol, 0.5% acetonitrile, and 0.1% phosphoric acid in water. HPLC method was used for quantitative and qualitative analysis of organic acids: column – Alltima C18 250×4.6 *mm*, 5 *μm*; detector laser wavelength – 210 *nm*; flow velocity – 0.7 *mL*/*min*; column temperature – 30°C; mobile phase – 0.5% CH_3OH / 0.5% CH_3CN / 0.1% H_3PO_4 / 98.9% H_2O.

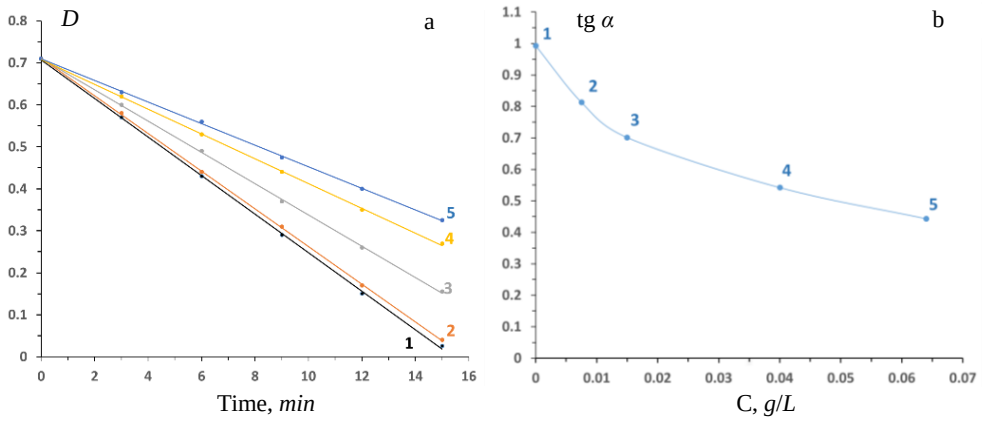
Results and Discussion.

Spectroscopic and Chromatographic Measurements. The extracts were previously diluted 50 times with the appropriate solvents and subjected to spectral analysis. According to the spectral studies the most effective extracts, which have the highest absorption intensities, were 1st, 4th, 5th, and 6th extraction methods. According to the literature data [9, 10], the absorptions obtained correspond to the organic acids present in the extracts, the presence of which was confirmed and quantitatively determined by a HPLC study. Based on the data of calibration curves of oxalic and tartaric acids, the amounts of those in the selected extracts are shown in Tab. 2.

Table 2

Quantitative data of some organic acids and their quantity in extracts

Extraction methods	Organic acid	Retention time, *min*	Intensity, AU	Quantity, *mg*/*mL*
1	oxalic acid	4.168	9551	0.012
4	oxalic acid	4.168	8564	0.012
5	oxalic acid	4.173	8567	0.011
6	oxalic acid	4.173	6122	0.009
4	tartaric acid	4.975	3159	0.02
5	tartaric acid	4.980	3193	0.012
6	tartaric acid	4.987	2028	0.012



Dependence of the absorption of PNDMA on the radiation period of hydrogen peroxide in the absence (1) and presence (2 – 0.0075 *g/L*, 3 – 0.015 *g/L*, 4 – 0.04 *g/L*, 5 – 0.0 *g/L*) of 7th extract (a). Dependence of the slopes of the plots given in (a) on the concentrations of the studied extract (b).

For the study of antioxidant properties, the method of competitive reactions, designed the study of antioxidant properties in aqueous systems, was used. Taking into account that only in 7th extraction method water is used as an extraction solvent, and PNDMA assay is valid for aqueous solutions, only this extraction method has

been used for furhher measurements. Figure (a) shows the dependence of the absorption of PNDMA at 440 *nm* as a function of radiation time in the absence (1) and presence (2 – 0.0075 *g*/*L*; 3 – 0.015 *g*/*L*; 4 – 0.04 *g*/*L*; 5 – 0.0 *g*/*L*) of 7th extract at different concentrations; Figure (b) shows the dependence of the reaction rate on the concentration of the studied extract.

From the dependence of the slopes of the plots presented in Figure (a) on the concentration of extract (b), the rate constant of the reaction between hydroxyl radicals and antioxidants present in the extract were determined using equation. The rate constants of the reaction between hydroxyl radicals and extract values are 1.3·108 $mol^{-1}\cdot L\cdot s^{-1}$ for 7th extract and 9.45·109 $mol^{-1}\cdot L\cdot s^{-1}$ for vitamin C. 7th extract shows significant antioxidant activity comparable with vitamin C.

Conclusion. Based on the conducted spectral analysis, it is evident that the quantity of extracted organic acids in the 1st, 4th, 5th, and 6th samples surpasses that in the 2nd, 3rd, and 7th samples. The levels of oxalic acid and tartaric acid within the extracts were determined using chromatography. Evaluation of antioxidant activity was performed quantitatively for the 7th extract using the method of competitive reactions. The rate constant of the reaction between hydroxyl radicals and extract ($1.3\cdot10^{8}$ $mol^{-1}\cdot L\cdot s^{-1}$) was compared with vitamin C ($9.45\cdot10^{9}$ $mol^{-1}\cdot L\cdot s^{-1}$) derived by the same method. It was shown that juniper extract possesses significant antioxidant activity, as the rate constant is approximately one order of magnitude lower than of vitamin C.

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ԳԻՀՈՒ ՊՏՈՒՂՆԵՐԻ ԵՎ ՍԵՐՄԵՐԻ ԷՔՍՏՐԱԿՏՆԵՐԻ ՊԱՏՐԱՍՏՈՒՄԸ ԵՎ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Բուսահումքում հակաօքսիդիչների ուսումնասիրությունը գիտական և կիրառական արդի խնդիրներից մեկն է: Սույն աշխատանքի նպատակն է դեղատնային վաճառքի գիհու պտուղների և սերմերի էքստրակտների ստացումը, մրցակցային ռեակցիաների եղանակով դրանց հակաօքսիդիչ հատկությունների ուսումնասիրությունը: Աշխատանքում կիրառվել են էքստրակցիայի մի շարք մեթոդներ, կատարվել են ստացված էքստրակտների հետազոտություններ սպեկտրաչափական և քրոմատոգրաֆիական մեթոդներով:

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ПРИГОТОВЛЕНИЕ И ИССЛЕДОВАНИЕ ЭКСТРАКТОВ ПЛОДОВ И СЕМЯН МОЖЖЕВЕЛЬНИКА

Изучение антиоксидантов среди растений является одной из современных научных и прикладных задач. Целью данной работы является получение экстрактов плодов и семян можжевельника для аптечной продажи, изучение их антиоксидантных свойств методом конкурентных реакций. В работе был использован ряд экстракционных методов, а также проведены исследования полученных экстрактов спектрометрическим и хроматографическим методами.