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INVESTIGATION OF THE POLYPHENOL COMPOSITION OF RED OAK (Quercus rubra L.) RAW MATERIALS

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The aim *of the work was to determine the main groups of polyphenolic compounds in Quercus rubra L. raw materials.*

Materials and methods. *The leaves and annual shoots of Northern Red Oak used for research were collected in August in Lisnyky village of Obuhiv district of Kyiv Oblast (Ukraine). Determination of the component composition and quantitative content of flavonoids (including separately catechins), hydroxycinnamic acids, and phenolic acids were carried out in the samples of air-shade-dried crushed raw material to a particle size of 3 mm by the method of high-performance liquid chromatography (HPLC). Agilent Technologies 1200 liquid chromatograph was used for liquid chromatography.*

Results. *Using the HPLC method, 18 polyphenolic compounds were identified in leaves and annual shoots of Northern Red Oak, in particular, flavonoids: rutin, quercetin-3-β-glucoside, luteolin, neohesperidin; catechins: catechin, epicatechin, epicatechin gallate, gallocatechin; hydroxycinnamic acids: chlorogenic, caffeic, trans-ferulic, trans-cinnamic, p-coumaric, hydroxyphenylacetic, benzoic, syringic, sinapic acids; phenolic acid is gallic acid. The dominant component among flavonoids is rutin (323.43 mg/100 g) (in the composition of catechins, epicatechin gallate (25.45 mg/100 g) prevails); among hydroxycinnamic acids in Northern Red Oak raw materials, chlorogenic acid (139.62 mg/100 g) and sinapic acid (74.64 mg/100 g) prevail.*

Conclusions. *The obtained results point to the prospects of further phytochemical and pharmacological studies of Quercus rubra raw materials, with the aim of creating new plant substances based on it with antioxidant, anti-inflammatory, and antiviral activity*

Keywords: *Northern Red Oak, Quercus rubra, leaves, shoots, flavonoids, catechins, hydroxycinnamic acids, high-performance liquid chromatography (HPLC)*

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1. Introduction

The search for promising types of raw materials of plant origin - potential sources of biologically active substances with antiviral, antitumor, anti-inflammatory and antioxidant effects, in particular polyphenols, remains an urgent issue of pharmaceutical science [1].

In this aspect, red oak (*Quercus rubra L.*) is a promising medicinal plant material, which is used in traditional medicine for colds and viral diseases to increase immunity and as an astringent [2].

The genus oak (*Quercus L.*), a family of *Fagaceae*, includes 464 species [3] distributed in temperate, subtropical and tropical regions. On the territory of Ukraine, there are 7 species of the genus in natural phytocenoses, species: common oak (*Quercus robur L.*), *Quercus petraea Liebl.*, *Quercus pubescens Willd.*, *Quercus polycarpa Schur*, *Quercus dalechampii Ten.*, *Quercus cerris L.*, *Quercus rubra L.*, of which the last 2 species are adventive, about 11 introduced oak species are cultivated in gardens, parks, and forest strips, including red oak [4].

The red oak (*Quercus rubra L., Fagaceae*) is an invasive plant introduced to Europe from North America [5], has acclimatized well in the territory of Ukraine, is resistant to diseases and actively occupies new territories, displacing the common oak from natural habitats, its raw material the base is constantly growing.

According to the literature, the composition of biologically active substances (BAS) of red oak is, to some extent close to common oak [6]. Therefore, it is of interest to conduct pharmacognostic studies of this species as a potential additional source of raw materials alongside the official species.

The official species of the genus is the common oak (*Quercus robur L.*), and the medicinal plant material is bark, the quality of which is regulated by the relevant article of SPhU "Oak Bark". Common oak bark contains tannins (up to 29 %), flavonoids (quercetin, rutin, apigenin, neohesperidin), gallic and ellagic acids (up to 1.6 %) [7]. Experimental and clinical data show that preparations from the bark of common oak exhibit a wide range of pharmacological effects: astringent, anti-inflammatory, antioxidant, antimicrobial, antispasmodic, hypotensive, radioprotective [8, 9].

According to the literature, the raw materials (bark, fruits) of a number of *Quercus* species contain

polyphenols, fatty and hydroxycinnamic acids, and procyanidins [10, 11], which, according to clinical and pharmacological studies, exhibit antibacterial, antimicrobial and antifungal effects against a wide range of microorganisms, and antitumor properties are also noted activity [12, 13].

The red oak species (*Quercus rubra L.*) is poorly studied in terms of BAS content. According to literature sources, 42 phenolic compounds were identified in red oak seeds, including ellagotannins, halotannins, phenolic glycosides, derivatives of hydroxybenzoic acid, and derivatives of ellagic acid [14]. Some studies have established that the fruits of *Quercus rubra* contain β-tocopherol, which is a rather rare phenomenon in the plant world, so the fruits of this species can be used as a source of this isomer of tocopherol [15]. It was established that aqueous extracts of *Quercus rubra* fruits could reduce the viability of HFL-1 (human fetal lung fibroblast) and DLD-1 (colorectal adenocarcinoma) cells and are able to inhibit the growth of gram-positive bacteria (*Bacillus cereus*) [16]. Aqueous and alcoholic extracts of red oak bark exhibit antioxidant and antimicrobial properties [17].

The leaves of *Quercus rubra* accumulate a significant amount of tannins, the content and ratio of which can vary depending on climatic conditions; in particular, the content of condensed tannins in green leaves ranges from 29 to 89 % of the total tannin content and 53–88 % in fallen leaves. About 69 % of hydrolyzed tannins with a predominant content of ellagotannins are contained in the green leaves of *Quercus rubra* (collected during the first week of September), the content of which decreases during the growing season to 11 % (in the leaves collected in the second week of October) [18]. *Quercus rubra* roots also contain condensed (75 %) and hydrolyzed (25 %) tannins. Among the hydrolyzed tannins, ellagotannins account for nearly 50 % of the total content, while the content of halotannins is only 5 % [19].

Given the presence of a significant raw material base, separate scattered literature data on the content of some polyphenolic compounds in raw materials, as well as information on antimicrobial, antifungal, and antitumor effects, the study of the spectrum of polyphenolic compounds in red oak raw materials seems promising.

Based on this, **the aim of our research** was to determine the main groups of polyphenolic compounds in *Quercus rubra* raw materials.

2. Research planning (methodology)

The planning of studies of medicinal plant material *Quercus rubra* is shown in Fig. 1.

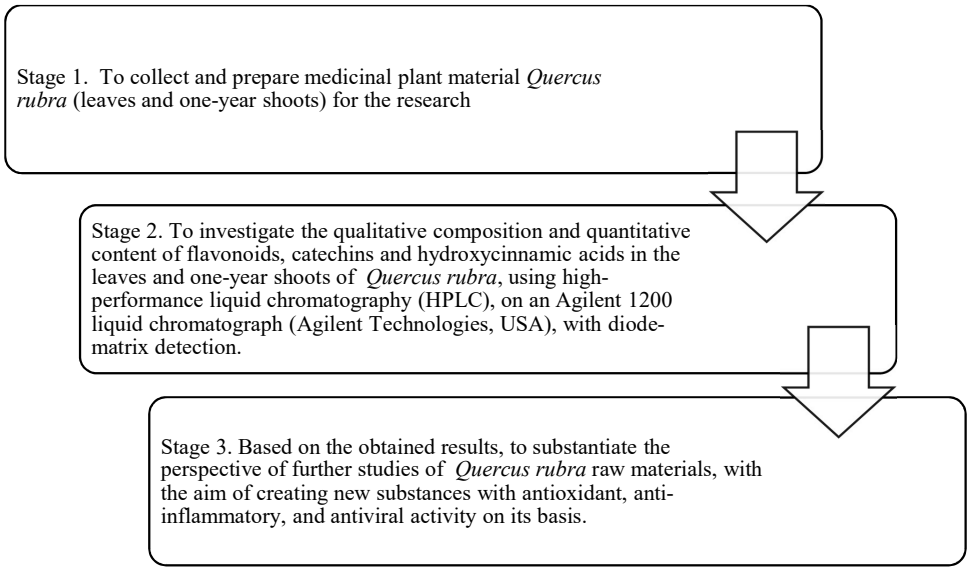

Fig. 1. Stages of the study of the content of polyphenolic compounds in the leaves and one-year shoots of the red oak *Quercus rubra*

3. Materials and methods

The object of the study was leaves and one-year shoots of red oak (*Quercus rubra L., Fagaceae*) collected in August 2020 in the Lisnyky village of Obuhiv district, Kyiv region, Ukraine.

Species affiliation was determined by Candidate of Biological Sciences, senior researcher of the National Museum of Natural History at the National Academy of Sciences of Ukraine O.F. Shcherbakova.

Determination of the component composition and quantitative content of flavonoids and (separately) catechins, hydroxycinnamic acids, and phenolic acids were carried out in samples of air-shade-dried raw materials crushed to a particle size of 3 mm by the HPLC method.

Sample preparation for analysis was carried out as follows.

For further study of flavonoids, a weight of raw material of each sample weighing 0.2–0.6 g was extracted with 10 ml of a 70 % solution of ethyl alcohol P in a Bandelin Sonorex Digitec ultrasonic bath at 80 °C for 5 hours in glass hermetic vials with Teflon lids. The obtained extract was centrifuged at 3000 rpm using an Eppendorf Centrifuge 5415 C and filtered through disposable membrane filters with pores of 0.22 μm (Sartorius, Minisart) [20].

To determine catechins, a weight of raw material of each sample weighing 0.2–0.6 g was extracted with 10 ml of a 70 % solution of ethyl alcohol P in a Bandelin Sonorex Digitec ultrasonic bath at 80 °C for 5 hours in glass hermetic vials with Teflon lids. The obtained extract was centrifuged at 3000 rpm using an Eppendorf Centrifuge 5415 C and filtered through disposable membrane filters with pores of 0.22 μm (Sartorius, Minisart) [21].

Extraction of hydroxycinnamic acids, phenolic acids and quinic acid from raw materials (weight of raw materials of each sample – 0.1–1.0 g) was carried out with 5–10 ml of 60 % methanol solution P in an ultrasonic bath Bandelin Sonorex Digitec at 80 °C for 4 hours in glass hermetic vials with Teflon lids. The obtained extract was centrifuged at 3000 rpm using an Eppendorf Centrifuge 5415 C and filtered through disposable membrane filters with pores of 0.22 μm (Sartorius, Minisart) [22].

The identification and determination of the quantitative content of polyphenols in red oak raw materials were carried out by the method of high-performance liquid chromatography in accordance with the requirements of the current edition of SPhU/EP, 2.2.29.

Liquid chromatography was performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) equipped with a diode array detector.

Sigma-Aldrich (Steinheim, Germany) HPLC quality solvents were used to prepare the mobile phases.

Stock solutions were prepared using 70 % ethanol P (flavonoids, catechins) or 60 % methanol P (hydroxycinnamic acids, phenolic acids, quinic acid) as an extractant. The concentration was selected experimentally, obtaining the correct value of the response, considering the achievement of the suitability parameters of the chromatographic system. Detection was carried out at the wavelengths at which the maximum absorption of certain groups of polyphenols in the UV region is observed (for flavonoids – 280 and 365 nm; for catechins, hydroxycinnamic acids, phenolic acids, quinic acid – 250 and 275 nm) [20–22].

When choosing the mobile phase, it was considered that the pH value and the content of the organic modifier are the main parameters when optimizing the composition of the mobile phase. For separation, a mobile phase was used - a mixture of acetonitrile and a 0.1 % solution of formic acid P in water P (flavonoids) or a mixture of methanol P and a 0.1 % solution of formic acid P in water P (catechins, hydroxycinnamic acids, phenolic acids, quinine acid) in a gradient mode, in order to achieve the required retention time of the corresponding compounds. By changing the pH value in the gradient mode, you can clearly separate all components.

To separate flavonoids, acetonitrile for chromatography P (*A*) and a 0.1 % solution of formic acid P in water P (*B*) was used as the mobile phase. Elution was carried out in the gradient mode: 0 min – *A* (30 %): *B* (70 %); 20 min – *A* (70 %): *B* (30 %); 22 min – *A* (100 %): *B* (0 %); 30 min – *A* (100 %): *B* (0 %). The separation was carried out on a Zorbax SB–C18 chromatographic column (3.5 μm, 150×4.6 mm) (Agilent Technologies, USA); the flow rate through the column was 0.25 ml/min. The flow rate of the mobile phase was chosen considering the pressure in the system, which for this type of column cannot exceed 400–600 bar and the type of mobile phase. Thermostat temperature 30 °C, injection volume 4 μl. Detection was carried out using a diode-matrix detector with signal registration at 280 and 365 nm and fixation of absorption spectra in the range of 210–700 nm [20].

To determine catechins, methanol P (*A*) and a 0.1 % solution of formic acid P in water P (*B*) were used as the mobile phase. Elution was carried out in the gradient mode: 0 min – *A* (20 %): *B* (80 %); 25 min – *A* (75 %): *B* (25 %); 27 min – *A* (100 %): *B* (0 %); 35 min – *A* (100 %): *B* (0 %). The separation was carried out on a Zorbax SB–C18 chromatographic column (3.5 μm, 150×4.6 mm) (Agilent Technologies, USA), the flow rate through the column was 0.25 ml/min, the temperature of the thermostat was 35 °C, the volume injections of 4 μl. Detection was carried out using a diode–matrix detector with signal registration at 250 and 275 nm and fixation of absorption spectra in the range of 210–700 nm [21].

To determine hydroxycinnamic acids, phenolic acids, and quinic acid, methanol P (*A*) and a 0.1 % solution of formic acid P in water P (*B*) was used as the mobile phase. Elution was performed in gradient mode: 0 min – *A* (25 %): *B* (75 %); 25 min – *A* (75 %): *B* (25 %); 27 min – *A* (100 %): *B* (0 %); 35 min - *A* (100 %): *B* (0 %). Separation was performed on a Zorbax SB-Aq chromatographic column (4.6 mm±150 mm, 3.5 μm) (Agilent Technologies, USA), column flow rate 0.5 ml/min, thermostat temperature 30 °C, volume injection container 4 μl. Detection was carried out using a diode-matrix detector with signal registration at 250 and 275 nm and fixation of absorption spectra in the range of 210–700 nm [22].

To assess the suitability of the chromatographic system, 20 μl of the comparison solution was injected, and 5 consecutive chromatograms were obtained.

According to SPhU/EP recommendations (2.2.29, 2.2.46), the chromatographic system is considered suitable if:

1) the symmetry coefficient (T) of the peak of the substance, calculated from the chromatograms of the reference solution, is 0.8–1.5;

2) the efficiency of the chromatographic column (*N*), calculated from the peak of the substance on the chromatogram of the comparison solution, will be at least 5,000 theoretical plates. After confirming the suitability of the system, in these conditions, the blank, tested solutions were injected, and calculations and evaluation of the obtained results were carried out.

Satisfactory linearity of the methods was achieved in the linear range (0.2–100 μg/ml for all types of compounds) and at the correlation coefficient (r^2>0.993).

Identification and quantitative analysis were carried out using standard control samples from Sigma-Aldrich (Steinheim, Germany): flavonoids (rutin, quercetin-3-β-glucoside, naringin, neohesperidin, quercetin, naringenin, kaempferol, luteolin, apigenin), catechins (pyrocatechin, catechin, epicatechin, epicatechin gallate and halocatechin), phenolic compounds (gallic acid, hydroxyphenylacetic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, trans-ferulic acid, sinapic acid, trans-cinnamic acid) and quinic acid [20–22].

The amount of compound (*X*) (μg/g) was determined by the formula:

$$X=c*V/m,$$

where *c* is the concentration of the compound determined chromatographically, μg/ml; *V* – the volume of extract, ml; m is the mass of the raw material from which the extraction was carried out, g.

4. Research results

Using the HPLC method, 18 polyphenol compounds were identified in the leaves and one-year shoots of red oak: flavonoids – rutin, quercetin-3-β-glucoside, luteolin, neohesperidin, separately catechins – catechin, epicatechin, epicatechin gallate, halocatechin; hydroxycinnamic acids – chlorogenic, caffeic, trans-ferulic, trans-cinnamic, p-coumaric, syringic, sinapic acids; phenolic acids – gallic, hydroxyphenylacetic, benzoic acids.

The results of the chromatographic separation of flavonoids are shown in Fig. 2, of catechins – in Fig. 3, of hydroxycinnamic acids – in Fig. 4, the quantitative content of individual compounds of a polyphenolic nature is shown in the Table 1–3.

Table 1

The content of flavonoids in raw materials (leaves+shoots) of red oak

No.	The name of the compound	Quantitative content, mg/100 g in terms of absolutely dry raw materials
1	Rutin	323.43
2	Quercetin-3-β-glucoside	7.05
3	Neohesperidin	16.12
4	Quercetin	7.97
5	Luteolin	11.96

As can be seen from the obtained results, the dominant component among flavonoids is rutin (323.43 mg/100 g), the content of neo hesperidin (16.12 mg/100 g) and luteolin (11.96 mg/100 g) is significantly lower.

Table 2

Content of catechins in raw materials (leaves + shoots) of red oak

No.	The name of the compound	Quantitative content, mg/100 g in terms of absolutely dry raw materials
1	Catechin	2.28
2	Epicatechin	1.54
3	Epigallocatechin gallate	25.45
4	Gallocatechin	1.67

The analysis of the obtained results shows that epicatechin gallate (25.45 mg/100 g) prevails among catechins in terms of quantitative content.

Table 3

The content of hydroxycinnamic and phenolic acids in raw materials (leaves + shoots) of red oak

No.	The name of the compound	Quantitative content, mg/100 g in terms of absolutely dry raw materials
1	Gallic acid	44.66
2	Hydroxyphenylacetic acid	29.61
3	Chlorogenic acid	139.62
4	Caffeic acid	5.34
5	Syringic acid	3.28
6	Benzoic acid	5.27
7	p-Coumaric acid	6.01
8	trans-Ferulic acid	8.05
9	Sinapic acid	74.64
10	trans-Cinnamic acid	2.28

The highest content among hydroxycinnamic and phenolic acids in red oak raw materials is established for chlorogenic acid (139.62 mg/100g), the content of sinapic (74.64 mg/100 g) and gallic acids (44.66 mg/100 g) is somewhat lower.

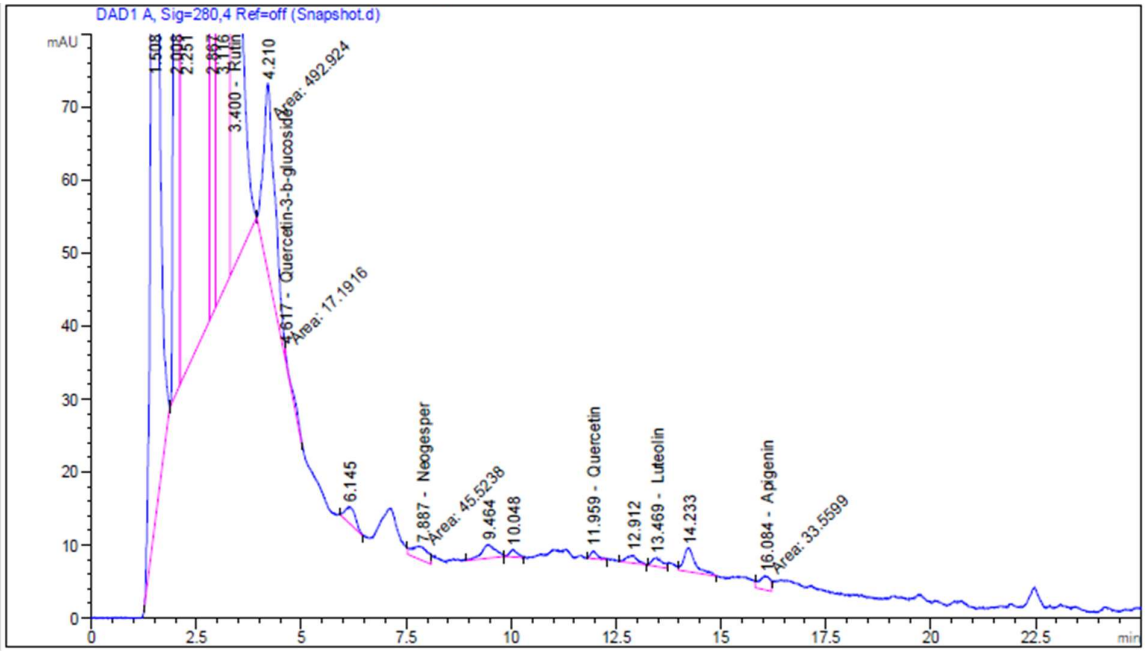

Fig. 2. Chromatogram of flavonoids of red oak leaves and shoots

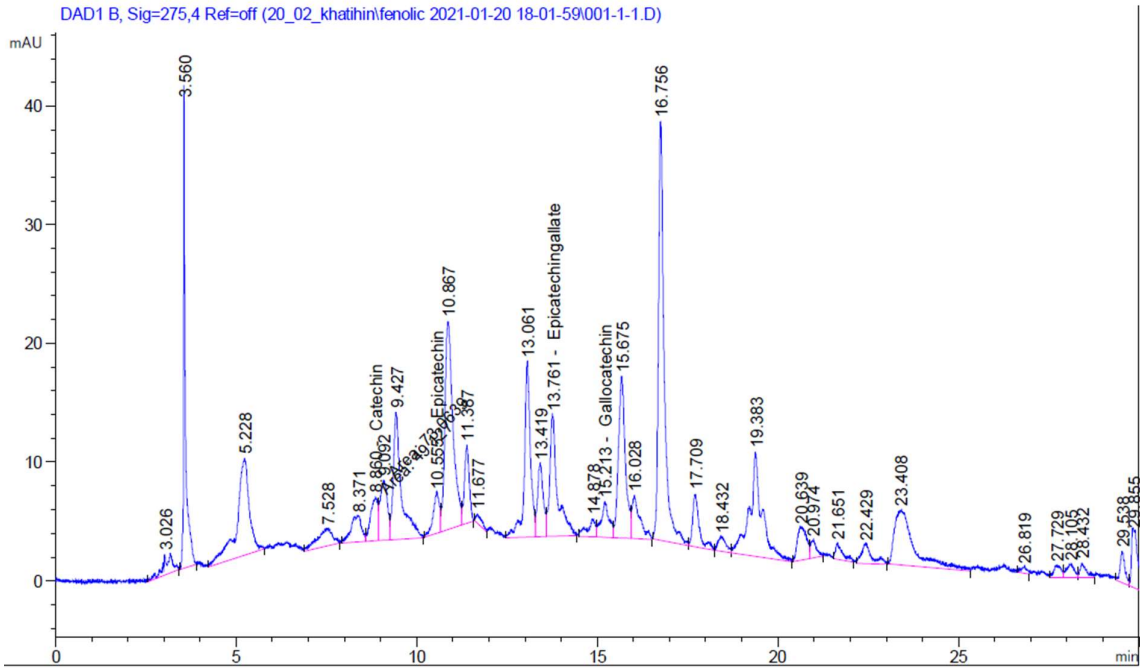

Fig. 3. Chromatogram of catechins of red oak leaves and shoots

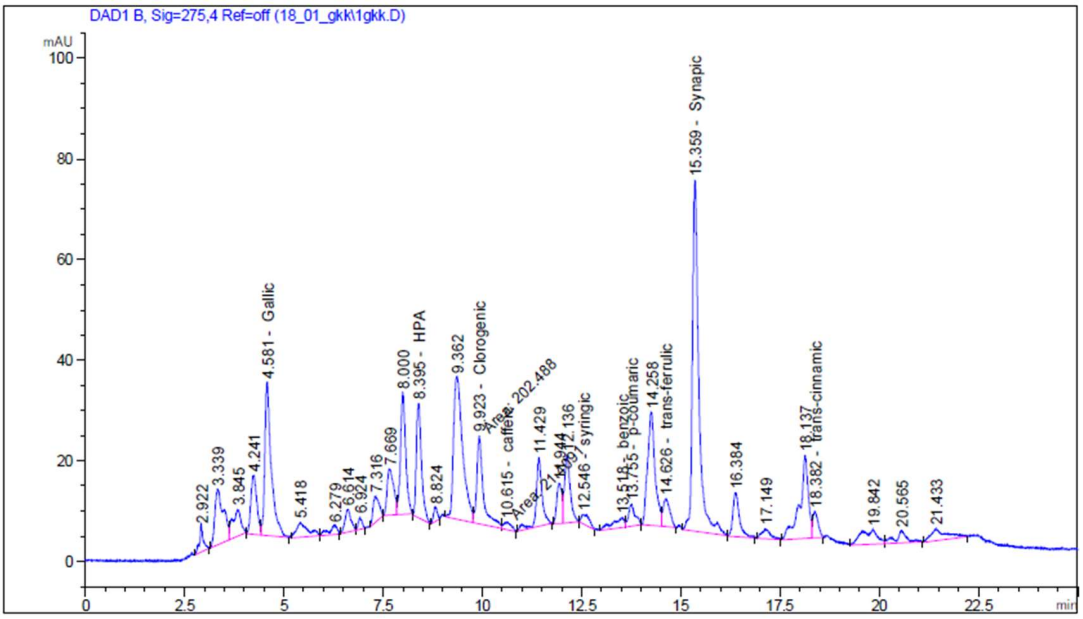

Fig. 4. Chromatogram of hydroxycinnamic acids of red oak leaves and shoots

5. Discussion of research results

According to the obtained results, in the studied raw material of red oak, rutin (323.43 mg/100 g) significantly prevails from the group of phenolic compounds in terms of quantitative content. It is known that rutin exhibits P-vitamin activity, increases the elasticity of blood vessels, and has pronounced antioxidant, antitumor, and antiradiation properties [23, 24].

In view of this, the leaves and annual shoots of red oak can be used as a raw material containing rutin and potentially having the above-mentioned pharmacological effects.

The content of chlorogenic acid (139.62 mg/100 g) in the raw material of red oak is quite high, which exhibits its antidiabetic, hepatoprotective, anti-inflammatory, analgesic and antibacterial properties and helps to reduce blood pressure [25, 26].

Sinapic acid, the content of which in the leaves and shoots of red oak is 74.64 mg/100 g, according to literature sources, shows antioxidant, anti-inflammatory, antimutagenic, antitumor, neuroprotective and antibacterial pharmacological effects [27].

Based on the obtained data on the polyphenolic composition of leaves and one-year shoots of red oak, this raw material can be considered promising for the further development of medicines with P-vitamin, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antiviral, analgesic and antibacterial effects.

The results of the research will be used to develop quality control methods for raw leaves and one-year shoots of red oak.

Study limitations. When studying the polyphenol composition in leaves and one-year shoots of red oak by the method of high-performance liquid chromatography (HPLC) in comparison with standards, the number of standards was limited, so not all polyphenolic compounds could be identified.

Prospects for further research. It is promising to develop phytopreparations with potential P-vitamin, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antiviral, analgesic, and antibacterial activity, standardized by the content of polyphenolic compounds from various types of red oak raw materials (leaves, one-year shoots, bark, fruits) and their determination pharmacological action. The results of the research will be used to develop quality control methods for promising red oak raw materials**.**

6. Conclusions

Using the HPLC method, 18 polyphenolic compounds were identified in the leaves and shoots of the red oak (*Quercus rubra*), and it was established that rutin (323.43 mg/100 g), chlorogenic acid (139.62 mg/100 g), and sinapic acid (74.64 mg/100 g).

The obtained results indicate the prospects of further phytochemical and pharmacological studies of *Quercus rubra* raw materials with the aim of creating new plant substances based on it with P-vitamin, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antiviral, analgesic and antibacterial activity.

The results of the conducted research will be used to develop quality control methods for promising *Quercus rubra* raw materials.

Conflict of interests

The authors declare that they have no conflict of interests in relation to this research, including financial, personal, authorship or other nature, which could affect the research and its results presented in this article.

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Availability of data

The manuscript has no associated data.

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