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STUDY OF FLAVONOID COMPOSITION AND BIOLOGICAL ACTIVITY OF LEAVES OF MAPLE SEMENOV ACER SEMENOVII

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Abstract

The results of a study of the content of polyphenolic compounds and their antioxidant activity in the plant - Acer semenovii - family Aceraceae Juss., growing in the mountainous regions of the Tashkent region of the Republic of Uzbekistan are presented. The collection of raw materials took place in the spring at the end of April - beginning of May and in the fall at the beginning of September 2022-2023. The conditions for the isolation of polyphenolic compounds from the leaves of Maple Semenov under various conditions were selected. It has been shown that the optimal content of polyphenols is extracted with 40% ethyl alcohol, followed by fractionation of the aqueous residue with ethyl acetate and precipitation with chloroform. It was shown that in plants collected in the spring, the yield of total polyphenols was 8.9% of the air-dry mass of the raw material. Chromatographic methods (BC and TLC) revealed that the leaves of plants collected in the spring contain more polyphenols than those of plants collected in early autumn. Compounds belonging to the class of poliphenolic compounds, flavonols, phenolic acids and flavan-3-ols were found in this plant.

Polyphenols such as rutin, quercetin, hyperoside, kaempferol, catechin and gallic acid were identified using HD and TLC methods.

The antioxidant activity of the sum of polyphenolic compounds was also studied in a model of lipid peroxidation (LPO) in rat liver mitochondria. It has been established that polyphenols have a protective effect on mitochondria, reducing the damaging effect of Fe /ascorbate and antioxidant activity depends on the concentration of the studied polyphenolic substances. Adding rutin to the incubation medium at a concentration of 5 μM inhibits lipid peroxidation processes by 32.0%, and at 10 μM - by 85.9% and at 20 μM - by 96.8%, compared to the control.

Keywords: Polyphenols, extraction, Acer Semenov, LPO, antioxidant activity.

Introduction

Semenov's maple (Latin name Ácer semenóvii) is a type of maple widely distributed in Central Asia, namely in the Tien Shan mountains, Eastern Pamir-Alai, Uzbekistan, Kazakhstan, Afghanistan and Iran. The plant was named after the Russian traveler Semenov-Tyan-Shansky, who first found this plant in the mountains of Central Asia [1]. Studying the results of the study and the ways of using this plant material, we can notice a huge variation in the use of this medicinal plant. Maple is very effective against bruises and liver metabolism disorders, and also in the treatment of various eye diseases and rheumatism [2, 3]. Maple exhibits a pronounced antiviral, tonic, and antibacterial effect against gram-negative and gram-positive bacteria and viruses. In addition, the leaves have a diuretic, antipyretic, wound-healing, choleretic, antiseptic, anti-inflammatory, analgesic, and tonic effect. Along with these beneficial properties, maple improves the digestive process, normalizes the functioning of the gastrointestinal tract, relieves inflammation of bacterial etiology, has a beneficial effect on the central nervous system, improves muscle function, normalizes blood circulation, stops bleeding, lowers blood pressure, and relieves joint pain. [4]. The juice contains organic acids (especially ascorbic acid). Saponins, traces of alkaloids, and tannins were found in fruits, bark, branches, and leaves. In addition, carbohydrates, alkaloids, aldehydes (alphahexene, beta-hexanoic), organic acids (acetic, succinic, phthalic), rubber, carotenoids (alpha-, beta-carotene, xanthophyll, etc.), nitrogen-containing compounds (methylamine) were found in the leaves etc.), vitamins C, E, phenol carbonic acids (salicylic, gallic), flavonoids, anthocyanins, higher fatty acids, lipids (phytinyl linolenate). Cyclotols, rubber and fatty oils were detected in the seeds. [5]. Their individual elements are substances such as tannins and polyphenol oxidase, ascorbic acid and ascorbate oxidases [6]. Polyphenol oxidase and peroxidase, in combination with phenolic substrates, are involved in the respiration process at intermediate stages of hydrogen transfer. The works contain

data indicating an increase in polyphenol oxidase activity in damaged tissues. The enzyme ascorbate oxidase is involved in the neutralization of reactive oxygen species, thereby protecting the plant organism and preventing the occurrence of oxidative stress [7,8,9]. Under laboratory conditions, the content of condensed tannins in the leaves of woody plants was determined by the permanganatometric method (Levental Kirsanov method). The quantitative content of ascorbic acid was determined in accordance with GOST 24556-89 [10,11]. The activity of polyphenol oxidase was determined by a spectrophotometric method based on measuring the optical density of the reaction products that are formed during the oxidation of catechol over a certain period of time [12].

In this regard, the purpose of our work is to study the chemical composition and biological activity of polyphenolic compounds in the leaves of the plant Ácer semenóvii. – Semenov maple, collected during different periods of the growing season. Comparison of the composition of plants growing on the territory of the Republic of Uzbekistan was carried out using two-dimensional paper and TLC methods.

Experimental part

Object of study.

The object of the study was the leaves of the Semenov maple (Ácer semenóvii). The raw materials were collected in the spring at the end of April - beginning of May and in the fall at the beginning of September 2022-2023. Place of selection and sampling - Tashkent region, Republic of Uzbekistan.

Isolation of polyphenols. In order to isolate the amount of polyphenols, methods of extraction of raw materials with organic solvents were used. We have optimized this method. For this purpose, a study was carried out to study the yield of the amount of polyphenols on the degree of grinding of the raw materials, the composition of the extractant, the extraction module, the extraction frequency, the ratio of raw materials to the extractant, the extraction temperature, the conditions of thickening, the treatment of the aqueous residue with organic solvents, the conditions for the precipitation of the amount of polyphenols and their drying. Based on the results obtained, an optimal method for isolating the amount of polyphenols was developed. It is as follows: air-dried crushed (to a size of 2-4 mm) leaves of Ácer semenóvii (100 g) are pre-extracted with chloroform to remove lipophilic substances. To do this, the raw material was placed in a 3-liter flask equipped with a reflux condenser and extracted with chloroform in a water bath in ratios from 1:4 to 1:12 (raw material: chloroform), at a temperature from 25 ° C to 75 ° C for 2 h, from one to five times

extraction of raw materials. After treating the raw material with chloroform, extraction was continued in aqueous ethyl alcohol at concentrations from 30% to 70%. Extraction was repeated from one to three times. Next, the resulting water-ethyl extracts were evaporated under vacuum on a rotary evaporator until the remainder of the aqueous part remained. Purified aqueous extracts were treated with ethyl acetate on a separatory funnel, in ratios from 1:1 to 1:6 (aqueous residue: ethyl acetate). Ethyl acetate fractions were concentrated and precipitated with chloroform in ratios from 1:1 to 1:5 (concentrate:precipitant), a flocculent precipitate was formed.

For the extraction of plant materials, we used solvents from JSC "Himreaktivkomplekt" (Uzbekistan), all other reagents were produced by Reakhim (Russia). UV spectra of polyphenols were taken in an alcohol solution on an EPS-3T device from Hitachi (Japan), IR spectra were taken on an IRTracer-100 device (Shimadzu, Japan) in the region of 400-3800 cm^{-1}.

The separation of polyphenols was carried out by column chromatography on polyamide and silica gel LS 100/40 (Czechoslovakia). To identify and determine the homogeneity of substances, the methods of BC (chromatography paper of the Filtrak brand) and TLC (on Silufol UV-254 plates (eluent benzene: acetone-9:4)) were used.

To separate and study the composition of polyphenols, the following solvent systems were used:

1). n-butanol-acetic acid-water (40:12:28);
2). 2% acetic acid;
3). n-butanol-acetic acid-water (4:1:5);
4). diethyl ether-ethyl acetate (7:3);
5). diethyl ether-ethyl acetate (4:6);

The following reagents were used as developers for spraying chromatograms:

1). 1% solution of vanillin in concentrated hydrochloric acid;
2). 1% aqueous and alcoholic solutions of $FeCl_3$;
3). A mixture of 1% aqueous solutions of $FeCl_3$ and $K_3[Fe(CN)_6]$;
4). Catechin reagent (1% solution of picric acid in 95% ethanol and 5% solution of KOH in 80% ethanol);

Study of the antioxidant properties of polyphenols using a model of mitochondrial swelling. Some polyphenols, exhibiting antiradical properties, overcome the oxidation of lipids and proteins in biological membranes. In order to identify the antioxidant properties of hawthorn polyphenols, the effect of different concentrations of the total polyphenols of the plant Ácer semenóvii, induced by the Fe^{2+}/ascorbate system of mitochondrial LPO, was studied. Mitochondria were isolated from the liver of rats weighing 180-200 g using the Schneider differential centrifugation method. The

animal was decapitated, the liver was removed and placed in a beaker with ice-cold isolation medium containing 250 mM sucrose, 10 mM Tris-chloride, 1 mM EDTA, pH 7.4. To release sucrose from mitochondria, they were resuspended with a buffer solution of potassium chloride (175 mM KCl, 25 mM Tris-chloride, pH 7.4) and centrifuged again [13]. Then we studied the process of lipid peroxidation of mitochondrial membranes in the Fe2+/ascorbate system. LPO was studied in the incubation medium: KCl - 125 mM, Tris-HCl - 10 mM, pH 7.4; $FeSO_4$ – 20 μM, ascorbate – 600 μM were added to the incubation medium; the final mitochondrial protein concentration was 0.5 mg/ml. Activation of LPO disrupts the functions of mitochondrial membranes and this process was recorded photometrically. Mitochondrial protein content was determined by the biuret method [14] using bovine serum albumin as a standard.

The discussion of the results

The process of isolating polyphenols from plant raw materials includes a number of stages: extraction of raw materials, processing of the extraction with organic solvents, evaporation, precipitation of the amount of polyphenols, purification, etc. etc. Increasing the efficiency of raw material use is achieved mainly at the first stage - extraction.

The yield of the sum of polyphenols was studied depending on: the composition of the extractant, the extraction module, the extraction frequency, the ratio of raw materials to extractant, the extraction temperature, the conditions of thickening, the treatment of the aqueous residue with organic solvents, the conditions for the precipitation of the sum of polyphenols and their drying [15]. To determine the optimal extraction module, the extractant raw materials were taken in ratios of 1:4, 1:6, 1:8, 1:10, 1:12. The extraction efficiency was judged by the yield of extractive substances, calculated as a percentage. The results obtained are shown in Fig. 1.

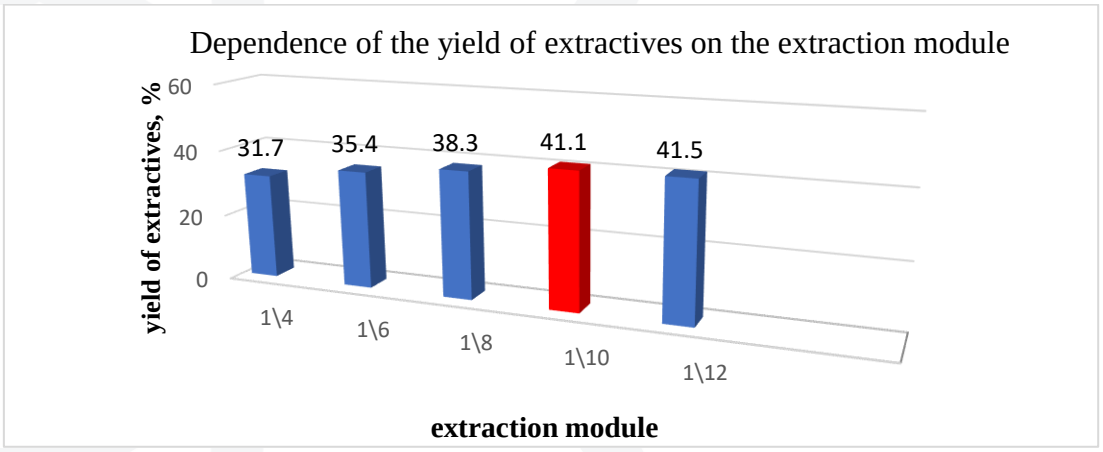

Fig. 1

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From the given data it is clear that the optimal ratio of raw materials: extractant is 1:10. The average yield of extractives was 41.1%. At a lower extraction module (1:4; 1:6; 1:8), the yield of extractives is not complete, and the use of a ratio of 1:12 leads to an increase in the extraction volume, which in turn leads to excessive consumption of organic solvents.In a similar way, we conducted research on the optimal extraction frequency. In this case, 1-, 2-, 3-, 4-, 5-fold extraction methods were used. The results obtained are shown in Fig. 2

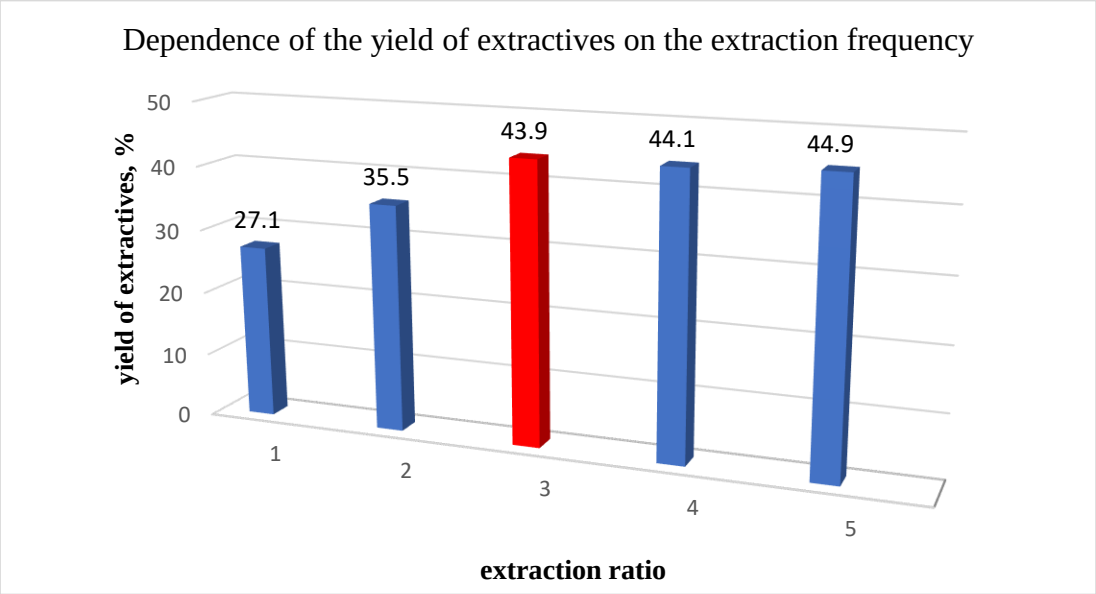

Fig. 2

The efficiency of extraction was judged by the yield of extractives. As can be seen from the data presented, the most optimal is 3-fold extraction. The average yield of extractives was 44.0%. With a low extraction frequency (1-, 2-fold), the yield of extractives is incomplete, and with an increase in the extraction frequency (4-, 5-fold), the yield of extractives changes slightly. At the same time, the extraction process takes a lot of time and the volume of the extract increases, which also further leads to an increase in the consumption of organic solvents.

To determine the optimal composition of the extractant, we used an aqueous solution of ethanol of different concentrations. 30%, 40%, 50%, 60%, 70% aqueous solutions of ethanol were used as an extractant. The results obtained are shown in Fig. 3.

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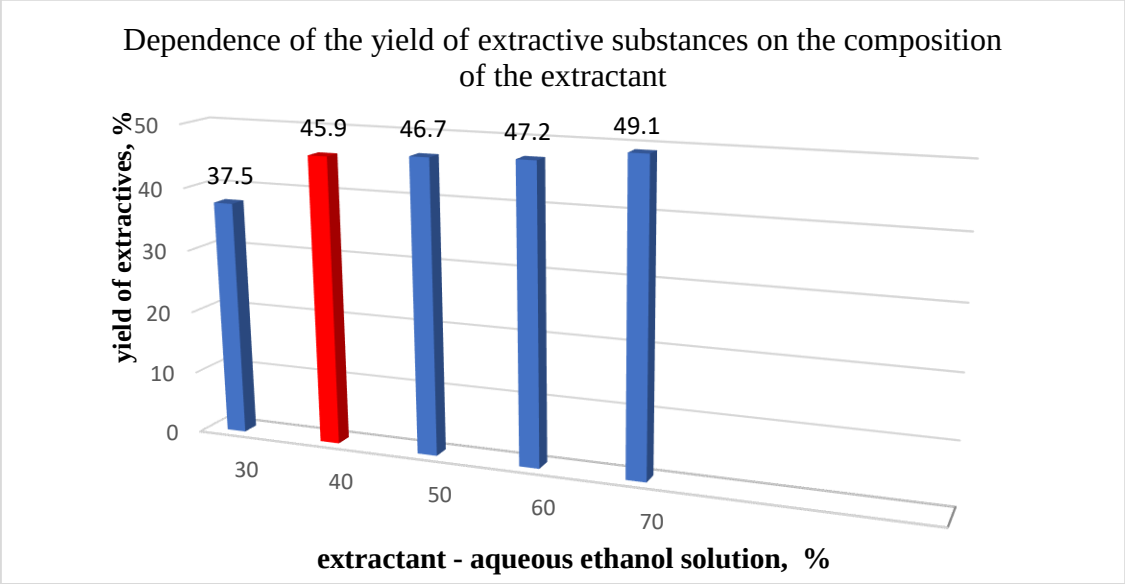

Fig. 3

As can be seen from the data presented, the most optimal extractant is 40% aqueous ethanol. At the same time, the average yield of extractives was 46.0%. The use of aqueous ethanol with a low concentration leads to incomplete extraction of extractives from raw materials, and the use of a higher concentration of ethanol does not lead to an increase in the yield of extractives.

In a similar way, we searched for the optimal extraction temperature. In this case, the extraction was carried out in the following temperature conditions: 20-25°C, 30-35°C, 40-45°C, 50-55 °C, 60-65 °C, 70-75 °C. From the data presented it is clear that the most optimal extraction temperature is 40-45 °C and under this regime the average value of extractives was 39.1%. At lower temperatures, the yield of extractive substances is incomplete, and when the extraction temperature increases to 70 °C and above, polyphenolic substances are hydrolyzed, which is confirmed by paper chromatography data.

In a similar way, the dependence of the yield of extractives on the extraction time was studied, which varied from 1 to 3 hours. It was found that the most optimal is a 2-hour extraction.

The resulting extracts were concentrated under vacuum to an aqueous residue. The extraction of polyphenols from the aqueous residue was carried out with ethyl acetate in a ratio of 1:1; 1:2; 1:3; 1:4; 1:5; 1:6. Ethyl acetate extracts were combined, dried over anhydrous Na_2SO_4 and concentrated. The results obtained are shown in Fig. 4.

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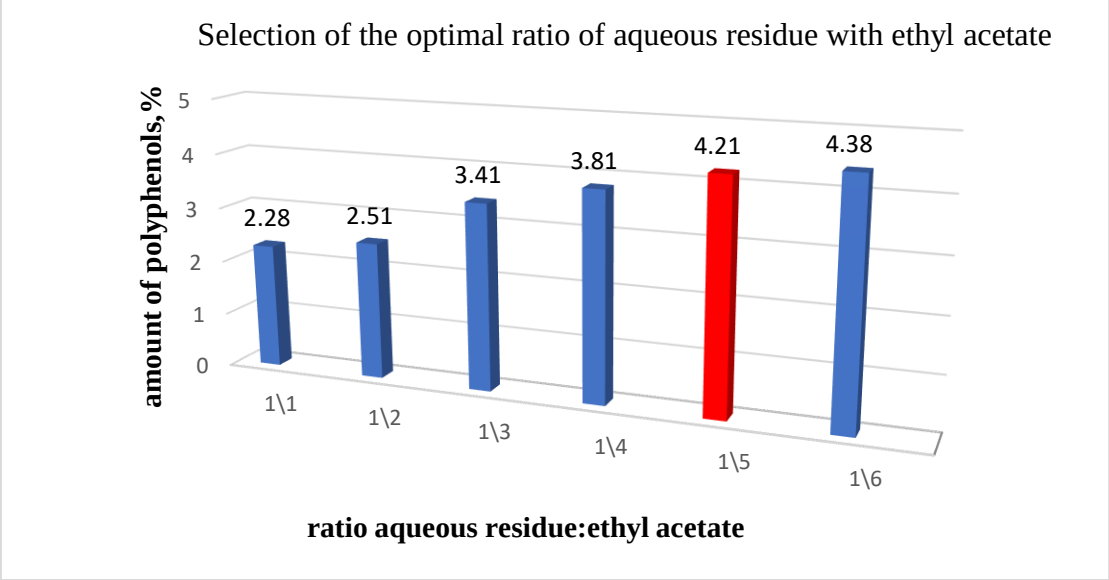

Fig. 4

From the above data it is clear that the optimal ratio of aqueous residue to ethyl acetate is 1:5. At low ratios of aqueous residue and solvent, incomplete extraction of polyphenols from the aqueous residue occurs. The yield of polyphenols increases slightly at a ratio of 1:6, but the solvent consumption increases.Chloroform was used to precipitate polyphenols from the ethyl acetate concentrate. The ratio of concentrate to precipitant was 1:1; 1:2; 1:3; 1:4; 1:5.

From the given data it is clear that the optimal ratio of concentrate and precipitant is 1:4. The average yield of total polyphenols was 4.28%. At a ratio of 1:5, the yield of polyphenols increases slightly, but the consumption of the precipitant increases significantly. At a low ratio of ethyl acetate concentrate to precipitant, incomplete precipitation of polyphenols occurs. The completeness of precipitation of polyphenols is determined by the appearance of turbidity or sediment upon additional addition of chloroform to the mother liquor.

Based on the results obtained, an optimal method for isolating the amount of polyphenols was developed. The raw material, crushed to 2-4 mm, was dried under a canopy. The dried raw materials were treated with chloroform to remove resinous substances, pigments and other related impurities. The material thus treated was dried under air draft to remove residual solvent, and the dried material was extracted three times with 40% ethyl alcohol in a ratio of 1:10. The resulting extracts were combined, concentrated under vacuum to a small volume, and the remaining aqueous residue was further treated with chloroform, then repeatedly with ethyl acetate (aqueous residue: ethyl acetate - 1:5).

The combined ethyl acetate extracts were dried over freshly calcined anhydrous sodium sulfate and concentrated under vacuum in a stream of nitrogen at 40-45°C [16,17]. From a concentrated ethyl acetate extract (1.5 L), polyphenols were precipitated by adding a fourfold amount of chloroform. The resulting flocculent precipitate was filtered on a glass filter, redissolved in absolute ethyl alcohol, concentrated and reprecipitated. The precipitate was filtered through a Schott funnel, dried in a vacuum drying cabinet, the yield of the total polyphenols was 8.28% from spring leaves.

Using the above method, the amount of polyphenols from the autumn leaves of the Semenov maple was also obtained and the resulting yield was 3.2%.

The sum of polyphenols obtained in this way from the ethyl acetate fraction of Semenov maple leaves is an amorphous powder of light brown color with an astringent taste. With ferric chloride the sum gives a blue color (developers 2 and 3), with a 1% solution of vanillin in concentrated hydrochloric acid - a bright red color (developer 1).

During a chromatographic study of the isolated fractions, it was found that the polyphenols of the ethyl acetate fraction are represented mainly by monomeric catechins with a small admixture.

Two-dimensional chromatography on paper in solvent systems 1 and 2 showed that the total polyphenols of the ethyl acetate fraction contain catechins, namely (+)-catechin, (-)-epicatechin.

5 g of the preparation of the sum of polyphenols from the ethyl acetate fraction was repeatedly ground in a mortar with wet diethyl ether (total volume - 1000 ml). In this case, only monomeric catechins, completely freed from condensation products and verified by paper chromatography, are transferred into the ether. The ether solution was chromatographed on a column (4.5x70 cm) with silica gel (100 g), using peroxide-free water-saturated diethyl ether as an eluent (system 4, 5). The separation was monitored using paper chromatography in system 1. As a result, flavan-3-ols were isolated in the individual state. The isolated flavan-3-ols were identified as: (+)-catechin, (-)-epicatechin.

(+)-catechin - 5,7,3',4'-tetrahydroxyflavan-3-ol. Mol mass 290, mp. 172-173°C, Rf 0.64 (system 1), λ_{max} = 280 (in ethanol), [α]¬D-16.90 (ethanol, s 1.05).

(-)-epicatechin – (5,7,3',4'-tetrahydroxyflavan-3-ol), Mol. weight 290, mp. 235°C, Rf 0.56 and 0.30 (systems 1 and 2), λ_{max} =276 nm (in ethanol), [α]-D-600 (acetone-water 1:1, s 1.22).

(-)-Epigallocatechingallate-(2R,3R)-3′,4′,5,5′,7-pentahydroxyflavan-3-yl gallate. $C_{22}H_{18}O_{11}$, mol mass 458.37, mp. 257-258 ° C, Rf 0.62 (system 1), λ_{max} max= 270 (in ethanol), $[\alpha]_D$ -14.60 (ethanol, s 1.05).

The remaining compounds were separated on a polyamide column using a water-ethanol mixture in various ratios as an eluent, and 4 compounds were isolated. Using physicochemical methods, the structures of these compounds were established.

Gallic acid - (3,4,5-trihydroxybenzoic acid). $C_7H_6O_5$, mol mass 458.37 white crystals from water, m.p. 221-223°C, Rf 0.51 in system 1 (n-butanol-acetic acid-water 4:1:5 – upper phase).

Rutin-(quercetin-3-rutinoside).-(eluted with 30% ethanol): $C_{21}H_{30}O_{16}$, mp. 190–192 °C (from CH_3OH), Rf 0.45 in system 2 (n-butanol-acetic acid-water 4:1:2). UV spectrum (C_2H_5OH, λmax, nm) 256, 264, 355 nm. Severe acid hydrolysis of 10% H_2SO_4 produces quercetin and rutinose (mp 187–188 °C). Acid hydrolysis of 1% H_2SO_4 (stepwise hydrolysis) produces quercetin (mp 312–313 °C), D-glucose, L-rhamnose, which was confirmed by thin layer chromatography with reliable witness samples.

Kaempferol – (3,5,7,4'-tetrahydroxoflavone) - light yellow crystalline substance of composition $C_{15}H_{10}O_6$, m.p. 268-270°C, mass spectrum (m/z) M^+286. UV spectrum λmax, (ethanol) 266.369 nm, which is typical for flavonoids; +NaOH:281.415 nm; +CH_3COOH 274.387 nm. The IR spectrum of the substance contains absorption bands of hydroxyl groups (3323-3277см$^{-1}$), carbonyl γ-pyrone (1662 cm^{-1}), aromatic C=C bonds (1591 cm^{-1}).

Quercetin - 3, 5, 7, 3', 4'-pentahydroxyflavone.- (eluted with 80% ethanol): $C_{15}H_{10}O_7$, mp. 310–312 °C (from CH3OH), UV spectrum (C_2H_5OH, λmax nm) 372, 264, 256. Rf 0.64 (system 2). IR spectrum (KBr, υ, cm^{-1}) 3380, 3300 (OH), 1665 (>C=O), 1615, 1565, 1515 (Ar), 815, 840 (p-substitution in ring “B”). When alkaline melting occurs, phloroglucinol and protocatechuic acid are formed.

Study of the antioxidant activity of polyphenols. Antioxidants, effectively influencing the functional parameters of cells and mitochondria, increase the activity of the enzymes superoxide dismutase and glutathione peroxidase, and inhibit lipid peroxidation (LPO).In the experiments, the maximum rate of mitochondrial swelling caused by the addition of Fe^{2+}/ascorbate, which initiates LPO, to the incubation medium was taken as 100%. In this case, lipid peroxidation products disrupt the barrier function of mitochondrial membranes, which leads to a sharp swelling of mitochondria . Under these conditions, the introduction of polyphenols into the incubation medium at a concentration of 5 μM leads to increased mitochondrial swelling by 16 ± 1.2% compared to the control, which indicates activation of the lipid peroxidation process in membranes. These data indicate that polyphenols exhibit pro-

oxidant properties at low concentrations. However, higher concentrations exhibited antioxidant properties. Thus, at concentrations of 10 μM, 20 μM and 30 μM, LPO was inhibited by 9±0.8%, 63.9±4.9% and 86±7.4% compared to the control, respectively . Moreover, the effect of polyphenols depended on their concentrations: the maximum inhibition of mitochondrial swelling was observed at a concentration of 40 μM. The polyphenol concentration causing half-maximal inhibition of the lipid peroxidation process (IC50) was 17.8 μM. The results obtained indicate that lower concentrations (5 μM) affect the mitochondrial membrane as a pro-oxidant, and higher concentrations act as an antioxidant. There is evidence in the literature that phenolic compounds can exhibit both antioxidant and prooxidant properties.

The addition of Fe2+/ascorbate to the incubation medium induces LPO; as a result, the functions of mitochondrial membranes are disrupted and they swell sharply compared to the control. Under conditions of induction of LPO by the Fe^{2+}/ascorbate system, the addition of rutin with a concentration of 5 μM to the incubation medium inhibits LPO processes by 32.0%, and at 10 μM concentration by 85.9% and at 20 μM concentration by 96.8% compared to the control [18,19,20,21].

Conclusions

A comparative study showed that the quantitative and qualitative composition of Ácer semenóvii (Semyonov maple) depends on the time or period of collection.

Physicochemical research methods were used to identify polyphenols isolated from Acer semenóvii. The antioxidant activity of the sum of polyphenolic compounds during lipid peroxidation in rat liver mitochondria was studied, and it was shown that polyphenols have a protective effect on mitochondria, reducing the damaging effect of Fe2+/ascorbate.

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