ХИМИЯ РАСТИТЕЛЬНОГО СЫРЬЯ. 2025. №2. С. 269–276.
KHIMIYA RASTITEL'NOGO SYR'YA, 2025, no. 2, pp. 269–276.
DOI: 10.14258/jcprm.20250216218

UDC 615.32:634.511:66.061.3

PREPARATION AND ANALYSIS OF PHYTOPREPARATIONS FROM THE WALNUT SEPTUM BIOMASS

© ***D. Chernikova[1*], Ju. Bazarnova[1], U. Gašić[2], S. Đurović[3]***

[1] Peter the Great St. Petersburg Polytechnic University, Graduate School of Biotechnology and Food Science, Polytechnicheskaya st., 29B, St. Petersburg, 195251, Russia, dasha511997@yandex.ru
[2] Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Bulevar Despota Stefana, 142, Belgrade, 11060, Serbia
[3] Institute of General and Physical Chemistry, Studentski trg, 12/V, Belgrade, 11158, Serbia

Recently, foreign researchers have been actively working on studying *Juglans regia* L. septum, which indicates their rich phytochemical composition and a wide range of biological properties. It has been shown that extracts from walnut septum have high antioxidant, antitumor, and antiflogistic activity, as well as antimutagenic, regenerating, and bactericidal potential. The work aimed to obtain a natural complex of phenolic compounds from the biomass of walnut septum of selected varieties to study the phytochemical composition of the obtained phytopreparations and their antioxidant properties. To extract phenolic compounds, samples of the biomass of walnut septum were mixed with an extraction mixture in a ratio of 1:30 by weight and subjected to ultrasonic treatment at 100 W with a frequency of 20–25 kHz for 10 minutes. The resulting aqueous ethanol extracts were condensed under vacuum, and their physicochemical characteristics and antioxidant properties were determined. The identification and analysis of phenolic compounds in the studied phytopreparations was done using the HPLC-MS/MS method. Seventeen phenolic compounds were found in the composition of the obtained phytoextracts, most of which belong to flavonoids. It was found that the total content of identified flavonoids and their glycosides in aqueous ethanol extracts obtained from the biomass of *Juglans regia* L. septum was 13.54 mg/g in a thick extract of phenolic compounds – 119.75 mg/g. The total content of the identified phenolic acids in aqueous-ethanol extracts from the biomass of the septum was 4.22 mg/g, in the thick extract – 69.43 mg/g. The antioxidant activity of the studied phytopreparations amounted to 94% inhibition of the DPPH radical. The results of the analysis and identification of the phytochemical composition indicate that the obtained phytopreparations from the biomass of walnut septum are a valuable source of phytomicronutrients as part of functional food ingredients and can also be considered pharmaceutical substances for therapeutic use.

Keywords: Juglans regia L., biomass of walnut septum, phytopreparations, phenolic compounds, water-ethanol extracts, thick extract of phenolic compounds, antioxidant activity, HPLC-MS/MS, DPPH.

For citing: Chernikova D., Bazarnova Ju., Gašić U., Đurović S. *Khimiya Rastitel'nogo Syr'ya*, 2025, no. 2, pp. 269–276. (in Russ.). https://doi.org/10.14258/jcprm.20250216218.

Introduction

A significant achievement of the last decade has been new data on the biological role of many phytomicronutrients related to essential nutrition factors, among which special importance is given to the products of secondary metabolism of higher plants. The functions of secondary plant metabolites are very diverse and have not yet been thoroughly investigated. Some belong to the components of the electron transport chains of respiration and photosynthesis, others act as regulators in the processes of growth and development, others participate in various redox processes of the plant cell, and others are used as a reserve energy material [1–4]. Unlike synthetic analogs, natural phenolic compounds are considered to be less toxic, and can be used as biologically active additives [5].

Recently, interest has increased in studying the phytochemical composition and biological properties of nutshell processing products, particularly walnut septum (*Juglans regia* L.) [6–10]. They represent a wooden septum inside the walnut kernel, which accounts for 4–5% of the total weight of a whole nut [11]. Septum of *Juglans regia*

* Corresponding author.

L. is a unique biological resource due to its rich chemical composition, represented by glycosides, alkaloids, naphthoquinones (juglone), tannins, and organic substances, phytoncides, fatty acids, and deserve special attention as a potential source of natural antioxidants [12, 13].

Aghapour SK.F. and Sisakhtnezhad S. [14] noted that aqueous decoctions obtained from *Juglans regia* L. septum suppress the cell cycle of bone marrow mesenchymal stem cells can prevent the development of beta cells producing pancreatic insulin by inhibiting the Pdx1 gene, and can promote glucose uptake by enhancing the regulation of Ins1 gene expression/2, Insr, and Glut1 derived from mesenchymal stem cells.

Rusu M.E., Fizesan I., Pop A. et al. [8] showed that the extract of phenolic compounds of *Juglans regia* L. septum has antimicrobial potential against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella enteritidis*, as well as cytotoxicity against normal (human gingival fibroblasts HGF) and cancer cells of human lung adenocarcinoma A549 and breast MCF-7, T47D-KBluc.

Genovese C., Cambria M.T., D'angeli F. et al. [15] discovered the ability of an ethanol extract from *Juglans regia* L. septum to remove viability, proliferation, and migration of cells in the human glioblastoma A172 cell line and counteract the growth of gram-positive bacteria.

The antitumor effect of extracts from *Juglans regia* L. septum was dictated by the ability to inhibit the surge in lipopolysaccharide-stimulated HGF cells by removing damage from proinflammatory cytokines (interleukin-1, 6 and 8) [8], as well as on an antitumor animal model using 3-month-old male Wistar rats [16].

Zangeneh A., Zangeneh M.M., and Goodarzi N. [17] reported that the protective effect of phytochemicals of an aqueous extract from the septum on hepatopathy in mice with streptozocin-induced diabetes is indicated, associated not only with a decrease in blood glucose levels but also with inhibition of liver damage in mice.

Rusu M.E., Georgiu C., Pop A. et al. [18], the antioxidant and anti-aging potential of dietary supplements with the introduction of *Juglans regia* L. kernel and septum extract was studied on a D-galactose-induced aging model and on a Wistar rat natural aging model. It was revealed that the example of the studied dietary supplements based on *Juglans regia* L. fruit products can significantly weaken the histopathological changes in the brain and liver caused by D-galactose, which, in its description, is due to a decrease in the level of reactive oxygen species and lipid peroxidation, as well as increased antioxidant activity of cells. Morphological and functional improvements in the brain and liver indicate that phytochemicals of the nucleus and septum of *Juglans regia* L. oxidize a favorable neuro- and hepatoprotective effect.

In Russia, the walnut septum is a poorly studied object. Therefore, the ice preparation is disposed of as a by-product together with the shell after the kernel is separated. The development of phytopreparations and functional food ingredients from the septum obtained from new breeding varieties, *Juglans regia* L zoned in the southern regions of the Russian Federation, is relevant.

The purpose of the work is to obtain a natural complex of phenolic compounds from the biomass of walnut septums of selected varieties and to study the phytochemical composition of the obtained phytopreparations and their antioxidant properties.

Materials and Methods

The work used walnut septum obtained from the breeding varieties "Valentine's Gift", "Burlyuk", "Alminsky" (Nikitsky Botanical Garden – National Scientific Center of the Russian Academy of Sciences, Republic of Crimea) of the harvest of 2021, 2022, and 2023 and water-alcohol extracts obtained from the biomass of septum by ultrasound (US) homogenization and maceration, as well as a thick extract of phenolic compounds (TEPC) obtained from aqueous ethanol extracts by removing the extractant.

The fractionation of raw materials crushed in a laboratory mill was carried out by the method of sieve analysis, taking into account the recommendations of foreign authors [7, 19, 20] using laboratory sieves (cell sizes 2, 1, 0.5, and 0.25 mm). As a result, four biomass fractions with particle sizes less than 2 mm, 1-2 mm, 0.5–1 mm, and 0.25–0.5 mm were obtained. For the analysis and extraction of phenolic substances, a biomass of septum with a particle size of 0.25–0.5 mm was used.

The moisture content of the obtained biomass was determined by drying to a constant mass of the sample in a thermoshock with forced ventilation SNOL 75/350 (Snol Term, Russia) at a temperature of 100±5 °C [21, 22].

To extract phenolic compounds, samples of walnut septum biomass were mixed with an extraction mixture in a ratio of 1 : 30 by weight [23–26] and subjected to ultrasonic (US) homogenization on a SCIENTZ-IID device

(SCIENTZ, China). 96% ethyl rectified alcohol and drinking water (manufacturer IDS Borjomi Russia) in a volume ratio of 50:50 were used to obtain the extraction mixture [26–29].

Technological modes of extraction of phenolic substances from the biomass of septum using US-homogenization are shown in Table 1.

After US-homogenization, the mixture was kept for 72 hours at room temperature without access to light in a hermetically sealed dark glass container. After that, it was separated from the meal by decantation and subsequent centrifugation.

The obtained aqueous-ethanol extracts of phenolic compounds were condensed using a LabTech EV311VAC rotary evaporator (Analyte, Russia) for 3.5–4 hours at a temperature between 45 and 55 °C.

Standardization of TEPS was carried out in accordance with the current regulatory documentation [30–34].

The quantitative determination of the total amount of biologically active and ballast substances extracted by the extraction mixture was carried out gravimetrically, in particular, by the single extraction method [32].

The identification and analysis of phenolic compounds in the studied phytopreparations were carried out by HPLC-MS/MS using the UHPLC Dionex Ultimate 3000 system, which is equipped with a diode array detector connected to a triple quadrupole mass spectrometer TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland).

The chromatographic separation process was performed at 40 °C on a Syncroniz C18 column (100 × 2.1 mm; particle size 1.7 microns; Thermo Fisher Scientific, Basel, Switzerland).

The mobile phase consisted of water containing 0.01% acetic acid (component A) and acetonitrile (component B), which were used in the following gradient elution: 5% B in the first 2.0 min, 2.0nd–12.0th minutes 5–95% B, 12.0th–13.0th minutes from 95 to 5% B, and 5% B until the 20th minutes. The flow rate was set to 0.3 mL/min, the detection wavelengths to 254 and 280 nm, and the injection volume was 5 μL.

Stock methanolic solutions of phenolic compounds at a concentration of 1000 mg/L were prepared. The stock solutions were mixed and diluted with water to obtain working solutions (concentrations of 0.01, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 mg/L).

A TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI) source was used. The vaporizer temperature was kept at 250 °C, and the ion source settings were as follows: spray voltage 4500 V, sheet gas (N2) pressure 27 AU, ion sweep gas pressure 0 AU, and auxiliary gas (N2) pressure 7 AU, capillary temperature 275 °C, skimmer offset 0 V, and capillary offset – 35 V.

The mass spectrometry data were acquired in the negative ionization mode in the m/z range from 100 to 1000.

Multiple mass spectrometric scanning modes, including full scanning (FS) and product ion scanning (PIS), were conducted to analyze the targeted compounds qualitatively. The collision-induced fragmentation experiments used argon as collision gas, while the collision energy varied depending on the compound. The time-selected reaction monitoring (tSRM) experiments for quantitative analysis were performed using two MS2 fragments for each compound that was previously defined as dominant in the PIS experiments.

Xcalibur software (version 2.2) was used for instrument control. Phenolics were identified and quantified according to the corresponding spectral characteristics: molecular ion, mass spectra, characteristic fragmentation, and characteristic retention time. The limits of detection (LOD) and quantification (LOQ) were calculated using standard deviations (SD) of the responses and the slopes of the calibration curves (S) according to LOD=3(SD/S) and LOQ=10(SD/S). The values of standard deviations and slopes were obtained from the calibration curves created in MS Excel.

The DPPH method was used to study the antioxidant properties of phytopreparations from walnut septum [35]. 50 μL of phytoextracts were mixed to 5 mL of the DPPH working solution. The kinetics of reducing the solution's optical density were recorded for 30 min at a wavelength of 517 nm on the SHIMADZU UV-1280 device (SHIMADZU, Japan). A working solution of DPPH was used as a control sample.

Results and Discussion

Table 2 shows the characteristics of a TEPC obtained by thickening water-ethanol extracts from the biomass of walnut septum.

The TEPC was an opaque mass of dark brown color with a pronounced woody-herbaceous aroma, tart taste, and a moisture content of 6.2%. The content of extractives is 285.0 mg/g, flavonoids – 147.5 mg/g, and phenolic

acids – 137.5 mg/g. The results also indicated negligible levels of heavy metals, which is quite significant from the human health perspective, i.e., extracts may be considered as free to use in food products. The presence of phenolic and polyphenolic compounds is also significant because they show various biological activities, such as antioxidant, antimicrobial, cytotoxic, and many others [36].

Figure shows the HPLC profiles of aqueous-ethanol extracts and thick extract obtained from walnut septum. The results of a chemical profile investigation of water-ethanol extracts and a TEPC indicate that the extraction conditions for obtaining a thick extract are selected precisely to not induce any visible changes in the chemical profile or decomposition of the thermolabile phenolic substances.

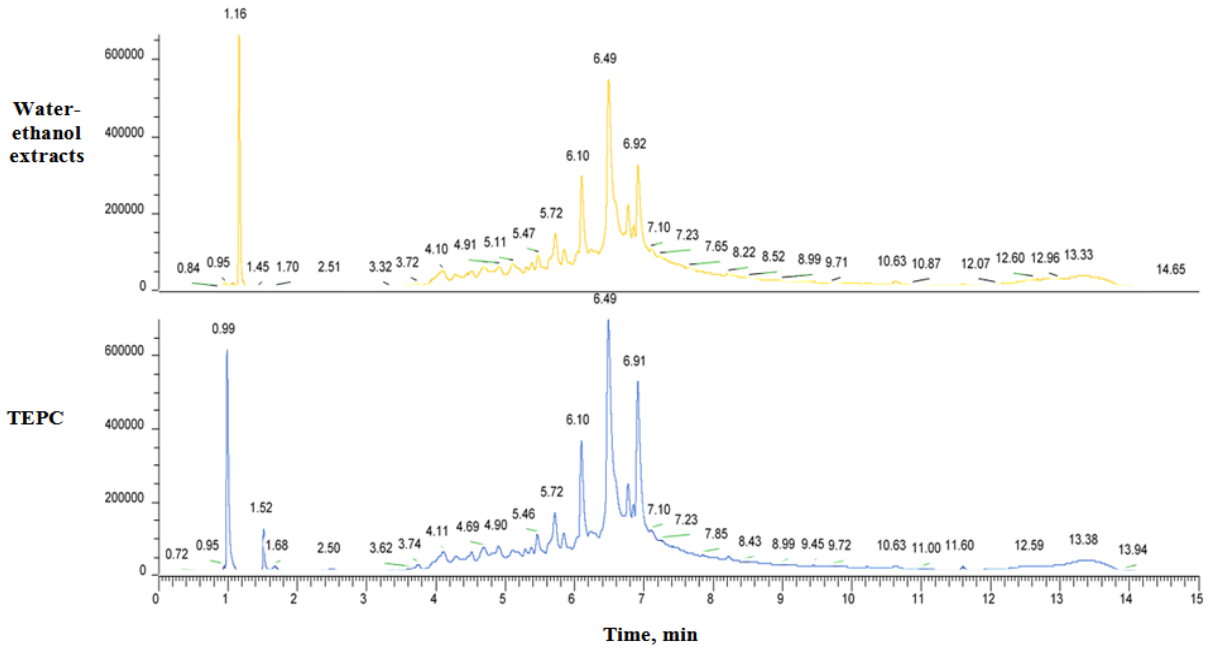
Information on the identified phenolic compounds is systematized in Table 3.

Table 1. Technological ranges of US-homogenization of biomass of walnut septum

№	Technological characteristics	Modes
1	Frequency of ultrasonic emitter, kHz	20±5
2	Power, W	100
3	Probe diameter, mm	6
4	Hydraulic module	1 : 30
5	Mixture temperature, °C	20±5
6	Processing time, min	10

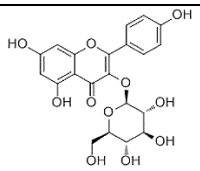
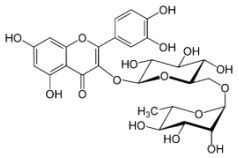
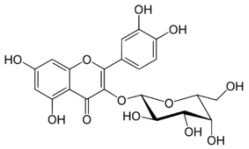
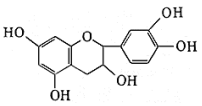
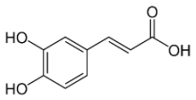
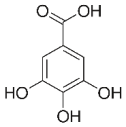
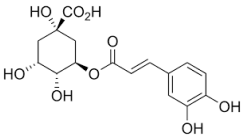
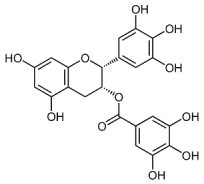
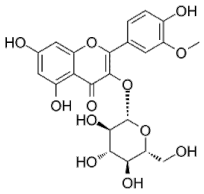
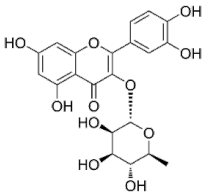
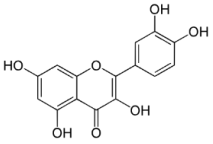
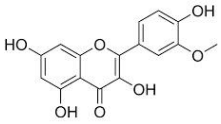
Table 2. Organoleptic and physicochemical characteristics of a thick extract of phenolic compounds from walnut septum

Organoleptic indicators	Description of the feature
Color	Dark brown
Transparency	Opaque
Smell	Woody-grassy
Taste	Tart
Physicochemical parameters	Meaning
Mass fraction of moisture, %	6.2±0.2
Mass fraction of ethyl alcohol, %	0.43±0.02
Density, g/cm^3	1.50±0.05
Extractive substances, mg/g	285.0±2.5
Flavonoids, mg/g	147.5±1.5
Phenolic acids, mg/g	137.5±1.5



HPLC-profiles of the obtained water-ethanol extracts and TEPC from walnut septum

Table 3. Characteristics of phenolic compounds found in the composition of water-ethanol extracts and TEPC

№	Phenolic compounds	Chemical structural formula	Calibration range, mg/L	R^2	LOD, mg/L	LOQ, mg/L
1	Kaempferol 3-*O*-glucoside		0.05–2.00	0.9913	0.12	0.41
2	Quercetin-3-*O*-rutinoside (rutin)		0.05–2.00	0.9923	0.09	0.31
3	Quercetin-3-*O*-galactoside (hyperoside)		0.05–2.00	0.9946	0.11	0.45
4	Catechin		0.05–2.00	0.9885	0.14	0.45
5	Caffeic acid		0.05–2.00	0.9983	0.11	0.38
6	Gallic acid		0.05–2.00	0.9920	0.16	0.55
7	Chlorogenic acid		0.05–2.00	0.9909	0.15	0.49
8	Epigallocatechin gallate		0.05–2.00	0.9942	0.15	0.51
9	Isorhamnetin-3-*O*-glucoside		0.05–2.00	0.9989	0.11	0.33
10	Quercetin-3-*O*-rhamnoside		0.05–2.00	0.9994	0.09	0.28
11	Quercetin		0.05–2.00	0.9971	0.08	0.27
12	Isorhamnetin		0.05–2.00	0.9972	0.09	0.21

12 phytochemical compounds were found in the composition of aqueous-ethanol extracts and thick extract, which belong to flavonols and their glycosides, flavan-3-ols and their gallates, hydroxycinnamic, hydroxybenzoic acids, and their derivatives, the structure of which molecules is C_6-C_3. Most of the identified compounds belong to the class of flavonoids, the structure of which molecules is C_6-C_3-C_6. The results obtained are in good agreement with the data of the authors Mateș L., Rusu M.E. and Popa D.-S., who report that the main phenolic substances of the septum of *Juglans regia* L. are hydroxybenzoic, hydroxycinnamic, hydroxyphenylacetic acids and their esters and flavonoids (flavones, flavonols, chalcones, anthocyanins, flavanones, flavanols) [12].

Structures presented in Table 3 indicate the presence of phenolic acids (gallic, chlorogenic, and caffeic acids) and glycosidic and aglycone forms of flavonoids in prepared extracts. Structures' elucidation was conducted according to their mass spectra, either comparing them to those of available standards or in available databases. Phenolic acids are characterized by losing the CO_2 group (gallic and caffeic acids), producing characteristic $[M-H-44]^-$ ion product. Chlorogenic acid (ester of caffeic and quinic acids) produces characteristic $[M-H-162]^-$ fragment, corresponding to quinic acid. Fragmentation of flavonoids may occur following different patterns. For example, rutin, kaempferol-3-*O*-glucoside, and quercetin give peaks at m/z 609, 447, and 301. Rutin loses the rutinoside unit and gives ion product $[M-H-308]^-$ at m/z 301. Quercetin gave two characteristic ions. The first is a deprotonated molecule $[M-H]^-$, while the second is a product of retro-Diels-Alder (RDA) fragmentation, including 1,3-scission in the C-ring. Kaempferol-3-*O*-glucoside is characterized by losing its glucoside fragment, giving a peak at m/z 285, which belongs to kaempferol aglycone [37–39].

Table 4 summarizes the results of systematized information on identified phenolic compounds in water-ethanol extracts and thick extracts and their content in the obtained phytopreparations.

Table 4. Composition of phenolic compounds of water-ethanol extracts and TEPC

Biologically active substances	The content of phenolic substances in phytopreparations, mg/g	
	Water-ethanol extracts	TEPC
Flavonoids and their glycosides		
Catechin	8.49±0.42	70.71±3.54
Epigallocatechin gallate	0.42±0.02	4.10±0.21
Quercetin	0.082±0.004	0.66±0.03
Quercetin-3-*O*-rhamnoside	2.30±0.12	23.77±1.19
Hyperoside	1.08±0.05	11.67±0.58
Rutin	0.077±0.004	0.56±0.03
Isoramnetin	0.84±0.04	5.22±0.26
Isoramnetin-3-*O*-glucoside	0.012±0.001	0.11±0.01
Kaempferol-3-*O*-glucoside	0.24±0.01	2.95±0.15
∑, mg/g	13.54±0.68	119.75±5.99
Phenolic acids		
Gallic acid	3.20±0.15	60.58±3.03
Chlorogenic acid	0.94±0.05	8.51±0.43
Caffeic acid	0.083±0.004	0.34±0.02
∑, mg/g	4.22±0.21	69.43±3.47

The total content of identified flavonoids and their glycosides in aqueous-alcohol extracts was 13.54 mg/g, while in TEPC was 119.75 mg/g, where catechin, quercetin-3-*O*-rhamnoside, and hyperoside (quercetin-3-galactoside) predominated. The total content of identified phenolic acids in aqueous-alcohol extracts and TEPC were 4.22 mg/g and 69.43 mg/g, respectively. The presented results do not indicate any significant changes after removing the solvent from the extract, leaving phenolic compounds in their native form.

The antioxidant activity of the obtained phytopreparations amounted to 94% inhibition of the DPPH radical, which correlates with experimental data obtained by the authors Mateș L., Rusu M.E., Popa D.-S., Medic A.,

Jakopic J. [9, 12, 16] indicates that phytopreparations obtained from the biomass of walnut septum are a valuable source of phytomicronutrients in the composition of functional food ingredients and can also be considered pharmaceutical substances for therapeutic use.

Efficiency in the scavenging of free radicals is commonly ascribed to polyphenolic compounds present in plants. Investigation of the structure-activity relationship revealed that functional groups and their position in the molecule significantly influence antioxidant activity. Studies showed that the presence of o-dihydroxy moiety in the B-ring, 2,3 double bond in conjugation with a 4-oxo functional group in the C-ring, and the existence of 3-OH and 5-OH groups together with a 4-oxo functional group in A and C-rings ensure maximal antioxidant activity. Similarly to flavonoids, the position and availability of hydroxyl groups in phenolic acids influence their antioxidant activity, whereas the free -OH group means higher antioxidant activity [40].

Conclusions

The thick extract obtained by thickening water-ethanol extracts from walnut septum is an opaque dark brown mass with a pronounced woody-herbaceous aroma, tart taste, and a moisture content of 6.2%. The content of extractive substances in TEPC is 285.0 mg/g, flavonoids – 147.5 mg/g, and phenolic acids – 137.5 mg/g.

Technological instruction TI 1089-001-02068574-2024 has been developed to obtain a TEPC from the walnut septum.

By the HPLC-DAD MS/MS gallic acid, hydroxycinnamic acids, quercetin and its derivatives, catechin, isoramnetin, and kaempferol-3-O-glucoside have been identified in the composition of TEPC. The total content of flavonoids and their glycosides in TEPC was 120 mg/g, gallic acid – 60.5 mg/g, and hydroxycinnamic acids – 8.9 mg/g. The antioxidant activity of TEPC, established by experimental methods, amounted to 94% inhibition of the DPPH radical.

The analysis of the physicochemical characteristics and phytochemical composition of a thick extract from walnut septum allows us to conclude about the prospects of its use as a pharmaceutical substance for therapeutic use.

Funding

This study was supported funding by Peter the Great St. Petersburg Polytechnic University, Graduate School of Biotechnology and Food Science, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade and Institute of General and Physical Chemistry.

Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

References

1. Godlewska-Żyłkiewicz B., Świsłocka R., Kalinowska M., Golonko A., Świderski G., Arciszewska Ż., Nalewajko-Sieliwoniuk E., Naumowicz M., Lewandowski W. *Materials*, 2020, vol. 13, no 19, article 4454. https://doi.org/10.3390/ma13194454.
2. Zagoskina N.V., Nazarenko L.V. *Vestnik Moskovskogo gorodskogo pedagogicheskogo universiteta. Seriya: Yestestvennyye nauki*, 2019, no. 2 (34), pp. 8–19. https://doi.org/10.25688/2076-9091.2019.34.2.1. (in Russ.).
3. Bazarnova Y.G. *Biologicheski aktivnyye veshchestva dikorastushchikh rasteniy i ikh ispol'zovaniye v pishchevykh tekhnologiyakh: monografiya* [Biologically active substances of wild plants and their use in food technologies: monograph]. St. Petersburg, 2016, 240 p. (in Russ.).
4. Larikova Yu.S., Malikova N.A. *Meditsina. Sotsiologiya. Filosofiya. Prikladnyye issledovaniya*, 2022, no. 6, pp. 138–141. (in Russ.).
5. Zhang Y., Cai P., Cheng G., Zhang Y. *Natural Product Communications*, 2022, vol. 17, no. 1. https://doi.org/10.1177/1934578X211069721.
6. Liu R., Zhao Z., Dai S., Che X., Liu W. *Journal of agricultural and food chemistry*, 2019, vol. 67, no 13, pp. 3811–3825. https://doi.org/10.1021/acs.jafc.8b06890.
7. Rusu M.E., Gheldiu A.-M., Mocan A., Moldovan C., Popa D.-S., Tomuta I., Vlase L. *Molecules*, 2018, vol. 23, no. 11, article 2814. https://doi.org/10.3390/molecules23112814.
8. Rusu M.E., Fizesan I., Pop A., Mocan A., Gheldiu A.M., Babota M., Vodnar D.C., Jurj A., Berindan-Neagoe I., Vlase L., Popa D.S. *Molecules*, 2020, vol. 25, no. 9, article 2187. https://doi.org/10.3390/molecules25092187.
9. Medic A., Jakopic J., Hudina M., Solar A., Veberic R. *Food chemistry*, 2021, vol. 352, article 129404. https://doi.org/10.1016/j.foodchem.2021.129404.
10. Li L., Song L., Sun X., Yan S., Huang W., Liu P. *Food chemistry*, 2019, vol. 286, pp. 669–677. https://doi.org/10.1016/j.foodchem.2019.02.054.
11. Zhan Y., Ma M., Chen Z., Ma A., Li S., Xia J., Jia Y. *Foods*, 2023, vol. 12, no. 18, article 3379. https://doi.org/10.3390/foods12183379.
12. Mateș L., Rusu M.E., Popa D.-S. *Antioxidants*, 2023, vol. 12, article 604. https://doi.org/10.3390/antiox12030604.
13. Hu Q., Liu J., Li J., Liu H., Dong N., Geng Y.-Y., Lu Y., Wang Y. *Food Science and Biotechnology*, 2020, vol. 29, no. 2, pp. 187–196. https://doi.org/10.1007/s10068-019-00655-z.
14. Aghapour SK.F., Sisakhtnezhad S. *Jentashapir Journal of Cellular and Molecular Biology*, 2021, vol. 12, no. 2, article e115014. https://doi.org/10.5812/jjcmb.115014.
15. Genovese C., Cambria M.T., D'angeli F., Addamo A.P., Malfa G.A., Siracusa L., Pulvirenti L., Anfuso C.D., Lupo G., Salmeri M. *International journal of oncology*, 2020, vol. 57, no. 5, pp. 1129–1144. https://doi.org/10.3892/ijo.2020.5130.
16. Fizeșan I., Rusu M.E., Georgiu C., Pop A., Ștefan M.G., Muntean D.M., Mirel S., Vostinaru O., Kiss B., Popa D.S. *Antioxidants*, 2021, vol. 10, no. 1, article 119. https://doi.org/10.3390/antiox10010119.

17. Zangeneh A., Zangeneh M.M., Goodarzi N., Najafi F., Hagh Nazari L. *Scientific Journal of Kurdistan University of Medical Sciences*, 2018, vol. 2, no. 1, pp. 26–37.
18. Rusu M.E., Georgiu C., Pop A., Mocan A., Kiss B., Vostinaru O., Fizesan I., Stefan M.-G., Gheldiu A.-M., Mates L., Moldovan R., Muntean D.M., Loghin F., Vlase L., Popa D.S. *Antioxidants*, 2020, vol. 9, no. 5, article 424. https://doi.org/10.3390/antiox9050424.
19. Zhang Y.G., Kan H., Chen S.X., Thakur K., Wang S., Zhang J.G., Shang Y.F., Wei Z.J. *Food chemistry*, 2020, vol. 321, article 126672. https://doi.org/10.1016/j.foodchem.2020.126672.
20. *OFS.1.5.3.0004 Opredeleniye podlinnosti, izmel'chennosti i soderzhaniya primesey v lekarstvennom rastitel'nom syr'ye i lekarstvennykh rastitel'nykh preparatakh* [GPA.1.5.3.0004 Determination of authenticity, pulverization and content of impurities in medicinal plant raw materials and medicinal herbal preparations]. (in Russ.)
21. *GOST 24027.2-80 Metody opredeleniya vlazhnosti, zol'nosti, ekstraktivnykh i dubil'nykh veshchestv, efirnogo masla* [GOST 24027.2-80. Methods for determination of moisture, ash content, extractive and tannin materials, essential oil]. Moscow, 2015, 10 p. (in Russ.)
22. *OFS.1.5.3.0007. Opredeleniye vlazhnosti lekarstvennogo rastitel'nogo syr'ya i lekarstvennykh sredstv rastitel'nogo proiskhozhdeniya* [GPA.1.5.3.0007 Determination of moisture content of medicinal plant raw materials and medicinal products of plant origin]. (in Russ.)
23. Yang J., Chen C., Zhao S., Ge F., Liu D. *Journal of Food and Nutrition Research*, 2014, vol. 2, no. 10, pp. 664–670. https://doi.org/10.12691/jfnr-2-10-2.
24. Medic A., Jakopic J., Hudina M., Solar A., Veberic R. *Journal of King Saud University – Science*, 2022, vol. 34, no. 3, article 101890. https://doi.org/10.1016/j.jksus.2022.101890.
25. Sha Y.H., Mao X.Y., Wu Q.Z., Zhang J., Cheng W.D. *Food Science*, 2021, vol. 42. pp. 91–98. https://doi.org/10.7506/spkx1002-6630-20200423-304.
26. Han H., Wang S., Rakita M., Wang Y., Han Q., Xu Q. *Food and Nutrition Sciences*, 2018, vol. 9, no. 8, pp. 1034–1045. https://doi.org/10.4236/fns.2018.98076.
27. Roselló-Soto E., Martí-Quijal F.J., Cilla A., Munekata P.E.S., Lorenzo J.M., Remize F., Barba F.J. *Molecules*, 2019, vol. 24, no. 4, article 797. https://doi.org/10.3390/molecules24040797.
28. Jahanban-Esfahlan A., Ostadrahimi A., Tabibiazar M., Amarowicz R.A. *Molecules*, 2019, vol. 24, no. 11, article 2133. https://doi.org/10.3390/molecules24112133.
29. Vieira V., Pereira C., Pires T.C., Calhelha R.C., Alves M.J., Ferreira O., Barros L., Ferreira I.C. *Industrial Crops and Products*, 2019, vol. 134, pp. 347–355. https://doi.org/10.1016/j.indcrop.2019.04.020.
30. *OFS.1.2.1.0010 Poterya massy pri sushke*. [GPA.1.2.1.0010 Weight loss during drying]. (in Russ.)
31. *OFS.1.2.1.0014 Plotnost'.* [GPA.1.2.1.0014 Density]. (in Russ.)
32. *OFS.1.5.3.0006 Opredeleniye soderzhaniya ekstraktivnykh veshchestv v lekarstvennom rastitel'nom syr'ye i lekarstvennykh rastitel'nykh preparatakh.* [GPA.1.5.3.0006 Determination of the content of extractive substances in medicinal plant raw materials and medicinal herbal preparations]. (in Russ.)
33. *OFS.1.5.3.0008 Opredeleniye soderzhaniya dubil'nykh veshchestv v lekarstvennom rastitel'nom syr'ye i lekarstvennykh preparatakh rastitel'nogo proiskhozhdeniya*. [GPA.1.5.3.0008 Determination of the content of tannins in medicinal plant raw materials and medicinal products of plant origin]. (in Russ.)
34. *OFS.1.2.3.0034 Opredeleniye etilovogo spirta v lekarstvennykh sredstvakh*. [GPA.1.2.3.0034 Determination of ethyl alcohol in medicines]. (in Russ.)
35. Adesanwo J.K., Makinde O.O., Obafemi C.A. *Journal of Pharmacy Research*, 2013, vol. 6, no. 9, pp. 903–907. https://doi.org/10.1016/j.jopr.2013.09.003.
36. Đurović S., Kojić I., Radić D., Smyatskaya A., Bazarnova J., Filip S., Tosti T. *International Journal of Molecular Science*, 2024, vol. 25, no. 6, article 3430. https://doi.org/10.3390/ijms25063430.
37. Vallverdú-Queralt A., Jáuregui O., Medina-Remón A., Andrés-Lacueva C., Lamuela-Raventós R.M. *Rapid Communications in Mass Spectrometry*, 2010, vol. 24, no. 20, pp. 2986–2992. https://doi.org/10.1002/rcm.4731.
38. Fabre N., Rustan I., de Hoffmann E., Quetin-Leclercq J. *Journal of the American Society for Mass Spectrometry*, 2001, vol. 12, no. 6, pp. 707–715. https://doi.org/10.1016/S1044-0305(01)00226-4.
39. Đurović S., Pavlić B., Šorgić S., Popov S., Savić S., Petronijević M., Radojković M., Cvetanović A., Zeković Z. *Journal of Functional Foods*, 2017, vol. 32, pp. 18–26. https://doi.org/10.1016/j.jff.2017.02.019.
40. Rice-Evans C., Miller N., Paganga G. *Free Radical Biology & Medicine*, 1996, vol. 20, no. 7, pp. 933–956. https://doi.org/10.1016/0891-5849(95)02227-9.

Received November 13, 2024
Revised December 23, 2024
Accepted February 18, 2025

Information about authors
Chernikova Daria – Assistant, dasha511997@yandex.ru
Bazarnova Julia – Doctor of Technical Sciences, Professor, Director, j.bazarnowa2012@yandex.ru
Gašić Uroš – Chief Researcher, Department of Plant Physiology, urosgasic@chem.bg.ac.rs
Đurović Saša – Candidate of Technical Sciences, Senior Researcher at the Chromatography Laboratory, sasatfns@uns.ac.rs