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HPLC Analysis of Hydro-Ethanolic Extracts from *Pastinaca sativa* L. Fruits.

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ABSTRACT

The objective of this work was to carry out high-performance liquid chromatography (HPLC) analysis of hydro-ethanolic extracts from the *Pastinaca sativa* L. fruits of three different cultivars for qualitative identification and quantitative assessment of the phenolic compounds present in extracts and for the optimal choice of ethanol concentration in hydro-ethanolic solution for extraction. In the study authors used ground fruits with the fraction of particles 0.1-0.5 mm of *Pastinaca sativa* L. fruits of the following cultivators: *Petrik*, *Student,* and *Globular*. For extraction, authors used hydro-ethanolic solutions with ethanol concentration 17±1, 34±1, 63±1, and 95±1 % m/m; the process of extraction was carried out at temperature 25±1 °C. Extraction method: 24-hour maceration. Plant raw material / extractant ratio (m/v): 1:5. Authors have demonstrated that xanthotoxol, bergapten, and xanthotoxin are dominating components among furanocoumarins in hydro-ethanolic extracts. For standardization of galenical drugs from *Pastinaca sativa* L. fruits, authors recommend using these standards. The range of optimal ethanol concentration in hydro-ethanolic solution for concurrent extraction of furanocoumarins, flavonoids and coumarins, as well as for selective extraction of furanocoumarins has been found.

Keywords: *Pastinaca sativa* L. fruits, HPLC analysis, furanocoumarins.

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INTRODUCTION

Apiacea family plants play an important role in human life, both, in the field of agriculture and medicine. Plant raw material or medicines from plants of this family are frequently used in cooking and medicine due to their flavor, as well as useful nutritional and pharmacological activities, for example: carminative, hypoglycemic, anxiolytic, hypolipidemic, lactogenic, spasmolytic, vasodilative, antimicrobial, expectorate, photosensitizing, etc. [1-4].

It is interesting to note that target substances of this plant family may differ as for their origin: fatty and essential oils, polysaccharides, proteins, furanochromones, furanocoumarins, coumarins, etc. [5].

One of interesting representatives of the family in the context of their study is *Pastinaca sativa* L. This plant is widely cultivated in agriculture. Furthermore, as a rule, its root is used for cooking as it is rich in vitamins, polysaccharides, proteins, and phenolic compounds. Fruits accumulate fatty and essential oils, flavonoids, and polyacetylene compounds [6], but it is furanocoumarins that are specific substances present in all parts of the plant including its fruits. Furanocoumarins exhibit spasmolytic, vasodilative, antimicrobial, photosensitizing and some other useful activities [7, 8].

It should be noted that a photosensitizing effect is typical for a very limited number of substances and it is used for treatment of certain dermatological diseases (such as vitiligo, psoriasis, some types of alopecia and mycosis). In addition, there are few medicines having a photosensitizing effect that are registered in the CIS countries and other countries of the world [9].

Thus, a study of plant raw material that contains substances with photosensitizing activity for the development of new drug products is a really vital task in pharmacy and medicine.

The **aim** of this work was to carry out high-performance liquid chromatography (HPLC) analysis of hydro-ethanolic extracts from the *Pastinaca sativa* L. fruits of different cultivars for qualitative identification and quantitative assessment of the phenolic compounds present in extracts and for the optimal choice of ethanol concentration in hydro-ethanolic solution for extraction.

MATERIALS AND METHODS

Plant raw materials

In our studies we used grinded fruits with a fraction of particles of 0.1-0.5 mm. Plant raw material was buying in “Kouel” company, Ukraine: *Pastinaca sativa* L., (*Petrik* cultivar), lot No. 45122, expiry date 08/2020C; “Pnsemena” company, Ukraine: *Pastinaca sativa* L., (*Student* cultivar), lot No. 075.34, expiry date 12/2021 and “Pnsemena” company, Ukraine: *Pastinaca* L., (*Globular* cultivar), lot No. 454124, expiry date 12/2021.

Preparation of extracts

For extraction, we used hydro-ethanol solutions with ethanol concentration 17±1, 34±1, 63±1, and 95±1 % m/m. determined with an alcoholmeter; the process of extraction was carried out at temperature 25±1 °C. Extraction method: 24-hour maceration. Plant raw material / extractant ratio (m/v): 1:5.

HPLC analysis

HPLC analysis was carried out using chromatograph «*Agilent Technologies 1200 Infinity*», made in USA. Chromatographic process was carried out under the following conditions: mobile phase (A): 1% water solution of formic acid, second mobile phase (B): ethanol in linear gradient elution regime; chromatographic column: *Supelco Ascentis express* C_{18} *2.7*µm *× 100* mm *× 4.6* mm; mobile phase velocity: 0.5 ml/min; temperature of chromatographic column: +35 °C; sample volume: 1 µl.

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Chemicals and reagents

Qualitative and quantitative analysis of substances were carried out with standards: xanthotoxol, xanthotoxin, bergaptol, bergapten (Sigma-Aldrich, Germany) and using scientific literature data [10]. Ethanol (pharmaceutical quality, Russia), formic acid (high purity, Russia), distilled water.

Statistical analysis

All the experiments were repeated in triplicates. Statistical calculations were carried out using MS Excel 2003.

RESULTS AND DISCUSSION

Table 1 shows data of area values for phenolic compounds obtained by HPLC analysis of extracts based on 17±1, 34±1, 63±1, and 95±1 % m/m hydro-ethanolic solutions from *Pastinaca sativa* L. fruit by example of *Petrik* cultivar.

Table 1: Area values for main compounds of extracts on different hydro-ethanol solutions from *Pastinaca sativa* L. fruit, *Petrik* cultivar

Compound (λ, nm)	Compound area, mAU·s [a] (Retention time, min)			
	Hydro-ethanolic solution			
	17 % m/m	34% m/m	63% m/m	95% m/m
Xanthotoxol (248.4 nm)	705±21 (21.4±0.5)	2,973±89 (21.7±0.5)	4,850±150 (21.9±0.5)	4,966±148 (22.3±0.6)
Sphondin (248.4 nm)	121±3 (22.5±0.6)	392±11 (22.4±0.6)	680±20 (22.8±0.4)	643±19 (23.1±0.5)
Unidentified furanocoumarin (248.4 nm)	69±2 (24.8±0.5)	363±10 (25.3±0.5)	660±20 (25.0±0.4)	674±20 (24.9±0.4)
Bergapten (248.4 nm)	82±2 (27.2±0.4)	625±18 (27.0±0.4)	1,390±40 (26.7±0.5)	1,479±44 (26.8±0.5)
Xanthotoxin (248.4 nm)	39.4±1.1 (34.1±0.4)	659±19 (34.0±0.4)	2,210±70 (33.7±0.4)	2,409±72 (33.4±0.5)
Umbelliferone derivative (360.4 nm)	-	82.5±2.4 (36.2±0.5)	330±10 (35.7±0.5)	365±10 (35.4±0.6)
p-coumaric acid derivative (310.4 nm)	159±4 (11.6±0.6)	126±3 (11.5±0.6)	192±6 (11.7±0.6)	65±2 (12.2±0.7)
Quercetin derivative (360.4 nm)	-	188±5 (17.1±0.8)	263±8 (16.4±0.8)	48.9±1.4 (17.0±0.9)
Kaempferol derivative (360.4 nm)	-	106±3 (20.2±0.5)	141±4 (19.8±0.6)	32.2±0.9 (19.3±0.6)

[a] Mean value and its confidence interval (Mean±SEM) are calculated with repeat counts n=3 and significance level P=0.95

As it can be seen from data of Table 1, hydro-ethanolic solutions with ethanol concentration ≥ 63 % m/m are good solvents for furanocoumarins and umbelliferone derivative. The content of these compounds in the extract increase with increase of ethanol concentration in the solution. Flavonoids (quercetin and kaempferol derivatives) and p-coumaric acid derivative have maximum content at 63 % m/m ethanol concentration in the hydro-ethanol solution. For other cultivars we got similar results.

For better visualization and substantiation of the rational choice of optimal ethanol concentration in hydro-ethanolic solution, we transformed the content of furanocoumarins, flavonoids, and coumarins to relative values by division their area in every type of hydro-ethanolic extract by their maximum value found in the experiment by HPLC method.

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Fig. 1 below presents the relationship between relative compound content (furanocoumarins, flavonoids, coumarins) in the extract and ethanol concentration in the hydro-ethanolic solution.

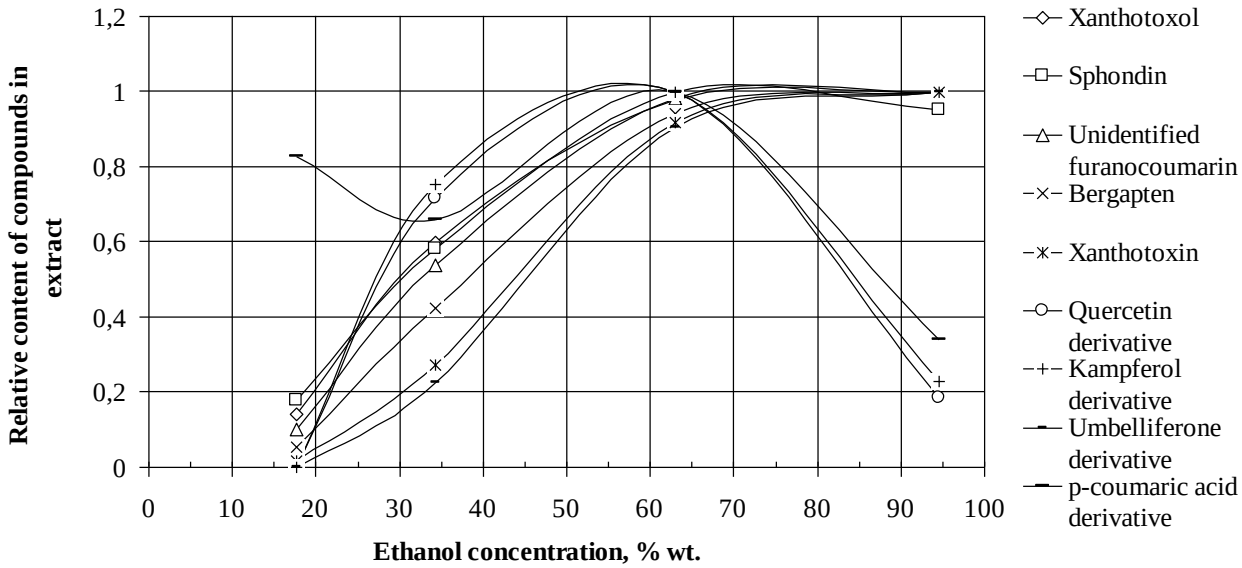

Figure 1: Relationship between relative content of some phenolic compounds in the extract and ethanol concentration in the hydro-ethanolic solution

As it can be seen from the data presented in Fig. 1, the maximum relative content of furanocoumarins falls within the range 80±15 % m/m of ethanol concentration in hydro-ethanolic solution. Wherein most furanocoumarins are characterized by increasing dependency to plateau and in the first approximation, this dependency is described well by S-type relation.

For flavonoids, we can clearly see a parabolic relation for their relative content in the hydro-ethanolic extract and ethanol concentration in the hydro-ethanolic solution with maximum value, which falls within the range of 58±10 % m/m.

Umbelliferone compound is characterized S-type relation with maximum ethanol concentration, which falls within the range of 80±15 % m/m.

The p-coumaric acid derivative has cubic relation of relative content in the extract from ethanol concentration in the solution and has two types of extremum: minimum at 34 % m/m ethanol concentration and maximum at 63 % m/m ethanol concentration.

It is interesting to note that the common point of intersection (isobestic point) for phenolic compounds falls within the range of ethanol concentration in hydro-ethanolic solution 67.5±2.5 % m/m.

Thus, to obtain a hydro-ethanolic extract with the maximum content of furanocoumarins, flavonoids and coumarins at the same time, we recommend using hydro-ethanolic solution with the concentration of 67.5±2.5 % m/m. But for the purpose of selective extraction of furanocoumarins, hydro-ethanolic solution with ethanol concentration not less than 80 % m/m can be used.

HPLC profile of phenolic compounds was similar in all cases, so chromatography data only to extract from *Petrik* cultivar is presented below.

Fig. 2 presents a chromatogram-profile of the extract from *Pastinaca sativa* L. fruits, *Petrik* cultivar, based on 63 % m/m hydro-ethanolic solution at the wavelength of 248.4 nm with UV-spectra of the main types of furanocoumarins found in the extract.

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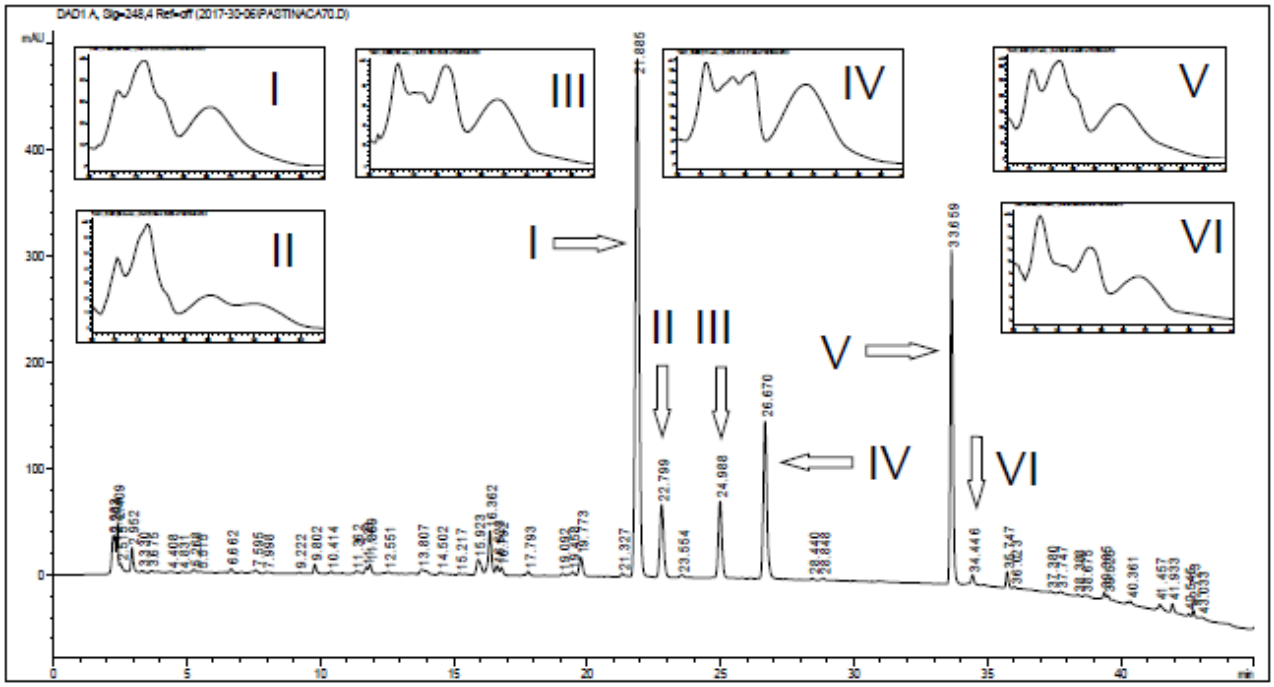

Figure 2: A chromatogram-profile of the extract from *Pastinaca sativa* L. fruits, *Petrik* cultivar, based on 63 % m/m hydro-ethanolic solution at 248.4 nm with UV-spectra of furanocoumarins: I - xanthotoxol, II - sphondin, III – unidentified furanocoumarin, IV - bergapten, V - xanthotoxin, VI - isopimpinellin

As it can be seen from Fig. 2, furanocoumarins dominate among the phenolic compounds detected in the hydro-ethanolic extract from *Pastinaca sativa* L. fruits, *Petrik* cultivar.

Furthermore, three compounds, namely I, IV and V, dominate among furanocoumarins, which are identified as xanthotoxol, bergapten and xanthotoxin, respectively.

They are followed by compounds II and III identified as sphondin and unidentified furanocoumarin, respectively. Bergaptol was not found in the extract.

It should be noted, that the results obtained in regards to furanocoumarins content in *Pastinaca sativa* L. fruits correlate to the data of work [7], but there are certain differences. For example, three compounds mentioned above, which dominate among furanocoumarins (xanthotoxin, bergapten and imperatorin), were identified, but in our studies, we identified xanthotoxol in large quantities instead of imperatorin. Probably, it may be explained by another cultivation location or plant's genetic characteristics, but in general, this difference is not significant.

Fig. 3 presents a chromatogram-profile of the extract from *Pastinaca sativa* L. fruits, *Petrik* cultivar, based on 63 % m/m hydro-ethanolic solution at a wavelength of 360.4 nm with UV-spectra of the main types of phenolic compounds found in the extract.

As it can be seen from data of Fig. 3, in addition to furanocoumarins there are other phenolic compounds in the hydro-ethanolic extract from *Pastinaca sativa* L. fruits, among which compounds VIII and IX dominate; they are quercetin derivative (probably, hyperin) and kaempferol derivative (probably, pasternoside), respectively.

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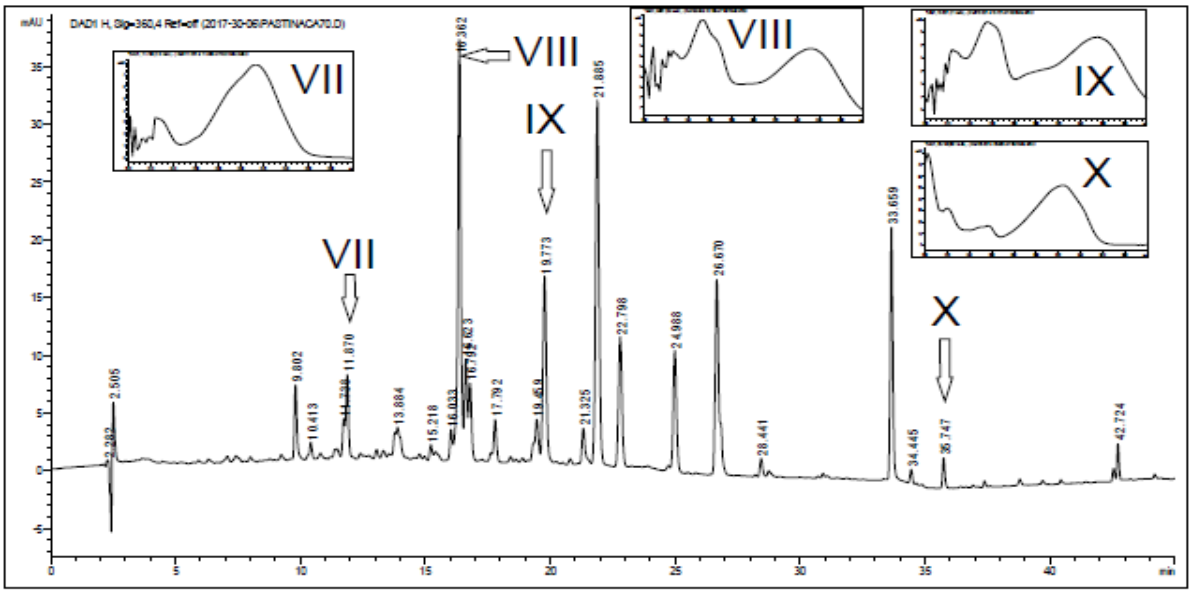

Figure 3: A chromatogram-profile of the extract *Pastinaca sativa* L. fruits, *Petrik* cultivar, based on 63 % m/m hydro-ethanolic solution at 360.4 nm with UV-spectra of phenolic compounds: VII – p-coumaric acid derivative, VIII – quercetin derivative, IX – kaempferol derivative, X – umbelliferone derivative

Compounds VII and X are present in minor quantities; they are p-coumaric acid and umbelliferone derivatives, respectively.

Table 2 shows data of area values for main compounds obtained by HPLC analysis of extracts based on 63±1 % m/m hydro-ethanolic solution from three types of *Pastinaca sativa* L. fruit cultivars.

Table 2: Area values for main compounds of extracts on 63 % m/m ethanol from three types of *Pastinaca sativa* L. fruit cultivars

Compound (λ, nm)	Compound area, mAU·s [a] (Retention time, min)		
	***Petrik* cultivar**	***Globular* cultivar**	***Student* cultivar**
Xanthotoxol (248.4 nm)	4,850±150 (21.9±0.5)	4,760±140 (21.4±0.5)	4,610±140 (20.4±0.6)
Sphondin (248.4 nm)	680±20 (22.8±0.4)	330±10 (22.4±0.6)	330±10 (22.6±0.5)
Unidentified furanocoumarin (248.4 nm)	660±20 (25.0±0.4)	690±20 (25.5±0.5)	690±20 (24.8±0.4)
Bergapten (248.4 nm)	1,390±40 (26.7±0.5)	1,480±40 (27.1±0.4)	1,460±40 (26.9±0.5)
Xanthotoxin (248.4 nm)	2,210±70 (33.7±0.4)	3,780±110 (34.3±0.4)	3,700±110 (33.5±0.5)
Isopimpinellin (248.4 nm)	79±2 (34.4±0.4)	150±5 (35.1±0.5)	96±3 (34.1±0.4)
Umbelliferone derivative (360.4 nm)	330±10 (35.7±0.5)	237±7 (36.3±0.5)	245±7 (35.8±0.6)
p-coumaric acid derivative (360.4 nm)	192±6 (11.7±0.6)	125±4 (11.8±0.6)	10.0±0.3 (12.6±0.7)
Quercetin derivative (360.4 nm)	263±8 (16.4±0.8)	274±8 (17.0±0.8)	153±4 (17.2±0.9)
Kaempferol derivative (360.4 nm)	141±4 (19.8±0.6)	164±4 (20.4±0.5)	27.0±0.8 (19.0±0.6)

[a] Mean value and its confidence interval (Mean±SEM) are calculated with repeat counts *n*=3 and significance level *P*=0.95

As it can be seen from data of table 2, furanocoumarins that occupy up to 95 % of the peak integral area dominate among phenolic compounds identified. It should be noted that xanthotoxol, xanthotoxin and bergapten are attributable to up to 85 % of the peak integral area. It is equivalent to about 1.7 mg/ml of furanocoumarins' integral concentration in the extract. And remaining 5 % of the peak integral area correspond to compounds of quercetin, kaempferol, p-coumaric acid, and umbelliferone.

Thus, for the purposes of quantitative description and standardization of galenical drugs from *Pastinaca sativa* L. fruits, xanthotoxol, bergapten and xanthotoxin can be used.

It is interesting to compare this content of total furanocoumarins 1.7 mg/ml (xanthotoxol, xanthotoxin and bergapten) in hydro-ethanolic extracts that are obtained by us with their content in "Ammifurinum" drug product that is obtained from *Ammi majus* L. fruits and is standardized by total furanocoumarins based on isopimpinellin, xanthotoxin and bergapten - 3.0 mg/ml [6]. As it can be seen, the content of furanocoumarins in hydro-ethanolic extracts obtained by us is almost 2-times less than their content in "Ammifurinum" drug product. But using respective types of extraction, the content of furanocoumarins in hydro-ethanolic extracts from *Pastinaca sativa* L. fruits can be increased to the level of the reference drug.

As can be seen from the data above, research and development of a galenical drugs technology on the basis of *Pastinaca sativa* L. fruit is most promising and will be our next step.

CONCLUSION

Using HPLC method, qualitative and quantitative content of phenolic compounds in hydro-ethanolic extracts from *Pastinaca sativa* L. fruits have been analyzed. In the result of experiments, authors have demonstrated that xanthotoxol, bergapten, and xanthotoxin are dominating components among furanocoumarins in hydro-ethanolic extracts from *Pastinaca sativa* L. fruits, whereas the content of sphondin and unidentified furanocoumarin is much less, and isopimpinellin's content is minor. Bergaptol has not been found in the extract. For standardization of galenical drugs from *Pastinaca sativa* L. fruits, authors recommend using a standard compound such as xanthotoxol, bergapten, and xanthotoxin. The range of optimal ethanol concentration in hydro-ethanolic solution for concurrent extraction of furanocoumarins, flavonoids, and coumarins, as well as for selective extraction of furanocoumarins has been found.

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Author Contribution

First author (Kuznietsova, V.Yu.): Carrying out an extraction process.
Second author (Shimorova Y.E.): Drafting the manuscript.
Third author (Boyko, N.N.): Concept and design of the study. Author's contribution is determinant and involves direct participation in all stages of the study.
Fourth author (Pisarev, D.I.): Carrying out HPLC analysis of hydro-ethanolic extracts.
Fifth author (Zhilyakova, E.T.): Formatting of the manuscript.
Sixth author (Novikov, O.O.): Data interpretation.

Conflict of Interests

Authors declare that they have no conflict of interests.

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