1. Introduction

Genus Parthenocissus Planch. – a representative of the Parthenocissaceae. J. Wen et Z. D. Chen (Vitaceae) includes tribe in various sources from 12 [1] to 13 [2, 3], sometimes 14 species [4], most of which are common in East Asia and South Asia, three species penetrate to North America through the intercontinental disjunction. In Ukraine, 3 species of Parthenocissus are cultivated: East Asian P. tricuspidata (Siebold et Zucc.) Planch. and North American – P. quinquefolia (L.) Planch. and P. vitacea (A.Kern.) Fritsch (=P. inserta (A. Kern.) Fritsch) [5], which are widely used as decorative vines for vertical landscaping. Representatives of the genus have a wide pharmacological activity, in particular cytostatic, antimicrobial, antiviral, antidiabetic, diuretic, anti-inflammatory, anticonvulsant, hypocholesterolemic, are used for rheumatism, arthritis, problems with the gastrointestinal tract [6].

With a broad understanding of P. quinquefolia s.l. (incl. P. vitacea) – Virginia creeper was mistakenly considered an invasive species in Ukraine, mainly due to non-recognition of P. vitacea as a specific species. Regarding P. quinquefolia s.str. cases of its naturalization in semi-natural and natural systems in Ukraine were not confirmed [7] but were noted in the flora of China [4]. The closely related and morphologically similar species P. quinquefolia and P. vitacea differ in the structure of the inflorescence [2, 8], leaves, fruit sizes, and the number of seeds in the fruit [5]. Detailed pharmacological and phytochemical studies of Parthenocissus species allow establishing new reliable diagnostic features for identifying closely related taxa.
In the chemical aspect, the raw material of *Parthenocissus* species is poorly studied. It is known that, in addition to flavonoids, steroids, fatty acids and triterpenes, the species of the *Vitaceae* family (*Vitis* sp., *Parthenocissus* sp.) are also characterized by phytalexins – derivatives of stilbene and resveratrol, which have antioxidant [9, 10], anticarcinogenic [11], fungicidal, antibacterial [6, 12], antiatherosclerotic action and have a therapeutic and preventive effect on the cardiovascular system [13–15].

From a phytochemical point of view, among the *Parthenocissus* species, *P. tricuspidata* is the most studied, from the stems of which dimers of resveratrol – tricuspidanol A were isolated [16]; isoampelopsin F, e-viniferin, pallidol [17]; from the roots of *P. laetevirens* Rehder – quadrangularin A and parthenocisin A [18]. Using the method of two-mode centrifugal partition chromatography, stilbenoids were isolated from 500 mg of *P. tricuspidata* shoot extract: resveratrol (13.9 mg) and its glucoside – trans-piceid (13.5 mg), as well as flavonoids: catechin (40.2 mg), aromadendrin-3-O-β-D-glucopyranoside (46.6 mg), engeletin (17.6 mg) [19]. Derivatives of caffeic acid were isolated from the leaves of *P. tricuspidata*: flavonoids: quercetin, methyl ether of quercetin-3-O-β-D-glucuronide and its glucosides, kaempferol, 3,5,7,4′-O-tetramethylkaempferol; phytosterols: β-sitosterol glucoside, 2α-hydroxyursolic and 2,24-dihydroxyursolic acids [20, 21]. In the lipophilic extract of green and red leaves of this species, the presence of chlorophylls, aglycones of flavonoids, simple phenols, as well as a significant content of carotenoids, the presence of which in the raw material is predicted to have anti-inflammatory and wound-healing activity, was established [22]. Among the phenolic compounds found in the stems of *P. tricuspidata*: protocatechuic, benzoic, caffeic, caffeylglycolic acids, methyl ether of caffeylglycolic acid, methyl 3,4-dihydroxycinnamate, 3,4',5-trihydroxybenzoic acid was found in the shoots; hydroxylated stilbene derivatives and their dimers – piceatannol, resveratrol, resveratrol trans-dehydrodromid, cyphostemin A and B, pallidol, glycosides of flavonoids – quercetin-3-O-α-L-rhamnopyranoside, myricetin-3-O-α-L-rhamnoside [25]. Also, the raw material of *P. quinquefolia* was distinguished by the content of anthocyanins (whole plant) [29]; delphinidin, petunidin, cyanidin, malvidin, peonidin and pelargonidin were found in the fruits [30]. Phytosterols β-sitosterol and stigmasterol, flavonoid rutin, and anthraquinone semside C were also isolated from the raw material (whole plant) [6].

A wide spectrum of pharmacological activity of flavonoids and phenolic compounds of *P. tricuspidata* has been shown, in particular, their antimicrobial, antioxidant, antithrombotic, anti-inflammatory and antitumor effects [21, 23, 26], they reduce the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation, for example, quercetin [31]. *P. quinquefolia* is used in folk medicine in a number of countries around the world for the treatment of diseases of the gastrointestinal tract, as well as a hemostatic, antitumor, anti-inflammatory, antiabetic, antiviral agent [13, 32]. Extracts of the bark and stem of *P. quinquefolia* have significant antioxidant activity, so it can be used to treat cancer and slow down the aging process [9, 32].

Considering the significant distribution, ease of cultivation and the potential perspective of medical use of *P. quinquefolia*, it is urgent to expand the information on the chemical composition and establish the pharmacological activity of the raw material of Virginia creeper growing in Ukraine. Pharmacognostic and pharmacological studies of the species will contribute to the expansion of the nomenclature of medicinal plants and the raw material base for the creation of new medicinal products.

An important group of BASs for the pharmaceutical industry are flavonoids – one of the most diverse and widespread groups of phenolic compounds that are found in many plants and have a wide range of biological effects: antioxidant, P-vitamin, choleretic, antispasmodic, diuretic, hypoglycemic, sedative, estrogenic, antitumor [33, 34]. Many studies confirm that herbal products are potential agents due to the absence of undesirable side effects and high tolerability regardless of the age of patients [35]. Therefore, it is little-studied plants with experience in folk medicine that can become a promising source of raw materials for obtaining new herbal preparations.

**The aim of the research** was to determine the component composition and quantitative content of flavonoids, including catechins, in the leaves, shoots and fruits of *Virginia creeper* (*P. quinquefolia*) for further standardization of raw materials and establishing the antioxidant activity of water-alcohol extracts of the investigated types of raw materials.

**2. Research planning (methodology)**

Stages of research of medicinal plant material *P. quinquefolia*:

Stage 1. Prepare plant material (leaves, shoots and fruits) of *P. quinquefolia* for the research.

Stage 2. Obtain water-alcohol extracts of leaves with shoots and fruits of *P. quinquefolia*.

Stage 3. Investigate the component composition and quantitative content of flavonoids, including catechins, by the method of high-performance liquid chromatography (HPLC) in the plant material of *P. quinquefolia*.

Stage 4. To determine the antioxidant activity of extracts of leaves with shoots and fruits of *P. quinquefolia* by spectrophotometric method.

Stage 5. Based on the obtained results, justify the prospects of using the leaves, shoots and fruits of *P. quinquefolia* as a source of biologically active substances with vaso-strengthening, antioxidant, antihistamine and anti-inflammatory effects.
3. Materials and methods

The object of the research is the leaves, shoots and fruits of Virginia creeper (*P. quinquefolia*). The raw materials were harvested in 2020 in Lisnyky village of the Obukhiv district of the Kyiv Oblast (Ukraine): leaves and shoots – in the flowering phase (July), fruits – during full ripeness (September–October).

The species affiliation was determined by Ph. D., senior researcher of the National Museum of Natural History of the National Academy of Sciences of Ukraine O. F. Shcherbakova.

Determination of the component composition and quantitative content of flavonoids, including catechins, was carried out in samples of air-shade-dried raw materials crushed to a particle size of 3 mm by the HPLC method.

Sample preparation of raw materials for the determination of flavonoids was carried out as follows: an exact weight of raw materials (leaves and shoots, fruits) of *P. quinquefolia* 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 hermetic glass vials with a Teflon cap. The obtained extract was centrifuged at 3000 rpm using an Eppendorf Centrifuge 5415 C and filtered through disposable membrane filters with 0.22 μm pores (Sartorius, Minisart) [36].

To determine catechins, a weight of raw material (leaves and shoots, fruits) of *P. quinquefolia* weighing 0.2–0.6 g was extracted with 10 ml of a 70 % solution of ethyl alcohol P in an ultrasonic bath Bandelin Sonorex Digitec at 80 °C for 5 hours in glass sealed vial with Teflon cap. 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) [37].

The identification and determination of the quantitative content of flavonoids in the raw material (leaves and shoots, fruits) of Virginia creeper (*P. quinquefolia*) was 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 grade solvents were used to prepare the mobile phases. Basic solutions were prepared using 70 % ethanol P 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 individual groups of polyphenols under investigation is observed in the UV region (for flavonoids – 280 and 365 nm; for catechins – 250 and 275 nm) [36, 37].

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. To carry out the 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) in a gradient mode, to achieve the required retention time of the corresponding compounds. By changing the pH value in the gradient mode, you can achieve a clear separation of all components.

To separate flavonoids, acetonitrile for chromatography 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 (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 [36].

Methanol (A) and a 0.1 % solution of formic acid in water (B) were used as the mobile phase for the determination of catechins. 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 [37].

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

According to the recommendations of SPhU/EP (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 comparison 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 performed using standard solutions of flavonoids (rutin, quercetin-3-β-glycoside, naringin, neohesperidin, quercetin, naringenin, kaempferol, luteolin, apigenin) and catechins (pyrocatechin, catechin, epicatechin, epicatechin gallate, and halocatechin) [36, 37].
Quantitative content \((X)\) (μg/g) was determined by the formula:

\[
X = \frac{c \times v}{m}
\]

where: 
- \(c\) – concentration of the compound, determined chromatographically, μg/ml;
- \(v\) – volume of extract, ml;
- \(m\) – weight of raw material from which extraction was carried out, g.

The antioxidant activity of water-alcohol extracts of leaves with shoots and fruits of *P. quinquefolia* was determined by the spectrophotometric method according to the method [38], which is based on establishing the kinetics of the inhibition reaction of autoxidation of adrenaline *in vitro*. It is known that during the autoxidation reaction of adrenaline in an alkaline environment at room temperature, a reaction product is formed that has an absorption maximum at 347 nm, which is significantly ahead of the formation time of adrenochrome, the absorption maximum of which is 480 nm. This technique is proposed for determining the antioxidant activity of various types of medicinal plant raw materials and preparations based on them [38].

**Reference solution.** To 4 ml of 0.2 M sodium carbonate buffer solution (pH=10.65), 0.2 ml of 0.1 % adrenaline hydrochloride solution was added, thoroughly and quickly mixed, placed in a spectrophotometer ULAB 108UV (China), optical density \((A_1)\) was determined after 30 seconds, after 3, 7 and 10 minutes at a wavelength of 347 nm in a cuvette with a thickness of 10 mm.

**Test solution.** 0.06 ml of the studied extract and 0.2 ml of 0.1 % adrenaline hydrochloride were added to 4 ml of the buffer solution (pH=10.65), mixed and the optical density \((A_t)\) was measured, similarly to the above method.

**Compensation solution.** A buffered solution (pH=10.65) of the extract without adrenaline \((A_c)\).

Antioxidant activity (AOA) of the studied extracts in percentage was calculated according to the formula:

\[
AOA = \frac{A_1 - (A_t - A_c) \times 100}{A_1}
\]

where:
- \(A_1\) – optical density of the reference solution;
- \(A_t\) – optical density of the test solution;
- \(A_c\) – optical density of the compensation solution.

The result was taken as the average arithmetic value of five consecutive measurements, the statistical processing of the obtained results was carried out using the Student’s test to determine the standard deviation at the significance level of 95 % according to the monograph of SPhU “5. 3. N. 1. Statistical analysis of the results of a chemical experiment.”

### 4. Research results

Several compounds of a polyphenolic nature were identified by the HPLC method in the leaves, shoots and fruits of Virginia creeper, in particular: flavones and flavonols – rutin, quercetin, quercetin-3-β-glycoside, naringin; catechins – epicatechin, catechin, halocatechin, epicatechin gallate, and their quantitative content was determined.

The results of chromatographic separation of flavones and flavonols in the studied raw materials are shown in Fig. 1, 2 and in Table 1, catechins – in Fig. 3, 4, and Table 2.

According to the obtained results, among the flavones and flavonols in the leaves, shoots and fruits of the Virginia creeper the quantitative content is dominated by rutin (24.39 mg/100 g and 23.05 mg/100 g, respectively) and quercetin (21.43 mg/100 g and 21.73 mg/100 g, respectively). It was established that the leaves and shoots of this type of Virginia creeper contain naringin (13.01 mg/100 g), which is not found in the fruits, and, at the same time, the fruits are characterized by the presence of luteolin (11.33 mg/100 g), which is not contained in leaves and shoots.

Among the catechins in the leaves, shoots and fruits of the Virginia creeper, the dominant components are epicatechin (3.34 mg/100 g and 3.89 mg/100 g, respectively) and catechin (2.85 mg/100 g and 3.16 mg/100 g, respectively). It is also worth noting that leaves and fruits accumulate three times more halocatechin (3.08 mg/100 g) compared to fruits (1.05 mg/100 g).

As a result of the study of the antioxidant activity of the studied raw materials by the method of UV-spectrophotometry, it was established that with the exposure time, the optical density of the comparison solution (pure adrenaline solution) increased, while the optical density of the tested solutions (adrenaline solution with water-alcohol extracts of leaves with shoots and fruits *P. quinquefolia*) decreased after 7 min of exposure (Fig. 5). The obtained results indicate that the extract of the leaves with shoots and the extract of the fruits of *P. quinquefolia* can reduce the process of autoxidation of adrenaline and show antioxidant activity. The results of the spectrophotometric study are given in Table 3.

#### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound name</th>
<th>Quantitative content, mg/100 g, based on absolutely dry raw materials</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves+shoots</td>
</tr>
<tr>
<td>1</td>
<td>Routine</td>
<td>24.39</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin-3-β-glycoside</td>
<td>16.45</td>
</tr>
<tr>
<td>3</td>
<td>Naringin</td>
<td>13.01</td>
</tr>
<tr>
<td>4</td>
<td>Quercetin</td>
<td>21.43</td>
</tr>
<tr>
<td>5</td>
<td>Luteolin</td>
<td>–</td>
</tr>
</tbody>
</table>

#### Table 2

<table>
<thead>
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<th>No.</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaves+shoots</td>
</tr>
<tr>
<td>1</td>
<td>Catechin</td>
<td>2.85</td>
</tr>
<tr>
<td>2</td>
<td>Epicatechin</td>
<td>3.34</td>
</tr>
<tr>
<td>3</td>
<td>Epicatechin gallate</td>
<td>1.32</td>
</tr>
<tr>
<td>4</td>
<td>Galocatechin</td>
<td>3.08</td>
</tr>
</tbody>
</table>
Fig. 1. Chromatogram of flavones and flavonols of leaves and shoots of *Parthenocissus quinquefolia*

Fig. 2. Chromatogram of flavones and flavonols of fruits of *Parthenocissus quinquefolia*

Fig. 3. Chromatogram of catechins of leaves and shoots of *Parthenocissus quinquefolia*
Table 3

<table>
<thead>
<tr>
<th>Antioxidant activity</th>
<th>Time, min</th>
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<th>4</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf and shoot extract</td>
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<td>16.1±0.26</td>
<td>28.5±0.28</td>
<td>48.8±0.39</td>
<td>50.4±0.37</td>
</tr>
<tr>
<td>Fruits extract</td>
<td></td>
<td>15.9±0.16</td>
<td>27.8±0.98</td>
<td>47.0±0.85</td>
<td>49.3±0.48</td>
</tr>
</tbody>
</table>

5. Discussion of research results

As a result of a chromatographic study, the content of 9 phenolic compounds in the leaves, shoots and fruits of Virginia creeper was identified and quantified, in particular 5 flavones and flavonols and 4 catechins, and it was established that rutin, quercetin, epicatechin, catechin dominate in both types of the studied raw materials. The obtained data are of scientific interest because it is known that the discovered compounds have a pronounced pharmacological effect. Thus, rutin has a powerful effect on blood vessels: it reduces the fragility and permeability of capillaries and is used in the treatment of hemorrhoids, varicose veins, and prevention of protection of brain vessels [39, 40]. Quercetin is a powerful antioxidant, has antihistaminic, anti-inflammatory effects, its pharmacological activity in the treatment of patients with COVID-19 [41] due to inhibition of viral proteases has been established [42, 43]. Catechin and epicatechin have anti-inflammatory, anti-tumor potential, exhibit antioxidant properties, proven effectiveness in the treatment of acute respiratory distress syndrome and neurodegenerative diseases.

According to the results of determining the antioxidant activity of the extracts of leaves with shoots and fruits of Virginia creeper, it was established that this raw material has a pronounced antioxidant activity. Comparing the obtained results with the antioxidant activity of quercetin, determined by the same method, which was 35.7% maximum [38], it can be concluded that the raw material of P. quinquefolia due to the rich composition of polyphenolic compounds, namely, the content of rutin, quercetin, quercetin-3-β-glycoside, luteolin, catechins, is quite promising for the creation of phytoreparations with antioxidant and antiradical effects.

This conclusion is also confirmed by the research of other scientists, in particular, S. Faisal et al. (2018), A. A. Mohamed et al. (2021) and others, which established that the raw materials of P. quinquefolia (shoots, bark, leaves, fruits) exhibit pronounced antioxidant activity due to the presence of a sum of flavonoids, hydroxycinnamic acids, and stilbenes [9, 26, 32].

Thus, the data of studies of the content of polyphenolic compounds, in particular, flavonoids, as well as the...
antioxidant activity of leaves, shoots, and fruits of *P. quinquefolia*, obtained by us, correlate with the corresponding data of literary sources [9, 22, 25–27], and confirm the perspective of using these raw materials as having anti-radical activity.

**Study limitations.** When studying the polyphenolic composition of leaves, shoots and fruits of the five-leaved maidenhair grape 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.** The obtained results make it possible to consider the raw materials of five-leaved ivy of Ukraine as a potential source of flavonoid compounds in order to expand the raw material base of medicinal plants for the creation of new medicinal products that have antioxidant and antiradical effects.

**6. Conclusions**

The content of polyphenolic compounds in leaves, shoots and fruits of Virginia creeper was investigated by HPLC. As a result of the study, 9 phenolic compounds were identified: rutin, quercetin, quercetin-3-β-glycoside, naringin, epicatechin, catechin, galloacetin, epicatechin gallate.

It has been established that rutin, quercetin, epicatechin, and catechin predominate in the leaves, shoots, and fruits of Virginia creeper.

The antioxidant activity of extracts of leaves with shoots and fruits of the Virginia creeper was investigated by the reaction of inhibition of autooxidation of adrenalin *in vitro*. It was established that the studied raw material is characterized by a high antioxidant activity.

Therefore, the obtained research results confirm the perspective of further phytochemical and pharmacological studies of *P. quinquefolia* raw materials with the aim of creating on its basis phytopreparations with anti-oxidant, anti-inflammatory, P-vitamin, antiviral, anti-neurodegenerative, antitumor effects and with a potential therapeutic effect in the treatment of symptomatic and asymptomatic infection of COVID-19.

**Conflict of interests**

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

**Financing**

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**Data availability**

The manuscript has no associated data.

**Use of artificial intelligence technologies**

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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Olena Konovalova, Doctor of Pharmaceutical Science, Professor, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

Yashchuk Bohdana*, Assistant, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

Iryna Hurtovenko, PhD, Associate Professor, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

Olha Shcherbakova, PhD, Senior Researcher, Department of Botany, National Science and Natural History Museum of National Academy of Sciences of Ukraine, Bohdana Khmelnytskoho str., 15, Kyiv, Ukraine, 01601, PhD, Associate Professor, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

Mariia Kalista, PhD, Researcher, Department of Botany, National Science and Natural History Museum of National Academy of Sciences of Ukraine, Bohdana Khmelnytskoho str., 15, Kyiv, Ukraine, 01601, PhD, Associate Professor, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

Natalia Sydora, Doctor of Pharmaceutical Science, Associate Professor, Professor, Department of Pharmaceutical and Biological Chemistry, Pharmacognosy, Private Higher Educational Establishment “Kyiv Medical University”, Boryspilska str., 2, Kyiv, Ukraine, 02099

*Corresponding author: Yashchuk Bohdana, e-mail: b.yashchuk@kmu.edu.ua