CHOOSING THE OPTIMAL EXTRACTION METHOD FOR EUROPEAN MISTLETOE

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Introduction. Given the significant demand for naturally sourced medicinal products, the pharmaceutical industry is focused on plants that grow or are cultivated within the country of production. Among such plants is European Mistletoe, which grows abundantly in Ukraine and possesses a substantial raw material base [1].

European Mistletoe (Viscum album L.) belongs to the genus Viscum, family Loranthaceae (Viscaceae). It is a perennial evergreen plant that parasitizes on the branches of many trees. Varieties of Mistletoe grow slowly but persistently; their natural demise is determined by the death of the host plant. With its modified and branched root system, Mistletoe penetrates under the bark and into the wood of the host tree, forming numerous haustoria. As a parasitic plant, it establishes a system through which nutrients and water are transferred from the host to the parasite. The stems, forming a spherical shrub, are forked, woody, jointed, bare, and easily breakable at nodes. The leaves are opposite, smooth, leathery, and green in color. The fruit is a false spherical monocotyledonous berry, green when unripe and turning white upon ripening (Fig. 1). European Mistletoe is widespread in Ukraine and other European countries [2, 3, 4, 5].

The use of European Mistletoe for medicinal purposes can be traced back to the times of Hippocrates, as indicated by literary sources. Currently, it is recognized as a pharmacopoeial raw material in several European countries and exhibits diverse pharmacological effects [1, 4, 6, 7, 8].

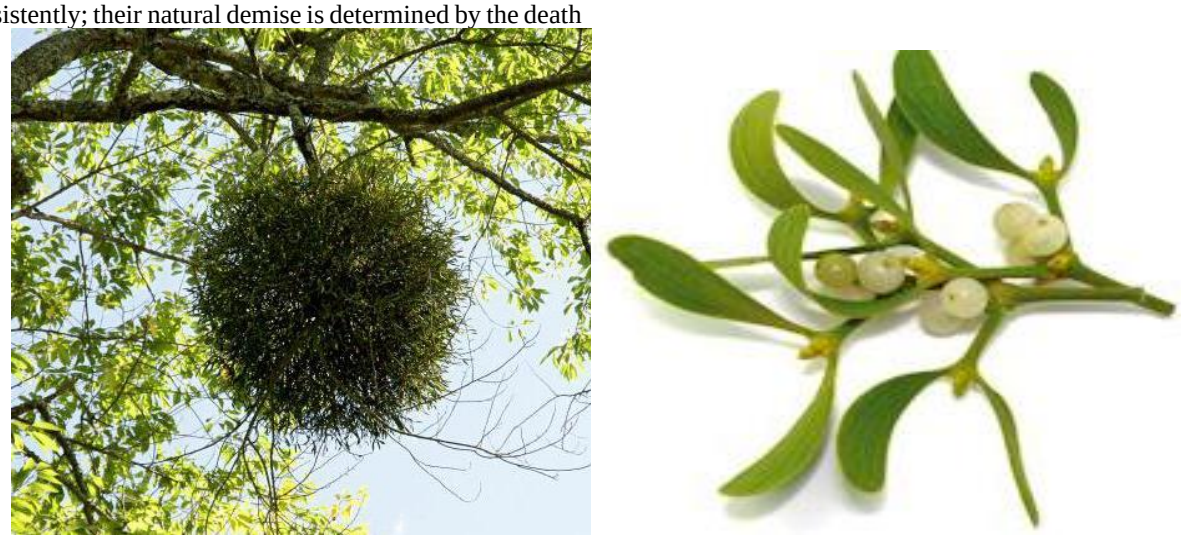

Fig. 1. General view and individual branches, leaves, and berries of European Mistletoe (Viscum album)

In folk medicine, the shoots with leaves and berries of European Mistletoe (Viscum album) are utilized, recommended particularly for cases of intestinal atony. A liquid extract from young leaves is employed in cases of pulmonary, uterine, hemorrhoidal, and nasal bleeding. Spirit-water extracts from Mistletoe dilate blood vessels, exhibit hypotensive and sedative effects, hence are recommended for hypertension, angina pectoris, and "contracted" kidney (nephrosclerosis) treatment. Furthermore, Mistletoe is widely used as an anticonvulsant agent in epilepsy and vertigo [2, 4, 9, 10].

Thanks to its "gentle" impact on the human body (in contrast to aconite and henbane), European Mistletoe is quite extensively utilized in Western Europe as a homeopathic anti-tumor remedy [8, 9, 11].

In scientific medicine, V. album preparations are hardly used, although modern preclinical and pilot clinical studies confirm the effectiveness of European Mistletoe for the treatment and prevention of cardiovascular risks. Foreign researchers have experimentally established that certain bioactive compounds (BAC) within Mistletoe expand blood vessels, lower arterial pressure, and reduce triglyceride levels in serum. In an experiment, the administration of aqueous extract of Viscum album to animals over an 8-week period optimized enzyme systems involved in lipid accumulation and atherosclerosis development. The study observed an increase in carnitine palmitoyltransferase and a decrease in fatty acid synthase [3, 4, 7].

It has been established that European Mistletoe (Viscum album) contains in its composition traces of alkaloids (viscotoxin, viscemin), nitrogen-containing compounds, phenols, flavonoids (rutin, quercetin, ramnetin), terpenoids, triterpenoid saponins, inositol, choline, fatty acids (oleic, ursolic), alcohols (ceryl), carotene, flavones, resins, rubber, wax, vitamins (PP, E, and C), various minerals (Cr, Cu, Fe, Ni, Zn, K, Mg, Mn, Na, S, Se), and more. Recent studies have identified and quantitatively determined over 110 components of different natures [2, 3, 4, 5].

It is widely known that atherosclerosis is referred to as a chronic arterial disease, which, according to literary sources, accounts for approximately 50 % of all deaths in

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Western societies. This pathology is most commonly associated with lipid processes (accumulation of low-density lipoproteins and residual lipoprotein particles) and the development of an active inflammatory process in the blood vessels, leading to heart attacks, ischemic heart disease, stroke, and peripheral artery disease [12, 13].

Due to the presence of polyphenols and flavonoids in European Mistletoe, experimental studies have successfully demonstrated a reduction in cholesterol levels in the body and triglycerides in serum. This beneficially impacted blood vessels and heart function, normalized blood pressure, and helped lower the risk of developing diabetes [4, 14].

Polyphenolic compounds in the body contribute to the formation of a thin layer of compacted protein, thereby exhibiting membrane-stabilizing effects. By densifying cell membranes, they exert an anti-inflammatory action within the walls of capillaries, while the polyphenolic molecules, through interaction with free radicals, function as natural antioxidants, thus normalizing lipid metabolism within the body [3, 7].

The flavonoid quercetin serves as the aglycone of numerous plant flavonoid glycosides, including rutin, and is categorized within the vitamin group P. Due to its capillary-stabilizing properties, attributed to its antioxidant and membrane-stabilizing effects, quercetin reduces capillary permeability. The anti-inflammatory effect of quercetin is achieved through the blockade of the lipoxygenase pathway of arachidonic acid metabolism, reducing the synthesis of leukotrienes, serotonin, and other inflammatory mediators.

Quercetin's cardioprotective attributes are linked to the enhancement of energy supply to cardiomyocytes through its antioxidant impact and improved blood circulation. Diuretic, spasmolytic, and anti-sclerotic properties have also been experimentally identified. Quercetin has the ability to normalize blood pressure and stimulate insulin release, accelerate platelet aggregation, and suppress thromboxane synthesis [1, 10, 14].

The mentioned circumstances warrant further in-depth research into the development of extract-based formulations from European Mistletoe, which would enable their endorsement as independent agents for the prevention and treatment of hypertension, angina, and vascular atherosclerosis [12, 14].

While some researchers consider V. album as potentially toxic material, a comprehensive analysis of contemporary publications has not revealed any substantial correlation in the use of tinctures or extracts when administered in prescribed dosages and under medical supervision. Instances of toxicity are predominantly associated with prolonged use of highly purified Mistletoe berry extracts, often administered as injections for the treatment of malignant neoplasms. It is important to note that the contraindications for European Mistletoe preparations are primarily limited to pregnancy and low blood pressure [11, 15].

According to the State Register of Medicinal Products of Ukraine, there are currently seven registered medicinal products containing European Mistletoe (Viscum album): an external ointment with anti-bruising and anti-inflammatory effects, homeopathic tablets, and two complex tinctures of Ukrainian production designed for cardiovascular disease treatment. The domestic pharmaceutical company PJSC "CHERVONA ZIRKA "CHEMICAL & PHARMACEUTICAL PLANT" has developed two substances from European Mistletoe, differing in extractant and composition of active pharmaceutical ingredients. However, the pharmaceutical market of Ukraine lacks both domestic and foreign medicinal products solely utilizing this raw material [16, 17].

Therefore, the existing evidence base from foreign researchers allows for the assumption that the use of phytotherapeutic agents for safe and relatively prolonged treatment would be an optimal approach. The domestic development of a European Mistletoe preparation as an independent remedy with anti-atherosclerotic properties is a relevant and contemporary topic. This is especially crucial for Ukraine, which, according to WHO data, ranks 2nd globally and 1st in Europe in terms of the number of deaths caused by cardiovascular diseases, accounting for 67% of the total mortality rate [18, 19, 20].

Research Objective. According to the available literature sources, extraction-based medicinal products from European Mistletoe are obtained through maceration using 40-90 % ethanol as the extractant, with a "raw material: extract" ratio (DER) of 1:10. Under these mentioned conditions, the amount of extracted substances is relatively small. Therefore, the aim of this study was to select a rational technology for obtaining an ethanol extract of European Mistletoe for the subsequent development of a preparation with anti-atherosclerotic properties. This work sets two main research tasks: the selection of an effective extraction method and the determination of optimal extraction conditions to maximize the extraction of selected bioactive substances of specific direction.

Materials and Methods. During the investigation of technological parameters, both pharmacopoeial and generally accepted non-pharmacopoeial methods were employed, as detailed further in the article. All obtained results were subjected to statistical analysis (P = 95 %, n = 5) [21].

The research focuses on selected shoots with leaves of European Mistletoe, collected in the Kharkiv region during October-November 2021. According to literature, European Mistletoe is commonly used in its fresh form, as drying the plant material can lead to processes that increase the toxicity of the initial material. The maximum content of beneficial BAS is observed from mid-autumn to winter. Therefore, fresh plant material collected in autumn was used for the study [2].

In the development of extraction technology from medicinal plant material, it is essential to consider its physicochemical and technological properties. These properties help determine the best extraction conditions and maximize the extraction of BAS, ensuring the therapeutic effect of the chosen pharmacological direction.

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Published data on some technological properties of V. album vary slightly in different studies [5, 22].

To determine the optimal extraction conditions for European Mistletoe, we studied the following indicators of the raw material: particle size, specific and bulk density, apparent density, porosity, pore volume, free volume of the layer, and the coefficient of extraction solvent absorption by the raw material [23, 24]. The fineness of the raw material characterizes the degree of tissue disruption of the raw material and the specific contact area between phases necessary for determining the optimal extraction conditions. The specific mass was determined as the ratio of the mass of absolutely dry fragmented plant raw material to the volume of the plant material.

The bulk mass was determined as the ratio of the mass of fragmented plant raw material at natural humidity to its total volume, which includes pores, cracks, and capillaries filled with air.

The bulk density was determined as the ratio of the mass of fragmented plant raw material at natural humidity to the occupied volume of the raw material, which includes pores of particles and voids between them. The porosity of the raw material characterizes the presence of voids inside the particles of the raw material and is determined as the ratio of the difference between the specific and bulk masses to the specific mass. The layer porosity characterizes the presence and size of voids between the particles of the fragmented plant material and is determined as the ratio of the difference between the bulk mass and bulk density to the bulk mass. The free volume of the layer characterizes the relative volume of free space within a unit of the raw material layer (voids inside particles and between them) and is determined as the ratio of the difference between the specific mass and bulk density to the specific mass.

The solvent absorption coefficient characterizes the amount of solvent filling intercellular pores, vacuoles, air voids in the plant material and not removed from the marc (exhausted. plant material). This parameter was calculated based on the difference in volume between the extraction agent used to pour the calculated weight of the raw material and the volume obtained after draining the extract and pressing the marc.

To obtain extracts, the following methods were used: "classical" maceration and extraction with variable pressure (EVP). As an extraction solvent in the maceration extraction method, solutions of 40% and 70% ethanol were employed, prepared from purified 96% ethanol and water. When using EVP, only 40% ethanol was utilized. This ethanol concentration was chosen based on the results of previous studies indicating the highest yield of substances with antiatherosclerotic properties (polyphenolic compounds, flavonoids, etc.). The ratio of "raw material: extract" (DER) was set at 1:10.

The determination of the content of extractive substances in the extracts was carried out by calculating the dry residue according to the methodology of the State Pharmacopoeia of Ukraine [21].

The qualitative and quantitative assessment of the obtained extracts was conducted using several methods [3, 21]:

1) Qualitative determination of polyphenolic compounds was performed through color reactions of identification with ferric chloride and sulfuric acid or by adding iron-ammonium alum;

2) Quantitative determination of the sum of polyphenolic compounds, expressed as tannin, was accomplished through titration with a 0.02 M potassium permanganate solution in the presence of indigosulfonic acid;

3) Qualitative identification of flavonoids was conducted under UV light at a wavelength of 365 nm after adding a solution of aluminum chloride in ethanol and using color reactions with a solution of vanillin in hydrochloric acid;

4) Quantitative determination of the sum of flavonoids, expressed as quercetin, was carried out using the spectrophotometric method at a wavelength of 400 nm with a path length of 10 mm after adding a 3 % solution of aluminum chloride in ethanol and a 0.1 M solution of hydrochloric acid, compared to a standard sample.

Results and discussion

In order to select the optimal method for extracting BAC from European Mistletoe, the technological properties of the raw material were initially investigated. The results of the technological parameter studies are presented in Table 1.

Table 1. Technological Parameters of European Mistletoe Raw Material

№	Technological parameter	Determination result
1	Fineness, mm	5±2
2	Specific mass, g/cm³	1.28±0.02
3	Bulk mass, g/cm³	0.49±0.03
4	Bulk density, g/cm³	0.53±0.02
5	Porosity of raw material, g/cm³	0.31±0.03
6	Layer porosity, g/cm³	0.41±0.04
7	Free volume of layer, g/cm³	0.59±0.02
8	Solvent absorption coefficient (40 % ethanol), ml/g	1.35 ±0.01

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The studied technological parameters of the raw material can be further utilized for determining the optimal mass and volume of raw material and solvent for infusion. The amount of solvent needed to fill the gaps between the raw material particles is determined by bulk density and porosity. This volume of the liquid phase is within the mass transfer zone, allowing calculations for equilibrium extraction methods. The need for compacting the raw material in the extractor is determined by bulk density and porosity indicators.

Fresh shoots with leaves were comminuted using a knife mill and extracted using fundamentally different methods [24].

The maceration method has been long known as the simplest one, requiring no complex equipment. However, it has significant drawbacks: incomplete extraction, prolonged extraction time, and thus, it is not widely used nowadays. Another possible reason for the low yield of extractive substances in our case is the use of fresh raw material. It is known that a living cell is in a state of turgor, and the cell membrane of fresh plant material is a semi-permeable barrier that does not allow external substances dissolved in the cell sap to pass through. In such cases, a necessary condition for obtaining substances from a living cell is the fine comminution of the raw material, but the obtained extract is contaminated with a significant amount of ballast and accompanying substances, the removal of which increases the time and complexity of the process [23, 24]. In our research, maceration was used as a comparative method.

The maceration extraction process was carried out following the recommendation of the State Pharmacopoeia of Ukraine. In a laboratory extractor, an appropriate amount of comminuted European Mistletoe shoots was loaded, the calculated amount of solvent was added, and the mixture was infused for 7 days at room temperature with periodic stirring. After the infusion period, the extract was drained, the remnants of the solvent from the marc were removed by pressing, and the extract was allowed to settle at a temperature of 8±2°C for 2 days. The transparent portion of the obtained extract was further used for qualitative and quantitative analysis [21, 23].

For maximum extraction of BAS from plant raw materials, researchers conduct studies dedicated to intensifying known or developing new extraction methods. One such method is extraction with variable pressure (EVP) [25].

EVP was carried out at room temperature using the "Timatic Micro 0.5" laboratory extractor from the Italian company "Technolab," the external appearance of which is shown in Fig. 2.

Fig. 2. Timatic Micro Extractor

This technology is based on the dual action of initially applying excessive pressure followed by pressure reduction (compression-decompression) and percolation. The extraction cycle alternates between a dynamic phase, achieved through programmed pressure, and a static phase to facilitate the transfer of substances into the solvent. During the primary dynamic phase, pressure is generated within the re-circulation of the extractant to ensure efficient penetration of the raw material. This process prevents the formation of free channels and product oversaturation [26].

The fundamental operation principle of Timatic Micro is illustrated in Fig. 3.

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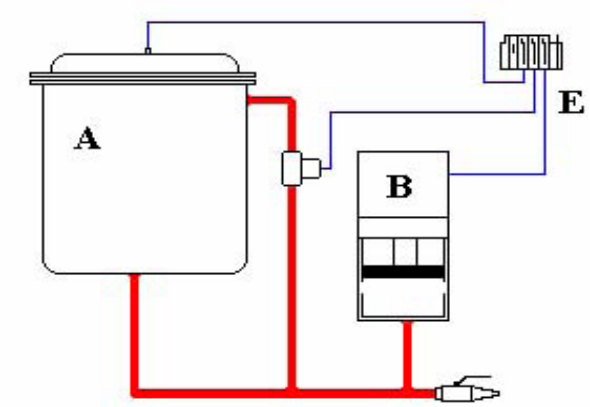

Fig. 3. Operational scheme of the Laboratory Extractor Timatic Micro:
A – extraction chamber; B – active piston; C – pneumatic valve

During the application of excess pressure, the extractant penetrates inside the plant cell by compressing the gas bubbles contained within them and dissolves the BAC. Upon pressure reduction, the gas expands again, displacing the formed solution of substances from the cell. Through percolation, the plant material particles are washed by the extractant, and the dissolved BAC are convectively transported from the particles to the entire volume of the extractor chamber. This mechanism accounts for the high intensity of the extraction process using variable pressure [26].

The extractor is equipped with a powerful microprocessor for data storage and automatic execution of production cycles. It ensures continuous monitoring of all cycles and stages, capable of storing large data arrays with user-defined programs. The number of cycle repetitions is selected by choosing the desired value.

Since the extraction process in this equipment is carried out at room temperature, the obtained extract retains the natural properties and characteristics of the active components. The degree of raw material exhaustion is significantly higher compared to traditional extraction methods.

The operation of this extractor model utilizes small quantities of raw material and extractant. The method also offers the advantage of obtaining pre-purified extract rapidly, while preventing contamination and oxidation of substances during the extraction process [26].

Extractions using this method were carried out in the following sequence: a portion of crushed Mistletoe was placed in a filtration bag, which was loaded into the extraction chamber. The calculated amount of extractant was added, and the plant material was extracted under specified conditions.

Excess pressure in the extractor is achieved only when the working chamber is fully loaded with plant material and extractant. The loading of the working chamber was calculated using the technological parameters of the raw material provided in Table 1.

To determine the optimal conditions for extraction, the following parameters were used: pressure – 5 atm for all samples, while compression time was varied from 2 min to 3 min, decompression time from 2 min to 3 min; the number of cycles ranged from 20, 30, 40 to 60; total process time from 120 min to 240 min. The enumerated parameters are presented in Table 2.

Table 2. Parameters for extraction with variable pressure

Sample Number	Pressure, atm	Compression time, min	Decompression time, min	Number of cycles	Total process time, min
3	5	2	2	30	120
4	5	3	3	20	120
5	5	2	2	60	240
6	5	3	3	40	240

Since it has been established that the maceration method using 40% ethanol extracts a greater amount of the selected target BAC compared to the use of 70% ethanol, only 40% ethanol was used in the experiments for extraction with variable pressure (EVP). The "raw material: extract" ratio (DER) was set at 1:10 for all samples extracted using the EVP method.

An advantage of the EVP method is that during extraction, the plant material is enclosed within a filtration bag. The obtained extract is discharged under excess pressure, passing through the filtration bag, thereby acting as a preliminary purification step and eliminating the need for additional purification methods. The extracts obtained after the extraction process were utilized for determining qualitative and quantitative indicators, the results of which are presented in Table 3.

Based on the presented results in Table 3, it can be concluded that changing the compression time and the decompression time from 2 minutes (samples 3 and 5) to the compression time and the decompression time 3 minutes (samples 4 and 6) leads to an increase in the

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amount of extractable substances, polyphenols, and flavonoids.

Table 3. Comparative evaluation of extracts from European Mistletoe obtained using different extraction methods

№	Extraction Method	Ethanol content in extractant, %	Content of		
			extractives, %	polyphenols, calculated as tannin, %	flavonoids, calculated as quercetin, %
1	Maceration	40	2.52±0.03	5.634±0.051	0.158±0.004
2	Maceration	70	2.38±0.07	5.220±0.033	0.146±0.003
3	EVP	40	3.20±0.05	6.216±0.044	0.198±0.004
4	EVP	40	3.29±0.06	6.761±0.039	0.206±0.003
5	EVP	40	3.33±0.04	6.430±0.062	0.223±0.005
6	EVP	40	3.39±0.03	6.902±0.056	0.232±0.006

Increasing the compression and decompression times within the specified range does not result in an increase in the extraction duration, so the number of cycles was reduced to keep the total extraction time the same. Therefore, it can be asserted that increasing the compensation and decompression time is a factor that enhances the yield of BAC under these experimental conditions.

The next factor varied was the total extraction time, which was changed from 120 minutes (samples 3 and 4) to 240 minutes (samples 5 and 6). With the increase in extraction duration, there is an increase in the yield of extractives, polyphenols, and flavonoids. However, the increase in extractives yield was minimal when doubling the extraction duration, indicating that the extraction time factor is not influential under these conditions. This can be explained by the high efficiency of the EVP method: the extraction process occurs much faster than in maceration, and the extract quickly saturates with bioactive substances almost to equilibrium concentration within 120 minutes. Further extraction of BAC from the plant material significantly slows down.

Thus, the obtained research results suggest that the most optimal technology for obtaining liquid extracts from fresh European Mistletoe shoots and leaves is extraction under variable pressure conditions. The optimal extraction parameters for European Mistletoe shoots and leaves can be considered as follows: fineness – 5±2 mm, "raw material: extract" ratio (DER) – 1:10, pressure – 5 atm, compression time – 3 min, decompression time – 3 min, number of cycles – 20, total process time – 120 min.

Conclusions:

1. As a result of the conducted research, the method of extraction under variable pressure has been chosen as the optimal technique for obtaining European Mistletoe extract.
2. The efficient extraction conditions have been experimentally established.
3. A qualitative and quantitative assessment of the obtained extract has been performed, confirming the correctness of selecting the EVP method for Viscum album extraction.

Selection of the optimal extraction method for European Mistletoe

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Introduction. The therapeutic properties of European Mistletoe (Viscum album L.) have been known since ancient times. This plant contains various biologically active compounds that contribute to its diverse pharmacological effects. Extracts from European Mistletoe have been found to exhibit direct cytotoxic and immunomodulating properties, making them suitable candidates for antitumor agents. The presence of polyphenolic and other compounds that dilate blood vessels, lower blood pressure, and reduce triglyceride levels in serum suggests that other extraction preparations from European Mistletoe could be recommended as standalone agents for the prevention and treatment of hypertension, angina, and vascular atherosclerosis. **Objective.** The study aimed to select a rational method for obtaining alcohol extract from European Mistletoe for the further development of a preparation with anti-atherosclerotic properties. The research had two main objectives: the selection of an effective extraction method and the determination of optimal extraction conditions for maximum recovery of the targeted BAC. **Materials and Methods.** Fresh shoots with leaves of European Mistletoe were selected as the subject of the study. After comminution and determination of key technological properties, extraction was carried out using the maceration method and extraction under variable pressure in the laboratory extractor "Timatic Micro 0.5" from the Italian company "Technolab". **Results and Discussion.** Comparative experimental studies were conducted to determine the optimal extraction method and main technological parameters for the extraction of the raw material. It was established that extracts obtained by the extraction method under variable pressure exhibited better quality indicators. The optimal extraction parameters for European Mistletoe shoots and leaves were determined as follows: fineness – 5±2 mm, "raw material: extract" ratio (DER) – 1:10, pressure – 5 atm, compression time – 3 min, decompression time – 3 min, number of cycles – 20, total process time – 120 min. **Conclusions.** Based on the conducted research, the extraction method under variable pressure was selected as the optimal approach for

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obtaining extracts from European Mistletoe. The effective extraction conditions were experimentally determined. Qualitative and quantitative evaluation of the obtained extract was conducted, confirming the correctness of choosing the extraction method for Viscum album.
Keywords: European Mistletoe, cardiovascular diseases, extract, extraction under variable pressure, Timatic Micro extractor.

References:

1. Phytotherapy. Medicinal plants. Mistletoe. URL: http://ua.oovacia.com.ua/index.php/fitoterapiya/52-likarski-roslini/117-omela-tsilyushchi-vlastivosti-pokazannya-retsepti.
2. Complete Atlas of Medicinal Plants / comp. I.S. Alekseev. Donetsk: Gloria Trade LLC. 2013. 400 p.
3. Trutaev SI. Development of the composition and technologies of tincture on the basis of vegetative raw material for treatment of an atherosclerosis. Abstract of thesis for the Candidate of Pharmacy Degree in speciality 15.00.01. Kharkiv, 2010. 20 p.
4. Committee on Herbal Medicinal Products (2012) Assessment report on Viscum album L., herba. Committee on Herbal Medicinal Product. London. URL: https://www.ema.europa.eu/en/ documents/herbal-report/final-assessment-report-viscum-album-l-herba_en.pdf.
5. Viscum album. URL: https://www.britannica.com/plant/mistletoe.
6. Voloshyn OI., Bachuk-Ponych NV., Bobkovych KO. et al. Viscum album – promising medical herb with diverse mechanism of action, clinical and pathophysiological aspects of application in internal medicine and oncology (literature review). Phytotherapy. Journal. 2019. № 1. P. 18-23. URL: http://nbuv.gov.ua/UJRN/Fch_2019_1_6
7. Poruthukaren KJ., Palatty PL., Baliga MS. et al. Clinical evaluation of Viscum album mother tincture as an antihypertensive: a pilot study. J Evid Based Complementary Altern. Med. 2014. № 19(1). P. 31-35. DOI: 10.1177/2156587213507726
8. Kienly GS., Kiene H. Influence of Viscum album L. (European Mistletoe) Extracts on Qualiti life in cancer patients: A systemic Review of Controlled Clinical Studies. Integrative Cancer Ther. 2010. Vol. 9(2). P. 142-157.
9. Voloshyn OI., Vasiuk VL, Voloshyna LO et al. Fundamentals of Phytotherapy and Homeopathy. 2nd edition. Chernivtsi: «Misto». 2017. P. 137-145.
10. Vicas SI., Rugina D., Socaciu C. Antioxidant Activity of European Mistletoe (Viscum album). URL: https://www.researchgate.net/publication /221920678_Antioxidant-Activity_of_European_Mistletoe_Viscum_album#pf2.
11. Melzer J., Iten F., Hostanska K. et al. Efficacy and Safety of Mistletoe Preparations for Patients with Cancer Disease. Forschende Komplementär medizin. 2009. Vol. 16. P. 217-226.
12. Zupanets IA., Tattis A., Shebeko SK. et al. Atherosclerosis: therapeutic and preventive possibilities of herbal medicines. Clinical pharmacy. 2016. Vol. 20, № 3. P. 18-22. URL: http://nbuv.gov.ua/UJRN/KlPh_2016_20_3_6
13. Tashchuk VK, Polyanska OS. Atherosclerosis is a problem of modern civilization. URL: https://www.bsmu.edu.ua/uk/news/digest/4838-ateroskleroz-tse-problema-suchasnoi-tsivilizatsii.
14. Szurpnicka A., Kowalczuk A., Szterk A. Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. Arch. Pharm. Res. 2020. № 43. P. 593-629.
15. Kienle GS., Grugel R., Kiene H. Safety of higher dosages of Viscum album L. in animals and humans-systematic review of immune changes and safety parameters. Complement Altern Med. 2011. №11. P. 72.
16. State Register of Medicines of Ukraine. URL: http://www.drlz.com.ua.
17. Compendium. Reference book of medicines. URL: https://compendium.com.ua/uk/
18. The World Health Organization. URL: http://www.who.int.
19. It became known what Ukrainians die from: in the first place are cardiovascular diseases, in the second – oncology. URL: https://ukranews.com/news/733617-v-2020-godu-ukraintsy-bolshe-vsego-umirali-ot-problem-s-serdtsem-i-sosudami-a-takzhe.
20. Cardiovascular diseases claim the lives of about 400,000 Ukrainians annually. URL: https://www.apteka.ua/article/427963.
21. State Pharmacopoeia of Ukraine. SE "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products". 2nd edition. Kharkiv. State "Ukrainian Scientific Pharmacopoeia Center for the Quality of Medicinal Products". 2015. Vol. 1. 1128 p.
22. Tertychko AV., Trutaev SI. Study of the technological parameters of the raw materials of white mistletoe for the production of tincture. Ensuring the health of the nation and the health of the individual as a priority function of the state: Materials of the International Scientific and Practical Conference. Odesa. January 22-23, 2021. Odesa. Public organization "Southern Foundation of Medicine". 2021. P. 25-28.
23. Gladukh IeV., Ruban OA., Sayko IV. et al. Industrial technology of drugs: a basic textbook for students of higher education (pharmaceutical faculties). Kharkiv: NPhU: Original, 2016. 632 p. URL: https://dspace.nuph.edu.ua/handle/123456789/23539?locale=en
24. Gladukh IeV., Sayko IV., Sichkar AA. et al. Theoretical foundations of pharmaceutical technology: manual. Kharkiv: NPhU, 2016. 203 p.
25. Soldatov D., Stepanenko S., Sayko I. et al. Research of Vitis Vinifera Leaves Extraction Process on Timatic Laboratory Extractor. Scripta Scientifica Pharmaceutica. 2019. 6(1). P. 45-52. DOI: http://dx.doi.org/10.14748/ssp.v6i1.5654
26. Timatic Micro. URL: http://www.timatic.it/index.php/en/products/extractors-of-active/ timatic-micro.