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of the Institute of Plant Biology and Biotechnology

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PHYTOCHEMICAL ANALYSIS AND DEVELOPMENT OF PRODUCTION OF BIOLOGICALLY ACTIVE COMPLEX ON THE BASIS OF RAW *MELISSA OFFICINALIS L*

Abstract. In this study, a comprehensive study of wild and cultured plants of the genus *Melissa (Melissa officinalis L)* was developed. It was determined the purity of the raw materials: moisture, total ash, ash insoluble in 10% HCl, sulphate ash, extractives. Macro- and microelement composition of total ash by atomic absorption spectroscopy was analyzed. The analysis of the component composition of the main classes of natural substances was conducted. the basic technological parameters of obtaining biologically active complex of the studied plant species by varying the nature of the extractant, its ratio of raw materials, time, and frequency extraction were worked out.

Key words: *Melissa officinalis L*, extractives, moisture content, total ash, ash insoluble in HCl , sulphate ash, macro- and microelement composition, atomic absorption spectroscopy, phytochemical analysis.

One of the priorities of the development of home science and practice of chemistry of natural compounds is the more complete use of own resources of wild and cultivated plant raw materials and the creation of effective drugs based on it, affordable and not inferior in quality to foreign analogues. Among natural biologically active compounds used for the treatment of upper respiratory tract diseases, special attention should be paid to plants of the family *Lamyaceae Lindl* (clear flow) [1].

The genus *Melissa (melissa)* includes, from 2 to 10 species. It grows like a weed plant in orchards, roads, fields, occasionally wild in the lower belt of mountains, and this plant is also cultivated. The most valuable species is *Melissa officinalis L* (lemon balm), home to which is the eastern Mediterranean region. Cultivate melissa medicinal in many countries of the world, where it is registered as pharmacopoeial plants.

In the culture this plant is introduced throughout Europe and North America and in Kazakhstan the plant is introduced in the regions like South Kazakhstan (Shymkent), Zhambyl (Taraz), Kyzylorda, Almaty regions. Wild species of *Melissa officinalis L* are common in Central and Southern Europe, the Caucasus, Middle and Near Asia, North Africa and North America [2].

The biological value of the raw material of lemon balm is caused by a complex of biologically active substances, such as ether compounds, phenolic substances, vitamins.

Phenolic compounds of *Melissa officinalis* are represented by phenolcarboxylic acids and their derivatives, flavonoids and coumarins. Analysis of literature data shows that, in the lemon balm medicated grown in Europe, n-coumaric, ferulic, kaftaric and coffee acids have been identified. Other researchers have identified rosemary, coffee and protocatechuic acids.

In addition, flavonoids, the glycosides of luteolin and apigenin, are characteristic of this plant. The aqueous extract of lemon balm contains hydrolysable tannins in an amount of 4.32% and flavonoids in an amount of 2.06% [3].

Healing properties of the aerial part of the lemon balm are caused by the high content of essential oil. Its most characteristic components are monoterpenes - citral, geraniol, nerol, citronellol, citronellal. Melissa essential oil also contains linalool, geranilacetate, myrcene, n-cymene, beta-cariophyllene oxide, beta-cariophyllene and other terpenoids, and more than 200 compounds have been isolated and described in total.

The second group of components of essential oil are phenylpropanoids, among which the most characteristic is rosmarinic acid. Phenylpropanoids are a class of plant organic compounds of the aromatic series, which are synthesized in a shikimate way, mainly through the amino acid phenylalanine. A characteristic structural fragment is a benzene ring with a branched three-carbon chain attached to it. Phenylpropanoids have a wide range of functions - protection from herbivorous animals and microbial diseases, protection against ultraviolet light, serve as structural components of cellular stains, pigments, act as signal molecules. Phenylpropanoids are also represented by ethyl ester of rosmarinic acid, caffeic acid, chlorogenic acid, n-coumaric acid, ferulic acid and synapic acids. The content of rosmarinic acid in melissa leaves is from 0.54 to 1.79% [4].

Melissa leaves also contain triterpenes - ursolic and oleanolic acids (0.50% and 0.17%, respectively) and their derivatives, terpenoids - nerol, geraniol, nerolic acid glucosides. They found bitterness, coumarins (esketuin), up to 5% tannins, succinic acid, mucus, tetrasaccharide stachyose (a combination of two galactose residues with glucose and fructose), carotene (0.007-0.01%), vitamins C (0.15 %), B1, B2, E [1, 2].

Melissa is widely used in medicine, in the perfumery, cosmetics and food industries in many countries. Raw melissa has sedative, spasmolytic, immunomodulating, antidepressant, antihistamine, antioxidant, anti-inflammatory and antimicrobial effects. In addition, it was found that this plant has antiviral activity against viral infections, such as smallpox, influenza, herpes [5].

Medicines, which include lemon balm, have pronounced soothing, antispasmodic and carminative properties. It has been established that melissa shows an easy hypnotic effect. Such pharmacological activity is mainly due to the components of the essential oil. Sedative and spasmolytic effects are manifested with the application of small doses of lemon balm, and the subsequent increase does not enhance these effects [6].

In the seeds of lemon balm it contains up to 20% of fatty oil.

Melissa tincture shows protective effect with experimental stomach ulcer. At the same time, it is established that it enhances the motility of the stomach, has choleretic and haemostatic properties. In the experimental animals, the antispasmodic effect of melissa has been established. Its tincture reduces the tension of the smooth muscles of the intestine, shows bronchodilator properties.

Melissa essential oils show anti-inflammatory, bacteriostatic and antiviral properties. Japanese scientists conducted a study of the antimicrobial activity of essential oil components of the plant *Melissa officinalis L* against a number of pathogenic fungi and microbacteria of tuberculosis. The most active were aldehydes (citral, citronelal), and less active alcohols (geraniol) essential oil of the plant [7].

The purpose of the research work is to substantiate the possibility of using the cultivated and wild-growing species *Melissa officinalis L*, introduced under the conditions of the Almaty region to obtain an extract with the subsequent study of the chemical composition.

The objects of the study are samples of a plant of the family *Lamiaceae* genus *Melissa (Melissa)* and its appearance *Melissa officinalis L* (Melissa officinalis). Raw materials individually cultivated at the experimental site of the laboratory of medicinal plants of the institute of phytointroduction and botany under the Ministry of Education and Science of the Republic of Kazakhstan, the city of Almaty and the wild type of lemon balm prepared in Almaty region.

Experimental part and discussion

The selection of raw material is determined by its former prisms and personal indications (to exert, to keep, to contain explosive substances).

All the indications of the distribution were determined by the methods of the SP RK, the Euphemistic Map and other literature sources [8, 9]. The data are presented in table 1.

As can be seen from the data presented in Table 1, the resistance of the plant in a plant of the cultivated species (5.36%) is greater than that of the plant in the sample of the wild-growing species (4.42%) less.

The content of extractives in medicinal plant raw materials is an important numerical indicator that determines its good quality, especially for those types of raw materials in which the quantitative determination of active substances is not carried out.

Table 1 – Numerical requirement for publication of the type of *Melissa officinalis L*

References are requested	Plant *Melissa officinalis* L	
	Cultured	Wild-growing
Implication, %	5,36	4,42
Total wt, %	7,91	10,60
Sulphate Salt, %	15,36	15,74
HCl of ash, %	13,80	14,68
Containment of detective substances (70% alcohol)	41,40	38,03

Depending on the chemical composition of the medicinal plant material and the solvent used, certain active and concomitant substances pass into the extraction.

The solvent, which should be taken when determining extractive substances, is indicated in the relevant specification for this type of raw material. Usually it is the same solvent that is used when preparing a tincture or extract from this raw material. Most often it is ethyl alcohol (50 or 70%) or water.

From the data of table 1, it follows that the greatest amount of extractive substances is extracted by the cultivated plant species.

In the plant raw material, the ash of total, sulfate ash, ashes is insoluble in 10% HCl, which is the residue after treatment with the total HCl ash and consists mainly of silicates, which are a natural component for some objects, but more often the result of contamination of the raw materials with sand, earth and stones. Thus, the increased content of insoluble in hydrochloric acid part of the ash indicates a significant content in the vegetable raw material of the mineral impurity. The amount of sulfate ash is commensurate with the content of metals in plants that form sulfate-insoluble in water. The content of all ash species in the aerial part of the plant does not exceed the maximum acceptable value for pharmacopoeial samples.

The next parameter of the definition is the mineral composition. The investigated plant species have a high ash content.

Mineral elements by their content in the plant are divided into macroelements, microelements and ultramicroelements. The macroelements include Na, K, Ca, Mg, their content in the ash is measured in hundredths of a percent. Microelements: Zn, Cu, Ni, Mn, Fe.

The aboveground part contains macro elements: K, Ca, Na, Mg; microelements: Mn, Fe, Cu, Zn, Ni. *Potassium and sodium* play a leading role in regulating the water-salt balance and acid-base balance of the body. *Calcium* plays a huge role in the life of the human body. The human body contains 1000-1200 g of calcium, 99% - is included in bone tissue, dentin, enamel of the teeth, and 1% plays an extremely important role as intracellular calcium, blood calcium and tissue fluid, that is, it plays an important role in the formation of bones. *Magnesium* participates in many processes occurring in the body - in energy production, glucose assimilation, neurotransmission, protein synthesis, bone tissue building, regulation of relaxation and tension of blood vessels and muscles. *Manganese* affects the development of the skeleton, participating in the process of osteogenesis, and therefore is necessary for normal growth. Manganese participates in the reactions of immunity, in blood and tissue respiration, supports reproductive functions, participates in the regulation of carbohydrate and lipid metabolism. *Zinc* is part of the active center of several hundred metal-enzymes. It is necessary for the functioning of DNA and RNA polymerases, which control the processes of transmission of hereditary information and the biosynthesis of proteins, and thereby the reparative processes in the body. *Nickel* is involved in stimulating the processes of hematopoiesis, the activation of certain enzymes. It has a high ability to enhance redox processes in tissues. Nickel in combination with cobalt, iron, copper participates in the processes of hematopoiesis, and independently - in the exchange of fats, providing cells with oxygen. In certain doses, it activates the action of insulin. *Iron* is the most important trace element, takes part in respiration, hematopoiesis, immunobiological and oxidation-reduction reactions, is part of more than 100 enzymes [10, 11].

In the total ash, the content of macro- and microelements was determined by atomic absorption spectroscopy. The data are presented in table 2, 3.

From the data of tables 2 and 3, it should be noted that the greatest amount of macro- and microelements is in the plant *Melissa officinalis L*, a wild-growing species. In the above-mentioned mass

Table 2 – Macroelements content - K, Ca, Na, Mg

Macroelements	Plant *Melissa officinalis L*	
	Cultured, %	Wild-growing, %
K	$1.173 \cdot 10^{-3}$	$0.737 \cdot 10^{-3}$
Na	$0.802 \cdot 10^{-3}$	$0.221 \cdot 10^{-3}$
Ca	$0.639 \cdot 10^{-3}$	$0.178 \cdot 10^{-3}$
Mg	$0.313 \cdot 10^{-3}$	$1.401 \cdot 10^{-3}$

Table 3 – Micronutrients content of Fe, Zn, Mn, Cu, Ni

Microelements	Plant *Melissa officinalis L*	
	Cultured, %	Wild-growing, %
Cu	$0.716 \cdot 10^{-3}$	$0.394 \cdot 10^{-3}$
Fe	$4.387 \cdot 10^{-3}$	$1.266 \cdot 10^{-3}$
Mn	$0.361 \cdot 10^{-3}$	$0.677 \cdot 10^{-3}$
Ni	0	$0.502 \cdot 10^{-3}$
Zn	$0.486 \cdot 10^{-3}$	$0.335 \cdot 10^{-3}$

of the cultivated and wild-growing species, the predominant micelles are Fe. In the cultivated form of the plant, an increased content of macroelements as K, Na, Ca and a in the wild form Mg and K is noted. The content of heavy metals does not exceed the maximum permissible norms [11].

A comparative phytochemical analysis of the above-ground mass of the plant on the main classes of biologically active substances was carried out. The data are presented in table 4.

Table 4 – Phytochemical analysis of the distribution of *Melissa officinalis L* cultivated and wild-growing species

BAS	Developers	*Melissa officinalis L*	
		Cultured	Wild-growing
Carbohydrates	o-toloidin	green	green
Tannins	ZHAK	blue	blue
	$FeCl_3$	blue	blue
Flavonoids	NH_3	yellow	yellow
	$AlCl_3$	bright yellow	bright yellow
	SiHNO	orange-red	orange-red
Cataracts	$KMnO_4$	bleaching	bleaching
Alkaloids	Phosphoric acid	bleaching	bleaching
Amino acids	ninhydrin	purple	purple
Carboxylic acids	urea	brown	brown
	$MgAc_2$	–	–

Through phytochemical analysis, using diagnostic gums in the aerial part of a plant of the type *Melissa officinalis L*, there were discovered the main groups of biological active substance such as tannins, amino acids, alkaloids, phenolic compounds, organic acids, flavonoids, carotenoids [12].

Method of paper chromatography in the use of reliable samples in the following types of pockets identified carbohydrates and amino acids.

The optimal technology for isolating the extract from the melissa plant was developed taking into account the requirements of the SP RK for the processing of plant raw materials [13, 14].

An important parameter in the technology of obtaining a plant extract is the ratio of raw materials and solvent from 1: 4 to 1: 8. Over 5 g of the aboveground part was extracted with different volumes of

50%, 70%, 90% ethyl alcohol. The constant factors of the extraction process were: extraction time (24 hours) and temperature (23-25 °C). These parameters for obtaining the plant extract are shown in table 5.

Table 5 – Determination of the optimum extractant for the extraction of the raw materials

m, g const	Solvents	t, hour const	T, °C const	m(r): v (ml)	The amount of dry extract, % *Melissa officinalis L*	
					Cultured	Wild-growing
5	50% ethanol	24	23-25°C	1:4	0,528	0,783
				1:6	2,501	2,670
				1:8	5,124	8,574
5	70% ethanol	24	23-25°C	1:4	2,604	3,211
				1:6	3,25	4,454
				1:8	7,94	10,264
5	90% ethanol	24	23-25°C	1:4	0,447	0,695
				1:6	1,121	1,880
				1:8	3,344	3,212

From these tables it follows that the optimal extractant was 70% ethanol. The greatest yield of the percentage of the extract shows extraction with 70% ethanol at a feed: extractant ratio of 1: 8, the amount of dry extract in cultivated form was 7.94%, and in the wild 10.264%.

Another important parameter in the technology of extracting extracts is the ratio of the selected extract with the custome. To determine the optimum volume, the selected extractor changes the content of the raw material and the filter from 1: 5 to 1: 8. To 5 g of the nasis part of the cultivated and wild-growing species *Melissa officinalis L* 70% ethyl alcohol. With this constant factor, the process of extraction was: time of extraction (24 hours) and temperature (23-25 °C). The data are presented in table 6.

Table 6 – Determination of the optimum ratio of raw material and extractant

Mass of raw materials, g const		5	5	5
Extraction time, hour, const		24	24	24
Extraction temperature, ° C const		23-25°C	23-25°C	23-25°C
Ratio of raw materials (g) and extractant (ml)		1:4	1:6	1:8
V2 volume of the filtered extract, ml	Cultured	3,5	5,0	9,5
	Wild-growing	4,0	6,7	11,3
Extraction amount,%	Cultured	1,76	3,25	5, 24
	Wild-growing	2,24	4,62	6,89

With the selected extract (70% ethanol), the optimum value of the extract 1:8 is shown in cultivated form, the amount of the extract is 5.24%, and in the dicorbic form 6.89%; at a temperature of 24-25 °C and a time of 24 hours.

The accuracy of the parameters "raw-extractor" is determined primarily by economic measures, as for a business, but the amount of the used extractor is significant.

Determination of the extraction time is an important parameter, so it is necessary to determine when the entire complex of biological active substances is recovered. The influence of the extraction time of the raw material on the yield of the extract was studied. Extraction was carried out with 70% ethanol at a raw material: extractant ratio of 1: 8. The data are presented in table 7.

On the basis of these arguments and the data obtained, the following regime is proved to be optimal: extraction of 70% by ethanol in 24 hours at a temperature of no more than 23-25 °C with raw materials: extractant ratio 1: 8. Under this regime, the amount of extract was 0.3939% cultivated, 0.2902% in the wild.

Table 7 – Determination of extraction time

Extractant (ml), const		70% ethanol	70 % ethanol	70 %ethanol
Ratio of raw materials (g) and extractant (ml), const		1:8	1:8	1:8
Time, (hour)		24	48	72
V2 volume of the filtered extract, ml	Cultured	17	2	7
	Wild-growing	13	8	11
Extraction amount, %	Cultured	0.3939	0.0605	0.1365
	Wild-growing	0.2902	0.2785	0.2336

Acknowledgement. According to the results of the research work, a comparative analysis of the chemical composition of the above-ground part of the cultivated and wild-growing species of the family *Melissa (Melissa officinalis L)* of the family *Lamiaceae* was performed; Technological parameters were worked out: different concentrations of extractants; dependence of the ratio of raw materials - solvent; the process dependence on time and the number of extractions. The optimum condition for obtaining a plant substance is an extractant of 70% ethyl alcohol, the ratio of extractant and raw material is 1: 8, extraction time is 24 hours, temperature is 23-25 °C.

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MELISSA OFFICINALIS L ӨСІМДІГІ НЕГІЗІНДЕ ФИТОХИМИЯЛЫҚ АНАЛИЗ ЖАСАУ ЖӘНЕ БИОЛОГИЯЛЫҚ БЕЛСЕНДІ КЕШЕН АЛУ

Аннотация. Бұл зерттеуде *Melissa (Melissa officinalis L)* тұқымдасына жататын өсімдіктің мәдени және жабайы түрлерінің кешенді негізі жетілдірілді. Шикізат сапалылығы: ылғалдылық, жалпы күлділік, HCl-да ерімейтін күлділік, сульфатты күлділік, экстрактивті заттар анықталды. Атомдық-абсорбциялық спектроскопиялық әдіспен жалпы күлділіліктің макро- және микроэлементтік құрамы талданды. Талдау компоненттік

құрамның негізгі кластары табиғи заттар. Зерттелініп отырған өсімдік түрлерінен экстрагенттің табиғатын, оның шикізатпен қатынасын, экстрактілеу уақыты мен жиілігін өзгерте отырып, биологиялық белсенді кешенді алу технологиясы өңделді.

Түйін сөздер: *Melissa officinalis L*, экстрактивті заттар, ылғалдылық, жалпы күлділік, HCl да ерімейтін күлділік, сульфатты күлділік, макро- және микроэлементтік құрамы, атомдық-абсорбциялық спектроскопия, фитохимиялық анализ.

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ФИТОХИМИЧЕСКИЙ АНАЛИЗ И РАЗРАБОТКА ПОЛУЧЕНИЯ БИОЛОГИЧЕСКИ АКТИВНОГО КОМПЛЕКСА НА ОСНОВЕ СЫРЬЯ *MELISSA OFFICINALIS L*

Аннотация. В данном исследовании были разработаны основы комплексного исследования культивированного и дикорастущего растения рода *Melissa (мелисса)*. Определены доброкачественности сырья: влажность, общая зола, зола не растворимая в 10 % HCl, сульфатная зола, экстрактивные вещества. Проанализирован макро- и микроэлементный состав общей золы методом атомно-абсорбционной спектроскопией. Проведен анализ компонентного состава на основные классы природных веществ. Отработаны основные технологические параметры получения биологически активного комплекса из исследуемых видов растений варьированием природы экстрагента, его соотношением с сырьем, времени и кратности экстракции.

Ключевые слова: *Melissa officinalis L*, экстрактивные вещества, влажность, общая зола, зола не растворимая в HCl, сульфатная зола, макро- и микроэлементный состав, атомно-абсорбционная спектроскопия, фитохимический анализ.

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