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STUDY OF CARATINOID, FLAVONOID, POLYPHENOL COMPOUNDS OF DICOTYLEDONOUS NETTLE (*URTICA DIOCA L.)*

Abstract. The article is dedicated to the study of chemical composition of dicotyledonous nettle (*Urtica dioca L.),* which belongs to genus *Asteraceae*, grows in the Medeu mountainous region of Kazakhstan and to optimize the efficient isolation of biologically active compounds and to analyze them. In addition, the qualitative composition and quantitative content of dicotyledonous nettle's (*Urtica dioca L.*) bioactive compounds (BAC) is shown. In particular, pectin, phenolic acids, tannins, anthocyanins, flavonoids and polyphenols are found and quantified in the composition of domestic medicinal raw nettles. In general, a simple physicochemical method for the separation of flavonoids and carotenoids belonging to the group BAC is proposed. As a result of the study, from the leaves and stems of the nettle a compound belonging to the P-vitamin group $C_{15}H_{10}O_7$ and from the leaves a compound belonging to the carotenoid group $C_{15}H_{10}O_2$ were isolated.

Keywords: *Urtica dioca L.*, dicotyledonous nettle, flavonoids, carotenoids, tannins, phenolic acids, IR, BAC

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ЕКІҮЙЛІ ҚАЛАҚАЙ *(URTICA DIOCA L.)* ҚҰРАМЫНДАҒЫ ПОЛИФЕНОЛДЫ ҚОСЫЛЫСТАРДЫ, ФЛАВОНОИДТАРДЫ, КАРОТИНОИДТАРДЫ ЗЕРТТЕУ

Аннотация. Мақала жекелеген биологиялық белсенді заттардың тиімді таралуын оңтайландыру мақсатында Қазақстанның Медеу тау ауданында өсетін күрделі гүлдер тұқымдасына жататын екіүйлі қалақайдың *(Urtica dioca L.)*

химиялық құрамын зерттеуге арналған. Өсімдіктің *(Urtica dioca L.)* сапалық және сандық құрамы көрсетілген. Атап айтқанда, екіүйлі қалақай өсімдігінің отандық дәрілік шикізатында пектин, фенол қышқылдары, илегіш заттар, антоциандер, флавоноидтар және полифенолдар табылды. ББЗ тобына жататын флавоноидтар мен каротиноидтарды алудың қарапайым физика-химиялық әдісі ұсынылды. Зерттеу нәтижесінде қалақай өсімдігінің жапырағы мен сабағынан Р дәрумені тобына жататын $C_{15}H_{10}O_7$ және қалақай өсімдігінің жапырағынан каратиноид тобына жатады $C_{15}H_{10}O_2$ қосылыстары бөлініп алынды.

Түйін сөздер: Urtica dioca L., қалақай екіжақты, флаваноид, каратиноид, илегіш заттар, фенол қышқылдары, ИҚ, ББЗ

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ИССЛЕДОВАНИЕ ПОЛИФЕНОЛЬНЫХ СОЕДИНЕНИЙ, ФЛАВОНОИДОВ, КАРОТИНОИДОВ КРАПИВЫ ДВУДОМНОЙ *(URTICA DIOCA L.)*

Аннотация. Статья посвящена изучению химического состава крапивы двудомной *(Urtica dioca L.),* растения, относящегося к роду сложных цветков, произрастающего в Медеуском горном районе Казахстана, с целью оптимизации эффективного распределения отдельных биологически активных веществ. Указан качественный и количественный состав растения *(Urtica dioca L.)*. В частности, в отечественном лекарственном сырье двудомного растения крапивы обнаружены и записаны пектиновые вещества, фенольные кислоты, дубильные вещества, антоцианы, флавоноиды и полифенолы. Предложен простой физико-химический метод получения флавоноидов и каротиноидов, принадлежащих к группе БАВ. В результате исследования из листа и стебля растения крапивы выделены соединения $C_{15}H_{10}O_7$, относящиеся к Р-витаминной группе, и $C_{15}H_{10}O_2$, относящиеся к каратиноидной группе, из листа растения крапивы.

Ключевые слова: *(Urtica dioca L.),* крапива двудомная, флаваноид, каратиноид, дубильные вещества, фенольные кислоты, ИК, БАВ

Introduction.

Currently, one of the key tasks of pharmaceutical science in the Republic of Kazakhstan is the development and introduction of import-substituting drugs, including medicines from plant raw materials. The Republic of Kazakhstan is rich with safe and affordable domestic raw materials, large reserves of herbs used in traditional medicine for centuries.

However, not all species of medicinal herbs, including the genus *Asteráceae*, are used in official medicine. Therefore, it is necessary to conduct a comprehensive study on the development and standardization of medicines based on medicinal raw materials under the initiative "Modern technologies and production of medicines".

In this regard, dicotyledonous nettle (*Urtica dioca L.)* is of particular interest as a raw material for medicines. Its demand is due to the high content of biologically active compounds such as phenolic acids, anthocyanins, flavonoids, polyphenols.

Dicotyledonous nettle (*Urtica dioca L.)* is a genus of the nettle family. There are 45 representatives of nettles on the planet, including 850 species. There are more than 40 species in temperate and tropical regions, and 3 species in Kazakhstan. It grows in the garden, woods, in shady, moist places along the road [4-5].

Biologically active compounds are one of nature's most valuable compounds derived from plants, which are obtained naturally and synthetically from plants. Nowadays it is important to get medicines from plants. This is due to the fact that medicines derived from environmentally safe raw materials, biologically active compounds are widely used in special natural foods, pharmaceuticals, medicine, household chemicals, agriculture. Biologically active compounds are substances obtained in different ways, which are potential sources of drugs that restore the pathologically altered functions of animals and humans [7]. M. Goryaev, L. Klyshev, M. Kukenov, T. Chumbalov and other scientists have studied a number of medicinal plants of Kazakhstan and obtained biologically active compounds from them.

Polyphenols are polyhydric phenols and their derivatives. Polyphenols prevent the process of photosynthesis, growth, asthma and various infectious diseases [8].

Today, about six thousand polyphenols have been isolated from plants. Polyphenols are found not only in useful plants, but also in vegetables and fruits as well. In addition to 1 g of antioxidant components, the human organism gets a number of vitamins, including about 100 mg of ß-carotene, vitamins C and E per day. Polyphenols play an important role in the biological, metabolic processes in the plant Kingdom. In plants tannins (floroglucin, pyrogallol, etc.) in the form of glucosides and essential oils are widely spread. Polyphenols are found in many foods. The products formed during their oxidation (for example, quinone) give food a delicious aroma and aromatic structure. Industrial polyphenols include catecholeamine and some hormones and mediators (adrenaline and noradrenaline) [8-10].

Polyphenols are divided into three types: tannins, lignins and flavonoids. The latter type of polyphenols is widespread and 10 types, composition and structure have been identified. These are: flavonoids, flavonols, flavonones, catechins, isoflavanoids, proanthocyanidine and anthocyanidine.

Polyphenols prevent aging by protecting human skin from sunlight, ozone and other toxins and. Studies by Canadian scientists have shown that polyphenols are found in red wine and are important in the treatment of red gums diseases. Polyphenols in grapes characterize antioxidant, antimutagenic, antibacterial activity of p-vitamins. Polyphenols in the blood vessels, improve blood circulation. Serves as a fundamental element that supports skin tissue. Therefore, doctors warn that excessive consumption

of alcohol can have a negative effect on the organism. In addition, excessive use of polyphenols causes kidney and liver disease [9-13].

Flavonoids are phenolic compounds. Most flavonoids form groups of pigments that give color to different parts of plants and combine in different amounts, giving a magical color to plant life. Others are the founders of flexible things. Flavonoids (lat. Flavo-yellow) are found in many medicinal plants and even in ordinary tea, and they are capable of antiseptic effect and PP-vitamin activity. They carry many powerful antioxidants (substances that counteract the oxidation of body tissues and body fluids, as well as substances that stop the aging of the organism and cells and form metabolic processes), the well-known vitamins E and C. Flavonoids retain their beneficial properties even after drying and extracting the plant. These substances are used in the preparation of antiseptics, dyes in the pharmaceutical industry. Flavonoids are used for therapeutic purposes in cleansing the bile ducts, expectoration, heart disease and cleansing the human body of radioactive substances. Their ability to suppress cancer is also being studied [5-17].

The purpose of the study: to determine the physicochemical composition and quantitative content of polyphenolic compounds, flavonoids, carotenoids in medicinal plant dicotyledonous nettle (*Urtica dioca L.)* growing wild in Kazakhstan.

Practical part

The object of the study was a wild-growing dicotyledonous nettle harvested in April-May 2018 and September-October 2019 in the Medeu mountainous area of Almaty.

Hydrogen index of aqueous, alcoholic solutions of dicotyledonous nettle (leaves, stems, roots) was determined by pH-meter "I-160 MI", refractive index by refractometer IRF-454B, density was determined by pycnometric method.

Moisture and ash content of dicotyledonous nettle by gravimetric method, acidity, ascorbic acid, pectin, tannins by titrimetric method, protein by Kjeldahl method, fiber by weight method according to A.E. Ermakov's modification, crude oil content in Soxhlet extractor, amount of disaccharides, monosaccharides, polyphenols, flavonoids, anthocyanins were determined on a photocalorimeter KFK-2.

Polyphenol compounds were extracted with water to separate from plant raw materials due to their high hydrophilic properties, the extract was washed with water by adsorption on activated carbon to remove lipophilic resins. Separation of the purified fraction was carried out by silica gel, cellulose column chromatography.

During the scientific data discussion, the simplest method of BAC isolation on the basis of the next scheme was used.

The raw material was extracted with 95% ethanol. To neutralize the organic acids, the solution was heated by adding sodium bicarbonate (10: 1). Distilled water was used as standard solution. After extraction, the raw material was filtered, the alcohol-water residue of the extract was pumped out and the filtrate was dried. The filtrate was dissolved in chloroform. The aqueous solution was treated 7-8 times in a filter funnel until an alcohol-water precipitate of the same amount of chloroform was formed. Further evaporation of chloroform (chloroform fraction) was carried. The residue from the extraction was heated in a water bath until the chloroform was removed, dried and treated with ethyl acetate [7-9].

The plant was extracted with pure alcohol to separate flavonoids. After evaporation of the obtained alcohol extract, hot water was poured into the residue and after cooling, non-polar compounds (chlorophyll, oils, essential oils, etc.) were removed from the aqueous phase with chloroform. From the aqueous phase, flavonoids were isolated alternately with ethyl acetate and butanol. Column chromatography was used to separate the components in each fraction. Silica gel and cellulose were obtained as sorbents. A specific method was used to isolate individual flavonoids. Extraction was carried out with hot water to separate the rutin from the bud. After cooling, rutin precipitated from the solution. It was filtered and recrystallized to alcohol [14,18].

The raw material for carotenoid separation was processed and extracted in acetone. The extract was divided into two parts, each of which was processed with 200 ml of petroleum ether. In order to eliminate xanthophyll, the petroleum ether was purified from ethanol and acetone by washing with 80% ethanol, water several times. Then comes the finely ground chlorophyll. Then anhydrous sodium sulfate was then filtered through 50 g of talc. Carotene crystallized when the filtrate was evaporated at 40°C and the fatty part was treated with absolute alcohol.

Results and discussion

Table 1. Physical properties of nettle

Raw material name	pH				n(refractive index)				p, g/cm^3			
	in water	ethanol,%			in water	ethanol,%			in water	ethanol, %		
		40	70	90		40	70	90		40	70	90
leaves	8,544	6,07	6,51	6,06	1,3310	1,3515	1,3600	1,3580	1,0022	0,9635	0,9587	0,8589
stems	7,6	6,08	6,47	5,65	1,3320	1,3510	1,3590	1,3536	1,0036	0,9042	0,8913	0,8909
roots	5,7	5,9	5,7	8,54	1,3320	1,355	1,36	1,33	1,2507	0,9612	0,8973	0,8470

According to Table 1, the pH of alcoholic solutions of dicotyledonous nettle (leaves, stems, roots) is weakly acidic, and the pH of aqueous solutions is close to neutral. The density of the solution in water is higher than the density of the solution in alcohol.

Table 2. Chemical composition of nettle

Raw material name		Leaf	Stems	Roots
Humidity, %		6,5	4,5	6,5
Ashes, %		0,877	0,9	0,96
Extractivity, %		4,1	3,7	3,9
Pectin substances, %	Water soluble	3,5	1,3	0,58
	Insoluble in water	3,7	1,4	0,6
Phenolic acids, %	Gallic acid	5,5	1,9	1,8
	Caffeic acid	5,2	2,01	1,7
Илегіш заттар, %	Condensed	4,5	2,15	0,37
	Hydrolyzed	4,45	2,9	0,36
Acidity, %		0,399	0,227	0,30
Anthocyanins, %		0,2	0,04	0,07
Flavonoids, %		2,5	1,05	0,75
Polyphenols, %		2,970	3,1003	2,40

Extraction was carried out in water and 80% ethanol for 2 hours. The extractivity of nettle leaves is 1.1 times higher than the stem.

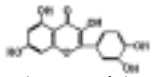
Pectins are 3.5 times higher in the leaves than in the stems, and water-insoluble pectins are 4.1 times higher in the leaves than in the stems. The content of phenolic acids, gallic acid in the leaves is 3.3 times higher than in the stems, and the content of caffeic acid in the leaves is 2.3 times higher than in the stems. And the roots are high in pectin and polyphenols.

Tannin substances are natural phenolic compounds with different molecular weights. The study results show that the amount of tannins in the leaves is 2 times higher than in the stems, and the amount of tannins in the roots is less.

Organic acids are 2 times more in the leaves than in the stems. Compared to the leaves and roots, the content of organic acids in the leaves is 1.6 times higher.

The amount of anthocyanins in the leaves is 4.1 times higher than in the stems and leaves. The content of flavonoids is 2 times lower, and in the roots is 2.5 times lower than in the leaves, the content of polyphenols is similar.

Table 3. Microanalytic index of polyphenols separated from nettle leaves

Nettle	Output, %	Melting point, ºC	Calculated, %		Molecular formula	Found, %	
			C	H		C	H
Leaves	8	179^0	59,55	3,3	$C_{15}H_{10}O_7$  (quartzetin)	58,65	3,7

The formula of polyphenols isolated from the leaves and stems of nettle is $C_{15}H_{10}O_{7.}$. It is a compound belonging to the group of P-vitamins. Melting point 179^0C.

According to scientific data, polyphenols are found at 3400–3450 cm^{-1} IR spectrum of polyphenols isolated from nettle leaves. The peaks were 3500 cm^{-1} for O-H group, 3000 cm^{-1} for CH_3 group, 1540 cm^{-1} for C-C group, 1384 cm^{-1} for C-O group. That is, the structure of the released polyphenols corresponds to the literature [10,21].

Шикізат	**O%**	**Zn%**	**Al%**	**Si %**	**P %**	**S %**	**Ca %**	**Mg %**	**K %**	**Na %**	**Ba %**
ҚОСҮЙЛІ ҚАЛА-ҚАЙДЫҢ *жапырағы*	48,73	6,80	1.43	6.97	2.31	2	22	2,65	9.81		0.01
ҚОСҮЙЛІ ҚАЛА-ҚАЙДЫҢ *сабағы*	47,13	6,31	3.50	2.59	2.17	1.53	22.16	0,21	15.83	0.21	0.01

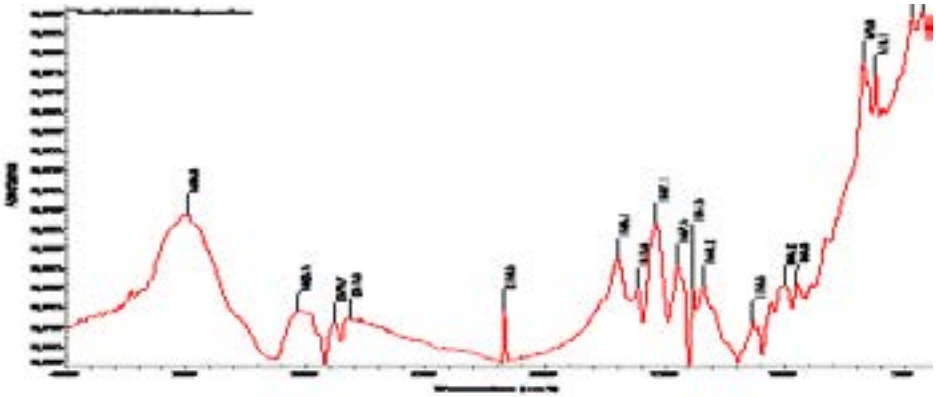
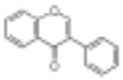


Fig. 1 - IR spectrum of polyphenols isolated from nettle leaves

Table 4. Elemental analysis of flavonoids separated from nettle leaves

Nettle	Output, %	Melting point, ºC	Calculated, %		Molecular formula	Found, %	
			C	H		C	H
Leaves	8	192^0	81	4,2	$C_{15}H_{10}O_2$  (phenyl)	80,2	4

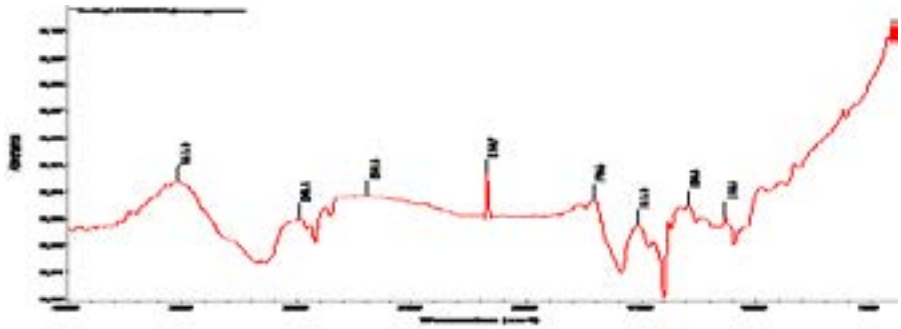


Fig. 2 - IR spectrum of flavonoids obtained from nettle leaves

Formula of flavonoids isolated from nettle leaves: $C_{15}H_{10}O_2$. It is an unsaturated vitamin belonging to the carotenoid group. Melting point – 192^0C. The frequency of oscillations of the IR spectrum 2900 cm^{-1} indicates the valence oscillations of the group C-H, 1600 cm^{-1} - the relationship between C = C-, 1300 cm^{-1} C-O. The oscillation frequency of 570 cm^{-1} corresponds to the methyl group [21].

Table 5. Elemental analysis of carotene

Nettle	Output, %	Melting point, ºC	Calculated, %		Molecular formula	Found, %	
			C	H		C	H
Leaves	9	183^0	89,55	10,44	$C_{40}H_{56}$ (β-carotene)	83,10	6,76

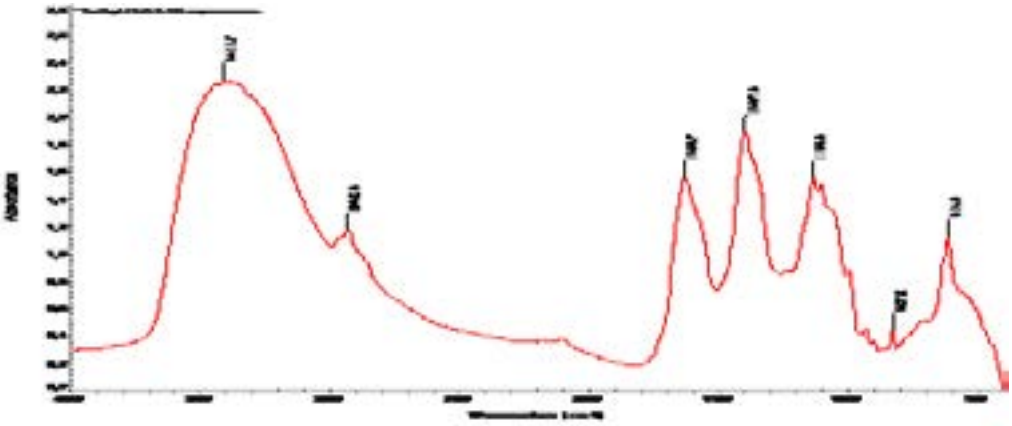


Fig. 3 - IR spectrum of carotene isolated from nettle leaves

The formula for carotene extracted from the leaves of the nettle plant is $C_{40}H56$. It is an unsaturated vitamin belonging to the group of carotenoids. Melting point 183°C. Melting point was determined on an electronic heater "Boetius". If the IR spectrum corresponds at 3632 cm-[1] to O-H group, the frequency of oscillations 3012 indicates the valence oscillations of H-N group, 1540 cm-1-the relationship between C = C- and the frequency of oscillations 1384 cm^{-1}–C-O linkage. In addition, the oscillation frequency of 618 cm^{-1} corresponds to the methyl group.

Conclusion

Biologically active compunds were isolated from the nettle, the composition and structure of which were identified by modern physicochemical methods.

In the future, nettle will be used in the production of domestic phytopreparations, effective medicines.

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