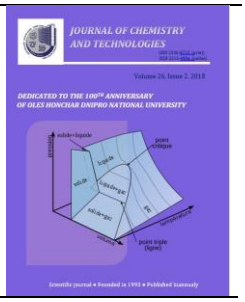
Journal of Chemistry and Technologies, 2018, *26*(2), 39-44

Journal of Chemistry and Technologies

pISSN 2306-871X (Print), ISSN 2313-4984 (Online)

journal homepage: http://chemistry.dnu.dp.ua



UDC 615.451.1:[615.322:582.736.1].07:544.3

STUDY OF NATIVE EXTRACTS FROM RAW LUPIN YELLOW AND ALFALFA YELLOW (LIKE-SICKLE OR ROMANIAN) BY CHROMATO MASS SPECTROMETRY

Olena V. Grechana*[1], Anatoly G. Serbin[2]

[1]Zaporizhia State Medical University, Mayakovsky Avenue, 26, Zaporizhia, 69000, Ukraine

2National University of Pharmacy, Pushkinskaya street, 53, Kharkiv, 61000, Ukraine

Received 30 March 2018; revised 13 December 2018; accepted 15 December 2018

Abstract

We have not found enough the scientific pharmacognostic works study Lucerne and Lupine that belong to the legume family (Fabaceae L.) despite wide dissemination and use them. That's why we studied by comparative and pharmacognosy representatives the common herbs from the Ukrainian flora. We have conducted a comparative analysis of the chemical composition and quantitative content of coumarins and other biologically active substances of primary and secondary biosynthesis in native extracts from the above-ground parts of the family Fabaceae L. - L. luteus L. and M. falcata L. subsp. romanica (Prodan) O. Schwähr & Klink. by gas chromatography-mass spectroscopy method. It identified 22 and 38 components, respectively. A complex of biologically active compounds includes higher hydrocarbons, terpenoids, fatty acids etc. Among them – derivatives of 4 oxycoumarin: 0.20% coumarin in the raw material of Lupinus luteus L.; there are dihydrocoumarin 1.60% and 13.13% in the raw material of grass Medicago falcata L. subsp. romanica (Prodan) O. Schwarz & Klink.

Keywords: Gas Chromatography-Mass Spectroscopy; Coumarins; Phytochemistry Analysis; Fabaceae; Lupinus; Medicago

ВИВЧЕННЯ НАТИВНИХ ЕКСТРАКТІВ СИРОВИНИ ЛЮПИНУ ЖОВТОГО ТА ЛЮЦЕРНИ ЖОВТОЇ (СЕРПОПОДІБНОЇ АБО РУМУНСЬКОЇ) МЕТОДОМ ХРОМАТО-МАС-СПЕКТРОМЕТРІЇ

Олена В. Гречана[1]*, Анатолій Г. Сербін*[2]

[1]Запорізький державний медичний університет, проспект Маяковського, 26, Запоріжжя, 69000, Україна

[2]Національний фармацевтичний університет, вулиця Пушкінська, 53, Харків, Харківська область, 61000, Україна

Анотація

Проведено порівняльне фармакогностичне вивчення двох представників родини бобових флори України з використанням методу газової хромато-мас-спектрометрії при аналізі нативного екстракту сировини люпину жовтого та люцерни жовтої. Ідентифіковано 22 та 38 компонентів відповідно. Біологічно активні сполуки включають вищі вуглеводні, терпеноїди, жирні кислоти, похідні 4-оксикумарину тощо. У сировині трави Lupinusluteus L. вміст кумарину склав 0.20%, у сировині трави Medicago falcata L. subsp. romanica (Prodan) O. Schwarz & Klink. було знайдено дигідрокумарину – 1.60 % та кумарину – 13.13 %.

Ключові слова: хромато-мас-спектрометрія; кумарини; фітохімічний аналіз; бобові; люпин; люцерна

**Corresponding author: tel.: 380974665610; e-mail: 1310grechanaya@ukr.net*

doi:10.15421/081812

Journal of Chemistry and Technologies, 2018, *26*(2), 39-44

ИЗУЧЕНИЕ НАТИВНЫХ ЭКСТРАКТОВ СЫРЬЯ ЛЮПИНА ЖЕЛТОГО И ЛЮЦЕРНЫ ЖЕЛТОЙ (СЕРПОВИДНОЙ ИЛИ РУМЫНСКОЙ) МЕТОДОМ ХРОМАТО-МАСС СПЕКТРОМЕТРИИ

Елена В. Гречаная*[1], Анатолий Г. Сербин[2]

[1]*Запорожский государственный медицинский университет, проспект Маяковского, 26, Запорожье, 69000, Украина*

[2]*Национальный фармацевтический университет, улица Пушкинская, 53, Харьков, 61000, Украина*

Аннотация

Проведено сравнительное фармакогностическое исследование двух представите лей сімейства бобовых флоры Украины с использованием метода газовой хромато-масс-спектрометрии при анализе нативного экстракта сырья люпина желтого и люцерны желтой. Идентифицированы 22 и 38 компонентов соответственно. Биологически активные соединения включают высшие углеводороды, терпеноиды, жирные кислоты, производные 4 оксикумарина и др. В сырье травы Lupinus luteus L. обнаружено кумарина 0.20%, дигидрокумарина в сырье травы Medicago falcata L. subsp. romanica (Prodan) O. Schwarz & Klink. найдено – 1.60 %, кумарина – 13.13 %.

Ключевые слова: хромато-масс-спектрометрия; кумарины; фитохимический анализ; бобовые; люпин; люцерна.

Introduction

Thegenera of Lucerne and Lupine are among them ostancient crop plants belonging to the Legume family (Fabaceae L.). There are 24 species in the alfalfa genus in Ukraine (61 species was counted and described). It described about 200 herbaceous, semi-sweet and bush, one-year and perennial, wintering species of lupine [1–18].

All types of alfalfa are divided into three groups by the number of chromosomes: diploid, tetraploid and hexaploid. In the one-year-old lupine, three kinds are distinguished – yellow, blue, or narrow-leaved and white. There are also perennial forms of lupine, which are characterized by valuable agrotechnical and fodder properties [4; 10; 16].

Hippocrates, Dioscorides, Avicenna, Galen, Botanist Theophrastus, and others in their ancient books mentioned lupine as a useful food and medicinal plant [3; 25]. The earliest culture of Alfalfa Yellow (Like-sickle or Romanian) began to grow many millennia ago, presumably at the same time when wheat – as a perennial drought-tolerant and very winter-resistant herbaceous plant, a height of 50-80 cm [4; 17].

Lucerne is cultivated in Ukraine in the forest-field zone – it occupies about 121 thousand hectares in 15 regions, lupine has crops of 250 thousand hectares. Gentle green grass species are a valuable feed for pets. It is harvested in hay and made from hay flour [1; 3; 9; 21].

In folk medicine, all the aboveground part of alfalfa is used in diseases of the intestines, stomach, thyroid gland, to improve metabolism, normalize the state of the circulatory system, lower cholesterol, increase hemoglobin in blood, and so on. Leaves and fruits of alfalfa contain mineral elements (potassium, calcium, fluorine, etc.), carbohydrates, proteins, fatty acids, essential oils, pectin, plant steroids, enzymes, chlorophylls, alkaloids, hormonal substances, carotene [2; 4; 15]. Lupine is used in folk medicine in cases of abscesses, birthmarks, to excite appetite, as a remedy for worms and abdominal and liver pain. With cosmetic purposes people use it for growth of hair and against wrinkles. With lupine yellow, a large selection work was carried out – it holds the first place among the other cultivars in terms of seed protein content and green mass. In addition, its seed contains up to 5% fat [8; 13; 14].

The purpose and objectives of the study

Ukraine has registered a number of biologically active nutritional supplements, which contain different contents of biologically active substances derived from lupine and alfalfa with recommendations for internal or external use. Despite the wide dissemination and use, there is no scientific complex workwith pharmacological studies of representatives of plants of these genera, the study of the content of groups of compounds of true coumarins and their 4-hydroxy derivatives, the presence of which should be characteristic of this tribe [6; 19; 20; 22; 24].

Therefore, we conducted a comparative analysis of the chemical composition and quantitative content of coumarins and other biologically active substances of primary and secondary biosynthesis in native extracts from the above-ground parts of the family Fabaceae L. – L. luteus L. and M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink.

Journal of Chemistry and Technologies, 2018, *26*(2), 39-44

Experimental

Materials

The above-ground part (vegetative material) was harvested during the period of active flowering – (May-June) in the suburbs of Zaporizhzhya (town of Primorsk). We dried it under a canopy [5; 7; 18; 23].

Analysis of the native raw material. The plant material was weighed and the target substances were extracted with methylenechloride for a certain time. The extract was evaporated and analyzed by GC-MS method [4; 11; 12].

Methods of research

An Agilent gas chromatograph (Agilent Technologies, Santa Clara, USA) equipped with an Aglient 5973 mass selective detector was used for the separation of analytes. Helium was used as a carrier. Agilent J&W DB-5capillary GC column with an internal diameter of 0.25 mm and length of 30 m was used for the analysis. The NIST 05 and WILEY 2007 mass spectrum libraries with a total number of spectra of over 470000 in conjunction with the programs for identifying AMDIS and NIST were used to identify the components.

For the quantitative calculations, the internal standard method was used. The calculation of the content (mg / 1000 g) of the components was carried out according to the formula:

$C = K_1 \cdot K_2$,

where:

K_1 = (S_1 – area of the peak of the test substance; S2 is the peak area of the standard);

K_2 = (50 – mass of the internal standard (μg) introduced into the sample; m – weight of a sample (g)).

Results and discussion

Qualitative composition and quantitative content of biologically active compounds of dried raw material of L. luteus L. and M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink. (native extraction) was determined by gas-liquid chromatography with mass spectrometric detection. The results obtained are presented in Table 1.

As a result of gas chromatographic investigation of the native extracts of dried raw material both of samples, 22 and 38 compounds were identified, respectively (Table 1) and some of true coumarins were identified among them. The content of coumarin in the raw herbs L. luteus L. was 0.20% and the content of coumarin in the raw herbs M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink was 13.13%. Also the content of dihydrocoumarine in the raw herbs M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink was 1.60 %.

Table

Comparative characteristic of the component composition of raw material Lupinus luteus L., Medicago falcata L. subsp. romanica (Prodan) O. Schwarz & Klink., harvested in Zaporozhye region, Primorsk (May-June 2010 - 2016)

Native extraction of raw materials Lupinus luteus L.			Native extraction of raw materials Medicago falcata L. subsp. romanica (Prodan) O. Schwarz& Klink.		
No. s / n	Component	Quantitative content (%)	No. s / n	Component	Quantitative content (%)
1	2	3	4	5	6
1	tetradecane	0.36	1	hexanoic acid	0.13
2	pyrrole 2.5 dion	0.15	2	cis, trans-neophytadiene	0.35
3	dodecanic acid	0.31	3	nonakozan	5.57
4	coumarin	0.20	4	nonanal	0.05
5	tetradecanic acid	0.41	5	internal standard	26.15
6	neophythadiene	0.44	6	2-phenoxyethanol (impurity)	0.13
7	hexahydrofarnesil-acetone	0.26	7	tetradecane	0.35
8	phthalate	0.40	8	pyrrole 2.5 dion	5.57
9	phytol	0.74	9	pentadecane	0.05
10	linolenic acid	8.75	10	phytol	26.15
11	pentacosan	0.28	11	dodecanic acid	0.25
12	lupanin	0.86	12	phthalate	0.14
13	phthalate	0.15	13	hexahydrofarnesil-acetone	0.20
14	neophythadiene	0.16	14	dihydroactinidiolide	0.11
15	nonakozan	3.01	15	coumarin	2.77
16	gentriacontan	11.66	16	benzyl alcohol	0.27

1	2	3	4	5	6
17	kempestherol	0.94	17	loliloid	0.36
18	stigmasterol	3.95	18	dihydrocoumarin (melitol)	0.46
19	γ sitosterol	0.16	19	oleic acid	0.30
20	β amirin	1.54	20	stearic acid	13.13
21	lupeole	1.22	21	linoleic acid	0.03
22	α amirin	0.41	22	linolenic acid	1.60
			23	pentacosan	0.66
			24	heptacosan	0.25
			25	isopropylilaurate (impurity)	0.19
			26	octacosan	0.16
			27	pentadecanoic acid	1.60
			28	trans-neophytadien	0.58
			29	eicosanol	15.05
			30	gentriacontan	1.34
			31	kempestherol	0.38
			32	stigmasterol	0.77
			33	γ sitosterol	3.08
			34	β amirin	0.14
			35	lupeole	0.20
			36	α amirin	0.15
			37	octadecane	0.07
			38	cis-neophytadiene	2.24

The extracts of both types of raw materials contained the representatives of higher hydrocarbons, including tetradecane (0.36% and 0.14%), pentacosan (0.28% and 0.66%), nonacosan (3.01% and 5.57%), gentriacontan (11.66% and 1.34%). Combined native extracts contained certain amounts of terpenoids, including hexahydrofarnesil-acetone (0.26% and 0.46%), kempestherol (0.94% and 0.38%), stigmasterol (3.95% and 0.77%), γ-sitosterol (0.16% and 3.08%), β amirine (1.54% and 0.14%), lupeole (1.22% and 0.20%), phytol (0.74% and 2.77%) and α-amirine (0.41% and 0.15%). Fatty acids were found in both extracts, including dodecanic acid (0.31% and 0.27%) and linoleic acid (8.75% and 0.85%).

Lupinus extracts differed: neophytadiene (0.44% + 0.16%), lupanin (0.86%) and tetradecanoic acid (0.41%). Quite a significant amount of the following compounds were present in alfalfa raw materials: loliloid (1.60%), dihydroquamarine (1.60%), stearic acid (1.65%), pentadecanoic acid (1.60%), eicosanol (15.05%), gentriacontan (1.34%), and sitosterol (3.08%).

In our opinion, the correlation of the ratios of the quantities of compounds identified in the extracts from the studied plants with the values of the quantities of the derivatives of 4-oxycoumarines should occur and we've worked about this problem.

Conclusions

The pharmacognostic analysis of native extracts of Lupinus luteus L. and Medicago falcata L. subsp. romanica (Prodan) O. Schwarz & Klink was conducted 22 and 38 components were identified, respectively.

In native extracts of L. luteus L. and M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink, there were present also components that are the representatives of higher hydrocarbons (tetradecane, pentacosan, nonacosan, gentriacontan), terpenoids (hexahydrofarnesil-acetone, cempesterol, stigmasterol, γ-sitosterol, β amirin, lupole, phytol and α-amirin), fatty acids (dodecanic acid and linoleic acid). Among them were found derivatives of 4oxycoumarins. The content of L. luteus L. coumarine in the raw material of herbs was 0.20% while in the raw material of grass M. falcata L. subsp. romanica (Prodan) O. Schwarz & Klink the contents of dihydrocouamarine and coumarin were equal 1.60% and 13.13%, respectively.

The compostion of lupine extracts was different and included neophytadiene, lupanin and tetradecanoic acid.

The raw materials of alfalfa were characterized by the presence of quite significant quantities of the following compounds: – loliloid, dihydrocouamarin, stearic and pentadecanoic acids, eicosanol, gentriacontan and γ-sitosterol.

Biblography

[1] Агрокарта України, карта посівів, посівні площі, агросправочник online, agromap [Електронний ресурс]: Агрокарта України 2017. – Режим доступу: http://www.4sg.com.ua/.

[2] Бубенчиков Р. А. Изучение антиоксидантной активности листьев растений рода лопух и травы фиалки полевой методами ТСХ и хемилюминисцентного анализа / Р. А. Бубенчиков, И. Л. Дроздова // Вестник Ставропольского государственного университета. – 2005. – № 42. – С. 173 – 176.

[3] Георгиевский В. П. Биологически активные вещества лекарственных растений / В. П. Георгиевский, Н. Ф. Комиссаренко, С. Е. Дмитрук. – Новосибирск: Наука, 1990. – 336 с.

[4] Гречана О. В. Фармакогностичне вивчення люцерни жовтої (серпоподібної або румунської) Medicago falcate L. subsp. romanica (Prodan.) O. Schwarz & Klink. / О. В. Гречана // Одеський медичний журнал. – 2016. – №2 (154). – С. 24 – 28.

[5] Губин К. В. Изучение химического состава надземной части Urtica cannabina L. флоры Сибири / К. В. Губин, М. А. Ханина // Химия растительного сырья. – 2009. - №2. – С. 89–92.

[6] Касьянова А. Ю. Влияние внесения сульфата кобальта на содержание кумаринов в Angelica archangelica L. / Касьянова А. Ю., Башкирова Р. М., Кудашкина Н. В. // Химия растительного сырья. – 2004. – №1. – С. 41–45.

[7] Ложкин А. В. Природные кумарины: методы выделения и анализа /А. В. Ложкин, Е. И. Саканян // Хим.-фармац. журн. – 2006. – Т. 40, № 6. – С. 47–55.

[8] Майсурян Н. А. Люпин / Н. А. Майсурян, Л. И. Атабекова – М.: Колос, 1974. – 463 с.

[9] Немерешина О. Н. О некоторых аспектах рационального использования лекарственных растений Предуралья / О. Н. Немерешина, Н. Ф. Гусев, В. Н. Зайцева // Известия ОГАУ. – 2009. – № 2 (22). – С. 308–311.

[10] Петрук В. А. Продуктивность люцерны на корм и семена / В. А. Петрук // Аграрная наука. – 2008. – № 2. – С. 16–18.

[11] Попик А. І. Дослідження кумаринів та іридоїдів Syringa vulgaris / А. І. Попик, В. В. Король, В. С. Кисличенко // Фармацевтичний часопис. – 2009. – №4. – С. 18–21.

[12] Сотникова О. П. Ідентифікація та кількісне визначення суми кумаринів у водному екстракті з трави буркуну лікарського /О. П. Сотникова, А. Г. Котов // Фармацевтичний журнал. – 2005. - № 6. – С. 70–73.

[13] Федько И. В. Перспектива использования растений народной медицины при фитотерапии туберкулеза / И. В. Федько // Вестник Томского государственного педагогического университета. – 2013. – №8 (136) – С. 210–212.

[14] Юдин В. Фитотерапия: прошлое, настоящее и будущее / В. Юдин // Укр. Мед. Часопис. – 2010. – № 2 (76). – С. 13–18.

[15] Barilli E. Clarification on Host Range of Didymella pinodes the Causal Agent of Pea Ascochyta Blight. /E. Barilli, M. J. Cobos, D. Rubiales // Front Plant Sci. – 2016. – № 13 (7). – P. 592. doi: 10.3389/fpls.2016.00592.

[16] Cevheri C. Element Content, Botanical Composition and Nutritional Characteristics of Natural Fourage of Şanliurfa, Turkey /C. Cevheri, C. Kụ́ç̣ụk, M. Avci, V. Atamov // Journal: Food, Agriculture and Environment. – 2013. – Vol. 11, I. 3&4 – P. 790–794.

[17] El Haouardi M. Medicinal Plants with Antiplatelet Activity. / M. El Haouari, J. A. Rosado // Phytother. Res. – 2016. - № 30 (7). P. 1059–1071. doi: 10.1002/ptr.5619.

[18] FAOSTAT, Production, Crops. Food and Agriculture Organization of the United Nations. Available online at: http://www.fao.org/countryprofiles/index/en/?iso3=UKR (Accessed 21.12.18).

[19] Cieśla Ł. Free Radical Scavenging Activities of Polyphenolic Compounds Isolated from Medicago Sativa and Medicago Truncatula Assessed by Means of Thin-Layer Chromatography DPPH Rapid Tests /Ł. Cieśla, I. Kowalska, W. Oleszek [et al.] //Phytochemical Analysis. – 2013. – Т. 24, № 1. – P. 47–52.

[20] Grechana O. V. The Study of Free Coumarins in the Plant Raw Material of Medicago Falcata L. subsp. Romanica (Prodan) O. Schwarz & Klink. / O. V. Grechana //Вісник фармації. – 2014. – № 1 (77). – С. 40–44. doi:10.24959/nphj.14.1942

[21] Legume Genomics: Understanding Biology Through DNA and RNA Sequencing / J. A. O'Rourke, Y. T. Bolon, B. Bucciarelli, C. P. Vance //Ann. Bot. – 2014. – №113(7). – 1107–1120. doi: 10.1093/aob/mcu072.

[22] Lacy A. Studies on Coumarins and Coumarin-Related Compounds to Determine their Terapeutic Role in the Treatment of Cancer/A. Lacy, R. O'Kennedy // Current Pharmaceutical Design. – 2004. – № 10. – P. 3797–3811.

[23] Zielińska-Dawidziak M. Legume seeds and cereal grains' capacity to accumulate iron while sprouting in order to obtain food fortificant. /M. Zielińska-Dawidziak, H. Staniek, E. Król [et al] // Acta Sci Pol Technol Aliment. – 2016. – №15 (3). – P. 333–338. doi: 10.17306/J.AFS.2016.3.32

[24] Basset G. J. Phylloquinone (Vitamin K): Occurrence, Biosynthesis and Functions. /G. J. Basset, S. Latimer, A. Fatihi [et al] // Mini Rev Med Chem. – 2017. – №17 (12). – P. 1028–1038. doi: 10.2174/1389557516666160623082714

[25] Ohkura N. Possible antithrombotic effects of Angelica keiskei (Ashitaba) /N. Ohkura, G. Atsumi, K. Ohnishi [et al] // Pharmazie. – 2018. – №73 (6). – P. 315–317. doi: 10.1691/ph.2018.8370. Review

References

[1] Agricultural Map of Ukraine, Map of Cities, Online Locations, Online Agro Directory, Agromap: Agricultural Map of Ukraine 2017. http://www.4sg.com.ua/

[2] Bubenchicov, R. A., Drozdova, I. L. (2005) [*Research of Antioxidant Activity of the Genus Arctium L. Leaves and Viola Arvense L. by the Methods of TLS and Chemoluminescense*]. *Vestnik Stavropolskogo Gosudarstvennogo Universiteta*, 42, 173–176 [in Russian].

[3] Georgiievskiy, V. P., Komissarenko, N. F., Dmitruk, S. E. (1990). *[Biologically Active Substances of Medicinal Plants]*. Novosibirsk, USSR: [in Russian].

[4] Grechana, O. V. (2016). The Study of Free Coumarins in the Plant Raw Material of Medicago Falcata L. subsp. Romanica (Prodan) O. Schwarz & Klink. *Odesskii Medichnii Zhurnal,* 2 (154) 24 – 28 [in Ukrainian].

[5] Gubin, K. V., Khanina, M. A. (2009) *[Study of the Chemical Composition of the aerial Part of the Urtica Dioica L. in*

the Flora of Siberia]. Khimia Rastitel'nogo Sir'ya, 2, 89–92 [in Russian].

[6] Kasianova, A. Yu., Bashkirova, R. M., Kudashkina, N. V. (2004) *[The Effect of Adding Cobalt Sulfate on the Content of Coumarins in Angelica archangelica L.]. Chimiia Rastitelnogo Syr'ya*, 1, 41 – 45 [in Russian].

[7] Lozgkin, A. V., Sakanyan, E. I. (2006) [*Natural Coumarins: Isolation and Analysis Methods*]. *Chem. Farm. Journal, 40 (6),* 47 – 55 [in Russian].

[8] Majsuryan, N. A., Atabekova, L. I. (1974). *[Lupine].* Moskow, USSR: [in Russian].

[9] Nemereshina, O. N., Husev, N. F., Zaytseva, V. N. (2009). *[On Some Aspects of the Rational Use of Medicinal Plants in the Ural]*. *Izvestiia OGAU*, 2 (22), 308 – 311 [in Russian].

[10] Petruk, V. A. (2008). *[The Productivity of Alfalfa for Feed and Seeds]*. *Agrarnaya Nauka*, 2, 16 – 18 [in Russian].

[11] Popik, A. I., Corol, V. V., Kislichenko, V. S. (2009) [*The Koumarin sand Iridoids Siringa Vulgaris Research*] *Farmatsevtychnyi Chasopys,* 4, 18 – 21 [in Ukrainian].

[12] Sotnikova, O. P., Kotov, A. G. (2005). *[Identification and Quantitative Determination of the Amount of Coumarins in Aqueous Extract from Medicinal Herb Melilot]*. *Farmatsevtichniy Zhurnal*, 6, 70–73 [in Ukrainian].

[13] Fedko, I. V. (2013) *[The Prospect of Using Traditional Medicine Plants in Phytotherapy of Tuberculosis]. Vestnik Tomskogo Gosudarstvennogo Pedagogicheskogo Universiteta*, 8 (136), 210 – 212 [in Russian].

[14] Yudin, V. (2010) *[Herbal medicine: past, present and future]. Ukr. Med. Chasopys,* 2 (76), 13–18 [in Ukrainian].

[15] Barilli, E., Cobos, M. J., Rubiales, D. (2016). Clarification on Host Range of Didymella pinodes the Causal Agent of Pea Ascochyta Blight. *Front Plant Sci.,* 13 (7), 592. doi: 10.3389/fpls.2016.00592

[16] Cevheri, C., Kụ́ç̣ụk, C., Avci, M., Atamov, V. (2013) Element Content, Botanical Composition and Nutritional Characteristics of Natural Fourage of Şanliurfa, Turkey. *JFAE*, 11 (3&4), 790–794.

[17] El Haouardi, M., Rosado, J. A. (2016). Medicinal Plants with Antiplatelet Activity. *Phytother. Res.,* 30 (7), 1059–71. doi: 10.1002/ptr.5619.

[18] FAOSTAT (2017). FAOSTAT, Production, Crops. Food and Agriculture Organization of the United Nations. Available online at: http://www.fao.org/countryprofiles/index/en/?iso3=UKR (Accessed 21.12.18).

[19] Cieśla, Ł., Kowalska, I., Oleszek, W., Stochmal, A. (2013). Free Radical Scavenging Activities of Polyphenolic Compounds Isolated From Medicago Sativa and Medicago Truncatula Assessed by Means of Thin-Layer Chromatography DPPĤ Rapid Tests. *Phytochemical Analysis*,24(1), 47–52.

[20] Grechana, O. V. (2014). The Study of Free Coumarins in the Plant Raw Material of Medicago Falcata L. subsp. Romanica (Prodan) O. Schwarz & Klink. *Visnik Farmatsii*, 1 (77), 40–44 [in Ukrainian]. doi: 10.14739/2409-2932.2016.2.70696

[21] O'Rourke, J. A., Bolon, Y. T., Bucciarelli, B., Vance, C. P. (2014). Legume Genomics: Understanding Biology Through DNA and RNA Sequencing. *Ann. Bot.,* №113 (7), 1107–1120. doi: 10.1093/aob/mcu072.

[22] Lacy, A., O'Kennedy, R. (2004). Studies on Coumarins and Coumarin-Related Compounds to Determine their Terapeutic Role in the Treatment of Cancer. *Current Pharmaceutical Design,* 10, 3797–3811.

[23] Zielińska-Dawidziak, M., Staniek, H., Król, E., Piasecka-Kwiatkowska, D., Twardowski, T. (2016). Legume Seeds and Cereal Grains' Capacity to Accumulate Iron While Sprouting in Order to Obtain Food Fortificant. *Acta Sci Pol Technol Aliment*, 15 (3), 333 - 338. doi: 10.17306/J.AFS.2016.3.32

[24] Basset, G. J., Latimer, S., Fatihi, A., Soubeyrand, E., Block, A. (2017) Phylloquinone (Vitamin K1): Occurrence, Biosynthesis and Functions. *Mini Rev Med Chem*., 17(12), 1028–1038. doi: 10.2174/1389557516666160623082714

[25] Ohkura, N., Atsumi, G., Ohnishi, K., Baba, K., Taniguchi, M. (2018). Possible antithrombotic effects of Angelica keiskei (Ashitaba). *Pharmazie*, 73(6), 315–317. doi: 10.1691/ph.2018.8370. Review