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«ХАЛЫҚ» ЖҚ

ХАБАРЛАРЫ

ИЗВЕСТИЯ

РОО «НАЦИОНАЛЬНОЙ
АКАДЕМИИ НАУК РЕСПУБЛИКИ
КАЗАХСТАН»
ЧФ «Халык»

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В 2016 году для развития и улучшения качества жизни казахстанцев был создан частный Благотворительный фонд «Халык». За годы своей деятельности на реализацию благотворительных проектов в областях образования и науки, социальной защиты, культуры, здравоохранения и спорта, Фонд выделил более 45 миллиардов тенге.

Особое внимание Благотворительный фонд «Халык» уделяет образовательным программам, считая это направление одним из ключевых в своей деятельности. Оказывая поддержку отечественному образованию, Фонд вносит свой посильный вклад в развитие качественного образования в Казахстане. Тем самым способствуя росту числа людей, способных менять жизнь в стране к лучшему – профессионалов в различных сферах, потенциальных лидеров и «великих умов». Одной из значимых инициатив фонда «Халык» в образовательной сфере стал проект Ozgeris powered by Halyk Fund – первый в стране бизнес-инкубатор для учащихся 9-11 классов, который помогает развивать необходимые в современном мире предпринимательские навыки. Так, на содействие малому бизнесу школьников было выделено более 200 грантов. Для поддержки талантливых и мотивированных детей Фонд неоднократно выделял гранты на обучение в Международной школе «Мирас» и в Astana IT University, а также помог казахстанским школьникам принять участие в престижном конкурсе «USTEM Robotics» в США. Авторские работы в рамках проекта «Тәлімгер», которому Фонд оказал поддержку, легли в основу учебной программы, учебников и учебно-методических книг по предмету «Основы предпринимательства и бизнеса», преподаваемого в 10-11 классах казахстанских школ и колледжей.

Помимо помощи школьникам, учащимся колледжей и студентам Фонд считает важным внести свой вклад в повышение квалификации педагогов, совершенствование их знаний и навыков, поскольку именно они являются проводниками знаний будущих поколений казахстанцев. При поддержке Фонда «Халык» в южной столице был организован ежегодный городской конкурс педагогов «Almaty Digital Ustaz.

Важной инициативой стал реализуемый проект по обучению основам финансовой грамотности преподавателей из восьми областей Казахстана, что должно оказать существенное влияние на воспитание финансовой грамотности и предпринимательского мышления у нового поколения граждан страны.

Необходимую помощь Фонд «Халык» оказывает и тем, кто особенно остро в ней нуждается. В рамках социальной защиты населения активно проводится

работа по поддержке детей, оставшихся без родителей, детей и взрослых из социально уязвимых слоев населения, людей с ограниченными возможностями, а также обеспечению нуждающихся социальным жильем, строительству социально важных объектов, таких как детские сады, детские площадки и физкультурно-оздоровительные комплексы.

В копилку добрых дел Фонда «Халык» можно добавить оказание помощи детскому спорту, куда относится поддержка в развитии детского футбола и карате в нашей стране. Жизненно важную помощь Благотворительный фонд «Халык» оказал нашим соотечественникам во время недавней пандемии COVID-19. Тогда, в разгар тяжелой борьбы с коронавирусной инфекцией Фонд выделил свыше 11 миллиардов тенге на приобретение необходимого медицинского оборудования и дорогостоящих медицинских препаратов, автомобилей скорой медицинской помощи и средств защиты, адресную материальную помощь социально уязвимым слоям населения и денежные выплаты медицинским работникам.

В 2023 году наряду с другими проектами, нацеленными на повышение благосостояния казахстанских граждан Фонд решил уделить особое внимание науке, поскольку она является частью общественной культуры, а уровень ее развития определяет уровень развития государства.

Поддержка Фондом выпуска журналов Национальной Академии наук Республики Казахстан, которые входят в международные фонды Scopus и Wos и в которых публикуются статьи отечественных ученых, докторантов и магистрантов, а также научных сотрудников высших учебных заведений и научно-исследовательских институтов нашей страны является не менее значимым вкладом Фонда в развитие казахстанского общества.

С уважением,
Благотворительный Фонд «Халык»!

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ISOLATION OF FLAVONOIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FROM PLANT OF GENUS *THYMUS SERPYLLUM L.*

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Annotation. Plant species of the genus *Thymus Serpyllum L*. are recognized in the scientific literature as the richest sources of secondary metabolites. The purpose of this study is isolation the chemical composition of plant materials and develop a method for obtaining new sources of biologically active substances, establishing their chemical structure. This article presents the results of a study of the chemical composition of the aerial parts of plants of the genus *Thymus Serpyllum L.* of the *Lamiaceae* family, collected during the fruiting period in the Altai region of Kazakhstan. A result of the studies, the content of the complex of flavonoids of the plant was determined by extraction methods (maceration 70 % EtOH, 72 hours, 1:9) and adsorption column chromatography with silica gel from a plant of the genus *Thymus Serpyllum L*. The

quantitative content of flavonoids is 1.02–1.13 %. Using paper chromatography and thin layer chromatography, a qualitative analysis was carried out and a pure substance, cynaroside, was isolated by high-performance liquid chromatography (RP-HPLC) with an ODS-H80 sorbent. The structure of the obtained individual substance was identified by modern physical and chemical methods on the basis of chemical transformations and results, the studied data of IR, UV, ^{1}H - NMR and ^{13}C - NMR, mass-spectrometry.

Keywords: *Lamiaceae, Thymus Serpyllum L.*, extraction, adsorption column chromatography, silica gel, high-performance liquid chromatography, flavonoids, cinaroside

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THYMUS SERPYLLUM L. ТЕКТІ ӨСІМДІК ҚҰРАМЫНАН ЖОҒАРЫЭФФЕКТИВТІ СҰЙЫҚТЫҚ ХРОМАТОГРАФИЯ ӘДІСІМЕН ФЛАВОНОИДТАРДЫ АНЫҚТАУ

Аннотация. Ғылыми әдебиеттерде *Thymus Serpyllum L.* текті өсімдік түрлері екіншілік метаболиттердің ең бай көздері ретінде танылған. Зерттеудің мақсаты өсімдік шикізатының химиялық құрамын зерттеу және биологиялық белсенді заттардың жаңа көздерін алу әдісін жасау, олардың химиялық құрылымын анықтау. Бұл мақалада Қазақстанның Алтай өңірінде өсетін жеміс беру кезеңінде жиналған *Lamiaceae* тұқымдасына жататын *Thymus Serpyllum L*. текті өсімдігінің жер үсті бөліктерінің химиялық құрамының зерттеу нәтижелері берілген. Зерттеу нәтижесі бойынша *Thymus Serpyllum L.*текті өсімдіктің флавоноидты кешендер құрамы экстракция (мацерация 70% EtOH, 72 сағ, 1:9) әдістерімен және силикагелді адсорбциялық бағаналы хроматография әдісімен анықталды. Флавоноидтардың сандық мөлшері 1,02–1,13 %. Қағазды және жұқа қабатты хроматографияларды қолдана отырып, сапалық талдау жүргізілді және ODS-H80 сорбенті бар жоғары өнімді сұйық хроматография (RP-HPLC) арқылы таза зат цинарозид бөлініп алынды. Бөлінген жеке заттың құрылысы заманауи физика-химиялық әдістердің: ИҚ, УК, ^{1}Н - ЯМР және ^{13}С – ЯМР, масс-спектрометрия мәліметтері бойынша анықталды.

Түйін сөздер: *Lamiaceae*, *Thymus Serpyllum L.*, экстракция, адсорбционды бағаналы хроматография, силикагель, жоғары эффективті сұйықтық хроматографиясы, флавоноидтар, цинарозид

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ОПРЕДЕЛЕНИЕ ФЛАВОНОИДОВ МЕТОДОМ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ ИЗ РАСТЕНИИ РОДА *THYMUS SERPYLLUM L.*

Аннотация. Виды растений рода *Thymus Serpyllum L.* в научной литературе признаны богатейшими источниками вторичных метаболитов. Цель данного исследования –изучение химического состава растительного сырья и разработка способа получения новых источников биологически активных веществ, установление их химического строения. В данной статье приведены результаты исследования химического состава надземных частей растений рода *Thymus Serpyllum L.* семейства *Lamiaceae*,собранных в период плодоношения в Алтайском краю Казахстана. В результате проведенных исследований установлено содержание комплекса флавоноидов методами экстракции (мацерация 70 % EtOH, 72 ч., 1:9) и адсорбционной колоночной хроматографии с силикагелем из растении рода *Thymus Serpyllum L.* Количественное содержание флавоноидов составляет 1,02–1,13 %. С помощью бумажной и тонкослойной хроматографии проведен качественный анализ и выделен чистое вещество — цинарозид методом высокоэффективной жидкостной хроматографии (RP-HPLC) с сорбентом ODS-H80. Структура полученного индивидуального вещества идентифицирована современными физико–химическими методами на основании химических превращений и результатов, изученных данных ИК, УФ, ^{1}H – ЯМР и ^{13}C – ЯМР, масс-спектрометрия.

Ключевые слова: *Lamiaceae, Thymus Serpyllum L.,* экстракция, адсорбционная колоночная хроматография, силикагель, высокоэффективная жидкостная хроматография, флавоноиды, цинарозид

Introduction

More than six thousand plant species are found on the territory of the Republic of Kazakhstan, most of which can produce the necessary medicines. Until this time, only 130 species of them were used as raw materials for the production of medicines. The flora of Kazakhstan is very rich in useful plants, including medicinal plants, which are especially important. Among the drugs used in medical practice, herbal products occupy an important place (Pavlov, 1960: 10).

From this point of view, plants of genus *Thymus L.* of *Lamiaceae* family are of

undoubted interest In Kazakhstan, the official plants of the *Thymus* genus are 15 species, which are recommended for use as an expectorant, antioxidant, antimicrobial and analgesic (Arti, 1998: 8; Duzbayeva, 2020: 10).

Currently, in modern medicine, *Thymus* is used as a liquid extract, showing anti-inflammatory, antibacterial activity and treating respiratory diseases. The basis of all the listed therapeutic effects are polyphenolic compounds (flavonoids) contained in the plant. Flavonoids are present in plants and are responsible for many biological properties, including antioxidant activity (Mata, 2007: 8). In the course of quantitative and qualitative analysis, investigation of secondary methobolytes in the chemical composition of *Thymus* revealed that plant raw materials are rich in various phenolic compounds. For the most part, the composition of essential oils with a terpene compound mixture containing simple phenols - thymol and carvacrol - has been studied a lot. The types and importance of the pharmacological effects of *Thymus* essential oils have been proven by many studies. (Aziz, 2008: 9). In recent years, the study of polyphenolic compounds, especially plant raw materials containing flavonoids, has increased, because the effects of biological activity of flavonoids are diverse and harmless to the body. One of the plants rich in polyphenolic compounds is *Thymus Serpyllum L.* of family *Lamiaceae* (Jia, 2010: 6).

Thymus serpyllum L.s.l. — a perennial plant, the shoots in the lower part are lignified with creeping stems and erect peduncles up to 15 cm high. The leaves are opposite, short-petioled, oblong-oval. The flowers are collected at the ends of the branches in a capitate inflorescence. It is confined mainly to the steppe zone, rocks, rocky and gravelly slopes (Klokov, 1954: 7).

Methods and materials

Objects of research are aboveground parts of family *Lamiaceae* of genus *Thymus Serpyllum L.* during the fruiting period in September 2020, East-Kazakhstan. Raw materials are dried in dry air and stored in a paper box. A herbarium sample of the plant is stored at the Department of Chemistry of the East Kazakhstan University named after S. Amanzholov.

70 % alcohol was used as an extract to separate the flavonoid complex from the raw materials. 1 kg of raw materials is extracted by simple maceration for 72 hours with aqueous - alcohol in a ratio of 1:9. The resulting extract was washed with hexane, chloroform, ethyl acetate and butanol as the polarity increased and four working extracts were obtained. Butanol extraction for the separation of polyphenolic compounds was studied by silica gel sorbent-based adsorption column chromatography (fractions 18–87).

Determination of the quantitative content of flavonoids by quercetin

1 g ground the plant *Thymus Serpyllum L.* is placed in a flask with a capacity of 150 ml and 30 ml of 90 % aqueous alcohol with 1 % HCl is poured on top. The flask is connected to the reverse refrigerator and boiled in a water bath for 30 minutes. After cooling, it is filtered into a flask with a capacity of 100 ml. The extraction is repeated twice with the same solution and the solution in the flask is brought to the mark with 90 % alcohol (solution A).

We take 2 ml of Solution A in a flask with a capacity of 25 ml, pour 1 ml of 1 % solution of aluminum chloride in 95 % alcohol and bring the volume of the solution to the mark with alcohol. After 20 minutes, the optical density of the solution is measured on a 10 mm thick cuvette, a spectrophotometer at a wavelength of 430 nm. As a comparative solution, 2 ml of a solution supplied with 95 % alcohol in a flask with a capacity of 25 ml is used (Muzychkina, 2004: 11).

The content of flavonoids in absolute dry raw materials is calculated by quercetin using the formula:

$$X = \frac{D \times 3 \times 100 \times 100}{764.6 \times 2 \times M \times (100 - W)}$$

where:

D - optical density of the solution,

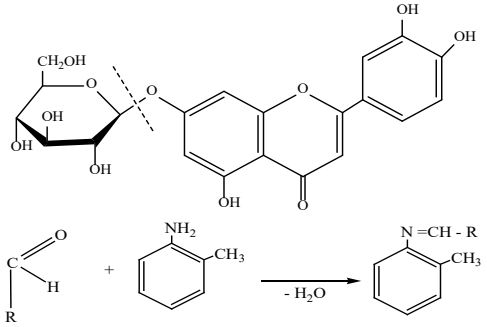
M - raw material weight, g;

W - lost mass during drying of raw materials, %.

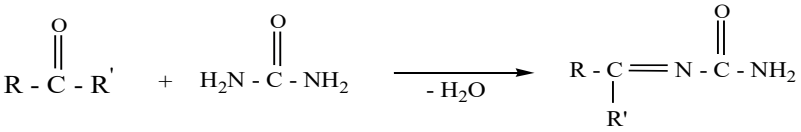
764.6 – Absorption rate of quercetin complex in the presence of aluminum chloride at 430 nm.

Acid hydrolysis on glycosides of flavonoids:

Dissolve 10 mg of the substance in 5 ml of 2 % HCl aqueous or alcohol solution, connect the flask to the refrigerator and heat it in an aqueous bath for 2 hours. Then we cool the solution and bring the reaction mixture with water to a neutral medium. Aglyconeis extracted and separated by ethyl acetate and sugars remain in the aqueous part. To determine which sugars are present, the aqueous part is performed in a single — system chromatography in the Butanol-ethyl acetate-water system, using j-toluidine and urea as determinants. The aqueous part is placed on paper chromatography (Harborne, 1965: 14).



o-toluidine



urea

Flavonoids carried out by thin layer chromatography on Sorbfil plates in hexane-ethyl acetate solvent relations 9:1, 8:2, 7:3, 1:1. After processing chromatograms with 5 % alcohol aluminum chloride, the presence of flavonoids at a wavelength of 365 nm of UV light was detected. Flavonoid compounds were separated by system MEOH-H_2O (1:1) solution in the column with sorbent ODS-H80 (150 × 20 nm × 5 microns) in preparative recycling HPLC (Tokyo, Japan).

Results and Discussion

According to the methodology of the I edition of the state Pharmacopoeia of the Republic of Kazakhstan, the quantitative content of flavonoids in the composition of plant raw materials was determined by the method of spectrophotometry, as a result of which the composition of the plant collected during flowering was 1.02 % and during fruiting – 1.13 %.

Butanol extract from plant raw materials chromatography in column chromatography with silica gel sorbent and washed with hexane-ethyl acetate by gradually increasing the polar eluent (9:1, 9,5:0,5, 8:2, 7:3, 6:4, 1:1) system, were obtained fractions consisting of flavonoid complexes. In qualitative analysis of fractions, thin layer chromatography was analyzed by observing yellow spots with UV-light, $Ce(SO_4)_2$ detectors and similar fractions with the same Rf values were combined. As a result, 38–42 fractions (DM) were examined in the ratio of methanol-water (1:1) solvents in the ODS-H80 column by the method of rotational phase high- -performance liquid chromatography (RP-HPLC) and obtained a pure substance (Figure 1) (Ramesh, 2017: 15).

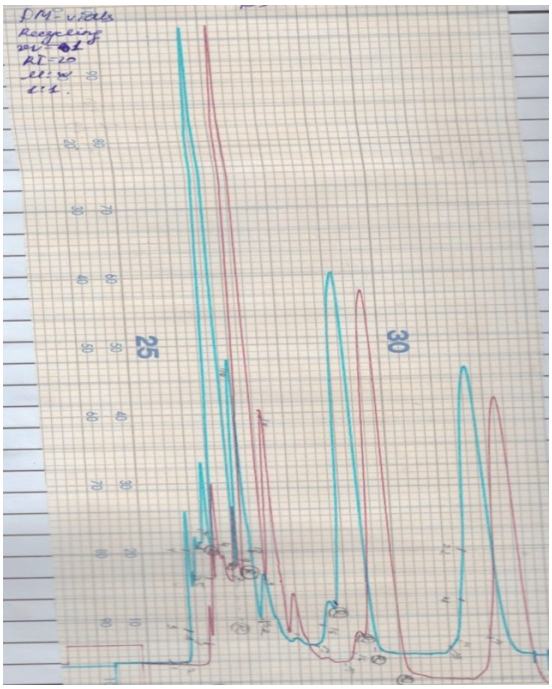


Figure 1 - Chromatogram of pure matter separated by the method of rotational phase high performance liquid chromatography (RP-HPLC)

Looking at the thin layer chromatography on n-butanol-acetic acid-water (4:1:5) system flavonoid was found that there was only one spot (Figure 2) (Wagner, 1996:7).

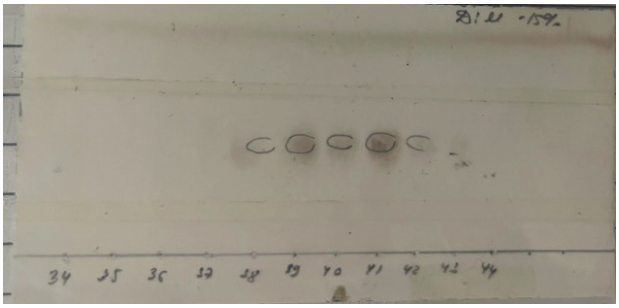
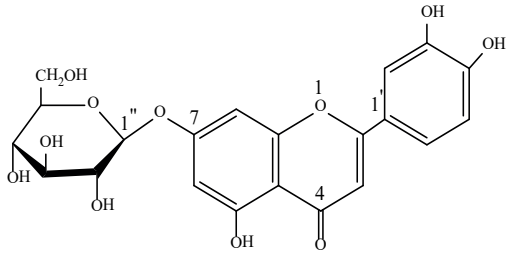


Figure 2 - Chromatogram of substances in thin layer chromatography

Table 1 – Chromatographic description of a flavonoid from the composition of a plant of the genus *Thymus Serpyllum L.*

№	Rf	Spot Color in the visible area	Spot Color in UV light	Fluorescence after 5 % aluminum chloride treatment	Conclusion
butanol-acetic acid-water (4:1: 5)					
1	0,53	Dark yellow	Brown	Light yellow	Flavon or 7-glycoside of flavonol

The structure of the separated individual substance was identified using a modern physico-chemical method - NMR: ^{1}H, ^{13}C, 2D NMR: HMBC, HSQC, COSY, NOESY, according to NMR spectra and DEPT-135 in the lower region during the spin system anomeric proton resonance δ5.05 (1H, d, J = 7.0 Hz) showed that the compound is luteolin. The chemical shift of H-6 and H-8 (0.2+0.3 ppm respectively) proves that sugar is bound at C-7 place.



Cynaroside

^{1}H NMR (500 MHz, Pyridine, δ ppm.) 6.73 (1H, s, H-3), 6.60 (1H, s, H-6), 6.73 (1H, s, H-8), 7.41 (1H, s, H-2'), 6.78 (1H, d, J = 8.5 Hz, H-5′), 6.79 (1H, d, J = 8.9 Hz, H-6′), 5,15 (1H, d, J=7.1, H-1″), 3.19-3.46 (1H, t, H-2″), 3.19-3.46 (1H, t, H-3″), 3.26 (1H, t, J=8.9, H-4″), 3.08 (1H, m, H-5″), 3.81 (1H, d, J=12.0 Hz, H-6″a), 3.61 (1H, d, J=12.8 Hz, H-6″b).

^{13}C NMR (100 MHz, Pyridine, δ ppm) 164.8 (C-2), 103.8 (C-3), 181.9 (C-4), 160.2 (C-5), 102.6 (C-6), 164.5 (C-7), 96.1 (C-8), 162.2 (C-9), 106.1 (C-10), 123.5 (C-1'),

115.8 (C-2'), 147.7 (C-3'), 152.2 (C-4'), 118.2 (C-5'), 121.0 (C-6'), 100.9 (C-1"), 75.2 (C-2"), 76.1 (C-3"), 70.3 (C-4"), 77.2 (C-5"), 61.4 (C-6") (Zoua, 2018:3).

As a result of acid hydrolysis, luteolin and β-glucose were released. In the course of analysis by physico-chemical methods, comparing with the literature, it was proved that the compound is cinaroside.

Conclusion

1 The quantitative content of the flavonoid complex was determined by the methods of extraction and adsorption column chromatography of silica gel sorbent from the plant of genus *Thymus Serpyllum L.* growing in the Altai region.

2 In thin layer chromatography with UV light and cerium sulfate, it showed the yellow spot and the RF value of the compounds belong to the flavonoid group.

3 The flavonoid complex was analyzed by the method of rotational phase high performance liquid chromatography (RP-HPLC) and it was observed that a separate substance was released on the chromatogram. As a result of the analysis by the physico-chemical methods (NMR: ^{1}H, ^{13}C, 2D NMR: HMBC, HSQC, COSY, NOESY, DEPT-135), it was proved that the individual substance is cinorazide.

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