https://doi.org/10.53360/2788-7995-2024-4(16)-43

IRSTI: 31.23.15, 31.23.39

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OPTIMIZING THE EXTRACTION METHOD OF TANNINS FROM *ALHAGI KIRGISORUM SCHRENK* AND THEIR BIOACTIVITY

Abstract: *Plants are a raw material source for obtaining various biologically active complexes, and their isolation can be used to obtain new drugs with diverse biological activities. As sources of biologically active substances, plants of the genus Alhagi, the species Alhagi Kirgisorum Schrenk (zhantak – Kyrgyz camel thorn), are of great interest. Alhagi Kirgisorum Schrenk, which was collected during the flowering period in the Shelek district, Almaty region, in 2023, is an above-ground part of the plant species. Analysis of biologically active substances in plant raw materials, consideration of effective technological parameters for extracting biologically active complexes from plants using ultrasonic extraction, and study of the pharmacological activity of the obtained medicinal preparations are important issues. In order to isolate the complex of tannins from plant raw materials: 50% ethanol solvent and ultrasonic extraction and maceration methods, a ratio of raw materials: extractant – 1:8 was proposed. The composition of this plant is very rich in secondary metabolites, including tannin substances, and it is known that their share is 4,92%. The complex obtained by using two different methods: maceration and ultrasonic extraction was subjected to component analysis, and it was found that the extract obtained by ultrasonic extraction contained more tannin substances. The anticholinesterase biological activity of the complex showed 48,20±4,39% against AChE, 4,21±0,39% against BChE, antidiabetics by the α-Amylase inhibitory activity against was 2807,63±0,10, α-Glucosidase inhibitory activity was 183,51±0,80. Also, against oxidation process: $ABTS^{+}$ assay 12,52±2,16 (6,25-50 μg/mL), DPPH assay 15,70±0,46 (3,125-25 μg/mL), CUPRAC 20,82±0,02 (6,25-50 μg/mL) and according to β- carotene/linoleic acid analysis showed 38,73±0,75 (3,125-25 μg/mL) activity.*

Key words: *Alhagi Kirgisorum Schrenk, minerals, polyflavans, biologically active complexes, extraction, pyrocatechol.*

Introduction

Plants are a raw material source for obtaining various biologically active complexes, and their isolation can be used to obtain new drugs with diverse biological activities. Therefore, herbal medicines play an important role in treating several diseases. To expand the arsenal of domestic medicines and the production of herbal medicines, systematic research on plant resources is necessary [1].

Interest in obtaining substances from secondary metabolism in plants is growing in various fields: food, agricultural, chemical, and pharmaceutical industries. Close attention to plant metabolites is due to the diversity of their biological activities, environmental safety, and the absence of side effects. Plant objects are an irreplaceable source for obtaining many practically necessary and important substances; they have various types of pharmacological activity and require clarification of their structures to identify a certain «structure-activity» relationship [2-3].

As sources of biologically active substances, plants of the genus *Alhagi*, the species *Alhagi Kirgisorum Schrenk* (zhantak – Kyrgyz camel thorn), are of great interest.

In 1894, the German scientist Schrenk discovered a special type of *Alhagi* plant in the Balkhash region. This type of *Alhagi* grows only in Kazakhstan. In the 1800s, the Kazakhs were called Kyrgyz, and hence, this type of *Alhagi* was called Schrenk's Kyrgyz *Alhagi* (*Alhagi Kirgisorum Schrenk*). According to botanists, this species grows only in Kazakhstan, and it is considered endemic. In Central Asia, the common, *Persian*, and rare-leaved *Alhagi* species are grown. The

Alhagi species Maurorum grows in Mongolia, whereas the Sparsipolia species grows in China and Azerbaijan.

From this plant raw material, the biologically active substance «Alkhidin» (RK-M-3-№ 004762) and medicines based on it were created, developed, and registered in the Republic of Kazakhstan: alkhidine ointment (RK-M-3-№ 005155), syrup «Zhantak» (RK-M-3-№ 005301), and camel thorn tincture (RK-M-3-№ 005302), as a new domestic medicine.

The state programme for the development of the pharmaceutical and medical industry is aimed at reducing the dependence of Kazakhstan's healthcare on imports and providing safe medicines; therefore, the development of new methods for isolating biologically active complexes and phytochemical and pharmacological studies of domestic conventional herbal medicines is undoubtedly an urgent task [4].

The object of the study was the above-ground parts of plants of the genus *Alhagi*, the species *Alhagi Kirgisorum Schrenk* (zhantak – Kyrgyz camel thorn) collected during the flowering period in the Shelek district, Almaty region in 2023.

The purpose of this work was to perform a component analysis of plant raw materials, select optimal parameters to obtain the biologically active complex «Alkhidin» using the ultrasonic extraction method, and study the pharmacological activity of the developed herbal medicine.

Scientific novelty of the work

At the Al-Farabi Kazakh National University, a biologically active complex called «Alkhidine» was obtained via percolation method the *Alhagi Kirgisorum Schrenk* plant, which exhibits anti-inflammatory, wound-healing, hepatoprotective, and anti-oxidative properties. Currently, the scope of our work is within the framework of the AP19680131 project «Оптимизация способа получение активного комплекса и разработка новых лекарственных средств из растений семейства маревых и бобовых». Ultrasonic extraction and sublimation drying were used to achieve optimal outcomes.

Practical part

Materials and equipment

Plant materials, ethanol 96%, ultrasonic extractor, rotary evaporator, membrane pump, household refrigerator, freeze-drying (sublimation) apparatus, spectrophotometer, and distile water.

Research methods

The plant material collected during flowering was dried out of the sun and ground to a diameter of 4 mm. Extraction was performed to further study the ground raw material. The extraction was carried out using 2 different methods and compared.

Method 1 involves maceration.

Advantages:

– Simplicity and low price

– No special equipment is required

Disadvantages:

– Extraction time is long

– low efficiency for poorly soluble compounds

Method 2 involves ultrasonic extraction

Advantages:

– Speed and efficiency.

– Prevents the decomposition of thermolabile compounds at low temperatures

Disadvantages:

– The need for special equipment

– Due to ultrasonic extraction, if the temperature rises, some connections may be broken or the necessary substances oxidised.

The use of high-energy extraction methods, including microwave or ultrasonic extraction, has shown good results for the isolation of natural bioactive compounds from plant-based medicinal and food raw materials [5].

The ultrasonic extraction parameters that affect polyflavan release and antioxidant activity of extracts are ultrasound temperature, frequency, power, and extraction time [6].

The biologically active complexes obtained using two methods (maceration, ultrasonic extraction) was qualitatively evaluated.

The original qualitative analysis was conducted using the following algorithm:

A) 10 ml of a solution of 6,0% hydrochloric acid in 96% ethyl alcohol was added to 0,1 g of dry powder and heated for 20 min in a flask under reflux in a water bath. After cooling, a dark brown, red precipitate of phlobaphene is observed (can be diluted 1:1 with water).

Phlobaphene does not have a dark brown, the appearance of the colour is explained by the adsorption of proanthocyanidin (proanthocyanidins) on its surface.

B) add 5 ml of 96% ethyl alcohol and distilled water (in a volume ratio of 2:8) to 0,1 g of dry powder, in the presence of 2 ml of a solution of 1% vanillin in 70% sulphuric acid; A red condensation product of vanillin is formed with the phloroglucin ring of polyflavan (polyflavans) [7].

Polyflavan was determined using the following methodology:

Quantitative determination of polyflavans. Briefly, 0,1 g (precisely weighed) drug is placed in a conical flask with a capacity of 100 ml, 20 ml of 6% hydrochloric acid in ethyl alcohol 96% was added, the flask was connected to a reflux condenser, placed in a boiling water bath, and heated for 15 min with thorough stirring. The resulting solution was filtered through cotton wool into a 100 ml volumetric flask, and the volume of the solution was adjusted to the mark using 6% hydrochloric acid in ethyl alcohol 96% and mixed. 1 ml of the resulting solution was diluted with ethyl alcohol 96% to 10 ml.

The optical density of the solution was measured using a spectrophotometer at a wavelength of 550 nm in a cuvette with a layer thickness of 10 mm. Ethyl alcohol 96% was used as the reference solution.

The concentration of polyflavans in solution in milligrammes per 1 millilitre (mg/ml) is determined using a calibration graph constructed using cyanocobalamin solutions. The content of polyflavans in the preparation in percent (X) is calculated using the following formula:

$$X = \frac{C \times 100 \times 10 \times 100}{m \times 1 \times 1000}$$

Here, C – is the amount of polyflavan detected as shown in the calibration graph;

M – is the measured mass of the drug, g.

Results and discussion

Using one- and two-dimensional paper chromatography in different solvent systems with the help of special reagents, it was determined that tannins, ursolic acid, flavonol mono-, di-, and triglycoside, amino acids, and carbohydrates are the main groups of biologically active substances on the surface of the plant species Alhagi kirgisorum Schrenk.

During the determination of the main parameters of the studied raw material, it was found that the moisture content of the raw material reached 7,54%, and the share of extracted substances reached 41,05%. The ash level was 10,25%, and the sulphate ash level was 8,32%.

Quantitative numbers of biologically active substances: amino acids, carbohydrates, flavonoids, organic acids, and tannins in plant raw materials were determined (Figure 1).

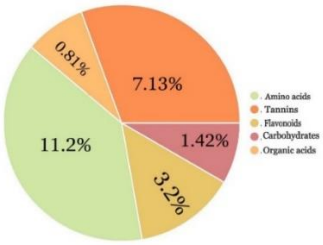

Figure 1 – Quantitative content of biologicallyactive substances in the plant Alhagi kirgisorum Schrenk

Alhagi kirgisorum Schrenk identified mineral residues of the plant type. The presence of Ni in the raw materials was confirmed for the first time.

As a result of the HPLC analysis of the phenolic compounds in the extract obtained by the maceration method. (pyrocatechol 1.0 mg/g, epicatechin 2.34 mg/g, rutin 1.04 mg/g and fisetin 11.24 mg/g) were determined (the results of the analysis are shown in Figure 2).

Table 1 – Mineral substances in *Alhagi kirgisorum Schrenk* (μg/ml)

	Na	K	Ca	Mg	Zn	Cu	Fe	Ni	Mn
Alhagi kirgisorum Schrenk	226,13	1414,44	652,86	359,20	1,59	0,51	11,61	0,36	3,37

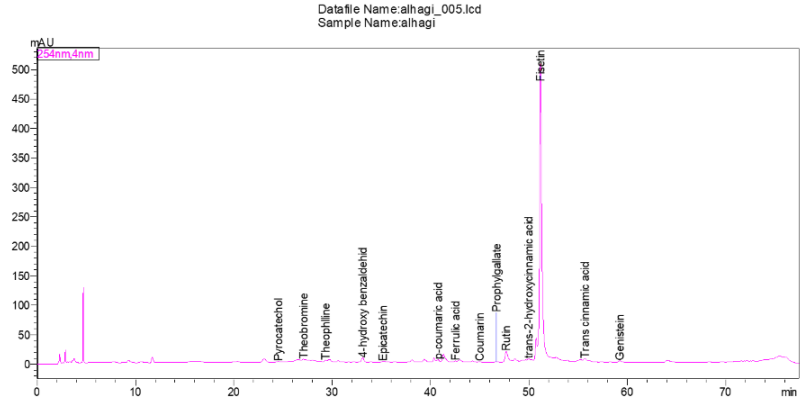

Figure 2 – HPLC-DAD chromatogram of complex from *Alhagi kirgisorum Schrenk* at 254 nm. Mobile phase 0,1% acetic acid-methanol (gradient elution)

In previous studies, extraction of the biologically active complex alkhydin was carried out using the classic maceration method. Acetone was used as the extractant solution, ethyl alcohol was used to precipitate the alkhydin, and the extraction time was measured in hours. The polyflavan content in the drug obtained by the maceration method was 12,95%.

An ultrasonic extraction method was developed for the first time to optimise the extraction of alkyl compounds. The parameters considered during this extraction are: effective solution, time, temperature, and extraction order.

50%-70% ethyl alcohol was used in the solution calculation, extraction time was 60-120 minutes, brown extract obtained at room temperature (20-25 ^{o}C), filtered, concentrated in a rotary evaporator and dried in a concentrate sublimation apparatus.

The drug obtained via ultrasonic extraction is a light brown amorphous powder. It dissolves well in water and has a musky smell.

The next step was to determine the polyflavan compounds (30,6-52,4%) in the powder obtained by ultrasonic extraction.

The drug was sent to Mugla Sitka Kocman University in the Republic of Turkey for biological activity testing [8-10]. The research results were described as follows. Anticholinesterase biological activity showed 48,20±4,39% against AChE, 4,21±0,39% against BChE, α-Amylase inhibitory activity against diabetes was 2807,63±0,10, α-Glucosidase inhibitory activity was 183,51±0,80. Also against oxidation process: $ABTS^{+}$ assay 12,52±2,16 (6,25-50 μg/ml), DPPH assay 15,70±0,46 (3,125-25 μg/ml), CUPRAC 20,82±0,02 (6,25-50 μg/ml) and activity of 38,73±0,75 (3,125-25 μg/ml) according to carotene/linoleic acid analysis.

Conclusion

Alhagi Kirgisorum Schrenk (zhantak – Kyrgyz camel thorn) is endemic and grows only in Kazakhstan. First, the authenticity indicators of plant raw materials were studied, and it was determined that the moisture content reached 7,54%, the ash level reached 10,25%, and the share of extracted substances reached 41,05%. Sufficient amounts of K, Na, Ca, Mg, Zn, and Fe minerals were found in plant raw materials. The composition of this plant is rich in secondary metabolites, including tannin substances, and its share is 4,92%. Component analyses of the complex obtained using two different methods (maceration and ultrasonic extraction) were performed, and it was found that the drug obtained by ultrasonic extraction contained more tannin substances. The anticholinesterase biological activity of the complex of tannins showed 48,20±4,39% against AChE, 4,21±0,39% against BChE, α-Amylase inhibitory activity against was 2807,63±0,10, α-Glucosidase inhibitory activity was 183,51±0,80. Also against the oxidation process: $ABTS^{+}$ assay 12,52±2,16 (6,25-50 μg/ml), DPPH assay 15,70±0,46 (3,125-25 μg/ml), CUPRAC 20,82±0,02 (6,25-50 μg/ml) and according to *β*- carotene/linoleic acid analysis showed 38,73±0,75 (3,125-25 μg/mL) activity.

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Funding

The research work was funded by the project AP19680131 under grant funding from the Scientific Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

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ALHAGI KIRGISORUM SCHRENK ӨСІМДІГІНЕН ТЕРІ ИЛЕГІШ ЗАТТАРДЫ АЛУ ЖОЛЫН ОҢТАЙЛАНДЫРУ ЖӘНЕ ОЛАРДЫҢ БИОБЕЛСЕНДІЛІГІ

Өсімдіктер әр түрлі биологиялық активті кешендерді алудың шикізкт көзі болып табылады, олардың оқшауланууы биологиялық белсенділіктің кең спектіріне ие жаңа препараттарды алуға мүмкіндік береді. Биологиялық белсенді заттардың көзі ретінде Alhagi туысының өсімдігі, Alhagi Kirgisorum Schrenk түрі (Жантақ өсімдігінің қырғыздық түрі) үлкен қызығушылық тудырады. 2023 жылы Алматы облысы, Шелек ауданында гүлдену кезеңінде жиналған Alhagi Kirgisorum Schrenk өсімдік түрінің жер үсті бөлігі. Өсімдік шикізатының құрамындағы биологиялық белсенді заттарды талдау, ультрадыбыстық экстракция әдісін қолданып, жантақ өсімдігінен биологиялық активті кешен алудың тиімді технологиялық параметрлерін қарастыру және алынған дәрілік препараттардың фармакологиялық белсенділігін зерттеу маңызды мәселе. Өсімдік шикізатынан тері илегіш заттар кешенін бөліп алу үшін: 50% этанол еріткіші және ультрадыбысты экстракция мен мацерация әдістері, шикізат:экстрагент – 1:8 қатынасы ұсынылды. Аталған өсімдік құрамы екіншілік метаболиттерге өте бай, соның ішінде тері илегіш заттар осы түрде көп кездеседі және бұл ретте олардың үлесі 4,92 % екені белгілі болды. Екі түрлі әдісті: мацерация және ультрадыбысты экстракцияны қолдану арқылы қол жеткізілген кешенге компонентті талдау

жүргізіліп, ультрадыбысты экстракция әдісімен алынған препарат құрамында тері илегіш заттардың мөлшері көбірек екені анықталды. Алынған кешенді антихолинэстераза бойынша биологиялық белсенділікке тексергенде AChE-ге қарсы 48,20±4,39%, BChE-ге қарсы 4,21±0,39% көрсетсе, сусамырға қарсы α-амилазаны тежеу қызыметі 2807,63±0,10, α-глюкозидазаны тежеу қызметі 183,51±0,80 болды. Сондай-ақ тотығу үрдісіне қарсы: ABTS^{+} талдау 12,52±2,16 (6,25-50 мкг/мл), DPPH талдау 15,70±0,46 (3,125-25 мкг/мл), CUPRAC 20,82±0,02 (6,25-50 мкг/мл) және β-каротин/линол қышқылын талдау бойынша 38,73±0,75 (3,125-25 мкг/мл) белсенділік көрсетті.

Түйін сөздер: *Alhagi Kirgisorum Schrenk, минералдар, полифлавандар, биологиялық белсенді кешендер, экстракция, пирокатехол.*

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ОПТИМИЗАЦИЯ СПОСОБ ПОЛУЧЕНИЯ ДУБИЛЬНЫХ ВЕЩЕСТВ ИЗ *ALHAGI KIRGISORUM SCHRENK* И ИХ БИОАКТИВНОСТЬ

Растения являются сырьевым источником для получения различных биологически активных комплексов, а их выделение может быть использовано для получения новых лекарственных средств с разнообразной биологической активностью. Как источники биологически активных веществ, огромный интерес представляют растения рода Alhagi, вида Alhagi Kirgisorum Schrenk (киргизский вид верблюжья колючка). Alhagi Kirgisorum Schrenk, собранный в период цветения в Шелекском районе Алматинской области в 2023 году, представляет собой надземную часть вида растения. Важными вопросами являются анализ биологически активных веществ в растительном сырье, рассмотрение эффективных технологических параметров извлечения биологически активных комплексов из растений с помощью ультразвуковой экстракции, изучение фармакологической активности полученных лекарственных препаратов. Для выделения комплекса дубильных веществ из растительного сырья: 50% растворителя этанола и ультразвуковой экстракции и мацерации методами предложено соотношение сырье:экстрагент – 1:8. Состав этого растения очень богат вторичными метаболитами, в том числе дубильными веществами, известно, что их доля составляет 4,92%. Комплекс, полученный с помощью двух разных методов: мацерации и ультразвуковой экстракции, был подвергнут компонентному анализу, и было установлено, что экстракт, полученный с помощью ультразвуковой экстракции, содержит больше дубильных веществ. Антихолинэстеразная биологическая активность комплекса составила 48,20±4,39% в отношении AChE, 4,21±0,39% в отношении BChE, противодиабетических средств по ингибирующей активности α-амилазы составила 2807,63±0,10, ингибирующей активности α-глюкозидазы 183,51±0,80. Также против процесса окисления: анализ ABTS^{+} 12,52±2,16 (6,25-50 мкг/мл), анализ DPPH 15,70±0,46 (3,125-25 мкг/мл), CUPRAC 20,82±0,02 (6,25-50 мкг/мл) и по анализ β-каротина/линолевой кислоты показал активность 38,73±0,75 (3,125-25 мкг/мл).

Ключевые слова: *Alhagi Kirgisorum Schrenk, минералы, полифлаваны, биологически активные комплексы, экстракция, пирокатехин.*

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Received 14.08.2024
Revised 23.09.2024
Accepted 24.09.2024

https://doi.org/10.53360/2788-7995-2024-4(16)-44

IRSTI: 31.15.29

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COMPARATIVE STUDY OF PHOTOCATALYTIC HYDROGEN EVOLUTION ON G-C_3N_4 DECORATED WITH NIS AND NIS_2 CO-CATALYSTS VIA ION EXCHANGE PRECIPITATION METHOD

Abstract: *NiS and NiS_2 co-catalysts were decorated on the surface of g-C_3N_4 through ion exchange reaction by precipitation method. Synthesized double systems were investigated using XRD, FT-IR, SEM, TEM, and TEM elemental mapping. XRD and FT-IR analyses showed the presence of g-C_3N_4 in the composition of g-C_3N_4/NiS and g-C_3N_4/NiS_2, however the presence of nickel sulfides was not identified. SEM analysis showed that double systems have heterogeneous systems, the stacked flat sheets with wrinkles and an irregular shape morphology and rough surface, where the presence of irregular shape pores is visible. TEM proved the presence of irregularly*