Agrobiodiversity for Improving Nutrition, Health and Life Quality

Research Article

OPEN ACCESS

Alhagi kirghisorum Schrenk: technological aspects of its thick extract for the pharmaceutical application

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Article Details:
Received: 2022-04-27
Accepted: 2022-05-23
Available online: 2022-05-31

DOI: https://doi.org/10.15414/ainhlq.2022.0009

The elaboration of medicinal substances with high pharmacological activity is the primary task of the pharmaceutical industry. In this regard, medicinal plant raw materials as a source of valuable biologically active substances are of great interest. The Kyrgyz camelthorn (*Alhagi kirghisorum* Schrenk) is a plant representing the genus of plants of the Fabaceae family growing in the deserts. The therapeutic properties of the desert plant are characterized by hemostatic, wound-healing, choleretic and astringent effect; bactericidal effect on staphylococci, streptococci and dysentery bacillus is manifested. The monograph on the herb of *Alhagi kirghisorum* is included in the State Pharmacopoeia of the Republic of Kazakhstan. The article presents the results of the research on the substantiation and development of the technology of a thick extract of *Alhagi kirghisorum*, the study of the chemical composition of the obtained thick extract of camel thorn and presents the results of studying the antimicrobial properties of this thick extract. The choice of 70% ethanol as an extractant has been experimentally substantiated, the dynamics of the extraction process was studied and the amount of extractant necessary for complete depletion of the raw materials in the extraction process was established as 1 : 5 (raw material : extractant). The presence of flavonoids was confirmed by common color reactions. The assay of the sum of flavonoids was performed by spectrophotometry. It has been found that the thick extract obtained by extraction with 70% ethanol has a higher quantitative content of flavonoid substances compared to the aqueous one. The microbiological studies (method of "wells") of *Alhagi kirghisorum* thick extract obtained by the extraction with 70% ethanol compared to ethanolic solution of chlorophyllipt showed that our extract had a moderate activity against of *Staphylococcus aureus* (zone diameters were 21.2 ±0.6 and 20.6 ±0.5 mm, respectively) and more pronounced antimicrobial activity against *Bacillus subtilis* (20.0 ±0.6 and 13.6 ±0.5 mm). Relative to the gram-negative culture of *Escherichia coli*, the activity of the thick extract of *Alhagi kirghisorum* was 21.6 ±0.5 mm while ethanolic solution of chlorophyllipt was not active. Therefore, the thick extract *Alhagi kirghisorum* is a prospective active substance for the development of herbal antibacterial preparations.

Keywords: Kyrgyz camelthorn, flavonoids, extraction dynamics, antimicrobial activity

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Introduction

The creation of highly effective and available medicines is the most important task of the pharmaceutical industry. Herbal preparations possess different biological properties, since they contain complexes of chemical compounds that simultaneously exhibit a combined therapeutic effect. Currently, wild medicinal plants are an integral part of natural wealth. Medicinal herbal raw materials are a powerful source of active substances for the manufacture of herbal medicinal products with high therapeutic activity. One of the well-known plants that have long been used in folk medicine in Kazakhstan is the wild plant *Alhagi kirghisorum*. The therapeutic properties of camelthorn are mentioned in the Canon of Medicinal Science of the famous Abu Ali Ibn Sina, better known as Avicenna (Sokolov and Shestakov, 2015; Varshochi and Asadollahi, 2015).

Kyrgyz camelthorn (*Alhagi kirghisorum* Schrenk) is a plant from the Fabaceae family, in folk medicine it is called "jantak" or "yantak". In folk medicine, the aerial parts (herb) of the *Alhagi kirghisorum*, less often the fruits and roots are used. Infusions and decoctions from *Alhagi kirghisorum* herb are used as a diuretic and sweating agent. Sometimes they are taken to soften the cough in the case of cold. Infusions, decoctions or fresh juice are taken for the treatment of gastrointestinal diseases, mainly chronic diarrhea and dysentery. Extracts from the aerial parts of camelthorn have an antimicrobial effect, and they exert a pronounced bactericidal effect on streptococci, staphylococci, and dysentery rods (Ahmad et al., 2010; Neamah, 2012; Asghari et al., 2016; Tavassoli et al., 2020). Decoctions are successfully used in the form of throat rinses in acute tonsillitis. Sometimes, *Alhagi kirghisorum* decoction is used in folk medicine for the treatment of hemorrhoids (baths, rinsing), for the external treatment of eczema, abscesses, putrefactive wounds and ulcers (washing, compresses). The decoction is used to treat patients with colitis, dysentery, gastric ulceration and gastritis, liver diseases, as a choleretic (Awaad Amani et al., 2006; Marashdah and Al-Hazimi 2010; Burasheva et al., 2012).

Our research was aimed at substantiating the technology of liquid extract in order to ensure the maximum extraction of biologically active substances from the raw material is relevant.

Material and methodology

Plant material

The aerial parts of the *Alhagi kirghisorum* (herb), which were harvested in the end of July to mid-August, was used for this research. Harvesting was carried out in the Almaty region, plant identification was performed according to the Pharmacopoeia monograph (2014). The upper, non-wooden part of the shoots was cut together with leaves and flowers, making a cut at a height of at least 8–10 cm from the ground. The raw materials were dried in the shade, spread in a layer 2–3 cm thick. Dried to a characteristic crack of the raw material at break. Damaged, blackened and browned parts of *Alhagi kirghisorum* were removed. The raw materials were crushed using a grass cutter; fractionated using a set of sieves, a fraction of raw materials with a size of 0.5–5.0 mm was used in the work.

Method for determining extractible substances

About 1 g of the crushed raw material (accurate weight) sieved through a sieve with holes of 1 mm diameter placed in a 200–250 mL conical flask. Later 50 mL of the extractant were added. The flask was closed with a stopper, weighed, and left for 1 h. The flask is then connected to a reflux condenser, heated maintaining a low boil for 2 h. After cooling, the flask with the content is closed again with a stopper, weighed and the loss in mass is replenished with the extractant. The content of the flask is thoroughly shaken and filtered through a dry paper filter into a dry 150–200 mL conical flask. 25 mL of the filtrate is pipetted onto a cup and the dry residue is determined according to the method of the State Pharmacopeia of Ukraine (SPU, 2015) (2.8.16). The percentage content of extractables (X) in terms of absolutely dry raw material is calculated by the formula:

$$X = \frac{m \times 200 \times 100}{m_1 (100 - W)}$$

where: m – the weight of the dry residue (g); m_1 – filtrate weight (g); W – weight loss during raw material drying (%)

Method for obtaining liquid extractions from the medicinal raw materials

Liquid extracts were obtained by the percolation method. The method includes three consecutive stages: wetting of the raw material (swelling of the raw material), infusion and actually percolation. The

essence of the method is that the raw material is loaded into the percolator and poured with the extractant until the formation of a "mirror", after which the infusion takes place during the 1st day. For the extraction process, we selected a fraction of camelthorn herb of 0.5–5.0 mm. After infusion, liquid extracts are drained (tempered) and the extractant is simultaneously fed at the same rate to create a high concentration difference in the raw material and in the extraction medium, which is the driving force of the extraction process. The percolation rate in our case was 1 drop per 1 second. In the process of percolation, consecutive drains were collected in an amount equal to the weight of the raw material (100 mL), in a ratio of 1 : 1 (weight-volume ratio).

The determination of the dry residue content in liquid extracts (drains) was carried out using the express moisture analyzer "SARTORIUS MA-150". The test was performed at a temperature of 105 °C in accordance with SPU 2.8.16.

Study of the dynamics of biologically active substances extraction from the plant raw material

The content of dry residue (An, g) in individual portions of liquid extracts V_n obtained with a corresponding extractant at an appropriate "raw material : extract" ratio was calculated by the formula:

$$A = \frac{\varpi_n \times V_n}{100}$$

where: V_n – the volume of a separately collected portion of the liquid extract obtained by the corresponding extractant with a step of the "raw material : extract" ratio of 1 : 1 (mL); ω_n – dry residue in a separately collected portion of liquid extract n (%)

Determination of the content of dry residue (B_n, g) in the total extracts V_{n+1} obtained by the corresponding extractant at the appropriate ratio of "raw material : extract" obtained at the stage was carried out by the formula:

$$B_n = \sum_{n=1}^{n} A_n$$

where: A_n – the dry residue in a separately collected portion of the extract V_n (g)

Determination of the dry residue (C_n, %) in the total extracts V_{n+1} obtained by the corresponding extractant at the appropriate ratio "raw material : extract" at the stage was carried out by the formula:

$$C = \frac{B_n}{V_{n+1}} \times 100$$

where: V_{n+1} – the volume of the total extract at the stage (mL); B_n – the content of dry residue in total extracts V_{n+1} (g)

Determination of the yield of extractives (absolutely dry extract) (D_n, %) from the extracted raw materials at each of the extraction stages with the appropriate extractant at the appropriate ratio "raw material : extract", was carried out by the formula:

$$D_n = \frac{B_n}{m_c} \times 100$$

where: m_c – the weight of the raw material loaded into the extractor (g); B_n – the content of dry residue in total extracts V_{n+1} (g)

Method for assay of the number of flavonoids in thick extracts of *Alhagi kirghisorum* herb

The assay of the sum of flavonoids was performed according to the method of SPU 2.1-2.2.25.

The quantitative content of the sum of flavonoids in the thick extract of camelthorn was determined by absorption spectrophotometry in the ultraviolet and visible region after the complexation reaction with aluminum chloride in terms of rutin. The test samples for spectrophotometric studies were prepared according to the following procedure.

Stock solution

Place 1.0 g (exact weight) of thick camelthorn extract in a 50 mL volumetric flask, dissolve in 25 mL of alcohol (70%, v/v) R, adjust the volume to the mark with the same solvent, stir. Filter through the "blue tape" filter.

Test solution

Place 2.0 mL of the stock solution into a 25 mL volumetric flask, add 1.0 mL of aluminum chloride R and adjust the volume to the mark with 5% (v/v) of glacial acetic acid R in ethanol R (70%), stir.

Compensation solution

Place 2.0 mL of the test solution into a 25 mL volumetric flask and adjust the volume to the mark with 5% (v/v) of glacial acetic acid R in ethanol R (70%), stir.

Comparison solutions

Were prepared in similar way, the initial sample (exact sample) of which was: for rutin – 0.0243 g, for quercetin – 0.0501 g, for gallic acid – 0.0398 g. The absorbance of the test solutions is measured 30 minutes after preparation for the compensation solution on Evolution 60 S (USA) spectrophotometer in a cuvette with a layer thickness of 1 cm at a wavelength of 410 nm. The content of the sum of flavonoids (in terms of rutin) in 1 g of thick extract is calculated by the formula:

$$X = \frac{D \times 50 \times 25 \times m_{st} \times 2 \times 100}{D_{st} \times m_n \times 2 \times 50 \times 25}$$

where: D – the absorbance of the test solution; D_{st} – the absorbance of the standard sample solution; m_n – the weight of the dense extract sample (g); m_{st} – the weight of the standard sample (g)

Chromatographic studies were carried out using Sorbfil plates (Russia). The test samples together with the comparison samples were applied to the start line of chromatographic plates in an amount of 5 µg. The plate with the applied samples was dried in air, then placed in a chromatographic chamber containing the solvent system glacial acetic acid:water:ethyl acetate (20 : 20 : 60) and then chromatographed in an ascending manner. Once the eluent front reached the edge of the plate, it was taken out and dried in air.

Microbiological methods of research of *Alhagi kirghisorum* thick extract

The antimicrobial activity of the test samples of the thick extract was studied *in vitro* by the method of diffusion into agar ("wells" method). This method is based on the ability of the active substances to diffuse into the agar previously cropped with cultures of microorganisms. As test cultures, pure cultures from the American Test Culture Collection (ATCC) were used: gram-positive microorganisms – *Staphylococcus aureus* ATCC 25293 and spore *Bacillus subtilis* ATCC 6633, gram-negative culture *Escherichia coli* ATCC 25922. Antifungal activity was determined in relation to yeast-like fungi *Candida ablicans* ATCC 885-653. An indicator of antimicrobial activity is the size of the inhibition zone that is formed in the nutrient medium around the wells in Petri dishes.

Statistical analysis

The calculation of metrological characteristics of analytical methods was performed in accordance with the requirements of the SPU monograph "*Statistical analysis of the results of chemical experiment N*" (2018). Statistical assessment of microbiological data are reported as mean ± SEM and were analyzed using STATISTICA 6 software with one-way ANOVA. *P* values less than 0.05 was assumed statistically significant.

Results and discussion

The scientific interest in *Alhagi* genus plants is due on the one hand to the rich composition of pharmacologically active substances with a wide range of therapeutic activity, and on the other – an extensive raw material base, as plants of this genus are wild and adapted to adverse growth conditions (Awaad et al., 2011).

According to numerous publications, among the biologically active substances in *Alhagi* plants, phenols, flavonoids, alkaloids, terpenoids, polysaccharides and fatty acids have been identified (Laghari et al., 2010; Laghari et al., 2012; Muhammad et al., 2015). The publications highlight the results of the study of antioxidant, anti-inflammatory, hepatoprotective, antibacterial, antidiarrheal and urolithic activity of *Alhagi* plant extracts with the content of total biologically active substances and some isolated individual substances (Muhammad et al., 2015; Nishanbaev et al., 2019). Analysis of the data shows that the most studied are *A. maurorum* and *A. pseudalhagi* both in terms of chemical composition and pharmacological action (Nishanbaev et al., 2019).

The works of Burasheva et al. (2012) are devoted to photochemical research of *A. kirgisorum*, according to which *A. kirgisorum* contains amino acids, condensed tannins, flavonoids, carbohydrates, carotene. Khalmatov (1960) found that the leaf of *A. kirgisorum* contains a significant amount of ascorbic acid – 1088.57 mg% in terms of dry matter.

The dependence of the chemical composition of the obtained plant extracts and their pharmacological activity on the used extractant is obvious, as the substances have different solubilities in polar and non-polar solvents (Marashdah and Al-Hazimi, 2010; Nishanbaev et al., 2019).

Thus, when determining the content of extractables in the raw material, a water-alcohol solution of various concentrations (40%, 50%, 60%, 70%, 80%) and purified water were used as the extractants. Ethyl alcohol is the most used extractant due to its high ability to dissolve biologically active substances. We have studied the extracting ability of hydroalcoholic

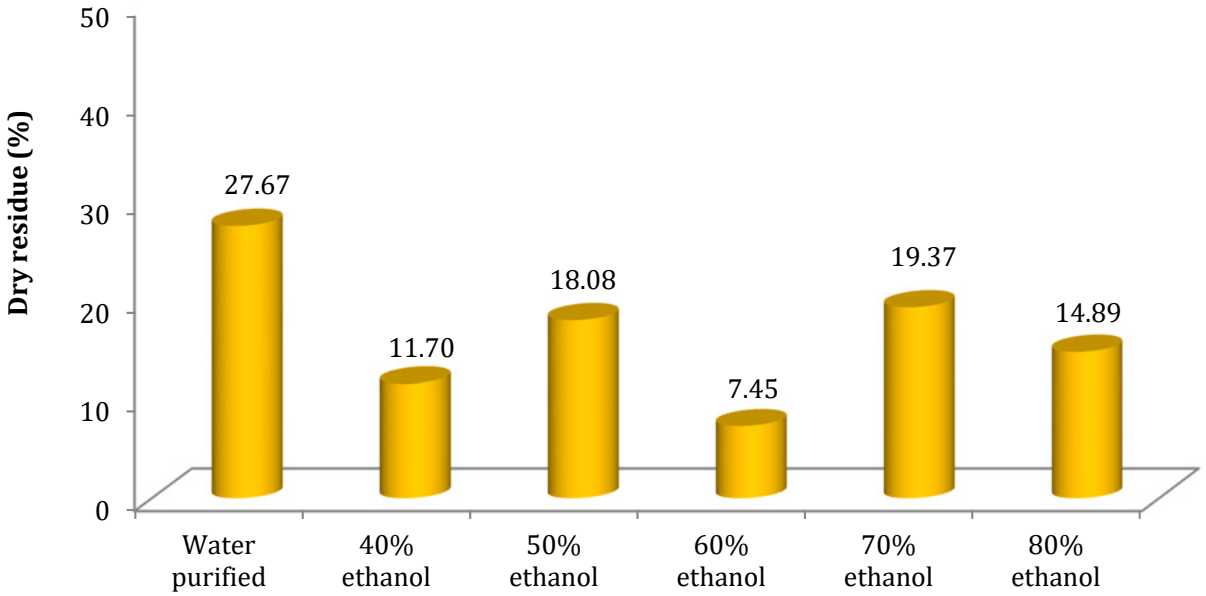

Figure 1 Amount of extractives depending on the solvent

solutions of various concentrations and purified water, the results are shown in Figure 1.

According to the above data, the maximum extraction of extractives (27.67%) is achieved with the use of purified water. Water-alcohol solutions extract from camelthorn herb from 7.45 to 19.37% of extractables.

In view of the fact that biologically active substances have different solubility, it was decided to use both purified water and 70% aqueous-alcoholic solution for further research. The extraction with 70% ethanol was performed at room temperature, with purified water at two temperature modes: room temperature and at 90 ±0.5 °C. The extraction of biologically active substances was carried out by the method of percolation. The efficiency of percolation as an extraction method is based on the high difference in the concentration of biologically active substances in the raw material and in the extractant, which is the motive force of the extraction process (Jones and Kinghorn, 2012; Blicharski and Oniszczuk, 2017). The advantage of this method is also that the pharmaceutical enterprises

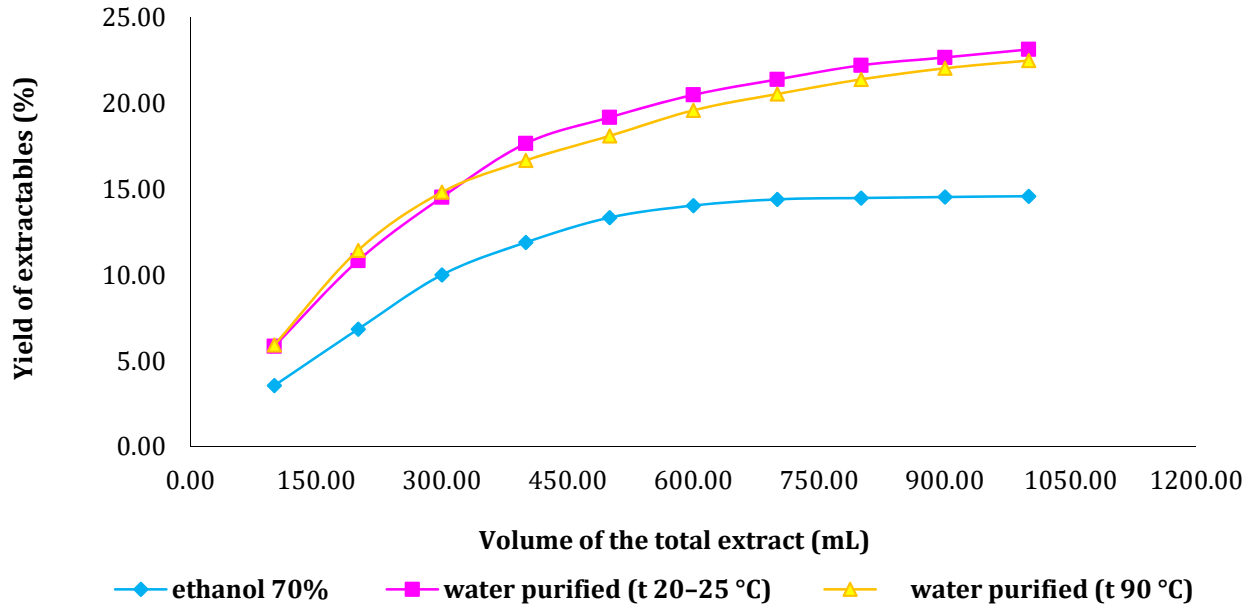

Figure 2 Dynamics of the yield of extractives in liquid extracts

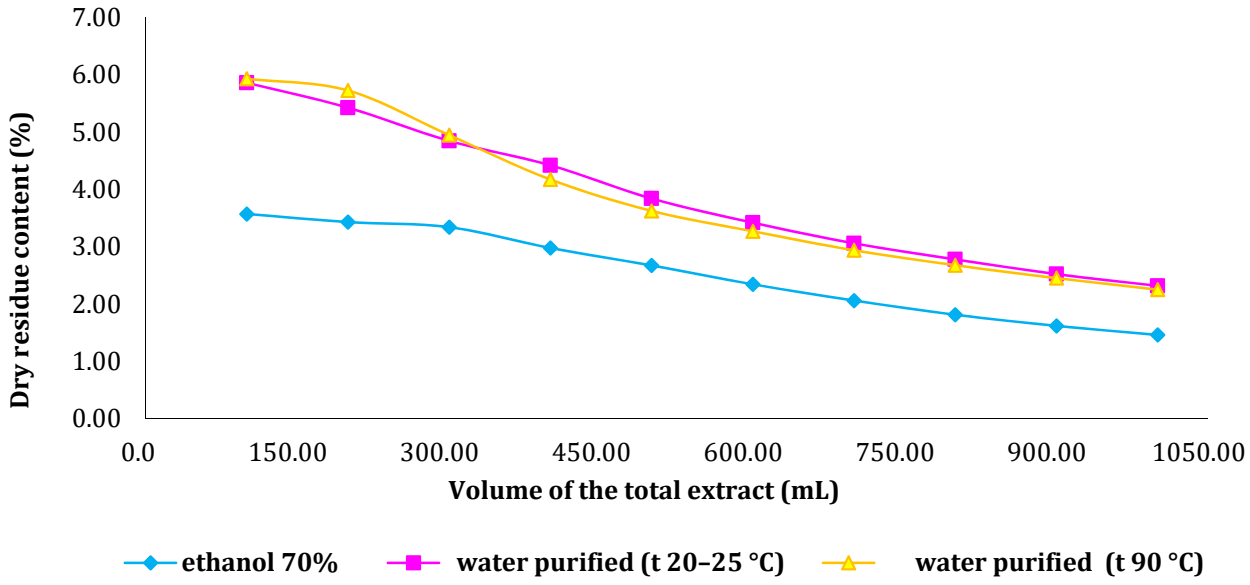

Figure 3 Dynamics of reduction of dry residue in liquid extracts

of Kazakhstan are well equipped with an appropriate equipment for the implementation of technology into serial production.

The study of the dynamics of the extraction process by percolation method was performed in accordance with the standard algorithm and the following criteria were calculated that characterize the extraction process: the content of dry residue in separately collected volumes of liquid extract (drains) and in the total extracts collected at each subsequent extraction stage (C_n, %); the content of extractives in the total extracts (D_n, %). The results of the study of the extraction process dynamics are shown in Figure 2 and 3.

Based on the data presented in Figure 2 and 3, it can be seen that at the extraction multiplicity from 1 to 5 at each extraction stage, there is a significant increase in the amount of extractable. A further increase in the extraction multiplicity slightly increases the yield of biologically active substances. Thus, it is rational to use the raw material : extractant ratio as 1 : 5 in percolation.

Thick extracts were obtained by condensing the resulting liquid extracts on a laboratory vacuum evaporator. The evaporation was carried out at a temperature of 45–50 °C, vacuum – 0.06 MPa. Under such conditions, the maximum possible preservation of biologically active substances at condensation is achieved (Blicharski and Oniszczuk, 2017).

The pharmacopoeial monograph for *A. kirgisorum* herb (State Pharmacopoeia of the Republic of Kazakhstan, 2014) provides data on its standardization by the content of tannins, which should be not less than 2.0%. However, numerous publications on phytochemical studies of plants of *Alhagi* genus cover data on the content of flavonoid substances and pharmacological action related to these groups of substances (Laghari et al., 2010; Laghari et al., 2012; Olas et al., 2015; Nishanbaev et al., 2019; Nishanbaev et al., 2020).

Flavonoids have a wide range of pharmacological activity, and show capillary-stabilizing, choleretic, diuretic, hepatoprotective, sedative, anti-inflammatory, anti-ulcer, hemostatic, bactericidal, hypotensive, hypoglycemic and antioxidant effects (Kukhtenko, 2016; Hudz et al., 2017a, b). Therefore, the paper is focused on the qualitative and quantitative analysis of flavonoids in the thick extract. Qualitative and quantitative analysis are important components of comprehensive research in the development of herbal medicines.

In the process of chemical analysis of thick extracts, pharmacopoeial methods were used to study the qualitative composition of the obtained thick extracts. For the study, aqueous and alcoholic (70%) solutions of thick extracts were diluted to a ratio of 1 : 10, which were subjected to qualitative analysis for the presence of flavonoids. The presence of biologically active substances of flavonoid structure was confirmed by generally accepted color reactions with the following reagents: concentrated hydrochloric acid (cyanidin test), 10% sodium hydroxide solution, 10% iron (III) chloride solution, 10% lead acetate solution. The results of experimental studies are presented in Table 1.

Table 1 Qualitative reactions to flavonoid structure substances

Reaction	Staining in reactions			Conclusion
	thick extract (aqueous) with water	thick extract (aqueous) with ethanol	thick extract (ethanol) with ethanol	
Cyanidin test	pink	pink	pink	red to raspberry coloring flavonols
KOH (without heating)	brown	green with sediment	green with sediment	thick extract (aqueous): chalcones and aurons thick extract (ethanol): flavones, flavonols, flavanones, flavanonols
KOH (after heating)	yellow with brown-red precipitate	brown	brown	
$FeCl_3$	black-green	black-green	brown-black	flavones, flavonols, flavanones, flavanonols, chalcones, aurons, catechins
Lead acetate	clear solution with loose precipitate	yellow coloring with sediment	yellow with sediment	flavones, chalcones, aurons containing a free o-hydroxyl group in ring B

As can be seen from the above data, thick extracts obtained by extraction with both aqueous (at room temperature and at a temperature of 90 °C) and alcoholic extracts contain both flavane and flavone derivatives. The qualitative reactions confirmed the presence of flavonoids in the extracts.

To compare the qualitative composition of thick extracts, we used the method of thin-layer chromatography according to SPU (2015) 2.1–2.2.26. Determination of the presence of phenolic compounds (phenolic acids) and flavonoids (flavonols) was carried out using comparison samples: rutin, quercetin and gallic acid.

The results of the analysis of chromatograms in daylight are presented in Figure 4 and indicate that the analyzed alcoholic extract contains substances of flavonoid structure similar to quercetin and rutin, since the chromatogram shows spots that correspond in color and location to the spots in the chromatogram of the mixture of rutin and quercetin. The TLC method did not reveal flavonoid nature substances in the aqueous extract in this concentration.

When studying the absorption spectra of the analyzed extracts obtained after interaction with aluminum chloride solution (Figure 5) it was found that in the thick extract obtained by extraction with water, the substances of the flavonoid structure are contained, but in a small amount, whereas in an alcoholic extract the amount of such substances is much higher 2.9%. Such an amount of flavonoid substances correlates with the data of Burasheva et al. (2012) and Nishanbaev et al. (2019).

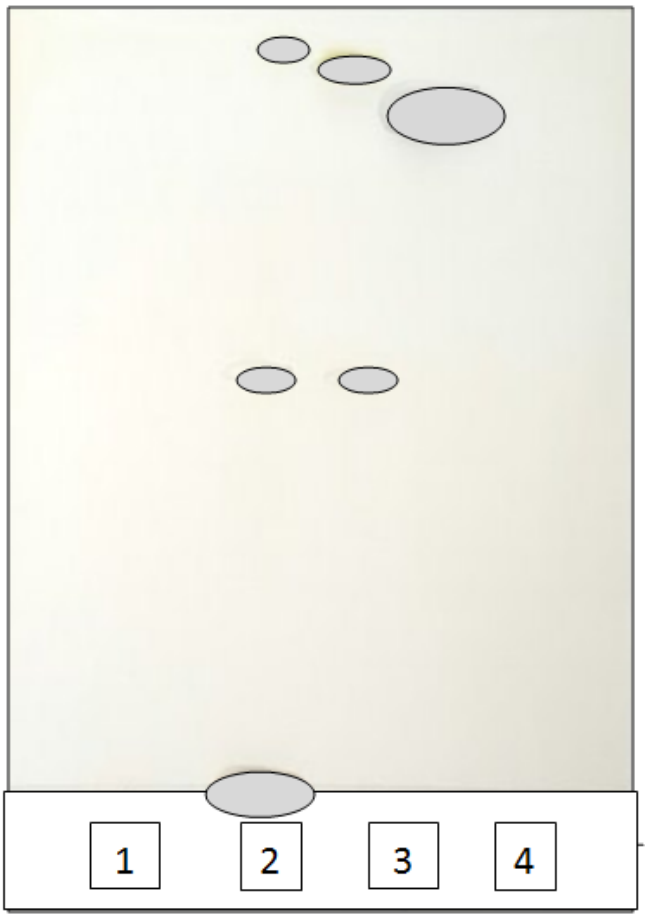

Figure 4 Scheme of the TLC chromatogram of the analysis of thick extracts of *Alhagi kirghisorum* Schrenk
1 – obtained by extraction with purified water; 2 – obtained by extraction with 70% ethanol; 3 – a solution of rutin with quercetin (1 : 1)

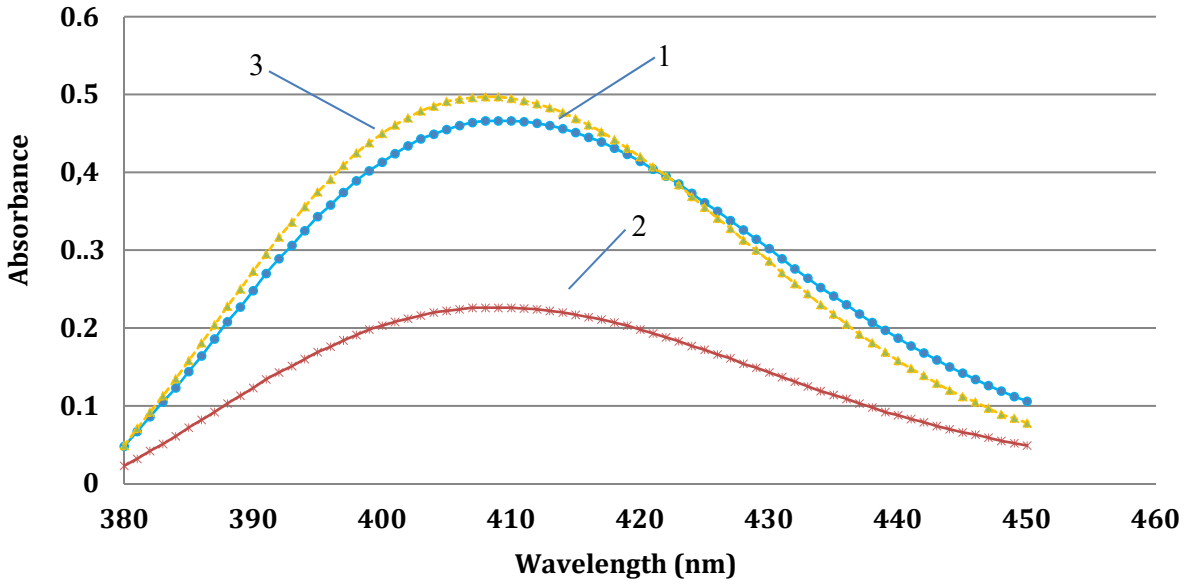

Figure 5 Absorption spectra of the sum of flavonoids in the thick extracts of *Alhagi kirghisorum* Schrenk obtained by extraction with 70% ethanol (1), purified water (2), and a standard sample of rutin (3) after reaction with aluminum chloride solution

It was found that extracts of *Alhagi* genus plants, obtained using ethanol or methanol with subsequent fractionation (*n*-hexane, chloroform, ethyl acetate, n-butanol) have antibacterial properties (Bakht et al., 2014; Ahmad et al., 2015). According to Orynkul et al. (2016) studies of antibacterial activity of the composition of camel thorn water extract with biopolymers polyhexamethylene guanidine hydrochloride (metacide) and β-C1 have shown fungicidal activity against crops pathogen *Puccinia recondita*.

Therefore, the next step was to investigate the antimicrobial properties of the thick extract of *Alhagi kirghisorum* obtained by extraction with 70% ethanol. The research results are given in Table 2.

The results obtained indicate that the test samples of thick extract of *Alhagi kirghisorum* compared to the alcohol solution of chlorophyllipt 10 mg.mL^{-1} have a moderate (culture of *Staphylococcus aureus* (growth retardation zone diameters 21.2 ±0.6 mm and 20.6 ±0.5 mm, respectively) and more pronounced (culture of *Bacillus subtilis* – 20.0 ±0.6 mm and 13.6 ±0.5 mm) antimicrobial activity; relative to the gram-negative bacterium *Escherichia coli* the activity of the thick extract of camelthorn amounted to 21.6 ±0.5 mm, while the alcohol solution of chlorophyllipt showed no activity relative to this microorganism.

The data of studying the antimicrobial activity of *Alhagi kirghisorum* thick extract obtained by extracting with 70% of ethanol, indicate the prospect of the elaboration of herbal medicinal products for the treatment of infectious diseases of the oral cavity or wounds, for the treatment of human diseases caused by bacterial infection.

Table 2 Antimicrobial activity of the test samples

Samples	Test cultures of microorganisms			
	S. aureus	*B. subtilis*	*E. coli*	*C. ablicans*
	diameters of the microbial growth inhibition zones (mm)			
Thick camelthorn extract	21.2 ±0.7	20.0 ±0.6	21.6 ±0.5	–
Ethyl alcohol (control)	–	–	–	–
Alcohol solution of chlorophyllipt 10 mg.mL^{-1} (reference drug)	20.6 ±0.5	13.6 ±0.5	–	–

Note: "–" – no zone of microbial growth inhibition.

Conclusions

Thus, the effect of the extractant nature on the yield of biologically active substances from the medicinal plant raw material at each step of extraction was studied. The dynamics of the extraction process were studied and the amount of the extractant required for the complete depletion of the raw material during the extraction process was set as 1 : 5 (raw material to the extractant). The use of 70% ethanol as an extractant was experimentally justified. The reaction on flavonoids confirmed their presence. The microbiological studies indicated that the test samples of thick extract of *Alhagi kirghisorum* compared to the alcohol solution of chlorophyllipt 10 $mg.mL^{-1}$ had a moderate activity against *Staphylococcus aureus* and more pronounced activity against *Bacillus subtilis*. The thick extract of camelthorn was active against *Escherichia coli* while ethanolic solution of chlorophyllipt was not active.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article does not contain any studies that would require an ethical statement.

Acknowledgments

The publication was prepared with the active participation of researchers in International Network Agrobio*Net*.

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