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Original Article

Pharmacognostical Investigations of *Hedysarum Caucasicum Bieb*. (Fabaceae) - An Ethnomedicinal Plant of Northern Caucasus, Russia, Determination of Mangiferin and Antibacterial Potentials

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ABSTRACT

The main active component of members of the genus Hedysarum is xanthone C-glycoside - mangiferin which was found in the above-ground part of 17 species of Hedysarum. Mangiferin is contained in plants of the genus Hedysarum can serve as a chemotaxonomic marker of this genus, it has antiviral activity against DNA-containing viruses: Herpes simplex virus, Varicella zoster, Cytomegaloviruses, also has immunostimulatory properties (stimulates cellular and humoral immunity). We have prepared the morphological and anatomical studying, phytochemical research availability of tannines, flavonoids, xanthones, the free organic acids, the sugars and amino acids among which in the significant amount glutamic acid, aspartic acid and an arginine collect is established. The maintenance of the sum of xanthones made 7.12%. As a result of a research of amino-acid structure of a grass of *Hedysarum caucasicum Bieb.* presence at the significant amount of glutamic acid (13.58 g/kg), aspartic acid (13.61 g/kg), an arginine (14.99 g/kg) is revealed. In a grass of *Hedysarum caucasicum Bieb.* The quantitative maintenance of the sum of xanthones in terms of a specific indicator of a mangiferin is established. The technology of receiving a liquid extract by means of 80% of ethanol is developed, standardization is carried out it. It is established that extract of *Hedysarum caucasicum* Bieb. shows the antimicrobial activity concerning Shigella sonnei, Bacillus subtilis and B.anthracoides.

Key words: *Hedysarum, Hedysarum caucasicum Bieb., Mangiferin*

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INTRODUCTION

The creation of antiviral and immunomodulatory drugs from medicinal plants is a pressing task of modern pharmacy. Thus, the preparation "Alpizarin," which contains xanthone glycoside - mangiferin, which has pronounced antiviral activity, has been created from *Hedysarum*.

The task - to study the above-ground part of the *Hedysarum caucasicum Bieb.* as a new additional raw material source of mangiferin and thus to expand the raw material base of the drug "Alpizarin." The purpose of the work was to investigate pharmacognostical studies of the grass of the *Hedysarum caucasicum Bieb.*, to develop a dosage form and to standardize it. To achieve this goal, research objectives were defined: to investigate morphological and anatomical analysis of raw materials; carry out commodity analysis of raw materials; to prepare phytochemical study of raw materials; to develop a dosage form technology; to standardize the dosage form; to investigate preliminary examination of antibacterial activity of the obtained medicinal form from the herb of the *Hedysarum caucasicum Bieb.*

The family Fabaceae Lindl. (Legumes) has about 650 genera and over 18,000 species, which range of growth covers all continents of the globe. Within its range, species of the legume family are characterized by a variety of habitats.[1-54]

Life form of species of the family Fabaceae Lindl. - perennial herbaceous plants, semi-plants, rarely - trees, there are tree or herbaceous lians.[44] The leaves are compounds, imparipennates with stipules. Infloresences racemeuses. The flowers are zygomorphic. The near-wind is double, the calice is most often actinomorphic, consists of 5 converged calice, the crown consists of 5 petals, two of which grow into a "boat," one large - a "sail" and two small petals - "vests" remain free. The android consists of 10 stamens, 9 of which usually grow together and 1 stamen remains free, gynecium monocarpic.[54] The fruit of legumes is monocarpium; one-, two- or multi-family legume, very diverse in morphological and anatomical features. Seeds without endosperm or with scant endosperm. Spare nutrients are deposited directly in the seed. Outside the seeds are covered with dense shiny seed peel.[10]

The genus *Hedysarum* L. combines about 285 species, which are predominantly common in Eurasia. The genus Hedysarum is distributed mainly in the Europe, but some species are also found in Asia.[44] *H. caucasicum* Bieb. grows in all highland areas of the North Caucasus, in the alpine zone, up to 3500 m.[10] This species is endemic, growing on the highland meadows of the Caucasus: in the Pre-Caucasus, Western and Eastern Transcaucasia, in Daghestan. It

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is described from the Caucasus.[44] C. Linnaeus (1753), B. A. Fedchenko (1902) were engaged in systematics of the species of Hedysarum L. The species *Hedysarum caucasicum* Bieb. we are investigating, first was described in 1808.[14] In Flora Caucasus in 1873, the species was classified as *H. obscurum var. caucasicum* Trautv.[14,50] The origin of the name of this plant is related to the shape of the fruit. Beans in appearance resemble coin meetings, and the Latin name *Hedysarum* comes from the Greek words «hedys» aroma, which literally means pleasantly smelling.[44]

The species of this genus are perennial herbs, shrubs, or semi-plants of seamless or stem-shortened plants. They grow both in forests - on raw meadows, on river banks, and on alpine and subalpine meadows, on rocky sprouts, in steppes.[10] Stems are often highly branched, branching, sometimes completely undeveloped and the flower arrow comes out of shortened shoots developing at the neck of the rhizome. Leaves are unparalleled, common 5-9-paired, less common 1-3-paired or even consisting of just one unparalleled leaf. The genus is characterized by simple racemeuse flowers - brushes; The calice is bell-shaped, its teeth are search-and-take longer than the tube. Wings are slightly or 2 times shorter than a boat, less often longer than it. The beans are artenic, and sometimes part of the seed does not develop and the bob consists of 1 to 3 artens. Sprouts are flat-compressed or slightly convex, smooth, bare or more often dried, mesh or with transverse ribs, often shrunk with short or longer bristles.[44]

According to Flora of the USSR, the *Hedysarum caucasicum* Bieb.- a plant 30 - 60 cm high; stems are straight or ascending, not shortened, olfactory; leaves 7 are 12-pair, elliptical or egg-like long-lasting-watt, with sharpness at the top, 12 - 16 mm long, 7 - 9 mm wide. Flowers (without brush) are longer than leaves; brushes are not very thick, o 25 to 35 flowers; the lower tooth of the cup is equal to the tube, the rest are shorter; the crown is dark - magenta, 16 - 18 mm long. The fruit is a legume of 3 to 6 arthropods, naked or desiccated.[10]

The microstructure of the aerian parts of some species of the Hedysarum was studied by Ladygina E.Y. *et al.*[21] The features of anatomical structure of leaves of five species from the section *Gamotion* Basin. were studied. These species containing mangiferin have been found to have bright golden yellow fluorescence of epidermis cells, which can be used to detect mangiferin directly in plant material.[16]

Detailed information on the main diagnostic signs of the *Hedysarum alpinum*, which is the main raw material source for the preparation of the preparation "Alpizarin".[9] The onthogenesis of species Hedysarum usually includes four periods and ten age states. In the example of H. austrosibiricum, age spectra can be divided into 4 types, which probably reflect ecological-phytocenotic growth conditions.[30]

During three years of life, alpine individuals undergo the following age conditions: in the first year - seedlings, juvenile, immature and more than 60% of individuals adult vegetative; In the second year, more than 90% of individuals enter a young reproductive state, and in the third year, all individuals transition to a reproductive state. Seeds of tested plants need pre-sowing treatment. Mechanical scarification and treatment with concentrated sulfuric acid are the best ways to disrupt the rest of both species.[28,30,45] The flowering phase of the Hedysarum caucasicum - the beginning and the middle of July, the fruiting phase - the end of July - the beginning of August.[44] For the genus Hedysarum, the following set of chromosomes is characteristic: 14, 16 and 48. For Hedysarum caucasicum chromosome number is 14.[49]

MATERIAL AND METHODS

As the object of the study was used the grass of *Hedysarum caucasicum* Bieb. family legumes (Fabaceae) collected in the flowering phase on the southeast slope of Mount Alibek at an altitude of 2,200 m (Dombay District, Caucasus mountains). The identification of the collected raw materials was carried out by the Doctor of Biological Sciences, Professor of the Department of Botany, as well as the Director of the Ecological-Botanical Station of the Russian Academy of Sciences, A. D. Mikheev. Samples of the herbarium of this species collected by the author are located in the departments of pharmacognosy and botany. For carrying out micromorphological analysis of vegetative organs, the *Hedysarum caucasicum* used conventional methods.[54] The sections obtained manually by the blades were stained with the following reagents: fluoroglucine, 50% sulfuric acid solution. Segments of anatomical sections were studied using a BIOLAM microscope with magnification of x9 lenses; h40; Slice segments were photographed with a SONY DSC-T3 digital camera at x3 magnification.

High performance liquid chromatography, photocolorimetry, complexonometry and chromato spectrophotometry are most commonly used to quantify mangiferin content in plant raw materials as well as biological fluids.[6,39,40,51] The photocolorimetric definition of mangiferin in both the raw material and crystalline powder has a number of advantages, such as shortening the analysis steps and replacing the deficient vegetable, tetrahydrofuran, with dioxane. The method is based on mangiferin's ability to produce a complex compound with chlorine iron. Mangiferin was determined from a calibration plot of the solution optical density versus mangiferin concentration. When comparing spectrophotometric and photocolorimetric methods, almost the same results were obtained.[46]

As for the current method of high performance liquid chromatography, according to literature, separation of xanthone glycosides by HPLC method could not be achieved on sorbents containing amino and cyano groups. The best results were obtained on reverse phase C18 sorbents, and methanol-water, ethanol-water, acetonitrile-acetic acid was used as the mobile phase. In aqueous systems, the shape of the mangiferin peak deteriorated. To quantify mangiferin in biological fluids, a sensitive reverse phase HPLC technique is proposed.[3,55-62]

The mobile phase was acetonitrile and a 3% CH_3COOH solution at a ratio of 16:84, chromatographed at a wavelength of 254 nm using an external standard method.[3,7] Furthermore, in the quantitative determination of mangiferin by HPLC, a system consisting of acetonitrile, water and phosphoric acid was used as the mobile phase. The selected conditions allowed to achieve clear separation of mangiferin and isomangiferin peaks on chromatogram.[51] In addition to the previous systems, methanol, tetrahydrofuran or acetonitrile, an aqueous solution of phosphoric acid in various ratios.[55-62] are used as the mobile phase in HPLC gradient elution. Of the latest techniques for the quantitative determination of mangiferin, liquid chromatography followed by mass spectrometric determination was used, and the method is characterized by speed and quality.[59]

The chromate-spectrophotometric examination is based on sequential chromatography and spectrophotometry. The raw material is treated with the following extractant system - acetone: water in ratio 1:1 with acidification with 5% hydrochloric acid followed by chromatography in the system with 15% acetic acid. After viewing the chromatogram in UV light and eluting the desired cellulose sites with mangiferin zones. The optical density of the solutions was measured at 372 nm. The mangiferin content was calculated from the specific absorption index.[39]

There area number of intense absorption bands in the UV spectrum of mangiferin. The most convenient to quantify a substance is a band with a maximum at 369 nm and a specific absorption coefficient of 295 ± 0.92. In the field of working concentrations, the absorption of mangiferin solutions is subject to Lambert-Ber law.[40] Analysis of literary sources has shown that the genus Hedysarum L. combines about 285 species, which are predominantly common in Eurasia. *H. caucasicum* Bieb. It is endemic, growing in the highland meadows of the Caucasus.

In a literary search, it has been found that chemical study information relates mainly to H. alpinum and H. flavescens. Information on the chemical study of *H. caucasicum* is fragmented and insufficient. Plants of the genus Hedysarum are widely used in folk and waitinal medicine, as an antibacterial, antiviral, immunomodulatory, anti-inflammatory agent. The following methods of analysis are used to identify and quantify the main active substances of xanthones: spectrophotometry, photocolorimetry, chromatographic methods of analysis (TLC, HPLC, mass spectrometry). The study of theoretical bases of extraction of medicinal raw materials allows to find optimal conditions of technology for creation of medicinal forms on the basis of herbal of Caucasus.

RESULTS AND DISCUSSION

Morphological study. Life form - a perennial herbaceous plant 40-50 cm high, underground organs reach 30 cm length. The escape is elongated, branched, straight-standing or raised, in the lower part of the dressing (Figure 1.1). The leaves are imparipennate, with sills. The number of leaflets located on rachis varies from 11-to 15. The leaves have an egg shape, a rounded base, a whole edge and a spiky top (Figure 1.3). Decaying of leaflets is insignificant. The flower is simple botriode, brush. The flower is zygomorphic. The calice consists of 5 sepales, a moth-type crown, the color of the petals pink-purple. The androceum consists of 9 converged tangles and 1 free. Gynecium is monocarpic. Fruit: according to morphogenetic classification of fruits it refers to monocarp, according to morphological - legume, flat, oblong in shape, consists of rounded arthropods. Number of squads from 3 to 5 (Figure 1.2).

The leaf is amphistomatic. The upper epidermal has weak and almost straight anticline cell walls. The stomatal apparate is anomocytical, surrounded usually by 3-5 cells. Trichomes were not detected on the abaxial side of the leaf (Figure 2.1). The lower epidermal has strong anticline cell walls. The trichomes are formed by simple single-cell hairs located either in the region of the veins or on the edge of the leaf plate (Figure 2.3-2.4). When considering the sheet microreparation from the surface, rhombic calcium oxalate crystals located in large veins are found. They form a characteristic crystalline lining of the veins. On the cross-section the sheets have a characteristic dorsoventrale structure (Figure 3.1.-3.2.).

The palisade mesophyll localizes only under the upper epidermis in one layer, its cells are stretched, tightly pressed to each other. The mechanical fabric is a collenchym which is located both under the upper and lower epidermis in the region of the core. In the central part of the main core there is a large collateral conducting bundle, from the dorsal side to the conducting bundle there is a sclerenchym of pericyclic origin. The leaflets are arranged on small cherries, which have cylindrical shape on cross section. The covering tissue of the cherry is represented by an epidermal, under it there is a collenchym in 1-2 layers, the main volume occupies chlorenchym. Collateral conducting bundle surrounded by sclerenchym is located in central part of transversal section. The rachis at the bottom has a grooved shape with a deep horseshoe spoon on the abaxial side. Collateral conducting bundles 6-8 are arranged. It is interesting that idioblasts with yellow content are found in the phloem, in the pericyclic zone, in the parenchyma of the core adjacent to the xylem portion of the conductive beam (Figure 3).

The stem has a polyhedral shape on the cross section (Figure 4). A cavity may form in the central part of the stem. Under the epidermal continuous ring in 1-2 layers of cells there is a plate-type collenchym. In the projection regions, the number of layers of collenchym cells increases to 5-7. Chlorenchym is located following collenchym in discontinuous regions in 2-3 layers of cells. The pericyclic zone is represented by sclerenchym fibers arranged in discontinuous areas above the conducting beams. Conducting system of bundle type (Figure 4.1). Conducting bundles of open collateral type in amount 14-16. The cambium forms secondary conductive beams and thick-walled parenchyma, which is subsequently strongly ligated. Idioblasts are found, as in the cherry, in the phloem part of the conducting beam, in the pericyclic zone, as well as in the parenchyma of the core.

The above-mentioned micromorphological characteristics of above-ground organs of the Hedysarum caucasicum can be used in preparing of the regulatory documentation for the grass as an additional raw material source of xanthone glycoside mangiferin.

Determination of humidity

By the moisture content of the raw material is meant a loss in mass due to hygroscopic moisture and volatile substances, which is determined by drying to a constant mass. Medicinal plant raw materials should

Figure 1: Appearance of the *Hedysarum caucasicum* collected in the flowering phase (09-07-2018) on the southeast slope of Mount Alibek at an altitude of 2,200 m (Dombay District, Caucasus mountains). 1- plant structure, 2- inflowercence, 3- fruits, 4- leaf structure

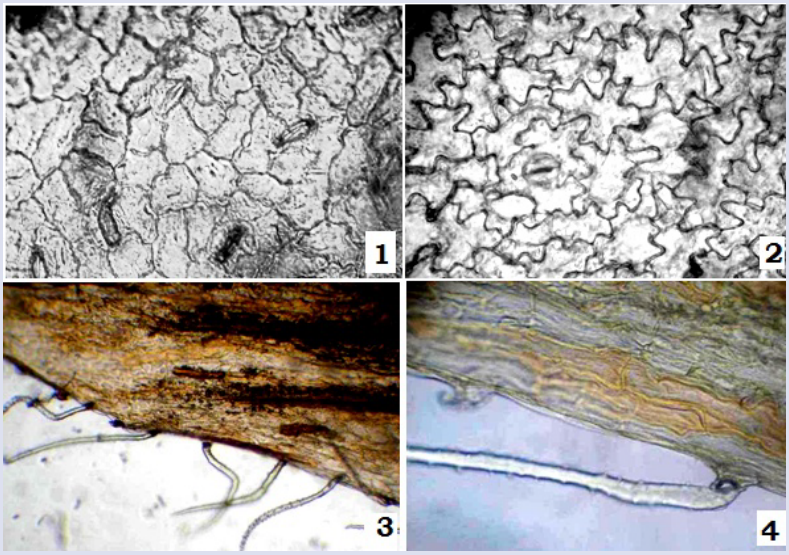

Figure 2: Micromorphological structure of the leaf of the *Hedysarum caucasicum* adaxial epidermis, 2—abaxial epidermis, 3-4 – trichomes

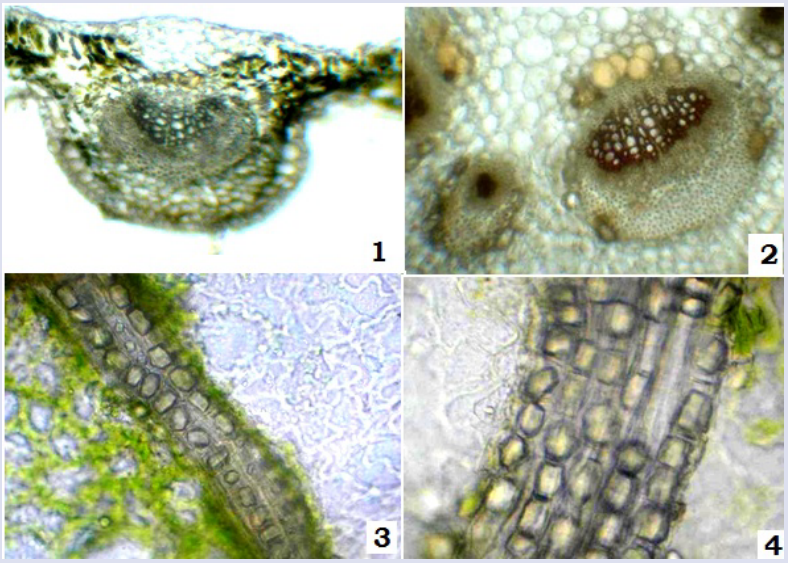

Figure 3: Micromorphological structure of the leaf of the *Hedysarum caucasicum* 1- cross section of the leaf, 2-vascular bundle with some idioblasts, 3-4 – druses on the leaf vein

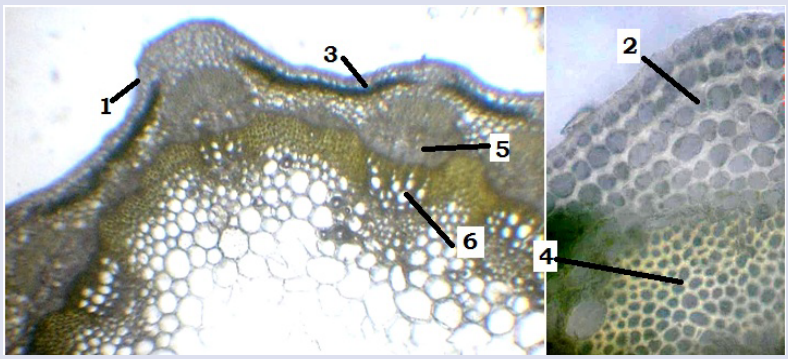

Figure 4: Micromorphological structure of the stem of the *Hedysarum caucasicum* on the cross –section 1- epidermis, 2- collenchym, 3- chlorenchym, 4 – sclerenchym, 5- phloem, 6- xylem

not contain moisture above permissible standards, as at high humidity during storage conditions are created, which contribute to deterioration of its quality. Moisture content of the analysed raw material was determined by the method of drying by GF XIV.[12] The weight loss on drying was 8.60% (average of two parallel determinations).

Determination of total ash

The ash of plant raw materials refers to the residue of inorganic substances obtained after burning the raw materials and then calcining the residue to a constant weight. Plant ash (total ash) consists of a mixture of various inorganic substances in the plant itself and mineral impurities (earth, sand, dust, stones) that can enter the raw materials when collected and dried. The common ash most commonly contains the following elements: Na, K, Ca, Mg, Fe, Si, F, P, C, which are in the form of oxides or salts of carbonic, phosphoric, sulfuric and other acids. Ash determination of total (x) was carried out.[12] The total ash content was 4.04% (average of two parallel determinations).

Determination of ash insoluble in 10% hydrochloric acid solution

In plant raw material the determination of total ash and ash insoluble in 10% hydrochloric acid solution, which is the residue after treatment of total ash with hydrochloric acid and consists of silicates, which for some objects are a natural constituent. Thus, the increased content of the hydrochloric acid insoluble portion of the ash indicates a significant mineral impurity content in the plant feed. The content of ash insoluble in hydrochloric acid was 1.34% (average of two parallel determinations).

Determination of content of extractive substances

Extractive substances of medicinal raw materials are referred to as complex of organic and inorganic substances extracted from vegetable raw materials by appropriate solvents and quantified as dry residue. The extractive substances were determined according to the procedure described.[12] The content of extractive substances was 24.56% (average of two parallel definitions).

Microbiological purity test

For research, the grass of the *Hedysarum caucasicum* Bieb., collected in the flowering phase on the southeast slope of Mount Alibek was used. According to amendments No. 3 to the article of GF XI of the publication "Methods of microbiological control of medicines," introduced in 19.06 2003, the studied plant raw materials belong to the category 4Б - medicinal plant preparations and medicinal plant raw materials "angro," prepared without the use of boiling water. The requirements for this category are as follows:

- Total number of aerobic bacteria - no more than 105 in 1 g or 1 ml;
- Total number of fungi - not more than 104 in 1 g or in 1 ml;
- *Enterobacteria* and other Gram-negative bacteria - no more 103 1 g or 1ml;
- Absence of *Escherichia coli* -in 1 g or 1ml;
- Absence of *Salmonella* in 10 g or 10 ml.

Before preparing the dosage form, the studied vegetable raw material of the *Hedysarum caucasicum* Bieb. was tested for microbiological purity. The results are shown in Table 1.

The presented results make it possible to conclude that according to the indicator "microbiological purity," the sample of vegetable raw materials of the ***Hedysarum caucasicum Bieb.,*** presented for analysis, meets the requirements for medicinal vegetable raw materials "angro," used without thermal treatment.

High-quality phytochemical analysis of ***Hedysarum caucasicum Bieb.***

Determination of tanning substances

About 1.0 g of raw material was poured with 100.0 g of water, heated for 20-30 minutes in a water bath and filtered. The following reactions were carried out with the resulting solution.[13]

- Several drops of iron ammonium alum were added to 2 ml of the solution, black and green staining appeared, indicating the presence of condensed tanning agents;

A few drops of a 1% solution of quinine hydrochloric acid were added to 2 ml of the solution, and opalescence appeared.

Determination of polysaccharides

For qualitative detection of polysaccharides, water extraction was prepared from 2.0 g of roots in a water bath for 30 minutes. Then it was filtered off and the filter was washed with hot water. The recovery was evaporated to 1/5 volume and three times the volume of 96% ethanol was added thereto. As a result, a loose curd precipitate of the polysaccharide complex was formed. The precipitate was separated, dissolved in water, reprecipitated, washed with alcohol, dried. The obtained polysaccharide complex is an amorphous mass soluble in water. In the composition of water-soluble polysaccharides of the penny, mucous substances predominate.[5]

Definition of the restoring sugars

About 1.0 of the milled raw material was placed in a 25 ml flask, poured with 10 ml of water and refluxed for 0.5 hour. The solution was filtered through gauze. Wash with water. 5 ml of the resulting solution was transferred to a tube and 15 ml of 95% ethyl alcohol was added. Precipitation of the bulk precipitate was observed. The solution was filtered, the precipitate was transferred to a test tube, 5 ml of diluted

Table 1: Microbiological Purity Test Results herbs of *Hedysarum caucasicum Bieb.*

party No.	Date of the analysis	Content in 1 mg			Absence in 1 mg	Absence in 10 mg
		Saprophyte forms of bacteria (no more than 100000)	Enterobacter (no more than 1000)	Mushrooms (no more than 100)	Escherichia coli	Salmonella
1	02.10 2006	Not exceeds 1000	Not exceeds 100	Not exceeds 1000	Not it is revealed	Not it is revealed

hydrochloric acid was added, boiled for several minutes, 5 ml of Feling reagent was added and boiled again, and orange-red staining was observed.[12]

Determination of free organic acids

A 1:10 decoction was prepared from grass while heating in a water bath for 1 hour. Broth was filtered. 5 drops of digestion were placed in the tube and adjusted to 1 ml with purified water. 1 drop of the methyl red indicator was added, red staining was observed, indicating the presence of organic acids.[13]

Definition of amino acids

For qualitative detection of amino acids reaction with 0.1% solution of ninhydrin in butanol on filter paper was used, characteristic blue-violet staining appeared in formation of Rueman complex.[13]

Determination of flavonoids and xanthones

In order to determine flavonoids, it was necessary to obtain an alcohol extract from the raw material. Extraction was carried out with 80% ethyl alcohol. About 1 g of the feed was placed in a 25 ml flask, 10 ml of 80% ethyl alcohol was added and heated in a water bath for 10-15 minutes under reflux. The resulting solution was filtered through a paper filter after cooling. Reactions were carried out with the resulting solution:

Cyanidine sample: 0.1 g of magnesium dust, 2 ml of concentrated hydrochloric acid were added to 1 ml of extraction, heated in a water bath for 2-3 minutes, after some time red-orange staining was observed;

2 drops of 2% basic lead acetate solution were added to 1 ml of the recovery, and yellow-lemon staining appeared;

1 ml of a 10% ammonia solution was added to 1 ml of the recovery, and yellow staining turned orange on heating appeared.

1 ml of a 2% solution of aluminum chloride in 96% ethyl alcohol was added to 1 ml of the recovery, and lemon-yellow staining was observed.[13,22]

Qualitative reactions with these reagents showed the presence of flavonoid substances in the grass of the ***Hedysarum caucasicum***, which allowed us to use the chromatography method for further analysis, which is widely used for their detection and identification. Chromatographic separation of the sum of flavonoids and xanthones was carried out in the preparation of the extracts, and ethyl alcohol 96, 80, 60, 40% concentration was used as the extractant.

0.05 ml of the ***Hedysarum caucasicum Bieb.*** extracts were applied to a 40x40cm Watman chromatographic paper and subjected to ascending chromatography in a solvent system: butanol - glacial acetic acid - water in a ratio of 4:1:5 [17.52] compared to witness substances. The witness mangiferin was obtained from tablets "Alpizarin 0.1g" manufactured by ZAO Pharmcentre "VILAR." Witnesses hyperoside and campferol were provided by PyatGFA 's Department of Organic Chemistry. When viewing the chromatogram in UV light, three main spots were found in extracts of the following concentrations of ethanol 96.80.60%. 1 spot corresponds to mangiferin, 2 to hyperoside, 3-campferol. Further, chromatograms were sprayed with alcohol solution AlCl3 and a change in stain color was observed (Table 2, Figure 1.4).

Study of antimicrobial activity of liquid extract from the grass of the ***Hedysarum caucasicum Bieb.*** The antimicrobial effect of extract was determined by agar diffusion (wells method). The method is based on evaluating the inhibition of the growth of test organisms by certain concentrations of the test agent. To test antimicrobial activity, we used 24-hour test cultures grown on beveled meat-and-python agar (MPA). Microbial cultures with MPA were washed with 2-3 ml of physiological saline and a suspension containing 500 million microbial bodies in 1 ml according to the turbidity standard.[48]

On the surface of peptone agar in petri dishes of the same diameter was sown with a solid lawn of standard suspensions of used test cultures. To this end, 2 ml of slurry was placed in a petri dish, the slurry was evenly distributed over the surface, and excess slurry was completely removed. By sterile drill with a diameter of 6 mm did 6 holes ("wells") at distance of 2.5 cm from the center and at identical distance from each other. In 3 wells, 0.1 ml of the test solution (concentration of xanthone sum in terms of mangiferin 0.022 g/ml) in a suitable solvent was added through one, in this case a liquid extract (ethanol 80%) was used. A sterile filter was placed in the three remaining wells to prevent condensate from entering the wells. All petri dishes were placed in the thermostat (37°C) for 18-20 hours strictly horizontally to obtain round zones of microflora growth suppression.

After incubation, the diameter of the growth suppression zones was measured with a millimeter ruler. The results were evaluated by the diameter of the growth retardation zones around the "well," including the diameter of the "well" itself:

- Absence of growth retardation zone - the tested culture is not sensitive to the given concentration of the preparation;
- Diameter of the growth retardation zone 10 mm - moderate sensitivity of the culture to the given concentration of the preparation;
- Diameter of growth retardation zone is more than 10 mm - high sensitivity of the tested culture to the given concentration of the preparation.
- Distilled water was used as solvent.

The results of determining the antimicrobial effect of liquid extract from the ***Hedysarum caucasicum Bieb.*** herb are given in Table 2.

The results of the study were confirmed by photographs taken at the end of the experience. The results of the microbiological studies carried out show that the liquid extract of the Caucasus penny has a pronounced antibacterial effect on Gram-positive cocci, pathogenic enterobacteria - the most current agents of dysentery – Shigella sonnei 3d, without depressing the representative of normal microflora – Escherichia coli 675, and a moderate antibacterial action subtilis L2 B. anthracoides

Table 2: Antimicrobial effect of liquid extract from grass *Hedysarum caucasicum Bieb.*

Structure of the small cavities	Diameter of growth retardation zone of microorganisms test cultures, mm Test cultures							
	Staphylococcus ceureus (209)	Staphylococcus aureus	Staphylococcus epidermidisWood-46;	Escherichiacoli 675;	Shigella sonnei 3d;	Salmonella typhimurium;	Bacillus subtilis L_2;	Bac.anthracoides -1
Extract	14	15	16	8	18	12	10	10

-1).[62-76] Thus, the liquid extract of the *Hedysarum caucasicum Bieb.* has a wide spectrum of antibacterial action and can be used in the development of a dosage form of antibacterial action.

CONCLUSION

1. As a result of morphological and anatomical examination of the *Hedysarum caucasicum Bieb.*, diagnostic signs of raw materials were established: amphistomatic sheet, anomocytical and anysocytical stomatal type, on the epidermal there are simple single-cell hairs. Stem on cross section has polyhedral shape. Idioblasts localize in parenchymal cells.
2. The main commodity indices of the *Hedysarum caucasicum Bieb.*'s grass are determined: humidity (8.6%), total ash (4.05), ash content insoluble in 10% hydrochloric acid solution (1.34%), content of extractive substances (24.56%), and microbiological purity of the raw materials.
3. Using various methods of qualitative determination of biologically active substances (color reactions, paper and thin layer chromatography), the presence of tanning substances, free organic acids, reducing sugars, as well as amino acids was found.
4. The quantitative content of the sum of xanthones in terms of specific mangiferin in the grass of the *Hedysarum caucasicum Bieb.* was 4.2% in terms of air-dry mire.
5. Glutamic acid (13.58 g/kg), aspartic acid (13.61 g/kg), arginine (14.99 g/kg) have been found to accumulate in a significant amount of *Hedysarum caucasicum Bieb.*grass.
6. The technology of obtaining liquid extract with the help of 80% ethyl alcohol has been developed, technological parameters of raw materials have been determined: content of extractive substances in raw materials (26.59%); Finished product removal factor (2.48); Feed absorption coefficient (3.00); Internal juice formation rate (3.37); Coefficient of volume increase at dissolution of extractive substances (2,013); Bulk density (0.21); Dry feed filling ratio (3.7); Swollen feed filling ratio (1.3); Displacement factor (1.7). Extract of liquid grass of Caucasus penny is obtained with ratio of phases 1:2 in a battery of 6 diffusers.
7. The quantitative content of the sum of xanthones in terms of the specific value of magneferin in the extract of liquid grass of the *Hedysarum caucasicum Bieb.* by UV spectrophotometry was determined, it was 1.57%.
8. Antimicrobial activity of the liquid herb extract of the *Hedysarum caucasicum Bieb***.** been proved with respect to Shigella sonnei, Bacillus subtilis and B. anthracoides -1.

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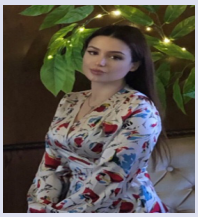

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