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The population status of Lagochilus setulosus Vved. and its biochemical composition

Arystanbek Eshibaev[1], Zhanar Aimenova[2,*], Lyazzat Akynova[3], Lyazzat Nurseitova[3], Aizhan Kopabaeva[3]

[1]ShymkentUniversity, Shymkent, 160031, Zhibekzholy street 131, Kazakhstan
[2]Muhtar Auezov South Kazakhstan University, Shymkent, 160012, Tauke Khan avenue 5, Kazakhstan
[3]South KazakhstanState Pedagogical University,Shymkent, 160012,
Baitursinov street 13, Kazakhstan
[*]corresponding author e-mail: aimenova.zhanara@gmail.com

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Abstract. This article describes endemic species of the genus *Lagochilus* growing in the territory of South Kazakhstan – *Lagochilus setulosus* Vved. Its biochemical composition, namely qualitative and quantitative composition of biologically active compounds was investigated. The established composition of substances indicates that this plant species can be used in pharmaceutical biotechnology to create a biological preparation with a hemostatic effect based on *Lagochilus setulosus*.

Keywords: Lagochilus setulosus, South Kazakhstan, leaves and flowers, hemostatic agent, biological active compounds, deterpenes, lagochilin, lagochirzin.

1. Introduction

Wild flora of South Kazakhstan is represented by more than 3000 species of vascular plants. According to scientists, 25% of them are medicinal plants. Many species are of practical value for the purposes of traditional and alternative medicine. However, the medicinal properties of many of them still remain unexplored.

There are more than 3000 plant species in the flora of South Kazakhstan, of which 553 are of interest as valuable natural sources of medicinal substances (Flora of Kazahstan, 1964). However, most of these plant species are still insufficiently known. Only a limited number of plant species are used in the domestic pharmaceutical and cosmetic industries. In this regard, the priority direction of research is to identify potential sources of biologically active compounds that will provide a long-term reliable raw material base for the pharmaceutical and cosmetology industries of Kazakhstan. Identification of the content of the main groups of important biologically active compounds in growing plant species will allow rational use of plant resources and will make it possible to significantly expand the range of herbal medicinal raw materials. Some of these plants are plants of the genus *Lagochilus*.

Plants of the genus *Lagochilus* belong to the *Lamiaceae* family and are represented by 35 species distributed mainly in Central Asia: in Kazakhstan, Iran, Afghanistan, Russia, Mongolia and China (Rehinger & Hedzh, 1982). Some plants of this genus are used as hemostatic agents, for the treatment of allergic dermatoses (Panossian & Wikman, 1982), and are also used as a medicine for clot retraction (Akopov, 1954) and glaucoma (Kadyroza, 1955). Among

the various plant species of the genus *Lagochilus*, *Lagochilus inebrians* Bunge has long been used in folk medicine of the East as a medicinal plant due to its sedative properties (Perry et al., 2002).

However, according to Akopov I.E., *Lagochilus setulosus* has the most pronounced stimulating effect on the blood clotting process (Akopov, 1981). However, this type of plant is still insufficiently known and requires further biological and chemical research.

To solve this problem, we have already performed studies on the macro-and micromorphological features of *Lagochilus setulosus* (Aimenova et al., 2015a).As a result of the research, the anatomical structure of this plant was established using the method of scanning electron microscopy. Gandular trichomes were found.

Also was performed a comparative research of the macro – and microelement composition of *Lagochilus inebrians* and *Lagochilus setulosus* (Aimenova et al., 2014). As a result of research, it was found that these plants differ in the quantitative content of macro-and microelements. According to the content of trace elements, the plant *Lagochilus inebrians* surpasses *Lagochilus setulosus*, but the quantitative content of iron, magnesium, lithium, and aluminum is 2-3 times higher in *Lagochilus setulosus*. At the same time, of all the identified macro – and microelements in *Lagochilus setulosus*, as well as in *Lagochilus inebrians*, the percentage content contains the most calcium, which in the presence of lagochirzin fully implements the process of plasma hemostasis.

The next, necessary stage of the study of this plant species is the study of its distribution area, as well as the study of its biological composition.

2. Objects and methods

For the research, the flowers and leaves of *Lagochilus setulosus* were collected during the mass flowering period in July. To do this, the tops of the stems of plants 20-30 cm long were cut off, leaving 1-2 plants per $1m^2$ for seeding and population renewal. Drying was carried out in the shade in a well-ventilated room, laying it out in a thin layer on a tarpaulin. Then, after drying, the stems were threshed with a wooden stick, separated and discarded. Then the yellowed and browned parts were removed and the raw materials were crushed in a mechanical shredder. Thus, the finished raw material contained flowers and leaves that retained their natural color.

Qualitative determination of the content of the main groups of biologically active substances in the phytomass of *Lagochilus setulosus* was performed according to the following methods:

1) The presence of diterpenes was determined by paper chromatography (Belenkij et al., 1983).
2) The presence of tannins was determined by a specific gelatin deposition reaction (Sumina et al., 2006).
3) The presence of essential oil was determined by the method described in State Pharmacopeia X (State Pharmacopoeia of USSR, 1968).
4) The presence of cardiac glycosides was determined by Keller-Kiliani Reaction (Syvkinet al., 1999).
5) The presence of saponins was determined by the reaction to foaming (Ladygina et al., 1983).
6) The presence of phenolglycosides was determined as follows (Petrov, 1978).
7) The presence of iridoids was determined by the method described in State Pharmacopeia XI (State Pharmacopoeia of USSR, 1989).
8) The presence of flavonoids was determined by cyanidin sample (Bandyukova, 1965).
9) The presence of coumarins was determined as follows: for the preparation of extraction from vegetable raw materials 2 g of crushed raw materials (crushed flowers and leaves of plants), 20 ml of ethyl alcohol was poured and boiled for 15 minutes with a reflux. After cooling, filtered. To 3-5 ml of alcoholic extract was added 10 drops of 10% KOH in methanol and heated for 5 min in a water bath (solution turned yellow), then added 5 drops of freshly prepared diazo reagent of Pauli Kutochku. Further, the solution acquired a cherry color, which indicates the presence of coumarins.
10) The presence of anthracene derivatives was determined by the following method (Kurkin et al., 2016).
11) The presence of sugars was determined by the method (Fialkov, 1946).
12) The presence of alkaloids was determined by the method (Ladygina, 1983).
13) The presence of resinous substances was determined by the decrease in the mass of the sample after exhaustive extraction in the soxlet apparatus with a capacity of 1 l for 20 h and subsequent drying to a constant weight at a temperature of 105°C. Further, the method of fractionation using chloroform and acetone solvents was used to separate resinous substances (Kuznetsov et al., 2004).

Quantitative determination of the content of the main groups of biologically active substances of *Lagochilus setulosus* performed according to the following methods:

1) essential oil by the method of (State Pharmacopoeia of the USSR, 1968).
2) resinous substances by the method: the leaves and flowers of *Lagochilus setulosus* crushed to a particle size of 1-2 mm and subjected to extraction of non-polar solvent, hexanal to conventional Soxhlet extractions for the separation of resinous substances. The yield of resinous substances depending on the terms of raw mate-

rial preparation and storage was 6-9% of the weight of the absolutely dry residue.

3) diterpene lagochilin according to the method: 10 g of finely powdered raw material (flowers and leaves of *Lagochilus setulosus* was placed in a 250 ml flask and 100 ml chloroform was poured. The flask with the contents was attached to the reflux and heated in a water bath for 1 hour. The extract was then cooled and filtered. In the same way chloroform extraction was repeated 5-6 times (from one raw material). Chloroform extracts were combined and chloroform distilled to produce a dry residue. 10 ml of distilled water was added to the dry residue and heated (5 min) in a water bath, adding 15 ml of 10% NaOH and heated in a water bath for 30 min. The water-alkaline mixture, after cooling, was repeatedly treated with ethyl ether (5-6 times). The combined essential extracts were concentrated to 10 ml and left for the crystallization of lagochilin, which was separated by filtration through a suspended filter, dried and weighed. Lagochilin recrystallization was performed with acetone.
4) diterpene lagochirzin by the method of water-alkaline solution, after removal of lagochilin neutralized with 20% H_2SO_4 solution until slightly acid reaction (pH=5) and the mixture was repeatedly treated with chloroform (5-6 times). Chloroform extract was combined, concentrated and chloroform had tetroxide in distilled. The dry residue was obtained in an amount of 0.22 g. It was passed through a column with 10 g of silica gel (column diameter 2 cm, height 20 cm) and eluted with a mixture of ethyl ether and petroleum ether (40:1). A total of 20 fractions (5 ml) were obtained; 5-12 fractions containing lagochirzin (chromatography on plates "Sorbfil") were combined, evaporated, the dry residue was dried and weighed.
5) flavonoids by the following technique: the optical density of eluates was measured on a spectrophotometer in a cuvette with a layer thickness of 1 cm at a wavelength of 363 nm against the background of the eluate of the idle experiment. The percentage of routine in raw materials in terms of absolutely dry raw materials was calculated by the formula:

$$x = \frac{K_1 V_1 V_3 D_{363} 100}{V_2 D_{см}^{1\%} m(100-w)\times 0{,}667 l} \quad (1)$$

where:
K_1 – the ratio of elution (1.195);
V_1 – volume of ethanol extract after dissolution of dry residue; ml;
V_2 – the volume of ethanol extract was taken for use on chromatogram, ml;
V_3 – the volume of eluate;
D_{363} – optical density of solution at λ = 363 nm;
D1%/1cm – the rate of absorption of rutinat λ = 363 nm (268.4);
m – the weight of the portion of the raw material, g;
w – loss in weight of raw materials during drying, %;
0.067 – conversion factor to 20 ml of extract;
l – layer thickness, cm.

6) iridoids by the following technique: the content of the sum of iridoids in terms of harpagid and absolutely dry raw materials (%) was calculated by the formula:

$$x = \frac{A\times 100\times 10\times 25\times 100}{56\times m\times 20\times 5\times(100-W)} - \frac{A\times 25000}{56\times m\times(100-W)}, \quad (2)$$

where:
A – optical density of the tested solution;
m – raw material linkage, g;
W – humidity of raw materials;
5. 56 – the rate of absorption of the reaction products with hydroxylamine and iron (III) chloride.

7) coumarins according to the technique. The optical density of the solutions was measured at the following wavelengths: isopimpinellin – 313 nm, bergapten – 311 nm. The percentage of izopimpinellin and bergapten was calculated by the formula:

$$x = \frac{V_1 V_3 D_2 1{,}045\times 100}{V_2 m E_{1\,см}^{1\%}(100-w)}, \quad (3)$$

where:
V_1 – extraction volume, ml;
V_2 – volume of extraction applied to the chromatogram, ml;
V_3 – the volume of eluate, ml;
m – the weight of the portion of the raw material, g;
$E\frac{1\%}{1см}$ – specific absorption rate: isopimpinellin-518, bergapten-651;
D_2 – the optical density of the eluate;
w – loss in weight of raw materials during drying, %.

8) phenolglycosides by the following technique: 1 ml of 0.1 N. iodine solution corresponds to 0.01361 arbutin. The percentage of arbutin in plant material x in terms of absolutely dry raw materials was calculated by the formula:

$$x = \frac{V 0{,}01361\times 2\times 100\times 100}{m(100-w)}, \quad (4)$$

where:
V – volume of 0.1 N. iodine solution consumed for titration, ml;
m – the weight of the portion of the raw material, g;
w – loss in weight of raw materials during drying, %.

9) alkaloids by the following technique: The percentage in terms of absolutely dry raw materials x was calculated by the formula:

$$x = \frac{(15-V)0{,}005780\times100\times100}{m(100-w)}, \quad (5)$$

where:
V – the volume of 0.02 n NaOH used for titration, ml;
m – the weight of the sample of raw materials corresponding to the volume of ether extraction, g;
w – loss in weight of raw materials during drying, %.

10) sugars by the method of Willstatter and Sudle. The optical density of the studied solution was recorded on a spectrophotometer at a wavelength λ = 582 nm. The sugar content in the sample was determined by the calibration curve based on glucose. To do this, 50 ml of a solution containing 10 mg/ml of glucose was prepared, and then the remaining solutions were obtained by dilution according to Table 1.

Table 1. Method of dilution of the solution to obtain a glucose calibration curve

Number	Content of glucose, mg/ml	Amount of initial glucose solution, ml	Amount of water, ml
1	0.5	0.5	9.5
2	1.0	1.0	9.0
3	2.5	2.5	7.5
4	5.0	5.0	5.0
5	7.5	7.5	2.5
6	10.0	10.0	0

The number of reducing sugars (x) was calculated by the formula:

$$x = \frac{c\times V}{m}\times 100\%, \quad (6)$$

where:
c – sugar content in the sample, found on the calibration curve;
V – the volume of the extract obtained from the sample;
m – weight of the sample in grams.

For isolating diterpene lagochirzin of phytomass *Lagochilus setulosus* we used the following method: 10 g of finely ground raw materials (flowers and leaves of *Lagochilus setulosus*) was placed in a 250 ml flask and 100 ml chloroform was poured. The flask with the contents was attached to the reflux and heated in a water bath for 1 hour. The extract was then cooled and filtered. In the same way chloroform extraction was repeated 5-6 times (from one raw material). Chloroform extracts were combined and chloroform distilled to produce a dry residue.

To the dry residue, 10 ml of distilled water was added and heated (5 min) in a water bath, then 15 ml of 10% NaOH was added and heated in a water bath for 30 min. The water-alkaline mixture, after cooling, was repeatedly treated with ethyl ether (5-6 times). The combined essential extracts were concentrated to 10 ml and left for the crystallization of lagochilin, which was separated by filtration through a suspended filter, dried and weighed. Lagochilin recrystallization was performed with acetone. The aqueous-alkaline solution, after removal of lagochilin was neutralized with 20% H_2SO_4 solution to a slightly acidic reaction (pH=5) and the mixture was repeatedly treated with chloroform (5-6 times). Chloroform extract was combined, concentrated and chloroform had tetroxide in distilled. The dry residue was obtained in an amount of 0.22 g. the Dry residue was passed through a column with 10 g of silica gel (column diameter 2 cm, height 20 cm) and eluted with a mixture of ethyl ether-petroleum ether (40:1); 20 fractions (5 ml) were obtained. 5-12 fractions contained lagochirzin (chromatography on plates "Sorbfil"), which were combined, evaporated, the dry residue was dried and weighed.

3. Results and discussion

The results of the taxonomic analysis established the distribution areas of plants of the genus *Lagochilus* in three floristic regions of the Turkestan region (Aimenova et al., 2015b).

At the same time, *Lagochilus setulosus* grows in the soil and climatic conditions of the foothill zone of the western Tien Shan, which is characterized by a moderate temperature regime, moisture availability and typical serozem.

Morphological features of *Lagochilus setulosus* are associated with the conditions of the place of growth of this species, which determines its characteristic macro- and micromorphological features. These features include the modification of the young leaves of the plant into spines, a reduction in the size of the leaves, the growth of sharp spines at the end of the leaves and sepals. The leaf epidermis is covered with a thick layer of cuticle, the mesophyll consists of 4 compressed layers of palisade tissue. The vegetative and generative organs are covered with glandular trichomes that produce a specific sticky substance around the seeds that keeps them moist.

Lagochilus setulosus is a semi-shrub, reaching a height of 20-60 cm. The root of the plant stem, the stems at the base stiffening, thin, glabrous or sparsely and protruding

bristly, later white, shiny, 30-80 cm tall; leaves rhombic in outline, broadly ovate.

We found four populations of *Lagochilus setulosus* in the Kazygurt district (Turkistan Region in Southern Kazakhstan, whose administrative center is the village of Kazygurt). The patches of plants vary from 1.3 to 4.5 ha. However, the density of plants in the area (coordinates 41.759°N 69.41°E) is not high and it does not exceed 1.2±0.1 plants per m^2 (Fig. 1).

The projective cover of the soil with vegetation is no more than 15.3±1.3%. Plants are found in individual specimens, bushy shape, height 35-40±1.7 cm in accordance with Figure 2 A, B.

The results of qualitative analyses of biological active substances presence are given in Table 2.

As it can be seen from Table 2, in the phytomass of *Lagochilus setulosus* were identified 9 specific qualitative reactions to the presence of biologically active substances, which are important organic compounds used in pharmaceuticals, perfumery and medicine. However, among these substances we have not found tannins, anthracenes, saponins and cardiac glycosides. Qualitative reactions to these compounds showed negative results.

In the next stage of our research, the presence of previously identified biologically active substances in the phytomass of plants was tested in experiments with thin-layer paper chromatography. As studies have shown, all qualitative reactions were reliable, all of the biologically active substances listed in table 3 were identified by comparing the standard samples – «witnesses».

Figure 1. The total area of the populations of *Lagochilus setulosus* in Kazygurt district

A) general view of the plant; B) type of flowering plant shoot

Figure 2. Appearance of *Lagochilus setulosus* plants

Table 2. Results of preliminary phytochemical screening of flowers and leaves of *Lagochilus setulosus*

Number	Biologically active substance	Qualitative reaction
1	tannins	not identified
2	phenolic glycosides	Identified
3	anthracenediones	not identified
4	sugars	Identified
5	saponines	not identified
6	essential oil	Identified
7	cardiac glycosides	not identified
8	flavonoids	Identified
9	coumarins	Identified
10	resinous substance	Identified
11	diterpenes	Identified
12	iridoids	Identified
13	alkaloids	identified

As shown by the results of quantitative studies in the phytomass of plants *Lagochilus setulosus* maximum measure have sugar (of 8.81%), resinous substances (of 8.45%), phenolic glycosides (2.75%) and diterpenes (2.43%). The content of the remaining compounds is below one percent – 0.11-0.81% (Table 3).

The established quantitative indicators of biologically active substances characterize the high pharmaceutical value of raw materials of plants of this species. Since these substances are usually synthesized in plants in small quantities, although they play a very important role in the metabolism of the body. The obtained quantitative indicators of biological active substances in plants of *Lagochilus setulosus* consistent with the results of other authors' research.

In the article of Akramov et al. (2019) is shown comparative study on the chemical composition and biological activities of the essential oils of three *Lagochilus* species collected from Uzbekistan. There are *L. gypsaceus*, *L. inebrians* and *L. setulosus*. *L. gypsaceus* was chosen by him to the study its phytochemical composition, which should be related to its most relevant biological properties. Studies of *L. gypsaceus* have revealed iridoid glycosides, diterpenes, flavonoids and sterols. Regarding diterpenoids – which, according to the conducted studies, determine the hemostatic function of plants of the genus *Lagochilus*, in *L. gypsaceus* only the diterpene lagochilin was found, while lagochirzin in its pure form is present in *L. setulosus* which grows in the territory of South Kazakhstan. Lagochirzin, which was previously synthesized from lagochilin as a result of multi-stage reactions, was performed by Zainutdinov on *L. inebrians*. *L. setulosus* is one of the rare plant species of this genus that contains lagochirzin in its pure form. This fact suggests a higher hemostatic activity of *L. setulosus* in comparison with *L. gypsaceus* (Zainutdinov, 1993).

Table 3. Results of the study of the quantitative content of the main groups of biologically active substances in the biomass of leaves and flowers of *Lagochilus setulosus* plants

Number	Biologically active substance	Quantitative content in % to weight of sample
1	Essential oil	0.12 ± 0.004
2	Resinous substances	8.45 ± 0.250
3	Diterpenes Lagochilin Lagochirzin	 0.75 ± 0.005 1.68 ± 0.023
4	Flavonoids	0.35 ± 0.002
5	Iridoids	0.81± 0.014
6	Coumarins	0.11± 0.001
7	Phenolic glycosides	2.75±0.011
8	Alkaloids	0.70±0.002
9	Sugars (in total)	8.81± 0.044

Phenolic glycosides, which were also found in *L. setulosus*, suggest the antioxidant activity of this plant. Since the literature data provides a clear link between the strong correlation between the total phenol content and the antioxidant (DPPH, CUPRAC, and FRAP) properties of the plant of the genus *Lagochilus* (Akramov et al., 2019).

In the study of Ebrahimi et al. (2020) also were investigated the total of 17 medicinal plants with hemostatic activity. The most frequently studied plant families were *Compositae*, *Lamiaceae*, *Fabaceae*, and *Asteraceae*. The majority of the plants were prepared in the form of aqueous or organic extracts of leaf, rhizome, flower, bark, pollen or the whole plant, and for some plants the active components were isolated. As a result, it was shown that the hemostatic activity of plant extracts is mainly attributed to several mechanisms, including coagulation stimulation via increasing the factor XII activity and plasma fibrinogen levels, the fibrinolysis inhibition, vascular or smooth muscle constriction and platelet aggregation. *Ageratum conyzoides* and *Typha latifolia* are the plants with the most in vivo and in vitro evidence of hemostatic activity. It is propounded that the bioactive compounds which are often

involved in the bleeding control are categorized as tannins, saponins, glycosides and other phenolics. Moreover, the anti-fibrinolytic effect of browplasminin (tannin), 8-O-acetyl shanzhiside methylester (iridoid glycoside) and lignin were confirmed. Other isolated hemostatic compounds include glycoconjugate from *Lythrum salicaria*, saponins from *Panax notoginseng*, and Gallic acid, vanillic acid and luteolin from *Sedum aizoon*.

4. Conclusion

Thus, the distribution areas of plants of the genus *Lagochilus* were established in three floristic regions of the Turkestan region. Qualitative and quantitative analyses of the given biomass were carried out. The established composition of substances characterizes this type of plant as promising for use in pharmaceutical biotechnology in the creation of a biological drug with a hemostatic effect based on *Lagochilus setulosus.*

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