Biological study of gynostemma pentaphyllum (thunb.) Makino

Nizomiddin Kodirov[11], *Elvira* Galiakhmetova[2], *Alfina* Nizamova[2], *Natalya* Kudashkina[2], *Renata* Galiakhmetova[2], and *Svetlana* Rashitovna[2]

[1]Samarkand State Medical University, st. A. Temura 18. B, 140100, Samarkand, Uzbekistan
[2]Bashkir State Medical University of the Ministry of Health of Russia, Ufa, Russian Federation

Abstract: The article is devoted to the pharmacognostic study of the herb Gynostemma pentaphyllum (Thunb.) Makino. The morphological and anatomical characteristics of the Gynostemma herb have been established. A quantitative assessment of diagnostically significant signs was carried out using a microscopic method. Using various analytical methods of phytochemical analysis, the quantitative content of the main groups of biological active substances was determined: tannins, ascorbic acid, free organic acids, polysaccharides, saponins and flavonoids. A high content of biologically active substances (saponins and flavonoids), which are pharmacologically active components in the herb Gynostemma pentaphyllum, was revealed. Key words: Gynostemma pentaphyllum, anatomical and diagnostic features, saponins, flavonoids, gypenosides.

1 Introduction

Gynostemma pentaphyllum is widely distributed under the Chinese name "Southern Ginseng" and is native to India, Nepal, Bangladesh, Sri Lanka, Laos, Myanmar, Korea and Japan [1-3]. Typical habitats of this species are bushes, forests, roadsides, lowlands, and also occurs on mountain slopes at an altitude of 300-3200 m above sea level with an optimal air temperature of 15-30 ˚C and an optimal relative illumination of 65-75% [4].

Gynostemma is found in the Russian Federation *pentaphyllum* on the territory of Kunashir Island (Far East) (Fig. 1), where it grows on the northeastern border of the range in coniferous-deciduous forests among shrubs and along river valleys [5-6]. Gynostemma quinquefolia is included in the “List of flora objects included in the Red Book of the Sakhalin Region” [7].

In order to preserve natural reserves, it is relevant to study species introduced in different climatic conditions that are not inferior in phytochemical composition and pharmacological activity.

Wild gynostemma is a dioecious perennial herbaceous plant with a creeping rhizome and a climbing, leafy and pubescent stem at the nodes. The leaves are simple, round or ovate, thin, bristly-pubescent, petiolate, with 3-5 ovate or narrowly elliptical pointed toothed lobes. The stem has branching tendrils at the nodes [8-12]. Gynostemma is a frost-

[1] Corresponding author: nizomiddinkodirov@mail.ru

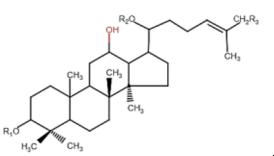
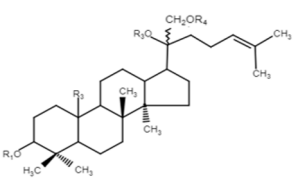
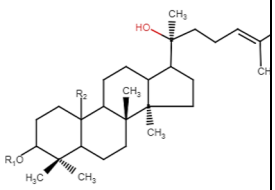
© The Authors, published by EDP Sciences. This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (https://creativecommons.org/licenses/by/4.0/).

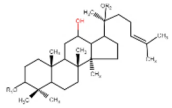
resistant plant, but when cultivated in a temperate climate zone, for better preservation of the plant, it is necessary to cover the soil with foliage or non-woven material [4].

Fig. 1. Distribution area of the Gynostemma species pentaphyllum (borrowed from open access The International Plant Names Index)

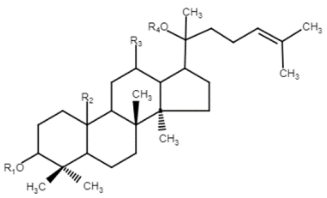
The following groups of compounds have been identified in the aerial parts of the plant: terpenoids , steroids, polysaccharides, flavonoids, amino acids, carotenoids, chlorophylls, organic acids, phenolcarboxylic acids, trace elements, fatty acids, nitrogen -containing compounds (allantoin), vitamins [13-24]. Main contribution to the pharmacological action *of Gynostemma pentaphyllum* contribute saponins (gypenosides) (Table 1)

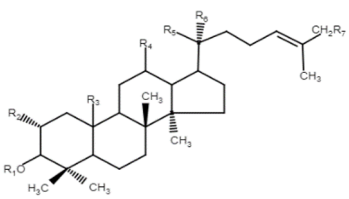
Table 1. Structures of the active components *of Gynostemma pentaphyllum*

Connection structures											
											
Gypenosides	R_1	R_2	R_3	Hypenosis ides	R_1	R_2	R_3	R_4	Gypenosides	R_1	R_2
I	- glc -(glc)- rham	- glc-glc	H	XXII	- glc-glc	CH2OH_ _	- glc-xyl	H	XXVII	- glc-glc	CH2OH_ _
II	glc -(glc)- rham	- glc-glc	H	XXIII	- glc-glc	CH2OH_ _	H	glc	XXVIII	- glc-glc	CHO
III	- glc-glc	- glc-glc	H	XXIV	- glc-glc	CHO	H	glc	XXIX	- ara-glc	CHO

IV	- glc-glc	- glc-xyl	H	XXV	- ara-glc	CHO	H	glc			
V	- glc-glc	-glc - rham	H	XXVI	- ara-glc	CHO	glc	H	Gypenosides	R_1	R_2
VI	glc -(glc)- rham	glc	H	XXX	glc	CH2OH_	H	H	A	- glc -(rham)- glc	- glc-glc
VII	-glc -rham	-glc - rham	H	XXXI	- glc-glc	CH2OH_	H	H	B	- glc -(rham)- glc	-glc - rham
VIII	- glc-glc	glc	H	XXXII	glc	CH2OH_	H	- glc - glc	E	- glc-glc	-glc - rham
IX	Glc	- glc-xyl	H	XXXIII	- glc-glc	CHO	H	H	F	- glc -(rham)- glc	-glc _
X	Glc	-glc - rham	H	XXXIV	- glc-glc	CHO	-glc - rham	H	G	-glc -rham	-glc - rham
XI	-glc -rham	glc	H	XXXV	- glc-glc	CHO	- glc-xyl	H	I	-glc _	- glc-xyl
XII	Glc	glc	H						J	-glc _	-glc - rham
XIII	H	- glc-xyl	H						K	-glc -rham	-glc _

Structures connections											
XIV	H	-glc - rham	H						M	H	-glc _
XV	- glc-xyl	- glc-xyl	H						N	H	-glc - rham
XVI	-glc- _ _	-glc - rham	H								
XVII	Glc	- glc-glc	H								
XVIII	- glc -(glc)- rham	-glc - rham	OH								
XIX	- glc-glc	-glc - rham	OH								
XX	- glc -(glc)- rham	- glc-glc	OH								
XXI	H	- glc-xyl	OH								





Gypenosides	R_1	R_2	R_3	R_4	Gypenosides	R_1	R_2	R_3	R_4	R_5	R_6	R_7
XXXVI	- ara-glc	CHO	H	-glc - rham	XLII	- glc-glc	OH	CH_3	OH	-oglc -glc	CH_3	H
XXXVII	- ara-glc	CHO	H	- glc-xyl	XLIII	- glc-glc	OH	CH_3	OH	-oglc - rham	CH_3	H
XXXVIII	- glc-glc	CH2OH_	H	H	XLIV	glc	OH	CH_3	OH	-oglc -glc	CH_3	H
XXXIX	- glc-glc	CH2OH_	OH	H(20R)	XLV	glc	OH	CH_3	OH	-oglc - rham	CH_3	H

XL	- glc-glc	CHO	OH	H(20R)	XLVI	- glc-glc	OH	CH_3	OH	- oglc	CH_3	H
XLI	- glc-glc	CH2OH_ _	H	H(20R)	XLVII	- glc-glc	OH	CH_3	OH	-oglc - rham	CH_3	OH
					XLVIII	- ara -(glc) - rham	H	CHO	H	OH	CH_2 - oglc	H
					XLIX	- ara -(xyl) - rham	H	CHO	H	OH	CH_2 - oglc	H

Note. glc – glucose, rham – rhamnose , xyl – xylose, ara – arabinopyranose .

Due to such a rich presence of bioactive components *of Gynostemma pentaphyllum* has versatile biological activity: hypolipidemic , immunostimulating, hypoglycemic, antiulcer , antiallergic, nootropic, antioxidant, anti-inflammatory, antitumor, cardioprotective , bronchoprotective , hepatoprotective , antithrombic [15, 25-34]. Information and literary analysis showed an extensive cluster of knowledge about *Gynostemma pentaphyllum* , native to Southeast Asia.

Purpose of the study : pharmacognostic study *of* Gynostemma pentafolia *pentaphyllum* (Thunb .) M akino) .

2 Experimental part

2.1. Materials

The object of research was samples of the herb Gynostemma quinquefolia - *Gynostemma pentaphyllum* (Thunb .) Makino , introduced into the territory of the Republic of Bashkortostan. The grass (the above-ground part of plants up to 2.5-3 m long) was harvested in the summer during the flowering period and subjected to air-shade drying (Fig. 2).

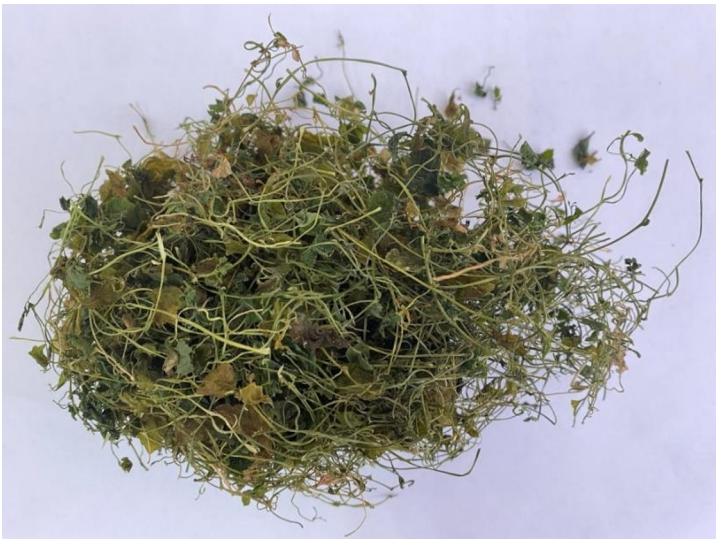


Fig. 2. *Gynostemma Raw Material pentaphyllum*

2.2. Methods

Using information search in systems such as Google , the scientific library of the Belarusian State Medical University, the electronic library, the State Pharmacopoeia of various publications, literature data was obtained.

Windows 10 software. The reliability of differences between experimental values was determined using the Student's test (P = 95%) of the State Fund XIV [35].

3 Results and discussion.

3.1. Morphological analysis of raw materials

The appearance of the grass was determined with the naked eye using a magnifying glass model " Brauberg " and a binocular microscope model MBS-1, the size of the grass was determined with a measuring ruler.

Grass is the above-ground part of a herbaceous vine with a long, leafy, branched, grooved stem. The leaves are simple, thin, deeply palmately dissected, bristly-pubescent on both sides and along the edge of the leaf blade. By the nature of attachment to the stem - long-petiolate, leaf arrangement alternate. The petioles are thin with a groove on the upper side (Fig. 3).

The nodes contain 1 to 5 whitish tubercles, from which branching shoots and inflorescences develop. In the nodes you can also observe 3-8 light hairs, which are visible to the naked eye, reaching 2 mm (Fig. 4). Starting from the 2-3 lower leaves, tendrils appear in the axils, forked at the apex.

The flowers are small, dioecious, collected in racemose inflorescences (Fig. 5).

The color of the stems is light green; The color of the leaves is green on the upper side and gray-green on the lower side .

The smell of the herb is weak, grassy, and intensifies when rubbed. The taste is bitter, turning into sweetish.

Fig. 3. Fragment of *a* leafy stem *G. pentaphyllum*

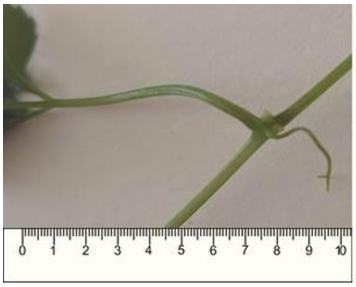


Fig. 4. Stem fragment *G. pentaphyllum* with tubercles and a forked mustache

Fig. 5. Fragment of *inflorescence G. pentaphyllum*

3.2. Anatomical analysis of raw materials

Temporary micropreparations of *Gynostemma herb pentaphyllum* (leaf blade, petiole, stem, tendril and flower) for research to study the authenticity of the plant were prepared according to the methods of the State Pharmacopoeia (SP) XI V edition [35]. For microscopic examination, optical instruments were used: microscopes of the Mini-Med 501 and Micromed R-1 models, a microvisor of the MVZ-103 model, a wide-angle and ultra-wide-angle 12 MP camera on an Apple phone . iPhone 11.

When examining the leaf from the surface, one can see: convoluted cells of the upper epidermis with clearly visible thickened walls (Fig. 6, 7) and less convoluted cells of the lower epidermis (Fig. 8).

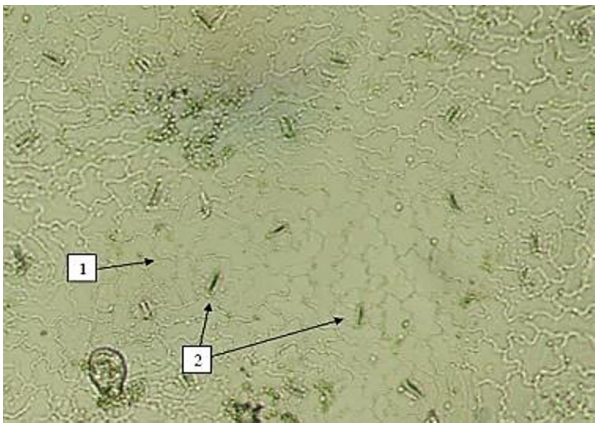


Fig. 6. Fragment of the upper epidermis of *leaf G. pentaphyllum* (magnitude 10/0.2) (1 – epidermal cells; 2 – stomatal apparatus)

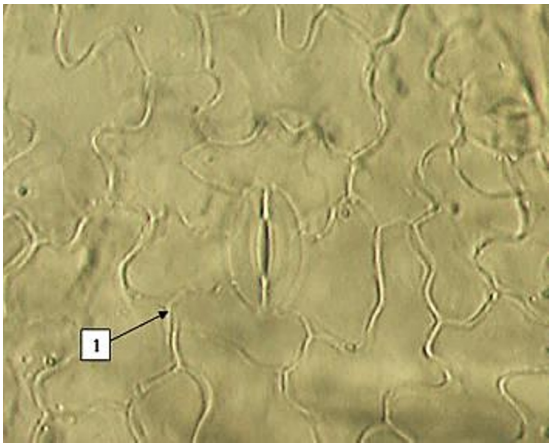


Fig. 7. Fragment of the upper epidermis of *leaf G. pentaphyllum* (uv . S 40/0.65) (1 – stomatal apparatus)

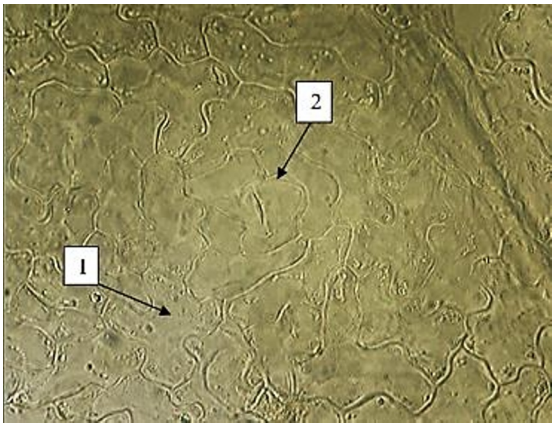


Fig. 8. Fragment of the lower epidermis of *leaf G. pentaphyllum* (magnification 10/0.25) (1 – cells epidermis ; 2 – stomatal apparatus)

The stomatal apparatus is anomocytic type, numerous, which is observed on both sides of the leaf blade (amphistomatic type).

Over the entire surface, as well as along the veins and edges of the leaf (transitioning into teeth), simple, large, multicellular (4-10), thick-walled, often curved hairs are visible, which cause the pubescence of the leaf blade . At the points of attachment to the epidermis, simple hairs form a rosette with a round ridge in the center (Fig. 9).

Small capitate hairs are present in large numbers over the entire surface, consisting of a short two-cell stalk and a one-, rarely two-cell, spherical or oval head (Fig. 10, 11).

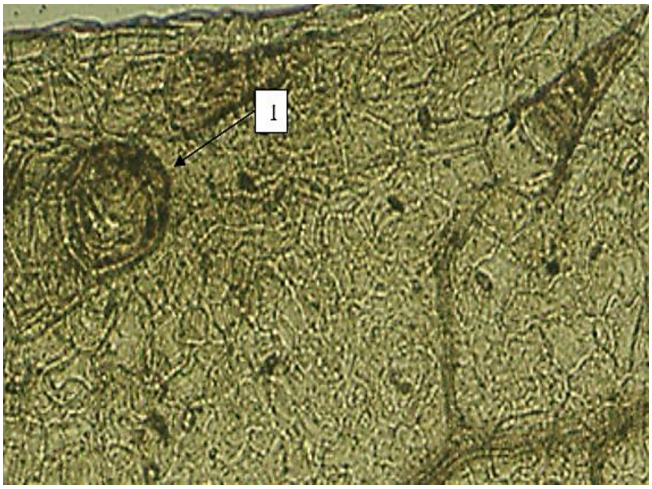


Fig. 9. Fragment of the epidermis *of G. pentaphyllum* (magnitude 10/0.25): 1 – place of attachment of a simple hair to the epidermis

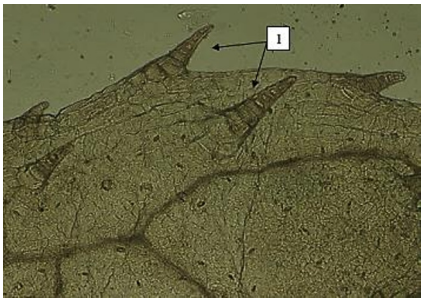


Fig. 10. Fragment of the epidermis *G. pentaphyllum* (magnitude 10/0.25) (1 – covering trichomes)

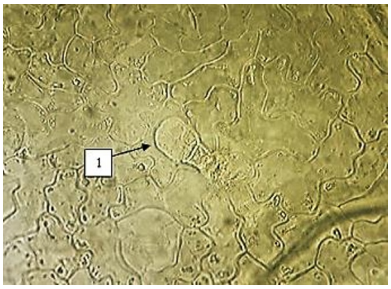


Fig. 11. Fragment of the lower *epidermis G. pentaphyllum* (uv . S 40/0.65) (1 – capitate hair

The leaf petiole in a transverse section on the adaxial side has a characteristic notch. Under the epidermis, collenchyma is located in several layers. The conducting system is of a bundle type, the number of conducting collateral bundles varies from 3 large and 2 small or less.

Phloem is oriented towards the outer side, xylem - towards the inner zone of the petiole. All vascular bundles are reinforced on the phloem side. There is a parenchymal lining around the vascular bundle (Fig. 12).

Microscopy of the petiole from the surface is represented by epidermal isodiametric polygonal cells with uniform thickening of the cell wall.

The stem has a grooved structure in cross section (Fig. 13). Under the epidermis in several layers, especially along the ribs, there is collenchyma, then sclerenchyma, which forms a ring of several layers of cells, varying from 2 to 4. The central part of the stem is occupied by parenchyma cells . Vascular-fibrous bundles of the collateral type are located in a circle, 4 large and 4-5 small along the ribs.

The surface epidermis of the stem is represented by polygonal rectangular cells elongated along the stem with a uniform cuticle. There are rare stomata of a rounded shape, the type of stomatal apparatus is anomocytic .

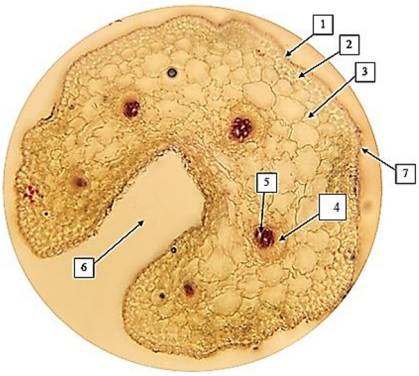


Fig. 12. Cross section of *petiole G. pentaphyllum* (magnitude 10/0.25) (1 – epidermis; 2 – collenchyma; 3 – parenchyma cells; 4 – phloem; 5 – xylem; 6 – adaxial side; 7 – abaxial side)

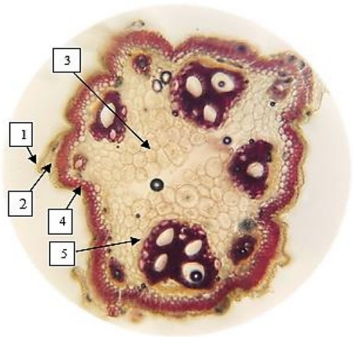


Fig. 13. Cross section of *stem G. pentaphyllum* (magnitude 10/0.25) (1 – epidermis; 2 – collenchyma; 3 – parenchyma; 4 – sclerenchyma; 5 – vascular-fibrous bunch)

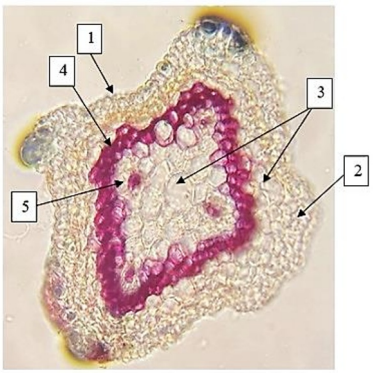


Fig. 14. Transverse section of the antennae G. _ *pentaphyllum* (magnitude 10/0.25) (1 – epidermis; 2 – collenchyma; 3 – parenchyma; 4 – sclerenchyma; 5 – vascular-fibrous bundle)

The antennal epidermal cells are rectangular and elongated from the surface, and in cross section microscopy resembles the structure of the stem, collateral bundles in the amount of 4 are located in the corners (Fig. 14).

The surface epidermis of the pedicel has polygonal cells with straight walls and a smooth cuticle. The hairs are simple, multicellular with a slightly curved apex and an expanded base; capitate with a single-celled short stalk and a two-celled head (Fig. 15).

When examining a flower preparation from the surface, one can see: epidermal cells of different shapes, from round, polygonal to elongated with straight walls; stomata are sparse, oval in shape, the stomatal apparatus is of anomocytic type; hairs are simple multicellular (5-9 cells) and capitate, consisting of a short one- and three-cell stalk and a one-, rarely two-cell oval head. The pollen is round-oval, smooth (Fig. 16).

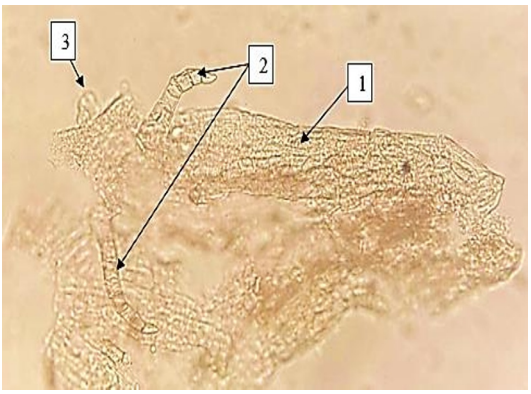


Fig. 15. Fragment of the surface of the *peduncle G. pentaphyllum* (magnitude 10/0.25) (1 – epidermis; 2 – simple hairs; 3 – capitate hair)

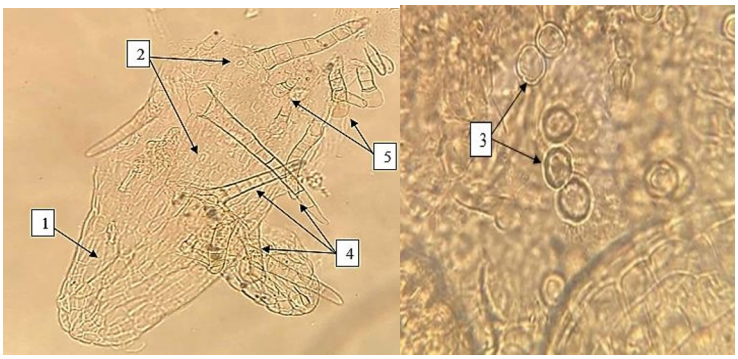


Fig. 16. Fragment of the surface of a flower *G. _ pentaphyllum* (magnitude 10/0.25; S 40/0.65) (1 – epidermis; 2 – stomatal apparatus; 3 – pollen grains; 4 – simple hairs; 5 – capitate hairs)

Quantitative assessment of the manifestation of diagnostically significant characteristics (DSC) was carried out not only to determine the authenticity of the herb, but also for standardization and quality control using a microscopic method of analysis.

To count anatomical features (significant and insignificant), we used grass powders passing through a sieve with holes with a diameter of 0,5 мм. Characteristics were determined at various magnifications 4/0.10; 10/0.25; S 40/0.65 in 15 replicates on a glass slide previously marked into squares. The number of diagnostically significant particles, the number of diagnostically insignificant particles, and the total amount of particles were calculated . Sample analysis was carried out according to the method developed by I.A. Samylina and O.G. Potanina [36, 37].

Results of a study on the manifestation of DZP in the herb *Gynostemma pentaphyllum* are presented in Table 2.

Table 2. Metrological characteristics of the method for calculating manifestation diagnostically significant signs of the herb *Gynostemma pentaphyllum*

N	F	$\bar{x}$	$S_{\bar{x}}$	P, %	T(P,f)	ε_{α}	$\varepsilon_{отн}$,%
15	14	71.08	0.63	95	2.15	1.35	1.91

The study revealed that the incidence of DZD in the herb Gynostemma pentafolia is 71.08±1.35%.

3.3. Quantitative content of biologically active substances in raw materials

The study of the quantitative content of components in the herb Gynostemma pentafolia was carried out using various methods of phytochemical analysis.

3.3.1. Titrimetric express method

Determination of tannins . An accurately weighed 2.0 g of crushed raw material, sifted through a sieve with 3 mm holes, is placed in a conical flask with a capacity of 500 ml, poured with 250 ml of purified water heated to a boil and refluxed on an electric stove with a closed spiral for 30 minutes at periodic stirring. The resulting extract is cooled to room temperature and filtered through cotton wool into a 250 ml volumetric flask so that particles of the raw material do not enter the flask, the volume of the solution is adjusted to the mark with water and mixed. 25.0 ml of the resulting aqueous extract is placed in a conical flask with a capacity of 1000 ml, 500 ml of water, 25 ml of indigo sulfonic acid solution are added and titrated with constant stirring of potassium permanganate with a 0.02 M solution until golden yellow.

indigosulfonic acid solution are placed in a conical flask with a capacity of 1000 ml and titrated with constant stirring of potassium permanganate with a solution of 0.02 M until it turns golden yellow.

1 ml of potassium permanganate solution 0.02 M corresponds to 0.004157 g of tannins in terms of tannin.

The content of the amount of tannins in terms of tannin in absolutely dry raw materials in percent (X) is calculated using the formula:

$$x = \frac{(V-V_1)\times 0{,}004157\times 250\times 100\times 100}{a\times 25\times(100-W)},$$

where

V – volume of potassium permanganate solution 0.02 M, used for titration of the aqueous extract, ml;

V 1 is the volume of potassium permanganate solution 0.02 M used for titration in the control experiment, ml;

0.004157 – amount of tannins corresponding to 1 ml of potassium permanganate solution 0.02 M (in terms of tannin), g;

a – weighed portion of raw materials or medicinal herbal preparation, g;

W – humidity of medicinal plant raw materials or medicinal herbal preparation, %;

250 – total volume of aqueous extraction, ml;

25 – volume of aqueous extract taken for titration, ml.

Determination of ascorbic acid. An accurate sample of 20.0 g of crushed raw material, sifted through a sieve with 3 mm holes, is placed in a conical flask with a capacity of 500 ml, 300 ml of water is added, infused for 20 minutes, and then filtered. Add 1 ml of the resulting filtrate, 1 ml of a 2% hydrochloric acid solution, 13 ml of water into a conical flask with a capacity of 100 ml, mix and titrate from a microburette with a 0.001 M solution of sodium 2,6-dichlorophenolindophenolate until a pink color appears, which does not disappear within 30 - 60 With. Titration is continued for no more than 2 minutes.

The content of ascorbic acid in terms of absolutely dry raw materials in percent (X) is calculated using the formula:

$$x = \frac{V \times 0{,}000088 \times K \times 300 \times 100 \times 100}{a \times 1 \times (100 - W)},$$

where

0.000088 – amount of ascorbic acid corresponding to 1 ml
0.001 M solution of sodium 2,6-dichlorophenolindophenolate, g;
V – volume of 0.001 M solution of sodium 2,6-dichlorophenolindophenolate,
used for titration, ml;
a – sample of raw materials, g;
W – raw material humidity, %;
K – correction factor.

Determination of organic acids. An accurate sample of 25.0 g of crushed raw material, sifted through a sieve with 3 mm holes, is placed in a conical flask with a capacity of 250 ml, 200 ml of water is added and kept for 1 hour in a boiling water bath, then cooled, transferred quantitatively into a volumetric flask with a capacity 250 ml, bring the volume of extraction to the mark with water and mix. Take 10 ml of the extract, place it in a 500 ml flask, add 300 ml of freshly boiled water, 1 ml of 1% phenolphthalein solution, 2 ml of methylene blue alcohol solution and titrate with 0.1 M sodium hydroxide solution until a purple-red color appears in the foam.

The content of free organic acids in terms of malic acid in absolutely dry raw materials in percent (X) is calculated by the formula:

$$x = \frac{V \times 0{,}0067 \times 250 \times 100 \times 100}{a \times 10 \times (100 - W)},$$

where

0.0067 – amount of malic acid corresponding to 1 ml of 0.1 M sodium hydroxide solution, g;
V – volume of 0.1 M sodium hydroxide solution used for titration, ml;
a—weight of raw material, g;
W – raw material humidity, % [35, 38].

Indicators for the content of tannins in terms of tannin in the herb Gynostemma quinquefolia ranged from 1.06±0.03% to 1.09±0.02%, ascorbic acid – from 0.130±0.003% to 0.134±0.003% and free organic acids – from 1.06±0.02% to 1.08±0.02% (Fig. 17).

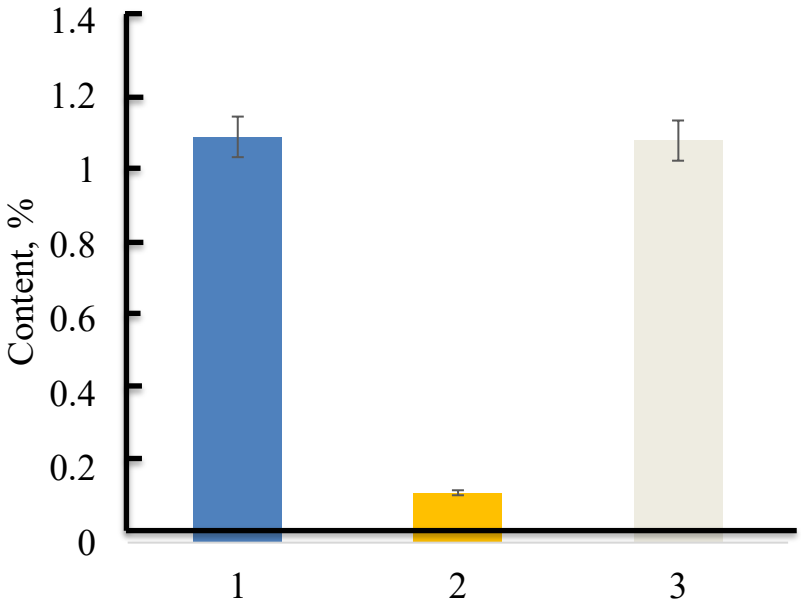


Fig 17. Results of determining the content of tannins (1), ascorbic acid (2) and organic acids (3) in the grass *of G. p entaphyllum*

3.3.2. Gravimetric method

The content of polysaccharides was determined after their fractional separation into: water-soluble polysaccharides (PSPS), pectin substances (PS), hemicellulose A (HCa) and hemicellulose B (HCb) [39].

Before isolating the polysaccharide complex, lipophilic compounds were removed from the raw material by extraction with a polar solvent in a Soxhlet apparatus . To do this, 10.0 g of gynostemma herb (analytical mass), crushed to a particle size of 0.5 mm, was loaded into a bag of filter paper, which was placed in a Soxhlet apparatus . Extracted with chloroform until the raw material was completely depleted.

Removal of substances of phenolic nature in the same raw material (after its preliminary drying) was carried out by extraction with ethyl alcohol 70% for 30 minutes. Then the polysaccharide fractions were obtained in turn: VPPS; PV; GCa and GCb .

HPPS was isolated by extraction of purified raw materials (from lipophilic substances and phenolic compounds) with hot water in a ratio of 1:10 when heated to 90 °C for 2 hours. The extraction process was repeated three times. The resulting extracts were combined and concentrated in evaporation dishes in a water bath. Precipitation of VPPS was carried out by treatment with 95% ethyl alcohol in a 3-fold volume. Within 24 hours, an amorphous flocculent precipitate formed, which was filtered, washed with ethyl alcohol 70% and 95%, chloroform, dried and weighed.

The extraction of PV was carried out from the air-dried meal remaining after obtaining the VPPS. To do this, we used the method of infusion in a mixture of oxalic acid and ammonium oxalate 0.5% in a 1:1 ratio for 2.5 hours at a temperature of 80-85 ºC. The PV content was also determined gravimetrically . To do this, they were precipitated from the extraction with a 5-fold amount of 95% ethyl alcohol. The resulting precipitate was filtered and washed with ethyl alcohol of various concentrations and chloroform, dried and weighed.

To extract HCa and HCb , air-dried meal, after extracting VPPS and PV, was treated with a 10% sodium hydroxide solution in a ratio of 1:5 at room temperature for 24 hours and filtered. Glacial acetic acid was added to the filtrate to pH 6-7 (checked with litmus paper) and the resulting HCA precipitate was filtered through a filter for fine-porous sediments. Next, to form a GCB precipitate, the resulting filtrate was precipitated with a 2-fold volume of 95% ethyl alcohol. The formed HCB precipitate was also washed with ethyl alcohol and dried.

Dried polysaccharide complexes after fractional separation showed different colors and solubilities (Table 3).

Table 3. Characteristics of polysaccharide fractions isolated from the herb *Gynostemma pentaphyllum*

Faction name	**Description**	**Solubility**	**Chemical reactions**
VRPS	amorphous flaky powder of light brown color	forms a cloudy milky solution in water; *"+" in acids and alkalis; *"-" in organic solvents	precipitation reactions with acetone, ethyl alcohol
PV	light gray amorphous powder	*"++" in water to form a viscous solution	precipitation reaction with 1% aluminum sulfate solution
GC_A and GC_B	amorphous yellowish-brown powders	*"++" in water and alkali	-

Note. Soluble – “+”, highly soluble – “++”, insoluble – “-”

Analysis polysaccharides from the herb Gynostemma pentafolia showed an average yield of carbohydrate complex: VPPS - 2.36 ± 0.04% ; PV – 1.35 ±0.03% ; HCA and HCb – 1.64 ±0.03% and 1.72 ±0.04% , respectively. The predominant role in the polysaccharide complex under study is occupied by the water-soluble fraction (Fig. 16).

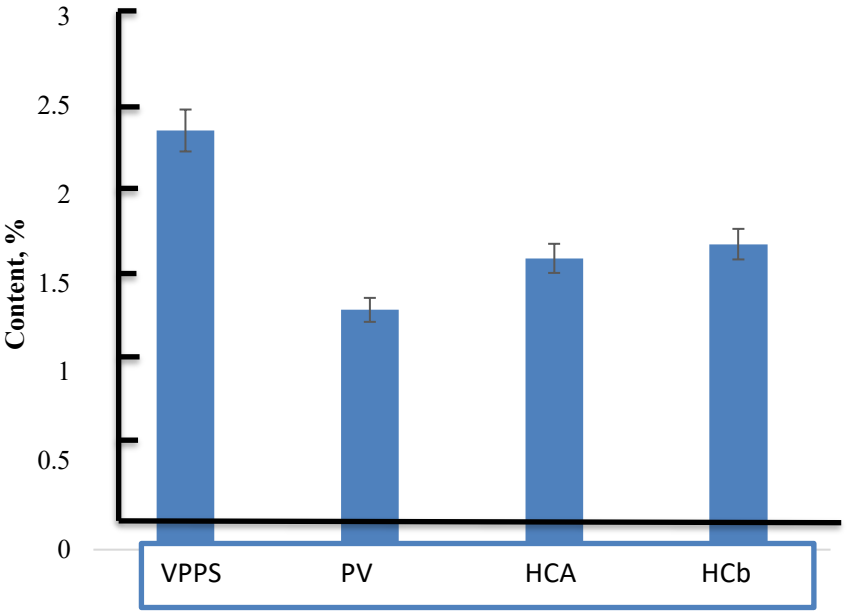


Fig.. 18. Results of determining the content of polysaccharides in grass *G. pentaphyllum*

3.3.3. UV-visible spectrophotometric method

Initially, a spectral analysis of extracts from the herb *G ynostemma was carried out pentaphyllum* in the wavelength range from 220 to 500 nm . Absorption maxima were identified corresponding to the triterpene saponin - β- escin and the flavonoid - rutin (Fig. 19, 20).

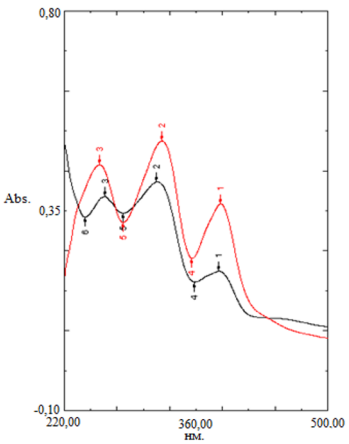


Fig. 19 – Spectra of a solution of CO β - aescin (red) and an aqueous- *alcoholic* extract from the herb *G. pentaphyllum* (black) with concentrated sulfuric acid

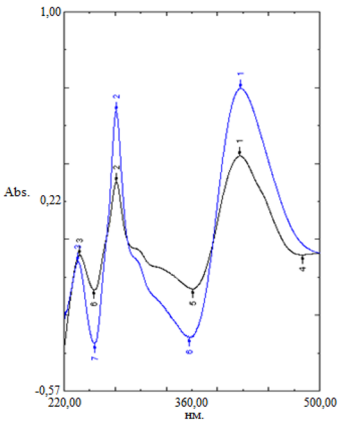


Fig. 20 – Spectra of a solution of CO rutin (blue) and aqueous- *alcoholic* extract from the herb *G. pentaphyllum* (black) with complexing additive

Then the quantitative content of the sum of saponins and flavonoids in aqueous-alcoholic extracts *of G ynostemma was determined pentaphyllum* using a Shimadzu UV-1800 device.

About the determination of saponins. An analytical sample of raw materials is crushed to a particle size that passes through a sieve with holes 7 mm in diameter. An accurate weighing of 1.0 g of crushed raw material is placed in a flask with a ground section with a capacity of 100 ml, 30 ml of 70% ethyl alcohol is added. The flask is stoppered and weighed on a tare scale with an accuracy of ±0.01. The flask is connected to a reflux condenser and heated over boiling water for 90 minutes. Then the flask is cooled to room temperature, closed with the same stopper, weighed again and the missing extractant is replenished to the original mass. The extract is filtered through a paper filter ("red stripe"). 5 ml of the resulting extract is evaporated to dryness in a water bath in a porcelain cup. After cooling, the dry residue is dissolved in 5–6 ml of water, quantitatively transferred to a layer of silica gel 1–1.5 cm high formed on a glass filter, and eluted with 10–15 ml of water. The aqueous eluate is discarded. Then elute with 95% alcohol into a 10 ml volumetric flask to the mark and mix (solution A).

1 ml of test solution A is added to a 25 ml flask, 5 ml of concentrated sulfuric acid is added and heated in a boiling water bath for 10 minutes. After cooling, the optical density of the solution is measured using a spectrophotometer at a wavelength of 322 nm in a cuvette with a layer thickness of 10 mm. 95% ethyl alcohol is used as a reference solution.

In parallel, under the same conditions, the optical density is measured with 1 ml of a 0.05% solution of a standard sample (SS) of β- escin .

The content of the sum of saponins (X) in terms of β- escin and absolutely dry raw materials (in%) is calculated using the formula:

$$x = \frac{D \times m_0 \times 30 \times 10 \times 6 \times 1 \times 100 \times 100}{D_0 \times m \times 5 \times 1 \times 25 \times 6 \times (100 - W)}, \text{ Where}$$

D – optical density of the test solution;
D_0 – optical density of β- escin CO solution ;
m – mass of raw materials, g;
m_0 – mass of CO β- escin , g;
W – weight loss during drying of raw materials, % [35, 40].

Determination of flavonoids. An accurate weighing of 1.0 g of crushed and sifted raw material through a sieve with a hole size of 3 mm is placed in a flask with a ground glass with a capacity of 200 ml, 100 ml of 70% ethyl alcohol are added, weighed, a reflux water cooler is connected to the flask, the contents of the flask are heated for 30 minutes on water bath, noting the time from the beginning of boiling, then the flask is disconnected, artificially cooled, weighed and, if necessary, brought to the original weight with the appropriate alcohol. Part of the extraction is filtered, discarding the first portions of the filtrate.

Place 2.0 ml of filtrate into two volumetric flasks with a capacity of 25 ml, add 4 ml of a 5% solution of aluminum chloride in 70% alcohol to one flask, and add 0.1 ml of 30% acetic acid to the other flask, adjust the volume in the flasks to 70 % alcohol to the mark, mix and after 30 minutes measure the optical density of the solution with aluminum chloride on a spectrophotometer in a cuvette with a layer thickness of 1 cm at a wavelength of 412 nm against the background of a solution acidified with acetic acid.

In parallel, under the same conditions, an experiment is carried out with 1.5 ml of a 0.1% solution of rutin in 95% alcohol.

$$x = \frac{D \times 100 \times C_0 \times 100 \times 100}{D_0 \times V \times a \times (100 - W)}$$, Where

D is the optical density of the test solution;
D_0 - optical density of rutin CO solution;
C_0 - rutin content in the standard sample in terms of dry matter, g;
V is the volume of extract taken for analysis, ml;
a is the mass of raw materials taken for extraction, g;
W is the loss in mass of raw materials during drying, % [41].

The content of triterpene saponins in the herb Gynostemma pentafolia ranged from 11.53±0.17% to 11.85±0.04%, and flavonoids - from 1.30±0.05% to 1.31±0.04%, which indicates their high number (Fig. 21).

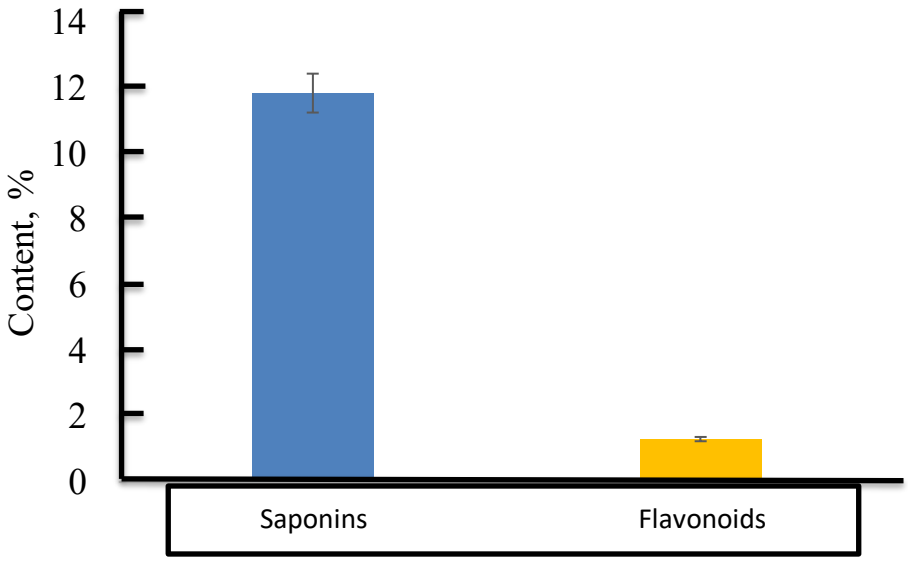


Fig. 21. Results of determination of the content of saponins and flavonoids in the grass *G. p entaphyllum*

4 Conclusion

Diagnostic morphological and anatomical characteristics of the grass *G ynostemma have been experimentally established pentaphyllum* :

– in micropreparations from the surface: the structure of the epidermis from the upper and lower sides, the stomatal apparatus, trichomes (simple and glandular hairs);

– in micropreparations of cross sections: the structure of the conduction system (collateral bundles), the presence of mechanical tissues (collenchyma and sclerenchyma) and storage parenchyma.

Using a microscopic method, diagnostically significant signs were identified in the grass of Gynostemma pentaphyllum, the occurrence of which is 71.08±1.35%.

Using phytochemical analysis, the content of tannins (from 1.06 ± 0.03% to 1.09 ± 0.02%), ascorbic acid (from 0.130 ± 0.003% to 0.134 ± 0.003%), free organic acids (from 1.06 ±0.02% to 1.08 ±0.02%), polysaccharides (VPS – 2.36 ±0.04% ; PV – 1.35 ±0.03% ;

GC $_A$ and GC $_B$ – 1 .64 ±0.03% and 1.72 ±0.04% , respectively), saponins (from 11.53±0.17% to 11.85±0.04%) and flavonoids (from 1.30±0. 05% to 1.31±0.04%).

Saponins and flavonoids are pharmacologically active components of the raw material and, due to their high content, can expand the indications for the use of the herb *G ynostemma pentaphyllum* .

References

1. Kharkevich , S.S. Rare plant species of the Soviet Far East and their protection / S.S. Kharkevich , N.N. Kachura. – M.: Nauka, 1981. – 232 p.
2. Kharkevich , S.S. Vascular plants of the Soviet Far East / S.S. Kharkevich . – L.: Nauka, 1996. – T. 8. – 383 p.
3. Ohwi , J. Flora of Japan / J. Ohwi // Washington: Smithsonian Institution, 1965. – 1081 p.
4. Zhou, J. Encyclopedia of Chinese Herbs Vol 2 / J. Zhou, G. Xic , X. Yan. – China, 1996. – P. 1878-1882.
5. Barkalov, V.Yu. Flora of the Kuril Islands / V.Yu. Barkalov. – Vladivostok: Dalnauka , 2009. – 468 p.
6. Probatova , N.S. Genus Gynostemma – *Gynostemma* // Vascular plants of the Soviet Far East. – St. Petersburg: Nauka, 1987. – P. 136-137.
7. Eremin, V.M. Red Book of the Sakhalin Region: Plants and Mushrooms / V.M. Eremin. – Kemerovo: Technoprint , 2019. – 354 p .
8. Keraudren-Aymonin , M. Cucurbitaceae. Flora du Cambodge du Laos et du Viet-Nam / MK Keraudren-Aymonin . – 1975. – Vol. 15. – P. 1-114.
9. Liu, X. Tissue culture and plantlet regeneration of *Gynostemma pentaphyllum* / X. Liu // Chinese Medicinal Materials. – 1989. – Vol. 12, No. 6. – P. 8-10.
10. Wilde de WJJO *Gynostemma* (Cucurbitaceae) in Thailand and Malesia / WJJO de Wilde, BEE Duyfjes // Blumea Journal of Plant Taxonomy and Plant Geography . – 2007. – Vol. 52, No. 2. – P. 263-280.
11. Wu, C.Y. A Study on the Genus *Gynostemma* Bl. (Cucurbitaceae) from China / CY Wu, SK Chen // Systematics and Evolution. – 1983. – Vol. 21. – P. 355-369.
12. Wu, M. Pharmacognosy of *Gynostemma pentaphyllum* and *Cayratia japonica* / M. Wu, R. Jiang // China journal of Chinese materia medica . – 1987. – No. 9 – P. 22-25.
13. Belenovskaya , L.M. *Gynostemma pentaphyllum* (Cucurbitaceae): component composition and biological activity / L.M. Belenovskaya , A.L. Budantsev , N.V. Bityukova // Plant resources . – 2018. – T. 54, No. 4. – P. 443-495.
14. Plant resources of Russia: Wild flowering plants, their component composition and biological activity / A.L. Budantsev , L.M. Belenovskaya , N.A. Medvedev [and others]. – St. Petersburg; M.: Partnership of scientific publications KMK, 2009. – T. 2. – 520 p.
15. Melikulov , O.Zh. , Kodirov , N.D. , & Baymuradov , E.S. (2022). And use barberry V pharmacotherapy . Oriental renaissance: Innovative, educational, natural and social sciences, 2 (Special Issue 4-2), 911-913.
16. Meliqulov , OJ, Kodirov , ND 1.4-benzodiazepinning tibbiyotda qo'llanadigan vositalari // ORIENSS. 2022. No. 8. URL: https://cyberleninka.ru/article/n/1-4-benzodiazepinning-tibbiyotda-qollanadigan-vositalari.

17. Characterization and antioxidant activities of acidic polysaccharides from *Gynostemma pentaphyllum* (Thunb .) Makino / B. Li, X. Zhang, M. Wang, L. Jiao // Carbohydrate polymers. – 2015. – Vol. 127. – P. 209-214.
18. Immunomodulatory and antioxidant effects of polysaccharides from *Gynostemma pentaphyllum* Makino in immunosuppressed mice / X. Shang, Y. Chao , Y. Zhang [et al.] // Molecules. – 2016. – Vol. 21, No. 8. – P. 1085.
19. Conjugated linolenic acids and nutraceutical components in Jiaogulan (*Gynostemma pentaphyllum*) seeds / C. Zou, H. Shi , X. Liu [et al.] // LWT Food Science and Technology. – 2013. – Vol. 68. – P. 111-118.
20. Analysis of amino acids, vitamins and chemical elements in *Gynostemma pentaphyllum* (Thunb .) Makino / S. Deng, X. Li, B. Chen [et al.] // Hunan Yike Daxue Xuebao . – 1994. – Vol. 19, No. 6. – P. 487-490.
21. Tsai, Y.C. Preparation of carotenoids and chlorophylls from *Gynostemma pentaphyllum* (Thunb .) Makino and their antiproliferation effect on hepatoma cell / YC Tsai, WB Wu, BH Chen // Journal of medicinal food. – 2010. – Vol. 13, No. 6. – P. 1431 -1442.
22. Concentrations of trace elements in organic fertilizers and animal manures and feeds and cadmium contamination in herbal tea (*Gynostemma pentaphyllum* Makino) / N. Rangkadilok , J. Satayavivad , S. Nookabkaew , N. Prachoom // Agricultural and food chemistry. – 2016. – Vol. 64, No. 16. – P. 3119-3126.
23. Conjugated linolenic acids and nutraceutical components in Jiaogulan (*Gynostemma pentaphyllum*) seeds / C. Zou, H. Shi , X. Liu [et al.] // LWT Food Science and Technology. – 2013. – Vol. 68. – P. 111-118.
24. Xu, Zl . Chemical constituents of *Gynostemma pentaphyllum* /Zl. Xu, Fq Yang, Zn Xia // Natural Product Research and Development. – 2013. – Vol. 8. – P. 1067 - 1069.
25. Protective effects of gypenosides against fatty liver disease induced by high fat and cholesterol diet and alcohol in rats / R. Qin, J. Zhang , C. Li [et al.] // Archives of pharmacal research. – 2012. – Vol. 35. – P. 1241 - 1250.
26. Mechanism of action of gypenosides on type 2 diabetes and non-alcoholic fatty liver disease in rats / Q. He, J.-K. Li , F. Li [et al.] // World journal of gastroenterology. – 2015. – Vol. 21, No. 7. – P. 2058-2066.
27. Immunomodulatory and antioxidant effects of polysaccharides from *Gynostemma pentaphyllum* Makino in immunosuppressed mice / X. Shang, Y. Chao , Y. Zhang [et al.] // Molecules. – 2016. – Vol. 21, No. 8. – P. 1085.
28. Phytopreventative effects of *Gynostemma pentaphyllum* against acute indomethacin-induced gastrointestinal and renal toxicity in rats / C. Hesse , V. Razmovski-Naumovski , C. C Duke [et al.] // Phytotherapy research. – 2007. – Vol. 11, No. 6. – P. 523-530.
29. Bronchodilatory effects of the aqueous extract of *Gynostemma pentaphyllum* and gypenosides III and VIII in anaesthetized guinea-pigs / C. Circosta , R. De Pasquale , D. R Palumbo , F. Occhiuto // Pharmacy and pharmacology. – 2005. – Vol. 57, No. 8. – P. 1053-1058.
30. Wang , TX. Bioassay-guided isolation and identification of anticancer and antioxidant compounds from *Gynostemma pentaphyllum* (Thunb .) Makino/ TX. Wang , M.M. Shi , JG. Jiang // RSC Advances . – 2018. – Vol. 8, No. 21. – P. 23181-23190.
31. Triterpenoid herbal saponins enhance beneficial bacteria, decrease sulfate-reducing bacteria, modulate inflammatory intestinal microenvironment and exert cancer

preventive effects in ApcMin /+ mice / L. Chen, MS Brar, FC Leung, WL Hsiao // Oncotarget . – 2016. – Vol. 7 , No. 21. – P. 31226-31242.

32. Gypenosides suppress growth of human oral cancer SAS cells in vitro and in a murine xenograft model: the role of apoptosis mediated by caspase-dependent and caspase-independent pathways / KW Lu, J.-C. Chen , T.-Y. Lai [et al.] // Integrative cancer therapies. – 2012. – Vol. 11 , no. 2 . – P. 129 - 140.
33. The influence of certain types of medicinal plant raw materials on the hemostasis system *in vitro* / N.V. Kudashkina , E.Kh. Galiakhmetova , L.I. Bashirova [and others] // Traditional medicine. – 2021. – No. 1 (64). – pp. 38-42.
34. Gypenosides ameliorate carbon tetrachloride-induced liver fibrosis by inhibiting the differentiation of hepatic progenitor cells into myofibroblasts / J. Chen, X. Li , Y. Hu [et al.] // The American journal of Chinese medicine . – 2017. – Vol. 45 , no. 5 . – P. 1061 - 1074.
35. State Pharmacopoeia of the Russian Federation XIV edition. [Electronic resource] // Federal Electronic Medical Library, 2018. – Access mode: http :// www . femb . ru /
36. Samylina, I.A. Pharmacognosy: Atlas: textbook / I.A. Samylina, O. G. Anosova. – M.: GEOTAR-Media, 2010. – T. 1. – 188 p.
37. Samylina, I.A. Pharmacognosy: textbook / I.A. Samylina, G.P. Yakovlev. – M.: GEOTAR-Media, 2013. – 976 p.
38. State Pharmacopoeia of the USSR: Vol . 1: General methods of analysis / USSR Ministry of Health. – 11th ed., add. – M.: Medicine, 1987. – 336 p.
39. Kochetkov, N.K. Chemistry of biologically active natural compounds / N.K. Kochetkov. – M.: Chemistry, 1970. – 378 p .
40. Kurkin, V.A. Determination of saponins in ginseng roots / V.A. Kurkin, A.S. Akushskaya // Pharmacy. – 2012. – No. 3. – pp. 18-20.
41. Pat. SU 1 507 394 A1 USSR, IPC A 61 K 35/78. Method for quantitative determination of flavonoids in plant raw materials / V.V. Belikov, N.T. Kolesnik; applicant and patent holder All-Union Scientific Research Institute of Chemistry and Technology of Medicines – No. 4301201/28-14; stated _ 08/25/1987; publ . 09/15/1989, Bulletin . No. 34.
42. Menglikulov, B., Umarov, S., Safarov, A., Zhyemuratov, T., Alieva, N., & Durmanov, A. (2023). Ways to increase the efficiency of transport logistics-communication services in Uzbekistan. In E3S Web of Conferences (Vol. 389, p. 05036). EDP Sciences. https://doi.org/10.1051/e3sconf/202338905036
43. Narinbaeva, G., Menglikulov, B., Siddikov, Z., Bustonov, K., & Davlatov, S. (2021). Application of innovative technologies in agriculture of Uzbekistan. In E3S Web of Conferences (Vol. 284, p. 02009). EDP Sciences. https://doi.org/10.1051/e3sconf/202128402009
44. Menglikulov, B., Dusmuratov, R., & Mamadiyorov, D. (2021). Improvement of methodological aspects to calculate the cost of dairy products: a review. In E3S Web of Conferences (Vol. 258, p. 04036). EDP Sciences. https://doi.org/10.1051/e3sconf/202125804036
45. Uralovich, K. S., Toshmamatovich, T. U., Kubayevich, K. F., Sapaev, I., Saylaubaevna, S. S., Beknazarova, Z., Khurramov, A. (2023). 'A primary factor in sustainable development and environmental sustainability is environmental education', Caspian Journal of Environmental Sciences, 21(4), pp. 965-975. doi: 10.22124/cjes.2023.7155