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Physico Chemical and Pharmacogenetic Study of Inula Macrophylla and Inula Rhizocephalan Herbs

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Abstract

Taking into account the information revealed by the scientific literature on the rich composition of the BAS target fragment of the metabolome of I. Macrophylla and I. Rhizocephala and their high biological activity, the development of a liquid extract for obtaining an industrial standardized dosage form of the drug for topical use was considered the most appropriate. The use of the entire leafy shoot of a grass plant as a type of raw material with a less laborious stage in the industrial harvesting of LRS was proposed as the initial I. Macrophylla and I. Rhizocephala in the preparation of a liquid extract. The dried leaves and roots of I. Macrophylla and I. Rhizocephala are used by the population in Tajikistan as a means of traditional medicine to obtain aqueous extracts that are used for local wound treatment.

Keywords: extractant; flavonoids; tannins; saponins; alkaloids; herbs; extract

Introduction

I. Macrophylla and root extract. I Rhizocephala is characterized by the presence of a complex of essential oils that have a beneficial effect on the functional state of the respiratory tract. These compounds promote the active passage of phlegm from the lung tissue, which makes elecampane an effective agent in the treatment of cough. Rhizocephala, has a beneficial effect on the intestinal microflora, contributing to the normalization of digestion and the fight against dysbiosis and constipation. In addition, the plant stimulates the passage of bile, optimizes the functioning of the liver and gallbladder, and also has a beneficial effect on the urinary system. Traditionally, the root of I. macrophylla and I. Rhizocephala, used to treat asthma, bronchitis, cough, diarrhea, nausea and intestinal parasites. The purpose of this work is the physicochemical and pharmacognostic study of the herb inula macrophylla and inula rhizocephala.

Material and Methods of Research

The work was carried out on biochemistry and medical biology of the Khatlon State Medical University, Avicenna TSMU, State Institution "Scientific Research Pharmaceutical Center of the Ministry of Health and North-West of the Republic

of Tajikistan", Tajik National University and Kursk State Medical University.

Results and Discussion

Substantiation of the Choice of The Type of Raw Materials I. Macrophylla and I. Rhizocephala

The results of the analysis of the data of traditional medicine in Tajikistan, the assessment of possible chemotherapeutic and pharmacological activities of ALS contained in the metabolome of the plant, the conduct of our own phytochemical studies of raw materials, showed the prospects for the use of the herbs I. Macrophylla and I. Rhizocephala for medical purposes. The use of this raw material to develop a technology for obtaining a liquid extract from it was the basis for the development of methods for establishing the authenticity and quality indicators of the dried herb of the plant. The creation of the layout of the draft regulatory document for the grass I. Macrophylla and I. Rhizocephala was carried out in accordance with the requirements of the State Fund of the Russian Federation XIII ed. to medicinal plant raw materials of a similar morphological group.

Assessment of the Content of Extractives in The Herb I. Macrophylla and I. Rhizocephala

Table 1: Content of extractives extracted by various solvents in samples of the herb *I. Macrophylla* and *I. Rhizocephala*.

Extractant	Content Of Extractives in Two Types of Raw Materials <i>I. Macrophylla</i> and <i>I. Rhizocephala</i> , %	
	Grass Harvested in The Flowering Phase	Grass Harvested Before Flowering
Purified Water	30,32 ± 1,22	30,12 ± 0,68
40% Alcohol	30,54 ± 0,47	31,20 ± 0,49
70% Alcohol	28,35 ± 0,35	31,17 ± 0,75
90% Alcohol	22,81 ± 0,86	25,56 ± 0,87

The results of identifying the most optimal extractant are presented in Table 1. Water and water-alcohol mixtures with a known concentration of alcohol were used as extractants. At the same time, work was carried out with another type of raw material elecampane grass collected during flowering, in a comparative aspect.

From those presented in Table 1. The data show that during extraction with water and 40% alcohol, the maximum extraction of extractives is observed, which is associated with the intensive transition of polar substances. When extracting raw materials with water-ethanol mixtures with a high concentration of alcohol, along with some hydrophilic compounds, less

polar representatives of the plant metabolome also pass, which, according to the literature, suggests that they are active in the [1-4]. Study of the content of target ALS in the raw materials of *I. macrophylla* and *I. Rhizocephala*. As you know, phenolic compounds are the most significant group of ALS, which is responsible for the antioxidant effect of drugs from medicinal plant raw materials. Among phenolic compounds, flavonoids are responsible for experiencing the biological effects described in the scientific literature. For the express determination of active substances, the content of phenolic compounds in terms of chlorogenic acid was determined by the UV-SPM method in Table 2.

Table 2: Influence of alcohol concentration on the yield of phenolic substances from two types of raw materials *I. Macrophylla* and *I. Rhizocephala*.

Alcohol Concentration, %	Phenolic Content in Terms of Chlorogenic Acid	
	Grass Harvested in The Flowering Phase	Grass Harvested Before Flowering
40	3,022 ± 0,01	7,584 ± 0,02
50	3,322 ± 0,01	7,592 ± 0,01
60	3,345 ± 0,02	7,589 ± 0,02
70	3,351 ± 0,01	7,604 ± 0,02
80	3,350 ± 0,015	7,350 ± 0,01
90	3,316 ± 0,01	7,352 ± 0,02

From the data presented in Table 2, it can be seen that the content of phenolics in the grass collected before flowering is almost twice as high, and at the same time

their maximum content is observed when extracted with 70% alcohol.

Table 3: Effect of feedstock: extractant ratio on the recovery of phenolic substances from LRSI *I. Macrophylla* and *I. Rhizocephala*.

Ratio of Raw Materials: Extractant Alcohol 70 %	Phenolic Content in Terms of Chlorogenic Acid	
	Grass Harvested in The Flowering Phase	Grass Harvested Before Flowering
1:5	2,055±0,01	5,652±0,01
1:10	3,315±0,01	7,513±0,01
1:20	3,345±0,01	7,523±0,01
1:30	3,337±0,01	7,585±0,01
1:50	3,358±0,01	7,604±0,02
1:100	3,363±0,01	7,602±0,02
1:150	3,359±0,01	7,602±0,02

According to the data presented in Table 3, the content of phenolic compounds is also twice as high in grass harvested before flowering, and is revealed as

the maximum possible at a ratio of raw material to extractant from 1:10. With an increase in the ratio, their yield is practically insignificant for the

technology of obtaining drugs. In the study of the effect of the grindability of raw materials, I. Macrophylla and I. Rhizocephala used particles of raw

materials passing through a sieve with holes of 0.2 size; 0,5; 1; 2 and 3 mm.

Table 4: Influence of the degree of grinding of raw materials on the extraction of phenolic substances.

Extraction Time, Min	Phenolic Content in Terms of Chlorogenic Acid, %	
	Grass Harvested in The Flowering Phase	Grass Harvested Before Flowering
15	3,118±0,01	7,348±0,02
30	3,244±0,01	7,409±0,01
45	3,349±0,01	7,598±0,02
60	3,351±0,01	7,603±0,01
75	3,351±0,02	7,602±0,01

From the results presented in Tables 4-5, it follows that the content of phenolic substances in the grass I. Macrophylla and I. Rhizocephala, collected before flowering, exceeds the same indicator for the grass collected during the flowering phase by more than 2 times. Since phenolic compounds are one of the target groups of ALS, the expediency of choosing the herb I. Macrophylla and I. Rhizocephala, collected before flowering, as a raw material and object for further research, was confirmed. Thus, the optimal conditions for the extraction of phenolic compounds from I. macrophylla and I. rhizocephala are as follows: 1:10 ratio of raw material to extractant, 70% ethanol extractant, 0.2-3.0 mm grinding degree, 60 min extraction time.

Qualitative Reactions

In order to select the most reliable qualitative reactions in order to establish the authenticity of the herb I. macrophylla and I. rhizocephala, several variants of extractions from raw materials were prepared - with the help of water (aqueous extraction) and different concentrations of water-alcohol mixtures. Qualitative reactions were carried out with the extracts obtained to confirm the literature data on the content of the target groups of ALS in the studied raw materials (flavonoids, tannins, saponins and alkaloids). The results of these tests are presented in Tables 5 and 6.

Table 5: Results of Determination of ALS Groups Using Qualitative Reactions in Various Extracts from Raw Materials.

Type of Reaction	Reaction Result	Extractant			
		Water	40% Alcohol	70% Alcohol	90% Alcohol
Flavonoids					
Cyanidin Test According to Briant	Division Into 2 Phases. Aqueous Layer - More Intense Coloring	++	++	+++	+++
		Predominance of Glycosides Over Aglycones			
With Iron (III) Chloride Solution	Black and Green Coloring	+	+	++	++
With an Alcoholic Solution of Alkali 10%	Yellow Staining	++	++	++	++
Tannins					
With Protein Solution	Manifestation of Turbidity	+++	++	++	+
With Iron-Ammonium Alum	Black And Green Coloring	+++	++	++	+
Saponins					
Foaming	Formation of Abundant and Stable Foam	++	+++	+++	+++
Chemical Group of Saponins (Test Tubes with HCL and NaOH Solutions)	Foam Formation	Persistent Foam in Both Tubes Triterpene Saponins			

Note: The amount of "+" indicates the intensity of coloring, sediment and the size of the foam column.

Qualitative reactions to the presence of alkaloids in the raw material of I. Macrophylla and I. Rhizocephala were carried out using general alkaloid reagents. The results of this work are presented in Table 6.

Table 6: Results of Determination of the Presence of Alkaloids in Various Extracts from *I. Macrophylla* and *I. Rhizocephala* Raw Materials.

Extractant	Name of the total alkaloid reagent / result					
	Sedimentary Reagents				Color Reagents	
	Wagner - Bouchard	Scheibler	Dragendorf	Tannin solution	H ₂ SO ₄ Conc	Sodium nitroprusside
	Brown color	Yellowish sediment	Orange brown sediment	White sediment	Purple when heated	Reddish-brown sediment
Water	++	+	++	-	+	++
Ethanol 40%	++	++	++	-	++	++
Ethanol 70%	+++	++	++	-	+++	++
Ethanol 90%	+++	++	++	-	++	++

Numerical indicators of the herb *I. Macrophylla* and *I. Rhizocephala*

In the production of the extract, crushed raw materials are used, the fractional composition of which is given in Table 7.

Table 7: Fractional composition of particles of chopped grass *I. Macrophylla* and *I. Rhizocephala*.

Raw Material Particles Remaining on A Sieve with Holes in Size	Fraction Content %
3 mm	0,28±0,002
2 mm	41,65±0,42
1 mm	32,23±0,82
0,5 mm	15,68±0,06
0,1 mm	8,32±0,08
Particles That Have Passed Through a Sieve with 0.1 mm Holes	1,84±0,04

The data in Table 7 show that in the crushed raw materials *I. macrophylla* and *I. Rhizocephala*, 90% are particles larger than 2 mm, but not less than 0.5 mm. The fraction of crushed raw materials from fine and dusty particles is at the level of 10%. The results of determining some numerical indicators of the raw materials *I. macrophylla* and *I. Rhizocephala* are shown in Table 8. As can be seen from Table 8, the

moisture content of *I. Macrophylla* and *I. Rhizocephala* grass did not exceed 6%, which corresponds to the level of values of no more than 14% for the dried 83 official raw materials of the morphological group. The total ash content in the grass is about 10%, and ash insoluble in a 10% HCl solution is up to 1%.

Table 8: Some numerical indices of the grass *I. Macrophylla* and *I. Rhizocephala*.

Numerical Indicator	Content %
Humidity	4,60±0,3
Total Ash	11,34±0,4
Ash, insoluble in 10% HCl solution	0,745±0,02

As a result of studies on the presence of heavy metals and arsenic in raw materials, as well as pesticide residues, the data presented in Table 9 were obtained.

Table 9: Results of determination of heavy metals, arsenic and residual pesticides in raw materials of *I. Macrophylla* and *I. Rhizocephala*.

Determinable Indicators	Grass <i>I. Macrophylla</i> and <i>I. Rhizocephala</i>	Maintenance Standards, No More Than ...
Heavy Metals, Mg/Kg		
Cadmium	0,048±0,006	1,0
Mercury	0,018±0,004	0,1
Lead	0,725±0,002	6,0

Arsenic	0,002±0,001	0,5
Pesticides, Mg/Kg		
DDT and Its Metabolites	Less Than 0.001	0,10
HCH (α, β, γ Isomers)	Less Than 0.001	0,10

The study of the herb *I. macrophylla* and *I. Rhizocephala* for compliance with safety standards for the indicators "Content of residual pesticides" and "Content of heavy metals and arsenic" showed that the raw material meets the requirements for the purity of medicinal products in the State Branch of the Russian Federation XIII ed. When studying the features of the development of *I. Macrophylla* and *I. Rhizocephala* by foreign scientists [5,6,7,8], it was found that elecampane accumulates heavy metals and arsenic in the underground parts, but such elements do not penetrate into the above-ground organs of the plant. Based on this, it is proposed to use elecampane to clean soils from pollution caused by anthropogenic impact.

Development of Methods for The Quantitative Determination of Active Substances in *I. Macrophylla* and *I. Rhizocephala* Raw Materials Acceptable for End-To-End Standardization

The data of the literature allow us to consider phenolic compounds, and in particular, flavonoids, as active ingredients of the herbs *I. macrophylla* and *I. Rhizocephala*. The results of the study of ALS of the target low-molecular fragment of the metabolome confirm their presence in the studied raw material. When developing a liquid extract from the herb *I. Macrophylla* and *I. Rhizocephala* estimated the content of the total amount of phenolic compounds to analyze the efficiency of the head stage of the technological process.

Development of a Method for Determining the Content of The Sum of Phenolic Compounds

Solution of CO chlorogenic acid. A precise load of CO chlorogenic acid (about 0.01 g) is placed in a 50

ml volumetric flask, the volume of the solution is brought to the mark with 70% alcohol and mixed. Place 1 ml of the first solution in a 25 ml volumetric flask and bring the volume of the solution to the mark with 70% alcohol, mix. The solution is used freshly prepared. Tested solution. 1.0 g of dry chopped (no more than 3 mm) herb *I. Viscosa* is placed in a conical flask of 250 ml with a section. Add 50 ml of 70% alcohol, add to the return refrigerator and heat in a boiling water bath for 1 hour (after boiling). The mixture is cooled, filtered into a 50 ml volumetric flask through a paper (blue ribbon) pleated filter, and the volume is brought to 70% with alcohol. Place 0.5 ml of the tested solution in a 25 ml volumetric flask, bring it to the mark with 70% alcohol and mix. The optical density of the resulting solution is measured on a spectrophotometer at the absorption maximum at 328±2 nm in the thickness of the absorbing layer of 10 mm. 70% alcohol is used as a reference solution. The optical density of the chlorogenic acid solution is measured.

The content in percentage (X) of phenolic compounds in terms of chlorogenic acid is calculated according to the formula:

$$x = \frac{A \cdot m_0 \cdot 50 \cdot 1 \cdot 50 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 0,5 \cdot 50 \cdot 25 \cdot P \cdot (100 - W)}$$

where A_0 is the optical density of the CO solution of the chlorogenic acid solution;

A is the optical density of the tested solution;

m_0 is the mass of chlorogenic acid, g,

m is the mass of the LRS, g,

W is the weight loss on drying, %

P is the content of the main substance in the CO solution of chlorogenic acid.

The UV spectrum of extraction from elecampane raw materials in the region of 280-350 nm coincides with that of chlorogenic acid rice for analytical purposes, the maximum absorption at 328 nm was chosen.

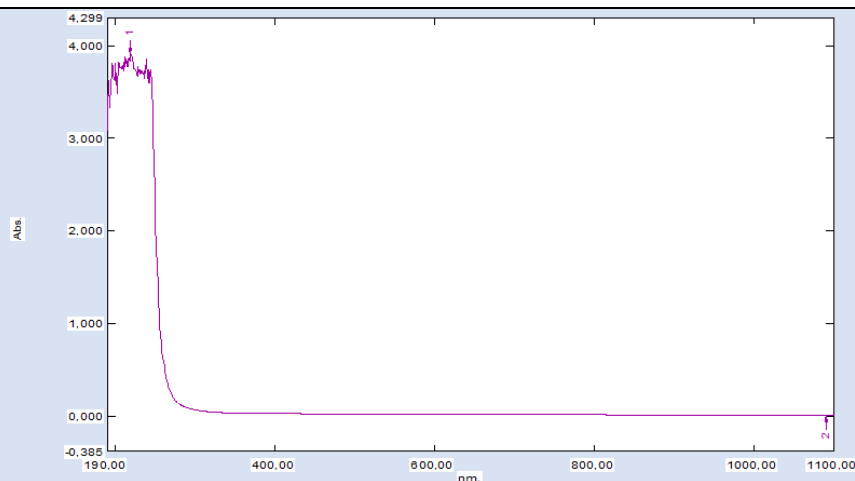


Figure 1: UV spectra of extraction of *I. Macrophylla* and *I. Rhizocephala* from grass (1), and a solution of CO chlorogenic acid (2).

In the studied samples of the herb *I. Macrophylla* and *I. Rhizocephala*, the content of phenolic compounds in terms of chlorogenic acid ranged from 6.87 to 7.63%. According to GPM.1.1.0012.15, validation was carried out for specificity, linearity, correctness and interlaboratory precision. The validation report is presented in Appendix 5. Validation of the developed method for the quantitative determination of phenolic compounds in terms of chlorogenic acid showed the consistency of the results of the analysis of the sum of phenolic compounds in terms of chlorogenic acid. Since the detailed composition of the sum of phenolic compounds by the UPLC-MS method revealed the presence of a significant number of flavonoids that make a significant contribution to the manifestation of anti-inflammatory activity stated in the literature and predicted by *insilico*, a method for quantifying the number of flavonoids in terms of Rutin was developed.

Development of A Method for Determining the Content of The Number of Flavonoids in The Raw Materials of The Herb *I. Macrophylla* and *I. Rhizocephala*

Flavonoids were determined after a complexation reaction with aluminum chloride, which is selective for this group of phenolic compounds and gives a spectrum shift to the long-wavelength region, which makes it possible to measure the content of mainly flavonoids in the presence of other associated groups of phenolic substances in the extraction from the raw material. When comparing the absorption spectra of flavonoid complexes from LRS *I. macrophylla* and *I. Rhizocephala* and RSO Rutin, it was found that in the range of 404-412 nm the absorption maxima coincide, which determined the choice of the analytical wavelength of 410 nm (Figure 2).

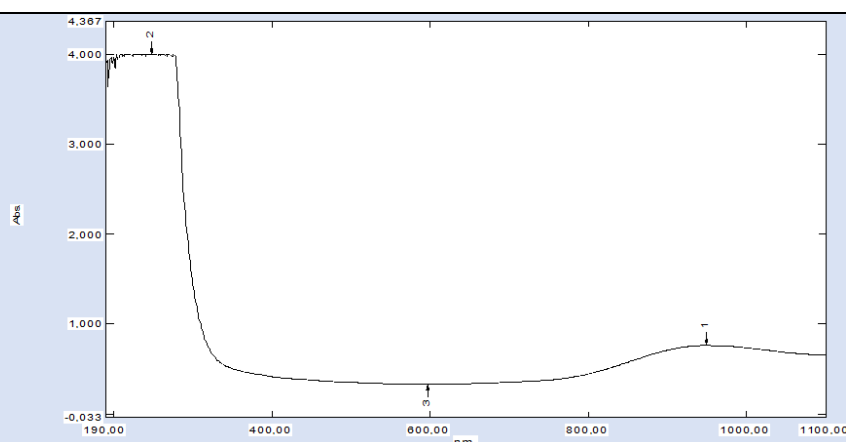


Figure 2: UV spectrum solutions of flavonoid complexes extracted from the herb *I. Macrophylla* and *I. Rhizocephala* (1) and Rutin with the reagent AlCl_3 (2).

Rutin standard sample solution. About 0.2 g (exact weighting) of CO rutin is placed in a 25 ml volumetric

flask, the volume of the solution is brought to the mark with 70% alcohol and stirred (solution A CO

rutin). The shelf life of the solution is 1 month. Place 2.0 ml of A CO rutin solution in a 25 ml volumetric flask, add 3 ml of 3% alcohol aluminum chloride solution, bring it to the mark with 70% alcohol, stir and stand for 40 minutes (B CO rutin solution). Tested solution. 1.0 g of dry raw materials of the herb I. Macrophylla and I. Rhizocephala (no larger than 3 mm), placed in a conical flask of 250 ml with a section, add 50 ml of 70% alcohol, and heat with a reverse refrigerator in a boiling water bath for 1 hour (from the moment the contents boil). The mixture is cooled, filtered into a 50 ml volumetric flask through a paper filter (blue tape) and the volume is brought to the mark with 70% alcohol (Test solution). 2 ml of the tested solution is placed in a 25 ml volumetric flask, 3 ml of 3% alcohol aluminum chloride solution is added, brought to the mark with 70% alcohol, stirred and kept for 40 minutes. Optical density is measured at a wavelength of 410 nm in a cell with 88 with an absorbing layer thickness of 10 mm. A reference solution is prepared: 2 ml of the tested extract is placed in a 25 ml volumetric flask, 0.5 ml of diluted acetic acid is added, brought to the mark with 70% alcohol, stirred and the optical density is measured. In parallel, they measure the optical density of the CO rutin solution prepared similarly to the test extraction solution.

The content of the sum of flavonoids in % in terms of rutin is calculated according to the formula:

$$x = \frac{m_0 \cdot 50 \cdot 2 \cdot 25 \cdot 100 \cdot 100}{A_0 \cdot m \cdot 2 \cdot 100 \cdot 25 \cdot P \cdot (100 - W)}$$

A_0 is the optical density of the CO solution rutin;
 A is the optical density of the solution of the tested extraction;
 m_0 is the weight of CO rutin in g,
 m is the weight of the raw material in g,
 W is the loss in mass during the drying of raw materials,
 P is the content of the main substance in CO rutin.

The content of the amount of flavonoids in terms of rutin in the herb I. Macrophylla and I. Rhizocephala is from 1.9 to 2.2 %, i.e., among phenolic compounds, the share of flavonoids is about 30%. Validation of the developed method for quantitative determination of the number of flavonoids in terms of rutin, in the raw material I. Macrophylla and I. Rhizocephala is presented in the form of a report in Appendix 6. The methods proposed for quantitative assessment of the quality of I. Viscosa herb are validated and allow them to be evaluated positively in all parameters. It can be concluded that the use of the developed methods for assessing the content of the sum of phenolic substances and flavonoids gives results that meet the requirements of regulatory documents for LRS and preparations based on it. Substantiation of the biological activity of ALS I. Macrophylla and I. Rhizocephalainsilico. In order to identify the main ALS of the target fragment of the metabolome I. Macrophylla and I. Rhizocephala, their possible potential targets and pharmacological activity, the PASS web service [9-14] was used, the results of which are presented in Table 10.

Table 10: Insilico prediction of pharmacological activity of ALS identified in I. Macrophylla and I. Rhizocephala.

ALS identified by UHPLC	Main predicted properties according to PASS online	Pa	Pi
Cynarin	Antibacterial, Anti-inflammatory, Antifungal	0,641; 0,629; 0,595	0,007; 0,026; 0,019
Padmatin	Membrane integrity agonist, Antioxidant, Skin whitener, Anti-inflammatory Antiviral	0,967; 0,909; 0,785; 0,691; 0,586	0,002; 0,003; 0,001; 0,017; 0,014
Luteolin 7-O-glucuron	Hemostatic, Membrane integrity agonist, Free radical scavenger, Histamine release inhibitor, Capillary fragility treatment, Antioxidant, Antiprotozoal (Leishmania), Antiviral, Antifungal, Antihemorrhagic Anti-inflammatory	0,980; 0,975; 0,966; 0,893; 0,844; 0,837; 0,798; 0,757; 0,732; 0,704; 0,611	0,001; 0,002; 0,001; 0,003; 0,000; 0,003; 0,005; 0,004; 0,008; 0,003; 0,007
Gispiduline	Chlordecone reductase inhibitor, Membrane permeability inhibitor, Membrane integrity agonist, HIF1A expression inhibitor, Aldehyde oxidase inhibitor Anaphylatoxin receptor antagonist Kinase inhibitor, Antimutagenic, Insulysin inhibitor, Choleric, Histamine release inhibitor, Antiprotozoal (Leishmania) Antioxidant, Antiseptic, Hemostatic, Histamine release stimulant, Anti-inflammatory, Spasmolytic, Antimycobacterial	0,965; 0,950; 0,945; 0,938; 0,930; 0,927; 0,909; 0,906; 0,760; 0,751; 0,725; 0,705; 0,675; 0,660; 0,628; 0,622;	0,002; 0,002; 0,004; 0,004; 0,003; 0,003; 0,003; 0,002; 0,004; 0,003; 0,004; 0,009; 0,004; 0,006; 0,004; 0,007;

		0,627; 0,569; 0,535	0,027; 0,017; 0,015;
Inuviscolide	Antimycotic, Antiprotozoal (Amoeba) Antibacterial	0,643; 0,542; 0,523	0,014; 0,007; 0,014
Chlorogenic acid (3-caffeoylquinic acid)	Membrane Protector, Antioxidant, Antieczematic, Antiproliferative	0,940; 0,809; 0,808; 0,782	0,004; 0,003; 0,017; 0,004
Tomentosin	Antiprotozoal (Leishmania), Antifungal	0,848; 0,621	0,004; 0,016

The screening of the biological activity of these compounds, the presence of which in the extract from elecampane raw materials is confirmed by the literature, showed that they may be carriers of pharmacological effects inherent in drugs from *I. Macrophylla* and *I. Rhizocephala*, known in traditional medicine and confirmed by modern studies.

Determination of The Authenticity of Raw Materials *I. Macrophylla* and *I. Rhizocephala* by UV Spectrophotometry and TLC

In the UV spectrum of extraction (70% alcohol from the LRS *I. macrophylla* and *I. Rhizocephala*), two absorption maxima were recorded at wavelengths of 298 and 329 nm (Figure 3). This circumstance suggests the presence of 72 phenolic compounds in the raw material, in particular phenol carboxylic acids and flavonoids. The obtained absorption spectrum can serve as an additional qualitative characteristic for establishing the authenticity of LRS *I. macrophylla* and *I. rhizocephala*.

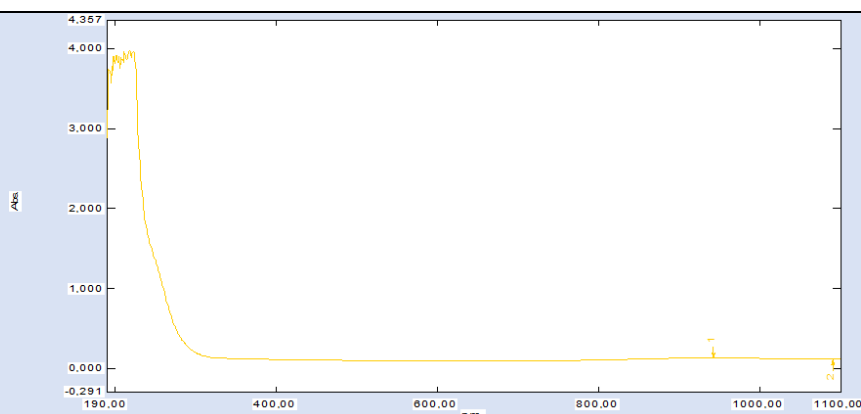


Figure 3: UV Spectrum of Extraction from Raw Materials *I. Macrophylla* and *I. Rhizocephala*.

With the help of TLC, the composition of the ALS of the target metabolome fragment in the raw material *I. viscosa* in the n-butanol-ONION-water system (4:1:1) was studied. Adsorption zones were detected in UV at 365 nm before and after chromatogram treatment with diphenylboryloxyethylamine (1% in methanol) and PEO-400 (5% in methanol) sequentially.

The test extract from LRS was applied to the chromatogram, and the following CO solutions were used as standards: caffeic acid, rutin, gallic and chlorogenic acids in ethanol. Solutions of the standards, on the chromatogram, appear as zones with an Rf of about 0.58 for rutin, with an Rf of about 0.76 gallic acid; with Rf about 0.51 chlorogenic acid and with Rf about 0.75 caffeic acid. Several blue zones (phenolcarboxylic acids) are visible on the chromatogram of the test extraction, the most intense of which have an Rf of about 0.51 and 0.75, which is identical to chlorogenic and caffeic acids, respectively.

Zones of brownish glow merging with zones of blue color, one of which corresponds to the rutin standard, the other is close to the zone of the gallic acid standard. The red zone, chlorophyll, is visible closer to the mobile phase front. After spraying the chromatogram with reagent solutions in daylight, three intensely stained flavonoid zones appear on it: with an Rf of about 0.58 (corresponding to rutin) and with an Rf of about 0.69 and with an Rf of about 0.78 (two unidentified substances). On the chromatogram at a wavelength of 365 nm, the blue-greenish adsorption zones belong to phenolcarboxylic acids, and the orange zones correspond to flavonoids (Rf about 0.58, 0.68 and 0.77). The violet fluorescence on chromatogram 74 has a zone of Adsorption of Co Gallich Asid, Booth You Correspondence Zones, Is Wickley Steined on To Chromatogram of You Tested Extraction, To You of Oter Substance on To Sones.

Thus, based on the study of the composition of biomass, extractives extracted by water and 70% alcohol, both types of raw materials are comparable, but the content of phenolic compounds of about 7% in the grass harvested before flowering confirms the expediency of choosing it as LRS. Also, a comparative study of the two possible types of raw materials made it possible to optimize such significant factors for the technology as the degree of dispersion of raw material particles, the concentration of the extractant, the ratio of raw materials to the extractant and the extraction time. Thus, the study of the extraction of *I. macrophylla* and *I. rhizocephala* from the raw material by the TLC method made it possible to establish the presence of phenolic substances, the prevailing content (judging by the area of the zones and their intensity of staining and fluorescence) among which are phenolcarboxylic acids, in particular, chlorogenic, caffeic and gallic acids, as well as flavonoids (rutin).

Development of The Composition and Technology of The Application Dosage Form with The Extract of The Herb *I. Macrophylla* and *I. Rhizocephala* "Inulan Gel"

Based on the data obtained in the experiments in vivo and in vitro, the presence of pronounced antimicrobial and anti-inflammatory activity of the developed drug "Inulan" was established, which indicates the possibility of developing its application form of gel. Recently, species of the genus *Inula* (in particular, *Inula grandifolia*) have been studied as a source of antioxidants and are used to remove free radicals from the body, in the treatment of diseases of the cardiovascular, nervous system, as well as metabolism [15-18].

Development of The Composition of The Application Form

The development of the composition of the application form with *I. Macrophylla* and *I. Rhizocephala* extracts involved the choice of the RAP polymer grade, the establishment of the concentration for a specific thickener. Taking into account that the extract contains at least 48 wt% alcohol, it is necessary to ensure the stability of the dispersed 106 system and maintain the ALS in a dissolved form, preventing condensation in the presence of an aqueous gel base of the RAP. The use of alcohols as co-solvents will make it possible to obtain LF "Gel" and should not adversely affect the viscosity characteristics acceptable for application to damaged skin (80-120 Pa·s, at 9 rpm). [19-22]. To obtain the gel base of the drug "Inulan", rarely-cross-linked acrylic polymers - RAP - were used. RAP-based gels are the basis of most modern applications used both for transdermal administration of drugs (NSAIDs - Nimulide gel, nice gel, antihistamines - Fenistil, etc.), and for the treatment of the first phase of the wound process (Furagel). The development of the gel composition involves the selection of the polymer grade, the establishment of the working concentration of RAP in the presence of the active substance, as well as the study of the effect of various explosives, in particular, glycerol, PG and PEO-400 alcohols. The presence of alcohols in the composition of various dermatological gels makes it possible to exert a unidirectional effect with the drug on the components of the pathological process and to ensure the physical and chemical stability of the active substances, as well as the microbiological purity of the drug in sufficient quantities Table 11. The results of studying the effect of cosolvents on the viscosity and appearance of gels are presented.

Table 11: Formulations of gel samples with Inulan based on Arespol 1.5%.

Components	Compositions, Content of Components %							
	2	11	12	13	14	15	16	17
Arespol	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5
Trometamol	To Rn 6,5-7,5							
Glycerin	-	10	0	10	0	20	10	10
PG	-	-	10	10	10	-	-	10
Alcohol (70%)	-	-	-	-	10	-	-	-
ПЭО-400					10	10	20	10
Inulan	10	10	10	10	10	10	10	10
Purified Water	Before 100							
Result of the Visual Assessment of The Compositions								

Appearance	Ointment -Like Mass of Brown-Green Color	Pronounced Emulsion (Not Transparent) Systems of Brown-Green Color			Medium Opalescence Brown-Green Gel Systems			
Dynamic Viscosity At 9 Rpm, Pals	100±4,0	90±3,6	90±3,6	80±3,2	40±1,6	60±2,4	50±2,0	80±3,2

Glycerin in dermatological ointments has a moisturizing effect and increases the comfort of use, prevents the drying of the LF. PG is a promoter of absorption and is used in the composition of application drugs in a concentration of up to 60%. PEO-400, as an osmotically active component, is relevant in formulations intended for the treatment of the first phase of the wound process with exudate and the risk of infection. The biopharmaceutical role of solvents was taken into account. The acceptance criteria were considered to be a satisfactory appearance - a transparent homogeneous gel with weak opalescence and dynamic viscosity in the range of 80-120 Pals, at 9 rpm. The results presented in Table 11 indicate a negative effect of alcohols on the

dynamic viscosity of gel samples at an Arespol content of 1.5%. At the same time, the total content of alcohols (PG, PEO-400 and glycerol) from 10 to 30 individually and in combinations does not allow to obtain a transparent, non-opalescent gel. The introduction of ethyl alcohol 70% allows for increased transparency, but leads to a sharp liquefaction (model 14), which excluded it from the samples. Sample 17 was recognized as the most successful according to the combination of the selected criteria. Increasing the content of Arespol to 2.0% increased the viscosity of the gel samples and at the same time the alcohol content to 35-40%, the results are presented in Table 12.

Table 12: Formulations of gel samples with Inulan based on Arespola gel 2.0%.

Components	Compositions, Component Content, %					
	18	19	20	21	22	23
Arespol	2,0	2,0	2,0	2,0	2,0	2,0
Trometamol	To pH 6,5-7,5					
Glycerin	15	20	10	5	5	5
PG	-	-	-	-	30	15
PEO-400	15	20	30	30	-	15
Inulan	10	10	10	10	10	10
Purified Water	To 100					
Appearance	Opalescent gel	Transparent gel with bubbles	Clear liquid gel with bubbles and	Gel with slight opalescence	Non-homogeneous gel	Homogeneous gel with a slight opalescence of brown-green color
Dynamic viscosity at 9 rpm, Pals	65-70	45±1,8	30±1,2	70±2,8	85±3,4	85±3,4

As can be seen from the results of the experiment presented in Table 12. Sample 2, with an alcohol content of 35%, fully met the viscosity criteria and was more transparent than the other samples. It was also interesting to establish the effect of the extract itself

on the appearance and viscosity of the gel form. Table 13 shows the results of the selection of the concentration of Arespol 1.5% or 2.0%, extract 5.0%, 8.0%, 10.0% and alcohols 20-40%.

Table 13: Compositions of samples of gels with Inulin and various polymer and alcohol contents.

Components	Compositions, Component Content, %						
	24	25	26	27	28	29	30
Arespol	1,5	1,5	2,0	2,0	2,0	2,0	2,0
Trometamol	1,5	1,5	2,0	2,0	2,0	2,0	2,0
Gylicern	-	-	5	-	5	5	15
PG	-	10	10	15	15	20	20
PEO-400	-	10	5	15	-	5	10
Alcohol 40%	-	10	-	-	-	-	-
Inula	5	5	5	8	10	8	8
Purified Water	Up to 100						
Result of the visual assessment of the compositions							

Appearance	Opalescent, Homogeneous Gel	Relatively transparent	Transparent gel	Transparent gel	Not homogeneous, with slight opalescence	Not homogeneous, with slight opalescence	Transparent gel
Dynamic viscosity at 9 rpm, Pa.s	70±2,8	85±3,4	160±6,4	100±4,0	180±7,2	180±7,2	100±4,0

The results obtained in Table 13 allow us to consider samples 17 acceptable in appearance. In general, the experiments carried out on the selection of the composition of Inulan gel indicate the wide possibilities for obtaining application LF's based on RAP and require further in-depth substantiation of formulations for various therapeutic tasks. For further

work in the course of this study, the composition (sample 1) of the application form based on Arespol 1.5% and the content of Inulan, glycerol, PG and PEO-400 10% each was selected.

Composition of Inulan-gel per 100.0 g

Components	Content g
Liquid extract of <i>I. viscosa</i>	10,0
Arespol (TU 2219-005-29053342-97)	1,5
Trometamol (LSR-001567/08-140308, 2008)	To pH 6,0-7,0
Glycerin (FS.2.2.0006.15)	10,0
Propylene glycol (Ph.Eur.)	10,0
Polyethylene glycol-400 (TU 2483-007-71150986-2006)	10,0
Purified water (FS.2.2.0020.15)	To 100

Thus, the presence of anti-inflammatory activity of the target fragment of the metabolome of *I. Macrophylla* and *I. Rhizocephala*, which are part of the developed preparation gel "Inulan", was confirmed by inhibition of the rate of the enzymatic reaction catalyzed by iNOS. According to the identified anti-inflammatory activity in working concentrations of the experimental sample, the content of gel "Inulan" in the composition of the application LF for local use should be about 1.0-1.5%.

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