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Research, Scientific, Chemical-Pharmaceutical, Physicochemical Studies of Medicinal Plants and Polymers in the Southern Regions of Tajikistan

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Dried leaves and roots of I nula Rhizocephala macrophylla and I nula are used by people in Tajikistan as traditional medicine to obtain aqueous extracts for topical wound treatment. Given the rich composition of the target metabolome fragment in the scientific literature, Given the high biological activity of I. macrophylla and I. rhizocephala, the development of a liquid extract for the production of an industrial standardized dosage form for topical use was deemed the most feasible approach. The use of the entire leafy shoot of I. macrophylla and I. rhizocephala for the liquid extract was proposed, as a raw material, reducing the labor-intensive stage of industrial medicinal plant material procurement.

Research, Training in Pharmaceutical Chemistry, Physico-Chemical Properties of Medicinal Plants and Polymer Plants in the Southern Regions of Tajikistan

Dried leaves and roots of I. macrophylla and I. rhizocephala are used by the population in Tajikistan as a folk medicine for extracting juice and treating wounds. Based on the scientific literature on the rich content of biologically active substances, the targeted fragment of the metabolome of I. macrophylla and I. rhizocephala, and their high biological activity, the development of a liquid extract to obtain a standardized industrial form of a medicinal product for topical use is appropriate. When obtaining a liquid extract of I. macrophylla

and I. rhizocephala, using the entire plant shoots and leaves as raw materials is a relatively laborious task for the preparation of medicines.

Research, Scientific, Chemical-Pharmaceutical, Physical-Chemical Studies of Medicinal Plants and Polymers in the Southern Regions of Tajikistan

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. 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.

Relevance

Thanks to their prebiotic properties, I. macrophylla and I. rhizocephala have a beneficial effect on intestinal microflora, helping to normalize digestion and combat dysbiosis and constipation. Furthermore, the plant stimulates bile flow, optimizes liver and gallbladder function, and has a beneficial effect on the urinary system. Traditionally, the roots of I. macrophylla and I. rhizocephala are used to treat asthma, bronchitis, cough, diarrhea, nausea, and intestinal parasites. The root extract of I. macrophylla and 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 removal of sputum from the lung tissue, making elecampane an effective remedy for coughs.

Target Research

Physicochemical and biological study of the herbs inula macrophylla and inula rhizocephala.

Material and Methods

The work was carried out at the departments of biochemistry and medical biology of the Khatlon State Medical University, the State Educational Institution "TSMU named after Abuali Ibni Sino, State Institution "Scientific Research Pharmaceutical Center of the Ministry of Health and Social Protection of the Republic of Tatarstan".

Results and Discussion

I. macrophylla and I. rhizocephala raw materials . The results of an analysis of traditional medicine data from Tajikistan, an assessment of the possible chemotherapeutic and pharmacological activities of the biologically active substances contained in the plant's metabolome, and our own phytochemical studies of the raw materials demonstrated the potential for medicinal use of I. macrophylla and I. rhizocephala herbs. The use of this raw material for the development of a technology for producing a liquid extract from it served as the basis for developing methods for establishing the authenticity and quality indicators of the dried herb of the plant. The draft regulatory document for I. macrophylla and I. rhizocephala herbs was developed in accordance with the requirements of the State Pharmacopoeia of the Russian Federation, 13th edition, for medicinal plant materials of a similar morphological group.

Evaluation of the Content of Extractive Substances in the Grass of I. Macrophylla and I. Rhizocephala

as extractants. Comparative studies were also conducted with another type of elecampane raw material—herb collected during flowering. The results revealed the optimal extractant. are presented in Table 1.

Table 1: Content of Extractive Substances Extracted by different Solvents in Samples of I. Macrophylla and I. Rhizocephala Grass.

Extraction agent	**Content of extractive substances in two types of raw materials I. macrophylla and I. rhizocephala, %**	
	Grass collected during the flowering phase	Grass collected 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 data presented in Table 1 demonstrate that extraction with water and 40% alcohol yields maximum extractive substances, which is associated with the intensive transfer of polar substances. Extraction of raw materials with water- ethanol mixtures with high alcohol concentrations also results in the transfer of some hydrophilic compounds, along with less polar representatives of the plant's metabolome. This, according to literature data, suggests that they contain active substances [1-4].

To study the content of target biologically active substances (BAS) in I. macrophylla and I. rhizocephala raw materials. Phenolic compounds are known to be the most significant group of BAS responsible for the antioxidant effects of medicinal plant materials. Among phenolic compounds, flavonoids are responsible for the biological effects described in the scientific literature. To rapidly determine the active ingredients, the phenolic content was determined as chlorogenic acid using the UV- SFM method (Table 2).

Table 2: The 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 collected during the flowering phase	Grass collected 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 is evident that the phenolic content in the grass collected before flowering is almost twice as high, and their maximum content is observed when extracted with 70% alcohol.

Table 3: The Influence of the Raw Material: Extractant Ratio on the Extraction of Phenolic Substances from the Medicinal Plant Materials of I. Macrophylla and I. Rhizocephala

Ratio of raw material:extractant alcohol 70%	Phenolic content in terms of chlorogenic acid	
	Grass collected during the flowering phase	Grass collected before flowering
1:5	2.0 55 ±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 herbs collected before flowering, and is maximal at a raw material: extractant ratio of 1:10. As the ratio increases, their yield is practically insignificant for the technology of producing the preparations.

When studying the influence of fineness for the analysis of raw materials of I. macrophylla and I. rhizocephala, we used raw material particles passing through a sieve with openings of 0.2; 0.5; 1; 2 and 3 mm.

Table 4: The Influence of the Degree of Raw Material Grinding on the Extraction of Phenolic Substances

Extraction time, min	Content of phenolic substances in terms of chlorogenic acid, %	
	Grass collected during the flowering phase	Grass collected 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

The results presented in Tables 4–5 indicate that the phenolic content of I. macrophylla and I. rhizocephala collected before flowering is more than twice that of I. macrophylla and I. rhizocephala collected during flowering. Since phenolic compounds are one of the target groups of BAS, the appropriateness of selecting I. macrophylla and I. rhizocephala collected before flowering as a raw material and subject for further research is confirmed .

Thus, the optimal conditions for the extraction of phenolic compounds from the medicinal plant raw materials of I. macrophylla and I. rhizocephala are: the ratio of raw material and extractant is 1:10, the extractant – 70% ethanol, grinding degree 0.2–3.0 mm, extraction time – 60 min. The obtained data are necessary for the development of methods for the quantitative determination of biologically active substances in medicinal plant materials.

Qualitative Reactions of I. Macrophylla and I. Rhizocephala

To select the most reliable qualitative reactions for establishing the authenticity of I. macrophylla and I. rhizocephala herbs , several extracts were prepared from the raw materials—using water (aqueous extraction) and different concentrations of aqueous-alcoholic mixtures. Qualitative reactions were conducted with the resulting extracts to confirm the literature data on the content of target groups of biologically active substances (BAS) 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 Biologically Active Substance Groups Using Qualitative Reactions in Various Extracts from Raw Materials I. Macrophylla and I. Rhizocephala

Type of reaction	The result of the reaction	Extraction agent			
		Water	40% alcohol	70% alcohol	90% alcohol
Flavonoids					
Briand's cyanidin test	Separation into two phases. The aqueous layer produces more intense coloration.	++	++	+++	+++
		Predominance of glycosides over aglycones			
With a solution of iron (III) chloride	Black and green coloring	+	+	++	++
With an alcohol solution of 10% alkali	Yellow coloring	++	++	++	++
Tannins					
With protein solution	Manifestation of turbidity	+++	++	++	+
With ferric 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 number of "+" indicates the intensity of coloring, sediment and the size of the foam column.

Qualitative tests for the presence of alkaloids in I. macrophylla and I. rhizocephala raw materials were conducted using general alkaloid reagents. The results obtained from this study are presented in Table 6.

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

Extraction agen	Name of the general alkaloid reagent / result					
	Precipitation 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%	+++	++	++	-	++	++

Grass Numerical Values I. macrophylla and I. rhizocephala.

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

Table 7: Fractional Composition of Particles of Crushed Grass I. Macrophylla and I. Rhizocephala

Raw material particles remaining on a sieve with holes of 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 passing through a sieve with holes of 0.1 mm	1.84±0.04

Table 7 data show that 90 % of the crushed I. macrophylla and I. rhizocephala raw materials are larger than 2 mm but not smaller than 0.5 mm. The fraction of fine and dust-like particles in the crushed raw materials is approximately 10%.

The results of determining some numerical indicators of raw materials I. macrophylla and I. rhizocephala are presented in Table 8.

As can be seen from Table 8, the moisture content of I. macrophylla and I. rhizocephala grasses did not exceed 6%, which corresponds to a maximum of 14% for dried 83 official raw materials of this morphological group. The total ash content of the grasses is approximately 10%, and the ash content 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
General ash	1 1, 34 ±0.4
Ash insoluble in 10% HCl solution,	0.745 ± 0.02

As a result of conducting research on the presence of heavy metals and arsenic in raw materials, as well as residual amounts of pesticides, 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

Determined indicators	Grass I. macrophylla and I. rhizocephala	Content standards, no more than...
Heavy metals, mg/kg		
cadmium	0.0 48 ±0.006	1.0
mercury	0.018±0.004	0.1
lead	0.7 25 ±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

I. macrophylla and I. rhizocephala herbs for compliance with safety standards for "Residual pesticide content" and "Heavy metal and arsenic content" showed that the raw materials meet the requirements for the purity of medicinal plant materials in the State Pharmacopoeia of the Russian Federation, 13th edition. When studying the developmental characteristics of I. macrophylla and I. rhizocephala, foreign scientists found that sticky elecampane accumulates heavy metals and arsenic in its underground parts, but such elements do not penetrate the aboveground parts of the plant [5-8]. Based on this, it was proposed to use sticky elecampane to cleanse 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

I. macrophylla and I. rhizocephala as active ingredients. – phenolic compounds, particularly flavonoids . Results from a biologically active substance (BAS) study of the target low-molecular-weight metabolome fragment confirm their presence in the studied raw materials. During the development of a liquid extract from I. macrophylla and I. rhizocephala herbs, the content of all phenolic compounds was assessed to analyze the effectiveness of the initial stage of the technological process.

Development of a Method for Determining the Content of Phenolic Compounds

Chlorogenic acid solution. An accurately weighed sample of chlorogenic acid solution (approximately 0.01 g) is placed in a 50 ml measuring flask, diluted to the mark with 70% alcohol, and mixed. Place 1 ml of the first solution in a 25 ml measuring flask, dilute to the mark with 70% alcohol, and mix. Use the solution freshly prepared. Test solution: 1.0 g of dry, crushed (no more than 3 mm) herb I. Place the viscosa in a 250 ml conical flask with a ground-glass joint. Add 50 ml of 70% alcohol, attach to a reflux condenser, and heat in a boiling water bath for 1 hour (after boiling). Cool the mixture, filter it into a 50 ml measuring flask through a pleated paper filter (blue tape), and make up to the mark with 70% alcohol.

Place 0.5 ml of the test solution in a 25 ml measuring flask, dilute to the mark with 70% alcohol, and mix. The optical density of the resulting solution is measured spectrophotometer at the absorption maximum at 328 ± 2 nm in a 10 mm thick absorbing layer. 70% alcohol is used as the reference solution.

chlorogenic acid solution is measured. The percentage content (X) of phenolic compounds, calculated as chlorogenic acid, is calculated using the formula:

$$x = \frac{A \cdot mo \cdot 50 \cdot 1 \cdot 50 \cdot 100 \cdot 100}{A0 \cdot m \cdot 0{,}5 \cdot 50 \cdot 25 \cdot P \cdot (100 - W)}$$

where A0 is the optical density of the solution of chlorogenic acid solution;
A - optical density of the test solution;
m0 is the mass of chlorogenic acid, g,
m - mass of medicinal plant material, g,
W - loss in mass on drying, %
P is the content of the main substance in the solution of chlorogenic acid.

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

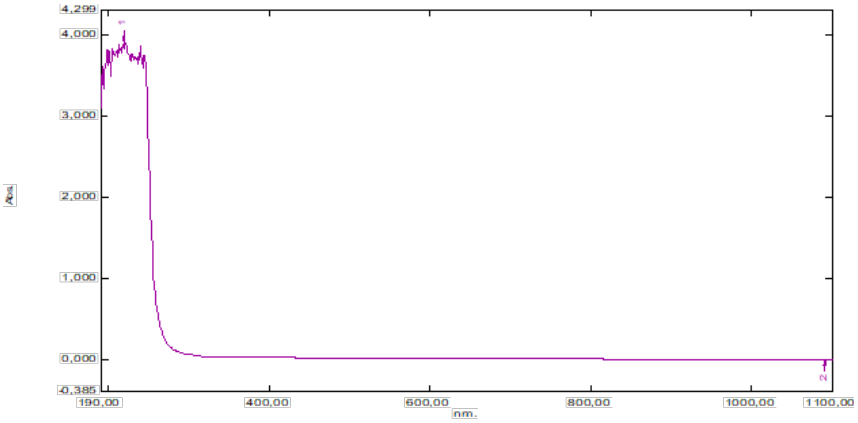

Figure 1: UV spectra of the extract from the grass of I. macrophylla and I. rhizocephala (1), and a solution of chlorogenic acid (2)

In the studied samples of I. Macrophylla and I. Rhizocephala herbs, the content of phenolic compounds in terms of chlorogenic acid ranged from 6.87 to 7.63%.

According to OFS.1.1.0012.15, validation was carried out for specificity, linearity, accuracy and intra-laboratory precision. The validation report is presented in Appendix 5. Validation of the developed method for the quantitative determination of phenolic compounds calculated as chlorogenic acid demonstrated the consistency of the results of the analysis of the total phenolic compounds calculated as chlorogenic acid.

Since the detailed analysis of the composition of the sum of phenolic compounds by the UHPLC-MS method revealed the presence of a significant amount of flavonoids that make a significant contribution to the manifestation of anti-inflammatory activity, as stated in literary sources and predicted in silico, a method for the quantitative determination of the sum of flavonoids in terms of rutin was developed.

Flavonoid Content in the Raw Materials of I. Macrophylla and I. Rhizocephala Herbs

Flavonoids were determined after a complexation reaction with aluminum chloride, which is selective for this

group of phenolic compounds and produces a spectrum shift to the long-wave region, which allows one to measure the content of predominantly flavonoids in the presence of other accompanying groups of phenolic substances in the extract from the raw material.

When comparing the absorption spectra of flavonoid complexes from the medicinal plant raw materials of I. macrophylla and I. rhizocephala and the RSO of 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 (Fig. 2)

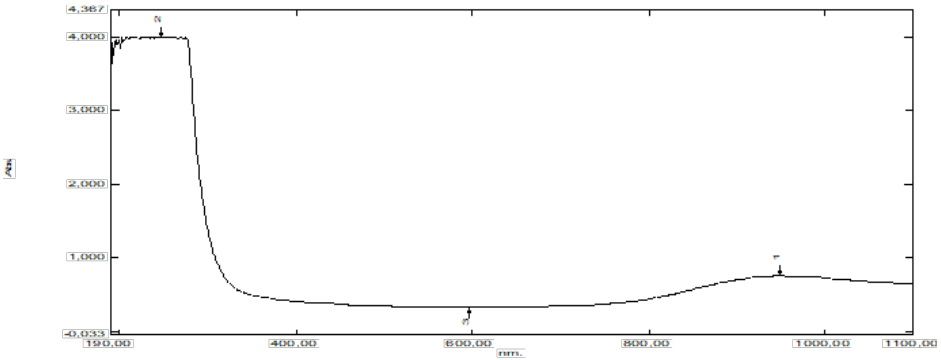

Figure 2: UV spectra of solutions of flavonoid complexes extracted from the herbs of I. macrophylla and I. rhizocephala (1) and rutin with the AlCl3 reagent (2)

Rutin Standard Sample Solution. Place approximately 0.2 g (accurately weighed) of rutin standard sample in a 25 ml measuring flask, dilute to the mark with 70% alcohol, and mix (rutin standard sample solution A). The shelf life of the solution is 1 month.

Place 2.0 ml of solution A of rutin SO into a 25 ml measuring flask, add 3 ml of 3% alcohol aluminum chloride solution, bring to the mark with 70% alcohol, mix and let stand for 40 minutes (solution B of rutin SO).

Test Solution: 1.0 g of dried I. macrophylla and I. rhizocephala grass (no more than 3 mm in size) is placed in a 250 ml conical flask with a ground-glass joint. 50 ml of 70% alcohol is added, and the mixture is heated under reflux in a boiling water bath for 1 hour (from the moment the contents begin to boil). The mixture is cooled, filtered into a 50 ml measuring flask through a paper filter (blue tape), and the volume is made up to the mark with 70% alcohol (test solution).

Two milliliters of the test solution are placed in a 25-mL volumetric flask, 3 mL of 3% aluminum chloride solution in alcohol is added, the solution is made up to the mark with 70% alcohol, mixed, and left for 40 minutes. The optical density is measured at a wavelength of 410 nm in an 88-gauge cuvette with a 10- mm absorbance layer. A reference solution is prepared: two milliliters of the test extract are placed in a 25-mL volumetric flask, 0.5 mL of dilute acetic acid is added, the solution is made up to the mark with 70% alcohol, mixed, and the optical density is measured. At the same time, the optical density of a rutin standard solution, prepared in the same way as the test solution, is measured.

The content of the total flavonoids in %, calculated as rutin, is calculated using the formula:

$$x = \frac{\cdot\ m0 \cdot 50 \cdot 2 \cdot 25 \cdot 100 \cdot 100}{A0 \cdot m \cdot 2 \cdot 100 \cdot 25 \cdot P \cdot (100 - W)}$$

A0 is the optical density of the solution of rutin solution;
A - optical density of the solution of the test extract;
m0 is the mass of rutin CO in g,
m is the mass of the raw material sample in g,
W - loss in mass during drying of raw materials,
P is the content of the main substance in the rutin solution.
The content of total flavonoids in terms of rutin in the herb of I. macrophylla and I. rhizocephala is from 1.9 to 2.2%, i.e. among phenolic compounds, the proportion of flavonoids is about 30%.

The validation of the developed method for the quantitative determination of the amount of flavonoids in terms of rutin in the raw materials of I. Macrophylla and I. Rhizocephala is presented in the form of a report in Appendix 6.

The methods proposed for quantitatively assessing the quality of I. viscosa herb have been validated and yield positive results across all parameters. It can be concluded that the use of the developed methods for assessing the total phenolic and flavonoid content yields results that comply with regulatory requirements for medicinal plant materials and products containing them.

Substantiation of the Biological Activity of I. Macrophylla and I. Rhizocephala in Silico

The conducted screening of the biological activity of the indicated compounds, the presence of which in the extract from elecampane raw materials is confirmed by literature data, showed that they can be carriers of the pharmacological effects inherent in the remedies from I. macrophylla and I. rhizocephala, known in traditional medicine and confirmed by modern research.

Establishing the Authenticity of I. Macrophylla and I. Rhizocephala Raw Materials by UV Spectrophotometry and TLC Methods

The UV spectrum of the extract (70% alcohol from I. macrophylla and I. rhizocephala medicinal plant raw materials) showed two absorption maxima at wavelengths of 298 and 329 nm (Fig. 3). This suggests the presence of 72 phenolic compounds, particularly phenolic carboxylic acids and flavonoids, in the raw material. The resulting absorption spectrum can serve as an additional qualitative characteristic for establishing the authenticity of I. macrophylla and I. rhizocephala medicinal plant raw materials.

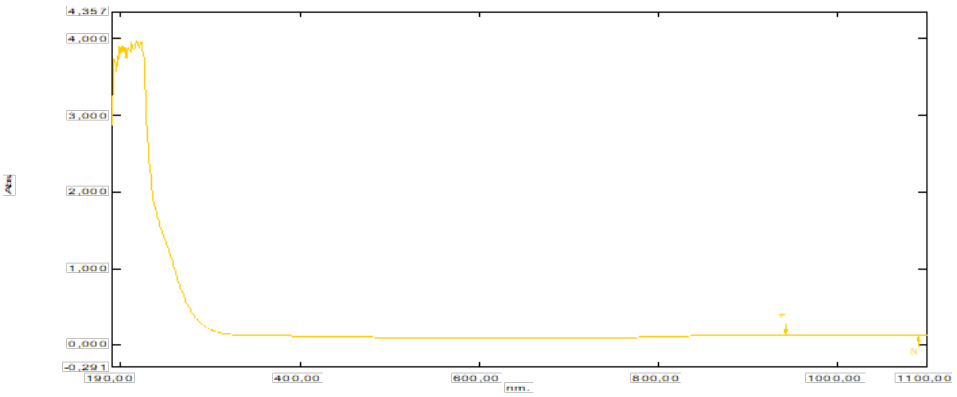

Figure 3: UV spectrum of the extract from I. macrophylla and I. rhizocephala raw materials

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

To the chromatogram, using the following standard solutions: caffeic acid, rutin, gallic acid, and chlorogenic acid in ethanol. The standard solutions appear on the chromatogram as zones with an Rf of about 0.58 for rutin, an Rf of about 0.76 for gallic acid, an Rf of about 0.51 for chlorogenic acid, and an Rf of about 0.75 for caffeic acid. The chromatogram of the tested extract shows several blue zones (phenolic carboxylic acids), the most intense of which have an Rf of about 0.51 and 0.75, which is identical to chlorogenic and caffeic acids, respectively. Brownish glow zones merging with blue zones, 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 front of the mobile phase.

After spraying the chromatogram with reagent solutions in daylight, three intensely colored zones of flavonoids appear: with an Rf of about 0.58 (corresponding to rutin), and with an Rf of about 0.69 and an Rf of about 0.78 (two unidentified substances). In the chromatogram at a wavelength of 365 nm, the blue-greenish adsorption zones belong to phenolic acids, and the orange zones correspond to flavonoids (Rf of about 0.58, 0.68 and 0.77). The violet fluorescence in the chromatogram is due to the adsorption zone of CO gallic acid, but the corresponding zone in the chromatogram of the test extract is weakly colored due to the overlap with the zones of other substances.

Thus, based on a study of the biomass composition and extractive substances extracted with water and 70% alcohol, both raw materials are comparable. However, the phenolic compound content of approximately 7% in the herb collected before flowering confirms its suitability as a medicinal plant raw material. Furthermore, a comparative study of the two possible raw materials allowed for the optimization of such significant technological factors as the degree of dispersion of the raw material particles, the concentration of the extractant, the raw material-to-extractant ratio, and the extraction time.

Thus, the study of the extract from the raw materials of I. macrophylla and I. rhizocephala by the TLC method made it possible to establish the presence of phenolic substances, the predominant content of which (judging by the area of the zones and their intensity of coloration and fluorescence) are phenolic carboxylic acids, in particular chlorogenic, caffeic and gallic, as well as flavonoids (rutin) [9-14].

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