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Quantitative And Qualitative Analysis Of A Liquid Extract Obtained On The Basis Of Wormwood

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Abstract: ***The work is devoted to the development of the technology of composition and the study of the chemical composition of the liquid extract based on bitter wormwood - Artemisia Absinitum L. grown in the territory of Uzbekistan. The addition of tansy and pumpkin seeds to the composition of wormwood makes it possible to obtain liquid dosage forms with an enhanced anthelmintic effect. The study used methods of spectrophotometry, thin layer chromatography, qualitative chemical analysis, ionization plasma analysis, etc. The liquid extract was standardized by quantitative determination of flavonoids. A number of factors have been identified that influence the optimal technology for obtaining a liquid extract from plant materials. A method has been developed for the quantitative determination of the amount of flavonoids using spectrophotometry in the wavelength range of 350-450 nm. The content of the sum of flavonoids in extracts varies in terms of a standard sample of rutin in the range from 1.2860 to 1.5871% (mg / ml). For elemental analysis, the method of inductively coupled plasma mass spectrometry was used, on the basis of which important macro- and microelements were found in the composition of the liquid extract.***
The studied indicators of the microbiological purity of the extract comply with the requirements of the State Pharmacopoeia XI issue 2.

Key words: bitter wormwood, tansy, pumpkin seeds, spectrophotometry, extractant, method, extract, technology, flavonoids, macro-elements, micro-elements, microbiological purity, quantity.

1. INTRODUCTION

Artemisia absinthium L. is known in scientific and folk literature as bitter wormwood, a perennial shrub that grows in large numbers in Central Asia. The herb is used as an antimicrobial, anti-inflammatory, antiviral, hypoglycemic, wound-healing, insecticidal agent, and is also used to treat a number of diseases in medicine. Comparative study of Artemesia Armeniaca Lam., Artemisia Latiffollalideb. and Artemisia Absinthium L. showed the presence in all types of polysaccharides, tannins, alkaloids, saponins, vitamins and flavonoids. In all objects tannin, gallic, chlorgenic and caffeic acids were found. Proline, valine, leucine and essential oils were identified from the sum of the amino acid composition [1]. The review [2] showed that bitter wormwood has antioxidant, antifungal, antimicrobial, anthelmintic, antiulcer, anticarcinogenic, hepatoprotective, neuroprotective, antidepressant, analgesic, immunomodulatory and cytotoxic effects. With prolonged use of essential oil, toxic and mental disorders with some clinical manifestations are observed. The chemical composition and

components of bitter wormwood, extracts in various solvents are of interest in medicine and biology [3-5]. The influence of dry wormwood and mallow on gastrointestinal parasites of small ruminants Haemonchus contortus was evaluated. Aqueous extracts of both plants showed a strong ovicidal effect on H. contortus in in vitro tests [6-7]. Artemisia absinthium L. has found application as an anthelmintic agent and as an insectoacaricide against ticks [8,9].

Preparations of tansy have antihelminthic (against ascaris and pinworms), anti-lambliosis, choleretic. antispasmodic and astringent action. They increase appetite, acidity of gastric juice, improve food digestion, have a positive effect on the metabolic functions of the liver in experimental hepatitis, have a bactericidal and bacteriostatic effect, and have insecticidal properties. There is evidence of the effectiveness of tansy - Tanacetum vulgare as an anti-inflammatory, antimicrobial and antihelminthic plant [10-12]. Pumpkin seeds are active against tapeworms (bovine, pork, dwarf tapeworms, broad tapeworm, etc.). The anthelminthic component is believed to be found primarily in a thin gray-green shell inside the pumpkin seed. The antihelminthic effect of pumpkin seeds against various parasites has been revealed [13-15].

Usually the addition to bitter wormwood of other medicinal plants such as tansy, pumpkin seeds is observed to increase the antihelminthic effect. In this regard, we obtained a liquid extract from wormwood, tansy and pumpkin seeds.

The aim andtask. The aim of the study was to obtain a liquid extract from bitter wormwood, tansy flowers and pumpkin seeds with control of the active ingredients in the liquid extract.

Objective of the study. The objects of research were the grass and leaves of wormwood, tansy flowers, pumpkin seeds grown in Uzbekistan.

2. MATERIALS AND METHODS

The extracts were obtained by the percolation method. Bitter wormwood: tansy flowers: pumpkin seeds in the extraction process were taken at a ratio of 1: 1: 1. Determination of the content of active substances in the extract was carried out according to the developed method, the SF method (UV spectrophotometer "Agilent Technology-8253", Germany). The determination of the dry residue was carried out according to the method described in the general article "Tinctures" (GF XI, issue 2, p. 148). Determination of the ethyl alcohol content was carried out according to the boiling point of the tincture and with a portable density meter ("Densito 30PX" METTLER TOLEDO, Switzerland). The method of quantitative determination of micro and macroelements was carried out by the method of inductively coupled plasma mass spectrometry (ICP-MS). Methods for testing microbiological purity were carried out according to the given method (GF XI issue 2. Change 2. Category 3 B).

EXPERIMENTAL PART

Herbs of wormwood and tansy flowers were ground to 5 mm, and pumpkin seeds to 0.5 mm. In order to establish the optimal concentration of the extractant, extracts were obtained using ethyl alcohol in 40%, 70% and 90% concentrations. The obtained extracts were studied in terms of dry residue and alcohol content according to the methods given in the normative and technical documentation.

The authenticity of the extracts was determined according to the following procedure: 10 ml of the preparation is evaporated in a porcelain cup in a water bath to 1 ml, 0.2 ml of ammonia solution, 0.1 g of a mixture of calcium oxide and talc (1: 1) are added and stirred until a powdery mass is obtained, which is treated with 10 ml of ether. The ether extracts are placed in a porcelain cup, the ether is distilled off to dryness. To the residue is added 0.5 ml of concentrated sulfuric acid; a brownish-red color appears, which turns into violet (glycosides) when 95% alcohol is added.

To 10 ml of the preparation was added 10 ml of water, mixed thoroughly, then shaken in a separatory funnel for 3 min with diethyl ether 2 times in portions of 10 ml. After separation of the layers, the ether extract was decanted. The first ether extract was discarded. The second ether extract was carefully evaporated in a water bath under vacuum until the solvent was completely removed. The remainder of the ether extract was dissolved in 2 ml of 95% alcohol, and 2 ml of concentrated hydrochloric acid were carefully added; then heated gently. The green color of the liquid gradually turns into red-brown (sesquiterpene lactones of the azulene series).

1 ml of the preparation was diluted with water to 1000 ml. 1 ml of the resulting solution is again diluted with water to 40 ml (1: 4000); 10 ml of this solution, when rinsed slowly, produces a bitter sensation (bitterness).

For the quantitative analysis of flavonoids in the obtained extracts, the UV spectrophotometric method was used. The flavonoid rutin was used as a standard, the absorption maximum of which most closely matches the absorption maximum of the complex with aluminum chloride in the test sample. The optical density was measured on an Agilent Technology-8253 UV spectrophotometer (Germany) in the wavelength range of 350–450 nm in cuvettes with a layer thickness of 10 mm.

Preparation of the test solution. 1 ml of the test preparation was placed in a 25 ml volumetric flask, 5 ml of 96% ethyl alcohol, 5 ml of a 5% solution of aluminum chloride in 70% ethyl alcohol were added. After 10 min, 2 ml of a 5% solution of acetic acid in 70% ethyl alcohol was added, the volume of the solution was brought to the mark with 70% ethyl alcohol, and the mixture was stirred.

After 30 min, the optical density of the resulting solution was measured on a spectrophotometer at a wavelength of 408 nm in a cuvette with a layer thickness of 10 mm, using as a compensation solution - a solution that consists of 1 ml of the drug, 5 ml of 96% ethyl alcohol and 2 ml of a 5% solution of acetic acid. acid in 70% ethyl alcohol, which is placed in a 25 ml volumetric flask and brought to the mark with 70% ethyl alcohol.

Preparation of a working standard sample (RSS) solution. Approximately 25 mg (accurately weighed) of RSO rutin, dried at 135 ° C to constant weight, was placed in a 100 ml volumetric flask, dissolved in 80 ml of 96% ethyl alcohol by heating in a water bath, after cooling, the volume of the solution was adjusted with the same alcohol ethyl 96% to the mark and mixed.

In parallel, after 30 min, the optical density of the solution was measured, which contains 1 ml of a reference solution (a solution of a standard sample of rutin), prepared similarly to the test solution, using as a compensation solution - a solution that consists of 1 ml of a reference solution, 2 ml of a 5% solution of acetic acid in 70% ethyl alcohol, which is placed in a 25 ml volumetric flask and brought up to the mark with 70% ethyl alcohol.

The content of the sum of flavonoids (X) in the preparation when recalculated for rutin in milligrams in 1 ml was calculated by the formula:

$$X = \frac{A \cdot m_0 \cdot P \cdot 1 \cdot 25}{A_0 \cdot 100 \cdot 100 \cdot 25 \cdot 1} = \frac{A \cdot m_0 \cdot P}{A_0 \cdot 10000}$$

Where:

A - is the optical density of the test solution;

A_0 is the optical density of the reference solution;

m_0 — weight of a sample of rutin CO, in mg;

P - content of rutin in CO, marked in the quality certificate, in percentage.

The determination of the content of flavonoids in the composition of the liquid extract was evaluated by chromatography. On the starting line of a 5x15 cm chromatographic plate, 0.02 ml of the test solution and 0.02 ml of the test solution and 0.01 ml of a standard solution of rutin are applied as a dot. The plate is placed in a chamber (pre-saturated for 3 hours) with

a mixture of solvents benzene-ethyl acetate in a ratio of 3: 1 and chromatographed in an ascending manner. When the front of solvents passes 13 cm from the start line, the plate is removed from the chamber, dried under traction for 30-35 minutes and viewed in UV light, sprayed with a 2% alcohol solution of aluminum chloride. In the chromatogram of the test solution, in addition to the main adsorption zone, the presence of additional blue adsorption zones with Rf about 0.64 and two dark spots with Rf 0.22 and 0.44 is allowed.

Preparation of the test solution. 2 ml of the test drug was placed in a 25 ml volumetric flask, 4 ml of a 2% solution of aluminum chloride in 95% ethanol, 1 drop of diluted acetic acid were added, the volume of the solution was brought to the mark with 95% ethyl alcohol and stirred.

Preparation of a working standard sample (RSS) solution. Approximately 0.05 g (accurately weighed) of PCO rutin, pre-dried at 135 ° C for 3 hours, is dissolved in 85 ml of 95% ethanol in a 100 ml volumetric flask with heating in a water bath, cooled, quantitatively transferred into a volumetric flask with a capacity 100 ml, bring the volume of the solution to the mark with the same alcohol and mix.

Method for quantitative determination of micro and macro elements by inductively coupled plasma mass spectrometry (ICP-MS). 0.0500-0.5000 g accurately weighed the test sample is weighed on an analytical balance and transferred to Teflon autoclaves. Then, the autoclaves are filled with a corresponding amount of purified concentrated mineral acids (nitric acid (chemically pure) and hydrogen peroxide (chemically pure)). The autoclaves are closed and placed on a Berghof microwave digester with MWS-3 + software. After decomposition, the contents in the autoclaves are quantitatively transferred into 50 or 100 ml volumetric flasks and the volume is adjusted to the mark with 0.2% nitric acid. The determination of the substance under study is carried out on an ICP MS instrument or a similar instrument, the optics of an emission spectrometer with an inductively coupled argon plasma. In the construction of the sequence of analyzes, indicate the amount in mg and the degree of its dilution in ml. After receiving the data, the true quantitative content of the substance in the test sample is automatically calculated and entered by the device in the form of mg / kg or μg / g with error limits - RSD in%.

3. RESULTS AND ITS DISCUSSION

In order to ensure the completeness of the extraction, the medicinal plant raw materials and the extractant were taken at different ratios.

Table 1 shows the dependence of the yield of flavonoids from herb wormwood, flowers of tansy and pumpkin seeds (1: 1: 1) on the concentration of ethanol (40%, 70% and 96%) in the ratio of raw materials and ethanol at 1: 5, 1:10 , 1: 2 (by percolation method).

Table 1. The influence of alcohol concentration, the ratio of raw materials and extractants on the qualitative indicators of extraction (percolation method)

Alcohol concentration, %	**Raw material and extractant ratio**	**Quality indicators extracts**		
		content alcohol,%	**dry residue,%**	**density, (g / ml)**
40%	1:2	32,0	2,0	0,9680
	1:5	34,0	2,35	0,9660
	1:10	28,0	0,79	0,9582
	1:2	64,0	2,31	0,9012

70%	1:5	62,0	2,63	0,9004
	1:10	61,0	1,78	0,9110
96%	1:2	89,0	1,28	0,8367
	1:5	87,0	1,04	0,8277
	1:10	81,0	0,56	0,8146

As shown by the research results, of the studied options, the technology option with the use of 40% and 70% alcohol as an extractant with the percolation method, respectively, turned out to be optimal.

As a result of qualitative reactions, characteristic results were obtained in all studied samples, confirming the presence of glycosides, sesquiterpene lactones of the azulene series, bitterness.

The optimal ratio of raw materials and extractant is 1: 2 and 1: 5, which confirms the quantitative content of extractives in the extraction.

Table 2. Results of determination of flavonoid content by chromatographic method*

№	Extracts	Rf	
1	in 40% ethanol in the ratio 1:10	0,44	-
2	in 40% ethanol in the ratio 1: 5	0,55	-
3	in 70% ethanol in the ratio 1: 2	0,39	0,58
4	in 70% ethanol in the ratio 1: 5	0,54	-
	RSO rutin	0,48	

* solvent system.

When the front of solvents (Table 2) passed 13 cm from the start line, the plate was removed from the chamber, dried under traction for 30-35 minutes and viewed in UV light, sprayed with a 2% alcohol solution of aluminum chloride. A spot corresponding to a routine, a yellow-greenish color with an Rf of 0.48, appeared on the chromatograms of all studied samples. On the chromatogram of the test solution - extract in 70% ethanol in a ratio of 1: 2, in addition to the main adsorption zone, additional blue adsorption zones with Rf about 0.58 and dark spots with Rf 0.39 are allowed. The results of the study showed that the highest content of flavonoids is observed in the process of extraction obtained with the method of percolation in 70% ethanol in a ratio of 1: 2.

The results of the metrological characteristics of the method for quantitative determination of the content of the sum of flavonoids in the liquid extract are shown in Table 3. The quantitative determination of the sum of flavonoids using spectrophotometry in the wavelength range of 350-450 nm has been developed. The content of the sum of flavonoids in extracts varies in terms of a standard sample of rutin in the range from 1.2860 to 1.5871% (mg / ml).

Evaluated and compared in terms of the content of active substances, extracts obtained with different ratios and extractants by the percolation method. The content of active substances in the extracts was determined by UV spectrophotometry. The flavonoid rutin was used as a standard, the absorption maximum of which most closely matches the absorption maximum of the complex with aluminum chloride in the sample under study. The optical density was measured on an Agilent Technology-8253 UV spectrophotometer (Germany) in the wavelength range of 350–450 nm in cuvettes with a layer thickness of 10 mm.

The research results in the selected extracts showed that the highest content of flavonoids is observed in the process of extraction obtained with the method of percolation in 70% ethanol in a ratio of 1: 2. Studies to measure the optical density of active substances showed that UV spectra of CO of rutin and liquid extracts are observed at a wavelength of 408 nm on a spectrophotometer in the wavelength range of 350-450 nm in cuvettes with a layer thickness of 10 mm.

Table 3. Metrological characteristics of the method for quantitative determination of the content of the sum of flavonoids in extracts

X:Y	1:5	1:5	1:5	1:10	1:2
Alcohol concentration,%	40	70	96	40	70
$X_{average}$	0.6909	1.2860	0.4491	0.2491	1.5671
S^2	0.0000	0.0000	0.0000	0.0000	0.0000
S	0.0005	0.0031	0.0005	0.0005	0.0083
S_x	0.0003	0.0018	0.0003	0.0003	0.0048
P,%	98	98	98	98	98
t,(P,F)	6.97	6.97	6.97	6.97	6.97
E	0.5242	1.6847	0.8064	1.4539	3.3113
$\bar{\varepsilon}$	0.3026	0.9727	0.4656	0.8394	1.9118

X-herbal raw materials, U-extractant

Figure 1.2 shows the UV absorption spectra of a standard solution of rutin and extract.

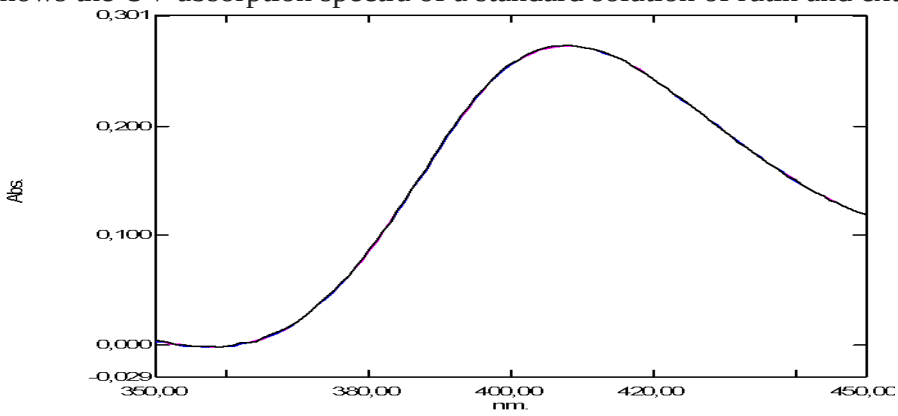

Fig. 1. UV spectrum RSO rutin

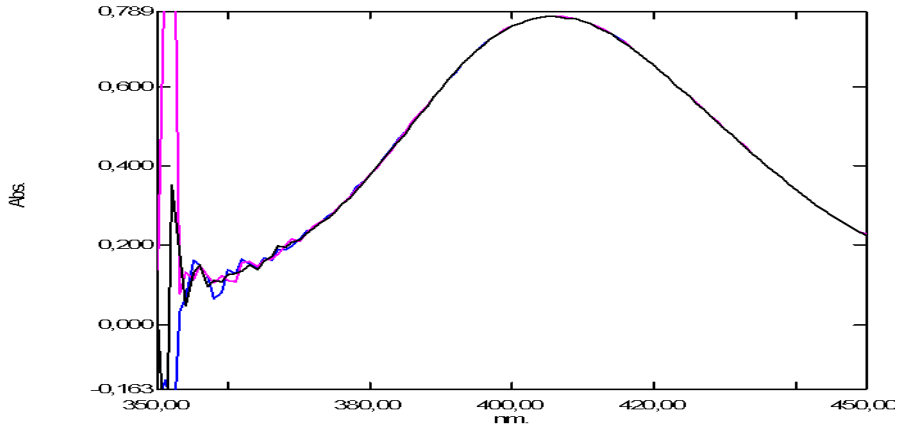

Fig. 2. UV spectrum of liquid extract of wormwood: tansy: pumpkin seeds at a ratio of 1: 1: 1.

The results of the quantitative determination of micro and macroelements by the ISP MC method of a liquid extract obtained in 70% ethanol in the ratio of plant material: extractant 1: 2 (percolation method) are shown in Table 4.

Table 4. Quantification of the content of macro and microelements

Element	**Content in the extract, mg / l**	**Element**	**Content in the extract, mg / l**
Potassium, K	2708,701	Vanadium, V	0,383
Phosphorus, P	330,376	Lithium, Li	0,118
Sodium, Na	217,908	Manganese, Mn	0,087
Silicon, Si	172,799	Silver, Ag	0,029
Magnesium, Mg	159,655	Nickel, Ni	0,031
Calcium Ca	114,771	Bismuth, Bi	0,002
Barium, Ba	26,671	Cobalt, Co	0,002
Aluminum, Al	25,611	Cadmium, Cd	0,001
Iron, Fe	14,644	Lead, Pb	0,001
Sulfur, S	5,678	Uranus, U	0,0006
Boron, B	1,712	Arsenic, As	0,003
Zinc, Zn	1,284	Molybdenum, Mo	0,0002
Selenium, Se	0,320	Germany, Ge	0,0002
Honey, Cu	0,295	Gaul, Ga	0,0004

Analysis of macro- and micronutrients showed that the sample contained a large amount of potassium - 2708.701 mg / l, phosphorus - 330.376 mg / l, sodium - 217.908 mg / l, silicon - 172.799 mg / l, magnesium - 159.655 mg / l, calcium - 114.771 mg / l. , as well as trace elements boron, zinc, copper and selenium.

Therefore, in the development of medicines, and especially of plant origin, an assessment of microbiological risks is a prerequisite. At the same time, given the direct relationship between the safety of a drug and the microbiological indicators of its contamination, it is necessary to

strictly control the quality of microbiological tests, which must be as accurate and reliable as possible.

Determination of the microbiological purity of the liquid extract obtained with the method of percolation in 70% ethanol in a ratio of 1: 2 was carried out in the laboratory of microbiology of the testing center of medical devices of LLC Scientific Center for Standardization of Medicines, according to the method of GF XI issue 2 and change No. 2, Category 3B, according to indicator "microbiological purity". The tests were carried out in sterile box conditions, at a room temperature of 25ºC and an air humidity of 54%. The test results are shown in table 5.

Table 5. Indicators of microbiological purity of liquid extract

№	Norm on ND	Test results	Conclusion
1.	The total number of aerobic bacteria is not more than $10 * ^{4}$ in 1g	40 KOE	Complies with ND
2.	The total number of mushrooms is not more than 200 per 1g	20 KOE	
3.	Enterobacteriaceae and other gr - bacteria no more than 100 per 1g	Absent	
4.	Escherichia coli, Ps aeruginosa, Staph. aureus - must be absent in 1g	Absent	
5.	Salmonella - must be missing at 10 g	Absent	Complies with ND

The liquid extract from bitter wormwood meets the requirements of GF XI, issue 2. Change 2. Category 3B for the above indicators.

A liquid extract from bitter wormwood, tansy flowers and pumpkin seeds are of interest as anthelmintic agents.

4. CONCLUSION

It has been established that the ratio of vegetable raw materials and extractant, alcohol concentration affects the yield of extractive substances during the extraction process. The extracts obtained by various ratios by the percolation method were evaluated and compared in accordance with the normative documentation for the content of active substances, for dry residue, density, alcohol content.

A quantitative determination of the sum of flavonoids using spectrophotometry in the wavelength range of 350-450 nm has been developed. The content of the sum of flavonoids in extracts varies in terms of a standard sample of rutin in the range from 1.2860 to 1.5871% (mg / ml).

The amount of macro and microelements has been determined. Analysis of macro- and micronutrients showed that the sample contained a large amount of potassium - 2708.701 mg / l, phosphorus - 330.376 mg / l, sodium - 217.908 mg / l, silicon - 172.799 mg / l, magnesium - 159.655 mg / l, calcium - 114.771 mg / l. And other important trace elements.

The results obtained can be used to develop extracts with anthelmintic properties.

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