OBTAINING A CHEMICAL COMPOSITION FROM DRY EXTRACT OF ARTICHOKE (CYNARA SCOLYMUS L.L.)

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Abstract*. The article presents data on the study of obtaining a chemical composition from a dry extract of the medicinal plant artichoke spiny, as well as some biological active substances in the dry extract.*

Metrological characteristics of the results of quantitative determination of hydroxycinnamic acids have been developed. The results showed that the dry extract contained hydroxycinnamic acids such as flavonoids, tannins, and amino acids. The amount of hydroxycinnamic acids in terms of chlorogenic acid is 7.12% on average.

Thus, the results obtained indicate a fairly high content of hydroxycinnamic acids in the dry extract. And the content of tannins in the dry extract averaged 6.39%. Quantitative determinations of some biologically active substances by HPLC (High Performance Liquid Chromatography (High Pressure Liquid Chromatography) in the dry extract of prickly artichoke showed a high content of chlorogenic acid, cinaroside and caffeic acid.

Keywords*: dry extract, nucleic acids, biological active substances, hydroxycinnamic acid, chlorogenic acid, caffeic acid, chromatogram.*

Introduction. It is known that the rapid development of the pharmaceutical industry in Uzbekistan has sharply increased the number of enterprises operating in this area, and very large areas have been allocated for sowing medicinal plants. Inexperienced and irregular collection of raw materials of medicinal plants for many years has led to a sharp reduction in the supply of medicinal plants from nature. Some of these medicinal plants even had to be included in the Red Book [1].

This situation naturally raised the problem of supplying raw materials for medicinal plants to rapidly developing pharmaceutical industry of our country.

In addition, pharmaceutical industry enterprises in our country have a great need for raw materials of about 40 types of medicinal plants [2].

However, the pharmaceutical industry of our country uses raw materials of medicinal plants that are imported from abroad, since very little of them are grown in our country. One such plant is the spiny artichoke (CYNARA SCOLYMUS L.L.) belonging to the Atseraceae Dumort family. It is believed that the prickly artichoke is a promising and valuable plant, unusual for Uzbekistan. According to data, the wet weight of Cynara scolymus L.L. contains 18% of protein, 15% of protein, 1.92% of inulin, as well as vitamins and other organic substances necessary for the life and development of living organisms [3].

Rutin, quercetin, luteolin and other substances belonging to the group of flavonoids are synthesized from prickly artichoke raw materials, imported for the pharmaceutical industry of our country. Therefore, the development of technologies for growing this plant is of great theoretical and practical importance. [1,2,3]

The objective. Prickly artichoke (CYNARA SCOLYMUS L.L.) is a relatively new plant for our region. The composition of prickly artichoke is rich in biologically active substances. The

main active ingredients are hydroxycinnamic acids: caffeic, chlorogenic, neochlorogenic and 1,5-di-0-caffeoylquinic acids. According to literature data, the plant contains from 0.1% to 1% of flavonoids.

The dominant flavonoids are lutealin-7-glycoside (cinaroside) and luteolin-7-rutinoside (scolymoside) [1]. Also, the leaves of the prickly artichoke contain a large amount of sesquiterpene lactones, organic acids, in particular malic, citric and lactic acids, potassium salts, etc. [2].

In addition, it is known that the aerial part of the prickly artichoke contains about 3% of protein, from 7 to 15% of carbohydrates, 0.4 mg% of carotene, 3-11 mg% of vitamin C, fats, nucleic acids, vitamins B1, B2, P, PP, K, E, 1.27% of fiber, tannins and minerals. Carbohydrates contain inulin. A difficulty in standardizing plant raw materials and preparations from artichoke is also the choice of various standard substances, for example, dry leaves should contain 1.7-4.2% of ortho-dioxyphenols (in terms of caffeic acid), 0.9-1.4% of phenol carbonates acids (in terms of cynarine) and 0.02-1.4% of caffeylquinic acids (in terms of chlorogenic acid). Medicines based on prickly artichoke exhibit a wide range of pharmacological effects: hepatoprotective, choleretic, diuretic, hypoazotemic, hypocholesterolemic, antiatherosclerotic, hypotensive, laxative, antitoxic, metabolic, normalizing digestive and metabolic functions [1, 2, 3].

The purpose of this work is to study the chemical composition of a dry extract obtained from the leaves of the prickly artichoke grown in Uzbekistan.

Materials and research methods. In appearance, the dry extract is a brown powder with a characteristic odor.

For qualitative identification of the main biologically active substances, the dry extract was dissolved in purified water in a mass-volume ratio of 1:10, slightly heated in a water bath until the powder was completely dissolved.

Qualitative detection of hydroxycinnamic acids was carried out by the paper chromatography method; a 2% solution of acetic acid was used as the mobile phase. The chromatogram was dried in air and examined under UV light. Under UV light, blue fluorescence appeared, indicating the presence of hydroxycinnamic acids [1].

Detection of flavonoids was carried out with sodium hydroxide (NaOH). To 1 ml of an aqueous solution of the dry extract prepared according to the above method, a few drops of sodium hydroxide solution were added. The appearance of a yellow color was observed [1].

When several drops of ferric ammonium alum were added to 1 ml of the test solution, a dark green color appeared, indicating the presence of tannins [4].

Qualitative detection of amino acids was carried out using the ninhydrin reaction. During the analysis, equal volumes of the test solution and a freshly prepared 0.1% ninhydrin solution were mixed and carefully heated. Upon cooling, the appearance of red-violet color was observed, indicating the presence of amino acids [5,6,7].

Quantitative determination of the amount of hydroxycinnamic acids in terms of chlorogenic acid was carried out using the SF method. An exact weighed portion of the dry extract (0.08825 g) was dissolved in 50 ml of 50% ethyl alcohol (solution A). From solution A, 0.5 ml was taken into a 25 ml flask and the volume of the flask was adjusted to the mark with 50% ethyl alcohol. The optical density of the solution was measured on an SF-46 spectrophotometer at a wavelength of 329 ± 2 nm. The reference solution was 50% ethyl alcohol [3].

In parallel, under similar conditions, the optical density of the RSO solution of chlorogenic acid was measured.

Preparation of chlorogenic acid RSO. An exact weighed portion (0.00700 g) of chlorogenic acid was transferred to a 50 ml flask and dissolved with 50% ethyl alcohol. 0.5 ml was taken from the solution into a 25 ml flask and the volume of the flask was adjusted to the mark with 50% ethyl alcohol.

The content of the total hydroxycinnamic acids in percent, in terms of chlorogenic acid, was calculated using the formula:

$$X = \frac{D_1 * m\,0 * C}{D_0 * m1}$$

D_1 - optical density of alcoholic extract from artichoke leaves;

D_0 - optical density of RSO of chlorogenic acid;

m1—weight of dry extract, g;

m0 - sample weight of the standard sample, g;

C – purity of the standard sample, not less than 97%

Quantitative study of some biologically active substances in the dry extract was carried out by reverse-phase HPLC (High Performance Liquid Chromatography (High Pressure Liquid Chromatography)) on an Agilent Technologies 1100 series instrument equipped with a G1379A degasser and a VWD G1314 variable wavelength spectrophotometric detector.

Column Zorbax Eclipse XDB-C8 (4.6x250 mm) with a particle size of 5 μm, precolumn Zorbax Eclipse XDB-C8 (2.1x12.5 mm) with a particle size of 5 μm (SF - detector, column and precolumn manufactured by Agilent Technologies Inc., USA), mobile phase: solution A - 10% acetonitrile in 0.1% phosphoric acid (pH-2.2), solution B - 50% acetonitrile in 0.1% phosphoric acid (pH-2.2).

Separation was carried out using a linear concentration gradient of solution B from 0 to 100% over 25 min. The flow rate was 1 ml/min, the column temperature was room temperature (20ºC), the pressure in the starting conditions of the gradient was from 90 to 120 bar, peak detection was carried out at UV 300 nm. The injection volume per column is 10 μ (table 1).

Table 1.

Preparation of standard samples

№	Substance (standard)	Solvent, dissolution conditions	Initial concentration, μg/ml	Final concentration, μg/ml
1	Chlorogenic acid	Methanol	1300	130
2	Caffeine	Methanol: HCOOH (95:5)	2000	200
3	Riboflavin	96% ethanol (heating at 30ºC)	500	50
4	Caffeic acid	Methanol (heating at 50ºC)	1100	110
5	Rutin	96% ethanol (heating at 30ºC)	2000	200
6	Cinaroside	Methanol (heating at 30ºC)	1100	110
7	Skutelyarin	96% ethanol	600	60

		(heating at 50ºC)		
8	Salicylic acid	Methanol	2200	220
9	Luteolin	96% ethanol (heating at 30ºC)	1000	100
10	Quercetin	Methanol	1200	120
11	Cinnamic acid	Methanol	1000	100

To carry out the analysis, an aqueous solution of the dry extract was used (hot water, ratio 1:100, ultrasonic bath). [8]. The obtained data are shown in Table 2 and Figure 1.

The quantitative content of tannins in the dry extract of prickly artichoke (Table 4) was determined by permanganatometry according to the GF method [7,8].

The results obtained are shown in tables 1, 3, 4 and in Fig. 1.

Table 2.

Metrological characteristics of the results of quantitative determination of hydroxycinnamic acids

Optical density, D	Found hydroxycinnamic acids, %	Metrological characteristics
0,111	7,17	$\overline{X}$=7,122 S=0,0501 S$\bar{x}$=0,0224 ΔX=0,128 $\Delta\overline{X}$=0,057 $\mathcal{E}$=1,79 $\bar{\mathcal{E}}$=0,80
0,110	7,11	
0,110	7,11	
0,111	7,17	
0,109	7,05	

The amount of hydroxycinnamic acids in the dry extract of prickly artichoke, in terms of chlorogenic acid, was 7.12%. The data indicates a fairly high content of hydroxycinnamic acids in the dry extract.

Table 3.

The quantitative composition of some biologically active substances of the dry extract of spiny artichoke was determined by HPLC (High Performance Liquid Chromatography (High Pressure Liquid Chromatography))

№	Identified substances	Retention time, min	Peak area rel. units or mAU*s	Contents substances µg/ml
1	Chlorogenic acid	7,505	869,53	67,2616
2	Caffeine	7,654	633,48	648,3990
3	Riboflavin	8,576	14,81	3,8428
4	Caffeic acid	9,276	1429,76	31,4565
5	Rutin	10,336	6,12	$8{,}9026^{-1}$
6	Cinaroside	11,167	361,24	41,5262
7	Skutelarin	12,160	69,79	4,6810

8	Salicylic acid	16,722	25,94	3,0347
9	Luteolin	17,011	14,25	$7,1974^{-1}$
10	Quercetin	17,312	3,90	$3,2354^{-1}$
11	Cinnamic acid	19,108	7,77	$2,2691^{-1}$

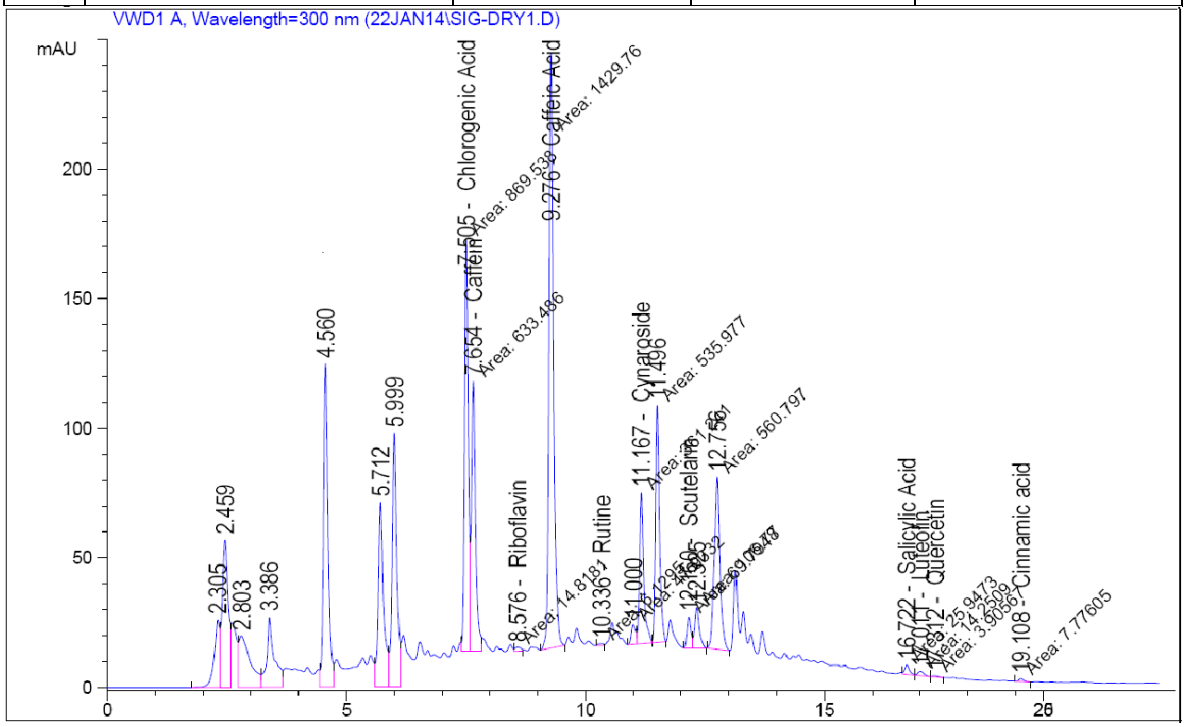


Fig. 1. Chromatogram of artichoke dry extract

Thus, the results of the quantitative determination of some BAS (Biologically active substances) in the dry extract of prickly artichoke showed a high content of chlorogenic acid, caffeine, cynaroside and caffeic acid.

Table 4

№	Tannins found, %	Metrological characteristics
1	6,35	$\overline{X}=6,39$
2	6,37	$S=0,03162$
3	6,39	$S_{\overline{x}}=0,01414$
4	6,41	$\Delta X=0,081263$
5	6,43	$\Delta\overline{X}=0,04160$
		$\varepsilon=1,27$
		$\overline{\varepsilon}=0,65$

According to research results (Table 3 and 4), the content of tannins in the dry extract is 6.39% on average . The relative error of determination is 0.65%, which allows the use of this technique for the quantitative determination of tannins when standardizing the dry extract.

Conclusions. Thus, according to the results of qualitative analysis, hydroxycinnamic acids, flavonoids, tannins, and amino acids were found in the dry extract. The amount of hydroxycinnamic acids in terms of chlorogenic acid is 7.12% on average. The results obtained indicate a fairly high content of hydroxycinnamic acids in the dry extract. According to research results, the content of tannins in the dry extract averaged 6.39%. The results of the quantitative

determination of some biologically active substances by HPLC in the dry extract of spiny artichoke (CYNARA SCOLYMUS L.L.) showed a high content of chlorogenic acid, cinaroside and caffeic acid.

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