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THE STUDY OF THE CHEMICAL COMPOSITION OF THE LIQUID EXTRACT BASED ON GLOBE ARTICHOKE

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ABSTRACT

The qualitative and quantitative composition of the liquid extract based on globe artichoke introduced in Uzbekistan has been studied. According to the data the amount of acids in terms of chlorogenic acid amounted to 2, %. The results of the research indicate a relatively high content of acids in the liquid extract. The average content of tannins in the preparation is 2.36%. Data for the quantitative determination of some biologically active substances by HPLC (high performance liquid chromatography) method has showed the high content of chlorogenic and caffeic acids, cynaroside. According to the results of elemental analysis of the drug, it contains such important bio-elements as sodium, potassium, calcium, magnesium, phosphorus, iron, sulfur, manganese, zinc and copper.

Keywords: liquid extract, hydroxy-cinnamic acids, tannins, flavonoids, biologically active substances (BAS).

INTRODUCTION

Globe artichoke is a relatively new plant for Uzbekistan. The composition of biologically active compounds of globe artichoke and preparations based on it is about 3% protein, 7 to 15% carbohydrates, 0.4 mg% carotene, 3-11 mg% vitamin C, fats, nucleic acids, vitamins B1, B2, P, PP, K, E, fiber 1.27%, tannins, sesquiterpene lactones and minerals. In the composition of carbohydrates include inulin. The main active ingredients are hydroxy-cinnamic acids: caffeic, chlorogenic, neochlorogenic and 1,5 - di-0-caffeylquinic acids. According to the literature sources, the plant contains from 0.1% to 1% flavonoids. The dominant flavonoids are luteolin-7-glycoside (cynaroside) and luteolin-7-rutinoside (solenoid) [1,2]. It is known that when standardizing plant raw materials and artichoke preparations, it is difficult to choose various standard substances, for example, dry leaves should contain 1.7-4.2% ortho-dioxyphenols (in terms of caffeic acid), 0.9-1.4 % phenolcarboxylic acids (in terms of cinarin) and 0.02-1.4% caffeoylquinic acids (in terms of chlorogenic acid). Medicinal preparations based on globe artichoke are used as a hepatoprotective, choleretic, diuretic, hypo-azotemic, hypocholesterolemic, anti-atherosclerotic, hypotensive, laxative, antitoxic, metabolic, normalizing the functions of digestion and metabolism [1, 2, 3].

The purpose of this work is to study the chemical composition of a liquid extract obtained from raw materials of globe artichoke grown in Uzbekistan.

Experimental part. In appearance, the liquid extract is the brown liquid with a characteristic smell and taste.

For qualitative identification of the main active biologically active substances, the liquid extract was diluted with purified water in a mass-volume ratio of 1:10 and thoroughly mixed.

Qualitative detection of hydroxy-cinnamic acids was performed by the biochemical method, and a 2% solution of acetic acid was used as the mobile phase. Chromatogram air-dried and examined under UV-light. Blue fluorescence appeared under UV-light, indicating the presence of hydroxy-cinnamic acids [1].

Detection of flavonoids was performed with caustic soda. To 1 ml of a dilute solution of a liquid extract prepared according to the above method, a few drops of a solution of caustic soda were added. The appearance of yellow staining was observed, indicating the presence of flavonoids [1].

When a few drops of iron-ammonium alum were added to 1 ml of the test solution, a dark green color appeared, which indicated the presence of tannins [4]. Qualitative detection of amino acids was performed using the ninhydrin reaction. During the analysis, equal volumes of the test solution and freshly prepared 0.1% ninhydrin solution were mixed and carefully heated. Upon cooling, a red – purple staining was observed, indicating the presence of amino acids [5]. Quantitative determination of the amount of hydroxy-cinnamic acids in terms of chlorogenic acid was performed using the spectrophotometric method. An exact sample of the liquid extract was placed in a 50 ml flask and the volume was adjusted to the mark with 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 with 50% ethyl alcohol to the mark. The optical density of the solution was measured on a spectrophotometer at a wavelength of 329±2 nm. The comparison solution was 50% ethyl alcohol [3].

In parallel, the optical density of a working standard sample solution of chlorogenic acid was measured under similar conditions.

Preparation of working standard samples of chlorogenic acid. The exact sample (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 sum of hydroxy-cinnamic acids as a percentage, in terms of chlorogenic acid, was calculated by the formula:

$$X = \frac{Д_1 \cdot m_0 \cdot C}{Д_0 \cdot m_1}$$

Д1 - optical density of the test solution;

Д0 - optical density of working standard samples of chlorogenic acid;

m1- suspension of liquid extract, g;

m0 - the suspension of standard sample, g;

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

The results are shown in the Table 1.

Table 1. Metrological characteristics of the results of the assay of hydroxy-cinnamic acids

The optical density, D	hydroxy-cinnamic acids found, %	Metrological characteristics
0,354	2,10	$\bar{X}$= 2,09 S= 0,0091 $S_{\bar{x}}$ = 0,0041 ΔX= 0,023 $\Delta \bar{X}$= 0,010 ε= 1,10 $\bar{\varepsilon}$= 0,48
0,353	2,098	
0,354	2,10	
0,352	2,093	
0,353	2,098	

The amount of hydroxy-cinnamic acids in the liquid extract of globe artichoke, in terms of chlorogenic acid, was 2.09%. The data indicates a high content of hydroxy-cinnamic acids in the extract.

Quantitative study of some biologically active substances of the drug was performed by reverse-phase HPLC on an Agilent Technologies 1100 series device 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 microns, pre-column Zorbax Eclipse XDB - C8 (2,1x12,5 mm) with a particle size of 5 microns (SF detector, column and pre - column 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 performed using a linear gradient of the solution concentration from 0 to 100% for 25 minutes. The flow rate is 1 ml/min, the column temperature is room (20 ° C), the pressure at the starting gradient conditions is from 90 to 120 bar, and the peaks were detected at UV 300 nm. The volume of injection per column is 10 µl.

Preparation of standard samples was carried out as follows: the exact weight of standard samples was weighed on analytical scales and dissolved in the appropriate solvent (Table 2). Then a standard mixture was prepared from equal volumes (200 µl) of the initial solutions. Standard solutions were used to check or refine the calibration (Table 2).

Table 2. Preparation of standard samples.

No.	Substance (standard	Solvent, the conditions of dissolution	Initial concentration, mcg/ml	Final concentration, mcg/ml
1	Chlorogenic acid	Methanol	1300	130
2	Caffeine	Methanol: HCOOH (95:5)	2000	200
3	Riboflavin	96% ethanol (heating at 30 ° C)	500	50
4	Caffeic acid	Methanol (heating at TEM. 50 ° C)	1100	110
5	Ruthin	96% ethanol (heating at 30 ° C)	2000	200
6	Cynaroside	Methanol (heating at 30 ° C)	1100	110
7	Scutellarin	96% ethanol (heating at 50 ° C)	600	60
8	Salicylic acid	Methanol	2200	220
9	Luteolin	96% ethanol (heating at 30 ° C)	1000	100
10	Quercetin	Methanol	1200	120
11	Cinnamic acid	Methanol	1000	100

A diluted aqueous solution of the liquid extract (1:10 ratio, agitator) was used for the analysis. The data obtained are shown in the Table 3 and Picture 1.

Table 3. Quantitative composition of some biologically active substances of the liquid extract of globe artichoke established by high performance liquid chromatography method

No	Identified substances	Holding time, min	Peak area, rel. units or mAU*s	Substance content, mcg/ml
1	Chlorogenic acid	7,452	66181,08	5119,32
2	Riboflavin	8,615	741,41	192,27
3	Caffeic acid	9,226	15647,42	344,26
4	Ruthin	10,228	559,15	81,21
5	Cynaroside	11,112	24740,00	2843,97
6	Scutellarin	12,108	2283,97	153,18
7	Salicylic acid	16,665	2598,46	303,91
8	Luteolin	16,750	444,22	22,43
9	Quercetin	17,111	381,11	31,57
10	Cinnamic acid	19,048	1887,93	55,09

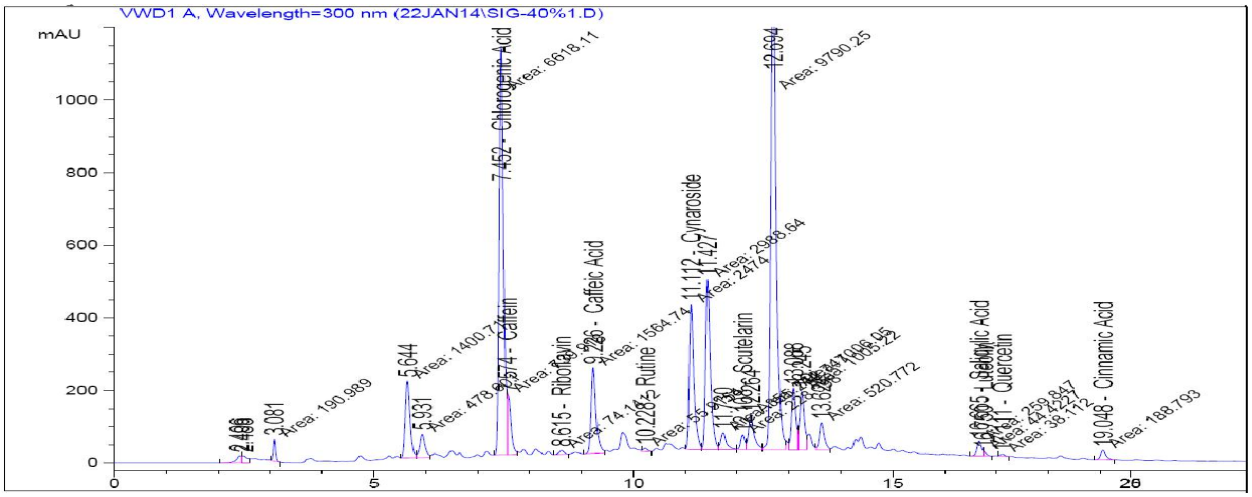

Figure 1. The chromatogram of artichoke liquid extract

Thus, the results of quantitative determination of some BAS in the liquid extract of globe artichoke have showed a high content of chlorogenic acid, cinaroside and caffeic acid. The quantitative content of tannins in the liquid extract of globe artichoke (Table 4) was determined by permanganatometry according to the GF method [4].

Table 4. The results of quantitative determination of tannins in the liquid extract of globe artichokes by the method of permanganatometry

No.	Tannins found, %	Metrological characteristics
1	2,33	$\bar{X}=2,36$ $S=0,02397$ $S_{\bar{x}}=0,01071$ $\Delta X=0,06160$ $\Delta\bar{X}=0,02754$ $\varepsilon=2,61$ $\bar{\varepsilon}=1,16$
2	2,35	
3	2,36	
4	2,38	
5	2,39	

According to the results of research (Table 4), the content of tannins in dry extract is on average 2.36%. The relative error of determination is 1.16%, which allows using this method for quantitative determination of tannins, when standardizing dry extract. To determine the micro-impurities of heavy metals, exact samples (0.5 g) from the object (liquid extract) were decomposed in a mixture of nitric and perchloric acids (8ml: 2ml) in a Milestone microwave oven with programming power from 250 to 500 W and temperature from 180 to 220 0C. The resulting solutions were quantitatively transferred to 100 ml volumetric flasks and then used for direct injection into the spray chamber of the device.

The elemental composition of mineral substances of the extract has been analyzed using the ICP-MS device (inductively coupled plasma mass spectrometer) AT 7500a. Device parameters: plasma power 1200 W, integration time 0.1 sec, rotation speed of the peristaltic pump–0.1 rpm. A multi-element (27-component) standard solution with a target component content of 1.0 mg/l was used as the standard (Table 5)

Table 5. The elemental composition of the liquid extract of globe artichoke

Elemental composition	Element content, mg/kg	Elemental composition	Element content, mg/kg
Li	1.00	Zn	5.20
Be	<0.017	As	0.19
Na	1.30	Se	1.10
Mg	1.00	Br	2.00
Al	1.70	Rb	4.50
P	730.00	Sr	7.90
S	110.00	Mo	0.54
K	14.00	Pd	<0.028
Ca	540.00	Ag	0.75
V	0.049	I	7.40
Cr	0.46	Ba	0.75
Mn	3.00	Pt	<0.016
Fe	19.00	Au	0.19
Co	0.11	Hg	0.10
Ni	0.36	Pb	0.21
Cu	8.70	Bi	0.022

The results of elemental analysis of the drug have indicated such bio-elements as sodium, potassium, calcium, magnesium, phosphorus, iron, sulfur, manganese, zinc and copper, which have the pharmacological effect of this drug.

CONCLUSIONS

According to the results of qualitative analysis, hydroxy-cinnamic acids, flavonoids, tannins, and amino acids have

been found in the liquid extract. The amount of acids in recalculation on chlorogenic acid was 2.0% on average. The results obtained indicate a high content of hydroxy-cinnamic acids in the extract. According to research results, the average content of tannins in the preparation was 2.36%. The results of quantitative determination of some biologically active substances by high performance liquid chromatography method in the liquid extract of globe artichoke have showed a high content of chlorogenic acid, cinaroside and caffeic acid. According to the results of elemental analysis of the drug, it contains such important bio-elements as sodium, potassium, calcium, magnesium, phosphorus, iron, sulfur, manganese, zinc and copper.

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