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ARTISHOK *(CYNARA SCOLYMUS L.)* BARGI TARKIBIDAGI POLIFENOL VA VITAMINLARNI ANIQLASH

ОПРЕДЕЛЕНИЕ ПОЛИФЕНОЛОВ И ВИТАМИНОВ В ЛИСТЬЯХ АРТИШОКА (*CYNARA SCOLYMUS* L.)

DETERMINATION OF POLYPHENOLS AND VITAMINS IN ARTICHOKE *(CYNARA SCOLYMUS* L.) LEAVES

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Annotatsiya

Maqolada artishok (Cynara scolymus L.) o‘simligining 3 hil navi tanlab olindi va o‘simlikning quritilgan barglari tarkibidagi suvda eruvchan vitaminlar hamda polifenol birikmalar miqdori YuSSX usuli orqali o‘rganildi. O‘simlik barglari tarkibida uchrovchi B_1, B_2, B_3 B_6, B_9,C vitaminlar hamda rutin, gall, salisil, kversitin va appegenin kabi ba’zi turdagi polifenol birikmalar miqdori taqqoslandi.

Аннотация

В статье представлены результаты исследования количеств водорастворимых витаминов и полифенольных соединений в сушеных листьях трех выбранных сортов артишока (Cynara scolymus L.) с использованием метода YSSX. Сравнены количества витаминов B1, B2, B3, B6, B9, C, а также некоторых типов полифенольных соединений, таких как рутин, галловая кислота, салициловая кислота, кверцетин и апигенин, содержащихся в листьях растения.

Abstract

This article presents the study of the amounts of water-soluble vitamins and polyphenolic compounds in the dried leaves of three selected varieties of artichoke (Cynara scolymus L.) using the YSSX method. The quantities of vitamins B1, B2, B3, B6, B9, C, as well as certain types of polyphenolic compounds such as rutin, gallic acid, salicylic acid, quercetin, and apigenin present in the plant leaves were compared.

Kalit so‘zlar: *Tikanliartishok, vitaminlar, polifenollar, flavonoid,nav, xalqtabobati, tibbiyot.*

Ключевые слова: *артишок, витамины, полифенолы, флавоноиды, сорт, народная медицина, медицина.*

Key words: *artichoke, vitamins, polyphenols, flavonoids, variety, traditional medicine, medicine.*

INTRODUCTION

Currently, more than a thousand species of medicinal plants are known, which are found in mountains, forests, deserts and steppes. For example, we can mention the artichoke plant, which is one of the medicinal plants in our country.

Currently, there are more than 140 types of artichoke, of which only about 40 have nutritional value. The leaves of the plant are green or grey-green, and some species have large pinnate cuts and a spiny shape. The artichoke plant can be propagated vegetatively in march-april [1].

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The pharmacological effect of medicinal preparations prepared from the leaves of the artichoke plant is due to the presence of phenol-bricomcynarin in their composition, as well as the combination of this substance with phenoxy acids, flavonoids and other substances. A number of active substances of the plant, such as cynarin, caffeine, chlorogen, neochlorogen, collectively exhibit antipyretic and hepatoprotective activity, activate the removal of urea and toxins from the body, including nitro compounds, alkaloids, and heavy metal salts [2]. In addition, these active substances have a positive effect on lipid metabolism in the body. As a result, the amount of cholesterol and urea in the blood decreases [3].

Vitamin C, carotenoids and other groups of vitamins, including B vitamins, nicotinic acid and vitamin E, inulin from carbohydrates contained in prickly artichoke extracts, actively participate in metabolic processes in the body and prevent the accumulation of excess fat in the body, which, in turn, leads to death, which has a positive effect on preventing excess weight gain [4].

Preparations based on artichoke raw materials are used in folk medicine and medicine mainly as an expectorant, antioxidant, hepatoprotective, diuretic, and stimulant of intestinal motility.

All artichoke-based drugs cause an increase in the secretory function of the liver; this condition increases the secretion of fluid from the gallbladder and stimulates the synthesis of fatty acids. The reflector increases the motility of the digestive system [5]. Continuous use of the drug increases the detoxification ability of the liver. For this reason, herbal medicines have a laxative effect and normalize metabolism. Artichoke-based preparations are also widely used for dyspeptic diseases caused by functional disorders of bile secretion [6-7].

In modern folk medicine, the leaves, flowers, roots and seeds of the artichoke plant are used in the form of decoctions, and their infusions are used for heart disease, as an antihypertensive, choleretic agent, and also for liver diseases. Artichoke seeds filled with alcohol extract are used for gastrointestinal diseases. A water infusion of artichoke roots is an effective means of lowering blood sugar levels. In modern scientific medicine, artichoke is recommended for nutrition of weakened patients with liver diseases. Sinaropicrin, the main component of artichoke, has antioxidant properties. Thanks to this, synaropicrin prevents the development of oxidation, stress and aging of the skin under the influence of UV radiation. Artichoke prevents DNA damage caused by genotoxic chemicals. Artichoke leaf extract inhibits the enzyme xanthine oxidase due to its luteolin content. In addition, it has been established that this substance has an antiuremic effect, and its leaves exhibit antiproliferative properties. The polyphenols contained in the plant have been proven to have anti-cancer effects. Plant seeds contain up to 30% oils [8-9].

A number of Uzbek scientists worked on the development of hepatoprotective and choleretic agents based on the raw materials of this plant and the study of their pharmacological activity: Kh.U. Aliev, R.T. Tulyaganov, A.A. Abzalov, A.Yu. Ibragimov, Kh.M. Komilov, N.K. Olimov, A.K. Saidvaliev, K.A. Ubaidullaev, T.A. Mirrakhimov conducted scientific research.

This paper presents the results of a study of the amount of some water-soluble vitamins in artichoke leaves using HPLC.

MATERIALS AND METHODS

3 varieties of the thorny artichoke (Cynara scolymus L.) plant "Krasavets" (Russia), "Artishok ispansky" (Russia) and locally cultivated "Artishok kolyuchiy" were selected and the water-soluble content of the dried leaves of the plant vitamins were checked by the following method.

Used reagents and equipment. Vitamins B_1, B_2, B_6, B_9 and C were obtained from "DSM Nutritional Products GmbH" (Germany), B_{12} from "Rhydburg Pharmaceuticals" (Germany). Gallic acid "Macklin" (China). Water, acetonitrile, chemically pure grade acetic acid and sodium hydroxide reagents were used.

The amount of water-soluble vitamins in the sample was determined using a LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Solutions of vitamins B_1 (CAS 70–16–6), B_6 (CAS 65–23–6) and B_{12} (CAS 68–19–9), C (CAS 50–81–7) (100 mg/l) of each vitamin an amount of 5 mg was prepared by dissolving in 50 ml of HPLC pure water. Standards of vitamins B_2 (CAS 83-88-5) and B_9 (CAS 59-30-3) were prepared by dissolving 5 mg in 50 ml of 0,025% sodium hydroxide solution. Then, 200 mkl of all vitamins were mixed and a solution with a concentration of 16,67 mg/l

of each vitamin was prepared. By diluting it, solutions with a concentration of 3,333 mg/l, 0,667 mg/l and 0,133 mg/l were prepared, poured into a vial and used for analysis.

Preparation of plant extract. The sample to be tested for the extraction of water-soluble vitamins was weighed with an accuracy of 0,01 g on an NV222 scale manufactured by OHAUS (USA), placed in a 50 ml conical flask, and 25 ml of 0,1 N HCl solution was added. The mixture was extracted in an ultrasonic bath of GT SONIC-D3 (China) at a temperature of 60°C for 20 minutes. The mixture was then cooled, filtered and made up to 25 ml with water in a volumetric flask. 1,5 ml of the extract was filtered through a 0,45 mkm syringe filter and placed in a vial and used for analysis.

Chromatographic conditions. Standard solutions and sample extracts LC-40 Nexera Lite high performance liquid chromatograph consisting of LC-40D pump, SIL-40 autosampler, SPD-M40 photo-diode array detector (PDA) and LabSolutions ver. 6,92 software was analyzed. Shim pack GIST C18 (150 × 4,6 mm; 5 mkm, Shimadzu, Japan) reversed-phase column and a gradient mobile phase consisting of acetonitrile (A) and a 0,5% solution of acetic acid in water (B) (Table - 1) was used. The injection volume was 10 mkl, the flow rate was 0,9 ml/min, and the temperature of the column thermostat was set at 35°C. Analytical signal (peak area) of each vitamin was recorded at three wavelengths 265, 291, 550 nm.

Table – 1.

Mobile phase gradient software.

Time, min.	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Finish	

Table – 2.

A mobile phase gradient program for the determination of vitamin C.

Time, min	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	0	100
2	0	100
6	50	50
6,01	0	100
15	Finish	

Used reagents and equipment. Gallic acid from "Macklin" (China), salicylic acid from "Rhydburg Pharmaceuticals" (Germany), quercetin, apigenin, kaempferols from "Regal" (China), rutin from natural sources were isolated by extraction and column chromatography methods. HPLC grade water, acetonitrile, chemically pure grade acetic acid and sodium hydroxide reagents were used.

The amount of polyphenols in the plant was determined using a LC-40 Nexera Lite high-performance liquid chromatograph manufactured by Shimadzu, Japan.

Preparation of standard solutions. Gallic acid (5,2 mg), salicylic acid (5,2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), kaempferol (5 mg) were dissolved in 96% ethanol in an ultrasonic bath for 20 minutes. and transferred to a 50 ml flask and made up to the mark with ethanol. 200 mkl of each solution was taken and mixed, and a total of 4 different solutions were prepared by diluting them. Each solution was poured into a vial and used for analysis.

Preparation of plant extract. For the extraction of phenolic compounds, 1 g of the test sample was weighed with an accuracy of 0,01 g on a NV222 scale manufactured by the OHAUS company (USA), placed in a 50 ml conical flask, and 25 ml of 96% ethanol was added. The mixture was extracted in an ultrasonic bath of GT SONIC-D3 (China) at a temperature of 60°C for 20 minutes. Then the mixture was cooled, filtered and made up to 25 ml with ethanol in a volumetric

flask. 1,5 ml of the extract was centrifuged at a speed of 7000 rpm in a Mini-7 centrifuge (BIOBASE, China) and filtered through a 0.45 mkm syringe filter and used for analysis.

Determination of phenolic compounds. Standard solution, sample extract Shim pack GIST C18 (150 × 4.6 mm; 5 mkm, Shimadzu, Japan) reverse phase column and a gradient mobile phase consisting of acetonitrile (A) and 0.5% acetic acid in water (B) (Table 4) was used. The injection volume was set at 10 mkl, the flow rate at 0,5 ml/min, and the column thermostat at 40°C. The analytical signal (peak area) of phenolic compounds was recorded at 300 nm (Figure 3).

Table – 3.

Mobile phase gradient software

Time	Acetonitrile (A), %	0,5 % acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

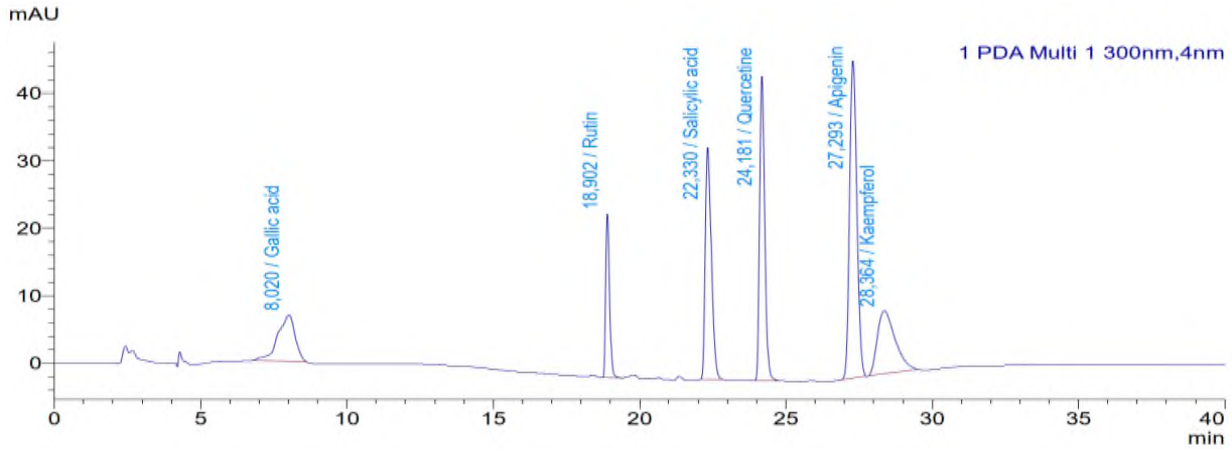
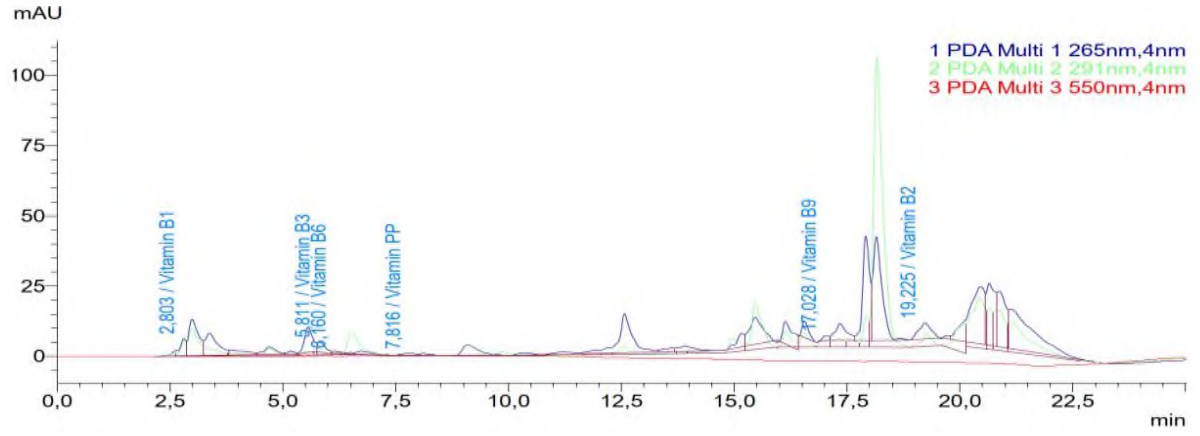


Figure 1. Chromatogram of polyphenol standards at 300 nm

RESULTS AND DISCUSSION

Determination of vitamins in artichoke extract. A ground plant leaf with an average mass of 1 g was extracted in 0.1 N HCl and chromatogram was obtained (Figure 1) and the results were processed and presented in Table 4.



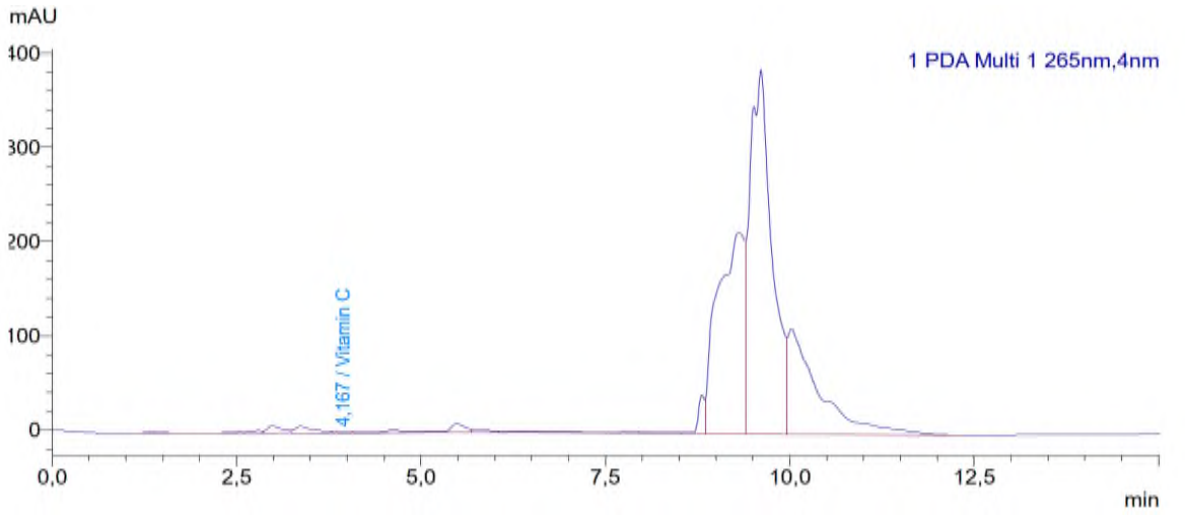


Figure - 2. Chromatogram for determining vitamins in the “Artichoke Ispanskiy” extract

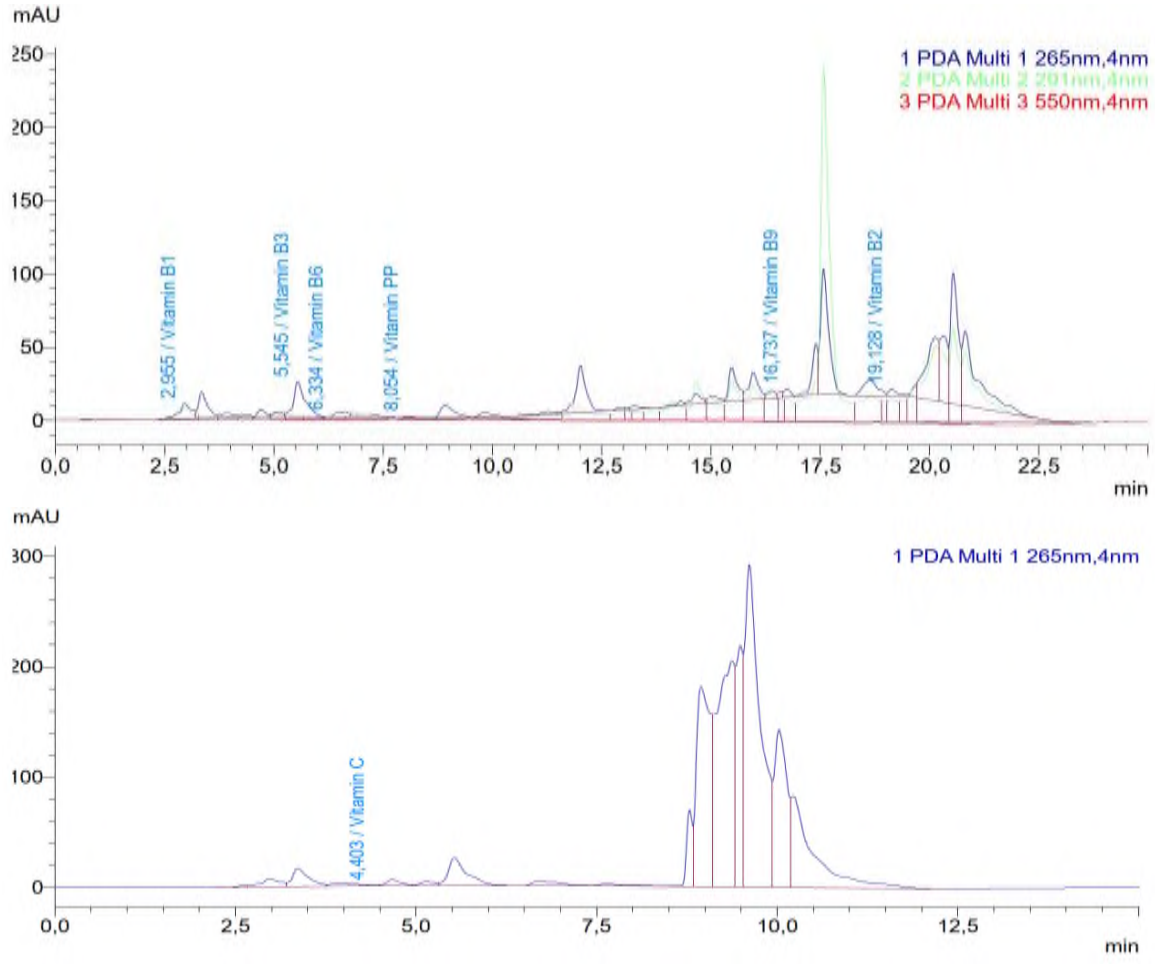


Figure 3. Chromatograms for determination of vitamins contained in "Krasavets" extract.

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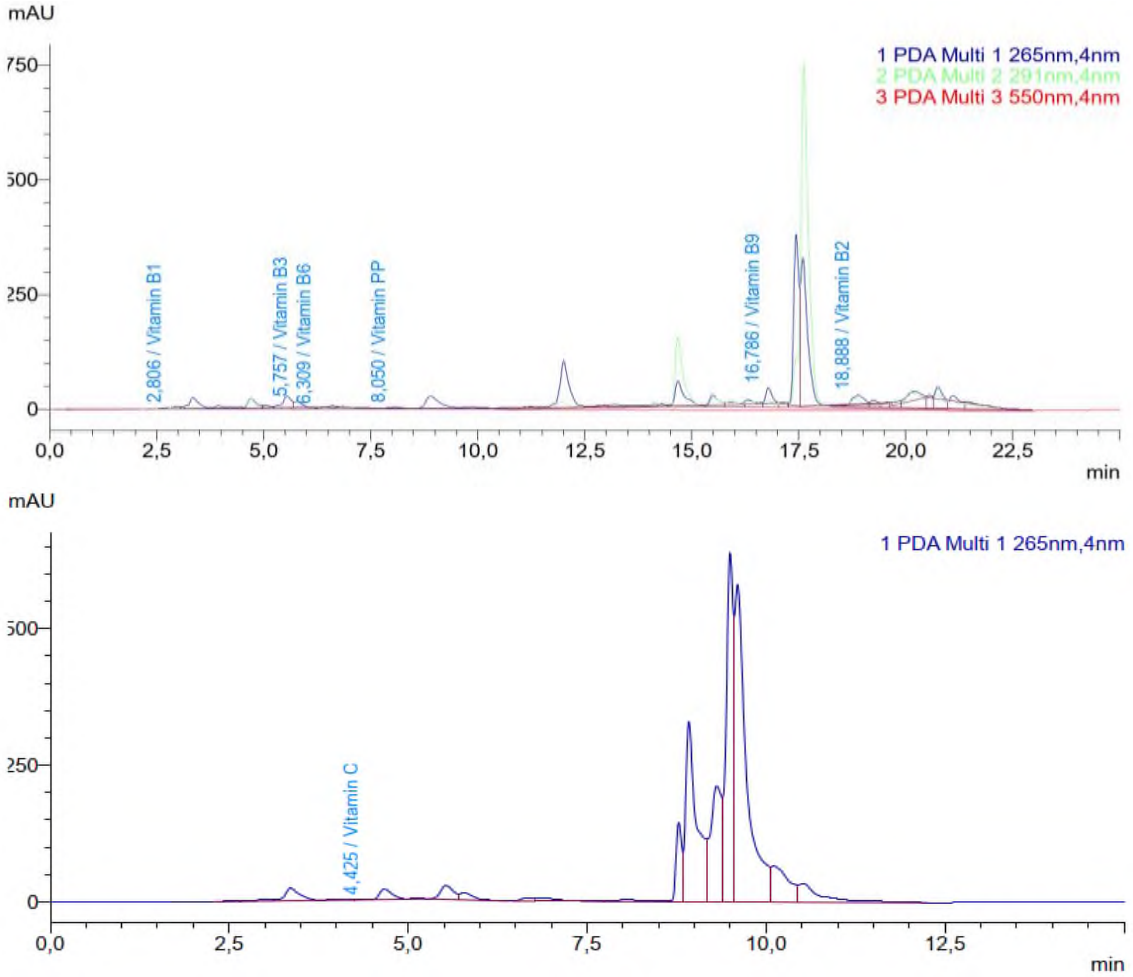

Figure 4. Chromatograms for determining the vitamins contained in the “Artichoke kolyuchiy” extract

Table – 4.

Amount and retention times of vitamins in the extract.

The name of vitamins	Retention time, sec	Concentration, mg/100 gr			
		“Artichoke Ispanskiy”	“Krasavets”	“Artichoke kolyuchiy”	Information provided in the literature [10]
Vitamin B_1	2.8-2.95	2,232	6,201	1,003	0,01
Vitamin B_3	5.54-5.81	1,557	11,633	4,051	-
Vitamin PP	7.81-8.05	0,446	0,317	1,428	1,91
Vitamin B_9	16.73-17.02	0,57	1,737	10,355	0,001
Vitamin B_2	18.88-19.22	1,841	1,088	5,975	-
Vitamin B_6	6.16-6.33	0,128	1,249	0,403	0,012
Vitamin C	4-16-4.42	0,898	0,134	0,17	0,12

Determining the amount of phenolic compounds in artichoke leaf extracts. A chromatogram of the sample extract weighing 1 g was taken (Figures 2-4) and based on the results, the amount of phenolic compounds in 100 g of the sample was calculated using the following formula and presented in Table 3.

$$X = \frac{C_{phen} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Here, X is the amount of phenol compounds in 100 grams of extract, mg;

C_{phen}– the concentration of the phenol compound in the extract determined by the HPLC method, mg/l;

$V_{extract}$– volume of sample extract, l;

M_{sample} – sample mass withdrawn for extract preparation.

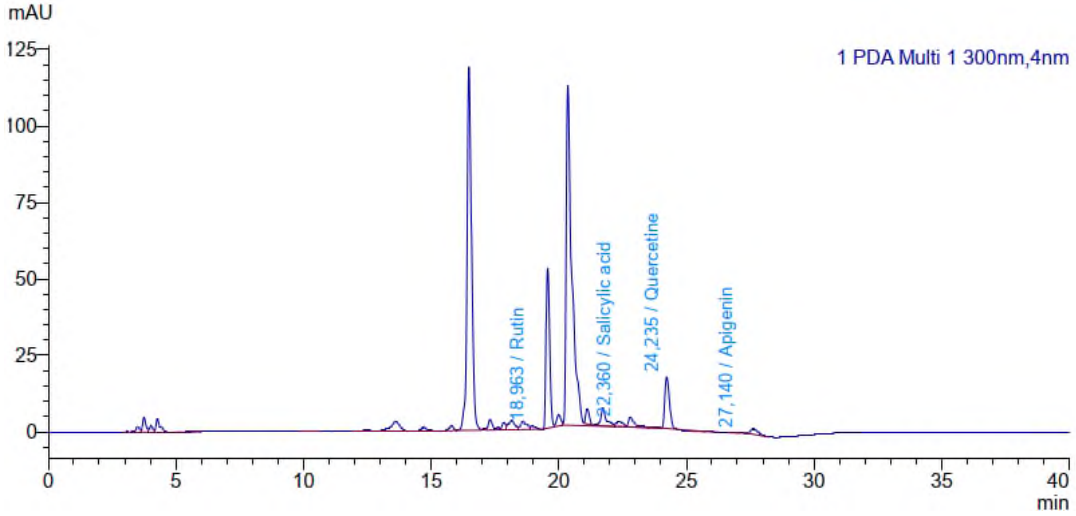

Figure 5. Chromatogram for the determination of polyphenols in the extract obtained from the leaf variety "Artishok ispansky".

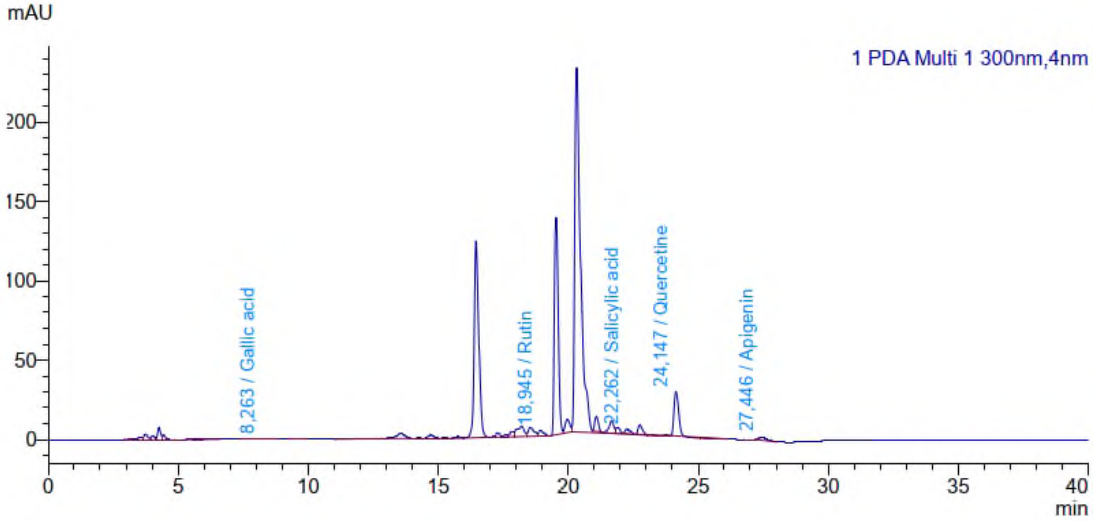

Figure 6. Chromatogram for the determination of polyphenols in the extract from the "Krasavets" leaf variety.

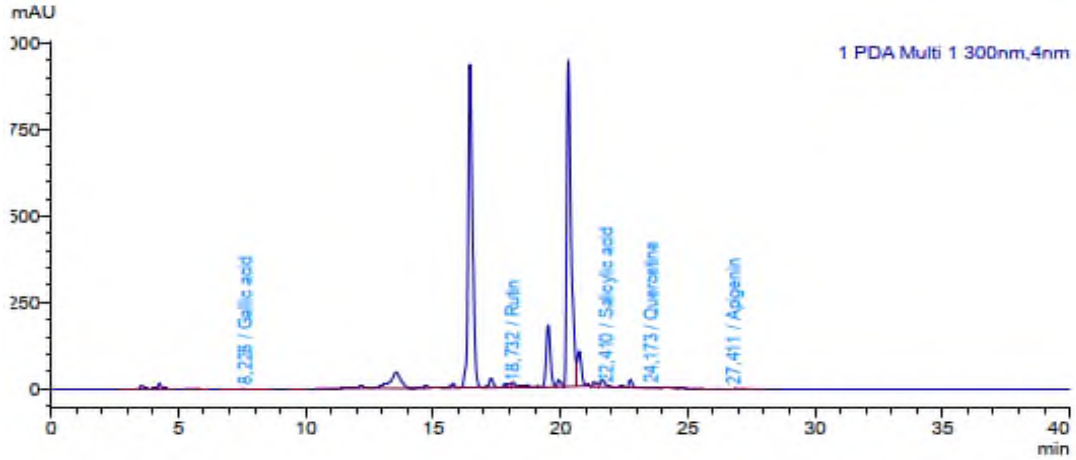

Figure 7. Chromatogram for the determination of polyphenols in the extract obtained from the leaf variety "Artishok Kolyuchy".

Table – 5. Amount and retention times of polyphenols in the extract.

Phenol compound name	Retention time, sec	Concentration, mg/100 gr		
		“Artichoke Ispanskiy”	“Krasavets”	“Artichoke kolyuchiy”
Gallicacid	8.22-8.26	-	0,424	0,221
Rutin	18.73-18.96	0,987	3,768	5,92
Salicylicacid	22.26-22.41	1,06	1,635	1,298
Quercetine	24.14-24.23	5,963	9,653	1,357
Apigenin	27.14-27.44	0,025	0,851	0,78

CONCLUSION

According to the results of the conducted research, 3 varieties of Artichoke plant were studied. In particular, the amount of water-soluble vitamins and polyphenols was determined from the barra leaves of the 2 types of "Krasavets", "Artishok ispansky" grown in Russia and the thorny artichoke "Artishok kolyuchiy" (Cynara scolymus L.) grown in Andijan region. All studied samples were qualitatively studied using HPLC. It was found that the leaves of plant varieties contain 7 types of water-soluble vitamins: B1, B2, B3, B6, B9, PP and C. According to it, 100 g of plant leaves contain the highest amount of vitamin C - 2,245 mg in the "Spanish Artichoke" variety, the highest amount of B3 - 29,083 in the "Krasavets" variety, and vitamin B9 - 25,888 mg in the "Kolyuchy" variety. When studying the water-soluble vitamins obtained from the leaves of Cynara scolymus L. in the literature by the following method (Chromatography conditions: Chromatograph-Agilent 1200 (USA), mobile phase (gradient mode) - acetonitrile buffer solution pH=2.92 (4%: 96%) 0-6 min., (10%:90%) 6-9 min., (20%:80%) 9-15 min., (4%:96%) 15-20 min. injection volume 20 The velocity of the mobile phase is 1000 ml/min - column Eclipse XDB - diode matrix, wavelength 272 nm, 254 nm, 297 nm and 360 nm [10] It was found that there is a small amount of boron. As an example, it can be seen that vitamin B1 is 100 times less than the locally grown "Artishok Kolyuchy" variety.

Polyphenols in the composition of 100 g of plant leaves were found to contain the highest amount of quercetin 24,133 mg in "Krasavets" variety, and 14,800 mg of rutin in "Artishok Kolyuchy" variety.

In conclusion, it can be said that it is possible to grow other varieties of Cynara scolymus L. locally and obtain new types of goods based on this.

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