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Study of Nepeta Olgai Regel L. Phenol Compounds by HPLC Method

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Abstract In this study, flavonoids of the plant Nepeta Olgae Regel, belonging to the Laminaceae family, collected in Chust (mountain slopes of Gowa) and Kosonsoy (mountain slopes and hills) were studied. In the process of studying the qualitative and quantitative flavonoid composition of Nepeta Olgae Regel L. plants, thin-layer (TLC) and high-performance liquid chromatography (HPLC) were used to identify 6 compounds that were assigned to this class of compounds. Among them, the most predominant were rutin, salidroside, dihydroquercitin and quersitin.

Keywords Extractive substances, Flavonoids, Nepeta Olgae Regel L., High performance liquid chromatography, Qualitative and quantitative analysis

1. Introduction

One of the priority directions in the search for new biologically active substances is the study of wild plant species of Uzbekistan, whose resources are quite abundant [1-3].

This approach ensures a reliable long-term raw material base, allows for the rational use of these plant resources, and provides the opportunity to expand the range of medicinal plant raw materials and medicines based on them.

From this perspective, plants of the Lamiaceae family are of particular interest. The Lamiaceae family includes about 200 genera and 3500 species, and is found almost worldwide. These are perennial herbaceous plants, many of which are medicinal, aromatic, essential oil-bearing, and ornamental, and are of great significance [2-4].

In connection with this, many species of plants belonging to the Lamiaceae family (Lamiaceae) have long been included in the medicinal plant raw material arsenal of foreign countries. In Uzbekistan, there are many species and reserves of such medicinal plants [1-3].

One such promising and poorly studied plant is Nepeta Olgae Regel L., a species belonging to the Lamiaceae family, which is found in the flora of the Namangan region [1-2].

2. Objective of the Study

The aim of the study is to investigate the flavonoids of the plant Nepeta Olgae Regel (L.) found in Uzbekistan's Namangan region.

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3. Materials and Methods

The aerial parts of the plant were collected and air-dried in the laboratory for two weeks and then ground into a fine powder using a sterilized mechanical grinder. After this, the qualitative parameters (moisture content, ash content, and extractive substances) were studied [5-6]. The results are presented in Tables 1 and 2.

Widely used methods in the literature for the separation of extractive substances include the use of different solvents according to their polarity, to separate substances into fractions with similar polarity (chloroform, ethyl acetate, and n-butanol). These fractions were extracted sequentially with these solvents and used for further research. The extraction scheme of flavonoids from Nepeta Olgae Regel L. is shown in Figure 1.

Flavonoids in the extractive substances were determined by liquid chromatography. 5-10 g of the sample were weighed and placed in a 300 ml flat flask. 50 ml of 70% ethanol solution was added. The mixture was heated to 70–80°C while stirring for 1 hour and then mixed at room temperature for 2 hours. The mixture was then cooled and filtered. The remaining portion was extracted twice with 25 ml of 70% ethanol. The filtrates were combined and brought to the mark with 70% ethanol in a 100 ml volumetric flask. The resulting solution was centrifuged at 6000–8000 rpm for 20–30 minutes. The solution was taken from the upper part for analysis.

Flavonoids in chloroform, ethyl acetate, butanol, and aqueous fractions isolated from the aerial parts of Nepeta Olgae Regel L. were determined by high-performance liquid chromatography (HPLC). The results obtained are presented in Table 3 and Figures 1–2.

In the literature, phosphate, acetate buffer systems, and acetonitrile are used as eluents for determining steroids and

flavonoids by HPLC. In this study, a phosphate buffer system and acetonitrile were used.

The experiment was conducted on an Agilent-1200 HPLC system using an Agilent Eclipse XDB-C18 5 μm, 4.6x150 mm column. Elution was performed in isocratic mode. Phosphate buffer systems and acetonitrile were used as mobile phases. The flow rate was 1.0 ml/min, and the injection volume was 10 μl. The measurement was carried out at wavelengths of 320 nm, 254 nm, and 276 nm. The gradient was as follows: 1-6% B/min (0-2.5 min); 6-30% B/min (2.51-40 min); 30-60% B/min (40.1-45 min); 60-60% B/min (45.1-50 min); 60-0% B/min (50.1-55 min).

Standard solutions of dihydroquercetin, quercetin, luteolin, rutin, sinaroside, and salidroside, prepared at a concentration of 10 μg/ml, were used for identification based on the literature data.

The composition and quantity of the flavonoids obtained are presented in Figures 1–6 and Table 4.

4. Results and Discussion

The qualitative parameters of Nepeta Olgae Regel L. were determined according to the requirements of the State Pharmacopoeia (GOST 22839, ГОСТ 24027.2) [5-6].

As seen from the data presented in Table 1, the hygroscopic moisture content of Nepeta Olgae Regel L. does not exceed the limit of 12-15%, which is allowed for all plants, typically for pharmacopoeial samples [4-6]. Therefore, the raw material of Nepeta Olgae Regel L. is of high quality, and medicinal substances can be extracted from it.

Table 1. Quality indicators of the plant Nepeta Olgae Regel L.

Plant organ	Collection location	Plant mass (g)	Ash content			Moisture content %
			g	%	10% HCl	
Above ground part	Slope of Gova mountain	63,49	4,22	6,65	2,18	12,11
	Slope of Kosonsay mountain	60,14	4,31,	7,16	2,35	11,84
Roots	Slope of Gova mountain	55,83	4,19	7,50	2,61	12,41
	Slope of Kosonsay mountain	54,51	4,47	8,20	2,86	12,62

Table 2. Extractive substances of the plant Nepeta Olgae Regel L.: Dependence of the extraction process on the solvent medium

Solvent	Amount of extractable substances, %			
	In the flowering phase		Before flowering	
	Slope of Gova mountain	Slope of Kosonsay mountain	Slope of Gova mountain	Slope of Kosonsay mountain
Distilled water	18,64	18,14	18,82	18,08
40 % ethanol	41,18	36,26	35,92	35,16
70 % ethanol	65,21	61,84	62,10	62,46
96 % ethanol	20,32	20,82	22,61	24,01
40 % methanol	22,31	22,64	22,85	22,66
70 % methanol	17,24	18,14	20,21	22,24
Chloroform	10,12	10,09	14,65	12,61
Ethyl acetate	21,01	22,32	22,26	21,08

Table 3. Results of qualitative determination of biologically active compounds in the extract of the plant Nepeta Olgae Regel L.

Type of qualitative reaction	Reaction result	Water	Alcohol		
			40%	70%	96%
Flavonoids					
Cyanid in test according to Brayant	A separation 2 phase occured. The aqueous phase is more colored.	++	++	+++	+++
Cyanid in test (conc. HCl+Zn(Mg))	Pink colors.	++	++	++	++
Iron (III) chloride solution	The formation is brown-black-green in color.	+	+	++	++
Heating with ammonia	Golden yellow color.	+	+	++	++
C 10% alcoholic solution of alkali	With the formation of yellow- brown colors	++	++	++	++
Mg, HCl+H_2O	Red color	+	+	++	+
$AlCl_3$	Dark yellow color	+	+	+	+

Note. The number of "+" signs indicates the intensity of the coloration.

To determine the amount of undesirable impurities in the plant raw material, the ash insoluble in 10% HCl is measured. According to the data presented in Table 1, this indicator ranges from 2.86% to 2.18%, which is comparable to the best pharmacopoeial samples.

Water and water-alcohol mixtures of a specific concentration were used as extractants. The results for determining the optimal extractant for extracting the plant extract before and during flowering are presented in Table 2.

The data presented in Table 2 show that the maximum amount of extractive substances was released during the extraction with 70% ethyl alcohol.

When extracting the plant with a water-ethanol mixture of high concentration, both hydrophilic compounds and less polar representatives of plant metabolites, i.e. active substances, are extracted together. This process is also mentioned in the literature [7].

For the analysis of the quality of the investigated plant Nepeta Olgae Regel L., the most reliable qualitative reactions were selected, and the qualitative reactions were conducted based on literary data. The results are presented in Table 3.

Based on the results of the qualitative reactions, the presence of phenolic compounds—flavonoids—was established in the extracts of Nepeta Olgae Regel L.

The qualitative and quantitative content of flavonoids in the aerial part of the plant Nepeta Olgae Regel L. was determined using high-performance liquid chromatography.

Figure 1 presents a flowchart of the sequential extraction of flavonoids from Nepeta Olgae Regel L. in various solvents.

The composition and quantity of the obtained flavonoids are presented in Figures 2–7 and in Table 4.

Standard samples of flavonoids and their chromatographic parameters were used in the analysis of the flavonoid composition of the aerial part of Nepeta Olgae Regel L. The standard samples included quercetin, rutin, luteolin, dihydroquercetin, synerozide, and salidroside. The quantitative content of flavonoids was determined by calculating the area of the corresponding peaks in the HPLC chromatograms. In addition to the standard flavonoids, other peaks were observed on the chromatogram. Due to the lack of corresponding standard solutions, it was not possible to identify which flavonoids these peaks belonged to.

The results from Table 4 show that the aglycones of flavonoids (dihydroquercetin, quercetin) migrate to the chloroform fraction, while the aglycones of flavonoids with high polarity and glycosides with low polarity migrate to the ethyl acetate fraction. The n-butanol fraction predominantly contains highly polar flavonoid glycosides (luteolin, rutin, synerozide, and salidroside).

It can be seen that in the chloroform fraction of Nepeta Olgae Regel L., dihydroquercetin and quercetin are aglycones, i.e., relatively non-polar, although they are found in small amounts in the chloroform fraction. At the same time, it was established that rutin and salidroside are present in higher quantities in the butanol extract compared to other solvent fractions. The fractions with ethyl acetate and butanol contain flavonoids such as dihydroquercetin, quercetin, luteolin, rutin, synerozide, and salidroside.

Thus, for the first time, the presence of flavonoids such as dihydroquercetin, quercetin, rutin, synerozide, and salidroside was confirmed in the aerial part of Nepeta Olgae Regel L.

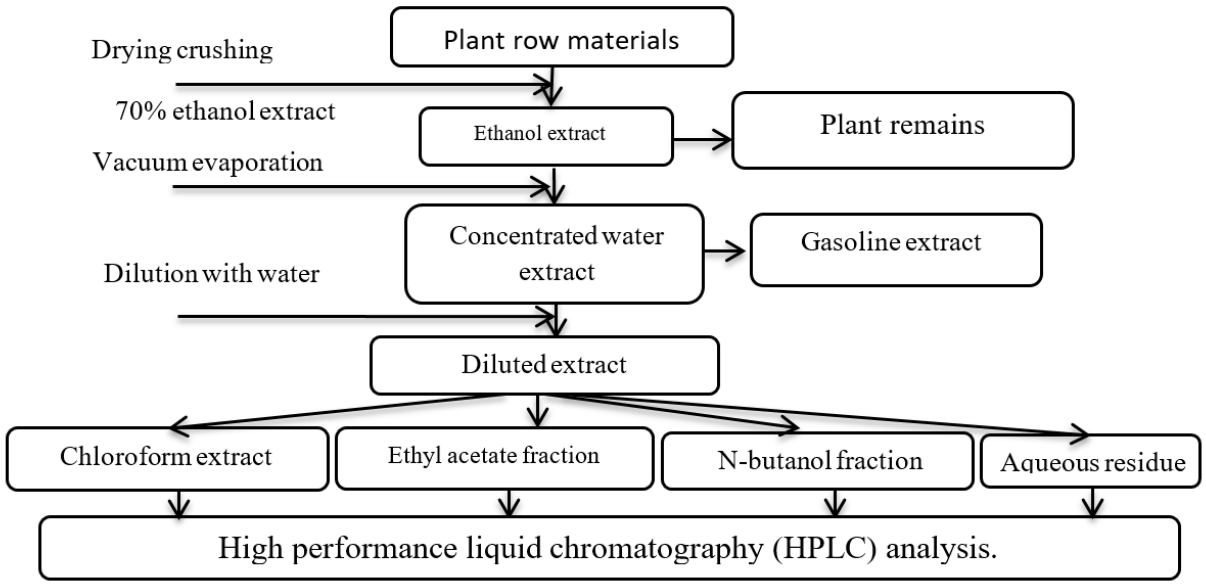

Figure 1. Scheme of fractionation of plant extracts of Nepeta Olgae Regel L. based on their solubility in various solvents

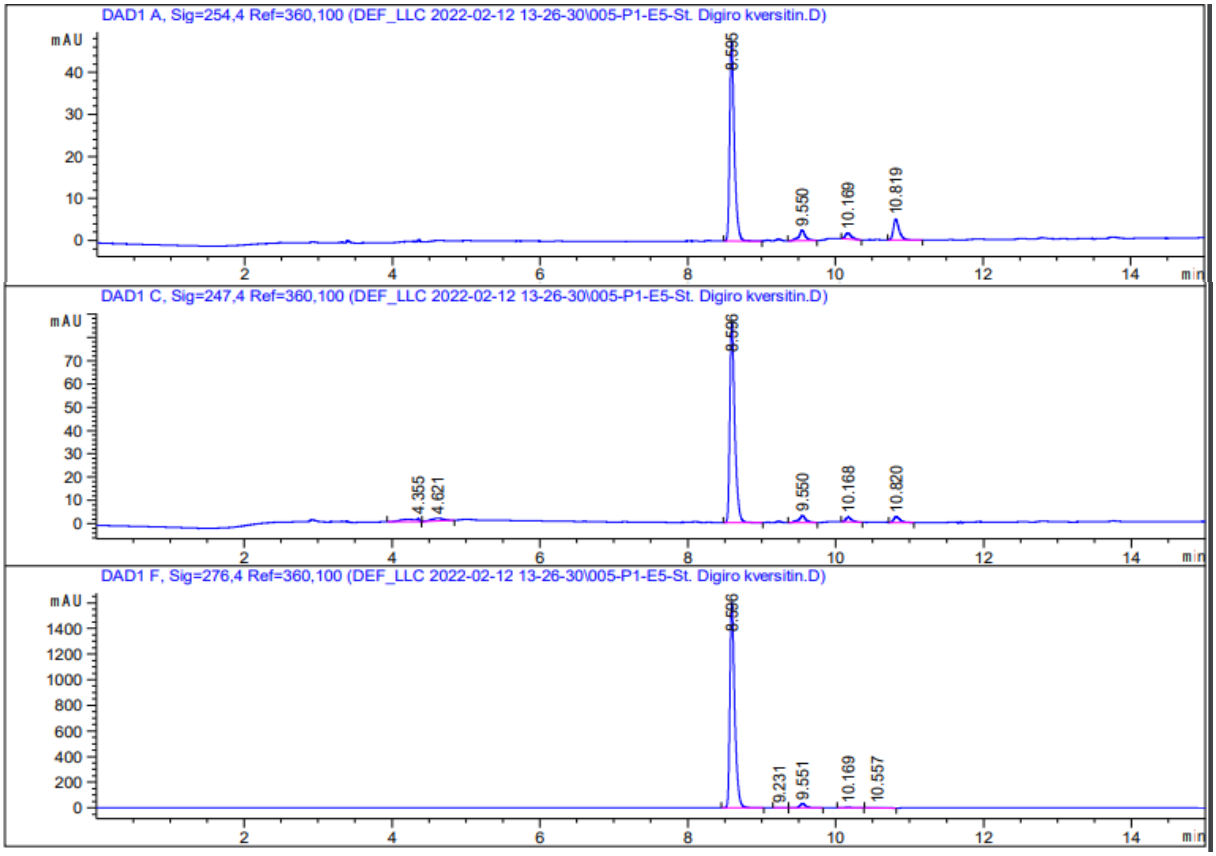

Figure 2. HPLC analysis of the flavonoid dihydroquercetin

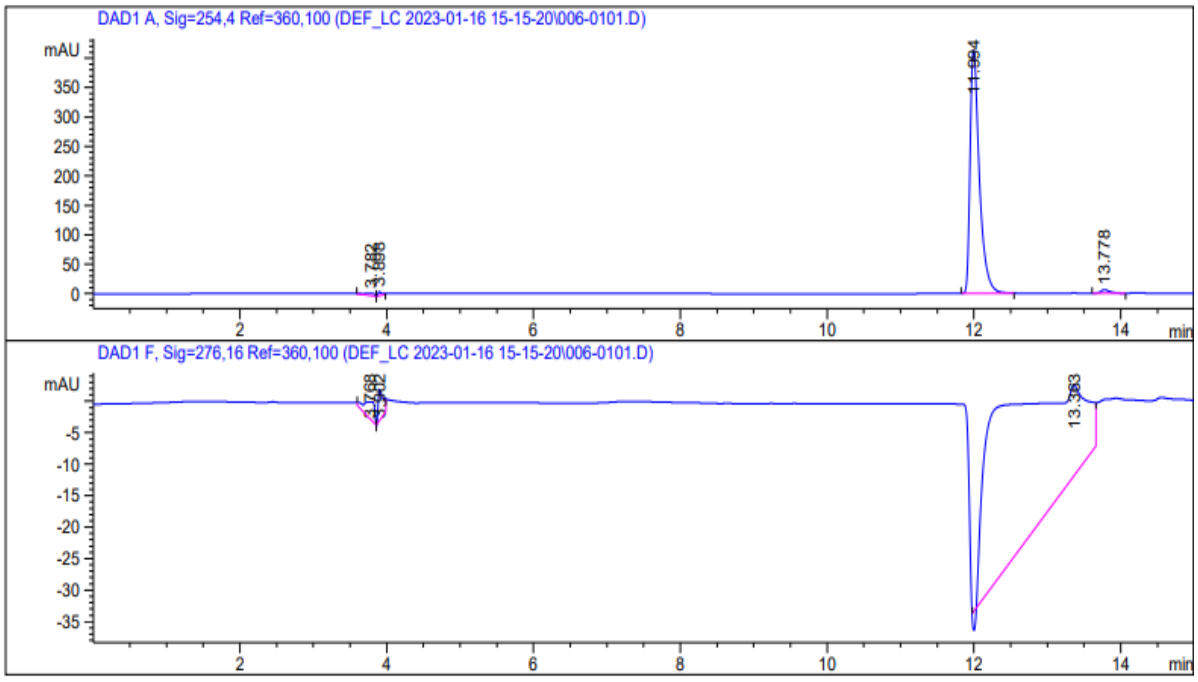

Figure 3. HPLC analysis of the flavonoid Luteolin

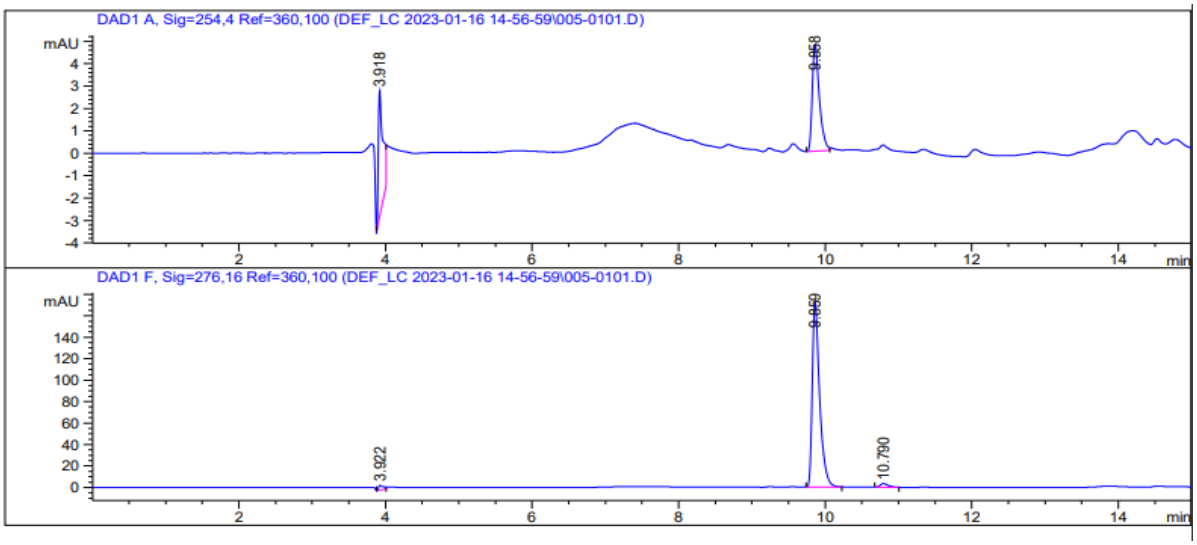

Figure 4. HPLC analysis of the flavonoid Quercetin

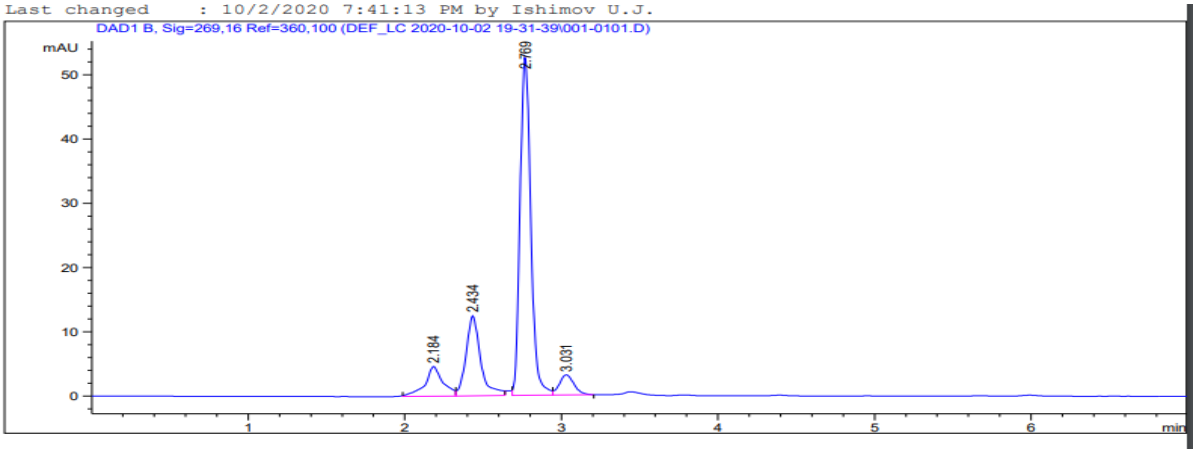

Figure 5. HPLC analysis of the flavonoid Rutin

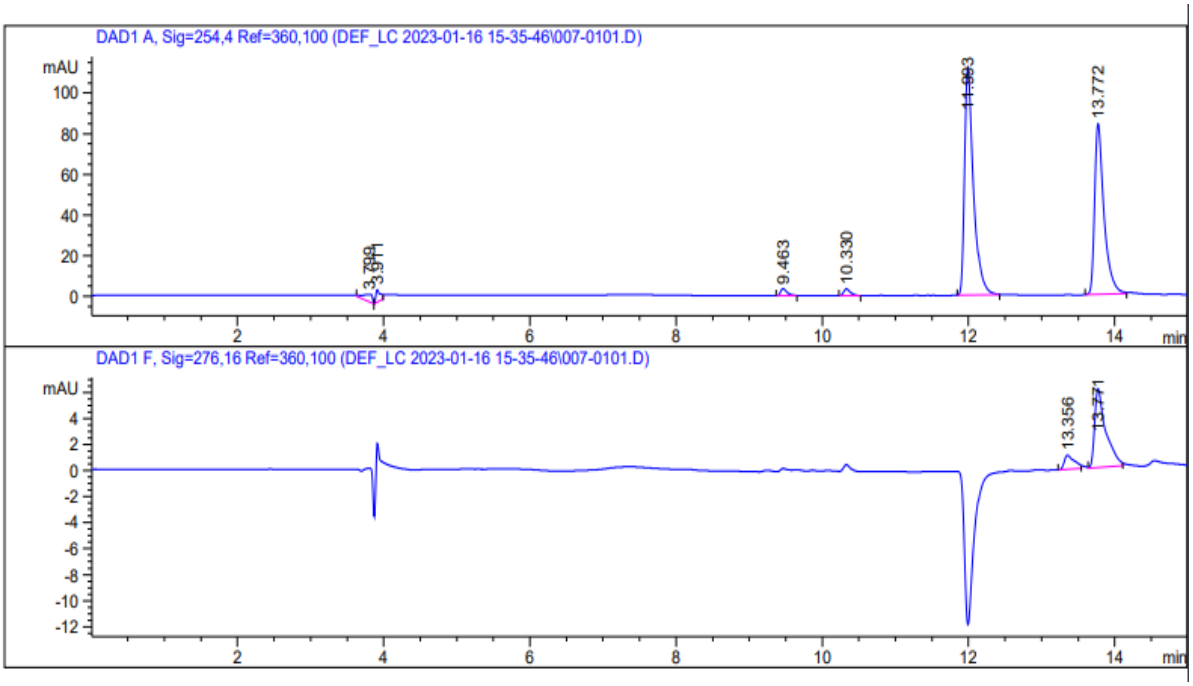

Figure 6. HPLC analysis of the flavonoid Synerozide

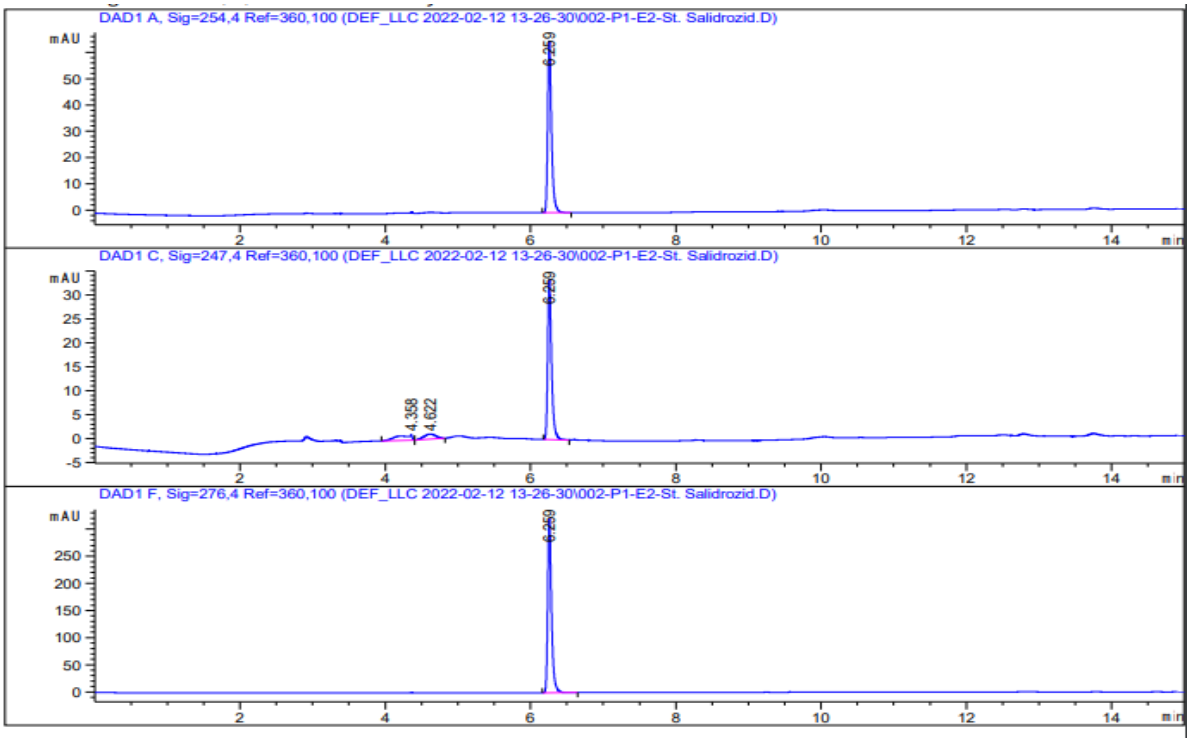

Figure 7. HPLC analysis of the flavonoid Salidroside

Table 4. Quantitative indicators of flavonoids in the aerial part of the plant Nepeta Olgae Regel L.

Flavonoids	№1 Chloroform fraction	№2 Ethyl acetate fraction	№3 Butanol fraction	№4 Water residue
	Concentration mg/g			
Dihydroquer-citine	0,598	3,93	0,97	0,576
Luthionine	0	0	0,77	0,454
Quercetino	0,132	1,13	0,904	0,61
Routine	0	1,41	1,58	0,17
Sinarozido	0	0	0,296	0,1
Salidrozido	0	0	3,21	0

5. Conclusions

Using high-performance liquid chromatography, the composition and quantity of flavonoids in the aerial part of Nepeta Olgae Regel L. were studied for the first time. The content of flavonoids dihydroquercetin, luteolin, quercetin, rutin, synerozide, and salidroside was determined.

Thus, the raw material of the plant Nepeta Olgae Regel L. has high quality, and medicinal substances can be extracted from it.

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