

# Phenolic Compounds and Antioxidant Potential of Wild-Growing Plant Materials of the North Caucasus Region

Anzaur Adamovich Skhalyakhov, Hazret Ruslanovich Siyukhov, Zareta Talbievna Tazova, Ludmila Victorovna Lunina, Irina Guchevna Mugu

**Abstract:** *The article presents the results of studying the qualitative composition and quantitative content of some groups of phenolic compounds in 11 types of medicinal plants growing in the foothills of the North Caucasus, and provides the estimates of the antioxidant activity of extracts from these plants. The qualitative and quantitative content of phenolic compounds was determined using a Kapel-105M capillary electrophoresis system, and the total antioxidant activity of the extracts was measured on a Tsvet Yauza-01-AA device with an amperometric detector.*

*In the studied plant samples, the total content of tannins was determined, eight phenolcarboxylic acids were identified and quantified, as well as quercetin and rutin — two of the most important flavonols.*

*The highest total content of phenolcarboxylic acids (11,776.2 mg/kg), as well as the highest antioxidant activity were noted in the aqueous extract obtained from *Echinacea purpurea* (lat. *Echinacea angustifolia*).*

*The direct relationship between the antioxidant activity of the studied medicinal raw material and the content of phenolic compounds has been experimentally established as follows: the higher is the concentration of phenolic substances, the higher is the antioxidant activity.*

*The results of this study provide new information on the composition and content of phenolic compounds in some types of wild-growing plant raw materials of the North Caucasus and the antioxidant activity of extracts based thereon that will facilitate the use of the studied plants as a potential source of natural antioxidants in the production of functional materials.*

**Keywords:** *medicinal raw material, phenolcarboxylic acids, rutin, quercetin, tannins, extract, antioxidant activity.*

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## I. INTRODUCTION

Phenolic compounds are widely spread in wild-growing plant raw material. The substances of this group account for up to 2 – 3 % of the mass of organic matter of plants and in some cases for up to 10 % or more. Currently there are more than two thousand naturally occurring phenolic compounds with various chemical structures [1, 2].

The biological role of phenolic compounds is primarily due to antioxidant properties.

It is known that under the action of stress factors free radicals are generated in the living body that can cause damage to functionally important molecules. The ability of antioxidants to interact with free radicals and convert them into inactive products can reduce the severity of the injury acting as a protective agent. A physicochemical regulatory system operates in the living organism that maintains the necessary level of free-radical reactions, regulates the exchange of membrane lipids and the rate of consumption of antioxidants. When the antioxidants level rises, the oxidation processes in the cell membranes slow down.

Numerous studies [3, 4] found that regular consumption of foods rich in phenolic compounds reduced the risk of cardiovascular, neurodegenerative, and cancer diseases due to their antioxidant activity, protection, and regeneration of other dietary antioxidants (e.g., vitamins C and E), and the chelation of prooxidant metal ions.

Currently, both natural and synthetic antioxidants are used for the enrichment of food with phenolic compounds. However, in respect of the synthetic antioxidants, the scientific literature provides enough information about possible negative effects on the human body [5]. Therefore, the interest of scientific researchers has been drawn, first and foremost, to natural antioxidants, with medicinal plants being their key sources [6-8].

The main physiologically active substances in the class of natural phenolic compounds include phenolcarboxylic acids and flavonoids. Their qualitative composition and quantitative content in plant materials are different depending on the species, location, and climatic factors.

Even though plants of the North Caucasus region are well-known for their biological value [9, 10], the scientific literature contains little information about their phenolic composition and antioxidant activity.

**Scope** of the research is studying the qualitative composition and quantitative content of certain groups of phenolic compounds (phenolcarboxylic acids, rutin, quercetin, tannins) in 11 species of medicinal plants growing in the North Caucasus, and assessing their antioxidant potential.

### II. MATERIALS

The study material was air-dried raw materials of the aerial parts of the following medicinal plants growing in the foothills of the Republic of Adygea, namely: common thyme (thyme) (Lat. *Thymus serpyllum* L), common origanum (Lat. *Origanum vulgare*), big-sting nettle (Lat. *Urtica dioica* L., leaves); common bilberry (Lat. *Vaccinium myrtillus* L., leaves); *Echinacea Purpurea* (Lat. *Echinacea angustifolia*); black currant (lat. *Ribes nigrum*, leaves); red clover (Lat. *Trifolium pratense* L., blossom truss); briar (Lat. *Rosa majalis*, fruits); walnut (Lat. *Juglans regia* L., leaves); common duckweed (Lat. *Lemna minor*, leaves); blessed milk thistle (lat. *Silybum marianum*), and aqueous extracts from plants under research.

To obtain the extracts, each type of plant raw material was ground at a temperature of 18 – 20° C to a particle size of 1 – 2 mm, poured with prepared water ( $t = +36 \pm 20^\circ \text{C}$ ) at the ratio of raw material/extractant of 1:10. During maceration, all extracts were subjected to ultrasonic treatment under the following extraction conditions: ultrasound exposure intensity – 100 W/cm<sup>2</sup>; ultrasound treatment time – 10 seconds; discreteness – every 10 minutes; and total extraction time – 60 minutes.

Ultrasonic exposure was carried out using a Volna series ultrasonic technological apparatus, model UZTA-0.4/22-OM.

### III. PROPOSED METHODOLOGY

#### A. General description

The mass concentration of phenolcarboxylic acids, rutin, and quercetin was determined as per [11, 12] (StP00668034-23-15-2009, 2009; STO 00668034-097-20018 10) using the Kapel-105M capillary electrophoresis system based on the separation of the components of the dissolved sample in a quartz capillary under the influence of an electric field and registration of output signals corresponding to each component on the electrophoregram. The range of working detection wavelengths was 190 – 380 nm.

The total content of tannins was determined according to (GOST 24027.2-80) [13] by the titrimetric method.

The antioxidant activity of the extracts was measured as per (GOST R 54037-2010) [14] by the amperometric method on the Tsvet Yauza-01-AA device designed to determine the total content of antioxidants.

The essence of this method consists in measuring the electric current arising at oxidation of the substance under research (or mixes of substances) on the surface of the working electrode at a constant potential of 1.3 V. With this

potential, only -OH groups of natural phenolic antioxidants are oxidized [15-17]. A calibration dependence of the signal of the reference sample (gallic acid) on its concentration was preliminarily constructed. Using the calibration obtained, the signals from the research extract were compared with the signals of the reference sample, gallic acid. The relative standard deviation (relative standard deviation of 5 – 6 identical readings of the device) was no more than 5 % [17].

#### B. Algorithm

Determining the content of phenolcarboxylic acids, rutin, and quercetin. Distilled water (hydraulic module 1:10,  $t$  of water 36°C) was poured in a portion of the dry test material  $m = 1.0$  g. It then stood for 24 hours with periodic stirring every eight hours. Then filtering was made through an ashless filter. The resulting solution was diluted with distilled water in a volume 10 times the volume of the solution and centrifuged at 6,000 rpm for 3 – 4 minutes. After that, the finished sample was placed in the Kapel-105M capillary electrophoresis device for analysis as per the previously constructed calibration dependence.

To prepare a working electrolyte, 75 mg of boric acid weighed to the nearest 0.1 mg were placed in a volumetric flask with the capacity of 25 cm<sup>3</sup>, and 20 cm<sup>3</sup> of distilled water were added. The solution obtained was dissolved in a water bath at 70°C, cooled, and 0.3 cm<sup>3</sup> of 0.1n sodium hydroxide was added, mixed, and brought to the mark with distilled water. The detection wavelength was 254 nm.

Subsequently, the mass concentration of each component was calculated as per the established calibration characteristics using the following formula (1):

$$X = k \cdot C, \text{ mg/dm}^3 (\text{mg/kg}), (1)$$

where  $k$  was the sample dilution ratio, defined as follows:  $k = M/m$  ( $M$  was the volume of the sample;  $m$  was the volume of the sample taken for testing),

$C$  was the concentration of the component found on the calibration graph, mg/kg.

Determination of the total content of tannins. The analytical sample of the raw material was crushed and sieved through a sieve with holes of 3 mm in diameter, after which a sample weighing 2 g was taken with an error of not more than 0.001 g. Subsequently, according to the procedure (GOST 24027.2-80) [12], the sample was prepared and titrated with 0.1 n of potassium permanganate solution to a golden yellow color, comparing it with the color of the control test solution.

The content of tannins ( $X$ ) in percent in absolutely dry raw materials was calculated by the formula provided in (GOST 24027.2-80) [12].

Measurements of the total content of water-soluble antioxidants on the Tsvet Yauza-01-AA device were carried out according to the instructions for this type of device.

All experiments were carried out in triplicate.

#### C. Flow chart

The research algorithm is presented in Figure 1.

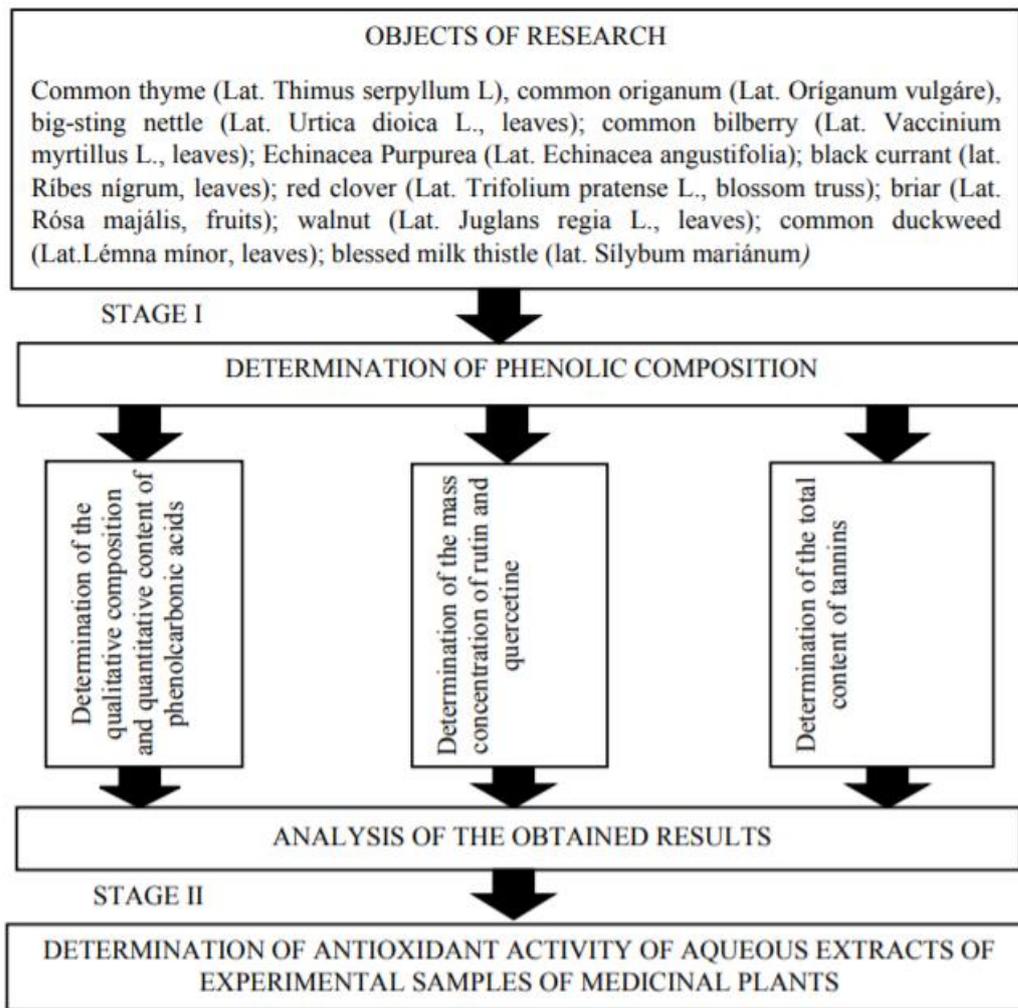


Fig. 1: Research Algorithm

IV. RESULT AND DISCUSSION

At the first stage of the study, the qualitative and quantitative composition of phenolcarboxylic acids, the

content of rutin and quercetin in the experimental samples of medicinal plant materials were determined.

The results are shown in Figures 2 – 12 and in Tables 1 – 2.

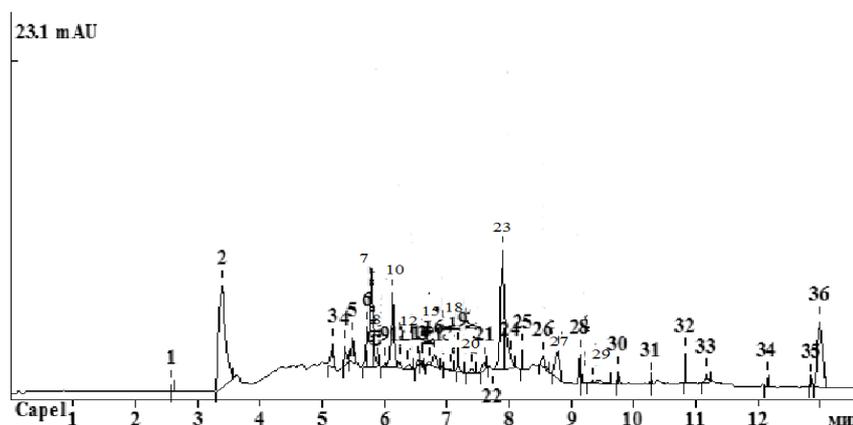


Fig. 2: Electrophoregram of sample No. 1/Common thyme (Lat. Thimus serpyllum L)/, phenolcarboxylic acids: 7 – chlorogenic acid, 8 – salicylic acid, 10 – rutin, 12 – genzitolovaya, 15 – quercetin, 18 – cinnamic acid, 20 – coumarin, 23 – syringic acid, 27-caffeic acid, 29-gallic acid

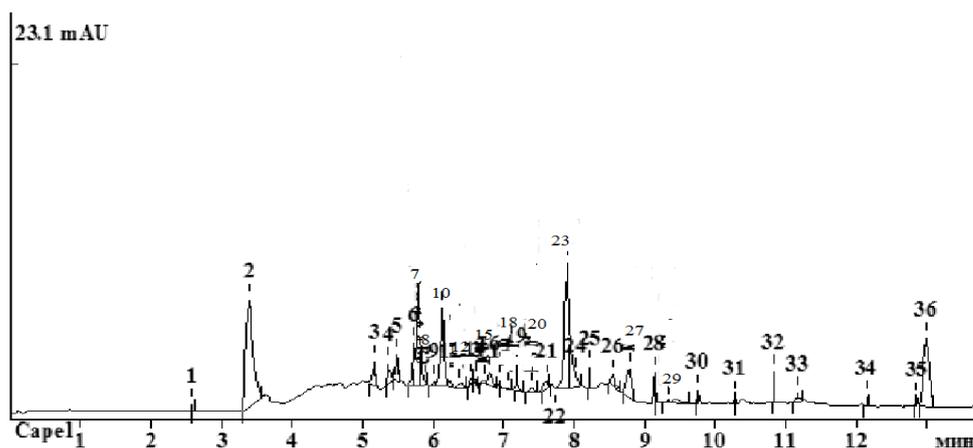


Fig. 3: Electrophoregram of sample No. 2/Common origanum (Lat. Origanum vulgare)/, phenolcarbionic acids:

8 – chlorogenic acid, 12 – salicylic acid, 13 – rutin, 16 – genzitolovaya, 18 – quercetin, 21 – cinnamic acid, 23 – coumarin, 28 – syringic acid, 34 – caffeic acid, 35 – gallic acid

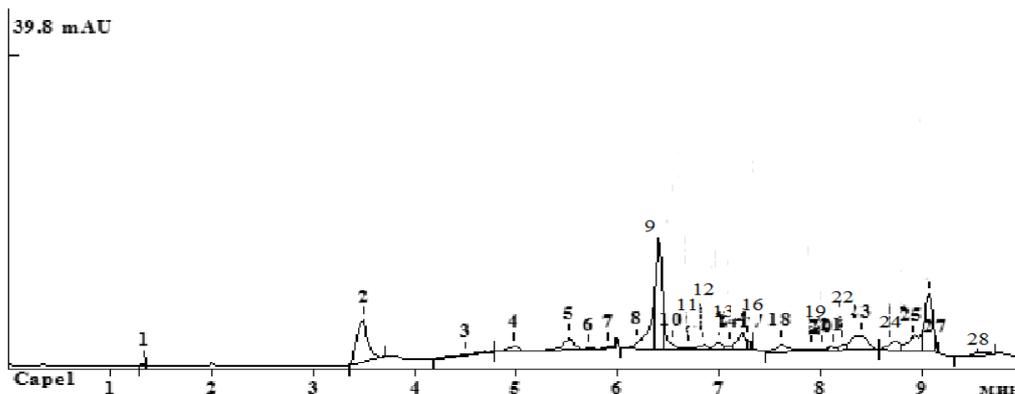


Fig. 4: Electrophoregram of sample No. 3/Big-sting nettle (Lat. Urtica dioica L., leaves)/, phenolcarbionic acids:

9 – chlorogenic acid, 11 – salicylic acid, 12 – rutin, 13 – genzitolovaya, 16 – quercetin, 19 – cinnamic acid, 22 – coumaric acid, 24 – syringic acid, 27 – caffeic acid, 28 – gallic acid

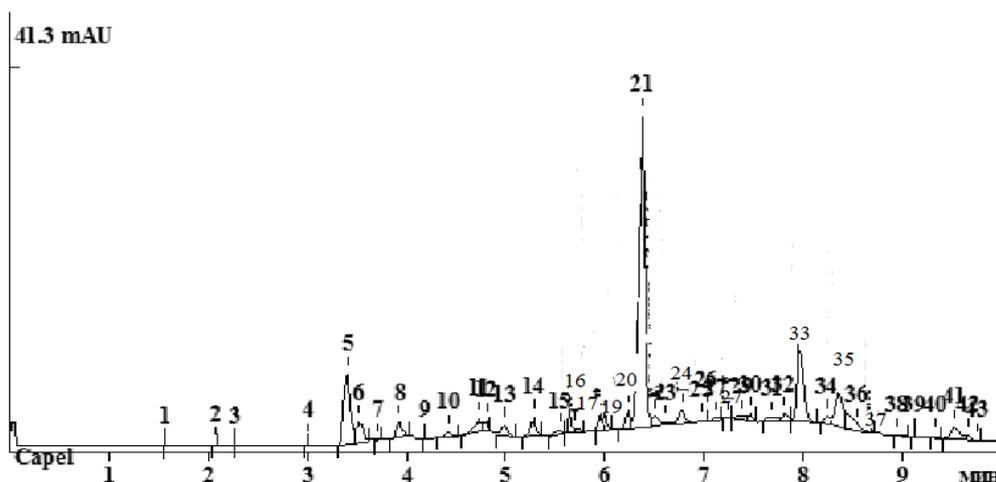
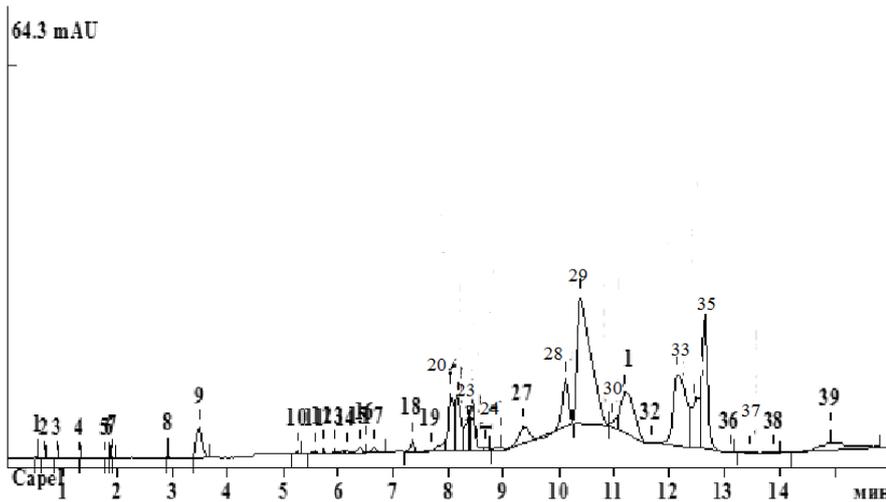
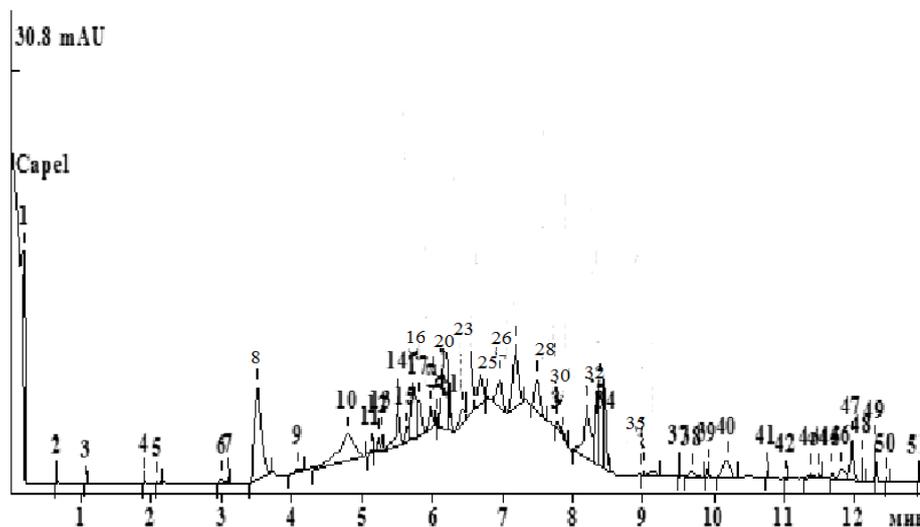


Fig. 5: Electrophoregram of sample No. 4/Common bilberry (Lat. Vaccinium myrtillus L., leaves)/, phenolcarbionic acids:

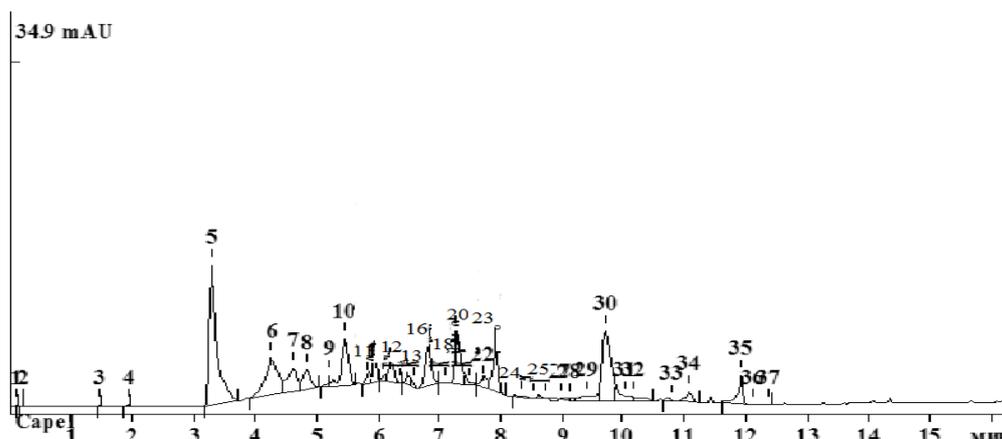
16 – chlorogenic acid, 17 – salicylic acid, 19 – rutin, 20 – genzitolovaya, 22 – quercetin, 24 – cinnamic acid, 27 – coumaric acid, 33 – syringic acid, 35 – caffeic acid, 37 – gallic acid



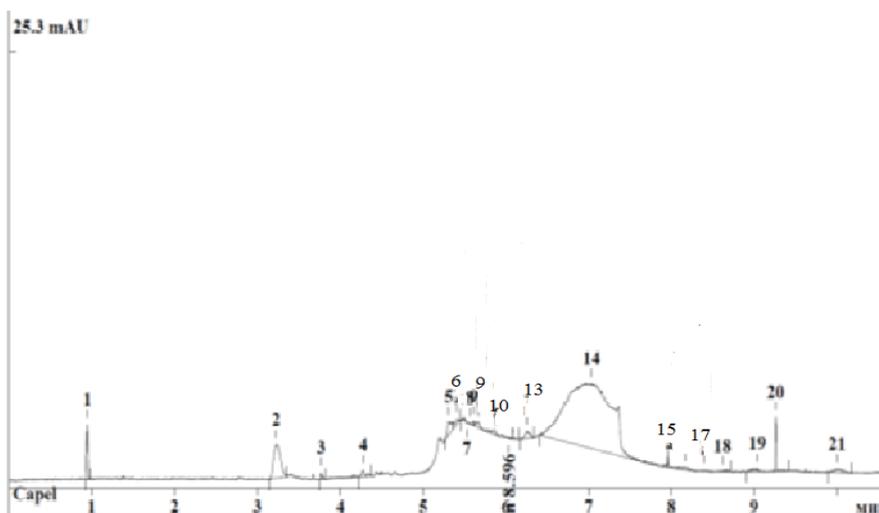
**Fig. 6: Electrophoregram of sample No. 5/Echinacea Purpurea (Lat. Echinacea angustifolia)/:**  
20 – chlorogenic acid, 23 – salicylic acid, 24 – genzitolovaya, 28 – cinnamic acid, 30 – coumaric acid, 33 – syringic acid, 35 – caffeic acid, 37 – gallic acid



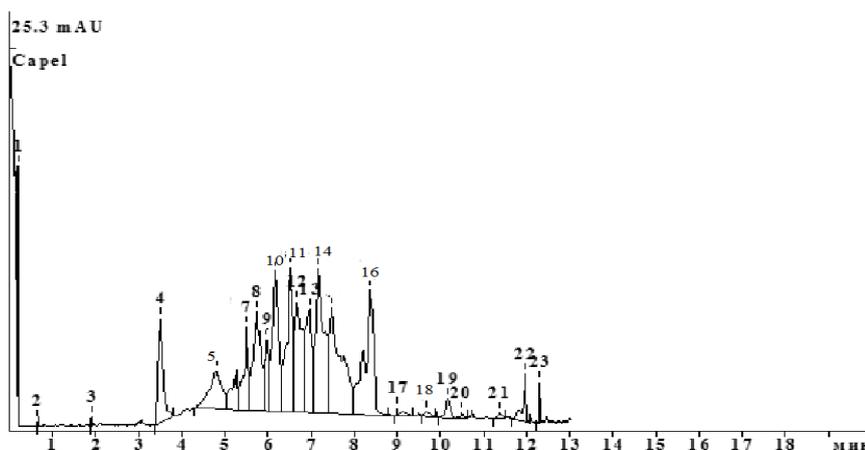
**Fig. 7: Electrophoregram of sample No. 6/Black currant (Lat. Ribes nigrum, leaves):**  
16 – chlorogenic acid, 17 – salicylic acid, 20 – rutin, 23 – genzitolovaya, 25 – quercetin, 26 – cinnamic acid, 28 – coumaric acid, 30 – syringic acid, 32 – caffeic acid, 35 – gallic acid



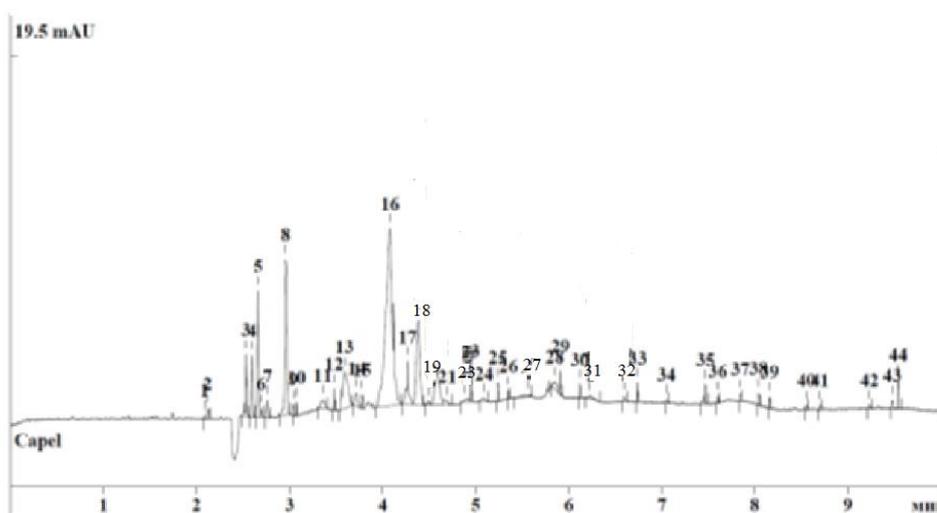
**Fig. 8: Electrophoregram of sample No. 7/Red clover (Lat. Trifolium pratense L., blossom truss)/:**  
11 – chlorogenic acid, 12 – salicylic acid, 13 – genzitolovaya, 16 – quercetin, 18 – cinnamic acid, 20 – coumaric acid, 23 – syringic acid, 24 – caffeic acid, 25 – gallic acid



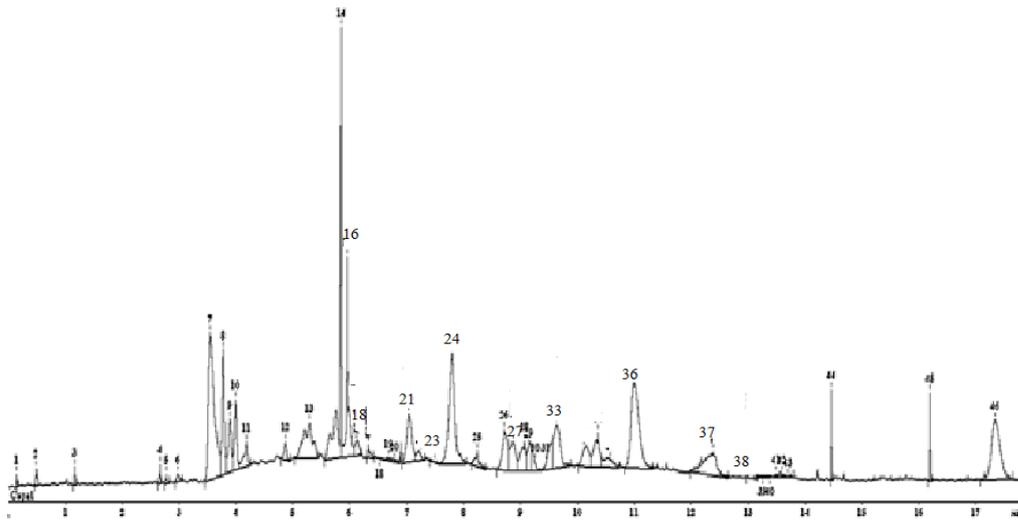
**Fig. 9: Electrophoregram of sample No. 8/Briar (Lat. Rósa majális, fruits):**  
 6 – chlorogenic acid, 9 – salicylic acid, 10 – rutin, 13 – p-coumaric acid, 15 – caffeic acid, 17 – gallic acid



**Fig. 10: Electrophoregram of sample No. 9/Walnut tree (Lat. Juglans regia L., leaves):**  
 5 – ascorbic acid, 10 – chlorogenic acid, 15 – orotic acid, 11 – nicotinic acid, 16-caffeic acid, 18 – gallic acid



**Fig. 11: Electrophoregram of sample No. 10/ Common duckweed (Lat. Lémna mínor, leaves):**  
 18 – chlorogenic acid, 19 – salicylic acid, 21 – rutin, 23 – quercetin, 27 – p-coumaric acid, 31 – caffeic acid, 32 – gallic acid



**Fig. 12: Electrophoregram of sample No. 11/Milk thistle (lat. Silybum marianum)/**

16 – chlorogenic acid, 18 – salicylic acid, 21 – rutin, 23 – genzitolovaya, 24 – quercetin, 27 – cinnamic acid, 33 – p-coumaric, 36 – syringic acid, 37 – caffeic acid, 38 – gallic acid

**Table 1: Mass concentration of phenolcarmonic acids in the experimental samples of plant raw materials, mg/kg**

Name of the identified phenolcarmonic acids	Common thyme (Lat. Thimus serpyllum L)	Common origanum (Lat. Origanum vulgare)	Big-sting nettle (Lat. Urtica dioica L., leaves)	Common bilberry (Lat. Vaccinium myrtillus L., leaves)	Echinacea Purpurea (Lat. Echinacea angustifolia)	Black currant (lat. Ribes nigrum, leaves)
Chlorogenic	556	1,341	1,454	136	1,300	485
Salicylic	70	26.7	35	40.2	603	131
Genzitolovaya	38.7	12.7	127	166	13.7	150
Cinnamic	7.2	68	53	138	1,483	266
Coumaric	24	35.5	110	9.2	328	285
Syringic	827	200	224	818	4,533	59.7
Caffeic	231	311	854	486	3,498	827
Gallic	38.5	27	96.7	6.5	17.5	48.6
Sum	1,792.4	2,021.9	2,953.7	2,161.72	11,776.2	2,252.3

**Table 2: Mass concentration of phenolcarmonic acids in the experimental samples of plant raw materials, mg/kg**

Name of the identified phenolcarmonic acids	Red clover (Lat. Trifolium pratense L., blossom truss):	Briar (Lat. Rósa majális, fruits):	Walnut (leaves) (Lat. Juglans regia L., leaves)	Common duckweed (Lat. Lémna minor, leaves)	Milk thistle (lat. Silybum marianum)
Chlorogenic	281	92.93	4,603	207	43
Salicylic	54	39.24	1954	17	12
Genzitolovaya	8.2	0	105	11.6	0.2
Cinnamic	19.5	0	45	9.3	103
Coumaric	113.6	63.56	14.3	5.9	186
Syringic	700	0	351	0	427
Caffeic	17.8	34.18	403	4.6	185
Gallic	0	7.05	19.5	0.1	0.3
Sum	1,194.1	236.96	7,494.8	255.5	956.5

It follows from the above data (Fig. 2 – 12 and Tables 1 – 2) that in nine out of 11 samples, eight phenolcarmonic acids were identified, namely chlorogenic, salicylic, genzitolovaya, cinnamic, coumaric, syringic, caffeic, and gallic acids. The blossom truss of the red clover (Lat. *Trifolium pratense L.*) lacked gallic acid, the briar fruits (Lat. *Rósa majális, fruits*) – genzitolovaya, cinnamic, syringic acids, and the leaves of the common duckweed (Lat. *Lémna minor*) – syringic acid.

The pharmacological properties of phenolcarmonic acids

are mainly conditioned upon strong antioxidant effect [18, 19]. It was established that in the experimental samples, chlorogenic phenolcarmonic acid dominated. Its biological role is to minimize the risks of developing malignant tumor formations, increase the elasticity of the vascular walls, normalize blood sugar levels, improve skin condition, prevent diseases of the cardiovascular system and liver, etc.

The content of chlorogenic acid in the experimental samples decreases in the following series: Walnut (Lat. *Juglans regia* L., leaves (4,603 mg/kg) → Big-sting nettle (Lat. *Urtica dioica* L., leaves (1,454 mg/kg) → Common origanum (Lat. *Origanum vulgare* (1,341 mg/kg) → Echinacea Purpurea (Lat. *Echinacea angustifolia*(1,300 mg/kg) → Common thyme (Lat. *Thimus serpyllum* L(556 mg/kg) → Black currant (lat. *Ribes nigrum*, leaves (485 mg/kg) → Red clover (Lat. *Trifolium pratense* L., blossom truss (281 mg/kg)→ Common duckweed (lat. *Lémna minor*,

leaves) (207 mg/kg) → Common bilberry (lat. *Vaccinium myrtillus* L., leaves (136 mg/kg) → Briar (lat. *Rósa majális*, fruits (92.93 mg/kg) → Milk thistle (Lat. *Silybum maríanum*) (43 mg/kg).

The highest total content of phenolcarbonic acids was observed in the Echinacea purpurea (lat. *Echinacea angustifolia* (11,776.2 mg/kg) herb.

The results of the experimental studies to determine the mass concentration of rutin and quercetin in the experimental samples are presented in Table 3.

**Table 3: The content of rutin and quercetin in the experimental samples of plant materials, mg/kg**

Samples	Content, mg/kg	
	Rutin	Quercetin
Common thyme (Lat. <i>Thimus serpyllum</i> L)	391	22
Common origanum (Lat. <i>Origanum vulgare</i> )	230	36.8
Big-sting nettle (Lat. <i>Urtica dioica</i> L., leaves)	93.2	287
Common bilberry (Lat. <i>Vaccinium myrtillus</i> L., leaves)	124	130
Echinacea Purpurea (Lat. <i>Echinacea angustifolia</i> )	583	83.7
Black currant (lat. <i>Ribes nigrum</i> , leaves)	39.1	286
Red clover (Lat. <i>Trifolium pratense</i> L., blossom truss):	328	63.5
Briar (Lat. <i>Rósa majális</i> , fruits)	47.66	8.60
Walnut (leaves) (Lat. <i>Juglans regia</i> L., leaves)	5280	250
Common duckweed (Lat. <i>Lémna minor</i> , leaves)	306	0
Milk thistle (Lat. <i>Silybum maríanum</i> )	159	411

The analysis of the data obtained (Table 3) indicates that the content of rutin (vitamin P) in the plant raw materials ranges from 47.66 mg/kg in the briar fruits to 5,280 mg/kg in walnut leaves. Considering that the daily need for routine (vitamin P) is 35 – 50 mg per day, in three out of ten experimental samples its concentration in 100 g of raw material meets or exceeds the established norm, and the maximum excess of the daily requirement (on average 10 times) is noted in walnut leaves.

Concerning quercetin, the range of variation of this flavonol is much smaller than that of rutin – 0 (Common duckweed (lat. *Lémna minor*, leaves) – 411 mg/kg (Milk

thistle (lat. *Silybum maríanum*).

According to numerous data [1, 2, 7, 20], rutin and quercetin have anti-inflammatory, bactericidal, immunostimulating and anti-allergic properties, improve the elasticity of blood vessels, prevent heart attacks and strokes, enhance the effects of vitamin C, etc.

When studying the total content of tannins in the experimental samples, it was found that the digital values of this indicator (Table 4) were in the range of 20.0 g/kg (Common thyme, blossom truss of the red clover) – 138 g/kg (common bilberry leaves).

**Table 4: The total content of tannins in the experimental samples, g/kg**

Samples	The total content of tannins, g/kg
Common thyme (lat. <i>Thimus serpyllum</i> L)	20.0
Common origanum (lat. <i>Origanum vulgare</i> )	68.0
Big-sting nettle (lat. <i>Urtica dioica</i> L., leaves)	27.0
Common bilberry (lat. <i>Vaccinium myrtillus</i> L., leaves)	136.0
Echinacea Purpurea (lat. <i>Echinacea angustifolia</i> )	48.0
Black currant (lat. <i>Ribes nigrum</i> , leaves)	82.0
Red clover (lat. <i>Trifolium pratense</i> L., blossom truss)	20.0
Briar (lat. <i>Rósa majális</i> , fruits)	31.0
Walnut (leaves) (lat. <i>Juglans regia</i> L., leaves)	68.0
Common duckweed (lat. <i>Lémna minor</i> , leaves)	48.0
Milk thistle (lat. <i>Silybum maríanum</i> )	81.0

Tannins belong to the group of polyoxyphenol compounds with high pharmacological activity. They contribute to the best absorption of beneficial compounds, can suppress the activity of pathogens, form sediments with alkaloids, cardiac glycosides, salts of heavy metals, and

remove them from the body.

At the second stage of the research, the antioxidant activity indices were determined in the extracts obtained from the experimental samples of medicinal plants (Figure 13).

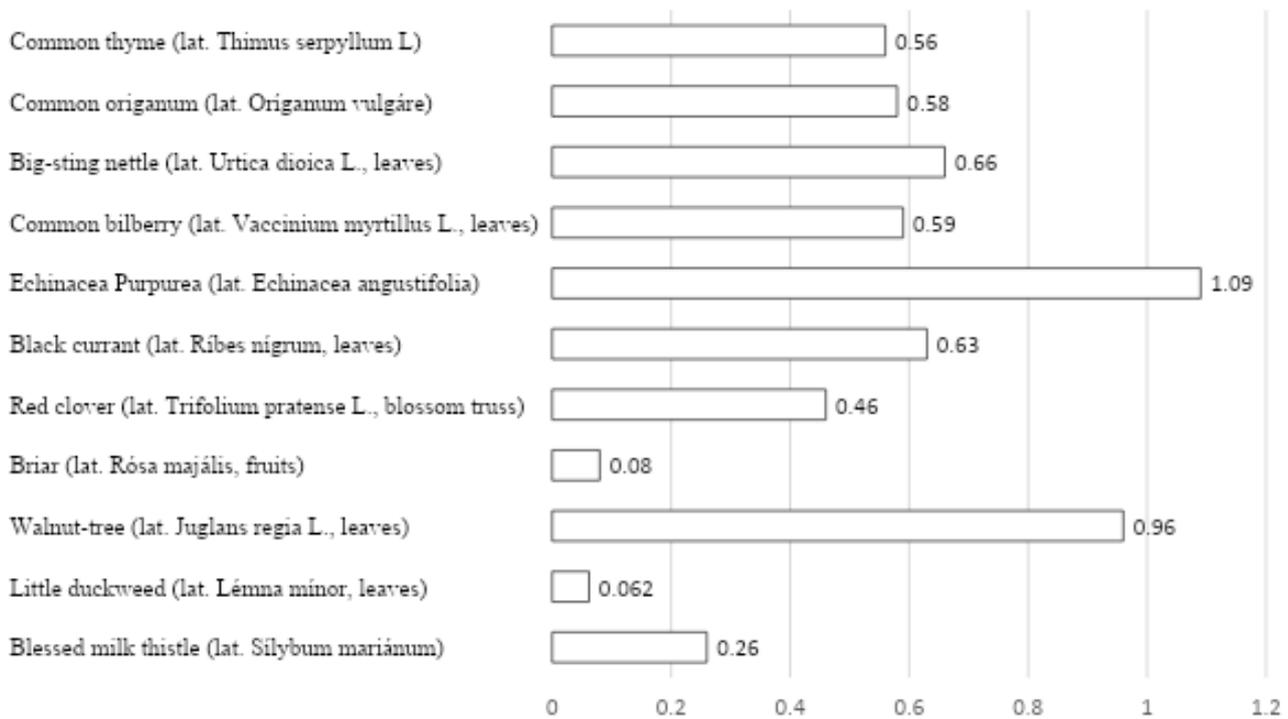


Fig. 13: Antioxidant activity of samples of plant raw materials in terms of gallic acid, g/dm<sup>3</sup>

The data in Figure 13 show that the highest antioxidant activity is observed in the Echinacea purpurea grass (1.06 g/dm<sup>3</sup>), and walnut leaves (0.96 g/dm<sup>3</sup>). This indicator is slightly lower in the leaves of big-sting nettle (0.66 g/dm<sup>3</sup>), common bilberry (0.58 g/dm<sup>3</sup>), and the common thyme grass (0.56 g/dm<sup>3</sup>). The minimum antioxidant activity was observed in the common duckweed leaves (0.062 g/dm<sup>3</sup>).

## V. CONCLUSION

1. The analysis of the qualitative composition and quantitative content of some groups of phenolic compounds in 11 species of wild-growing medicinal plants of the North Caucasus has shown that such plants as the common thyme (lat. *Thimus serpyllum* L), big-sting nettle (lat. *Urtica dioica* L., leaves); common bilberry (lat. *Vaccinium myrtillus* L., leaves); Echinacea Purpurea (lat. *Echinacea angustifolia*); black currant (lat. *Ribes nigrum*, leaves); red clover (lat. *Trifolium pratense* L., blossom truss), and walnut (lat. *Juglans regia* L., leaves) have sufficiently high concentration of phenolic compounds and can be used as enriching ingredients for the production of functional antioxidant products.

2. The experimental studies have shown a direct relationship between the antioxidant activity of the studied medicinal plants and the content of phenolic compounds therein: the higher is the concentration of phenolic substances in plant materials, the greater is their antioxidant activity.

It has been established that the antioxidant potential decreases in the following series: Echinacea Purpurea (lat. *Echinacea angustifolia*) > Walnut (lat. *Juglans regia* L., leaves) > Big-sting nettle (lat. *Urtica dioica* L., leaves) > Black currant (lat. *Ribes nigrum*, leaves) > Common bilberry (lat. *Vaccinium myrtillus* L., leaves); Common origanum (lat. *Origanum vulgare*) > Common thyme (lat.

*Thimus serpyllum* L), Red clover (lat. *Trifolium pratense* L., blossom truss) > Milk thistle (lat. *Silybum marianum*) > Briar (lat. *Rosa majalis*, fruits) > Common duckweed (lat. *Lémna minor*, leaves) due to the different concentration of phenolic compounds in the study objects. The research was carried out within the framework of the Federal Target Program "Research and Development in Priority Areas for the Development of the Russian Science and Technology Complex for 2014 – 2020" on the topic "Development of technologies for the production of high-quality and safe functional drinks using biologically active components of unconventional vegetable raw materials of the North Caucasus region", Agreement No. 14.574.21.0174. Unique identifier of the work (project) RFMEF157417X0174).

## NOVELTY STATEMENT

The scientific novelty of the research lies in the fact that the phenol composition of 11 species of medicinal plants of the North Caucasus region has been studied for the first time and their antioxidant potential has been evaluated as well.

## AUTHOR'S CONTRIBUTION

H. R. Siukhov developed the study and supervised it, participated in data analysis and interpretation of results, as well as in the writing of the main part of the article. A.A. Skhalyakhov participated in the development of the research algorithm, collection and analysis of data, interpretation of results and writing the main part of the article. Z.T. Tazova participated in the development of the research algorithm, selection of research methods, data analysis, interpretation of results and writing the main part of the article. L.V.

Lunina participated in the development of the research algorithm, selection of research methods, data analysis, interpretation of results and writing the main part of the article. I.G. Mugu conducted a literature review, collected additional information, participated in data analysis and preparation of the manuscript.

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