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ANTIRADICAL ACTIVITY AND CONTENT OF PHENOLIC COMPOUNDS OF STINGING NETTLE (URTICA DIOICA L.)

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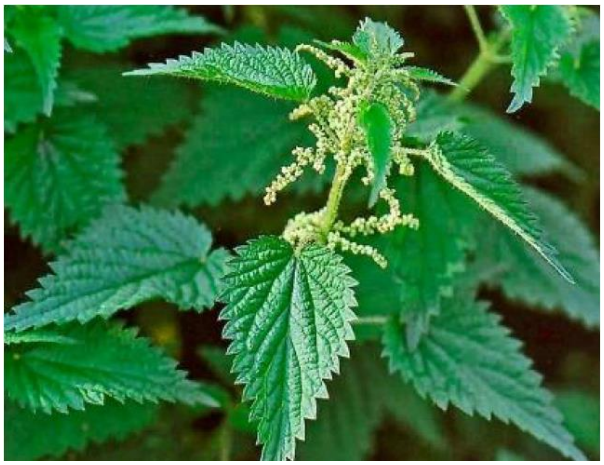
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Urtica dioica L., extraction, antiradical activity, 2,2-diphenyl-1-picrylhydrazyl, HPLC, flavonoid, quercetin, rutin

Annotation
The antiradical activity of an extract obtained from the dried and ground aerial part of the dioecious nettle (Urtica dioica L.) by extraction in 96% ethanol using an ultrasonic bath for 20 minutes was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The quantitative content of phenolic compounds (flavonoids) was determined by high-performance liquid chromatography (HPLC). The resulting extract exhibited antiradical activity, with an IC_{50} value of 314.64 µL. Chromatographic analysis showed that 100 g of dried Urtica dioica L. contains 7.3 mg of quercetin, 6.4 mg of rutin, 0.7 mg of salicylic acid, and 0.1 mg of apigenin.

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Introduction. Among the medicinal plants used in traditional medicine, stinging nettle (*Urtica dioica L.*) occupies a special place (figure 1). It is a perennial plant of the nettle family that grows in various regions of the world, depending on altitude, terrain, climate, and other environmental factors. It is predominantly found in temperate climate zones of Asia, Europe, and North America, as well as in cooler regions of Africa [1, 2]. The nettle family (*Urticaceae*) belongs to the class of dioecious plants. In terms of morphological structure, it is closely related to the mulberry family. Among its members, the following species are the most widespread: Urtica dioica, Urtica urens, and Urtica hyperborea. These species have been recorded in the flora of Uzbekistan, with Urtica dioica and Urtica urens being the most well-known and widely studied species with practical significance [1].



Stinging nettle (*Urtica dioica L.*) contains a large number of biologically active compounds in its leaves and roots, including polyphenols (flavonoids, gallic acid, caffeic acid, ellagic acid), phytosterols (beta-sitosterol), terpenoids, saponins, tannins, alkaloids, vitamins (A, C, K, B group), and biogenic elements (calcium, potassium, iron, magnesium, zinc, silicon, phosphorus), among others. Because of these components, preparations made from this plant exhibit anti-inflammatory, diuretic, anticancer, antihypertensive, and antioxidant properties. They can also be used in the treatment of hemorrhoids, heavy menstrual bleeding, anemia, cancer, bladder stones, gout, and for the prevention of dizziness, bleeding, arthritis, and possess antimicrobial, analgesic, antiviral, and anticolitic effects [3, 4, 2].

This article presents the results of evaluating the antiradical activity of the ethanol extract from the aerial parts of Urtica dioica L. using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method and determining the flavonoid content using the UV-Vis spectrophotometric method.

Materials and Methods

Assessment of Antiradical Activity. In ethanol solution, the stable purple radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) has the property of discoloration when exposed to various antiradical (antioxidant) compounds, due to its ability to pair its unpaired electron. This property is widely used in the spectrophotometric evaluation of antiradical activity [5, 6, 7]. In this study, an improved method of DPPH• free radical inhibition, originally proposed by Blois [6] and further modified in [7], was used to determine the antiradical activity of the nettle extract.

Preparation of the Working DPPH• Solution. A 7.92 mM solution of DPPH• in 96% ethanol was prepared in a 100 mL volumetric flask. The solution was protected from light and stored in a dark place at room temperature, wrapped in aluminum foil.

Preparation of the Extract from the Aerial Part of Urtica dioica L. To prepare the extract, 0.75 g of plant material was extracted in 25 mL of 96% ethanol using an ultrasonic bath for 20 minutes. The resulting extract was filtered through a syringe filter with a pore size of 0.45 µm [8] and used for experiments to determine antiradical activity and phenolic compound content.

Determination of the Plant's Antiradical Activity. Into a 4 mL quartz cuvette with a wall thickness of 10 mm, 3 mL of the ethanolic DPPH solution and 100 µL of ethanol were added. The cuvette was then placed into a spectrophotometer, and measurements of optical density (D_1) at a wavelength of 517 nm were taken every 5 minutes over 30 minutes using a YOKE K7000 spectrophotometer. The measured D_1 value remained unchanged over time and was recorded as 1.067.

Evaluation of the Antiradical Activity of the Urtica dioica L. Extract. To evaluate the antiradical activity of the Urtica dioica L. extract, four separate cuvettes (10 mm path length) were prepared, each containing 25, 50, 75, or 100 µL of the filtered plant extract. To each cuvette, 3 mL of the DPPH solution was added. The mixtures were thoroughly stirred and brought to a total volume of 4.1 mL using ethanol. The resulting solutions in the cuvettes were immediately mixed, and the optical density at 517 nm (D_2) was measured using the abovementioned method. The measured optical density values were then used to calculate the antiradical activity using the following formula:

$$ARF, \% = \frac{D_1 - D_2}{D_1} \cdot 100$$

The antiradical activity of the ethanol extract of Urtica dioica L. was calculated and is presented in table 1.

Table 1. Values of optical density of the extract from the aerial parts of Urtica dioica L. added to various volumes of the DPPH solution at a wavelength of 517 nm, and the calculated antiradical activity (ARF).

Volume of the extract, µL	Time, min	Optical density, D2	ARF%	Volume of the extract, µL	Time, min	Optical density, D2	ARF%
25	0	1,067	0,00	75	0	1,067	0,0
	5	1,02	4,40		5	0,95	11,0
	10	1,019	4,50		10	0,946	11,3
	15	1,018	4,59		15	0,941	11,8
	20	1,016	4,78		20	0,934	12,5
	25	1,016	4,78		25	0,932	12,7
	30	1,016	**4,78**		**30**	0,93	**12,8**
50	0	1,067	0,00	100	0	1,067	0,00
	5	0,986	7,59		5	0,927	13,12
	10	0,983	7,87		10	0,921	13,68
	15	0,981	8,06		15	0,915	14,25
	20	0,977	8,43		20	0,905	15,18
	25	0,976	8,53		25	0,902	15,46
	30	0,975	**8,62**		**30**	0,9	**15,65**

Chromatographic Analysis. To determine the content of phenolic compounds in the ethanol extract from the aerial parts of Urtica dioica L., a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph was used [9]. For the chromatographic analysis of phenolic compounds, standard solutions were employed, and the analysis of the Urtica dioica L. extract was performed using a Shim pack GIST C18 reverse-phase column (150 × 4.6 mm; 5 µm, Shimadzu) with a gradient mobile phase consisting of acetonitrile (A) and a 0.5% aqueous solution of acetic acid (B) (Table 2).

Table 2. Gradient program for the mobile phase

Tim	Acetonitrile (A), %	0.5% acetic acid solution (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

The injection volume was 10 µL, the flow rate was 0.5 mL/min, and the column thermostat temperature was 40 °C. Chromatograms of the mixture of standard phenolic compound solutions and the extract of the studied plant were recorded using a UV detector at a wavelength of 300 nm (Figures 2 and 3).

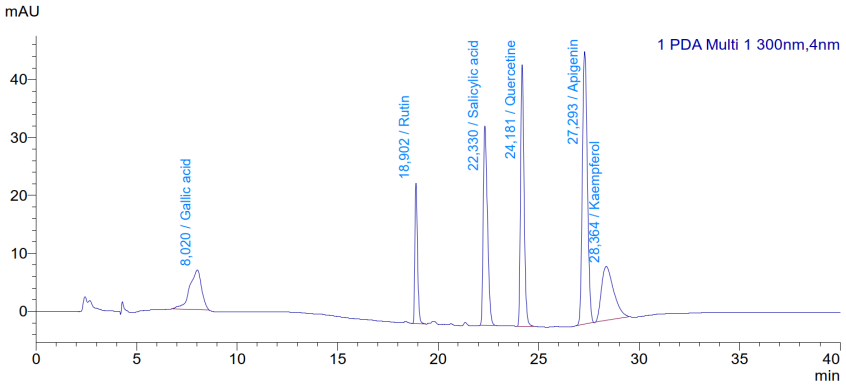


Figure 2. Chromatogram of standards at 300 nm

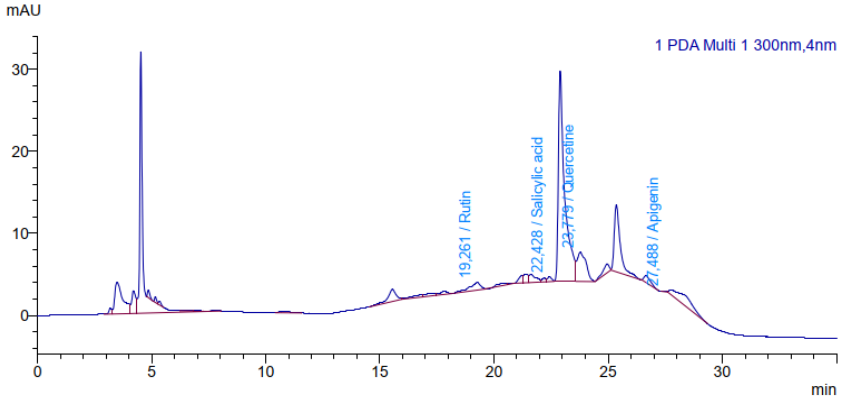


Figure 3. Chromatogram for the determination of polyphenols in the sample extract

Results and Discussion

Results of Determining Antiradical Activity. The obtained results of the evaluation of the antiradical activity of the ethanol extract of the aerial part of the plant Urtica dioica L. are presented in Table 2. To calculate the IC_{50} — the concentration that inhibits 50% of the DPPH solution — a graph was plotted based on the values of antiradical activity (ARF, %) after 30 minutes and the volume of the added sample, as well as using the trendline function drawn through the data.

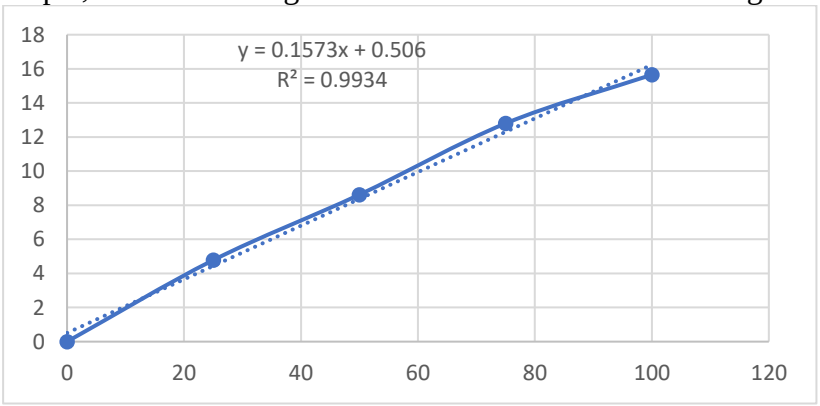


Figure 3. Graph showing the relationship between ARF% values and the volumes of Urtica dioica L. extract measured after 30 minutes, including the best-fitting straight line and the trendline function, as well as the R^2 value, which indicates the closeness of the fit to the experimental data points

The trendline function on the graph has the form y = mx + b, and the volume of extract exhibiting 50% ARF (%) was calculated using the formula (IC_{50}):x = (y - b) / m [10]:

$$IC_{50} = \frac{(50 - 0.506)}{0{,}1573} = 314{,}64\ mkl$$

This value indicates the presence of antiradical activity in the ethanol extract of Urtica dioica L., which in turn confirms the plant's medicinal properties.

The amount of phenolic compounds in the extract was determined using chromatographic analysis. Based on the chromatograms shown in Figures 2 and 3, the retention times of the phenolic compounds, their concentrations in the extract, and the calculated values were determined using the following formula:

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

Where: X — the amount of phenolic compounds in 100 g of the dried aerial part of Urtica dioica L., in mg;

C_{phen} — the concentration of phenolic compounds in the plant extract determined by HPLC, in mg/L;

V_{extra} — volume of the plant extract, in L;

m_{sample} — the mass of the sample used for extract preparation, in g.

The calculation results include data on the amount of phenolic compounds in the extract and their retention times, as presented in table 3.

Table 3. Content of Phenolic Compounds in the Extract and Their Retention Times

Name of Phenolic Compound	Retention Time, min	Concentration, mg/L	Amount, mg/100 g
Gallic acid	Not detected	0	0,0
Rutin	19,261	2,56	6,4
Salicylic acid	22,428	0,263	0,7
Quercetin	23,779	2,898	7,3
Apigenin	27,488	0,036	0,1
Kaempferol	Not detected	0	0,0

Based on Table 3, it can be seen that phenolic compounds such as gallic acid and kaempferol were not detected in Urtica dioica L. However, apigenin, salicylic acid, quercetin, and rutin were present. Notably, quercetin and rutin were found in higher amounts in stinging nettle (*Urtica dioica*), with their biologically active content being 7.3 mg and 6.4 mg, respectively.

Indian researchers [11] also performed chromatographic analysis of Urtica dioica L. collected in the northern foothills of the Himalayas and reported a high content of rutin and quercetin in the plant. Among all polyphenols, quercetin and rutin generally constitute the major portion of flavonoids found in our daily diet. Their presence in extracts confirms their therapeutic efficacy.

The presence of these key bioactive compounds in Urtica dioica L. may contribute to the pharmacological effects of nutraceuticals derived from this plant.

Conclusion. The evaluation of the antiradical activity of Urtica dioica L. using the DPPH free radical scavenging method showed that the plant exhibits antiradical activity. The minimum extract volume required to inhibit 50% of radicals (IC_{50}) was 314.64 µL, confirming the medicinal properties of the plant.

The analysis of phenolic compounds revealed that the aerial parts of Urtica dioica L. are rich in flavonoids such as quercetin and rutin. Based on these findings, we recommend the use of the aerial part of Urtica dioica L. for the treatment of diabetes, inflammation, prostate cancer, and liver diseases.

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