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# EVALUATION OF THE ANTIRADICAL ACTIVITY AND QUANTIFICATION OF PHENOLIC COMPOUNDS IN THE ETHANOL EXTRACT OF TANACETUM VULGARE

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**Keywords:**
Tanacetum vulgare, antiradical activity, DPPH, spectrophotometry, phenolic compounds

**Annotation**

The article presents the results of a study on the antiradical activity of aqueous and alcoholic extracts from the aerial part of Tanacetum vulgare, evaluated using spectrophotometric analysis based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The content of phenolic compounds was also quantified using high-performance liquid chromatography (HPLC). It was found that the alcoholic extract of Tanacetum vulgare exhibits high antiradical activity, with an $IC_{50}$ value of 19.96 µL. This is attributed to the high content of flavonoids such as rutin (73.29 mg), quercetin (25.84 mg), kaempferol (16.69 mg), and apigenin (9.97 mg) in 100 grams of the plant.

**How to Cite**

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**Introduction.** Yarrow, one of the medicinal plants, has been used to treat many diseases [1, 2]. Yarrow (Asteraceae) is a perennial herbaceous plant, called Achillea filipendulina. This plant is mainly found naturally in Central and Southwest Asia, including Kazakhstan, Iran, Afghanistan, Turkey, and the Caucasus. The plant grows to a height of 60–120 cm. Its leaves are deeply divided, fern-like, toothed, and fragrant, giving it a unique appearance. Its flowers are small, densely arranged in flat inflorescences, and bloom in bright yellow. The flowering period lasts from mid-summer to late summer. It grows well in regions such as Tashkent, Samarkand, Bukhara, Kashkadarya, Navoi, Surkhandarya, especially in sunny and arid areas [1]. In folk medicine, parts of the Achelia plant are widely used to treat diseases such as nervous, joint, cardiovascular system, intestinal and stomach, and gallstones [2].

This article analyzes the results of determining the antiradical activity and the content of phenolic compounds of aqueous and alcoholic extracts of the above-ground part of Achelia (Achelia Filipindulina). Free radicals in the body mainly originate from active forms of oxygen and nitrogen [3, 4] and are formed under the influence of various endogenous systems, various physicochemical conditions or pathophysiological conditions in our body. Free radicals can negatively change lipids, proteins and DNA, which is one of the main causes of aging and a number of diseases [5]. Antiradical or antioxidant active compounds that catalytically quench free radicals include chelating compounds, hormones, water- and fat-soluble vitamins, thiol-containing amino acids and polypeptides, phenolic compounds, flavonoids, carotenoids, etc. [6]. Most of these compounds inhibit the chain reaction of free radical formation and prevent the development of oxidative stress.

**Materials and methods.**

Antiradical activity was assessed in vitro using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical quenching method [7] [8].

A 0.812 mM DPPH• solution in ethanol was prepared in a 100 ml volumetric flask, wrapped in aluminum foil, and kept in the dark at room temperature for 30 minutes. In a 4 ml quartz cuvette with a thickness of 1 cm, 3 ml of DPPH solution and 100 µl of ethanol (blank sample) were added and the optical density (D1) of the solution was measured every 30 seconds at 517 nm for 10 minutes using a YOKE (China) K7000 spectrophotometer.

To obtain the ethanol extract of Dastarbaş, 1 g of the sample was boiled in 25 ml of 96% ethanol in an ultrasonic bath with a power of 100 W for 20 minutes in a 50 ml round-bottomed flask equipped with a reflux condenser. The extract was cooled and filtered through a 0.45 µm syringe filter for analysis. To assess the antiradical activity of the primer, 25, 50, 75, 100 µl of the sample extract was mixed with 3 ml of DPPH solution, and the optical density (D2) of the solutions obtained in the above procedure at a wavelength of 517 nm was measured. Ethanol was added to the remaining part to bring the total volume of the solution in the cuvette to 3.1 ml. The antiradical activity of the samples was calculated in percent using the following formula:

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

**Chromatographic analysis.** The content of polyphenols in Achelia Filipindulina (Achelia Filipindulina) was determined on a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph. 1.5 ml of the extract was centrifuged at 7000 rpm in a Mini-7 centrifuge (BIOBASE, China) and filtered through a 0.45 µm syringe filter and used for analysis.

**Chromatographic determination of phenolic compounds.** Chromatographic analysis of a mixture of standard solutions and an alcoholic extract of the aerial part of Achelia Filipindulina was carried out using a Shim pack GIST C18 (150 × 4.6 mm; 5 µm, Shimadzu) reversed-phase column using a gradient mobile phase consisting of acetonitrile (A) and a 0.5% solution of acetic acid in water (B) (Table 1).

Table 1. Mobile phase gradient program.

| 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 | |

The infection volume was set to 10 µl, the flow rate was 0.5 ml/min, and the column thermostat was set to 40 °C. Chromatograms recorded at a wavelength of 300 nm (Figures 1-2).

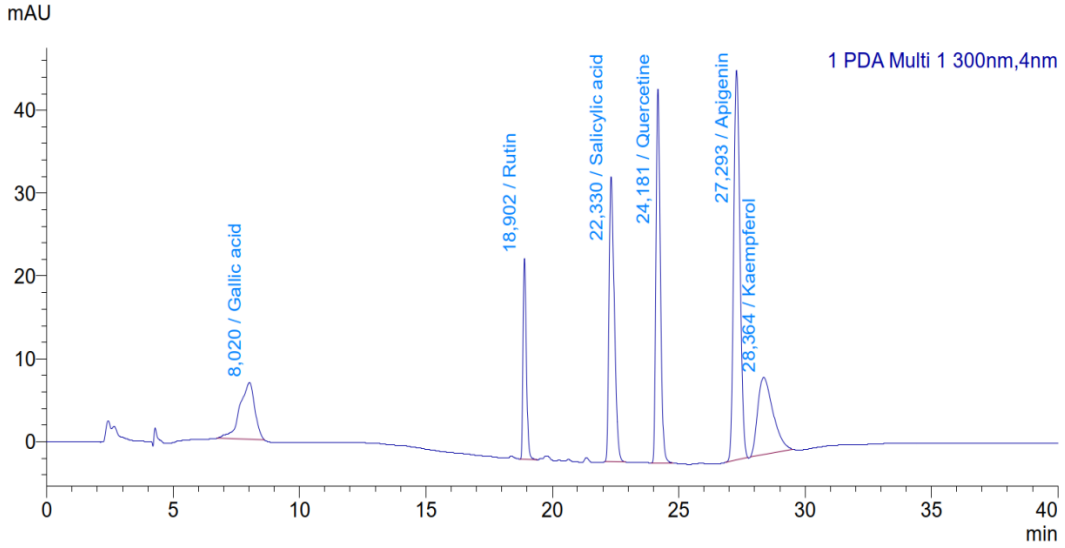



Figure 1. Chromatogram at 300 nm of a mixture of standard solutions.

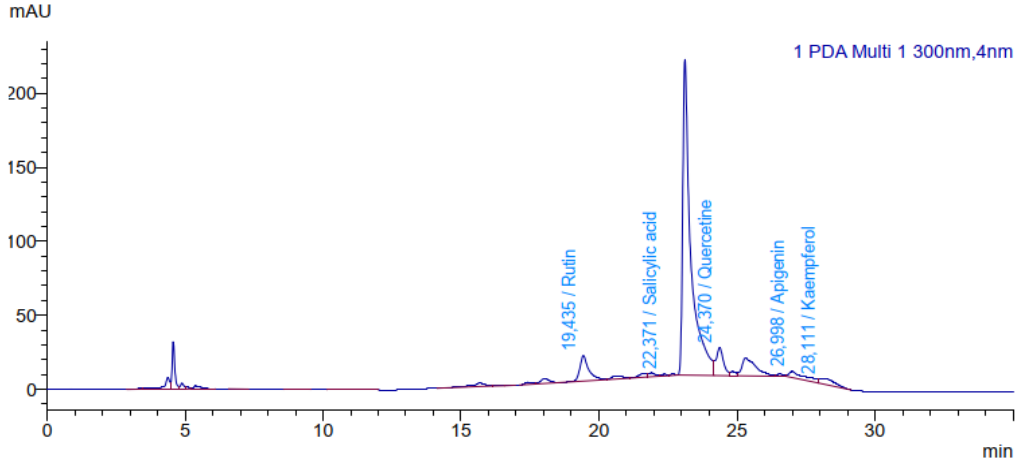



Figure 2. Chromatogram for the determination of polyphenols in the alcoholic extract of Dastarbosh.

**Results and discussion.**

**Results of determination of antiradical activity.** The results obtained for the evaluation of the antiradical activity of the alcoholic extract of the Dastarbas plant are presented in Table 2.

Table 2. Optical density values measured at a wavelength of 517 nm and calculated antiradical activity (ARA) of the DPPH solution and the added alcoholic extract of Dastarbas (diluted 10 times).

| Volume, mkl | Time, min. | Optic density, D | ARA, % | Volume, mkl | Time, min. | Optic density, D | ARA, % |
|---|---|---|---|---|---|---|---|
| 25 | 0 | 0,957 | 0,00 | 75 | 0 | 0,957 | 0,0 |
| | 5 | 0,887 | 7,31 | | 5 | 0,808 | 15,6 |
| | 10 | 0,881 | 7,94 | | 10 | 0,796 | 16,8 |
| | 15 | 0,877 | 8,36 | | 15 | 0,788 | 17,7 |
| | 20 | 0,874 | 8,67 | | 20 | 0,783 | 18,2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 25 | 0,872 | 8,88 | | 25 | 0,778 | 18,7 |
| | **30** | 0,87 | 9,09 | | **30** | 0,773 | 19,2 |
| 50 | 0 | 0,957 | 0,00 | 100 | 0 | 0,957 | 0,00 |
| | 5 | 0,847 | 11,49 | | 5 | 0,78 | 18,50 |
| | 10 | 0,837 | 12,54 | | 10 | 0,76 | 20,59 |
| | 15 | 0,829 | 13,38 | | 15 | 0,744 | 22,26 |
| | 20 | 0,823 | 14,00 | | 20 | 0,732 | 23,51 |
| | 25 | 0,819 | 14,42 | | 25 | 0,722 | 24,56 |
| | **30** | 0,815 | 14,84 | | **30** | 0,715 | 25,29 |

To calculate the $IC_{50}$ of a 10-fold diluted extract, i.e. the sample volume that provides 50% quenching of the DPPH solution, the following graph was constructed based on the antiradical activity (ARA, %) values at 30 minutes in each experiment and the volume of alcohol samples added (Figure 3) and calculated based on the trend line function applied to it.

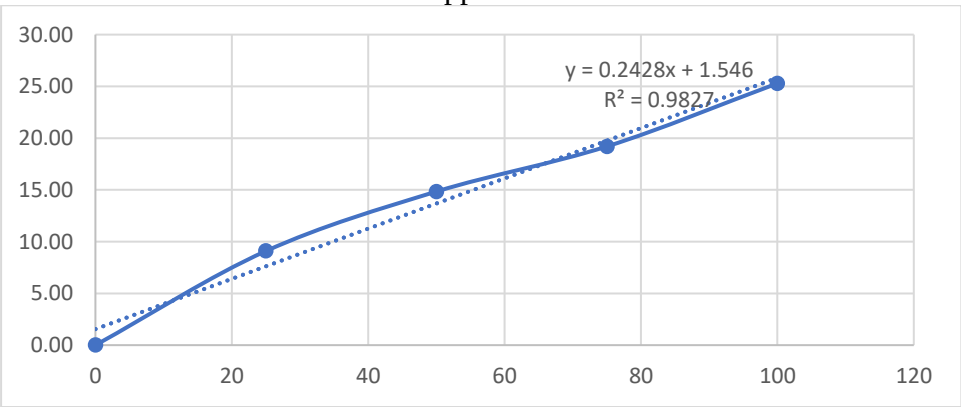



Figure 3. Graph of the relationship between the ARA% values and the volumes determined at 30 minutes of the 10-fold diluted alcohol extraction of the base.

The trend line plotted on the graph was calculated from the function formula y=mx+b, and the volume expressing 50% ARA, % ($IC_{50}$) was calculated based on the formula x=(y-b)/m [8]:

$$IC_{50} = \frac{(50-0.2428)}{1.546} = 199.56\ mkl.$$

Therefore, considering that the obtained value is the result of a 10-fold dilution of the alcoholic extract of the rootstock, it was found that the alcoholic extract obtained by boiling the aboveground part of this plant in 96% ethanol for 20 minutes has a very high antiradical activity, and the IC50 value is 19.95 µl. Based on this, we concluded that the aboveground part of this plant can be used in medicine as a syphon with high antiradical properties.

**Results of chromatographic determination of the amount of phenolic compounds.** Based on the analysis of the chromatograms shown in Figures 1 and 2, the amount of phenolic compounds in 1 g of dried rootstock was calculated using the following formula and is presented in Table 3.

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

Here, X is the amount of phenolic compounds in 100 grams of fruit, mg;

$C_{phen}$ is the concentration of phenolic compounds in the extract determined by the HPLC method, mg/l;

$V_{extract}$ is the volume of the sample extract, l;

$m_{sample}$ is the mass of the sample taken for extract preparation.

Table 3. Retention times, concentrations and amounts of polyphenols in the extract of the rootstock plant.

| Phenol compound name | Holding time, sec | Concentration, mg/l | Amount in 100 g of sample, mg |
|---|---|---|---|
| Gallic acid | Not detected | 0 | 0,000 |
| Rutin | 19,435 | 29,317 | 73,293 |
| Salicylic acid | 22,371 | 0,607 | 1,518 |
| Quercetine | 24,37 | 10,337 | 25,843 |
| Apigenin | 26,998 | 3,987 | 9,968 |
| Kaempferol | 28,111 | 6,674 | 16,685 |

**Conclusion.** The results of this study showed that the content of flavonoid compounds, especially rutin and quercetin, in the composition of the plant is high. These flavonoids are known for their strong antioxidant properties, which are useful in the prevention and treatment of a number of diseases, such as cardiovascular disease, diabetes, and inflammation. Due to the high concentration of flavonoids in the plant, we recommend using the plant as a raw material for the production of food additives based on the plant.

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