A contribution to the knowledge of the species *Dipsacus sylvestris* Huds.

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Abstract: This study aimed to investigate the content of phenols, flavonoids, and antioxidant activity in *Dipsacus sylvestris* from different localities. Based on the spectrophotometric determination, the extract from the flower Vujan Mountain exhibited the highest phonolic content (98.50 ± 3.18 mg GAE/g), while the leaf extracts from Lake Gruza (48.81 ± 1.76 mg RU/g), contained the highest amount of flavonoids. Additionally, the extract from the root of Vujan Mountain (3.12 ± 0.01 μg/mL) demonstrated the highest capacity to neutralize DPPH radicals. These results suggest that, despite the extensive historical use of the root of *D. sylvestris* as a medical plant, its stem, leaves and flower may also possess valuable biological properties. The presented results may serve as a basis for further research on *D. sylvestris* for potential applications in the pharmaceutical industry.

Keywords: *Dipsacus sylvestris*, Phenols, Flavonoids, Antioxidant activity

1. Introduction

Dipsacus sylvestris Huds. is a herbaceous biennial plant belonging to the Dipsacaceae family, native to Europe, and Asia and it is widespread and invasive. It grows in wet areas, next to swamps, and streams, as well as alon gside roads, in dusty and dry areas. [1] The plant can reach a height of up to 2.5 meters, with prickly stems, a distinctive cone-shaped flower head, and leaves downward-pointing prickly. [2] Some of the research has shown that the root plant can be used in the treatment of various conditions, including fibromyalgia, bone fracture, Lyme disease, and especially cancer and Alzheimer's disease [3,4]. It is also known for its use in treating skin diseases such as warts and fistulas. The Infusion made from the plant root is used to strengthen the stomach, stimulate appetite, alleviate liver obstruction, and treat jaundice [5].

However, although there is a wealth of information about the traditional use of *D. sylvestris*, there is no literature data on its chemical composition. So, this study aimed to investigate the total phenols and flavonoid content, and antioxidant activity of the aboveground part and root methanol extracts of *D. sylvestris*.

2. Materials and methods

2.1 Plant material and extracts preparation

The plant material was collected in July 2020 in the flowering phase, (the aboveground parts and roots i.e. herba et radix) in the Vujan Mountain (43°57'54.6"N 20°26'15.9"E), and Lake Gruza (43°55'19.7"N 20°40'30.9"E). Plant material was identified and the voucher specimen of *D. sylvestris* was deposited in the Herbarium at the University of Kragujevac, Faculty of Science, Department of Biology and Ecology. The aboveground part and root were cleaned, separated, and dried at room temperature in a well-ventilated dark room. The dried and finely ground aboveground part and the root were extracted with methanol by the maceration process, and the resulting extract was filtered and evaporated in a vacuum.

2.2 Spectrophotometric determination of total phenols and flavonoid content of the extracts

Total phenols were determined spectrophotometrically by the Folin-Ciocalteu method as described by Peter et al. (2011) [6], and the results were expressed in gallic acid equivalents (mg GAE/g extract). $AlCl_3$ solution was used as a reagent for spectrophotometric determination of total flavonoid content by was described by Quettier-Deleu et al. (2000) [7], whereby results were expressed in rutin equivalents (mg RU/g extract).

2.3 Determination of the antioxidant activity of the extracts

The free radical scavenging activity of samples methanol solutions was analyzed using 2,2-diphenyl-1-picrylhydroxyl (DPPH) according to the Takao et al. (1994) [8], and the results were expressed as ascorbic acid equivalents. The percent DPPH inhibition was calculated by the equation (Eq. 1). Antioxidant activity was expressed as the 50% inhibitory concentration (IC_{50} values in μg/mL)

$$\% \text{ inhibition} = ((A \text{ of control} - A \text{ of sample})/(A \text{ of control})) \times 100 \quad (1)$$

3. Results and discussion

Based on the reviewed literature, these are the first results dealing with the examination of the total phenols and flavonoids, aboveground parts, and root methanol extracts of *D. sylvestris*. The results of the spectrophotometric determination of the content of total phenols are shown in Table 1. In this work, the extraction of all *D. sylvestris* samples was performed with methanol. The higher content of total phenols in all parts of the plant

species *D. sylvestris* from the Vujan Mountain was constant in relation to the plant species collected from Lake Gruza. Based on the obtained results, it can be noticed that the extract Vujan Mountain had a high total phenols content (98.50 ± 3.18 mg GAE/g) from the flower and the Lake Gruza extract (51.61 ± 2.99 mg GAE/g) from the stem. The lowest content of total phenols was constant from Vujan Mountain (61.67 ± 1.92 mg GA/g) from the stem and Lake Gruza (31.27 ± 3.91 mg GA/g) from the root.

Table 1. The amount of total phenols determined in methanol *D. sylvestris* extracts presented as equivalents of gallic acid, mg of GA/g extract.

	Root	Stem	Leaf	Flower
Vujan Mountain	67.00 ± 2.73	61.67 ± 1.92	62.67 ± 1.82	98.50 ± 3.18
Lake Gruza	31.27 ± 3.91	51.61 ± 2.99	49.61 ± 3.43	48.28 ± 4.13

Values are mean ± standard deviation of triplicate analyses.

The results of the spectrophotometric determination of the content of total flavonoids are shown in Table 2. In this work, the extraction of all *D. sylvestris* samples was performed with methanol. Based on the obtained results, it can be observed that the leaf extracts, from both locations contained the highest amount of total flavonoids, Lake Gruza (48.81 ± 1.76 mg RU/g), and Vujan Mountain (42.90 ± 1.85 mg RU/g). The lowest content of total flavonoids was constant in root extracts from both locations, Lake Gruža (1.33 ± 0.22 mg RU/g), and Vujan Mountain (0.93 ± 0.52 mg RU/g).

Table 2. The amount of total flavonoids determined in methanol *D. sylvestris* extracts presented as equivalents of rutin, mg of RU/g extract.

	Root	Stem	Leaf	Flower
Vujan Mountain	0.93 ± 0.52	5.55 ± 0.18	42.90 ± 1.85	6.71 ± 2.77
Lake Gruza	1.33±0.22	10.07±0.50	48.81 ± 1.76	3.71 ± 0.25

Values are mean ± standard deviation of triplicate analyses.

Both from Vujan Mountain and Lake Gruza, *D. sylvestris* extracts showed a good radical scavenging activity in vitro Table 3.

Table 3. Antioxidant activity of investigated *D. sylvestris* extracts, IC_{50} values (μg/mL).

	Root	Stem	Leaf	Flower
Vujan Mountain	3.12 ± 0.01	11.05 ± 0.45	203.09 ± 3.56	350.81 ± 3.56
Lake Gruza	138.68 ± 1.92	75.55 ± 1.21	394.9 ± 3.36	351.76 ± 2.36

Values are mean ± standard deviation of triplicate analyses.

The lower IC_{50} value reflects greater activity. The highest antioxidant activity was measured in an extract from Vujan Mountain (3.12 ± 0.01 μg/mL, from root) and (11.05 ± 0.45 μg/mL, from the stem), while the lowest capacity to neutralize DPPH radicals was measured in an extract Lake Gruza (519.76 ± 2.36 μg/mL) from the flower. Compared to

the results published by Witkowska-Banaszczak (2018) [9], our results show much higher IC_{50} values in the extract of Vujan Mountain (3.12 ± 0.01 μg/mL) from the root, while the mentioned study reported IC_{50} value of (86.01 μg/mL) from the root. However, much lower IC_{50} values were constant in the extract from Vujan Mountain (203.09 ± 3.56 μg/mL) and Lake Gruza (394.9 ± 3.36 μg/mL) from the leaves, compared to the results of the mentioned study, IC_{50} (143.1 μg/mL).

4. Conclusions

The results of this investigation revealed that *D. sylvestris* methanol extracts from Vujan Mountain and Lake Gruza possess a high amount of total phenols and have significant (extracts root and stem) antioxidant activity. The higher content of total phenols in all parts of the plant species *D. sylvestris* from the Vujan Mountain was constant compared to the plant species collected from Lake Gruza. These results suggest that, while the root of *D. sylvestris* has been extensively used since ancient times, its stem, leaves and flower may also possess valuable biological properties. The presented results may serve as a basis for further research on *D. sylvestris* for potential application in pharmaceuticals.

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