

Qualitative content of selected phenolic compounds in different extracts of plant species *Iris pumila* L.

Gorica Đelić¹, Milica Pavlović¹, Vesna Veličković^{2,*}

¹ University of Kragujevac, Faculty of Science, Department of Biology and Ecology, Radoja Domanovića 12, 34000 Kragujevac, Serbia; e-mail: gorica.djelic@pmf.kg.ac.rs; milica.novakovic@pmf.kg.ac.rs

² University of Kragujevac, Faculty of Technical Science, Svetog Save 65, 32000 Čačak, Serbia; e-mail: vesna.velickovic@ftn.kg.ac.rs

DOI: 10.46793/ICCBi23.555DJ

* Corresponding author

Abstract: The aim of this paper is to determine the content of selected phenolic compounds and to find the appropriate optimal extraction agent for the extraction of phenolic compounds from the plant species *Iris pumila* L. The content of total phenols and flavonoids in the rhizome and aboveground parts of the plant was determined by the application of the spectrophotometric method in the examined extracts obtained by the maceration method. By comparatively analyzing the application of two solvents for extraction, we concluded that the application of methanol in the conventional maceration technique contributes to a higher yield of phenolic compounds. Using methanol as a solvent, it was found that the content of selected phenolic compounds in the rhizome (28.57 mg/g extract total phenolic and for total flavonoids 24.21 mg/g extract) compared to ethyl acetate as solvent. Tested extracts of parts of *Iris pumila* L. plant contain high levels of phenolic compounds and are suitable for use as a source of compounds with pronounced antioxidant activity.

Keywords: extraction, maceration, spectrophotometry, phenolics compounds

1. Introduction

Iris pumila L. (bearded dwarf iris) is a perennial herbaceous plant of the family irises (*Iridaceae*). It is a perennial entomophilic plant that inhabits the meadow-steppe regions of Central and Southeastern Europe. [1] The natural habitats of this species in Serbia are its northern and eastern parts, and it is most common in Deliblato sandstone, which consists of a complex of sand dunes in southern Banat. [2] It blooms in April, the flowering period lasts about two weeks. The flowers of the plant are single and distinctly polymorphic in relation to color. [3] The leaves of irises or irises are fleshy, erect, lanceolate, and light green. Ordinary irises inside the flower have clusters of tiny hairs and are therefore often referred to as bearded irises. [3]

Iris pumila as a plant species has been studied in several morphological and cytological studies, but it has not been studied in terms of the content of phenolic compounds. [4, 5, 6] In recent decades, there has been an increase in the interest of the

general scientific public in natural antioxidant compounds and plant species, which are a significant source of these compounds. Numerous scientific studies confirm that natural phenolic compounds (polyphenols) exhibit significant antioxidant potential and are very good antioxidants. [7, 8] Plants are its main source. Given that synthetic antioxidants exhibit a number of side effects and adverse effects due to long-term use, interest and demand for the application of natural antioxidants are on the rise. [9] Among the natural antioxidants, a large and very wide, diverse group of phenolic compounds stands out. These compounds also called natural polyphenols (among which the most important are flavonoids, phenolic acids, anthocyanins, etc.) exhibit a number of positive properties, and due to their antioxidant activity show antiviral [7], antibacterial [8], anticancer effects [9]. Plant antioxidants can be extracted from a variety of plant materials, mainly dried by the extraction process - a method of mass transfer by means of solvents from the solid phase to the liquid. Mass transfer depends on many factors, such as the type of solvent, extraction length, temperature, and polarity of the solvent. [10] This study aimed to investigate selected phenolic compounds and to find the appropriate optimal extraction agent for the extraction of phenolic compounds from the aerial part and rhizome of *Iris pumila*.

2. Materials and methods

2.1 Plant material and extracts preparation

The rhizome and the above-ground part of the iris were collected from the locality Ovcar-Kablar Gorge. Dried parts of the plant are ground in a blender, and as such used in the further procedure. We prepared the extracts by maceration method. 50 ml of solvent and 10 g of herbal drugs were used. Ethyl acetate and methanol (Table 1) were used as solvents. Maceration lasted three days, after which the resulting extracts were dried using a rotary vacuum-evaporator. The extracts are stored in Petri dishes, in the refrigerator at a temperature of + 4 degrees Celsius.

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

The content of the total phenols was determined by the spectrophotometric method according to Folin-Ciocalteu. [11] The method is based on the reduction of Folin-Ciocalteu reagents in the presence of phenolic compounds. Phenolic compounds by dissociation produce a proton and a phenoxide ion that reduces folin-Ciocalteu reagent to phenol-MoW11O40-ion that is colored blue. The intensity of color is measured spectrophotometrically, at the wavelength $\lambda = 765$ nm. The absorbance is measured at $\lambda = 765$ nm. The results of the analysis are shown in Table 1.

The content of total flavonoids was determined by the modified method by Brighente. [12] The method is based on the property of flavonoids to build metalcomplexes with metals, of which the complex with aluminum is particularly

important. [12] The absorbance is measured at $\lambda = 430$ nm. The analysis was done in three repetitions. Gallic acid, a series of standard solutions with a concentration of 0.05-1 mg/mL, was used as standard, the results obtained were expressed as gallic acid equivalents (mg GAE/g \pm SD). All measurements were made on the UV-VIS spectrophotometer, brand DR 3900 Hach Lange produced in 2022. All analyses were done in three repetitions. The results of the analysis are shown in Table 2.

3. Results and discussion

Table 2 presents the results of determining the content of total phenols in the aerial parts and rhizomes of the plant species *Iris pumila* L. by the method of spectrophotometry.

Table 1. The content of total phenols in the various parts of the *Iris pumila* L.

Sample*	A	Total phenolics (mg GAE/g \pm SD)
1.	0.029	25.62 \pm 0.05
2.	0.034	25.66 \pm 0.06
3.	0.088	26.14 \pm 0.17
4.	0.364	28.57 \pm 0.71

*tags :1 -ethyl-acetate-aerial part. 2-ethyl-acetate-rhizome. 3-methanol-above-ground part. 4-methanol rhizome. A- absorbance

The extract obtained by the maceration process where it was used as a solvent methanol contains the largest amount of total phenols (28.57 mg GAE/g). This content is higher in relation to the content of the total phenols of the aboveground part using the same solvent and compared to the rhizome extract in ethyl acetate (25.62 mg GAE/g). where the lowest amount of total phenols was recorded. Methanol is a more efficient solvent for extracting phenolic compounds from plants.

Table 2. The content of flavonoids in the various parts of the *Iris pumila* L.

Sample*	A	Total flavonoids (mg GAE/g \pm SD)
1.	0.009	1.88 \pm 0.02
2.	0.012	2.05 \pm 0.03
3.	0.029	5.87 \pm 0.13
4.	0.121	24.21 \pm 0.67

*tags :1 -ethyl-acetate-aerial part. 2-ethyl-acetate-rhizome. 3-methanol-above-ground part. 4-methanol rhizome. A- absorbance

Based on the results it is noticeable that higher concentrations of flavonoids are represented in extracts in which methanol was used as a solvent in the extraction process. The highest concentration of flavonoids is in rhizome extract in methanol (24.21

mg GAE/g) which is about twelve times higher than the concentration of flavonoids present in the aboveground extract obtained using methanol (5.87 mg GAE/g). The aboveground extract in ethyl acetate shows three times lower content compared to the extract where methanol is used as a solvent (1.88 mg GAE/g).

4. Conclusions

The results obtained in the paper show that extracts of different parts of the plant species *Iris pumila* L. contain significant amounts of total phenols. The content of total phenols is highest in rhizome extract obtained by the maceration process (84.60 mg/g extract) when methanol was used as a solvent. This extract contains the largest amount of total flavonoids (24.21 mg/g extract). Based on the study it can be concluded that methanol has proven to be a better solvent in extraction by maceration process of different parts of plant species *Iris pumila* L. The application of this solvent ensures an increase in efficiency - methanol yields higher concentrations of phenolic compounds in the extract of different parts of the plant species *Iris pumila* L.

References

- [1] L. F. Randolph., *The geographic distribution of European and eastern Mediterranean species of bearded Iris*, *Iris Year Book*, (1955). 35–46.
- [2] M. Gajić., *Flora Deliblatske peščare*, Prirodno matematički fakultet OOUR Institut za biologiju. Novi Sad, (1983).
- [3] S. Đ. Budečević., *Morfološko variranje. fenotipska plastičnost i fluktuirajuća asimetrija oblika svetnih organa kod Iris pumila L.*, Doktorska disertacija Beograd, (2018).
- [4] E.G.B. Luscombe., *Iris pumila* L. I. - *The Iris Year Book*. (1972) 149-157.
- [5] J. Mitra., 1: *Karyotype analysis of bearded iris*, *Botanical Gazette*, 117 (1956) 265-293.
- [6] L. F. Randolph, K. Heinig., *Cytology and breeding behavior of dwarf and tall-bearded Iris hybrids*, *Bulletin of the American Iris Society* (1951) 1-8.
- [7] M.R. Loizzo, A.M. Saab, R. Gambari, J. Cinatl, H.W. Doerr, *Phytochemical analysis and in vitro antiviral activities of the essential oils of seven Lebanon species*, *Chemistry & Biodiversity*, 5 (2008) 461-470.
- [8] B. Khameneh, R. Diab, K. Ghazvini, B.S.F. Bazzaz, *Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them*, *Microbial Pathogenesis*, 95 (2016) 32-42.
- [9] I. C. Arts, P.C. Hollman *Polyphenols and disease risk in epidemiological studies*, *The American Journal of Clinical Nutrition*, 81 (2005) 317S-25S
- [10] Q.W. Zhang, L.G. Lin, W.C. Ye., *Techniques for extraction and isolation of natural products. A comprehensive review*, *Clinical Medicine*, 13 (2018) 20.
- [11] V.L. Singleton, J.A. Rossi, *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents*, *Enology and Viticulture*, 16 (1965) 144-158.
- [12] I.M.C. Brighente, M. Dias, L.G. Verdi, M.G. Pizzolatto, *Antioxidant activity and total phenolics content of some Brazilian species*, *Pharmaceutical Biology*, (2007) 156-161.