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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF WATER-ETHANOL EXTRACTS OF DANDELION (*TARAXACUM OFFICINALE*)

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Summary

Dandelion or *Taraxacum officinale* is a well - known medical plant that is a source of various nutrients and biologically active substances, and its polyphenolic compounds are considered responsible for the high biological activity of dandelion and its antioxidant, anti - inflammatory and atitumor effects. Aques - ethanol extracts were obtained using various maceration extraction techniques at room and boiling temperature, ultrasonic and Soxhlet extraction. In this study, all parts of the plant (roots, leaves, stems, flowers) previously dried at room temperature were analysed. The content of mineral elements of the plant was determined by AAS, antioxidant activity by DPPH (2,2 - diphenyl - 1 - pycryl hydrazyl) and FRAP (Ferric Reducing Antioxidant Power) method, flavonoids (method with $AlC1_3$), vitamin C, carotenoids and chlorophyll were determined spectrophotometrically. The content of total phenols was determined by the Folin - Ciocalteu method. The content of phenolic compounds is higher in the outer parts of the plant (flowers and leaves) than in the root. The results of chemical analysis show that all its components have special qualities in terms of chemical composition. The best antioxidant activity was shown by the water - ethanol extract obtained using Soxlet extraction.

Keywords: chemical composition, antioxidant activity, *Taraxacum officinale*

Introduction

Dandelion (*Taraxacum officinale*) is a wild plant from the Asteraceae family where recent research shows a pronounced biological potential due to the significant content of bioactive compounds. This plant is rich in polyphenolic compounds dominated by hydroxycinnamic acids (Sun et al., 2014), caffeic acid, flavonoids-apigenin and luteolin-7-glucoside, phytosterols-sitosterol and stigmasterol, carotenoids (Kuštrak, 2005). Also, it is rich in vitamin C and minerals such as potassium, iron, sodium and phosphorus, as well as essential oils, fatty acids (Erhatić et al., 2014) and inulin of variable content. In spring, the root contains only 1-2%, and in autumn up to 40% inulin (Kuštrak, 2005), and in October the root contains the most taraxerin and levulin (Erhatić et al., 2014). A review paper (Di Napoli and Zucchetti, 2021) shows that twelve medicinal properties of *Taraxacum officinale* are often cited in the scientific literature. These properties include diuretic, hepatoprotective, anticholitic, immunoprotective, antiviral, antifungal, antibacterial, antiarthritic, antidiabetic, antihypertensive, antioxidant and anticancer effects. The presence of bioactive compounds in living organisms is of great importance because aerobic organisms in vivo continuously generate free radicals and reactive oxygen species. Free radicals are usually very reactive and in increased concentrations can lead to cell and tissue damage, which can be the cause of a large number of diseases (Nikolić et al., 1998). The body defends itself against free radicals with natural antioxidants that are introduced into the body through food, natural or synthetic substances, and have the ability to resist oxidation or inhibit reactions initiated by reactive species. These compounds differ in chemical structure and have different mechanisms of action. Therefore, various antioxidants are needed to protect the cell from various biomolecules in vivo (Gutteridge and Halliwell, 2010). These are compounds that are present in low concentrations and act as "scavengers" of free radicals, transforming them into stable non-radical products, complexing metal ions, preventing their catalytic function in the processes of lipid peroxide degradation and free radical formation, degrading lipid peroxides, acting as reducing agents, " scavengers " of singlet oxygen, inhibit some enzymes, show synergistic effects, to release hydrogen and chelate metals (Ivanova et al., 2005). Root and young tops are mainly used for medicinal purposes (Grieve, 1931; Rasool and Sharma, 2014; Stewart-Wade et al., 2002). The results of current research show that dandelion root is a valuable source of dietary fiber and natural antioxidants and could be successfully used in foods that can improve digestion and prevent diseases caused by oxidative stress (Petkova et al., 2015). The young leaves of *Taraxacum officinale* are also used as

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food in salads, drinks and vegetable dishes, due to their nutritional value. Studies show that *Taraxacum officinale* leaves contain high concentrations of fiber, minerals, vitamins, and essential fatty acids (Escudero et al., 2003; Di Napoli and Zucchetti, 2021).
The aim of this work is to carry out the extraction of polyphenols from dandelion (*Taraxacum officinale*) with an aqueous solution of ethanol 50% (v/v) as a solvent. The root, stem, leaf and flower were used as samples. Several extraction methods were used: using maceration of samples at room temperature, boiling temperature, ultrasonic extraction and Soxlet extraction. After extraction with samples, antioxidant activity was determined.

Materials and methods

Sampling was performed in 2020. at the end of April and the beginning of May. All parts of the dandelion plant (*Taraxacum officinale*) were sampled. They were then dried to room temperature and stored in paper bags. Samples were ground using an IKA Tube Mill 100 control laboratory grinder and six replicates were taken for each analysis.

The homogenized sample was subjected to maceration extraction techniques at room temperature and boiling temperature, ultrasonic and Soxhlet extraction, with aqueous ethanol solution 50 % (v/v) as solvent. Classic extraction of macerations at room temperature was performed for 240 minutes. After extraction, the liquid extracts were separated from the plant material by vacuum filtration on a Büchner funnel and transferred to vials. Boiling extraction for 30 minutes and ultrasonic extraction (WiseClean WUC) were performed for 30 minutes, the samples were filtered on a Büchner funnel and transferred to vials. Soxhlet extraction was performed in a Soxhlet apparatus with 25 g of plant material each. The extraction lasted 160 minutes. All obtained extracts were stored in a refrigerator at + 4 °C until further analysis.
In the prepared samples, total phenols were determined by the Folin-Ciocalteu method (Dewanto et al., 2002), and the results were calculated from the calibration curve of gallic acid (Figure 1). The concentration of total phenols is calculated according to the equation of direction obtained by Excel, with gallic acid concentrations (mg/L) plotted on the abscissa and absorbance values measured on the photoLlab 6600 UV-VIS WTW spectrophotometer at 765 nm.

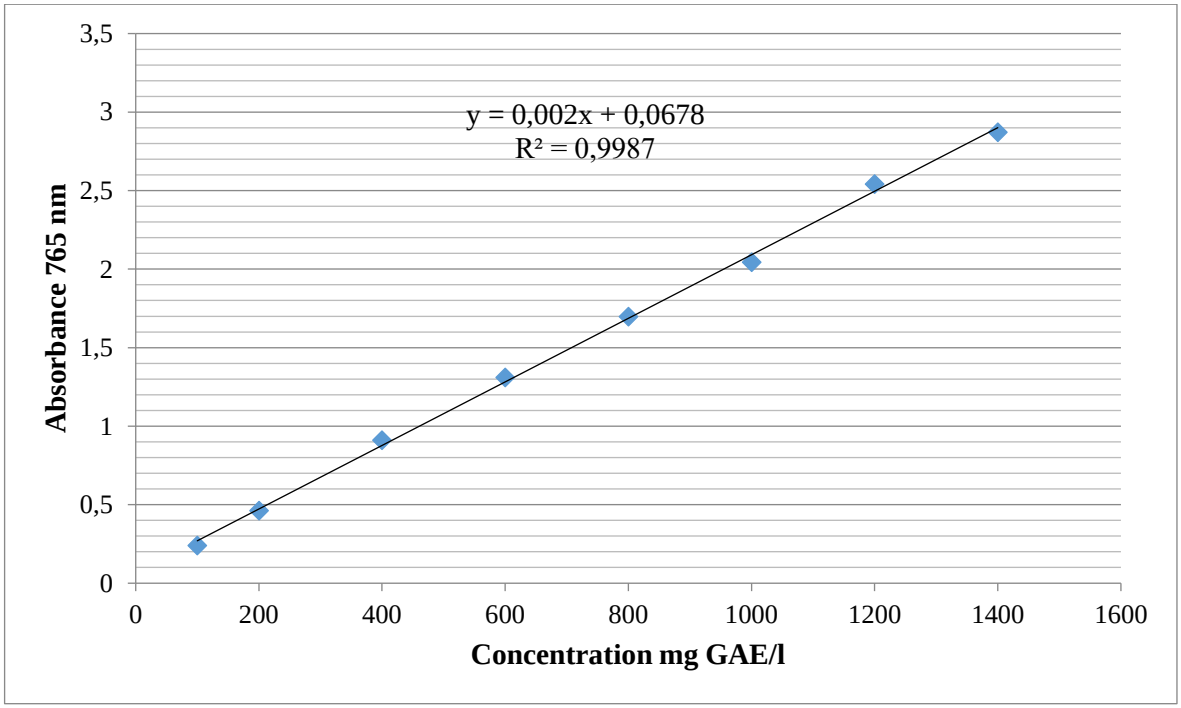

Figure 1. Standard diagram for determination of total phenols

A modified colorimetric method with $AlCl_3$ was used to determine total flavonoids (Khlifi et al., 2011), and a standard quercetin solution was used to construct the calibration direction (Figure 2).

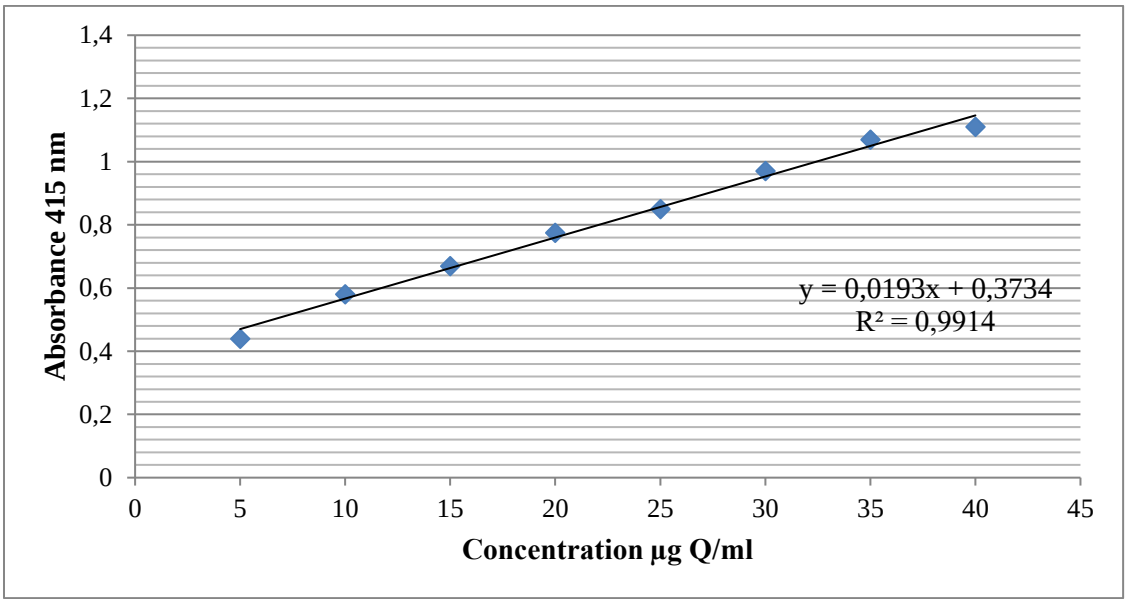

Figure 2. Standard diagram for the determination of flavonoids

The DPPH method was used to determine antioxidant capacity. DPPH (2,2-diphenyl-1-picrylhydrazyl) is one of the most stable organic nitrogen radicals with maximum absorption in UV-VIS at 517 nm. The DPPH radical is a stable nitrogen radical whose solution is dark purple, and with the addition of antioxidants the solution fades as the free radical is reduced to light yellow diphenylpicrylhydrazine, which is monitored spectrophotometrically via a drop in absorbance at 517 nm. The sample was incubated for 15 minutes at 37 °C in the dark. Decreases in absorbance at 517 nm were measured using a spectrophotometer compared to a blank containing methanol. The standard curve was constructed with a Trolox concentration between 0.005 and 1.0 mM. Results are expressed in mM Trolox/g dry weight (Ivanov et al., 2015).

FRAP test: 0.1 ml of test extracts were added to 3 ml of FRAP reagent (0.3 M) acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 20 mM $FeCl_3 \times 6H_2O$ (10: 1: 1, v/v/v) and allowed to stand for 10 min at 37 °C in the dark. Absorbance was measured at 593 nm (Benzie and Strain, 1996). Results from both antioxidant methods were expressed as mM Trolox/g dry weight.

Carotenoids and chlorophylls from dandelion leaves, stems and flowers were extracted from 1 g of fresh sample homogenized with 5 ml of 80% aqueous acetone for 30 min, followed by centrifugation (Hettich centrifuge UNIVERSAL 320) at 4500 rpm for 10 minutes (Guan et al., 2006). The supernatant was diluted 10-fold using 80% aqueous acetone and absorbance was measured at 460, 647 and 664 nm using a photoLab 6600 spectrophotometer.

The obtained absorbance values were included in the Lichtenthaler formula to calculate the pigment concentrations (Lichtenthaler, 1987):

$$\text{Total chlorophyll} = 7.15A664 + 18.71A647 \quad (1)$$

$$\text{Total carotenoids} = (1000A_{460} - 1.82Ca - 85.2Cb)/198 \quad (2)$$

where is: A = absorbance

$$Ca \text{ (chlorophyll a)} = 12{,}25A_{664} - 2{,}79A_{647} \quad (3)$$

$$Cb \text{ (chlorophyll b)} = 21.50A_{647} - 5.10A_{664} \quad (4)$$

The ascorbic acid content was determined according to Bendritter (Bendritter et al., 1998). About 0.5 g of tissue and 10 mL of distilled water were pipetted into the previously weighed and labeled tubes with a screw cap, and then the tubes were centrifuged for 15 minutes at 3000 g at 4 °C. After centrifugation, pipette 300 µL of aqueous extract into 2 mL tubes. To the aqueous extract was added 100 µL of 13.3% trichloroacetic acid, 25 µL of distilled water and 75 µL of DNPH (2,6-Dichlorophenolindophenol Sodium salt Hydrate) reagent. After the addition of the reagent, incubate for one hour in a water bath at 37 °C. Using the same procedure, blank samples were prepared for each sample without the addition of DNPH reagent. At the end of the incubation, DNPH reagent and 500 µL of 65% sulfuric acid were added to all blanks in all samples. All mixtures are mixed once more. Absorbance was measured spectrophotometrically

(photoLab 6600 spectrophotometer) at 520 nm, while ascorbic acid concentration was determined from a calibration curve with known concentrations.
The content of calcium and magnesium mineral elements was performed on an atomic absorption spectrophotometer "Perkin Elmer" AAnalyst -800, whose results are expressed in mg / kg (ppm) "Analytical Methods" FP-3 Analysis of Meat and Meat Products (2000), "Perkin Elmer" AAnalyst -800.

Results and discussion

Previous research shows that the total yield of extracted substances, the content of total phenols, flavonoids and antioxidant activity depend on the applied extraction technique and solvent. According to the literature, methanol is more efficient for the extraction of phenol from plant material, however, we used an aqueous solution of ethanol in this work due to less toxicity.
The results of testing the antioxidant activity of ethanolic extracts of dandelion *(Taraxacum officinale)* indicate that this plant is rich in compounds that have antioxidant activity. In research Nowak et al. (2019), raw dandelion plant material consisted of fresh and dried leaves, flowers and roots. Extracts were prepared for analysis using an ultrasonic bath (extraction time 15, 30 and 60 min) in water and 40% (v/v), 70% (v/v) and 96% (v/v) ethanol mixtures used as solvents. They found that the antioxidant activity of dandelion depended on the type of raw material used, as well as the type of solvent and extraction time. The highest DPPH activity was found to be dried flower extracts prepared in 70% ethanol for 30 min. With the FRAP method, the highest reduction capacity was observed for dried leaf extracts in 40% ethanol 30 minutes.

Table 1. Antioxidant activity of dandelion measured by DPPH and FRAP method, obtained by different extraction techniques

	Sample	DPPH mg Trolox/g dry weight	FRAP mg Trolox/g dry weight
		±SD	
DANDELION	Flower (ST)	118.24±4.02	89.27±2.87
	Flower (TK)	118.55±3.56	88.16±1.59
	Flower (UE)	127.98±3.87	97.25±2.17
	Flower (SE)	130.49±2.18	103.14±2.34
	Leaf (ST)	127.31±1.95	98.23±1.24
	Leaf (TK)	121.18±2.43	99.34±2.05
	Leaf (UE)	129.38±4.65	107.16±2.13
	Leaf (SE)	135.14±3.16	119.27±0.97
	Stem (ST)	99.43±1.99	90.28±1.84
	Stem (TK)	98.27±1.65	92.13±1.63
	Stem (UE)	104.53±2.53	103.55±0.99
	Stem (SE)	109.28±3.17	108.14±1.82
	Root (ST)	52.75±0.65	45.34±0.73
	Root (TK)	50.89±0.88	46.17±0.44
	Root (UE)	56.48±1.09	49.18±1.25
	Root (SE)	61.36±2.01	53.54±1.42

Results from both antioxidant methods were expressed as mM Trolox/g dry weight, SD = Mean of 6 measurements, ST = sample maceration extractions at room temperature, TK = boiling temperature, UE = ultrasonic extraction, SE = Soxlet extraction.

The influence of solvents on the extraction of phenolic compounds, from dandelion leaves, was studied in the research of Petkova et al. (2015). *Taraxacum officinale* subsequent extracts of aqueous root showed the highest antioxidant activity DPPH test 83.1 ± 3.2 mg TE/g dry matter; FRAP test 46.9 ± 1.3 mg TE/g dry matter, while aqueous extracts showed highly defined activity only by FRAP test 52.9 ± 0.3 mg TE/g dry matter.
According to research performed by Ivanov (2015), the results indicated that total phenolics, chicoric acid concentration, DPPH, FRAP and CuPRAC values were higher in 50% ethanol extract of *Taraxacum officinale* leaves: 33.90 ± 0.57 mg GAE/g dry matter, 3.1 g/100g dry matter, 136.3 mM TE/g dry matter (DPPH method), 131.5 mM TE/g dry matter (FRAP method) and 407.8 mM TE/g dry matter (CuPRAC method). Based on the values obtained in Table 1, it can be seen that dandelion leaves (*Taraxacum officinale*) extracts obtained by Soxlet extraction with aqueous ethanol 50% (v/v) as solvent, compared to the above authors, show approximately equal values of antioxidant activity DPPH method, while other values are much lower. Extraction is the most important phase in polyphenol isolation. The solubility of polyphenols depends on the degree of polymerization, interaction with other components and the polarity of the solvent Mujica et al. (2009), where the lipophilicity/hydrophilicity of the compounds in plant

material should be taken considered. The choice of extraction conditions, primarily the type of extraction, duration and temperature significantly affect the antioxidant activity. According to our research, it was found that in the case of classical extraction maceration at room temperature and with increasing temperature, the value of antioxidant activity did not change significantly, the assumption is due to the duration of extraction. Also, thermal processing conditions might result in the loss of natural antioxidants because heat may accelerate their oxidation and other degenerative reactions.

Kenny et al. (2014) found maximum quantities the ethyl acetate crude extract (*Taraxacum officinale* - root) demonstrated the highest antioxidant activity for both the DPPH (227.728 ± 11.849 mg TE/g) and FRAP (463.066 ± 3.942 mg TE/g) assays. This extract also contained the highest phenolic content (228.723 ± 2.392 mg GAE/g), which is significantly higher than the value of our results (Table 1).

Table 2. Total phenol and flavonoid content in dandelion flowers, leaves, stems and roots (sample maceration extractions at room temperature)

	Sample	Total phenols (mg GAE/g dry matter)	Flavonoids (mg quercetin/g dry matter)
		±SD	
DANDELION	Flower	26.08±0.67	4.98±0.51
	Leaf	30.05±0.89	2.26±0.31
	Stem	23.89±0.75	-
	Root	4.23±0.43	-

Milek and Legath (2015) isolated phenolic compounds from dandelion flower and leaves by ultrasonic extraction. Methanol, ethanol and acetone were used as solvents in a concentration of 70%. In the isolation of phenolic compounds from the leaves, the highest yields were given by extraction with acetone, followed by methanol and ethanol. They found 362.14 ± 6.76 μM quantities of total phenolics in the extracts of *Taraxacum officinale*.

According to research by Ghaima et al. (2013), the content of total phenols in dandelion leaves extracted with ethyl acetate was determined to be 10.2 mg GAE/g dry matter, which is much lower than our results (Table 2). Much higher values are reported by Stylianon et al. (2014) who extracted phenolic components by maceration in dandelion leaf and root. For the content of total phenols in the leaf, they state the values of 52.29 ± 0.0178 mg GAE/g, and in the root 9.61 ± 0.1397 mg GAE/g. Ivanov (2015) found maximum quantities of total phenolics in 50% ethanol extract of *Taraxacum officinale* leaves: 33.90 ± 0.57 mg GAE/g dry weight.

The influence of solvents on the extraction of phenolic compounds from dandelion leaves was also studied in the research of Petkova et al. (2015). Values of total phenols ranged from 4.5 to 9.2 mg GAE/g dry matter. The highest total content of polyphenols and total flavonoids was registered by subsequent extraction of water (9.2 ± 0.3 mg GAE/g and 1.7 mg EQ/g dry matter, which is significantly less than the value of our results. Khan et al. (2019) also determined the content of total phenols. They found varied from 41.47 mg/g to 691.6 mg/g quantities of total phenolics in the extracts of *Taraxacum officinale*. The maximum phenolic contents were found in hydro-alcoholic extract (691.6 mg/g GAE) in comparison with aqueous extract.

The results of current investigation demonstrate that dandelion is a valuable source of natural antioxidants and could be successfully used in foods with the potential to improve digestion and prevent from oxidative stress related diseases.

Table 3. Amount of vitamin C, chlorophyll and carotenoids in dandelion flowers, leaves, stems and roots (sample maceration extractions at room temperature)

	Sample	**Vitamin C** [mg/kg]	Total chlorophyll [mg/kg]	Total carotenoids [mg/kg]
		±SD		
DANDELION	Flower	58.54±0.85	-	43.56±0.44
	Leaf	106.49±1.27	427.18±3.87	198.29±1.56
	Stem	96.89±1.13	47.21±0.48	24.87±0.66
	Roof	18.02±0.56	-	-

The research by Pădureț et al. (2016) found that the highest content of vitamin C in dandelion leaves is 121,862 mg/kg. These values are slightly higher compared to our results (Table 3). This research shows the results of Pădureț et al. (2016), which is, that the leaf contains a higher proportion of vitamin C than in other analyzed parts of the dandelion plant.

The chlorophyll value of 87.62 mg/kg was determined according to the results of Pădureț et al. (2016). Our results showed lower values compared to the above results. The chlorophyll content in the stem was 47.21 mg/kg.

Table 4. Average values of calcium and magnesium mineral elements (sample maceration extractions at room temperature)

	Sample	**Ca** [mg/kg]	Mg [mg/kg]
		±SD	
DANDELION	Flower	328.33±8.16	5.19±0.35
	Leaf	448.26±9.23	6.31±0.49
	Stem	366.28±8.08	4.23±0.38
	Roof	408.21±10.12	2.17±0.27

The leaves of dandelion had magnesium and calcium levels of 6.31±0.49 and 448.26±9.23 mg/kg, respectively. According to research by Biel et al. (2017), dandelion leaves contain an average of 0.24 g magnesium and 0.67 g calcium/ 100 g dry matter. According to the available data in literature (Harrington et al., 2006), dandelion leaves contain an average of 0.353 g Mg and 0.96 g Ca/100 g dry matter. This discrepancy may result from the genetic, climatic, and soil differences. According to Šelih et al. (2014), the elemental composition in plants depends on internal factors, the application of fertilizers, and local fertility conditions because the elements are absorbed from the soil.

According to Escudero et al., (2003) 100 g of dandelion leaves contain of vitamin C 121 mg/kg, chlorophyll 450.784 mg/kg, carotene 206.429 mg/kg and minerals 499.7 mg/kg Ca and 6.299 mg/kg Mg. These values are slightly higher compared to our results (Tables 3 and 4). *Taraxacum officinale* leaves are rich in iron, calcium, magnesium, phosphorus, vitamins A and C, the B vitamins (thiamine and riboflavin). This plant is one of richest vegetable source of beta-carotene 0.84 mg/g compared to 0.61 mg/g of carrots tissue Gail (1994).

Conclusions

Based on the obtained results, it can be concluded that the dandelion or *Taraxacum officinale* plant contains more phenolic compounds in the outer parts of the plant. The choice of extraction conditions, primarily the type of extraction and temperature affect the antioxidant activity. Based on the obtained results, it can be concluded that the applied extraction technique had an impact on the values of antioxidant activity. In our research, the highest antioxidant capacity was achieved using Soxlet extraction. According to the literature, methanol is more efficient for the extraction of phenol from plant material, however, we used an aqueous solution of ethanol in this work due to less toxicity.

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