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The antioxidant activity of ethanolic and aqueous extracts of dandelion (*Taraxacum officinale* L.)

Aktywność antyoksydacyjna wyciągów etanolowych i wodnych z mniszka lekarskiego (*Taraxacum officinale* L.)

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ABSTRACT

Introduction: Dandelion (*Taraxacum officinale* L.) has been commonly used in traditional and contemporary medicine due to its diuretic and hepatoprotective properties. It contains polyphenols, vitamins and terpenes – i.e. compounds with antioxidant potential. Natural antioxidants protect organisms against oxidative stress, an important factor in the ageing process and in the pathogenesis of neoplastic, cardiovascular, neurodegenerative and some other diseases.

Materials and methods: Raw dandelion plant material consisted of fresh and dried leaves, flowers and roots, harvested from a natural site. The extracts for analysis were prepared 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. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods.

Results: 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 for dried flower extracts prepared in 70% ethanol for 30 min. With FRAP method, the highest reduction capacity was observed for dried leaf extracts in 40% ethanol for 30 min.

Conclusions: The antioxidant potential of ethanolic and aqueous dandelion extracts was observed. This finding suggests the usefulness of this plant as a source of antioxidants to be used in pharmaceutical and cosmetic industries.

Keywords: dandelion; antioxidant activity; DPPH; FRAP; ultrasound-assisted extraction; ethanolic extracts; aqueous extracts.

ABSTRAKT

Wstęp: Mniszek lekarski (*Taraxacum officinale* L.) znalazł zastosowanie zarówno w ludowej, jak i współczesnej medycynie jako surowiec moczopędny oraz działający ochronnie na miąższ wątroby. Zawiera on m.in. polifenole, witaminy i terpeny, które wykazują działanie przeciwutleniające. Substancje będące naturalnymi antyoksydantami chronią organizm przed stresem oksydacyjnym, odgrywającym istotną rolę w procesie starzenia oraz patogenezie nowotworów, choroby wieńcowej czy chorób neurodegeneracyjnych.

Materiały i metody: Materiał roślinny stanowiły świeże i suszone liście, kwiaty oraz korzenie mniszka lekarskiego zebrane ze stanowiska naturalnego. Wyciągi sporządzono metodą ekstrakcji wspomaganej ultradźwiękami, trwającej 15, 30 oraz 60 min, przy czym jako rozpuszczalniki zastosowano 40% (v/v), 70% (v/v) i 96% (v/v) etanol oraz wodę. Aktywność antyoksydacyjną oznaczano metodami 2,2-difenylo-1-pikrylohydrazylu (DPPH) oraz oznaczania zdolności redukowania jonów żelaza (FRAP).

Wyniki: Działanie przeciwutleniające mniszka lekarskiego było zależne od użytego surowca, zastosowanego rozpuszczalnika, a także czasu ekstrakcji. W przypadku metody DPPH najwyższą aktywność wykazywały wyciągi z suszonych kwiatów przygotowane w 70% etanolu (czas ekstrakcji – 30 min). Przy zastosowaniu metody FRAP najwyższe działanie przeciwutleniające zaobserwowano dla wyciągów z suchych liści przygotowanych w alkoholu 40% (czas ekstrakcji – 30 min).

Wnioski: Etanolowe i wodne wyciągi z mniszka lekarskiego wykazują działanie antyoksydacyjne. Może to sugerować ich przydatność w przemyśle kosmetycznym i farmaceutycznym.

Słowa kluczowe: mniszek lekarski; aktywność antyoksydacyjna; DPPH; FRAP; ekstrakcja wspomagana ultradźwiękami; ekstrakty etanolowe; ekstrakty wodne.

INTRODUCTION

Dandelion (*Taraxacum officinale* L.) is a perennial plant belonging to the Asteraceae family. It is deeply rooted, meaning that even after cutting the aerial parts, it is able to regenerate new growth. A typical specimen of dandelion contains 5–10 flowers that transform into seeds containing hairy down fluff to allow dispersal by the wind. Due to the content of biologically active ingredients in the leaves, flowers and roots, its traditional use has been documented in many countries [1]. The largest crops of dandelion in Europe can be found in Poland, Bulgaria, Romania and Hungary. Moreover, it can be often found

as a wild growing weed, highly resistant to adverse weather conditions. It is estimated that in moderate regions there are about 2,800 varieties of this plant [2]. Dandelion, both in its raw and processed form, is well tolerated by humans and practically no adverse effects can be observed after its use. In some countries, its leaves are consumed in raw form as ingredients in salads, while roasted root can be applied as a substitute for coffee [3]. Biologically active compounds have been found in various parts of this plant. The roots are rich in phenolic and terpene compounds, sesquiterpene lactones, fructosans and inulin. The leaves contain substances belonging to the group of flavonoids, phenolic acids, coumarins and vitamins, especially vitamin A [4]. The flowers are rich in active compounds, including caffeic acid, chlorogenic acid, as well as luteolin derivatives [2]. Numerous studies confirm a number of pro-health properties of the dandelion. Due to the content of the mentioned chlorogenic acid and taraxasterols or sesquiterpene lactones, dandelion has a beneficial effect in the treatment of humans suffering from type 2 diabetes [2]. Moreover, its anti-inflammatory and antioxidant effects are also confirmed, as well as immunostimulating, antiallergic or diuretic properties [5, 6]. Its beneficial health-promoting effect on the gastrointestinal tract and metabolism is well known both in humans and animals [7, 8]. In addition, many reports on the antioxidant activity of extracts from various parts of *Taraxacum officinale* can be found. This activity is mainly due to the presence of phenolic compounds, especially flavonoids [5, 9]. The content of compounds with a radical scavenging activity is important due to their possibility to decrease so called oxidative stress, which is responsible for a number of adverse effects on living organisms, for instance Parkinson's and Alzheimer's diseases as well as cardiovascular and neoplastic disorders [10, 11]. In addition, oxidative stress is a significant factor in the development of metabolic diseases, among others, diabetes [12], as well as certain mental disorders [13].

The aim of the study was to evaluate and to compare the antioxidant activity of extracts, alcoholic and aqueous, of fresh and dried parts of the dandelion, such as the flower, leaf and root, harvested while the plant is flowering.

MATERIALS AND METHODS

2,4,6-tripyridyl-S-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma Aldrich, USA; iron (III) chloride hexahydrate and iron (VI) sulphate (II) heptahydrate – from Merck, Darmstadt, Germany; glacial acetic acid, anhydrous sodium acetate, 36% hydrochloric acid (all of analytical purity) and ethyl alcohol – from Chempur, Piekary Śląskie.

The dandelion was harvested from a natural location close to Szczecin. The leaves, flowers and root of the plant were used to obtain extracts in 40% (v/v), 70% (v/v), 96% (v/v) ethanol to water baths. To prepare the extracts, 5% of plant raw material was added to each of the 3 solvent baths, followed by ultrasound-assisted extraction for 15, 30 or 60 min. The antioxidant activity was assessed using DPPH and ferric reducing antioxidant power (FRAP) methods, as previously described [14, 15, 16].

Statistical analysis of the results was performed using one-way analysis of ANOVA variance, with a significance level $\alpha = 0.05$. Inter-group differences were determined by Tukey test ($n = 3$). The Pearson correlation coefficient between the antioxidant activity determined by the DPPH and FRAP method, was calculated in mg trolox/g of raw material for particular parts of the plant. All the calculations were performed in Statistica 12 (StatSoft).

RESULTS

Tables 1 and 2 present the antioxidant activities of the extracts, taking into account the part of the plant used to obtain the extract as well as the extraction time and the solvent applied. In Figure 1, the vertical lines represent standard deviation (SD), and the bars marked with different letters differ significantly within the part of the plant (significance level: $\alpha = 0.05$; $n = 3$).

The antioxidant potential of the evaluated samples ranged from 0.010 ±0.003 to 3.37 ±0.01 mg trolox/g of raw material, and corresponded to values from 1.87 ±0.08% to 72.52 ±0.25% RSA (radical scavenging activity). The highest activity was found for the extract of dried dandelion flowers, extracted in 70% ethanol for 30 min. A high activity was also observed for the extracts of dried leaves in the same solvent – from 2.80 ±0.04 to 3.18 ± 0.04 mg trolox/g of raw material. Taking into account the type of raw material evaluated, in the majority of cases, higher values were obtained for the extracts of dried leaves, flowers and roots compared to their fresh form. The root was characterized by a lower activity than the other parts of the plant, the mean was 23.13% RSA, while for the leaf – 35.22% RSA, and the flower – 30.16% RSA. The lowest DPPH radical scavenging capacity was found for the aqueous fresh leaves extracted for 30 min (0.011 ±0.002 mg trolox/g of raw material) and for 15 min (0.05 ±0.03 mg trolox/g of raw material), and corresponded to 1.87 ±0.08% and 2.67 ±0.67% RSA, respectively. The antioxidant activities of leaf and flower extracts prepared in water are usually much lower than the ethanol extracts (Tab. 1, Fig. 1).

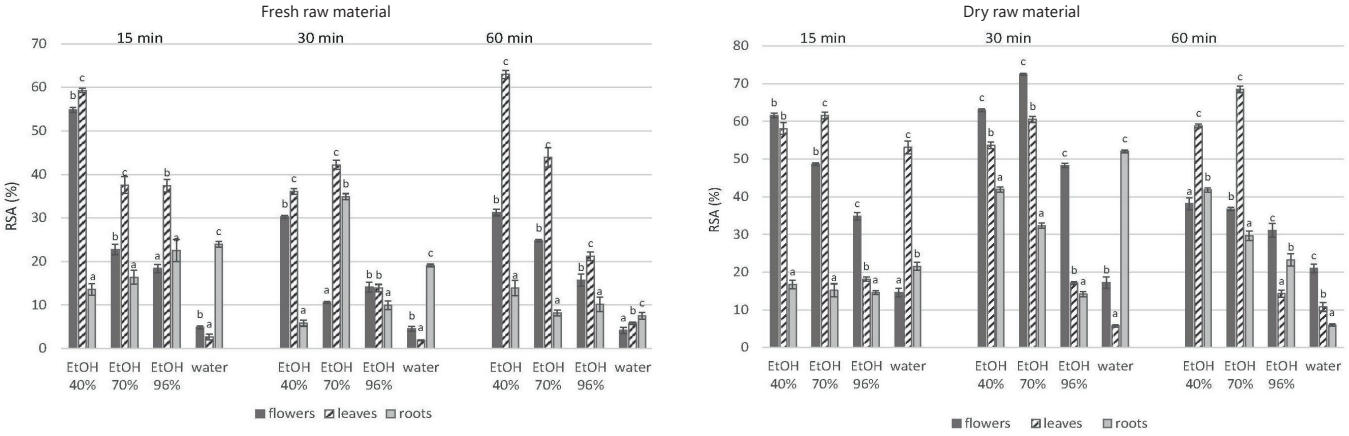
The iron ion reduction capacities, determined by the FRAP method and expressed as trolox equivalents (mg trolox/g of raw material) are summarized in Table 2.

The reduction capacity varied between 0.79 ±0.02 and 28.92 ±0.27 mg trolox/g of raw material. The highest activities were observed for the extracts of dried leaves prepared in 40% ethanol – 25.18 ±0.39 15 min; 28.92 ±0.27 30 min; 27.63 ±0.95 mg trolox/g of raw material 60 min. Similar to the previous method, the reduction capacity of the extracts of dried dandelion leaf were higher than the other parts of the plant – on average 10.04 mg trolox/g of raw material, versus the flower – 3.79 mg trolox/g of raw material and only 2.75 mg trolox/g of raw material for the root. High reduction properties were also found for extracts obtained from dried flowers, between 1.73 ±0.15 and 10.13 ±0.13 mg trolox/g of raw material. The lowest antioxidant

TABLE 1. Mean (±SD) antioxidant activity of ethanolic and aqueous extracts of roots, flowers and dandelion leaves, determined by DPPH technique and expressed as trolox equivalents (mg trolox/g of raw material)

Raw material		Solvent	Trolox equivalent (mg trolox/g raw material)		
			duration of ultrasound-assisted extraction (min)		
			15	30	60
Flower	**fresh**	**ethanol 40% (v/v)**	**2.53 ±0.03 d**	**1.36 ±0.02 d**	**1.41 ±0.03 d**
		ethanol 70% (v/v)	1.00 ±0.06 c	0.43 ±0.01 b	1.10 ±0.01 c
		ethanol 96% (v/v)	0.80 ±0.04 b	0.59 ±0.06 c	0.67 ±0.07 b
		water	0.16 ±0.01 a	0.14 ±0.03 a	0.12 ±0.03 a
	dried	ethanol 40% (v/v)	2.85 ±0.03 c	2.91 ±0.02 c	1.74 ±0.08 b
		ethanol 70% (v/v)	2.23 ±0.02 c	3.37 ±0.01 d	1.67 ±0.02 b
		ethanol 96% (v/v)	1.58 ±0.05 b	2.22 ±0.03 b	1.40 ±0.09 b
		water	0.62 ±0.05 a	0.74 ±0.07 a	0.92 ±0.05 a
Leaf	fresh	ethanol 40% (v/v)	2.74 ±0.02 c	1.64 ±0.03 c	2.91 ±0.04 d
		ethanol 70% (v/v)	1.71 ±0.09 b	1.93 ±0.05 d	2.01 ±0.10 c
		ethanol 96% (v/v)	1.70 ±0.07 b	0.58 ±0.04 b	0.93 ±0.05 b
		water	0.05 ±0.03 a	0.01 ±0.00 a	0.20 ±0.01 a
	dried	**ethanol 40% (v/v)**	**2.68 ±0.07 c**	**2.47 ±0.04 c**	**2.71 ±0.03 c**
		ethanol 70% (v/v)	2.85 ±0.04 c	2.80 ±0.04 b	3.18 ±0.04 c
		ethanol 96% (v/v)	0.79 ±0.03 a	0.74 ±0.02 b	0.60 ±0.05 b
		water	2.45 ±0.08 b	0.20 ±0.02 a	0.44 ±0.06 a
Root	fresh	ethanol 40% (v/v)	0.57 ±0.06 a	0.20 ±0.03 a	0.58 ±0.08 b
		ethanol 70% (v/v)	0.70 ±0.07 a	1.58 ±0.03 d	0.35 ±0.03 a
		ethanol 96% (v/v)	0.99 ±0.12 b	0.40 ±0.05 b	0.41 ±0.08 a
		water	1.06 ±0.03 b	0.83 ±0.02 c	0.32 ±0.04 a
	dried	ethanol 40% (v/v)	0.72 ±0.05 a	1.91 ±0.03 c	1.91 ±0.03 d
		ethanol 70% (v/v)	0.65 ±0.08 a	1.46 ±0.03 b	1.33 ±0.06 c
		ethanol 96% (v/v)	0.62 ±0.02 a	0.60 ±0.03 a	1.03 ±0.08 b
		water	0.95 ±0.05 b	2.39 ±0.02 d	0.21 ±0.01 a

Mean values marked with different letters differ significantly within the applied solvent for extraction (significance level: α = 0.05; n = 3)



The vertical lines represent standard deviation (SD). Bars marked with different letters differ significantly within a part of the plant (significance level: α = 0.05; n = 3)

FIGURE 1. Mean ±standard deviation (SD) antioxidant activity of aqueous and ethanolic (EtOH) extracts of fresh and dried dandelion parts, extracted for 15, 30 or 60 min, determined by DPPH method and expressed as %RSA

TABLE 2. Mean ±standard deviation (SD) of Fe^{3+} ion reduction capacity of ethanolic and aqueous extracts of roots, flowers and dandelion leaves, determined by FRAP technique and expressed as trolox equivalents (mg trolox/g of raw material)

Raw material		Solvent	Trolox equivalent (mg trolox/g raw material)		
			duration of ultrasound-assisted extraction (min)		
			15	30	60
Flower	fresh	ethanol 40% (v/v)	3.01 ±0.19 c	4.08 ±0.32 d	5.93 ±0.29 b
		ethanol 70% (v/v)	2.37 ±0.02 b	1.16 ±0.11 a	5.55 ±0.14 b
		ethanol 96% (v/v)	1.17 ±0.11 a	1.75 ±0.10 b	5.35 ±0.29 b
		water	2.81 ±0.09 c	2.55 ±0.20 c	2.64 ±0.25 a
	dried	ethanol 40% (v/v)	4.01 ±0.14 c	10.13 ±0.13 c	5.66 ±0.13 d
		ethanol 70% (v/v)	6.96 ±0.10 d	8.75 ±0.08 c	2.35 ±0.18 b
		ethanol 96% (v/v)	2.40 ±0.07 a	1.73 ±0.15 a	2.74 ±0.08 c
		water	3.11 ±0.19 b	2.93 ±0.11 b	1.93 ±0.06 a
Leaf	fresh	ethanol 40% (v/v)	7.58 ±0.40 d	7.40 ±0.42 d	16.67 ±0.15 d
		ethanol 70% (v/v)	3.08 ±0.20 b	5.35 ±0.07 b	10.76 ±0.36 c
		ethanol 96% (v/v)	3.84 ±0.05 c	3.58 ±0.19 c	5.69 ±0.11 b
		water	2.29 ±0.08 a	2.41 ±0.37 a	3.38 ±0.23 a
	dried	ethanol 40% (v/v)	25.18 ±0.39 d	28.92 ±0.27 d	27.63 ±0.95 d
		ethanol 70% (v/v)	8.38 ±0.64 b	21.95 ±0.11 c	22.46 ±0.09 b
		ethanol 96% (v/v)	1.16 ±0.06 a	2.85 ±029 b	2.54 ±0.32 a
		water	13.03 ±0.10 c	1.96 ±0.02 a	12.94 ±0.08 d
Root	fresh	ethanol 40% (v/v)	1.30 ±0.10 b	1.65 ±0.05 b	4.56 ±0.08 c
		ethanol 70% (v/v)	1.36 ±0.04 b	1.01 ±0.09 a	3.66 ±0.14 a
		ethanol 96% (v/v)	0.79 ±0.02 c	2.13 ±0.13 c	3.27 ±0.21 a
		water	3.32 ±0.06 a	3.26 ±0.09 d	2.71 ±0.15 b
	dried	ethanol 40% (v/v)	1.05 ±0.05 a	5.59 ±0.09 c	5.88 ±0.08 c
		ethanol 70% (v/v)	1.05 ±0.05 a	3.03 ±0.09 b	3.58 ±0.03 b
		ethanol 96% (v/v)	0.91 ±0.11 a	1.16 ±0.04 a	1.90 ±0.03 a
		water	1.23 ±0.04 b	9.94 ±0.06 d	1.75 ±0.11 a

Mean values marked with different letters differ significantly within the applied solvent for extraction (significance level: $\alpha = 0.05$; $n = 3$)

activity, measured by the FRAP method, was found for dried root extracts prepared in 96% ethyl alcohol for 15 min – 0.79 ±0.02 mg trolox/g of raw material and 0.91 ±0.11 mg trolox/g of raw material for fresh root extracts. The activity of the aqueous extract of the dried root of 9.94 ±0.06 mg trolox/g of raw material, extracted for 30 min, was significantly higher compared to the extracts of this part of dandelion prepared in other solvents (Tab. 2).

Figure 2 presents the relationship between the results obtained by both methods used to evaluate the antioxidant activity for extracts of individual parts of the plant. A statistically significant relationship was found between the activities determined with both methods, the Pearson correlation coefficients were $r = 0.726$ ($p < 0.0001$), $r = 0.610$ ($p < 0.002$) and $r = 0.656$ ($p < 0.001$) for leaf, flower and root extracts, respectively.

DISCUSSION

In the present study the antioxidant properties of alcoholic and aqueous extracts of various parts of the dandelion, harvested during flowering, were determined. To prepare *Taraxacum officinalis* extracts, ultrasound-assisted extraction for 15, 30 or 60 min was applied using ethanol at 3 concentrations – 40%, 70%, 96% (v/v) and water as extractants. The dandelion is a plant with antioxidative capacity, however, the obtained results prove that this activity differed depending on several factors including the plant part, the solvent used, as well as the duration of extraction (Tab. 1 and 2). Moreover, the drying process of the raw material could also influence the results. The highest antiradical activity, measured by the DPPH method, was demonstrated by extracts from dried dandelion flowers made in 70% ethanol for

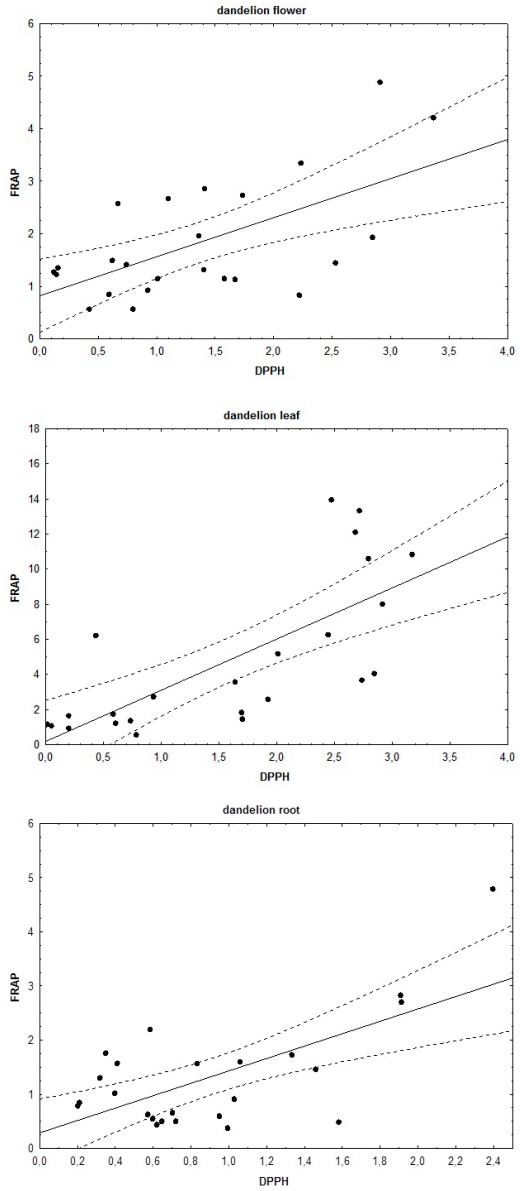


FIGURE 2. The relationship between the antioxidant activity assessed with DPPH technique and iron reduction capacity evaluated with FRAP method for the extracts of all dandelion parts

30 min, and by extracts from dried leaves in the same solvent for 60 min (Fig. 1). In our study, the ability to reduce iron Fe^{3+} was also determined using the FRAP method. It was found that the most valuable extracts were obtained from dried dandelion leaves in 40% ethanol, regardless of extraction time (Tab. 2).

Many reports proved a therapeutic effect of all parts of the dandelion, however, due to the slightly different composition, they may have a variety of therapeutic applications. The root and leaf could be used mainly in liver failure and in gastric hyposecretion. In addition, these raw materials, especially the root, due to inulin content, are often included in herbal mixtures and are used in the early stage of diabetes to decrease blood sugar levels. The dandelion flower is rich in polysaccharides, and this makes it useful as an anti-inflammatory factor [4]. Due to the valuable composition, mainly the content of polyphenolic compounds, this plant has antioxidant activity [17, 18]. The content of phenolic compounds depends on the part of the plant, with a higher content found in the leaves [4, 19]. In our study, the antioxidant activity of the extracts of individual parts of the dandelion was different. In most cases, the highest antioxidant activity was found for both fresh and dried leaf extracts, followed by the flower, and the lowest potential was found for root extracts (Fig. 1). The antiradical activity of various dandelion parts was also observed by others, who studied leaf [3, 18, 19, 20], root [17, 21], flower [17, 22], stalk [17, 20, 23], as well as fruit extracts [24].

In our study, the antioxidant activity of the dandelion extracts depended on the solvent used for extraction as well as its concentration, i.e. the water content. The antioxidant potential of the aqueous extracts of leaves and flowers was lower compared to ethanolic, particularly with those prepared in 40% and 70% alcohol to be of the highest potential. Similar results were obtained by Ivanov, who observed a higher antioxidant activity of dandelion leaves extracts prepared in 50% ethanol determined by DPPH and FRAP methods compared to extracts in undiluted ethanol. In addition, the samples extracted with diluted alcohol contained the largest amount of chicoric acid, one of the most important active compounds found in dandelion leaves [19]. Tsai et al. observed the highest content of chicoric acid in *Echinacea purpurea* L. flower extracts prepared in 50% ethyl alcohol, this plant is also characterized by a high content of this valuable ingredient. The authors applied as extractant, water and 25%, 50%, 75% and 95% ethanol [25]. Chicoric acid is one of the main phenolic compounds to be responsible for the high antioxidant activity of *Echinacea purpurea*, so, probably diluted ethanol is a better extractant compared to water and undiluted ethanol [26]. Ghaima et al. demonstrated a 44% inhibition of lipid peroxidation by dandelion dry leaf extracts using 95% ethyl acetate as extractant [18]. Similar results were obtained by Sengul et al. They observed an antioxidative potential of 43% for methanolic extracts of dandelion stems [23].

It should be added that dandelion is often administered orally as alcoholic and aqueous extracts. In our study, an attempt was made to assess the antioxidant activity of both ethanolic and aqueous extracts of this plant in order to evaluate which solvent is most preferable as an extractant. It was found that ethanolic extracts were characterized by a much higher antioxidant potential in most cases. Choi et al. applied dandelion root and leaf in dietotherapy as an antiatherosclerotic raw material to protect cells from oxidative stress [27]. Rabbits were fed on a high-cholesterol diet containing 1% dandelion leaf or root for 4 weeks. The treatment with dandelion led to positive antioxidant and hypolipidemic effects. Due to its antioxidative potential it may prevents endothelial cell injury caused by oxygen-radical action [27]. Root aqueous extracts can play a significant role as hepatoprotective agents in alcoholic liver damage. In an *in vivo* study by You et al., an aqueous dandelion root extract was administered to 8-week old mice. The animal diet included ethanol as a potential liver-damaging factor. An intake of this compound is connected with oxidative stress via, among others, the production of reactive oxygen species. As a result, in the group of animals with dietary supplementation of dandelion aqueous extracts, a hepatoprotective effect was demonstrated by a significant reduction of blood

aspartate and alanine aminotransferases, alkaline phosphatase and lactate dehydrogenase activity [28]. The main component of the root is inulin (40–50%), with probiotic, antidiabetic and anti-sclerotic activity [29, 30]. In our study a lower antioxidant activity compared to the leaves and flowers was observed. This may be partly due to the different chemical composition of these parts of the plant. However, it should be taken into account that the plants for this study were harvested at the beginning of May, during flowering, while the highest content of inulin, a potential antioxidant in the root, could be observed in July [29]. In addition, a high content of flavonoids, i.e. substances with antiradical activity, was found in the leaf and flower extracts, which may explain the high antioxidant activity of these plant organs [26, 31].

CONCLUSIONS

1. The extracts of the individual dandelion parts were characterized by different antioxidant activities, determined by DPPH and FRAP. In the majority of cases, the leaf and flower ethanolic extracts were characterized by a higher antioxidant potential.
2. The extracts of dried flower in 70% ethanol for 30 min were the most valuable products taking into account antioxidant activity.
3. Due to its properties, the dandelion could be considered a valuable raw material to be applied in cosmetics and pharmaceuticals.

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