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ORIGINAL ARTICLE

Total Phenolic Content and Antioxidant Properties of *Taraxacum officinale* Extracts Obtained with Different Solvents

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ABSTRACT
Total phenolic content and antioxidative properties of Taraxacum officinale leaves and flowers extract was studied. Extracts was made with different solvents: classical organic and surfactant solutions. The results showed that all examined extracts contain some polyphenols and have antioxidant potential against DPPH radical and reducing power (Fe^{3+} - Fe^{2+}). Leaf extracts made with organic solvents had the highest content of phenolic compounds and radical scavenging potential. Surfactant assisted extraction gave extracts with similar phenolic content and good antioxidative properties. Data from this study suggest that the investigated properties of T. officinale depend on solvent system used to extraction.
Keywords: *antioxidants, polyphenols, reducing power, Taraxacum officinale*

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Introduction

Dandelion (*Taraxacum officinale* Web.) is common perennial plant from *Asteraceae*family, widely distributed all over Europe. It has several medicinal uses, such as diuretic [1,2], hepatoprotective[3,4,5] and anti-inflammatory agent [6,7,8]. Moreover, there are literature reports about its anticancer effect [9,10]. Dandelion root (*Radix Taraxaci*), leaf (*Folium Taraxaci*) and flower (*FlosTaraxaci*) are listed in many pharmacopoeias. The main secondary metabolites of this plant are terpenes and polyphenols. Terpenes, present mainly in dandelion root, can be divided into two groups: sesquiterpene lactones (germacranolides, guaianolides and eudesmanolides) and triterpenes (taraxasterol, ψ-taraxasterol and their derivatives) [11]. Polyphenols are represented by phenolic acids (e.g. caffeic, chlorogenic, chicoric, ferulic, vanillic), flavonoids (quercetin, luteolin, chrysoeriol, apigenin and their glycosides) and coumarins: chicoriin, aesculetin and scopoletin) [11]. Phenolic content results in antioxidant properties of *Taraxacum* extracts.

Extraction of plant polyphenols is usually carried out using traditional organic solvents, such as ethanol, methanol, acetone or ethyl acetate. However, new methods of extraction are still developed, in order to reduce organic solvents consumption. A relatively new application for surfactants is micellar extraction of various compounds both polar and nonpolar which are solubilized in micelles. The micelle-mediated extraction can be good alternative to conventional extraction with organic solvents which often are toxic and flammable [12].

The aim of this study was to determine total phenolic content and radical scavenging activity of several *Taraxacum officinale* extracts obtained with different solvent systems – organic and surfactant solutions.

MATERIALS AND METHODS

Extracts preparation

T. officinale leaves and flowers was collected in spring 2014 from natural population in the region of Rzeszów (south-west Poland). The collected plant material after identification was air dried in darkness at ambient temperature (20-25^{o}C). After pulverization an accurately weighted mass (0.5 g) of leaves or flowers was placed in screw cap test tube and 20 ml of appropriate solvent was added. The following solvents were used: methanol, ethanol, acetone (70% v/v), and Triton X-100, Tween 20, Nonidet P-40

(2% v/v). Extraction was carried out in ultrasonic bath (Sonorex RK 31, Bandelin, Germany) for 30 min at ambient temperature. After centrifugation (10 min,6500×g) the supernatant was collected and crude extracts was analyzed.

Determination of total phenolic contents in the plant extracts

The *T. officinale* extracts were analyzed for total phenolics content by the Folin-Ciocalteau procedure [13]. The reaction mixture was prepared by mixing 0.1 ml of crude extract and 0.2 ml of 1:10 Folin-Ciocalteau reagent dissolved in water. After 5 min equilibration 0.8 ml of 700 mM Na_2CO_3 solution was added. After incubation for 1 hour at room temperature in darkness, the absorbance of the mixture was read at 765 nm against a blank (without extract) using spectrophotometer (UV-160A, Shimadzu, Japan). The total phenolic content was expressed as (+)-catechin equivalents (mg CE/ml of extract) based on prepared standard curve ($y = 0.0034x + 0.176$, $R^2 = 0.9898$). The samples were prepared in triplicate and the mean value of absorbance was calculated.

Free radical scavenging active test (DPPH)

The radical scavenging activity was evaluated using the method described by Brand-Williams et al. [14] with slight modifications. 3.9 ml of 0.1 mM solution of DPPH radical in methanol was placed in 1 cm cuvette and the absorbance value A_0 was read against blank (methanol) at 515 nm. The extract (0.1 ml) was then pipetted into the cuvette. The decrease of absorbance was monitored at 1 min interval up to 15 minute. The scavenging ability was expressed as the inhibition of DPPH radical percentage after 15 min, according to the formula:

% inhibition = $[(A_0 – A_A) / A_0] \times 100$,

Where A_0 is the absorbance of DPPH solution (t = 0) and A_A is the absorbance with plant extract after 15 min. The samples were prepared in triplicate and the mean value of inhibition was calculated. The reaction was carried at ambient temperature.

Reducing power assay

The reducing power of crude extract was determined according to the method of Oyaizu [15] with slight modifications. 0.5 ml of crude plant extract was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of potassium ferricyanide (1% w/v). The mixtures were incubated at 50°C for 20 min. Then 1 ml of trichloroacetic acid (10% w/v) was added and mixtures were centrifuged (10 min, 3000 rpm). The supernatants was collected and mixed with 1.5 ml of distilled water and 0.1 ml of $FeCl_3$ (0.01 % w/v). The absorbance of the mixture was read at 700 nm. Higher absorbance indicated greater reducing power of examined extract. The samples were prepared in triplicate and the mean value of reducing power was expressed as ascorbic acid equivalents (mg AAE/ml of extract) based on prepared standard curve ($y = 0.0013x - 0.0738$, $R^2 = 0.9744$).

RESULTS AND DISCUSSION

The results of total phenolic content, anti-radical activity against DPPH radical and reducing power determination are summarized in Tab.1.

The highest phenolic content was observed for leaf extract made with 70% acetone. Methanolic and ethanolic extracts revealed similar values. Generally, leaves extracts have shown higher values of polyphenol content than flowers extracts and also percent of DPPH radical scavengingand reducing power were higher for this extracts. All examined extracts have shown antioxidant potential, both in DPPH and reducing power experiments. The highest DPPH scavenging activity was observed for ethanolic leaf extract (89.52%). The absorbance at 700 nm had high values for all leaves extracts, especially made with Triton X-100 and Tween 20. It indicates, that this surfactant assisted extracts show the highest reducing power. In case of flowers extracts the reducing power was slightly lower for micelle-mediated extracts, but still had relative high value. Analytical methods used in this research allow the assessment of complex plant extracts ability to free radicals disposal. Reactive oxygen and nitrogen species fulfill important roles in biological systems, but disequilibrium between their formation and utilization may have negative consequences for living cells and organisms. Natural antioxidants are valuable and often used in different medical or food preparations. Obtained results confirm radical-scavenging potential of *T. officinale*, described earlier by several authors [16,17, 18].

Table 1. Total phenolic content, radical scavenging activity and reducing power of *T. officinale* crude extracts.

Extract		Total phenolic content [mg CE/ml of extract]	% inhibition of DPPH	Reducing power [mg AAE/ml of extract]
Flower	Methanol 70%	0.414±0.030	68.13±0.07	1056.26±2.22
	Ethanol 70%	0.372±0.019	51.41±0.05	997.28±1.78
	Acetone 70%	0.385±0.008	78.61±0.04	1017.54±1.33
	Triton X-100 2%	0.229±0.010	32.34±0.09	870.10±0.89
	Tween 20 2%	0.178±0.018	26.41±0.07	673.44±0.44
	Nonidet P-40 2%	0.122±0.025	20.50±0.54	577.03±1.78
Leaf	Methanol 70%	0.553±0.014	86.28±0.02	1027.80±0.89
	Ethanol 70%	0.533±0.040	89.52±0.03	1126.26±2.22
	Acetone 70%	0.586±0.007	84.56±0.01	1075.23±0.00
	Triton X-100 2%	0.410±0.009	84.00±0.03	1164.97±5.77
	Tween 20 2%	0.475±0.017	87.23±0.04	1230.62±2.66
	Nonidet P-40 2%	0.230±0.004	64.91±0.19	1140.87±0.44

Data are presented as the mean ± SD values of triplicate determinations.

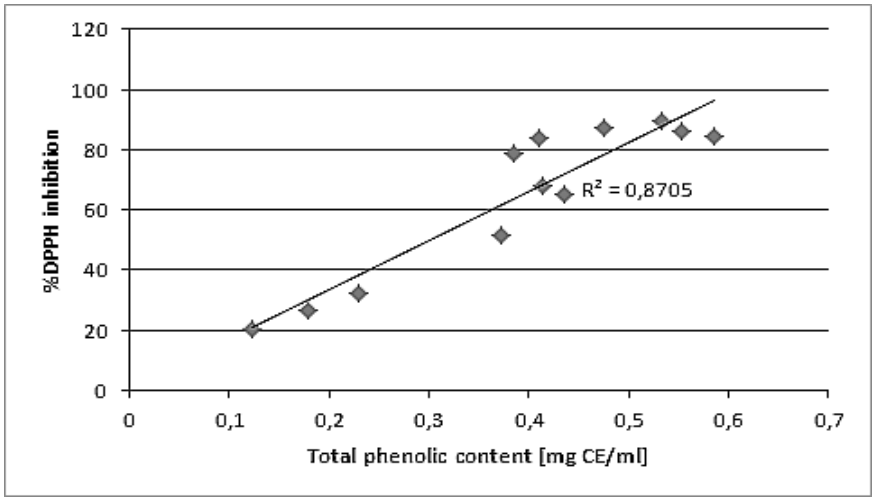

Figure 1. Correlationbetween total phenolic content and DPPH scavenging activity of*T. officinale* extracts

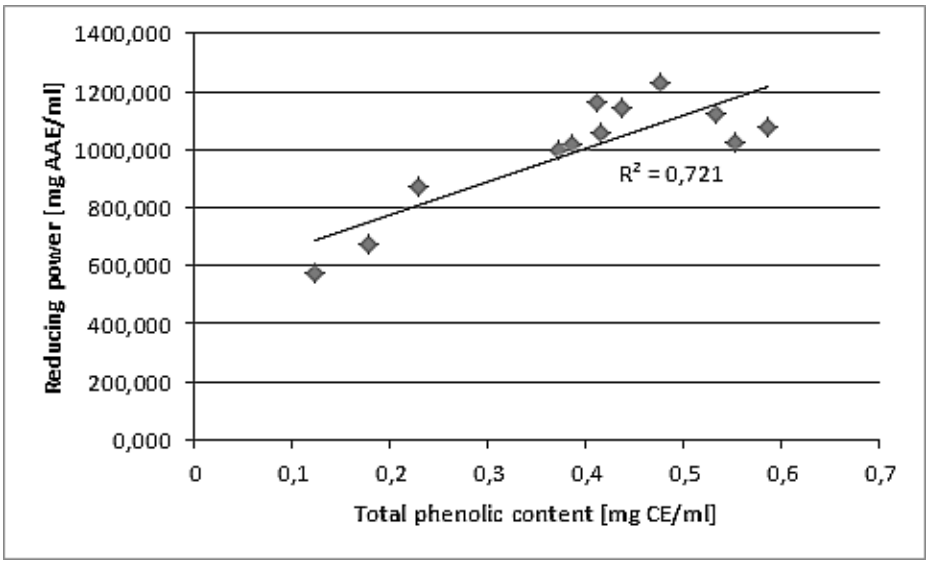

Figure 2. Correlationbetween total phenolic content and reducing power of *T. officinale* extracts

Fig.1 and 2 demonstrate the correlation between total phenolic content and antioxidant activity of *T. officinale* extracts. Based on obtained results it can be concluded, that polyphenols are main antioxidants isolated from dandelion flowers and leaves. This class of plant secondary metabolites is the most important group of natural antioxidants [19]. They can act as free radical acceptors or as chain breakers. This mechanism are important in natural antioxidant systems. It should be remembered, that also other classes of phytochemicals can act as antioxidant agents, such as carotenoids [19].
Surfactants solutions as extraction solvents proved to be effective for obtaining polyphenol-rich extracts of *T. officinale.* Surfactant made extracts shown comparable to classical organic solvents values of total phenolic content, DPPH radical scavenging activity and reducing power. It indicates the possibility of use such solvents systems in plant polyphenols extraction. There are some earlier reports about using surfactants for some polyphenols extraction, e.g. isoflavones form *Puerariae radix*[20], esculin and esculetin from *cortex fraxini*[21] or chlorogenic acid, quercetin and rutin from honeysuckle [22]. Such method was also applied for complex antioxidant extraction from elderberry blossom [23]. Using of surfactant assisted extraction allow to reduce use of organic solvents, which are toxic and flammable. Extracts obtained with this method may be used in pharmaceutical, cosmetic and food industry [12].

CONCLUSIONS

The results of this research suggest that ethanolic, methanolic and acetone extracts from *T. officinale* leaves and flowers contain some polyphenols and possess antioxidant properties. A quite new extraction technique using surfactant solutions has also been shown to be effective for obtainingantioxidants from plant material. This method should be further optimized and may find applications in plant secondary metabolites technology.

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