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Investigation of the Antioxidant Capacity of *Taraxacum Officinale L.* Leaf Extract

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Abstract: The aim of this study is to investigate the potent antioxidant capacities of *Taraxacum officinale* leaf extract. Asteraceae family, the genus Taraxacum is widespread in the Mediterranean countries, with approximately 2000 species worldwide. *Taraxacum officinale*, a species that stands out in phytotherapy, is a perennial plant with yellow flowers commonly found in the warm regions of Asia and Europe. Initially used in folk medicine for liver diseases, it has been observed over time that it is also used for various diseases such as dyspepsia and urinary system disorders. *T. officinale* contains numerous phenolic compounds contributing to antioxidant, anti-inflammatory, and antimicrobial activities. This research aimed to evaluate the antioxidant capacities of aqueous and ethanol-water extracts prepared from *T. officinale* leaves. Antioxidant capacities were assessed using spectrophotometric methods in DPPH, FRAP, and CuPRAC forms. The results showed that the DPPH, FRAP, and CuPRAC values were higher in the 50% ethanol extract of T. officinale leaves: 137.1 mg Trolox/g (DPPH method), 132.7 mg Trolox/g (FRAP method), and 409.9 mg Trolox/g (CuPRAC method). In conclusion, *Taraxacum officinale* leaves clearly demonstrate to be a rich source of polyphenols with high antioxidant properties. It has no seious side effect or toxicity. Considering its lack of serious side effects and toxicity, when formulated into herbal medicine, it could be effective in the treatment of various diseases, especially common liver and bile diseases, based on its biological activity studies.

Keywords: Taraxacum officinale, In vivo antioxidant, DPPH, FRAP, CUPRAC.

Introduction

The Asteraceae family is one of the largest families of flowering plants, comprising almost 1000 genera and approximately 20,000 species (Tanker et al., 1993). The genus Taraxacum consists of approximately 2000 species and is a member of the Asteraceae family. In a study, it was found that many subspecies belong to this genus, divided into approximately 30-57 varieties (Schutz et al., 2006). There are 45 species of this genus in Türkiye (Soest, 1975).

Traditionally used for many diseases for years (Schutz et al., 2006), it has also been observed clinically used (Blumenthal, 1998; ESCOP Monographs, 2003; WHO Monographs, 2007; PDR for Herbal Medicines, 2008) in diseases such as dyspepsia, liver, gallbladder, and urinary tract diseases, and *Taraxacum officinale* is seen to enter our lives today only as a dietary supplement in various forms. However, it is a plant with many bioactivities, especially strong anti-inflammatory, antioxidant, and hepatoprotective properties. When considering its side effects and toxicity, it no serious side effects or toxicity reported. In light of this information, it is evaluated that if it is converted into herbal medicine format, it can be effective in the treatment of many diseases, especially cancer, liver, and bile duct diseases, which are commonly seen today, due to its strong antioxidant, anti-inflammatory, anti-cancer, and hepatoprotective effects. According to the results of bioactivity studies conducted on the plant, *Taraxacum officinale* has been reported to have analgesic, antiallergic (Schutz et al., 2006), antidepressant (Li et al., 2014) , anti-inflammatory (ESCOP Monographs, 2003; Schutz et al., 2006 ;

WHO Monographs, 2007; Jeon et al., 2008; Koh et al., 2010; Awortwe et al., 2011; Zhang et al., 2012; Park et al., 2014, Piao et al., 2015), anti-hyperglycemic (ESCOP Monographs, 2003; Schutz et al., 2006), anticarcinogenic (Schutz et al., 2006; Sigstedt et al., 2008; Choi & Kim, 2009), antimicrobial (ESCOP Monographs, 2003; Astafieva et al., 2012; Astafieva et al., 2015; Rehman et al., 2016), antimutagenic (Di Giorgio et al., 2015), antioxidant (ESCOP Monographs, 2003; Schütz et al., 2006; Park et al., 2011; Colle et al., 2012; Park et al., 2014; Kenny et al., 2015; Yang et al.,2015; Lis and Olas, 2019;Majewski et al., 2020), antispermic (Tahtamouni et al., 2011; Tahtamouni ve ark., 2016), antithrombotic (ESCOP Monographs, 2003; Schutz et al., 2006), diuretic (ESCOP Monographs, 2003; Schütz et al., 2006 ; WHO Monographs, 2007;Clare et al., 2009), hypolipidemic(Zhang et al., 2008; Choi et al., 2010; Gonzalez et al., 2014; Kim et al., 2014), immunological(WHO Monographs,2007; Jinchun & Jie, 2011; Lee et al., 2012), choleretic (ESCOP Monographs, 2003; Schutz et al., 2006) , and prebiotic effects (Schutz et al., 2006), as well as hepatoprotective (Domitrović et al., 2010; Mahesh et al.,2010; Park et al., 2010; Colle et al., 2012;Gulfrazl et al., 2014; Hfaiedh et al.,2016), nephroprotective and neuroprotective properties it is used in hepatitis treatment. Extracts contain lipotropic substances that can improve the functionality of hepatocytes. Many other health benefits have been attributed to the use of T. officinale extracts or the plant itself.

T. officinale contains many phenolic compounds that contribute to antioxidant, anti-inflammatory, and antimicrobial activities (Park et al., 2011; Colle et al., 2012; Kenny et al., 2015; Martinez et al., 2015). In a study by Williams and colleagues, the flavonoid and phenolic fractions responsible for the bioactivity of *Taraxacum officinale* were comprehensively addressed (Williams et al., 1996). The main phytochemicals found in the plant can be listed as follows: carotenoids; flavonoids (e.g., quercetin, luteolin-7-glucoside); phenolic acids (e.g., caffeic acid, chlorogenic acid, chicoric acid); polysaccharides (e.g., inulin); sesquiterpene lactones (e.g., taraxinic acid, taraxacoside, 11p,13-dihydrolactucin, ixerin D); sterols (e.g., taraxasterol, β-sitosterol, stigmasterol); triterpenes (e.g., α-amyrin) (Singh et al., 2008; Amin Mir et al., 2013).

The aim of this study is to investigate the potent antioxidant capacity of *Taraxacum officinale* leaf extract using two different methods and to contribute to the natural prevention of oxidative stress, which is implicated in many diseases, in the future.

Materials and Methods

Plant Material: *Taraxacum officinale* in the leafy stage were collected from the Gaziantep Region, Türkiye, and the above-ground parts were harvested and washed before being dried in the shade.

Extraction Procedure: The dried plant samples were ground in the laboratory at room temperature using a laboratory grinder. The powdered sample (50 g of dandelion leaves) was weighed and extracted in 1 L of ethanol (96%) for two weeks at room temperature. The mixture was occasionally shaken during the waiting period. Then, it was filtered (Whatman No. 4 filter) and strained. After removing the solvent using a rotary evaporator, the extracts were stored at +4°C in a refrigerator (Colle et al., 2012).

Antioxidant Activity (AOA): Firstly, a DPPH solution was prepared for DPPH detection. For this purpose, 0.002 mg of DPPH was weighed and dissolved in 50 ml of methanol to prepare a DPPH solution with a final concentration of 0.2 mM.

The scavenging capacities of the extracts for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical were determined (Hatano et al., 1988). Addition of DPPH solution to the extracts results in a decrease in optical density absorbance at 517 nm, and the discoloration of the extracts indicates their radical scavenging activity (Baydar et al., 2011).

To test tubes containing 0.1 mg/100µl of extract solution, prepared DPPH solution (final concentration 0.2 mM) was added. For the control, 1.0 ml of dH_2O was added to the test tube instead of the extract. After incubating the samples at room temperature and in darkness for 30 minutes, absorbances were measured at 517nm. The free radical scavenging activity was calculated using the following equation:results were expressed as mM Trolox® equivalent (TE) per mg Trolox/g.

Percentage Inhibition was calculated using the following formula:

% Inhibition = [(AControl 517 nm - ASample 517 nm) / AControl 517 nm) x 100].
(AControl: absorbance of control and ASample: absorbance of the sample)

Ferric Reducing Antioxidant Power (FRAP):

The FRAP assay was performed according to Benzie and Strain (1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1 immediately before use. The 300 mM acetate buffer was prepared by mixing 0.31 g sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$) with 1.6 mL acetic acid and diluting to a final volume of 100 mL with distilled water. The TPTZ solution was prepared by dissolving 10 mM TPTZ in 40 mM HCl.

For the assay, 100 µL of extract was mixed with 900 µL of water and 2 mL of FRAP reagent. After incubating the mixture at room temperature in the dark for 30 minutes, the absorbances were measured against a blank at 593 nm. The results were expressed as mg Trolox equivalent (TE) per gram of sample.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC):

The CUPRAC analysis was conducted according to the method described by Apak et al. (2004). In a test tube, 1 mL of $CuCl_2$ solution (0.01 M), neocuproine (7.5 mM), and 1 M ammonium acetate buffer (pH 7.0) solutions were added. After adding 0.1 mL of extract to the test tube, 1 mL of distilled water was added. All samples were then incubated in the dark at room temperature for 1 hour, and the absorbance values were measured at 450 nm. The results were expressed as mg Trolox equivalent (TE) per gram of sample.

Results

In our study, we decided to evaluate the antioxidant activities of T. officinale leaf ethanol extracts using two methods: a complex method based on both hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms (DPPH), and two methods based solely on the SET mechanism (FRAP and CUPRAC) (Table 1). The antioxidant activities of plant extracts are generally attributed to the presence of phenolic acids. Therefore, T. officinale's 50% ethanol extracts exhibited the highest antioxidant activities. Data regarding the DPPH radical scavenging activity and metal reduction activity of extracts obtained from T. officinale and their antioxidant activities determined in vitro are presented. These results express the total antioxidant capacity for each extract in mg Trolox/g (Table 1).

Table 1. Antioxidant activity in different extracts from *T. officinale*.

Extracts	Radical scavenging activity		Metal reducing activity	
	DPPH mg Troloks/g	EC_{50}, mg/ml	FRAP mg Troloks/g	CuPRAC mg Troloks/g
96% ethanol extract	28.8 ± 0.6	16.7	27.1 ± 0.6	95.9 ± 0.2
50% ethanol extract	137.1 ± 3.7	3.9	132.7 ± 2.3	409,9± 5.6
Water extract	57.1 ± 1.4	8.6	43.9 ± 3,4	186.7 ± 2.5
Standart				
Gallic acid		0.198		

Discussion

Extraction yield is a measure of the efficiency of the solvent in extracting specific components from raw materials. Considering our radical scavenging activity and EC50 values, it can be concluded that T. officinale exhibits strong antioxidant activity. Similarly, Paduret et al. found an inhibition capacity of 80.664% for T. officinale methanolic extract in a 50% DPPH solution. The ethanol extract of T. officinale flowers recorded 90.27 ± 0.5% inhibition at a concentration of 100 g/mL (Padureţ et al., 2016). In another study, an aqueous extract showed an IC50 value of 4.48 µg/mL, lower than our findings (Hu & Kitts, 2005). Tettey et al. reported T. officinale as a potent antioxidant in their study. García-Carrasco et al. detected the highest DPPH radical scavenging activity in leaf extract with an EC50 value of 1.9 µg/mL, consistent with our findings. Leaf extract provided the highest antioxidant capacity with a value of 302.3 ± 26.3 µmol TE/g, indicating good antioxidant activity in the FRAP assay (García-Carrasco et al., 2015). Furthermore, findings from González-Castejón et al. are consistent with our results (González-Castejón et al., 2012). The antioxidant activity of T. officinale is attributed to phenolic hydroxyl groups and activation of endogenous antioxidant enzymes (Park et al., 2014). Specifically applying Taraxacum officinale roots and leaves to rats leads to the development of an endogenous antioxidant profile (Kuntz & Kuntz, 2008; Harvey & Ferrier, 2017). Our results for antioxidant capacities using

the DPPH, FRAP, and CuPRAC methods for *Taraxacum officinale* leaf parts are consistent with previous findings.

Conclusion

In conclusion, Taraxacum officinale leaves clearly demonstrate being a rich source of polyphenols with potential application in scavenging free radicals and reducing metal. Upon consideration of side effects and toxicity, it appears to have no serious adverse effects or toxicity. Given the biological activity studies of this plant, if formulated into a herbal medicine, it could be effective in treating various diseases, particularly common liver and bile diseases.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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