




Antioxidant activity and three phenolic compounds from the roots of *Taraxacum gracilens* Dahlst.

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ABSTRACT

Background and Aims: The genus *Taraxacum* is a member of the family Asteraceae. *Taraxacum* species are widely distributed in the warmer temperate zones of the Northern Hemisphere. The aim of this study was to evaluate the phenolic contents and antioxidant activity of the ethanol extract and its chloroform and ethyl acetate fractions from the roots of *Taraxacum gracilens* Dahlst. and isolate some of its phenolic compounds.

Methods: The roots of *T. gracilens* Dahlst. were first macerated with EtOH and fractionated to obtain chloroform and ethyl acetate fractions, and then phenolic compounds were isolated by column chromatography. The polyphenolic contents and antioxidant activities of the fractions were evaluated by measuring their abilities to inhibit lipid peroxidation induced by Fe³⁺-ascorbate, their reducing powers, and their hydrogen-donor activities.

Results: Three phenolic compounds [vanillic acid (**1**), caffeic acid (**2**) and luteolin (**3**)] were isolated from the roots of *T. gracilens* Dahlst. The ethyl acetate fraction from the ethanol extract of *T. gracilens* Dahlst. roots showed the highest antioxidant activity due to its richest phenolic contents, whereas the ethanol extract containing the least phenolics was the weakest in activity.

Conclusion: The fractions have potential to act as antioxidant agents. *T. gracilens* Dahlst. roots were investigated for the first time in literature data in terms of phenolic contents and antioxidant activity.

Keywords: *Taraxacum gracilens* dahlst., Root, Phenolic compounds, Antioxidant activity

INTRODUCTION

Plants of the genus *Taraxacum* F. H. Wigg. are members of the family Asteraceae, Cichorioideae subfamily, Lactuceae tribe. *Taraxacum* species are widely distributed in the warmer temperate zones of the Northern Hemisphere and are cosmopolitan, perennial weeds of the Asteraceae family commonly found in meadows, gardens, roadsides, and uncultivated areas (Schütz, Carle & Schieber, 2006; Martinez et al., 2015). *Taraxacum* species, the name is derived from the Greek words "taraxis" for inflammation and "akeomai" for curative, are known as dandelion (Schütz et al., 2006).

Taraxacum genus has long been used in folk medicine in the form of decoctions and infusions of its roots, leaves, or flowers due to its pharmacological effects as a mild laxative, aperitive, stimulant, tonic, and diuretic (Schütz et al., 2006; Martinez et al., 2015; Stefano, Bruna, Virginia & Giuliano Bonanomi, 2019). Many phytochemicals have been found in the plants of *Taraxacum*

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genus, such as sesquiterpenoids, phytosterols, triterpenoids, flavonoids, phenolic acids, organic acids, inulin, indole alkaloids, coumarins, and lignans (Schütz et al., 2006; Martinez et al., 2015; Hu, 2018).

The aim of this study was to evaluate the phenolic contents and antioxidant activity of the ethanol extract and its chloroform and ethyl acetate fractions from the roots of *T. gracilens* Dahlst. and isolate some of its phenolic compounds.

MATERIAL AND METHODS

Plant material

The plant material was collected from Istanbul in April 2013. A voucher specimen (ISTE 81948) identified by Prof. Dr. Aynur Sari is deposited at the Herbarium of the Faculty of Pharmacy, Istanbul University, Turkey.

Extraction and isolation

The air-dried roots of *Taraxacum gracilens* Dahlst. (540 g) were exhaustively macerated with ethanol (EtOH) at room temperature. After solvent evaporation, the yield of EtOH extract was dissolved in methanol/water (MeOH/H₂O) (1:2), and then successively extracted with chloroform (CHCl₃) and ethyl acetate (AcOEt). The ethyl acetate and chloroform fractions were dried in vacuo yielding 8.0329 g and 8.2133 g, respectively.

The ethyl acetate fraction was separated by silica gel column chromatography using a stepwise gradient of CHCl₃ and MeOH to give 8 fractions. Fraction 1 (0.1511 g) was subjected to preparative thin layer chromatography (prep. TLC) (silica gel; CHCl₃/ MeOH 95:5) and then to column chromatography (Sephadex LH-20; MeOH) to provide pure **1** (4.3 mg). Fraction 7 (0.3847 g) was subjected to column chromatography (Sephadex LH-20; MeOH and Acetone) and then to prep. TLC (silica gel; Toluol/AcOEt/HCOOH 5:4:1) to provide pure **2** (10.9 mg) and **3** (6.9 mg), respectively.

Antioxidant activity

Antioxidant activity of the ethanol extract, ethyl acetate, and chloroform fractions from the ethanol extract of *T. gracilens* roots was assayed by 4 different methods: total phenols assay by Folin-Ciocalteu (FCR) (Slinkard & Singleton, 1977), ferric ion reducing antioxidant power (FRAP) (Benzie & Strain, 1996), DPPH free radical activity (Brand-Williams, Cuvelier, & Berset, 1995), and thiobarbituric acid test using the lipid peroxidation of liposomes (LPO) (Duh, Tu, & Yen, 1999). The antioxidant activities exhibited by the fractions evaluated by these assays reflect the capacity of fractions to act as electrons or hydrogen atom donors, a necessary requirement for antioxidant function in biological systems.

Statistical analysis

Results were expressed as the mean ± the standard deviation of triplicate analysis. Statistical comparisons were performed using the Student's t-test. Differences were considered significant at $p < 0.05$.

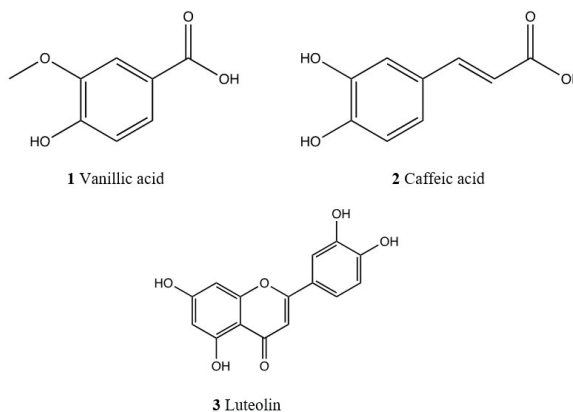
RESULTS AND DISCUSSION

Dandelion extract may be prepared as the whole plant or as different parts, such as roots, leaves, stem, and flowers. The ex-

traction procedure and solvents were properly designed and selected in order for the desired bioactive compounds to be efficiently extracted and the chemical composition of the produced extracts to be identified. The phytochemical research of the *Taraxacum* root extracts reported the presence of phenolic compounds, sesquiterpenes, triterpenes, and phytosterols (Schütz et al., 2006). Among the phenolic compounds, hydroxycinnamic acid derivatives like chlorogenic acid, caffeic acid, ferulic acid, and coumaric acid are generally reported (Williams, Goldstone, & Greenham, 1996; Ivanov, 2014; Kenny, Smyth, Hewage & Brunton, 2014).

Compared to the roots, dandelion leaves and flowers are more enriched in flavonoids (luteolin and its glycoside derivatives, chrysoeriol) and coumarins (cichoriin and aesculin), but hydroxycinnamic acid derivatives (caffeic, chlorogenic, chicoric, and monocaffeoyltartaric acids) were also reported to be present (Williams et al., 1996; Budzianowski 1997).

In this study, phytochemical investigations resulted in the isolation of vanillic acid (**1**), caffeic acid (**2**), and luteolin (**3**) which are new for the species *Taraxacum gracilens* Dahlst. roots. Their structures were established conclusively by ultraviolet visible spectroscopy (UV) and nuclear magnetic resonance (1H-NMR) spectra analyses in comparison with literature data.



All these compounds had already been identified in *T. officinale* and *T. formosanum* root extracts (Leu, Wang, Huang & Shi, 2005; Kenny et al., 2014; Jedrejek, Lis, Rolnik, Stochmal & Olas, 2019). This results are in agreement with a study reported by Kenny, Smyth, Hewage & Brunton (2015) where chlorogenic acid was the most abundant chemical compound in a dandelion root extract, followed by caffeic acid. Also, ferulic, syringic, vanillic, coumaric, and gallic acids were identified. Research about the antioxidant properties of these compounds have revealed strong antioxidant activities (Kenny et al., 2014; Kenny et al., 2015). Therefore, the antioxidant activities of the ethyl acetate extract could be explained by the presence of these compounds.

The highest total phenolic level was detected in the ethyl acetate fraction of the *T. gracilens* root ethanol extract. The lowest total phenolic level was detected in the ethanol extract of the root (Table 1). The order of total phenolic content was established as follow: Ethyl Acetate fraction > Chloroform fraction > Ethanol extract ($p < 0.05$).

Table 1. Total phenolic compounds (PC) (as gallic acid equivalents), total flavonoids (as catechin equivalents), and EC₅₀ values of *T. gracilens* root ethanol extract and the fractions obtained from EtOH extract.

Extract	Anti-LPO EC ₅₀ * (mg/mL)	DPPH EC ₅₀ * (mg/mL)	FRAP** (mM)	Phenol (mg/g extract)	Flavonoid (mg/g extract)
Chloroform fraction	6.36±0.03 ^d	5.04±0.24 ^d	0.76±0.04 ^b	26.14±1.69 ^b	14.33±1.03 ^b
Ethyl acetate fraction	1.28±0.11 ^c	0.83 ±0.14 ^c	3.94±0.13 ^d	122.54±9.28 ^c	110.85±5.59 ^c
Ethanol extract	20.39±0.55 ^e	9.01 ±0.26 ^e	0.49±0.02 ^e	16.85±0.65 ^d	9.33±1.02 ^d
Quercetin	0.059±0.001 ^f	0.034±0.002 ^f	3.24±0.13 ^a	-	-

*EC₅₀ value: The effective concentration at which the anti-LPO activity was 50% and DPPH radicals were scavenged by 50%; EC₅₀ value was obtained by interpolation from linear regression analysis; **Expressed as mM ferrous ions equivalents
Values were the means of three replicates ± standard deviation. Values with different letters in the same column were significantly (p<0.05) different.

From the effective concentrations (EC₅₀) (Table 1), it was seen that among the three fractions selected for the study, the ethyl acetate fraction showed the highest antioxidant activity due to its richest phenolic content. This activity may be contributed to significant ferric reducing power (3.94 mM Fe²⁺/g extract), DPPH radical scavenging activity (EC₅₀=0.83 mg/ml), and inhibition of lipid peroxidation on liposomes (EC₅₀=1.28 mg/ml).

These results are in accordance with the literature data. Kenny et al. (2014) also demonstrated high antioxidant activity in the reducing power and free radical activity assays, which are correlated with a high content of total phenolics of the extracts of *Taraxacum officinale* roots (Kenny et al., 2014).

The vanillic acid and caffeic acid compounds, although mono-nuclear aromatic ones, have *ortho* and *para* OH groups leading to higher activity. The presence of at least one free OH group, attached to either ring A or B, is necessary for the antioxidant activity (Pratt, 1976).

CONCLUSION

The aim of this study was to isolate phenolic compounds from the roots of *T. gracilens* Dahlst. and investigate antioxidant activity of the ethanol extract and the ethyl acetate and chloroform fractions obtained from the ethanol extract of the roots. As a result, luteolin, vanillic acid, and caffeic acid were isolated from *T. gracilens* roots.

It is concluded that the ethyl acetate and chloroform fractions as well as the ethanol extract acted as antioxidant agents i.e. reducing the thiobarbituric acid reactive substances (TBARS) produced from lipid peroxidation and showing the proton and electron-donating ability. So this herbal drug may serve as a dietary source of phenolic substances, which may act as antioxidants for disease prevention and general health promotion through improved nutrition.

T. gracilens Dahlst. roots were investigated for the first time in the literature data in terms of phenolic contents and antioxidant activity.

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Conflict of Interest: The authors have no conflict of interest to declare.

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