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Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A phytochemical study

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ABSTRACT

This study examined the antioxidant and anti-tyrosinase activity and phenolic profiles of three extracts (ethyl acetate, methanol and water) obtained from different anatomical parts (bulb, stem and seed) of *Ornithogalum narbonense*. Antioxidant activity of the extracts was evaluated using DPPH*, ABTS+*, FRAP, CUPRAC, metal chelating and phosphomolybdenum assays. Tyrosinase inhibitory activity was measured using the modified dopachrome method with L-DOPA as the substrate. Phenolic content of the extracts varied according to plant part and extraction solvent and included HPLC–DAD, epicatechin, rutin, ferulic, protocateuchic *p*-hydroxybenzoic, chlorogenic, caffeic, benzoic and rosmarinic acids. The ethyl acetate extract of *O. narbonense* bulb samples demonstrated the most antioxidant and anti-tyrosinase activity and had the highest phenolic content. All other extracts showed moderate antioxidant and anti-tyrosinase activity. *O. narbonense* extracts, especially ethanol extract of *O. narbonense* bulbs, may have dietary and medicinal applications.

1. Introduction

Plant-based products – including foods, pharmaceuticals and dietary supplements – capable of protecting humans against oxidative damage and disease have recently attracted worldwide interest (Huang et al., 2005). Oxidative stress results from an imbalance between the body's production of free radicals and its ability to counteract their harmful effects through antioxidants, which neutralize free radicals by either providing the extra electrons needed to complete them or by breaking them down to render them harmless. Numerous studies have shown antioxidants to reduce the risk of chronic and degenerative diseases such as cancer and heart disease by reducing oxidative damage (Halliwell and Gutteridge, 1984).

The high levels of antioxidant activity of plant-derived natural chemical substances, especially phenolic compounds, have led to their use in the food and pharmaceutical industries. Apart from their antioxidative properties, plant-based materials may be valued for their antimicrobial, antiviral and anti-inflammatory activities (Rice-Evans et al., 1997), and those substances that have been shown to inhibit pharmaceutical enzymes such as tyrosinase are of particular interest for their potential nutritional, nutraceutical and pharmaceutical applications (Shi et al., 2005; Farhoosh et al., 2008).

Ornithogalum is a genus comprised of 150 bulbous species belonging to the Asparagaceae family that are distributed throughout temperate climates in Europe, Asia and Africa (Bryan, 1989). In Turkey, the genus is represented by 36 species, some of which, such as *Ornithogalum* narbonense and *Ornithogalum sigmoideum*, are used as foodstuffs (Kızılarslan and Özhatay, 2012; Dogan et al., 2004). Other *Ornithogalum* species have important medicinal properties; for example, *Ornithogalum thyrosides* and *Ornithogalum caudatum* bulbs are known to possess antimicrobial, cytotoxic, anticarcenogenic and antioxidant properties stemming from the presence of steroidal gylcosides and monoterpenes (Kuroda et al., 2002; Bai et al., 2005).

However, to the best of our knowledge, there are no reports in the literature investigating the phytochemical composition and biological activities of *O. narbonense*. Therefore, the present study examined the pharmacological properties of *O. narbonense* in terms of the antioxidant and tyrosinase-inhibitory potential of three extracts (ethyl acetate, methanol and water) obtained from different anatomical parts (bulb, stem and seed) of *O. narbonense* and developed phenolic profiles of these extracts using HPLC–DAD.

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Table 1
Abbreviations, extraction yield, total phenolic content, DPPH and ABTS scavenging activities of *O. narbonense* extracts (values expressed are means ± S.D. of three parallel measurements)*.

Plantparts	Solvent	Yield (%)	Total phenolic content (mg GAEs/g extract)[a]	DPPH scavenging activity (mg TEs/g extract)[b]	ABTS scavenging activity (mg TEs/g extract)[b]	Abbreviation
BULB	Ethyl acetate	2.03	21.05 ± 0.33a	18.16 ± 0.09a	30.32 ± 1.62a	Bulb-EA
	Methanol	8.98	10.33 ± 0.26d	12.60 ± 0.30c	18.16 ± 1.21b	Bulb-Met
	Water	20.69	4.79 ± 0.29f	4.02 ± 0.55f	7.52 ± 0.64c	Bulb-W
STEM	Ethyl acetate	1.27	14.59 ± 0.20b	16.80 ± 0.18b	19.24 ± 0.51b	St-EA
	Methanol	5.62	12.49 ± 0.19c	16.56 ± 0.27b	20.24 ± 1.02b	St-Met
	Water	10.60	8.11 ± 0.06e	9.51 ± 0.58d	18.51 ± 0.25b	St-W
SEED	Ethyl acetate	1.83	12.91 ± 0.76c	3.66 ± 0.43f	0.88 ± 0.65d	S-EA
	Methanol	6.71	9.77 ± 0.30d	7.03 ± 0.54e	7.59 ± 1.23c	S-Met
	Water	12.91	4.48 ± 0.30f	3.73 ± 0.38f	8.78 ± 1.89c	S-W

* Different letters in the same column indicate significant difference ($p < 0.05$).
[a] GAEs – gallic acid equivalents.
[b] TEs – trolox equivalents.

2. Materials and methods

2.1. Plant material

The herbal parts of *O. narbonense* L. was collected from Bingol village, (1380 m, 39°43′38″N, 37°06′08″E) Sivas – Turkey when the end of flowering season (June 2013). Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr. Murad Aydın Sanda, from the Department of Biology, Selcuk University. The voucher specimen was deposited at the KNYA Herbarium of Department of Biology, Selcuk University, Konya – Turkey. The plant materials (stem, bulb and seed) were carefully separated and dried at the room temperature.

2.2. Preparation of the solvent extracts

To produce solvent extracts, the parts (5 g) of *O. narbonense* were macerated with 100 mL of solvent with ethyl acetate or methanol at room temperature for 24 h. Ethyl acetate and methanol were then removed with a rotary evaporator. For water extract, the air-dried samples (5 g) were extracted by boiling deionized water (100 mL) for 15 min. The water extract was freeze–dried. All extracts were stored at +4 °C until analyzed. The yields of solvent extracts from different parts of *O. narbonense* are shown in Table 1.

2.3. Quantification of phenolic compounds by RP-HPLC

Phenolic compounds were evaluated by reversed-phase high-performance liquid chromatography (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan). Detection and quantification were carried out with a LC-10ADvp pump, a diode array detector, a CTO-10Avp column heater, SCL-10Avp system controller, DGU-14A degasser and SIL-10ADvp auto sampler (Shimadzu Scientific Instruments, Columbia, MD). Separations were conducted at 30 °C on Agilent® Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 μm particle size). The eluates were detected at 278 nm. The mobile phases were (A): 3.0% acetic acid in distilled water and (B): methanol. For analysis, the samples were dissolved in methanol, and 20 μL of this solution was injected into the column. The elution gradient applied at a flow rate of 0.8 mL/min was: 93% A/7% B for 0.1 min, 72% A/28% B in 20 min, 75% A/25% B in 8 min, 70% A/30% B in 7 min and same gradient for 15 min, 67% A/33% B in 10 min, 58% A/42% B in 2 min, 50% A/50% B in 8 min, 30% A/70% B in 3 min, 20% A/80% B in 2 min B in 5 min until the end of the run. Phenolic compositions of the extracts were determined by a modified method of Caponio et al. (1999). Protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (−)-epicatechin, ferulic acid, benzoic acid, rutin, rosmarinic acid and apigenin were used as standard. Peaks identified by comparing retention times and UV spectra with authentic standards. Concentrations of individual phenolic compounds were determined based on peak areas and calibration curves derived from corresponding authentic phenolic compounds. The amount of each phenolic compound was expressed as μg per gram of the extract.

2.4. Total phenolic content

The total phenolic content was determined by employing the methods given in the literature (Slinkard and Singleton, 1977), with slight modification. Sample solution (0.25 mL) was mixed with diluted Folin–Ciocalteu reagent (1 mL, 1:9) and shaken vigorously. After 3 min, Na_2CO_3 solution (0.75 mL, 1%) was added and the sample absorbance was read at 760 nm after a 2 h incubation at room temperature. The total phenolic content was expressed as gallic acid equivalent (GAE/g extract).

2.5. Total antioxidant activity by phosphomolybdenum method

The total antioxidant activity of the samples was evaluated by phosphomolybdenum method according to Zengin et al. (2014) with slight modification. Sample solution (0.3 mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm after a 90 min incubation at 95 °C. The total antioxidant capacity was expressed as Trolox equivalent (TE/g extract).

2.6. Radical scavenging activity

2.6.1. DPPH* radical scavenging activity

The effect of the samples on 1,1-diphenyl-2-picrylhydrazyl (DPPH*) radical was estimated according to Sarikurkcu (2011). Sample solution (1 mL) was added to a 4 mL of a 0.004% methanol solution of DPPH*. The sample absorbance was read at 517 nm after a 30 min incubation at room temperature in dark. DPPH* radical scavenging activity was expressed as Trolox equivalent (TE/g extract).

2.6.2. ABTS cation radical scavenging activity

The scavenging activity aganist ABTS cation (2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid) radical was measured according to the method of Re et al. (1999) with slight modification. Briefly, ABTS+* radical cation was produced directly by reacting 7 mM ABTS+* solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12–16 h in dark at the room