Original Article

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Phenolic composition of *Anchusa undulata* L. subsp. *hybrida* (Ten.) Coutinho from Turkey

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ABSTRACT

Phenolic compounds are a major class of plant secondary metabolites. These compounds represent an important component of human diets and exhibit a wide range of biological effects, including antioxidant, anti-mutagenic, and anti-carcinogenic. To date, no study has been reported on the phenolic composition of *Anchusa undulata* subsp. *hybrida*. In this study, the phenolic constituents from the methanolic extract of *A. undulata* subsp. *hybrida* were analyzed by high-performance liquid chromatography-diode array detector. Thirteen compounds were identified. The major components include rosmarinic acid, benzoic acid, and rutin. Present study showed that *A. undulata* subsp. *hybrida* can be considered as good source of these components for food and drug applications.

KEY WORDS: *Anchusa, Boraginaceae*, phenolic component, rosmarinic acid

INTRODUCTION

The genus *Anchusa* (*Boraginaceae*) consists of about 170 taxa native in worldwide (Selvi and Bigazzi, 2003). In Turkey, 15 species are classified in the genus (Davis, 1988). Some *Anchusa* members are used in different countries (including Turkey) for several ailments (wound healing, diuretic agent, demulcent, analgesic, sedative, and hypotensive). Several chemical studies were performed on *Anchusa* species, which possess some triterpene glycosides and flavonoids (Ozcan, 2008). Nevertheless, there is only one report on antioxidant, enzyme inhibitory effect, and the fatty acid contents of the seed of *Anchusa undulata* subsp. *hybrida* in the literature (Sarikurkcu *et al*., 2015). However, no report is available for phenolic components of *A. undulata* subsp. *hybrida*. Therefore, data presented here will be the first record on *A. undulata* subsp. *hybrida*. In the present work, therefore, the aim was to evaluate phenolic components of *A. undulata* subsp. *hybrida*.

MATERIALS AND METHODS

Plant Material

A. undulata L. subsp. *hybrida* (Ten.) Coutinho plant was collected in 2012 from Mugla University Campus, Mugla-Turkey at its flowering season. Taxonomic identification of the plant material was confirmed by the senior taxonomist Dr. Olcay Ceylan, in Department of Biology, Mugla University. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Mugla University, Mugla-Turkey (Voucher No: B6055).

Preparation of the Methanol Extract

The air-dried samples (20 g) were extracted by using a Soxhlet extractor for 5 h with 250 ml of methanol under reflux conditions. Methanol was removed with a rotary evaporator to obtain an extract in the yield of 12.81% (w/w).

Quantification of Phenolic Compounds by Reversed-phase High-performance Liquid Chromatography (RP-HPLC)

Phenolic compounds were evaluated by RP-HPLC (RP-HPLC, Shimadzu Scientific Instruments, Kyoto, 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 autosampler (Shimadzu Scientific Instruments, Columbia, MD, USA). Separations were conducted at 30°C on Eclipse XDB C-18 reversed-phase column (250 mm × 4.6 mm length, 5 µm particle size, Agilent, Santa Clara, CA, USA). 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 were 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 100% 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). Gallic acid, protocatechuic acid, (+)-catechin, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, (−)-epicatechin, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, sinapinic acid, benzoic acid, *o*-coumaric acid, rutin, naringin, hesperidin, rosmarinic acid, eriodictyol, *trans*-cinnamic acid, quercetin, naringenin, luteolin, kaempferol, and apigenin were used as standards. Identification and quantitative analysis were done by comparison with standards. The amount of each phenolic compound was expressed as mg per gram of extract using external calibration curves, which were obtained for each phenolic standard.

RESULTS AND DISCUSSION

HPLC analysis of the phenolic components from the methanolic extract of *A. undulata* subsp. *hybdrida* aerial parts enabled the identification of 13 compounds. However, 8 constituents (gallic acid, chlorogenic acid, epicatechin, syringic acid, naringin, hesperidin, naringenin, kaempferol) were not detected. The name and amount of each constituent are listed in Table 1. Among the identified constituents, rosmarinic acid (5.48 mg/g extract), benzoic acid (3.60 mg/g extract), rutin (2.57 mg/g extract), and catechin (1.25 mg/g extract) were determined as major components in the tested extract [Figure 1]. Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Wang *et al*., 2004). This is commonly found in species of the *Boraginaceae*. This component exhibits a broad spectrum of biological activities. Different studies have shown that antioxidant, anti-microbial, antiviral, anti-carcinogenic, and anti-allergic properties of rosmarinic acid (Parnham and Kesselring 1985; Hooker *et al*., 2001; Huang and Zheng 2006). In this sense, finding new plants containing a high amount of rosmarinic acid is very important to introduce as novel bioactive agents. Thus, *A. undulata* subsp. *hybrida* may be considered as a new source of rosmarinic acid for food, cosmetics, and pharmaceutical industries. Again, benzoic acid have an anti-microbial potential, thus, is widely used as food and cosmetic preservatives (Borawska *et al*., 2008). Rutin is a common dietary flavonoid and exhibit important pharmacological activities, including anti-oxidation, anti-inflammation, and anti-diabetic (Baldisserotto *et al*., 2015). In this sense, *A. undulata* subsp.

Table 1: Phenolic constituents of the methanolic extract from *A. undulata* subsp. *hybdrida*

Phenolic components	(mg/g extract)
Gallic acid	nd
Protocatechuic acid	0.22±0.004
Catechin	1.25±0.06
p-hydroxybenzoic acid	0.84±0.01
Chlorogenic acid	nd
Caffeic acid	0.49±0.01
Epicatechin	nd
Syringic acid	nd
Vanillin	0.06±0.003
p-coumaric acid	0.08±0.002
Ferulic acid	0.04±0.001
Benzoic acid	3.60±0.05
Rutin	2.57±0.03
Naringin	nd
Hesperidin	nd
Rosmarinic acid	5.48±0.09
Cinnamic acid	0.04±0.001
Quercetin	0.08±0.005
Naringenin	nd
Kaempferol	nd
Apigenin	0.01±0.002

nd: Not determined

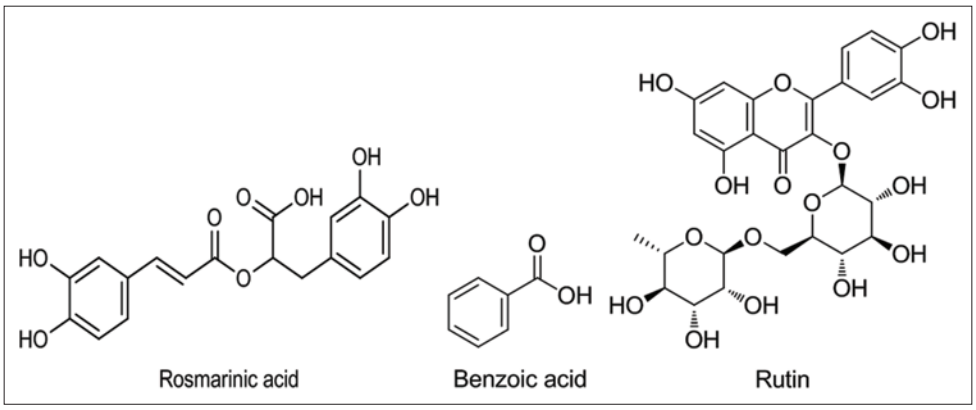

Figure 1: Major components of the methanolic extract from *A. undulata* subsp. *hybdrida*

hybrida can be considered as a source of biologically active compounds. Again, new biologically active compounds were reported for *Anchusa strigosa* by Braca *et al*. (2003).

CONCLUSION

The present report is the first comprehensive study of the polyphenolic content of methanolic extracts of *A. undulata* subsp. *hybrida*. This study indicated that the methanolic of *A. undulata* subsp. *hybrida* might be regarded as biologically active compounds, including rosmarinic acid, benzoic acid, and rutin.

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