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Quantitative Analysis of Bioactive Compounds by High-Performance Liquid Chromatography in *Origanum bilgeri*

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ABSTRACT

Origanum genus is an aromatic and medicinal plant with significant bioactive compounds. *Origanum* species have been used as a folk medicine for years. In this study, the aerial part of *Origanum bilgeri* P.H. Davis was extracted with hexane and methanol successively, the methanol extract was used for quantitative analysis of bioactive compounds by High-Performance Liquid Chromatography. Rosmarinic acid was found as a major product (26.61 mg/g extract). The other major compounds were detected as catechin hydride (5.65 mg/g extract), trans-cinnamic acid (2.65 mg/g extract), and resveratrol (1.33 mg/g extract). Consequently, *Origanum bilgeri* may be a valuable material for drug development and food.

Key words: *Origanum bilgeri*, Natural products, Quantitative analysis

Yüksek Performanslı Sıvı Kromatografisi ile *Origanum bilgeri*'deki Biyoaktif Bileşiklerin Kantitatif Analizi

ÖZET

Origanum cinsi önemli biyoaktif bileşikler içeren aromatik ve tıbbi bir bitkidir. *Origanum* türleri yıllardır halk ilacı olarak kullanılmaktadır. Bu çalışmada, *Origanum bilgeri* P.H. Davis sırasıyla heksan ve metanol ile ekstrakte edildi, metanol ekstraktı, Yüksek Performanslı Sıvı Kromatografisi ile biyoaktif bileşiklerin kantitatif analizi için kullanıldı. Ana ürün olarak rosmarinik asit bulundu (26.61 mg/g ekstrakt). Diğer ana bileşikler kateşin hidrit (5.65 mg/g ekstrakt), trans-sinnamik asit (2.65 mg/g ekstrakt), resveratrol (1.33 mg/g ekstrakt) olarak tespit edildi. Sonuç olarak, *Origanum bilgeri* ilaç geliştirme ve gıda için değerli bir materyal olabilme potansiyeline sahiptir.

Anahtar kelimeler: *Origanum bilgeri*, Doğal ürünler, Kantitatif analiz

INTRODUCTION

Medicinal plants have been used effectively for traditional medicine since ancient times due to their bioactive compound contents (Demirtas et al., 2013; Elmastas et al., 2004; Topçu et al., 1999). *Origanum* genus belonging to the Lamiaceae family contains 43 species and 18 hybrids (Erenler, Adak, et al., 2017). These species are medicinal plants consisting of significant secondary metabolites and essential oils employed in the pharmaceutical, cosmetic, and food industries (Erenler et al., 2016). These species have been used in folk medicine to relieve conditions such as stomachache, bronchitis, indigestion, inflammatory diseases, asthma, and diabetes (Erenler, Meral, et al., 2017). The benefits of *Origanum* species on human health have been attributed to their bioactive compound content (Elmastas et al., 2018). In the last decade, there has been a growing trend toward the use of aromatic herbs, including *Origanum*, as feed additives in animal nutrition (Franz et al., 2010). Growth-promoting antibiotics have been banned in the European Union since 2006, and natural products have been effectively consumed as feed additives (Elmastaş et al., 2015; Phillips, 2007). In addition, due to the strong antioxidant effect of *Origanum* species, it has started to be used in foods instead of synthetic antioxidants (Zhang et al., 2014). In contrast, spices have been used in traditional cooking for many years and there is no concern about their safety (Erenler et al., 2015). In addition, since these spices are widely consumed in most countries, there is no legal obstacle to their use in food production. Phytochemical studies have shown that *Origanum* species contain flavonoids, and phenolic acids (Erenler et al., 2018). Scientific research shows that the basic physiological function of phytochemicals is to serve plants as a plant defense mechanism against pathogens, pests, herbivores, UV light, and oxidative stress (Erenler et al., 2016). The compound content in plants depends on various factors such as cultivar, weather, soil condition, climate, water stress, and harvesting time (Türkmen et al., 2014). Due to the biological activity of flavonoids and phenolic acids, identification, and quantification of these compounds in *Origanum bilgeri* are important (Karan et al., 2022; Saidi et al., 2022). According to reports from various *Origanum* species, flavones are the most abundant subgroup of flavonoids, followed by flavonols, flavanones, and flavanols. The most common phenolic acids found in *Origanum* species include hydroxycinnamic and hydroxybenzoic acid derivatives, as well as other phenolics. Rosmarinic acid, apigenin, luteolin, quercetin, scutellarein, and their derivatives have been identified as the major flavonoids and phenolic acids found in *Origanum* species (Lin et al., 2007).

Flavonoids and phenolic acids are found in leaves, flowers, and stems of plants and protect plants against pathogens, insect attack, UV radiation, and injury (Karan, Altuner, et al., 2017). Phenolics help plants adapt to the environment and survive (Karan & Erenler, 2017). Phenolic acids act as starting compounds for stilbenes, chalcones, flavonoids, lignans, and anthocyanins. On the contrary, flavonoids have various functions in plants such as pigmentation, stimulation of nitrogen-fixing nodules, growth regulation, UV protection, and disease resistance (Kumar et al., 2013).

Material and Methods

Plant Material and Extraction

Origanum bilgeri was cultivated in the Aromatic and Medicinal Plant Field of Tokat Gaziosmanpasa University.

Extraction

The plant material (30 g) was extracted with hexane (200 mL). After filtration, the solvent was separated, and the plant material was re-extracted with methanol (200 mL). The mixture was filtrated, and the solvent was removed by a rotary evaporator to yield the crude extract.

Quantitative Analysis of Bioactive Compounds

Quantitative analysis of bioactive compounds was carried out by High-Performance liquid chromatography (HPLC, Agilent 1260 infinity) with ACE Generix, 4.6 mm × 250 mm, 5 µm column. Diode-Array Detection (DAD) detector was used for compound detection, the injection volume was adjusted to 10 µL and the flow rate was set to 0.8 mL/min. The gradient system was adjusted as A: 0.1% phosphoric acid in water and B: acetonitrile. The gradient program was adjusted as: 0 min, 80% A; 0–5 min, 75% A; 6–10 min, 65% A; 11–20 min, 60% A; 21–30 min, 55% A; 31–35 min, 50% A; 36–45 min, 45% A; 46–50 min.

RESULTS and DISCUSSION

Due to the importance of bioactive compounds in plants, qualitative and quantitative analysis of corresponding compounds in the plant serve for the development of drugs. In this study, quantitative analysis of flavonoids and phenolic acids was determined by HPLC analysis. Rosmarinic acid was determined as a major product (26.61 mg/g extract). Therefore, *Origanum bilgeri* has been identified as an important source of rosmarinic acid. The other major compounds were detected as catechin hydride (5.65 mg/g extract), trans-cinnamic acid (2.65 mg/g extract), resveratrol (1.33 mg/g extract) as well as minor products, chlorogenic

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acid (0.33 mg/g extract), rutin (0.52 mg/g extract), naringenin (0.36 mg/g extract), o-coumaric acid (0.51 mg/g extract), quercetin (0.06 mg/g extract) (Table).

These compounds were reported to display significant biological activities and they have been used in drug formulations. Rosmarinic acid is a caffeic acid and 3,4-dihydroxyphenyllactic acid ester. Rosmarinic acid was reported to have significant biological activities including antioxidative, antimutagenic, astringent, anti-inflammatory, antibacterial, and antiviral properties (Petersen et al., 2003). Cinnamic acid and its phenolic analogs are natural substances. Chemically, the 3-phenyl acrylic acid functionality in cinnamic acids provides three main reactive sites: substitution at the phenyl ring, addition at the unsaturation, and carboxylic acid functionality reactions. Because of these chemical properties, cinnamic acid derivatives have received considerable attention in medical research Cinnamic acid derivatives have been reported to be used in the treatment of cancer, bacterial infections, diabetes, and neurological disorders, among other things (De et al., 2011).

Table Quantitative analysis of compounds in *Origanum bilgeri* (mg/g extract)

Compounds	Quantity
Chlorogenic acid	0.33
Catechin hydride	5.65
Caffeic acid	nd
4-Hydroxybenzoic acid	0.30
Vanillin	nd
p-Coumaric acid	nd
Rutin	0.52
Ferulic acid	0.05
Hydroxy cinnamic acid	0.07
Naringin	0.36
o-Coumaric acid	0.51
Rosmarinic acid	26.61
Salicylic acid	0.08
Resveratrol	1.33
Quercetin	0.06
trans-Cinnamic acid	2.65
Naringenin	0.24
Chrysin	nd
Flavone	0.09

nd: not detected

Conclusion

Origanum bilgeri contains bioactive compounds displaying considerable biological activities. Moreover, rosmarinic acid is the major compound in this plant. Hence, *O. bilgeri* can be an effective agent for the pharmaceutical and food industries. In addition, this plant may be a good source of rosmarinic acid to isolate the corresponding compound. This plant should be cultivated, and a stress study should be carried out to increase the quantity of rosmarinic acid as well as other bioactive compounds.

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