

Phenolic compounds from section *Majorana* (Mill.) Benth of *Origanum* L. species extracts via validated LC-MS/MS method

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Abstract: *Origanum majorana* L., (*OM*), *O. onites* L. (*OO*) and *O. syriacum* L. (subsp. *bevanii* (Holmes) Greuter & Burdet) (*OS*) are belong to *Majorana* (Mill.) Benth. section of *Origanum* L. genus. In this study, quantities of some phenolic compounds; gallic acid, syringic acid, *p*-hydroxy benzoic acid, caffeic acid, (*E*)-ferulic acid, chlorogenic acid, rosmarinic acid, pyrogallol and vanillin; in the chloroform (C), acetone (Ac) and methanol (M) extracts prepared from *Origanum* species collected from Türkiye were investigated via liquid chromatography and tandem mass spectrometry (LC-MS/MS). The relative standard deviations (RSD) of the phenolics were found as follows; 4.85, 8.39, 4.78, 8.04, 5.21, 5.45, 3.73, 5.47, respectively. Curcumin was used as an internal standard. In the extracts, (*E*)-ferulic acid and caffeic acid were found to be the most abundant compounds. *O. syriacum* was determined as the richest species in terms of phenolic acids.

Keywords: *Origanum*; section *Majorana* (Mill.) Benth.; *Origanum majorana* L.; *O. onites* L.; *O. syriacum* L. (subsp. *bevanii* (Holmes) Greuter & Burdet); LC-MS/MS. © 2022 ACG Publications. All rights reserved.

1. Sample Source

As a continuation of our work on *Origanum* species [1-7] section *Majorana* (Mill.) Benth., were studied. This section consists of three species distributed in Mediterranean basin countries *Origanum majorana* L., *O. onites* L. and *O. syriacum* L. (subsp. *bevanii* (Holmes) Greuter & Burdet) were studied. Localities, coordinates, herbarium numbers of the voucher species are given in the supporting information (Table S1). The species were identified by Dr. Tuncay Dirmenci at Balıkesir University.

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2. Previous Studies

Origanum L. is a well-known aromatic genus in the Lamiaceae family and consists of 42 species (49 taxa) and 22 hybrids in the world [8-9]. The genus *Origanum* divided into ten sections and species of eight sections are distributed in Türkiye. Therefore, Türkiye and East Aegean Islands are important centers of diversity for the whole genus and Türkiye has 21 species with 3 subspecies (24 taxa) and 13 hybrids. Among them, 25 taxa are endemic. Due to the valuable components they contain, the genus of *Origanum* has been the subject of many studies [1,2].

The members of Section *Majorana* of *Origanum* are widely preferred by people to use in folk medicine, or in daily consumption as a food additive [10, 11]. *O. onites* is cultivated in a wide area in Türkiye. It is also collected from nature. The total amount obtained from the cultivated areas is around 15 thousand tons. Around 86% of this production is provided from the agricultural areas in Denizli province [12, 13]. An important part of the spice known as *Oregano* in the world originates from *O. onites*. *O. majorana* (cultivated in small areas) and *O. syriacum* subsp. *Bevanii* are mostly collected from nature and about 50-100 tons in Turkey [12-14].

Having many advantages over traditional analytical techniques such as short analysis time, high specificity, and sensitivity, the liquid chromatography (LC)–mass spectrometry (MS) method has become a popular method to determine the secondary metabolite in plant extracts [4-7, 15-18]. In our previous study, we reported a new validated LC-MS/MS method for quantification of phenolics of some plant extracts including *Origanum species* [3-7] and phenolic acids such as rosmarinic acid, caffeic acid and gallic acid are mainly found in *Origanum* extracts [2].

3. Present study

Aerial part of the all the plant samples were collected after the flowering period in 2013 from Southern part of Anatolia (Table 1 in supporting information), then plant samples were air-dried. Approximately 100 g of powdered aerial parts of the plant were extracted with chloroform (C), acetone (Ac), and methanol (M), respectively. The crude extracts were filtered and evaporated after the extraction. LC-MS experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Türkiye) mass spectrometry equipped with a Troyasil C18 (Istanbul Türkiye) HPLC column (150 x 3 mm i.d., particle size: 3mm). The optimum ESI parameters were determined according to previous studies in the literature [3-7, 15-22]. Curcumin (97 %, in 100 mg/L concentration) was used as an internal standard for the quantification of phenolics in the samples. For LC-MS/MS analysis standards of phenolics were bought from Merck or Sigma Aldrich. Stock and working solutions were prepared by using HPLC grade methanol and water (see supporting information S1).

The LC-MS/MS parameters and amount of the substances as mg/kg of the reported compounds in *Majorana* Section of *Origanum* extracts are given in Table S2, and Table 1, respectively. Method validation and uncertainty calculations were performed based on EURACHEM Guide and NIST documents [23, 24]. Table 2 Summarized the LOD (limit of detection) and LOQ (limit of quantification) values of the LC-MS/MS methods which were calculated to be 0.5-50 mg/L, and the LODs and LOQs were determined to be 3 and 10 times bigger than standard deviation, respectively. Detailed procedure was given in the supplementary material.

The quantities of seven phenolic acids (gallic acid, syringic acid, p-hydroxybenzoic acid, caffeic acid, (*E*)-ferulic acid, chlorogenic acid, rosmarinic acid) and two simple phenolics (pyrogallol and vanillin) were determine in the extracts. *O. syriacum* was found to be as rich in phenolic acids. Acetone and methanol extracts of the species contained more components, while chloroform was found to be lower. While the main component was determined as (*E*)-ferulic acid in the range of 10.09-347.18 mg/kg for the chloroform and methanol extracts of all three species and the acetone extract of the OO species, this component was determined as caffeic acid for the AC extract of OS (1215.83±240.60 mg/kg) and OM (253.06±50.08 mg/kg) species. The results are given in Table 1. The LC-MS/MS chromatogram of the determined phenolics are given Figure S1 (see supporting information)

In the present study, quantities of some phenolics mainly have phenolic acid structures were characterized via LC-MS/MS. Plants are important phenolic sources which have many bioactivities such as antioxidant, antimicrobial, anti-inflammatory and it is widely consumed among the people for many purposes. The use of experiments and parameters of validation and linearity of the LC-MS/MS method can provide an effective and easy way to determine the amounts of secondary metabolites, which are the greatest richness of plants. In conclusion, this study determined the phenolic acids of the three most important *Origanum* species known as “*Marjoram*”, which are very important both in terms of commercial use and production in Türkiye and in the world.

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Supporting Information

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