Vrije Universiteit Brussel

# UPLC-PDA-ESI-QTOF-MS/MS and GC-MS analysis of Iranian Dracocephalum moldavica L

Fattahi, Azin; Shakeri, Abolfazl; Tayarani-Najaran, Zahra; Kharbach, Mourad; Segers, Karen; Heyden, Yvan Vander; Taghizadeh, Seyedeh Faezeh; Rahmani, Hanieh; Asili, Javad

*Published in:*
Food Science and Nutrition

*DOI:*
10.1002/fsn3.2396

*Publication date:*
2021

*License:*
CC BY

*Document Version:*
Final published version

Link to publication

*Citation for published version (APA):*
Fattahi, A., Shakeri, A., Tayarani-Najaran, Z., Kharbach, M., Segers, K., Heyden, Y. V., Taghizadeh, S. F., Rahmani, H., & Asili, J. (2021). UPLC-PDA-ESI-QTOF-MS/MS and GC-MS analysis of Iranian Dracocephalum moldavica L. *Food Science and Nutrition*, *9*(8), 4278-4286. https://doi.org/10.1002/fsn3.2396

**Copyright**
No part of this publication may be reproduced or transmitted in any form, without the prior written permission of the author(s) or other rights holders to whom publication rights have been transferred, unless permitted by a license attached to the publication (a Creative Commons license or other), or unless exceptions to copyright law apply.

**Take down policy**
If you believe that this document infringes your copyright or other rights, please contact openaccess@vub.be, with details of the nature of the infringement. We will investigate the claim and if justified, we will take the appropriate steps.

Download date: 17. Apr. 2024

Check for updates

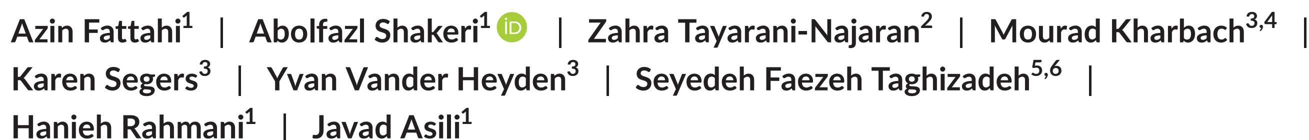
Received: 24 December 2020 | Revised: 28 April 2021 | Accepted: 23 May 2021

DOI: 10.1002/fsn3.2396

Food Science & Nutrition Open Access WILEY

ORIGINAL RESEARCH

# UPLC–PDA-ESI–QTOF–MS/MS and GC-MS analysis of Iranian *Dracocephalum moldavica* L.



**Azin Fattahi**[1] | **Abolfazl Shakeri**[1] | **Zahra Tayarani-Najaran**[2] | **Mourad Kharbach**[3,4] | **Karen Segers**[3] | **Yvan Vander Heyden**[3] | **Seyedeh Faezeh Taghizadeh**[5,6] | **Hanieh Rahmani**[1] | **Javad Asili**[1]

[1]Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling, Center for Pharmaceutical Research (CePhaR), Vrije Universiteit Brussel (VUB), Brussels, Belgium

[4]Biopharmaceutical and Toxicological Analysis Research Team, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V-Rabat, Morocco

[5]Department of Horticultural Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

[6]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

**Correspondence**

Javad Asili, Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: asilij@mums.ac.ir

**Funding information**

Mashhad University of Medical Sciences (Mashhad, Iran) Research Council, Grant/Award Number: 960960

## Abstract

*Dracocephalum moldavica* L. is a significant component in the Iranian food basket. This study aimed to investigate the bioactive compounds and biological activities of different extracts obtained from *D. moldavica* aerial parts. From the aerial parts, a crude methanolic (MeOH) extract and its four sub-fractions, that is, petroleum ether (Pet), ethyl acetate (EtOAc), n-butanol (n-BuOH), and aqueous (water) extracts were obtained. The total phenolic and flavonoid contents as well as the antioxidant and cytotoxic activities of the extracts were determined. Moreover, the phytochemical profiles of the essential oil (EO) and of those extracts with the highest antioxidant activity measured by GC/MS and UPLC–PDA-ESI–QTOF–MS/MS. Results showed that the highest concentrations of phenols and flavonoids as well as the most potent antioxidant potential according to the DPPH method were determined in the EtOAc and MeOH extracts with $IC_{50}$ values of 22.0 and 34.4 µg.ml$^{-1}$, respectively. Quantitative analysis of these extracts was subsequently performed by UPLC–PDA-ESI–QTOF–MS/MS. Both extracts contained mainly rosmarinic acid, caffeic acid, and 2-hydroxycinnamic acid, which may be responsible for their high antioxidant activity. Moreover, none of the extracts showed cytotoxic effects against MCF7, SW48, and a normal cell line of mouse embryonic fibroblast cells (NIH/3T3) in the tested concentrations (up to 400 µg.ml$^{-1}$). Additionally, GC-MS analysis showed that oxygenated monoterpenes (55.4%) were the main constituents of the EO of *D. moldavica*.

**KEYWORDS**

antioxidant activity, cytotoxic activity, *Dracocephalum moldavica*, essential oil, GC-MS, UPLC-MS

Azin Fattahi and Abolfazl Shakeri contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2021 The Authors. *Food Science & Nutrition* published by Wiley Periodicals LLC

## 1 | INTRODUCTION

The daily intake of sufficient vegetables has an important role in preventing several diseases (Barends et al., 2019). *D. moldavica* (Moldavian balm) is a common edible vegetable used daily for the preparation of many Iranian dishes. It belongs to the Lamiaceae family, is up to 80 cm tall, and is native to central Asia (Yousefzadeh et al., 2018). *D. moldavica* preparations are used in food and in pharmaceutical industries as food additive, tea, and herbal remedy. Traditionally, the plant is applied as analgesic, anticonvulsive, anti-inflammatory, sedative, wound healing, and in the treatment of cardiovascular disorders (Yousefzadeh et al., 2013). In the Mexican traditional medicine, it is used for the treatment of nervous diseases (Martinez-Vazquez et al., 2012), while in traditional Chinese medicine (TCM), it is mainly used in the treatment of liver disorders, headache, stomach problems, and congestion (Jiang et al., 2014). Furthermore, in TCM in a clinical trial the aqueous extract of *D. moldavica* was shown to be effective in the treatment of cardiovascular disease, asthma, fatigue insomnia, and neurasthenia (N. Yu et al., 2015).

Phytochemical investigations on the aerial parts of *D. moldavica* have demonstrated the presence of several bioactive compounds, including terpenoids, phenolic compounds (rosmarinic and caffeic acid derivatives), flavonoids (kaempferol, quercetin, esculetin, diosmetin, acacetin, apigenin, luteolin, cirsimaritin, salvigenin, santa flavone, agastachoside, and their glycosides), alkaloids, iridoids, and coumarins (Sultan et al., 2008; Yang et al., 2014; Zeng et al., 2010). Phenolic compounds, especially phenolic acid derivatives, such as rosmarinic and caffeic acids, were associated with the high antioxidant potential of *D. moldavica* (Weremczuk-Jeżyna et al., 2013). Various analytical methods are developed for the identification and quantification of bioactive compounds in medicinal plants. However, in these samples, there are some limitations, including the complexity, the structural diversity, and the low content of bioactive compounds (Adnani et al., 2012). In this regard, the choice of an appropriate technique is important. The application of UPLC-ESI-MS in the identification of natural compounds has attracted much attention because of its high resolution for the separation of complicated samples, analysis speed, sensitivity, selectivity, specificity, and reduced solvent consumption (Chen et al., 2010). As it is a significant component in the Iranian food basket, *D. moldavica* was selected for this study. To the best of our knowledge, there is no comprehensive study on this edible vegetable plant. Therefore, for the comprehensive identification and quantification of the chemical composition of *D. moldavica*, UPLC–DAD-ESI–QTOF–MS/MS was used as a powerful tool for the separation of low molecular weight and nonvolatile samples, and GC/MS for the separation of volatile and thermally stable compounds. As biological activities, we evaluated the antioxidant and cytotoxic abilities of different plant extracts. Our study established a new approach to explore comprehensively the chemical components of *D. moldavica* extracts using UPLC–PDA-ESI–QTOF–MS/MS. The obtained results broaden our knowledge about the structural diversity of the components in Moldavian balm for a better understanding of the possible role of the constituents on biological properties as well as for further research in food and pharmaceutical issues.

## 2 | MATERIAL AND METHODS

### 2.1 | Plant material

*D. moldavica* was purchased from a local market in Mashhad city (Khorasan Razavi province, Northeastern of Iran) in September 2017. The plant material was identified by M. Souzani (Department of Pharmacognosy, Mashhad University of Medical Sciences) and a voucher specimen (10,169) was deposited in the herbarium of the Department of Pharmacognosy, Mashhad University of Medical Sciences.

### 2.2 | Preparation of the extracts

The aerial parts were washed with tap water and dried. For extraction of plant materials, all solvents were purchased from Dr. Mojallali Industrial Chemical Complex Co. 400 g dried material was powdered and macerated in methanol (analytical grade, 99.5%) for 24 hr (3 times, 1 L) at room temperature. The obtained extract was filtered using filter papers (Whatman® No.1, Merck) and the organic solvent concentrated under a vacuum. Then, the entire extract was suspended in water (50 ml) and partitioned with Pet (200 ml), EtOAc (200 ml), and n-BuOH (200 ml), successively. Afterward, the solvents were evaporated under reduced pressure to get the different subfractions. To prepare the EO, the aerial parts of the plant were subjected to hydrodistillation (Clevenger-type apparatus, Pyrexfan Co) for 3 hr. The obtained EO was dried over anhydrous sodium sulfate (Merck) and stored in the dark until further testing.

### 2.3 | Total phenolic content (TPC)

The TPC was measured colorimetrically with a standard Folin–Ciocalteu method (Slinkard & Singleton, 1977). The extract (20 µl) was mixed with 1,160 µl distilled water and 100 µl Folin-Ciocalteu reagent (Merck). After 5 min, 300 µl sodium bicarbonate (20%, Merck) solution was added to the mixture and kept at room temperature for 2 hr. Absorbance was read at 760 nm using a Biotech Plate Reader (BioTek Instruments). A calibration curve (5–80 µg/ml) was built with gallic acid (Sigma-Aldrich), and TPC expressed in mg gallic acid per gram dried extract (mg GAE $g^{-1}$).

### 2.4 | Total flavonoid content

The TFC was determined by the aluminum chloride colorimetric method (Chang et al., 2002). After mixing 500 µl extract with 100 µl aluminum chloride (10%, Merck)), 1,500 µl ethanol (95%), 100 µl

potassium acetate (1 M, Merck), and 2,800 µl distilled water, the mixture was kept at room temperature for 30 min and the absorbance measured at 415 nm. The results were expressed as mg quercetin (≥95%, Merck) equivalents per gram dried extract (mg QE $g^{-1}$).

## 2.5 | Antioxidant activity

### 2.5.1 | 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

The free radical scavenging activity of extracts was tested by a DPPH test (Mensor et al., 2001). Briefly, 100 µl of different extract concentrations (12.5–400 $\mu g.ml^{-1}$) was added to 100 µl freshly prepared 0.1 mM DPPH (Merck) solution in methanol. After 30 min of reaction at 37°C in the darkness, the absorbance of the sample was measured at 518 nm. Ascorbic acid was applied as positive control. In this method, DPPH (100 µl) + methanol (100 µl) are used as blank. The antioxidant capacity was then calculated using the following Equation (1):

$$AA\% = \left[ \left( A_{blank} - A_{sample} \right) / A_{blank} \right] \times 100 \quad (1)$$

### 2.5.2 | β-carotene linoleic acid bleaching (BCB) assay

The BCB assay was conducted according to the standard method (Kulisic et al., 2004). In brief, *β*-carotene (0.1 mg, ≥93%, Merck) was dissolved in 0.5 ml chloroform and mixed with 10 mg linoleic acid (≥99%, Merck) and 100 mg Tween-40. Then, the chloroform was evaporated at 50°C, distilled water (25 ml) was added and the mixture sonicated for 1 min. An initial absorbance was recorded at 470 nm (time =0 min). Aliquots of the *β*-carotene/linoleic acid solution (200 µl) were mixed with the prepared extracts (50 µl) and incubated at 50°C. The absorbance was measured at 470 nm after 120 min incubation. Antioxidant activity of the extracts was calculated by Equation (2):

$$\%\ \text{Inhibition} = \left[ \left( A_{A(120)} - A_{C(120)} \right) / \left( A_{(C0)} - A_{C(120)} \right) \right] \times 100 \quad (2)$$

where $A_{A(0)}$ and $A_{A(120)}$ are the absorbances of sample at times 0 and 120 min, while $A_{C(0)}$ and $A_{C(120)}$ are the absorbances of control after 0 and 120 min.

## 2.6 | Cytotoxic activity

Human breast cancer cell line MCF7, colorectal cancer cell line SW48, and a normal cell line mouse embryonic fibroblast cells NIH 3T3 were provided by the National Cell Bank of Iran (Pasteur Institute). They were kept with 10% (v/v) fetal bovine serum (FBS) (Gibco), penicillin/streptomycin at 100 IU/ml and 2 mM L-glutamine. Cultures were incubated with 5% $CO_2$ in a humidified atmosphere at 37°C. The cytotoxic effect of the prepared extracts was assessed using the AlamarBlue® (BioSource Invitrogen) proliferation assay. Briefly, cells were seeded in 96-well plates at a density of $1 \times 10^4$. The cells were treated with different concentrations of extract (100 µl, 50–400 $\mu g.ml^{-1}$) after overnight growth. After 48 hr treatment, 20 µl AlamarBlue® reagent was added to each well. After 2 to 4 hr, the absorbance at 600 nm was measured on a Biotech Plate Reader (BioTek Instruments). Doxorubicin (0.1, 0.5 and 2 $\mu g.ml^{-1}$) was chosen as a positive control. $IC_{50}$ values were calculated from Boltzmann sigmoidal concentration–response curve nonlinear regression fitting models (Lyles et al., 2008).

## 2.7 | Chemical profiles and phytochemical content

### 2.7.1 | Gas chromatography–mass spectrometry (GC-MS)

The GC-MS analyses were performed using a Agilent 5,975 apparatus with a HP-5ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness) interfaced with a quadruple mass detector and a computer equipped with Wiley 7n.l library. Instrumental conditions: oven temperature gradient: 50°C during 5min, 50°C–250°C at 3°C /min and 250°C during 10 min; injector temperature 250°C; injection volume, 1 µl; split ratio, 1:20; carrier gas, Helium at 1.0 ml/min; ionization potential, 70 eV; ionization current, 150 µA; ion source temperature, 280°C; mass range, 35–465 m/z. The constituents of the oils were identified by calculation of their retention indices under temperature programmed conditions for n-alkanes ($C_8$-$C_{23}$) and the oil on the HP-5ms column (van Den Dool & Dec. Kratz, 1963). Identification of individual compounds was made by comparison of their mass spectra and retention indices (RI) with those of authentic samples and those given in the literature (Adams, 2007).

### 2.7.2 | Ultra-performance liquid chromatography coupled with a photo diode array detector and electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-PAD/ESI-QTOF/MS)

An Acquity Ultra-Performance Liquid Chromatograph (UPLC, Waters) coupled to a photo diode array detector (PDA, Waters) and an electrospray ionization quadrupole time-of-flight tandem mass-spectrometer (ESI–QTOF/MS; Waters) was used. Chromatographic separation was done using an Acquity UPLC column (UPLC® BEH $C_{18}$, 100 mm × 2.1 mm, 1.7 µm, Waters). A binary mobile phase was used, mobile phase A (ultra-pure water with 0.1% formic acid) and mobile phase B (acetonitrile with 0.1% formic acid). Formic acid and acetonitrile were UPLC-MS grade from Actu, OSS, The Netherlands. A gradient separation was applied; 10% B, 0 min; 70% B, 30 min; 100% B, 33.33 min; 100% B, 38.33 min; 10% B, 41.67 min; 10% B, 50 min. The column temperature was maintained at 40°C, flow rate at 0.5 ml/min, wavelength range between 210 and 400 nm, and 10 µl sample was injected.

The ESI operating conditions for MS spectra acquisition in negative mode were as follows: capillary voltage, 2.6 kV; cone, 40 V; desolvation temperature 500°C; and source temperature, 150°C. The desolvation and cone gas flow rates were 0 and 1,000 L/h, respectively. Nitrogen (99.80% N28, Air Liquide, Auderghem, Belgium) was used for both desolvation and cone gas. Sample analysis was done independently in $MS^E$ acquisition (E is the collision energy) applying a full scan mode (50–1200 m/*z* range), in 1 s scan time. The precursor mass spectra acquisition was done in two continuous modes, a no collision energy mode, and a high collision energy (15–35 eV). Leucine enkephalin (Sigma-Aldrich) was used as internal reference (LockSpray™) to calibrate the ESI source. The data were acquired by a MassLynx™ 4.1 software (Waters).

### 2.7.3 | Sample preparation

Plant extract, 4 mg, was dissolved in 2.0 ml water/methanol (1:1; v/v) and then mixed for 10 min. Then, the sample was filtered using a membrane filter (0.20-μm) prior to injection.

### 2.7.4 | Identification and quantification of compounds

Compounds were identified and quantified in accordance to the retention times and mass spectral data (mass-to-charge (*m/z*), molecular peaks and their fragmentation) of the calibration standards. The analyte concentration was calculated using calibration curves of pure standards (Sigma-Aldrich). Stock solution of each pure calibration standard (1 $mg.ml^{-1}$) was prepared in methanol, and dilutions were made at 6 levels (1, 5, 10, 25, 50, 100 $\mu g.ml^{-1}$) for the calibration curves. Results were expressed as $\mu g.g^{-1}$ pure extract. The quantification was done in duplicate.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Essential oil composition

Seventy compounds, representing 99.6% of the EO of *D. moldavica*, were identified (Table 1). The main components were geranial (25.5%), estragole (16.0%), and geranyl acetate (15.2%). The majority of the compounds in the EO were oxygenated monoterpenes (55.4%). Golparvar et al., (2016) reported that *D. moldavica* EO collected from Kamu Mountain, Isfahan province, Iran, was dominated by geranyl acetate (36.62%), geraniol (24.3%), neral (16.2%), and geranial (11.2%). In a study by Yousefzadeha et al. (2018), geraniol, geranial, nerol, and geranyl acetate were the major constituents of the EO of *D. moldavica* collected from five habitats in the north-west of Iran (Salmas, Urmia, Khoy, Maragheh, and Tabriz). Fallah et al., (2018) found that the major components of the EO of *D. moldavica* were geranyl acetate, neral, linalool acetate and geraniol. In another study

**TABLE 1** Volatile components in the EO of *Deracocephalum moldavica*

| No | Compound | RI[1] | Percentage (%) |
|---|---|---|---|
| 1 | Benzaldehyde | 962 | *t*[2] |
| 2 | 1-octen-3-ol | 982 | 0.1 |
| 3 | 6-methyl-5-hepten-2-one | 988 | 0.4 |
| 4 | Myrcene | 992 | 0.1 |
| 5 | 2E,4E-heptadienal | 1,011 | *T* |
| 6 | *ρ*-cymene | 1,026 | *T* |
| 7 | Limonene | 1,030 | 0.1 |
| 8 | *cis*-ocimene | 1,041 | 0.2 |
| 9 | Benzene acetaldehyde | 1,045 | 0.1 |
| 10 | *trans*-ocimene | 1,052 | 0.1 |
| 11 | Bergamal | 1,058 | 0.1 |
| 12 | *cis*-linalool oxide | 1,074 | 0.1 |
| 13 | Terpinolene | 1,089 | 0.1 |
| 14 | *trans*-linalool oxide | 1,090 | 0.1 |
| 15 | Linalool | 1,101 | 1.3 |
| 16 | 1-octen-3-yl acetate | 1,115 | 0.1 |
| 17 | Allo-ocimene | 1,133 | 0.1 |
| 18 | *trans*-chrysanthemal | 1,154 | 0.1 |
| 19 | Citronellal | 1,156 | 0.1 |
| 20 | Nerol oxide | 1,159 | 0.1 |
| **21** | **Methyl chavicol (estragole)** | **1,204** | **16.0** |
| 22 | 4-methylene isophorone | 1,220 | 0.1 |
| 23 | Nerol | 1,232 | 0.3 |
| 24 | Neral | 1,254 | 9.7 |
| 25 | Geraniol | 1,258 | 0.5 |
| **26** | **Geranial** | **1,280** | **25.5** |
| 27 | Unknown | 1,302 | 0.2 |
| 28 | Geranyl formate | 1,306 | 0.4 |
| 29 | Neryl acetate | 1,365 | 1.2 |
| 30 | α-copaene | 1,378 | 1.0 |
| 31 | Nerolic acid | 1,378 | 0.2 |
| 32 | β-bourbonene | 1,389 | 0.3 |
| **33** | **Geranyl acetate** | **1,390** | **15.2** |
| 34 | Geranic acid | 1,406 | 0.2 |
| 35 | Methyl eugenol | 1,410 | 0.2 |
| 36 | β-caryophyllene | 1,423 | 0.6 |
| 37 | Unknown | 1,430 | 0.1 |
| 38 | β-copaene | 1,434 | *T* |
| 39 | Dihydro-β-ionone | 1,443 | *T* |
| 40 | Aromadendrene | 1,446 | *T* |
| 41 | α-humulene | 1,457 | 0.2 |
| 42 | E-β-farnesene | 1,461 | *T* |
| 43 | α-amorphene | 1,483 | 0.1 |

(Continues)

**TABLE 1** (Continued)

| No | Compound | RI[1] | Percentage (%) |
|---|---|---|---|
| **44** | Germacrene D | 1,486 | 0.3 |
| 45 | E-β-ionone | 1,490 | 0.5 |
| 46 | E,E-α-farnesene | 1,510 | 0.1 |
| 47 | γ-cadinene | 1517 | 0.1 |
| 48 | δ-cadinene | 1526 | 0.3 |
| 49 | β-thujaplicinol | 1537 | 0.2 |
| 50 | α-calacorene | 1547 | 0.2 |
| 51 | E-ρ-methoxy cinnamaldehyde | 1572 | 0.5 |
| 52 | Spathulenol | 1583 | 1.8 |
| 53 | Caryophyllene oxide | 1588 | 1.3 |
| 54 | *n*-hexadecane | 1601 | *T* |
| 55 | Ledol | 1606 | 0.2 |
| 56 | 1,10-di-epi-cubenol | 1622 | 1.1 |
| 57 | Epi-α-muurolol | 1648 | 0.2 |
| 58 | 3-thujopsanone | 1655 | 0.2 |
| 59 | 2Z,6E-farnesol | 1,730 | 0.5 |
| 60 | 2E,6E-farnesol | 1748 | 0.6 |
| 61 | Tetradecanoic acid | 1,770 | 0.4 |
| 62 | Neophytadiene | 1842 | 0.6 |
| 63 | Hexahydrofarnesyl acetone | 1851 | 0.5 |
| 64 | Methyl hexadecanoate | 1925 | 0.1 |
| 65 | Isophytol | 1952 | 0.2 |
| 66 | Dibutyl phthalate | 1968 | 0.3 |
| 67 | *n*-hexadecanoic acid | 1972 | 3.2 |
| 68 | Eicosane | 2001 | 0.1 |
| **69** | ***cis*-phytol** | **2,122** | **9.7** |
| 70 | Ethyl linoleate | 2,161 | 1.7 |
| | **Major Compound Groups** | | |
| | Monoterpene hydrocarbon | | 0.6 |
| | Oxygenated monoterpene | | 55.4 |
| | Sesquiterpen hydrocarbon | | 3.4 |
| | Oxygenated sesquiterpene | | 6.7 |
| | Diterpenoide | | 9.9 |
| | Phenyl propanoides | | 16.7 |
| | Miscellaneous | | 6.9 |
| | **Total Identified** | | **99.6** |

*Note:* Major compounds are shown in bold.
[1]RI: Retention Index on the HP-5 MS column.
[2]*t*: trace (<0.1%).

(Fallah et al., 2018), geranial (29.0%–41.5%), geranyl acetate (24.7%–34.8%), and neral (21.9%–28.6%) were the main components of the EO of *D. moldavica*. Still different results were reported by some other researchers, who found that linalool (Hussein et al., 2006) and citral (Nikitina et al., 2008; Shuge et al., 2009) are the predominant components of *D. moldavica* EO. Such differences in EO composition are common and might be due to physiological variations as well as ecological and genetic factors, seasonal and climatic conditions, harvest period, and the distillation technique applied (Shakeri et al., 2019).

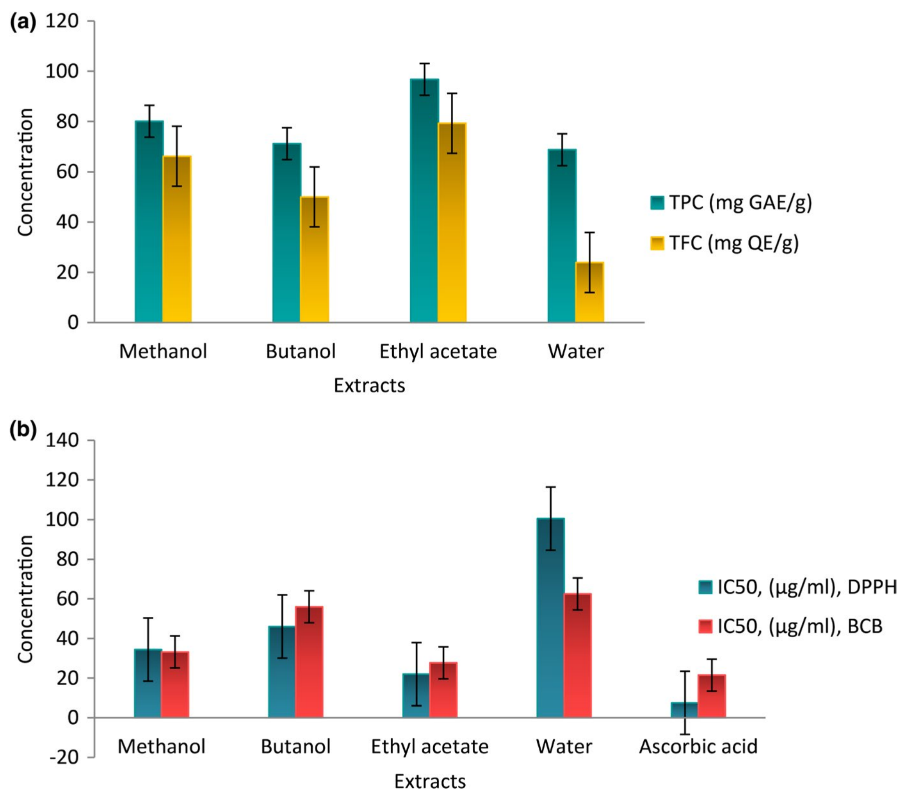
## 3.2 | Total phenolic (TPC) and total flavonoid contents (TFC)

The total phenolic content (TPC) of extracts from *D. moldavica* is most commonly estimated by the Folin-Ciocalteu method. In this analytical method, phenolic compounds are deprotonated and form phenolate ions that react with the Folin–Ciocalteu reagent (phosphomolybdate and phosphotungstate), resulting in a blue color, which absorbs visible light with a maximum around 765 nm (Vazquez et al., 2015), while the method for the determination of TFC is based on the formation of flavonoid–aluminum complexes with a maximum absorbance at 410–430 nm (Pękal, 2014). TPC and TFC of the extracts are presented in Figure 1a in aqueous and EtOAc extracts, respectively. The highest TPC was determined in EtOAc extract (96.8 ± 1.5 mg GAE $g^{-1}$), followed by the MeOH extract, 80.1 ± 2.3 mg GAE $g^{-1}$. The lowest TPC was measured in the aqueous extract, 68.8 ± 2.4 mg GAE $g^{-1}$. TFC was in the range from 23.9 ± 1.2 (in aqueous extract) to 79.3 ± 2.5 mg QE $g^{-1}$ (in EtOAc extract). In the literature, the antioxidant activity and TPC of a 70% aqueous MeOH extract of *D. moldavica* was evaluated by (Weremczuk-jeżyna et al., 2017). The TPC of the aerial parts of *D. moldavica* was 110.1 mg GAE $g^{-1}$, which was higher than observed in our study. In another study, by Aprotosoaiea et al. (2016), the TPC of the aerial parts of *D. moldavica* was 289.55 ± 2.63 mg of GAE $g^{-1}$, which was also higher than found for the MeOH extract in our study. Furthermore, Dastmalchi et al., (2007) observed a higher TPC for the 80% MeOH extract of the aerial parts of Iranian *D. moldavica* (488.4 ± 1.8 mg/g), but lower amounts for the EtOAc extracts compared to our samples.

## 3.3 | Antioxidant activity and UPLC/ESI-QTOF-MS analysis

Among the extracts of *D. moldavica*, the EtOAc one exhibited the strongest scavenging activity with an $IC_{50}$ value of 22.0 ± 2.1 μg.$ml^{-1}$ which is less active than ascorbic acid as positive control ($IC_{50}$ = 7.5 ± 0.2 μg.$ml^{-1}$) (Figure 1b). Antioxidant activity was also found in the MeOH extract ($IC_{50}$ = 34.4 ± 2.5 μg.$ml^{-1}$). The potent free radical scavenging activity of the MeOH extract of *D. moldavica* confirmed Dastmalchi et al., (2007), who revealed that the MeOH extract was a significantly better scavenger than quercetin. It is also in accordance with another study which reported scavenging effects of the MeOH extract of *D. moldavica* in the DPPH assay ($EC_{50}$ = 23.10 ± 0.10 μg.$ml^{-1}$) (Aprotosoaie et al., 2016). In the BCB method, the EtOAc extract again exerted the strongest β-carotene inhibition activity (94% inhibition, at 150 μg.$ml^{-1}$) followed by the MeOH (82%), n-BuOH (75%), and aqueous (59%) extracts (Figure 1b). In the present study, UPLC/

**FIGURE 1** Total phenolic and total flavonoid contents (a) and antioxidant activities (b) of *Deracocephalum moldavica* extracts




ESI-QTOF-MS was carried out on the extracts with the highest antioxidant activity to find the compounds potentially responsible for the antioxidant activity. The antioxidant activity of the MeOH and especially the EtOAc extracts of *D. moldavica* was in accordance with their amounts of phenolic acids. The UPLC/ESI-QTOF-MS analysis (Table 2) revealed that the MeOH extract of *D. moldavica* contains high amounts of phenolic acids, including rosmarinic acid (34,407 ± 694 µg.g$^{-1}$) and 2-hydroxycinnamic acid (15,124 ± 2000 µg.g$^{-1}$), and of 4-hydroxycoumarin (5,216 ± 95 µg.g$^{-1}$). In the literature, rosmarinic acid was also found to have the highest concentration in a MeOH extract of an Iranian *D. moldavica* (89,083 ± 1,380 µg.g$^{-1}$) (Dastmalchi et al., 2007). In our study, a much higher concentration of rosmarinic acid (75,508 ± 1,044 µg.g$^{-1}$) than in the MeOH extract was found in the EtOAc extract, followed by caffeic acid (69,678 ± 5,578 µg.g$^{-1}$), 3-hydroxybenzoic acid (35,368 ± 2,803 µg.g$^{-1}$), and 2-hydroxycinnamic acid (23,466 ± 2,122 µg.g$^{-1}$). It is evident from our results that the compounds most responsible for high antioxidant capacity of *D. moldavica* were phenolic acids such as rosmarinic acid, caffeic acid, hydroxycinnamic acids, and hydroxycinnamic acid. The antioxidant activity of rosmarinic acid, an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, has already been demonstrated both in vitro and in vivo by many researchers (Adomako-Bonsu et al., 2017; Nicolai et al., 2016; Tsai et al., 2019).

## 3.4 | Cytotoxic activity

Extracts of *D. moldavica* in a concentration range from 50 to 400 µg.ml$^{-1}$ were assayed for their cytotoxic activity against two human cancer cell lines, SW-48 and MCF-7, and against a normal cell line, NIH/3T3. None of the extracts (50–400 µg.ml$^{-1}$) exhibited cytotoxic activity, suggesting potential safety of the plant. This is in accordance with a study by Yu et al., (2019) who did not found a significant cytotoxic effect of the EtOAc extract of *D. moldavica* (33.3% growth inhibition at 100 µg.ml$^{-1}$) against human epidermal keratinocyte (HaCaT) cells. To the best of our knowledge, there is no other published data on the cytotoxicity of *D. moldavica* extracts.

# 4 | CONCLUSION

The antioxidant and cytotoxic activities of different extracts of *D. moldavica*, that is, EtOAc, MeOH, n-BuOH and aqueous extracts, the total phenolic and flavonoid contents as well as the phytochemical profiles of the EO and the extracts were determined. The EtOAc and MeOH extracts were found to possess remarkable antioxidant activity in the DPPH and BCB assays. GC-MS analysis showed that the majority of the compounds in the EO were oxygenated monoterpenes (55.4%). Further, UPLC–QTOF–MS analysis allowed identifying 37 metabolites, mainly pertaining to phenolic acids. Rosmarinic acid occurs in high amounts in the EtOAc and MeOH extracts of *D. moldavica* and may be responsible for most of the antioxidant activity. Our UPLC/PDA-MS analysis focused on the quantification of some specific phenolic compounds. Thus, further studies are required to identify other compounds that may be present in significant amounts, but were not determined. None of the extracts, even at high concentrations (400 µg.ml$^{-1}$), showed considerable cytotoxicity, which suggests potential safety of the plant to be used as a natural preservative in food.

**TABLE 2** Phenolic compounds quantified in the evaluated extracts from *Deracocephalum moldavica*, presented as mean ±standard deviation (μg.g$^{-1}$)

| | Compounds | MeOH extract | EtOAC extract | Molecular formula | Molecular weight (M) | HPLC ESI-MS (*m/z*) | |
|---|---|---|---|---|---|---|---|
| | | | | | | RT (min) | [M-H]$^{-}$ |
| 1 | Malic acid | 255 ± 59 | 73.7 ± 46.6 | C4H6O5 | 134.087 | 0.94 | 133.014 |
| 2 | Quinic acid | 463 ± 29 | 72.3 ± 3.8 | C7H12O6 | 192.167 | 0.96 | 191.120 |
| 3 | Succinic acid | 4,527 ± 902 | 5,072 ± 131.2 | C4H6O4 | 118.088 | 1.21 | 117.018 |
| 4 | Citric acid | 5,101 ± 397 | 46.2 ± 3.8 | C6H8O7 | 192.123 | 1.22 | 191.102 |
| 5 | Pyrogallol | 2.4 ± 0.7 | 11.75 ± 0.2 | C6H6O3 | 126.111 | 1.24 | 125.024 |
| 6 | Gallic acid | 17 ± 1.2 | 79.4 ± 2.4 | C7H6O5 | 170.022 | 1.33 | 168.90 |
| 7 | Pyrocatechol | 6.7 ± 0.04 | 141 ± 8 | C6H6O2 | 110.112 | 1.95 | 109.028 |
| 8 | 3–4-Hydroxybenzoic acid | 56.7 ± 0.15 | 1,151 ± 62.7 | C7H6O4 | 154.121 | 2.01 | 153.010 |
| 9 | Catechin | 0.76 ± 0.24 | 0.20 ± 0.03 | C15H14O6 | 290.271 | 2.21 | 289.064 |
| 10 | Chlorogenic acid | 1,359 ± 100 | 288 ± 16 | C16H18O9 | 354.311 | 2.37 | 353.202 |
| 11 | 4-Hydroxybenzoic acid | 70 ± 5.2 | 2,867 ± 240 | C7H6O3 | 138.122 | 2.8 | 137.050 |
| 12 | 3-Hydroxybenzoic acid | 535 ± 486 | 35,368 ± 2,803 | C7H6O3 | 138.122 | 2.83 | 137.025 |
| 13 | Esculetin | 31 ± 1.7 | 888.9 ± 0.52 | C9H6O4 | 178.143 | 3.03 | 177.018 |
| 14 | Vanillic acid | 97.8 ± 29 | 755.5 ± 29.65 | C8H8O4 | 168.148 | 3.13 | 167.036 |
| 15 | Syringic acid | 39 ± 1.9 | 107.4 ± 2.7 | C9H10O5 | 198.174 | 3.17 | 197.045 |
| 16 | Caffeic acid | 3,019 ± 44 | 69,678 ± 5,578 | C9H8O4 | 180.159 | 3.19 | 179.035 |
| 17 | Epicatechin | 0.33 ± 0.01 | 0.48 ± 0.28 | C15H14O6 | 290.271 | 3.84 | 289.064 |
| 18 | 4-Hydroxycinnamic acid | 80 ± 3.6 | 1587 ± 80.8 | C9H8O3 | 164.160 | 4.54 | 163.042 |
| 19 | 3-Hydroxycinnamic acid | 121 ± 10.6 | 2,146 ± 90 | C9H8O3 | 164.160 | 4.56 | 163.042 |
| 20 | Rutin | 668 ± 8.8 | 530 ± 43.3 | C27H30O16 | 610.153 | 4.71 | 609.1 |
| 21 | Sinapic acid | 0.96 ± 0.2 | 16.7 ± 1.10 | C11H12O5 | 224.212 | 4.88 | 223.061 |
| 22 | Ferulic acid | 10 ± 7.0 | 416 ± 0.80 | C10H10O4 | 194.186 | 5.05 | 193.050 |
| 23 | 2- Hydroxycinnamic acid | 15,124 ± 2000 | 23,466 ± 2,122 | C9H8O3 | 164.160 | 5.14 | 163.042 |
| 24 | Tannic acid | 4,069 ± 2,101 | 73.45 ± 11.50 | C76H52O46 | 1701.206 | 5.31 | 1,700.080 |
| 25 | Naringin | 965 ± 17.7 | 11 ± 2.4 | C27H32O14 | 580.539 | 5.84 | 579.173 |
| 26 | Benzoic acid | 662 ± 54.25 | 3,268 ± 20 | C7H6O2 | 122.123 | 5.97 | 121.031 |
| 27 | Quercitrin | 26 ± 8.4 | 321.4 ± 42 | C21H20O11 | 448.38 | 6.02 | 447.120 |
| 28 | Hesperidin | 1,030 ± 251 | 789.7 ± 513 | C28H34O15 | 610.565 | 6.24 | 609.172 |
| 29 | Rosmarinic acid | 34,407 ± 694 | 75,508 ± 1,044 | C18H16O8 | 360.318 | 6.94 | 359.054 |
| 30 | 4-Hydroxycoumarin | 5,216 ± 95 | 7,215 ± 158 | C9H8O3 | 164.160 | 7.04 | 163.042 |
| 31 | Salicylic acid | 3.20 ± 0.10 | 20.92 ± 0.07 | C7H6O3 | 138.122 | 7.38 | 137.025 |
| 32 | Resveratrol acid | 1.3 ± 0.04 | 53.13 ± 3.3 | C14H12O3 | 228.247 | 8.24 | 227.072 |
| 33 | Luteolin | 5.6 ± 2.85 | 7.5 ± 0.2 | C15H10O6 | 286.239 | 8.87 | 285.040 |
| 34 | Quercitin | 1.5 ± 0.12 | 12.4 ± 1.2 | C15H10O7 | 302.238 | 9.11 | 301.000 |
| 35 | Naringenin | 33.4 ± 4.2 | 114.9 ± 0.6 | C15H12O5 | 272.256 | 10.77 | 271.061 |
| 36 | Hesperetin | 9.9 ± 0.36 | 19.9 ± 9.8 | C16H14O6 | 302.282 | 11.04 | 301.015 |
| 37 | Kaempferol | 38.7 ± 17.6 | 134 ± 4.5 | C15H10O6 | 286.239 | 11.12 | 285.040 |

Abbreviation: ND, not detected.

## ACKNOWLEDGMENTS

This research was financially supported by grants from the Mashhad University of Medical Sciences (Mashhad, Iran) Research Council [grant number 960960].

## CONFLICT OF INTEREST

No conflict of interest was reported by the authors.

## AUTHOR CONTRIBUTION

**Azin Fattahi:** Investigation (equal). **Abolfazl Shakeri:** Conceptualization (equal); Investigation (equal); Writing-original draft (lead). **Zahra Tayarani-Najaran:** Software (equal). **Mourad Kharbach:** Methodology (equal). **Karen Segers:** Methodology (equal). **Yvan Vander Heyden:** Methodology (equal). **Seyedeh Faezeh Taghizadeh:** Formal analysis (equal); Software (equal). **Hanieh Rahmani:** Investigation (equal). **Javad Asili:** Conceptualization (equal); Funding acquisition (equal).

## ORCID

*Abolfazl Shakeri* https://orcid.org/0000-0002-0676-6619

## REFERENCES

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectrometry (Vol. 456)*. Allured Publishing Corporation.

Adnani, N., Michel, C. R., & Bugni, T. S. (2012). Universal quantification of structurally diverse natural products using an evaporative light scattering detector. *Journal of Natural Products*, *75*(4), 802–806. https://doi.org/10.1021/np300034c

Adomako-Bonsu, A. G., Chan, S. L., Pratten, M., & Fry, J. R. (2017). Antioxidant activity of rosmarinic acid and its principal metabolites in chemical and cellular systems: Importance of physico-chemical characteristics. *Toxicology in Vitro*, *40*, 248–255. https://doi.org/10.1016/j.tiv.2017.01.016

Aprotosoaie, A. C., Mihai, C. T., Vochita, G., Rotinberg, P., Trifan, A., Luca, S. V., & Miron, A. (2016). Antigenotoxic and antioxidant activities of a polyphenolic extract from European *Dracocephalum moldavica* L. *Industrial Crops and Products*, *79*, 248–257. https://doi.org/10.1016/j.indcrop.2015.11.004

Barends, C., Weenen, H., Warren, J., Hetherington, M. M., de Graaf, C., & de Vries, J. H. M. (2019). A systematic review of practices to promote vegetable acceptance in the first three years of life. *Appetite*, *137*, 174–197. https://doi.org/10.1016/j.appet.2019.02.003

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182.

Chen, Z., Wu, J. B., Liao, X. J., Yang, W., & Song, K. (2010). Development and validation of an UPLC-DAD-MS method for the determination of leonurine in Chinese motherwort (*Leonurus japonicus*). *Journal of Chromatographic Science*, *48*(10), 802–806. https://doi.org/10.1093/chromsci/48.10.802

Dastmalchi, K., Damien Dorman, H. J., Laakso, I., & Hiltunen, R. (2007). Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. *LWT - Food Science and Technology*, *40*(9), 1655–1663. https://doi.org/10.1016/j.lwt.2006.11.013

Fallah, S., Rostaei, M., Lorigooini, Z., & Abbasi Surki, A. (2018). Chemical compositions of essential oil and antioxidant activity of dragonhead (*Dracocephalum moldavica*) in sole crop and dragonhead- soybean (Glycine max) intercropping system under organic manure and chemical fertilizers. *Industrial Crops and Products*, *115*, 158–165. https://doi.org/10.1016/j.indcrop.2018.02.003

Golparvar, A. R., Hadipanah, A., Gheisari, M. M., & Khaliliazar, R. (2016). Chemical constituents of essential oil of *Dracocephalum moldavica* L. and *Dracocephalum kotschyi* Boiss. from Iran. *Acta Agriculturae Slovenica*, *107*(1), 25–31.

Hussein, M. S., El-Sherbeny, S. E., Khalil, M. Y., Naguib, N. Y., & Aly, S. M. (2006). Growth characters and chemical constituents of *Dracocephalum moldavica* L. plants in relation to compost fertilizer and planting distance. *Scientia Horticulturae*, *108*(3), 322–331. https://doi.org/10.1016/j.scienta.2006.01.035

Jiang, J., Yuan, X., Wang, T., Chen, H., Zhao, H., Yan, X., Wang, Z., Sun, X., & Zheng, Q. (2014). Antioxidative and cardioprotective effects of total flavonoids extracted from *Dracocephalum moldavica* L. against acute ischemia/reperfusion-induced myocardial injury in isolated rat heart. *Cardiovascular Toxicology*, *14*(1), 74–82. https://doi.org/10.1007/s12012-013-9221-3

Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, *85*(4), 633–640. https://doi.org/10.1016/j.foodchem.2003.07.024

Lyles, R. H., Poindexter, C., Evans, A., Brown, M., & Cooper, C. R. (2008). Nonlinear model-based estimates of IC(50) for studies involving continuous therapeutic dose-response data. *Contemporary Clinical Trials*, *29*(6), 878–886. https://doi.org/10.1016/j.cct.2008.05.009

Martinez-Vazquez, M., Estrada-Reyes, R., Martinez-Laurrabaquio, A., Lopez-Rubalcava, C., & Heinze, G. (2012). Neuropharmacological study of *Dracocephalum moldavica* L. (Lamiaceae) in mice: Sedative effect and chemical analysis of an aqueous extract. *Journal of Ethnopharmacology*, *141*(3), 908–917. https://doi.org/10.1016/j.jep.2012.03.028

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*(2), 127–130. https://doi.org/10.1002/ptr.687

Nicolai, M., Pereira, P., Vitor, R. F., Reis, C. P., Roberto, A., & Rijo, P. (2016). Antioxidant activity and rosmarinic acid content of ultrasound-assisted ethanolic extracts of medicinal plants. *Measurement*, *89*, 328–332. https://doi.org/10.1016/j.measurement.2016.04.033

Nikitina, A. S., Popova, O. I., Ushakova, L. S., Chumakova, V. V., & Ivanova, L. I. (2008). Studies of the essential oil of *Dracocephalum moldavica* cultivated in the Stavropol region. *Pharmaceutical Chemistry Journal*, *42*(4), 203–207. https://doi.org/10.1007/s11094-008-0092-z

Pękal, A. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Analytical Methods*, *7*, 1776–1782. https://doi.org/10.1007/s12161-014-9814-x

Shakeri, A., D'Urso, G., Taghizadeh, S. F., Piacente, S., Norouzi, S., Soheili, V., Asili, J., & Salarbashi, D. (2019). LC-ESI/LTQOrbitrap/MS/MS and GC–MS profiling of *Stachys parviflora* L. and evaluation of its biological activities. *Journal of Pharmaceutical and Biomedical Analysis*, *168*, 209–216. https://doi.org/10.1016/j.jpba.2019.02.018

Shuge, T., Xiaoying, Z., Fan, Z., Dongqing, A., & Tao, Y. (2009). Essential oil composition of the *Dracocephalum moldavica* L from Xinjiang in China. *Pharmacognosy Research*, *1*(4), 172–174.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, *28*(1), 49–55.

Sultan, A., Aisa, H., & Eshbakova, K. (2008). Flavonoids from *Dracocephalum moldavica*. *Chemistry of Natural Compounds*, *44*(3), 366–367. https://doi.org/10.1007/s10600-008-9065-4

Tsai, C.-F., Wu, J.-Y., & Hsu, Y.-W. (2019). Protective effects of rosmarinic acid against selenite-induced cataract and oxidative damage in rats. *International Journal of Medical Sciences*, *16*(5), 729–740. https://doi.org/10.7150/ijms.32222

van Den Dool, H., & Dec. Kratz, P. (1963). A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *Journal of Chromatography A*, *11*, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X

Vázquez, C. V., Rojas, M. G. V., Ramírez, C. A., Chávez-Servín, J. L., García-Gasca, T., Ferriz Martínez, R. A., García, O. P., Rosado, J. L., López-Sabater, C. M., Castellote, A. I., Montemayor, H. M. A., & de la Torre Carbot, K. (2015). Total phenolic compounds in milk from different species. Design of an extraction technique for quantification using the Folin-Ciocalteu method. *Food Chemistry*, *176*, 480–486. https://doi.org/10.1016/j.foodchem.2014.12.050

Weremczuk-jeżyna, I., Grzegorczykkarolak, I., Frydrych, B., Hnatuszko-konka, K., Gerszberg, A., & Wysokińska, H. (2017). Rosmarinic Acid Accumulation and Antioxidant Potential of *Dracocephalum moldavica* L. Cell Suspension Culture. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *45*(1), 215–219. https://doi.org/10.15835/nbha45110728

Weremczuk-Jeżyna, I., Grzegorczyk-Karolak, I., Frydrych, B., Królicka, A., & Wysokińska, H. (2013). Hairy roots of *Dracocephalum moldavica*: Rosmarinic acid content and antioxidant potential. *Acta Physiologiae Plantarum*, *35*(7), 2095–2103. https://doi.org/10.1007/s11738-013-1244-7

Yang, L.-N., Xing, J.-G., He, C.-H., & Wu, T. (2014). The phenolic compounds from *Dracocephalum moldavica* L. *Biochemical Systematics and Ecology*, *54*, 19–22. https://doi.org/10.1016/j.bse.2013.12.009

Yousefzadeh, S., Daryai, F., Mokhtassi-Bidgoli, A., Hazrati, S., Yousefzadeh, T., & Mohammadi, K. (2018). Morphological, essential oil and biochemical variation of *Dracocephalum moldavica* L. populations. *Journal of Applied Research on Medicinal and Aromatic Plants*, *10*, 59–66. https://doi.org/10.1016/j.jarmap.2018.06.005

Yousefzadeh, S., Modarres-Sanavy, S. A. M., Sefidkon, F., Asgarzadeh, A., Ghalavand, A., & Sadat-Asilan, K. (2013). Effects of Azocompost and urea on the herbage yield and contents and compositions of essential oils from two genotypes of dragonhead (*Dracocephalum moldavica* L.) in two regions of Iran. *Food Chemistry*, *138*(2), 1407–1413. https://doi.org/10.1016/j.foodchem.2012.11.070

Yu, H., Liu, M., Liu, Y., Qin, L., Jin, M., & Wang, Z. (2019). Antimicrobial Activity and Mechanism of Action of *Dracocephalum moldavica* L. Extracts Against Clinical Isolates of *Staphylococcus aureus*. *Frontiers in Microbiology*, *10*(1249), 1–10. https://doi.org/10.3389/fmicb.2019.01249

Yu, N., He, C., Awuti, G., Zeng, C., Xing, J., & Huang, W. (2015). Simultaneous determination of six active compounds in Yixin Badiranjibuya Granules, a Traditional Chinese Medicine, by RP-HPLC-UV Method. *Journal of Analytical Methods in Chemistry*, *2015*, 974039–974047. https://doi.org/10.1155/2015/974039

Zeng, Q., Jin, H. Z., Qin, J. J., Fu, J. J., Hu, X. J., Liu, J. H., Yan, L., Chen, M., & Zhang, W. D. (2010). Chemical constituents of plants from the genus *Dracocephalum*. *Chemistry & Biodiversity*, *7*(8), 1911–1929. https://doi.org/10.1002/cbdv.200900188

**How to cite this article:** Fattahi A, Shakeri A, Tayarani-Najaran Z, et al. UPLC–PDA-ESI–QTOF–MS/MS and GC-MS analysis of Iranian *Dracocephalum moldavica* L.. *Food Sci Nutr.* 2021;9:4278–4286. https://doi.org/10.1002/fsn3.2396