ORIGINAL RESEARCH

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

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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 \mu g.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 \mu g.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
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, anti-convulsive, 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-Żeżyńska 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...
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 μg 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):

\[ AA\% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \]  

(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 μg 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):

\[ \% \text{ Inhibition} = \left( \frac{A_{\text{C}_{120}} - A_{\text{C}_{0}}}{A_{\text{C}_{120}}} \right) \times 100 \]  

(2)

where \( A_{\text{C}_{0}} \) and \( A_{\text{C}_{120}} \) are the absorbances of sample at times 0 and 120 min, while \( A_{\text{C}_{0}} \) and \( A_{\text{C}_{120}} \) 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% CO₂ 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 × 10⁴. The cells were treated with different concentrations of extract (100 μl, 50–400 μg 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 μg ml⁻¹) was chosen as a positive control. IC₅₀ 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 quadrupole mass detector and a computer equipped with Wiley 7n.l library. Instrumental conditions: oven temperature gradient: 50°C during 5 min, 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 (C₈–C₂₃) 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 C₁₈, 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; 100% B, 41.67 min; 100% 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 MSX 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 each pure calibration standard (Sigma-Aldrich). Stock solution of each pure calibration was done in duplicate. Results were expressed as μg.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 Yousefzadeh et al. (2018), geraniol, geranial, neral, 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

| No | Compound                     | RI1 | Percentage (%) |
|----|------------------------------|-----|----------------|
| 1  | Benzaldehyde                 | 962 |                |
| 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  | β-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 | Citronellial                 | 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-bourbonone                 | 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 | α-copaene                    | 1,434 | T            |
| 39 | Dihydro-β-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)
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 Aprotosoaie et al. (2016), the TPC of the aerial parts of *D. moldavica* was 289.55 ± 1.5 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/
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 hydroxyphenylacetic 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 find 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 phe

![FIGURE 1 Total phenolic and total flavonoid contents (a) and antioxidant activities (b) of Deracocephalum moldavica extracts](image-url)
## 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) | 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     | 168.900|
| 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 ± 5.78 | 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 ± 1.1    | 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 | C7H6O2046         | 1701.206             | 5.31     | 1700.080|
| 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 ± 51.3  | 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   | 199.9 ± 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.
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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-Najarani: Software (equal). Mourad Kharbach: Methodology (equal). Karen Segers: Methodology (equal). Yvan Vander Heyden: Methodology (equal). Seyyedeh Faezeh Taghizadeh: Formal analysis (equal); Software (equal). Hanieh Rahmani: Investigation (equal). Javad Asili: Conceptualization (equal); Funding acquisition (equal).

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