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Original article

Phytochemistry and antioxidant activity of *Lallemantia iberica* aerial parts

N. Khosravi Dehaghi[1], A.R. Gohari[2], S.S. Sadat-Ebrahimi[3], H. Naghdi Badi[4], Y. Amanzadeh[5*]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Alborz University of Medical Sciences, Karaj, Iran.*
[2]*Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[3]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[4]*Institute of Medicinal Plants, Iranian Academic Center for Education, Culture & Research (ACECR),Karaj, Iran.*
[5]*Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*

Abstract

Background and objectives: *Lallemantia iberica* (Lamiaceae) is a medicinal plant distributed in different parts of Iran. This research, has evaluated the phytochemical constituents and antioxidant activity of the aerial parts of the plant. **Methods:** Different chromatographic methods such as column chromatographies using Silica gel (normal and reversed phases), Sephadex LH-20 and HPLC were used for isolation of the compounds from the ethyl acetate and methanol extract of *L. iberica* aerial parts. The structures of the isolated compounds were elucidated using ^{1}H-NMR, ^{13}C-NMR and EI-MS. Antioxidant activity of the extracts were also evaluated in DPPH and FRAP tests. **Results:** Two sterols, β-sitosterol acetate (1), β-sitosterol (2), one triterpenoic acid, ursolic acid (3), one polyphenol, rosmarinic acid (4) and six flavonoides, Luteolin-7-*O*-glucoside (5), 4'-methoxy-luteolin-7-*O*-glucoside (6), apigenin-7-*O*-glucoside (7), Luteolin (8), diosmetin (9), apigenin (10) were isolated and identified from the ethyl acetate and methanol extracts. The antioxidant activity of the ethyl acetate (IC_{50} 189.95±2.8 μg/mL) and the methanol extracts (IC_{50} 140±1.2 μg/mL) were compared to the standard antioxidant, BHA (IC_{50} 100±1.6 μg/mL) in DPPH method. The reducing power of the ethyl acetate (300.28 μmol Eq $FeSO_4.7H_2O$/mg DW), the methanol extract (553.14 μmol Eq $FeSO_4.7H_2O$/mg DW) and BHA (558.36 μmol Eq $FeSO_4.7H_2O$/mg of standard) were elucidated in FRAP assay. **Conclusion:** The results introduce *L. iberica* as a medicinal plant with valuable constituents which are responsible of different pharmacological activities.

Keywords: antioxidant activity, DPPH, FRAP, *Lallemantia iberica*, phytochemistry

Introduction

Genus *Lallemantia* belongs to the family Lamiaceae which has 46 genera and 410 species and subspecies in Iran [1]. It is distributed in different parts of Iran, especially in north and north-west. This genus has five different species including *peltata*, *royleana*, *iberica*, *baldshuanica* and *canescen* [2-4]. Seeds of *Lallemantia iberica* are known as "*Balangue shahri*" in Persian. Its synonyms are *Lallemantia sulphurea* and *Dracocephalum ibericum* (Bieb.) [4,5]. The seeds are normally used as stimulant, diuretic and expectorant [1]. The mucilage of the seeds is used for treatment of some nervous, hepatic and renal diseases and it was used as

Available at: http://rjpharmacognosy.ir
*Corresponding author: amanzadehy@sina.tums.ac.ir, Tel: +9826- 32567175, Fax: +9826-32567178

general tonic in the Iranian Traditional Medicine [5,6]. Constituents of the *L. iberica* essential oil have been previously studied by GC and GC/MS in two different stages. The major compounds in the flowering stage were β-cubeben (19.55%), linalool (18.71%) and spathulenol (18.04%). In post-flowering stage, caryophylene oxide (38.77%), linalool (15.15%) and germacrene-D (7.03%) were the major constituents [6]. Another study about the essential oil of the plant, explained that *p*-cymene (22.1%), isophytol (19.8%) and t-cadinol (11.1%) were the major constituents [7]. A new putrescine bisamide phenolic glycoside, lallenmantoside, together with a known phenolic glycoside, cucurbitoside D , were also isolated from the seeds of *L.iberica* [8]. The aim of the present study was to identify the chemical composition of the plant and its antioxidant activity.

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Experimental

Instrumentation

The ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Brucker Avance 500 DRX (USA) (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with tetramethylsilane as the internal standard, chemical shifts were given in δ (ppm). EI-MS spectra were measured on an Agilent Technology (Palo Alto CA, USA) instrument with a 5973 Network mass selective detector (MS model). Silica gel 35-70 and 230-400 mesh (Merck, Germany), Sephadex LH-20 (Fluka, Switzerland) were applied for column chromatography. Silica gel $60F_{254}$ pre-coated plates (Merck, Germany) and silica gel 60 RP-18 F_{254} plates (Merck, Germany) were used for the TLC. The HPLC was Knauer Wellchrom system (Germany) that was connected to a PDA detector 2600 and pump was Smart line 1000, including 10 mL pump head stainless steel EA4300 model, semi-preparative column (250×20 mm, eurospher 100-7 C_{18}). The injection volume was 2 mL.

Materials

2,2-diphenyl-picrylhydrazyl (DPPH), Butylated hydroxyanisole (BHA) and 2,4,6-tripyridyl-*s*-triazine (TPTZ) were bought from Sigma-Aldrich (Germany). Sodium carbonate, sodium acetate, ferrous sulphate and $FeCl_3$ were prepared from Scharlau, Spain. Other chemicals and all solvents were purchased from Merck (Germany)..

Plant material

Lallemantia iberica (Bieb.) Fisch & C.A. Mey (Lamiaceae) was cultivated in Institute of Medicinal Plants Research (ACECR), Karaj, Halejerd, Iran. A voucher specimen (6713-TEH) was deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction

The dried aerial parts (2 Kg) were powdered and macerated with EtOAc and MeOH at room temperature for three times every 48 hours. The obtained methanol and ethyl acetate extracts were concentrated using a rotary evaporator at 37 °C. The ethyl acetate (107 g) and methanol extracts (200 g) were refrigerated.

Isolation and purification of the compounds

Ninety grams of the ethyl acetate extract was moved to silica gel column and eluted with *n*-hexane, *n*-hexane/$CHCl_3$ (7:3, 5:5, 4:6), $CHCl_3$, $CHCl_3$/EtOAc (3:7) and EtOAc to get five fractions (A-E). Silica gel column chromatography of fraction B (10 g) with a gradient mixture of *n*-hexane/ $CHCl_3$ (5:5, 2:8), $CHCl_3$/ EtOAc (5:5), EtOAc yielded nine fractions (B_1-B_9). Fraction B7 (100 mg) was moved to a Sephadex LH-20 column and eluted with $CHCl_3$/ MeOH (3:7) to get five fractions (B7a-B7e). Compound **1** (13 mg) was isolated from the fraction B7c on a Sephadex LH-20 column ($CHCl_3$/MeOH 3:7). Fraction C (12 g) was subjected to silica gel column chromatography with *n*-hexane/$CHCl_3$ (4:6, 3:7), $CHCl_3$/EtOAc (5:5) and EtOAc to get seven fractions (C_1-C_7). Silica gel column chromatography of the fraction C_6 (350 mg) with a gradient mixture of *n*-hexane: EtOAc (9:1, 8:2) yielded ten fractions (C_6a-C_6j). Compound **2** (22mg) was isolated from the fraction C_6g (98 mg) with a silica gel column chromatography (*n*-hexane/EtOAc 8:2, EtOAc). Fraction D (12 g) was subjected to silica gel column chromatography with $CHCl_3$, $CHCl_3$/EtOAc (9:1, 8:2, 5:5, 2:8) and EtOAc to get twenty fractions (D_1-D_{20}). Fraction D_{10} (180 mg) was moved to silica gel column chromatography and eluted with $CHCl_3$/EtOAc (7:3, 5:5) and EtOAc to get eight fractions (D_{10}a-D_{10}h). Compound **3** (12 mg) was isolated from fraction D_{10}f (80mg) on silica gel column chromatography ($CHCl_3$/EtOAc 5:5, 3:7, EtOAc). One hundred g methanol extract was moved to a silica gel column chromatography and eluted with $CHCl_3$, EtOAc, EtOAc/ MeOH (7:3, 3:7) and MeOH to get seven fractions (A-G). Fraction E (8 g) was subjected to silica gel column chromatography with EtOAc/MeOH (6:4) to get five fracions (E_1-E_5). Fraction E_2 was subjected to Sephadex LH-20 and eluted with MeOH/H_2O (4:6) to get seven fractions (E_2a-E_2g). Fraction E2e (450 mg) was moved to Sephadex LH-20 and eluted with MeOH to get eight fractions (E_{2e1}- E_{2e8}). Compound **4** (12 mg) was isolated from fraction E_{2e6} (68 mg) over the RP silica gel column chromatography twice with MeOH/H_2O (4:6, 7:3) as the eluent. Fraction C was moved to silica gel column chromatography and eluted with EtOAc/MeOH (5:5) to get eight fractions (C_1-C_8). Two times sephadex LH-20 column chromatography of the fraction C_6 (800 mg) with MeOH resulted in six fractions (C_{6c1}-C_{6c6}). Six compounds [**5** (27 mg), **6** (25.4 mg), **7** (17 mg), **8** (20 mg), **9** (13.5 mg), **10** (11.7 mg)] were achieved from fraction C_{6c5} (350 mg) by HPLC on RP C_{18} column that was eluted with MeOH/Aceton (UV350 nm) at 350 nm. Gradient time program for isolation has been presented in table1.

Table 1. Gradient time program of HPLC for isolation of fraction C_{6c5} of *Lallemantia iberica*

Time	%H_2O	%CH_3COCH_3
0	70	30
30	70	30
40	60	40
60	60	40
90	50	50
100	50	50
120	30	70
140	30	70
141	70	30
150	70	30

Free radical-scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay is a popular method in natural product antioxidant studies. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers [9]. The assay was carried out according to Sarker *et al*. [10,11]. The stock solution of DPPH was prepared at the concentration of 8.0×10^{-2} mg/mL in methanol. The extract dilutions were made in methanol to get the concentrations of 5.0×10^{-1}, 2.5×10^{-1}, 1.25×10^{-1}, 6.25×10^{-2}, 3.13×10^{-2} and 1.6×10^{-2} mg/mL. The prepared solutions of extracts (2.0 mL each) were mixed with DPPH solution (2.0 mL). After thirty minutes, UV absorbances of the solutions were recorded at 517 nm. Butylated hydroxytoluene (BHT), a synthetic antioxidant, was used as the positive control. Inhibition of DPPH free radical was calculated as:

Inhibition % =100-[(Sample absorption-control absorption)/Blank absorption] ×100

The concentration that caused 50% decrease in the initial DPPH radical concentration was defined as IC_{50}. The experiments were repeated tree times and the IC_{50} values were expressed as Mean ± SD.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of the plant extracts were evaluated according to the method of Benzie and Strain [12]. The FRAP reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3$ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10 (v/v). Fifty µL of each diluted extracts were mixed with 3 mL of freshly prepared FRAP reagent and the reaction mixtures were incubated at 37 °C for 30 min. The absorbance was determined at 593 nm against distilled water as blank. Aqueous solutions of ferrous sulfate (100–2000 µM) were used for

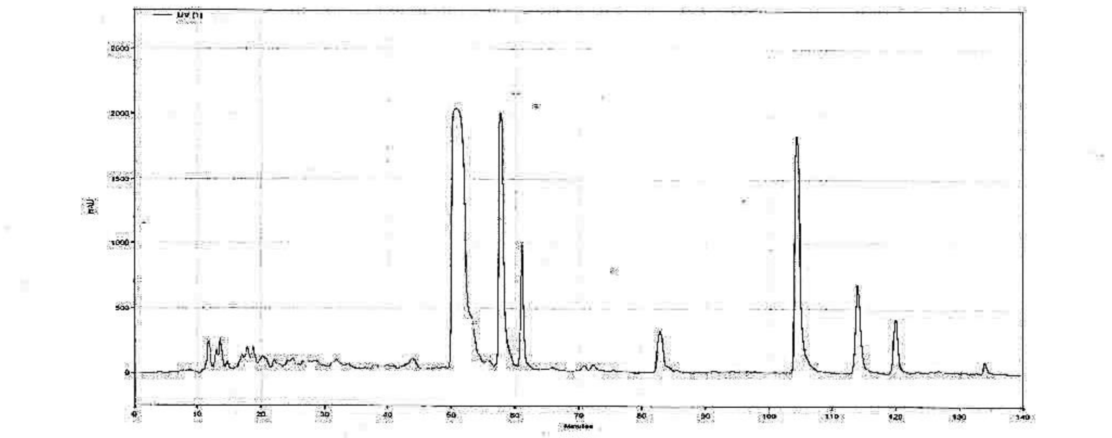

Figure 1. HPLC chromatogram with retention times of constituents (5-10) of *Lallemantia iberica* aerial parts at 350 nm

calibration. Triplicate measurements were taken and the FRAP values were expressed as μmol of Fe (II)/g dry weight of plant powder [13,14]

Results and Discussion

Phytochemical studies of the ethyl acetate and methanol extracts of the aerial parts of *L. iberica* resulted in isolation of β-sitosterol acetate (**1**), β-sitosterol (**2**), ursolic acid (**3**), rosmarinic acid (**4**), luteolin-7-*O*-glucoside (**5**), 4'-methoxy-luteolin-7-*O*-glucoside (**6**), apigenin-7-*O*-glucoside (**7**), luteolin (8), diosmetin (9), apigenin (10) (figure 2).

The structures of the isolated compounds were determined using ^{1}H-NMR, ^{13}C-NMR and EI-MS spectral analyses, and also by comparison with published information. Some of the structures of isolated compounds such as β-sitosterol acetate (1), β-sitosterol (2), ursolic acid (3), rosmarinic acid (4), luteolin (5), apigenin (10) have been previously reported from other plants [15-23]; however, this is the first report about the isolation of compounds 1-10 from the aerial parts of *L. iberica*. ^{1}H-NMR, ^{13}C-NMR, MS data for compounds 6-9 have been presented as follows:

Compound (6): luteolin-7-*O*-glycoside; ^{1}H-NMR (500 MHz , DMSO d6): δH 6.80 (1H, *s*, H-3), 6.44 (1H, *d*, J = 2 Hz, H-6), 6.76 (1H, *d*, J = 2 Hz, H-8), 7.42 (1H, *bs*, H-2'), 6.90 (1H, *d*, J = 8.4 Hz, H-5′), 7.44 (1H, *dd*, J = 8.4 Hz, H- 6′), 12.98 (1H,*s*, OH-5), 5.06 (1H, *d*, J = 7.4 Hz, Glu H-1"), 3.17-5.06 (6H, *m*, sugar protons) [15, 16].^{13}C-NMR (125 MHz, DMSO d6): δC 182.03 (C-4), 164.58 (C-2), 163.03 (C-7), 161.25 (C-5), 157.04(C-9), 150.06 (C-4′), 145.90 (C-3′), 121.43 (C-1′), 119.28 (C-6′), 116.10 (C-5′), 113.62 (C-2′), 105.42 (C-10), 103.22 (C-3), 99.62 (C-6) 94.84 (C-8) and sugar: 99.94 (C-1"), 73.19 (C-2"), 77.22 (C-5"), 76.44 (C-3"), 69.62 (C-4"), 60.68 (C-6")[15-17]. EI-MS: m/z (%): 286 [aglycon fragment] $^{+}$, 152 [A1] $^{+}$, 137 [B2] $^{+}$, 109 [B2- CO]$^{+}$ [15].

Compound (7): 4'-methoxy-luteolin-7-*O*-glucoside; ^{1}H-NMR (500 MHz ,DMSO d6): δH 6.85 (1H, *s*, H-3), 6.45 (1H, *d*, J = 2 Hz, H-6), 6.82 (1H, *d*, J = 2 Hz, H-8), 7.46 (1H, *bs*, H-2′), 7.10 (1H, *d*, J = 8.4 Hz, H-5′), 7.57 (1H, *bd*, J = 8.4 Hz, H- 6′), 12.95 (1H,*s*, OH-5), 3.87 (3H,*s*, O-Me), 5.05 (1H, *d*, J = 6.6 Hz, Glu H1"), 3.5-5.05 (6H, *m*, sugar protons) [15].^{13}C NMR (125MHz,DMSO d6): δC 182.00(C-4), 164.12 (C-2), 163.03 (C-7), 161.14 (C-5), 157.00(C-9), 151.34 (C-4′), 146.82 (C-3′), 118.02 (C-1′), 122.88 (C-6′), 112.13 (C-5′), 113.13 (C-2′), 105.40 (C-10), 103.83 (C-3), 99.58 (C-6) 94.81 (C-8) and sugar: 99.86 (C-1"), 73.11 (C-2"), 77.17 (C-5"), 76.40 (C-3"), 69.52 (C-4"), 60.60 (C-6"),55.82(4′- O-Me) [17]. **Compound (8):** diosmetin; ^{1}H-NMR (500 MHz, DMSO d6): δH 6.75 (1H, *s*, H-3), 6.19 (1H, *d*, J = 1.85 Hz, H-6),

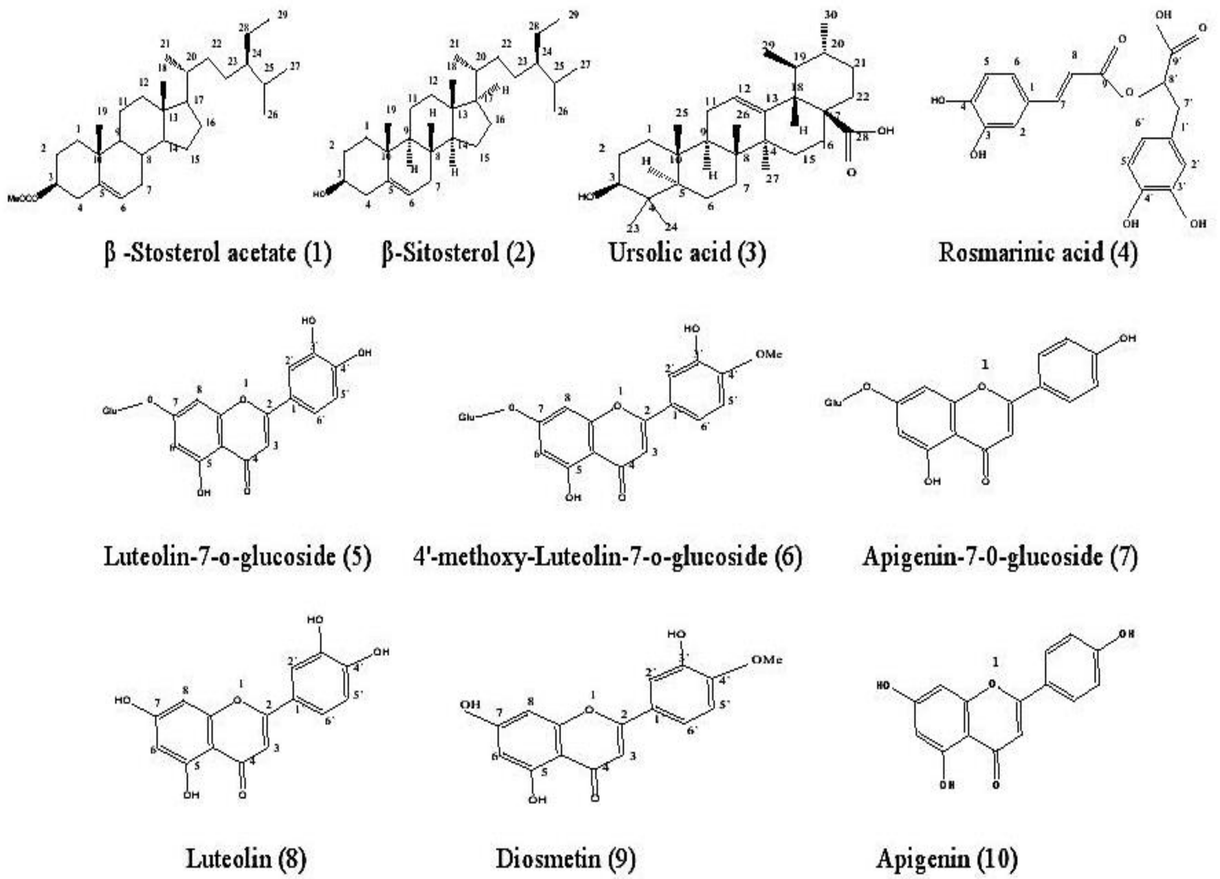

Figure 2. Structures of the isolated compounds (1-10) form the aerial parts of *Lallemantia iberica*

6.47 (1H, *d*, J = 1.95 Hz, H-8),7.43 (1H, *d*, J=2.25Hz, H-2′), 7. 08 (1H, *d*, J = 8.5 Hz, H-5´), 7.53 (1H, *bd*, J = 8.4 Hz, H- 6´), 12.93 (1H, *s*, OH-5), 3.86 (3H, *s*, 4′-*O*-Me) [18]. ^{13}C NMR (125 MHz , DMSO d6): δC 181.62(C-4), 163.45 (C-2), 164.41 (C-7), 161.41 (C-5), 157.29 (C-9), 151.10 (C-4′), 146.78 (C-3′), 122.99 (C-1′), 118.64 (C-6′), 112.15 (C-5′), 112.92 (C-2′), 103.48 (C-10), 103.47 (C-3), 98.90 (C-6) ,93.90 (C-8) ,55.73 (4′- *O*-Me) [17]. **Compound (9):** apigenin-7-*O*-glycoside; ^{1}H-NMR (500 MHz ,DMSO d6): δH 7.94 (2H, *d*, J = 8.4Hz, H-2′ ,6′), 6.95(2H, *d*, J = 8.4Hz, H-5′, 3′), 6.87 (1H, *s*, H-3), 6.83 (1H, *bs*, H-8), 6.44 (1H, *bs*, H-6), 12.93(1H,*s*, OH-5), 5.06 (1H, *d*, J = 7.4 Hz, Glu H-1"), 3.17-5.06 (6H, *m*, sugar protons) [15]. ^{13}C-NMR (125 MHz , DMSO d6): δC 181.97 (C-4), 164.23 (C-2), 162.93 (C-7), 156.91 (C-4′), 161.34 (C-5), 161.08 (C-9), 128.14 (C- 2'), 128.58 (C-6′), 121.00 (C-1′), 115.97 (C-3′), 116.56 (C-5'), 105.31 (C-10), 103.08 (C-3), 99.88 (C-6), 94.81 (C-8) and sugar: 99.49 (C-1"), 77.15 (C-5"), 76.42 (C-3"), 73.07 (C-2"), 69.52 (C-4"), 60.58 (C-6") [15,17]. EI-MS: m/z (%) 270 [aglycon fragment] $^{+}$, 256 [270 -CH2] $^{+}$, 153 [A1+H] $^{+}$, 121 [B2] $^{+}$, 94 [B2 +H -CO]$^{+}$ [15].

The results of the antioxidant activity of the extracts have been reported in table 2.

There are limited studies about the genus

Lallemantia. One study has explained that *L. royleana* seeds decreased the serum cholesterol and triglyceride levels in hypercholesterolemic animals [24].

Table 2. Antioxidant activity of different extracts of *L. iberica* by different methods

Samples	DPPH free radical scavenging activity IC_{50} (μg/mL)	FRAP value (μmol Fe^{2+}/ mg DW)
Methanol extract	140±1.2	553.14
Ethyl acetate extract	189.95±2.8	300.28
BHA	100±1.6	558.36

In Another research, antifungal and antibacterial activities of *L. royleana* essential oil were investigated. It has been introduced as antimicrobial additive in foods [25]. The methanol extract of *L. iberica* has shown antinociceptive effect in male rats [26]. In the present research, the constituents of *L. iberica* have been investigated and there are different studies about the biological activities of its isolated components. For example some studies have confirmed the biological activities of β-sitosterol such as its effects in prostate hyperplasia, and breast cancer [27,28]. In previous researches, the antioxidant and anti-inflammatory activities of ursolic acid were shown [29,30]. Antiviral, antibacterial, anti-inflammatory and antioxidant activities of rosmarinic acid were investigated in different previous works [31]. Some studies have elucidated the biological activities of flavonoids such as their antioxidant, anti-atherosclerose, anti-inflammatory, antitumor, antiviral and antifertility properties [32,33]. Accordingly, most isolated compounds from *L. iberica* have shown antioxidant effects and our results confirmed the antioxidant activity of *L. iberica*.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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