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Isolation of secondary metabolites from the Iranian medicinal plant *Eremurus persicus*

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Abstract: *Eremurus persicus* (Jaub. & Spach) Boiss. belonging to Xanthorrhoeaceae family is an endemic medicinal plant widely distributed in Iran. Its leaves have been traditionally used as a food and also as medicinal plant. Regarding the widespread application of *E. persicus* in Iranian folk medicine, and the insignificant investigation of its components, this study aimed at the isolation and identification of major secondary metabolites of this plant. By applying various chromatographic techniques, corchoionoside A (**1**), 4-amino-4-carboxychroman-2-one (**2**), isoorientin (**3**), ziganein 5-methyl ether (**4**), auraptene (**5**), and imperatorin (**6**) were isolated from the EtOAc and $CHCl_3$ fractions of the crude extract. Except isoorientin (**3**), all the identified phytoconstituents were reported for the first time from *Eremurus* genus.

Keywords: *Eremurus persicus*; Iranian medicinal herb; isoorientin; secondary metabolites.

1 Introduction

The genus *Eremurus* (Xanthorrhoeaceae, syn. Asphodelaceae family) comprises 50 species, mainly distributed in Central and Western Asia [1–4]. Some species have been used in Turkish, Iranian and Chinese folk medicine. The roots of *Eremurus chinensis* Fedtsch. and *Eremurus anisopterus* (Kar. & Kir.) Regel have been used to treat rheumatism and physical weaknesses in China [3, 5]. The leaves of *Eremurus spectabilis* (Bieb.) Fedtsch. have traditionally been consumed in Turkish and Iranian folk medicine as food additive [6], to treat scabies, rheumatism, diabetes, for intestinal, liver and stomach disorders [7, 8]; to cure haemorrhoids, hypertension [9], eye inflammation, constipation, colon pain, and also in case of snake bite or scorpion sting [7, 10, 11]. *E. spectabilis* has been used in Iranian folk medicine as a remedy of jaundice, pimples, bone fractures, dermal infections, and for its purported anti-hyperlipidemic effect [12, 13].

The roots of *Eremurus persicus* (Jaub. & Spach) Boiss. are used in Turkish folk medicine for relieving rheumatism, gastrointestinal disorders [14], against scabies [7] and inflammatory skin conditions [15, 16]. This species is widely distributed and consumed in Iran [10]. The leaves are traditionally eaten with rice [17], also used as a remedy of constipation, diabetes, different disorders of liver, stomach and the genitourinary system [18, 19], atherosclerosis, inflammation-related diseases, as well as against fungal skin diseases [18, 20–22], and as diuretic [23].

Among *Eremurus* species, biological and pharmacological activities of different extracts of *E. spectabilis* populations have been widely investigated. Antioxidant and antiradical activities of roots and leaves [9, 22, 24–26], antiproliferative effects [11, 22, 26, 27], cytotoxic, NO production inhibitory activity [28], antibacterial [4, 25, 26] and gastroprotective properties [29] of *E. spectabilis* extracts have been reported in the literature. Analysis of antioxidant potential of root extract of *E. chinensis* Fedtch. [27], protein tyrosine phosphatase inhibitory activity of *Eremurus altaicus* (Pall.) Stev. [30], and *in vivo* hypoglycemic effect of the methanol extracts of *Eremurus himalaicus* [31] have also been documented.

In case of *E. persicus*, chloroform extract of aerial parts was previously demonstrated to have antiglycation [10], also anti-inflammatory [1], antiradical, anti-inflammatory and antiproliferative effects [22] of ethanolic root extract, dihydrofolate reductase of methanolic extract [32], antifungal of aerial parts methanolic extract [21], antileishmanial of root ethanolic extract [33], antibacterial, and cytotoxic activities of methanolic extract aerial part [19] have been evaluated.

Furthermore, antioxidant, antimicrobial, anticancer, acetylcholinesterase inhibitory activities and anti-dermatophyte effects of its essential oil were previously studied [34]. These activities somewhat explain the folk

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medicinal uses (e.g. in diabetes and fungal skin diseases) of the plant.

The analysis of phytochemical composition *Eremurus* spp. revealed the presence of anthranoid derivatives as characteristic components in the genus. Chrysophanol [5, 28, 30, 35], chrysophanol 8-methyl ether, aloesaponol III 8-methyl ether, 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene [5, 30, 35], along with methyl linolenate, β-sitosterol, isoorientin and inosine [28], a bi-anthraquinone glycoside 2-acetyl-1,8-dimethoxy-3-methylnaphthalene, a pre-anthraquinone 10-(chrysophanol-7′-yl)-10-hydroxychrysophanol-9-anthrone [5], altaicusin A, emodin [30], aloesponol III, and (R)-aloechrysone [35] were isolated from different species of *Eremurus* genus. Total phenolic contents of various extract of *E. spectabilis* [9, 22, 25] were also evaluated by Folin-Ciocalteu's method.

The phytochemistry of *E. persicus* has been scantily studied. Prior to our work only four secondary metabolites, namely, (R)-(−)-aloesaponol III 8-methyl ether [33], helminthosporin [36], 5,6,7-trimethoxy-coumarin [10], and 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene [36] were isolated from the leaves of *E. persicus*.

Regarding the widespread application of *E. persicus* in Iranian folk medicine, and the lack of phytochemical data, this study aimed at the isolation and identification of major secondary metabolites of aerial parts of *E. persicus*.

2 Materials and methods

2.1 Plant material

Aerial parts of *E. persicus* (1.8 kg) were harvested by J.M. in Iran (Neyriz, Fars, Iran) in July 2018 in flowering stage. The plant was identified by Dr. Mohammad Jamal Saharkhiz (Department of Horticultural Science, Faculty of Agriculture, Shiraz University, Iran), and a voucher specimen (no. 880) has been deposited in the Herbarium of Department of Pharmacognosy, University of Szeged.

2.2 General procedures

NMR spectra were recorded in CD_3OD and $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). The peaks of the residual solvents (δ_H 3.31 and 7.26, δ_C 49.0 and 77.2, respectively) were taken as reference. The data were acquired and processed with MestReNova v6.0.2e-5475 software. Chemical shifts are expressed in parts per million and coupling constant values (J) are reported in Hz. Analytical grade solvents were used in the isolation procedure (Molar Chemicals Kft, Halasztelek, Hungary). Pure compounds were isolated by using flash chromatography (FC, Biotage® Instrument, Isolera™ Spektra Systems with ACI™ and Assist) with integrated UV–Vis, using normal phase flash columns (10, 50, 80, and 100 g) (Biotage® SNAP cartridge, KP-Sil), medium pressure liquid chromatography (MPLC, silica gel 60, 0.045–0.063 mm, Merck, Darmstadt, Germany), gel chromatography (Sephadex® VR LH-20, Pharmacia, Uppsala, Sweden), normal (Silica gel 60, Merck, Darmstadt, Germany) and reverse phase (Silica gel 60 RP-18 F254s, Merck, Darmstadt, Germany) preparative thin layer chromatography (PTLC and RP-PTLC, respectively), centrifugal PTLC (Silica gel 60 GF254, Merck, Darmstadt, Germany) (CPTLC).

2.3 Isolation of phytochemicals

The plant material (flowers, stems and leaves) were shade-dried at room temperature, then grinded, and extracted with methanol (50 L). The obtained crude extract (127.75 g) was filtrated and concentrated under reduced pressure.

The extract was dissolved with methanol–water 1:1 (1 L) and was subjected to liquid-liquid partition with *n*-hexane (4 × 1 L), $CHCl_3$ (4 × 1 L), and EtOAc (4 × 1 L). The EtOAc soluble extract (3.5 g) was initially separated by flash chromatography with a gradient solvent system composed of increasing ratio of $CHCl_3$–MeOH (1:1) from 20 to 100% in *n*-hexane. Column fractions with similar TLC patterns were combined into six major fractions E_1–E_6. By applying flash chromatography with increasing concentration of $CHCl_3$–MeOH (9:1) from 10 to 100% in *n*-hexane, E_1 was separated to six fractions (E_{11}–E_{16}). E_{15} was further chromatographed by flash chromatography using the same solvent system to afford four subfractions (E_{151}–E_{154}). E_{153} was separated to 60 subfractions with CPTLC by increasing MeOH in toluene (0–100%). Subfraction 39–49 was purified to yield compound **1** (1.41 mg) by RP-PTLC (MeOH–H_2O 1:1).

E_{16} was subjected to CPTLC with two gradient solvent systems; first with increasing ratio of solvent mixture of EtOAc–acetone (1:1) 50–100% in *n*-hexane, then increasing MeOH (0–50%) in EtOAc–acetone (1:1). From the obtained six fractions (E_{161}–E_{166}), E_{166} was further fractionated by means of Sephadex LH–20 (eluent: MeOH) to obtain compound **2** (1.24 mg).

MPLC was used for the separation of fraction E_2 with increasing concentration of MeOH (0–100%) in EtOAc–*n*-hexane (1:2). From the eight collected fractions (E_{21}–E_{28}), E_{24} was subsequently chromatographed to 40 subfractions by Sephadex LH–20 (eluent: MeOH) to obtain 40 fractions. Subfractions 25 and 26 contained compound **3** (22.22 mg).

The $CHCl_3$-soluble fraction (4.7 g) was separated to seven major fractions (C_1–C_7) by applying MPLC eluting with EtOAc in *n*-hexane (0–50%), then with increasing ratio of MeOH (0–100%) in EtOAc–*n*-hexane (1:1). By using Sephadex LH–20 (eluent: MeOH), C_4 was also separated to nine sub-fractions, and sub-fractions 22–24 afforded compound **4** (1 mg). C_7 was fractionated by Sephadex LH–20 (eluent: CH_2Cl_2–MeOH 1:1) to get three major fractions (C_{71}, C_{72} and C_{73}). C_{72} was purified with the same method to yield five fractions (C_{721}–C_{725}). By applying CPTLC with MeOH in toluene (10–100%) as a gradient solvent system, 38 subfractions were afforded. Subfraction 1–12 was chromatographed by PTLC with toluene–MeOH (9.8:0.2), and pure compounds **5** (1.03 mg) and **6** (0.95 mg) were finally isolated.

3 Results

3.1 Isolation of secondary metabolites

The use of successive chromatographic techniques led to the isolation of six pure compounds. The obtained compounds were identified by 1D (^{1}H, ^{13}C) and 2D (^{1}H–^{1}H COSY,

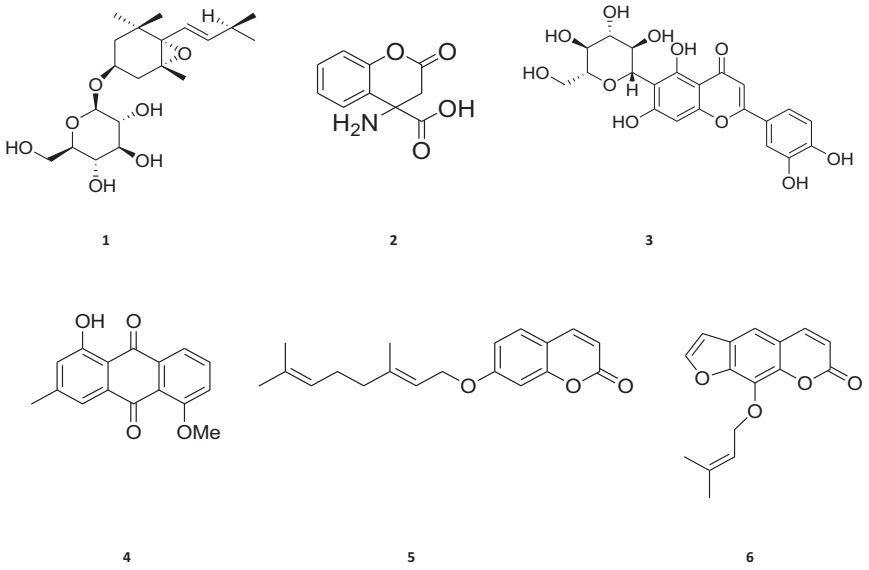

Figure 1: The chemical structures of the isolated phytochemicals from *Eremurus persicus*.

HSQC, HMBC, NOESY) NMR spectral analysis, and by comparison of the 1D NMR data with those reported in the literature.

A rare glucoside aliphatic alcohol, corchoionoside A (**1**) [37, 38], a rare dihydro-coumarin, 4-amino-4-carboxychroman-2-one (**2**) [39, 40], the flavone *C*-glycoside isoorientin (**3**) [41], a very scarce anthraquinone, ziganein 5-methyl ether (**4**) [42], and two coumarin derivatives, namely, auraptene (**5**) [43, 44], and imperatorin (**6**) [45] were isolated from *E. persicus*. With the exception of isoorientin (**3**), all the compounds are reported for the first time in the *Eremurus* genus. The chemical structures of the isolated compounds are presented in Figure 1.

4 Discussion

The phytochemical constituents of *Eremurus* species have been rarely investigated. In general, isoorientin and methylnaphthalene derivatives have been identified as the predominant phytoconstituents of the plants belonging to this genus [5, 28, 30, 35]. In our study, by extraction of $CHCl_3$ and EtOAc soluble fractions, six pure compounds including the megastigmane glycoside corchoionoside A (**1**), 4-amino-4-carboxychroman-2-one (**2**), isoorientin (**3**), ziganein 5-methyl ether (**4**), auraptene (**5**), and imperatorin (**6**) were obtained from *E. persicus*. Among the identified compounds, only isoorientin (**3**) has been previously reported from the *Eremurus* genus, while all the other secondary metabolites are new in the genus.

The laxative effect of *E. persicus* is related to its anthranoid constituents. Here we report the presence of a rare member of this group, ziganein 5-methyl ether (**4**), which was previously reported only from *Aloe hijazensis* [42]. 4-Amino-4-carboxychroman-2-one, previously identified only from *Prunus domestica* and *Centipeda minima* [39, 40], may play role in the antioxidant effect of the plant. The pharmacological effects of corchoionoside A (**1**) have not been studied so far, however, considering the antidiabetic effect of megastigmanes [46], the presence of this type of compounds in the plant may contribute to the *in vitro* antiglycation activity of *E. persicus*, and could be related to the folk medicinal use of it as antidiabetic remedy [47]. Isoorientin (**3**) was identified as a major compound of this species. Antinociceptive, anti-inflammatory [48], antiproliferative [49, 50], and gastroprotective effects [29] of this compound were reported previously. These activities are in accordance with the previously reported anti-inflammatory and anti-proliferative effects of the plant [1, 22]. Hopefully our results will contribute to the better understanding of the mechanisms of action of this plant.

Author contributions: J. M. did the phytochemical experimental work and drafted the manuscript, N. K., J. H. and Y.-C. T. recorded and analyzed the NMR spectra, D. C. checked and completed the manuscript.

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