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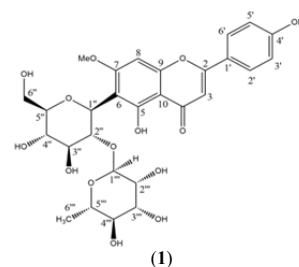
In the first phytochemical investigation of non-volatile secondary metabolites from the Kurdish traditional plant *Iris persica* L., (-)-embinin was isolated from flowers and leaves, isovitexin from flowers, *trans*-resveratrol-3-*O*- β -D-glucopyranoside from rhizomes and tectorigenin from bulbs. The complete NMR spectra of embinin are reported for the first time. In an MTT assay, embinin showed an inhibition activity higher than the well-known antitumor drug cisplatin against five of the six tested human tumor cells. Moreover, embinin showed a significant DPPH radical scavenging activity (IC₅₀ value of 112.16) comparable to the reference antioxidant ascorbic acid. The remarkable biological activities exhibited by the extracts of *Iris persica* and isolated compounds have validated the uses of *I. persica* in the traditional medicine of Kurdistan.

Keywords: (-)-Embinin, *Iris persica*, Cytotoxicity, Antiradical activity, Kurdish medicinal plant.

Iris persica L. grows rather widely in Halgurd mountain (Choman) and Korek mountain (Rawanduz) of Kurdistan/Iraq, where it is called "Sausan". The plant is commonly employed in the Kurdish traditional medicine for curing wound inflammations and tumors. As part of our ongoing project on scientific validation of Kurdistan traditional plants [1], flowers, leaves, bulbs and rhizomes of *I. persica* were collected in Korek mountain and submitted to the first phytochemical investigation of non-volatile secondary metabolites. Several *Iris* species have demonstrated to contain a wide variety of bioactive natural products [2]. Therefore, the cytotoxic and antioxidant activities of some isolated compounds and extracts have also been tested.

Repeated MPLC chromatographic separation on reversed-phase columns of the residue resulting from evaporation of the chlorophyll-free methanolic extract of flowers, afforded (-)-embinin {(-)-5-hydroxy-7,4'-dimethoxyflavone-6-*C*-[*O*-(α -L-rhamnopyranosyl)-1 \rightarrow 2- β -D-glucopyranoside] (1) and 6-*C*-glucosylapigenin (isovitexin) [3]. Embinin (1) was also isolated from the methanolic extract of leaves. The stilbene derivative, *trans*(*E*)-resveratrol-3-*O*- β -D-glucopyranoside [(*E*)-piceid, (*E*)-polydatin] [4] and the isoflavone tectorigenin [5] were isolated from the methanolic extracts of rhizomes and bulbs, respectively. The structures of isolated compounds were established by extensive 1D- and 2D-NMR experiments and comparison of the spectroscopic data with the literature. (-)-Embinin (1), isovitexin, *trans*-resveratrol-3-*O*- β -D-glucopyranoside, and tectorigenin have been isolated for the first time from *I. persica*, though they have previously been isolated from other *Iris* species.

Embinin (1) was first isolated by Hirose in 1962 from *Iris tectorum*, but the structure was incorrectly determined at that time [6a]. Subsequently, embinin was reisolated from *I. germanica* [6b] and from *Siphonoglossa sessilis* [6c], and eventually the structure was correctly elucidated as 5-hydroxy-7,4'-dimethoxyflavone-6-*C*-[*O*-(α -L-rhamnopyranosyl)-1 \rightarrow 2- β -D-glucopyranoside (1). The ¹H NMR data of an embinin derivative [6c] and the NMR spectra of embinin in a mixture with apigenin 4'-methyl ether 6-*C*-glycoside-



2"-*O*-rhamnoside [7] are found in the literature; however, to the best of our knowledge, the complete NMR data of a pure isolated sample of embinin has not yet been reported. Therefore, in this paper we report the full assignment of ¹H and ¹³C NMR spectral data of 1.

The effects of two isolated compounds, embinin (1) and tectorigenin, on the proliferation of six human tumor cell lines, MCF7 and SkBr3 breast, endometrial Ishikawa, ovarian BG-1, mesothelioma IST-MES1 and lung A549 cells, were evaluated in comparison with standard drug, *cis*-diamminedichloroplatinum (II) (cisplatin) by an MTT assay [8]. Tumor cells were treated for 48 h with increasing concentrations of tested compounds. IC₅₀ (μ M) values are reported in Table 1. Tectorigenin exhibited weak activity against all six lines, with IC₅₀ values >50. In contrast, 1 showed a remarkable cytotoxicity, which was even higher than cisplatin, against five cell lines, i.e., MCF7, SkBr3, Ishikawa, BG-1, and A549 tumor cells (Table 1). To the best of our knowledge this is the first report of the cytotoxic properties of embinin (1). It is worth noting that the high cytotoxicity of 1 stands in striking contrast with the activities from weak to moderate shown by some acyl embinin derivatives [6d-f].

Table 1: Cytotoxicity (MTT assay) of isolated compounds from *Iris persica*.

Sample	IC ₅₀ (μ M) \pm S.D					
	MCF7	SkBr3	IST-MES1	BG-1	Ishikawa	A549
Embinin (1)	6 (\pm 3)	4 (\pm 1)	10 (\pm 3)	8 (\pm 2)	7 (\pm 2)	9 (\pm 1)
Tectorigenin	>50	>50	>50	>50	>50	>50
Cisplatin	17 (\pm 4)	10 (\pm 2)	10 (\pm 3)	12 (\pm 3)	12 (\pm 2)	13 (\pm 2)

Radical scavenging activity (DPPH test): In search of new source of natural antioxidants, the free radical scavenging effects of embinin (**1**) and extracts of flowers, leaves, bulbs, rhizomes of *I. persica*, corresponding to the quenching efficiency of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, were evaluated by a UV spectroscopic assay [9]. Ascorbic acid was used as the reference compound. The DPPH radical scavenging activity of the MeOH extract of the flowers (IC₅₀ 75.14 µg/mL) was higher than that of ascorbic acid (IC₅₀ 112.74 µg/mL), indicating that this extract is a potential source of potent antioxidant compounds. The MeOH extract of the leaves also showed a significant DPPH radical scavenging activity (IC₅₀ 139.76 µg/mL), while the DCM (IC₅₀ 153.90 µg/mL) and MeOH (IC₅₀ 201.87 µg/mL) extracts of the bulbs, as well as the MeOH extract (IC₅₀ 211.03 µg/mL) of rhizomes were only moderately active. The scavenging activity of **1** (IC₅₀ = 112.16 µg/mL) was in nice agreement with the activity of the extracts of flowers and leaves; however, the higher activity displayed by the extract of flowers clearly indicated that other more potent antioxidant compounds, still unidentified, likely occur in the extract. In conclusion, the biological activities determined for the extracts and pure compounds isolated from *I. persica* have validated the traditional medicinal use of this plant in Kurdistan.

Plant material and General experimental procedures: *Iris persica* L. (voucher specimen No. 7229, deposited at the Education Salahaddin University Herbarium (ESUH)) was collected in April 2014 from Korek Mountain in Iraqi Kurdistan region. The plant was identified Prof. Dr. Abdul Hussain Al-khayyat at Salahaddin University-Erbil/Iraq.

For details on the extraction of flowers, leaves, bulbs and rhizomes, as well as the isolation of individual compounds, see the

Supplementary Material. For most experimental techniques and procedures, see reference [10].

(-)-Embinin (**1**)

MP: 179-182°C

[α]_D²⁰: -9.52 (c 0.010, MeOH).

R_f: 0.53 on a RP-18 TLC plate eluted with MeOH-H₂O (8:2).

IR (KBr): 3414, 1704, 1654, 1606, 832 cm⁻¹.

UV λ_{max} (MeOH) nm (log ε): 272, 333.

¹H NMR (300 MHz, pyridine-*d*₅, 22°C): δ 1.24, 1.27* (3H, d, J = 6.2 Hz, H₃-6''), 3.21, 3.30* (1H, m, H-5''), 3.58, 3.75* (3H, s, 4'-OMe), 3.74, 3.82* (3H, s, 7-OMe), 4.07-4.82 (3H, m, H-2'', 3'', 4''), 4.20-4.59 (5H, m, H-3'', 4'', 5'', 6''), 5.20 (1H, t, J = 9.2 Hz, H-2''), 5.62, 5.72* (1H, d, J = 9.8 Hz, H-1''), 6.30, 6.43 (1H, d, J = 1.1 Hz, H-1''), 6.91, 6.94* (1H, s, H-8), 7.01, 7.04 (1H, s, H-3), 7.09 (2H, d, J = 8.9 Hz, H-3', 5'), 7.93 (2H, d, J = 8.9 Hz, H-2', 6'), 14.31 (1H, s, 5-OH).

¹³C NMR (75 MHz, pyridine-*d*₅, 22°C) δ 18.9 (CH₃, C-6''), 55.9 (CH₃, 7-OMe), 56.6 (CH₃, 4'-OMe), 63.9 (CH₂, C-6''), 70.0 (CH, C-5''), 72.9 (CH, C-2''), 73.3 (CH, C-4''), 74.1 (CH, C-1''), 74.1 (CH, C-3''), 74.1 (CH, C-4''), 76.6 (CH, C-2''), 82.1 (CH, C-5''), 83.4 (CH, C-3''), 91.5 (CH, C-8), 102.6 (CH, C-1''), 105.1 (CH, C-3), 106.4 (C, C-10), 111.8 (C, C-6), 115.2 (2xCH, C-3', 5'), 129.0 (2xCH, C-2', 6'), 124.5 (C, C-1'), 158.4 (C, C-9), 163.4 (2xC, C-7, 4'), 164.5 (C, C-2), 164.8 (C, C-5), 183.6 (CO, C-4). The number of protons attached to the corresponding carbons were determined by DEPT experiments. *Signals attributed to minor rotamer.

LC-ESI-MS (positive-ion mode): *m/z* 629 [M+Na]⁺.

LC-ESI-MS/MS of the ion at *m/z* 629: *m/z* 611 [(M+Na)-18 (H₂O)]⁺, 483 [(M+Na)-146 (rhamnose)]⁺, 465 [483-18 (H₂O)]⁺. Anal. Calcd for C₂₉H₃₄O₁₄: C, 57.42; H, 5.65. Found C, 57.32; H = 5.77.

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