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Isoflavonoids from the rhizomes of *Iris hungarica* and antibacterial activity of the dry rhizomes extract

Isoflavonoides de los rizomas de *Iris hungarica* y actividad antibacteriana del extracto de rizomas seco

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The author have declared that no financial relationships with any organizations that might have an interest in the submitted work; nor any other relationships or activities that could appear to have influenced the submitted work.

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Aim: The aim of the work was isolation and identification of the phenolic compounds from the rhizomes of *Iris hungarica*.

Materials and methods: To establish by chemical and spectral methods for the structures of phenolic compounds, which were isolated from the rhizomes of *Iris hungarica* Waldst. et Kit. (*Iridaceae* family). Compounds were obtained by column chromatography on silica gel and their structures were determined by UV, IR, MS, ^{1}H-NMR spectra methods. Preliminary screening of antibacterial activity was determined.

Results: From the ethanolic extract of the rhizomes of *I. hungarica*, which is widespread in Ukraine, for the first time two new for this species isoflavones, tectorigenin and tectoridin and xanthone mangiferin, together with known isoflavonoids daidzein, genistein, formononetin were isolated. The dry extract of the rhizomes of *I. hungarica* at a concentration of 1% has shown the highest inhibitory activity for Gram-positive bacteria and fungi.

Keywords: isoflavonoids; tectorigenin; tectoridin; mangiferin; *Iris hungarica;* antibacterial activity.

RESUMEN

Objetivo: El objetivo del trabajo es el aislamiento y la identificación de los compuestos fenólicos de los rizomas de *Iris hungarica*.

Materiales y métodos: Se utilizaron métodos químicos y espectrales para conocer las estructuras de compuestos fenólicos que se aislaron de los rizomas de *Iris hungarica* Waldst. et Kit. (Familia de *Iridaceae*). Los compuestos se obtuvieron mediante cromatografía en columna sobre gel de sílice y se determinaron sus estructuras mediante análisis de sus espectros por UV, IR, MS, 1H-RMN. Se determinó el cribado preliminar de la actividad antibacteriana.

Resultados: Se aislaron por primera vez de los rizomas de *Iris hungarica* (común en Ucrania) dos isoflavonas, tectorigenina y tectoridina (nuevas para esta especie), el xantonoide mangiferina y los isoflavonoides daidzeina, genisteina y formononetina. El extracto seco de los rizomas de *I. hungarica* a una concentración de 1% ha mostrado la actividad inhibitoria más alta para bacterias y hongos Gram-positivos.

Palabras claves: isoflavonoides; tectorigenina; tectoridina; mangiferina; *Iris hungarica*; actividad antibacteriana.

INTRODUCTION

Iris hungarica Waldst. et Kit. (syn. *Iris aphylla* L.) is a perennial rhizomatous plant, growing to 30 cm high with a long rhizome belong to the family *Iridaceae* and widely distributed in most parts of the world[1]. Geographical distribution in Europe[2,3]: Spain, Czech Republic, France, Germany, Hungary, Italy, Poland, Romania, Ukraine[4,5].

Previous phytochemical investigations of the *Iris* plants have resulted in the isolation of a variety of compounds including flavonoids[6], isoflavones (irigenin, nigricin, irisflorentin, iriskumaonin, irilon, iriflogenin, etc.)[7] and their glycosides; C-glucosylxanthones[8], quinones[9], triterpenoids[10], stilbene glycosides[11]. The rhizomes of irises also contain carbohydrates, fatty oil, organic acids, tannins and essential oil, which is used in perfumery and cosmetics[12,13], while the leaves are a source of ascorbic acid and vitamins[11].

The dried rhizomes of iris has been used in folk medicine of European countries as a diaphoretic for bronchitis, in dental practice – in order to accelerate teething in infants; as anti-inflammatory for the treatment of pancreatic and salivary glands, and the vegetative neurosis[8,14]. The iris essential oil has expectorant properties[10,13]. The compounds isolated from iris species have piscicidal, antineoplastic[8], antiplasmodial, antituberculosis[15,16], antioxidant[17], antimicrobial, anti-tumor[18] effects.

The literature data analysis of the chemical composition of the rhizomes of *I.hungarica* has shown that the plant is almost not studied. Thus, the aim of the work was the isolation and identification of phenolic compounds from the rhizomes of *I. hungarica (Figure 1)*.

a

b

Figure 1. General view (a) of the living plants and raw materials (b) of *I. hungarica*

MATERIALS AND METHODS

General experimental procedure

^{1}H-NMR spectra (200 MHz) were recorded on a Varian Mercury-VX-200 in DMSO-d_6 with TMS as an internal standard. Low-resolution mass spectra were measured on a GC–MS Varian 1200L (ionizing voltage 70 eV) instrument. UV spectra were recorded on a Carl Zeiss Specord M-80 in EtOH. IR spectra were recorded on a Tensor 27 UR-20 spectrophotometer. Column chromatography (CC) was carried out on silica gel 75 – 150 mesh. Chromatographic testings were carried out on pre-coated thin-layer chromatography (TLC) plates (silica gel 60 F_{254} Merck, Silufol UV_{254}) and paper Filtrak. Compounds on the TLC plates were detected by the characteristic fluorescence in UV-light at the wavelength 365 and 254 nm before and after ammonium vapours, 2% alcoholic solution of aluminium chloride as a spraying reagent. Melting point (°C) was determined on a Kofler block. The recrystallization of selected substances was carried out in 96% ethanol with addition 2-3 drops of water. The substances were dried under vacuum (10-2 mm Hg) over P_2O_5 at 110 – 115°C for 5 h.

Plant Material

The rhizomes of *Iris hungarica* Walds. et Kit. were collected from M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kiev, Ukraine, in May of 2015 and were air-dried. The plant material complies with the description of the Flora Ukraine[4]. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department and Botany Department, National University of Pharmacy, Kharkiv, Ukraine.

Extraction and isolation of compounds

Air-dried rhizomes of *I. hungarica* (1.0 kg) were extracted with ethanol (EtOH) (70%, 5 L) in a percolator for 24 h[19,20]. The extraction was repeated thrice under the same conditions. The aqueous EtOH extracts were combined, filtered, evaporated in a rotary evaporator to 0.5 L of aqueous residue, and left for 1 d. The supernatant liquid was separated. The resulting extract was worked up successively with chloroform ($CHCl_3$), ethyl acetate (EtOAc), and butanol (*n*-BuOH). The resulting extracts were evaporated in vacuum. The qualitative composition of $CHCl_3$, EtOAc and BuOH fractions controlled by paper and thin-layer chromatography in a solvent system butanol – acetic acid – water (BuOH – HAc – H_2O) (4:1:2) (R_{f1}) and chloroform – methanol ($CHCl_3$ – MeOH) (9:1) (R_{f2}).

For further analysis the acid hydrolysis of sum of the phenolic compounds was conducted with 5% H_2SO_4 (2 ml) for 5 h in the water bath. The solution was neutralized with Na_2CO_3 and extracted with EtOAc. Analysis of the sub-

stances was carried out by paper (PC) and (TLC) . By the characteristic fluorescence in UV-light, R_f value and colour of spots on chromatograms after they were exposed to ammonia vapors in comparison with authentic samples and literature data were identified as daidzein, formononetin, genistein[21,22].

EtOAc extract was evaporated by heating under vacuum to complete stripping of solvent and subjected to CC (80×4 cm) on silica gel and eluted with gradient: $CHCl_3$ and ethanol-mixtures with increasing ethanol concentration (0-100%) to afford 50 fractions. Compound 1 (80 mg) detected in fractions of chloroform-ethanol (9:1), compound 2 (85 mg) – chloroform-ethanol (8:2) and compound 3 (100 mg) – chloroform-ethanol (5:1).

Spectral data

Tectorigenin (1) $C_{16}H_{12}O_6$, yellow amorphous powder, m.p. 230-231 °C. M 300 g/mol. MS m/z: 300 (M^+). R_{f1} 0.90, dark; R_{f2} 0.57, dark; UV λ_{max} (C_2H_5OH) nm: 276, 338. IR (KBr), ν, cm^{-1}: 3478 (-OH), 1640 (C=O), 1610, 1512 (C=C), 1063 (-OCH_3). ^{1}H-NMR (200 MHz, DMSO-d_6) δ, ppm: 13.05 (1H, s, 5-OH), 10.75 (1H, s, 7-OH), 9.55 (1H, s, 4'-OH), 8.25 (1H, s, 2-H), 7.35 (2H, d, *J* = 8 Hz, H-2′, 6′), 6.82 (2H, d, *J* = 7.5 Hz, H-3′, 5′), 6.45 (1H, s, 8-H), 3.75 (3H, s, 6-OCH_3).

Tectoridin (2) $C_{22}H_{22}O_{11}$, yellow amorphous powder, m.p. 273-274 °C. M 462,41 g/mol. MS m/z: 300 (–$C_6H_{11}O_5$) (M^+). R_{f1} 0.97, dark; UV λ_{max} (C_2H_5OH) nm: 267, 332. IR (KBr), ν, cm^{-1}: 3373 (-OH), 1658 (C=O), 1612, 1518 (C=C), 1089 (-OCH_3), 812 (*n*-substitution in the ring «B»). ^{1}H-NMR (200 MHz, DMSO-d_6) δ, ppm: 12.91 (1H, s, 5-OH), 9.59 (1H, s, 4′-OH), 8.45 (1H, s, 2-H), 7.38 (2H, d, *J* = 10 Hz, H-2′, 6′), 6.86 (1H, s, 8-H), 6.79 (2H, d, *J* = 10 Hz, H-3′,5′), 5.41 (1H, d, *J* = 7.2 Hz, 1″-H), 5.13 (2H, d, *J* = 7.2 Гц, 2″-CH_2OH), 3.75 (3H, s, 6-OCH_3), 3.68 – 3.65 (2H, m, 6″-H), 3.43 – 3.40 (1H, m, 5″-H), 3.28 – 3.24 (2H, m, 2″,3″-H), 3.16 – 3.12 (1H, m, 4″-H).

Mangiferin (3) $C_{19}H_{18}O_{11}$, yellow amorphous powder, m.p. 269-271 °C. M 422.35 g/mol. R_f 0.55, dark yellow. UV λ_{max} (C_2H_5OH) nm: 369, 318, 259, 241. IR (KBr), ν, cm^{-1}: 1650 (C=O), 3100 – 3700 (OH-), 1591, 1565, 1494 (C=C), 1065 (Ar–O–Ar str.), 1051 cm^{-1} (RCH2OH O-H str.). ^{1}H-NMR (200 MHz, DMSO-d_6) δ, ppm: 13.77 (1H, s, 1-OH), 10.65 (2H, s, 6,7-OH), 9.85 (1H, s, 3-OH), 4.86 (2H, s, 3′, 4′-OH), 4.49 (1H, s, 6′-OH), 3.87 (1H, d, 2′-OH), 7.38 (1H, s, 8-H), 6.83 (1H, s, 5-H), 6.35 (1H, s, 4-H), 4.60 (1H, d, $3J_{H1'/H2'}$ = 9.52, 1′-H), 4.05 (1H, t, $3J_{H2'/H3'}$ = 9.23 Hz, 2′-H), 3.69 (1H, d, $3J_{H6'/H5'}$ = 2.55 Hz, 6′-H), 3.41 (1H, dd, $3J_{H6'/H5'}$ = 5.22 Hz, 6′-H), 3.18 (3H, m, $3J_{H3'/H4'}$ = 9.19 Hz, $J_{H4'/H5'}$ = 9.17 Hz, 3, 4′, 5′-H).

Genistein (4) $C_{15}H_{10}O_5$, white amorphous powder, m.p. 290-292 °C. M 270.24 g/mol. MS m/z: 270 (M^+). R_{f1} 0.70, dark; UV λ_{max} (C_2H_5OH) nm: 260, 325. IR (KBr), ν, cm^{-1}: 3726, 3410, 3104 (-OH), 1651 (C=O), 1615, 1569, 1424, 1503 (C=C), 885 (*n*-substitution in the ring „B"). ^{1}H-NMR (200 MHz, DMSO-d_6) δ, ppm: 10.75 (1H, s, 7-OH), 8.42 (1H, s, 2-H), 6.72 (1H, dd, *J* = 1.7 Hz, 6-H), 6.47 (1H, d, *J* = 1.7 Hz, 8-H), 7.40 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.83 (2H, d, *J* = 8.5 Hz, H-3′, 5′), 9.64 (s, OH-4′), 12.94 (1H, s, OH-5).

Daidzein (5) $C_{15}H_{10}O_4$, amorphous powder, m.p. 320-322 °C. M 254.24 g/mol. MS, m/z: 254 (M^+). R_{f1} 0.88, blue; UV λ_{max} (C_2H_5OH) nm: 250, 260sh, 302. IR (KBr), ν, cm^{-1}: 3666, 3171 (-OH), 1631 (C=O), 1595, 1518, 1188, 1460 (C=C), 887, 820 (*n*-substitution in the ring "B"). ^{1}H-NMR (200 MHz, DMSO-d_6) δ, ppm: 10.80 (1H, s, 7-OH), 9.60 (s, OH-4′), 8.31 (1H, s, 2-H), 8.05 (1H, d, 5-H), 7.14 (1H, dd, *J* = 1.7 Hz, 6-H), 7.23 (1H, d, *J* = 1.7 Hz, 8-H), 7.41 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.82 (2H, d, *J* = 8.5 Hz, H-3′, 5′).

Formononetin (6) $C_{16}H_{12}O_4$, amorphous powder, m.p. 255-261 °C. M 268.27 g/mol. MS, m/z: 268 (M^+). R_{f1} 0.94, blue; R_{f2} 0.8, blue; UV λ_{max} (C_2H_5OH) nm: 250, 300sh. IR (KBr), ν, cm^{-1}: 2923 (OCH_3), 1596 (C=O), 1458, 1373 (C=C), 840 (*n*-substitution in the ring "B"). ^{1}H-NMR(200 MHz, DMSO-d_6) δ, ppm: 10.81 (1H, s, 7-OH), 8.35 (1H, s, H-2), 7.98 (1H, d, *J* = 9.0 Hz, H-5), 7.50 (2H, d, *J* = 9.0 Hz, H-2′,6′), 6.97 (2H, d, *J* = 9 Hz, H-3′, 5′), 6.95 (1H, dd, *J* = 9.0 Hz, *J* = 2.4 Hz, H-6), 6.87 (1H, d, *J* = 2.4 Hz, H-8), 3.79 (3H, s, 4′-OCH_3).

Antibacterial activity

In vitro antibacterial activity was determined by agar well diffusion method[23]. According to the WHO recommendations[24] the following test-strains were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636, *Candida albicans* ATCC 885/653. The inoculum suspension was prepared using a Densi-La-Meter apparatus (made by PLIVA-Lachema, at the wavelength of 540 nm). The cultures were synchronized using low temperature conditions (4°C). The microbial load was 10^7 cells per 1 ml of the medium and was determined according to McFarland standard[25]. The 18 to 24-hour culture of microorganisms was used for the test. Mueller-Hinton agar was used ("HIMedia Laboratories Pvt. Ltd India", expiration date to XII 2016, India) for bacteria. The strains of *Candida albicans* were cultivated using Sabouraud agar ("HIMedia Laboratories Pvt. Ltd India", expiration date to XI 2016, India). The obtained dry extract of rhizomes was dissolved in distilled water. The concentration of drugs was 1.0%, 0.5% and 0.25%. The pure solvents used as a control, in concentrations that correspond to their content in the preparations. The antibacterial activity was assessed by measuring zones of inhibition of the corresponding microorganism.

Extraction procedure of plant for bioassay

The powder plant material 100 g was extracted with distilled water (1 liter) on a water bath for 2 hours. This extracts was cooled and filtered. The extraction of raw remainder was repeated under the same conditions. The aqueous extracts were combined, evaporated in a rotary evaporator for dry extract obtained.

Statistic analysis

Statistical analysis of the results was conducted in accordance with the requirements of the State Pharmacopeia of Ukraine[19] in Excel XS application.

RESULTS

The ethyl acetate extract of the rhizomes of *I. hungarica* was subjected to repeated chromatography on columns of silica gel to obtain compounds **1-6**. Compounds soluble in ethanol, benzene, chloroform and are poorly soluble in water, diethyl ether and petroleum ether.

Chromatographic data. TLC analysis of compounds **1, 2** and **4** using system: *n*-butanol – acetic acid – water (4:1:2) produced a spots with dark fluorescence that was darkens by ammonia vapor. This was characteristic of 5-hydroxyisoflavones[21]. Also, **5** and **6** in the same conditions become bright blue fluorescence after processing by ammonia vapors, it's characteristic for isoflavones without OH-group at C-5. Chromatographic analysis of **3** ($R_{f\ 1}$ 0.55) produced a spot with dark-yellow fluorescence. The processing of ammonia vapor spots becomes an orange, which is characteristic for dibenzo-γ-piron; after 2% $AlCl_3$ spots gets green color, that characteristically for xanthones nature of the compound.

Spectral data. The UV spectrums of isolated compounds **1, 2, 4-6** in ethanol showed only one absorption peak between 250-276 nm and "shoulder" 300-338 nm suggesting the isoflavone skeleton. This is in accordance with the hydroxyl pattern of the B-ring of compounds (4′-OH).

The UV spectrum of **3** showed the λ max absorptions at 369, 318, 259 and 241 nm suggesting the xanthones skeleton. The UV absorption maxima of **3** on addition of anhydrous NaOAc indicated the characteristic bathochromic shift of the presence of free hydroxyl group at C-3; under of the influence of sodium acetate and 3% solution of boric acid is the characteristic bathochromic shift, which is typical for two OH-group of ring B at *ortho* position[21].

The flavonoid nature of the compounds is confirmed by the IR spectrums, which indicated the presence of hydroxyl group between 3100-3800 cm^{-1}, as well the bands at 1640-1660 cm^{-1} (C=O, γ-piron), 1420-1620 cm^{-1} (C=C, aromatic ring), while at **1**, **2** and **6** presence of a signal of methoxy group is noted. The signal of intense absorption at 840 cm^{-1} in the isoflavones, which characterizes the substitution at C-4. There are three absorption bands C-H carbohydrate residues at 1100-1010 cm^{-1} that characterizes a pyranose form and band at 812 cm^{-1} – β-glycosidic linkage at **2** exhibited. Likewise, the IR spectrum **3** showed intense absorption at 1051 cm^{-1} (R-CH_2OH O-H str.).

The structure of the compounds is confirmed by the ^{1}H NMR spectrums. The ^{1}H-NMR data of the isolated isoflavones showed the presence of singlet signals of the hydroxyl groups at δ 12.91 – 13.05 ppm (1H, s, 5-OH), 10.75 – 10.81 ppm (1H, s, 7-OH), 9.55 – 9.64 ppm (1H, s, 4′-OH), also signals of methoxy group at δ 3.75 ppm (3H, s, 6-OCH_3) for compounds **1 – 2** and δ 3.79 ppm (3H, s, 4′-OCH_3) for **6**. The proton resonance for isoflavones C-2 were located between δ 8.25 – 8.45 ppm (1H, s), which also confirmed the nature of the rings. The signals of aromatic protons are registered at δ 7.35 – 7.50 ppm (2H, d, *J* 8.5 – 10.0 Hz, H-2′,6′) and 6.79 – 6.97 ppm (2H, d, *J* 7.5 – 10.0 Hz, H-3′,5′). It also exhibited a signals at δ 6.45 – 7.23 ppm (1H, s, 8-H, compounds **1-2**), (1H, d, *J* 1.7 – 2.4 Hz, 8-H, compounds **4-6**), δ 6.72 – 7.14 ppm (1H, dd, J_1 1.7 – 2.4 Hz, J_2 9.0 Hz, 6-H) only for **4-6**. In additions, at **5, 6** the proton signal at C-5 at δ 7.98 – 8.05 ppm (1H, d, *J* 8.5-9.0 Hz) resound.

Antibacterial activity. Antimicrobial activity of the dry extract of the rhizomes of *I. hungarica* against the standard test-strains of gram-positive and gram-negative bacteria and fungi is given in Table 1.

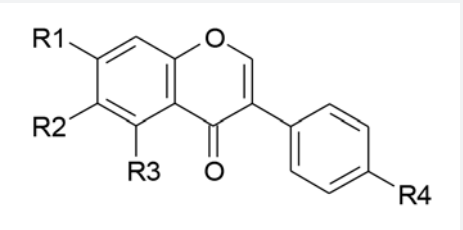
Table 1. Antimicrobial activity of *I. hungarica* dry rhizomes extract.

Name of the microorganisms	Diameter of zone of inhibition (mm) at different concentration (%)			Diameter(mm) of zone of inhibition of control
	1.0%	0.5%	0.25%	
Staphylococcus aureus ATCC 25923	15.60 ± 0.92	-	-	-
Escherichia coli ATCC 25922	14.60 ± 0.80	-	-	-
Proteus vulgaris ATCC 4636	13.60 ± 0.10	-	-	-
Pseudomonas aeruginosa ATCC 27853	13.10 ± 0,13	-	-	-
Basillus subtilis ATCC 6633	16.00 ± 0.12	-	-	-
Candida albicans ATCC 653/885	16.30 ± 0.60	-	-	
Note "-"growth of microorganism. The difference is relatively accurate to control, ($p \leq 0,05$).				

DISCUSSION

The present work on the ethanolic extracts of the rhizomes of *I. hungarica* has resulted in the isolation and characterization of new for this species compounds **1**, **2**, **3** along with known compounds **4 – 6**.

Compounds **1-3** were isolated as a yellow amorphous powder. The mass spectrum of compounds **1** and **2** showed the molecular ion peaks at m/z 300 (M^+) and m/z 300 (–$C_6H_{11}O_5$) (M^+), in agreement with the molecular formulas $C_{16}H_{12}O_6$ and $C_{22}H_{22}O_{11}$, respectively.



1: $R_1 = R_3 = R_4 = OH$; $R_2 = OCH_3$;
2: R_1 = O-Glu; $R_2 = OCH_3$; $R_3 = R_4 = OH$;
4: $R_1 = R_3 = R_4 = OH$; $R_2 = H$;
5: $R_1 = R_4 = OH$; $R_2 = R_3 = H$;
6: $R_1 = OH$; $R_2 = R_3 = H$; $R_4 = OCH_3$.

Figure 2. Structures of isoflavones and isoflavon glycosides from *I. hungarica*

The IR spectrum of **1** and **2** indicated the presence of hydroxyl group at 3478 and 3373 cm^{-1}, respectively, which occurred as a strong and broad band. The presence of carbonyl functionality and -OCH_3 stretching were indicated by the absorptions at 1640-1658 and 1063-1089 cm^{-1}, respectively. The UV absorption maxima of **1** and **2** at 276 and 267 nm, respectively pointed to the presence of isoflavone nucleus. In addition, by the ^{1}H-NMR spectrums the proton resonance for isoflavone C-2 was located at δ 8.25 – 8.45 ppm, which also confirmed the nature of the rings. The ^{1}H-NMR spectrum of **1** revealed the presence of three singlet signals of the hydroxyl groups at C-5, C-7 and C-4′ position, and signals of methoxy group at C-6. The aromatic region integrated for the presence of six aromatic protons, with the occurrence of the two sets of doublets each integrated for two protons. The doublet at 8.0 was assigned to the two equivalents protons at H-2′ and H-6′ and the other doublet at 7.5 to the two equivalent protons at H-3′ and H-5′. It also exhibited an aromatic singlet at 6.45 ppm was assigned to H-8, indicating that only one hydrogen atom was present on the A-ring of the isoflavone. NMR spectrum is not contradicted to shown structure of **1**.

The data of chemical analysis, the spectral characteristics of the compound **1** are identical with literature data on the structure of 4′,5,7-trihydroxy-6-methoxy isoflavone or tectorigenin. It was isolated from the rhizomes of *I. hungarica*[16,26] at first. From literature data known that the antibacterial activity of tectorigenin to against methicillin-resistant *Staphylococcus aureus*[27,28] was established.

Structure of **2** have established similarly. Acid hydrolysis of **2** with 10% H_2SO_4 gave aglycone (tectorigenin) and *D*-glucose and which were identifed by co-PC and co-TLC. The ^{1}H-NMR spectrum **2** also showed a pair of doublets of aromatic protons at H-2′, H-6′ δ 7.38 ppm and H-3′, H-5′ at δ 6.79 ppm, each with coupling constant of 10 Hz, respectively, characteristic of a *p*-substituted benzene ring and a characteristic one proton signal for C-8 position. Also, it indicated the presence of two singlet signals of the hydroxyl groups at C-5 and C-4′ and signals of methoxy groups at C-6.The ^{1}H-NMR spectrum unlike to the spectra of aglycone as tectorigenin further noted the presence of groups of

signals corresponding to the presence of six protons, which confirms that glycoside has monoside nature. The signal of anomeric proton of *D*-glucose is shown at δ 5.41 ppm as a doublet with a spin-spin interaction constant of 7.2 Hz, which characterizes the presence of β- linkage of the sugar and a pyranose carbon skeleton oxidative cycle. On the basis of these data, we concluded that **2** is tectoridin (4',5-dihydro-6-methoxy-7-(o-β-D-glucoside) isoflavone), which isolated from the rhizomes of *I. hungarica* at first[16].

After removal of several isoflavonoids by elution with a mixture of chloroform-ethanol (20:1), the column was eluted with a mixture of chloroform-ethanol (5:1) to afford a compound **3.** It was recrystallized from EtOH to give pale yellow amorphous powder with a molecular weight of 422.35 g/mol. The melting point (anhydrous) of **3** has been reported to be 271˚C[29]. It is soluble in solvents such as DMSO, water and methanol. It's gave a positive reaction on the phenolic hydroxyl groups greenish to 3% $FeCl_3$[21].

The mass spectrum of the compound **3** showed a pseudomolecular peak at *m/z* 423 (M^+) corresponding to the molecular formula $C_{19}H_{18}O_{11}$ and a loss of 120 atom mass units in the MS^2 event, a fragmentation behavior typical of C-glycosides. Other prominent peaks were found to occur at m/z 405, 369, 357, 327, 303 and 273. In contrast to flavonoid O-glycosides, C-glycosides do not generate abundant aglycone ions[30].

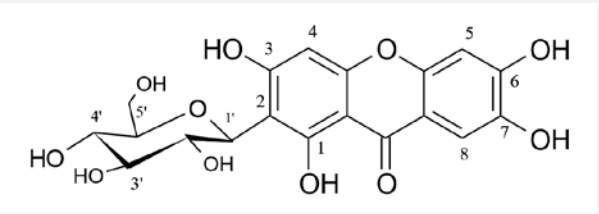


Figure 3. The chemical structures of the compound 3.

A spin-spin interaction constant of anomeric proton (*J* 9.5 Hz) and resistance of the compound **3** to the acid hydrolysis indicate that it is a xanthones 3-*C*-glycosides. Chemical, spectral and literature data on mangiferin indicate that compound **3** has got structure 1,3,6,7-tetrahydroxyxanthone *C*-2-β-D-glucoside[15]. It is a well known natural xanthones and from this type of irises species has been obtained for the first time. Mangiferin have important and broad pharmacological activity, including antidiabetes, antiviral, antitumor, immunomodulatory, antioxidant, anti-inflammatory and vascular modulatory activity[15,29,31].

The results of the antimicrobial activity screening of *I. hungarica* rhizomes the dry extract at a concentration of 1% inhibits the growth of all taxonomic groups of microorganisms. The most sensitive microorganisms to preparations proved to *Basillus subtilis*, *Staphyloccocus aureus* and *Candida albicans* diameter of a growth inhibition 15-17 nm. Preparations of the rhizomes to *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* showed relatively low (diameter of a growth inhibition 13-15 mm) antibiotic susceptibility of a culture. The high activity was observed mostly against the gram-positive strains and fungi, while gram-negative bacteria were resistant for the extract tested. Other preparations in a 0.5% and 0.25% concentration activity did not show. So, the pharmacological activity of *Iris* plants due to the composition of their biologically active compounds.

CONCLUSIONS

Two isoflavones, tectorigenin and tectoridin and xanthone mangiferin, together with known isoflavonoids daidzein, genistein, formononetin were isolated for the first time from the ethyl acetate extract of the rhizomes of *Iris hungarica*, by column chromatography. The structures of the compounds were established by chemical and spectral methods. The antibacterial activity screening of the dry extract of the rhizomes of *I. hungarica* showed high activity for the gram-positive strains and fungi, while gram-negative bacteria were resistant for the extract tested.

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