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Chemical composition, antibacterial, antioxidant and tyrosinase inhibitory activities of glycosides from aerial parts of *Eryngium tricuspdatum* L.

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Abstract.

Two new phenolic glucosides, together with six known compounds, were isolated from the aerial part of *Eryngium tricuspdatum* L. (Apiaceae). The structures of the new compounds were established as 2-hydroxy-3,5-dimethyl-acetophenon-4-*O*- β -D-glucopyranoside (**1**) and 2,3-dimethyl-4-hydroxymethylphenyl-1-hydroxymethyl-*O*- β -D-glucopyranoside (**2**) on the basis of detailed spectroscopic data including MS, 1D, and 2D NMR. The antibacterial, tyrosinase inhibitory and DPPH radical scavenging activities of hydromethanolic extract, fractions, and the eight isolated compounds were evaluated. The antibacterial assay showed a moderate activity for magnolioside (**4**) against *Staphylococcus aureus* CIP 53.154. Compound **7** (quercetin 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 6)-*O*- β -D-galactopyranoside) had moderate DPPH radical scavenging activity whereas compounds **2** exhibited good inhibitory effect against mushroom tyrosinase.

Keywords: *Eryngium tricuspdatum*, Apiaceae, antibacterial activity, antioxidant activity tyrosinase inhibitory activity.

1. Introduction

Eryngium tricuspdatum L. is a species belonging to the large family of Apiaceae, comprising more than 3000 species. *Eryngium* species is the largest genus with, approximately 250 species, distributed worldwide, mainly in Eurasia, North of Africa and South America (Merghache et al., 2014). About 7 species can be found in Algeria (Quezel and Santa, 1963). Plants of this genus are known for their rich content in secondary metabolites. Indeed, numerous studies undertaken on this genus have revealed presence of flavonoids (Khalfallah et al., 2014), saponins (Erdem et al., 2015), polyacetylene (Ayoub et al., 2006) and monoterpen glycosides (Nacef et al., 2008). Anti-inflammatory (Kupeli et al., 2006), cytotoxic (Yurdakok and Baydan, 2013), antioxidant (Le Claire et al., 2005), antimicrobial (Ndip et al., 2007), and antidiabetic (Celik et al., 2011) activities have been reported from *Eryngium* species. In folk medicine, various *Eryngium* species are used against inflammatory disorders or

as antitussive, diuretic, appetizer, stimulant, and aphrodisiac (Suciu and Pârvu, 2012).

Eryngium tricuspdatum L. grows in North Africa, Spain, Sardinia and Sicily (Merghache et al., 2014, Tahri et al., 2011). The decoction of its roots is effective against poisoning and constipation (Bamm and Douira, 2002). The chemical composition and the antibacterial, antifungal and antioxidant activities of the aerial parts essential oil of *E. tricuspdatum* have been reported recently (Merghache et al, 2014). The essential oil showed significant antibacterial and antifungal property against *S. aureus*, *E. faecalis*, *P. aeruginosa* (MIC 9 μ g/mL) and *C. albicans* (MIC 4.6 μ g/mL) and moderate DPPH radical-scavenging activity and ferric reducing-antioxidant power. In continuation of our phytochemical and bioactivity studies on the Algerian plants, herein we reported the isolation, structural identification and bioactivities (antibacterial, antioxidant, and tyrosinase inhibitory) of extracts and compounds from the aerial parts of *E. tricuspdatum*.

2. Results and discussion

The 50 % MeOH extract afforded two new phenolic glycosides (**1**) and (**2**) as well as six known compounds, benzyl-1-*O*- β -D-glucopyranoside (**3**) (Wen et al., 2012), magnolioside (**4**) (Tanja et al., 2005), asyngangoside (**5**) (Kanchanapoom and Ruchirawat, 2007), citroside B (**6**) (Yang et al., 2012), quercetin 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 6)-*O*- β -D-galactopyranoside (**7**) (Nawwar et al., 1989), and kaempferol 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 6)-*O*- β -D-galactopyranoside (**8**) (Hiller et al., 1980).

Compound **1** was obtained as a colorless amorphous solid. The negative HR-ESI-MS spectrum showed a molecular ion peak at m/z 341.1241 [M-H]⁻ and the molecular formula was deduced as C₁₆H₂₂O₈ (calcd 341.1236). The UV spectrum revealed absorption bands at 268 and 326 nm. Analysis of the ¹H and ¹³C NMR spectra of **1** revealed the presence of an anomeric proton at δ_H 4.75 (d, $J=7.7$ Hz) correlated in the HSQC spectrum with an anomeric carbon at δ_C 103.9 (Table 1). The sugar unit was assigned as a β -D-glucopyranosyl unit according to the COSY, HSQC, and HMBC correlations as well as the coupling constant values of all oxymethine protons (Yashiro et al., 2001) (Table 1). The relative configuration of β -D-glucopyranosyl moiety was further confirmed by analysis of the ROESY correlations [22] from the α -axial protons H-1/H-3 and H-1/H-5. The ¹H NMR and ¹³C NMR spectra of **1** (Table 1) revealed also the presence of an acetyl group at δ_H 2.60 (3H, s), one aromatic proton at δ_H 7.60 (1H, s), and two methyl signals at δ_H 2.30 (3H, s) and 2.35 (3H, s). The signal at δ_H 2.60 correlate in the HSQC spectrum with carbon at δ_C 25.3, and in the HMBC spectrum with carbon at δ_C 115.0 and the carbonyl carbon at δ_C 205.0. Five signals of quaternary carbons were observed at δ_C 115.0, 120.0, 122.0, 159.9 and 160.0, the two at δ_C 160.0 and 159.9 were consistent with the aromatic carbons bearing a hydroxyl substituent. The signal at δ_C 129.7 showed correlation with the singlet at δ_H 7.60 in the HSQC experiment. The anomeric carbon at δ_C 103.9 exhibited correlation with the quaternary carbon at δ_C 159.9 in the HMBC experiment, revealing the position of the glucose moiety on the aromatic ring. The methyl signals at δ_C 8.4 and 15.8 correlated in the HSQC experiment with their corresponding protons at δ_H 2.30 and 2.35. The HMBC spectra showed

correlations between the protons at δ_H 2.30 and carbons at δ_C 120.0, 160.0, and 159.9, and between the protons at δ_H 2.35 and carbons at δ_C 122.0, 129.7 and 159.9 (Fig. 2), assigning the positions of the two methyl groups. The aromatic proton (δ_H 7.60) correlated in the HMBC spectra with carbons at δ_C 205.0, 160.0, 159.9, and 15.8. Further interpretation of 2D NMR especially HMBC data (Fig. 2) established that compound **1** was an acetophenone derivative substituted by two methyls and two hydroxyl groups (Kuang et al., 2008, Vendetti et al., 2014) (Fig. 1). To exclude a different position of the substituents (Ar-CH₃ and Ar-CO-CH₃), a ROESY experiment was performed and showed rOe correlations between the aromatic proton H-6' and the methyl protons H₃-2 and H₃-8' and between the anomeric proton H-1'' and the two methyl protons H₃-3' and H₃-8'. These evidences were consistent with the structure of **1** as 2-hydroxy-3,5-dimethyl-acetophenon-4-*O*- β -D-glucopyranoside

Compound **2** was obtained as a colorless solid. The [M + Na]⁺ ion at m/z 351.1412 (calcd for C₁₆H₂₄O₇Na; 351.1420) in the HR-ESI-MS spectra was consistent with the molecular formula C₁₆H₂₄O₇. The UV spectrum revealed absorption band at 276 nm. The ¹H and ¹³C NMR spectra of **2** (Table 1) showed the presence of a β -D-glucopyranosyl moiety from the anomeric signals at δ_C 101.6 and δ_H 4.32 (d, $J = 7.7$ Hz). The ¹³C NMR spectrum exhibited 16 distinct carbon resonances, 6 of which were assigned for the β -D-glucopyranosyl unit. The ¹H NMR spectrum of the aglycone of **2** exhibited two AB type aromatic protons at δ_H 7.25 and 7.18 (each 1H, d, $J = 7.8$ Hz), two methyl groups at δ_H 2.29 and 2.33 connected to an aromatic ring, and two pairs of methylene protons bearing oxygen functions [δ_H 4.65 (2H, s), 4.69 and 4.99 (each 1H, d, $J = 11.5$)] (Table 1). Analysis of the ¹H and ¹³C NMR, HSQC and HMBC spectra allowed assignment to **2** of two methyls [δ_C 13.5 (CH₃-9) and 13.9 (CH₃-8)], two hydroxymethyls [δ_C 62.7 (C-10) and 69.5 (C-7)], two methines [126.7 (C-6) and 124.8 (C-5)] and four quaternary carbons [134.6 (C-1), 137.5 (C-2), 138.7 (C-3) and 134.6 (C-4)] indicating the presence of a 1,2,3,4-tetrasubstituted aromatic ring. From these observation, compound **2** was similar to 2,3,4-trimethylphenylalcohol-*O*- β -D-glucopyranoside, previously isolated from *Prangos tschimganica* [23]. The only difference

was the presence of a hydroxymethyl group in **2**, instead a methyl group. The HMBC spectrum showed correlations between H₂-10 (δ_{H} 4.65) and C-3, C-4, and C-5 which allowed assignment of CH₂-10 in position C-4 whereas HMBC correlations between H₂-7 (δ_{H} 4.69 and 4.99) and C-1, C-2, and C-6 placed C-7 in position C-1 (Fig. 2). The HMBC correlations between H₃-8 and C-1, C-2 and C-3 allowed assignment of CH₃-8 in position C-2 whereas HMBC correlations between H₃-9 and C-2, C-3 and C-4 placed the CH₃-9 in

position C-3. In addition, an HMBC correlation observed between the anomeric proton H-1' and C-7 (δ_{C} 69.5) indicated that the glucopyranosyl unit was linked at C-7. These assignments were supported by the observation of the rOe effects between H-6/H-7, H-7/H-8, H-8/H-9, H-9/H-10, H-10/H-5 and H-1'/H-7 in the ROESY spectrum. Therefore, compound **2** was determined to be 2,3-dimethyl-4-hydroxymethyl-1-hydroxymethylphenyl-*O*- β -D-glucopyranoside.

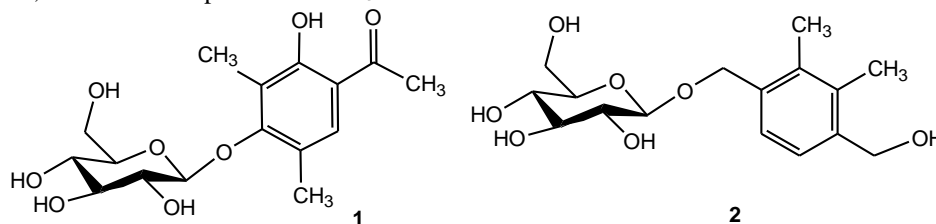


Figure 1: Chemical structure of compounds **1** and **2** isolated from *E. tricuspidatum*.

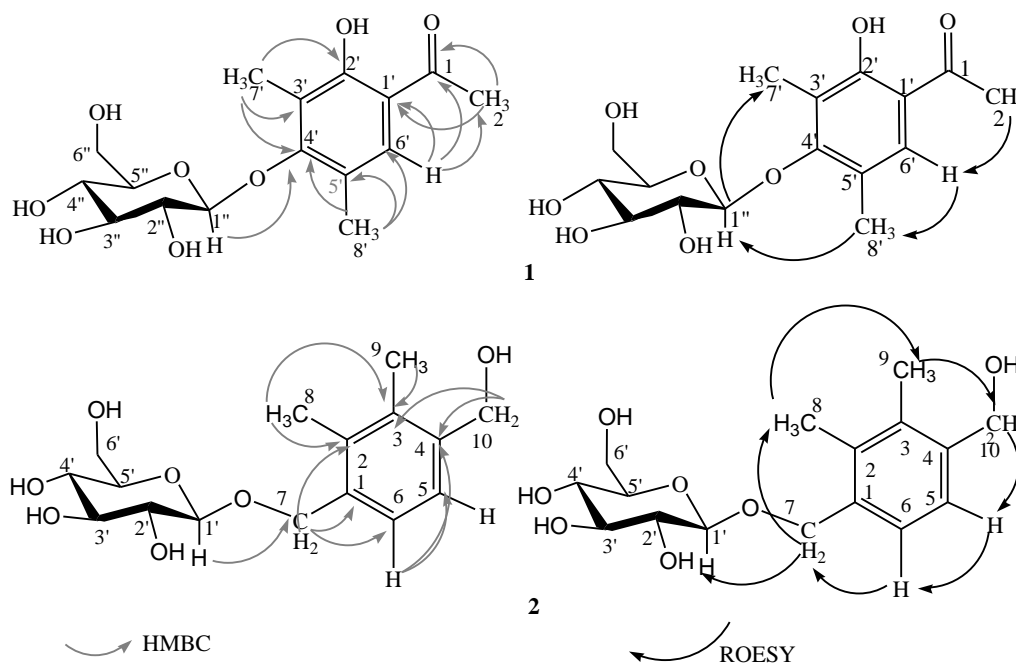


Figure 2: Key HMBC and ROESY relationships of compounds **1** and **2**

Table 1. ¹H NMR and ¹³C NMR data CD₃OD for compounds **1** and **2**.

1			2		
δ_H m (J in Hz)	δ_C	HMBC (H to C)	δ_H m (J in Hz)	δ_C	HMBC (H to C)
1	-	205.0	-	134.6	-
2	2.60 s	25.3	C-1, C-1'	-	137.5
1'	-	115.0	-	-	138.7
2'	-	160.0	-	-	134.6
3'	-	120.0	-	-	124.8
4'	-	159.9	-	-	126.7
5'	-	122.0	-	-	69.5
6'	7.60 s	129.7	C-1, C-2', C-8'	4.99 d (11.5)	C-1, C-2, C-6, C-1'
7'	2.30 s	8.4	C-2', C-3', C-4'	2.33 s	C-1, C-2, C-3
8'	2.35 s	15.8	C-4', C-5', C-6'	2.29 s	C-2, C-3, C-4
			10	4.65 s	62.7
					C-3, C-4, C-5
glc			glc		
1''	4.75 d (7.7)	103.9	C-4'	4.32 d (7.7)	101.6
2''	3.54 t (7.8)	74.3	C-1'', C-2''	3.26 t (7.8)	73.7
3''	3.45 t (7.9)	76.5	C-2'', C-4''	3.33 t (7.9)	76.8
4''	3.40 t (7.9)	70.1	C-3''	3.29 t (7.9)	70.4
5''	3.17 m	76.7	C-4''	3.80 m	76.6
6''	3.67 dd (11.8,5.7)	61.3	C-5''	3.71 dd (11.8,5.7)	61.6
	3.79 dd (11.80,2.1)		C-5''	3.71 dd (11.8,2.1)	

Several biological activities of *Eryngium* species e.g., anti-inflammatory, antioxidant, and antimicrobial, were reported. Moreover, the aerial parts essential oil showed antibacterial and antifungal and DPPH radical-scavenging activities. Thus, the hydromethanolic extract, fractions I-V, and compounds **1-8** were tested for their antibacterial, antioxidant and tyrosinase inhibitory activities.

The hydromethanolic extract and fractions I-V were initially tested for their antibacterial capacity against *Staphylococcus aureus* CIP 53.154 by using TLC bioautography method (Table 2). The results showed a high activity for the fraction I and a milder activity for the fractions IV and V comparable with the reference gentamicin. Despite these positive results for three fractions, the extract did not show anti-staphylococcal activity. It could be due to the presence of a small percentage of antibacterial compounds in 50% MeOH extract. Subsequently, the test was repeated for eight compounds isolated from fractions I, II and III, and only the compound **4**, obtained from the fraction I, was moderately active. In our opinion, it is possible that the antimicrobial activity of fraction I is a synergistic effect of the major and minor constituents, present in the fraction.

Table 2. Biological activities of 50% MeOH extract, fractions I-V, and compounds **1-8**.

	Antibacterial activity against <i>S.aureus</i> inhibition zone at 50 μ g (in cm)	DPPH radical scavenging activity IC ₅₀ (μ g/mL)	Mushroom tyrosinase inhibition IC ₅₀ (μ g/mL)
50% MeOH extract	na	180 \pm 10	1900
Fraction I	1.5	nd	nd
Fraction II	na	170	1920
Fraction III	na	160	1850
Fraction IV	0.5	nd	nd
Fraction V	0.5	nd	nd
1	na	nd	1950
2	na	nd	80.0
3	na	nd	nd
4	0.5	nd	nd
5	na	nd	nd
6	na	nd	nd
7	na	20.0	nd
8	na	150	nd
Gentamicin ^a	1.0		
Ascorbic acid ^a		6.3	
Kojic acid ^a			20.0

na : not active at 50 μ g

nd : 50% inhibition not achieved at the concentration of 200 μ g/mL.

^aUsed as a positive control.

The DPPH radical scavenging activity of 50% MeOH extract, fractions I-V, and compounds **1-8** was measured and their IC₅₀ are listed in Table 2. In this assay, antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picrylhydrazine. Hydromethanolic

extract and fractions I, II and III showed low DPPH radical scavenging activity (IC_{50} 160-180 $\mu\text{g/mL}$). Only compound **7** exhibited moderate scavenging activity on DPPH radical with IC_{50} of 20 $\mu\text{g/mL}$, compared with the reference ascorbic acid (IC_{50} 6.3 $\mu\text{g/mL}$). The seven other compounds showed low or no antiradical activity.

As initially stated, we measured the mushroom tyrosinase inhibitory effects of 50% MeOH extract, fractions I-V and compounds **1-8**, isolated from *E. tricuspidatum*. We found that the two new compounds (**1** and **2**) could reduce mushroom tyrosinase activity with dose-dependent trends (Table 2). Compound **2** exhibited good tyrosinase inhibitory effect (IC_{50} 80 $\mu\text{g/mL}$), compared to the reference kojic acid (IC_{50} 20 $\mu\text{g/mL}$).

In summary, two new phenolic glucosides, along with six known compounds, were isolated from the aerial parts of *E. tricuspidatum*. The antibacterial assay showed a high activity for the fraction I and a milder activity for compound **4**. Compound **7** had moderate antioxidant abilities in DPPH scavenging activity. Compound **2** showed a good *in vitro* mushroom tyrosinase inhibitory activity, compared to kojic acid.

3. Experimental

3.1. General experimental procedures

The optical rotations were recorded on a PerkinElmer 341 Polarimeter. The UV spectra were obtained in methanol on a Shimadzu UV-2450 spectrophotometer. 1D and 2D NMR spectra were recorded in CD_3OD on a Bruker Avance DRX III 500 MHz spectrometer (^1H at 500 MHz and ^{13}C at 125 MHz). 2D-NMR experiments were performed using standard Bruker microprograms. Chemical shifts (δ) are reported in ppm using the internal solvent resonances at δ_{H} 3.33 and δ_{C} 47.6 (CD_3OD). HR-ESI-MS experiments were performed using a Micromass Q-TOF instrument, equipped with a pneumatically assisted electrospray ion source (Manchester, UK). Silica gel 60 F_{254} precoated aluminium plates (0.2 mm, Merck) were used for TLC analysis. The TLC and PTLC spots were visualized under UV light (254 and 366 nm) followed by spraying with 50% H_2SO_4 and heating. All solvents were AR grade. Column chromatography was carried out on Kieselgel 60 (63-200 mesh) or LiChroprep RP-18 (40-63 μm) Merck. High Performance Flash chromatography was performed on a Grace

Reveleris system equipped with dual UV and ELSD detection using Grace® cartridges (Silica gel or RP-C₁₈) and a flow rate of 30 mL/min. The chromatograms were monitored at 205, 225, 250, and 360 nm. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and a Chromeleon software. RP-C₁₈ column (Phenomenex 250x15 mm, Luna 5 μ) was used for semi preparative HPLC with a binary gradient eluent (H_2O (pH 2.4 with TFA); MeCN) and a flow rate of 5 mL/min; the chromatogram was monitored at 205, 225, 250, and 350 nm. Absorbance (A) values in the DPPH free radical scavenging and antityrosinase assay were read on a Fluostar omega microplate reader (BMG labtech).

3.2. Plant material

The aerial parts of *E. tricuspidatum* were collected in Mars 2013 from Constantine (North Eastern Algerian). A voucher specimen (Et.03.13) has been deposited in the Herbarium of the Department of Chemistry, Université des frères Mentouri-Constantine, and authenticated by Prof. Gérard De Belair (University of Annaba, Algeria).

3.3. Extraction and isolation

The dried aerial parts (1000 g) of *E. tricuspidatum* were macerated in 50% MeOH (3x5L, 24h) at room temperature. After filtration and concentration under low pressure, 60 g of MeOH 50% extract was obtained. A part of the 50% MeOH extract (45 g) was subjected to Diaion HP-20 resin column chromatography eluted with 25, 50, 75, and 100% MeOH.

Fraction eluted with 50% MeOH (5.2 g) was subjected to RP-C₁₈ vacuum liquid chromatography (VLC) using a gradient of MeOH- H_2O (20, 40, 60, 80, and 100% MeOH) to give fractions I-V, successively. Fraction I (2 g) was subjected to Flash chromatography over silica gel, eluted by a gradient system of CHCl_3 -MeOH (0-30 % MeOH), in 30 min, to afford 14 fractions (I₁- I₁₄). Fractions I₅ and I₇ corresponds to compounds **3** (4.4 mg) and **4** (16 mg), respectively. Fractions I₁₁ (32 mg) was purified by semi-prep HPLC (32 % MeCN) to afford compounds **5** (R_t 9.57 min, 2.1 mg) and **1** (R_t 19.13 min, 1.8 mg). Fractions II-III (2.2 g) were subjected together to Flash chromatography, over silica gel, eluted by a gradient system of CHCl_3 -MeOH (0-40 % MeOH), in 30 min, to afford 19

fractions [(II-III)₁- (II-III)₁₉]. Fractions (II-III)₁₅ (37 mg) was purified by semi-prep HPLC (25 % MeCN) affording compounds **2** (R_t 11.87 min, 2 mg) and **6** (R_t 15.19 min, 2.1 mg). Fraction (II-III)₁₇ (180 mg) was subjected to Flash chromatography, over RP-C₁₈, eluted with H₂O-MeOH (5-45 % MeOH) in 30 min and then purified by semi-prep HPLC (20 % MeCN) to yield compounds **7** (R_t 9.06 min, 1.6 mg) and **8** (R_t 10.06 min 6.5 mg).

2-hydroxy-3,5-dimethyl-acetophenon-4-*O*-β-D-glucopyranoside (**1**)

Colorless amorphous powder. $[\alpha]_D^{20}$ -49 (*c* 0.2, MeOH). UV_{max} (MeOH) 268 (2.7), 326 (0.98). ¹H NMR and ¹³C NMR (CD₃OD), Table 1. HR-ESI-MS: *m/z* 341.1236 [M-H]⁻ (calcd for C₁₆H₂₁O₈).

2,3-dimethyl-4-hydroxymethyl-1-hydroxymethylphenyl-*O*-β-D-glucopyranoside (**2**)

Colorless amorphous powder. $[\alpha]_D^{20}$ -153.8 (*c* 0.13, MeOH). UV_{max} (MeOH): 276 (0.4). ¹H and ¹³C NMR (CD₃OD), Table 1. HR-ESI-MS: *m/z* 351.1420 [M + Na]⁺ (calcd for C₁₆H₂₄O₇Na).

3.4. Sugar analysis and determination of absolute configuration

A part of fractions I-III (100 mg each) was refluxed with TFA 2N (15 mL) for 4 h. After filtration, the mixture was extracted with EtOAc (3 x 10 mL) and the acid aq layer was evaporated. The residue was purified by prep. HPLC, on a Rezex ROA column with H₂SO₄ 2.5 mM, as solvent, to yield glucose and galactose. The monosaccharide fractions were then neutralized with NaOH 50 mM and freeze-dried. The residues were solubilized in pyridine and soln. were filtrated and then evaporated. Glucose and galactose were dissolved in hexane-EtOH-TFA (50:50:1) by ultrasonication. The solutions were analyzed by chiral HPLC with a Chiralpak IC, using a mixture of hexane-EtOH-TFA (80:20:0.1), as solvent. By comparison with authentic D or L monosaccharide samples, the configurations were identified as D-glucose (R_t 19.32 min) and D-galactose (R_t 19.39 min).

3.5. Biological activities

3.5.1. Bioautography for antibacterial activity

This method is used mostly to identify the compounds responsible for the antibacterial activity in complex extracts. In this study, an immersion bioautography method was employed

as a preliminary antibacterial evaluation (Abedini et al., 2013). An aliquot of extract/fraction/compound (2 mg) was solubilized in 1 mL methanol. The resulting solutions (25 μL) were spotted onto Merck 60 F₂₅₄ pre-coated silica gel plates (10 x 10 cm). Gentamicin (50 μg) was also spotted on the plates as a positive control. The TLC plates were directly dried without migration and sterilized. The plates were then covered by Mueller-Hinton (MH) agar medium containing a *Staphylococcus aureus* 53.154 suspension (10⁵ bacteria/mL) in square Petri dishes. After incubation 24 h at 37 °C, bacterial growth was revealed by a 2 mg/mL solution of thiazolyl blue tetrazolium bromide (MTT) and growth inhibition zones were measured. White stains indicate where reduction of MTT to the colored formazan did not take place due to the presence of extracts that inhibited bacterial growth.

3.5.2. Free radicals scavenging activity

The antioxidant activity of our target compound was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method (Bendaikha et al., 2014). Briefly, 5 μL of different concentrations of the samples (dissolved in DMSO) were added to 95 μL of DPPH solution (158 μM, dissolved in EtOH 50%). The reaction proceeded for 30 min at 37 °C on a 96-well microplate. The absorbance was then read at λ 515 nm. The percentage of inhibition was calculated using the following equation: % inhibition $[(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100$. DPPH solution in EtOH 50% was used as a control. The curve of the % scavenging activity against the concentration of sample was prepared by MS Excel based program to obtain the IC₅₀ (concentration required to obtain a 50% antioxidant effect). All the tests were conducted in triplicate. Ascorbic acid was used as a positive control agent.

3.8. Tyrosinase enzyme assay

Tyrosinase activity inhibition was determined spectrophotometrically according to the method described previously (Bendaikha et al., 2014). Different concentrations of test compounds were prepared in 10% DMSO in aqueous solution and 100 μL of each concentration were added to 96-well plate and then 100 μL of 135 U/mL mushroom tyrosinase in phosphate buffer solution (PBS, pH 6.8) were added. After pre-incubation at 25 °C for 10 min, 100 μL of L-dopa (0.5 mM, PBS pH 6.8) were added into 96-well plate. The reaction mixture was incubated for another 5 min at 25 °C. The amount of dopachrome in the

mixture was determined by the measurement of the absorbance of each well at 475 nm. Kojic acid was used as positive control agent. The inhibitory percentage of tyrosinase was calculated according to the following equation: % inhibition = $\{[(A - B) - (C - D)] / (A - B)\} \times 100$ (A: Ab at 475 nm without test substance; B: Ab at 475 nm without test

substance and tyrosinase; C: Ab at 475 nm with test substance; D: Ab at 475 nm with test substance, but without tyrosinase). All the tests were conducted in triplicate and IC₅₀ was determined by interpolation of concentration % inhibition curve obtained by MSEXcel based program.

Supporting Information

HR-ESI-MS, ¹H and ¹³C NMR, COSY, HMBC and ROESY spectra for compounds **1** and **2**.

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