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Three new dihydroisocoumarin glycosides with antimicrobial activities from *Scorzonera aucheriana*

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ABSTRACT

Three new dihydroisocoumarin glycosides (*iso*-scorzopygmaecoside, scorzoaucherioside I, and scorzoaucherioside II) together with five known compounds (scorzopygmaecoside, scorzocreticoside II, 3,5-O-dicaffeoyl-*epi*-quinic acid, 3,5-O-dicaffeoylquinic acid, and 3,4-dihydroxyphenyl caffeate) were isolated from the aerial parts of the *Scorzonera aucheriana* collected from Sivas province in Turkey. The isolated compounds' structures were elucidated based on NMR (^{1}H, ^{13}C/APT, COSY, HMBC, HSQC, and TOCSY), UV, FT-IR, and LC-QTOF-MS data. These compounds have been isolated for the first time from *S. aucheriana*. An antimicrobial assay against ten microorganisms was applied to all isolated compounds. *Iso*-scorzopygmaecoside, scorzoaucherioside I, and 3,4-dihydroxyphenyl caffeate showed notable anti-pseudomonal activity. *Iso*-scorzopygmaecoside and scorzoaucherioside II exhibited strong activity against *Mycobacterium smegmatis* with MIC values of 25.6 μg/mL and 21.2 μg/mL, respectively.

1. Introduction

The plant world is the source of a wide variety of natural compounds with different chemical structures that show biological activity in a wide spectrum. It is thought that there are approximately 350,000 plant species on earth, one third of which have not yet been discovered. Natural resources are of great importance in the treatment of diseases and the protection of health. Natural compounds have been the primary source of drug precursors until recent years (Cseke et al., 2006).

Scorzonera L. belongs to the Asteraceae family, and there are 52 species which 32 of which are endemic in Turkey. *S. aucheriana* is a perennial herb and is popularly known as Ice Tekesakalı (Çoşkunçelebi et al., 2015). *Scorzonera* L. species are used in complementary medicine as consumed as a vegetable in the world and Turkey (Baytop, 1999). It is known that different *Scorzonera* species are used as pain relievers with analgesic, anti-inflammatory, and wound healing effects (Baytop, 1999; Çoşkunçelebi et al., 2015; Davis, 1975). *Scorzonera hispanica* has been used traditionally used in Europe as a cure for cold, a mucolytic, a stomachic, against lung diseases, to increase appetite, a diuretic, for its antipyretic effects, against snake bites, and it has been used as a substitute fo coffee to prepare drinks with a similar bitter taste (Zidorn et al., 2000a, b). *Scorzonera humilis* L. is used for wound healing and gastrointestinal disorders (Zidorn et al., 2000a,b). *Scorzonera mongolica* Maxim., and *Scorzonera austriaca* Willd. were used to treat fever, boils, and breast inflammation (Wang et al., 2009a, b; Wang et al., 2010a,b). *Scorzonera divaricata* Lipsch in the treatment of ulcers and malignant stomach tumors; *Scorzonera pseudodivaricata* Turcz type is used in febrile conditions caused by pulmonary edema, diuretic, and viral infections (Tsevegsuren et al., 2007). *S. pseudodivaricata* and *S. divaricata* species are used for medicinal purposes among the public in Mongolia (Tsevegsuren et al., 2007).

As a result of the isolation and purification studies performed on *Scorzonera* species, sesquiterpenoids, bisabolol, and curcumenal derivatives from *S. hispanica* species, flavanoid glycosides, a sesquiterpene lactone, and guaianolides from *S. austriaca* species (Granica et al., 2015; Wu et al., 2011; Zidorn et al., 2000a,b), dihydroisocoumarins, hydrangenol, scorosotomentocin 4′-O-*β*-glycopyranoside, caffeoyl-derived chlorogenic acid methyl ester, 4,5-O-dicaffeoylquinic acid, 4,5-O-dicaffeoylquinic acid methyl ester, and 3,5-caffeic acid from *Scorzonera latifolia* (Bahadır et al., 2010; Sarı, 2012), tyrolobibenzyls compounds from *S. humilis* species (Zidorn et al., 2000a,b), triterpene erythrodiol fatty acid esters (Wang et al., 2010a), triterpene derivatives (Wang et al., 2010a,b). dehydrocostus lactone, *β*-sitosterol, cholesterol, 5*α*,8*α*-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3*β*-ol, glycerol-1-octadecanoate,

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1-linoloylglycerol, stearic acid, palmitic acid, sucrose, luteoline-5, 3′-dimethyl ether, diisobutyl-O-phthalate, and dibutyl-O-phthalate from *S. mongolica* species (Wang et al., 2009a, b), flavonoid, quinic acid and stilbene derivatives from *S. radiata* species (Wang et al., 2010a,b; Wang et al., 2009c, 2012), phenolic compounds from the *Scorzonera judaica* (Bader et al., 2011), and flavonoids, caffeic acids and triterpenes from the *Scorzonera aristata* species (Jehle et al., 2010) have been reported. Chlorogenic acids, scorzoveratrin, and scozoveratrozide derivatives have been isolated from the *Scorzonera veratrifolia* Fenzl species (Sarı, 2010). Studies conducted on *S. divaricata* species, a sesquiterpene lactone, pyridinium alkaloid, amino substituted sesquiterpene lactone, bisabolol sesquiterpene, tirusallane triterpene, quinic acids were isolated (Yang et al., 2013). Triterpenoid compounds and phenolic derivatives from *Scorzonera undulata* species have been reported (Athmouni et al., 2015; Brahim et al., 2013). Also, in another phytochemical study of *S. undulata*, β-amirin acetate, methyl oleanate, methyl ursolate, stigmasterol, β-sitosterol, galangustine, coumarin-O-β-glycoside, and acteoside were isolated (Harkati et al., 2010). Methyl ester of scorocretizine, 8-O-β-d-glucoside, scorocreticoside, dihydroisocoumarin, scozocretizin, scozocreticoside I and II from *Scorzonera cretica* species (Paraschos et al., 2001; Saeed, 2006), scopoletin, xanthotoxin, and flavonoids from *Scorzonera alexandrina* B. (Donia, 2016), flavonoids and sesquiterpenoids from *Scorzonera ruprechtiana* (Li and Wang, 2010), dihydroisocoumarins, phthalide, stilbenes from *Scorzonera tomentosa* (Sarı et al., 2007) and finally flavonoid glucosides from the *Scorzonera columnae* (Menichini et al., 1994) have been isolated. Considering the literature studies, it is seen that *Scorzonera* species are quite rich in terpenic and phenolic compounds (Sareedenchai and Zidorn, 2010; Tsevegsuren et al., 2007). In biological activity studies performed on the *Scorzonera* species, compounds showing antioxidant activity, cytotoxic activity, and antinociceptive activity were obtained (Bahadır et al., 2010; Tsevegsuren et al., 2007; Wang et al., 2012).

Literature survey revealed no secondary metabolite isolation, structure determination, and biological/pharmacological activity study on *S. aucherana*. In our ongoing biological activity-oriented work, it is of great importance in terms of clarifying the phytochemical content of the *S. aucheriana* species, which has such a chemical diversity as well as its biological activities and adding new compounds to the scientific world.

2. Material and methods

2.1. Plant materials

S. aucheriana DC (Asteraceae) was collected from Sivas, Yarağıl, between Zara-Divriği, calcareous rocks, 1496 m, 13 vii 2010, 39 45 044 N - 037 42 034 E, Makbul & Coşkunçelebi 244 in Turkey. The plant was identified by using Flora of Turkey (Davis, 1975) and taxonomical conspectus of Turkish *Scorzonera* (Çoşkunçelebi et al., 2015). The voucher was deposited in the Herbarium of Biology (KTUB) at Karadeniz Technical University, Turkey. The plant was cleaned to remove impurities, dried, and stored in an air-tight container before use.

2.2. Chromatographic and instrument methods

Optical rotations were measured on Automatic AA-5 Series polarimetry. UV spectra were obtained with a Spectrostar nano BMG labtech spectrometer. Infrared spectra were obtained with a PerkinElmer 1600 FT-IR (ATR) (4000−400 cm^{-1}) spectrometer. The mass spectral analyses were carried out on an Agilent 6230A LC-QTOF-MS. Melting points were determined using Thermo-var apparatus fitted with a microscope and are uncorrected. ^{1}H and ^{13}C NMR, along with 2D NMR spectra, were obtained on a Bruker 400 MHz NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C), using TMS as an internal standard. CD_3OD and $CDCl_3$ were used as NMR solvents. ACD NMR program was used for the elucidation of isolated compounds. Chemical shifts were expressed in δ (ppm), and coupling constants (*J*) were reported in hertz (Hz). CC was carried out on silica gel (Kieselgel 60, 320–400 mesh), RP18 silica gel, and preparative thin layer chromatography (PTLC, Silica gel HF_{254}, 20 × 20 cm, 0.5 mm,). TLCs were carried on silica gel (Kieselgel 60 F_{254}, Merck) plates, and the spots were visualized by UV lamp or spraying with 20 % H_2SO_4 and heating.

2.3. Extraction and isolation

Dried powdered aerial parts of *S. aucheriana* (833 g) were macerated with methanol (4 L) three times for 72 h at room temperature on a vibratory. Total extract was evaporated under vacuum at a temperature not exceeding 40 °C using a chiller (−10 °C) to yield crude extracts (60.6 g). The crude methanol extract (42.96 g) was dissolved in MeOH-H_2O (2:8) and then successively fractionated with *n*-hexane (50 mL x 3), chloroform (50 mL x 3), ethyl acetate (50 mL x 3) and water (80 mL) to yield 12.9 g, 1.8 g, 4.6 g and 20.9 g, respectively. All the fractions showed moderate antimicrobial activities, thus EtOAc and water fractions were then studied. The crude ethyl acetate fraction (4.6 g) was subjected to CC (Kieselgel 60, 320–400 mesh) using increased polarity of n-hexane- EtOAc (98:2, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 40:60, 30:70, 20:80, 10:90, 0:100 mL, each), and EtOAc-methanol (98:2, 97:3, 95:5, 93:7, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100) solvent mixture to give 106 fractions (~35 mL, each, EA).

After the TLC control, some of the fraction were combined and EA-Fr 92-96 (135 mg) was further separated by PTLC (silica gel HF_{254}, 20 × 20, 0.5 mm, 2 plates) using EtOAc-MeOH-H_2O (70:30:4.5) mobile phase to provide A, B, C, and D bands. Band A afforded compound **1** (16 mg). After the TLC control, EA fractions 69-74 were combined (435 mg) and further purified by repeated CC (Kieselgel 60, 320-400 mesh) with increasing polarity of *n*-hexane-$CHCl_3$ (100:0, 96:4, 92:8, 88:12, 85:15, 80:20, 75:25, 65:35, 60:40, 50:50, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95, and 0:100 mL, each), and *n*-hexane-EtOAc solvent mixture (100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 30:70, 10:90, and 0:100 mL, each) to give 42 subfraction (SFr) (~25 mL, each). After the TLC control, some of the fraction were combined and EA-SFr 24-25 (120 mg) was further separated by PTLC (silica gel HF_{254}, 20 × 20, 0.5 mm, 2 plates) using EtOAc-MeOH-H_2O (80:20:2) mobile phase to provide A, B, C, and D bands. Band C and D afforded compounds **2** (41.4 mg), and **8** (6.1 mg), respectively.

The crude water fraction (10.8 g) was subjected to reverse phase CC (RP-18, silica gel) using decreased polarity of H_2O:MeOH (100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 88:12, 86:14, 82:16, 80:20, 78:22, 75:25, 70:30, 65:35, 60:40, 55:45, 50:50, 45:55, 40:60, 35:65, 30:70, 20:80, 10:90, and 0:100 mL) mobile phase to give 79 fraction (10 mL, each, W). After the TLC control, some of the fraction were combined and W-Fr 71 afforded compounds **4** (8.1 mg). W-Fr 59-62 was further separated by Sephadex CC (Sephadex LH-20) using isocratic flow of methanol (100 %) mobile phase to give 24 subfraction (10 mL, each). After the TLC control, W-SFr 10-20 was subjected to CC (Kieselgel 60, 320-400 mesh) using isocratic flow of EtOAc-MeOH-H_2O (80:20:2) mobile phase to give 23 subfraction. W-S_2Fr 5 afforded compound **3** (9.3 mg).

W-Fr 68-70 was further separated by Sephadex CC (Sephadex LH-20) using isocratic flow of methanol (100 %) mobile phase to give 20 subfractions (10 mL, each). W-SFr 12 afforded compound **5** (4.7 mg). W-Fr 59-62 was further separated by Sephadex CC (Sephadex LH-20) using isocratic flow of methanol (100 %) mobile phase to give 30 subfractions (10 mL, each). W-S_2Fr 23 and 25 afforded compounds **6** (5.2 mg) and **7** (7.8 mg), respectively. Compounds **1-3** gave a yellow color with vanillin-sulfuric acid reagent (0.5 g vanillin in 100 mL sulfuric acid-ethanol (80:20) on heating the plates at 110 °C for 2 min.

2.3.1. Acid hydrolysis of compounds *1-3*

A solution of each compounds **1-3** (12.1 mg, 10.8 mg, 5.4 mg, respectively) was refluxed with 2 N HCl (2 mL) in dioxane (3 mL) for 45

Table 1
NMR data for compounds **1** and **2** (CD_3OD, 400 MHz NMR).

1				2		
No	C (APT)	^{1}H (COSY)	^{13}C (HSQC)	C (APT)	^{1}H (COSY)	^{13}C (HSQC)
1	C	–	175.6	C	–	172.2
3	CH	5.37 (d, *J* = 13 Hz, 1 H)	79.3	CH	5.38 (dd, *J* = 12.5, 1.9 Hz, 1 H)	79.4
4	CH_2	3.17 (dd, *J* = 16, 13 Hz, H4a) 2.96 (d, *J* = 16 Hz, H4b)	36.4	CH_2	3.20 (dd, *J* = 16, 12.5 Hz, H4a) 2.93 (d, *J* = 16 Hz, H4b)	36.1
4a	C	–	143.1	C	–	143.5
5	CH	6.22 (bs, 1 H)	112.9	CH	6.33 (d, *J* =1.9 Hz, 1 H)	111.7
6	C	–	162.7	C	–	162.4
7	CH	6.59 (bs, 1H)	107.7	CH	6.66 (d, *J* = 1.9 Hz, 1 H)	106.7
8	C	–	166.6	C	–	166.2
8a	C	–	100.1	C	–	101.4
1'	C	–	132.6	C	–	131.0
2′, 6′	CH	7.44 (d, *J* = 8.2 Hz, 2 H)	127.5	CH	7.44 (d, *J* =8.6 Hz, 2 H)	127.6
3′, 5′	CH	6.97 (d, *J* = 8.1 Hz, 2 H)	113.5	CH	6.97 (d, *J* =8.7 Hz, 2 H)	113.5
4'	C	–	159.9	C	–	159.9
4'-OCH_3	CH_3	3.83 (s, 3 H)	54.3	4'-OCH_3	3.83 (s, 3 H)	54.3
Gluc.						
1	CH	4.76 (d, *J* =8.0 Hz, 1 H)	103.7	CH	4.78 (d, *J* =8 Hz, 1 H)	103.6
2	CH	3.37 (m, 1 H)	75.9	CH	3.61 (m, 1 H)	76.4
3	CH	3.60 (m, 1 H)	73.6	CH	3.36 (m, 1 H)	70.2
4	CH	3.62 (m, 1 H)	70.4	CH	3.50 (m,1 H)	75.9
5	CH	3.52 (m, 1 H)	76.3	CH	3.52 (m, 1 H)	72.5
6	CH_2	4.12 (d, *J* =11.2 Hz, 1 H) 3.66 (d, *J* =11.5 Hz, 1 H)	67.5	CH_2	4.08 (d, *J* =11.2 Hz, 1 H) 3.64 (m, 1 H)	67.4
Api.						
1	CH	5.10 (bs, *J* = 2.4 Hz, 1 H)	110.2	CH	5.09 (bs, *J* = 2.4 Hz, 1 H)	110.0
2	CH	4.02 (d, *J* = 2.4 Hz, 1 H).	76.6	CH	4.04 (d, *J* = 2.4 Hz, 1 H).	76.4
3	C	–	79.2	C	–	79.2
4	CH_2	3.83, 4.02 (d, *J* = 9.8, 2 H)	73.7	CH_2	3.78, 4.03 (d, *J* = 9.8, 2 H)	73.6
5	CH_2	3.62 (s, 2 H)	64.1	CH_2	3.64 (s, 2 H)	64.1
Glycerol						
1				CH_2	3.53 (m, 2 H)	63.0
2				CH	3.54 (m, 1 H)	73.7
3				CH_2	3.53 (m, 2 H)	63.0

min. The reaction mixtures were then concentrated under reduced pressure to remove dioxane. It was then diluted with H_2O (3 mL) and aglycones extracted with ethyl acetate (3 x 5 mL). After evaporation of ethyl acetate yielded aglycone of compounds **1–3** (3.8 mg, 3.3 mg, and 1.2 mg, respectively). Optical activity of each aglycone of compounds **1-3** were measured and observed values were found to be $[\alpha]_d^{24}$ +18.3 (c 0.06, methanol), $[\alpha]_d^{24}$ +19.4 (c 0.05, methanol), and $[\alpha]_d^{24}$ 0.0 (c 0.03, methanol), respectively. Optical activities of the glycone extract after the acid hydrolysis of the compounds **1-3** were also examined, and observed values were found to be $[\alpha]_d^{24}$ +57 (c 0.001, methanol), $[\alpha]_d^{24}$ +53 (c 0.0011, methanol), and $[\alpha]_d^{24}$ +66 (c 0.0013, methanol), respectively.

The aqueous layers were adjusted to pH 7 with 2 N NaOH and evaporated to dryness. The residues were extracted with MeOH to obtain monosaccharides fractions subjected to TLC (silica gel, n-butanol-acetone-water 4:3:1). The sugars were detected with authentic samples (D-glucose and L-rhamnose) on a silica gel plate by spraying of a solution of vanillin-sulfuric acid reagent (0.5 g vanillin in 100 mL sulfuric acid-ethanol (80:20). HPLC analysis for the glycone fractions of compound **1-3** were also carried out at Agilent 1100 Series HPLC instrument equipped with a RID detector using Inertsil NH_2 (250 mm × 4.6 mm, 5 μm) analytical column. The mobile phase was MeCN/H_2O (85:15). The sample injection volume was 20 μL with the 1.0 mL/min flow rate. The column furnace temperature was set at 25 °C. Identification of l-rhamnose and d-glucose present in the monosaccharides of compound **1-3** was carried out by comparing their retention times with those of authentic samples (t_R (min): 7.24 (L-rhamnose) and 14.60 (D-glucose)).

2.3.2. Compound **1** (iso-scorzopygmaecoside)

Yellowish, m.p. 52-55 °C; $[\alpha]_d^{24}$-46.8 (c 0.0064, Methanol); R_f: 0.65 (AcOEt-MeOH-H_2O, 70:30:4); UV (Methanol) λ_{max} (nm): 225 (2.54), 268 (2.33), 291 (2.18). FT-IR (ATR, cm^{-1}): 3290 (−OH), 2936, 2846 (−CH), 1592 (C = O), 1425, 1353 (C = C) 1249, 1071(C-O); $C_{27}H_{32}O_{14}$, positive LC-QTOF-MS: *m/z* (%) $[M + Na]^+$ 603.1674 (100), calc. 603.1679; $[M + Na+H]^+$ 604.1763 (30), calc. 604.1767; $[M+2\ Na-H]^+$; 625.1501 (12), calc. 625.1508; Aglycone: positive LC-QTOF-MS: *m/z* (%) $[M+2\ H]^+$ 288.2906 (12), calc. 288.2910; ^{1}H and ^{13}C NMR data see Table 1.

2.3.3. Compound **2** (Scorzoaucherioside I)

Yellowish, m.p. 58−59 °C; $[\alpha]_d^{24}$ -43.4 (c 0.0039, Methanol); R_f: 0.70 (AcOEt-MeOH-H_2O, 80:20:2); UV (Methanol) λ_{max} (nm): 207 (2.79), 237 (2.69), 278 (2.35); FT-IR (ATR, cm^{-1}): 3330 (-OH), 2922(-CH), 1681, 1587 (C = O), 1517, 1343 (C = C), 1254, 1066 (C-O); $C_{30}H_{38}O_{16}$, positive LC-QTOF-MS: *m/z* (%) $[M\text{-}CH_3OH\text{-}H_2O]^+$ 604.1605 (35), calc. 603.1605; $[M\text{-}2H_2O\text{-}H]^+$ 617.1792 (8), calc. 617.1790; [M-Apio-3 H]$^+$ 501.1496 (12), calc. 501.1446; Aglycone: positive LC-QTOF-MS: *m/z* (%) $[M+2\ H]^+$ 288.2921 (16), calc. 288.2910; ^{1}H and ^{13}C NMR data see Table 1.

2.3.4. Compound **3** (Scorzoaucherioside II)

Yellowish, m.p. 75−78 °C; $[\alpha]_d^{24}$ +24.8 (c 0.0086, Methanol); R_f: 0.44 (AcOEt-MeOH-H_2O, 7:2:1); UV (Methanol) λ_{max} (nm): 206 (2.75), 222 (2.40), 250 (2.06), 271 (2.00); FT-IR (ATR, cm^{-1}): 3335 (−OH), 2946, 2835 (−CH), 1653 (C = O), 1449 (C = C), 1119 (C-O); $C_{64}H_{80}O_{34}$, positive LC-QTOF-MS: *m/z* (%) $[M\text{-Glycerol} + CH_3OH\text{-}H]^+$ 1348.4412 (10), calc. 1348.4418; $[M\text{-Glc-Api-Glu}+H_2O+2\ H]^+$ 1044.9794 (18),

Table 2
NMR data for compound **3** (CD_3OD, 400 MHz).

No	C (APT)	^{1}H (COSY)	^{13}C (HSQC)	No	C (APT)	^{1}H (COSY)	^{13}C (HSQC)
Aglycone I				Gluc-I			
1	C	–	164.5	1	CH	5.00 (d, *J* =7.6 Hz, 1 H)	102.5
3	CH	5.46 (d, *J* = 13.0 Hz, 1 H)	79.6	2	CH	3.54 (m, 1 H)	76.5
4	CH_2	3.33 (m, H4a) 3.12 (d, *J* = 16 Hz, H4b)	36.0	3	CH	3.37 (m, 1 H)	70.1
4a	C	–	144.2	4	CH	3.55 (m,1 H)	76.6
5	CH	6.81 (, d, *J* = 2 Hz, 1 H)	109.1	5	CH	3.55 (m, 1 H)	73.4
6	C	–	162.4	6	CH_2	4.12 (d, *J* =11.2 Hz, 1 H) 3.62 (m, 1 H)	67.4
7	CH	6.98 (1H, d, *J* = 2 Hz)	104.5	Glycerol			
8	C	–	160.7	1	CH_2	3.59, 3.95 (m, 2 H)	61.1
8a	C	–	108.3	2	CH	3.78 (m, 1 H)	73.6
1′	C	–	130.4	3	CH_2	3.59, 3.95 (m, 2 H)	61.1
2′, 6′	CH	7.45 (d, *J*=8.0 Hz, 2 H),	127.6	Api.			
3′, 5′	CH	7.00 (d, *J*=8.0 Hz, 2 H)	113.6	1	CH	5.01 (bs, 1 H)	109.5
4’	C	–	160.1	2	CH	4.00 (d, *J* = 2.4 Hz, 1 H).	77.3
4’-OCH_3	CH	3.84 (3H, s)	54.4	3	C	–	79.6
				4	CH_2	3.78, 4.02 (d, *J* = 9.8, 2 H)	73.6
				5	CH_2	3.62 (s, 2 H)	64.0
Aglycone-II (C6-O-C6’)				Gluc-II			
1	C	–	164.4	1	CH	5.07 (d, *J* =7.2 Hz,1 H),	99.6
3	CH	5.46 (d, *J* = 13.0 Hz, 1 H)	79.6	2	CH	3.74 (m, 1 H)	75.8
4	CH_2	3.33 (m, H4a) 3.12 (d, *J* = 16 Hz, H4b)	36.0	3	CH	3.37 (m, 1 H)	72.5
4a	C	–	143.9	4	CH	3.60 (m, 1 H)	68.5
5	CH	6.81 (d, *J* = 2 Hz, 1 H)	108.6	5	CH	3.54 (m, 1 H)	77.2
6	C	–	162.3	6	CH_2	4.08 (d, *J* =11 Hz, 1 H) 3.59 (m, 1 H)	66.6
7	CH	6.98 (d, *J* = 2 Hz, 1 H)	104.1	Rham			
8	C	–	160.7	1	CH	4.73 (bs, 1 H)	100.8
8a	C	–	108.2	2	CH	3.94 (m, 1 H)	70.7
1’	C	–	130.4	3	CH	3.74 (m, 1 H)	71.1
2′, 6′	CH	7.45 (d, *J* =8.0 Hz, 2 H)	127.6	4	CH	4.00 (m, 1 H)	75.8
3′, 5′	CH	7.00 (d, *J* =8.0 Hz, 2 H)	113.6	5	CH	3.52 (m, 1 H)	69.9
4’	C	–	160.1	6	CH_3	1.26 (d, *J* =6.0 Hz, 3 H)	16.6
4’-OCH_3	CH_3	3.84 (3H, s)	54.4	Gluc-III			
				1	CH	5.11 (d, *J* =7.5 Hz, 1 H)	99.6
				2	CH	3.42 (m, 1 H)	76.0
				3	CH	3.55 (m, 1 H)	73.3
				4	CH	3.37 (m, 1 H)	70.2
				5	CH	3.54 (m, 1 H)	76.6
				6	CH_2	3,87 (d, *J* =12 Hz, 1 H) 3.68 (m, 1 H)	61.7

calc. 1044.9790; Aglycone: positive LC-QTOF-MS: *m/z* (%) $[M + Na-2H]^+$ 575.1981 (20), calc. 575.1979; ^{1}H and ^{13}C NMR data see Table 2.

2.4. Antimicrobial activity assessment (Agar-well diffusion method)

All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: 3 g (-); *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, 4 g (+); *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Listeria monositogenes NCTS11994, Bacillus cereus* 709 Roma, 1 no gram; *Mycobacterium smegmatis* ATCC607, and 2 fungi; *Candida albicans* ATCC60193, and *Saccharomyces cerevisiae* RSKK 251. The antimicrobial screening test using the agar-well diffusion method as adapted was used earlier (Barry, 1999; Woods, 2011). Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted approximately 106 colony forming units (CFU) per ml. They were “flood-inoculated” onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans* SDA was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Extract stock solutions were prepared at different concentrations (1.100–80.200 µg/mL) according to the amount of material obtained. The 1/10 dilution of each solvent was used as a control.

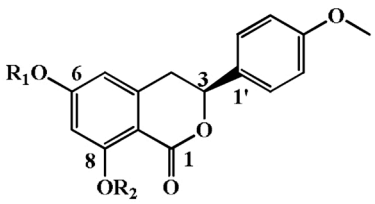
2.5. Minimal inhibition concentration (MIC) assay

The antimicrobial properties of compounds **1–8** were investigated quantitatively in respective broth media by using double microdilution, and the minimal inhibition concentration (MIC) values (µg/mL) were examined (Barry, 1999; Woods, 2011). The antibacterial and antifungal assays were carried out in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH. 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. The microdilution test plates were incubated for 18 h at 35 °C. Brain Heart Infusion broth (BHI) (Difco, Detriot, MI) was used for *M. smegmatis* and incubated for 48−72 h at 35 °C. The MIC was defined as the lowest concentration that showed no growth. Ampicillin (10 mg/mL), streptomycin 10 mg/mL, and fluconazole (2 mg/mL) were used as a standard antibacterial and antifungal drug, respectively. The 1/10 dilution of each solvent was used as a control.

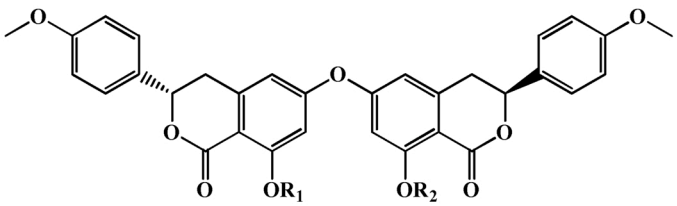
3. Results and discussion

3.1. Structure elucidation

The chromatographic separation and spectroscopic analysis (1D and 2D NMR, LC-QTOF-MS, UV, FT-IR) of crude ethyl acetate and water fractions of MeOH extract of *Scorzonera aucheriana* indicated the



1-2



3

Comp.	R_1	R_2
1	O-*β*-D-glucopyranosyl-(6⟶1)-O-*β*-D-apiofuranoside	-H
2	O-*β*-D-glucopyranosyl-[(4⟶2)-O-glyceryl)]-(6⟶1)-O-*β*-D-apiofuranoside	-H
3	O-*β*-D-glucopyranosyl-[(4⟶2)-O-glyceryl)]-(6⟶1)-O-*β*-D-apiofuranoside	O-*β*-D-glucopyranosyl-(6⟶1)-O-*α*-L-rhamnopyranosyl-(4⟶1)-O-*β*-D-glucopyranoside

Fig. 1. Chemical structure of isolated new compounds from *S. aucheriana*.

presence of three new dihydroisocoumarins glycoside compounds (**1**-**3**). In addition to these, two known scorzopygmaecoside (**4**) (Şahin et al., 2020), and scorzocreticoside II (**5**) (Paraschos et al., 2001) and three known caffeic acid derivatives such as 3,5-O-dicaffeoyl-*epi*-quinic acid (**6**), 3,5-O-dicaffeoylquinic acid (**7**) (Kim and Lee, 2005; Sarı et al., 2019; Wang et al., 2012), and 3,4-dihydroxyphenyl caffeate (**8**) (Zhang et al., 2008) were isolated from this plant for the first time (Fig. 1). Dihydroisocoumarins are phenolic compounds related to isocoumarin, and its glycosides are known in the literature (Paraschos et al., 2001; Şahin et al., 2020).

Compound **1** was isolated as a yellow amorphous solid. Its molecular formula was determined as $C_{27}H_{32}O_{14}$ by LC-QTOF-MS (*m/z* $[M + Na]^+$ 603.1674 (100), calc. 603.1679). The UV spectrum of **1** showed absorption maxima at 291, 268, and 225 nm and closely resembled that of dihydroisocoumarin glycoside. The ^{1}H NMR spectrum of compound **1** showed a proton at *δ* 5.37 ppm (d, *J* = 13 Hz, H-3) coupled with two methylene protons at *δ* 3.17 (dd, *J* = 16, 13 Hz, H-4a) and 2.96 (d, *J* = 16 Hz, H-4b) ppm, a methoxy group at *δ* 3.83 ppm (s, 3 H), and two meta-coupled aromatic protons on a disubstituted A-ring at *δ* 6.22 (bs, 1H, H-5) and *δ* 6.59 ppm (bs, 1H, H-7). These two protons (H-5 and H-7) were located at *δ* 6.52 ppm (s, H-5)/*δ*C 112.92 ppm (C-5), and *δ* 6.81 ppm (s, H-7)/*δ*C 103.7 ppm (C-7) in the scorzopygmaecoside (Şahin et al., 2020). Chemical shifts of H-5 and H-6 showed 0.3 ppm variation. Thus sugar substituted positions (C-6 and C-8) could vary each other. The sharp ^{1}H NMR signal at *δ* 8.04 ppm indicated the presence of the hydroxyl group at the C-8 position of the aglycone. Additionally, the presence of ortho-coupled aromatic protons (*δ* 6.97, d, *J* = 8.1 Hz, H-3′, 5′ and *δ* 7.44, d, *J* = 8.2 Hz, H-2′,6′) suggested a disubstituted B-ring. The ^{13}C NMR spectrum showed the presence of one carbonyl group (*δ* 175.0, C-1) characteristic of dihydroisocoumarins glycoside (Paraschos et al., 2001; Şahin et al., 2020). Moreover, the ^{13}C NMR and DEPT spectra for the aglycone moiety of compound **1** revealed 12 aromatic carbons (three oxygenated quaternary, three quaternary, and six protonated), one methylene, one oxygenated methine, and one methoxy group. In the HMBC spectrum, a ^3J correlation between the methylene protons (H-4) and C-4a (*δ* 143.1) confirmed the dihydroisocoumarin skeleton. The B-ring connectivity to the benzopyranone unit was indicated from the HMBC spectrum by the correlations of C-3 (*δ* 80.1) with H-2′ and H-6′. The protons of the methoxy group were correlated with C-4' (*δ* 159.9). Furthermore, ^{1}H NMR spectra of compound **1** gave two anomeric proton peaks at *δ* 4.76 ppm (d, *J* =8.0 Hz, Glc H-1)/*δ*C 99.8 ppm (Glc C-1), and *δ* 5.10 ppm (bs, *J* = 2.4 Hz, Api H-1)/*δ*C 110.19 ppm (Api C-1) which showed the glycoside as in dimeric form. The rest of the proton's connectivities were assigned by the 2D COSY, and TOCSY spectra and the NMR data are listed in Table 1. The anomeric proton at *δ*H 4.76 ppm of the glucose moiety showed an HMBC correlation with C-6 (*δ*C 162.35 ppm) in ring A of the dihydroisocoumarin. Besides, the aromatic protons signal of the dihydroisocoumarin at *δ* 6.22 ppm (bs, H-5) and *δ* 6.59 ppm (bs, H-7) showed an HMBC correlation with Glu C-1 (*δ*C 99.8 ppm), thus showing the *O*-glycosidic linkage was at the C-6 position of compound **1**. In the ^{1}H-^{1}H COSY spectrum, a cross-peak was observed between the anomeric proton at *δ* 4.76 ppm (d, *J* =8.0 Hz, Glc H-1) to H-6″ at *δ* 3.80/4.08 ppm (m, Glc H-6'a/e) for the *β*-glucopyranose ring system. The chemical shifts of the second sugar unit with the anomeric proton at *δ* 5.10 ppm (bs, *J* = 2.4 Hz, 1H, Api H-1))/*δ*C 110.19 ppm (Api C-1) and others Api H-2 (4.02, d, *J* = 2.4 Hz, 1 H), Api H-4 (3.81, 4.02, d, *J*=9.8

Hz, 1 H), and Api H-5 (3.62, s, 2 H) indicated that it was an apiofuranose system with an β-anomeric configuration as in literature (Wang et al., 2012). Apiose was linked to the Glu H-6 due to the ^{13}C NMR data of Glu C-6 at 67.5 ppm, which was shifted downfield. Thus, apiose was assigned as the terminal position of the glycoside of compound **1**. The linkage of the glycone moieties to the dihydroisocoumarin aglycone was established from HMBC data, which allows unambiguous assignment of the sugar position linkage. The anomeric proton at δ 4.76 ppm of the glucose moiety showed an HMBC correlation with C-6 (δC 162.71 ppm) in ring A dihydroisocoumarin, thus establishing the *O*-glycosidic linkage at C-6 of the dihydroisocoumarin nucleus.

Relative stereochemistry at the C-3 position of the aglycone obtained after the acidic hydrolysis of the compound **1** was determined to be S configuration from the observed optical activity value ($[\alpha]_d^{24}$ +18.3) (Şahin et al., 2020; Paraschos et al., 2001). The optical activity of pure d-glucose and d-apiose are known as $[\alpha]_d^{24}$ +52 and $[\alpha]_d^{24}$ +8, respectively. Optical activity for the glycone fraction of compound **1** was obtained to be $[\alpha]_d^{24}$ +57. Thus, the configuration of sugars part of compound **1** was assigned as D. TLC, and HPLC analysis of the glycone with the authentic sample (D-glucose) was also showed d-glucose.

After the interpretation of all data, new compound **1** was (3*S*)-6-[O-β-d-glucopyranosyl-(6→1)-O-β-d-apiofuranosyl]-8-hydroxy-3-(4-methoxyphenyl)-3,4-dihydro-1*H*-isochromen-1-one and given the trivial name as *iso*-scorzopygmaecoside (Şahin et al., 2020). Various substituted phenyl dihydroisocoumarins are known in the literature (Çiçek et al., 2018).

Compound **2** was isolated as a yellowish powder, and its molecular formula was determined as $C_{30}H_{38}O_{16}$ by LC-QTOF-MS (*m/z* [M-CH_3OH-H_2O]$^+$ 604.1605 (35), calc. 603.1605). The UV spectrum of **1** showed absorption maxima at 278, 237, and 207 nm and closely resembled that of compound **1** and dihydroisocoumarin glycoside (Paraschos et al., 2001; Şahin et al., 2020). ^{1}H and ^{13}C NMR spectra indicated a similar substitution pattern in compound **2** compared with that of **1**. The data observed in the ^{1}H and ^{13}C NMR spectra showed a strong similarity with compound **1** and Scorzocreticoside I (Paraschos et al., 2001) but had variation in the glycone region of compound **2**. The anomeric proton at δ 4.78 (d, J =8.0 Hz), 5.09 (d, J = 2.4 Hz), and the other sugar protons' splitting pattern gave evidence of a β-glucose and apiose, which was the same as glycone part of compound **1**. However, two extra CH (73.68 ppm) and 2xCH_2 (62.98 ppm) peaks were observed in the 60−80 ppm region of the ^{13}C/APT NMR spectra. It was determined that this group was glycerol, and as a result of HMBC correlation and literature comparison, the glycerol unit was attached from the C-2 position to the C-4 position of glucose. The ^3J correlation between the anomeric proton and C-6 (δ 163.2) in the HMBC spectrum indicates the attachment site of glucose. The carbon at position C-8 was identified by its ^2J correlation with only one aromatic proton at δ 6.66 (H-7), while C-6 (δ 165.8) showed two ^2J correlations with both H-5 (δ 6.33) and H-7 (δ 6.66). Furthermore, the hydroxyl signal at δ 8.02 ppm indicated the presence of a free hydroxyl group on C-8.

Stereochemistry at the C-3 position of the aglycone obtained after the acidic hydrolysis of the compound **2** was also determined to be S configuration from the observed optical activity value ($[\alpha]_d^{24}$ +19.4) as in compound **1** (Şahin et al., 2020; Paraschos et al., 2001). The optical activity of the sugar fraction of compound **2** was found to be $[\alpha]_d^{24}$ +53, which was similar to compound **1**. Therefore, the configuration of sugars in the glycone part of compound **1** was assigned as D.

Consequently, new compound **2** was assigned as (3*S*)-6-{O-β-d-glucopyranosyl-[(4→2)-O-glyceryl)]-(6→1)-O-β-d-apiofuranosyl}-8-hydroxy-3-(4-methoxyphenyl)-3,4-dihydro-1*H*-isochromen-1-one and given the trivial name as Scorzoaucherioside I (**2**).

The third new bis-dihydroisocoumarin glycosides (**3**) was isolated as a yellowish powder, and its molecular formula was determined as $C_{64}H_{80}O_{34}$ by LC-QTOF-MS (*m/z* [M-Glycerol + CH_3OH-H]$^+$ 1348.4412 (10), calc. 1348.4418). The UV spectrum was similar to that of compounds **1** and **2**. Comparison of the ^{1}H and ^{13}C NMR data of **3** with the isolated compounds **1**, **2**, **4–5** and literature (Donia, 2016; Harkati et al., 2010; Li and Wang, 2010; Paraschos et al., 2001; Saeed, 2006; Sarı et al., 2007; Şahin et al., 2020; Çiçek et al., 2018), the aglycone of compound **3** found to be bis-dihydroisocoumarin derivatives linked at C6-O-C6' position as 6,6′-oxybis[3,4-dihydro-8-glcosyl-3-(4-methoxyphenyl)-1*H*-2-benzopyran-1-one. The proton peaks for the aglycone part of compound **3** were overlapped. However, some of the carbon peaks of the aglycone of **3** appeared very close to each other (Table 2). In the ^{1}H NMR spectrum for the aglycone part of compound **3** showed 4 protons (2xH-2′,6′) at δ 7.45 ppm, 6 protons (2xH-3′,5' and 2xH-7) at δ 7.0 ppm, 2 protons at δ 6.81 (2xH-5) ppm and 2 protons at δ 5.46 (2xH-3) ppm were integrated. Observation of these peaks and some of them in the lower area than other dihydroisochroman compounds indicates that the aglycone is in bis form. The ^{1}H NMR spectrum for the aglycone parts of **3** was comparable to that of **1**-**2** and **4**-**5** (Paraschos et al., 2001; Şahin et al., 2020), but the presence of five anomeric protons and carbons at δ 5.00 (Glu-I H-1)/δC 102.5 ppm (Glu-I C-1), δ 5.01 (Api. H-1) δC 109.5 ppm (Api. C-1), δ 5.07 (Glu-II H-1)/δC 99.6 ppm (Glu-II C-1), δ 4.73 (Rha. H-1)/δC 100.8 ppm (Rha. C-1), and δ 5.11 (Glu-III H-1)/ δC 99.6 ppm (Glu-III C-1) were indicative of a disaccharide and three saccharides in the glycone parts of compound **3**. The attachment site of glucose I-O-apiose and glucose II-O-rhamnose-O-glucose III to the aglycons I and II was determined similarly to compounds **1** and **2**. The numerous signals between 3.10−411 ppm corresponded to the protons of the five sugars in **3**. The sugar proton assignment was confirmed by its ^3J correlations between H-1' to H-6'/5' in the COSY spectra. The ^{13}C NMR spectrum confirmed the presence of added sugar compared to **1** and **2** (29 peaks in **3** compared to 11/13 peaks in **1** and **2**, respectively). The glucose I and II were attached to the aglycones, observed from the glucose I and II anomeric protons (δ 5.00 and 5.07) to the C-8/C-8′ carbons (δ 160.8, 162.3) of **3**, respectively. The characteristic downfield shift of C-6 of glucose I and II (δ 66.6 and 67.4) indicated that an apiose and a rhamnose unit was attached to this position. And the third glucose was seemed to be in the terminal position of the three saccharide chain of **3**. Moreover, the first glycone part of **3** was the same as in compound **2**. These were confirmed by the ^3J correlation of apiose H-1 with glucose I C-6 in the HMBC spectrum.

The aglycone portion obtained after the acidic hydrolysis of compound **3** was not optically active. Thus, relative stereochemistry at C-3/C-3′ positions of bis-aglycone was assigned as S/R, respectively. Optical activity of pure d-glucose, d-apiose, and l-rhamnose are known as $[\alpha]_d^{24}$ +52 and $[\alpha]_d^{24}$ +8, and $[\alpha]_d^{24}$ +7, respectively. The optical activity of the glycone fraction of compound 3 was found to be $[\alpha]_d^{24}$ +66. Sugars could not be purified individually due to the small quantity. Thus, sugars in the glycone part of compound 3 were assigned as d-glucose, d-apiose, and l-rhamnose. Some of the sugars in the glycone of compound **3** were compared on TLC and HPLC with the standard samples (d-glucose and l-rhamnose) that we had (Yokosuka et al., 2013).

Consequently, compound **3** was assigned as (3*S*,3′*R*)-8-{O-β-d-glucopyranosyl-[(4→2)-O-glyceryl)]-(6→1)-O-β-d-apiofuranosyl}-3-(4-methoxyphenyl)-6-{[3-(4-methoxyphenyl)-1-oxo-8-[O-β-d-glucopyranosyl-(6→1)-O-α-l-rhamnopyranosyl-(4→1)-O-β-d-glucopyran-osyl]-3,4-dihydro-1*H*-isochromen-6-yl]oxy}-3,4-dihydro-1*H*-isochromen-1-one and given the trivial name as scorzoaucherioside II (**3**). In the literature, a cyclic form of dimeric phenyl dihydroisocoumarin, which was known as tragoponol, has been mentioned (Zidorn et al., 2010; Çiçek et al., 2018). Compound **3** was identified as the dimeric dihydroisocoumarin, which was belongs to a new type of dimer, a phenol ether, as opposed to the known macrocyclic dilactone tragoponol (Zidorn et al., 2010; Çiçek et al., 2018).

All the NMR data of **4** and **5** isolated from *Scorzonera aucheriana* revealed known compounds scorzopygmaecoside (Şahin et al., 2020) and scorzocreticoside II (Paraschos et al., 2001), respectively. 4′-Methoxy-dihydroisochromone compounds have hydroxyl groups at the C6 and C8 positions. When sugars are attached to the C-8 position, the carbonyl carbon peak resonates at 161.0–164.0 ppm. Besides, the

Table 3
Screening for the antimicrobial activity of isolated compounds (**1**-**8**).

Com.	Stock Sol. (μg/mL)		Microorganisms, inhibition zone (mm), and minimal inhibition concentration (MIC, μg/mL)									
			Gram (-)			**Gram (+)**				**Tub.**	**Fungi**	
			Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Lm*	*Bc*	*Ms*	*Ca*	*Sc*
1	80200	mm	–	–	6	8	8	8	–	10	–	–
		MIC	–	–	4100	2050	2050	2050	–	25.6	–	–
2	13500	mm	–	–	10	–	–	–	–	–	–	–
		MIC	–	–	377.5	–	–	–	–	–	–	–
3	3400	mm	–	–	–	–	–	–	–	10	–	–
		MIC	–	–	–	–	–	–	–	21.2	–	–
4	2700	mm	–	–	–	–	6	–	–	–	–	–
		MIC	–	–	–	–	135	–	–	–	–	–
5	8000	mm	–	–	–	10	12	–	–	8	8	10
		MIC	–	–	–	400	200	–	–	800	800	400
6	1100	mm	–	–	–	–	–	–	–	–	–	–
		MIC	–	–	–	–	–	–	–	–	–	–
7	7000	mm	–	–	–	–	–	–	–	–	–	–
		MIC	–	–	–	–	–	–	–	–	–	–
8	2900	mm	–	–	6	–	–	–	–	10	8	–
		MIC	–	–	290	–	–	–	–	145	290	–
Amp.	10	mm	10	10	18	10	35	15		–	–	–
		MIC	10	18	128	35	10	15				
Strep.	10	mm								35		
		MIC								4		
Flu	5	mm									25	25
		MIC									8	8

Ec: *Escherichia coli*, *Yp*: *Yersinia pseudotuberculosis*, *Pa*: *Pseudomonas aeruginosa*, *Sa*: *Staphylococcus aureus*, *Ef*: *Enterococcus faecalis*, *Lm*: *Listeria monositogenes*, *Bc*: *Bacillus cereus*, *Ms*: *Mycobacterium smegmatis*, *Ca*: *Candida albicans*, *Sc*: *Saccharomyces cerevisiae*, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (-): no activity: Tub.: Tuberculosis.

C-8a quaternary carbon peaks have been observed between 105−108 ppm. When sugars are bound at the C-6 position, the carbonyl carbon peak was seen at the lower area around 171.0–175.0 ppm, and the C-8a carbon peak is in the upper field around 100 ppm. Thus, the observation of C-1 and the C8a carbon peaks in the up and downfield area, glycone could be substituted at the C-6 or C-8 position of 4'methoxy substituted dihydroisocoumarin compounds. Also, the observation of the HMBC correlation and the proton peak formed by the chelation of the C-8 free hydroxyl group in the downstream area indicates the positions of the glycoside binding. Thus, glycone parts of compounds **1** and **2** are located at the C-6 position, and glycosylation of compounds **4** (Şahin et al., 2020) and **5** (Paraschos et al., 2001) were located at the C-8 position of dihydroisocoumarin.

Analysis of NMR data of **6**, **7**, and **8** showed known compounds 3,5-O-dicaffeoyl-*epi*-quinic acid (Kim and Lee, 2005; Sarı et al., 2019; Wang et al., 2012), 3,5-O-dicaffeoylquinic acid (Kim and Lee, 2005; Sarı et al., 2019; Wang et al., 2012), and 3,4-dihydroxyphenylcaffeate (Zhang et al., 2008), respectively.

3.2. Antimicrobial activity

The antimicrobial properties of all isolates were investigated against ten microorganisms. Compounds **1**, **2**, and **8** showed potent anti-pseudomonal activity. It was determined that compound **2** was selective and had activity at low concentrations of 290−377 μg/mL. Compound **4** gave low dose activity against Gram-positive bacteria *E. faecalis*. However, compounds **1** and **5** have been observed to have low activity against Gram-positive bacteria. It was determined that the most sensitive microorganism against the tested compounds **1–8** was *M. smegmatis*. Compounds **1** and **3** had high levels of anti-tuberculosis activities. Compound **3** had shown only selective activity against *M. smegmatis*.

Besides, compounds **5** and **8** showed low anti-tuberculosis activities in the range of 145−800 μg/mL. There was no activity against yeast fungi for the tested compounds, but a low level of antifungal activity was observed in compounds **5** and **8** in the range of 290−800 μg/mL concentration (Table 3).

4. Conclusion

The chemical and biological investigation of the *S. aucheriana* were herein reported. All the isolated compounds (**1**-**8**) were tested against 10 microorganisms. Activity guided ethyl acetate and water fraction of a crude methanol extract of the *S. aucheriana* gave eight natural compounds, which are named as *iso*-scorzopygmaecoside (**1**), scorzoaucherioside I (**2**), scorzoaucherioside II (**3**), scorzopygmaecoside (**4**), scorzocreticoside II (**5**), 3,5-O-dicaffeoyl-*epi*-quinic acid (**6**), 3,5-O-dicaffeoylquinic acid (**7**), and 3,4-dihydroxyphenyl caffeate (**8**). They were characterized to be three new 4'-methoxy-dihydroisocoumarins derivatives (**1**-**3**), two known dihydroisocoumarins (**4**, **5**), two known quinic acid derivatives (**6**, **7**), and one known phenolic caffeate (**8**). All compounds (**1**-**8**) were isolated and identified for the first time from this species. Dihydroisocoumarins, dicaffeoylquinic acid and dihydroxyphenyl caffeate type of natural compounds are very common to *Scorzenera* species and show various biological activities (Donia, 2016; Harkati et al., 2010; Li and Wang, 2010; Paraschos et al., 2001; Saeed, 2006; Sarı et al., 2007; Şahin et al., 2020; Çiçek et al., 2018). Compounds **1** and **3** have shown strong anti-tuberculose activity against *M. smegmatis* with MIC values of 25.6 μg/mL and 21.2 μg/mL, respectively.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2021.02.010.

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