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Research Paper

Phytochemical analysis and antimicrobial, antioxidant, and cytotoxic activities of *Scorzonera aucheriana* DC (Asteraceae)

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ABSTRACT

The present study is mainly focused on the isolation and structure elucidation of four new (**1–4**), two known astragalin (**5**), and kaempferol-3,7-di-O-β-D-glucopyranoside (**6**) from the aerial parts of *Scorzonera aucheriana* DC. The structures of the isolated compounds were elucidated based on 1D and 2D NMR (^{1}H, ^{13}C/APT, COSY, HMBC, and HSQC), UV, FT-IR, and HRESI-MS data. The total phenol and flavonoid contents and the antioxidant activity of *S. aucheriana* crude methanol extract and its fractions (*n*-hexane, chloroform, ethyl acetate, and water) were investigated. The highest antioxidant activities against DPPH and FRAP were observed in the ethyl acetate fraction of *S. aucheriana*. The antimicrobial activity of the methanol extract, its solvent fractions, and isolated compounds (**1–6**) was evaluated against 10 microorganisms. The chloroform and ethyl acetate fractions exhibited antimicrobial activity against all tested microorganisms. Compounds **1–3**, **5**, and **6** exhibited anti-tuberculosis activity with MICs ranging from 30.9 μg/mL to 250 μg/mL. Compounds **2**, **4**, and **5** have also demonstrated moderate antifungal activity, with MICs ranging from 110 μg/mL to 765 μg/mL against *C. albicans*, a pathogenic yeast. Compounds **1–3** showed almost no cytotoxic activity in L-929 cells.

1. Introduction

The genus *Scorzonera* L. (Asteraceae, formerly Compositae) comprises approximately 180 species worldwide. *Scorzonera aucheriana* DC. belongs to the genus Scorzonera, which comprises more than 52 species, including 32 endemics to Türkiye [1]. This species is spread throughout the Mediterranean and central Asia and is popularly known as 'Buz Tekesakalı' in Türkiye [2]. Members of the genus *Scorzonera* are consumed as vegetables and used in traditional medicine worldwide, including in Türkiye [1,3,4]. Although *S. auceriana* has been transferred to the genus *Gelesia* Cass. (*Gelasia aucheriana* (DC.) Zaika, Sukhor. & N. Kilian) based on new molecular phylogenetic and carpological data [5], since taxonomic debates regarding members of the Scorzonera genus continue, the name *S. auceriana* has been preferred in this article.

Pharmacological research on the genus *Scorzonera* began in 1992 with the species *Scorzonera glabra* Rupr. [6]. Pharmacological studies have been conducted on crude extracts or isolated compounds from various organs of *Scorzonera* species [7]. To date, numerous biological activities such as anti-inflammatory, antinociceptive effect, wound-healing, skin protection effect, antitumor and cytotoxic activities, hepatoprotective activity, antimicrobial and anti-biofilm activities, quorum sensing-regulated effect, anti-ulcerogenic and antidiarrheal effects, laxative, hypolipidemic effect, enzyme, antioxidant, repairing cerebral ischemia, protective effect on acute myocardial infarction, sedative and antidepressant, anti-stress, and immunomodulatory effects have been evaluated *in vitro* and *in vivo* across 56 species [7]. They are traditionally used to treat diabetes mellitus, herpes zoster, pregnancy resistance, arteriosclerosis, hypertension, rheumatism, colds, lung diseases, ulcers, kidney diseases, snake bites, stomach tumors, and pulmonary edema [7–9].

The bioactive naturally occurring spirocyclic dienone compounds Spirocalcardine A and B, Argustosine D, Hopeanol, Glaziovine, and Spirocacillene A have been isolated from plants, trees, and bacteria [10,11]. Spiroindans compounds have been reported from the *Cannabis sativa* plant [12]. Sulfur-containing spiro secondary metabolites have been reported in the Asteraceae, Alliaceae, and Brassicaceae families

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Table 1
NMR data of compounds **1–3** (CD_3OD, 400/100 MHz NMR, ppm, *J*, Hz).

No	APT	1		2		3	
		^1H (COSY)	^{13}C (HSQC)	^1H (COSY)	^{13}C (HSQC)	^1H (COSY)	^{13}C (HSQC)
1, 4′	C	–	52.1	–	52.1	–	52.2
2	CH	7.07 (d, 9.8, 1H)	155.1	7.09 (d, 9.6, 1H)	154.7	7.05 (d, 9.8, 1H)	154.8
3	CH	6.26 (d, 9.8, 1H)	126.2	6.27 (d, 9.6, 1H)	126.3	6.23 (d, 9.8, 1H)	126.1
4	C	–	187.6	–	187.6	–	187.9
5	CH	6.26 (d, 9.8, 1H)	126.2	6.27 (d, 9.6, 1H))	126.3	6.29 (d, 9.8, 1H)	126.5
6	CH	7.07 (d, 9.8, 1H)	155.1	7.09 (d, 9.6, 1H)	154.7	7.12 (d, 9.8, 1H)	155.5
2′	CH	7.06 (s, 1H)	105.9	7.06 (s, 1H)	105.9	6.49 (s, 1H)	100.7
3a′	C	–	122.6	–	123.2	–	121.1
5′	CH_2	2.24 (brs, 2H).	37.8	2.27*, (t, 8.0, 2H)	37.5	2.27*, 2H	37.0
6′	CH_2	3.57 (brs, 2H),	33.1	3.53*, 2H	33.3	3.49*, 2H	33.4
6a′	C	–	152.8	–	149.6	–	149.8
1″	C			–	151.5	–	165.4
2″	C			–	123.2	–	158.4
3″	CH			6.80 (d, 8.1, 1H)	114.7	6.80 (d, 3.2, 1H)	114.6
4″	CH			6.71 (d, 8.1, 1H)	115.1	–	129.3
5″	C			–	148.3	6.98 (d, 8.3, 1H)	129.1
6″	CH			7.08 (brs, 1H)	129.6	6.68 (d, 8.3, 1H)	114.5
7″	CH_2			3.42 (s, 2H)	70.7	3.85 (s, 2H)	60.9
1‴	CH			4.31 (d, 7.8, 1H)	102.9	4.96**	99.3
2‴	CH			3.28*, 1H	76.7	3.20*, 1H	76.7
3‴	CH			3.53*, 1H	73.7	3.25*, 1H	73.7
4‴	CH			3.30*, 1H	70.2	3.42*, 1H	73.5
5‴	CH			3.42*, 1H	78.1	3.30*, 1H	77.2
6‴	CH_2			3.90*, 3.71*, 2H	61.3	3.86*, 3.72*, 2H	60.9
1⁗	CH					4.85 (d, 7.8, 1H)	100.5
2⁗	CH					3.42*, 1H	76.6
3⁗	CH					3.52*, 1H	73.2
4⁗	CH					3.38*, 1H	70.1
5⁗	CH					3.40*, 1H	76.8
6⁗	CH_2					3.90*, 3.76*, 2H	60.9

* Signal patterns are unclear due to overlapping.
** Under the HOD (HSQC).

[13]. Ten sulfur-containing spirochetal glycoside compounds have been reported from the above-ground parts of the *Breynia fruticosa* plant [14].

The chemical constituents of the *Scorzonera* genus have been extensively studied in recent years. Many different secondary metabolites such as volatile oils, monoterpenes, diterpenes, sesquiterpenes, triterpenoids, steroids, quinic acid derivatives, flavonoids, coumarins, lignans, stilbene derivatives, benzylphthalides and kava lactones, phenylpropanoids and their derivatives, phenolic acids and other phenolics, aliphatic acids, phthalic acids, and alkanes, vitamin, sugars and glycerol derivatives, alkaloids, amino acid composition, and polysaccharides were reported on a review manuscript [7].

Phytochemical investigation of *S. aucheriana* led to the isolation and structural elucidation of four new natural compounds and two known flavonoids. In addition, the antimicrobial activities of the crude methanol extract, its fractions, and the isolated compounds (**1**–**6**) are presented. The antioxidant activities of the methanol extract and its fractions were studied. Cytotoxicity of the isolated compounds **1–3** against L929 cells was also investigated.

2. Materials and methods

2.1. Plant materials

S. aucheriana was collected from Sivas Province, Zara District, Yarağıl region, on calcareous rocks at 1496 m (July 13th, 2018, 39° 45′ 044 N - 037° 42′ 034 E) in Türkiye. Materials were identified using Flora of Türkiye and The East Aegean Islands [3] and relevant literature of Turkish *Scorzonera* [1]. The voucher was deposited in the Herbarium of Biology (KTUB, Makbul & Coşkunçelebi 244) at Karadeniz Technical University, Turkey. The plant was cleaned to remove impurities, dried, and stored in an air-tight container before use.

2.2. Chemicals, chromatography, and instrument methods

Butylated hydroxyanisole (BHA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, gallic acid (GA), iron (III) chloride ($FeCl_3$), methanol, chloroform, ethyl acetate, *n*-hexane, petroleum ether, dichloromethane, trichloroacetic acid (TCA), sodium carbonate (Na_2CO_3), and quercetin (QE) were purchased from Sigma-Aldrich (St. Louis, MO). Optical rotations were measured using an AA-5 Series automatic polarimeter. UV spectra were obtained with a Spectrostar nano BMG Labtech spectrometer. Infrared spectra were obtained using a PerkinElmer 1600 FT-IR spectrometer (ATR) (4000–400 cm^{-1}). The mass spectral analyses were performed using an Agilent 6230 **A** HRESI-MS. Melting points were determined using a Thermo-var apparatus fitted with a microscope, and the results were uncorrected. Using TMS as an internal standard, ^1H and ^{13}C NMR, along with 2D NMR spectra, were obtained on a Bruker 400 MHz NMR spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C/APT). CD_3OD and $CDCl_3$ were used as NMR solvents. The ACD NMR program was used to elucidate the structures of the isolated compounds. Chemical shifts were expressed in δ (ppm). Coupling constants (*J*) were reported in hertz (Hz). Column chromatography (CC) was performed using silica gel (Kieselgel 60, 320–400 mesh), RP18 silica gel, and PTLC (Silica gel HF_{254}, 20 cm × 20 cm, 0.5 mm). TLCs were performed on silica gel (Kieselgel 60 F_{254}, Merck) plates, and the spots were visualized under UV light or by spraying with 20% H_2SO_4 and heating.

2.3. Extraction and isolation

The dried, powdered aerial parts of *S. aucheriana* (833 g) were extracted with methanol (4 L) three times for 72 **h** at room temperature on a shaker. The 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 × 3), chloroform (50 mL × 3), ethyl acetate (50 mL × 3) and water (80 mL) to give crude mixtures (12.9 g, 1.8 g, 4.6 g and 20.9 g, respectively) [15]. The water fraction (10.8 g) was subjected to reverse-phase CC (RP-18, silica gel) using a mobile phase with decreasing polarity of H_2O:MeOH (100:0 → 0:100), yielding 79 fractions (10 mL each, W—Fr). After the TLC control, fractions W—Fr 24, W—Fr 38, and W—Fr 54 afforded compounds **1** (23.9 mg), **2** (12.3 mg), and **3** (14.4 mg), respectively. The ethyl acetate fraction (4.6 g) was subjected to CC (Kieselgel 60, 320–400 mesh) using the increased polarity of *n*-hexane-AcOEt (100:0 → 0:100), and AcOEt-methanol (100:0 → 0:100) mobile phase to give 106 subfractions (EA-Fr, ~35 mL, each). After TLC control, EA-Fr 66–68 (158.8 mg) was combined and further separated by PTLC (silica gel HF_{254}, 20 × 20, 0.5 mm, 2 plates) using AcOEt-MeOH-H_2O (80:20:2) mobile phase to provide pure compound **4** (17.5 mg). Then, EA-Fr 64–65 were also combined (125.5 mg) and further purified by repeated CC (Kieselgel 60, 320–400 mesh) with increasing polarity of *n*-hexane-$CHCl_3$ (100:0 → 0:100), $CHCl_3$-AcOEt (100:0 → 0:100), and AcOEt-CH_3OH (100:0 → 0:100) mobile phase to give 69 subfractions (EA-SFr1, 25 mL, each). TLC control revealed that EA-SFr1 42 gave compound **6** (31.5 mg), and EA-SFr1 69–74 were combined and further purified by repeated CC (Kieselgel 60, 320–400 mesh) with increasing polarity of *n*-hexane-$CHCl_3$ (100:0 → 0:100), and *n*-hexane-AcOEt (100:0 → 0:100) mobile phase to give 42 subfractions (EA-SFr2, ~25 mL, each). After TLC control, EA-SFr2 20–22 (110.8 mg) was combined and further separated by PTLC (silica gel HF_{254}, 20 × 20, 0.5 mm, 2 plates) using an AcOEt-MeOH-H_2O (90:10:1) mobile phase to yield **5** (20.5 mg).

Compound **1** [sodium 4-oxo-5′,6′-dihydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta[d][1,3]-oxathiol]-2′-yl sulfate]: Yellowish, m.p. 155–158 °**C**; $[\alpha]_D^{24}$ + 17.7 (c 0.0015, methanol); R_f: 0.32 (AcOEt-MeOH-H_2O, 7:2:1); UV (Methanol) λ_{max} (nm): 224 (2.67), 245 (2.54), 302 (1.94). FT-IR (ATR, cm^{-1}): 3344 (-OH, due to moisture), 2917, 2837 (-CH), 1657 (C=O), 1447 (C=C), 1119, 1023 (C—O); HRESI-MS (*m/z*, %): $C_{11}H_9O_6S_2Na$: $[M + H]^+$ 325.2160 (3), calc. 325.2137; $[M + CO-H]^+$ 351.0115 (100), calc. 351.0182; $[M\text{-}SO_3Na + CH_3OH + H_2O]^+$ 271.0573 (65), calc. 271.0527; $[M + 2(^{34}S) + CO\text{-}H]^+$ 353.0210 (7), calc. 353.0267; and $[M + 4(^{34}S) + CO\text{-}H]^+$ 355.0690 (5), calc. 355.0775); ^1H and ^{13}C NMR data are shown in Table 1.

Compound **2** [5-Hydroxy-2-(4-oxo-5′,6′-dihydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta[d]-[1,3]-oxathiol]-2′-yl)-benzyl-O-*β*-D-glucopyranoside]: Yellowish, m.p. 137–139 °**C**; $[\alpha]_D^{24}$ + 36.3 (c 0.0011, methanol); R_f: 0.40 (AcOEt-MeOH-H_2O, 7:2:1); UV (Methanol) λ_{max} (nm): 226 (2.53), 248 (2.25), 294 (1.80). FT-IR (ATR, cm^{-1}): 3345 (-OH), 2918, 2837 (-CH), 1655 (C=O), 1415 (C=C) 1118, 1023 (C—O); HRESI-MS (*m/z*, %): $C_{24}H_{26}O_{10}S$: $[M + H]^+$ 507.2605 (100), calc. 507.2665; $[M + 2\ (^{34}S)]^+$ 508.4142 (8), calc. 508.4087; ^1H and ^{13}C NMR data are presented in Table 1.

Compound **3** [(4-(Hydroxymethyl)-1-(4-oxo-5′,6′-dihydrospiro [cyclohexa-2,5-diene-1,4′-cyclopenta[d]-[1,3]oxathiol]-2′-yl)phenyl-2-O-*β*-D-glucopyranosyl-(2 → 1)-O-*β*-D-gluco-pyranoside]: Yellowish, m.p. 146–149 °**C**; $[\alpha]_D^{24}$ + 45.6 (c 0.001, $CHCl_3$); R_f: 0.58 (AcOEt-MeOH-H_2O, 70:30:4); UV (Methanol) λ_{max} (nm): 221 (2.03), 250 (1.78), 296 (1.66). FT-IR (ATR, cm^{-1}): 3327 (-OH), 2917, 2853 (-CH), 1659 (C=O), 1596, 1447 (C=C) 1263, 1074 (C—O); HRESI-MS (*m/z*, %): $C_{30}H_{36}O_{15}S$, $[M + H]^+$ 669.1838 (40), cal. 669.1844; $[M + Na]^+$ 691.1669 (100), cal. 691.1663; $[M + 2\ (^{34}S) + Na]^+$ 693.3787, calc. 693.3754; ^1H and ^{13}C NMR data are shown in Table 1.

Compound **4** (7-O-Acetyl quercetin-3-O-*β*-D-galactopyranoside): Yellowish, m.p. 243–245 °**C**; $[\alpha]_D^{24}$–29.4 (c 0.0034, methanol); R_f: 0.75 (AcOEt-MeOH-H_2O, 40:10:1); UV (Methanol) λ_{max} (nm): 257 (3.16), 274 (3.07), 352 (2.94), λ_{max} (nm): 257 (3.16), 274 (3.07), 352 (2.94), FT-IR (ATR, cm^{-1}): 3679, 3340 (-OH), 2972, 2850 (-CH), 1620 (C=O), 1570, 1407 (C=C) 1249, 1065 (C—O); HRESI-MS (*m/z*, %): $C_{23}H_{22}O_{13}$: $[M + H]^+$ 507.1139 (100), calc. 507.1131; $[M\text{-}OAc]^+$ 463.0878 (75), calc. 463.0870. ^1H and ^{13}C NMR data are shown in Table 3.

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: *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Lisssteria monositogenes NCTS11994, Bacillus cereus* 709 Roma, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC60193, and *Saccharomyces cerevisiae* RSKK 251. The antimicrobial screening test, using the agar-well diffusion method as adapted, was employed earlier [16,17]. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted to approximately 10^6 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 into the agar using a sterile corn borer, and 50 μL of the extract was delivered into each well. The plates were incubated for 18 h at 35 °**C**. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Extracts and isolated compound stock solutions were prepared at different concentrations (1.100–221.100 μg/mL) according to the amount of material obtained. The 1/10 dilution of each solvent was used as a control.

2.4.1. Minimal inhibition concentration (MIC) assay

The antimicrobial properties of the *n*-hexane, chloroform, ethyl acetate, water fractions, methanol extract, and compounds **1–6** were investigated quantitatively in respective broth media by using double microdilution, and the minimal inhibition concentration (MIC) values (μg/mL) were examined [16,17] (Table 4). 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 at which no growth was observed. Ampicillin (10 mg/mL), streptomycin (10 mg/mL), and fluconazole (2 mg/mL) were used as standard antibacterial and antifungal agents, respectively. The 1/10 dilution of each solvent was used as a control.

2.5. Antioxidant activities

2.5.1. DPPH radical scavenging assay

The DPPH radical-scavenging activities of the n-hexane, chloroform, ethyl acetate, water fractions, and methanol extract were investigated using previously reported methods [18,19] (Table 5). Butylated hydroxytoluol (BHT) (50–25–12.5–6.25 μg/mL) was used as the positive control, and an evaluation mixture without extract served as the negative control. Briefly, a DPPH solution (6 × 10^{-5} M) and extracts at different concentrations were mixed and incubated for 30 min at **25** °**C** in the dark. The absorbance of the solution was measured at 517 nm using a spectrophotometer. IC_{50} values, representing the concentration at which 50% of DPPH radicals were quenched, were derived from the absorbance-concentration graphs (Table 5).

2.5.2. Ferric reducing antioxidant power (FRAP) assay

FRAP of the *n*-hexane, chloroform, ethyl acetate, water fractions, and methanol extract was investigated using previously reported methods (Albayrak et al., 2010; Kirby et al., 1997) (Table 5). Trolox (1000–500–250-125-62.5 μg/mL) was used as a positive control. Extracts, phosphate buffer (pH 6.6), and potassium ferricyanide ($K_3[Fe(CN)_6]$) (10%) were separately added and incubated for 20 min at 50 °**C**. After incubation, trichloroacetic acid (TCA) (10%) was added to the solution. The solutions were combined with distilled water and $FeCl_3$ (0.15%) and incubated for 30 **min** at 30 °**C** in the dark. Then, the absorbance was measured at 593 nm using a spectrophotometer. The

FRAP of extracts was presented as μg trolox equivalent (TE) per mg of sample (Table 5).

2.6. Total phenolic content (TPC)

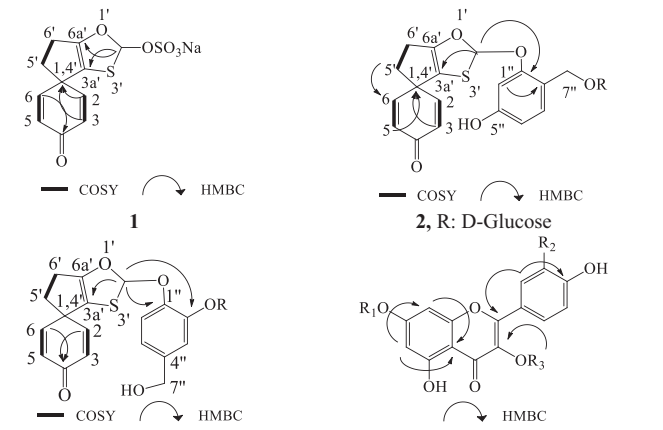
The total phenolic content was investigated using the method described by Agbor et al. [20] (Table 5). Gallic acid (GA) (500–250–125-62.5-31.25 μg/mL) was used as a positive control. The extracts, Folin-Ciocalteu reagent, water, and Na_2CO_3 (7%) were separately added and incubated for 2 h at 25 °C in the dark. Then, the absorbance of the solution was measured at 760 nm. The total phenolic contents of the *n*-hexane, chloroform, ethyl acetate, and water fractions, as well as the methanol extract, are μg gallic acid equivalent (GAE) per mg of sample in Table 5.

2.7. Total flavonoid content (TFC)

The total flavonoid content was investigated using the method described by Arvouet-Grand et al. [21] (Table 5). Quercetin (Q) (500–250–125-62.5-31.25 μg/mL) was used as a positive control. The extracts, methanol, and $AlCl_3$ (3%) were separately added and incubated for 10 min at 25 °C in the dark. Then, the absorbance of the solution was measured at 415 nm. The total flavonoid contents of the *n*-hexane, chloroform, ethyl acetate, and water fractions, as well as the methanol extract, are μg quercetin equivalent (QE) per mg of sample in Table 5.

2.8. Cell viability assay

Stock solutions **1–4** were prepared by dissolving the compounds in methanol to achieve a concentration of 100 mg/mL. Dilution series of 10, 1, 0.1 mg/mL were prepared from these stock solutions (Logarithmic doses were chosen). Aliquots of the stock solution and the diluted solutions were then added to the prepared medium at a final volume ratio of 0.1% (*v*/v), yielding final compound concentrations of 100, 10, 1, and 0.1 μg/mL. The cytotoxic effects were evaluated on L-929 mouse fibroblast cells (ATCC®CCL-1™) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Serva, Germany) assay. L-929 cell line was cultured in high glucose Dulbecco's Modified Eagle's medium (CAT: 41966–029), which was used to culture the mouse fibroblast cell line L-929 (ATCC # CCL-1) at 37 °C and 5% CO_2 atmosphere, including 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. The safety of the extract was evaluated using the MTT assay. MTT assay is widely used to measure cytotoxicity under *in vitro* conditions; it is a rapid, simple, and highly accurate quantitative colorimetric method [22]. The MTT assay is based on viable cells reducing a yellow, water-soluble tetrazolium salt to an insoluble, purple-colored formazan precipitate by NADH-dependent succinate dehydrogenase [23]. The cells were sown in a 96-well microplate. After 24 h, cells are incubated with methanol extracts with a concentration range (0.1–100 μg/mL). Cells incubated in 0.1% methanol were used as the solvent control. Cells incubated with 3 mM sodium lauryl sulfate (SLS) were used as the positive control. After 24 h, MTT solution was added to the 96-well plate in DMEM with a final concentration of 0.5 μg/mL. After 3 h of incubation, the plate was shaken on an orbital shaker for 10 min. The absorbance of the formed formazan crystals was measured at 570 and 690 nm [22,24]. The absorbance values measured for each concentration of the extracts were proportioned as a percentage of the absorbance value of the solvent control group. Thus, the percentage of cell viability was calculated for each compound concentration (Fig. 2). The studies were carried out in duplicate. The results were given as the mean % viability value ± standard deviation for each extract. A one-way ANOVA test was used to assess whether there was a statistically significant difference between the control group and the extracts. The Tukey test was used as the *post hoc* test. All tests were performed using GraphPad Prism 5.1. The significance level was set at $p < 0.05$ [25].



Comp.	-R_1	-R_2	-R_3
4	-$COCH_3$	-OH	-D-Gal
5	-H	-H	-D-Glu
6	-D-Glu	-H	-D-Glu

Fig. 1. Chemical structure of isolated compounds (**1–6**) from *S. aucheriana*.

3. Results and discussion

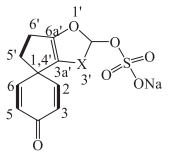
3.1. Chemical structure

Four new compounds (**1–4**), two known astragalin (**5**), and kaempferol-3,7-di-O-*β*-D-glucopyranoside (**6**) [26] were isolated and identified from the aerial parts of *S. aucheriana* (Fig. 1).

Compound **1** was obtained as a white amorphous powder from an aqueous extract of *S. aucheriana* by RP-C_{18} column chromatography, and its NMR (^{1}H, ^{13}C/APT, COSY, HSQC, and HMBC), FT-IR, UV, and HR-ESI-MS spectra are available in supplementary data figs. S1-S9, respectively. The molecular formula of **1** was determined as $C_{11}H_9O_6S_2Na$ by positive HRESI-MS, which displayed a protonated ion at *m/z* 325.2160 $[M + H]^+$ (calc. 325.2137), indicating 7 degrees of unsaturation. The FT-IR spectrum of **1** indicated the presence of an *α,β*-unsaturated carbonyl group (1657 cm^{-1}), double bonds (1447 and 1515 cm^{-1}), and a C—O functionality (1023 cm^{-1}). The FT-IR spectrum of **1** exhibited a signal at 3344 cm^{-1}, which could be due to moisture in the sample.

The ^{1}H NMR spectrum of **1** showed two doublet signals at δ_H 7.07 ppm (d, $J = 9.8$ Hz, H-2,6) and at δ_H 6.26 ppm (d, $J = 9.8$ Hz, 2H, H-3,5) for the cyclohexadienone unit of **1**. ^{1}H NMR spectrum also revealed peaks at δ_H 7.01 ppm (s, 1H, H-2′), δ_H 2.24 ppm (brs, 2H, H-5″), and δ_H 3.57 ppm (brs, 2H, H-6′), which had HSQC correlation to the carbon resonances at δ_C 105.9 ppm, δ_C 37.8 ppm and δ_C 33.1 ppm for the C-2′, C-5′, and C-6′, respectively [12,27,28]. The ^{13}C NMR and APT spectra revealed the presence of two sp^2 protonated carbons (δ_C 155.1 ppm, C-2,6 and δ_C 126.2 ppm, C-3,5), two methylene (δ_C 37.8 and 33.1 ppm), one methin (δ_C 105.9 ppm), and four quaternary carbons (δ_C 187.6 (C=O), 152.8, 122.6, and 52.1 ppm). A quaternary carbon peak at δ_C 52.1 ppm (C-1 and C-4′) was highly specific for the spiro carbon between the 2,5-cyclohexadiene-4-one and cyclopentenyl units as in the natural cannabispiradienone [12,27,28]. NMR data of **1** revealed that the partial structure in **1** was like cannabispiradienone [27–29], indicating **1** possessed a spiro cyclopentenyl cyclohexadienone core, with the obvious differences at the unsaturated cyclopentene unit with —O— and —S— substitution at C-3a′ (δ_C 122.6 ppm) and C-6a′ (δ_C 152.8 ppm). The HMBC cross peak correlations from H-2′ (7.01 ppm) to C-3a′ (122.6 ppm) and C-6a′ (152.8 ppm), from H-3,5 (δ_H 6.26 ppm) to C-2,6 (δ_C 155.1 ppm), C-1/4′ (δ_C 52.1 ppm) and C-4 (δ_C 187.6 ppm), and from H-2,6 (δ_H 7.07) to C-3/5 (δ_C 126.2 ppm), C-1/4′ (δ_C 52.1 ppm) and C-4 (δ_C 187.6 ppm) supported the proposed structure of **1**. Complete assignment of the proton and carbon resonances of **1** as reported in Table 1. The

Table 2
ACD ^{1}H and ^{13}C NMR data of compound **1** with modifications X as S, O, and N.



1: X = S or O or NH

No	APT	1		X					
				S		O		N	
		^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1,4′	C	–	52.1	–	51.5	–	50.3	–	48.9
2,6	CH	7.07, d, 2H	155.1	7.08, d, 2H	149.6	6.85, d, 2H	147.1	6.72, d, 2H	147.5
3,5	CH	6.26, d, 2H	126.2	6.42, d, 2H	127.4	6.48, d, 2H	128.6	6.41, s, 2H	130.1
4	C	–	187.6	–	187.2	–	187.2	–	187.2
2′	CH	7.01, s, 1H	105.9	8.20, s, 1H	105.0	7.90, s, 1H	112.6	6.64, s, 1H	99.5
3a′	C	–	122.6	–	119.1	–	163.3	–	125.0
5′	CH_2	2.24, brs, 2H	37.8	1.70, 2H	36.2	1.46, 2H	35.0	1.51, 2H	34.6
6′	CH_2	3.57, brs, 2H	33.1	2.37, 2H	25.7	2.94, 2H	24.0	2.86, 2H	20.8
6a′	C	–	152.8	–	151.4	–	148.3	–	151.7

literature reports carbon peaks of the 1,3-oxathiole ring in the 2,2′-spirobi-(1,3-benzoxathiole) at δ_C 122.7 ppm and δ_C 144.1 ppm, respectively [30,31]. ACD NMR (^{1}H and ^{13}C) data analysis of **1** showed the best overlap with the 1,3-oxathia structure of **1** (Table 2). Thus, sulfur and oxygen-bearing sp^2-hybridized quaternary carbon (C=C) resonances on the cyclopentene unit of **1** were ascertained as C-3a′ at δ_C 122.6 ppm and C-6a′ at δ_C 152.8 ppm [31]. The proton and carbon chemical shift values of H-2′ and C-2′ (δ_H 7.01 ppm, δ_C 105.9 ppm) in **1** are in the downfield region, indicating that it is in acetal form, with oxygen and sulfur in a sulfate ester substitution. The high polarity of this compound, together with the chemical shift of the low-field ^{13}C signal for the acetal C-2′ (δ_C 105.9 ppm), suggested the presence of a sodium sulfate group [32]. This idea was also consistent with the presence in the ^{1}H NMR for the acetal H-2′ at δ_H 7.01 ppm. The FT-IR spectrum **1** also displayed bands at 1210 and 1110 cm^{-1}, attributed to the sulfate moiety [33,34]. The mass spectrum of **1** gave $[M\text{-}SO_3Na + CH_3OH + H_2O]^+$ peak at 271.0573 (65), calc. 271.0527 resulted the loss of $NaSO_3$- unit of **1**. In addition, $[M + 2(^{34}S) + CO\text{-}H]^+$ peak 353.0210 (5) (calc. 353.0267) and $[M + 4(^{34}S) + CO\text{-}H]^+$ peak 355.0690 (7) were observed from the ^{34}S isotope in the mass spectrum of **1**. The structure of **1** was interpreted as that of a sodium sulfate ester based on the mass and NMR data. The observed optical rotation of **1** was found $[\alpha]_D^{24}$ + 17.7. Complete assignment of the proton and carbon resonances of **1** as reported in Table 1. Thus, the planar structure of **1** was established as sodium 4-oxo-5′,6′-dihydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta-[d][1,3]oxathiol]-2′-yl sulfate.

Compound **2** had a molecular formula of $C_{24}H_{26}O_{10}S$, with 12 degrees of unsaturation, as determined by HRESI-MS data (*m/z*, %; $[M + H]^+$ 507.2605, cal. 507.2665). The FT-IR spectrum indicated the presence of an -OH (3345 cm^{-1}), carbonyl (1655 cm^{-1}), double bonds (1415 cm^{-1}), and C—O functionality (1023 cm^{-1}). The UV spectrum exhibited absorption maxima at λ (nm) 226 (2.53), 248 (2.25), and 294 (1.80), characteristic of the 1,2,4-trisubstituted benzyl and cyclohexadienone moieties of compound **2**. NMR (^{1}H, ^{13}C/APT, COSY, HSQC, and HMBC), FT-IR, UV, and HR-ESI-MS spectra of **2** are available in supplementary data figs. S10-S18, respectively. ^{1}H NMR spectrum for the aromatic region of **2** revealed an ABX system at δ_H 6.80 (d, J = 8.1 Hz, H-3″), δ_H 6.71 ppm (d, J = 8.1 Hz, H-4″), and δ_H 7.08 (brs, H-6″), which were assigned to the 2,4-dihydroxy benzyl alcohol. ^{1}H NMR spectrum of **2** also gave AX system at δ_H 7.09 ppm (d, J = 9.6 Hz, H-2,6) and δ_H 6.27 ppm (d, J = 9.6 Hz, H-3,5) as in compound **1**, which were assignable for the cyclohexa-2,5-dien-1-one unit. ^{1}H NMR spectra of **2** gave H-5′ and H-6′ signals at δ_H 2.27 ppm (t, 2H, J = 8.0 Hz, H-5′) and δ_H 3.53 ppm (m, 2H, H-6′) for the cyclopentene ring, which was linked to 2,5-cyclohexadiene-4-one at C-1/4′ position with the spiro structure [12,35] as in compound **1**. Furthermore, the ^{1}H NMR spectrum of **2** showed a single anomeric proton signal, assignable to H-1 of the glucosyl moiety at δ_H 4.31 ppm (d, 1H, J = 7.8 Hz). The ^{13}C NMR spectrum of **2** showed a single anomeric carbon signal, detected at δ_C 102.9 ppm (C-1, HSQC). The coupling constant (7.8 Hz) of the anomeric proton indicated a *β*-glucopyranosyl unit, and all the ^{1}H and ^{13}C NMR data for the glycone part of **2** were in good agreement with the literature [35]. Benzylic –CH_2- carbon peaks shifted to downfield (δ_C 70.7 ppm), which indicated that D-glucose is substituted at this position. The HMBC correlations from H-2′ (δ_H 7.06 ppm) to C-1″ (δ_C 151.5 ppm) indicated that the spiro unit was linked to the C-1″ position of the trisubstituted phenyl ring of **2**. Complete assignment of the proton and carbon resonances of **2** as reported in Table 1. In addition, $[M + 2\ (^{34}S)]^+$ peak 508.4142 (calc. 508.4112) was also observed from the ^{34}S isotope in the mass spectrum of **2**. The optical activity of compound **2** was found to be $[\alpha]_D^{24}$ + 36.3. Thus, from these observations, compound **2** was elucidated as 5-hydroxy-2-(4-oxo-5′,6′-dihydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta [d][1,3]-oxathiol]-2′-yl)-benzyl-O-*β*-D-glucopyranoside.

Compound **3** was isolated as a yellowish oil, and its molecular formula was determined as $C_{30}H_{36}O_{15}S$ by HRESI-MS, which gave a protonated ion peak at *m/z* $[M + H]^+$ 669.1838 (40), cal. 669.1844; and a sodium addition peak at *m/z* $[M + Na]^+$ 691.1669 (100), cal. 691.1663. The UV spectrum of **3** showed absorption maxima at λ (nm) 221 (2.03), 250 (1.78), 296 (1.66). NMR (^{1}H, ^{13}C/APT, COSY, HSQC, and HMBC), FT-IR, UV, and HR-ESI-MS spectra of **3** are available in supplementary data figs. S19-S28, respectively. 1D and 2D NMR spectra of **3** were very similar to those of compound **2**. The NMR data for **3** differed only in displaying additional signals for a second glucose and minor shift differences in the phenyl moiety due to the different linkage. Spiro units of **3** are the same as those in compounds **1** and **2**, which were substituted at the C-1″ position of the trisubstituted phenyl ring, as indicated by the HMBC correlation between H-2′ (δ_H 6.49 ppm) and C-1″ (δ_C 165.4 ppm). ^{1}H NMR spectrum of compound **3** showed a typical proton signals for the phenyl units, which showed an ABX system for the 1,2,4-trisubstituted benzyl ring at δ_H 6.80 ppm (d, J = 3.2 Hz, 1H, H-3″), δ_H 6.98 ppm (d, J = 8.3 Hz, 1H, H-5″), and δ_H 6.68 ppm (d, J = 8.3 Hz, 1H, H-6″). The ^{13}C NMR and APT spectra of **3** showed 30 carbon resonances, 12 of which were assigned to the glycone and the remainder to the aglycone. Twelve carbons for the glycone part of **3** were assigned to two glucose units

Table 3
NMR data of compound **4** (CD_3OD, 400/100 MHz NMR, ppm).

No	C (APT)	^{1}H (COSY)	^{13}C (HSQC)	HMBC
2	C	–	156.4	
3	C	–	134.1	
4	C	–	178.9	
5	C	–	161.1	
6	CH	6.09 (s, 1H)	100.8	C7, C8, C10
7	C	–	161.2	
8	CH	6.25 (s, 1H)	94.9	C6, C9, C10
9	C	–	157.5	
10	C	–	102.1	
1′	C	–	121.4	
2′	CH	7.86 (d, J = 1.6 Hz)	116.1	C2, C3′, C4′
3′	C	–	144.5	
4′	C	–	148.7	
5′	CH	6.86 (d, J = 8.4 Hz)	114.7	C3′, C4′, C6′
6′	CH	7.59 (dd, J = 8.4, 1.6 Hz)	121.4	C2, C2′, C4′
1″	CH	5.03 (d, J = 7.8 Hz)	104.6	C3
2″	CH	3.83*, 1H	71.8	
3″	CH	3.55*, 1H	73.8	
4″	CH	3.86*, 1H	68.5	
5″	CH	3.48*, 1H	75.7	
6″	CH_2	3.67*, 3.55*, 2H	60.4	
COO	C	–	177.2	
-CH_3	CH_3	1.91 (s, 3H)	22.8	COO

* Signal patterns are unclear due to overlapping.

based on the NMR data. Two anomeric proton signals in the ^{1}H NMR spectra of **3** appeared at δ_H 4.96 ppm (under the HOD, Glu-I, H-1, HSQC) and at δ_H 4.85 ppm (d, J = 7.8 Hz, Glu-II, H-1). The ^{13}C NMR spectrum of **3** revealed anomeric carbons at δ_C 99.29 ppm (Glu-I, C-1) and δ_C 100.50 ppm (Glu-II, C-1). The HMBC spectrum of **3** showed cross-peak correlations from H-2′ (δ_H 6.49 ppm) to C-1″ (δ_C 165.4 ppm, phenyl) and Glu-II H-1 (δ_H 4.85 ppm) to Glc-I C-2 (δ_C 76.7 ppm), showing (2 → 1) linkage, and from Glu-I H-1 (δ_H 4.96 ppm) to C-2″ (δ_C 158.4 ppm), establishing the glycone connection of **3**. Complete assignment of the proton and carbon resonances of **3** as reported in Table 1. In addition, $[M + 2 (^{34}S) + Na]^+$ peak 693.3787 (calc. 693.3746) was also observed from the ^{34}S isotope in the mass spectrum of **3**. The absolute configuration of **3** was determined to be $[\alpha]_D^{24}$ + 45.6. Therefore, new compound **3** was identified as (4-(hydroxymethyl)-1-(4-oxo-5′,6′-dihydrospiro[cyclohexa-2,5-diene-1,4′-cyclopenta-[d]-[1,3]oxathiol]-2′-yl)phenyl-2-O-β-D-glucopyranosyl-(2 → 1)-O-β-D-gluco-pyranoside through the comparison of several physical and spectroscopic data with the literature [15,35].

Spirocarbocycles are important structural motifs in natural products, including drugs and ligands [36]. Spiro-containing compounds have been reported from a wide range of biological sources, and spirocyclic compounds containing heteroatoms, such as spirooxindoles, are well-known [10–12,37,38]. One of the earliest isolated spiro products was anhydrous β-retinol [36,39], and cannabispiradienone [35]. Sulfur-containing spiro secondary metabolites have been reported in the Asteraceae, Alliaceae, and Brassicaceae families [13]. Ten sulfur-containing spirochetal glycoside compounds have been reported from the above-ground parts of the *Breynia fruticosa* plant [14]. Two sulfides from *Scorzonera calyculata* [40] and sulfated sequiterpens sulfoscorzonin C, B, E, and D were reported from *S. divaricate* [41,42].

Compound **4** was isolated as a yellow, amorphous powder. The positive HRESI-MS gave a protonated ion peak at *m/z* $[M + H]^+$ 507.1139 (100), calc. 507.1131 and a fragment ion peak at *m/z* $[M-OAc]^+$ 463.0878 (75), calc. 463.0870, corresponding to a molecular formula of $C_{23}H_{22}O_{13}$. NMR (^{1}H, ^{13}C/APT, COSY, HSQC, and HMBC), FT-IR, UV, and HR-ESI-MS spectra of **4** are available in supplementary data figs. S29-S35, respectively. ^{1}H NMR spectrum of **4** gave the typical proton signals of quercetin moiety, that is, those of an AMX spin system for the 1′,3′,4′-trisubstitttion of ring B at δ_H 7.86 ppm (d, J = 1.6 Hz, 1H, H-2′), δ_H 6.86 ppm (d, J = 8.4 Hz, 1H, H-5′), and δ_H 7.59 ppm (dd, J =

Table 4
Screening for the antimicrobial activity of crude extract, solvent fractions, and isolated compounds (**1–6**) from *S. aucheriana*.

Extract, Fractions, Comp. No	Stock Sol. (μg/mL)		Microorganisms, inhibition zone (IZ, mm), and minimal inhibition concentration (MIC, μg/mL)									
			Gram (−) Bac.			*Gram (+) Bac.*				*No Gr.*	*Fungi*	
			Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Lm*	*Bc*	*Ms*	*Ca*	*Sc*
Methanol	69,800	IZ	–	–	18	11	12	12	10	12	–	–
		MIC	–	–	436	436	436	436	872	436	–	–
n-Hexane	53,300	IZ	–	–	–	–	6	–	–	–	–	–
		MIC	–	–	–	–	2665	–	–	–	–	–
$CHCl_3$	25,000	IZ	30	30	20	20	10	10	8	30	25	30
		MIC	156	156	156	78	312	312	625	156	156	156
EtOAc	37,800	IZ	24	12	30	12	15	14	22	20	8	10
		MIC	118	472	59	236	236	236	118	118	945	945
Water	221,100	IZ	–	–	–	16	–	–	–	–	–	–
		MIC	–	–	–	144	–	–	–	–	–	–
1	9900	IZ	–	–	–	–	6	–	–	15	–	10
		MIC	–	–	–	–	495	–	–	30.9	–	123
2	15,300	IZ	–	–	–	–	6	–	–	10	6	6
		MIC	–	–	–	–	765	–	–	47.5	765	765
3	7000	IZ	–	–	–	10	–	–	–	8	–	6
		MIC	–	–	–	87	–	–	–	175	–	350
4	8300	IZ	–	–	–	6	–	–	–	–	8	–
		MIC	–	–	–	415	–	–	–	–	207	–
5	1100	IZ	–	–	10	–	–	–	–	10	8	–
		MIC	–	–	45	–	–	–	–	45	110	–
6	10,000	IZ	–	–	8	6	6	–	–	10	8	8
		MIC	–	–	500	1000	1000	–	–	250	500	500
Amp.	10	IZ	10	10	18	10	35	15	15	–	–	–
		MIC	10	18	>128	35	10	15	15			
Strep.	10	IZ								35		
		MIC								4		
Flu	5	IZ									25	25
		MIC									8	8

Ec: Escherichia coli, *Yp*: *Yersinia pseudotuberculosis*, *Pa*: *Pseudomonas aeruginosa*, *Sa*: *Staphylococcus aureus*, *Ef*: *Enterococcus faecalis*, *Lm*: *Lisssteria monositogenes NCTS11994*, *Bc*: *Bacillus cereus* 702 Roma, *Ms*: *Mycobacterium smegmatis*, *Ca*: *Candida albicans*, *Sc*: *Saccharomyces cerevisiae*, Amp.: Ampicillin, Strep.: Streptomycin, Flu.: Fluconazole, (—): no activity.

8.4, 1.6 Hz, 1H, H-6′). Typical protons for H-6 and H-8 in ring A were observed at δ_H 6.09 ppm (1H, s) and δ_H 6.25 ppm (1H, s), respectively. The ^{13}C and APT spectra of **4** showed resonances for 15 carbons, indicating the presence of a quercetin moiety, except for the —Ac and monosaccharide moieties. An anomeric proton signal at δ_H 5.03 (d, J = 7.8 Hz, H-1″) and other oxygenated methine and methylene signals of the sugar moiety at δ_H 3.87–3.07 (m) were observed. Also, the carbon signals of the sugar moiety were observed at δ_C (ppm) 102.1 (C-1″), 71.8 (C-2″), 73.8 (C-3″), 68.5 (C-4″), 75.7 (C-5″), and 60.4 (C-6″), suggesting the presence of galactopyranoside units [43–44]. In the HMBC spectrum of **4**, a cross peak from Gal H-1 (δ_H 5.02 ppm) to C-3 (δ_C 134.1 ppm) established the connection between quercetin and β-galactose at the C-3 position of **4** [45–47]. The ^{1}H and ^{13}C NMR spectra revealed the presence of an acetate group. The HMBC spectrum of **4** exhibited a cross-peak correlation from the acetate methyl proton (δ_H 1.91 ppm, -CH₃) to the acetate carbonyl carbon (δ_C 177.2 ppm, C═O), and other $^3J_{HC}$ HMBC correlations for the acetate for the aglycone and glycone units of **4** were not observed. ^{1}H and ^{13}C NMR data of **4** are highly similar to those of quercetin-3-*O*-β-D-galactopyranoside [45] except that the C-7 carbon signals are shifted upfield by at least 4 ppm. This indicates that the acetate group is attached at the C-7 position of **4**. Complete assignment of the proton and carbon resonances of **4** as reported in Table 3. Thus, the structure of the new compound **4** was identified as 7-*O*-acetyl quercetin-3-O-β-D-galactopyranoside. In the literature, the isomer of compound **4**, 7-*O*-acetyl quercetin-3-*O*-β-D-glucopyranoside, has been reported only in mass spectrometric studies [48].

In addition to the above four compounds, two known astragalin (quercetin-3-*O*-β-D-glucopyranoside) (**5**) and kaempferol-3,7-di-O-β-D-glucopyranoside (**6**) [26] were also isolated from this plant. All the compounds (**1–6**) were isolated for the first time from the aerial parts of *S. aucheriana*.

3.2. Biological activity

The antimicrobial activities of extracts, fractions, and isolated compounds (**1–6**) obtained from *S. aucheriana* are in Table 4. Chloroform and ethyl acetate fractions exhibited antimicrobial activity against all tested microorganisms (Gram-negative, Gram-positive, non-Gram bacteria, and yeasts) [16,17,49,50]. The chloroform fraction exhibited the most potent antimicrobial activity against the Gram-positive bacterium *S. aureus* (78.1 μg/mL) and the least activity against the Gram-positive spore-forming bacterium *B. cereus* (625 μg/mL); however, it did not exhibit overall selective activity. The ethyl acetate fraction generally exhibited higher activity against bacteria than against yeast and showed good anti-pseudomonal activity (59.1 μg/mL). The *n*-hexane and water fractions do not exhibit specific activities, and the very low activity of the *n*-hexane fraction suggests that it lacks antimicrobial activity or is present at insufficient concentration. On the other hand, the water fraction exhibits anti-staphylococcal activity against *S. aureus* with both methods (16 mm/anti-tuberculosis activity 144.4 μg/mL), which is a notable feature. It is ineffective against *E. faecalis* (Gram-positive), which has a similar morphology but is resistant to this antibiotic. This indicates that it may employ a different mechanism of action from those of known antibiotics. The methanol extract showed moderate activity against the non-fermentative gram-negative bacillus *P. aeroginosa*, gram-positive bacteria, and *M. smegmatis*.

The antimicrobial properties of isolated compounds **1–6** were also investigated against ten microorganisms. Compounds **1–6** showed anti-tuberculosis activity, and among them, **1** and **2** are the most effective, showing selective anti-tuberculosis activity with 30.9 μg/mL and 47.5 μg/mL, respectively. Additionally, compound **2** exhibits anti-pseudomonal activity. Compound **3** exhibited low-dose activity against the Gram-negative bacterium *P. aeruginosa*. It was determined that compound **3** exhibits specific, low-dose (87 μg/mL) anti-staphylococcal activity. It is thought that all substances exhibit low antifungal activity, and that compounds **4** and **5** are essential for *C. albicans*, the pathogenic yeast. Compound **4** exhibited low antimicrobial activity against *E. faecalis*.

Table 5
Antioxidant activities (DPPH, FRAP), total phenolic, and total flavonoid of the solvent fractions and crude methanol extract from *S. aucheriana* (n = 3)[a].

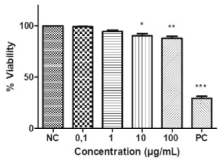
Extract, Fractions	DPPH[b]	FRAP[c]	TPC[d]	TFC[e]
Methanol	0.047 ± 0.002	440.91 ± 6.42	37.73 ± 0.37	50.00 ± 0.50
n-Hexane	0.207 ± 0.003	1.37 ± 0.01	5.46 ± 0.01	0.46 ± 0.01
$CHCl_3$	0.064 ± 0.001	106.37 ± 0.50	16.36 ± 0.16	5.91 ± 0.06
EtOAc	0.043 ± 0.001	197.28 ± 1.30	5.91 ± 0.55	164.54 ± 0.65
Water	0.052 ± 0.001	784.09 ± 6.27	44.09 ± 0.40	15.46 ± 0.15
BHT	0.032 ± 0.001			

MTT assays were twice conducted; NC: Negative control. PC: Positive control.
[a] Data are the mean ± SD.
[b] IC_{50} value (mg/mL).
[c] Presented as trolox equivalents (μg TE/mg dried extract).
[d] Presented as gallic acid equivalents (μg GAE/mg dried extract).
[e] Presented as quercetin equivalents (μg GAE/mg dried extract).

The literature reports the antimicrobial and antifungal activities of tirolobibenzyl A, B, C, and D compounds isolated from *S. humilis*, but no efficacy was observed [51]. Scorzolongin I, scorzolongin II, and scorzolongin III isolated from *S. longiana* exhibited notable antimicrobial activity against *E. coli, Y. pseudotuberculosis, C. albicans,* and *S. cerevisiae* [50]. Sulfocorzonine D, isolated from *S. divaricata*, showed antibacterial activity against *Clostridium perfringens*, comparable to the standards erythromycin and streptomycin [41]. The anti-pseudomonal activity of isoscorzopygmaecoside, scorzoaucherioside I, and 3,4-dihydroxyphenyl caffeate, isolated from S. aucheriana, was reported. These compounds showed moderate activity against *M. smegmatis,* with MIC values of 25.6 μg/mL and 21.2 μg/mL, respectively [49].

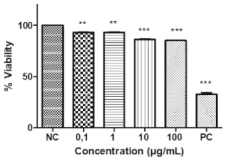
The highest total phenolic and flavonoid in *S. aucheriana* were 44.09 μg/mg and 164.54 μg/mg, corresponding to gallic acid and quercetin equivalents in the water and ethyl acetate fractions, respectively (Table 5). The antioxidant properties of the crude methanol extract and its fractions of *S. aucheriana* were determined using the DPPH and FRAP methods. In the DPPH assay, the ethyl acetate fraction was the most effective against the BHT-positive control, with an IC_{50} of 0.032 mg/mL, compared with 0.043 mg/mL for the control. DPPH analysis revealed IC_{50} values of 0.047 mg/mL for the crude methanol extract and 0.052 mg/mL for the water fraction. The least antioxidant activity was observed in the *n*-hexane fraction. In the FRAP assay, the water fraction (784.09 μg/mg) showed the highest effect, and the FRAP effects of the crude methanol extract and ethyl acetate fraction were determined to be 440.91 μg/mg and 197.28 μg/mg, respectively.

The results showed that the highest antioxidant activity among the *S. aucheriana* fractions was observed in the ethyl acetate fraction, followed by the water fraction (Table 5). Antioxidant activities vary with the amounts of phenolic and flavonoid compounds present in fractions, which vary in abundance [52]. The ethyl acetate fraction of *S. aucheriana* had the highest total phenolic and flavonoid content. Considering the activities observed using the DPPH method, a significant correlation was found with the amounts of total phenolic and flavonoid substances. These activities are attributed to the phenolic components. It is well established in the literature that one of the primary components responsible for the antioxidant activity of plants is the high content of phenolic compounds [53]. In addition, differences in results across methods suggest that these may be due to distinct mechanisms of action in the reactions. Solvent extracts of Scorzonera species have been extensively reported, and their activities have been evaluated



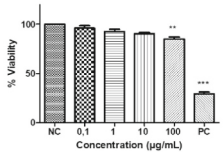
1

MTT assay of compound **1** at concentrations of 100 µg/mL and 10 µg/mL significantly reduced L-929 cell viability compared to the negative control group (post-test Tuckey, *: p<0,05; **: p<0,01; ***: p<0,001, R^2=0.9963).



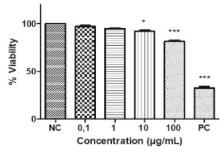
2

MTT assay of compound **2** at concentrations of 100 µg/mL, 10 µg/mL, 1 mg/mL, and 0.1 mg/mL significantly reduced L-929 cell viability compared to the negative control group (post-test Tuckey, **: p<0,01; ***: p<0,001, R^2=0.9986).



3

MTT assay of compound **3** at a concentration of 100 µg/mL significantly reduced L-929 cell viability compared with the negative control group (post-test Tuckey, **: p<0,01; ***: p<0,001, R^2=0.9943).



4

MTT assay of compound **4** at concentrations of 100 µg/mL and 10 µg/mL significantly reduced L-929 cell viability compared with the negative control group (post-test Tuckey, *: p<0,05; ***: p<0,001, R^2=0.9977).

Fig. 2. Cytotoxic activities of compounds **1–4** in L-929 cells.

against ABTS, DPPH, FRAP, NO, MCC, DMPD, and BHT [7].

Compounds **1** and **2** show selective anti-tuberculosis activity; thus, the cytotoxic activity of **1–4** against L-929 cells was investigated (Fig. 2). The results showed that compounds **1–3** exhibited no or weak growth inhibition at the tested concentrations (0.1–100 µg/mL). Compounds **1–4** exhibit nearly identical cytotoxic activities against L-929 cells [22–25]. Cell viability (%) values ranged from 82 ± 7.5 to 95 ± 7.8% at concentrations up to 100 µg/mL, following 24 h exposure.

In the literature, the cytotoxic activity of thyrolobibenzyl A, B, C, and D, isolated from *S. humilis*, was investigated against P388 cells (Mouse leukemia cell line). However, no cytotoxic activity was reported [52]. Cytotoxicity of sulfoscorzonine D and sulfoscorzonine E, and glucosaluzanin C isolated from *S. divaricata* were evaluated against HepG2, K562, and Hela with IC_{50} values of 4.21 µg/mL, 6.53 µg/mL, and 8.15 µg/mL, respectively [41]. Cytotoxic activities of quinic acid derivatives isolated from *S. divaricate* were tested against HL-60 (Human promyelocytic leukemia) and Hep-G2 (Human hepatoma) cell lines with an IC_{50} value of 14.6 µg/mL [54]. Sulfoscorzonine A-C and scorcodivaricin A-C isolated from *S. divaricata* were tested against HL-60, HeLa, HepG2, and SMMC-7721. Scorcodivaricin B exhibited significant cytotoxic activity with IC_{50} values of 24.4 µM, 24.5 µM, 36.2 µM, and 66.7 µM, respectively [42]. The cytotoxic activities of platyphylloside, scorzoneric acid, and scorzonerin, isolated from *S. divaricata* and *S. pseudodivaricata* were investigated against the mouse lymphoma cell line (L5175Y). They exhibited over 90% cell growth inhibition [8]. Biological activity studies and comparative literature indicate that the isolated compounds warrant further pharmacological investigation.

4. Conclusion

The ethyl acetate and water fractions of *S. aucheriana* were used to identify six compounds. Three of them were cannabispiradienone derivatives (**1–3**), and the others were flavonoid derivatives (**4–6**). Studies on the antioxidant and antimicrobial activity of the solvent fraction obtained from the methanol extract of *S. aucheriana* revealed the best activity in chloroform and ethyl acetate fractions. The antimicrobial properties of compounds **1** and **2** exhibited selective anti-tuberculosis activity with MIC values of 30.9 µg/mL and 47.5 µg/mL, respectively. All substances exhibited low antifungal activity, with compounds **4** and **5** being particularly active only against *C. albicans*, a pathogenic yeast fungus. Compounds **1–4** have demonstrated no cytotoxicity in L-929 cells, making them promising candidates for further research as potential natural anti-tuberculosis agents.

CRediT authorship contribution statement

Nurettin Yaylı: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization. **İshak Erik:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Conceptualization. **Büşra Korkmaz:** Writing – original draft, Project administration, Conceptualization. **Gözde Bozdal:** Writing – review & editing, Project administration, Formal analysis, Conceptualization. **Can Özgür Yalçın:** Writing – original draft, Methodology. **Kamil Coşkunçelebi:** Writing – original draft, Validation, Funding acquisition, Conceptualization. **Serdar Makbul:** Validation, Conceptualization. **Şengül Alpay Karaoğlu:** Writing – review & editing, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2026.107088.

Data availability

Data will be made available on request.

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