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Abietane diterpenoids from *Sideritis montana* L. and their antiproliferative activity

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ABSTRACT

The present study aimed at the phytochemical and pharmacological investigation of *Sideritis montana* L. (Lamiaceae). Two new abietane diterpenes [sideritins A (**1**) and B (**2**)] were isolated from the methanol extract of the plant. Six known compounds [pomiferin E (**3**), 9α,13α-*epi*-dioxyabiet-8(14)-en-18-ol (**4**), paulownin (**5**), 6-methoxysakuranetin (**6**), 3-oxo-α-ionol (**7**) and 4-allyl-2,6-dimethoxyphenol glucoside (**8**)] were also obtained from the plant. The structures were determined by means of HREIMS and NMR experiments. The antiproliferative effect of the isolated compounds was investigated on human cancer cell lines (HeLa, SiHa and C33A) at 10 and 30 μM concentrations, using the MTT assay. The results demonstrated that pomiferin E (**3**) and 6-methoxysakuranetin (**6**) displayed considerable activity [inhibition (%) ± SEM: 46.93 ± 2.35 on HeLa (pomiferin E), and 51.52 ± 2.45 on C33A (6-methoxysakuranetin)] at 30 μM concentration.

1. Introduction

The genus *Sideritis* (Lamiaceae family) includes more than 150 species, which are distributed widely in the Mediterranean area [1]. These plants are traditionally used as remedies for several disorders, such as anti-ulcerative, vulnerary, anticonvulsant, and analgesic agents. Infusions and decoctions prepared from *Sideritis* species are consumed frequently, since the extracts of plants possess different pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, spasmolytic and carminative effects [2].

Previously iridoid glycosides (ajugol, ajugoside and melittoside), a flavonoid (diosmetin), and a phenylethanoid glycoside (verbascoside) have been isolated from *Sideritis montana* L. The volatile oil of the plant contains considerable amounts of sesquiterpenes, such as germacrene D and bicyclogermacrene. The triterpenoid constituents (ergosterol, stigmasterol and β-sitosterol) of *S. montana* seeds have also been identified by HPLC. Up to now, only one diterpenoid, siderol was described from the plant, but its detailed spectroscopic analysis was not reported [3]. The investigation of secondary metabolites of *S. montana* subsp. *montana* resulted in the identification of flavonoids (isoscutellarein derivatives), chlorogenic acid, methylarbutin and iridoids (*e.g.* harpagide, melittoside). The essential oil of the plant was mainly characterized by sesquiterpene hydrocarbons (germacrene D and bicyclogermacrene) [4].

Recently, the effect of hydroalcoholic extracts prepared from *S. euboea* and *S. scardica* (named as Greek mountain tea) was tested in Alzheimer's β-amyloidosis mouse models and investigated their activities on memory and learning processes. It was observed that daily oral treatment of the extracts enhanced cognition in aged, non-transgenic as well as in APP-transgenic mice. These results support the traditional use of *Sideritis* species in the prevention of age-related problems (*e.g.* dementing disorders like Alzheimer's disease) in elderly individuals [5]. The essential oil of *S. montana* subsp. *montana* showed moderate cytotoxicity on A375, MDA-MB 231 and HCT116 cell lines, and weak antioxidant activity [4].

The aim of the present study was to perform a preparative phytochemical work with *S. montana*, and to investigate the antiproliferative properties of the isolated compounds.

2. Experimental

2.1. General

Vacuum liquid chromatography (VLC) was carried out on silica gel

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(15 μm, Merck); LiChroprep RP-18 (40–63 μm, Merck) stationary phase was used for reversed-phase VLC; column chromatography (CC) was performed on polyamide (MP Biomedicals). Preparative thin-layer chromatography (preparative TLC) was performed on silica gel 60 F_{254} plates (Merck) as well on reversed-phase silica gel 60 RP-18 F_{254} plates (Merck). Rotation planar chromatography (RPC) was carried out on silica gel 60 GF_{254} with a Chromatotron instrument (Model 8924, Harrison Research). Centrifugal partition chromatography (CPC) was performed on Armen SCPC apparatus (Armen Instrument Sas, Saint-Avé, France) equipped with a gradient pump, a 10 mL sample loop, an ASC/DSC valve, a 250 mL column, a UV detector, and an automatic fraction collector. The system was controlled by Armen Glider software.

NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker Avance DRX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). The signals of the deuterated solvents were taken as references. The chemical shift values (δ) were given in ppm and coupling constants (J) are in Hz. Two-dimensional (2D) experiments were performed with standard Bruker software. In the COSY, HSQC and HMBC experiments, gradient-enhanced versions were used. The high resolution MS spectra were acquired on a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer equipped with ESI ion source in positive ionization mode. The resolution was over 1 ppm. The data were acquired and processed with MassLynx software. All solvents used for CC were of at least analytical grade (VWR Ltd., Szeged, Hungary).

2.2. Plant material

Sideritis montana was collected during the flowering period in July 2013, near Öskü (Hungary). Botanical identification of the plant material was performed by one of the authors, Dr. Gyula Pinke (Department of Botany, University of West Hungary, Mosonmagyaróvár, Hungary) and a voucher specimen (No 822) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

2.3. Extraction and isolation

The air-dried whole plant of *S. montana* (2.8 kg) was percolated with MeOH (60 L) at room temperature. The crude methanol extract was concentrated under reduced pressure (637.4 g) and subjected to solvent–solvent partitioning with *n*-hexane, $CHCl_3$, and EtOAc. 5 × 1.5 L solvent was used for each partitioning.

The concentrated *n*-hexane soluble fraction (S1) (49.5 g) was separated by polyamide open column chromatography with gradient system of MeOH–H_2O [2:3, 3:2, 4:1, 1:0 (3, 2.5, 3.5 and 2 L, respectively), each eluent was collected as a fraction]. The fraction obtained from the polyamide column with MeOH–H_2O 3:2 (S1/2) (2.43 g) was subjected to vacuum liquid chromatography on silica gel (VLC, Kieselgel GF_{254}, Merck) with a gradient system of cyclohexane–EtOAc–MeOH [from 9:1:0 to 5:5:1 (200 mL/eluent), and finally with MeOH (150 mL); volume of collected fractions were 20 mL] to yield the major fractions S1/2/1–6. The fractions were combined according to their TLC patterns, using cyclohexane–EtOAc–MeOH (20:10:1) as solvent system (detection at 254 and 366 nm, and at daylight after spraying with vanillin-sulfuric acid reagent and heating at 120 °C for 5 min).

Fraction S1/2/2 (48.7 mg) was separated by reversed-phase VLC, which was eluted with a gradient system of MeOH–H_2O [from 2:3 to 9:1 (100 mL/eluent), and finally MeOH (100 mL); volume of collected fractions was 10 mL] to yield six subfractions. Compound **3** (4.2 mg) was obtained from subfraction S1/2/2/5 (13.6 mg) by preparative TLC on silica gel 60 F_{254} plates using toluene-acetone (8:2) as solvent system.

Fraction S1/2/3 (38.0 mg) was also purified by reversed-phase VLC, a gradient system of MeOH–H_2O [from 3:7 to 9:1 (100 mL/eluent), and finally MeOH (100 mL)] was used as eluent; (volume of collected fractions was 10 mL) to afford five subfractions. From subfraction S1/2/3/3 (14.3 mg) compound **5** (4.7 mg) was purified by preparative TLC on silica gel 60 F_{254} plates using toluene–acetone (8:2) as solvent system.

Reversed-phase VLC was used for the separation of fraction S1/2/4 (117.1 mg). The fraction was eluted by a gradient system of MeOH–H_2O [from 3:7 to 9:1 (120 mL/eluent), and finally MeOH (150 mL), volume of collected fractions was 10 mL] to afford nine subfractions. By the use of preparative TLC on silica gel 60 F_{254} plates using toluene–acetone (8:2) as solvent system compound **1** (5.7 mg) and compound **4** (3.1 mg) were isolated from subfractions S1/2/4/3 (11.1 mg) and S1/2/4/6 (9.3 mg), respectively.

Fraction S1/2/5 (148.3 mg) was also chromatographed by reversed-phase VLC, which was eluted with a gradient system of MeOH–H_2O [from 3:7 to 9:1 (150 mL/eluent), and finally MeOH (100 mL); volume of collected fractions was 10 mL] to afford five combined fractions. Fraction S1/2/5/1 (24.1 mg) was purified by the use of preparative TLC on silica gel 60 F_{254} plates using toluene–acetone (8:2) as solvent system to yield compound **2** (7.2 mg) and compound **6** (4.6 mg).

The $CHCl_3$-soluble fraction (S2) (35.5 g) was chromatographed on a polyamide column with gradient system of MeOH–H_2O [1:4, 2:3, 3:2, 4:1, 1:0 (2.5, 2.5, 3, 3.5, and 2 L, respectively)] to give nine combined fractions (S2/1-9). Fraction S2/1 (5.73 g) was further chromatographed by VLC on silica gel with a gradient system of $CHCl_3$–MeOH [from 100:1 to 1:1 (500 mL/eluent), and finally with MeOH (400 mL); volume of collected fractions were 50 mL] to yield twelve major fractions (S2/1/1-12). The fractions were concentrated and monitored by TLC using $CHCl_3$–MeOH (95:5 and 9:1) and EtOAc–EtOH–H_2O (25:4:3) as solvent system. Subfraction S2/1/4 (225.4 mg) was separated by RPC on silica gel 60 GF_{254} with the use of CH_2Cl_2–MeOH gradient elution [from 99:1 to 7:3 (150 mL/eluent), and finally with MeOH (100 mL); volume of collected fractions were 20 mL] to yield seven subfractions. Compound **7** (3.9 mg) was purified from subfraction S2/1/4/4 (30.2 mg) using preparative TLC on reversed-phase silica gel 60 RP-18 F_{254} plates with MeOH–H_2O (7:3) as solvent system.

Fraction S2/1/9 (960.3 mg) was chromatographed with CPC, using a two-phase solvent system consisting of $CHCl_3$–MeOH–H_2O 10:3:7 (1000 rpm, 10 mL/min flow rate, 90 min) in the ascending mode. After combination eight subfractions were obtained. From subfraction S2/1/9/4 (24.5 mg) compound **8** (4.7 mg) was isolated by the use of preparative TLC on reversed-phase silica gel 60 RP-18 F_{254} plates with MeOH–H_2O (7:3) as eluent.

2.3.1. Sideritin A (1)

Yellow amorphous solid; $[\alpha]_D^{26}$ + 47 (*c* 0.1, MeOH); ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 285.2217 $[M–H_2O + H]^+$ (calcd for $C_{20}H_{29}O$, 285.2213).

2.3.2. Sideritin B (2)

Yellow amorphous solid; $[\alpha]_D^{26}$ − 7 (*c* 0.2, MeOH); ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 359.2198 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2193) providing the molecular formula, $C_{20}H_{32}O_4$.

2.3.3. Pomiferin E (3)

^{13}C NMR ($CDCl_3$, 125 MHz) δ 199.3 (C=O, C-7), 152.7 (C, C-9), 147.1 (C, C-13), 132.7 (CH, C-12), 130.5 (C, C-8), 125.1 (CH, C-14), 123.4 (CH, C-11), 65.0 (CH, C-2), 50.5 (CH_2, C-3), 48.7 (C, C-4), 46.9 (CH_2, C-1), 39.4 (C, C-10), 36.0 (CH_2, C-6), 34.8 (CH, C-15), 33.6 (CH_3, C-19), 32.6 (CH, C-5), 23.8 (CH_3, C-16), 23.7 (CH_3, C-17), 24.4 (CH_3, C-20), 22.0 (CH_3, C-18).

2.4. Bioassay

Antiproliferative effect of the isolated compounds (**1–7**) were measured *in vitro* on human cervical cancer cell lines (HeLa, SiHa, and C33A) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

Table 1
NMR data for sideritin A (**1**).

position	**1**[a]		**2**[b]	
	δ_H (*J* in Hz)	δ_C, type	δ_H (*J* in Hz)	δ_C, type
1α	1.04, t (11.8)	47.8, CH_2	1.70, m	41.6, CH_2
1β	2.45, brd (11.7)		1.79, m	
2	3.81, m	63.0, CH	3.85, m	65.1, CH
3α	1.04, t (10.6)	50.5, CH_2	1.14, m	51.3, CH_2
3β	1.69, brd (10.6)		1.75, m	
4		34.0, C		35.2, C
5	1.20, brd (12.7)	48.4, CH	1.56, m	42.6, CH
6α	2.01, dd (12.3, 7.1)	29.3, CH_2	2.26, m	29.0, CH_2
6β	1.55, m		1.56, m	
7	4.53, dd (10.0, 7.1)	69.4, CH	4.71, dd (10.1, 2.8)	67.3, CH
8		138.9, C		146.6, C
9		146.3, C		81.2, C
10		39.3, C		41.0, C
11(α)	7.12, d (8.2)	123.6, CH	2.12, m	23.4, CH_2
11β			1.55, m	
12	7.03, d (7.7)	124.8, CH	1.99, 2H, m	24.9, CH_2
13		145.0, C		79.8, C
14	7.33, brs	125.3, CH	6.47, s	133.7, CH
15	2.81, sept (6.9)	33.1, CH	1.94, m	32.4, CH
16	1.17, 3H, d (7.2)	23.9, CH_3	1.01, 3H, d (8.0)	17.4, CH_3
17	1.16, 3H, d (7.2)	24.0, CH_3	0.99, 3H, d (8.0)	17.5, CH_3
18	0.90, 3H, s	22.4, CH_3	0.99, 3H, s	24.0, CH_3
19	0.94, 3H, s	33.2, CH_3	0.95, 3H, s	33.1, CH_3
20	1.16, 3H, s	26.1, CH_3	1.26, 3H, s	20.0, CH_3
2-OH	4.45, d (4.8)			
7-OH	5.19, d (7.3)			

[a] Recorded in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C).
[b] Recorded in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C).

bromide (MTT) colorimetric assay. These cell lines were purchased from the European Collection of Cell Cultures (Salisbury, UK) and maintained in minimal essential medium supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and an antibiotic-antimycotic mixture, in a humidified atmosphere of 5% CO_2 at 37 °C. All of the chemicals, if otherwise not specified, were purchased from Sigma-Aldrich Ltd. (Budapest, Hungary). The cytotoxicity tests were carried out in 96-well microtitre plates, using 5000 cells/well for HeLa and SiHa and 10,000 cells/well for C33A cells, which were allowed to adhere overnight before the drugs were introduced. The original medium was then removed, 200 µL culture medium containing the compounds of interest was added and the cells were incubated for 72 h. The tested extracts and compounds were dissolved in DMSO. The final concentration of DMSO never exceeded 0.3%, and therefore had no essential effect on the cell growth. Next, the living cells were assayed: aliquots (20 µL at 5 mg/mL) of the MTT stock solution were pipetted into each well and reduced by viable cells to an insoluble formazan product during a further 4 h. The precipitated formazan crystals were solubilized in 100 µL DMSO by gentle shaking for 60 min. The absorbance was determined at 545 nm with an enzyme-linked immunosorbent assay reader [6]. In this way the cell growth or drug toxicity was determined. All *in vitro* experiments were carried out on two microplates with five parallel wells. Based on our previous antiproliferative activity experiments, cisplatin (IC_{50} 12.43 µM, 7.84 µM, and 3.69 µM, on HeLa, SiHa, and C33A cells, respectively), a clinically used anticancer agent, was used as the reference substance [7].

3. Results and discussion

In the course of our study, the phytochemical and pharmacological investigations of *Sideritis montana* L. were performed. The dried whole plant material (2.8 kg) was powdered and extracted with MeOH at room temperature. After concentration, the extract was dissolved in 50% aqueous MeOH, and solvent–solvent partition was performed with *n*-hexane, $CHCl_3$, and finally with EtOAc. The *n*-hexane, and $CHCl_3$ fractions were purified with a combination of different chromatographic techniques to yield eight compounds (**1–8**) (Fig. 1). The structure elucidation of the compounds was carried out by extensive spectroscopic analysis, using 1D and 2D NMR (^{1}H–^{1}H COSY, HSQC, HMBC, NOESY) spectroscopy, HRESIMS experiments and comparison of the spectral data with literature values.

Compound **1** was obtained as an amorphous solid with $[\alpha]_D^{26}$ + 47 (*c* 0.1, MeOH). Its HRESIMS proved the molecular formula $C_{20}H_{30}O_2$ through the presence of a peak at *m/z* 285.2217 $[M–H_2O + H]^+$ (calcd for $C_{20}H_{29}O$, 285.2213) and supported by the hydrogen and carbon atom counts in the NMR spectra. The ^{1}H NMR spectrum (Table 1) displayed signals of two *ortho*-coupled aromatic protons (δ_H 7.12 d and 7.03 d), and one aromatic proton as a broad singlet (δ_H 7.33), five methyls, three methylenes, four sp^3 methines and signals of protons belonging to two hydroxyl groups. In the JMOD (*J*-modulated spin-echo experiment) spectrum, the presence of 20 carbon signals was detected (Table 1) indicating this compound to be a diterpene. In the ^{1}H–^{1}H COSY spectrum, correlations were observed between protons at δ_H 3.81 m and 1.04 t and 2.45 brd (H-2/H-1α, H-1β and H-3α), δ_H 1.55 m and 1.20 brd and 4.53 dd (H-6β/H-5 and H-7), δ_H 2.01 dd and 4.53 dd (H-6α/H-7), δ_H 7.12 d and 7.03 brd (H-11/H-12), and δ_H 2.81 sept and 1.16 d (3H) and 1.17 d (3H) (H-15/H_3-16 and H_3-17) (Fig. 2).

These structural parts and quaternary carbons were connected by inspection of the long-range H–C correlations observed in the HMBC spectrum (Fig. 2). The two- and three-bond correlations between H-1, H-5, H-6, H-11 and H_3-20 and the quaternary carbon C-10; H-1, H-5, H-12, H-14, and H-20 and the quaternary carbon C-9; H-6, H-7, H-11 and C-8; and finally H-11, H-14, H-15 and C-13 revealed that the structure forms an abietane skeleton, frequently occur in different Lamiaceae species. Two of the methyl groups (δ_H 0.94 s and 0.90 s) were placed at C-4 on the basis of their HMBC correlations with the quaternary carbon at δ_C 34.0 (C-4), and δ_C 50.5 (C-3) and δ_C 48.4 (C-5). Another methyl group was connected to C-10 according to its long-range correlation H_3-20/C-10. The linkage of hydroxy groups to C-2 and C-7 were confirmed by the chemical shift of the tertiary carbons ($\delta_{C\text{-}2}$ 63.0 and $\delta_{C\text{-}7}$ 69.4).

The NOESY correlations confirmed the stereostructure of compound **1**. Overhauser effects were detected between H-1α/H-3α, OH-2/H-1α, H-2/H_3-18 and H-20, H-6α/H-19, OH-7/H-6β, and H-7/H-5 and H-6α. All of the above evidence confirmed the structure of **1**, named as sideritin A.

Compound **2** was isolated as an amorphous powder with $[\alpha]_D^{26}$ − 7

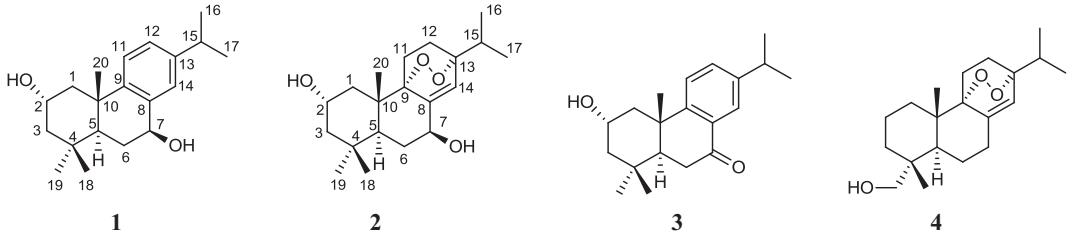
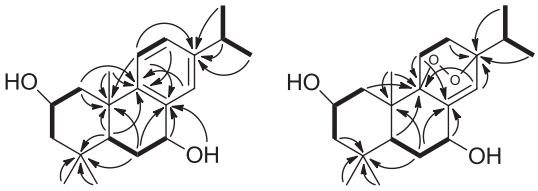


Fig. 1. Structures of compounds **1–4**.



1 2

Fig. 2. Diagnostic COSY (▬) and HMBC correlations (H → C) for **1** and **2**.

(*c* 0.2, MeOH). Its HRESIMS provided the molecular formula, $C_{20}H_{32}O_4$, through the presence of a peak at *m/z* 359.2198 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2193). The ^1H NMR spectrum (Table 1) showed signals characteristic of three tertiary methyls (δ_H 0.95 s, 0.99 s, and 1.26 s), one isopropyl (δ_H 1.94 m, 0.99 d and 1.01 d) and one olefinic proton signal (δ_H 6.47 s). The JMOD spectrum (Table 1) confirmed the presence of a trisubstituted double bond (δ_C 133.7 and 146.6), and also showed signals attributed to the presence of two quaternary oxygenated carbons (δ_C 81.2 and 79.8). Thus, analysis of its ^1H and ^{13}C NMR data suggested that **2** is based on an *epi*-dioxyabietene structure [8].

According to the correlations observed in the $^1H-^1H$ COSY spectrum the same structural parts could be deduced as in case of **1** with exception of the aromatic *ortho* protons which are replaced by the two correlated methylenes [δ_H 1.99 m (2H), 2.12 m (1H) and 1.55 m (1H)]. The long-range H−C correlations observed in the HMBC spectrum (Fig. 2) between H-14 and C-7, C-9, C-12, C-13, and C-15 proved that an oxygen functionality was present between the C-9 and C-13, which was identified as an endoperoxide with regard to the molecular formula. According to their HMBC correlations methyl groups were placed at C-4 and C-10, and hydroxyl groups at C-2 and C-7, similarly as in case of **1**.

The NOESY correlations further confirmed the structure of compound **2**. Overhauser effects were detected between H-5/H-6α, H-7 and H_3-19, H_3-19/H-6α, and between H_3-20/H-1β, H-2, H-6β and H-11β. The relative stereochemistry at the epoxide for C-9 and C-13 in **2** was established as α,α by NOESY correlations between H_3-20 and H-11β, similarly as it was observed in case of angustanoic acid B [9]. All these evidence confirmed the structure of **2**, named sideritin B.

Besides the two new diterpenes, sideritin A (**1**) and B (**2**), two known diterpenoids, pomiferin E (**3**) [10], 9α,13α-*epi*-dioxyabiet-8(14)-en-18-ol (**4**) [8], the lignan paulownin (**5**) [11], the flavanone 6-methoxysakuranetin (**6**) [12], the megastigmane 3-oxo-α-ionol (**7**) [13], and 4-allyl-2,6-dimethoxyphenol-glucoside (**8**) [14] were also isolated from the methanol extract of *S. montana*. All of the compounds were isolated for the first time from the plant, however 6-methoxysakuranetin (**6**) was previously reported from other *Sideritis* species (*S. sventenii*) [12]. Since the genus *Sideritis* is a rich source of flavonoids, the presence of 6-methoxysakuranetin (**6**) in the plant was expectedly [3]. Compounds **1–3** contain hydroxyl group at C-2, which is rare in nature; such type of components were isolated previously from *Salvia pomifera* and *Cryptomeria fortune*[10,15]. Although great deals of diterpenoids were isolated from *Sideritis* species, until now these compounds have not been detected or isolated from *S. montana*[16]. Four compounds were obtained earlier from other Lamiaceae species, 3-oxo-alfa-ionol (**7**) and 4-allyl-2,6-dimethoxyphenol-glucoside (**8**) were isolated from *Glechoma longituba*[17,18], pomiferin E (**3**) was identified from *S. pomifera*[10] and compound **4** from *Hyptis suaveolens*[19]. The ^{13}C NMR data of **3** was published here for the first time (see Section 2.3.3).

Diterpenes can be considered as chemotaxonomic markers for plants belonging to the genus *Sideritis*. *S. montana* was categorized into the *Hesiodia* section. Plants belonging to this section produces triterpenes or sterols, but among their secondary metabolites diterpenoids were not found [20]. The isolation of diterpenoids with unusual 2-hydroxy substitution from *S. montana* confirms its close relationship with the section *Empedoclea*[3,20].

Previously, abietane-type diterpenes were tested for their anti-tumor-promoting activities, by measuring the inhibitory activity of the compounds on EBV-EA (Epstein-Barr virus early antigen) activation induced by TPA (12-*O*-tetradecanoylphorbol-13-acetate) and potent or moderate inhibitory effects were observed [21,22]. In our study, the antiproliferative properties of the isolated compounds were determined on three human cancer (HeLa, SiHa and C33A) cell lines, at 10 and 30 μM concentrations. In the performed assay, *in vitro* cell growth inhibitory effects were measured by the use of MTT assay. Among the isolated compounds, considerable inhibitory activities (above 40% growth inhibition) were measured for pomiferin E (**3**) on HeLa cell line (inhibition (%) ± SEM: 46.93 ± 2.35) and for 6-methoxysakuranetin (**6**) on C33A cells (inhibition (%) ± SEM: 51.52 ± 2.45), at 30 μM concentration. Moderate inhibitory effects (20–40% growth inhibition) were detected for sideritin A (**1**) on HeLa (28.34 ± 2.46%) and SIHA cells (26.87 ± 0.88%), pomiferin E (**3**) on SIHA cells (24.49 ± 2.22%) and compound **6** on HeLa (39.70 ± 2.64%) and SIHA (35.49 ± 2.49%) cells, at 30 μM concentration. Other compounds proved to be inactive on the tested cell lines.

Previously, 9α,13α-*epi*-dioxyabiet-8(14)-en-18-ol (**4**) was tested against A549 (human lung carcinoma), H-116 (human colon carcinoma), PSN1 (human pancreatic adenocarcinoma), T98G (human caucasian gioblastoma), and SKBR3 (human breast carcinoma) cell lines, but it had no inhibitory effect on them [8]. In our study, this compound was also proved to be inactive on the tested cell lines. The antiproliferative property of the other isolated compounds was not studied before.

In conclusion, our results allowed the identification of four abietane diterpenes (**1–4**) substituted with hydroxyl and endoperoxide groups, two of them [sideritins A (**1**) and B (**2**)] are new natural products. Moreover, a lignan (**5**), a flavanone (**6**) a methoxystigmane (**7**), and a phenol-glucoside (**8**) were also isolated from the plant. Finally, this was the first time when the antiproliferative properties of the above-mentioned compounds were established and remarkable activities were detected for pomiferin E (**3**) and 6-methoxysakuranetin (**6**).

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Conflict of interest

The authors declare no competing financial interest.

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