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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 ($\delta$) 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) $\delta$ 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