Fitoterapia 128 (2018) 97–101

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Cytotoxic diterpene quinones from *Salvia tebesana* Bunge

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ARTICLE INFO

Keywords:
Salvia tebesana
Abietane diterpenoids
Structure elucidation
Cytotoxic activity

ABSTRACT

A phytochemical investigation of the root extracts of *Salvia tebesana* Bunge led to the isolation of two new diterpenoids, tebesinone A (**1**) and tebesinone B (**2**), along with two known compounds, aegyptinone A (**3**) and aegyptinone B (**4**). The structures were established by spectroscopic analysis including 1D and 2D NMR and HRESIMS. The cytotoxic activities of the crude extracts and isolated compounds were evaluated against MCF-7, B16F10, PC-3 and C26 human cancer cell lines, in which compounds **2** and **3** showed significant cytotoxic activities (IC_{50} 2.1–10.3 μM).

1. Introduction

Salvia is one of the largest genera of the Lamiaceae family with over 900 species in the world which about 58 species are distributed in Iran, 17 of them are endemic [1]. The genus *Salvia* distributed extensively throughout tropical and temperate regions [2]. Some members of the genus *Salvia* are used in Iranian folk medicine. For example, *S. sclarea* as tonic, *S. macrosiphon* as antimicrobial, *S. hydrangea* and *S. mirzayanii* have antispasmodic and anti-inflammatory effects, respectively [3,4].

Phytochemically, a variety of compounds have been reported from the genus of *Salvia* including flavonoids, caffeoyl derivatives, sesquiterpenoids, diterpenoids, triterpenes, sesterterpenes, steroids and essential oils [3,5,6]. Diterpenoids extracted from *Salvia* species are mainly based on abietane and clerodane skeletons that produced mainly in the roots of plants [7,8]. The plant of this genus showed different biological activities such as antioxidant [9], antifungal [10], cytotoxic [11], diuretic [12], antibacterial [13], hemostatic, sedative and anticancer [14]. The anticancer and cytotoxic constituents of genus *Salvia* are related to terpenoids, while abietane diterpenoids are responsible for the antibacterial and antifungal activities. Phenolic compounds, especially caffeic acid derivatives were found as potent antiviral agents in *Salvia* species [3].

Salvia tebesana Bunge is one of the Iranian endemic species that distributed around Tabas, Iran and locally named “Maryamgoli Tabasi”. Recently, the essential oil of *S. tebesana* has been analyzed by GC–MS and α-pinene, 7-epi-α-eudesmol, caryophyllene oxide and (*E*)-caryophyllene have been detected as major constituents [15]. To the best of our knowledge in this study for the first time, two new diterpenoids, tebesinones A (**1**) and B (**2**) along with two known abietane diterpenoids including aegyotinone A (**3**) [16] and B (**4**) [17] were isolated from the roots of *S. tebesana*.

2. Experimental

2.1. General experimental procedures

UV spectra were recorded using a Shimadzu UV1650 spectrometer and infrared (IR) spectra were run on Spectrum Two FTIR spectrometer (Perkin Elmer, USA). NMR spectra were obtained using Bruker AVANCE III-300 spectrometer (Bruker, Germany). Optical rotations were recorded on a Polax-2L ATAGO, digital polarimeter at 25 °C (ATAGO Co. Ltd., Tokyo, Japan). High-resolution MS data were recorded on a Thermo Q Exactive plus Orbitrap mass spectrometer equipped with HESI source. The resolution was over 40,000. The data were acquired and processed with Thermo Xcalibur 4.0 software. Silica gel 230–400 mesh (Merck, Germany) was used for column chromatography (CC). Semipreparative HPLC was performed on a KNAUER liquid chromatograph system with a quaternary pump (Smartline Pump 1000) and semi-prep C18 column (onyx monolithic; 100 × 10 mm). Diode array detector (Smartline DAD 2800) and EZ Chrom Elite software were used for detection and processing of data respectively.

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https://doi.org/10.1016/j.fitote.2018.05.005
Received 9 April 2018; Received in revised form 6 May 2018; Accepted 12 May 2018
Available online 14 May 2018
0367-326X/

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** (300 MHz in $CDCl_3$, δ in ppm, J in Hz).

Position	1			2		
	δ_H	δ_C	HMBC	δ_H	δ_C	HMBC
1	2.67 t (6.4)	28.7	C-2,C-3,C-5,C-6	2.66 t (6.4)	28.1	C-2,C-3,C-5,C-6,C-10
2	1.79 m	19.2	C-4,C-6	1.82 m	19.1	C-4,C-5
3	1.58 m	37.9	C-1,C-2,C-6,C-18	1.62 m	37.8	C-1,C-2,C-18
4		35.0			34.8	
5		142.0			139.0	
6		152.1			145.2	
7	7.96 s	123.4	C-4,C-5,C-9,C-14	7.12 s	127.4	C-4,C-8,C-9,C-14
8		132.4			139.9	
9		126.4			126.4	
10		141.2			153.7	
11		180.5			182.5	
12		161.1		7.18 s	143.5	C-9,C-11,C-13,C-15
13		125.7			133.3	
14		182.6			182.2	
15	3.58 m	35.6	C-13	3.07 m	35.4	C-8,C-16,C-17
16a	4.24 dd (8.6, 6.5)	80.0	C-12,C-13,C-15,C-17	3.67 m	66.4	C-13,C-15,C-17
16b	4.74 t (9.4)			3.70 m		
17	1.32 d (6.9)	18.9	C-13,C-15,C-16	1.22 d (7.1)	15.5	C-13,C-15,C-16
18	1.27 s	31.2	C-1,C-3,C-6	1.29 s	31.2	C-3,C-6
19	1.27 s	31.2		1.29 s	31.2	
20	2.57 s	16.7	C-9,C-10,C-11	2.54 s	16.7	C-5,C-6,C-9

2.2. Plant material

The roots of *Salvia tebesana* Bunge were collected in April 2017 from its natural habitat near Tabas, South Khorasan Province. The plant was identified by Mr. M.R. Joharchi, from the Ferdowsi University of Mashhad Herbarium (FUMH). A specimen of this plant (No. 24864) was deposited in the herbarium of School of Pharmacy, Mashhad University of Medical Sciences.

2.3. Extraction and isolation

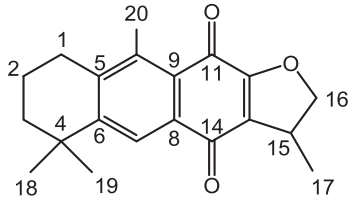
The dried roots (1500 g) were extracted three times with MeOH (each 24 h) at room temperature. The whole extract was filtered and the solvent was evaporated under vacuum affording crude extract (30 g). Methanol extract was further fractionated by solvent-solvent partition to give four different fractions [petroleum ether (40–60 °C), dichloromethane (CH_2Cl_2), butanol (*n*-BuOH) and water (H_2O)] according to Otsuka method [18]. The isolated fractions were concentrated in vacuo to yield residues of 4.2, 7.0, 4.5 and 10 g respectively. Based on the cytotoxic activity test the CH_2Cl_2 fraction (7 g) with high potency was subjected to silica-gel column chromatography (55 × 5 cm) eluted with petroleum ether–ethyl acetate (100:0 to 0:100) to give eighteen fractions (Fr.1–18) with the aid of TLC analysis. The fractions were separated via normal phase open columns and further purified by semipreparative HPLC-C18 column chromatography using MeOH-H_2O (20:80). Compound **1** (3 mg, t_R 8.46 min) was obtained from Fr.6 using a petroleum ether–EtOAc (80:20) as a yellow powder; Compound **2** (8 mg, t_R 9.86 min) was obtained from Fr.15 using a petroleum ether–EtOAc (60:40) as orange prism; Compound **3** (50 mg, t_R 9.23 min) was isolated from Fr.11 using a petroleum ether –EtOAc (70:30) as dark-orange prism and compound **4** (3 mg, t_R 8.78 min) obtained from Fr.17 using a petroleum ether –EtOAc (50,50) as dark red plates.

2.3.1. Tebesinone A (1)

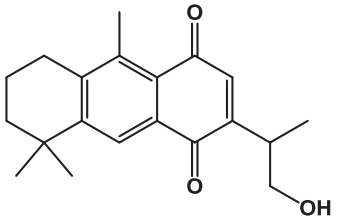
Yellow powder; $[\alpha]_D^{25}$ + 20 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 366 (1.15), 368 (1.15), 400 (1.00), 423 (0.54) nm; IR ν_{max} (MeOH) 2957, 2929, 1729, 1645, 1580, 1268, 1078, 1040 and 803 cm^{-1}; ^{1}H NMR, ^{13}C NMR and HMBC data see Table 1. HR-ESI-MS positive *m/z* 311.1640 $[M + H]^+$ (calcd for $C_{20}H_{23}O_3$, 311.1647).

2.3.2. Tebesinone B (2)

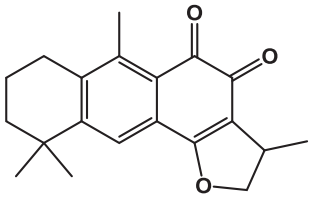
Orange prism; $[\alpha]_D^{25}$ + 5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 364 (4.75), 400 (3.38), 423 (3.72), 431(3.78) nm; IR ν_{max} (MeOH) 3437, 2962, 2929, 1655, 1580, 1259, 1027 and 798 cm^{-1}; ^{1}H NMR, ^{13}C



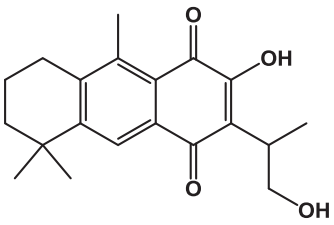
Tebesinone A (**1**)



Tebesinone B (**2**)



Aegyptinone A (**3**)



Aegyptinone B (**4**)

Fig. 1. Chemical structures of compounds **1–4**.