



Original Article

Cytotoxic diterpenoids from the roots of *Salvia lachnocalyx*
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ABSTRACT

Salvia lachnocalyx Hedge, Lamiaceae, is an endemic sage which grows naturally in the Fars Province of Iran. The phytochemical analyses of the roots of *S. lachnocalyx* led to the isolation of five known diterpenoids: ferruginol (**1**), taxodione (**2**), sahandinone (**3**), 4-dehydrosalvilimbino (**4**) and labda-7,14-dien-13-ol (**5**). Their chemical structures were elucidated using one (¹H and ¹³C) and two dimensional (COSY, HSQC and HMBC) NMR spectroscopic data as well as electron impact mass spectra. The cytotoxicity of the purified compounds was evaluated against three human cancer cell lines; MOLT-4 (acute lymphoblastic leukemia), HT-29 (colorectal adenocarcinoma) and MCF7 (breast adenocarcinoma) and all of the isolated compounds showed considerable cytotoxic activity against these cell lines. Compounds **2** and **3** (IC₅₀ range: 0.41–3.87 μg/ml) with endocyclic α,β-unsaturated carbonyl functional group, exhibited the highest cytotoxic activities compared to the other compounds (IC₅₀ range: 6.85–17.23 μg/ml). In conclusion, compounds **2** and **3** are presented as compounds that deserve further investigation of their biological activities.

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Introduction

The genus *Salvia*, Lamiaceae, consists of more than 900 species that are widely dispersed throughout the world. There are 61 *Salvia* species naturally growing in Iran, seventeen of them are endemic (Jamzad, 2012). Several species of *Salvia* are pharmacologically active and have been widely used in folk medicine (Wu et al., 2013; Firuzi et al., 2013; Jassbi et al., 2017). Bioassay-guided purification of chemical constituents of *Salvia* species has resulted in the isolation of many bioactive phytochemicals such as flavonoids, phenolics, terpenoids and steroids (Jassbi et al., 2017). Among them, more than 550 diterpenoids have been reported from different *Salvia* species (Kabouche and Kabouche, 2008), most of which have shown a broad spectrum of interesting biological activities and ecological roles, that include antimicrobial, anticancer, antiviral, antioxidant and anti-inflammatory activities (González, 2015; Jassbi et al., 2017). Among different classes of anticancer drugs, natural products remain a main source of potential drugs to treat human tumors. According to a recent publication on the role of natural products in the field of anticancer drugs, about 75% of small

molecules with anticancer effect have been directly or indirectly derived from natural products (Newman and Cragg, 2016).

Salvia lachnocalyx Hedge is an endemic sage which grows naturally in the Fars Province of Iran (Jamzad, 2012). Recently, three sesterterpene lactones, two sesterterpenoids, three flavonoids, two steroids, one norditerpenoid and one triterpenoid were reported from an acetone extract of the aerial parts of *S. lachnocalyx* (Farimani and Mazarei, 2014). Furthermore, the essential oil of *S. lachnocalyx* has been analyzed by GC-MS and bicyclogermacrene, α-pinene, sabinene and β-pinene have been detected as major constituents (Mirza and Bahernik, 2007). To the best of our knowledge, the present report is the first bioassay-guided phytochemical analyses of the roots of *S. lachnocalyx* for isolation and structural elucidation of its cytotoxic active natural products.

Material and methods

Reagents and chemicals

Fetal bovine serum (FBS), phosphate buffered saline (PBS), RPMI 1640 and trypsin were purchased from Biosera (Ringmer, UK). Acetonitrile (ACN, HPLC grade), dichloromethane (DCM), dimethyl sulfoxide (DMSO), methanol (MeOH), silica gel (70–230 mesh) for open column chromatography and pre-coated silica gel F₂₅₄ TLC aluminum sheets were obtained from Merck (Darmstadt,

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Germany). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma–Aldrich (St. Louis, MO, USA), while cisplatin and penicillin/streptomycin were provided from EBEWE Pharma (Unterach, Austria).

Instrumentation

The purified compounds were subjected to NMR spectroscopy measurements on a Bruker Avance 400 spectrometer (BioSpin GmbH, Rheinstetten, Germany) at resonance frequencies of 400.13 for ^1H and 100.6 MHz for ^{13}C . ^1H and ^{13}C NMR were measured in CDCl_3 with TMS as an internal standard. Mass spectra (EI-MS) were recorded on an Agilent 5975C inert GC/MSD instrument.

The optimization of separation conditions and the evaluation of purity percentage of the isolated compounds were assessed by analytical HPLC using different gradients of ACN and H_2O on a RP C18 HPLC column (Eurospher-100, 250×4.6 mm, Knauer, Berlin, Germany) using a Knauer system (Knauer), that consisted of a K-1800 pump and a K-2500 UV–Vis detector.

Separations on preparative HPLC (prep. HPLC) were developed on a Knauer system, that consisted of a K-1800 pump, with an RP C18 (Eurospher II 100-5 C18, 250×20 mm ID with pre-column 30×20 mm ID) column, eluted isocratically with ACN/ H_2O (85:15) at 20 ml/min. The K-2500 UV–vis detector was set at 210 nm

Optical rotations were measured on a Krüss P8000 polarimeter.

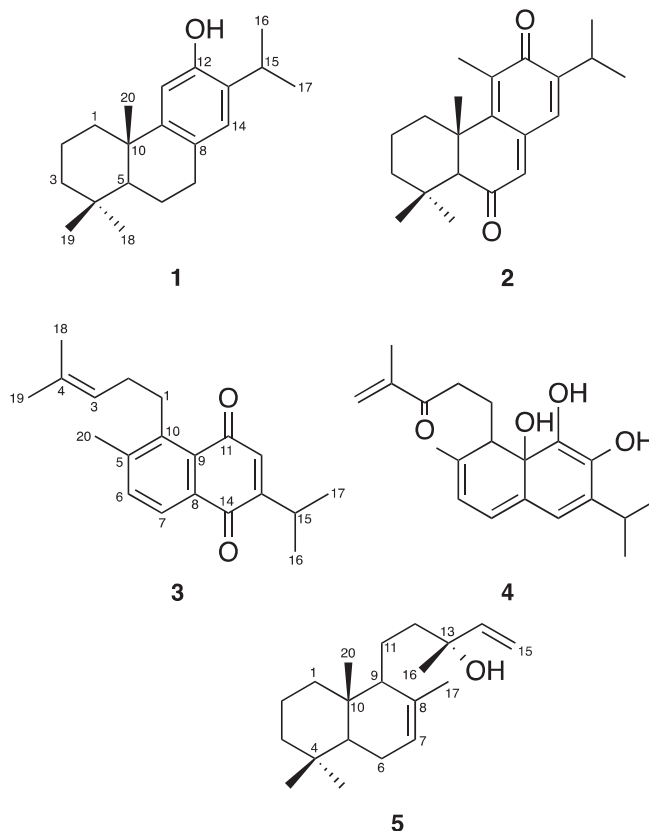
Plant material

The roots of *Salvia lachnocalyx* Hedge, Lamiaceae, were collected from Eghlid in the Fars Province, Iran, in May 2015 and identified by Mojtaba Asadollahi, plant taxonomist at the Medicinal and Natural Products Chemistry Research Center (MNCRC). A voucher specimen (No. 94-3-8-4) was deposited at the MNCRC, Shiraz University of Medical Sciences, Fars, Iran.

Extraction, fractionation and purification

The air-dried, ground roots of *S. lachnocalyx* (400 g) were extracted with DCM (3×21) at room temperature for two days. The extracts were filtered and the solvent was evaporated under reduced pressure at 40°C . The concentrated extract (6 g; yield = 1.5%, w/w) was subjected to open column chromatography (100×5 cm) over silica gel (110 g, 70–230 mesh). The elution of the column was performed using an *n*-hexane with gradient of DCM up to 100%, and then followed by increasing the polarity of the mobile phase to pure MeOH. This chromatographic separation afforded 49 fractions. After checking the purity of fractions by TLC, similar fractions were pooled to afford overall 22 fractions (F1–F22). Fraction F15, eluted with hexane-DCM (80:20), was evaporated to dryness (420 mg), reconstituted in MeOH, centrifuged at $5000 \times g$ for 10 min and filtered through a $0.45 \mu\text{m}$ syringe filter before HPLC analyses. The residue (70 mg) and the upper layer (350 mg) were stored separately. A part of the methanol soluble fraction ($20 \mu\text{l}$, $200 \mu\text{g/ml}$) was repeatedly analyzed by analytical HPLC with various solvent systems to optimize the separation conditions. The optimal conditions were used for the prep. HPLC and the fractions were collected manually according to the chromatographic profiles. Then, $20 \mu\text{l}$ of each of the fractions obtained by prep. HPLC were injected onto the analytical HPLC column to evaluate their purity. The purity was measured by HPLC peak-area normalized method. According to the analytical HPLC results, the fractions with identical retention times were combined and evaporated under reduced pressure to afford the pure natural products. Purification by prep. HPLC afforded compound **1** (13 mg, HPLC purity $\geq 98\%$), **2** (43 mg, HPLC purity $\geq 97\%$), **3** (144 mg, HPLC purity $\geq 95\%$), **4** (17 mg, HPLC purity $\geq 97\%$), and **5** (9 mg, HPLC purity $\geq 97\%$). The

pure phytochemicals were stored at -20°C until performing the spectroscopic experiments and cytotoxicity assays.



Cell lines and culture

HT-29 (human colorectal adenocarcinoma, Cell bank number: IBRC C10097) cell line was purchased from Iranian Biological Resources Center, Tehran, Iran, while MCF7 (human breast adenocarcinoma, Cell bank number: C135) and MOLT-4 (human lymphoblastic leukemia, Cell bank number: C149) cells were obtained from National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. The cells were cultured in sterile T25 flasks in RPMI 1640 medium supplemented with 10% v/v fetal bovine serum, penicillin (100 units/ml) and streptomycin (100 $\mu\text{g/ml}$). HT-29 and MCF7 cell lines were grown in monolayer cultures, while MOLT-4 cells were grown in suspension cultures in humidified air containing 5% CO_2 at 37°C .

Cytotoxicity assay

The inhibitory effect of purified compounds on cancer cell proliferation was assessed by the MTT reduction assay (Mosmann, 1983). This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan by the action of mitochondrial dehydrogenase enzymes in viable cells. The purified compounds were dissolved in DMSO, and then diluted in growth medium at least 400 times. Cells were seeded in 96-well plates at the density of 50,000 cells/ml in $100 \mu\text{l}$ of growth medium. After 24 h of incubation at 37°C , $50 \mu\text{l}$ of the medium was exchanged with $50 \mu\text{l}$ of test compounds diluted in fresh growth medium (3–4 different concentrations). After 72 h of further incubation, the medium of each well was replaced by RPMI without phenol red containing MTT 0.5 mg/ml and incubated at 37°C for 4 h. DMSO was used to solubilize the formed formazan crystals. The absorbance of wells was measured at 570 nm, with background

correction at 655 nm using a microplate reader and percentages of antiproliferative activity was calculated compared to untreated control wells. IC₅₀ value defined as the concentration that caused 50% of maximum inhibition of cell viability was calculated by plotting percentage of inhibition against compound concentration with CurveExpert software, version 1.3 for Windows.

Spectroscopic data of the constituents

Ferruginol (1): Yellow crystals; C₂₀H₃₀O; [α]_D²⁷ –25° (c 0.04, CHCl₃); ¹H NMR (CDCl₃, 400.13 MHz): δ = 6.83 (1H, s, H-14), 6.63 (1H, s, H-11), 4.48 (1H, br s, –OH), 3.12 (1H, heptet, *J* = 6.9 Hz, H-15), 2.84 (1H, dt, *J* = 14.4, 7.5 Hz, H-7a), 2.77 (1H, m, H-7b), 2.17 (1H, br d, *J* = 12.7 Hz, H-1 α), 1.85 (1H, dd, *J* = 12.1, 7.5 Hz, H-6a), 1.73 (1H, m, H-2a), 1.65 (1H, dd, *J* = 12.2, 6.7 Hz, H-6b), 1.58 (1H, m, H-2b), 1.47 (1H, brd, *J* = 13.5 Hz, H-3a), 1.40 (1H, dd, *J* = 12.7, 3.1 Hz, H-1b), 1.33 (1H, dd, *J* = 12.5, 2.3 Hz, H-5), 1.30 (1H, m, H-3b), 1.25 (3H, d, *J* = 6.9 Hz, H-16), 1.23 (3H, d, *J* = 6.9 Hz, H-17), 1.17 (3H, s, H-20), 0.94 (3H, s, H-18), 0.92 (3H, s, H-19); ¹³C NMR (CDCl₃, 100.6 MHz): δ = 150.7 (C-12), 148.7 (C-9), 131.6 (C-13), 127.3 (C-8), 126.1 (C-14), 110.9 (C-11), 50.3 (C-5), 41.7 (C-3), 38.9 (C-1), 37.5 (C-10), 33.4 (C-18), 33.2 (C-4), 29.7 (C-7), 26.8 (C-15), 24.8 (C-20), 22.7 (C-16), 22.5 (C-17), 21.6 (C-19), 19.3 (C-2), 19.2 (C-6); EI-MS: *m/z* (rel. int.): 286 [M]⁺ (100).

Taxodione (2): Dark yellow crystals; C₂₀H₂₆O₃; [α]_D²⁷ +37° (c 0.2, CHCl₃); ¹H NMR (CDCl₃, 400.13 MHz): δ = 7.56 (1H, s, –OH), 6.88 (1H, s, H-14), 6.20 (1H, s, H-7), 3.07 (1H, heptet, *J* = 6.9 Hz, H-15), 2.93 (1H, m, H-1 β), 2.59 (1H, s, H-5), 1.75 (1H, m, H-1 α), 1.69 (1H, m, H-2 β), 1.60 (1H, m, H-2 α), 1.39 (1H, m, H-3 β), 1.26 (6H, s, H-19, H-20), 1.20 (1H, m, H-3 α), 1.15 (3H, d, *J* = 6.9 Hz, H-16), 1.16 (3H, d, *J* = 6.9 Hz, H-17), 1.11 (3H, s, H-18); ¹³C NMR (CDCl₃, 100.6 MHz): δ = 201.0 (C-6), 181.7 (C-12), 145.3 (C-13), 145.0 (C-11), 139.9 (C-8), 136.1 (C-14), 133.9 (C-7), 125.6 (C-9), 63.0 (C-5), 42.9 (C-10), 42.6 (C-3), 37.0 (C-1), 33.2 (C-18), 32.9 (C-4), 27.1 (C-15), 22.0 (C-19), 21.8 (C-20), 21.6 (C-17), 21.2 (C-16), 18.5 (C-2); EI-MS: *m/z* (rel. int.): 314 [M]⁺ (100).

Sahandinone (3): Red crystals; C₂₀H₂₄O₂; ¹H NMR (CDCl₃, 400.13 MHz): δ = 7.35 (1H, d, *J* = 7.8 Hz, H-6), 7.07 (1H, s, H-12), 7.03 (1H, d, *J* = 7.8 Hz, H-7), 5.29 (1H, t, *J* = 7.1 Hz, H-3), 3.08 (2H, m, H-1), 3.01 (1H, heptet, *J* = 6.9 Hz, H-15), 2.39 (3H, s, H-20), 2.19 (2H, ddd, *J* = 6.8, 8.7, 7.8 Hz, H-2), 1.70 (3H, s, H-18), 1.60 (3H, s, H-19), 1.17 (3H, d, *J* = 6.9 Hz, H-16), 1.19 (3H, d, *J* = 6.9 Hz, H-17); ¹³C NMR (CDCl₃, 100.6 MHz): δ = 182.5 (C-11), 181.5 (C-14), 148.1 (C-10), 144.5 (C-13), 140.3 (C-12), 140.2 (C-5), 136.6 (C-6), 134.9 (C-9), 132.8 (C-4), 128.5 (C-8), 128.0 (C-7), 123.8 (C-3), 30.2 (C-1), 27.5 (C-2), 26.9 (C-15), 25.7 (C-18), 21.34 (C-16), 21.34 (C-17), 19.7 (C-20), 17.4 (C-19); EI-MS: *m/z* (rel. int.): 296 [M]⁺ (43), 227 (100).

4-Dehydrosalvilimbinal (4): Deep yellow crystals; C₂₀H₂₆O₄; [α]_D²⁷ –47° (c 0.1, CHCl₃); ¹H NMR (CDCl₃, 400.13 MHz): δ = 7.10 (1H, d, *J* = 7.6 Hz, H-7), 6.99 (1H, d, *J* = 7.6 Hz, H-6), 6.89 (1H, s, H-14), 4.91 (2H, br s, H-19), 2.97 (1H, heptet, *J* = 6.5 Hz, H-15), 2.94 (1H, m, H-2a), 2.65 (1H, m, H-2b), 2.44 (1H, dd, *J* = 11, 4.3 Hz, H-10), 2.30 (1H, m, H-1a), 2.25 (3H, s, H-20), 2.03 (3H, s, H-18), 1.81 (1H, ddd, *J* = 15.2, 10.7, 5.4 Hz, H-1b), 1.14 (3H, d, *J* = 6.5 Hz, H-16), 1.12 (3H, d, *J* = 6.5 Hz, H-17); ¹³C NMR (CDCl₃, 100.6 MHz): δ = 201.7 (C-3), 149.1 (C-4), 141.7 (C-12), 137.3 (C-11), 137.3 (C-5), 136.3 (C-8), 130.1 (C-7), 135.3 (C-14), 128.2 (C-13), 126.4 (C-6), 111.7 (C-19), 74.5 (C-9), 46.0 (C-10), 26.9 (C-15), 26.0 (C-2), 25.0 (C-1), 24.5 (C-18), 21.5 (C-17), 21.4 (C-16), 19.8 (C-20); EI-MS: *m/z* (rel. int.): 330 [M]⁺ (6), 225 (100).

Labda-7,14-dien-13-ol (5): Viscous colorless oil; C₂₀H₃₄O; [α]_D²⁷ +25° (c 0.1, CHCl₃); ¹H NMR (CDCl₃, 400.13 MHz): δ = 5.93 (1H, dd, *J* = 17.4, 10.8 Hz, H-14), 5.37 (1H, br s, H-7), 5.21 (1H, dd, *J* = 17.4, 1.2 Hz, H-15_{trans}), 5.06 (1H, dd, *J* = 10.8, 1.2 Hz, H-15_{cis}), 1.96 (1H, m, H-6a), 1.87 (1H, m, H-6b), 1.84 (1H, m, H-1a), 1.80 (1H, d, m, H-12a), 1.67 (3H, s, H-17), 1.57 (1H, m, H-9), 1.53 (1H, m, H-2a), 1.47 (1H,

m, H-11a), 1.45 (1H, dd, *J* = 5.6, 2.4 Hz, H-2b), 1.42 (1H, m, H-12b), 1.38 (1H, m, H-3a), 1.28 (3H, s, H-16), 1.21 (1H, m, H-11b), 1.16 (1H, m, H-5), 1.15 (1H, m, H-3b), 0.93 (1H, m, H-1b), 0.87 (3H, s, H-18), 0.85 (3H, s, H-19), 0.75 (3H, s, H-20); ¹³C NMR (CDCl₃, 100.6 MHz): δ = 145.1 (C-14), 135.3 (C-8), 122.2 (C-7), 111.7 (C-15), 73.6 (C-13), 55.2 (C-9), 50.2 (C-5), 44.9 (C-12), 39.2 (C-1), 42.3 (C-3), 37.0 (C-10), 33.16 (C-18), 32.9 (C-4), 27.5 (C-16), 23.8 (C-6), 22.2 (C-17), 21.8 (C-19), 21.2 (C-11), 18.8 (C-2), 13.6 (C-20); EI-MS: *m/z* (rel. int.): 290 [M]⁺ (1), 204 (100).

Results and discussion

Ferruginol (**1**), taxodione (**2**), sahandinone (**3**), 4-dehydrosalvilimbinal (**4**) and labda-7,14-dien-13-ol (**5**) were purified using silica gel-open column chromatography and RP 18 preparative HPLC analyses of the DCM extract from the roots of *S. lachnocalyx*. The structures of the purified compounds were elucidated using their EI-MS, 1D (¹H and ¹³C) and 2D (COSY, HSQC and HMBC) NMR spectroscopic data and by comparing them with those reported in the literature for the authentic compounds (Carman et al., 1973; Topcu et al., 1996; Tezuka et al., 1998; Rodríguez, 2003; Jassbi et al., 2006).

The ¹H and ¹³C NMR spectroscopic data of compounds **1** and **2** that were isolated from *Salvia chorassanica* and *S. deserta* (Tezuka et al., 1998; Tayarani-Najaran et al., 2013) fully matched with those reported in this study. The EIMS, ¹H and ¹³C NMR spectroscopic data recorded for compound **3** with a rearranged abietane diterpenoid structure, that were reported from the roots extracts of *S. sahendica* and *S. rhytidea* were in agreement with the respective spectroscopic data for sahandinone reported from *S. lachnocalyx* (Jassbi et al., 2006; Jassbi et al., 2016). Compound **4**, with the same rearranged carbon skeleton of compound **3**, was isolated from the roots of *S. limbata* and showed the same spectroscopic data as those reported here for compound **4**. The stereochemistry at C-9 and C-10, because of lack of ¹H NMR signal for C-9 (OH) could not be determined, in either of the reports (Topcu et al., 1996). Although the configuration at the above mentioned stereocenters have not been determined with NOESY or chiroptical methods, molecular mechanics calculations of the most stable conformers suggested β orientation of H-10 and OH-9 (Topcu et al., 1996).

The ¹H and ¹³C NMR spectroscopic data of compound **5** suggested a labdane-type diterpenoid which was similar to those reported in the literature for labda-7,14-dien-13-ol and the configuration at C-13 was determined to be *S* by measuring the optical rotation (+25°) which was similar to that observed in the literature (+20°), while the opposite epimer showed smaller magnitude; [α]_D –1.8° (Carman et al., 1973; Jassbi et al., 2016).

Assessment of cytotoxic activity of compounds isolated from the roots of *S. lachnocalyx* (**1–5**) in our study showed that all of these derivatives possessed reasonable cytotoxic effects against the tested cell lines (Fig. 1). Among the isolated compounds, **2** and **3** showed the highest activities with IC₅₀ values in the range of 0.41–3.87 μ g/ml in comparison to cisplatin as the positive control and MOLT-4, HT-29 and MCF7 cell lines were the most sensitive lines to the tested phytochemicals, respectively (Table 1).

Compound **1** has been reported in the resin of the Miro tree (*Podocarpus ferrugineus*) (Brandt and Neubauer, 1939) as well as several other species and has exhibited various biological activities, including antimicrobial, antiprotozoal, anti-inflammatory, antioxidant, cardioprotective, antihypertensive and also reported as a cytotoxic agent (Ulubelen et al., 2000; Ulubelen et al., 2002; Samoylenko et al., 2008; Chen et al., 2011; Yao et al., 2012; Ebrahimi et al., 2013; Rungsimakan and Rowan, 2014). Bioassay-guided isolation of the chemical constituents of the roots of *S. chorassanica* has led to the isolation of compound **1** with cytotoxic effect against

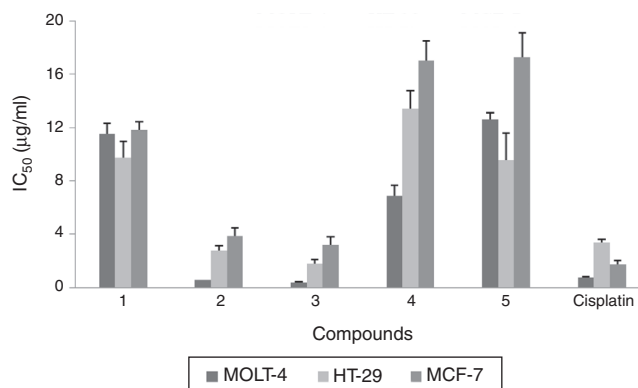


Fig. 1. Diterpenoids isolated from the roots of *S. lachnocalyx*.

Table 1
Anti-proliferative effects of diterpenoids isolated from the roots of *Salvia lachnocalyx* against human cancer cell lines.

Compound	IC ₅₀ (µg/ml)		
	MOLT-4	HT-29	MCF-7
1	11.5 ± 0.81	9.73 ± 1.23	11.82 ± 0.58
2	0.54 ± 0.05	2.76 ± 0.37	3.87 ± 0.62
3	0.41 ± 0.04	1.78 ± 0.33	3.20 ± 0.60
4	6.85 ± 0.80	13.43 ± 1.31	17.02 ± 1.48
5	12.3 ± 0.49	9.58 ± 2.01	17.23 ± 1.89
Cisplatin	0.75 ± 0.08	3.41 ± 0.23	1.7 ± 0.35

Values are presented as mean ± S.E.M. of 3–5 experiments. Cisplatin was tested as a reference cytotoxic agent.

two leukemia cancer cell lines, HL-60 and K562 cancer cells with IC₅₀ values of 19.51, 7.73 µM, respectively (Tayarani-Najaran et al., 2013). Compound 1 that was isolated from *S. sahendica* showed activity against MIAPaCa-2 (human pancreatic cancer cell line) with IC₅₀ = 25.9 µM (Fronza et al., 2011).

Compound 2 has been reported from the leaves of *Taxodium distichum* (Kupchan et al., 1969) and the roots of several members of *Salvia* species (Tezuka et al., 1998; Tayarani-Najaran et al., 2013) and has shown cytotoxic potential against various human cancer cell lines (Topcu et al., 2003; Kuźma et al., 2012; Burmistrova et al., 2013; Tayarani-Najaran et al., 2013). For instance, it inhibited the growth of BC1 (human breast cancer, IC₅₀ 1.2 µg/ml), LU1 (human lung cancer, IC₅₀ 5.1 µg/ml), COL2 (human colon cancer, IC₅₀ 0.7 µg/ml), LNCaP (human prostate cancer, IC₅₀ 0.7 µg/ml), P388 (mouse lymphoblastic leukemia, IC₅₀ 0.3 µg/ml), and A2780 (human ovarian cancer, IC₅₀ 9 µg/ml) cell lines (Topcu et al., 2003). In another study, compound 2 showed high anticancer activity against MOLT-3 (leukemia), MCF-7 (breast adenocarcinoma), SK-MEL-1 (melanoma), U937 (leukemia), HL-60 (leukemia) human cancer cell lines, with IC₅₀ values of 1.8, >30, 9.6, 3, 4.8 µM, respectively (Burmistrova et al., 2013).

Compound 3 was previously isolated as one of the major constituents from the roots of *S. sahendica* and *S. rhytidea* (Jassbi et al., 2006; Jassbi et al., 2016). Fronza and co-workers have also reported cytotoxic effects of compound 3 against human pancreatic cancer cell line (MIAPaCa-2) with an IC₅₀ value of 10.2 µM (Fronza et al., 2011).

Compound 4 was isolated from the roots of *Salvia limba* (Topcu et al., 1996). To the best of our knowledge, no biological activity is reported for this compound in the literature. Compound 5 was isolated from *Aster spathulifolius*, Asteraceae, and reported as a cytotoxic agent against four human cancer cell lines, SK-OV-3 (ovarian adenocarcinoma), SK-MEL-2 (malignant melanoma), XF498 (central nervous system tumor), and HCT15

(colon adenocarcinoma) cells with EC₅₀ values of 2.4, 8.7, 4.4, 9.5 and 9.1 µg/ml, respectively (Lee et al., 2005).

Comparison of our results with the previously reported findings reveals that this is the first study of cytotoxic effects of compounds 3–5 against MOLT-4, HT-29 and MCF7 human cancer cell lines. Furthermore, the cytotoxic activity of compound 1 against HT-29 and compound 2 against MOLT-4 and HT-29 cells also represent new findings.

Assessment of structure-activity relationships of the isolated compounds revealed that compounds 2 and 3, which contained endocyclic α,β-unsaturated carbonyl functional group showed higher activities (IC₅₀ values of 0.41–3.87 µg/ml) compared to the other compounds with the same abietane (1), rearranged-abietane (4) and labdane (5) skeletons, but no α,β-unsaturated carbonyls in their rings (IC₅₀ values of 6.85–17.23 µg/ml). Even compound 4 with an exocyclic unsaturated ketone was not as active as 3 with the same carbon skeleton.

Our current study and previous findings on several compounds with α,β-unsaturated carbonyl moiety, suggested that the presence of an α,β-unsaturated carbonyl group plays a role as a key pharmacophore for exhibiting cytotoxic activity (Nakayachi et al., 2004; Wu et al., 2012; Dai et al., 2015). In this regard, structure-activity studies have shown that α,β-unsaturated carbonyl function of the compounds facilitates the alkylation of cellular thiol groups in a Michael type addition reaction that causes cell damage and cytotoxicity (Amslinger, 2010; Nord et al., 2014).

Conclusion

In summary, five known diterpenoids were isolated from the DCM extract of the roots of *S. lachnocalyx* and their structures were elucidated by the spectroscopic data. The isolated diterpenoids showed *in vitro* cytotoxic activity against MOLT-4, HT-29 and MCF7 human cancer cell lines. Among them, compounds 2 and 3 with endocyclic α,β-unsaturated carbonyl functional group demonstrated a remarkable cytotoxic effect in comparison with cisplatin as a standard chemotherapeutic agent. The obtained evidence showed the roots of *S. lachnocalyx* merit further investigation as a potential source of biologically active compounds.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contribution

ARJ: Supervised the studies in analyzing natural products and determining the structures of natural products, helped in writing the paper, HHM: PhD Student; a part of his dissertation, OF: Supervised the studies in assessment of biological activity, contributed to the preparation and writing the manuscript; BS: Performed NMR spectroscopy and scientific advising; ITB: Scientific supporting the project and helping in editing the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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