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Original Article

Salvastearolide, a new acyl-glyceride, and other constituents from the seeds of *Salvadora persica*

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ABSTRACT

The chemical investigation of the *n*-hexane fraction of *Salvadora persica* L., Salvadoraceae, seeds afforded a new stearic acid ester, salvastearolide, together with five other phytosteroids identified as stigmasterol, β-sitosterol, Δ^7-campesterol, Δ^7-avenasterol and campesterol. Their structures were established on the basis of extensive spectroscopic methods including 1D and 2D NMR experiments and HRESI mass spectrometry. In addition, salvastearolide and the isolated fractions were tested for their cytotoxicity against human cancer cell lines MCF-7, MDA-MB-231 and HT-29. The *n*-hexane fraction exhibited significant anti-proliferative effect against human breast cancer cell line MCF-7 (IC_{50} 50 μg/ml), while salvastearolide possessed a weak cytotoxic effect against MCF-7 cells with IC_{50} 103.98 μg/ml.

Introduction

Salvadora persica L., known as miswak, is a shrub with a soft white wood and belongs to the Salvadoraceae family. It is distributed mainly in tropical and subtropical Asia. The aqueous extract of *S. persica* leaves possesses analgesic activity and decreases carrageenan-induced inflammation in rat paw (Ramadan and Alshamrani, 2016).

S. persica has been reported to have antiulcer activity (Sanogo et al., 1999) and antimicrobial activity (Almas, 1999; Sofrata et al., 2008). The aqueous extracts of *S. persica* enhance the growth of fibroblasts and inhibit the growth of cariogenic bacteria (Darmani et al., 2006).

The phytochemical study of *S. persica* led to the isolation of four benzylamides (Khalil, 2006). The characterization of the obtained compounds were confirmed through spectral analysis. Moreover, five different glycosides were isolated from the stems of *S. persica* and identified as sodium 1-*O*-benzyl-β-D-glucopyranoside-2-sulphate, 5,5′-dimethoxylariciresinol 4,4′-bis-*O*-β-D-glucopyranoside, syringin, liriodendrin and sitosterol 3-*O*-glucopyranoside. This investigation was the first report of syringin and lignan glycosides from the family Salvadoraceae (Kamel et al., 1992).

A new alkaloid named salvadoricine has been isolated from *S. persica*. Its chemical structure was established by spectroscopic methods and confirmed by synthesis (Malik et al., 1987).

The volatile roots oil of *S. persica* contain benzyl isothiocyanate with other constituents such as α-pinene, β-pinene, β-elemene, camphene, myrcene, limonene, δ-3-carene, benzyl nitrile, benzaldehyde, umbellulone, myristicin, γ-muurolene, terpinolene, longifolene and β-caryophyllene (Bader and Flamini, 2002).

GC-MS analysis of the volatile oil from the leaves of *S. persica* resulted in the isolation of thymoland β-caryophyllene, eucalyptol, isoterpinolene, benzyl nitrile, isothymol and eugenol (Alali and Al-Lafi, 2003).

We report here the antiproliferative effects of different extracts from *S. persica* seeds as well as the isolation and identification of several known steroidal compounds and a new monoglyceride derivative from the *n*-hexane fraction. The isolated compounds were characterized by NMR spectroscopic methods. Furthermore, the new isolated compound was tested for its cytotoxicity against breast cancer cell line MCF-7 *in vitro*.

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Materials and methods

Plant material

Seeds of *Salvadora persica* L., Salvadoraceae, were collected from Gabal Fiafa in Jazan, Saudi Arabia, on June 2016 and identified by Dr. Wael Kassim, Department of Botany, Jazan University, Saudi Arabia. A voucher specimen (108-42016) has been deposited in Jazan University Herbarium (JAZUH).

Isolation and characterization of compounds

The grounded air-dried seeds of *S. persica* (370 g) were extracted with hexane to remove fats, then with methanol thrice (3 × 2 l) at room temperature (24 h each). The combined methanol extracts were then concentrated under reduced pressure to give a crude residue (80.66 g). The residue was suspended in water and then partitioned successively with hexane, CH_2Cl_2, EtOAc and *n*-BuOH affording residues of 48.3, 458, 150 and 3.8 g, respectively.

Accurately weighed 5 g of the hexane residue was subjected to chromatographic separation over silica gel column chromatography eluted with CH_2Cl_2 to afford 10 fractions (1–10). Fractions 6, 7 and 8 were further combined and purified by preparative HPLC column chromatography to afford compounds **2–6**. Fraction 2 was purified by silica gel column chromatography eluted with hexane-ethyl acetate (15:1) followed by hexane-ethyl acetate (30:1) to give three subfractions (2A, 2B and 2C). Subfractions 2B and 2C (780 mg) were combined and purified by silica gel column chromatography with CH_2Cl_2–MeOH (20:1) to afford compound **1** (100 mg).

General experimental methods

Silica gel 60 was used for column chromatography. Thin layer chromatography analysis was carried out using silica gel 60 F254 plates. Vacuum liquid chromatography was carried out using silica gel 60 H. The chromatograms were visualized under UV light and then sprayed with anisaldehyde and vanillin reagents. High-performance liquid chromatography analysis was conducted using Waters 600E instrument with a diode array detector. Infrared spectra were recorded on a Bruker IFS instrument. ESIMS was performed on Finnigan MAT SSQ 7000 instrument. NMR spectra for the isolated metabolites were obtained on Bruker 400 spectrometer using TMS as an internal standard.

Cell culture

The human cancer cell lines, such as breast cancer cell lines (MCF-7 and MDA-MB-231), colon cancer cell line (HT-29) and nonmalignant human adipose-derived stem cells (ADSCs) were procured from Korean Cell Line Bank, Korea. Cancer cell lines were grown in DMEM supplemented with 1% antibiotics and 10% FBS at 37 °C in a humidified atmosphere containing 5% CO_2. ADSCs were cultured in MesenPRO RS™ medium supplemented with growth factors (Life Technologies) at the same atmosphere with 5% CO_2.

Antiproliferative assay

The effect of crude extracts as well as different fractions like hexane, dichloromethane, ethyl acetate and butanol on proliferation of both malignant and nonmalignant cells was evaluated by the MTT assay method (Mosmann, 1983). Briefly, the cells were seeded in 96-well plates at a density of 5000 cells/well in 100 μl respective culture medium. After overnight incubation, cells were treated with different concentrations of crude extracts as well as fractions of seeds and then incubated for 24 h at 37 °C with 5% CO_2. After incubation, the medium was replaced with 100 μl of MTT solution which was prepared freshly as 0.5 mg/ml in phenol red and serum-free DMEM, filtered through a 0.22-μm filter, was added to each well, and then the plates were incubated in dark for additional 4 h at 37 °C. Afterward, the media were removed from the 96-well plates, 100 μl of DMSO was added to each well and absorbance was measured at 570 nm using a microplate reader. The results are expressed as the percentage of cell viability in comparison with the control cells (cells without drug treatment). The cell viability of the control group without exposure to the drug was defined as 100%.

Table 1
^1H and ^{13}C NMR spectral data of compound **1** in $CDCl_3$ (δ in ppm, *J* in Hz).[a]

Position	δ_H (multiplicity)	δ_C
1	11.0 (*br.*, OH)	173.50
2	5.25 (*m*, 1H), 1.57 (*br.*, OH)	69.05
3	4.26 (*dd*, 1H, 4.4, 16.4 Hz), 4.12 (*dd*, 1H, 6.0, 18.0 Hz)	62.30
1′	–	173.09
2′	2.28 (*m*, 2H)	34.42
3′	1.55[b]	25.06
4′	1.22[b]	29.28
5′	1.22[b]	29.32
6′	1.22[b]	29.55
7′	1.22[b]	29.57
8′	1.22[b]	29.71
9′	1.22[b]	29.87
10′	1.22[b]	29.90
11′	1.22[b]	32.12
12′	1.22[b]	29.83
13′	1.22[b]	29.68
14′	1.22[b]	29.50
15′	1.22[b]	29.48
16′	1.22[b]	34.25
17′	1.22[b]	22.90
18′	0.85 (*t*, 3H, 6.8 Hz)	14.32

[a] The assignments were based upon 2D-COSY, DEPT, HSQC and HMBC spectra.
[b] Multiplicity is not clear for some resonances due to overlapping.

2-Hydroxy-3-(stearoyloxy) propanoic acid (*1*)

Solidified colourless oil, IR (KBr): 3550, 1741, 1725 cm^{-1}; ^1H NMR and ^{13}C NMR: ($CDCl_3$, 400 MHz for proton and 100 MHz for carbon): Table 1 (+) ESI-MS: *m/z* 373 $[M+H]^+$, HR-ESI-MS: 395.2839 $[M+Na]^+$ (calcd. 395.2773 for $C_{21}H_{40}O_5Na$).

Results and discussion

The cytotoxic activity of the obtained fractions as well as the crude methanol extract was evaluated using cancer cell lines such as human breast cancer cells MCF-7 and MDA-MB-231, and human colon cancer cells HT-29 by MTT colorimetric assay method (Mosmann, 1983). The adipose-derived stem cells (ADSCs) were used as a normal control in order to assess the toxic potential of the extracts. As shown in Fig. 1, the hexane fraction induced significant antiproliferative effect against human breast cancer cell line MCF-7, at a minimum concentration of 50 μg/ml, whereas the same concentration of the fraction did not influence the proliferation of other cancer cell lines as well as the normal stem cells, ADSCs. None of the other fractions showed significant antiproliferative activity against both normal and cancer cells.

The steroidal compounds (**2–6**) were isolated and identified based on their ^1H and ^{13}C NMR spectral data compared to those in the literature as stigmasterol, β-sitosterol (Chaturvedula and Prakash, 2012; Jain and Bary, 2010), Δ^7-campesterol, Δ^7-avenasterol and campesterol (Zhang et al., 2016).

Salvastearolide (**1**) was isolated as solidified colourless oil at room temperature. The ESI positive mode mass spectrum showed $(M+1)^+$ molecular peak at *m/z* 373, while the HR-ESIMS analysis

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