Fitoterapia 98 (2014) 137–142

Contents lists available at ScienceDirect

Fitoterapia

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

Triterpenoids of sour jujube show pronounced inhibitory effect on human tumor cells and antioxidant activity

Aimin Qiao [a], Yihai Wang [b], Limin Xiang [b], Zhenxue Zhang [c,*], Xiangjiu He [b,**]

[a] School of Life Science and Bio-pharmaceutical, Guangdong Pharmaceutical University, Guangzhou 510006, China
[b] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, China
[c] College of Chemical Engineering, Shenyang University of Chemical Technology, Shenyang 110142, China

ARTICLE INFO

Article history:
Received 16 June 2014
Accepted in revised form 22 July 2014
Available online 1 August 2014

Keywords:
Sour jujube
Triterpenoids
Structure identification
Antiproliferative activity
Antioxidant activity

ABSTRACT

Sour jujube is a common fruit and traditional medicine in China. Bioactivity-guided fractionation of sour jujube was used to determine the chemical identity of potent antiproliferative and antioxidant constituents. Four novel ursane-type triterpenoids, together with 8 known were isolated and identified. The new triterpenoids were elucidated to be 2α,3β,13β,23-tetrahydroxy-urs-11-en-28-oic acid (**3**), 2α,3β-dihydroxy-urs-20(30)-en-28-oic acid (**9**), 2α,3β,28-trihydroxy-urs-20(30)-ene (**10**), and 3β,12β,13β-trihydroxy-ursan-28-oic acid (**11**). Among the triterpenoids isolated, 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid (**7**), **9** and **10** showed high potent inhibitory activity toward the proliferation of HepG2 cells, which the IC_{50} values were lower than 5 μM. Compounds **9** and **10** also exhibited pronounced activity against MCF-7 cells, with IC_{50} value of 0.8 ± 0.03 and 1.5 ± 0.1 μM, respectively. Compound **10** showed high antioxidant activity with an EC_{50} of 0.8 ± 0.02 μM, which was 18.9 times higher than ascorbic acid in antioxidant capacity.

1. Introduction

Jujube (*Ziziphus jujuba*, Rhamnaceae) is widely distributed in the temperate and subtropical areas of the northern hemisphere, especially the inland region of north China. To date, about 700 cultivars of jujube have been found in China, and its cultivation area has reached 1.5 million hectares [1]. The annual output of fresh jujube fruits is about 400,000 tons.

Jujube is rich of biological components related to both nutritional and nutraceutical values, which is commonly served as food, food additive, and flavoring for thousands of years in China. Studies have found that jujube contains various nutrients, including vitamins, trace minerals, proteins, and sugars [2,3]. Jujube has also been found to have various secondary metabolites, such as triterpenoids [4–6], flavonoids [7,8], cerebrosides [9], and phenolic acids [10,11].

Jujube has many health benefits and has long been studied for its biological activities. The health benefits related to jujube consumption include antitumor, antioxidant, anti-inflammatory, immunostimulating, hepatoprotective effects, and others. Jujube extracts could inhibit the growth of human breast cancer cells (MCF-7, SKBR3), human hepatoma cells (HepG2), cervical cancer cells (HeLa), lung cancer cells (A549), and lymphoma cells (U937). Induction of apoptosis is one of the possible mechanisms for the antitumor activities of jujube extracts [12–16].

Sour jujube (*Ziziphus jujuba* var. *spinosa*) is one of the main varieties of jujube, which is widely distributed in northern China. The fruits of sour jujube (Chinese name "Suan-zao") are a common fruit and its seeds have been used as a traditional medicine in China with antitumor, sedative and hypnotic effects. However, the bioactive component of sour jujube is not clear. In continuing efforts to seek bioactive components from

* Correspondence author. Tel.: +86 24 8938 3622.
** Correspondence author. Tel./fax: +86 20 3935 2132.
E-mail addresses: zhangzhenxue02@hotmail.com (Z. Zhang), hexiangjiu@hotmail.com (X. He).

http://dx.doi.org/10.1016/j.fitote.2014.07.020
0367-326X/

high plants [17,18], bioactivity-guided fractionation of sour jujube was used to determine the identity of bioactive compounds, which could inhibit tumor cell growth. The objective of this research was to isolate and identify the bioactive compounds of sour jujube with potent antiproliferative and antioxidant activity.

2. Materials and methods

2.1. General

HPLC analysis and purification were performed on a Waters 600 instrument equipped with a PDA detector and the observing wavelength was set at 210 nm (Waters Corp., Milford, MA). All NMR spectra were measured on a Bruker AV-500 spectrometer (Bruker Inc., Fällanden, Switzerland) with routine sequences. ESI-MS spectra were recorded on a Bruker Esquire 2000 mass spectrometer (Bruker Inc., Fällanden, Switzerland). High-resolution ESI-MS spectra (HR-ESI-MS) were recorded on an Exactive spectrometer (Thermo Fisher Scientific) with Orbitrap technology with a routine protocol.

All chemicals used in the study, such as methanol, acetone, hexane, ethyl acetate, dichloromethane, and *n*-butanol, were of analytical grade and were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). The deuteriated pyridine for NMR measurement was purchased from Sigma-Aldrich, Inc. (St. Louis, MO).

2.2. Chromatographic materials

Silica gel for column chromatography, 230–400 mesh, and precoated silica gel 60 TLC plates were purchased from Merck KGaA (Darmstadt, Germany). Precoated Rp-18 TLC plates were obtained from Macherey-Nagel (Düren, Germany). Diaion HP-20 was purchased from Supelco, Inc. (Bellefonte, PA). ODS for open column chromatography and MPLC was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The semi-preparative HPLC column was a product of Agilent (Zorbax Rx-C18, 5 μm, 9.4 × 250 mm, Agilent).

2.3. Plant material

The fruits of sour jujube (*Ziziphus jujuba* var. *spinosa*) were purchased from the local herbal market and were harvested in the fall of 2006, and were identified by Prof. Xiangjiu He, School of Pharmacy at Guangdong Pharmaceutical University.

2.4. Extraction, isolation, and purification procedures of bioactive constituents from sour jujube

Dried sour jujubes (5.0 kg) were homogenized for 8 min with 80% acetone (1:5, w/v). The homogenates were filtered, and the filtrate was evaporated to dryness under vacuum at 45 °C. The residue was then resuspended in 30% methanol and subjected to an HP-20 column (620 × 80 mm), and eluted with 60% and 100% methanol. The methanol elution (81 g) was further purified by silica gel chromatography (230–400 mesh, 810 × 80 mm) and eluted with a CH_2Cl_2/MeOH gradient elution (the ratios of CH_2Cl_2–MeOH were from 100:0 to 0:100).

The CH_2Cl_2/MeOH (100:1) elution (3.2 g) was further subjected to silica gel column chromatography (310 × 25 mm) and eluted with hexane/ethyl acetate. Compound **4** (210.3 mg) was obtained from hexane/ethyl acetate (5:1) elution. The fraction eluted with hexane/ethyl acetate (3:1, 312.0 mg) was purified on a semi-preparative HPLC using the Zorbox column eluted isocratically with 85% methanol (containing 0.1% CF_3COOH) at a flow rate of 2.5 mL/min, and compounds **3** (5.5 mg), **5** (72.1 mg), **2** (8.5 mg) and **8** (6.2 mg) were obtained. The later part of the hexane/ethyl acetate (5:1) elution (512.0 mg) was subjected to a Sephadex LH-20 column (510 × 15 mm), followed by the semi-preparative HPLC (Zorbox-C18 column, 5 μm, 9.4 × 250 mm) using 80% methanol (containing 0.1% CF_3COOH) as mobile phase, and obtained compounds **1** (7.1 mg), **9** (5.5 mg) and **10** (6.7 mg). The CH_2Cl_2/MeOH (20:1) elution (4.2 g) of the methanol fraction from the HP-20 column was subjected to an ODS MPLC column (30 × 250 mm) and eluted with H_2O/MeOH, and the fractions were purified by HPLC; compounds **11** (4.3 mg), **12** (8.1 mg), **6** (11.0 mg) and **7** (7.5 mg) were obtained using 80% and 78% methanol (containing 0.1% CF_3COOH, pH 2.0) as mobile phase.

2.5. Measurement of inhibition activity on tumor cell proliferation

Antiproliferative activities against human liver cancer cells (HepG2) and human breast cancer cells (MCF-7, MDA-MB-231) of the pure triterpenoids isolated from sour jujube were measured by MTT assay. Briefly, cells were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco Life Technologies), 10 mM Hepes, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin and were maintained at 37 °C in 5% CO_2. A total of 2.5×10^4 cells in growth media were placed in each well of a 96-well flat-bottom plate. After 12 h of incubation, the growth medium was replaced by media containing different concentrations of the test samples. After 48 h of incubation, cell proliferation was determined by colorimetric MTT assay. Cell proliferation (percent) was determined at 48 h from the MTT absorbance (490 nm) reading for each concentration compared to the control. At least three replications for each sample were used to determine the cell proliferation.

2.6. Measurement of antioxidant activity using peroxyl radical scavenging capacity (PSC) assay and superoxide anion free radical (O_2^-) methods

Antioxidant activity of the triterpenoids was determined through the peroxyl radical scavenging capacity assay (PSC) described previously [19]. The median effective concentration (EC_{50}) was defined as the dose required to cause a 50% inhibition (PSC unit 0.5) for each pure compound. Results were expressed as μmol of vitamin C equivalents per μmol of pure compound ± standard deviation (SD) for triplicate analysis.

Superoxide anion radical scavenging activity was assayed by the NBT reduction method [20]. The reaction mixture used for the O_2^- scavenging activity assay containing Tris–HCl (pH 8.1, 50 mM, 445 μL), NADH (0.15 mM, 250 μL), PMS (0.03 mM, 50 μL), NBT (0.10 mM, 250 μL) and sample solution (5 μL), in the final volume of 1000 μL. The test samples were dissolved in DMSO. The reaction was conducted at 37 °C for 5 min, and