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Novel triterpenoids isolated from hawthorn berries functioned as antioxidant and antiproliferative activities

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ABSTRACT

Hawthorn berry is a common fruit native to Northern China. Its chemical identity of potent antiproliferative and antioxidant constituents was carried out. Fifteen triterpenoids, including 4 novels, were isolated and identified. The new triterpenoids were elucidated to be 3β,6β,18β-trihydroxy-olean-12-en-28-oic acid (**1**), 3β,6β,18β,23-tetrahydroxy-olean-12-en-28-oic acid (**2**), 2α,3β,6β,18β-tetrahydroxy-olean-12-en-28-oic acid (**3**), and 2α,3β,6β,18β,23-pentahydroxy-olean-12-en-28-oic acid (**4**), respectively. Antiproliferative and antioxidant activities of the triterpenoids were evaluated. Compounds **2**, **3** and **4** showed high potent inhibitory activity toward the proliferation of HepG2 and MCF-7 cells, with the EC_{50} values were lower than 5 μM. **1** and **10** also exhibited high antioxidant activity in PSC assay, which were 70 and 28 times higher than ascorbic acid in antioxidant capacity. These results showed the triterpenoids isolated from hawthorn berries have potent antiproliferative and antioxidant activity and may be responsible for the anticancer activities of hawthorn berries.

1. Introduction

Hawthorn berry (*Crataegus pinnatifida*) is a medicinal food product native to Northern China (where it derives the common name of Chinese Hawthorn), but also found spreading across a similar latitude to Japan, South Korea, Europe, and parts of North America.

Hawthorn berry has attracted increasing attention in the field of food, nutraceuticals, and phytomedicine because of its wide health benefits. It could improve coronary artery blood flow and the contractions of heart muscle, and is used widely in cardiovascular diseases like arrhythmia, myocardial infarction, and congestive heart failure (Degenring, Suter, Weber, & Saller, 2003; Long, Carey, Crofoot, Proteau, & Filtz, 2006; Zhang, Zhang, Yin, & Zhao, 2004). It also could reduce plasma lipids such as total cholesterol, triacylglycerides and LDL fraction (Andrade-Cetto & Heinrich, 2005). Hawthorn berry also may be employed as anti-inflammatory, gastro-protective, and antimicrobial agent (Kao et al., 2005; Tadic, Dobric, Markovic, Sofija, & Tanja, 2008). It could inhibit angiotensin converting enzyme (ACE) and reduce production of the potent blood

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vessel-constricting substance angiotensin II, which could act as hypotensive and diuretic agent (Schroder, Weiser, & Klein, 2003). Moreover, hawthorn berry showed high antioxidant and immunostimulating activity (Li, Yuan, & Rashid, 2009; Soberon, Sgariglia, Sampietro, Quiroga, & Vattuone, 2010; Zhang et al., 2001).

The leaves, flowers and berries of hawthorn contain a variety of flavonoids that appear to be primarily responsible for the cardiac actions of the plant. Flavonoids found in hawthorn plant include oligomeric procyanidins, vitexin, quercetin, and hyperoside. The action of these flavonoids on the cardiovascular system has led to the development of leaf and flower extracts, which are widely used in Europe. Other chemical constituents include tannins, nitrogen-containing compounds, triterpenoids, steroids, lignans. The triterpenoids in hawthorn are classified into tetracyclic triterpenoids and pentacyclic triterpenoids, such as ursolic acid, 2α,3β,19α-trihydroxyursolic acid, corosolic acid, cuneataol, cycloartenol, uvaol, oleanolic acid, crataegolic acid, butyrospermol, 24-methylene-24-dihydrolanosterol, betulin, and 18,19-seco-2α,3β-dihydroxy-19-oxo-urs-11,13(18)-dien-28-oic acid. The triterpenoids in hawthorn also showed considerable antitumor activity (Min et al., 2000; Wu, Peng, Qin, & Zhou, 2014).

However, the bioactive components of hawthorn that may be responsible for antitumor activity are not clear. In continuing efforts to seek bioactive components from medicinal plants, fruits and vegetables (He & Liu, 2007; Wang, Xiang, Wang, Tang, & He, 2013), bioactivity-guided fractionation of hawthorn berries was used to determine the identity of bioactive compounds with potent antiproliferative and antioxidant activity.

2. Materials and methods

2.1. Plant material

The hawthorn berries were purchased from the local herbal market and were harvested in fall, 2005, which were identified as *C. pinnatifida* by Prof. Xiangjiu He of the School of Pharmacy at Guangdong Pharmaceutical University. A voucher specimen is available at the School of Pharmacy, Guangdong Pharmaceutical University in Guangzhou (510006), China.

2.2. Reagents

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

2.3. Chromatographic materials and instrumentation

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).

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.

2.4. Extraction, isolation, and purification procedures of bioactive constituents from hawthorn berries

Dried hawthorn berries (8.0 kg) were soaked for 5 hrs and then homogenized for 10 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% ethanol and subjected to a HP-20 column (620 × 80 mm). Then the column was eluted with 60% and 95% ethanol. The 95% ethanol elution (40 g) was further purified by silica gel chromatography (230–400 mesh, 900 × 80 mm) and eluted with a CH_2Cl_2/MeOH gradient elution.

The CH_2Cl_2/MeOH (100:1) elution (2.1 g) was further subjected to silica gel column chromatography (280 × 25 mm) and eluted with hexane/ethyl acetate. Compound **13** (170.1 mg) was obtained from hexane/ethyl acetate (5:1) elution. The fraction eluted with hexane/ethyl acetate (2:1, 206.8 mg) was purified on a semi-preparative HPLC using the Zorbox column eluted isocratically with 86% methanol (containing 0.1% CF_3COOH) at a flow rate of 3.0 mL/min, and compounds **14** (6.0 mg), **15** (4.1 mg), **7** (8.0 mg) and **8** (4.7 mg) were obtained.

The CH_2Cl_2/MeOH (50:1) elution (3.1 g) of the methanol fraction was subjected to a Sephadex LH-20 column (1200 × 30 mm), eluted with 50% $CHCl_3$/MeOH, compounds **5** (1.2 g) and **6** (21.0 mg) were obtained after recrystallization.

The later part of the CH_2Cl_2/MeOH (50:1) elution (810.2 mg) of the methanol fraction was subjected to a Sephadex LH-20 column (500 × 15 mm), followed by the semi-preparative HPLC (Zorbox-C18 column, 5 μm, 9.4 × 250 mm) using 78% methanol (containing 0.1% CF_3COOH) as mobile phase, and got compounds **1** (6.1 mg), **2** (5.3 mg), **11** (7.2 mg) and **12** (7.2 mg). The CH_2Cl_2/MeOH (20:1) elution (1.3 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 **3** (4.7 mg), **4** (6.5 mg), **9** (12.0 mg) and **10** (7.8 mg) were obtained using 76% 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 hawthorn berries were measured by the MTT assay as described previously (Zeng et al., 2011). Briefly, cells were cultured in Dulbecco's