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Antibacterial oleanane-type triterpenoids from pericarps of *Akebia trifoliata*

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ABSTRACT

Three new oleanane triterpenoids, 2α,3β,29-trihydroxyolean-12-en-28-oic acid (**1**), 2α,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid (**2**) and 2α,3β,21β,22α-tetrahydroxyolean-12-en-28,29-dioic acid (**3**), and ten known ones, maslinic acid (**4**), arjunolic acid (**5**), oleanolic acid (**6**), 3-*epi*-oleanolic acid (**7**), stachlic acid A (**8**), serratagenic acid (**9**), gypsogenic acid (**10**), 2α,3β-dihydroxyol-ean-13(18)-en-28-oic acid (**11**), mesembryanthemoidigenic acid (**12**) and 12α-hydroxy-δ-lactone (**13**), were isolated from the pericarps of *Akebia trifoliata*, a new valued fruit crop in China. Their structures were elucidated on the basis of extensive spectroscopic analysis. Compounds **8**, **10**, **11** and **13** were isolated for the first time from the genus *Akebia*. All the compounds were tested for their antimicrobial activity against five bacterial strains. Compounds **4**, **6** and **11** showed significant antibacterial activity toward all the assayed microorganisms with MIC values ranging from 0.9 to 15.6 μg/mL, which were close or even more potent than the reference compound Kanamycin (MIC values ranging from 1.9 to 3.9 μg/mL).

1. Introduction

Akebia trifoliata (Thunb.) Koidz., belonging to the family Lardizabalaceae, is a liana plant mainly distributed in the east part of Asia, whose stems has been used as a diuretic and an antiphlogistic in traditional Chinese medicine for centuries (Li, Chen, Yao, Tian, & Huang, 2010). The fruits of *A. trifoliata*, commonly called 'Bayuezha' in China and can be processed for making fruit vinegar, sweet and juice, has long been consumed by local Chinese people as delicious food (Du et al., 2012; Wang et al., 2005). Since the flesh of the fruits is believed to be nutritive due to richness of saccharides, vitamins, human essential amino acids etc. (Li, Yao, Zhong, Chen, & Huang, 2010), *A. trifoliata* has now been rapidly developed and cultivated in large scale as an important economic crop for fruits in many places of China, including Hunan, Hubei, Jiangxi, Shaanxi and Chongqing provinces (Li, Chen, et al., 2010; Li, Yao, et al., 2010). The delicious taste and attractive appearance of the fruits are making it popular in local markets at the ripe season.

The nutritive flesh of the fruits of *A. trifoliata* is easily rotted by bacteria infection when exposed to the air. But in nature during the growth period, the flesh is physiologically covered by pericarps which is capable of effectively prevent the flesh from bacterial infection. Considering the fact that the pericarps of *A. trifoliata* is soft and fragile, which principally do not relies on lignification to construct solid physical barriers for defence, it is reasonable to deduce that rich defence related antibacterial chemicals would exist in the pericarps, and those bioactive chemicals might further be ideal candidates that could be developed as effective and safe antibacterial agents for food or fruit preservation. This perspective attracts our interest to investigate the potential antibacterial chemicals in the pericarps of *A. trifoliata*.

In recent years, phytochemical studies have revealed many triterpenoids and trierpenoid saponins (Gao & Wang, 2006; Mimaki et al., 2003; Wang, Lu, & Lin, 2004) from the stems of *A. trifoliata*, and some lignans (Guan et al., 2008) and phenolic compounds (Guan, Yu, & Guan, 2010) were also reported from this plant species. Very recently, a phytochemical study further revealed a series of triterpenoid saponins from the pericarps of *A. trifoliata* (Iwanaga, Warashina, & Miyase, 2012). However, these studies have so far mainly focused on the purification and structural elucidation of chemical constituents and few addressed on the bioactivities of the isolates. With the aim to clarify the potential antibacterial chemicals in the pericarps of *A. trifoliata*, we carried out a

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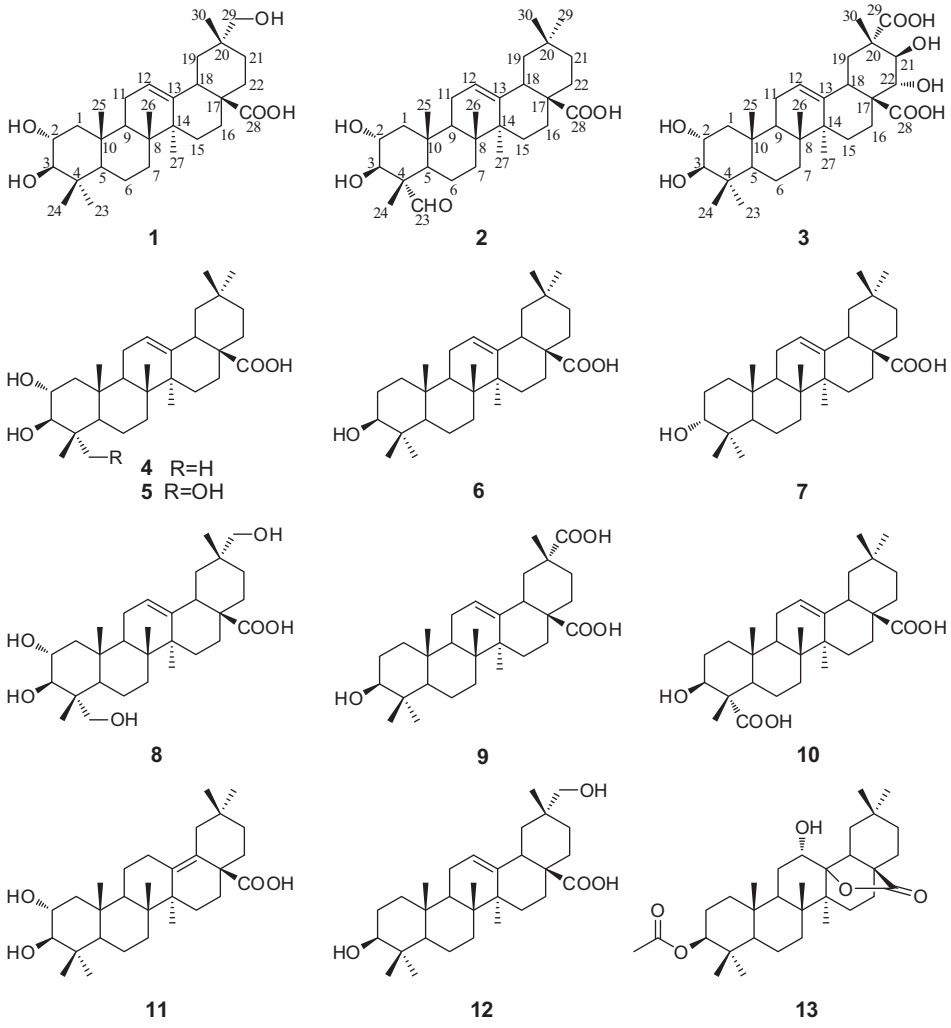

Fig. 1. Structures of compounds **1**–**13**.

phytochemical study on the pericarps of this plant, which led to the isolation of three new (**1**–**3**) and ten known oleanane-type triterpenoids (**4**–**13**) (Fig. 1). Herein, we report the isolation and structure elucidation of these compounds as well as their antibacterial activities against two gram-positive (*Staphylococcus aureus*, *Bacillus thuringiensis*) and three gram-negative (*Escherichia coli*, *Salmonella enterica* and *Shigella dysenteriae*) bacterial strains.

2. Materials and methods

2.1. Chemicals and reagents

Kanamycin sulfate, Resazurin and C_5D_5N were purchased from Sigma–Aldrich Co. (Louis, USA); Analytical grade petroleum ether (b.p. 60–90 °C), methanol, ethyl acetate, chloroform, *n*-butyl alcohol and acetone were obtained from Tianjin Fuyu Fine Chemical Industry Co. (Tianjin, China); HPLC grade methanol was purchased from J & K Chemical Ltd. (Beijing, China); For column chromatography (CC), Silica gel (80–100 and 200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), YMC ODS-A (50 μm, YMC Co. Ltd., Kyoto, Japan), Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden), and MCI gel CHP 20 P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan) were used. Fractions were monitored by precoated $HSGF_{254}$ TLC (Yantai Jiangyou silica gel Co. Ltd., Yantai, China), and spots were visualised by heating the silica gel plates sprayed with 10% sulphuric acid in ethanol (v/v).

2.2. Instruments

Preparative HPLC was conducted using a CXTH P3000 HPLC pump and a UV 3000 UV–Vis Detector with a Fuji-C18 column (10 μm-100 A); The performance of MPLC (medium pressure liquid chromatography) was a CXTH P3000 HPLC pump, a UV 3000 UV–Vis Detector and a C18 column (400 × 25 mm i.d., 500 × 50 mm i.d.); Electrospray ionization/mass spectroscopy (ESI/MS) date were obtained using a MDS SCIEX API 2000 LC/MS/MS system (Applied Biosystems, Forster City, USA); HR-ESI-MS data were measured on a Bruker maXis spectrometer (Bruker Daltonics, USA). NMR spectra were recorded on a Bruker advance 600 NMR spectrometer (Bruker, Switzerland) and a Bruker DRX-400 NMR spectrometer (Bruker Biospin, Rheinstetten, Germany); Optical rotations were measured on a Perkin-Elmer 341 polarimeter (Perkin-Elmer, Waltham, USA) with MeOH as solvent.

2.3. Plant material

The pericarps of *A. trifoliata* (9 kg) were collected at Liye of Longshan, Hunan province, PR China, in September 2009, identified