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Isolation, chemotaxonomic significance and cytotoxic effects of quassinoids from *Brucea javanica*

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ABSTRACT

A new quassinoid, bruceene A (**1**) along with seventeen known quassinoids (**2**–**18**) was isolated from the fruits of *Brucea javanica*. The structure of **1** was elucidated by extensive spectroscopic methods, and was further confirmed by single-crystal X-ray diffraction analysis. Isolation of similar quassinoids **1**–**3** as those in genus *Ailanthus* from genus *Brucea*, indicated the close chemotaxonomic relationship between these two genera, which further supported the phylogenetic study by DNA analysis. Compounds **5**, **7**, **10** and **12** with a 3-hydroxy-3-en-2-one moiety showed potent inhibitory activities against the MCF-7 and MDA-MB-231 cells with IC_{50} values in the ranges 0.063–0.182 μM and 0.081–0.238 μM, respectively; while glycosidation at 3-OH significantly decreased the cytotoxicity. It was also found that the most potent compound **7** induced apoptosis in MCF-7 cells via the intrinsic mitochondrial apoptotic pathway.

1. Introduction

Brucea javanica (L.) Merr. (Simaroubaceae family), an evergreen shrub, is widely distributed from southeast Asia to northern Australia. The fruit of this herb ("Ya-Dan-Zi" in Chinese) was used as a traditional Chinese medicine since the Ming Dynasty (1364–1644 AD) [1], and is currently recorded in the Pharmacopoeia of the People's Republic of China (2010 edition) for removing fever, treatment of malaria and amebic dysentery [2]. Phytochemical studies revealed that *B. javanica* is a rich source of quassinoids such as brusatol, bruceines and bruceosides [3,4], and oil-like lipids such as oleic, linoleic, palmitic, and stearic acids [5].

The oil-like lipid part of the fruit has been developed into several well-known pharmaceutical products, e.g. *B. javanica* oil soft capsule, oral *B. javanica* oil emulsion, and *B. javanica* oil injection as single or adjuvant treatments for proliferative diseases such as cancer [6].

The quassinoids from *B. javanica* were found to have potent antiviral activities against tobacco mosaic virus with IC_{50} values in the range of 3.42–5.66 μM [7]. Both bruceine A and D exhibited significant inhibitory activity against the *Dactylogyrus intermedius* in goldfish with EC_{50} values of 0.49 mg/L and 0.57 mg/L, respectively, which were more effective than the positive control mebendazole [8]. Bruceine E and D were reported to decrease the blood glucose concentration comparable to glibenclamide [9]. Besides the above biological activities reported, the most significant role of this category of compounds is for the prevention or treatment of cancer. Quassinoids were found to show cytotoxic effect against a variety of cancer cells [10,11]. Especially, chrysoeriol could selectively kill the leukemic cells and potentiate the amplification of ROS levels [12]. Brusatol could activate NF-κB and promote HL-60 cell differentiation [13]. Furthermore, brusatol could combat chemoresistance and enhance the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism [14].

Due to its pronounced clinical effect, *B. javanica* is widely cultivated in Guangxi and Hainan provinces of China; however, the fruits of *B. javanica* are used only for extraction of oil and the non-oil part containing the quassinoids is discarded.

To promote the utilization of the non-oil part, a systematic phytochemical study was performed. In this paper, we report the isolation and structural elucidation of one new quassinoid bruceene A (**1**), along with seventeen known quassinoids (**2**–**18**) (Fig. 1) and their inhibitory activities against the breast cancer cells MCF-7 and MDA-MB-231. In addition, the chemotaxonomic significance was discussed.

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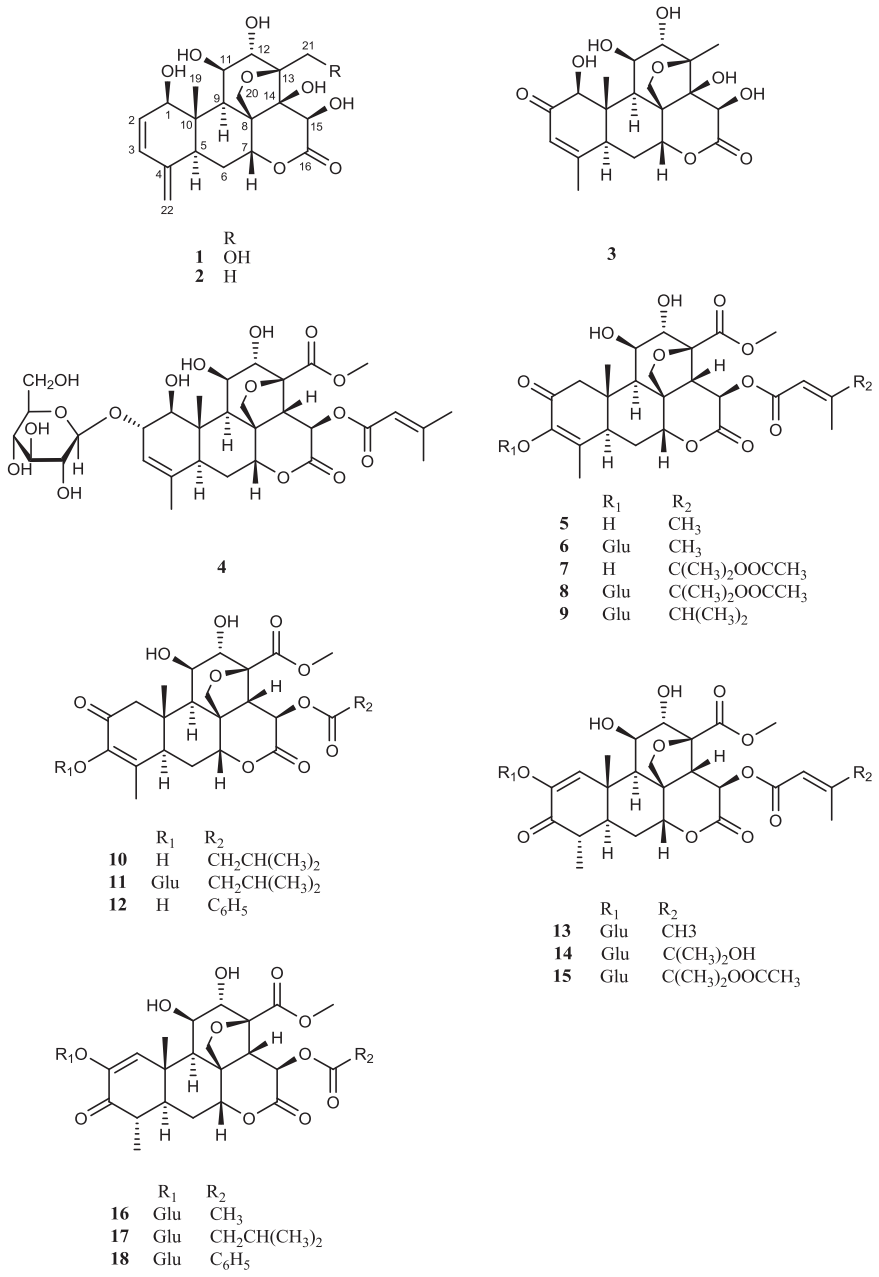

Fig. 1. Structural formulae of compounds **1**–**18**.

2. Experimental

2.1. General experimental procedures

Ultraviolet (UV) spectra were determined in $CHCl_3$ on a Jasco V-550 UV/vis spectrophotometer. ESI-MS spectra were carried out on a Finnigan LCQ Advantage Max ion trap mass spectrometer. HR-ESI-MS data were obtained on an Agilent 6210 ESI/TOF mass spectrometer. Optical rotation was recorded in $CHCl_3$ on Jasco P-1020 polarimeter at room temperature. Infrared (IR) spectra were measured on a Jasco FT/IR-480 plus Fourier Transform infrared spectrometer using KBr pellet. Nuclear magnetic resonance (NMR) spectra were measured on Bruker AV-300/400 spectrometers. Thin-layer chromatography (TLC) analyses were carried out using pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Plant, Qingdao, People's Republic of China).

2.2. Plant material

The fruits of *B. javanica* were bought from the Guangzhou Qingping herb medicine market in October of 2010, and were identified by Prof. Guang-Xiong Zhou (Jinan University). A voucher specimen (No. YDZ20101011) was deposited in the institute of Traditional Chinese Medicine and Natural Products, Jinan University, PR China.

2.3. Extraction and isolation

Dried powdered fruit of *B. javanica* (11 kg) was percolated with 95% EtOH (20 L × 3) at room temperature to afford 800 g of crude extract, which was suspended with water and then extracted with petroleum ether (500 mL × 3), chloroform (500 mL × 3), and *n*-BuOH