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Cytotoxic chalcones and antioxidants from the fruits of *Syzygium samarangense* (Wax Jambu)

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Abstract

Bioassay-guided fractionation of the methanolic extracts of the pulp and seeds of the fruits of *Syzygium samarangense* (Blume) Merr. and L.M. Perry led to the identification of four cytotoxic compounds and eight antioxidants on the basis of HPLC-PDA analysis, MS, and various NMR spectroscopic techniques. Three *C*-methylated chalcones, 2′,4′-dihydroxy-3′,5′-dimethyl-6′-methoxychalcone (**1**), 2′,4′-dihydroxy-3′-methyl-6′-methoxychalcone (stercurensin, **2**), and 2′,4′-dihydroxy-6′-methoxychalcone (cardamonin, **3**), were isolated and displayed cytotoxic activity ($IC_{50} = 10$, 35, and 35 μM, respectively) against the SW-480 human colon cancer cell line. Also a number of known antioxidants were obtained including six quercetin glycosides: reynoutrin (**4**), hyperin (**5**), myricitrin (**6**), quercitrin (**7**), quercetin (**9**), and guaijaverin (**10**), one flavanone: (*S*)-pinocembrin (**8**), and two phenolic acids: gallic acid (**11**) and ellagic acid (**12**).

Keywords: *Syzygium samarangense*; Chalcones; Flavonoids; Cytotoxic activity; Antioxidants

1. Introduction

Syzygium samarangense (Bloom) Merr. and L.M. Perry (Myrtaceae), known commonly as wax jambu, is an evergreen tree with origins in Asia. It produces a pink fleshy fruit which is eaten fresh. Many cultivars have been developed and they are grown throughout the tropical and subtropical parts of the world. The fruit is oblong, pear-shaped, and 5–12 cm in length, with four fleshy calyx lobes and 1–4 seeds (1–2 cm in diameter). The tree can be grown as an ornamental, and attains a height of seven meters. Wax jambu belongs to the same genus as *Syzygium aromaticum*, the source of cloves, a common spice.

In Malaysia, the green fruits of wax jambu are eaten raw with salt or cooked as a sauce. The flowers, which contain tannins, desmethoxymatteucinol, 5-*O*-methyl-4′-desmethoxymatteucinol, oleanic acid, and β-sitosterol, are used in Taiwan to treat fever and halt diarrhea (Morton, 1987).

Previous phytochemical studies of the leaves of *S. samarangense* have shown the presence of ellagitannins (Nonaka, Aiko, Aritake, & Nishioka, 1992), flavanones (Kuo, Yang, & Lin, 2004), flavonol glycosides (Kuo et al., 2004; Nair, Krishnan, Ravikrishna, & Madhusudanan, 1999), proanthocyanidins (Nonaka et al., 1992), anthocyanidins (Kuo et al., 2004; Nonaka et al., 1992),

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triterpenoids (Srivastava, Shaw, & Kulshreshtha, 1995), chalcones (Resurreccion-Magno, Villasenor, Harada, & Monde, 2005; Srivastava et al., 1995), and volatile terpenoids (Wong & Lai, 1996).

Ethanolic leaf extract exhibited immunostimulant activity (Srivastava et al., 1995), the hexane extract was found to relax the hypermotility of the gut (Ghayur et al., 2006), while the alcoholic extract of the stem bark showed antibacterial activity (Chattopadhayay, Sinha, & Vaid, 1998).

The immunomodulatory (Kuo et al., 2004), antihyperglycaemic (Resurreccion-Magno et al., 2005), spasmolitic (Amor, Villasenor, Ghayur, Gilani, & Choudhary, 2005), and prolyl endopeptidase inhibitor effects of chalcones **1** and **2** and the flavanone 5-*O*-methyl-4-desmethoxymatteucinol isolated from the leaves have also been reported (Amor, Villasenor, Yasin, & Choudhary, 2004).

Chalcones are a group of plant-derived polyphenolic compounds that are intermediates in the biosynthesis of flavonoids and are associated with several biological activities, including antiviral (Kiat et al., 2006), antifungal (Svetaz et al., 2004) anti-inflammatory (Lespagnol et al., 1972) and antioxidant (Han, Kang, Windono, Lee, & Seo, 2006). They also displayed anticancer and cytotoxic activity (Go, Wu, & Liu, 2005).

As part of a programme to find novel antioxidant and cytotoxic compounds from plants, (Yang et al., 2005), we have investigated the anticancer activity and antioxidant properties of wax jambu, and we report here the bioassay-guided identification of polyphenolic constituents and cytotoxic chalcones from the pulp and seeds of the fruits of *S. samarangense*.

2. Materials and methods

2.1. General methods

Optical rotation was measured on an Autopol III Automatic Polarimeter (Rudolph Research Analytical, Newburgh Hackttstown, NJ, USA); ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Avance 300, operating at 300 and 75 MHz, respectively. Spectra were obtained in CD_3OD or $CDCl_3$, with chemical shifts expressed in δ and coupling constant (J) in Hertz (Hz). Electrospray ionization mass spectrometry (ESI-MS) was performed with a ThermoFinnigan LCQ instrument (San Jose, CA, USA), equipped with Xcalibur software. Samples were dissolved in HPLC grade MeOH and introduced by direct injection. Nitrogen was used as both an auxiliary and sheath gas, the capillary voltage was 10 V, the spray voltage was 4.5 kV, the capillary temperature was 230 °C and the tube lens offset was 0 V. HPLC analyses were carried out on a Waters 2695 Separations Module (Milford, MA, USA) equipped with a model 996 photodiode array detector and Empower software, using a 250 × 4.6 mm i.d., 5 μm, Aqua C-18 column (Phenomenex, Torrance, CA, USA). Preparative HPLC was carried out using a Waters 600 controller with a Waters 486 tunable absorbance detector and Waters Empower software with a 250 × 21.20 mm i.d., 10 μm Luna C-18 column (Phenomenex Torrance, CA, USA) and the mobile phase consisted of 10% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 20 ml/min, with a linear gradient consisting of 40% A–30% A in 20 min run time and the column at room temperature.

A Molecular Devices (Sunnyvale, CA, USA) Versamax tunable absorbance detector was used for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antiradical assay, total flavonoid content (TFC), and total phenolic content measurements (TPC).

TLC analyses were performed on Si gel 60 F_{254} (1 mm layer thickness, EM Science, Darmstadt, Germany) and RP-18 F_{254} plates (1 mm layer thickness, EM Science, Darmstadt, Germany), with compounds visualized by spraying with a vanillin solution (1.0 g of vanillin in 10 ml of concentrated H_2SO_4 and 90 ml of EtOH) and heating at 50 °C. Sephadex LH-20 (25–100 μm) (Pharmacia Fine Chemicals, Piscataway, NJ, USA), silica gel (230–400 mesh) (EM Science, Darmstadt, Germany), Diaion HP-20 (HP-20) (Supelco, Bellefonte, PA, USA), and C-18 reversed-phase Si gel (40 μm) (J. T. Baker, Phillipsburg, NJ, USA) were used for column chromatography. Solvents for chromatography, HPLC-grade acetonitrile, MeOH, formic acid, and HPLC-grade water, were obtained from J.T. Baker (Phillipsburg, NJ, USA), and GR-grade MeOH, EtOAc and acetone from VWR Inc (Bridgeport, PA, USA). $AlCl_3$, DMSO, $FeCl_3$, anhydrous Na_2CO_3, $NaNO_2$, NaOAc, NaOH, trolox, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, 2,4,6-tri-pyridiyl-*s*-triazine, and the Folin–Ciocalteu phenol reagent were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ellagic acid, gallic acid, myricitrin, quercetin, and quercitrin were also obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Plant material

Fruits of *S. samarangense* were collected from the Fruit and Spice Park (Homestead, FL, USA) in June 2001. Fresh fruits were shipped to New York City by overnight courier and stored at −20 °C until extracted. A voucher specimen (Reynertson 17) was prepared, identified, and deposited at the Steere Herbarium of The New York Botanical Garden (Bronx, NY).

2.3. Extraction

The fresh frozen pulp (3.2 kg) of the fruits of *S. samarangense* were extracted twice with MeOH (5 l) at room temperature for 1 h per extraction. After the MeOH was removed *in vacuo*, the resulting dark extract (27.0 g) was suspended in water and sequentially partitioned with hexane (1 l, ×3), EtOAc (1 l, ×3), and *n*-BuOH (1 l, ×3), respectively. The EtOAc and *n*-BuOH partitions were concentrated *in vacuo* to give 3.6 g and 18.0 g of dark brown extracts, respectively.