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Research Article

Chemical Constituents of *Garcinia mangostana* Pulp and Seeds

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ABSTRACT
Chemical investigation of the dichloromethane extracts of the freeze-dried *Garcinia mangostana* Linn. led to the isolation of δ-tocotrienol (**1**), α-mangostin (**2**), 3-isomangostin (**3**), stigmasterol (**4**), triacylglycerols (**5**), a mixture of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6a**) and stigmasteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6b**) in about 3:2 ratio, from the pulp; and **4**, **5**, and linoleic acid (**7**) from the seeds. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **2**-**7** were identified by comparison of their NMR data with literature data.

Keywords: *Garcinia mangostana* Linn., δ-tocotrienol, α-mangostin, 3-isomangostin, β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters, stigmasteryl-3β-glucopyranoside-6′-*O*-fatty acid esters, stigmasterol, β-sitosterol, triacylglycerols, linoleic acid.

INTRODUCTION

Garcinia mangostana Linn., commonly known as mangosteen, is a natural source of xanthones which are antioxidants, anti-inflammatory[1,2], antifungal[3], and are also used for chemoprevention[4-6]. Extracts and xanthones isolated from *G. mangostana* have antioxidant, antitumor, anti-allergic, anti-inflammatory, antibacterial, antifungal, and antiviral properties[7]. The major constituent and most studied bioactive xanthone from *G. mangostana* is α-mangostin which at 10 µM showed complete inhibition of human leukemia cell line HL60 through the induction of apoptosis[5], while at 20 µM caused a cytotoxic effect as indicated by morphological findings[8]. α-Mangostin also showed significant activity against CEM-SS cell line with IC_{50} of 5.5 µg/mL[9] and exhibited the most potent effects against breast cancer (BC-1) cells and epidernoid carcinoma of the mouth (KB) with IC_{50} of 0.92 µg/mL and 2.08 µg/mL, respectively. α-Mangostin also preserves the myocardial membrane integrity and extenuates anomalous TNF-alpha and COX-2 expressions by mitigating ISO-induced oxidative stress and cellular damage effectively. Restoration of cellular normalcy is attributed to the cytoprotective role of α-mangostin[10]. Furthermore, it exhibited protective effect on lipid peroxidation and antioxidant tissue defense system during ISO-induced myocardial infarction in rats[10]. A strong inhibitory effect against Mycobacterium tuberculosis with MIC = 6.25 µg/mL was also exhibited by α- and β-mangostin and garcinone B[11]. α-Mangostin gave a minimum *S. aureus* inhibitory concentration of 1.57–12.5 µg/mL[12] and was found to be active against enterococci (VRE) and methicillin resistant S. aureus (MRSA) with MIC values of 6.25 and 6.25 to 12.5 µg/mL, respectively[13]. An earlier study reported that mangostin gave an MIC in the range of 12.5–50 µg/mL for bacteria and 1–5 µg/mL for fungi[14]. The mature rind extracts contained higher quantities of flavonoids and α-mangostin and exhibited higher activity against acne-producing bacteria than the young fruit rind[15]. The MIC of mangostin, 3-isomangostin, and gartanin against a normal strain of *S. aureus* are 15.6, 125, and 250 µg/mL, respectively. When these compounds were tested against 41 samples of penicillin-resistant strains of *S. aureus*, mangostin and 3-isomangostin gave MIC values of 1.56–12.5 µg/mL and 250 µg/mL, respectively[16]. We earlier reported the isolation of α-mangostin, gartanin and 3-isomangostin from the pericarp of *G. mangostin*[17]. We report herein the isolation of δ-tocotrienol (**1**), α-mangostin (**2**), 3-isomangostin (**3**), stigmasterol (**4**), triacylglycerols (**5**), a misxture of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6a**) and stigmasteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6b**) in about 3:2 ratio, from the pulp; and **4**, **5**, and linoleic acid (**7**) from the seeds. The structures of **1**-**3** and **6a** are presented in Fig. 1.

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was

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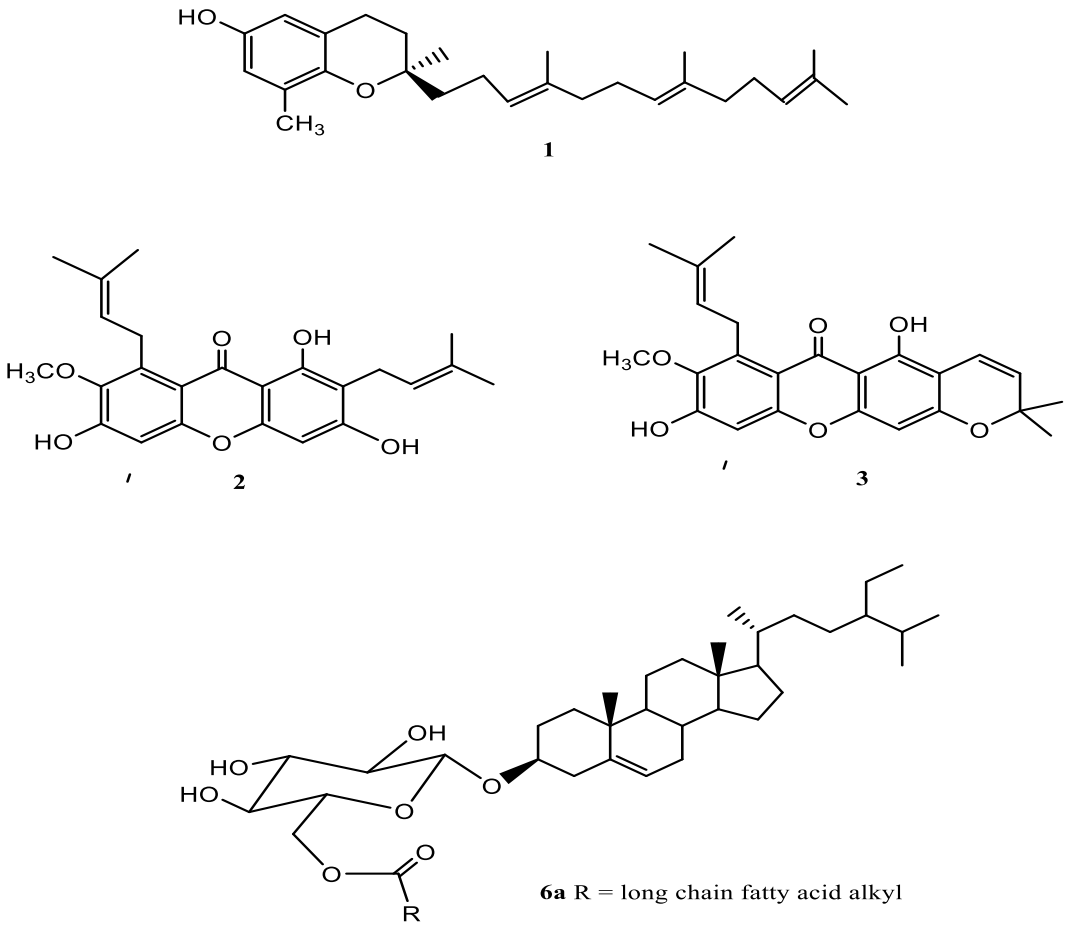

Figure 1: Chemical structures of δ-tocotrienol (**1**), α-mangostin (**2**), 3-isomangostin (**3**), and β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6a**) from *G. mangostana* pulp.

performed, with silica gel 60 (70-230 mesh). Thin layer chromatography, was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

Sample Collection

Garcinia mangostana Linn. fruit was collected from Davao, Philippines in October 2015. The fruit was authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

The freeze-dried pulp (143.3 g), and seeds (54.4 g) of *G. mangostana* were ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford crude extracts of pulp (1.96 g), and seeds (20.50 g) which were each chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone at 10% increment by volume as eluents. A glass column 18 inches in height and 1inch internal diameter was used for the fractionation of the crude extracts. Eleven 20 mL fractions were collected. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography of fractions from the crude extracts. 2 mL fractions were collected. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Isolation of Chemical Constituents of the Pulp

The CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford **5** (6 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **4** (3 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether to yield **3** (7 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (5 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **2** (12 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to afford **1** (15 mg) after washing with petroleum ether. The 80% and 90% acetone in CH_2Cl_2 fractions were combined and rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to afford a mixture of **6a** and **6b** (5 mg) after washing with petroleum ether.

Isolation of Chemical Constituents of the Seeds

The 20% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (5 ×) using 5% EtOAc in petroleum ether to afford **5** (45 mg). The 40% acetone in CH_2Cl_2

fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **4** (18 mg) after washing with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to yield **7** (5 mg).

δ-Tocotrienol (**1**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.24 (s, 2-Me), 1.78, 1.72 (H_2-3), 2.70 (m, H_2-4), 6.37 (d, *J* = 3.0 Hz, H-5), 4.46 (br s, 6-OH), 6.46 (d, *J* = 3 Hz, H-7), 2.11 (s, 8-Me), 1.55, 1.64 (H-1'), 2.08 (H_2-2'), 5.12 (t, *J* = 6.6 Hz, H-3'), 1.57 (s, 4'-Me), 1.96 (H_2-5'), 2.08 (H_2-6'), 5.13 (t, *J* = 6.6 Hz, H-7), 1.58 (s, 8'-Me), 1.96 (H_2-9'), 2.08 (H_2-10'), 5.12 (d, *J* = 6.6 Hz, H-11'), 1.58 (s, 12'-Me), 1.67 (s, 12'-Me),; ^{13}C NMR (150 MHz, $CDCl_3$): δ 75.3 (C-2), 24.0 (2-CH_3), 31.3 (C-3), 22.5 (C-4), 112.6 (C-5), 147.7 (C-6), 115.6 (C-7), 127.3 (C-8), 16.0 (8-CH_3), 121.2 (C-9), 145.9 (C-10), 39.7 (C-1'), 22.1 (C-2'), 124.4 C-3'), 135.1 (C-4'), 16.0 (4'-CH_3), 39.7 (C-5'), 26.7 (C-6'), 124.2 (C-7'), 134.9 (C-8'), 15.9 (8'-CH_3), 39.7 (C-9'), 26.6 (C-10'), 124.2 (C-11'), 131.2 (C-12'), 17.7 (12'-CH_3), 25.7 (12'-CH_3).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *G. mangostana* yielded **1** – **7**. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy. The NMR spectra of **1** are in accordance with data reported in the literature for δ-tocotrienol[18]; **2** for α-mangostin[17]; **3** for 3-isomangostin[17]; **4** for stigmasterol[19]; **5** for triacylglycerols[20]; **6a** for β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters[21], **6b** for stigmasteryl-3β-glucopyranoside-6′-*O*-fatty acid esters[22]; and **7** for linoleic acid[23]. The mixture of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6a**) and stigmasteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**6b**) in about 3:2 ratio was deduced from the intensities and integrations of the methyl protons at δ 0.66 (s) for β-sitosterol from **6a** and δ 0.68 (s) for stigmasterol from **6b**; and the olefinic protons for the stigmasterol from **6b** at δ 5.34, 5.00 and 5.12 and the olefinic proton of the β-sitosterol from **6a** at δ 5.34 . Literature search revealed that δ-tocotrienol (**1**) exhibited anti-cancer, anti-diabetic, anti-inflammatory, antioxidant, immune-stimulatory, neuroprotective, hepatoprotective and nephroprotective[24]. A review on the pharmacological potential of tocotrienols has been provided[24].

CONCLUSION

The dichloromethane extracts of the pulp and seeds of *G. mangostana* afforded stigmasterol (**4**) and triacylglycerols (**5**), while the pulp yielded a vitamin E, δ-tocotrienol (**1**) and two xanthones, α-mangostin (**2**) and 3-isomangostin (**3**) with reported diverse biological activities.

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