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Research Article

Chemical Constituents of *Flacourtia rukam* Zoli. & Moritzi Fruit

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ABSTRACT
Chemical investigation of the dichloromethane extract of *Flacourtia rukam* Zoli. & Moritzi (*Syn. Flacourtia euphlebia* Merr.) led to the isolation of monogalactosyl diacylglycerols (**1**), β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**2**), β-sitosterol (**3**) and triacylglycerols (**4**) from the pulp; **3** and chlrorophyll a (**5**) from the fruit peel; and **4** from the seeds. The structures of **1**-**5** were identified by comparison of their NMR data with literature data.

Keywords: *Flacourtia rukam* Zoli. & Moritzi, *Flacourtia euphlebia* Merr., Flacourtiaceae, monogalactosyl diacylglycerols, β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters, β-sitosterol, triacylglycerols, chlorophyll a

INTRODUCTION

Flacourtia rukam Zoll. and Mor. (*Syn. Flacourtia euphlebia* Merr.)[1], locally known as bitongol, is found in forest at low and medium altitude. The fruit of the cultivated *F. rukam* is edible and is used for making pies and jams, while the wild tree has sour fruit. The wood is used in the rural areas for house construction[2]. The juice of the leaves is applied to inflamed eye-lids. The immature fruit is employed as medicine against diarrhoea and dysentery. A decoction of the roots is taken by women after childbirth[1].

We report herein the isolation of monogalactosyl diacylglycerols (**1**), β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**2**), β-sitosterol (**3**), triacylglycerols (**4**), and chlorophyll a (**5**) from *F. rukam*. The chemical structures of **1**-**5** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **1**-**5** from *F. rukam*.

MATERIALS AND METHODS

General Experimental Procedure

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). Column chromatography was 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

The sample was collected from the Salikneta farm, San Jose Del Monte, Philippines in 2015. It was authenticated as *Flacourtia rukam* Zoli. & Moritzi at the Botany Division of the Philippine National Herbarium, National Museum, Philippines.

General Isolation Procedure

The crude extract was fractionated by silica gel chromatography using increasing proportions of EtOAc in petroleum ether as eluents. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained.

Isolation of the chemical constituents of the Pulp of F. rukam

The freeze-dried pulp of *F. rukam* (77.7 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.55 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The acetone fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to afford **4** (7 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield **3** (2 mg) after washing with petroleum ether. The 30% to 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford **2** (3 mg) after washing with petroleum ether. The 70% to 80% acetone in CH_2Cl_2 fractions were combined and rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2:2:6, v/v) to yield **1** (3 mg) after trituration with petroleum ether.

Isolation of the chemical constituents of the Peel of F. rukam

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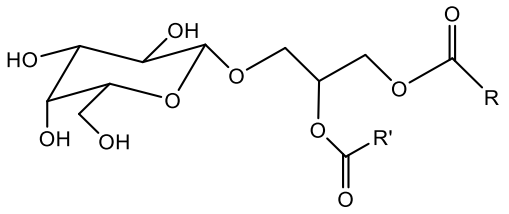
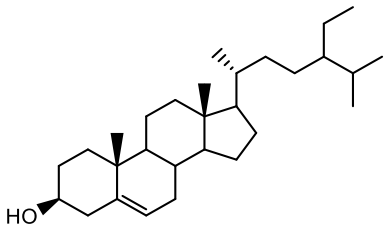


1 R, R' = long chain fatty acid alkyls



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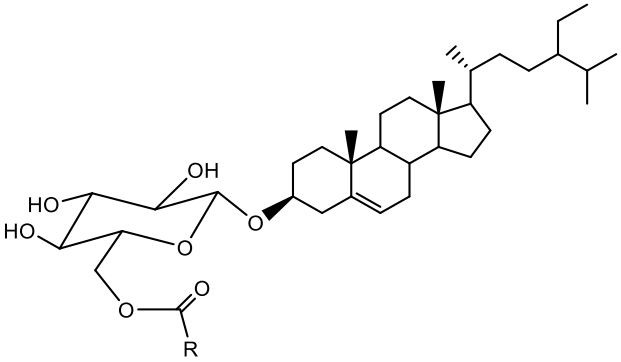
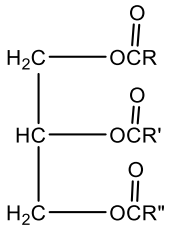


2 R = long chain fatty acid



4 R, R', R" = long chain fatty acid alkyls

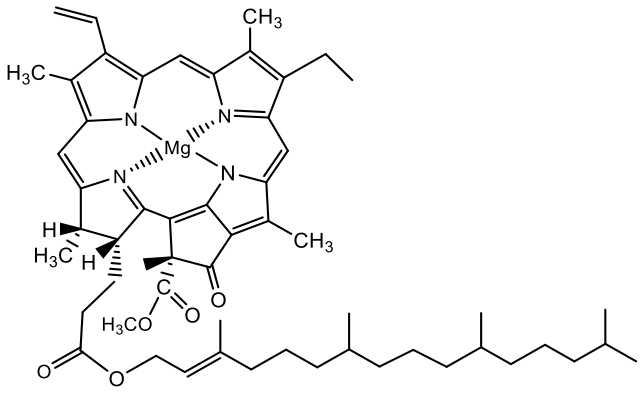


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Figure 1: Chemical structures of monogalactosyl diacylglycerols (**1**), β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**2**), β-sitosterol (**3**), triacylglycerols (**4**) and chlorophyll a (**5**) from *F. rukam*.

The freeze-dried peel of *F. rukam* (47.7g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (0.17 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to yield **3** (4 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **5** (7 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the chemical constituents of the Seeds of F. rukam

The freeze-dried seeds of *F. rukam* (57.7 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (3.96 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether to yield **4** (12 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the different parts of *F. rukam* yielded **1**–**5**. The NMR spectra of **1** are in accordance with data reported in the

literature for monogalactosyl diacylglycerols[3]; **2** for β-sitosteryl-3β-glucopyranoside-6′-O-fatty acid esters[4]; **3** for *β*-sitosterol[5]; **4** for triacylglycerols[5]; and **5** for chlorophyll a[6]. Literature search revealed that the compounds (**1-5**) isolated from *F. rukam* exhibited diverse biological activities. Monogalactosyl diacylglycerols (**1**) and dinogalactosyl diacylglycerols are the most widespread non-phosphorous polar lipids in nature, constituting about 80% of membrane lipids in plants and more than half of all lipids in algae[7,8]. These compounds were reported to exhibit a number of biological properties, such as anti-tumor[9,10], anti-viral[11], algicidal[12] and anti-inflammatory[13-16]. Monogalactosyl diacylglycerols were also found to show cytotoxic and anti-inflammatory activity in RAW 264.7 macrophage cells with IC_{50} values of 60.06 and 65.70 μg/mL, respectively[17]. Compound **1** was also reported to exhibit anti-inflammatory activity in human articular cartilage[14]. It inhibited the growth of human melanoma cells in a dose-dependent manner with an IC_{50} value of 114 μM[18].

β-Sitosteryl-3α-glucopyranoside-6'-*O*-palmitate (**2**) was reported to exhibit cytotoxicity against Bowes (melanoma) and MCF7 (breast) cancer cell lines with IC_{50} values of 152 μM and 113 μM, respectively[19]. Furthermore, **1** exhibited cytotoxicity against human stomach adenocarcinoma (AGS) cell line with 60.28% growth inhibition[20]. Compound **1** was found to exhibit potent anti-complement activity ($IC_{50} = 1.0 \pm 0.1$ μM) as compared to the positive control, tiliroside ($IC_{50} = 76.5 \pm 1.1$ μM)[21].

β-Sitosterol (**3**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells[22]. It was shown to be effective for the treatment of benign prostatic hyperplasia[23]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis[24]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake[25]. It has also been reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells[26].

Triacylglycerols (**2**) was reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma[27]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*[28]. Another study reported that triacylglycerols showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation[29].

Chlorophyll (**5**) and its various derivatives are used in traditional medicine and for therapeutic purposes[30]. Natural chlorophyll and its derivatives have been studied for wound healing[31], anti-inflammatory properties[32], control of calcium oxalate crystals[33], utilization as effective agents in photodynamic cancer therapy[34-36], and chemopreventive effects in humans[37,38]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided[39].

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