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Chemical Constituents of *Coix lacryma-jobi.*

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Coix lacryma-jobi* afforded triglyceride (**1**) and β-sitosterol (**2**) from the grains; **1** and a mixture of **2** and stigmasterol (**3**) in a 4:3 ratio from the stems; and **1** and phytyl fatty acid ester (**4**) from the leaves. Their structures were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Keywords: *Coix lacryma-jobi,* Poaceae, triglyceride, β-sitosterol, stigmasterol, phytyl fatty acid ester

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INTRODUCTION

Coix lacryma-jobi, also known as jobs tears and adlay, belongs to the grass family Poaceae. Adlay is commonly found throughout the Philippines and is consumed as a nutritional food. A recent study identified six antimutagenic constituents from adlay hull, with trans-coniferylaldehyde exhibiting the highest activity [1]. Flavonoids in adlay bran were reported to contribute to its anti-inflammatory effect [2]. The dehulled adlay exhibited antiulcer activity, and it was found that caffeic acid was one of the compounds responsible for its gastroprotective activity [3]. The potential active component of the ethyl acetate fraction of adlay bran ethanolic extract which retard carcinogenesis through an anti-inflammatory pathway was identified as ferulic acid [4]. Adlay exhibited high hypocholesterolemic and antioxidant activities due to a protective effect on cardiovascular health *in vivo* [5]. The dichloromethane extracts of *Coix lachryma* Linn. exhibited IC_{50} = 2.75 and 5.16 ppm against MCF"7 and KB cells, respectively [6]. The antitumor activity of adlay was attributed to palmitic, stearic, oleic and linoleic acids [7]. The water extract of adlay seed was found to exhibit anti-obesity effects through neuroendocrine modulation [8]. Adlay bran extract reduced the release of histamines and cytokines and suppressed the production of Akt which influenced the signal transduction in RBL-2H3 cells. These are the mechanisms of the anti-allergic effects of adlay [9]. The chloroform extract of the stems of adlay significantly reduced blood glucose level ($p<0.01$) as well as gluconeogenic enzyme activities such as G6Pase and F1,6BPase ($p<0.05$) on STZ-induced hyperglycemic mice [10]. Two compounds were isolated from the chloroform extract are β-sitosterol and stigmasterol which were reported to exhibit hypoglycemic properties [10-12]. Furthermore, adlay is capable of reversing the osteoporotic status in rats and may help in osteoporosis prevention [13]. Another study reported that adlay contains vitamin E, squalene, campesterol, stigmasterol, β-sitosterol, oleic acid and linoleic acid [14] which is of relevance to our present report.

We report herein the isolation and identification of chemical constituents of the dichloromethane extracts of *Coix lacryma-jobi*: triglyceride (**1**) and β-sitosterol (**2**) from the grains; **1** and a mixture of **2** and stigmasterol (**3**) in a 4:3 ratio from the stems; and **1** and phytyl fatty acid ester (**4**) from the leaves (Fig. 1).

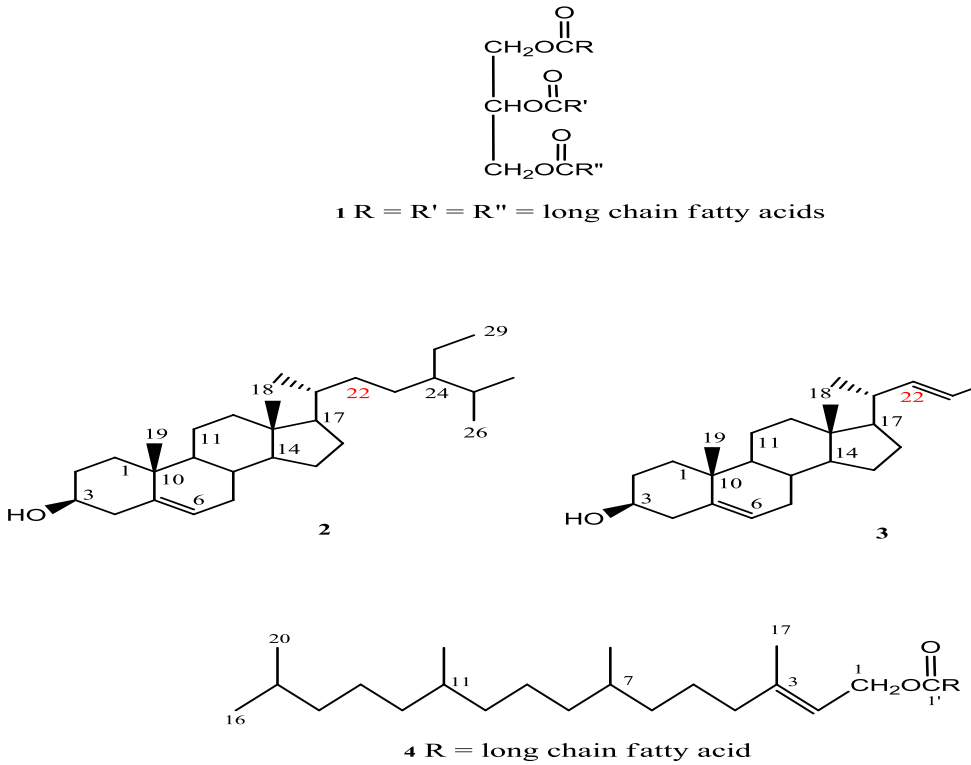

Figure 1: Chemical constituents of *Coix lacryma-jobi*: triglyceride (1), β-sitosterol (2), stigmasterol (3), and phytyl fatty acid ester (4).

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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 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 Benguet, Mountain Province, Philippines in September 2013. It was identified as *Coix lacryma-jobi* at the Bureau of Plant Industry, Manila, Philippines.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inches internal diameter was packed with silica gel. The crude extract from the twigs were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Fifty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation

The air-dried grains (24.74 g) of *Coix lacryma-jobi* was 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 (0.9 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to afford **1** (12 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford **2** (9 mg) after washing with petroleum ether.

The air-dried stems (167.7 g) of *Coix lacryma-jobi* was 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 (0.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to afford **1** (7 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford a mixture of **2** and **3** (5 mg) after washing with petroleum ether.

The air-dried leaves (49.85 g) of *Coix lacryma-jobi* was 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 (1.1 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (×) in 5% EtOAc in petroleum ether to afford **4** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 5% EtOAc in petroleum ether to afford **1** (10 mg).

Triglyceride (**1**): Triglyceride: ^1H NMR (600 MHz, $CDCl_3$): δ 4.27 (dd, 4.2, 12.0), 4.12 (dd, 6.0, 12.0, glyceryl CH_2O), 5.24 (glyceryl CHO), 2.29 (α-CH_2), 5.32 (olefinic H), 2.75 (double allylic CH_2), 2.00 (allylic, CH_2), 1.60 (β-CH_2), 1.23-1.35 (CH_2), 0.87 (t, 7.2, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl CH_2), 68.87 (glyceryl CH), 173.25 (C=O α), 172.82 (C=O β), 34.04 (C-2α), 34.18 (C-2β), 24.83 (C-3α), 24.86 (C-3β), 29.08 (C-4α), 29.04 (C-4β), 29.19 (C-5α), 29.26 (C-5β), 29.11 (C-6α), 29.16 (C-6β), 29.62 (C-7α), 29.65 (C-7β), 29.19 (both C-8), 130.00 (C-9α), 129.97 (C-9β), 128.05 (C-10α), 128.07 (C-10β), 25.62 (both C-11), 127.88 (C-12α), 127.87 (C-12β), 130.21 (both C-13), 27.19 (both C-14), 29.35 (both C-15), 31.52 (both C-16), 22.56 (both C-17), 14.06, 14.10 (both C-18).

β-Sitosterol (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.33 (dd, 1.8, 5.4, H-5), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.92 (d, 6.6, CH_3-21), 0.84 (d, 6.6, CH_3-26), 0.83 (d, 6.0, CH_3-27), 0.87 (t, 6.0, CH_3-29). ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.29 (C-4), 140.74 (C-5), 121.71 (C-6), 31.89 (C-7), 31.89 (C-8), 50.15 (C-9), 36.50 (C-10), 21.07 (C-11), 39.76 (C-12), 42.20 (C-13), 56.76 (C-14), 24.35 (C-15), 28.24 (C-16), 56.04 (C-17), 11.97 (C-18), 19.39 (C-19), 36.14 (C-20), 18.77 (C-21), 33.93 (C-22), 26.06 (C-23), 45.82 (C-24), 29.14 (C-25), 19.02 (C-26), 19.81 (C-27), 23.06 (C-28), 11.85 (C-29).

Stigmasterol (**3**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.33 (dd, 1.8, 5.4, H-6), 0.68 (s, CH_3-18), 0.99 (s, CH_3-19), 1.01 (d, 6.6, CH_3-21), 5.13 (dd, 8.4, 15.0, H-22), 5.00 (dd, 9.0, 15.0, H-23), 0.84 (d, 6.6, CH_3-26), 0.83 (d, 6.0, CH_3-27), 0.80 (t, 6.0, CH_3-29). ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.65 (C-2), 71.80 (C-3), 42.29 (C-4), 140.74 (C-5), 121.71 (C-6), 31.89 (C-7), 31.89 (C-8), 50.15 (C-9), 36.49 (C-10), 21.07 (C-11), 39.67 (C-12), 42.20 (C-13), 56.76 (C-14), 24.35 (C-15), 28.91 (C-16), 55.94 (C-17), 12.03 (C-18), 19.39 (C-19), 40.48 (C-20), 21.07 (C-21), 138.31 (C-22), 129.26 (C-23), 51.23 (C-24), 31.90 (C-25), 21.20 (C-26), 18.97 (C-27), 25.40 (C-28), 12.24 (C-29).

Phytyl fatty acid ester (**4**): δ 4.57 (d, 6.6, H_2-1), 5.34 (H-2), 2.03 (H_2-4), 0.87 (d, 6.6, CH_3-16), 1.67 (br s, CH_3-17), 0.85 (d, 6.6, CH3-18), 0.83 (d, 6.6, CH3-19), 0.87 (d, 6.6, CH_3-20), 2.27 (t, 7.8, H_2-2'), 1.60 (H_3-3'), 1.23-1.36 $(CH_2')_n$, 0.87 (t, 6.6, CH_3'-terminal); ^{13}C NMR (150 MHz, $CDCl_3$): δ 61.14 (C-1), 117.95 (C-2), 142.50 (C-3), 39.85 (C-4), 25.04 (C-5), 36.63 (C-6), 32.70 (C-7), 37.40 (C-8), 24.46 (C-9), 37.44 (C-10), 32.80 (C-11), 37.29 (C-12), 24.79 (C-13), 39.36 (C-14), 27.97 (C-15), 22.71 (C-16), 16.36 (C-17), 19.74 (C-18), 19.74 (C-19), 22.62 (C-20), 174.03 (C-1'), 34.42 (C-2'), 25.04 (C-3'), 29.53 (C-4'), 29.15-29.70 $(CH_2')_n$, 31.92 (CH_2'), 29.53 (CH_2'), 14.11 (CH_3'-terminal).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the air-dried grains of *Coix lacryma-jobi* afforded triglyceride (**1**) [15] and β-sitosterol (**2**) [15] from the grains; **1** and a mixture of **2** and stigmasterol (**3**) [15] in a 4:3 ratio from the stems; and **1** and phytyl fatty acid ester (**4**) [16] from the leaves. Their structures were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [15-16]. It is interesting to note that the triglycerides present in the grains, stems, and leaves have similar ratios of monounsaturated and double unsaturated fatty acids (about 1:1 ratio) as deduced from the integrations of the olefinic protons, double allylic methylene protons and allylic methylene protons at δ 5.32, 2.75, and 2.00, respectively. Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on the biological activities of **1**-**3**.

A study reported that antimicrobial tests on triglyceride (**1**) indicated that they exhibited antimicrobial activity against *S. aureus, P. aeruginosa, B. subtilis, C. albicans* and *T. mentagrophytes* [15]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [17].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [18]. It was shown to be effective for the treatment of benign prostatic hyperplasia [19]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [20]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [21]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [22]. The antihyperglycemic and insulin-releasing effects of β-sitosterol 3-beta-D-glucoside and β-sitosterol has also been reported [11].

Stigmasterol (**3**) lowered plasma cholesterol levels, inhibited intestinal cholesterol and plant sterol absorption, and suppressed hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [23]. It showed therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [24]. The thyroid inhibitory, antiperoxydative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma* has also been reported [12].

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