Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2015; 7(5): 356-359

ISSN- 0975 1556

Research Article

Secondary Metabolites from *Cycas lacrimans*

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Available Online: 31st August, 2015

ABSTRACT

Chemical investigation of the sarcotesta of *Cycas lacrimans*, a plant endemic to the Philippines afforded β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), a mixture of alkyl 4-hydroxy-*trans*-cinnamate esters (**2a**) and alkyl 4-hydroxy-*cis*-cinnamate esters (**2b**), β-sitosterol (**3**), unsaturated triacylglycerols (**4**), and methyl fatty acid esters (**5**). The structures of **1**-**5** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Cycas lacrimans,* Cycadaceae, β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, alkyl 4-hydroxy-*trans*-cinnamate esters, alkyl 4-hydroxy-*cis*-cinnamate esters, β-sitosterol, triacylglycerols, methyl fatty acid esters

INTRODUCTION

Cycas, the only currently known genus of the Family Cycadaceae, are considered as fossil plants though they may have evolved only about 12 million years ago[1]. Their long existence and persistence through time have sparked special interest in their biology and evolution. The cycads resemble palms in morphology thus are commonly called sago palm. They bear naked seeds and are dioecious (male and female as separate individuals). These are widely distributed in the Tropics, with species found in Asia, Africa, Southeast Asia, Pacific, and Australia[2]. They also grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats[3].

In the Philippines, there are eleven (11) cycad species namely, *C. aenigma* K. D. Hill & Lindstrom, *C. curranii* (J. Schust.) K. D. Hill, *C.edentata* de Laub., *C. lacrimans* Lindstrom & K. D. Hill, *C. nitida* K. D. Hill & Lindstrom, *C. riuminiana* Porte ex Regel, *C. saxatilis* K. D. Hill & Lindstrom, *C. sancti-lasallei* Agoo & Madulid, *C. wadei* Merr., *C. vespertilio* Lindstrom & K. D. Hill, and *C. zambalensis* Madulid & Agoo[3-5]. All species, except for *C. edentata*, are endemic to the country[4]. *C. revoluta*, a widely cultivated species, is an introduced species from Japan and Taiwan.

The demand of *Cycas* species for domestic and international horticultural trade, grassland and forest fires of anthropogenic origin, and conversion of their natural habitats to settlements and other land uses have threatened to varying degrees the wild populations of the genus[6]. Some of these species which have been assessed and evaluated in the 2010 IUCN Red List of Threatened Species are *C. curranii*[7], *C. wadei*[8] and *C. zambalensis* as Critically Endangered (CR)[7], *C. riuminiana* as Endangered (E)[7], and *C. saxatilis*as Vulnerable (V)[9].

This study is part of our research on the chemical constituents of *Cycas* species that are endemic to the Philippines. We earlier reported the isolation of isopimaran-19-ol (**I**) from the megasporophyll lamina; 9αH-isopimara-7,15-diene (**II**) and triacylglycerols (**III**) from the bark; **III**, oleic acid (**IV**), and 1,2-dioleylglycerol (**V**) from the leaflets; **III**, β-sitosterol (**VI**), and stigmasterol (**VII**) from the petiole and rachis; **VI** from the roots; and **III** and **VI** from the endotesta and sclerotesta of *C. lacrimans*[10]. In another study, we reported the isolation of **III**, **VI**, **VII**, and squalene (**VIII**) from the sarcotesta; **III**, **VI**, **VII**, and phytyl fatty acid esters (**IX**) from the endotesta; **III**, **VI**, **VII**, and β-sitosteryl fatty acid esters (**X**) from the sclerotesta; and **III** and **X** from the bark of *C. sancti-lasallei*[11]. Another *Cycas* species, *C. vespertilio* yielded **III**, a mixture of **VI** and **VII**, pinoresinol (**XI**), sesamin (**XII**), and paulownin (**XIII**) from the cone base; **III**, **VI**, **VII**, **XI**, **XIII**, and lariciresinol (**XIV**) from the cataphylls; **VI** from the megasporophyll lamina; **VI** and a mixture of *trans*-4-hydroxycinnamate fatty acid esters (**XV**) and *cis*-4-hydroxycinnamate fatty acid esters (**XVI**) from the unripe sarcotesta; and **III** and **VI** from the ripe sarcotesta[12]. We recently reported the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**XVII**) from the sarcotesta; **X**, unsaturated fatty acid methyl esters (**XVIII**), **VI** and **VII** from the endotesta; chlorophyll a (**XIX**) from the leaflets; and triacylglycerols (**III**) from the male cone of *C. edentata*[13].

We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), a mixture of alkyl 4-

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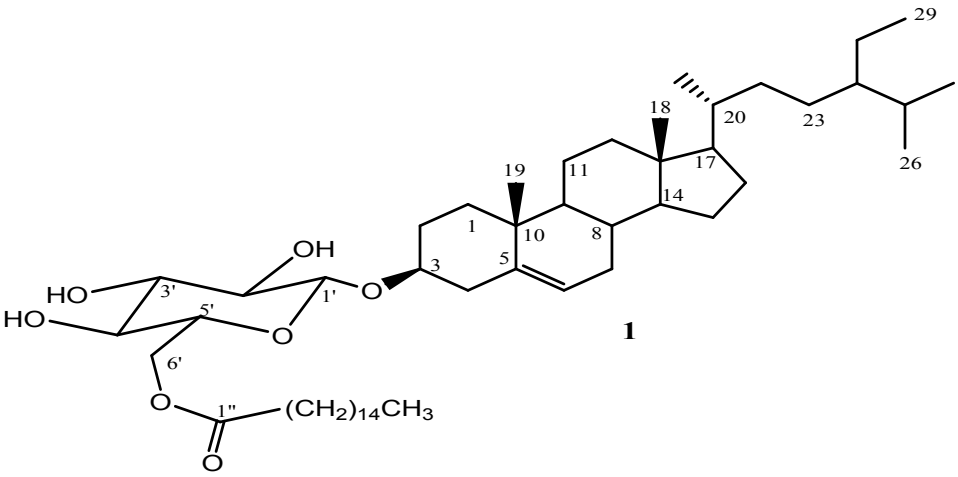

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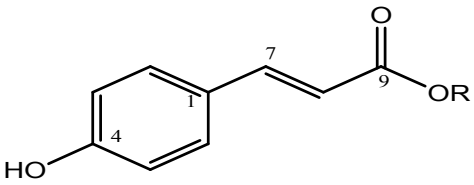

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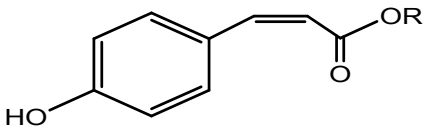

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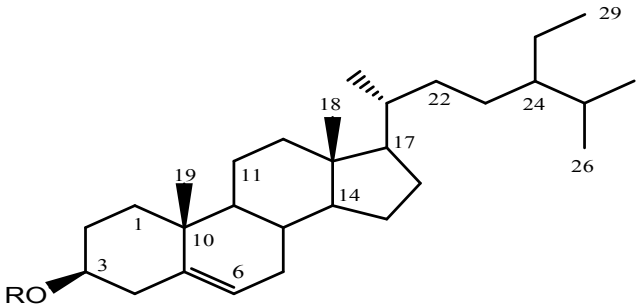

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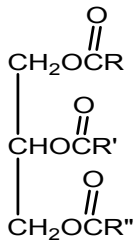

4 R, R', R" = long chain fatty acids

$CH_3(CH_2)nCOOCH_3$

5

Figure 1: Chemical structures of β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**), alkyl 4-hydroxy-*trans*-cinnamate esters (**2a**), alkyl 4-hydroxy-*cis*-cinnamate esters (**2b**), β-sitosterol (**3**), unsaturated triacylglycerols (**4**), and methyl fatty acid esters (**5**) from *C. lacrimans*.

hydroxy-*trans*-cinnamate esters (**2a**) and alkyl 4-hydroxy-*cis*-cinnamate esters (**2b**), β-sitosterol (**3**), unsaturated triacylglycerols (**4**), and methyl fatty acid esters from the sarcotesta of *Cycas lacrimans*. The structures of **1-5** were elucidated by comparison of their ^1H and/or ^{13}C NMR data with those reported in the literature. To the best of our knowledge this is the first report on the isolation of **1-2b** and **5** from *C. lacrimans*.

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

Cycas lacrimans sarcotesta were collected in 2013. Voucher specimens were collected and authenticated by

one of the authors (EMGA) and deposited in the De La Salle University- Manila Herbarium (DLSUH3113).

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Ten milliliter 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. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents of the Sarcotesta

The freeze-dried sarcotesta of *C. lacrimans* (105 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated 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 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to yield **5** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to afford **4** (5 mg). The more polar fractions were combined and rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield a mixture of **2a** and **2b** (3 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to yield **1** (5 mg).

In the second isolation. the freeze-dried sarcotesta of *C. lacrimans* (140 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (1.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) in 7.5% EtOAc in petroleum ether to afford **4** (12 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford **3** (15 mg) after washing with petroleum ether.

***β-Sitosteryl-3β-glucopyranoside-6'-O-palmitate* (1):** ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.55 (m, H-3), 5.34 (m, H-6), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.92 (3H, d, *J* = 6.0 Hz, CH_3-21), 0.82 (3H, d, *J* = 7.2 Hz, CH_3-26), 0.85 (d, *J* = 7.2 Hz, CH_3-27), 0.86 (t, *J* = 7.2 Hz, CH_3-29), 4.37 (d, *J* = 7.8 Hz, H-1'), 3.36 (dd, *J* = 7.5, 9.0, H-2'), 3.56 (dd, *J* = 9.0, 10.0, H-3'), 3.36 (dd, *J* = 9.0, 10.0 Hz, H-4'), 3.43 (m, H-5'), 4.23 (dd, *J* = 12.0, 2.0 Hz, H-6a'), 4.49 (dd, *J* = 5.0, 12.0 Hz, H-6b'), 2.34 (t, *J* = 7.5 Hz, H-2"); ^{13}C NMR (150 MHz; $CDCl_3$): δ 37.24 (C-1), 29.71 (C-2), 79.53 (C-3), 38.88 (C-4), 140.26 (C-5), 122.19 (C-6), 31.93, 31.85 (C-7, C-8), 50.15 (C-9), 36.72 (C-10), 21.06 (C-11), 39.74 (C-12), 42.31 (C-13), 56.74 (C-14), 24.28 (C-15), 28.24 (C-16), 56.05 (C-17), 11.84 (C-18), 19.34 (C-19), 36.13 (C-20), 18.77 (C-21), 33.93 (C-22), 26.05 (C-23), 45.81 (C-24), 29.13 (C-25), 19.01 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29), 101.18 (C-1'), 73.58 (C-2'), 75.87 (C-3'), 69.98 (C-4'), 73.98 (C-5'), 75.87 (C-6'), 174.80 (C-1"), 34.21 (C-2"), 24.94 (C-3"), 29.31 (C-4"), 29.52 (C-5"), 29.71 (C-6"), 29.71 (C-7"-C-12"), 29.36 (C-13"), 31.93 (C-14"), 22.69 (C-15"), 14.12 (C-16").

***Trans-4-hydroxycinnamate fatty acid esters* (2a)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.83 (d, *J* = 9.0 Hz, H-3, H-5), 7.43 (d, *J* = 9.0 Hz, H-2, H-6), 7.61 (d, *J* = 15.6 Hz, H-7), 6.28 (d, *J* = 15.6 Hz, H-8), 4.16 (t, *J* = 6.6 Hz, H-1'), 1.67 (2H, quintet, *J* = 6.6 Hz, H-2'), 1.23–1.38 (H-3'–H-n'), 0.88 (t, *J* = 6.6 Hz, Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 127.41 (C-1), 115.81 (C-3, C-5), 129.90 (C-2, C-6), 157.47 (C-4), 144.10 (C-7), 115.87 (C-8), 167.44 (C-9), 64.46/64.61 (C-1'), 28.24 (C-2'), 129.90 (=CH), 27.79 (allylic CH_2'), 22.69, 26.04, 29.06-29.70 $(CH_2')_n$, 31.92-31.65 (CH_2'), 14.12 (CH_3' terminal).

***Cis-4-hydroxycinnamate fatty acid esters* (2b)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.6 Hz, Me), 1.23–1.38 (m), 1.67 (2H, quintet, *J* = 6.6 Hz, H-2'), 4.09 (2H, t, *J* = 6.6 Hz, H-1'), 5.83 (1H, d, *J* = 12.6 Hz. H-8), 6.78 (2H, d, *J* = 9.0 Hz, H-3, H-5), 6.82 (1H, d, *J* = 12.6 Hz, H-7), 7.59 (2H, d, *J* = 9.0 Hz, H-2, H-6); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 127.11 (C-1), 114.90 (C-3, C-5), 132.33 (C-2, C-6), 156.48 (C-4), 143.02 (C-7), 117.42 (C-8), 166.59 (C-9), 64.46/64.61 (C-1'), 22.69, 26.04, 28.24 (C-2'), 129.90 (=CH), 29.06–29.70 $(CH_2')_n$, 31.92–31.65 (CH_2'), 14.12 (CH_3' terminal).

***β-Sitosterol* (3)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.51 (m, H-3), 2.28, 2.24 (H_2-4), 5.33 (dd, *J* = 5.4, 2.4 Hz, H-6), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.91 (d, *J* = 6.6 Hz, CH_3-21), 0.80 (d, *J* = 6.6 Hz, CH_3-26), 0.82 (d, *J* = 7.2 Hz, CH_3-27), 0.86 (t, *J* = 7.2 Hz, CH_3-29). ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.24 (C-1), 31.66 (C-2), 71.81 (C-3), 42.30 (C-4), 140.75 (C-5), 121.72 (C-6), 31.90, 31.91 (C-7, C-8), 50.12 (C-9), 36.14 (C-10), 21.07 (C-11), 39.76 (C-12), 42.30 (C-13), 56.76 (C-14), 24.30 (C-15), 28.24 (C-16), 56.04 (C-17), 11.85 (C-18), 19.39 (C-19), 36.50 (C-20), 19.02 (C-21), 33.93 (C-22), 29.14 (C-23), 45.82 (C-24), 26.06 (C-25), 18.77 (C-26), 19.81 (C-27), 23.06 (C-28), 11.97 (C-29).

***Triacylglycerols* (4)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.28 (2H, dd, *J* = 4.2, 12.0 Hz, glyceryl CH_2O), 4.12 (2H, dd, *J* = 6.0, 12.0 Hz, glyceryl CH_2O), 5.32 (1H, m, glyceryl CHO), 2.30 (6H, t, *J* = 7.2 Hz, α-CH_2), 5.33 (m, olefinic H), 2.75 (double allylic CH_2), 1.98-2.05 (allylic, CH_2), 1.23-1.35 (CH_2), 0.87 (t, *J* = 6.6 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 62.09 (glyceryl CH_2), 68.87 (glyceryl CH), 173.29, 173.25 (C=O α), 172.84 (C=O β), 34.03, 34.05, 34.19 (C-2), 24.84, 24.86 (C-3), 29.05, 29.08, 29.12 (C-4), 29.17, 29.20, 29.27 (C-5), 29.48 (C-6), 22.57, 22.68 (C-8), 130.23, 130.01, 129.70 (C-9), 127.74, 128.29, 129.68 (C-10), 25.61, 27.17, 27.20, 27.22, 29.32, 29.35, 29.36, 29.52, 29.62, 29.66, 29.70, 29.76 (CH_2), 31.52, 31.90, 31.92 (CH_2), 14.07, 14.11, 14.27 (terminal CH_3).

***Fatty acid methyl esters* (5)**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.86 (t, *J* = 6.6 Hz), 0.96 (t, *J* = 7.2 Hz), 1.23-1.35 (m), 1.53-1.61 (m), 2.02-2.06 (m), 2.28 (t, *J* = 7.5 Hz), 2.78 (t, J = 5.7 Hz), 3.65 (s), 5.30-5.37 (m); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.07, 14.12, 14.27, 20.54, 22.57, 22.69, 24.94, 24.96, 25.52, 25.60, 25.62, 27.15, 27.19, 27.19, 29.09, 29.11, 29.15, 29.25, 29.31, 29.34, 29.35, 29.45, 29.52,

29.58, 29.66, 29.70, 29.76, 31.52, 31.90, 31.92, 34.10, 34.12, 51.44, 127.10, 127.72, 127.90, 128.03, 128.24, 128.27, 129.75, 130.00, 130.05, 130.21, 130.27, 131.96, 174.32.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the sarcotesta of *C. lacrimans* afforded β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**)[14,15], a mixture of alkyl 4-hydroxy-*trans*-cinnamate ester (**2a**)[12] and alkyl 4-hydroxy-*cis*-cinnamate ester (**2b**)[12,16], β-sitosterol (**3**)[10,17], unsaturated triacylglycerols (**4**)[10], and methyl fatty acid esters (**5**)[18]. The structures of **1-5** were identified by comparison of their ^{1}H and ^{13}C NMR data with literature data.

β-sitosterol (**3**) isolated from the sarcotesta was also present in the petiole and rachis, roots, endotesta and sclerotesta of *C. lacrimans*[10], while triacylglycerols (**4**) obtained from the sarcotesta were also found in the bark, leaflets, petiole and rachis, roots, endotesta and sclerotesta of *C. lacrimans*[10]. Results of this study indicate that *C. lacrimans* shares similar chemical characteristics with other members of the family Cycadaceae: *C. edentata* which contained β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate (**1**) and methyl fatty acid esters (**5**)[13] and *C. vespertilio* which yielded alkyl 4-hydroxy-*trans*-cinnamate esters (**2a**) and alkyl 4-hydroxy-*cis*-cinnamate esters (**2b**)[12].

ACKNOWLEDGEMENT

A research grant from the Commission on Higher Education – Philippine Higher Education Research Network (CHED–PHERNet) of the Philippines is gratefully acknowledged.

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