ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Chemical Constituents of *Cycas nitida.*

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ABSTRACT

Chemical investigation of the dichlroromethane extracts of *Cycas nitida* afforded triacylglycerol(**1**),squalene (**2**), fatty acid methyl esters (**3**), a mixture of β-sitosterol (**4**) and stigmasterol (**5**), and fatty alcohol (**6**) from the bark;**1**, **4**,anda mixture of **3**and β-sitosteryl fatty acid ester (**7**)from the sarcotesta; **2** and chlorophyll a (**8**) from the leaflets; **2**, **4**, and **5** from the roots;**1**, anda mixture of**4**and **5**from the endotesta; a mixture of **4**and **5**from the petiole and rachis;**1** from the megasporophyll lamina;and **2**from the sclerotesta. The structures of **1**-**8** were identified by comparison of their NMR data with literature data.
Keywords: *Cycas,* Cycadaceae, triacylglycerol, squalene, fatty acid methyl ester, β-sitosterol, stigmasterol, fatty alcohol, β-sitosteryl fatty acid ester,chlorophyll a

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ISSN: 0975-8585

INTRODUCTION

Cycas resemble palms in morphology and are commonly called sago palm. They are considered as fossil plants though they may have evolved only about 12 million years ago [1]. They are widely distributed in the Tropics [2] where they grow on volcanic, limestone, ultramafic, sandy, or even water-logged soils in grassland and forest habitats [3]. The demand of *Cycas* species for domestic and international horticultural trade, grassland and forest fires, and conversion of their natural habitats to settlements and other land uses have threatened to varying degrees the wild populations of the genus [4]. Some of these threatened species are *C. curranii*[5], *C. wadei*[6] and *C. zambalensis* as Critically Endangered (CR) [5], *C. riuminiana* as Endangered (E) [5], and *C. saxatilis* as Vulnerable (V) [7].

There are no reported chemical and biological activity studies on *C. nitida*.However, a number of studies have been reported on the chemical constituents of other indigenous Philippine *Cycas.* We earlier reported the chemical constituents of the different parts of *C. sancti-lasallei*[8-11]*, C. vespertilio*[12, 13]*, C. zambalensis*[14]*, C. lacrimans*[15-17]*, C. aenigma*[18,19]*,* and *C. edentata* [20,21].

We report herein the isolation of triacylglycerol (**1**),squalene (**2**), fatty acid methyl esters (**3**), a mixture of β-sitosterol (**4**) and stigmasterol (**5**), and fatty alcohol (**6**) from the bark; **1**, **4**, and a mixture of **3** and β-sitosteryl fatty acid ester (**7**)from the sarcotesta; **2** and chlorophyll a (**8**) from the leaflets; **2**, a mixture of**4**, and **5** from the roots; **1** and **4** from the endotesta; **4** and **5** from the petiole and rachis;**1** from the megasporophyll lamina; and **2** from the sclerotesta. The structures of **1**-**8** are presented in Fig. 1.

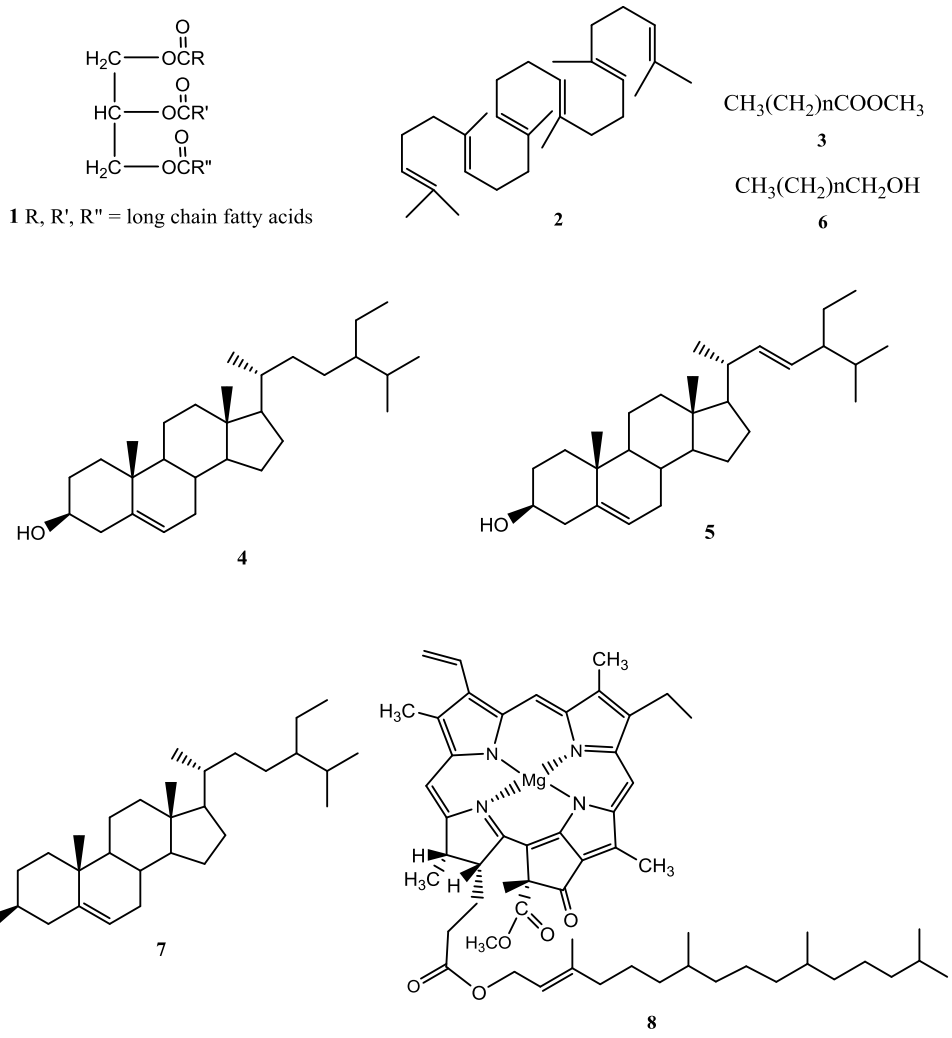

Figure 1: Chemical constituents of *Cycas nitida*: triacylglycerol (1),squalene (2), fatty acid methyl esters (3), β-sitosterol (4), stigmasterol (5), fatty alcohol (6), β-sitosteryl fatty acid ester (7),and chlorophyll a (8).

ISSN: 0975-8585

MATERIALS AND METHODS

General Experimental Procedure

^{1}H 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.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. Twenty milliliter fractions were collected. 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. 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.

Plant material

*Cycas nitida*leaflets, petiole, rachis, bark, roots, megasporophyll lamina and seeds were collected in 2014. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3120).

Isolation of the Chemical Constituents of the Bark

The air-dried bark (190 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2fraction was rechromatographed (2 ×) using petroleum ether to afford **2** (5 mg). The 10% acetone in CH_2Cl_2fraction was rechromatographed using 5% EtOAc in petroleum ether, followed by 7.5% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum ether were combined and rechromatographed (3 ×) using 5% EtOAc in petroleum ether to yield **3** (3 mg). The fractions eluted with 7.5% EtOAc in petroleum ether were combined and rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to afford**1** (10 mg). The 20% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield **6** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford a mixture of **4** and **5** (7 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sarcotesta

The air-dried sarcotesta (65 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether, followed by 7.5% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum were combined and rechromatographed using 5% EtOAc (3 ×) in petroleum ether to yield a mixture of **3** and **7** (5 mg). The fractions eluted with 7.5% EtOAc in petroleum were combined and rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to afford **1** (7 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford**4** (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Leaflets

The air-dried leaflets (135.8 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (4.3 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (3 ×) using petroleum ether to afford**2**(8 mg). The 30% acetone in CH_2Cl_2

fraction was rechromatographed (4 ×) using CH_2Cl_2 to afford**8** (10 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Roots

The air-dried roots (93 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.7 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford**2** (10 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 15% EtOAc in petroleum ether to afforda mixture of **4** and **5**(7 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Endotesta

The air-dried endotesta (85 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three 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 (10% increment) as eluents. The 20% EtOAc in petroleum ether fraction was rechromatographed (2×) using 2.5% EtOAc in petroleum ether to afford**1**(15 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **4** and **5** (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis (73.3 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **4** and **5** (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Megasporophyll Lamina

The air-dried megasporophyll lamina (65 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to afford **1** (15 mg).

Isolation of the Chemical Constituents of the Sclerotesta

The air-dried sclerotesta (70 g) of *C. nitida* were ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.15 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford**2** (9 mg).

RESULTS AND DISCUSSION

Silica gel chromatography of the CH_2Cl_2 extracts of *Cycas nitida* yielded triacylglycerol (**1**) [22],squalene (**2**) [23], fatty acid methyl esters (**3**) [24], a mixture of β-sitosterol (**4**) [25, 26] and stigmasterol (**5**) [26], and fatty alcohol (**6**) [27] from the bark; **1**, **4**,anda mixture of **3** and β-sitosteryl fatty acid ester (**7**) [28] from the sarcotesta; **2** and chlorophyll a (**8**) [29] from the leaflets; **2**, a mixture of**4**, and **5** from the roots; **1**, anda mixture of **4** and **5** from the endotesta; a mixture of **4** and **5** from the petiole and rachis;**1** from the megasporophyll lamina; and **2** from the sclerotesta. The structures of **1**-**8** were identified by comparison of their NMR data with literature data. The ratios of β-sitosterol (**4**) and stigmasterol (**5**)from the bark, roots, endotesta, and petiole and rachis are 2.5:1, 4:1, 6:1, and 5:1, respectively. These ratios were deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **5** at δ 5.13 (dd, *J* = 8.4, 15 Hz), 5.00 (dd, *J* = 8.4, 15 Hz), and 5.33 (dd, *J* = 1.8, 3.6 Hz) and the olefinic protons of **4** at 5.33 (dd, *J* = 1.8, 3.6 Hz) [28].

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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