ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Secondary Metabolites from *Cycas flabellata.*

Vincent Antonio S. Ng[1], Esperanza Maribel G. Agoo[2], and Chien-Chang Shen[3], and Consolacion Y. Ragasa[1,4]*.

[1]Chemistry Department and Natural Products Research and Drug Development, Center for Natural Sciences and Environmental Research, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines,
[2]Biology Department, De La Salle University 2401 Taft Avenue, Manila 1004, Philippines,
[3]National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan
[4]Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Binan City, Laguna 4024, Philippines,

ABSTRACT

Chemical investigation of the dichlroromethane extracts of *Cycas flabellata*, a plant endemic to the Philippines afforded 9αH-isopimara-7,15-diene (**1**), squalene (**2**), β-sitosterol (**3**), methyl fatty acid ester (**4**), and triacylglycerols (**5**) from the roots; **3** and **4** from the endotesta; **3** from the sclerotesta; and **2**, a mixture of **3** and stigmasterol (**6**) in a 1:1 ratio, and β-sitosterone (**7**) from the petiole and rachis. The structures of **1**-**7** were identified by comparison of their NMR data with literature data.

Keywords: *Cycas flabellata,* Cycadaceae, 9αH-isopimara-7,15-diene, squalene, β-sitosterol, methyl fatty acid ester, triacylglycerols, stigmasterol, β-sitosterone

Corresponding author

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].

A number of studies have been reported on the chemical constituents of 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], *C. riuminiana* [20], *C. nitida* [21, 22], *C. wadei* [23], *C. edentata* [24, 25] and *C. mindanaensis* [26].

We recently reported the isolation of squalene, phytyl fatty acid ester, lutein, and long chain 1-alkenes from the leaflets; a mixture of β-sitosterol and stigmasterol, triacylglycerols, and hydrocarbons from the bark; triacylglycerols and β-sitosteryl fatty acid esters from the sarcotesta; and a mixture of β-sitosterol and stigmasterol and β-sitosteryl fatty acid esters from the megasporophyll lamina of *Cycas flabellata* [27}.

We report herein the isolation of 9αH-isopimara-7,15-diene (**1**), squalene (**2**), β-sitosterol (**3**), methyl fatty acid ester (**4**), and triacylglycerols (**5**) from the roots; **3** and **4** from the endotesta; **3** from the sclerotesta; and **2**, a mixture of **3** and stigmasterol (**6**) in a 1:1 ratio, and β-sitosterone (**7**) from the petiole and rachis of *Cycas flabellata*. The structures of **1**-**7** are presented in Fig. 1.

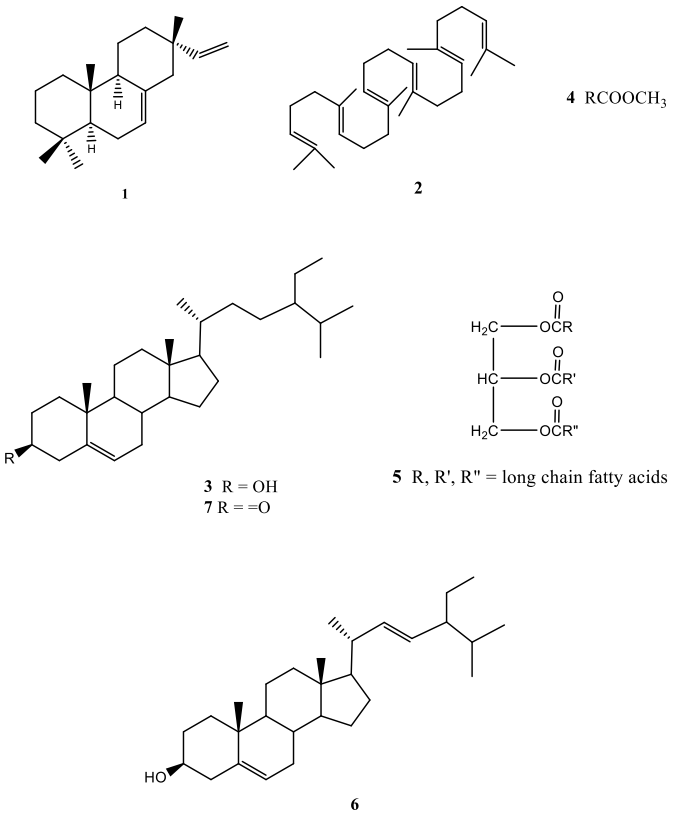

Fig. 1. Chemical structures of 9αH-isopimara-7,15-diene (1), squalene (2), β-sitosterol (3), methyl fatty acid ester (4), triacylglycerols (5), stigmasterol (6), and β-sitosterone (7) from *C. flabellata.*

ISSN: 0975-8585

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^{1}H 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 flabellata roots, endotesta, sclerotesta, and petiole and rachis were collected from Mati, Davao Oriental in June 2015. Voucher specimens were collected and authenticated by one of the authors (EMGA) and deposited in the De La Salle University-Manila Herbarium (DLSUH 3122).

Isolation of the Chemical Constituents of the Roots

The air-dried roots (89 g) *C. flabellata* 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 (0.25 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed (3 ×) using petroleum ether to afford **1** (3 mg). The more polar fractions were combined and rechromatographed (2 ×) using petroleum ether to afford **2** (4 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield **4** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to yield **5** (5 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **3** (7 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Endotesta

The air-dried endotesta (12 g) of *C. flabellata* 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 (0.3 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 2.5% EtOAc in petroleum ether to yield **4** (5 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **3** (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sclerotesta

The air-dried sclerotesta (52 g) of *C. flabellata* 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 (0.2 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 (2 ×) using 15% EtOAc in petroleum ether to yield **3** (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis (139 g) of *C. flabellata* 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 (0.65 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** (3 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield **7** (4 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield **3** (7 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the CH_2Cl_2 extracts of *Cycas flabellata* yielded 9αH-isopimara-7,15-diene (**1**) [15, 26], squalene (**2**) [26], β-sitosterol (**3**) [29, 30], methyl fatty acid ester (**4**) [16], and triacylglycerols (**5**) [31] from the roots; **3** and **4** from the endotesta; **3** from the sclerotesta; and **2**, a mixture of **3** and stigmasterol (**6**) [29, 30] in a 1:1 ratio, and β-sitosterone (**7**) [32] from the petiole and rachis of *Cycas flabellata*. The structures of **1**-**6** were identified by comparison of their NMR data with literature data.

These results indicate that *Cycas flabellata* shares similar chemical characteristics with other members of the family Cycadacea: *Cycas lacrimans* [15] and *Cycas edentata* [24] which afforded 9αH-isopimara-7,15-diene (**1**); *C. aenigma* [18, 19], *Cycas mindanaensis* [25], *C. nitida* [21], *C. riuminiana* [20], *C. sancti-lasallei* [8-11], *C. vespertilio* [12, 13], and *C. zambalensis* [14] which provided squalene (**2**); *C. sancti-lasallei, C. vespertilio, C. zambalensis, C. lacrimans, C. aenigma, C. riuminiana*, *C. nitida*, *C. wadei*, *C. edentata* and *C. mindanaensis* which yielded β-sitosterol (**3**), stigmasterol (**6**), and triacylglycerols (**5**) [8-25]; and *C. nitida* [21] and *C. lacrimans* [16] which contained methyl fatty acid ester (**4**).

ACKNOWLEDGEMENT

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

REFERENCES

[1] Nagalingum NS, Marshal CR, Quental TB, Tai HS, Little DP. Matthews S. Science **2011**; 334:796–799.
[2] Donaldson JS, Cycads. Status survey and conservation action plan. IUCN Gland, Switzerland and Cambridge, U.K. 2003.
[3] Madulid DA, Agoo EMG. Blumea 2009; 54:99–102.
[4] IUCN Red List of Threatened Species. Version 2010.4. <www.iucnredlist.org>. Downloaded on 09 February 2011.
[5] Agoo EMG, Madulid DA, Linis VC, Sambale E, In: IUCN 2010. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 16 December 2013.
[6] Hill KD. 2010. *Cycas wadei*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 26 December 2013.
[7] Bosenberg JD. 2010. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on16 December 2013.
[8] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. J Appl Pharm Sci 2015; 5(Suppl. 1):12-17.
[9] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Der Pharma Chemica 2015; 7(7):373-376.
[10] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Der Pharmacia Lettre, 2015; 7(9):168-179.
[11] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Int. J. Pharmacog. Phytochem. Res., 2015, 7(5), 884-887.
[12] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Braz. J. Pharmacog., 2015, 25(4), .
[13] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Int. J. Pharmacog. Phytochem. Res., 2015, 7(4), 727-731.
[14] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Chem. Nat. Compd., 2016, 52(1),
[15] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Int. J. Pharmacog. Phytochem. Res., **2015**, 7(3), 616-620.
[16] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Int. J. Pharm. Clin. Res., 2015, 7(5), 356-359.
[17] Ragasa CY, Ng VAS, Agoo EMG,Shen C-C. Int. J. Toxicol. Pharmacol. Res., 2015, 7(5), 259-263.
[18] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. J Appl. Pharm. Sci., 2015, 5(9), 32-36.
[19] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Res J Pharm Biol Chem Sci 2015; 6(6):267-270.

[20] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Der Pharma Chemica 2015; 7(10): 485-489.
[21] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Res J Pharm Biol Chem Sci 2015; 6(6):1305-1309.
[22] Ragasa CY, Ng VAS, Agoo EMG, Shen C-C. Der Pharmacia Lettre 2016; 8(1):148-152.
[23] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Der Pharma Chemica 2015; 7(11):294-298.
[24] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. J Pharm Sci Res 2015; 7(9):643-646.
[25] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Int J Pharm Sci Rev Res 2015; 33(2):107-109.
[26] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Der Pharma Chemica. 2015; 7(12):323-327.
[27] Ng VAS, Agoo EMG, Shen C-C, Ragasa CY. Der Pharma Chemica. 2016; 8(2):
[28] Tsai P-W, de Castro-Cruz K, Shen C-C, Ragasa CY. Pharm Chem J 2012; 46(4):225-227.
[29] Ragasa CY, Ng VAS, De Los Reyes MM, Mandia EH, Oyong GG, Shen C-C. Der Pharma Chemica 2014; 6(5):182-187.
[30] Ragasa CY, Ng VAS, Agoo EMG, Shen C-C. Int J Pharmacog Phytochem Res 2015; 7(3):616-620.
[31] Ragasa CY, Caro J, Shen C-C. Der Pharma Chemica 2015; 7(2):178-182.
[32] Prachayasittikul S, Suphapong S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasittikul V. Molecules 2009; 14:850-867.