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Chemical constituents of *Cycas wadei*

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Cycas wadei, a plant endemic to the Philippines, led to the isolation of δ-tocopherol (**1**), β-sitosterol (**2**), β-sitosteryl fatty acid ester (**3**), triacylglycerol (**4**) from the megasporophyll lamina; **2**, trilinolein (**5**), a mixture of linoleic acid and oleic acid, and hydrocarbons from the sarcotesta; **2**, chlorophyll a (**6**), fatty alcohol, and hydrocarbons from the leaflets; a mixture of **2** and stigmasterol (**7**),squalene (**8**), and fatty alcohol from the petiole and rachis; **2** and **4** from the endotesta; **3** and **4** from the sclerotesta; and a mixture of **2** and **7**, **8** and fatty alcohol from the roots ofCycas wadei. The structures of these compoundswereidentified by comparison of their NMR data with literature data.*

Keywords: *Cycas wadei*, Cycadaceae, δ-tocopherol, β-sitosterol, β-sitosteryl fatty acid ester, triacylglycerol, trilinolein, chlorophyll a, stigmasterol, squalene

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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]. The cycads resemble palms in morphology and are commonly called sago palm. 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]. Ten out of the eleven cycad species in the Philippines are endemic.

There are no reported chemical and biological activity studies on *C. wadei*. The most studied *Cycas* are *Cycas revoluta* and *C. circinalis* whichcontain the carcinogenic toxin cycasin [4, 5]. The methanolic extract of the leaflets of *C. circinalis* L. and the chloroform extract of *C. revoluta* yielded biflavonoids, lignans, flavan-3-ols, flavone-C-glucosides, nor-isoprenoids, and a flavanone. Three of the biflavonoids exhibited moderate activity against *S. aureus* and methicillin-resistant *S. aureus* [6]. Further studies on the chemical constituents of the leaves of *C. revoluta* and*C. circinalis* afforded lariciresinol, naringenin and biflavonoids which are derivatives of amentoflavone and hinokiflavone [7].

There are no reported chemical and biological activity studies on *C. wadei*. 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], *C. edentata*[20, 21], *C. riumimiana* [22], *C. curranii* [23],and *C. nitida*[24].

We report herein the isolation of δ-tocopherol (**1**), β-sitosterol (**2**), β-sitosteryl fatty acid ester (**3**), and triacylglycerol (**4**)from the megasporophyll lamina; **2**, trilinolein (**5**), fatty acids, and hydrocarbons from the sarcotesta; **2**, chlorophyll a (**6**), fatty alcohol, and hydrocarbons from the leaflets; a mixture of **2** and stigmasterol (**7**),squalene (**8**), and fatty alcohol from the petiole and rachis; **2** and **4** from the endotesta; **3** and **4** from the sclerotesta; and a mixture of **2** and **7**, **8** and fatty alcohol from the roots of*Cycas wadei.* The structures of **1**-**8** are presented in Fig. 1. This is the first report on the isolation of these compounds from *C. wadei*.

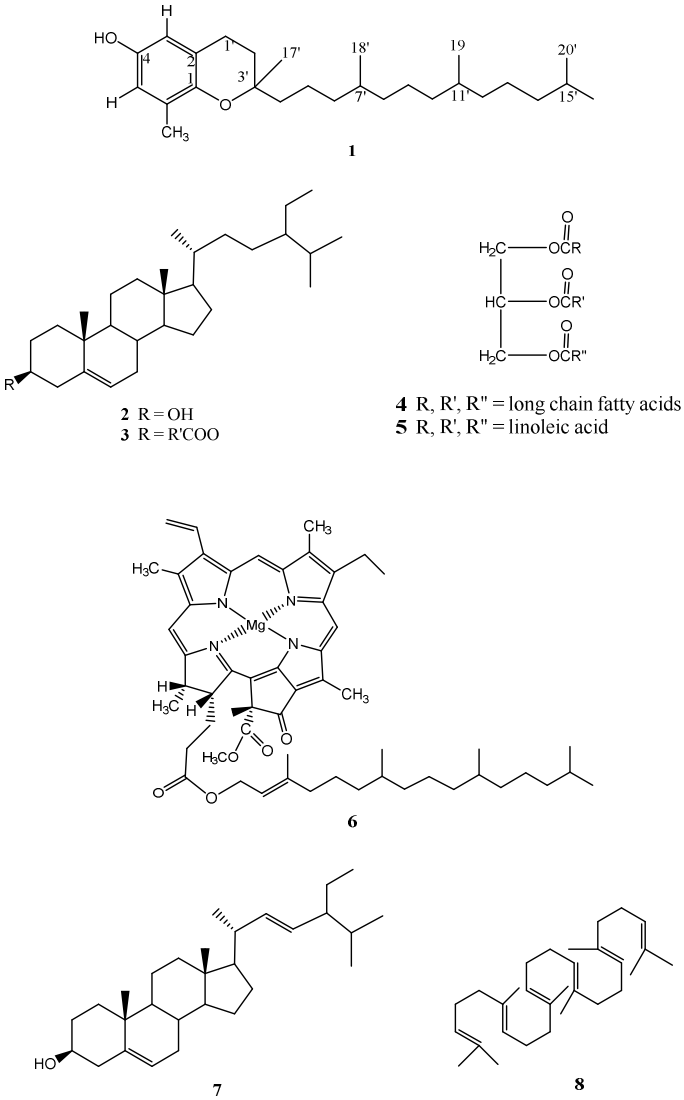

Fig. 1. Chemical structures of δ-tocopherol (1), β-sitosterol (2), β-sitosteryl fatty acid ester (3), triacylglycerol (4), trilinolein (5), chlorophyll a (6), stigmasterol (7), andsqualene (8) from *C. wadei*

MATERIALS AND METHODS

General Isolation Procedure

NMR spectra were recorded on a Varian VNMRSspectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for^{13}C NMR spectra. Column chromatography was performed withsilica gel 60 (70-230 mesh). Thin layer

chromatography was performed with plastic backed plates coated with silica gel F_{254}andthe plates were visualized by spraying with vanillin/H_2SO_4solution followed by warming.

Sample Collection

The megasporophyll lamina, sarcotesta, leaflets,of *Cycas wadei*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 3115).

Isolation of the Chemical Constituents of the Megasporophyll Lamina

The air-dried megasporophyll lamina of *C. wadei*(70 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (0.5 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed using 1% EtOAc in petroleum ether to yield **3** (3 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **4**(6 mg). The more polar fractions were combined and rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield **1**(2 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **2**(4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sarcotesta

The air-dried sarcotesta of *C. wadei*(164.2 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (2.1 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford hydrocarbons (10 mg) after washing with petroleum ether. The 10% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) in 2.5% EtOAc in petroleum ether to yield **5** (8 mg). The 40% acetone in CH_2Cl_2fraction was rechromatographed in 15% EtOAc in petroleum ether to afford **2** (4 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5 by volume) to afford a mixture of linoleic acid and oleic acid (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Leaflets

The air-dried leaflets of *C. wadei*(210 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (3.3 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford hydrocarbons(10 mg) after washing with petroleum ether. The 10% acetone in CH_2Cl_2fraction was rechromatographed in 5% EtOAc in petroleum ether to yield fatty alcohol (7 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **2** (5 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed (2 ×) using CH_2Cl_2toafford **6**(6 mg) after washing with petroleum ether, followed by Et_2O.

Isolation of the Chemical Constituents of the Petiole and Rachis

The air-dried petiole and rachis of *C. wadei*(50.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (0.35 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford **8** (3 mg). The 20% acetone in CH_2Cl_2fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to yield fatty alcohol (5 mg). The 40% acetone in CH_2Cl_2fraction was rechromatographed in 15% EtOAc in petroleum ether to afford a mixture of **2** and **7** (7 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Endotesta

The air-dried endotesta of *C. wadei*(141 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (0.3 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **4**(5 mg). The 40% acetone in CH_2Cl_2fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **2** (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sclerotesta

The air-dried sclerotesta of *C. wadei*(159.3 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (0.15 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using 1% EtOAc in petroleum ether to afford **3**(2 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **4**(4 mg).

Isolation of the Chemical Constituents of the Roots

The air-dried roots of *C. wadei*(15.5 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. Thesolvent was evaporated under vacuum to afford a crude extract (0.45 g) which was chromatographed using increasing proportionsofacetone in CH_2Cl_2 at 10% increment. The CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford **8** (4 mg). The 20% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to yield fatty alcohol (2 mg). The 40% acetone in CH_2Cl_2fraction was rechromatographed (3 ×) in 15% EtOAc in petroleum ether to afford a mixture of **2** and **7** (5 mg) after washing with petroleum ether.

δ-Tocopherol (**1**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.36 (d, *J* = 3 Hz, H-3), 6.45 (d, *J* =2.4 Hz, H-5), 2.10 (s, 6-CH_3), 2.67 (dd, J = 6.6, 12 Hz, H_2-1'), 1.76, 1.70(H_2-2'), 0.85 (d, *J* = 6.6 Hz, CH_3-16'), 1.25 (s, CH_3-17'),0.83 (d, *J* = 6.6 Hz, CH_3-18'), 0.82 (d, *J* = 6.6 Hz, CH_3-19'), 0.87 (d, *J* = 6.6 Hz, CH_3-20'), 4.19 (s, 4-OH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 146.06 (C-1), 121.29 (C-2), 112.54 (C-3), 147.64 (C-4), 115.57 (C-5), 127.35 (C-6), 16.05 (6-CH_3), 22.51 (C-1'), 31.31 (C-2'), 75.56 (C-3'), 39.93 (C-4'),20.97 (C-5'), 37.44 (C-6'), 32.68 (C-7'), 37.44 (C-8'), 24.43 (C-9'), 37.27 (C-10'), 32.79 (C-11'), 37.41 (C-12'), 24.79 (C-13'), 39.36 (C-14'), 27.97 (C-15'), 22.62 (C-16'), 24.10 (C-17'), 19.64, 19.74 (C-18', C-19'), 22.71 (C-20').

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of of *Cycas wadei*, a plant endemic to the Philippines, led to the isolation of δ-tocopherol (**1**) [25], β-sitosterol (**2**) [26], β-sitosteryl fatty acid ester (**3**) [27], triacylglycerol (**4**) [28] from the megasporophyll lamina; **2**, trilinolein (**5**) [29], a mixture of linoleic acid [30] and oleic acid [31], and hydrocarbons [32] from the sarcotesta; **2**, chlorophyll a (**6**) [33], fatty alcohol [34], and hydrocarbons from the leaflets; a mixture of **2** and stigmasterol (**7**) [26],squalene (**8**) [35], and fatty alcohol from the petiole and rachis; **2** and **4** from the endotesta; **3** and **4** from the sclerotesta; and a mixture of **2** and **7**, **8** and fatty alcohol from the roots of*Cycas wadei*. The structures of these compoundswere identified by comparison of their NMR data with those reported in the literature.

The major chemical constituent of the different parts of *C. wadei* is β-sitosterol (**2**) which is found in the megasporophyll lamina, sarcotesta, leaflets, petiole and rachis, endotesta,and roots of*C. wadei*, while δ-tocopherol (**1**) was found only in the megasporophyll lamina. Sterol **2** is also the major chemical constituent 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], *C. riumimiana* [22], *C. curranii* [23], and *C. nitida* [24].On the other hand, **1** was found only in *C. wadei*, while α-tocopherol, an analogue of **1**was isolated only from*C. ruminiana* [22]. Both δ-and α-tocopherols are natural tocopherols which are components of vitamin E with strong antioxidant property [36].

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REFERENCES

[1] N. S. Nagalingum, C. R. Marshal, T. B. Quental,H. S. Tai, D. P. Little,S. Matthews, *Science*, **2011**, 334, 796–799.
[2] J. S. Donaldson, Cycads. Status survey and conservation action plan. IUCN Gland, Switzerland and Cambridge, U.K.**2003**.
[3] D. A. Madulid, E. M. G. Agoo,*Blumea*, **2009**, 54, 99–102.
[4] K. Nishida,A. Kobayashi,T. Nagahama,*Bull. Agric. Chem. Soc. Japan*, **1955**,19, 77–84.
[5] G. L. Laqueur,O. Mickelsen,M. G. Whiting,L. T. Kurlad, *J. Natl. Cancer Inst.*,**1963**,31, 919–951.

[6] A. Moawad, M.Hetta, J. K.Zjawiony, M. R.Jacob, M.Hifnawy, J. P.Marais, D.Ferreira, *Planta Med.*, **2010**,76, 796–802.
[7] D. Ferreira,J. K. Zjawiony, A. Moawad,M. Hifnawy,M. Hetta,*Planta Med.*, **2009**,75, P-53.
[8] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*J. Appl. Pharm. Sci*.,**2015**, 5(Suppl. 1), 12−17.
[9] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, *Der Pharma Chemica*, **2015**, 7(7), 373−376.
[10] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*Der Pharmacia Lettre*,**2015**, 7(9), 168−171.
[11] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen,*Int. J. Pharmacog.Phytochem. Res*.,**2015**, 7(5).
[12] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, *Braz. J. Pharmacog*.,**2015**, 25, 526−528.
[13] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, *Int. J. Pharmacog. Phytochem. Res*.,**2015**, 7(4), 727−731.
[14] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C.Shen, *Chem. Nat. Compd*.,**2016**, 52(1),
[15] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen,*Int. J. Pharmacog. Phytochem. Res*., **2015**, 7(3), 616−620.
[16] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*Int. J. Pharm. Clin. Res*.,**2015**, 7(5), 356−359.
[17] C. Y. Ragasa, V. A. S. Ng, E. M. G. Agoo, C.-C. Shen,*Int. J. Toxicol. Pharmacol. Res*.,**2015**, 7.
[18]V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*J. Appl. Pharm. Sci*.,**2015**, 5(9), 32−36.
[19] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*Res. J. Pharm. Biol. Chem. Sci*.,**2015**, 6(6), 267−270.
[20] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*J. Pharm. Sci. Res*.,**2015**, 7(9),643−646.
[21] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Int. J. Pharm. Sci. Rev. Res*., **2015**, 33(2), 107−109.
[22] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Der Pharma Chemica*,**2015**, 7(10), 485–489.
[23] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Int. J.Pharm. Clin. Res.*, **2015**, 7(6).

[24] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Res. J. Pharm. Biol. Chem. Sci*.,**2015**,6(6), 1305–1309.
[25] J. K. Baker, C. W. Myers, *Pharm. Res.*, **1991**, 8(6), 763–770.
[26] C. Y. Ragasa, V. Ebajo Jr., V. A. S. Ng, M. M. De Los Reyes, C.-C. Shen, *Der Pharma Chemica*, **2014**, 6(6), 366–373.
[27] V. A. S. Ng, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa,*J. Appl. Pharm. Sci*.,**2015**, 5(Suppl 1), 12–17.
[28] C. Y. Ragasa, J. L. Caro, C.-C. Shen. *J. Appl. Pharm. Sci*., **2014**, 4(10), 7–11.
[29] L. B. Alemany, *Chem. Phys. Lipids*, **2002**,120, 33–44.
[30] Human Metabolome Database. Linoleic acid. Downloaded from http://www.hmdb.ca/spectra/ nmr_one_d/1471 on Nov. 27, 2013.
[31] C. Y. Ragasa, O. B. Torres, J. M. P. Gutierrez, H. P. B. C. Kristiansen, C.-C. Shen,*J. Appl. Pharm. Sci.*,**2015**, 5(4), 94–100.
[32] C. Y. Ragasa, V. D. Ebajo Jr., N. Lazaro-Llanos, R. Brkljača, S. Urban,*Der Pharma Chemica*,**2015**. 7(10), 473–478.
[33] C. Y. Ragasa, V. D. Ebajo Jr., M. M.De Los Reyes, E. H. Mandia, M. C. S. Tan, R. Brkljača, S. Urban,*J. Appl. Pharm. Sci*.,**2015**, 5(Suppl.2), 16–21.
[34] C. Y. Ragasa, M. P. Medecilo, C.-C. Shen,*Der Pharma Chemica*,**2015**, 7(7), 395–399.
[35] P.-W. Tsai, K. de Castro-Cruz, C.-C. Shen, C. Y. Ragasa,*Pharm. Chem. J*.,**2012**, 46(4), 225–227.
[36] Biological and antioxidant activities of tocopherols. Downloaded from www.rikenvitamin.com/foodingredients/**tocopherol**/property.html on November 8, 2015.