Chemical constituents of *Cycas flabellata*

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**ABSTRACT**

Chemical investigation of the dichloromethane extracts of *Cycas flabellata*, a plant endemic to the Philippines afforded squalene (1), phytyl fatty acid ester (2), lutein (3), and long chain 1-alkenes (4) from the leaflets; a mixture of β-sitosterol (5) and stigmasterol (6), triacylglycerols (7), and hydrocarbons (8) from the bark; 7and β-sitosteryl fatty acid esters (9) from the sarcotesta; and a mixture of 5and 6, 8, and 9from the megasporephyll lamina. The structures of 1-9 were identified by comparison of their NMR data with literature data.

**Keywords:** *Cycas flabellata*, Cycadaceae, squalene, phytyl fatty acid esters, lutein, 1-alkenes, β-sitosterol, stigmasterol, triacylglycerols, hydrocarbons, β-sitosteryl fatty acid esters

**INTRODUCTION**

*Cycas* are gymnosperms which resemble palms in habit 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. flabellata*. 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. riuminiana* [20], *C. nitida* [21], *C. wadei* [22], *C. edentata* [23, 24] and *C. mindanaensis* [25].
We report herein the isolation of squalene (1), phytol fatty acid ester (2), lutein (3), and long chain 1-alkenes (6) from the leaflets; a mixture of β-sitosterol (4) and stigmasterol (6), triacylglycerols (7), and hydrocarbons (8) from the bark; 7 and β-sitosteryl fatty acid esters (9) from the sarcotesta; and a mixture of 5 and 6, 8, and 9 from the megasporophyll lamina. The structures of 1-9 are presented in Fig. 1.

Fig. 1. Chemical structures of squalene (1), phytol fatty acid ester (2), lutein (3), long chain 1-alkenes (4), β-sitosterol (5), stigmasterol (6), triacylglycerols (7), hydrocarbons (8), and β-sitosteryl fatty acid esters (9) from *C. flabellata*.
MATERIALS AND METHODS

General Experimental Procedure
NMR spectra were recorded on a Varian VNMR S spectrometer in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C 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 F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ 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 Rₜ 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* leaflets, bark, sarcotesta, and megasporophyll lamina 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 Leaflets
The air-dried leaflets (252 g) of *C. flabellata* were ground in an osterizer, soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (5.2 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. The CH₂Cl₂ fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed using petroleum ether to afford 4 (3 mg). The more polar fractions were combined and rechromatographed (3 ×) using petroleum ether to afford 1 (5 mg). The 10% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield 2 (2 mg). The 60% acetone in CH₂Cl₂ fraction was rechromatographed (2 ×) using CH₂CN:Et₂O:CH₂Cl₂ (1:1:8, v/v) to yield 3 (6 mg) after washing with petroleum ether, followed by diethyl ether.

Isolation of the Chemical Constituents of the Bark
The air-dried bark (32.5 g) of *C. flabellata* were ground in an osterizer, soaked in CH₂Cl₂ 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 CH₂Cl₂ (10% increment) as eluents. The CH₂Cl₂ fraction was rechromatographed (2 ×) using petroleum ether to afford 8 (2 mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield 7 (8 mg). The 30% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield a mixture of 5 and 6 (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Sarcotesta
The air-dried roots (58 g) of *C. flabellata* were ground in an osterizer, soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.45 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. The 20% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 7.5% EtOAc in petroleum ether to yield 7 (5 mg). The 30% acetone in CH₂Cl₂ fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield a mixture of 5 and 6 (4 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Megasporophyll Lamina
The air-dried megasporophyll lamina (182 g) of *C. flabellata* were ground in an osterizer, soaked in CH₂Cl₂ 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 CH₂Cl₂ (10% increment) as eluents. The CH₂Cl₂ fraction was rechromatographed (2 ×) using petroleum ether to afford 8 (3 mg). The 10% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield 9 (4 mg). The 30% acetone in CH₂Cl₂,
fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield a mixture of 5 and 6 (7 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the CHCl₃ extracts of Cycas flabellata yielded squalene (1) [26], phytol fatty acid ester (2) [27], lutein (3) [28], and long chain 1-alkenes (4) [29] from the leaflets; a mixture of β-sitosterol (5) [30, 31] and stigmasterol (6) [30, 31], triacylglycerols (7) [32], and hydrocarbons (8) [33] from the bark; and a mixture of 5 and β-sitosteryl fatty acid esters (9) [8] from the sarcotesta; and a mixture of 5 and 6, and 9 from the megasporophyll lamina. The structures of 1-9 were identified by comparison of their NMR data with literature data.

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REFERENCES