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Research Article

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Triterpenes from *Ficus nervosa*

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ABSTRACT

*The dichloromethane extract of the air-dried leaves of Ficus nervosa afforded lupenone (**1**), β-friedelinol (**2**), squalene (**3**), β-sitosterol (**4**) and a mixture of cycloeucalenol (**5**), lupeol (**6**), α-amyrin (**7**), and β-amyrin (**8**). The structures of **1**-**8** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.*

Keywords: *Ficus nervosa*, Moraceae, lupenone, β-friedelinol, cycloeucalenol, lupeol, α-amyrin, β-amyrin, squalene, β-sitosterol

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INTRODUCTION

Ficus nervosa is grown at the De La Salle University, Manila campus as an ornamental tree. As part of our continuing studies on the chemical constituents of trees belonging to the genus *Ficus*, the chemical constituents of the leaves of *F. nervosa* were investigated. There is only one reported study on the chemical constituents of *F. nervosa* which reported the isolation of two new pyranocoumarins, 3-hydroxyxanthyletin and 3-methoxyxanthyletin, along with 22 known compounds: coumarins, flavonois, benzenoids, steroids such as β-sitosterol and stigmasterol, and a triterpenoid, oleanolic acid from the roots [1]. Our research group studied the chemical constituents of six *Ficus* species, four of which are endemic to the Philippines. The isolation of a new neohopane triterpene [2] and furanocoumarin derivatives, bergapten and oxypeucedanin hydrate [3] which exhibited antimicrobial properties from a local collection of *Ficus pumila* has been reported. In another study, the isolation of squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, lupeol fatty acid ester, lupenone, oleanone, and ursenone from the leaves of *Ficus pseudopalma* and lutein, lupeol acetate, β-carotene, phytol, α-amyrin fatty acid ester, squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, β-sitosterol and stigmasterol from the leaves of *Ficus ulmifolia* which are endemic to the Philippines were also reported [4]. Recently, chemical investigation of the dichloromethane extracts of the air-dried leaves of *Ficus triangularis* and a Philippine endemic tree, *Ficus linearifolia* led to the isolation of 11α,12α-epoxyurs-14-en-3β-yl acetate, β-amyrin, α-amyrin, squalene, β-sitosterol, stigmasterol, polyprenol, linoleic acid and lutein from *F. linearifolia* and ergosta-6,22-dien-3,5,8-triol, ergosterol, taraxerol, hop-22(29)-ene, squalene, β-sitosterol, stigmasterol, polyprenol, linoleic acid and lutein from *F. triangularis* [5]. Furthermore, another endemic tree, *Ficus odorata* afforded β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate, squalene, lutein, α-amyrin acetate, lupeol acetate, and β-carotene. β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate exhibited cytotoxicity against AGS cell line with 60.28% growth inhibition [6].

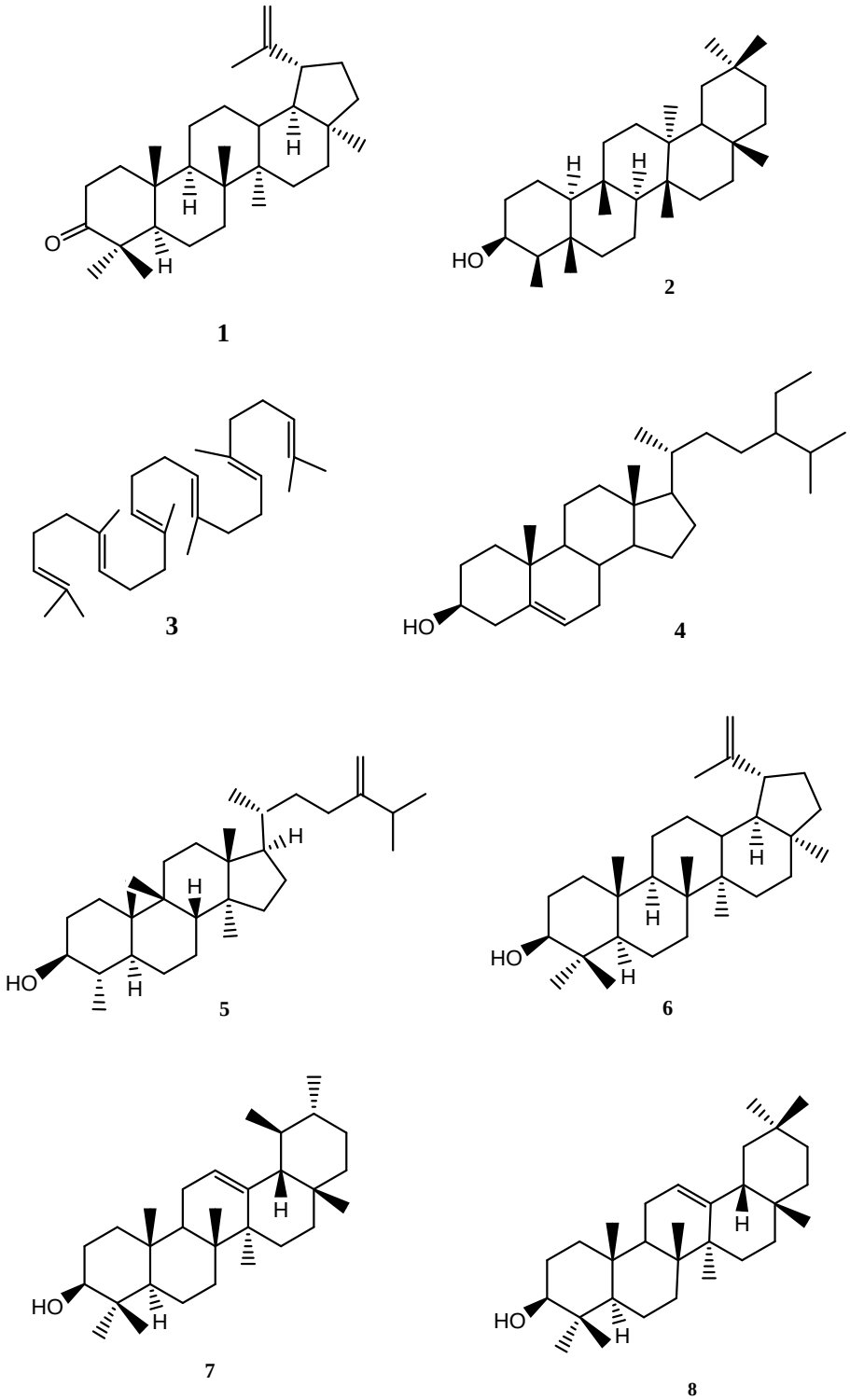

Fig. 1. Chemical constituents of *F. nervosa* leaves: lupenone (1), β-friedelinol (2), squalene (3), β-sitosterol (4), cycloeucalenol (5), lupeol (6), α-amyrin (7) and β-amyrin (8)

A recent study reported that phytochemical screening of the bark extract of *F. nervosa* showed the presence of alkaloids, glycosides, sugars and carbohydrates, terpenoids, anthocyanins, saponins, quinones, resins, flavonoids,

tannins, and phenolic compounds [7]. The results of preliminary phytochemical studies confirmed the presence of alkaloid, carbohydrate, glycoside , steroid, protein, tannin, terpenoid, flavonoid and phenol in the chloroform leaf extract of *F. nervosa* [8]. Another study investigated the effect of ethyl acetate and ethanolic leaf extracts of *F. nervosa* when administered to alloxan monohydrate induced diabetic albino rats. The results showed that the ethanolic extract at a dose of 250mg/kg BW has significant antihyperglycemic effect [9]. The anti-ulcer activity of chloroform, ethyl acetate and ethanolic extracts of *F. nervosa* bark in Winstar albino rats was also investigated. The results indicate that the ethanolic extract 200mg/kg significantly decreased the volume of gastric acid secretion, pH, free acidity, total acidity and ulcer index with respect to standard. The anti ulcer activity of *F. nervosa* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition [10]. Moreover, the ethanol extract (500 mg/kg, p. o.) of *F. nervosa* exhibited a remarkable hepatoprotective activity against paracetmol induced hepatotoxicity as judged from the serum markers for liver damage [11].

We report herein the isolation and identification of lupenone (**1**), β-friedelinol (**2**), squalene (**3**), β-sitosterol (**4**) and a mixture of cycloeucalenol (**5**), lupeol (**6**), α-amyrin (**7**) and β-amyrin (**8**) from the dichloromethane extract of the air-dried leaves of *F. nervosa* (Fig. 1).

EXPERIMENTAL SECTION

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.

Sample Collection and Isolation

The sample used in this study was collected from the De La Salle University, Manila campus in March 2013. It was authenticated as *Ficus nervosa* Heyne at the Botany Division, Philippine National Museum, Intramuros, Manila, Philippines.

The CH_2Cl_2 extract of the air-dried leaves (300 g) of *F. nervosa* was ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford the crude extract (11.2 g) which was chromatographed in increasing proportions of acetone in CH_2Cl_2 at 10% increment. A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Five 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. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (3×) in petroleum ether to afford **3** (8 mg). The 30% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (4×) in 7.5% EtOAc in petroleum ether to afford **1** (25 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (3×) in 7.5% EtOAc in petroleum ether to afford a mixture of **5-8** (15 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (5×) in 15% EtOAc in petroleum ether to afford **2** (5 mg) and **4** (7 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *F. nervosa* afforded lupenone (**1**), β-friedelinol (**2**), squalene (**3**), β-sitosterol (**4**) and a mixture of cycloeucalenol (**5**), lupeol (**6**), α-amyrin (**7**), β-amyrin (**8**) in a 1:1:1:0.5 ratio. The ratio of the triterpenes in the mixture was deduced from the ^{1}H NMR spectrum integrations in the olefinic region, e.g. δ 5.16 (β-amyrin) [12], δ 5.11 (α-amyrin) [12], δ 4.54 and 4.75 (lupeol) [13] and δ 4.64 and 4.67 (cycloeucalenol) [14, 15]. For the cycloeucalenol, the integrations for the cyclopropyl protons at δ 0.54 and 0.32 [14, 15] were also considered.

The structures of **1**-**8** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature for lupenone (**1**) [13], β-friedelinol (**2**) [16], squalene (**3**) [17], β-sitosterol (**4**) [18] and a mixture of cycloeucalenol (**5**) [14, 15], lupeol (**6**) [13], α-amyrin (**7**) [19] and β-amyrin (**8**) [19].

CONCLUSION

The dichloromethane extract of the air-dried leaves of *F. nervosa* afforded lupenone (**1**), β-friedelinol (**2**), squalene (**3**), β-sitosterol (**4**) and a mixture of cycloeucalenol (**5**), lupeol (**6**), α-amyrin (**7**) and β-amyrin (**8**). From our previous studies, the following compounds from *F. nervosa* have been previously isolated from other Philippine *Ficus* species: **1** from *F. pseudopalma* [4]; **3** from *F. linearifolia* [5], *F. triangularis* [5], *F. odorata* [6], *F. pseudopalma* and *F. ulmifolia* [4]; **4** from *F. ulmifolia* [4], *F. triangularis* [5] and *F. linearifolia* [6]; and **7** and **8** from *F. linearifolia* [5]. To the best of our knowledge this is the first report on the isolation of **2, 5** and **6** from Philippine *Ficus* species.

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