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Research Article

Secondary Metabolites from *Ficus ampelas* Burm.F.

Consolacion Y Ragasa[1,2*], Maria Roxanne Macuha[1,3], Mariquit M De Los Reyes[4,5], Emelina H Mandia[4], Chien-Chang Shen[6]

[1]*Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines.*
[2]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines.*
[3]*Food and Drug Administration, Civic Drive, Filinvest Corporate City, Alabang, Muntinlupa City 1781, Philippines*
[4]*Biology Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines*
[5]*Biology Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines.*
[6]*National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei, Taiwan.*

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ABSTRACT
Chemical investigation of the dichloromethane extracts of *Ficus ampelas* Burm.f. has led to the isolation of squalene (**1**), mixtures of β-amyrin fatty acid esters (**2a**) and α-amyrin fatty acid esters (**2b**) in a 1:2 ratio and β-sitosterol (**3a**) and stigmasterol (**3b**) in a 2:1 ratio, chlorophyll a (**4**), and saturated fatty acids (**5**) from the twigs; and **5**, β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**6**), and long-chain fatty alcohols (**7**) from the fruit. The structures of **1**-**7** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Ficus ampelas*, Moraceae, squalene, β-amyrin fatty acid esters, α-amyrin fatty acid esters, β-sitosterol, stigmasterol, chlorophyll a, saturated fatty acids, β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters, long-chain fatty alcohols, β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters

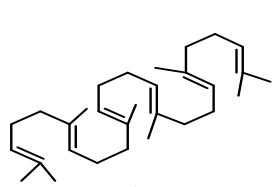
INTRODUCTION

Ficus ampelas Burm.f., locally known as *upling-gubat*, is an evergreen, small to medium-sized tree commonly found in primary and secondary lowland forests. This rather variable species is distributed in Ryukyu Islands, Taiwan, Philippines, Sumatra, Java, Sunda Islands, Sulawesi, the Moluccas and New Guinea[1]. The latex is used in the treatment of diarrhea and mouth sores, and employed as a diuretic. The fruits are eaten raw or cooked, while the leaves are used as sandpaper[1]. The leaves, applied externally, is used against fever, cough, colds, and is known to treat bacterial and fungal infections[2]. In an earlier study, we reported the isolation of ursolic acid and oleanolic acid from the twigs; and butyrospermol cinnamate and lutein from the leaves of *F. ampelas*[3].

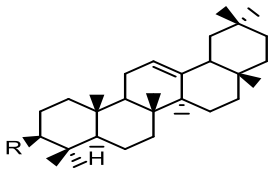
This study was conducted as part of our research on the chemical constituents of *Ficus* species found in the Philippines. Ten *Ficus* species, six of which are endemic to the Philippines have been studied[4-10]. We earlier reported the isolation of a new neohopane triterpene[4], furanocoumarin derivatives, bergapten and oxypeucedanin hydrate[5] which exhibited antimicrobial properties from *F. pumila*. In another study, we reported 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 *F. 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 *F. ulmifolia*[6]. Chemical investigation of the dichloromethane extracts of the leaves of two *Ficus species* 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 the leaves of *F. triangularis*[7]; and 3,5,4'-trihydroxy-6",6"- dimethylpyrano[2",3":7,6]flavanone, α-amyrin fatty acid ester, β-amyrin fatty acid ester, lupeol fatty acid ester, stigmast-4-en-3-one, β-sitosterol, and stigmasterol from the stems of *F. triangularis*[8]. *F. 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[9]. Recently, the isolation of lupenone, β-friedelinol, squalene, β-sitosterol, cycloeucalenol, lupeol, α-amyrin, and β-amyrin from *F. nervosa*[10], and 4-(2-hydroxyethyl)-2-methoxyphenol, β-sitosterol, *meso*-2,3-butanediol, (2*R*,3*R*)-2,3-butanediol and (2*S*,3*S*)-2,3-butanediol from *F. nota*[11] have been reported.

We report herein the isolation of squalene (**1**), β-amyrin fatty acid esters (**2a**), α-amyrin fatty acid esters (**2b**), β-

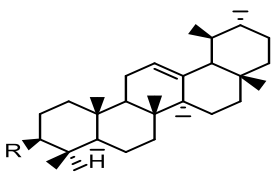
**Author for Correspondence: consolacion.ragasa@dlsu.edu.ph*



1



2a R = long-chain fatty acid esters



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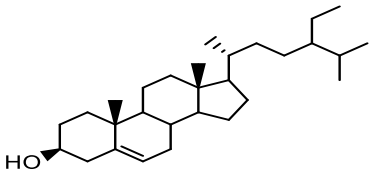
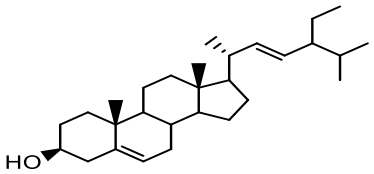
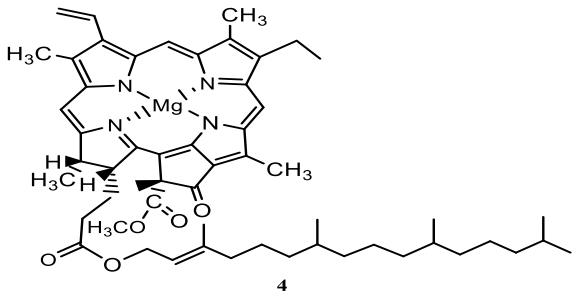


3a



3b



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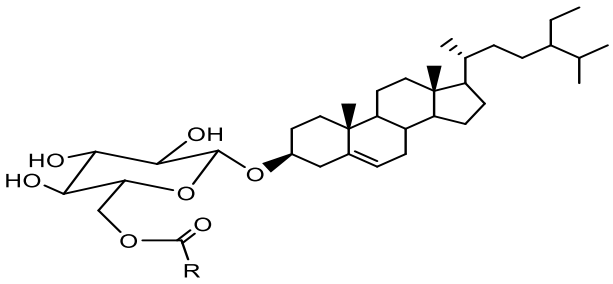

6 R = long-chain fatty acid alkyls

Figure 1: Chemical structures of squalene (**1**), β-amyrin fatty acid esters (**2a**), α-amyrin fatty acid esters (**2b**), β-sitosterol (**3a**), stigmasterol (**3b**), chlorophyll a (**4**), and β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**6**) from *F. ampelas*.

sitosterol (**3a**), stigmasterol (**3b**), chlorophyll a (**4**), saturated fatty acids (**5**) from the twigs; and **5**, β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**6**), and long-chain fatty alcohols (**7**) from the fruit of *F. ampelas*. To the best of our knowledge, this is the first report on the isolation of these compounds from *F. ampelas.*

MATERIALS AND METHODS

Sample Collection

The twigs and fruits of *F. ampelas* were collected from the riparian forest along Carmona River in the vicinity of the De La Salle University – Science and Technology Complex (DLSU-STC), Binan, Laguna in May 2015. The samples (collection #950) were authenticated by one of the authors (EHM).

General Isolation Procedure

A glass column 12 inches in height and 0.5 inch internal diameter was used for the chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. Five 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. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents from the Twigs of Ficus ampelas

The air-dried *F. ampelas* twigs (135 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.0 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed in petroleum ether to afford **1** (3 mg). The more polar fractions were combined and rechromatographed in 2.5% EtOAc in petroleum ether to yield **2a** and **2b** (4 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed using CH_2Cl_2. The less polar fractions were combined and rechromatographed using CH_2Cl_2 to afford a mixture of **3a** and **3b** (4 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed (2 ×) using CH_2Cl_2 to yield **4** (5 mg) after washing with petroleum ether, followed by Et_2O. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford **5** (2 mg).

Isolation of the Chemical Constituents from the Fruit of Ficus ampelas

The air-dried *F. ampelas* fruit (81.6 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (3.4 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume.

The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **7** (3 mg). The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to yield **5** (4 mg). The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to afford **6** (5 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the twigs and fruit of *F. ampelas* yielded **1**–**7**. The NMR spectra of **1** are in accordance with data reported in the literature for squalene[12]; **2a** for β-amyrin fatty acid esters[13]; **2b** for α-amyrin fatty acid esters[14]; **3a** for β-sitosterol[14]; **3b** for stigmasterol[14]; **4** for chlorophyll a[15]; **5** for saturated fatty acids[16]; **6** for β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters[17]; and **7** for long-chain fatty alcohols[18].

F. ampelas shares similar chemical characteristics with other members of the genus *Ficus* found in the Philippines: *F. pseudopalma, F. linearifolia, F. triangularis, F. odorata,* and *F. nervosa* which contained squalene (**1**); *F. pseudopalma, F. ulmifolia,* and *F. triangularis* which afforded β-amyrin fatty acid esters (**2a**); *F. ulmifolia* and *F. triangularis* which yielded α-amyrin fatty acid esters (**2b**); *F. ulmifolia, F. linearifolia, F. triangularis, F. nervosa,* and *F. nota* which provided β-sitosterol (**3a**); *F. ulmifolia*, *F. linearifolia* and *F. triangularis* which contained stigmasterol (**3b**); and *F. odorata* which yielded β-sitosteryl-3β-glucopyranoside-6β-*O*-fatty acid esters (**7**).

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