Secondary Metabolites from *Ficus ampelas* Burm.F.

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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-sitosteryl (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 as a diuretic. The fruits are eaten raw or cooked, while the leaves are used as sandpaper.² The leaves, applied externally, is used against fever, cough, colds, and is known to treat bacterial and fungal infections.³ In an earlier study, we reported the isolation of ursoic acid and oleanolic acid from the twigs; and butyrospermol cinnameate 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 studied⁴⁵¹⁰. We earlier reported the isolation of a new neohopane triterpene⁴, furanocoumarin derivatives, bergapten and oxypeucedanin hydrate⁵ which exhibited antimicrobial properties from *F. pumila*. In another study, we reported the isolation of squalene, polyrenol, 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, polyrenol, 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, polyrenol, linoleic acid and lutein from *F. linearifolia*; and ergosta-6,22-dien-3,5,8-triol, ergosterol, taxacerol, hop-22(29)-ene, squalene, β-sitosterol, stigmasterol, polyrenol, linoleic acid and lutein from the leaves of *F. triangularis*; and 3,5,4′-trihydroxy-6′,6″-dimethylpyran[2″,3″:7,6]lavanone, α-amyrin fatty acid ester, β-amyrin fatty acid ester, lupeol fatty acid ester, stig mast-4-en-3-one, β-sitosterol, and stigmasterol from the stems of *F. triangularis*.⁷ *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.⁸ Recently, the isolation of lupenone, β-friedelinol, squalene, β-sitosterol, cycloecualenal, lupeol, α-amyrin, and β-amyrin from *F. nervosa*, and 4-(2-hydroxyethyl)-2-methoxyphenol, β-sitosterol, meso-2,3-butanediol, (2R,3R)-2,3-butanediol and (2S,3S)-2,3-butanediol from *F. noto*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-
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$_2$Cl$_2$ 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

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.
The air-dried F. ampalas twigs (135 g) were ground in a blender, soaked in CH$_2$Cl$_2$ 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$_2$Cl$_2$ in 10% increments by volume. The 10% acetone in CH$_2$Cl$_2$ 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$_2$Cl$_2$ fraction was rechromatographed using CH$_2$Cl$_2$. The less polar fractions were combined and rechromatographed using CH$_2$Cl$_2$ 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$_2$Cl$_2$ to yield 4 (5 mg) after washing with petroleum ether, followed by Et$_2$O. The 50% acetone in CH$_2$Cl$_2$ 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 ampalas**

The air-dried F. ampalas fruit (81.6 g) were ground in a blender, soaked in CH$_2$Cl$_2$ 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$_2$Cl$_2$ in 10% increments by volume. The 40% acetone in CH$_2$Cl$_2$ 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$_2$Cl$_2$ fraction was rechromatographed (3 ×) using CH$_3$CN:Et$_2$O:CH$_2$Cl$_2$ (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. ampalas yielded 1–7. The NMR spectra of 1 are in accordance with data reported in the literature for squalene; 2a for β-amyrin fatty acid esters; 2b for α-amyrin fatty acid esters; 3a for β-sitosteryl; 3b for stigmasterol; 4 for chlorophyll a; 5 for saturated fatty acids; 6 for β-sitosteryl-3β-glucopyranoside-6β-O-fatty acid esters; and 7 for long-chain fatty alcohols. F. ampalas shares similar chemical characteristics with other members of the genus Ficus found in the Philippines: F. pseudopalma, F. linearifolia, F. triangulais, F. odorata, and F. nervosa which contained squalene (1); F. pseudopalma, F. ulmifolia, and F. nervosa which afforded β-amyrin fatty acid esters (2a); F. ulmifolia and F. triangulais which yielded α-amyrin fatty acid esters (2b); F. ulmifolia, F. linearifolia, F. triangulais, F. nervosa, and F. nota which provided β-sitosterol (3a); F. ulmifolia, F. linearifolia and F. triangulais which contained stigmasteryl (3b); and F. odorata which yielded β-sitosteryl-3β-glucopyranoside-6β-O-fatty acid esters (7).

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