

Chemical Constituents of *Ficus septica* Burm. F.

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Available Online: 15th November, 2016

ABSTRACT

Chemical investigation of the dichloromethane extracts of *Ficus septica* Burm.f. led to the isolation of β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (1), α -amyirin fatty acid esters (2), and a mixture of β -sitosterol (3a) and stigmasterol (3b) in a 5:2 ratios from the twigs; and 3a, β -amyirin (4), and long chain saturated fatty alcohols (5) from the leaves. The structures of 1-5 were identified by NMR spectroscopy.

Keywords: *Ficus septica*, Moraceae, β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters, α -amyirin fatty acid esters, β -sitosterol, stigmasterol, β -amyirin, fatty alcohols

INTRODUCTION

Ficus septica Burm. f., locally known as *hauili*, is a common fig found in thickets at low and medium altitudes from Northeast India, South China, Taiwan to Australia and throughout the Malasian region. In the Philippines, the decoction of the roots serves as toxin neutralizer, diuretic and asthma preventive agent, while a poultice is employed for boils. The fruits serve as laxative while their latex is used against herpes. Fresh leaves are sudorific, and when bruised with oil are used for headaches and applied externally as anti-rheumatic. A Philippine tribe, the Ifugaos, utilizes this plant for diarrhea, cough, malaria and stomach problems¹. One study has reported the isolation of seven triterpenes together with a rare triterpene derivative, 13,27-cycloursan-3 β -yl acetate, and two lignans from the non-alkaloidal fractions of the stem of *Ficus septica*². Other studies were conducted on the isolation and biological activities of alkaloids from *F. septica*. The new alkaloids: ficuseptines B–D, 10R,13aR-tylophorine N-oxide, 10R,13aR-tylocrebrine N-oxide, 10S,13aR-tylocrebrine N-oxide, 10S,13aR-isotylocrebrine N-oxide, and 10S,13aS-isotylocrebrine N-oxide and six known phenanthroindolizidine alkaloids were isolated from a methanol extract of the stems of *Ficus septica*³. A methanolic extract of *F. septica* leaves exhibited strong antibacterial and antifungal activities. Bioactivity-guided fractionation of this extract afforded two indolizidine alkaloids, 4,6-bis-(4-methoxyphenyl)-1,2,3-trihydroindolizidinium chloride and antofine⁴. The leaves of *F. septica* yielded phenanthroindolizidine N-Oxide, ficuseptine-A, together with eighteen known compounds.

Four of the isolated compounds, ficuseptine, (+)-tylophorine and a mixture of (+)-tylocrebrine and (+)-isotylocrebrine exhibited strong cytotoxic activity against NUGC and HONE-1⁵. Furthermore, the isolation of the alkaloids, ficuseptamines A, B, and C, together with 12 known alkaloids and an acetophenone derivative have been reported from a methanolic extract of the leaves of *F. septica*⁶. Two phenanthroindolizidine alkaloids, NSTPOG01 (tylophorine) and NSTPOG07 (ficuseptine-A) isolated from the leaves of *F. septica*, exhibited potent suppression of nitric oxide production. NSTPOG01 exerted its anti-inflammatory effects by inhibiting expression of the proinflammatory factors and related signaling pathways⁷. Twenty phenanthroindolizidine alkaloids, including ten new ficuseptines E-N and ten known compounds were isolated from the roots of *F. septica*. With the exception of dehydrotylophorine, all the compounds showed pronounced cytotoxic activity on gastric adenocarcinoma (NUGC) and nasopharyngeal carcinoma (HONE-1). In addition, 13aR-antofine exhibited ED₅₀ values of < 0.1 μ g/mL against L-1210, P-388, A-549 and HCT-8 cell lines⁸. The MeOH extract from the twigs of *F. septica* led to the isolation of a new secophenanthroindolizidine alkaloid and three known phenanthroindolizidine alkaloids. All compounds except the new alkaloid exhibited antimalarial activity against the 3D7 strain of *P. falciparum* with IC₅₀ values of 0.028–0.42 μ M. The new compound showed a moderate antimalarial activity⁹. 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

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to the Philippines have been studied¹⁰⁻¹⁸. We earlier reported the isolation of a new neohopane triterpene¹⁰, furanocoumarin derivatives, bergapten and oxypeucedanin hydrate¹¹ which exhibited antimicrobial properties from *F. pumila*. In another study, we reported the isolation of squalene, polyprenol, β -amyirin fatty acid ester, α -amyirin acetate, β -amyirin acetate, lupeol fatty acid ester, lupenone, oleanone, and ursenone from the leaves of *F. pseudopalma* and lutein, lupeol acetate, β -carotene, phytol, α -amyirin fatty acid ester, squalene, polyprenol, β -amyirin fatty acid ester, α -amyirin acetate, β -amyirin acetate, β -sitosterol and stigmasterol from the leaves of *F. ulmifolia*¹². Chemical investigation of the dichloromethane extracts of the leaves of two *Ficus* species led to the isolation of 11 α ,12 α -epoxyurs-14-en-3 β -yl acetate, β -amyirin, α -amyirin, 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*¹³; and 3,5,4'-trihydroxy-6",6"-dimethylpyrano[2",3":7,6]flavanone, α -amyirin fatty acid ester, β -amyirin fatty acid ester, lupeol fatty acid ester, stigmast-4-en-3-one, β -sitosterol and stigmasterol from the stems of *F. triangularis*¹⁴. *F. odorata* afforded β -sitosteryl-3 β -glucopyranoside-6'-*O*-palmitate, squalene, lutein, α -amyirin acetate, lupeol acetate, and β -carotene. β -Sitosteryl-3 β -glucopyranoside-6'-*O*-palmitate exhibited cytotoxicity against AGS cell line with 60.28% growth inhibition¹⁵. Recently, the isolation of lupenone, β -friedelinol, squalene, β -sitosterol, cycloeculenol, lupeol, α -amyirin, and β -amyirin from *F. nervosa*¹⁶; ursolic acid, oleanolic acid, butyrospermol cinnamate and lutein from *F. ampelas*¹⁷; 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. nota*¹⁸ have been reported. We report herein the isolation of β -sitosteryl-3 β -glucopyranoside-6'-*O*-fatty acid esters (1), α -amyirin fatty acid ester (2), and a mixture of β -sitosterol (3a) and stigmasterol (3b) in a 5:2 ratios from the twigs; and β -amyirin (4), 3a, and long chain saturated fatty alcohols (5) from the leaves of *F. septica*. The structures of 1–5 are presented in Fig. 1.

experimental

General Experimental Procedure

¹H NMR spectra were recorded in CDCl₃ on a Bruker Avance 400 in CDCl₃ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄ (Merck) and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming. All solvents used are analytical grade.

Sample Collection

Samples of the leaves and twigs of *Ficus septica* Burm.f. were collected from the De La Salle University – Science and Technology Complex (DLSU-STC) Complex, Leandro V. Locsin Campus, Biñan City, Laguna, Philippines in 2015. The samples 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 packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH₂Cl₂ (10% by volume increment) as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *R_f* value 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 of the twigs of *F. septica*

The air-dried twigs of *F. septica* (185.2 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (2.8 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment by volume. The 20% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield 2 (1 mg) after washing with petroleum ether. The 20% acetone in CH₂Cl₂ fractions was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford a mixture of 3a and 3b (3 mg) after washing with petroleum ether. The 40% acetone in CH₂Cl₂ fraction was rechromatographed (2 ×) using CH₃CN:Et₂O:CH₂Cl₂ (1.5:1.5:7, v/v) to yield 1 (2 mg) after trituration with petroleum ether.

Isolation of the chemical constituents of the leaves of *F. septica*

The air-dried leaves of *F. septica* (299.3 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (3.1 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment by volume. The 40% acetone in CH₂Cl₂ fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield 5 (4 mg). The more polar fractions were combined and rechromatographed (2 ×) using 15% EtOAc in petroleum ether to yield 3a (2 mg) after washing with petroleum ether. The 70% acetone in CH₂Cl₂ fraction was rechromatographed (3 ×) using CH₃CN:Et₂O:CH₂Cl₂ (0.5:0.5:9, v/v) to yield 4 (1 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the different parts of *F. septica* yielded 1–5. The NMR spectra of 1 are in accordance with data reported in the literature for β -sitosteryl-3 β -glucopyranoside-6'-*O*-fatty acid esters¹⁹; 2 for α -amyirin fatty acid ester²⁰, 3a for β -sitosterol²¹; 3b for stigmasterol²¹; 4 for β -amyirin²¹; and 5 for long chain saturated fatty alcohols²². The integrations of the ¹H NMR resonances for the olefinic protons of 3a at δ 5.33 (H-6)²¹ and 3b at δ 5.33 (H-6), 5.13 (dd, *J* = 8.4, 15.0 Hz, H-22) and 5.00 (dd, *J* = 8.4, 15.0 Hz, H-23)²¹ suggested that the ratio of 3a and 3b is about 5:2. Although no biological activity tests were conducted on the isolated

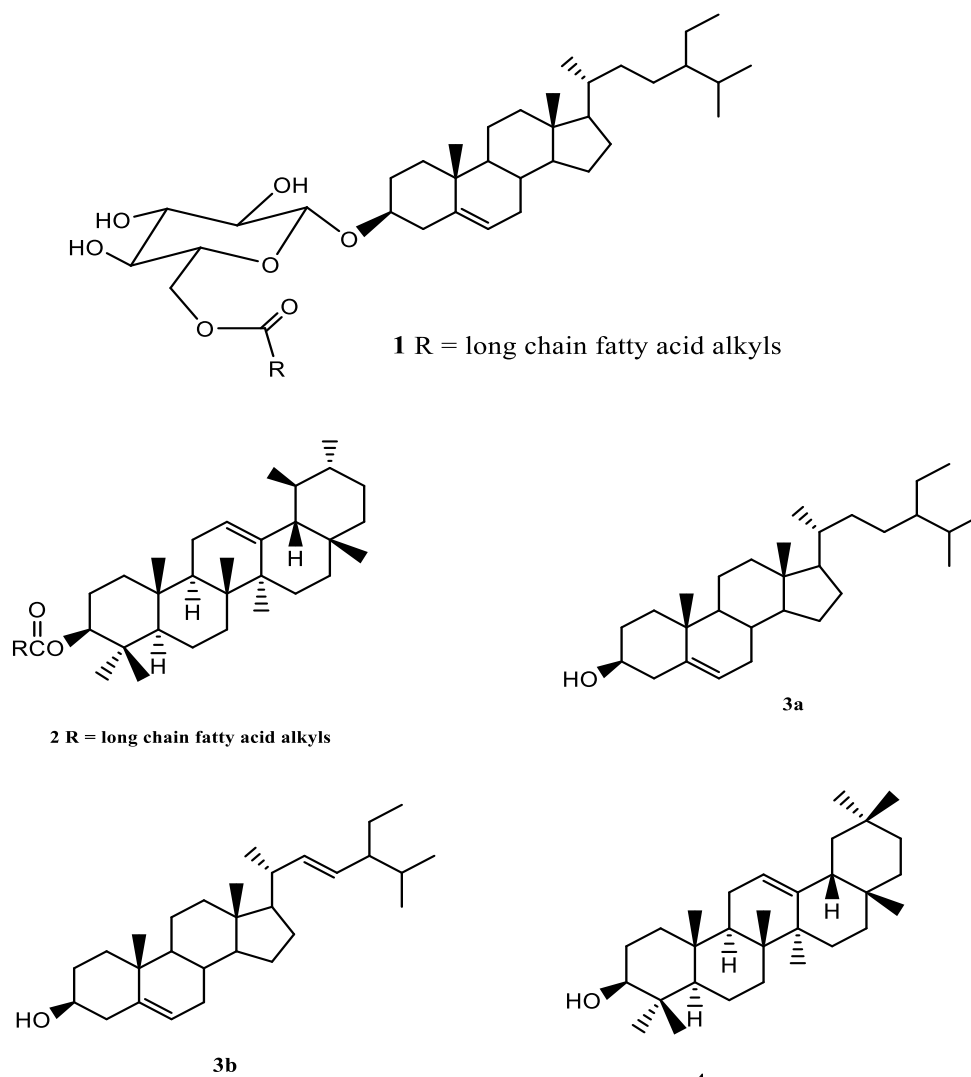


Figure 1: Chemical structures of β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (1), α -amyrin fatty acid ester (2), β -sitosterol (3a), and stigmasterol (3b), and β -amyrin (4) from *F. septica*.

compounds, a literature search of 1-5 revealed that these have diverse bioactivities. β -Sitosteryl-3 α -glucopyranoside-6'-O-palmitate (1) was reported to exhibit cytotoxicity against Bowes (melanoma) and MCF7 (breast) cancer cell lines with IC_{50} values of 152 μ M and 113 μ M, respectively²³. Furthermore, 1 exhibited cytotoxicity against human stomach adenocarcinoma (AGS) cell line with 60.28% growth inhibition²⁴. Compound 1 was found to exhibit potent anti-complement activity ($IC_{50} = 1.0 \pm 0.1 \mu$ M) as compared to the positive control, tiliroside ($IC_{50} = 76.5 \pm 1.1 \mu$ M)²⁵. On the other hand, α -amyrin, β -amyrin, and the 3-O-acyl derivatives of α -amyrin (2) and β -amyrin exhibited analgesic property²⁶⁻²⁷. β -Sitosterol (3a) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells²⁸. It was shown to be effective for the treatment of benign prostatic hyperplasia²⁹. It was also reported to attenuate β -catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis³⁰. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake³¹. It has also been reported to

induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells³². Stigmasterol (3b) shows therapeutic efficacy against Ehrlich ascites carcinoma in mice while conferring protection against cancer induced altered physiological conditions³³. It has been reported to lower plasma cholesterol levels, inhibit intestinal cholesterol and plant sterol absorption, and suppress hepatic cholesterol and classic bile acid synthesis in Wistar and WKY rats³⁴. In other studies, stigmasterol showed cytostatic activity against Hep-2 and McCoy cells³⁵, markedly inhibited tumour promotion in two stage carcinogenesis experiments³⁶, and exhibited antimutagenic³⁷, topical anti-inflammatory³⁸, antiosteoarthritic³⁹, and antioxidant⁴⁰ activities. α -Amyrin and β -amyrin (4) were reported to possess antiinflammatory⁴¹⁻⁴³ and analgesic⁴⁴⁻⁴⁵ properties. Triterpene 4 also showed antifungal activity against *A. rabiei* with an MIC value of 0.0156 mg/mL⁴⁶. The mixture of α -amyrin and 4 effectively reduced the elevated plasma glucose levels during the oral glucose tolerance test (OGTT). Furthermore, the mixture of these triterpenes at 100 mg/kg significantly decreased the VLDL and LDL

cholesterol and increased the HDL cholesterol⁴⁷. A review on the sources and biological activities of α -amyrin and 4 has been provided⁴⁸. Long chain saturated fatty alcohols (5) and lipids were reported to exhibit significant virucidal activities on RSV and parainfluenza virus⁴⁹. The antibacterial activity of fatty alcohols varied with the length of the aliphatic carbon chain. 1-Nonanol, 1-decanol and 1-undecanol exhibited bactericidal activity and membrane-damaging activity, while 1-dodecanol and 1-tridecanol showed the highest antibacterial activity, but had no membrane-damaging activity⁵⁰. In another study, saturated fatty alcohols, tetradecanol and pentadecanol exhibited the highest activity (MIC, 1.56 $\mu\text{g/ml}$) against a cariogenic bacterium, *Streptococcus mutans*⁵¹. The antimycobacterial activities of alcohols with C5 to C13 chain lengths were evaluated against *Mycobacterium smegmatis* mc2 155 and *M. tuberculosis* H37Rv. Results showed that the best activity was exhibited by a chain length of C10 which can partly be attributed to its ability to damage the complex cell envelope of *Mycobacteria*⁵².

CONCLUSION

The dichloromethane extracts of the twigs and leaves of *F. septica* yielded β -sitosterol-3 β -glucopyranoside-6'-*O*-fatty acid esters (1), α -amyrin fatty acid ester (2), β -sitosterol (3a), stigmaterol (3b), β -amyrin (4), and long chain saturated fatty alcohols (5). Most of the studies on *F. septica* reported on the alkaloids which were isolated from the leaves, stems and roots of the tree and their bioactivities. The non-alkaloidal compounds isolated in this study were reported to exhibit diverse bioactivities which may contribute to the known biological activities of *F. septica*.

ACKNOWLEDGEMENT

A research grant from De La Salle University Science Foundation, through the University Research Coordination Office, is gratefully acknowledged.

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