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Chemical Constituents of *Ficus ampelas*

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ABSTRACT

The dichloromethane extract of the air-dried twigs of *Ficus ampelas* afforded a mixture of ursolic acid (**1**) and oleanolic acid (**2**), while the leaves yielded butyrospermol cinnamate (**3**) and lutein (**4**). The structures of **1**-**3** were elucidated by extensive 1D and 2D NMR spectroscopy, while the structure of **4** was identified by comparison of its ^{1}H NMR data with those reported in the literature.

Keywords: *Ficus ampelas* Burm.f., Moraceae, ursolic acid, oleanolic acid, butyrospermol cinnamate, lutein

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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 forest. It 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].

There are no reported chemical and biological studies on *F. ampelas.* However, congeners of the tree have been studied for their chemical constituents and biological activities. As part of our continuing studies on the chemical constituents of trees belonging to the genus *Ficus,* the chemical constituents of the leaves and stems of *F. ampelas* were investigated. 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 *Ficus pumila* have been reported. A study of the chemical constituents of two endemic *Ficus* species, *Ficus pseudopalma* and *Ficus ulmifolia* led to the isolation of squalene, polyprenol, β-amyrin fatty acid ester, α-amyrin acetate, β-amyrin acetate, lupeol fatty acid ester, lupenone, oleanone, ursenone**,** lutein, lupeol acetate, β-carotene, phytol, α-amyrin fatty acid ester, β-sitosterol and stigmasterol [4]. Recently, chemical investigation of the dichloromethane extracts of the air-dried leaves of *Ficus triangularis* and an 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, lutein, ergosta-6,22-dien-3,5,8-triol, ergosterol, taraxerol and hop-22(29)-ene [5]. Moreover, another endemic tree, *Ficus odorata* yielded squalene, lutein, α-amyrin acetate, lupeol acetate, β-carotene and β-sitosteryl-3β-glucopyranoside-6'-*O*-palmitate which exhibited cytotoxicity against AGS cell line with 60.28% growth inhibition [6].

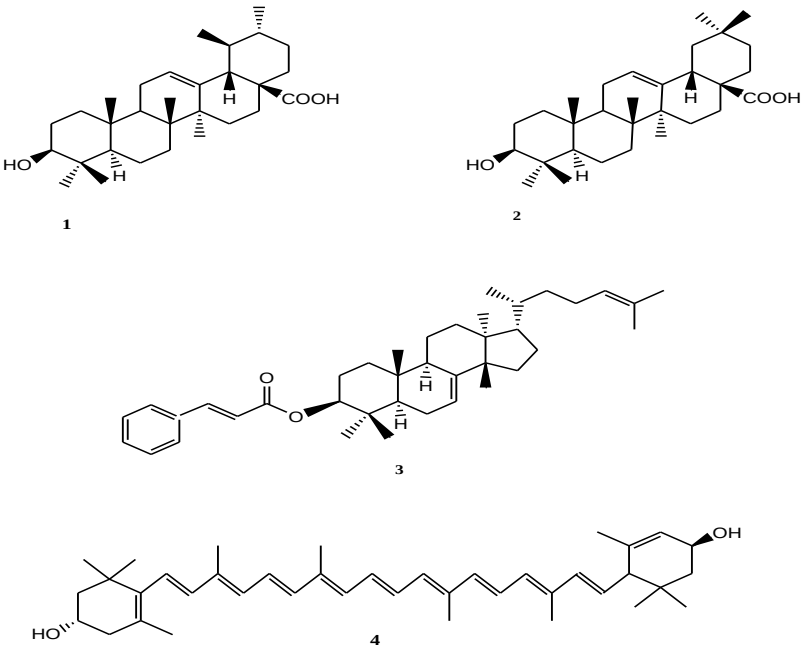

Figure 1. Chemical structures of compounds 1-4 from *Ficus ampelas.*

We report herein the isolation and structure elucidation of ursolic acid (1) and oleanolic acid (2) from the dichloromethane extract of the air-dried twigs of *F. ampelas.* The isolation and structure elucidation of butyrospermol cinnamate (3) and the isolation of lutein (4) from the leaves of *F. ampelas* are likewise reported. To the best of our knowledge this is the first report on the isolation of 1-4 (Figure 1) from *F. ampelas.*

MATERIALS AND METHODS

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

The leaves and twigs of *Ficus ampelas* Burm. f. were collected from a private farm in SitioUsiwan, Barangay Palola, Lucban, Quezon in March 2013. The farm includes a patch of secondary montane forest at 620 m asl amidst plantations of passion fruits and vegetables. Voucher specimens were collected and identified by one of the authors (EHM) with collection #901 and deposited at De La Salle University – Manila.

Isolation

The CH_2Cl_2 extract of the air-dried twigs (70.6) and leaves (75.6 g) of *F. ampelas* were ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford crude extracts of twigs (1.3 g) and leaves (6.9 g) which were each 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 80% acetone in CH_2Cl_2 fraction from the chromatography of the crude twigs extract was washed with petroleum ether and then rechromatographedusing 15% EtOAc in petroleum ether, followed by rechromatography (2 ×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to afford a mixture of 1and 2 (7 mg) after trituration with petroleum ether.

The 20% acetone in CH_2Cl_2 fraction from the chromatography of the crude leaves extract was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **3**(5 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction from the chromatography of the crude leaves extract was rechromatographed (4 ×) using

CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to provide 4 (10 mg) after washing with petroleum ether, followed by Et_2O.

RESULTS AND DISCUSSION

The structures of 1-3 were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR data with those of ursolic acid (1) [7], oleanolic acid (2) [7] and butyrospermol cinnamate (3) [8] reported in the literature. The structure 4 was identified by comparison of its ^{1}H NMR data with those reported in the literature for lutein [9].

Although no biological activity assays were conducted on the isolated compounds, literature search revealed that these compounds exhibited biological properties. A recent study identified the multiple cellular targets of ursolic acid (1) and underlines its application as a potent anti-inflammatory agent with the rapeutic potential [10]. Ursolic acid was found to induce apoptosis in tumor cells by activation of caspases and modulation of other pathways involved in cell proliferation and migration [11]. It decreases proliferation of cells and induces apoptosis, thereby inhibiting growth of tumor cells both *in vitro* and *in vivo* [12]. An earlier study reported that ursolic acid (1) and oleanolic acid (2) exhibited anti-tumor activity against human colon carcinoma cell line HCT15 with 1 showing stronger activity than 2 [13]. Oleanolic acid exhibited anti-inflammatory effects by inhibiting hyperpermeability, the expression of CAMs, and the adhesion and migration of leukocytes [14]. It showed anti-inflammatory activities through the inhibition of the HMGB1 signaling pathway [15]. Butyrospermol cinnamate (3) was reported to exhibit marked anti-inflammatory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 microg/ear) in mice and moderate inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) in Raji cells as a primary screening test for inhibitors of tumor promoters [8].

Dietary lutein (4) at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [16](Chew et al., 2003). Another study reported that the chemopreventive properties of all-*trans* retinoic acid (ATRA) and lutein may be attributed to their differential effects on apoptosis pathways in normal *versus* transformed mammary cells [17]. Furthermore, very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [18].

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