ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Triterpenes and Sterols from *Samanea saman.*

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ABSTRACT

The dichloromethane extract of *Samanea saman* afforded epilupeol (**1**), lupenone (**3**) and chlorophyll a (**3**) from the leaves; **2** and lupeol (**4**) from the peduncle; and **4,** unsaturated triglycerides (**5**), α-spinasterol (**6**), and α-spinasterone (**7**) from the twigs. The structures of **1**-**6** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature.

Keywords: *Samanea saman,* Fabaceae, epilupeol, lupenone, lupeol, α-spinasterol, α-spinasterone

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ISSN: 0975-8585

INTRODUCTION

Samanea saman (Jacq.) Merr. of the family Fabaceae is commonly known as acacia or rain tree. In the Philippines, it is widely planted as a shade tree. A decoction of the bark and leaves is used to treat diarrhea, acute bacillary dysentery, enteritis, colds, sore throat and headache. A decoction of fresh material is applied as external wash for anaphylactic dermatitis, eczema, skin pruritus [1]. *S. saman* was reported to exhibit potent antimicrobial, molluscicidal, nematicidal, hemolytic, and hypercholesterolemic properties [2]. Literature search on the chemical constituents of *S. saman* revealed the presence of octacosanol, α-spinasterol, β-D-glucose of α-spinasterol, kaempferol and pithecolobine from the different parts of the tree [3]. The volatile constituents of *S. saman* have been reported with palmitic acid (55.5%), 1,8-cineole (15.9%), and oleic acid (7.4%) as the major constituents [2, 4]. Another study reported the isolation of lupeol and epilupeol from the whole plant of *S. saman* [5].

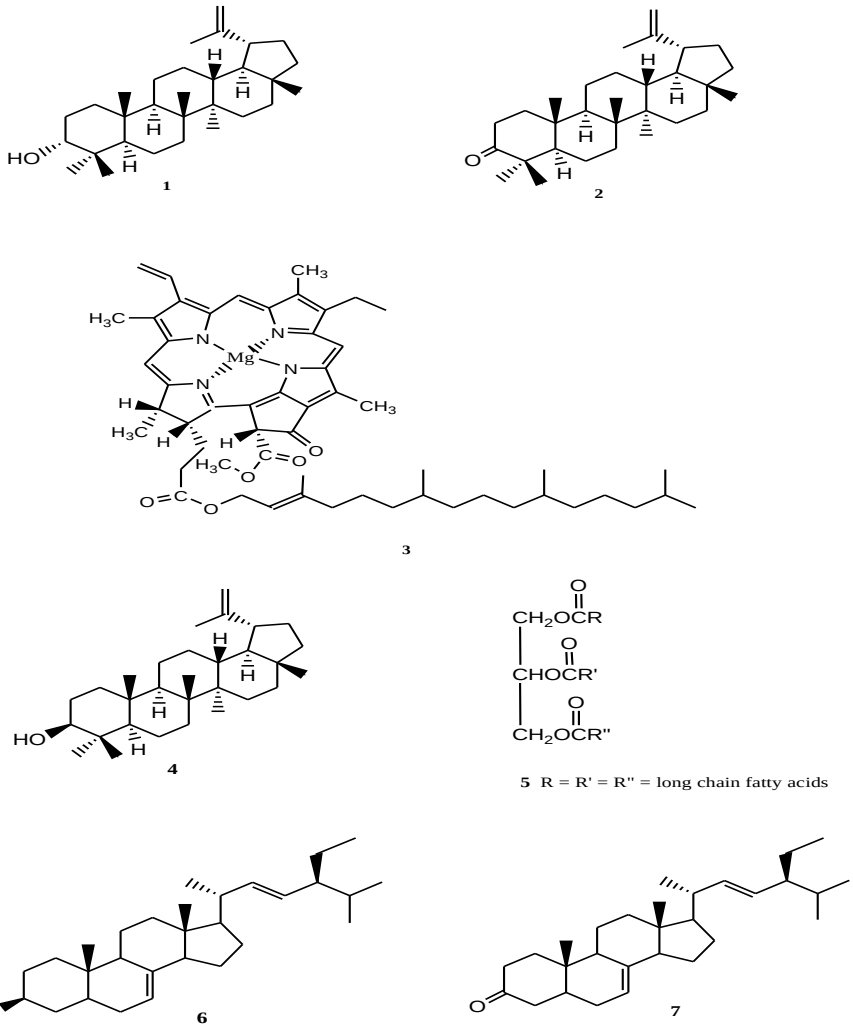

Fig. 1. Chemical constituents of *Samanea saman*: epilupeol (1), lupenone (2), chlorophyll a (3), lupeol (4), unsaturated triglycerides (5), α-spinasterol (6), and α-spinasterone (7).

ISSN: 0975-8585

This study was conducted as part of our research on the chemical constituents of trees found at the De La Salle University–Manila campus. We earlier reported the chemical constituents of *Barringtonia asiatica* [6-7], *Alstonia scholaris* [8], *Pterocarpus indicus* [9-10], and *Swietenia macrophylla* [11]. In this study, the isolation and identification of epilupeol (**1**), lupenone (**2**) and chlorophyll a (**3**) from the leaves; **2** and lupeol (**4**) from the peduncle; and **4**, unsaturated triglycerides (**5**), spinasterol (**6**), and spinasterone (**7**) from the twigs of *S. saman* are reported.

MATERIALS AND METHODS

General Experimental Procedures

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), while the TLC was performed with plastic-backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming.

A glass column (18 inches in height and 1.0 inch internal diameter) was packed with silica gel. The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10 % increments) as eluents. 100 mL fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* values were combined and rechromatographed. A glass column (12 inches in height and 0.5 inch internal diameter) was used for the rechromatography. 5mL fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Sample Collection

The sample was collected from the De La Salle University-Manila Campus in July 2013. It was identified as *Samanea saman* (Jacq.) Merr. at the Bureau of Plant Industry, Manila, Philippines.

Isolation of Chemical Constituents

The air-dried leaves (264 g), petioles (43 g) and twigs (165 g) of *S. saman* were separately ground in a blender, soaked in CH_2Cl_2 for three days and then filtered to afford crude extracts: leaves (4.34 g), petioles (0.42 g) and stems (1.56 g). The crude extracts were separately fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10 % increments) as eluents.

The 10% acetone in CH_2Cl_2 fractions from the chromatography of the crude leaves extract was rechromatographed (4 ×) in 5% EtOAc in petroleum ether to afford **2** (4 mg) after washing with petroleum ether. The 20% to 30% acetone in CH_2Cl_2 fractions from the chromatography of the crude petioles extract were combined and rechromatographed (3 ×) in

ISSN: 0975-8585

10% EtOAc in petroleum ether afford **1** (6 mg) after washing with petroleum ether. The 40% to 50% acetone in CH_2Cl_2 fractions from the chromatography of the crude leaves extract were combined and rechromatographed (4 ×) in 15% EtOAc in petroleum ether to afford **3** (4 mg) after washing with petroleum ether, followed by Et_2O.

The 30% to 50% acetone in CH_2Cl_2 fractions from the chromatography of the crude petioles extract were combined and rechromatographed (3 ×) in 10% EtOAc in petroleum ether, followed by 12.5% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were combined and rechromatographed (2 ×) in the same solvent to afford **4** (2 mg) after washing with petroleum ether. The fractions eluted with 12.5% EtOAc in petroleum ether were combined and rechromatographed (3 ×) in the same solvent to afford **2** (3 mg) after washing with petroleum ether.

The 10% acetone in CH_2Cl_2 fractions from the chromatography of the crude twigs extract was rechromatographed (3 ×) in 5% EtOAc in petroleum ether to afford **7** (2 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fractions from the chromatography of the crude twigs extract was rechromatographed (5 ×) in 5% EtOAc in petroleum ether to afford **5** (3 mg). The 30% to 40% acetone in CH_2Cl_2 fractions from the chromatography of the crude twigs extract were combined and rechromatographed (2 ×) in 10% EtOAc in petroleum ether, followed by 12.5% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were combined and rechromatographed (3 ×) in the same solvent to afford **4** (4 mg) after washing with petroleum ether. The fractions eluted with 12.5% EtOAc in petroleum ether were combined and rechromatographed (4 ×) in the same solvent to afford **6** (3 mg) after washing with petroleum ether.

Epilupeol (**1**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 33.24 (C-1), 25.39 (C-2), 76.26 (C-3), 37.52 (C-4), 49.02 (C-5), 18.27 (C-6), 34.13 (C-7), 41.02 (C-8), 50.20 (C-9), 37.28 (C-10), 20.77 (C-11), 25.11 (C-12), 38.01 (C-13), 42.90 (C-14), 27.37 (C-15), 35.58 (C-16), 43.01 (C-17), 48.23 (C-18), 48.03 (C-19), 151.04 (C-20), 29.84 (C-21), 40.00 (C-22), 28.24 (C-23), 22.13 (C-24), 15.91 (C-25), 15.96 (C-26), 14.62 (C-27), 18.00 (C-28), 109.29 (C-29), 19.28 (C-30).

Lupenone (**2**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 39.61 (C-1), 34.16 (C-2), 218.23 (C-3), 47.33 (C-4), 54.91 (C-5), 19.67 (C-6), 33.55 (C-7), 40.77 (C-8), 49.78 (C-9), 36.87 (C-10), 21.46 (C-11), 25.14 (C-12), 38.16 (C-13), 42.89 (C-14), 27.42 (C-15), 35.51 (C-16), 47.98 (C-17), 48.23 (C-18), 47.95 (C-19), 150.89 (C-20), 29.82 (C-21), 39.97 (C-22), 26.64 (C-23), 21.03 (C-24), 15.77 (C-25), 15.97 (C-26), 14.47 (C-27), 18.00 (C-28), 109.38 (C-29), 19.30 (C-30).

Lupeol (**4**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 38.70 (C-1), 27.42 (C-2), 79.01 (C-3), 38.86 (C-4), 55.29 (C-5), 18.32 (C-6), 34.28 (C-7), 40.83 (C-8), 50.43 (C-9), 37.17 (C-10), 20.92 (C-11), 25.14 (C-12), 38.05 (C-13), 42.83 (C-14), 27.44 (C-15), 35.58 (C-16), 43.00 (C-17), 47.99 (C-18), 48.30 (C-19), 150.99 (C-20), 29.85 (C-21), 40.00 (C-22), 27.98 (C-23), 15.36 (C-24), 16.11 (C-25), 15.97 (C-26), 14.54 (C-27), 18.00 (C-28), 109.31 (C-29), 19.30 (C-30).

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Spinasterol (**6**): ^{13}C NMR (150 MHz, $CDCl_3$): δ 37.13 (C-1), 31.46 (C-2), 71.06 (C-3), 37.98 (C-4), 40.24 (C-5), 29.65 (C-6), 117.45 (C-7), 139.56 (C-8), 49.43 (C-9), 34.21 (C-10), 21.54 (C-11), 39.54 (C-12), 43.28 (C-13), 55.11 (C-14), 23.01 (C-15), 28.51 (C-16), 55.88 (C-17), 12.04 (C-18), 13.04 (C-19), 40.83 (C-20), 21.37 (C-21), 138.17 (C-22), 129.42 (C-23), 51.24 (C-24), 31.92 (C-25), 21.09 (C-26), 19.02 (C-27), 25.40 (C-28), 12.25 (C-29).

RESULTS AND DISCUSSION

The dichloromethane extract of *Samanea saman* afforded epilupeol (**1**) [12], lupenone (**2**) [13] and chlorophyll a (**3**) [14] from the leaves; **2** and lupeol (**4**) [15] from the peduncles; and **4**, unsaturated triglycerides (**5**) [16], α-spinasterol (**6**) [17], and α-spinasterone (**7**) [18] from the twigs. The structures of **1**-**7** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in the literature [12-18].

Although bioassays were not conducted on the isolated compounds, there were previous studies that reported on their biological activities.

A mixture of lupenone (**2**) and caryophyllene oxide in a 1:4 ratio showed *in-vitro* typanocydal activity against epimastigotes forms of *T. cruzi* (IC_{50} = 10.4 µg/mL, FIC = 0.46) [19]. Triterpene **2** from *E. multiflora* stimulated melanogenesis in B16 murine melanoma cells through the inhibition of ERK1/2 activation, indicating that it can be used as a possible treatment for hypopigmentation diseases [20].

Lupeol (**4**) exhibited anticancer activities against pancreatic [21], prostate [22-23], ovarian [24], colorectal and myeloma [25], breast [26], stomach [27], cervical [26-28], lymphoma [28], leukemia [26, 29], melanoma and neuroblastoma [29], melanoma [25-27, 29-30], and lung [25-28, 30] cancers. Furthermore, **4** was also found to exhibit antimicrobial [31], anti-inflammatory [32], and anti-arthritic [33-34] properties.

α-Spinasterol (**6**) exhibited antiproliferative action against CACO-2 cell line with IC_{50} value of 60 nM/ml [35]. Moreover, **6** has significant therapeutic potential to modulate the development and/or progression of diabetic nephropathy [36]. It was also found to exhibit anti-angiogenic potential [37]. It was also reported to exhibit antioxidative [38], antinociceptive [39], anti-inflammatory [40], anti-ulcerogenic [41] and antitumor [42] effects.

ACKNOWLEDGMENT

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

ISSN: 0975-8585

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