

# 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),  $\alpha$ -spinasterol (6), and  $\alpha$ -spinasterone (7) from the twigs. The structures of 1-6 were identified by comparison of their <sup>1</sup>H and/or <sup>13</sup>C NMR data with those reported in the literature.

**Keywords:** *Samanea saman*, Fabaceae, epilupeol, lupenone, lupeol,  $\alpha$ -spinasterol,  $\alpha$ -spinasterone

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## 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*,  *$\alpha$ -spinasterol*,  *$\beta$ -D-glucose* of  *$\alpha$ -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].

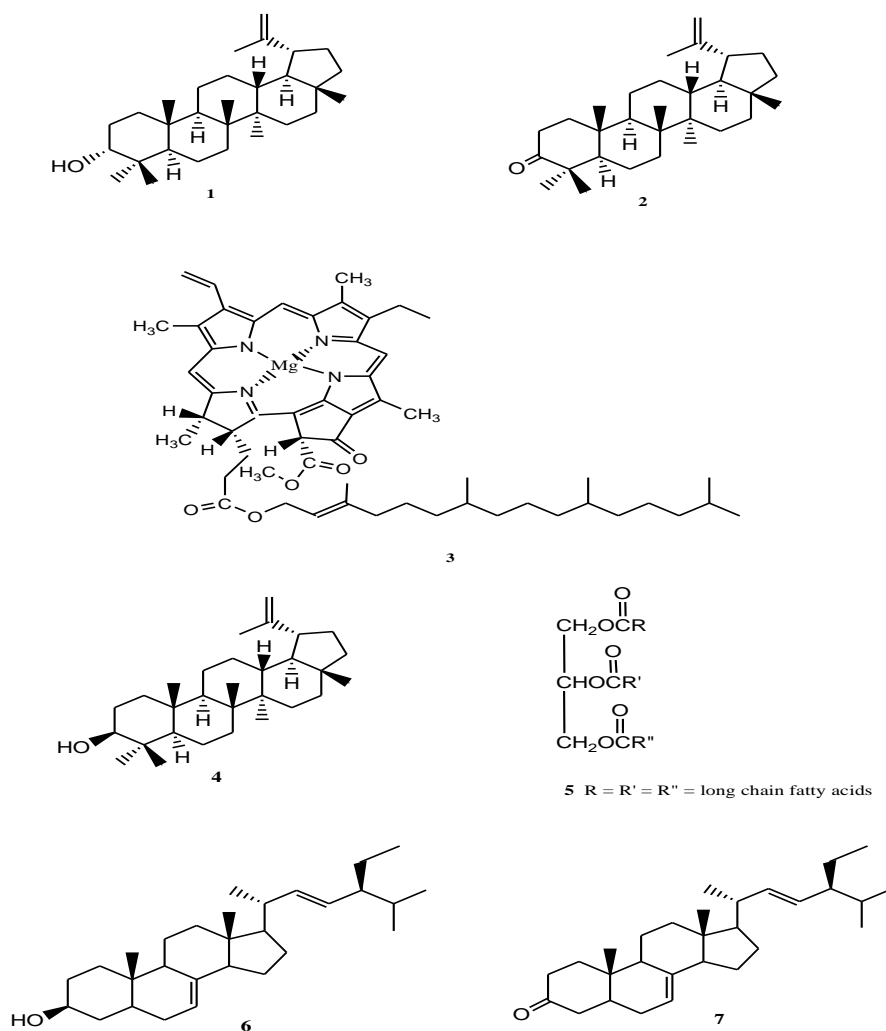


Fig. 1. Chemical constituents of *Samanea saman*: epilupeol (1), lupenone (2), chlorophyll a (3), lupeol (4), unsaturated triglycerides (5),  $\alpha$ -spinasterol (6), and  $\alpha$ -spinasterone (7).

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<sub>3</sub> at 600 MHz for <sup>1</sup>H NMR and 150 MHz for <sup>13</sup>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<sub>254</sub>. The plates were visualized with vanillin-H<sub>2</sub>SO<sub>4</sub> 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 *R<sub>f</sub>* 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<sub>2</sub>Cl<sub>2</sub> 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<sub>2</sub>Cl<sub>2</sub> 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<sub>2</sub>Cl<sub>2</sub> fractions from the chromatography of the crude **petioles** extract were combined and rechromatographed (3 ×) in

10% EtOAc in petroleum ether afford **1** (6 mg) after washing with petroleum ether. The 40% to 50% acetone in  $\text{CH}_2\text{Cl}_2$  fractions from the chromatography of the crude **leaves** extract were combined and rechromatographed (4  $\times$ ) in 15% EtOAc in petroleum ether to afford **3** (4 mg) after washing with petroleum ether, followed by  $\text{Et}_2\text{O}$ .

The 30% to 50% acetone in  $\text{CH}_2\text{Cl}_2$  fractions from the chromatography of the crude **petioles** extract were combined and rechromatographed (3  $\times$ ) 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  $\times$ ) 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  $\times$ ) in the same solvent to afford **2** (3 mg) after washing with petroleum ether.

The 10% acetone in  $\text{CH}_2\text{Cl}_2$  fractions from the chromatography of the crude **twigs** extract was rechromatographed (3  $\times$ ) in 5% EtOAc in petroleum ether to afford **7** (2 mg) after washing with petroleum ether. The 20% acetone in  $\text{CH}_2\text{Cl}_2$  fractions from the chromatography of the crude **twigs** extract was rechromatographed (5  $\times$ ) in 5% EtOAc in petroleum ether to afford **5** (3 mg). The 30% to 40% acetone in  $\text{CH}_2\text{Cl}_2$  fractions from the chromatography of the crude **twigs** extract were combined and rechromatographed (2  $\times$ ) 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  $\times$ ) 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  $\times$ ) in the same solvent to afford **6** (3 mg) after washing with petroleum ether.

**Epilupeol (1):**  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  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):**  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  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):**  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  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).

**Spinasterol** (6):  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ ):  $\delta$  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],  $\alpha$ -spinasterol (6) [17], and  $\alpha$ -spinasterone (7) [18] from the twigs. The structures of 1-7 were identified by comparison of their  $^1\text{H}$  and/or  $^{13}\text{C}$  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* typanocidal activity against epimastigotes forms of *T. cruzi* ( $\text{IC}_{50}$  = 10.4  $\mu\text{g}/\text{mL}$ ,  $\text{FIC}$  = 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-arthritis [33-34] properties.

$\alpha$ -Spinasterol (6) exhibited antiproliferative action against CACO-2 cell line with  $\text{IC}_{50}$  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.

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