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**Research Article**

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# Chemical constituents of *Schizostachyum lumampao*

**Consolacion Y. Ragasa[1*,2], Andrew Philip U. Chua[2] and Chien-Chang Shen[3]**

[1]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines*
[2]*Chemistry Department, De La Salle University, Taft Avenue, Manila, Philippines*
[3]*National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei, Taiwan*

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**ABSTRACT**

*The dichloromethane extract of the air-dried leaves of Schizostachyum lumampao (Blanco) Merr. afforded lutein (**1**), β-sitosterol (**2**), stigmasterol (**3**), chlorophyll a (**4**), phytol (**5**), (E)-3-alkenoic acid (**6**), triglycerides, fatty alcohols, and fatty acids, while the twigs yielded saturated fatty acid ester. The structures of **1**-**5** were confirmed by comparison of their $^{13}C$ NMR data with those reported in the literature. The structure of **6** was elucidated by 1D and 2D NMR spectroscopy.*

**Keywords**: *Schizostachyum lumampao*, Poaceae, buho, lutein, β-sitosterol, stigmasterol, chlorophyll a, phytol, (E)-3-alkenoic acid

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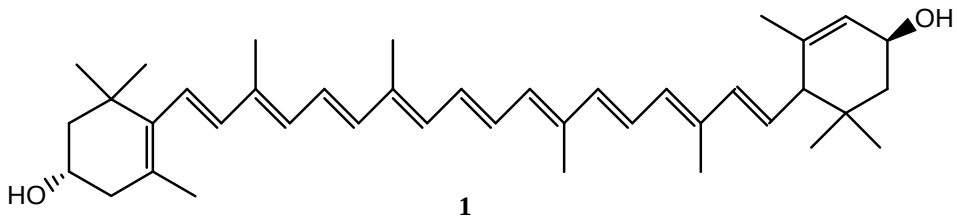
## INTRODUCTION

*Schizostachyum lumampao* (Blanco) Merr. of the family Poaceae is an endemic Philippine bamboo locally known as buho. This bamboo species is used as material for making surgical tools, agricultural and weaving implements, shelter constructions, socio-cultural activities and indigenous musical instruments [1]. Phytochemical screening of the ethanolic and aqueous leaf extracts of *S. lumampao* detected saponins, diterpenes, triterpenes, phenols, tannins, and flavonoids from both extracts, while phytosterols were only detected in the ethanolic extract [2]. There is no reported study on the chemical constituents and biological activity of *S. lumampao*.

We report herein the isolation of lutein (**1**), β-sitosterol (**2**), stigmasterol (**3**), chlorophyll a (**4**), phytol (**5**), (E)-3-alkenoic acid (**6**), triglycerides, fatty alcohols, and fatty acids from the leaves; and saturated fatty acid ester from the twigs of *S. lumampao*. This is the first report on the isolation of these compounds from *S. lumampao*.

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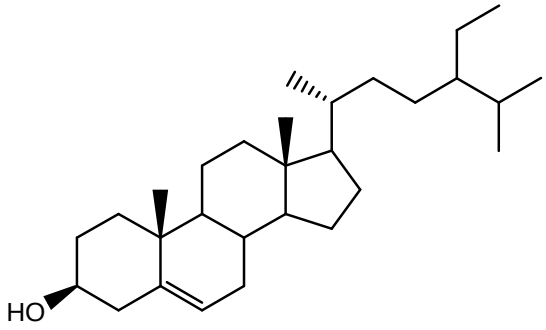


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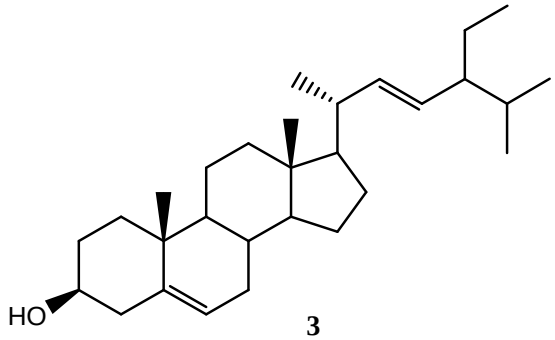


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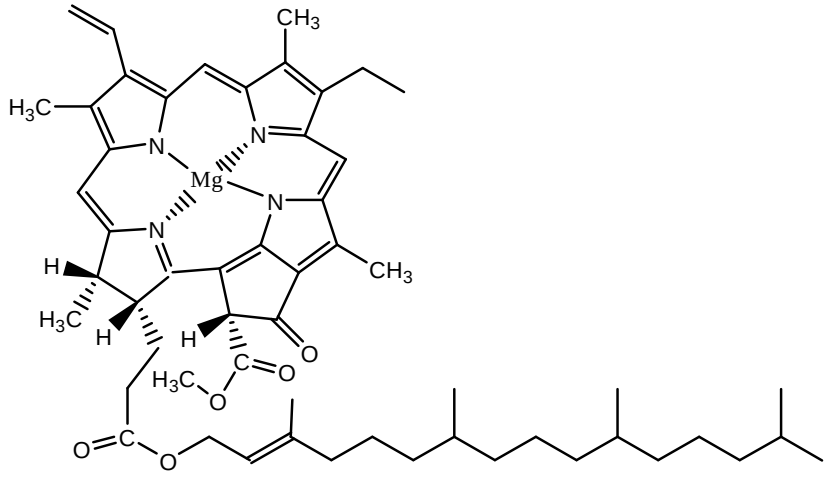


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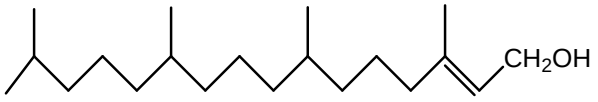


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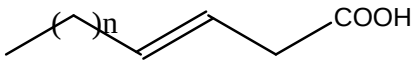


**6**

**Fig. 1. Chemical constituents of *S. lumampao* leaves: lutein (1), β-sitosterol (2), stigmasterol (3), chlorophyll a (4), phytol (5), and (E)-3-alkenoic acid (6)**

## EXPERIMENTAL SECTION

***General Experimental Procedures***

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for $^1H$ 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 sample was collected from Bataan, Philippines in October 2013. It was identified as *Schizostachyum lumampao* (Blanco) Merr. at the Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman, Quezon City.

***Isolation of Chemical Constituents***

The air-dried leaves (691 g) and twigs (173.8 g) of *S. lumampao* were separately ground in a blender, soaked in $CH_2Cl_2$ for three days, and then filtered. The filtrates were concentrated under vacuum to afford the crude extracts: 8.8 g (leaves) and 0.5 g (twigs). The crude extracts were fractionated by silica gel chromatography eluted with 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. Ten 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. Five milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

The 20% acetone in $CH_2Cl_2$ fraction from the chromatography of the crude leaf extract was rechromatographed by gradient elution in 5% EtOAc in petroleum ether, followed by10% EtOAc in petroleum ether, and finally 15% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether afforded triglycerides (12 mg) and fatty alcohols (10 mg), while the fractions eluted with 15% EtOAc in petroleum ether yielded phytol (**5**, 12 mg) and fatty acids (20 mg). The 30% acetone in $CH_2Cl_2$ fraction from the chromatography of the crude extract was rechromatographed in 15% EtOAc in petroleum ether, followed by $CH_2Cl_2$. The fractions eluted with 15% EtOAc in petroleum ether yielded (E)-3-alkenoic acid (**6**, 8 mg), while a mixture of **2** and **3** (18 mg) in 3:2 ratio was obtained from the fractions eluted with $CH_2Cl_2$, after washing with petroleum ether. The 40% acetone in $CH_2Cl_2$ fraction from the chromatography of the crude extract was rechromatographed in 15% EtOAc in petroleum ether, followed by $CH_2Cl_2$ to afford **4** (25 mg) after washing with petroleum ether, followed by $Et_2O$. The 50% acetone in $CH_2Cl_2$ fraction from the chromatography of the crude extract was rechromatographed (4×) in $Et_2O$:$CH_3CN$:$CH_2Cl_2$ (0.5:0.5:9 by volume ratio) to provide **1** (16 mg) after washing with petroleum ether, followed by $Et_2O$.

The $CH_2Cl_2$ and 10% acetone in $CH_2Cl_2$ fractions from the chromatography of the crude twigs extract were combined and rechromatographed by gradient elution in petroleum ether, followed by 5% EtOAc in petroleum ether, and finally 10% EtOAc in petroleum ether to afford saturated fatty acid ester (7 mg) after washing with petroleum ether.

## RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *Schizostachyum lumampao* afforded lutein (**1**), β-sitosterol (**2**), stigmasterol (**3**), chlorophyll a (**4**), phytol (**5**), (E)-3-alkenoic acid (**6**), triglycerides, fatty alcohols, and fatty acids. The structures of **1**-**5** were confirmed by comparison of their $^{13}C$ NMR data with those reported in the literature for lutein [3], β-sitosterol [4], stigmasterol [4], chlorophyll a [5] and phytol [6], respectively. The structure of **6** was elucidated by 1D and 2D NMR spectroscopy. The olefin at C-3 was deduced from the HMBC long-range correlations between H-3, $H_2$-2 and C-1; H-4, H-3 and C-2; and $H_2$-5, H-4 and C-3. A long chain (E)-3-alkenoic acid was indicated by a high intensity resonance at δ 1.24 for methylene protons. Compound **6** gave similar $^1H$ NMR data with those reported in the literature for trans-3-hexadecenoic acid [7]. The triglycerides [8], fatty alcohols [9], and fatty acids [9] were identified by similar $^1H$ NMR resonances with reported data. The dichloromethane extract of the twigs of *S. lumampao* yielded saturated fatty acid ester which was identified by comparison of its $^{13}C$ NMR data with those reported in the literature [10].

Although biological activities were not conducted on the isolated compounds from *S. lumampao*, literature search revealed that these compounds exhibited varied biological activities.

Dietary lutein (**1**), especially at 0.002%, inhibited tumor growth by selectively modulating apoptosis, and by inhibiting angiogenesis [11]. 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 [12]. A previous study reported that very low amounts of dietary lutein (0.002%) can efficiently decrease mammary tumor development and growth in mice [13].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [14]. It was shown to be effective for the treatment of benign prostatic hyperplasia [15]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [16]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [17]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [18].

Stigmasterol (**3**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [19]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [20].

Chlorophyll (**4**) and its various derivatives are used in traditional medicine and for therapeutic purposes [21]. Natural chlorophyll and its derivatives have been studied for wound healing [22], anti-inflammatory properties [23], control of calcium oxalate crystals [24], utilization as effective agents in photodynamic cancer therapy [25-27], and chemopreventive effects in humans [28-29]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [30].

Phytol (**5**) is an aromatic constituent in many fragrance compounds which may be found in cosmetics and non-cosmetic products [31]. It showed anticonvulsant activity by modulating neurotransmitter systems [32]. It exhibited antinociceptive and antioxidant activities [33], anti-inflammatory and antiallergic effects [34], immune stimulant [35], antimicrobial activity against *Mycobacterium tuberculosis* [36-37] and *Staphylococcus aureus* [38], antischistosomal effect against *Schistosomiasis mansoni* [39] and it is non toxic [40].

## CONCLUSION

Although *S. lumampao* has no known medicinal properties, the compounds isolated from the dichloromethane extract of the leaves of the plant were reported to exhibit varied biological activities [10-39]. Furthermore, the isolation of phytol, β-sitosterol and stigmasterol from the leaves of *S. lumampao* supported an earlier report on the presence of diterpenes and phytosterols from the leaves of the plant [2].

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