

Chemical Constituents of *Salacca wallichiana* Mart.

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ABSTRACT

Chemical investigation of the dichloromethane extracts of *Salacca wallichiana* Mart. led to the isolation of monogalactosyl diacylglycerols (**1**), β -sitosteryl-3 β -glucopyranoside-6'-*O*-fatty acid esters (**2**), β -sitosterol (**3**) and triacylglycerols (**4**) from the flesh of the fruit; **3** and β -sitosterone (**5**) from the female flower; a mixture of **3** and stigmaterol (**6**) from the roots and **2**, **4** and linoleic acid (**7**) from the seeds. The structures of **1-7** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Salacca wallichiana* Mart., Arecaceae, monogalactosyl diacylglycerols, β -sitosteryl-3 β -glucopyranoside-6'-*O*-fatty acid esters, β -sitosterol, triacylglycerols, β -sitosterone, stigmaterol, linoleic acid

INTRODUCTION

Salacca is a genus of about 20 species of palms native to Southeast Asia and the eastern Himalayas¹. *Salacca wallichiana* is one of the species of the genus *Salacca* which is found in Malaya, Myanmar, Sumatra, Thailand, and Vietnam². The plant which is locally known as paratugon could have been introduced to the Philippines from mainland Asia³. In Thailand, the plants are valued for their edible fruit and as a source of construction materials⁴. In Palawan, Philippines where the samples for this study were collected, the edible fruits are eaten by indigenous people in Southern Palawan, particularly the Pala'wan tribe. The rachis of this plant is utilized as wall divider and furniture, among others. The fruit of this plant was studied for its potential as a source of raw material for wine making and by products i.e. marmalade, prunes, candy, etc.⁵. Phytochemical studies on *S. wallichiana* revealed the presence of α -amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, steroids, and terpenoids. The moisture (11.83 %), ash (2.77 %), fibre (4.67 %), protein (0.21%), carbohydrate (80.52%) and vitamin C (91.72 mg/100g fresh weight) contents of *S. wallichiana* have been determined. The flesh of the fruit revealed the presence of K, Cl, Fe, Rb, S, Zn, Cu and Br, while the shell of the fruit contained K, Ca, Cl, Fe, S, Zn, Mn, Rb, Sr, Cu and Br.⁶ A number of chemical studies have been reported on the genus *Salacca*. Forty-six compounds were identified from *S. edulis* Reinw., among which methyl esters of branched-chain alkenoic and β -

hydroxy acids predominated. The most abundant components were methyl 3-hydroxy-3-methylpentanoate (25.0%) and methyl (*E*)-3-methylpent-2-enoate (23.4%)⁷. The snake fruit (*S. edulis* Reinw) Pondoh afforded methyl esters of butanoic acids, 2-methylbutanoic acids, hexanoic acids, pentanoic acids, and furaneol (4-hydroxy-2,5-dimethyl-3(2*H*)-furanone)⁸. GC-olfactometry was used to characterize the specific aroma of a pentane extract of snake fruit (*S. edulis* Reinw cv. *Pondoh*). Ten compounds, including two carboxylic acids, six methyl esters, an alcohol and a furaneol, were detected as the most characteristic odorants⁹. A study reported that the total phenolic and flavonoid contents of *S. zalacca* were in the range of 12.6-15.0 mg gallic acid equivalent/g and 4.9-7.1 mg catechin equivalent/g, respectively. Furthermore, *S. zalacca* exhibited antioxidant activity using DPPH assay¹⁰. We report herein the isolation of monogalactosyl diacylglycerols (**1**), β -sitosteryl-3 β -glucopyranoside-6'-*O*-fatty acid esters (**2**), β -sitosterol (**3**) and triacylglycerols (**4**) from the flesh of the fruit; **3** and β -sitosterone (**5**) from the female flower; a mixture of **3** and stigmaterol (**6**) from the roots and **2**, **4** and linoleic acid (**7**) from the seeds of *S. wallichiana*. The structures of **1-7** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of these compounds from *S. wallichiana*.

MATERIALS AND METHODS

General Experimental Procedure

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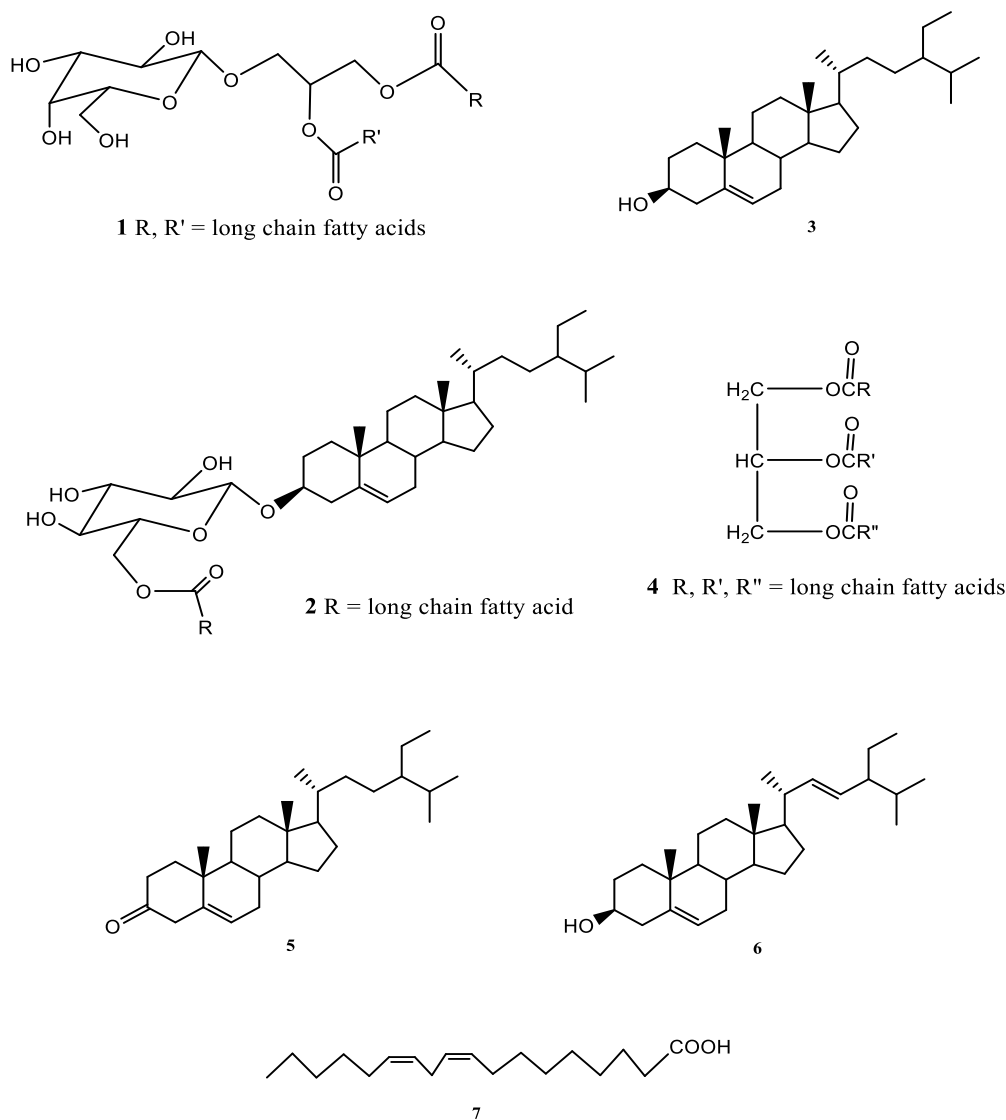


Figure 1: Chemical structures of monogalactosyl diacylglycerols (1), β -sitosterol-3-O-fatty acid esters (2), β -sitosterol (3), triacylglycerols (4), β -sitosterone (5), stigmasterol (6), and linoleic acid (7) from *S. wallichiana*.

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 F₂₅₄ and the plates were visualized by spraying with vanillin/ H_2SO_4 solution followed by warming.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. 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.

Plant material

The *Salacca wallichiana* Mart. fruits, flowers, roots and seeds were collected from the Western Philippines University campus, Aborlan, Palawan, Philippines in September 2015. The plant was identified by John Dransfield of the Royal Botanic Gardens Kew, UK³.

Isolation of the Chemical Constituents of the Flesh of the Fruit

The air-dried *S. wallichiana* flesh of the fruit (68.89 g) was ground in an osterizer, soaked in CH_2Cl_2 for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.71 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The CH_2Cl_2 and 10% acetone in CH_2Cl_2 fractions were combined and

rechromatographed using 5% EtOAc in petroleum ether to afford **4** (5 mg). The 20% acetone in CH₂Cl₂ fraction was rechromatographed using 15% EtOAc in petroleum ether to afford **3** (4 mg) after washing with petroleum ether. The 40% acetone in CH₂Cl₂ fraction was rechromatographed using CH₃CN:Et₂O:CH₂Cl₂ (2:2:6, v/v) to yield **2** (4 mg) after trituration with petroleum ether. The 60% to 70% acetone in CH₂Cl₂ fractions were combined and rechromatographed using CH₃CN:Et₂O:CH₂Cl₂ (2.5:2.5:5, v/v) to yield **1** (7 mg) after trituration with petroleum ether.

Isolation of the Chemical Constituents of the Female Flower

The air-dried *S. wallichiana* female flower (205.9 g) was ground in an osterizer, soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (1.99 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. The 20% acetone in CH₂Cl₂ fraction was rechromatographed by gradient elution using 5% EtOAc in petroleum ether, followed by 7.5% EtOAc in petroleum ether, and finally, 10% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum ether were rechromatographed using the same solvent to afford **5** (3 mg) after washing with petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether were rechromatographed using 15% EtOAc in petroleum ether to yield **3** (5 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Roots

The air-dried *S. wallichiana* roots (177.5 g) were ground in an osterizer, soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.81 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. The 10% acetone in CH₂Cl₂ fraction was rechromatographed using 15% EtOAc in petroleum ether to yield a mixture of **3** and **6** (6 mg) after washing with petroleum ether.

Isolation of the Chemical Constituents of the Seeds

The air-dried *S. wallichiana* seeds (122.1 g) were ground in an osterizer, soaked in CH₂Cl₂ for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (0.23 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ (10% increment) as eluents. The CH₂Cl₂ fraction yielded **4** (12 mg) after washing with petroleum ether. The 20% acetone in CH₂Cl₂ fraction was washed with petroleum ether to afford **7** (10 mg). The 40% acetone in CH₂Cl₂ fraction was rechromatographed using CH₃CN:Et₂O:CH₂Cl₂ (2:2:6, v/v) to yield **2** (6 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the different parts of *S. wallichiana* yielded **1–7**. The NMR spectra of **1** are in accordance with data reported in the literature for monogalactosyl diacylglycerols¹¹. **2** for β -sitosterol-3 β -glucopyranoside-6 β -O-fatty acid esters¹². **3**

for β -sitosterol¹³. **4** for triacylglycerols¹⁴. **5** for β -sitosterone¹⁵. **6** for stigmasterol¹³. and **7** for linoleic acid¹⁶.

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