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Research Article

Chemical Constituents of *Brassica rapa Chinensis* L. and *Brassica rapa* var. *Parachinensis* (Baily) Hanelt

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ABSTRACT

Brassica rapa chinensis L. and *Brassica rapa* var. *parachinensis* (Baily) Hanelt were studied for their chemical constituents. The dichloromethane extracts of the leaves of *Brassica chinensis* L. afforded β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**1**), β-sitosterol (**2**), chlorophyll a (**3**) and phytyl fatty acid esters (**4**), while the leaves of *Brassica rapa* var. *parachinensis* (Baily) Hanelt yielded **4**, monogalactosyl diacylglycerol (**5**) and lutein (**6**). The structures of **1**-**6** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Brassica rapa chinensis* L., *Brassica rapa* var. *parachinensis*, Brassicaceae, β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters, β-sitosterol, chlorophyll a, phytyl fatty acid esters, monogalactosyl diacylglycerol, lutein

INTRODUCTION

Brassica rapa chinensis L locally known as Petchay and also known as Pak choi is a popular vegetable in the Philippines. It belongs to the family Brassicaceae which exhibit anti-cancer properties due to their high content of glucosinolates which hydrolyze to form the bioactive isothiocyanates[1,2]. An earlier study on Pak choi (*Brassica campestris* L. ssp chinensis var. communis) reported the isolation of the phenolic compounds, K-3-*O*-sophoroside-7-*O*-glucoside, K-3,7-di-*O*-glucoside. K-3-*O*-sophoroside, K-7-*O*-glucoside, K-3-*O*-(caffeoyl)sophoroside-7-*O*-glucoside, K-3-O-(methoxycaffeoyl) sophoroside-7-*O*-glucoside, K-3-*O*-(sinapoyl)-sophoroside-7-*O*-glucoside, K-3-O-(*p*-coumaroyl)-sophoroside-7-*O*-glucoside, 3-caffeoylquinic acid, ferulic acid, sinapic acid, 2-disinapoylgentiobiose, 1-sinapoyl-2-feruloylgentiobiose, 1,2,2'-trisinapoylgentiobiose, and 1,2'-disinapoyl-2-feruloylgentiobios[3,4]. Another study reported that temperature affects the carotenoids and glucosinolate contents of Pak choi (*B. rapa* L. spp. chinensis var. communis)[5]. Furthermore, the carotenoids, ascorbic acid, minerals and total glucosinolates in leafy pakchoi (*B. rapa* L. *chinensis*) are affected by season and variety[6]. Pak choi (*B. rapa* L. ssp. *chinensis*) yielded quercetin (3.2-6.1 mg /100 g), isorhamnetin (8.1-35.1 mg/100 g) and kaempferol (36.0-102.6 mg/100 g)[7]. *Brassica rapa* var. *parachinensis* (Baily) Hanelt, also known as Choi sum is the Chinese flowering cabbage which is one of the most popular vegetables in China[8]. A previous study reported the levels of chlorophylls from *B. rapa chinensis* and *B. rapa* var. *parachinensis*[9].

This study is part of our research on the chemical constituents of vegetables belonging to the family Brassicaceae. We earlier reported the isolation of sterols, triglycerides and essential fatty acid constituents of *Brassica oleracea* varieties, *Brassica juncea* and *Raphanus sativus*[10]. We report herein the isolation of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**1**), β-sitosterol (**2**), chlorophyll a (**3**), and phytyl fatty acid esters (**4**) from *B. rapa chinensis* L., while the leaves of *B. rapa* var. *parachinensis* (Baily) Hanelt afforded **4**, monogalactosyl diacylglycerol (**5**) and lutein (**6**). The chemical structures of **1**-**6** are presented in Fig. 1,

MATERIALS AND METHODS

General Experimental Procedure

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.

Plant material

Brassica rapa chinensis L. and *Brassica rapa* var. *parachinensis* (Baily) Hanelt were collected from Benguet, Mountain Province, Philippines in October

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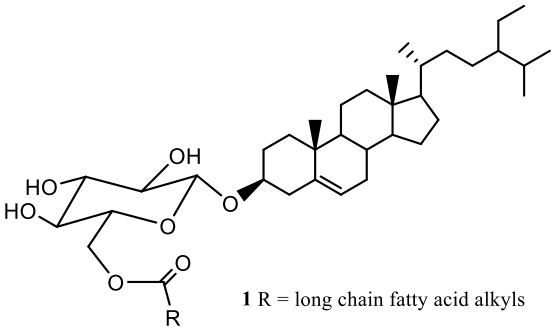

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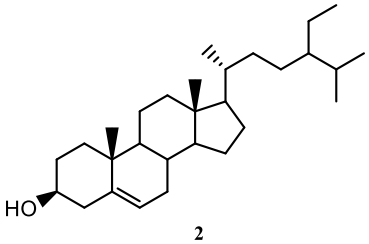

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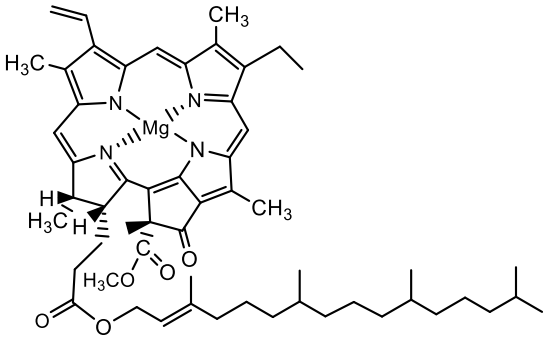

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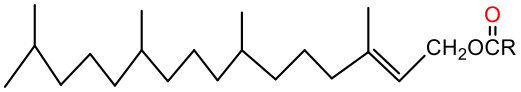

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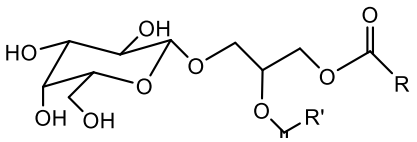

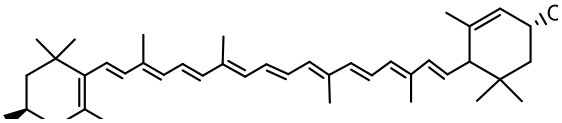

Figure 1: Chemical structures of β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid esters (**1**), β-sitosterol (**2**), chlorophyll a (**3**), and phytyl fatty acid esters (**4**) from *Brassica rapa chinensis* L.; and monogalactosyl diacylglycerol (**5**) and lutein (**6**) from *Brassica rapa* var. *parachinensis* (Baily) Hanelt.

2015 and authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 20 inches in height and 2.0 inches internal diameter was packed with silica gel. The crude extract from the leaves were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. One hundred milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *Rf* 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.

Isolation of the Chemical Constituents of B. rapa chinensis

The freeze-dried leaves of *B. rapa chinensis* (65.8 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 (2.03 g). The extract was chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone at 10% increment by volume as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 1% EtOAc in petroleum ether to afford **4** (2 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 10% EtOAc

in petroleum ether to yield **2** (5 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **3** (6 mg) after washing with petroleum ether, followed by Et_2O. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7, v/v) to yield **1** (3 mg) after trituration with petroleum ether.

Isolation of the Chemical Constituents of B. rapa var. parachinensis

The freeze-dried leaves of *B. rapa* var. *parachinensis* (94 g) were 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 (1.68 g). The extract was chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone at 10% increment by volume as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using petroleum ether to afford **4** (4 mg). The 80% acetone in CH_2Cl_2 fraction was rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v). The less polar fractions were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **6** (5 mg) after washing with petroleum ether, followed by Et_2O. The more polar fractions were combined and rechromatographed using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5, v/v) to afford **5** (4 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the leaves of *B. rapa chinensis* L. afforded **1-4,** while the leaves of *B. rapa* var. *parachinensis* (Baily) Hanelt yielded **4-6**. The NMR spectra of **1** are in accordance with data reported in the literature for β-sitosteryl-3β-glucopyranoside-6′-*O*-fatty acid ester[11]; **2** for β-sitosterol[12]; **3** for chlorophyll a [13]; **4** for phytyl fatty acid esters[14]; **5** for monogalactosyl diacylglycerol[15]; and **6** for lutein[16].

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