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# Chemical Constituents of *Brassica rapa* var. *parachinensis* (Baily) Hanelt. Flowers.

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**ABSTRACT**

Chemical investigation of the dichloromethane extract of the flowers of *Brassica rapa* var. *parachinensis* (Baily) Hanelt, also known as choi sum led to the isolation of β-sitosterol (**1**), chlorophyll a (**2**) and triacylglycerol (**3**). The structures of **1-3** were identified by comparison of their NMR data with those reported in the literature.

**Keywords:** *Brassica rapa* var. *parachinensis* (Baily) Hanelt, choi sum, β-sitosterol, chlorophyll a, triacylglycerol

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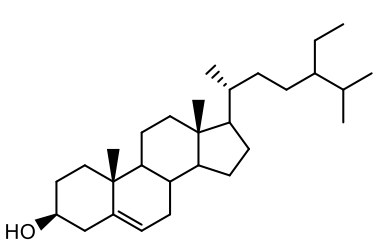
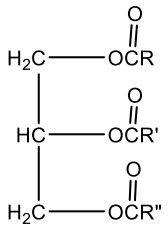
## INTRODUCTION

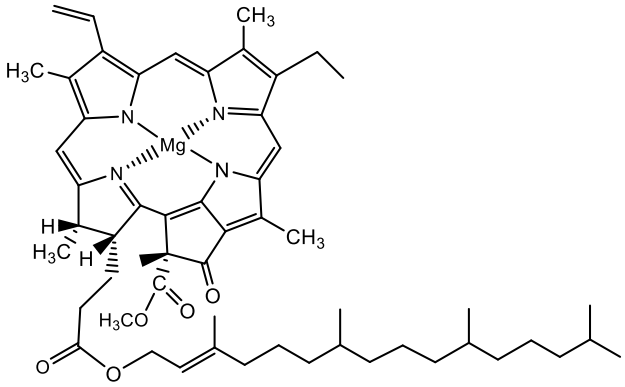
*Brassica rapa* var. *parachinensis* (Baily) Hanelt, also known as choi sum is the Chinese flowering cabbage which is one of the most popular vegetables in China [1]. It is cultivated in Benguet Mountain Province, Philippines and sold as a vegetable in local supermarkets and wet markets. A previous study reported the levels of chlorophylls from *B. rapa* var. parachinensis [2]. We earlier reported the isolation of phytyl fatty acid esters, monogalactosyl diacylglycerol and lutein from the leaves of *B. rapa* var. *parachinensis* (Baily) Hanelt [3]. We report herein the isolation of β-sitosterol (**1**), chlorophyll a (**2**) and triaylglycerol (**3**) from the flowers of *B. rapa* var. *parachinensis* (Baily) Hanelt. The chemical structures of **1**-**3** are shown in Fig 1.




**1**




**2** R, R', R" = linoleoyl, dilinolenoyl




**3**

**Figure 1: Chemical structures of β -sitosterol (1), chlorophyll a (2) and triacylglycerol (3) from *Brassica rapa* var. *parachinensis* (Baily) Hanelt.**

## 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* var. *parachinensis* (Baily) Hanelt was collected from Benguet, Mountain Province, Philippines in October 2015 and authenticated at the Botany Division, Philippine National Museum.

### General Isolation Procedure

A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Five 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. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

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

The freeze-dried flowers of *B. rapa* var. *parachinensis* (65.83 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 (1.85 g). The extract was chromatographed by gradient elution with using $CH_2Cl_2$, 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 10% EtOAc in petroleum ether to afford **2** (4 mg). The 20% acetone in $CH_2Cl_2$ fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **1** (3 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using $CH_3CN$:$Et_2O$:$CH_2Cl_2$ (0.5:0.5:9, v/v) to afford **3** (5 mg) after washing with petroleum ether, followed by $Et_2O$.

## RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the flowers of *B. rapa* var. *parachinensis* (Baily) Hanelt yielded **1**-**3.** The NMR spectra of **1** are in accordance with data reported in the literature for β-sitosterol [4]; **2** for triacylglycerol [5] and **3** for chlorophyll a [6]. The fatty acids attached to the triacylglycerol were identified as dilinolenic acid and linoleic acid based on resonance intensities and integrations for the methyl triplet at δ 0.96 (t, *J* = 7.8 Hz), the double allylic methylenes at δ 2.80 and the olefinic protons at δ 5.34 (m) for the alpha-linolenic acid; methyl triplet at δ 0.86 (t, *J* = 6.6 Hz), the double allylic methylene at δ 2.78 and the olefinic protons at δ 5.34 (m) for the linoleic acid [5].

Literature search revealed that **1-3** exhibited diverse biological activities. β-Sitosterol (**1**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [7]. It was shown to be effective for the treatment of benign prostatic hyperplasia [8]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench the radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [9]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [10]. It has also been reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [11].

Triacylglycerols (**2**) have been reported to significantly inhibit the tumor growth in the spleen of mice with intrasplenically implanted Lewis lung carcinoma [12]. Triacylglycerols exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T.* mentagrophytes [4]. Another study reported that triacylglycerols showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [13]. Linoleic acid belongs to the omega-6 fatty acids. It was reported to be a strong anticarcinogen in a number of animal models. It reduces the risk of colon and breast cancer [14] and lowers cardiovascular disease risk and inflammations [15]. Linolenic and linoleic acids inhibited parasites growth by 70% and 64% respectively, against *P. berghei* using the 4-day suppressive test. The two compounds, when used in combination, inhibited the parasites by 96% on day 4 of treatment [16]. Alpha-linolenic acid belongs to the omega-3 fatty acids. Omega-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA), and their fatty acid ethyl esters, exhibited strong antibacterial activity against various oral pathogens, including *Streptococcus mutans*, *Candida albicans*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis*. They also showed anti-inflammatory effects [17]. Peroxisome proliferator-activated receptor-γ (PPAR-γ) and cyclooxygenase-2 (COX-2) inhibition serve as two signaling pathways for the inhibitory effects of ALA on the human renal cell carcinoma (RCC) cell proliferation [18]. Another study reported that apoptosis of hepatoma cells was induced by the α-linolenic acidenriched diet which correlated with a decrease in arachidonate content in hepatoma cells and decreased cyclooxygenase-2 expression [19]. γ-Linolenic acid (GLA) and ALA exhibited greater than 90% cytotoxicity between 500 µM and 1 mM against all but two malignant micro-organ cultures tested in 5-10% serum. GLA and ALA killed tumor at concentrations of 2 mM and above in tests using 30-40% serum [20].

Chlorophyll (**3**) 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 [20], utilization as effective agents in photodynamic cancer therapy [24-27], and chemopreventive effects in humans [28-29]. A review on digestion, absorption and cancer preventive activity of dietary chlorophyll has been provided [30].

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