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Chemical Constituents of *Kappaphychus alvarezii* (Doty)

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ABSTRACT

*Chemical investigation of the dichloromethane extract of Kappaphychus alvarezii(Doty),led to the isolation of zeinoxanthin (**1**), β-carotene (**2**), chlorophyll a (**3**),cholesterol (**4**), phytyl fatty acid esters (**5**), triacylglycerols(**6**), and saturated fatty acids (**7**). The structures of **1**-**7**were identified by comparison of their NMR data with those reported in the literature.*

Keywords: Kappaphychus alvarezii, Solieriaceae, zeinoxanthin, β-carotene, chlorophyll a,cholesterol, phytyl fatty acid esters, triacylglycerols, saturated fatty acids

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INTRODUCTION

Kappaphychus alvarezii(Doty)(syn. *K. cottonii*;*Eucheuma cottonii*) is a species of red algae which is one of the most important commercial sources of carrageenans [1]. In the Northern Philippines, *K. alvarezii*is a popular edible seaweed used as salad and cooked with vegetables. A number of studies have been conducted on the chemical constituents and biological activities of *K. alvarezii*. High concentrations of saturated fatty acids with palmitic acid and stearic acid as the major constituents have been reported [2]. A new natural product, 2-carboxy-8-methoxy-naphthalene-1-ol was isolated from *K. alvarezii* [3].Another study reported that *K. alvarezii* is rich in protein(16.2% w/w), fiber (29.4% w/w), carbohydrates (27.4% w/w), unsaturated fatty acids (44.5%), saturated fatty acids (37.0%), and a good source of minerals, containing 0.16% calcium, 0.033% iron and 0.016% zinc [4]. *K. alvarezii* powder is used in the preparation of spice to enhance its nutritional quality due to the ash, protein, crude fiber, Vitamin E, niacin and Vitamin B2 contents of the seaweed [5]. The antioxidant properties *K. alvarezii* was attributed to carrageenan, polyphenols, β-carotene, and vitamins C and E which help scavenge free radicals [6]. Another study reported that high lectin contents (185-338 $\mu g \cdot g^{-1}$ dry alga) and hemagglutinating activity in rabbit blood (1281,365 $HU \cdot mL^{-1}$) were observed inthe three morphotypesof *K. alvarezii*[7]. The antioxidant potential of the ethanol and water extracts of *K. alvarezii* showed a radical-scavenging activity with an IC_{50} values of 3.03 $mg \cdot mL^{-1}$ and 4.76 $mg \cdot mL^{-1}$, respectively [8].Furthermore, *K. alvarezii* decreased the atherogenic index (AI) indicating that the seaweed had a protective effect and could reduce the risk of cardiovascular disease [6].

This study was conducted to investigate the chemical constituents of the dichloromethane extract of *K. alvarezii*, a popular edible seaweed in the Northern Philippines. We report herein the isolation of zeinoxanthin (**1**), β-carotene

(**2**), chlorophyll a (**3**),cholesterol (**4**), phytyl fatty acid esters (**5**), triacylglycerols (**6**), and saturated fatty acids (**7**) from *K. alvarezii*.The chemical structures of **1**-**6**are presented in Fig. 1.

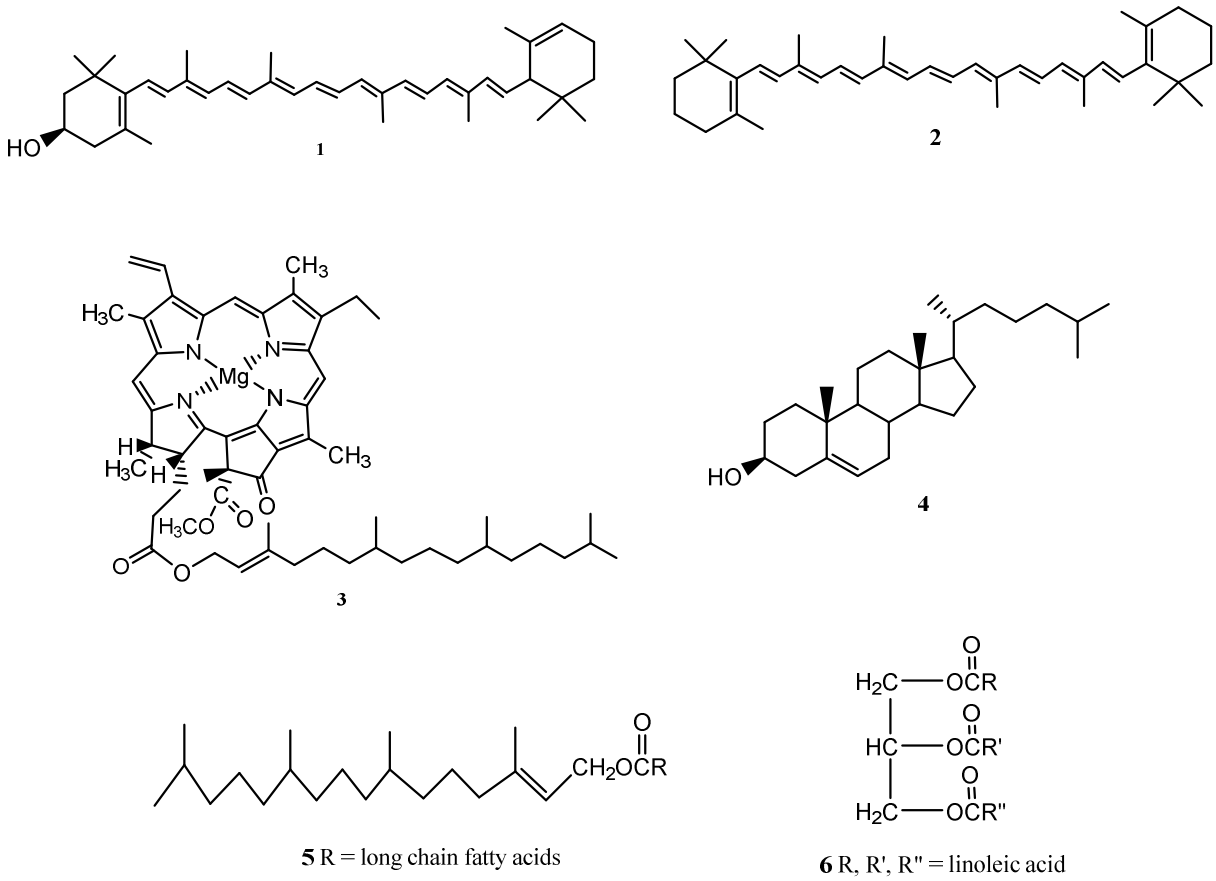

Fig. 1 Chemical structures of zeinoxanthin (1), β-carotene (2), chlorophyll a (3), cholesterol (4),phytyl fatty acid esters (5), and triacylglycerols (6)from*Kappaphychus alvarezii*

MATERIALS AND METHODS

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 $R_f$value 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.

Sample Collection

The seaweed was collected from Marinduque, Philippines in April 2016. The sample was authenticated as *Kappaphychus alvarezii*(Doty) at the Philippine National Museum.

Isolation

The freeze-dried (211.8 g) *K. alvarezii*was cut into small pieces, ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated from the filtrate under vacuum to afford a crude extract (0.7506g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as

eluents. The CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 5% EtOAc in petroleum ether to afford **2** (4 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **5**(3 mg).The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to yield **7** (4 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using15% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 7.5% EtOAc in petroleum ether to yield **6** (5 mg). The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **4**(6 mg).The 30% 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 yield afford **3** (5 mg) after washing with petroleum ether, followed by Et_2O. The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford **1**(2 mg) after washing with petroleum ether, followed by Et_2O.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *K. alvareziii*yielded **1**-**7**.The NMR spectra of **1** are in accordance with data reported in the literature forzeinoxanthin [9]; **2** for β-carotene [10]; **3** for chlorophyll a [11]; **4**for cholesterol [12]; **5** for phytyl fatty acid esters [13]; **6** for triacylglycerols [14] and **7** for saturated fatty acids [15].It is interesting to note that the major fatty acids in *K. alvarezii* are saturated fatty acids (**7**)which is in agreement with those reported in the literature [2]. Furthermore, the fatty acids attached to the triacylglycerols (**6**)are mostly saturated fatty acids.

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