Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (19):442-445
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Chemical Constituents of *Gracilaria tenuistipitata* var. liui Zhang and Xia

Consolacion Y. Ragasa[1,2,*], Virgilio D. Ebajo Jr.[1], Nancy Lazaro-Llanos[1], Robert Brkljača[3] and Sylvia Urban[3]

[1]Chemistry Department, De La Salle University, Taft Avenue, Manila, Philippines,
[2]Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines,
[3]School of Science (Discipline of Applied Chemistry and Environmental Science), RMIT University (City Campus), 124 La Trobe St Melbourne 3000, Victoria, Australia

---

ABSTRACT

*Chemical investigation of the dichloromethane extract of Gracilaria tenuistipitata var. liui Zhang and Xia has led to the isolation of monogalactosyl diacylglycerol (**1**), β-sitosterol (**2**), linoleic acid (**3**), and 2-hydroxyethyl benzoate (**4**). The structures of **1**-**4** were identified by NMR spectroscopy.*

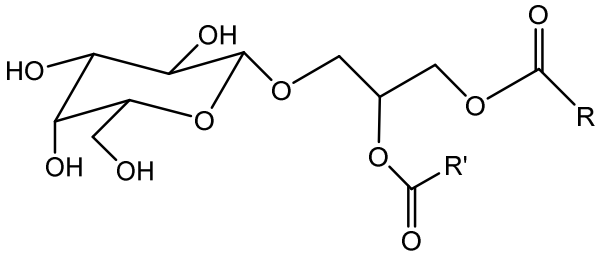
Keywords: *Gracilaria tenuistipitata*, Gracilariaceae, monogalactosyl diacylglycerol, β-sitosterol, linoleic acid, 2-hydroxyethyl benzoate

---

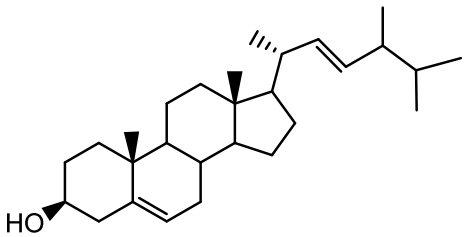
INTRODUCTION

Gracilaria tenuistipitata var. liui Zhang and Xia locally known as huganot is found in South-East Asia and China. It is used for extraction of good quality agar, in traditional medicine, and as a vegetable and fertilizer [1]. *G. tenuistipitata* was reported to contain β-carotene, zeaxanthin, β-cryptoxanthin and chlorophyll a [2]. In another study, *G. tenuistipitata* yielded saturated and monounsaturated fatty acid content [14:0, 16:0, 18:0, 18:1 (n-7) and 18:1 (n-9)] and the major pigments (violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll a and β-carotene) [3]. Furthermore, the ethanol extract of *G. tenuistipitata* concentration-dependently inhibited Hepatitis C virus replication via cyclooxygenase-2 suppression and reduced virus-induced inflammation [4].

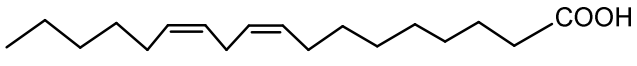
We report herein the isolation of monogalactosyl diacylglycerol (**1**), β-sitosterol (**2**), linoleic acid (**3**), and 2-hydroxyethyl benzoate (**4**) from *G. tenuistipitata* var. liui Zhang and Xia. To the best of our knowledge this is the first report on the isolation of **1**-**4** from *G. tenuistipitata*.



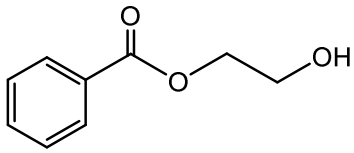
1 R, R' = long chain fatty acid alkyls



2



3



4

Fig. 1. Chemical structures of monogalactosyl diacylglycerol (1), β-sitosterol (2), linoleic acid (3), and 2-hydroxyethyl benzoate (4) from *G. tenuistipitata*.

MATERIALS AND METHODS

General Experimental Procedure

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). 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.

General Isolation Procedure

A glass column 18 inches in height and 1.0 inch internal diameter was used for the chromatography of the crude extracts. 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

Gracilaria tenuistipitata var. liui Zhang and Xia was collected from Bacoor Bay, Cavite, Philippines in October 2014. It was authenticated at the Philippine National Museum.

Isolation of the Chemical Constituents

The freeze-dried *G. tenuistipitata* (59.5 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 (0.2 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to yield **2** (3 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume ratio) to yield **3** (6 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1.5:1.5:7 by volume ratio) to afford **4** (2 mg) after washing

with petroleum ether. The 80% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using CH_3CN:Et_2O:CH_2Cl_2 (2.5:2.5:5 by volume ratio) to afford **1** (3 mg) after trituration with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of *G. tenuistipitata* has led to the isolation of **1-4**. The NMR spectra of **1** are in accordance with data reported in the literature for monogalactosyl diacylglycerol [5]; **2** for β-sitosterol [6]; **3** for linoleic acid [7]; and **4** for 2-hydroxyethyl benzoate [8].

Although no biological activity tests were conducted on the isolated compounds, a literature search of **1-3** revealed that these have diverse bioactivities.

Monogalactosyl diacylglycerols (**1**) and dinogalactosyl diacylglycerols are the most widespread non-phosphorous polar lipids in nature, constituting about 80% of membrane lipids in plants and more than half of all lipids in algae [9-10]. These compounds were reported to exhibit a number of biological properties, such as anti-tumor [11-12], anti-viral [13], algicidal [14] and anti-inflammatory [15-18]. Monogalactosyl diacylglycerols were also found to show cytotoxic and anti-inflammatory activity in RAW 264.7 macrophage cells with IC_{50} values of 60.06 and 65.70 μg/mL, respectively [19]. Compound **1** was also reported to exhibit anti-inflammatory activity in human articular cartilage [16]. It inhibited the growth of human melanoma cells in a dose-dependent manner with an IC_{50} value of 114 μM [20].

β-Sitosterol (**2**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [21]. It was shown to be effective for the treatment of benign prostatic hyperplasia [22]. 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 [23]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [24]. 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 [25].

Linoleic acid (**3**) 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 [26] and lowers cardiovascular disease risk and inflammations [27]. 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 [28].

Acknowledgement
A research grant from the De La Salle University Science Foundation through the University Research Coordination Office is gratefully acknowledged.

REFERENCES

[1] *Gracilaria tenuistipitata* (PROSEA) – Plant Use. Downloaded from uses.plantnet-project.org/en/*Gracilaria_tenuistipitata*_(PROSEA) on November 20, 2016.
[2] P. B. Torres, F. Chow, C. M. Furlan, F. Mandelli, A. Mercadante, D. Y. A. C. dos Santos, *Braz. J. Oceanogr.*, **2014**, 62(1), 57-63.
[3] E. Pinto, A. P. Carvalho, K. H. M. Cardozo, F. X. Malcata, F. M. dos Anjos, P. Colepicolo. *Rev. Bras. Farmacogn.*, **2011**, 21(2), 349-354.
[4] K. J. Chen, C. K. Tseng, F. R. Chang, J. I. Yang, C. C. Yeh, W. C. Chen, S. F. Wu, H. W. Chang, J. C. Lee. PLoS One. 2013;8(2):e57704.
[5] C. Y. Ragasa, V. A. S. Ng, N. Lazaro-Llanos, M. C. Tan, Brkljača R, Urban S. *Der Pharma Chemica*, **2015**, 7(7), 194-198.
[6] C. Y. Ragasa, G. S. Lorena, E. H. Mandia, D. D. Raga, C.-C. Shen. *Amer. J. Essent. Oils Nat. Prod.*, **2013**, 1(2), 7-10.
[7] C. Y. Ragasa, V. D. Ebajo Jr., R. G. Reyes, R. Brkljača, **S.** Urban. *Der Pharma Chemica*. **2015**, 7(10), 331-336.
[8] H. Sharghi, M. H. Sarvari. *J. Org. Chem.*, **2003**, 68, 4096-4099.
[9] S. V. Khotimchenko. *Chem. Nat. Compd*., **2002**, 38, 223–229.
[10] P. Dormann, C. Benning. *Trends Plant Sci*., **2002**, 7, 112–118.

[11] N. Maeda, Y. Kokai, T. Hada, H. Yoshida, Y. Mizushina. *Exp. Ther. Med.*, **2013**, 5, 17–22.
[12] N. Maeda, T. Hada, H. Yoshida, Y. Mizushina. *Curr. Med. Chem*., **2007**, 14, 955–967.
[13] L. M. Souza, G. L. Sassaki, M. T. V. Romanos, E. Barreto-Bergter. *Mar. Drugs*, **2012**, 10, 918–931.
[14] S. Hirao, K. Tara, J. Kuwano, F. Tanaka, F. Ishibashi. *Biosci. Biotechnol. Biochem.*, **2012**, 76, 372–374.
[15] A. Bruno, C. Rossi, G. Marcolongo, A. di Lena, A. Venzo, C. P. Berrie, D. Corda. *Eur. J. Pharmacol.*, **2005**, 7, 159–168.
[16] V. Ulivi, M. Lenti, C. Gentili, G. Marcolongo, R. Cancedda, F. D. Cancedda. *Arthritis Res. Ther.*, **2011**, 13, doi:10.1186/ar3367.
[17] A. H. Banskota, R. Stefanova, A. Sperker, R. Melanson, J. A. Osborne, S. J. B. O'Leary. *J. Appl. Phycol*., **2013**, 25, 951–960.
[18] A. H. Banskota, P. Gallant, R. Stefanova, R. Melanson, S. J. B. O'Leary. *Nat. Prod. Res.*, **2012**, 27, 1084–1090.
[19] G. Lopes, G. Daletos, P. Proksch, P. B. Andrade, P. Valentão. *Mar. Drugs*, 2014; 12, 1406-1418.
[20] T. L. Imbs. S. P. Ermakova, S. A. Fedoreyev, S. D. Anastyuk, T. N. Zvyagintseva. *Mar. Biotechnol.*, **2013**, 15, 606–612.
[21] A. B. Awad, M. Chinnman, C. S. Fink, P. G. Bradford. *Phytomed.*, **2007**, 14, 747–754.
[22] G. K. Jayaprakasha, K. K. Mandadi, S. M. Poulose, Y. Jadegoud, G. A. Gowda, B. S. Patil. *Bioorg. Med. Chem*., **2007**, 15, 4923-4932.
[23] A. A. Baskar, S. Ignacimuthu, G. Paulraj, K. Numair. *BMC Comp. Alt. Med.*, **2010**, 10, 24, 1-10.
[24] E. D. Jesch, J. M. Seo, T. P. Carr, J. Y. Lee. *Nutr Res*., **2009**, 29(12), 859-66.
[25] D. O. Moon, J. L. Kyeong, H. C. Yung, K. G. Young. *Int. Immunopharmacol.*, **2007**, 7, 1044-1053.
[26] J. Whelan. *Prostaglandins Leukot. Essent. Fatty Acids*, **2008**, 79(3-5), 165-167.
[27] P. Chan, G. N. Thomas, B. Tomlinson. *Acta Pharmacol. Sin.*, **2002**, 23(12), 1157-1162.
[28] P. Melariri, W. Campbell, P. Etusim, P. Smith. *Adv. Stud. Biol*., **2012**, 4(7), 333–349.