Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(6):229-232
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Sterols and triglyceride from the fruit of *Garcinia binucao*

Consolacion Y. Ragasa[1,2*], Oscar B. Torres[2], Elizabeth Marasigan[3] and Chien-Chang Shen[3]

[1]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines*
[2]*Chemistry Department, De La Salle University, Taft Avenue, Manila, Philippines*
[3]*Chiang Kai Shek College, 1477 Narra St, Tondo, Manila, Philippines*
[4]*National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei, Taiwan*

ABSTRACT

*Chemical investigation of the fruit of Garcinia binucao (Blanco) afforded a mixture of β-sitosterol (**1**) and stigmasterol (**2**) in a 3:2 ratio and monounsaturated and saturated triglycerides (**3**). The structures of **1-3** were identified by comparison of their ^{1}H NMR data with those reported in the literature.*

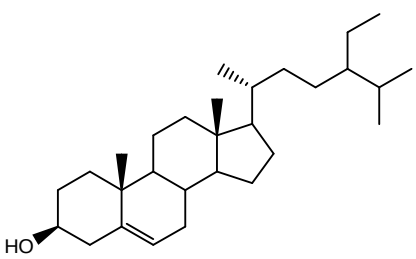
Keywords: *Garcinia binucao*, Clusiaceae, β-sitosterol, stigmasterol, triglycerides

---

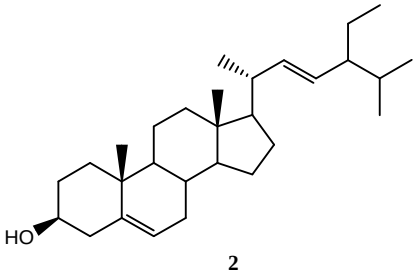
INTRODUCTION

Garcinia binucao (Blanco), locally known as batuan or binukaw is an indigenous Philippine tree. It is grown as a home garden tree and its fruits maybe eaten raw or used for souring local dishes [1]. *G. binucao* fruit is commonly used for various purposes such as food, forage, processing/preservation and condiment/ingredient [2]. An earlier study reported that *G. binucao* fruits have physicochemical properties and nutrient contents which are comparable to or even better than some fruits used for souring dishes [3]. Another study reported that *G. binucao* seeds were good sources of stearic and oleic acids [4]. Furthermore, the highest pectin qualities among the fruits tested were found in *Ficus nota* and *G. binucao* with an approximate gelatinizing degree of 140-200 and 80-120, respectively [5].

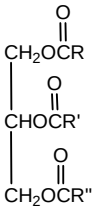
This study is part of our research on the chemical constituents of the genus *Garcinia* found in the Philippines. We earlier reported the isolation and structure elucidation of α-mangostin, gartanin and isomangostin from the pericarp of *Garcinia mangostana* L [6]. α-Mangostin exhibited high antibacterial activity against *S. aureus* [6]. We report herein the isolation and identification of β-sitosterol (**1**), stigmasterol (**2**), and triglycerides (**3**) (Fig. 1) from the fruits of *G. binucao*. To the best of our knowledge this is the first report on the isolation of **1-3** from *G. binucao.*



1



2



3 R, R', R" = long chain fatty acids

Fig. 1. Chemical constituents of *Garcinia binucao*: β-sitosterol (1), stigmasterol (2), and triglycerides (3)

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.

Sample Collection

Fruits of *Garcinia binucao* (Blanco) were collected from Sicogon Island, Carles, Iloilo, Philippines in March 2013. Voucher specimens were authenticated at the Botany Division of the 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 fruits were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane (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

The freeze-dried fruits (200 g) of *G. binucao* were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (12 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 5% EtOAc in petroleum ether to afford **3** (15 mg). The 40% to 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford a mixture of **1** and **2** (8 mg) after washing with petroleum ether.

β-Sitosterol (**1**): ^1H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.33 (dd, 1.8, 4.8, H-6), 0.66 (s, CH_3-18), 0.99 (s, CH_3-19), 0.92 (d, 6.6, CH_3-21), 0.84 (d, 6.6, CH_3-26), 0.83 (d, 6.0, CH_3-27), 0.87 (t, 6.0, CH_3-29).

Stigmasterol (**2**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.50 (m, H-3), 5.33 (dd, 1.8, 4.8, H-6), 0.68 (s, CH_3-18), 0.99 (s, CH_3-19), 1.01 (d, 6.6, CH_3-21), 5.13 (dd, 9.0, 15.0, H-22), 5.00 (dd, 9.0, 15.0, H-23), 0.84 (d, 6.6, CH_3-26), 0.83 (d, 6.0, CH_3-27), 0.80 (t, 6.0, CH_3-29).

Triglycerides (**3**): ^{1}H NMR (600 MHz, $CDCl_3$): δ 4.12 (H-1/H-3, 2H, dd, *J* = 6.0, 12.0 Hz); 4.27 (H-1/H-3, 2H, dd, *J* = 4.2, 12.0 Hz); 5.24 (H-2, m); 2.29 (H_2-2'); 1.60 (H_2-3'); 1.23-1.32 $(CH_2)_n$, 5.32 (CH=CH); 0.85 (CH_3, t, *J* = 6.6 Hz).

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the fruits of *G. binucao* yielded a mixture of β-sitosterol (**1**) [7] and stigmasterol (**2**) [8] in a 3:2 ratio and monounsaturated and saturated triglycerides (**3**) [9]. The 3:2 ratio of **1** and **2** was deduced from the integrations of the ^{1}H NMR resonances for the olefinic protons of **4** at δ 5.33 (dd, 1.8, 4.8, H-6) and **5** at δ 5.33 (dd, 1.8, 4.8, H-6), 5.13 dd (9.0, 15.0, H-22) and 5.00 dd (9.0, 15.0, H-23). The monounsaturated and saturated fatty acids esterified to glycerol could be oleic acid and stearic acid, respectively which were reported as major fatty acid constituents of *G. binucao* [4]. The structures of **1**-**3** were identified by comparison of their ^{1}H NMR data with those reported in the literature [7-9].

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

β-Sitosterol (**1**) was reported to exhibit growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [10]. It was shown to be effective for the treatment of benign prostatic hyperplasia [11]. It attenuated β-catenin and PCNA expression, as welll as quenched radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis [12]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [13]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake [14].

Stigmasterol (**2**) shows therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions [15]. It lowers plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats [16]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells [17], markedly inhibited tumour promotion in two stage carcinogenesis experiments [18], exhibited antimutagenic [19], topical anti-inflammatory [20], anti-osteoarthritic [21] and antioxidant [22] activities.

Triglycerides (**3**) exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes* [23]. Another study reported that triglycerides showed a direct relationship between toxicity and increasing unsaturation, which in turn correlated with increasing susceptibility to oxidation [24]. Oleic acid has been reported to be responsible for the reduction of blood pressure induced by olive oil [25]. It may hinder the progression of adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands [26]. Oleic acid inhibited cancer cell growth and survival in low metastatic carcinoma cells, such as gastric carcinoma SGC7901 and breast carcinoma MCF-7 cell lines [27].

CONCLUSION

Silica gel chromatography of the dichloromethane extract of the fruits of *G. binucao* yielded β-sitosterol (**1**), stigmasterol (**2**), and monounsaturated and saturated triglycerides (**3**) which were reported to exhibit diverse biological activities.

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

REFERENCES

[1] R. E. Coronel, **2011**. Important and Underutilized Edible Fruits of the Philippines. DA-BAR and UPLBFI. Manila, Philippines.
[2] R. T. Chua-Barcelo, *Asian Pac. J. Trop. Biomed*., **2014**, 4(Suppl 1), S525-38.
[3] E. S. Quevedo, A. C. Laurena, F. E. Merca, *Ann. Trop. Res*., **2013**, 35(2), 1-21.
[4] Z. A. M. Faisal, S. Sotheeswaran, C. Wijesundera, *J. Natl. Sci. Coun. Sri Lanka*, **1982**, 10(2), 221-3.
[5] H. W. Scharpenseel, *Landwirtschaftliche Forsch.* **1957**, 10, 191-4.
[6] C. Y. Ragasa, C. J. J. Crisostomo, K. D. C. Garcia, Shen, C.-C., *Philipp. Scient.*, **2010**, 47, 63-75.
[7] C. Y. Ragasa, V. A. S. Ng, M. M. De Los Reyes, E. H. Mandia, G. G. Oyong, Shen, C.-C., *Der Pharma Chemica*, **2014**, 6(5), 182-187.
[8] C. Y. Ragasa, J. L. Caro, L. G. Lirio, C.-C. Shen, *Res. J. Pharm. Biol. Chem. Sci.*, **2014**, 5(6), 344-348.
[9] C. Y. Ragasa, R. F. Galian, E. Lagueux, C.-C. Shen, *Res. J. Pharm. Biol. Chem. Sci*., **2014**, 5(6), 984- 987.
[10] A. B. Awad, M. Chinnman, C. S. Fink, P. G. Bradford, *Phytomed*., **2007**, 14, 747–754.
[11] G. K. Jayaprakasha, K. K. Mandadi, S. M. Poulose, Y. Jadegoud, G. A. Gowda, B. S. Patil, *Bioorg. Med. Chem.*, **2007**, 15, 4923–4932.
[12] A. A. Baskar, S. Ignacimuthu, G. Paulraj, K. Numair, *BMC Comp. Alt. Med*., **2010**, 10, 24.
[13] D. O. Moon, L. Kyeong-Jun, H. C. Yung, K. Gi-Young, *Int. Immunopharmacol*., **2007**, 7, 1044–1053.
[14] E. D. Jesch, J. M. Seo, T. P. Carr, J. Y. Lee, *Nutr. Res*., **2009**, 29(12), 859–66.
[15] T. Ghosh, T. K. Maity, J. Singh, *Orient. Pharm. Exp. Med.*, **2011**, 11, 41-49.
[16] A. K. Batta, G. Xu, A. Honda, T. Miyazaki, G. Salen, *Metabolism* **2006**, 55(3), 292-299.
[17] M. A. Gómez, M. D. García, M. T. Sáenz, *Phytother. Res*., **2001**, 15(7), 633-634.
[18] Y. Kasahara, K. Kumaki, S. Katagiri, K. Yasukawa, S. Yamanouchi, M. Takido, *Phytother. Res*., **1994**, 8(6), 327-331.
[19] L. J. Chu; P. J. Hee, B. Milos, K. Alexander, H. Y. Hwan, K. Byung-Soo, *Chem. Pharm. Bull*., **2005**, 53(5), 561-564.
[20] M. D. García, M. T. Sáenz, M. A. Gómez, M. A. Fernández, *Phytother. Res*., **1999**, 13(1), 78-80.
[21] O. Gabay, C. Sanchez, C. Salvat, F. Chevy, M. Breton, G. Nourissat, *Osteoarthritis Cartilage*, **2010**, 18(1), 106-116.
[22] S. Panda, M. Jafri, A. Kar, B. K. Meheta, *Fitoter*., **2009**, 80 (2), 123-126.
[23] 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.
[24] M. G. Ferruzzi, J. Blakeslee, *Nutr. Res*., **2007**, 27, 1–12.
[25] S. Teres, G. Barcelo-Coblijn, M. Benet, R. Alvarez, R. Bressani, J. E. Halver, P. V. Escriba, *Proc. Natl. Acad. Sci.* **2008**, 105(37), 13811–13816.
[26] W. B. Rizzo, P. A. Watkins, M. W. Phillips, D. Cranin, B. Campbell, J. Avigan, *Neurology* **1986**, 36(3), 357–61.
[27] S. Li, T. Zhou, C. Li, Z. Dai, D. Che, Y. Yao, L. Li, J. Ma, X. Yang, G. Gao, PLoS ONE, **2014**, 9(5), e97330. doi:10.1371/journal.pone.0097330.