Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(19):257-260
(http://derpharmachemica.com/archive.html)

Secondary Metabolites from *Dracontomelon dao* (Merr. & Rolfe)

Consolacion Y. Ragasa[1,2*], Julius Leonard A. Vivar [1], Mariquit M. De Los Reyes[3], and Ian A. van Altena[4]

[1]Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines
[2]Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines
[3]Biology Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines
[4]School of Environmental and Life Sciences, Faculty of Science and Information Technology, Chemistry, The University of Newcastle-Australia, Callaghan, NSW, 2308, Australia

---

ABSTRACT

*The dichloromethane extract of the leaves of Dracontomelon dao (Merr. & Rolfe) has led to the isolation of anacardic acid (**1**), β-sitosteryl-3β-glucopyranoside-6'-O-fatty acid esters(**2**), β-sitosterol (**3**), phytol (**4**), a mixture of phytyl fatty acid esters (**5**) and β-sitosteryl fatty acid esters (**6**), chlorophyll a (**7**), squalene (**8**),long-chain fatty alcohols (**9**), and long-chain hydrocarbons (**10**). The structures of **1**–**10**were identified by comparison of their NMR data with literature data.*

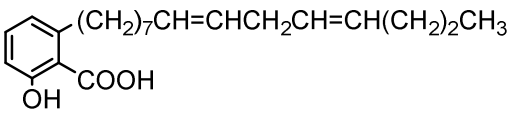
Keywords: *Dracontomelon dao*, Anacardaceae, anacardic acid, β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters, β-sitosterol, phytol, phytyl fatty acid esters, β-sitosteryl fatty acid esters, chlorophyll a, squalene, long-chain fatty alcohols, long-chain hydrocarbons

---

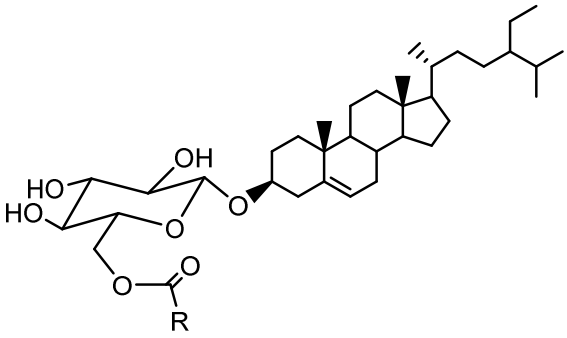
INTRODUCTION

Dracontomelon dao (Blanco) Merr. et Rolfe of the family Anacardiaceae is widely distributed throughout the South and Southeast Asia [1]. The mature fruits and kernel of the seeds are edible, while the flowers and young leaves are eaten as vegetables. The wood of *D.dao*is employed in light construction, and as timber and firewood [2]. The EtOAc extract of the leaves of *D. dao* showed strong anti-bacterial activity with an IC_{50} value of 98.5 µg/mL [3]. The crude methanolic extracts of the leaves, stem and root barks of*D. dao* exhibited a very good level of broad spectrum antibacterial activity, while the leaf extract exhibited antifungal activity [4]. The essential oil extracted from the skins of stem of *D. dao* yielded as major components, n-hexadecanoic acid (46.13%), octadecanoic acid (15.44%), (*E*)-9-octadecenoic acid (13.73%), and (*Z,Z*)-9,12-octadecadienoic acid (7.79%) [5].

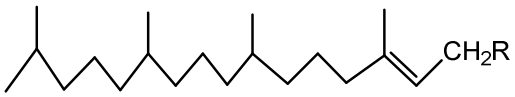
We report herein the isolation of anacardic acid (**1**), β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters(**2**), β-sitosterol (**3**), phytol (**4**), a mixture of phytyl fatty acid esters (**5**) and β-sitosteryl fatty acid esters (**6**), chlorophyll a (**7**), squalene (**8**), long-chain fatty alcohols (**9**), and long-chain hydrocarbons (**10**) from the leaves of *D. dao.* To the best of our knowledge this is the first report on the isolation of **1**–**10** from *D. dao*.



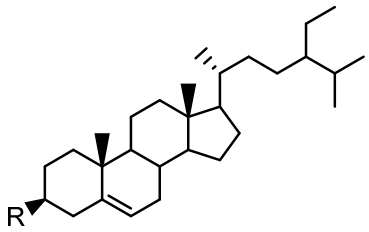
1



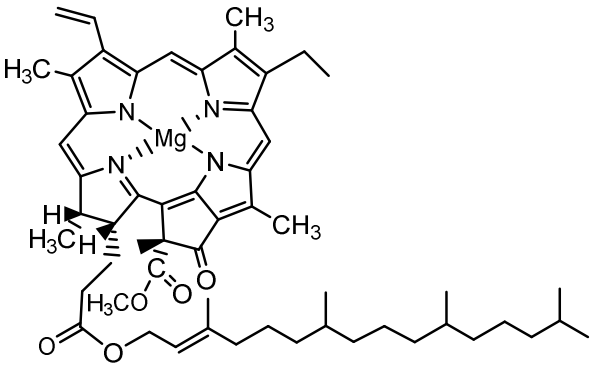
2 R = long-chain fatty acid alkyls



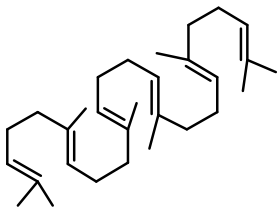
3 R = OH
5 R = long-chain fatty acid esters



4 R = OH
6 R = long-chain fatty acid esters



7



8

Fig. 1. Chemical structures of anacardic acid (1), β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters(2), β-sitosterol (3), phytol (4), phytyl fatty acid esters (5), β-sitosteryl fatty acid esters (6), chlorophyll a (7), and squalene (8) from the leaves of *D. dao*

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Ascend 400 in $CDCl_3$ at 400 MHz. Column chromatography was performed with silica gel 60 (70-230 mesh, Merck). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} (Merck) and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming. All solvents used are analytical grade.

Sample Collection

Samples of the petiole and twigs of *Dracontomelon dao* (Blanco) Merr. et Rolfe were collected from De La Salle University – Science and Technology Complex (DLSU-STC), Leandro V. Locsin Campus, Biñan City, Laguna, Philippines in March 2016. The samples were 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 chromatography. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume as eluents. 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 from the leaves of Dracontomelon dao

The air-dried *D. dao* leaves (199 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (6.20 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 in 10% increments by volume. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. The less polar fractions were combined and rechromatographed in petroleum ether to afford **10**(5 mg). The more polar fractions were combined and rechromatographed in 1% EtOAc in petroleum ether to yield **8** (10 mg).The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 2.5% EtOAc in petroleum ether to yield a mixture of **5** and **6** (4 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 5% EtOAc in petroleum ether to yield **9**(3 mg). The 40% acetone in CH_2Cl_2 fraction was rechromatographed using 7.5% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed (2 ×) using 10% EtOAc in petroleum ether to afford **4** (4 mg). The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to yield **7** (6 mg) after washing with petroleum ether, followed by Et_2O. The 50% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 10% EtOAc in petroleum ether to afford **3** (5 mg) after washing with petroleum ether.

The 60% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford **1** (3 mg) after washing with petroleum ether. The 70% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (1:1:8, v/v) to afford **2** (4 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of*D. dao* yielded **1**–**9**. TheNMR spectra of **1** are in accordance with data reported in the literature for anacardic acid [6]; **2** for β-sitosteryl-3β-glucopyranoside-6'-*O*-fatty acid esters[7]; **3** for β-sitosterol [8]; **4** for phytol [9]; **5** for phytyl fatty acid esters [10]; **6** for β-sitosteryl fatty acid esters [11]; **7** for chlorophyll a [12]; **8** for squalene [13]; **9** for long-chain fatty alcohols [14]; and **10**for long-chain hydrocarbons [15].

Dracontomelon dao extracts have been reported to exhibit antimicrobial properties. A number of studies were conducted on the antimicrobial activities of anacardic acid (**1**). The growth of two methicillin-resistant *Staphylococcus aureus* (MRSA strains was inhibited by 6 x 25 μg mL^{-1} of **1** [16]. The degree of unsaturation in the alkyl side chain of anacardic acids was reported to affect the antibacterial activity. The activity of methicillin against MRSA strains was significantly increased in combination with $C_{12:0}$-anacardic acid and the fractional inhibitory concentration index of this combination was 0.281 [17]. Furthermore, **1**inhibited spore germination in the fungus *Colletotrichum capsici*at 125–150 μg mL^{-1} [18]. In another study, anacardic acids exhibited activity against gram-positive bacteria, among them *Streptococcusmutans* and *Prorionibacterium acnes* were the most sensitive [19]. A review on the potential of anacardic acids and their semi-synthetic derivatives for antibacterial, antitumor, and antioxidant activities has been published [20].

The antibacterial activity of fatty alcohols (**8**)varied with the length of the aliphatic carbon chain. 1-Nonanol, 1-decanol and 1-undecanol exhibited bactericidal activity and membrane-damaging activity, while 1-dodecanol and 1-tridecanol showed the highest antibacterial activity, but had no membrane-damaging activity [21]. In another study, saturated fatty alcohols, tetradecanol and pentadecanol exhibited the highest activity (MIC, 1.56 μg mL^{-1}) against a cariogenic bacterium, *Streptococcus mutans* [22]. The antimycobacterial activities of alcohols with C5 to C13 chain

lengths were evaluated against *Mycobacterium smegmatis* mc2 155 and *M. tuberculosis* H37Rv. Results showed that the best activity was exhibited by a chain length of C10 which can partly be attributed to its ability to damage the complex cell envelope of *Mycobacteria* [23].

CONCLUSION

Dracontomelon dao extracts were reported to exhibit antimicrobial properties. Anacardic acid (**1**)and fatty alcohols (**8**)were isolated from the leaves of *D. dao* and have been reported to exhibit antimicrobial properties. Thus, these compounds may contribute to the antimicrobial properties of *D. dao* extracts.

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

REFERENCES

[1] F. Goeltenboth, A. Goeltenboth. Agroecological comparison of "rainforestation" farming sites on Leyte, Philippines. http://www.rainforestation.ph/resources/pdf/publications/Goeltenboth_and_Goeltenboth_2000_AgroEcological_Comparison.pdf on October 22, **2016**.
[2] Dao | Greenin Phiippines - RAFI.org.ph. Downloaded from rafi.org.ph/greenin-philippines/green-almanac/dao/ on October 22, **2016**.
[3] S. Liu, Y. Zhao, N. Zeng, T. Liu, Y. Zhang, B. Han, J. Li, L. Wang, R. Wang, M. Gong, Y. Li, X. Xiao. *J. Therm. Anal. Calorim*., **2014**, 116(1), 491–497.
[4] M. R. Khan, A. D. Omoloso. *Fitoterapia.*,**2002**, 73(4), 327–333.
[5] X.-F. Su, Z.-Y. Liang, Y.-X. Zhang. *Lishizhen Med. Mater. Med. Res.*,**2008**, 7, 045.
[6] C. Y. Ragasa, D. Borja, R. Bassig, J. A. Rideout. *Philipp. J. Sci.,* **2002**, 131(1), 9–15.
[7] V. A. S. Ng, E. M. Agoo, C.-C. Shen, C. Y. Ragasa. *J. Appl. Pharm. Sci*., **2015**, 5(Suppl 1), 12–17.
[8] 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.
[9] C. Y. Ragasa, E. S. C. Javier, I. G. Tan. *Philipp. J. Sci.*, **2003**, 132(1), 21–25.
[10] C. Y. Ragasa, J. L. Caro, L. G. Lirio, C. C. Shen. *Res. J. Pharm. Biol. Chem. Sci*., **2014**, 5(6), 344–348.
[11] C. Y. Ragasa, V. D. Ebajo Jr., M. M. De Los Reyes, E. H. Mandia, R. Brkljača, S. Urban. *J. Appl. Pharm. Sci*.,**2015**, 5(Suppl. 2), 16–21.
[12] C. Y. Ragasa, J. de Jesus. *Res. J. Pharm. Biol. Chem. Sci.*, **2014**, 5(3), 701–708.
[13]V. A. S. Ng, E. M. Agoo, C.-C. Shen, C. Y. Ragasa. *J. Appl. Pharm. Sci*.,**2015**, 5(Suppl. 1), 12-17.
[14] C. Y. Ragasa, M. P. Medecilo, C.-C. Shen. *Der Pharma Chemica*,**2015**, 7(7), 395–399.
[15] V. D. Ebajo Jr., R. Brkljača, S. Urban, C. Y. Ragasa. *J. Appl. Pharm. Sci.,* **2015**, 5(11), 69–72.
[16] H. Muroi, I. Kubo. *J. Appl. Bacteriol*., **1996**, 80(4), 387–94.
[17] I. Kubo, K.-I Nihei, K. Tsujimoto. *J. Agric. Food Chem.*,**2003**,51(26), pp 7624–7628.
[18] B. Prithiviraj, U. P. Singh, M. Manickam, A. B. Ray. *Can. J. Bot.*, **1997**, 75(1): 207-211.
[19] M. Himejima, I. Kubo. *J. Agric. Food Chem.*,**1991**,39(2), 418–421.
[20] F. B. Hamad, E. B. Mubofu. *Int. J. Mol. Sci*., **2015**, 16, 8569–8590.
[21] N. Togashi, A. Shiraishi, M. Nishizaka, K. Matsuoka, K. Endo, H. Hamashima, Y. Inoue. *Molecules*,**2007**,12(2),139–148.
[22] M. Hattori, K. Miyachi, S. Hada, N. Kakiuchi, F. Kiuchi, Y. Tsuda, T. Namba. *Chem. Pharm. Bull*., **1987**; 35(8), 3507–3510.
[23] K. Mukherjee, P. Tribedi, B. Mukhopadhyay, A. K. Sil. *FEMS Microbiol. Lett*.,**2013**, 338(2), 177–83.