

Chemical Constituents of *Dracontomelon Dao* (Blanco) Merr. et Rolfe

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ABSTRACT

Introduction: The leaves, twigs and flowers of *Dracontomelon dao* (Blanco) Merr. et Rolfe, an indigenous Philippine tree were investigated for their chemical constituents. **Methods:** The compounds were isolated by silica gel chromatography and their structures were identified by NMR spectroscopy. **Results:** Chemical investigation of *D. dao* led to the isolation of cardol (1), β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (2), β -sitosteryl fatty acid esters (3), and a mixture of β -sitosterol (4a) and stigmasterol (4b) from the petiole; 1, a mixture of 4a and 4b, anacardic acid (5), triacylglycerols (6), monoacylglycerol (7), long-chain fatty acid esters (8), and linoleic acid (9) from the twigs; and a mixture of 4a and 4b, 5, 6, 8, long-chain fatty alcohols (10), and long-chain hydrocarbons (11) from the flowers of *D. dao*. The structures of 1 and 5 were elucidated by extensive 1D and 2D NMR spectroscopy, while those of 2-4 and 6-11 were identified by NMR spectroscopy. **Conclusion:** This is the first report on the isolation of 1, 4b and 6-9 from *D. dao*.

Key words: *Dracontomelon Dao* (Blanco) Merr. et Rolfe, Anacardiaceae, Cardol, Anacardic Acid, 3-Alkylphenols, *B*-Sitosteryl-3 β -Glucopyranoside-6'-O-Fatty Acid Esters, *B*-Sitosteryl Fatty Acid Esters.

INTRODUCTION

Dracontomelon dao (Blanco) Merr. et Rolfe of the family Anacardiaceae, locally known as dao is an indigenous Philippine tree which is also widely distributed throughout the South and Southeast Asia.¹ The dao bark is used against dysentery. The mature fruits and kernel of the seeds are edible, while the flowers and young leaves are eaten as vegetables. The wood of *dao* is employed in light construction, timber and firewood.² The EtOAc extract of the leaves of *D. dao* was observed to exhibit strong anti-bacterial activity with an IC₅₀ of 98.5 μ g/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 was extracted from the skins of stem of *D. dao* by steam distillation. GC-MS analysis identified 13 compounds with the following 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 earlier reported the isolation of anacardic acid, β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters, β -sitosterol, phytol, phytol fatty acid esters, β -sitosteryl fatty acid esters, chlorophyll a, squalene, long-chain fatty alcohols, and long-chain hydrocarbons from the leaves of *D. dao*.⁶ We report herein the isolation of cardol (1), β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (2), β -sitosteryl fatty acid esters (3), and a mixture

of β -sitosterol (4a) and stigmasterol (4b) from the petiole; 1, anacardic acid (5), a mixture of 4a and 4b, triacylglycerols (6), monoacylglycerol (7), long-chain fatty acid esters (8) and linoleic acid (9) and from the twigs; and 4a-6, 8, long-chain fatty alcohols (10), and long chain-hydrocarbons (11) from the flowers of *D. dao*. The structures of 1-9 are presented in Figure 1. To the best of our knowledge this is the first report on the isolation of 1, 4b and 6-9 from *D. dao*.

MATERIALS AND METHODS

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRs spectrometer in CDCl₃ at 600 MHz for ¹H NMR and 150 MHz for ¹³C 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 F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

Sample Collection

Samples of the petiole, twigs and flowers of *Dracontomelon dao* (Blanco) Merr. et Rolfe were collected from De La Salle University – Science and Technology Complex (DLSU-STC) Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines in March

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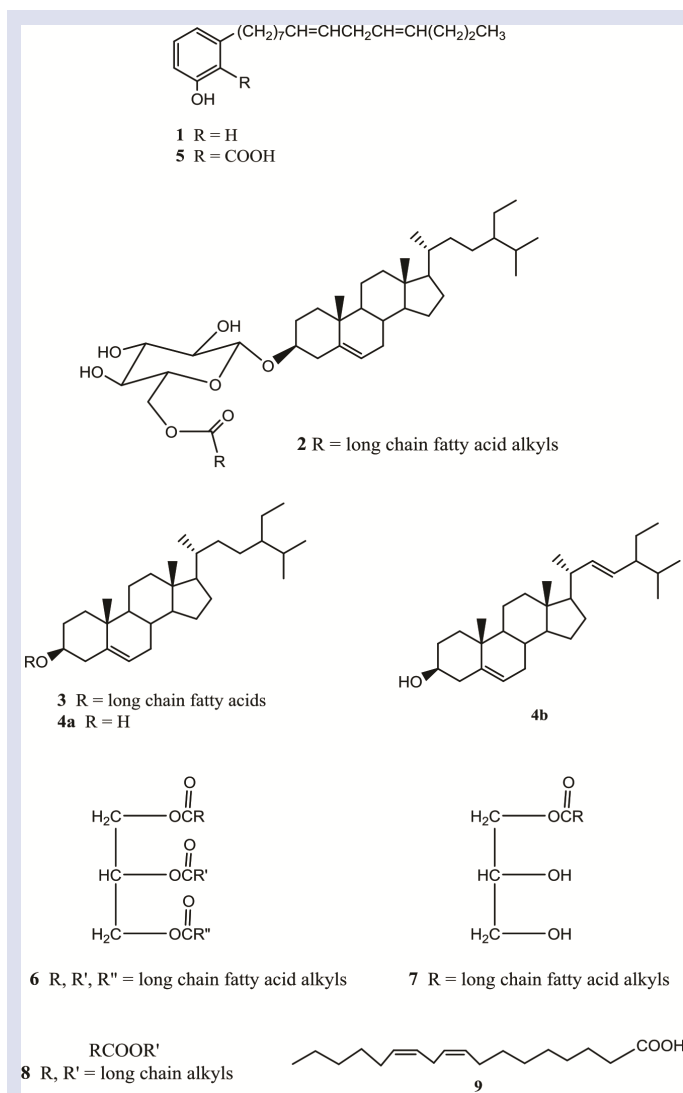


Figure 1: Chemical structures of cardanol (**1**), β -sitosteryl- β -glucopyranoside-6-*O*-fatty acid esters (**2**), β -sitosteryl fatty acid esters (**3**), β -sitosterol (**4a**), stigmasterol (**4b**), anacardic acid (**5**), triacylglycerols (**6**), monoacylglycerol (**7**), long-chain fatty acid esters (**8**) and linoleic acid (**9**) from *D. dao*.

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 petiole of *D. dao*

The air-dried *D. dao* petiole (179.3 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 (0.90 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by

volume. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2 \times) using 1% EtOAc in petroleum ether to afford **3** (2 mg). The first 30% acetone in CH_2Cl_2 fraction was rechromatographed using 10% EtOAc in petroleum ether. The less polar fractions were combined and rechromatographed using 10% EtOAc in petroleum ether to afford **1** (4 mg). The second 30% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether to yield **4a** and **4b** (3 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed (3 \times) using $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (1:1:8, v/v) to afford **2** (3 mg) after washing with petroleum ether.

Isolation of the chemical constituents from the twigs of *D. dao*

The air-dried *D. dao* twigs (87 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 (0.30 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was rechromatographed using petroleum ether. A second rechromatography was conducted using 1% EtOAc in petroleum ether to yield **8** (2 mg). The 10% acetone in CH_2Cl_2 fraction was rechromatographed by gradient elution using 5% EtOAc in petroleum ether; followed by 10% EtOAc in petroleum ether; then 15% EtOAc in petroleum ether; and finally 20% EtOAc in petroleum ether. The fractions eluted with 5% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to afford **1** (2 mg) and **9** (3 mg). The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to yield a mixture of **4a** and **4b** (6 mg) after washing with petroleum ether. The fractions eluted with 20% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to afford **6** (4 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 20% EtOAc in petroleum ether. The less polar fractions were rechromatographed using CH_2Cl_2 to yield **5** (3 mg) after washing with petroleum ether. The more polar fractions yielded **7** (2 mg) after washing with petroleum ether.

Isolation of the chemical constituents from the flowers of *D. dao*

The air-dried *D. dao* flowers (19 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 (0.300 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The CH_2Cl_2 fraction was rechromatographed using petroleum ether (2 \times) to afford **11** (5 mg) after washing with petroleum ether. The 10% acetone in CH_2Cl_2 fraction was rechromatographed using 1% EtOAc in petroleum ether to yield **6** (3 mg) and **8** (4 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed using 5% EtOAc in petroleum ether to afford **10** (5 mg) and a mixture of **4a** and **4b** (6 mg) after washing with petroleum ether. The 30% to 50% acetone in CH_2Cl_2 fractions were combined and rechromatographed (3 \times) using 20% EtOAc in petroleum ether to yield **5** (12 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *D. dao* yielded **1-11**. The NMR spectra of **1** are in accordance with data reported in the literature for cardanol;⁷ **2** for β -sitosteryl- β -glucopyranoside-6'-*O*-fatty acid esters;⁸ **3** for β -sitosteryl fatty acid ester;⁹ **4a** for β -sitosterol;¹⁰ **4b** for stigmasterol;¹⁰ **5** for anacardic acid;¹¹ **6** for triacylglycerols;¹² **7** for monoacylglycerol;¹⁰ **8** for long-chain fatty acid esters;¹³ **9** for linoleic acid;¹⁴ **10** for long-chain fatty alcohols;¹⁵ and **11** for long-chain hydrocarbons.¹⁶

CONCLUSION

The petiole, twigs, flowers and leaves of *D. dao* afforded phenolics, sterols and lipids. The following compounds were obtained from the

different parts of the tree: cardol (1) from the petiole; β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (2) from the petiole and leaves; β -sitosteryl fatty acid esters (3) from the petiole; β -sitosterol (4a) from the petiole, twigs, flowers and leaves; stigmasterol from the petiole, twigs and flowers; anacardic acid (5) from the twigs, flowers and leaves; triacylglycerols (6) from the twigs and flowers; monoacylglycerol (7) from the twigs; long-chain fatty acid esters (8) from the twigs and flowers; linoleic acid (9) from the twigs; long-chain fatty alcohols (10) and long-chain hydrocarbons (11) from the flowers.

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CONFLICT OF INTEREST

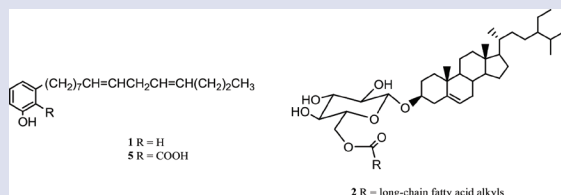
There is no conflict of interest.

List of Abbreviations: NMR – Nuclear Magnetic Resonance, EtOAc – Ethyl acetate, Et₂O – Diethyl ether.

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GRAPHICAL ABSTRACT



AUTHOR PROFILE



Consolacion Y. Ragasa: Obtained her Ph. D. in Chemistry from the University of the Philippines - Diliman. Dr. Ragasa is a Full Professor of the Chemistry Department and a University Fellow of De La Salle University - Manila. Her research focuses on the isolation and structure elucidation of the chemical constituents of Philippine plants. She has published extensively in natural products, phytochemistry and pharmacognosy journals. Dr. Ragasa was awarded the Pillar of Lasallian Excellence Award in Research (2013), National Research Council of the Philippines Achievement Award in Chemical Research (2003), Philippine Federation of Chemistry Societies Achievement Award in Chemical Research (2002), and St. Miguel Febres Cordero Research Award (SY 2000-2001). She is a member of the editorial board of several international and national journals.



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HIGHLIGHTS OF PAPER

- D. dao* yielded cardol (1), β -sitosteryl-3 β -glucopyranoside-6'-O-fatty acid esters (2), β -sitosteryl fatty acid esters (3), and a mixture of β -sitosterol (4a) and stigmasterol (4b) from the petiole; 1, a mixture of 4a and 4b, anacardic acid (5), triacylglycerols (6), monoacylglycerol (7), long-chain fatty acid esters (8), and linoleic acid (9) from the twigs; and a mixture of 4a and 4b, 5, 6, 8, long-chain fatty alcohols (10), and long-chain hydrocarbons (11) from the flowers of *D. dao*.
- The structures of 1 and 5 were elucidated by extensive 1D and 2D NMR spectroscopy, while those of 2-4 and 6-11 were identified by NMR spectroscopy.

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