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Research Article

TRITERPENES FROM *CERIOPS DECANDRA* (GRIFF.) W. THEOB.

JUDY PEREZ[1,2], CHIEN-CHANG SHEN[3], CONSOLACION Y. RAGASA[1,4]*

[1]Department of Chemistry, De La Salle University, Manila, Philippines. [2]Department of Natural Sciences, College of Science and Engineering, Ateneo de Naga University, Camarines Sur, Philippines. [3]Division of Chinese Medicinal Chemistry, National Research Institute of Chinese Medicine, Ministry of Health and Welfare, Taiwan. [4]Department of Chemistry, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Philippines. Email: consolacion.ragasa@dlsu.edu.ph

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ABSTRACT

Objective: To isolate and identify the chemical constituents of *Ceriops decandra.*

Methods: The chemical constituents of *C. decandra* (Griff.) W. Theob. were isolated by silica gel chromatography. The structures of the isolated compounds were identified by nuclear magnetic resonance (NMR) spectroscopy.

Results: Chemical investigation of the dichloromethane extracts of the leaves of *C. decandra* has led to the isolation of 3β-E-coumaroylbetulinic acid (1), lupeol fatty acid esters (2), betulonic acid (3), betulin (4), betulinic acid (5), lupeol (6), lupenone (7), and a mixture of 3β-E-feruloyllupeol (8) and 3β-Z-feruloyllupeol (9), and chlorophyll a (10). The structures of 1-10 were identified by comparison of their NMR data with literature data.

Conclusion: To the best of our knowledge, this is the first report on the isolation of 1-3 from *C. decandra.* Literature search revealed that the triterpenes isolated from *C. decandra* exhibited anticancer properties.

Keywords: *Ceriops decandra,* Rhizophoraceae, 3β-E-coumaroylbetulinic acid, Lupeol fatty acid esters, Betulonic acid, Betulin, Betulinic acid, Lupeol, Lupenone, 3β-E-feruloyllupeol, 3β-Z-feruloyllupeol.

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INTRODUCTION

Ceriops decandra of the family Rhizophoraceae, locally known as Malatangal, is a mangrove which commonly grows in the middle to landward parts of the mangrove swamp. The bark of this species was an important source of high-quality tannin. The sap of the bark of this tree is a source of a black dye which is used in the batik industry. A decoction of the bark is used to treat hemorrhages [1], while the bark, fruits, and leaves are employed for the treatment of hepatitis and ulcer [2]. Furthermore, *C. decandra* was reported to exhibit astringent and anti-hemorrhage properties and is used for the treatment of pain, ulcers, and hepatitis [3]. Several studies were reported on the chemical constituents of *C. decandra.* The leaves of *C. decandra* yielded lupenone, lupeol, betulinaldehyde, 3β-Z-coumaroyllupeol, 3β-E-coumaroyllupeol, 3-epi-betulinic acid, betulin, betulinic acid, 3β-E-feruloylbetulin, 30-nor-lup-3β-ol-20-one, 3β-E-caffeoyllupeol, lup-20(29)-en-3β,30-diol, 3β-hydroxylupan-29-oic acid, 3β,20-dihydroxylupane, oleanolic acid, ursolic acid, and 3β-E-feruloyllupeol and 3β-Z-feruloyllupeol [4] which are of relevance to our present report. Another study reported that the leaf extracts afforded α-amyrin, β-amyrin, lupeol, oleanolic acid, and ursolic acid [5]. The bark of *C. decandra* yielded decandrins A-K [6]; 2-(9-hydroxy-3a,5a,5b,8,8,11a-hexamethylicosahydro-1H-cyclopenta[a]chrysen-1-yl) propanoic acid (3β-hydroxylupan-29-oic acid) [7]; d-catechin, leucoanthocyanidins [4]; procyanidin [8]; decandrinin [9]; (-)-syringaresinol, (-)-pinoresinol, β-sitosterol, stigmasterol, palmitic acid, and 3,4-dihydroxybenzoic acid Et ester [10]; and 7,13-abietadien-3β-ol, 7-oxodehydro-abietinol, margocin, 3β-hydroxy-abieta-8,11,13-trien-7-one, 15,18-dihydroxyabieta-8,11,13-trien-7-one, 7β,18-dihydroxy dehydroabietanol, 4-epi-triptobezene L, 7α, 18-dihydroxydehydroabietanol, sabiperone E, 13β,18-dihydroxy-abiet-8(14)-ene-7-one, ent-labd-8(17),13E-dien-15-ol, ent-8(14)-pimarene-15R,16-diol, and (5S*,8S*,9S*,10R*,13S*)-3-hydroxy-16-nor-2-oxodolar-3-ene-15-oic acid [11]. Furthermore, the wood extract of *C. decandra* yielded 3β,13β-dihydroxy-8-abietaen-7-one and 3β-hydroxy-8,13-abietadien-7-one [12]. Other studies reported the isolation of ceriopsin A-D [13], ceriopsin E [14], and ceriopsin F and G [15] from the roots.

This study is part of our research on the chemical constituents of the mangroves found in the Philippines. We earlier reported the isolation of ursolic acid and squalene from the leaves and oleanolic acid, ursolic acid, α-amyrin cinnamate, β-amyrin cinnamate, β-sitosterol, and stigmasterol from the fruit; lupeol, oleanolic acid, ursolic acid, β-sitosterol, and stigmasterol from the twigs of *Sonnerata alba* [16]. We report herein the isolation of 3β-E-coumaroylbetulinic acid (1), lupeol fatty acid esters (2), betulonic acid (3), betulin (4), betulinic acid (5), lupeol (6), lupenone (7), and a mixture of 3β-E-feruloyllupeol (8) and 3β-Z-feruloyllupeol (9), and chlorophyll a (10). The chemical structures of 1-9 are presented in Fig. 1. To the best of our knowledge, this is the first report on the isolation of 1-3 from *C. decandra.*

METHODS

General experimental procedure

Nuclear magnetic resonance (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 (TLC) 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

Samples of the leaves of *C. decandra* (Griff.) W. Theob. were collected from the mangrove swamp of Caramoan, Camarines Sur Philippines in September 2016. The samples were authenticated at the Botany Division, Philippine National Museum.

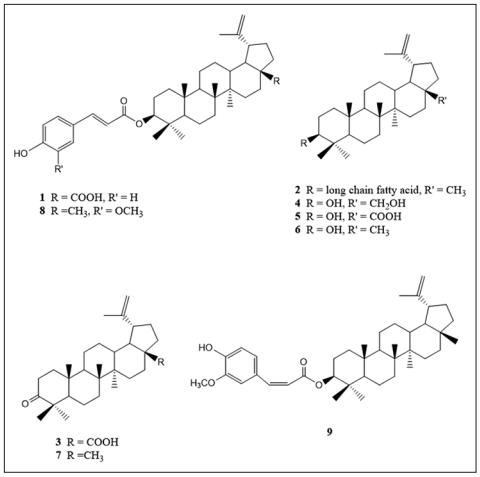

Fig. 1: Chemical structures of 3β-E-coumaroylbetulinic acid (1), lupeol fatty acid esters (2), betulonic acid (3), betulin (4), betulinic acid (5), lupeol (6), lupenone (7), 3β-E-feruloyllupeol (8) and 3β-Z-feruloyllupeol (9) from *C. decandra*

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. 5 ml fractions were collected. All fractions were monitored by TLC. 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. 1 ml fractions were collected.

Isolation of the chemical constituents from the leaves of *C. decandra*

The air-dried *C. decandra* leaves (223.8 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 (4.0 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The 10% and 20% acetone in CH_2Cl_2 fractions were rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford 2 (1.9 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to yield 8 (10.6 mg) and a mixture of 8 and 9 (1.7 mg) after washing with petroleum ether. The 50% 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 yield 1 (7.2 mg) and 7 (1.8 mg) after washing with petroleum ether. The more polar fractions were combined and rechromatographed using 15% EtOAc in petroleum ether to afford 3 (6.5 mg) and a mixture of 4 and 5 (2.0 mg) after washing with petroleum ether. The 60% acetone in CH_2Cl_2 fraction was rechromatographed using 12.5% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 12.5% EtOAc in petroleum ether were combined and rechromatographed in the same solvent to yield 6 (24.1 mg). The fractions eluted with 15% EtOAc in petroleum ether were combined and rechromatographed using the same solvent to afford 10 (9.3 mg) after washing with petroleum ether, followed by Et_2O. The more polar fractions were combined and rechromatographed (2 ×) using the same solvent to yield 4 (2.4 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of the leaves of *C. decandra* yielded 1-10. The NMR spectra of 1 are in accordance with data reported in the literature for 3β-E-coumaroylbetulinic acid (1) [17]; 2 for lupeol fatty acid esters [18], 3 for betulonic acid [19], 4 for betulin [20], 5 for betulinic acid [21], 6 for lupeol [16], 7 for lupenone [22], 8 for 3β-E-feruloyllupeol [2], 9 for 3β-Z-feruloyllupeol [2], and 10 for chlorophyll a [23].

Literature search revealed that the triterpenes isolated from *C. decandra* exhibited anticancer properties. Betulinic acid (5) and its derivatives, betulonic acid (3), 3β-O-(Z)-coumaroylbetulinic acid, and 3β-O-(E)-coumaroylbetulinic acid (1) were found to be catalytic inhibitors of Topo II activities with IC_{50} values ranging from 0.38 to 58 μM. The acylation of the OH group at C-3 of betulinic acid exhibited stronger Topo II inhibitory activity [24]. Reviews on anticancer properties of betulinic acid (5) and its derivatives have been provided [25-30]. A review on the anticancer and chemopreventive potential of betulin (4) *in vitro* and *in vivo* has also been provided [31].

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