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Research Article

Chemical Constituents of *Dioscorea luzonensis* Schauer

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ABSTRACT
Chemical investigation of the dichloromethane extracts of *Dioscorea luzonensis* Schauer, tubers led to the isolation of long chain alkyl *trans*-ferulates (**1**), a mixture of **1** and β-sitosterol (**2**), and fatty acids from the skin; and ursolic acid (**3**) and fatty acids from the inner portion. The structures of **1**–**3** were identified by comparison of their NMR data with literature data.

Keywords: *Dioscorea luzonensis*, Dioscoreaceae, alkyl *trans*-ferulates, β-sitosterol, ursolic acid, fatty acids.

INTRODUCTION

Dioscorea luzonensis, also known as wild yam or camangeg, is a plant endemic to The Philippines. It is a wild root crop that grows naturally in Ilocos province of The Philippines. The tubers of this plant are usually harvested during the early part of August and these are used as a vegetable and in making delicacies such as haleya[1]. The tuber has a unique brown color with fine roots on its surface and an elongated and irregular morphology. The tuber can also be eaten after 20 to 30 minutes boiling and has a unique taste and aroma. The skin of the tuber is usually considered to be inedible and it is removed when it is used as a food supplement. The inner portion of the tuber is the edible portion with uniform white color. *Dioscorea luzonensis* has no reported biological activities and chemical constituents. However, congeners of *D. luzonensis* have been studied for their chemical constituents. *Dioscorea bulbifera* yielded stigmasterol, mono-arachidin, 1,7-bis-(4-hydroxyphenyl)-1*E*,4*E*,6*E*-heptatrien-3-one, behenic acid, demethyl batatasin IV, 2,3'-di-hydroxy-4',5'-dimethoxybibenzyl, diosbulbins B, and D, docosyl ferulate, 7-bis-(4-hydroxyphenyl)-4*E*, 6*E*-heptadien-3-one, 5,3,4-trihydroxy-3,7-dimethoxy flavone, tristin, protocatechuic acid, and adenosine[2]. Another study reported the isolation of palmitic acid, □-sitosterol, oleic acid, □-sitosterol acetate, 5-(hydroxymethyl) furfural, nonanedioic acid, □-daucosterol, cyclo-(Phe-Tyr), cyclo-(Tyr-Tyr), 6-methyl citrate, 1,5-dimethyl citrate, trimethyl citrate from *Dioscorea opposite*[3]. Furthermore, another species, *Dioscorea collettii* var. hyplauca, afforded tricosanatine, 1,7-bis(4-hydroxyphenyl)-4,6-heptadien-3-one, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, and dioscin, dioscorone A, 3S-6,8-dihydroxy-3-phenyl-3,4-dihydroisocoumarin, diosgenin, gracillin, 3-*O*-α-L-rhamnopyranosyl-(1,2)-β-D-gucopyranoside-diosgenin, protodioscin, methyl protodiscin, protogracillin, methyl protogracillin, β-sitosterol, daucosterol, and palmitic acid[4]. Hydroxybenzoic acid, hydroxycinnamic acid and their derivatives were found in extracts of *Dioscorea hispida* Dennst tuber[5]. Moreover, the phenolic compounds cyanidin-3-glucoside and the procyanidin dimers B-1 and B-3 were reported from *Dioscorea alata*[6]. We report herein the isolation of long chain alkyl *trans*-ferulates (**1**), a mixture of **1** and β-sitosterol (**2**), and fatty acids from the skin of the tuber; and ursolic acid (**3**) and fatty acids from the inner portion of the tuber of *D. luzonensis*. The structures of **1–3** are presented in Fig.1. To the best of our knowledge this is the first report on the chemical constituents of *D. luzonensis*.

MATERIALS AND METHODS

General Experimental Procedure

^{1}H NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 400 in $CDCl_3$ at 400. 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 packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in dichloromethane

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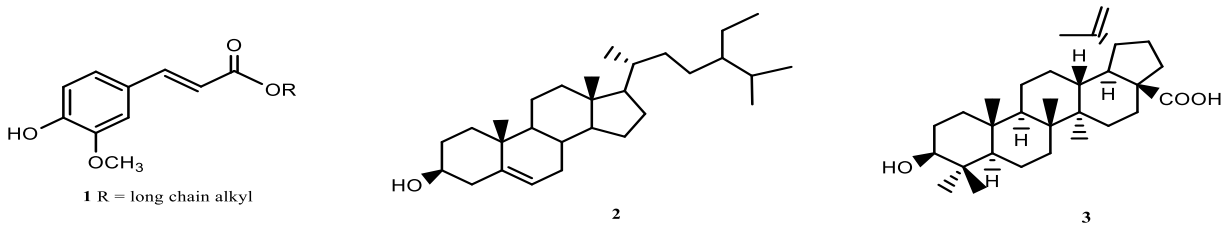

Figure 1: Chemical structures of compounds **1-3** from *D. luzonensis.*

(10% increment) as eluents. Twenty milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same R_f value 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

The tubers were bought from Batac Public Market in Batac, Ilocos Norte, Philippines in November 2014. The sample was authenticated by Flordeliz Rapacon Estira of Mariano Marcos State University, Batac, Ilocos Norte, Philippines.

Isolation of the Chemical Constituents of the Skin of the Tuber

The freeze-dried skin of the tuber (66.27 g) of *D. luzonensis* was cut into small pieces, ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated from the filtrate under vacuum to afford a crude extract (0.6 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to afford **1** (2 mg). The 20% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 7.5% EtOAc in petroleum ether to yield a mixture of **1** and **2** (3 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using 15% EtOAc in petroleum ether to afford fatty acids (5 mg).

Isolation of the Chemical Constituents of the Inner Portion of the Tuber

The freeze-dried inedible part of the tuber (301.96 g) of *D. luzonensis* were cut into about 1 cm, ground in an osterizer, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.02 g), which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increments by volume as eluents. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 20% EtOAc in petroleum ether to afford fatty acids (4 mg). The 50% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) using CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9, v/v) to yield **4** (2 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *D. luzonensis* yielded compounds **1-3** and fatty acids. The structures of **1-3** and fatty acids were identified by comparison of their NMR data with literature data. The NMR spectra are in accordance with data reported in the literature for alkyl *trans*-ferulates (**1**)[7]; β-sitosterol (**2**)[8], and ursolic acid (**3**)[9]. The fatty acid fraction was determined to contain both saturated and unsaturated fatty acids[10].

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