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Chemical Constituents of *Hoya paziae* Kloppenb.

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ABSTRACT

Chemical investigation of the dichloromethane extract of the leaves of *Hoya paziae* Kloppenb. led to the isolation of a mixture of lupeol (**1a**), α-amyrin (**1b**), and β-amyrin (**1c**) in a 2:3:1 and a mixture of lupeol fatty acid esters (**2a**), α-amyrin fatty acid esters (**2b**), and β-amyrin fatty acid esters (**2c**) in a 3:2:1 ratio. The structures of **1a**-**2c** were identified by comparison of their NMR data with those reported in the literature.

Keywords: α-amyrin, α-amyrin fatty acid esters, β-amyrin, β-amyrin fatty acid esters, Apocynaceae, *Hoya paziae*, lupeol, lupeol fatty acid esters

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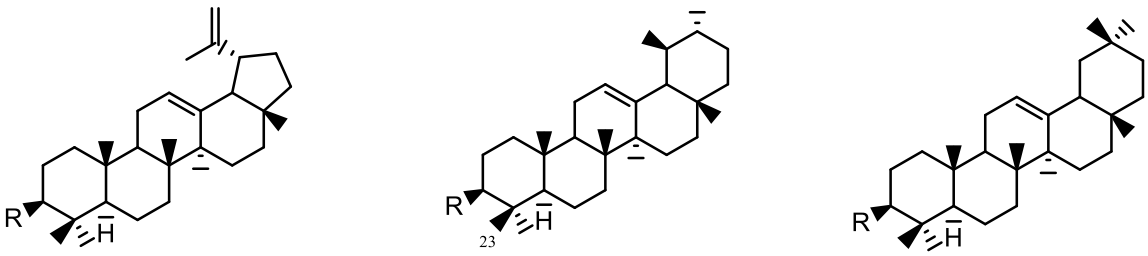
INTRODUCTION

As a genus of the family Apocynaceae, the *Hoya* have been utilized as a source of ornamental plants because of their waxy foliage and attractive flowers. At least 109 species of *Hoya* are found in the Philippines, with 88 of these considered as endemic to the country [1]. Most *Hoya* species are epiphytic vines, but *H. paziae* is an epiphytic shrub with scandent stems that tend to be pendent due to the weight of its branches and foliage. Its leaves are decussate instead of being opposite, while its flowers are white with a purplish red and white corona, and pleasantly scented during the first day after opening. With blooms lasting up to six days, this species, originally found in Antique, Oriental Mindoro, and Quezon provinces, is now commercialized locally and abroad as a hanging ornamental plant [1]. Although a few *Hoya* species have been cited being used in traditional medicine, *H. paziae* has not been mentioned as having medical applications for its different parts. The isolation of taraxerol, taraxeryl acetate, α-amyrin acetate, and β-amyrin acetate from the stems of *H. paziae* Kloppenb. was reported recently [2].

This study is part of our research on the chemical constituents of Philippine native hoyas. We earlier reported the isolation of lupenone and lupeol from the roots; lupeol, squalene and β-sitosterol from the leaves; and betulin from the stems of *H. mindorensis* Schlechter [3]. In another study, we reported the isolation of α-amyrin, β-amyrin, lupeol acetate, α-amyrin acetate, and β-amyrin acetate from the stems; and α-amyrin, bauerenol, squalene, lutein, β-sitosterol, and stigmasterol from the leaves of *H. multiflora* Blume [4]. Moreover, the isolation of β-amyrin cinnamate and taraxerol from the stems; and taraxerol, triglycerides, chlorophyll a, and a mixture of β-sitosterol and stigmasterol from the leaves of *H. wayetii* Kloppenb. has been reported [5]. Furthermore, the isolation of taraxerol, taraxerone, β-sitosterol, stigmasterol, α-amyrin cinnamate and β-amyrin cinnamate from the stems; taraxerol, taraxerone, and β-sitosterol from the roots; α-amyrin cinnamate and β-amyrin cinnamate from the flowers; and squalene, β-sitosterol, and saturated hydrocarbons from the leaves of *H. buotii* Kloppenb. has been reported [6]. We also reported the isolation of β-amyrin cinnamate, squalene, β-sitosterol, β-amyrin, α-amyrin, lupeol and saturated hydrocarbons from the leaves; and squalene, taraxerol, lupeol cinnamate, β-sitosterol and stigmasterol from the stems of *H. diversifolia* Blume [7]. Recently, we reported the isolation of taraxerol, β-sitosterol and stigmasterol from the stems of *H. pubicalyx* Merr. [8].

In this study, we obtained a mixture of lupeol (**1a**), α-amyrin (**1b**), and β-amyrin (**1c**) in a 2:3:1 ratio and another mixture of lupeol fatty acid esters (**2a**), α-amyrin fatty acid esters (**2b**), and β-amyrin fatty acid esters (**2c**) in a 3:2:1 ratio from the leaves of *H. paziae*. The chemical structures of **1a**-**2c** are presented in Fig. 1.



1a R = OH
2a R = long-chain fatty acid

1b R = OH
2b R = long-chain fatty acid

1c R = OH
2c R = long-chain fatty acid

Fig 1: Chemical structures of lupeol (1a), α-amyrin (1b), β-amyrin (1c), lupeol fatty acid esters (2a), α-amyrin fatty acid esters (2b), and β-amyrin fatty acid esters (2c) from the leaves of *Hoya paziae*.

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

Healthy of *H. paziae* were collected from propagated plants at the Philippine Nuclear Research Institute *Hoya* Germplasm Collection under MTA No. 2015-004 dated March 20, 2015. The clone with Accession Number PNRI-H.30 came from the Institute of Plant Breeding, University of the Philippines Los Baños (UPLB) as a stem cutting in 2008 when it was authenticated by Dr. Simeona V. Siar of UPLB, Laguna, Philippines. The original plant was sourced from Quezon province.

General Isolation Procedure

A glass column 18 inches in height and 1 inch internal diameter was used for the fractionation of the crude extracts. Eleven 20 mL fractions were collected. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography of fractions from the crude extracts. 2 mL fractions were collected. Fractions with spots of the same R_f values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. 1 mL fractions were collected.

Isolation of Chemical Constituents

The air-dried *H. paziae* leaves (53.2 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 (6.7 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 (2 ×) using 7.5% EtOAc in petroleum ether to afford a mixture of **2a**–**2c** (3 mg) after washing with petroleum ether. The 30% acetone in CH_2Cl_2 fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford a mixture of **1a**–**1c** (5 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the leaves of *H. paziae* yielded **1a**–**2c**. The NMR spectra of **1a** are in accordance with data reported in the literature for lupeol [9], **1b** for α-amyrin [10], **1c** for for β-amyrin [10]; **2a** for lupeol fatty acid esters [11]; **2b** for α-amyrin fatty acid esters [12]; and **2c** for β-amyrin fatty acid esters [12]. The 2:3:1 ratio of the mixture of lupeol (**1a**), *α*-amyrin (**1b**) and β-amyrin (**1c**) and 3:2:1 ratio of the mixture of lupeol fatty acid esters (**2a**), *α*-amyrin fatty acid esters (**2b**) and β-amyrin fatty acid esters (**2c**) were deduced from the intensities and integrations of the ^{1}H NMR resonances for the olefinic protons of **1a** at δ 4.66 (br d, *J* = 2 Hz, H_a-29) and 4.54 (br s, H_b-29) [9, 11], **1b** at δ 5.10 (t, *J* = 3.6 Hz, H-3) [10, 12] and **1c** at δ 5.16 (t, *J* = 3.6 Hz, H-3) [10, 12].

These results indicate that *H. paziae* shares similar chemical characteristics with other members of the genus *Hoya*: *H. diversifolia* [7] which yielded a mixture of lupeol (**1a**), α-amyrin (**1b**), and β-amyrin (**1c**) in a 1:2:4 ratio; *H. mindorensis* [3] which contained lupeol; and *H. multiflora* [4] which afforded α-amyrin (**1b**) and β-amyrin (**1c**). This is the first report on the presence of lupeol fatty acid esters (**2a**), α-amyrin fatty acid esters (**2b**), and β-amyrin fatty acid esters (**2c**) from Philippine native hoyas.

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