Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(5); 1042-1045

ISSN: 0975-4873

Research Article

Chemical Constituents of *Hoya wayetii* Kloppenb.

Virgilio D. Ebajo Jr.[1], Fernando B. Aurigue[2], Robert Brkljača[3], Sylvia Urban[3], Consolacion Y. Ragasa[1,4,*]

[1]*Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines*
[2]*Agriculture Research Section, Atomic Research Division, Philippine Nuclear Research Institute, Commonwealth Avenue, Diliman, Quezon City 1101, Philippines*
[3] *School of Applied Sciences (Discipline of Chemistry), Health Innovations Research Institute (HIRi) RMIT University, GPO Box 2476V Melbourne, Victoria 3001, Australia*
[4]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines*

Available Online:28th September, 2015

ABSTRACT
Chemical investigation of the dichloromethane extracts of *Hoya wayetii* Kloppenb. afforded β-amyrin cinnamate (**1**) and taraxerol (**2**) from the stems; and **2**, triglycerides (**3**), chlorophyll a (**4**), and a mixture of β-sitosterol (**5a**) and stigmasterol (**5b**) from the leaves. The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy, while those of **3**-**5b** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Hoya wayetii* Kloppenb., Apocynaceae, β-amyrin cinnamate, taraxerol

INTRODUCTION

Hoya plants of the family Apocynaceae are also called wax plants due to the waxy appearance of their leaves or flowers. There are at least 109 species of *Hoya* found in the Philippines, 88 of these are endemic to the country[1]. *Hoya wayetii* is endemic to the Philippines and was first collected from a village north of Baguio City, Benguet province, Luzon island[2]. It is also found in Sibuyan island of Romblon province in central Philippines[1]. The species is usually cultivated in hanging planters as an ornamental plant with fleshy, elongated leaves and clusters of short-lived, dark red flowers.

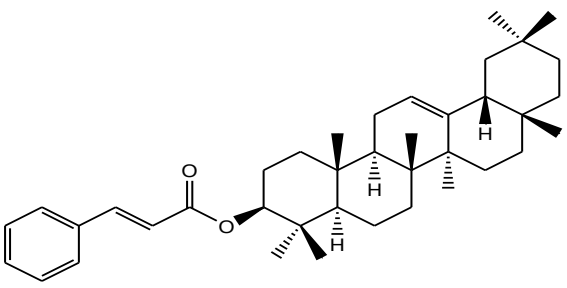
There are no reported chemical studies and biological activities on *H. wayetii*. However, a few hoya species have been studied for their chemical constituents. Gas chromatographic analysis on the chemical constituents of *Hoya naumannii* Schltr. led to the detection of the triterpenes β-amyrin, lupeol and α-amyrin and their 3,4-*seco*-3-oic acid methyl esters[3]. The isolation of pentacyclic triterpenols δ-amyrin, β-amyrin, lupeol and α-amyrin and their 3,4-*seco*-3-*nor*-2-ol derivatives (australinols A–D) from the leaf wax of *Hoya australis* R.Br. ex Traill have been reported[4]. Moreover, the β-amyrin derivative 5-isopropyl-10(2-methoxycarbonylethyl)des-A-olean-12-en and the taraxerol derivative 5-isopropyl-10(2-methoxycarbonylethyl)des-A-olean-14-en were isolated from *Hoya lacunosa* Blume[5]. The oligosaccharides 6-deoxy-3-*O*-methyl-β-allopyranosyl (1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaronic acid δ-lactone and 6-deoxy-3-*O*-methyl-β-allopyranosyl (1→4)-β-oleandropyranosyl(1→4)-β-cymaropyranosyl (1→4)-β-cymaronic acid δ-lactone and its sodium salt were isolated from *Hoya carnosa* R.Br.[6]. *Hoya* species yielded pregnanes, lipids, sterols, flavanols, triterpenes, sesquiterpenes and disaccharides. They were reported to exhibit antinematodal activity, hypo sensitization, immunological properties and phytotoxicity; used for the treatment of occupational asthma and sea-squirt asthma and allergies; and employed as antigens and insecticides[7]. A review on the chemical and pharmacological aspects of *Hoya* species has been provided[7]. This study was conducted as part of our research on the chemical constituents of the genus *Hoya*. 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* Schltr[8]. Recently, we reported the isolation of lupeol, 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[9].

We report herein the isolation of β-amyrin cinnamate (**1**) and taraxerol (**2**) from the stems; and **2**, triglycerides (**3**) and chlorophyll a (**4**) from the leaves of *H. wayetii*. The leaves also yielded a mixture of β-sitosterol (**5a**) and stigmasterol (**5b**). To the best of our knowledge this is the first report on the isolation of these compounds from *H. wayetii* (Fig. 1).

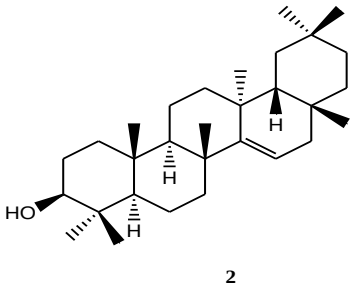
Experimental

^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were acquired in $CDCl_3$ on a 500 MHz Agilent DD2 NMR

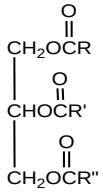
**Author for Correspondence*



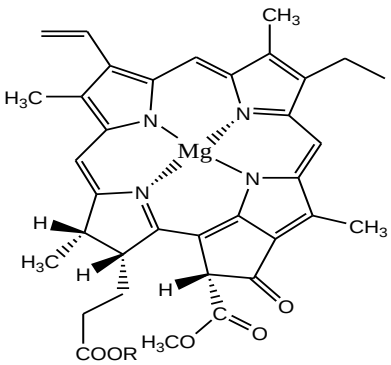
1



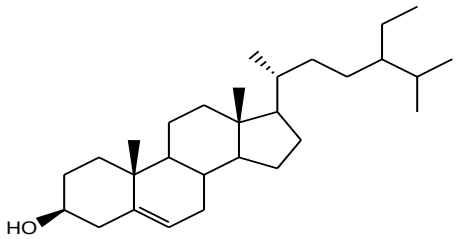
2



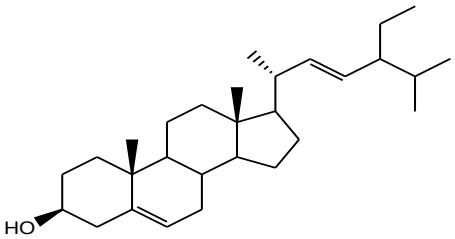
3 R, R', R" = long chain fatty acids



4 R = phytyl



5a



5b

spectrometer with referencing to solvent signals (δ 7.26 and 77.0 ppm). Two-dimensional NMR experiments recorded included gCOSY, HSQCAD, and gHMBCAD NMR experiments. 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.

Hoya wayetii was collected from the Philippine Nuclear Research Institute *Hoya* Germplasm Collection with Accession Numbers H.024 and H.098 under Material Transfer Agreement No. 2014-002 dated June 17, 2014. Both clones have been propagated from cuttings obtained from a natural resident of Sibuyan Island.

The air-dried leaves (38 g), and stems (24.6 g) of *H. wayetii* were ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrates were concentrated under vacuum to afford crude extracts of leaves (2.5 g), and stems (1.0 g) which were each chromatographed by gradient elution with CH_2Cl_2, followed by increasing amounts of acetone at 10% increment by volume as eluents. A glass column 12 inches in height and 0.5 inch internal diameter was used for the fractionation of crude extracts. Two milliliter 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. One milliliter fractions were collected.

The CH_2Cl_2 fraction from the chromatography of the crude stem extract of *H. wayetii* was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to afford **1** (9 mg) after washing with petroleum ether. The 40% acetone in CH_2Cl_2 fraction was rechromatographed (4 ×) using 15% EtOAc in petroleum ether to afford **2** (6 mg) after washing with petroleum ether.

The 10% acetone in CH_2Cl_2 fraction from the chromatography of the crude leaf extract of *H. wayetii* was rechromatographed (3 ×) using 5% EtOAc in petroleum ether to afford **3** (8 mg). The 30% acetone in CH_2Cl_2 fraction was rechromatographed using 15% EtOAc in petroleum ether. The less polar fractions from the second column were combined and rechromatographed in 15% EtOAc in petroleum ether to

afford **2** (10 mg) after washing with petroleum ether. The more polar fractions from the second column were combined and rechromatographed in 20% EtOAc in petroleum ether. The less polar fractions from the third column were combined and rechromatographed in 20% EtOAc in petroleum ether to afford a mixture of **5a** and **5b** (7 mg) after washing with petroleum ether. The more polar fractions from the third column were combined and rechromatographed in 20% EtOAc in petroleum ether to afford **4** (10 mg) after washing with Et_2O.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *Hoya wayetii* yielded β-amyrin cinnamate (**1**)[10] and taraxerol (**2**)[11] from the stems; and **2**, triglycerides (**3**)[12], chlorophyll a (**4**)[13], β-sitosterol (**5a**)[14], and stigmasterol (**5b**)[14] from the leaves. The structures of **1** and **2** were elucidated by extensive 1D and 2D NMR spectroscopy. The structures of **3**-**5b** were identified by comparison of their NMR data with those reported in the literature.

β-Amyrin cinnamate (**1**) was reported to inhibit the TPAS-induced inflammation (ID_{50} 0.27 μmol/ear; CI 95% 0.23-0.33 μmol/ear) which is more inhibitory than the positive control, indomethacin (ID_{50} 0.91 μmol/ear; CI 95% 0.23-0.33 μmol/ear)[10]. On the other hand, taraxerol (**2**) was reported to exhibit anti-inflammatory activity by selective COX-1 inhibition[15]. Another study reported that **2** downregulates the expression of proinflammatory mediators in macrophages by preventing NF-κB activation[16]. Furthermore, **2** was shown as a glucose transport inhibitor and stimulator of glycogen synthesis[17]. Moreover, **2** inhibited the growth of Hela and BGC-823 with IC_{50} of 73.4 μmol/L^{-1} and 73.3 μmol/L^{-1}, respectively[18].

CONCLUSION

Hoya wayetii is a Philippine endemic ornamental plant with no reported chemical studies and biological activities. This study reports on the isolation of β-amyrin cinnamate (**1**) and taraxerol (**2**) which were reported to exhibit high anti-inflammatory activity. Triterpene **2** was also reported to show anti-hyperglycemic and anti-cancer properties. The other isolated compounds are triglycerides[19], chlorophyll a[13], β-sitosterol[9] and stigmasterol[9] which were also reported to exhibit diverse biological activities. Thus, this ornamental plant contains compounds with medicinal applications.

ACKNOWLEDGMENT

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

REFERENCES

1. Aurigue FB. A Collection of Philippine Hoyas and Their Culture, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAANRRD). Department of Science and Technology (DOST), 2013; p 110.
2. Baas WJ, Van Berkel IEM. 3, 4-Seco-triterpenoid acids and other constituents of the leaf wax of *Hoya naumanii*. Phytochem 1991; 30(5):1625-1628.
3. Kloppenburg, R.D. Philippine *Hoya* Species: A Monograph. 2nd Edition. Orca Pub. Co., 1993; 507 pages.
4. Baas WJ, Van Berkel IEM, Versluis C, Heerma W, Kreyenbroek M.N. Ring-A fissioned 3, 4-seco-3-nor-triterpene-2-aldehydes and related pentacyclic triterpenoids from the leaf wax of *Hoya australis*. Phytochem 1992; 31(6):2073–2076.
5. Baas WJ. Dihydronyctanthic acid methyl ester and other 3, 4-seco-pentacyclic triterpenoids from *Hoya lacunosa*. Phytochem 1983; 22(12):2809–2812.
6. Yoshikawa K, Nishino H, Arihara S, Chang HC, Wang JD. Oligosaccharides from *Hoya carnosa.* J Nat Prod 2000; 63(1):146–148.
7. Pandey SC, Singh SS, Ghosh AC, Deepak D, Khare AJ. Chemistry and pharmacological aspects of *Hoya* species. Med Arom Plant Sci 2004; 26(4):775–783.
8. Ebajo Jr V, Shen, C-C, Ragasa CY. Triterpenes and sterol from *Hoya mindorensis.* Der Pharma Chemica 2014, 6(4):321–325.
9. Ebajo Jr V, Shen C-C, Ragasa CY. Terpenoids and Sterols from *Hoya multiflora* Blume. J Appl Pharm Sci 2015; 5(4):33-39.
10. Akihisa T, Kojima N, Kikuchi T, Yasukawa K, Tokuda H, Masters ET, Manosroi A, Manosroi J. Anti-inflammatory and chemopreventive effects of triterpene cinnamates and acetates from shea fat. J Oleo Sci 2010, 59, 273–280.
11. Mahato SB, Kundu AP. ^{13}C NMR Spectra of pentacyclic triterpenoids - A Compilation and some salient features. Phytochem 1994, 37(6), 1517–1575.
12. Ragasa CY, Chua APU, Mandia EH, Bernardo LO, Shen C-C. Chemical constituents of *Cardamine flexuosa.* Der Pharma Chemica 2015; 7(1):100–105.
13. Ragasa CY, Caro JL, Shen C-C. Triterpenes and sterol from *Artocarpus ovatus.* J Appl Pharm Sci 2014, 4(10):7–11.
14. Ragasa CY, Caro JL, Lirio LG, Shen C-C. Chemical constituents of *Coix lacryma-jobi.* Res J Pharm Biol Chem Sci 2014; 5(6):344–348.
15. Amir F, Wong KC, Eldeen I, Asmawi MZ, Osman H. Evaluation of biological activities of extracts and chemical constituents of *Mimusops elengi.* Trop J Pharm Res 2013; 12(4):591-596.
16. Yao X, Li G, Bai Q, Xu H, Lu C. Taraxerol inhibits LPS-induced inflammatory responses through suppression of TAK1 and Akt activation. Int Immunopharmacol 2013; 15(2):316-324.

17. Sangeetha KN, Sujatha S, Muthusamy VS, Anand S, Nithya N, Velmurugan D, Balakrishnan A, Lakshmi BS. 3β-taraxerol of *Mangifera indica*, a PI3K dependent dual activator of glucose transport and glycogen synthesis in 3T3-L1 adipocytes. Biochim. et Biophys Acta 2015; 1800(3):359-366.

18. Yang X, Li H, Chen H, Li P, Ye B. Chemical constituents in the leave of *Rhizophora stylosa* L and their biological activities. Yaoxue Xuebao 2008; 43(9):974-978.

19. Ragasa CY, Galian RF, Shen C-C. Chemical constituents of *Annona muricata.* Der Pharma Chemica 2014; 6(6):382-387.