Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2014, 6(4):321-325
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Triterpenes and sterol from *Hoya mindorensis*

Virgilio D. Ebajo Jr.[1], Chien-Chang Shen[2] and Consolacion Y. Ragasa[1,3*]

[1]*Chemistry Department De La Salle University, Taft Avenue, Manila, Philippines*
[2]*National Research Institute of Chinese Medicine, 155-1, Li-Nong St., Sec. 2, Taipei, Taiwan*
[3]*Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna, Philippines*

ABSTRACT

*Chemical investigation of the dichloromethane extract of the air-dried roots of Hoya mindorensis Schlechter afforded lupenone (**1**) and lupeol (**2**). The leaves yielded **2**, squalene (**3**) and β-sitosterol (**4**), while the stems afforded betulin (**5**). The structures of **1-5** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in literature.*

Key words: *Hoya mindorensis,* Apocynaceae, lupenone, lupeol, squalene, β-sitosterol, betulin

---

INTRODUCTION

Hoya plants are also called wax plants due to the waxy appearance of their leaves or flowers. There are at least 109 species of *Hoyas* found in the Philippines, 88 of these are endemic to the country [1]. The *Hoya mindorensis* is considered as one of the most beautiful red hoyas. It is endemic to the Philippines and was first found in Mindoro islands. The plant is an epiphytic vine with a smooth, round stem that branches and forms adventitious roots [1].

There are no reported chemical studies and biological activities on *H. mindorensis*. However, congeners of the plant have been studied for their chemical constituents. The oligosaccharides 6-deoxy-3-*O*-methyl-β-allopyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaropyranosyl(1→4)-β-cymaronic acid, and 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* [2]. 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* have been reported [3]. Furthermore, gas chromatographic analysis on the chemical constituents of *Hoya naumanii* led to the detection of the triterpenes β-amyrin, lupeol and α-amyrin and their 3,4-*seco*-3-oic acid methyl esters [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 lacunose* [5]. A review on the chemical and pharmacological aspects of *Hoya* species has been provided [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 [6].

This study was conducted as part of our research on the chemical constituents of plants endemic to the Philippines. We earlier reported the chemical constituents of *Tectona philippinensis* [7, 8], *Diospyros blancoi* [9], *Dillenia*

philippinensis [10], *Pycnarrhena manillensis* [11], *Broussonetia luzonicus* [12], *Atalantia retusa* [13], *Arenga tremula* [14], *Petersianthus quadrialatus* [115], *Artocarpus odoratissimus* [16], *Musa errans* [17] and *Myristica philippensis* [18]. The following endemic plants were also investigated: *A. pyramidalis* Cav. Pers. [19], *A. cf. elliptica* [20], and *A. squamulosa* [21] from the genus *Ardisia*; *C. cebuense* [22, 23], *C. griffithii* [24], *C. rupestre, C. nanophyllum* [25], *C. utile* [26], *C. iners* [27], and *C. trichophyllum* [28] from the genus *Cinnamomum*; and *F. pseudopalma, F. ulmifolia* [29], *F. odorata* [30], *F. linearifolia, F. triangularis* [31] and *F. nota* [32] from the genus *Ficus.*

We report herein the isolation of lupenone (**1**) and lupeol (**2**) from the roots; **2**, squalene (**3**) and β-sitosterol (**4**) from the leaves; and betulin (**5**) from the stems of *H. mindorensis*. To the best of our knowledge, this is the first report on the isolation of these compounds from *H. mindorensis.*

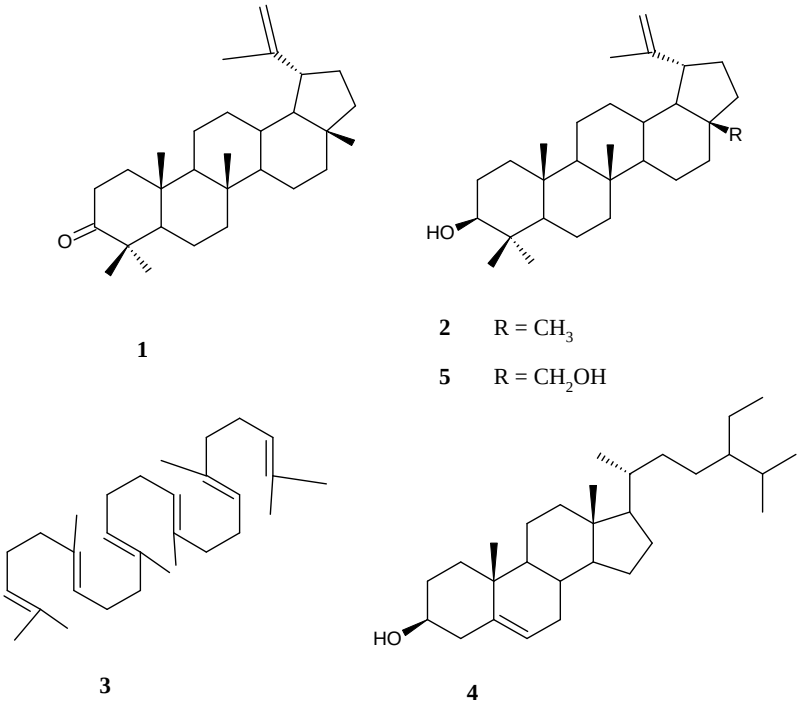

Figure 1: Compounds isolated from *Hoya mindorensis*: lupenone (1), lupeol (2), squalene (3), β-sitosterol (4), and betulin (5)

MATERIALS AND METHODS

General Experimental Procedure

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 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

Hoya mindorensis Schlechterat was collected from a garden in Pangasinan, Philippines in September 2013. Voucher specimens were authenticated at the Botany Division of the Philippine National Museum.

Isolation

The air-dried roots (4.1 g), leaves (16.8 g), and stems (8.8 g) of *H. Mindorensis* 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 roots (0.7 g), leaves (0.8 g), and stems (0.7 g) which were each chromatographed by gradient elution with petroleum ether, followed by increasing amounts of EtOAc (5%, 10%, 15%, and 20%) 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 10% EtOAc in petroleum ether fraction from the chromatography of crude roots extract was rechromatographed (3 ×) with 5% EtOAc to afford **1** (1 mg) after washing with petroleum ether. The 15% EtOAc in petroleum ether fraction was rechromatographed (5×) with 10% EtOAc in petroleum ether to afford **2** (3 mg) after washing with petroleum ether.

The 5% EtOAc in petroleum ether fraction from the chromatography of crude leaves extract was rechromatographed with 1% EtOAc in petroleum ether (6 ×) to afford **3** (5 mg). The 15% EtOAc fraction was rechromatographed with 10% EtOAc in petroleum ether (5 ×) to afford **2** (2 mg) after washing with petroleum ether. The 20% EtOAc in petroleum ether fraction was rechromatographed (5 ×) with 15% EtOAc in petroleum ether to afford **4** (3 mg) after washing with petroleum ether.

The 20% EtOAc fraction from the chromatography of crude stem extract was rechromatographed with 15% EtOAc in petroleum ether (6 ×) to afford **5** (6 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

The dichloromethane extract of the roots of *Hoya mindorensis* afforded lupenone (**1**) [6] and lupeol (**2**) [33], the leaves yielded squalene (**3**) [32], β-sitosterol (**4**) [32] and **2**, while the stems afforded betulin (**5**) [34]. The structures of **1**-**5** were identified by comparison of their ^{1}H and/or ^{13}C NMR data with those reported in literature [12, 33].

Although there is no reported biological acitivity for *H. mindorensis*, the compounds isolated from the plant were reported to possess diverse activities.

Lupenone (**1**) inhibits adipocyte differentiation by suppressing PPARγ and C/EBPα protein levels [34]. It also increases the tyrosinase enzyme expression via mitogen-activated protein kinase phosphorylated extracellular signal-regulated kinases 1 and 2 phosphorylation inhibition which results to stimulation of melanogenesis. This suggests that lupenone could be a possible treatment for hypopigmentation [35].

Lupeol (**2**) inhibits MCF-7 breast cancer cells proliferation by blocking the generation of succinyl-CoA and the reaction of substrate phosphorylation of tricarboxylic acid cycle of MCF-7 cells [36]. It can induce apoptotic cell death and inhibit migration and invasion of gallbladder carcinoma GBC-SD cells by suppressing EGFR/MMP-9 signaling [37].

Squalene (**3**) possesses cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties [38]. It significantly supress ACF formation and crypt multiplicity which could lead to possible chemopreventive activity against colon carcinogenesis [39].

β-Sitosterol (**4**) was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells [40]. It also possesses growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells [41].

Betulin (**5**) inhibits cell growth and induction of apoptosis in human melanoma cell line [42]. It also induces apaptosis of *Leishmania donovani*. Treatment of promastigotes with betulin led to mitochondrial membrane damage, activation of caspase-like proteases, and DNA fragmentation in *L. donovani* promastigotes [43].

Thus, *Hoya mindorensis* afforded triterpenes (**2**, **3** and **5**) and sterol (**4**) that were reported to exhibit anticancer properties.

Acknowledgement
One of the authors (VE) acknowledges a research and scholarship grant from the Department of Science and Technology, Science Education Institute, Accelerated Science and Technology Human Resource Development Program- National Science Consortium (DOST-SEIASTHRDP-NSC) of the Philippines.

REFERENCES

[1] F. B. Aurigue, A Collection of Philippine Hoyas and Their Culture, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD). Department of science and technology (DOST), **2013**, 195 pages.
[2] K. Yoshikawa, H. Nishino, S. Arihara, H. C. Chang, J. D. Wang, *J. Nat. Prod.*, **2000**, 63(1), 146-148.
[3] W. J. Baas, I. E. M. Van Berkel, C. Versluis, W. Heerma, M. N. Kreyenbroek, *Phytochem*., **1992**, 31(6), 2073-2076.
[4] W. J. Baas, I. E. M. Van Berkel, *Phytochem*., **1991**, 30(5), 1625-1628.
[5] W. J. Baas, *Phytochem*., **1983**, 22(12), 2809-2812.
[6] S. C. Pandey, S. S. Singh, A. C. Ghosh, D. Deepak, A. Khare, *J. Med. Arom. Plant Sci*., **2004**, 26(4), 775-783.
[7] C. Y. Ragasa, M. M. Tepora, D. L. Espineli, E. H. Mandia, J. A. Rideout, *J. Nat. Prod.*, **2008**, 71(4), 701-705.
[8] C. Y. Ragasa, J. J. Lee, M Lapina, E. Mandia, J. A. Rideout, *Nat. Prod. Res*., **2008**. 22(9), 820-824.
[9] C. Y. Ragasa, M. R. A. Puno, J. M. A. P. Sengson, C.-C. Shen, J. A. Rideout, D. D. Raga, *Nat. Prod. Res*., **2009**, 23(13), 1252-1258.
[10] C. Y. Ragasa, A. B. Alimboyoguen, C.-C. Shen, *Philipp. Scient*., **2009**, 46, 78-87.
[11] C. Y. Ragasa, M. M. Tepora, J. A. Rideout, *ACGC Chem. Res. Commun.*, **2009**, 23, 31-34.
[12] P.-W. Tsai, K. de Castro-Cruz, C.-C. Shen C-C, C. Y. Ragasa, *Phcog. J.*, **2012**, 4(31), 1-4.
[13] C. Y. Ragasa, D. L. Espineli, E. H. Mandia, D. D. Raga, M-J Don, C.-C. Shen, *Z. Naturforsch. B*, **2012**, 67b, 426-432.
[14] C. Y. Ragasa, Fortin D, Shen C-C. *Res. J. Pharm. Biol, Chem, Sci*., **2014**, 5(4), 1479-1485.
[15] C. Y. Ragasa, O. B. Torres, J. V. V. Tongco, R. A. Razal, C.-C. Shen, *Res. J. Pharm. Biol. Chem. Sci*., **2014**, *5*(4), 181-186.
[16] C. Y. Ragasa, V. A. S. Ng, J. H. Park, D. W. Kim, L. Cornelio, C.-C. Shen, *Res. J. Pharm. Biol. Chem. Sci*., **2014**, 5(4), 1081-1087.
[17] C. Y. Ragasa, A. T. Martinez, J. E. Chua, J. A. Rideout, *Philipp. J. Sci.*, **2007**, *136*(2), 167-171.
[18] C. Y. Ragasa, O. B. Torres, J. V. V. Tongco, R. A. Razal, C.-C. Shen, *J. Chem. Pharm. Res*., **2013**, 5(11), 614-616.
[19] D. D. Raga, A. B. Alimboyoguen, C.-C. Shen, C. Y. Ragasa, *Philipp. Agric. Scient*., **2011**, 94(2), 103-110.
[20] D. D. Raga, A. A. Herrera, C. Y. Ragasa, *Chin. J. Nat. Med*., **2013**, 11(2), 128-138.
[21] C. Y. Ragasa, D. L. Espineli, D. D. Raga, A. A. Herrera, C.-C. Shen, *Chem. Nat. Compd*., **2013**, 49(2), 388-389.
[22] R. S. Del Fierro, Q. M. A. Maquilang, R. A. S. Sanjorjo, M. D. Tradio, C.-C. Shen, C. Y. Ragasa, *J. Med.Plants Res.*, **2012**, 6(11), 2146-2149.
[23] C. Y. Ragasa, D. L. Espineli, E. M. G. Agoo, R. S. del Fierro, *Chin. J. Nat. Med*. **2013**, 11(3), 264-268.
[24] C. Y. Ragasa, D. L. Espineli, E. M. G. Agoo, C.-C. Shen, *Chem. Nat. Compd*., **2013**, 49(1), 127-128.
[25] C. Y. Ragasa, D. L. Espineli, E. M. G. Agoo, C.-C. Shen, *Chem. Nat. Compd*., **2013**, 49(4), 757-758.
[26] D. L. Espineli, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Chem. Nat. Compd*., **2013**, 49(4), 769-770.
[27] D. L. Espineli, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Chem. Nat. Compd*., **2013**, 49(5), 932-933.
[28] D. L. Espineli, E. M. G. Agoo, C.-C. Shen, C. Y. Ragasa, *Chem. Nat. Compd*., **2014**, 50(2), 389-390.
[29] C. Y. Ragasa, P.-W. Tsai, C.-C. Shen, *Philipp. J. Sci*., **2009**, 138(2), 205-209.
[30] P.-W. Tsai, K. de Castro Cruz, C.-C. Shen, C. Y. Ragasa, *Pharm. Chem, J*., **2012**, 46(4), 225-227.
[31] C. Y. Ragasa, O. B. Torres, C.-C. Shen, L. B. Bernardo, E. H. Mandia, K. de Castro Cruz, P.-W. Tsai, *Chem. Nat. Compd.*, **2014**, 50(1), 172-173.
[32] C. Y. Ragasa, A. B. Alimboyoguen, C.-C. Shen, *Der Pharma Chemica*. **2014**, 6(4),
[33] C. Ragasa, K. Lim, C.-C. Shen, D. D. Raga, *Phar. Chem. J.*, **2013**, 47(1), 54-58.
[34] E. K. Ahn, J. S. Oh, *Phytother. Res*., **2013**, 5, 761-766.
[35] M. O. Villareal, J. Han, M. Matsuyama, Y. Sekii, A. Smaoui, H. Shigemori, H. Isoda, *Planta Med*., **2013**, 79(3-4), 236-243.
[36] D. Shi, Y. Kuang, G. Wang, Z. Peng, Y. Wang, C. Yan, *Chinese Journal of Chromatography* **2014**, 32(3), 278-283.
[37] Y. Liu, T. Bi, G. Shen, Z. Li, G. Wu, Z. Wang, L. Qian, Q. Gao, *Cytotechnology*, **2014**, 19.
[38] K. H. S. Farvin, R. Anandan, S. Hari, S. Kumar, K. S. Shing, S. Mathew, T. V. Sankar, P. G. V. Nair, *J. Med. Food*, **2006**, 9(4), 531-536.
[39] C. V. Rao, H. L. N. Mark, B. S. Reddy, *Carcinogenesis*, **1998**, 19(2), 287-290.
[40] D. O. Moon, L. Kyeong-Jun, H. C. Yung, K. Gi-Young, *Int. Immunopharmacol*., **2007**, 7(8), 1044-1053.

[41] A. B. Awad, M. Chinnman, C. S. Fink, P. G. Bradford, *Phytomed*., **2007**, 14, 747-754.
[42] A. Orchel, A. Kulczycka, E. Chodurek, E. Bebenek, P. Borkowska, S. Boryczka, J. Kowalski, Z. Dzierzewicz, *Postepy Hig. Med. Dosw*., **2014**, 68(0), 191-197.
[43] P. Saudagar, V. K. Dubey, *Am. J. Trop. Med. Hyg*., **2014**, 90(2), 354-360.