

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/330218492>

# Triterpenes and Sterols from Leaves of *Hoya meliflua* Merr

Article in *Pharmacognosy Journal* · January 2019

DOI: 10.5530/pj.2019.1.9

CITATIONS

0

READS

100

4 authors:



**Vincent Antonio Santos Ng**

De La Salle University

50 PUBLICATIONS 184 CITATIONS

[SEE PROFILE](#)



**Raymond Malabed**

De La Salle University

15 PUBLICATIONS 9 CITATIONS

[SEE PROFILE](#)



**Fernando Aurigue**

Philippine Nuclear Research Institute

20 PUBLICATIONS 45 CITATIONS

[SEE PROFILE](#)



**Consolacion Ragasa**

De La Salle University

346 PUBLICATIONS 1,935 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Natural Products Chemistry [View project](#)



Graduate School [View project](#)

# Triterpenes and Sterols from Leaves of *Hoya meliflua* Merr.

Vincent Antonio S. Ng<sup>1,\*</sup>, Raymond S. Malabed<sup>2</sup>, Fernando B. Aurigue<sup>3</sup>, Consolacion Y. Ragasa<sup>1,4</sup>

Vincent Antonio S. Ng<sup>1,\*</sup>,  
Raymond S. Malabed<sup>2</sup>,  
Fernando B. Aurigue<sup>3</sup>,  
Consolacion Y. Ragasa<sup>1,4</sup>

<sup>1</sup>Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, PHILIPPINES.

<sup>2</sup>Department of Chemistry, Graduate School of Science, Osaka University, Osaka 560-0043, JAPAN.

<sup>3</sup>Agriculture Research Section, Atomic Research Division, Philippine Nuclear Research Institute-Department of Science and Technology, Commonwealth Avenue, Diliman, Quezon City 1101, PHILIPPINES.

<sup>4</sup>Chemistry Department, De La Salle University Science and Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, PHILIPPINES.

## Correspondence

Dr. Vincent Antonio S. Ng

Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, PHILIPPINES.

Phone no : +632-5360230

E-mail: vincent.ng@dlsu.edu.ph

## History

- Submission Date: 25-07-2018;
- Review completed: 04-10-2018;
- Accepted Date: 01-11-2018

DOI : 10.5530/pj.2019.1.9

## Article Available online

<http://www.phcogj.com/v11/i1>

## Copyright

© 2019 Phcog.Net. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



## ABSTRACT

**Introduction:** *Hoya* plants are also called wax plants due to the waxy appearance of their leaves and flowers. Most species are cultivated as ornamental plants. In the Philippines, there are at least 109 species of *Hoya*; 88 of these are endemic to the country. One of the endemic species is *Hoya meliflua* often confused with *H. diversifolia*, which can also be found in other countries. This study is part of our research on the chemical constituents of Philippine native *Hoyas*. **Methods:** The compounds were isolated by silica gel chromatography and identified by NMR spectroscopy. **Results:** Chemical investigation of the dichloromethane extract from the leaves of *Hoya meliflua* afforded squalene and mixtures of  $\beta$ -amyirin (**1a**),  $\alpha$ -amyirin (**1b**) and lupeol (**1c**) in about 1:1:0.25 ratio; oleanone (**2a**), ursenone (**2b**) and lupenone (**2c**) in about 1:1:0.3 ratio;  $\beta$ -amyirin cinnamate (**3a**),  $\alpha$ -amyirin cinnamate (**3b**) and lupenyl cinnamate (**3c**) in about 0.5:0.3:1 ratio; and  $\beta$ -sitosterol and stigmasterol in about 5:1 ratio. **Conclusion:** The results of our study indicate that *Hoya meliflua* shares similar chemical characteristics with other members of the genus *Hoya*. The triterpenes and sterols obtained from *H. meliflua* were also identified from other *Hoya* species. It is interesting to note that although most *Hoya* plants have no known biological activity and medicinal property, the compounds isolated from *H. meliflua* possess diverse bioactivities.

**Key words:** *Hoya meliflua*, Apocynaceae, Squalene,  $\beta$ -amyirin,  $\alpha$ -amyirin, Lupeol, Oleanone, Ursenone, Lupenone,  $\beta$ -amyirin Cinnamate,  $\alpha$ -amyirin Cinnamate, Lupenyl Cinnamate,  $\beta$ -sitosterol, Stigmasterol.

## INTRODUCTION

*Hoya* Br. is considered the largest genus of flowering plants in the family Apocynaceae. Commonly called wax plants due to the waxy appearance of their leaves and flowers, most species have ornamental value. A few have been reported to have medicinal properties or used in traditional medicine. In the Philippines, there are at least 109 species of *Hoya*; 88 of these are endemic to the country. One such endemic species is *Hoya meliflua* Merr. found in Apayao, Bataan, Laguna, La Union and Rizal in Luzon Island and in Leyte, Mindoro, Negros, Palawan and Panay islands in Central Philippines all at low altitudes.<sup>1</sup> New records of occurrence in other provinces, such as Laguna, have not been published yet. The Plant List.<sup>2</sup> states that *H. meliflua* is an unresolved name because some data suggest that it is synonymous with *H. diversifolia* Blume which is also found in other countries. This may be attributed to the confusion on the identity of the two Philippine native species.

This study is part of our research on the chemical constituents of Philippine native hoyas. We earlier reported the isolation of lupenone (**I**) and lupeol (**II**) from the roots; **II**, squalene (**III**) and  $\beta$ -sitosterol (**IV**) from the leaves; and betulin (**V**) from the stems of *H. mindorensis* Schlechter.<sup>3</sup> In another study, we reported the isolation of **II**,  $\alpha$ -amyirin (**VI**),  $\beta$ -amyirin (**VII**), lupeol acetate (**VIII**),  $\alpha$ -amyirin acetate (**IX**)

and  $\beta$ -amyirin acetate (**X**) from the stems; and **III**, **IV**, **VI**, bauerenol (**XI**), lutein (**XII**) and stigmasterol (**XIII**) from the leaves of *H. multiflora* Blume.<sup>4</sup> Furthermore, the isolation of  $\beta$ -amyirin cinnamate (**XIV**) and taraxerol (**XV**) from the stems; and **IV**, **XIII**, **XV**, triglycerides (**XVI**) and chlorophyll a (**XVII**) from the leaves of *H. wayetii* Kloppenb has been reported.<sup>5</sup> Moreover, the isolation of **IV**, **XIII**, **XIV**, **XV**, taraxerone (**XVIII**) and  $\alpha$ -amyirin cinnamate (**XIX**) from the stems; **IV**, **XV** and **XVIII** from the roots; **XIV** and **XIX** from the flowers; and **III**, **IV** and saturated hydrocarbons (**XX**) from the leaves of *H. buotii* has been reported.<sup>6</sup>

We also reported the isolation of **II**, **III**, **IV**, **VI**, **VII**, **XIV** and **XX** from the leaves; and **III**, **IV**, **XIII**, **XV** and lupeol cinnamate (**XXI**) from the stems of *H. diversifolia* Blume.<sup>7</sup> Chemical investigation of the dichloromethane extracts of *H. cumingiana* Decne. yielded **II**, **IV**, **VI**, **VII**, **XIII** and bauerenol (**XXII**) from the leaves; and **XV** from the stems.<sup>8</sup> Recently, we reported the isolation of **IV**, **XIII** and **XV** from the stems<sup>9</sup> and **I**, **IV**, **XIII**, 2-hydroxyethylbenzoate (**XXIII**) and fatty acid methyl esters (**XXIV**) from the leaves<sup>10</sup> of *Hoya pubicalyx*. In another study, the dichloromethane extracts of *H. paziae* Kloppenb yielded **IX**, **X**, **XV** and taraxeryl acetate (**XXV**) from the stems<sup>11</sup> and **II**, **VI**, **VII**, lupeol fatty acid esters

**Cite this article:** Vincent AS. NG, Malabed RS, Aurigue FB, Ragasa CY. Triterpenes and Sterols from Leaves of *Hoya meliflua* Merr. Pharmacogn J. 2019;11(1):48-52.

(XXVI),  $\alpha$ -amyrin fatty acid esters (XXVII) and  $\beta$ -amyrin fatty acid esters (XXVIII) from the leaves.<sup>12</sup> Furthermore, the dichloromethane extracts of *H. cagayanensis* afforded **I**, **II**, **IV**, **XIII**, **XX** and dihydrocinnaric acid (XXIX), from the stems; and 2-hydroxyethyl benzoate (XXX) from the leaves.<sup>13</sup> Recently, the dichloromethane extracts of *H. madulidii* yielded **II**, **IV**, **XIII**, **XIV**, **XIX**, **XX** and **XXI** from the stems and **III**, **IV**, **XIII**, **XVII** and **XX** from the leaves.<sup>14</sup>

In this study, the dichloromethane extract of the leaves of *Hoya meliflua* yielded squalene,  $\beta$ -amyrin (**1a**),  $\alpha$ -amyrin (**1b**), lupeol (**1c**), oleanone (**2a**), ursenone (**2b**), lupenone (**2c**),  $\beta$ -amyrin cinnamate (**3a**),  $\alpha$ -amyrin cinnamate (**3b**), lupenyl cinnamate (**3c**),  $\beta$ -sitosterol and stigmasterol (Figure 1).

## MATERIALS AND METHODS

### General Experimental Procedure

Sample spectra were obtained on a JEOL ECS 400 spectrometer with CDCl<sub>3</sub> as solvent. 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<sub>254</sub> and the plates were visualized by spraying with vanillin/H<sub>2</sub>SO<sub>4</sub> solution followed by warming.

### Sample Collection

*Hoya meliflua* leaves and stems were collected from healthy vines of a clone that has been cultivated for more than 10 years. Samples labeled as PNRI-H.CA were provided under Material Transfer Agreement No. 2016-004 dated June 24, 2016. The original plant came from Quezon Province, Philippines and authenticated by one of the authors (FBA).

### General Isolation Procedure

A glass column 12 inches in height with 0.5 inch internal diameter was used for the fractionation of the crude extract. Fractions of 10 mL volumes were collected and monitored by thin layer chromatography. Fractions containing spots with similar R<sub>f</sub> values were combined and rechromatographed using the appropriate solvent. Final purification was carried out using Pasteur pipette as the column, collecting 1 mL fractions. TLC-pure isolates were combined and after evaporation of the solvent, were subjected to NMR analysis.

### Isolation of Chemical Constituents of the Leaves

The air-dried leaves (252.8 g) were ground in a blender, soaked in CH<sub>2</sub>Cl<sub>2</sub> for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (7.8 g) which was chromatographed by gradient elution with CH<sub>2</sub>Cl<sub>2</sub>, followed by increasing amounts of acetone at 10% increment by volume as eluents.

The CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed (2 ×) using petroleum ether to afford squalene (12.8 mg). The 10% acetone in CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed (3 ×) using 1% EtOAc in petroleum ether to yield a mixture of **3a**, **3b** and **3c** (5.0 mg), after washing with petroleum ether. The 20% acetone in CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed (3 ×) using 2.5% EtOAc in petroleum ether to yield a mixture of **2a**, **2b** and **2c** (10.3 mg), after washing with petroleum ether. The 30% acetone in CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed (4 ×) using 7.5% EtOAc in petroleum ether to afford a mixture of **1a**, **1b** and **1c** (10.6 mg) after washing with petroleum ether. The 40% acetone in CH<sub>2</sub>Cl<sub>2</sub> fractions was rechromatographed (3 ×) using 10% EtOAc in petroleum ether to yield a mixture of  $\beta$ -sitosterol and stigmasterol (21.0 mg) after washing with petroleum ether.

## RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract from the leaves of *Hoya meliflua* afforded squalene and mixtures of  $\beta$ -amyrin (**1a**),  $\alpha$ -amyrin (**1b**) and lupeol (**1c**) in about 1:1:0.25 ratio; oleanone (**2a**),

ursenone (**2b**) and lupenone (**2c**) in about 1:1:0.3 ratio;  $\beta$ -amyrin cinnamate (**3a**),  $\alpha$ -amyrin cinnamate (**3b**) and lupenyl cinnamate (**3c**) in about 0.5:0.3:1 ratio; and  $\beta$ -sitosterol and stigmasterol in about 5:1 ratio. The structures of these compounds were identified by comparison of their NMR data with those reported in the literature.

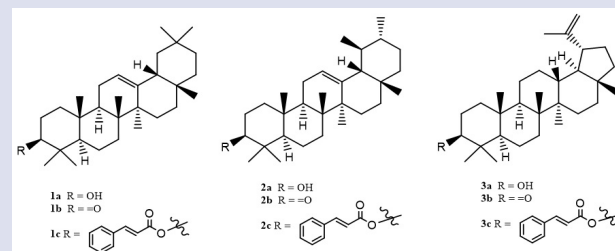
The NMR spectra of **1a** are in accordance with data reported in the literature for  $\beta$ -amyrin,<sup>4</sup> **1b** for  $\alpha$ -amyrin,<sup>4</sup> **1c** for lupeol,<sup>4</sup> **2a** for oleanone,<sup>15</sup> **2b** for ursenone,<sup>15</sup> **2c** for lupenone,<sup>16</sup> **3a** for  $\beta$ -amyrin cinnamate,<sup>6,17</sup> **3b** for  $\alpha$ -amyrin cinnamate,<sup>6,17</sup> **3c** for lupeol cinnamate,<sup>17</sup> squalene,<sup>18</sup>  $\beta$ -sitosterol<sup>18</sup> and stigmasterol.<sup>18</sup>

The ratio of about 1:1:0.25 for the mixture of  $\beta$ -amyrin (**1a**),  $\alpha$ -amyrin (**1b**) and lupeol (**1c**) was deduced from the intensities and integrations of the <sup>1</sup>H NMR resonances for the olefinic protons of **1a** at  $\delta$  5.16 (brs),<sup>7,12</sup> **1b** at  $\delta$  5.11 (brs)<sup>7,12</sup> and **1c** at  $\delta$  4.68 (brs) and 4.56 (brs).<sup>7,12,18</sup> The ratio of about 1:1:0.30 for the mixture of oleanone (**2a**), ursenone (**2b**) and lupenone (**2c**) was deduced from the intensities and integrations of the <sup>1</sup>H NMR resonances for the olefinic protons of **2a** at  $\delta$  5.19 (t, *J* = 3.2 Hz),<sup>15</sup> **2b** at  $\delta$  5.14 (t, *J* = 3.2 Hz)<sup>15</sup> and **2c** at  $\delta$  4.67 (brs) and 4.55 (brs).<sup>19</sup> The ratio of about 0.5:0.3:1 for the mixture of  $\beta$ -amyrin cinnamate (**3a**),  $\alpha$ -amyrin cinnamate (**3b**) and lupenyl cinnamate (**3c**) was deduced from the intensities and integrations of the <sup>1</sup>H NMR resonances for the olefinic protons of **3a**  $\delta$  5.18 (brs),<sup>6,17</sup> **3b** at  $\delta$  5.13 (brs),<sup>6,17</sup> and **3c** at  $\delta$  4.68 (br s) and 4.56 (br s).<sup>16</sup> The ratio of about 5:1 for the mixture of  $\beta$ -sitosterol and stigmasterol was deduced from the intensities and integrations of the <sup>1</sup>H NMR resonances for olefinic protons at  $\delta$  5.33 (brs, H-5) and methyl protons at  $\delta$  0.66 (s) for  $\beta$ -sitosterol and olefinic protons at  $\delta$  5.33 (brs, H-5), 5.13 (dd, *J* = 8.4, 15.2 Hz) and 5.00 (dd, *J* = 8.4, 15.2 Hz) and the methyl protons at  $\delta$  0.68 (s) for stigmasterol.<sup>18</sup>

*H. meliflua* is cultivated as an ornamental plant in the Philippines. There is no reported biological activity and medicinal property for the plant, but Blanco<sup>20</sup> mentioned as early as 1845 a record that *H. meliflua* causes "stoppage of birth," or perhaps as an abortifacient. However, literature search revealed that the compounds isolated from the plant possess diverse activities.

$\beta$ -Amyrin (**1a**) and  $\alpha$ -amyrin (**1b**) were reported to possess anti-inflammatory<sup>21-23</sup> and analgesic<sup>24-25</sup> properties

Triterpene **1a** showed antifungal activity against *A. rabiei* with an MIC value of 0.0156 mg/mL,<sup>26</sup> while **1b** was proposed as a possible biomarker for the fungal resistance of grape-vine leaves (*Vitis vinifera*).<sup>27</sup> The mixture of **1a** and **1b** effectively reduced the elevated plasma glucose levels during the oral glucose tolerance test (OGTT). Furthermore, the mixture of **1a** and **1b** at 100 mg/kg significantly decreased the VLDL and LDL cholesterol and increased the HDL cholesterol.<sup>28</sup> A review on the sources and biological activities of **1a** and **1b** has been provided.<sup>29</sup>



**Figure 1:** Chemical structures of  $\beta$ -amyrin (**1a**),  $\alpha$ -amyrin (**1b**), lupeol (**1c**), oleanone (**2a**), ursenone (**2b**), lupenone (**2c**),  $\beta$ -amyrin cinnamate (**3a**),  $\alpha$ -amyrin cinnamate (**3b**) and lupenyl cinnamate (**3c**).

On the other hand, **lupeol** (**1c**) exhibited **antiulcerogenic** and **diuretic** activity.<sup>30</sup> It prevented the formation of vesical calculi and reduced the size of the preformed stones in rats.<sup>31</sup> It also showed **antifungal** activity against *Fusarium oxysporum* and *Penicillium notatum*.<sup>32</sup> Triterpene **1c** significantly reduced the **451Lu** tumor growth in athymic nude mice,<sup>33</sup> inhibited the proliferation of **MDA-MB-231** human breast cancer cells in a dose dependent manner<sup>34</sup> and induced growth inhibition and apoptosis in hepatocellular carcinoma **SMMC7721** cells by down-regulation of the death receptor 3 (DR3) expression.<sup>35</sup> It exhibited potent **anti-inflammatory** activity in an allergic airway inflammation model by a significant reduction in eosinophils infiltration and in Th2-associated cytokines levels that trigger the immune responses in asthma.<sup>36</sup> Another study reported that **1c** and **lupeol acetate** have shown **hypotensive** activity.<sup>37</sup> A review on the biological activities of lupeol has been provided.<sup>38</sup>

**Lupenone** (**2c**) inhibited **adipocyte differentiation** by suppressing PPAR $\gamma$  and C/EBP $\alpha$  protein levels.<sup>39</sup> It also increased 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 **2c** could be a possible treatment for hypopigmentation.<sup>40</sup>

**$\beta$ -Amyrin cinnamate** (**3a**) was reported to **inhibit** the **TPAS-induced inflammation** (ID<sub>50</sub> 0.27  $\mu$ mol/ear; CI 95% 0.23–0.33  $\mu$ mol/ear) which is more inhibitory than the positive control, indomethacin (ID<sub>50</sub> 0.91  $\mu$ mol/ear; CI 95% 0.23–0.33  $\mu$ mol/ear).<sup>19</sup>  **$\alpha$ -Amyrin cinnamate** (**3b**), **lupeol cinnamate** (**3c**) and **3a** exhibited marked **anti-inflammatory** activity against **TPA-induced inflammation** (ID<sub>50</sub> = 0.15–0.75  $\mu$ mol/ear). Triterpene **3c** showed the highest activity with ID<sub>50</sub> of 0.15  $\mu$ mol/ear.<sup>12</sup> This triterpene at a dose of 10 mg/kg also exhibited anti-inflammatory activity on rat hind paw edema induced by carrageenan.<sup>12</sup> Furthermore, **3c** exhibited **inhibitory effect on skin tumor promotion** in an *in vivo* two-stage carcinogenesis test using DMBA as an initiator and TPA as a promoter.<sup>12</sup> Triterpenes **3a–3c** showed moderate **inhibitory effects** on **Epstein-Barr virus early antigen (EBV-EA)** in Raji cells as a primary screening test for inhibitors of tumor promoters.<sup>12</sup>

**Squalene** was reported to significantly **suppress** colonic **ACF** formation and crypt multiplicity which strengthened the hypothesis that it possesses chemopreventive activity against colon carcinogenesis.<sup>41</sup> It showed cardioprotective effect which is related to inhibition of lipid accumulation by its hypolipidemic properties and/or its antioxidant properties.<sup>42</sup> A recent study reported that **tocotrienols**, **carotenoids**, **squalene** and **coenzyme Q10** have **anti-proliferative effects on breast cancer cells**.<sup>43</sup> The preventive and therapeutic potential of squalene containing compounds on tumor promotion and regression have been reported.<sup>44</sup> A recent review on the bioactivities of squalene has been provided.<sup>45</sup>

**$\beta$ -Sitosterol** was observed to have **growth inhibitory effects** on human breast **MCF-7** and **MDA-MB-231** adenocarcinoma cells.<sup>46</sup> It was shown to be effective for the treatment of benign prostatic hyperplasia.<sup>47</sup> It was also reported to attenuate  $\beta$ -catenin and PCNA expression, as well as quench radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis.<sup>48</sup> It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake.<sup>49</sup> It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-t102 murine fibrosarcoma cells.<sup>50</sup>

**Stigmasterol** showed **therapeutic efficacy** against **Ehrlich** ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions.<sup>51</sup> It lowered plasma cholesterol levels, inhibited intestinal cholesterol and plant sterol absorption and suppressed hepatic cholesterol and classic bile acid synthesis in Wistar as well as WKY rats.<sup>52</sup> Other studies reported that **stigmasterol** showed **cytostatic** activity against **Hep-2** and **McCoy** cells,<sup>53</sup> markedly **inhibited tumour**

**promotion** in two stage carcinogenesis experiments,<sup>54</sup> exhibited **antimutagenic**,<sup>55</sup> topical **anti-inflammatory**,<sup>56</sup> **antiosteoarthritic**<sup>57</sup> and **antioxidant**<sup>58</sup> activities.

## CONCLUSION

The results of our study indicate that *Hoya meliflua* shares similar chemical characteristics with other members of the genus *Hoya*: *H. multiflora*,<sup>4</sup> *H. diversifolia*,<sup>7</sup> *H. cumingiana*,<sup>8</sup> and *H. paziae*,<sup>2</sup> which yielded  **$\beta$ -amyrin** (**1a**) and  **$\alpha$ -amyrin** (**1b**); *H. mindorensis*,<sup>3</sup> *H. multiflora*,<sup>4</sup> *H. diversifolia*,<sup>7</sup> *H. cumingiana*,<sup>8</sup> *H. paziae*,<sup>12</sup> *H. cagayanensis*,<sup>13</sup> and *H. madulidii*,<sup>14</sup> which contained **lupeol** (**1c**); *H. mindorensis*,<sup>3</sup> *H. pubicalyx*,<sup>12</sup> and *H. cagayanensis*,<sup>5</sup> which afforded **lupenone** (**2c**); *H. wayetii*,<sup>5</sup> *H. buotii*,<sup>6</sup> and *H. madulidii*,<sup>4</sup> which yielded  **$\beta$ -amyrin cinnamate** (**3a**); *H. buotii*,<sup>6</sup> and *H. madulidii*,<sup>4</sup> which afforded  **$\alpha$ -amyrin cinnamate** (**3b**); *H. diversifolia* and *H. madulidii*,<sup>14</sup> which provided **lupenyl cinnamate** (**3c**); *H. mindorensis*,<sup>3</sup> *H. multiflora*,<sup>4</sup> *H. wayetii*,<sup>5</sup> *H. buotii*,<sup>6</sup> *H. diversifolia*,<sup>7</sup> *H. cumingiana*,<sup>8</sup> *H. pubicalyx*,<sup>9,10</sup> *H. cagayanensis*,<sup>13</sup> and *H. madulidii*,<sup>14</sup> which contained  **$\beta$ -sitosterol** and **stigmasterol**; and *H. mindorensis*,<sup>3</sup> *H. buotii*,<sup>6</sup> *H. diversifolia*,<sup>7</sup> and *H. madulidii*,<sup>14</sup> which contained **squalene**. The most common constituents of the eleven native *Hoya* species studied are  $\beta$ -sitosterol and stigmasterol which are found in ten species. Of the twelve compounds isolated from *H. meliflua*, lupeol is found in eight species, squalene,  $\beta$ -amyrin and  $\alpha$ -amyrin in five species, lupenone and  $\beta$ -amyrin cinnamate in four species,  $\alpha$ -amyrin cinnamate and lupenyl cinnamate in three species. Oleanone and ursenone are reported for the first time from Philippine *Hoya* species.

It is interesting to note that although most *Hoya* plants have no known biological activity and medicinal property, the compounds isolated from *H. meliflua* possess diverse bioactivities.

Lastly, the difference in results of phytochemical analysis of *H. meliflua* is a proof that it is an entirely different and distinct species from *H. diversifolia*.

## ACKNOWLEDGEMENT

VASN would like to thank Prof. Michio Murata of Osaka University for his generous assistance with the NMR measurements. A research grant from the University Research Coordinating Office (URCO) of De La Salle University Manila (07N1TAY16-2TAY17) is gratefully acknowledged.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**CH<sub>2</sub>Cl<sub>2</sub>**: Dichloromethane; **EtOAc**: Ethyl acetate; **Et<sub>2</sub>O**: Diethyl ether.

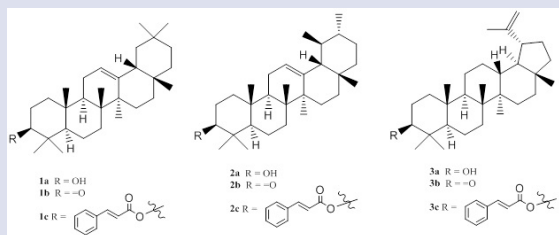
## REFERENCES

- Aurigue FB. A collection of Philippine hoyas and their culture. Los Baños, Laguna: PCAARRD-DSOST. 2013;195. (Book Series No.2/2013).
- The Plant List. Version 1.1. Published on the Internet; <http://www.theplantlist.org/> (accessed 13<sup>th</sup> July 2018). 2013.
- Ebajo JVD, Shen CC, Ragasa CY. Triterpenes and sterol from *Hoya mindorensis*. Der Pharma Chemica. 2014;6(4):321-25.
- Ebajo JVD, Shen CC, Ragasa CY. Terpenoids and sterols from *Hoya multiflora* Blume. J Appl Pharm Sci. 2015;5(3):33-9.
- Ebajo JVD, Aurigue FB, Brkljaca R, Urban S, Ragasa CY. Chemical constituents of *Hoya wayetii* Kloppenb. Int J Pharmacog Phytochem Res. 2015;7(5):1041-5.
- Ebajo JVD, Brkljaca R, Urban S, Ragasa CY. Chemical constituents of *Hoya buotii* Kloppenb. J Appl Pharm Sci. 2015;5(11):69-72.
- Panajon NM, Aurigue FB, Shen CC, Ragasa CY. Triterpenes and sterols from *Hoya diversifolia* Blume. J Appl Pharm Sci. 2016;6(6):79-82.
- Ragasa CY, Panajon NM, Aurigue FB, Brkljaca R, Urban S. Chemical constituents of *Hoya cumingiana* Decne. Int J Pharm Phytochem Res. 2016;8(12):2033-8.
- Panajon NM, Aurigue FB, Shen CC, Ragasa CY. Triterpenes and sterols from



- Hoya pubicalyx* Merr. Der Pharmacia Lettre. 2016;8(13):270-3.
10. Bolinget E, Panajon NM, Aurigue FB, Van AIA, Ragasa CY. Secondary metabolites from *Hoya pubicalyx* Merr. Res J Pharm Biol Chem Sci. 2017;7(2):678-81.
11. Borlagdan M, Aurigue F, Van AI, Ragasa CY. Triterpenes from *Hoya paziae* Kloppenb. Phcog J. 2016;8(5):487-9.
12. Perez JDV, Borlagdan MS, Aurigue FB, Van AIA, Ragasa CY. Chemical constituents of *Hoya paziae* Kloppenb. Res J Pharm Biol Chem Sci. 2017;7(2):736-9.
13. Ragasa CY, Borlagdan MS, Aurigue FB, Brkljaca R, Urban S. Chemical constituents of *Hoya cagayanensis* CM Burton. J Appl Pharm Sci. 2017;7(5):61-5.
14. Borlagdan M, Aurigue FB, Shen CC, Ragasa CY. Chemical constituents of *Hoya madulidii*. Chem Nat Compd. 2018;54(4):198-9.
15. DeCarvalho MG, Velandia JR, DeOliveira LF, Bezerra FB. Triterpenos isolados de *Eschweilera longipes* Miars (*Lecythidaceae*). Química Nova. 1998;21(6):720-43.
16. Tsai PW, DeCastro-Cruz K, Shen CC, Ragasa CY. Chemical constituents of *Broussonetia luzonensis*. Phcog J. 2012;4(31):1-4.
17. Akihisa T, Kojima N, Kikuchi T, Yasukawa K, Tokuda H, Masters ET, et al. Anti-inflammatory and chemopreventive effects of triterpene cinnamates and acetates from shea fat. J Oleo Sci. 2010;59(6):273-80.
18. Ragasa CY, Tan MCS, Fortin D, Shen CC. Chemical constituents of *Ixora philippinensis*. J Appl Pharm Sci. 2015;5(9):62-7.
19. Hisham A, Kumar GJ, Fujimoto Y, Hara N. Salacianone and salacianol, two triterpenes from *Salacia beddomei*. Phytochem. 1995;40(4):1227-31.
20. Blanco M. Flora de Filipinas: First English and modern Spanish edition. Intramuros, Manila: San Agustin Convent. 1993;3
21. Recio MC, Giner RM, Manes S, Rios JL. Structural requirements for the anti-inflammatory activity of natural triterpenoids. Planta Med. 1995;61(2):181-5.
22. Madeiros R, Otuki MF, Avellar MC, Calixto J. Mechanisms underlying the inhibitory actions of the pentacyclic triterpene-amyrin in the mouse skin inflammation induced by phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Eur J Pharmacol. 2007;55(9):227-35.
23. Okoye NN, Ajaghaku DL, Okeke HN, Ildigwe EE, Nworu CS, Okoye FBC. Beta-Amyrin and alpha-amyirin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharm Biol. 2014;52(11):1478-86.
24. Otuki C, Ferreira J, Lima F, Meyre-Silva C, Malheiros A, Muller L, et al. Antinociceptive properties of a mixture of  $\alpha$ -amyrin and  $\beta$ -amyrin triterpenes: evidence for participation of protein kinase C and protein kinase A pathways. J Pharmacol Exp Therapeutics. 2005;31(1):310-18.
25. Soldi C, Pizzolatti G, Luiz A, Marcon R, Meotti F, Miotob L, et al. Synthetic derivatives of the  $\alpha$ - and  $\beta$ -amyrin triterpenes and their antinociceptive properties. Bioorg Med Chem. 2008;16(6):3377-86.
26. Jabeen K, Javaid A, Ahmad E, Athar M. Antifungal compounds from *Melia azederach* leaves for management of *Ascochyta rabiei*, the cause of chick pea blight. Nat Prod Res. 2011;25(3):264-76.
27. Batovska DI, Todorova IT, Nedelcheva DV, Parushev SP, Atanasov AJ, Hvarleva TD, et al. Preliminary study on biomarkers for the fungal resistance in *Vitis vinifera* leaves. J Plant Physiol. 2008;165(8):791-5.
28. Santos FA, Frota JT, Arruda BR, DeMelo TS, DeCarvalho AA, DaSilva A, et al. Antihyperglycemic and hypolipidemic effects of  $\alpha$ -,  $\beta$ -amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. Lipids in Health and Disease. 2012;11(1):98.
29. Vázquez LH, Palazon J, Navarro-Ocaña A. The pentacyclic triterpenes,  $\alpha$ -,  $\beta$ -amyryns: A review of sources and biological activities, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Rao V (Ed.), ISBN: 978-953-51-0296-0, In Tech, Available from: <http://www.intechopen.com/books/phytochemicals-a-global-perspective-of-their-role-in-nutrition-and-health/the-pentacyclic-triterpenes-amyryns-a-review-of-sources-and-biological-activities>. 2012.
30. Vidyala L, Leni M, Varalakshmi P. Evaluation of the effect of triterpenes on urinary risk factors of stone formation in pyridoxine hyperoxaluric rats. Phytother Res. 2002;16(6):514-8.
31. Anand R, Patnaik GK, Kulshreshtha DK, Dhawan N. Antirolithiatic activity of lupeol, the active constituent of *Crateva nuriata*. Phytother Res. 1994;8(7):417-21.
32. Manzano PI, Miranda M, Abreu-Payrol J, Silva M, Sterner O, Peralta EL. Pentacyclic triterpenoids with antimicrobial activity from the leaves of *Vernonanthura patens* (Asteraceae). Emir J Food Agric. 2013;25(7):539-43.
33. Saleem M, Maddodi N, Zaid MA, Khan N, Hafeez B, Asim M, et al. Lupeol inhibits growth of highly aggressive human metastatic melanoma cells *in vitro* and *in vivo* by inducing apoptosis. Cancer Therapy: Preclinical. 2008;14(7):2119-27.
34. Lambertini E, Lampronti I, Penolazzi L, Khan MTH, Ather A, Giorgi G, et al. Expression of estrogen receptor gene in breast cancer cells treated with transcription factor decoy is modulated by Bangladeshi natural plant extracts. Oncol Res. 2005;15(2):69-79.
35. Zhang L, Zhang Y, Zhang L, Yang X, Lv Z. Lupeol, a dietary triterpene, inhibited growth and induced apoptosis through downregulation of DR3 in SMMC7721 cells. Cancer Investigation. 2009;27(2):163-70.
36. Vasconcelos JF, Teixeira MM, Barbosa-Filho JM, Lúcio ASSC, Almeida JRGS, Queiroz LP, et al. The triterpenoid lupeol attenuates allergic airway inflammation in a murine model. Intl Immunopharmacol. 2008;8(9):1216-21.
37. Saleem R, Ahmad SI, Ahmed M, Faizi Z, Zikr-ur Rehman S, Ali M, et al. Hypotensive activity and toxicology of constituents from *Bombax ceiba* stem bark. Biol Pharm Bull. 2003;26(1):41-6.
38. Gallo MBC, Sarachine MJ. Biological activities of lupeol. Intl J Biomed Pharm Sci. 2009;3(1):46-66.
39. Ahn EK, Oh JS. Lupenone isolated from *Adenophora triphylla* var. japonica extract inhibits adipogenic differentiation through the downregulation of PPAR $\gamma$  in 3T3-L1 cells. Phytother Res. 2013;27(5):761-6.
40. Villareal MO, Han J, Matsuyama M, Sekii Y, Smaoui A, Shigemori H, et al. Lupenone from *Erica multiflora* leaf extract stimulates melanogenesis in B16 murine melanoma cells through the inhibition of ERK1/2 activation. Planta Med. 2013;79(3-4):236-43.
41. Rao CV, Mark HLN, Reddy BS. Chemo preventive effect of squalene on colon cancer. Carcinogenesis. 1998;19(2):287-90.
42. Farvin KHS, Anandan R, Hari S, Kumar S, Shing KS, Mathew S, et al. Cardio protective effect of squalene on lipid profile in isoprenaline induced myocardial infarction in rats. J Med Food. 2006;9(4):531-6.
43. Loganathan R, Selvaduray KR, Nesaretan K, Radhakrishnan A. Differential and antagonistic effects of palm tocotrienols and other phytonutrients (carotenoids, squalene and coenzyme Q10) on breast cancer cells *in vitro*. J Oil Palm Res. 2013;25:208-15.
44. Desai KN, Wei H, Lamartiniere CA. The preventive and therapeutic potential of the squalene-containing compound, Roidec, on tumor promotion and regression. Cancer Lett. 1996;101(1):93-6.
45. Ronco AL, DeStéfani E. Squalene: a multi-task link in the crossroads of cancer and aging. Functional Foods in Health and Disease. 2013;3(12):462-76.
46. Awad AB, Chinnman M, Fink CS, Bradford PG. Sitosterol activates Fas signaling in human breast cancer cells. Phytomed. 2007;14(11):747-54.
47. Jayaprakasha GK, Mandadi KK, Poulse SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliata* (L.) Raf. Bioorg Med Chem. 2007;15(14):4923-32.
48. Baskar AA, Ignacimuthu S, Paulraj G, Numair K. Chemopreventive potential of  $\beta$ -sitosterol in experimental colon cancer model – an *in vitro* and *in vivo* study. BMC Comp Alt Med. 2010;10(1):24.
49. Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like1 in FHs 74 Int cells. Nutr Res. 2009;29(12):859-66.
50. Moon DO, Kyeong-Jun L, Yung HC, Gi-Young K. Sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. Intl Immunopharmacol. 2007;7(8):1044-53.
51. Ghosh T, Maity TK, Singh J. Evaluation of antitumor activity of stigmasterol, a constituent isolated from *Bacopa monnieri* Linn aerial parts against ehrlich ascites carcinoma in mice. Orient Pharm Exp Med. 2011;11(1):41-9.
52. Batta AK, Xu G, Honda A, Miyazaki T, Salen G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. Metabolism. 2006;55(3):292-9.
53. Gómez MA, García MD, Sáenz MT. Cytostatic activity of *Achillea ageratum* L. Phytother Res. 2001;15(7):633-4.
54. Kasahara Y, Kumaki K, Katagiri S, Yasukawa K, Yamanouchi S, Takido M. Carthami flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. Phytother Res. 1994;8(6):327-31.
55. Lim JC, Park JH, Budesinsky M, Kasal A, Han YH, Koo BS, et al. Antimutagenic constituents from the thorns of *Gleditsia sinensis*. Chem Pharm Bull. 2005;53(5):561-4.
56. García MD, Sáenz MT, Gómez MA, Fernández MA. Topical anti-inflammatory activity of phytosterols isolated from *Eryngium foetidum* on chronic and acute inflammation models. Phytother Res. 1999;13(1):78-80.
57. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Osteoarthritis Cartilage. 2010;18(1):106-16.
58. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol, isolated from *Butea monosperma*. Fitoter. 2009;80(2):123-26.

## GRAPHICAL ABSTRACT



## SUMMARY

- Chemical investigation of the dichloromethane extract from the leaves of *Hoya meliflua* afforded squalene and mixtures of  $\beta$ -amyrin (1a),  $\alpha$ -amyrin (1b) and lupeol (1c) in about 1:1:0.25 ratio; oleanone (2a), ursenone (2b) and lupenone (2c) in about 1:1:0.3 ratio;  $\beta$ -amyrin cinnamate (3a),  $\alpha$ -amyrin cinnamate (3b) and lupenyl cinnamate (3c) in about 0.5:0.3:1 ratio; and  $\beta$ -sitosterol and stigmasterol in about 5:1 ratio. The structures of these compounds were identified by comparison of their NMR data with those reported in the literature.

**Cite this article:** Vincent AS, NG, Malabed RS, Aurigue FB, Ragasa CY. Triterpenes and Sterols from Leaves of *Hoya meliflua* Merr. Pharmacog J. 2019;11(1):48-52.