Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(12); 2033-2038

ISSN: 0975-4873

Research Article

# Chemical Constituents of *Hoya cumingiana* Decne.

Consolacion Y Ragasa[1,2*], Nelson M Panajon[1,3], Fernando B Aurigue[4], Robert Brkljača[5], Sylvia Urban[5]

[1]*Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines,*
[2]*Chemistry Department, De La Salle University Science & Technology Complex, Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines,*
[3]*Chemistry Department, Central Luzon State University, Munoz, Nueva Ecija 3121, Philippines,*
[4]*Agriculture Research Section, Atomic Research Division, Philippine Nuclear Research Institute-Department of Science and Technology, Commonwealth Avenue, Diliman, Quezon City 1101, Philippines,* [5]*School of Science (Discipline of Chemistry), RMIT University (City Campus), Melbourne 3001, Victoria, Australia,*

*Available Online: 15th December, 2016*

**ABSTRACT**
Chemical investigation of the dichloromethane extracts of *Hoya cumingiana* Decne. yielded a mixture of α-amyrin (**1**), β-amyrin (**2**), bauerenol (**3**) and lupeol (**4**) in about 9:3:1:1 ratio and another mixture of β-sitosterol (**5**) and stigmasterol (**6**) in a 5:1 ratio from the leaves; and taraxerol (**7**) from the stems. The structures of **1**-**7** were identified by comparison of their NMR data with literature data.

**Keywords:** Apocynaceae, α-amyrin, β-amyrin, bauerenol, *Hoya cumingiana*, lupeol, β-sitosterol, stigmasterol, taraxerol

## INTRODUCTION

*Hoya* is the largest genus in the family Apocynaceae. Most *Hoya* species release milky white latex that is mildly poisonous and can irritate sensitive skin, but some species are used in local medicine[1]. At least 109 species of *Hoya* occur in the Philippines and 21 of these are considered indigenous[2]. One such species that was originally collected from Mindoro island, but is also found in Benguet, Bontoc, Bataan, Rizal, Camarines, and Albay provinces in Luzon and in Palawan island, Philippines, and likewise discovered in Java and Borneo (Kalimantan and Sabah) is *Hoya cumingiana* Decne.[1,2]. The plant is a terrestrial to epiphytic shrub with long pendulous or upright stems that branch at the base, and short-petioled decussate leaves[2]. The waxy flowers are clustered in a positively geotropic convex umbel and emit a pleasant sweet citrus scent. The contrasting colors (yellowish-greenish corolla and purplish-brown to crimson corona) of the flowers that last up to 8 days and the neat arrangement of the foliage make it a very attractive ornamental plant which is why it has been commercialized locally and internationally. Literature on the chemical constituents and traditional uses of *H. cumingiana* in medicine is lacking.

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* 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*[7]. Recently, the isolation of taraxerol, taraxeryl acetate, α-amyrin acetate, and β-amyrin acetate was reported from the stems of *H. paziae* Kloppenb[8].

In this study, the dichloromethane extracts of *H. cumingiana* yielded a mixture of α-amyrin (**1**), β-amyrin (**2**), bauerenol (**3**), and lupeol (**4**) in about 9:3:1:1 ratio and a mixture of β-sitosterol (**5**) and stigmasterol (**6**) in 5:1 ratio from the leaves; and taraxerol (**7**) from the stems. The chemical structures of **1**-**7** are presented in Fig. 1. To the best of our knowledge this is the first report on the isolation of **1**-**7** from *H. cumingiana.*

## MATERIALS AND METHODS

*General Experimental Procedure*

**Author for Correspondence: consolacion.ragasa@dlsu.edu.ph*

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*

Cuttings of *H. cumingiana* were harvested from healthy plants of the clone with Accession Number PNRI-H.01 cultivated at the Philippine Nuclear Research Institute *Hoya* Gerrmplasm Collection. It was authenticated by one of us (FBA) under Material Transfer Agreement No. 2015-003 dated January 20, 2015. The original planting material was obtained from Benguet province, Luzon island, Philippines in 1996.

*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 (10% increment) as eluents. Fifty 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. All fractions were monitored by thin layer chromatography. A glass column 12 inches in height and 0.5 inch internal diameter was used for further purification. Five milliliter fractions were collected. Rechromatography and final purifications were conducted using Pasteur pipettes as columns. Two milliliter fractions were collected.

*Isolation of the Chemical Constituents of the Leaves*

The air-dried leaves (93.8 g) of *H. cumingiana* were ground in an Osterizer blender, soaked in $CH_2Cl_2$ for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (7.10 g) which was chromatographed by gradient elution with $CH_2Cl_2$, followed by increasing amounts of acetone by 10% increments by volume. The 40% acetone in $CH_2Cl_2$ fraction was rechromatographed by gradient elution, starting with 10% EtOAc in petroleum ether, followed by 15% EtOAc in petroleum ether. The fractions eluted with 10% EtOAc in petroleum ether afforded a mixture of **1**-**4** (7 mg) after washing with petroleum ether. The fractions eluted with 15% EtOAc in petroleum ether yielded a mixture of **5** and **6** (4 mg) after washing with petroleum ether.

*Isolation of the Chemical Constituents of the Stems*

The air-dried stems (229.7 g) of *H. cumingiana* were ground in an Osterizer blender, soaked in $CH_2Cl_2$ for three days and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (10.3 g) which was chromatographed by gradient elution with $CH_2Cl_2$, followed by increasing amounts of acetone by 10% increments by volume. The 50% acetone in $CH_2Cl_2$ fraction was rechromatographed (2 ×) using 15% EtOAc in petroleum ether to afford **7** (9 mg) after washing with petroleum ether.

## RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extracts of *H. cumingiana* yielded **1**-**7**. The NMR spectra of **1** are in accordance with data reported in the literature for α-amyrin[9]; **2** for β-amyrin[9]; **3** for bauerenol[10]; **4** for lupeol[11]; **5** for β-sitosterol[12]; **6** for stigmasterol[12]; and **6** for taraxerol[6]. The α-amyrin (**1**):β-amyrin (**2**):bauerenol (**3**):lupeol (**4**) ratio is about 9:3:1:1 which was deduced from the intensities of the olefinic proton resonances at ô 5.06 for **1**[9], ô 5.12 for **2**[9], ô 5.42 ratio of about 9:3:1:1 or **3**[10], and ô 4.65 and 4.54 for **4**[11]. The ratio of 5:1 for β-sitosterol (**5**):stigmasterol (**6**) was deduced from the intensities of the olefinic proton resonances at ô 5.35 for **5**[12] and ô 5.35, 5.13 and 5.00 for **6**[13].

These results indicate that *H. cumingiana* shares similar chemical characteristics with other members of the genus *Hoya*: *H. multiflora*[4] which contained α-amyrin (**1**), β-amyrin (**2**) and bauerenol (**3**); *H. diversifolia*[7] which yielded β-amyrin (**2**); *H. mindorensis*[3] and *H. diversifolia*[7] which yielded lupeol (**4**); *H. mindorensis*[3], *H. multiflora*[4], *H. wayetii*[5], *H. diversifolia*[7] and *H. buotii*[6] which afforded *β*-sitosterol (**5**) and stigmasterol (**6**); *H. wayetii*[5], *H. buotii*[6], *H. diversifolia*[7] and *H. paziae*[8] which contained taraxerol (**7**).

Although no biological activity tests were conducted on the isolated compounds, a literature search of **1**-**7** revealed that these have diverse bioactivities.

α-Amyrin (**1**) and β-amyrin (**2**) were reported to possess antiinflammatory[13-15] and analgesic[16-17] properties. Triterpene **1** was proposed as a possible biomarker for the fungal resistance of grape-vine leaves (*Vitis vinifera*)[18]. On the other hand, **2** showed antifungal activity against *A. rabiei* with an MIC value of 0.0156 mg/mL[19]. The mixture of **1** and **2** effectively reduced the elevated plasma glucose levels during the oral glucose tolerance test (OGTT). Furthermore, the mixture of these triterpenes at 100 mg/kg significantly decreased the VLDL and LDL cholesterol and increased the HDL cholesterol[20]. A review on the sources and biological activities of **1** and **2** has been provided[9].

A mixture of **1**, **2** and bauerenol (**3**) obtained from *Ardisia* species exhibited angio-suppressive effects on duck chorioallantoic membrane (CAM)[21]; restricted inter-capillary length and reduced branch point with 100% CAM viability and embryo survivability and promoted intense expression of the von Willebrand factor (F8)[22]; was found toxic to *A. salina nauplii* after 48h of exposure and showed teratologic manisfestations on *Danio rerio* embryos[23]; and exhibited analgesic property in the acetic acid writhing test and hot plate assay[24]. Another study reported that a mixture of **1-3** from *Carmona retusa* exhibited 51% analgesic activity and showed 20% anti-inflammatory activity at dosage of 100 mg/kg mouse, while of 250 mg/kg mouse showed a 29% anti-diarrheal activity[25].

Lupeol (**4**) exhibited antiurolithiatic and diuretic activity[26]. It prevented the formation ofvesical calculi and reduced the size of the preformed stones in rats[27]. It also showed antifungal activity against *Fusarium oxysporum* and *Penicillium notatum*[28]. Triterpene **4** significantly

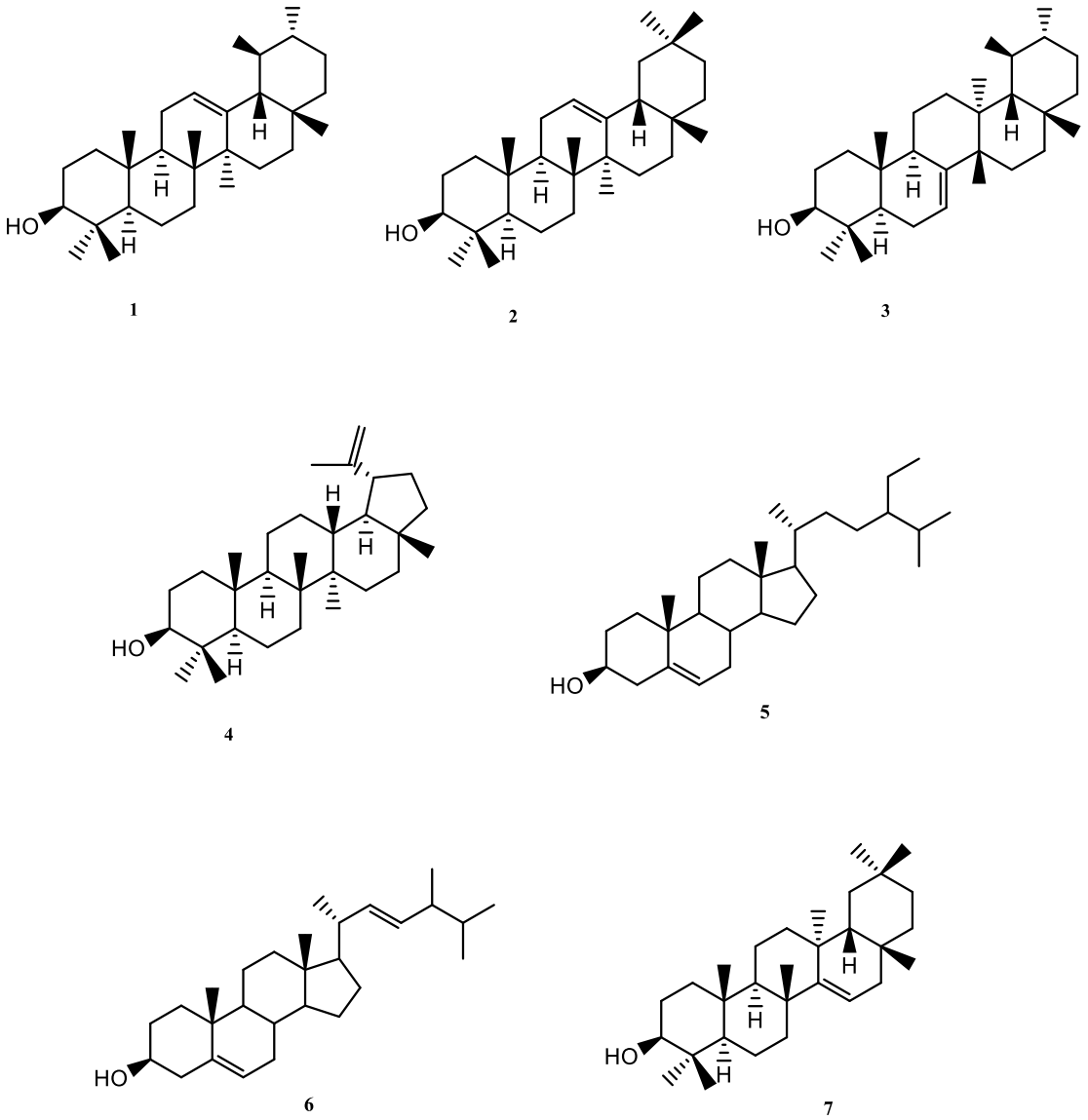


Figure 1: Chemical structures of α-amyrin (**1**), β-amyrin (**2**), bauerenol (**3**), lupeol (**4**), β-sitosterol (**5**), stigmasterol (**6**) and taraxerol (**7**) from *Hoya cumingiana.*

reduced the 451Lu tumor growth in athymic nude mice[29], inhibited the proliferation of MDA-MB-231 human breast cancer cells in a dose dependent manner[30], and induced growth inhibition and apoptosis in hepatocellular carcinoma SMMC7721 cells by down-regulation of the death receptor 3 (DR3) expression[31]. Triterpene **4** and lupeol acetate have shown hypotensive activity[32], while **4** also exhibited antidyslipidemic activity in hamster at 100 mg/Kg body weight[33]. It showed 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[34]. A review on the biological activities of **4** has been provided[35].

*β*-Sitosterol (**5**) was observed to have growth inhibitory effects on human breast MCF-7 and MDA-MB-231 adenocarcinoma cells[36]. It was shown to be effective for the treatment of benign prostatic hyperplasia[37]. It was also reported to attenuate β-catenin and PCNA expression, as well as quench radical *in-vitro*, making it a potential anticancer drug for colon carcinogenesis[38]. It can inhibit the expression of NPC1L1 in the enterocytes to reduce intestinal cholesterol uptake[39]. It was reported to induce apoptosis mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells[40].

Stigmasterol (**6**) showed therapeutic efficacy against Ehrlich ascites carcinoma bearing mice while conferring protection against cancer induced altered physiological conditions[41]. It lowered plasma cholesterol levels, inhibits intestinal cholesterol and plant sterol absorption, and suppresses hepatic cholesterol and classic bile acid synthesis in Winstar as well as WKY rats[42]. Other studies reported that stigmasterol showed cytostatic activity against Hep-2 and McCoy cells[43], markedly inhibited tumour promotion in two stage carcinogenesis experiments[44], exhibited antimutagenic[45], topical anti-inflammatory[46], antiosteoarthritic[47] and antioxidant[48] activities.

Taraxerol (**7**) was reported to exhibit anti-inflammatory activity by selective COX-1 inhibition[49]. Another study reported that **1** downregulates the expression of proinflammatory mediators in macrophages by preventing NF-κB activation[50]. Furthermore, **1** was shown as a glucose transport inhibitor and stimulator of glycogen synthesis[51]. Moreover, **1** inhibited the growth of Hela and BGC-823 with $IC_{50}$ of 73.4 μmol/L-1 and 73.3 μmol/L-1, respectively[52].

## CONCLUSION

The dichloromethane extracts of *H. cumingiana*, a plant indigenous to the Philippines, afforded α-amyrin (**1**), β-amyrin (**2**), bauerenol (**3**), lupeol (**4**), β-sitosterol (**5**), stigmasterol (**6**) and taraxerol (**7**) which were reported to exhibit diverse biological activities.

## ACKNOWLEDGEMENTS

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

## REFERENCES

1. Lamb A, Rodda M. A Guide to Hoyas of Borneo. Natural History Publications (Borneo) Sdn. Bhd., 2016. 204p.
2. Aurigue FB. A Collection of Philippine Hoyas and their Culture. Los Baños, Laguna: PCAARRD-DOST, 2013. 195p.
3. Ebajo Jr VD, C-C Shen, Ragasa CY. Triterpenes and sterol from *Hoya mindorensis*. Der Pharma Chemica 2014; 6(4):321-325.
4. Ebajo Jr VD, Shen C-C, Ragasa CY. Terpenoids and sterols from *Hoya multiflora* Blume. J Appl Pharm Sci 2015a; 5(3):33-39.
5. Ebajo Jr VD, Aurigue FB, Brkljača R, Urban S, Ragasa CY. Chemical constituents of *Hoya wayetii* Kloppenb. Int J Pharmacog Phytochem Res 2015b; 7(5):1041-1045.
6. Ebajo Jr VD, Brkljača R, Urban S, Ragasa CY. Chemical constituents of *Hoya buotii* Kloppenb. J Appl Pharm Sci 2015; 5(11):69-72.
7. Panajon NM, Aurigue FB, Shen C-C, Ragasa CY. Triterpenes and sterols from *Hoya diversifolia* Blume. J Appl Pharm Sci 2016; 6(6):79-82.
8. Borlagdan M, Aurigue F, van Altena I, Ragasa CY. Triterpenes from *Hoya paziae* Kloppenb. Phcog J 2016; 8(5):487-489.
9. Vázquez LH, Palazon J, Navarro-Ocaña A. The Pentacyclic Triterpenes α, β-amyrins: A Review of Sources and Biological Activities, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Dr Venketeshwer Rao (Ed.), 2012; 487-502.
10. Solis PN, Nakamura N, Olmedo D, Hattori M, Gupta MP. Parathesilactones and paratheiquinones from branches of *Parathesis amplifolia.* Pharm Biol 2006; 44(5):328-335.
11. Ragasa CY, Tan MCS, Fortin D, Shen C-C. Chemical constituents of *Ixora philippinensis. J. Appl. Pharm. Sci.,* 2015; 5(9):62-67.
12. Ragasa CY, Lorena GS, Mandia EH, Raga DD, Shen C-C. Chemical constituents of *Abrus precatorius.* Amer J Essent Oils Nat Prod 2013; 1(2):7-10.
13. Recio MC, Giner RM, Manez S, Rios JL. Structural requirements for the anti-inflammatory activity of natural triterpenoids. Planta Med 1995; 61 (2):181-185.
14. Madeiros R, Otuki MF, Avellar MC, Calixto JB. Mechanisms underlying the inhibitory actions of the pentacyclic triterpene-amyrin in the mouse skin inflammation induced by phorbol ester 12-Otetradecanoylphorbol-13-acetate. Eur J Pharmacol 2007; 55 (9):227-235.
15. Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FBC. beta-Amyrin and alpha-amyrin acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. Pharm Biol 2014; 52 (11):1478-1486.
16. Otuki C, Ferreira J, Lima F, Meyre-Silva C, Malheiros A, Muller L, Cani G, Santos A, Yunes R, Calixto J. Antinociceptive properties of a mixture of α-amyrin and β-amyrin triterpenes: evidence for participation of protein kinase C and protein kinase A pathways. J Pharmacol Exp Therapeutics 2005; 31 (1):310-318.
17. Soldi C, Pizzolatti G, Luiz A, Marcon R, Meotti F, Miotob L, Santos A. Synthetic derivatives of the α- and β-amyrin triterpenes and their antinociceptive properties. Bioorg Med Chem 2008; 16 (6):3377-3386.
18. Batovska DI, Todorova IT, Nedelcheva DV, Parushev SP, Atanassov AJ, Hvarleva TD, Djakova GJ, Bankova VS. Preliminary study on biomarkers for the fungal resistance in *Vitis vinifera* leaves, J Plant Physiol 2008; 165:791-795.
19. Jabeen K, Javaid A, Ahmad E, Athar M. Antifungal compounds from *Melia azederach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Nat Prod Res 2011; 25 (3):264-276.
20. Santos FA, Frota JT, Arruda BR, de Melo TS, de Carvalho AA, da Silva A, Brito GAdC, Chaves MH, Rao VS. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture, from *Protium heptaphyllum* in mice. Lipids in Health and Disease 2012; 11:98.
21. Raga DD, Herrera A, Shen C-C, Ragasa CY. Triterpenes from *Ardisia cf. elliptica* (subgenus Tinus) limit vascular density and promote von Willebrand factor expression on duck chorioallantoic membrane. Pharm Chem J 2013b; 47 (1):44-53.
22. Raga DD, Herrera AA, Dinah Espineli, Shen C-C, Ragasa CY. Triterpenes from *Ardisia squamulosa* C. Presl (Myrsinaceae) limit angiogenesis and the expression of Von Willebrand factor in duck chorioallantoic membrane. J Chem Pharm Res, 2013c; 5 (10):230-239.
23. Raga DD, Herrera AA, Alimboyoguen AB, Shen C-C, Ragasa CY. Effects of triterpenes from *Ardisia cf. elliptica* (subgenus Tinus) and sterols from *Ardisia pyramidalis* Cav Pers on *Artemia salina* and *Danio*

*rerio* toxicity and caudal fin regeneration. J Chem Pharm Res 2014a; 6 (3):1014-1022.
24. Raga DD, Herrera AA, Shen C-C, Ragasa CY. Analgesic triterpenes from *Ardisia cf. elliptica* (subgenus Tinus) (Myrsinaceae). Der Pharma Chemica 2014b; 6 (4):153-161.
25. Villasenor IM, Canlas AP, Faustino KM, Plana KG. Evaluation of the bioactivity of triterpene mixture isolated from *Carmona retusa* (Vahl.) Masam leaves. J Ethnopharmacol 2004; 92 (1):53-56.
26. Vidya 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):514-518.
27. Anand R, Patnaik GK, Kulshreshtha DK, Dhawan N. Antiurolithiatic activity of lupeol, the active constituent of *Crateva nuriala.* Phytother Res 1994; 8 (7):417-421.
28. Manzano PI, Miranda M, Abreu-Payrol J, Silva M, Sterner O, Peralta EL. Pentacyclic triterpenoids with antimicrobial activity from the leaves of *Vernon anthura* patens (Asteraceae). Emir J Food Agric 2013; 25 (7):539-543.
29. Saleem M, Maddodi N, Zaid MA, Khan N, Hafeez B, Asim M, Suh Y, Yun J, Setaluri V, Mukhtar H. Lupeol inhibits growth of highly aggressive human metastatic melanoma cells in vitro and in vivo by inducing apoptosis. Cancer Therapy: Preclinical 2008; 14:2119-2127.
30. Lambertini E, Lampronti I, Penolazzi L, Khan MTH, Ather A, Giorgi G, Gambari R, Piva R. Expression of estrogen receptor gene in breast cancer cells treated with transcription factor decoy is modulated by Bangladeshi natural plant extracts. Oncology Research 2005; 14: 69-79.
31. 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; 27:163-170.
32. Saleem R, Ahmad SI, Ahmed M, Faizi Z, Zikr-ur-Rehman S, Ali M, Faizi S. Hypotensive activity and toxicology of constituents from *Bombax ceiba* stem bark. Biol Pharm Bull 2003; 26: 41-46.
33. Reddy KP, Singh AB, Puri A, Srivastava AK, Narender T. Synthesis of novel triterpenoid (lupeol) derivatives and their in vivo antihyperglycemic and antidyslipidemic activity. Bioorg Med Chem Lett 2009; 19:4463-4466.
34. Vasconcelos JF, Teixeira MM, Barbosa-Filho JM, Lúcio ASSC, Almeida JRGS, Queiroz LP, Ribeiro-dos-Santos R, Soares MBP. The triterpenoid lupeol attenuates allergic airway inflammation in a murine model. Intl Immunopharmacol 2008; 8:1216-1221.
35. Gallo MBC, Sarachine MJ. Biological activities of lupeol. Intl J Biomed Pharm Sci 2009; 3 (1):46-66.
36. Awad AB, Chinnman M, Fink CS, Bradford PG. Sitosterol activates Fas signaling in human breast cancer cells. Phytomed. 2007; 14:747-754.
37. Jayaprakasha GK, Mandadi KK, Poulose SM, Jadegoud Y, Gowda GA, Patil BS. Inhibition of colon cancer growth and antioxidant activity of bioactive compounds from *Poncirus trifoliate* (L.) Raf. Bioorg Med Chem 2007; 4923-4932.
38. Baskar AA, Ignacimuthu S, Paulraj G, Numair K, Chemopreventive potential of β-sitosterol in experimental colon cancer model - an *in vitro* and *in vivo* study. BMC Comp Alt Med 2010; 10**:**24.
39. Jesch ED, Seo JM, Carr TP, Lee JY. Sitosterol reduces messenger RNA and protein expression levels of Niemann-Pick C1-like1in FHs 74 Int cells**.** Nutr Res 2009; 29(12):859-66.
40. Moon DO, Kyeong-Jun L, Yung HC, Gi-Young K. Int Immunopharmacol 2007; 7:1044-1053.
41. Ghosh T, Maity TK, Singh J. Evaluation of antitumor activity ofstigmasterol, a constituent isolated from *Bacopa monnieri* Linn aerial parts against ehrlich ascites carcinoma in mice. Orient Pharm Exp Med 2011; 11:41-49.
42. 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):292–299.
43. Gómez MA, García MD, Sáenz MT. Cytostatic activity of *Achillea ageratum* L. Phytother Res 2001; 15(7):633–634.
44. 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):327–331.
45. Lim J-C, Park JH, Budesinsky M, Kasal A, Han Y-H, Koo B-S, Lee S-I, Lee D-U. Antimutagenic constituents from the thorns of *Gleditsia sinensis*. Chem Pharm Bull 2005; 53(5):561–564.
46. 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.
47. 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):106–116.
48. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol, isolated from *Butea monosperma.* Fitoter 2009; 80(2):123–126.
49. 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.
50. Yao X, Li G, Bai Q, Xu H, Lu. Taraxerol inhibits LPS-induced inflammatory responses through suppression of TAK1 and Akt activation. Int Immunopharmacol 2013; 15(2):316-324.
51. 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.

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