

Research Article

Triterpenes from *Plumeria rubra* L. FlowersJariel Naomi B Bacar¹, Maria Carmen S Tan¹, Chien-Chang Shen², Consolacion Y Ragasa^{1,3}¹Chemistry Department, De La Salle University, 2401 Taft Avenue, Manila 1004, Philippines.²National Research Institute of Chinese Medicine, Ministry of Health and Welfare, 155-1, Li-Nong St., Sec. 2, Taipei 112, Taiwan.³Chemistry Department, De La Salle University Science & Technology Complex Leandro V. Locsin Campus, Biñan City, Laguna 4024, Philippines.Received: 8th Jan, 17; Revised: 8th Feb, 17; Accepted: 16th Feb, 17; Available Online: 25th February, 2017**ABSTRACT**

Chemical investigation of the dichloromethane extract of the white flowers of *Plumeria rubra* L. (syn. *Plumeria acuminata* W.T.Aiton) afforded a mixture of lupeol (**1**), α -amyirin (**2**) and β -amyirin (**3**) in about 8:2:1 ratio. The structures of **1-3** were identified by comparison of their NMR data with those reported in the literature.

Keywords: *Plumeria rubra*, *Plumeria acuminata*, Apocynaceae, lupeol, α -amyirin, β -amyirin.

INTRODUCTION

Plumeria rubra L. (syn. *Plumeria acuminata* W.T.Aiton) commonly known as frangipani and locally known as kalachuchi is grown as an ornamental tree throughout the Philippines. A number of studies were reported on the biological activities of the different parts of *P. rubra*. The ethanolic extract of *Plumeria rubra* flower and its butanol fraction were observed to possess significant anxiolytic potential using elevated plus model of anxiety¹. Another study reported that the methanolic flower extract of *P. rubra* exhibited antioxidant, cytotoxic and hypolipidemic activities². Furthermore, the methanolic extracts of the leaves and flowers of *P. rubra* inhibited the growth of 14 bacteria with the zones of inhibition between 12-28 mm. The *P. rubra* flowers extract was more active than the leaf extract against *Bacillus cereus* with a zone of inhibition of 28 mm³. In addition, the methanolic extracts of the flowers of *P. rubra* were reported to exhibit antioxidant and anti-inflammatory activities⁴. In Mexico, the flower infusions of *P. rubra* are used for the treatment of diabetes mellitus⁵. Many studies were conducted on the chemical constituents of the different parts of *P. rubra*. The flowers of *P. rubra* yielded nerolidols⁶, naphthalene⁷, linalool⁸, benzyl benzoate and methyl salicylate^{7,8}. In a recent study, benzyl salicylate (26.7%, 33.5%), benzyl benzoate (22.3%, 7.9%), geraniol (trace, 17.2%), (*E, E*)-geranyl linalool (9.4%, 0.2%), tricosane (8.3%, 1.1%), linalool (0.1%, 8.0%), nonadecane (7.0%, 3.8%), (*E*)-nerolidol (7.0%, 5.5%), and pentacosane (4.4%, 0.3%) as the major constituents of the flower oil and hydrodistilled volatile distillate, respectively⁹. Another study reported that the flower oil of *P. rubra* contained (*E*)-non-2-en-1-ol (15.7%), limonene (10.8%), phenylacetaldehyde (9.0%), *n*-tetradecanal (8.8%), γ -elemene (6.5%) and (*E, E*)- α -farnesene (6.1%)¹⁰. Moreover, the *P. rubra* flowers essential oil has yielded 2-methylbutan-1-ol, β -phenylethyl alcohol, nanodecane,

heneicosane, benzyl salicylate, tetradecanoic acid, octadecanoic acid and phenylacetaldehyde¹¹. In another study, the flowers of *P. rubra* were reported to contain resin, quercetin, and traces of kaempferol and cyanidin diglycosides; the fresh leaves and bark contain plumeride, resinic acid; and the bark also contain fulvoplumerin, a mixture of terpenoids and sterols plumieride. Furthermore, the latex coagulum from the branches gave caoutchoue and resin matter¹². Two new iridoid diastereomers were isolated from the flowers of *P. rubra* L. cv. *Acutifolia*, while the heartwood yielded plumericin, isoplumericin, 4-hydroxyacetophenone, plumeride, 13-coumaroylplumieride and protoplumericine. Significant amounts of immunoreactive cardiac glycoside were found to be present in *P. rubra*¹³. Four new iridoids, plumeridoids A, B, and C and epiplumeridoid C were isolated from the stem bark of *P. rubra* Linn. together with twenty-four known compounds: 1-(*p*-hydroxyphenyl)propan-1-one, isoplumericin, plumericin, dihydroplumericin, allamcin, fulvoplumerin, allamandin, plumieride, *p*-*E*-coumaric acid, 2,6-dimethoxy-*p*-benzoquinone, scopoletin, cycloart-25-en-3 β ,24-diol, 2,4,6-trimethoxyaniline, ajunolic acid, ursolic acid, oleanolic acid, β -amyirin acetate, betulinic acid, lupeol and its acetate, 2,3-dihydroxypropyl octacosanoate, glucoside of β -sitosterol, stigmaterol and β -sitosterol¹⁴. The bark of *P. rubra* contained plumieride¹⁵, fulvoplumerin¹⁶. The root contains plumiericine, β -dihydroplumericin, and isoplumericin¹⁷, β -dihydroplumericin, fulvoplumerin, plumeride, taraxasteryl acetate, lupeol, stigmaterol, oleanolic acid, and rubrinol which is an antibacterial triterpenoid¹⁸. Phytochemical constituents from the bark of *Plumeria rubra* were identified as stigmast-7-enol, lupeol carboxylic acid, lupeol acetate, urosolic acid, (2*R*,3*S*)-3,4'-dihydroxy-7,3',5'-trimethoxyflavan-5-*O*- β -D-glucopyranoside, and flavan-3-ol glycoside¹⁹. The

phytochemical constituents from the ethanolic extract of the leaves of *Plumeria acuminata* were reported as stigmast-7-enol²⁰, lupeol carboxylic acid²¹, lupeol acetate²², and urosolic acid²³. This study is part of our research on the chemical constituents of plants belonging to the family Apocynaceae. We earlier reported the chemical constituents of *Hoya cumingiana*²⁴, *Hoya wayetii*²⁵, and *Wrightia pubescens*²⁶. In this study, we obtained a mixture of lupeol (**1**), α -myrin (**2**) and β -amyrin (**3**) in about 8:2:1 ratio from the white flowers of *P. rubra*. To the best of our knowledge this is the first report on **1-3** as chemical constituents of the flowers of *P. rubra*. The structures of **1-3** are presented in Fig. 1.

MATERIALS AND METHODS

General Experimental Procedure

¹H spectra were acquired in CDCl₃ on a 600 MHz Varian VNMRs NMR spectrometer with referencing to solvent signals (δ 7.24). Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄ and the plates were visualized by spraying with vanillin/H₂SO₄ solution followed by warming.

Sample Collection

The white flowers of *Plumeria rubra* L. (syn. *Plumeria acuminata* W.T.Aiton) were collected from Mandaluyong City, Philippines in January 2016. The sample was authenticated at the Botany Division, Philippine National Museum.

General Isolation Procedure

A glass column 12 inches in height and 0.5 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH₂Cl₂ (10% by volume increment) as eluents. Five milliliter fractions were collected. All fractions were monitored by thin layer chromatography. Fractions with spots of the same *R_f* value were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

Isolation of the Chemical Constituents of *P. rubra* Flowers

The air-dried flowers of *P. rubra* (29.52 g) were ground in a blender, soaked in CH₂Cl₂ for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.5543 g) which was chromatographed using increasing proportions of acetone in CH₂Cl₂ at 10% increment by volume. The 20% acetone in CH₂Cl₂ fraction was rechromatographed (3 \times) using 10% EtOAc in petroleum ether to yield a mixture of **1-3** (4 mg) after washing with petroleum ether.

RESULTS AND DISCUSSION

Silica gel chromatography of the dichloromethane extract of the flowers of *P. rubra* yielded a mixture of lupeol (**1**), α -amyrin (**2**) and β -amyrin (**3**). The NMR spectra of **1** are in accordance with data reported in the literature for lupeol²⁷; **2** for α -amyrin²⁸; and **3** for β -amyrin²⁸. The **1:2:3** ratio is about 8:2:1 which was deduced from the intensities

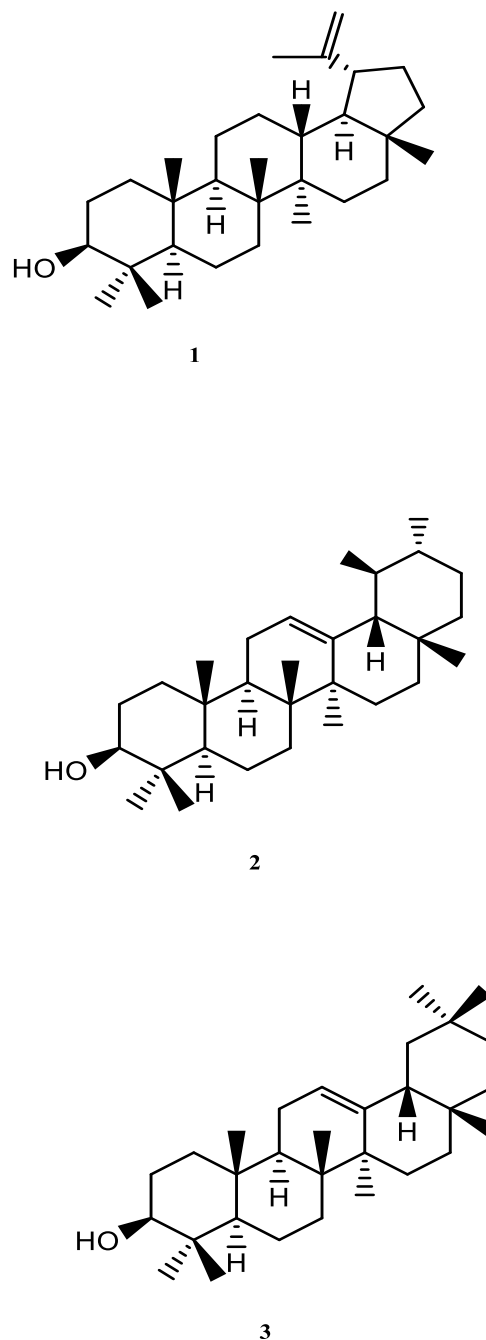


Figure 1: Chemical structures of lupeol (**1**), α -amyrin (**2**) and β -amyrin (**3**) from *P. rubra* flowers.

of the olefinic proton resonances at δ 4.65 and 4.54 for **1**²⁷, δ 5.08 for **2**²⁸, and δ 5.14 for **3**²⁸. Although no biological activity tests were conducted on the isolated compounds, a literature search of **1-3** revealed that these have diverse bioactivities. Lupeol (**1**) exhibited antirolithiatic and diuretic activity²⁹. It prevented the formation of vesical calculi and reduced the size of the preformed stones in rats³⁰. It also showed antifungal activity against *Fusarium oxysporum* and *Penicillium notatum*³¹. Lupeol significantly reduced the 451Lu tumor growth in athymic nude mice³², inhibited the proliferation of MDA-MB-231 human breast cancer cells in a dose dependent manner³³, and induced growth inhibition and apoptosis in

hepatocellular carcinoma SMMC7721 cells by down-regulation of the death receptor 3 (DR3) expression³⁴. Lupeol and lupeol acetate have shown hypotensive activity³⁵. 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 asthma³⁶. A review on the biological activities of lupeol has been provided³⁷. α -Amyrin (**2**) and β -amyrin (**3**) were reported to possess anti-inflammatory³⁸⁻⁴⁰ and analgesic⁴¹⁻⁴² properties. Triterpene **2** was proposed as a possible biomarker for the fungal resistance of grape-vine leaves (*Vitis vinifera*)⁴³. On the other hand, **3** showed antifungal activity against *A. rabiei* with an MIC value of 0.0156 mg/mL⁴⁴. The mixture of **2** and **3** 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 cholesterol⁴⁵. A review on the sources and biological activities of **2** and **3** has been provided²⁸. A mixture of **2**, **3** and bauerenol obtained from *Ardisia* species exhibited angio-suppressive effects on duck chorioallantoic membrane (CAM)⁴⁶; 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)⁴⁷; was found toxic to *A. salina nauplii* after 48h of exposure and showed teratologic manifestations on *Danio rerio* embryos⁴⁸; and exhibited analgesic property in the acetic acid writhing test and hot plate assay⁴⁹. Another study reported that a mixture of **2**, **3** and bauerenol 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 activity⁵⁰.

REFERENCES

1. Chatterjee M, Verma R, Lakshmi V, Sengupta S, Verma AK, Mahdi AA, Palit G. Anxiolytic effects of *Plumeria rubra* var. *acutifolia* (Poir) L. flower extracts in the elevated plus-maze model of anxiety in mice. *Asian J Psychiatr* 2013; 6(2):113-8.
2. Hafizur R, Vijaya BR, Soumya G, Sandeep KM, Geetika P, Sibi G. Antioxidant, cytotoxic and hypolipidemic activities of *Plumeria alba* L. and *Plumeria rubra* L. *Amer J Life Sci*. 2014, 2:11-15.
3. Egwaikhide PA, Okeniyi SO, Gimba CE. Screening for anti-microbial activity and phytochemical constituents of some Nigerian medicinal plants. *J Med Plants Res* 2009; 3(12):1088-91.
4. Sirisha K, Rajendra Y, Gomathi P, Soujanya K, Yasmeen N. Antioxidant and anti-inflammatory activities of flowers of *Plumeria rubra* L. f. *rubra* and *Plumeria rubra* f. *lutea*: A comparative study. *Res J Pharm Biol Chem Sci* 2013; 4(4):743-756.
5. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *J Ethnopharmacol* 2005; 99:325-348.
6. Matthias O, Hamburger, Geoffrey A, Cordell, Nijisiri R. Traditional medicinal plants of Thailand XVII. Biologically active constituents of *Plumeria rubra*. *J Ethnopharmacol* 1991; 33(3):289-92.
7. Zhu LF, Li YH, Li BL, Lu BY, Zang WL. Aromatic plants and essential constituents. Chinese Academy of Science, Guangzhou.1983; 89.
8. Pino JA, Ferres A, Alvarez D, Rosado. Volatiles of an alcoholic extract of flowers from *Plumeria rubra* L. var. *acutifolia*. *Flavour Fragr J* 1994; 9:343-45.
9. Goswami P, Chauhan A, Verma RS, Padalia RC. Chemical constituents of floral volatiles of *Plumeria rubra* L. from India. *Med Aromat Plants* 2016; S3: 005. doi:10.4172/2167-0412.S3-005.
10. Lawal OA, Ogunwande IA, Opoku AR. Chemical composition of essential oils of *Plumeria rubra* L. grown in Nigeria. *European J Med Plants* 2015; 6(1):55-61.
11. Sulaiman SF, Yaacob SS, Tan ML, Muhamma TST. Chemical components of the essential oils from three species of Malaysian *Plumeria* L. and their effects on the growth of selected microorganisms. *J. Biosci* 2008; 19:1-7.
12. Oladipupo A, Lawal I, Opoku Y. Chemical composition of essential oils of *Plumeria rubra* L. grown in Nigeria, *European J Med Plants*. 2015; 6:55-6.
13. Ye G1, Yang YL, Xia GX, Fan MS, Huang CG. Complete NMR spectral assignments of two new iridoid diastereoisomers from the flowers of *Plumeria rubra* L. cv. *acutifolia*. *Magn Reson Chem* 2008; 46:1195-7.
14. Kuigoua GM, Kouam SF, Ngadjui BT, Schulz B, Green IR, Choudhary MI, Krohn K. Minor secondary metabolic products from the stem bark of *Plumeria rubra* Linn. displaying antimicrobial activities. *Planta Med* 2010; 76(6):620-625.
15. Gupta M, Mazumder UK, Gomathi P, Selvan VT. Antiinflammatory evaluation of leaves of *Plumeria acuminata*. *BMC Complement Alternat Med* 2006; 6:36.
16. Gupta M, Mazumder UK, Gomathi P. Evaluation of antioxidant and free radical scavenging activities of *Plumeria acuminata* leaves. *J Biol Sci* 2008; 7:1361-7.
17. Merina AJ, Sivanesa D, Begum VH, Sulochana N. Antioxidant and hypolipidemic effect of *Plumeria rubra* L. in alloxan induced hyperglycemic rats. *E-J Chem* 2010, 7(1), 1-5.
18. Gupta M, Mazumder UK, Gomathi P. Evaluation of antipyretic and antinociceptive activities of *Plumeria acuminata* leaves. *J Medical Sci* 2007; 7:835-839.
19. Matthias O, Hamburger, Geoffrey A, Cordell, Nijisiri R. Traditional medicinal plants of Thailand. XVII. Biologically active constituents of *Plumeria rubra*. *J Ethnopharmacol* 1991; 33(3):289-92.
20. Nadkarni KM, Indian Materia Medica, Vol I, Popular Prakashan, Bombay, 1976, pp.561, 993.
21. Burkill IH, A Dictionary of the Economic Products of the Malay Peninsular. Crown Agents for the Colonies, London 1935; II(I-Z):1776-1778.
22. Omata A, Yomogida K, Nakamura S, Hashimoto S, Arai T, Furukawa K. Volatile components of *Plumeria*

- flowers. Part 1. *Plumeria rubra* forma *acutifolia* (Poir) Woodson cv. 'Common yellow'. *Flav Fragr J* 1991; 6:277-279.
23. Kamariah AS, Linda BL, Baser KHS, Ozek T, Demirci B. Composition of the essential oil of *Plumeria obtusa* L. *Flav Fragr J* 1999; 14:237-240.
 24. Ragasa CY, Panajon NM, Aurigue FB, Brkljača R, Urban S. Chemical Constituents of *Hoya cumingiana* Decne. *Int J Pharmacog Phytochem Res.* 2016; 8(12):2033–2038.
 25. Ebajo Jr VD, Aurigue FB, Brkljača R, Urban S, Ragasa CY. Chemical Constituents of *Hoya wayetii* Kloppenb. *Int J Pharmacog Phytochem Res.* 2015. 7(5): 1042–1045.
 26. Ragasa' CY, Ng VAS, De Los Reyes MM, Mandia EH, Shen C-C. An isoflavone from *Wrightia pubescens*. *Int J Pharmacog Phytochem Res.* 2015. 7(2):353–355.
 27. Ebajo Jr VD, Shen C-C, Ragasa CY. Terpenoids and sterols from *Hoya multiflora* Blume. *J Appl Pharm Sci* 2015; 5(3):33-39.
 28. Vázquez LH, Palazon J, Navarro-Ocaña A. The Pentacyclic Triterpenes α , β -amyryns: 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.
 29. 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.
 30. Anand R, Patnaik GK, Kulshreshtha DK, Dhawan N. Antiuro lithiatic activity of lupeol, the active constituent of *Crateva nuriala*. *Phytother Res* 1994; 8(7):417-421.
 31. 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):539-543.
 32. 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.
 33. 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. *Oncol Res* 2005; 14:69-79.
 34. 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.
 35. 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.
 36. 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.
 37. Gallo MBC, Sarachine MJ. Biological activities of lupeol. *Intl J Biomed Pharm Sci* 2009; 3(1):46-66.
 38. 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.
 39. 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.
 40. Okoye NN, Ajaghaku DL, Okeke HN, Ilodigwe EE, Nworu CS, Okoye FBC. beta-Amyrin and alpha-amyryn acetate isolated from the stem bark of *Alstonia boonei* display profound anti-inflammatory activity. *Pharm Biol* 2014; 52(11):1478-1486.
 41. 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 -amyryn and β -amyryn triterpenes: evidence for participation of protein kinase C and protein kinase A pathways. *J Pharmacol Exp Therapeutics* 2005; 31(1):310-318.
 42. Soldi C, Pizzolatti G, Luiz A, Marcon R, Meotti F, Miotob L, Santos A. Synthetic derivatives of the α - and β -amyryn triterpenes and their antinociceptive properties. *Bioorg Med Chem* 2008; 16(6):3377- 3386.
 43. 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.
 44. 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.
 45. 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 -amyryn, a triterpenoid mixture, from *Protium heptaphyllum* in mice. *Lipids in Health and Disease* 2012; 11:98.
 46. Raga DD, Herrera AA, 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* 2013; 47(1):44-53.
 47. Raga DD, Herrera AA, Espineli DL, 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.* 2013; 5(10):230-239.
 48. 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* 2014; 6(3):1014-1022.

49. Raga DD, Herrera AA, Shen C-C, Ragasa CY. Analgesic triterpenes from *Ardisia cf. elliptica* (subgenus *Tinus*) (Myrsinaceae). *Der Pharma Chemica* 2014; 6(4):153-161.
50. 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.