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Research Article

Triterpenes from *Plumeria rubra* L. Flowers

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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-amyrin (**2**) and β-amyrin (**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-amyrin, β-amyrin.

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 anxiety[1]. Another study reported that the methanolic flower extract of *P. rubra* exhibited antioxidant, cytotoxic and hypolipidemic activities[2]. 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 mm[3]. In addition, the methanolic extracts of the flowers of *P. rubra* were reported to exhibit antioxidant and anti-inflammatory activities[4]. In Mexico, the flower infusions of *P. rubra* are used for the treatment of diabetes mellitus[5]. Many studies were conducted on the chemical constituents of the different parts of *P. rubra*. The flowers of *P. rubra* yielded nerolidols[6], naphthalene[7], linalool[8], 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, respectively[9]. 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, tetradeconoic acid, octadecanoic acid and phenylacetaldehyde[11]. 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 caoutchove and resin matter[12]. 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-3β,24-diol, 2,4,6-trimethoxyaniline, ajunolic acid, ursolic acid, oleanolic acid, β-amyrin acetate, betulinic acid, lupeol and its acetate, 2,3-dihydroxypropyl octacosanoate, glucoside of β-sitosterol, stigmasterol and β-sitosterol[14]. The bark of *P. rubra* contained plumieride[15], fulvoplumerin[16]. The root contains plumericine, β-dihydroplumericin, and isoplumericin[17], β-dihydroplumericinic acid, fulvoplumerin, plumeride, taraxasteryl acetate, lupeol, stigmasterol, oleanolic acid, and rubrinol which is an antibacterial tritripenoid[18]. 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'dihdroxy-7,3',5'-trimethoxyflavan-5-*O*-β-D-glucopyranoside, and flavan-3-ol glycoside[19]. The

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phytochemical constituents from the ethanolic extract of the leaves of *Plumeria acuminata* were reported as stigmast-7- enol[20], lupeol carboxylic acid[21], lupeol acetate[22], and urosolic acid[23]. 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

^{1}H spectra were acquired in $CDCl_3$ 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 F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 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

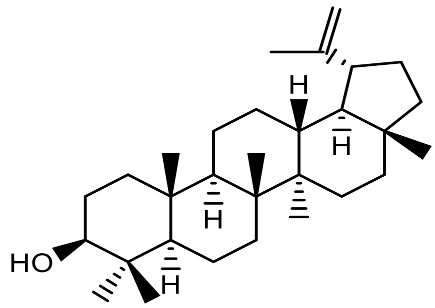
A glass column 12 inches in height and 0.5 inch internal diameter was packed with silica gel. The crude extracts were fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% by volume increment) as eluents. Five milliliter fractions were collected. All fractions 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

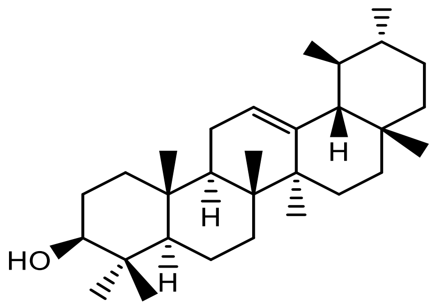
The air-dried flowers of *P. rubra* (29.52 g) were ground in a blender, soaked in CH_2Cl_2 for 3 days and then filtered. The solvent was evaporated under vacuum to afford a crude extract (1.5543 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment by volume. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3 ×) 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 lupeol[27]; **2** for α-amyrin[28]; and **3** for β-amyrin[28]. The **1**:**2**:**3** ratio is about 8:2:1 which was deduced from the intensities



1



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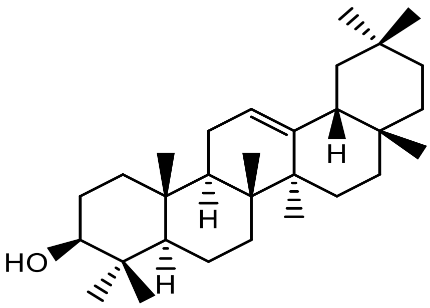

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Figure 1: Chemical structures of lupeol (**1**), α-amyrin (**2**) and β-amyrin (**3**) from *P. rubra* flowers.

of the olefinic proton resonances at ô 4.65 and 4.54 for **1**[27], ô 5.08 for **2**[28], and ô 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 antiurolithiatic and diuretic activity[29]. It prevented the formation of vesical calculi and reduced the size of the preformed stones in rats[30]. It also showed antifungal activity against *Fusarium oxysporum* and *Penicillium notatum*[31]. Lupeol significantly reduced the 451Lu tumor growth in athymic nude mice[32], inhibited the proliferation of MDA-MB-231 human breast cancer cells in a dose dependent manner[33], and induced growth inhibition and apoptosis in hepatocellular carcinoma SMMC7721 cells by down-regulation of the death receptor 3 (DR3) expression[34]. Lupeol and lupeol acetate have shown hypotensive activity[35]. 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[36]. A review on the biological activities of lupeol has been provided[37]. α-Amyrin (**2**) and β-amyrin (**3**) were reported to possess anti-inflammatory[38-40] and analgesic[41-42] 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/mL[44]. 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 cholesterol[45]. A review on the sources and biological activities of **2** and **3** has been provided[28]. 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 manisfestations on *Danio rerio* embryos[48]; and exhibited analgesic property in the acetic acid writhing test and hot plate assay[49]. 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 activity[50].

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