References

1. Vietnam Ministry of Health (2017), Vietnamese Pharmacopoeia V, Medical Publishing House, Part 2, 1206-1207. **2.** Phuong Thien Thuong, Nguyen Thi Ha Ly, Nguyen Thi Phuong, Nguyen Thi Bich Thu (2019), Selecting reference constituents of herbal origin for quality evaluation of herbal medicines in Vietnam: Contributions from scientific researches, *Vietnam Journal of Medicinal Materials*, 24(1), 3-14. **3.** Wang R., Chen W. H., Shi Y. P. (2010), *ent*-Kaurane and *ent*-Pimarane Diterpenoid from *Siegesbeckia pubescens. Journal of Natural Products,* 73(1), 17-21. **4.** Ying X., Zhang H., Fan C. Q., Yue J. M. (2004), Novel diterpenoids and diterpenoid glycosides from *Siegesbeckia orientalis* L., *Journal of Natural Products,* 67(9), 1517-1521. **5.** Wang J. P., Zhou Y. M., Ye Y. J., Shang X. M., Cai Y. L., Xiong C. M., Wu Y. X., Xu H. X. (2011), Topical anti-inflammatory and analgesic activity of kirenol isolated from *Siegesbeckia orientalis. Journal of Ethnopharmacology,* 137(3), 1089-1094. **6.** Karl L. (1998), Demonstrating the cosmetic activity of darutoside, esculoside and ursolic acid. *Cosmetics and Toiletries Magazine,* 113, 67. **7.** Department of Health Hong Kong Special Administrative Region (2005), *Hong Kong Chinese Materia Medica Standards*: Hong Kong. Vol. 8, 443-461. **8.** Health Ministry of China (2015), *Pharmacopoeia of the People Republic of China*: China. Vol. IA, 425. **9.** World Health Organization expert Committee on specifcations for pharmaceutical preparations (2017), *World Health Organization guidelines for selecting marker substances of herbal origin for quality control of herbal medicines*, 72-85. **10.** International Standard Oganization (2017), ISO Guide 35: 2017 *Reference Materials - Guidance for Characterization and Assessment of Homogeneity and Stability*, pp. 9-45. **11.** World Health Organization (2006), *General guidelines for the establishment, maintenance and distribution of chemical reference substance*, 59-83. **12.** ICH (1996), *Validation of Analytical Procedures: Text and methodology*, ICH Hamonised Tripartite Guideline. **13.** Phan Tong Son, Phan Minh Giang, Walter C. Taylor (2005), NMR studies of darutoside, a rare *ent*-pimarane glucoside, *Natural Product Research*, 19(5), 503-507.

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FLAVONOIDS FROM THE ROOT OF *MILLETTIA PULCHRA* KURZ COLLECTED IN AN GIANG PROVINCE

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Summary

Flavonoids from the Root of *Millettia pulchra* Kurz Collected in An Giang Province

Millettia pulchra Kurz (Fabaceae) was called as "Bạch chỉ nam" and it has been used in the traditional medicine in Vietnam for the treatment of skin allergy, rheumatism bone pain, digestive disorder, abnormal pain, and diarrhoea. Seven compounds were isolated and identified as (6a*R**,11a*R**)-maackiain (**1**), daidzein (**2**), genistein (**3**), 7,5′-dihydroxy-3′-methoxyisoflavone (**4**), pseudobaptigenin (**5**), 3-*O*-methylbutein (**6**), and pongamol (**7**) from the ethyl acetate fraction from the root of *Millettia pulchra* Kurz. Their structures were determined by the mean of spectroscopic methods (MS, 1D and 2D NMR).

Keywords: *Millettia pulchra, Flavonoid, Isoflavonoid, Chalcone.*

1. Introduction

Millettia pulchra Kurz is small shrubs belonging to the Fabaceae family. There are 150 species of the genus *Millettia* which are distributed in the tropical and sub-tropical area in the Indian region and Southeast Asian countries. In Vietnam, there are 25 species widely distributed from the North to the South [1]. The main components from the root of *M. pulchra* were flavonoids, especially isoflavonoids, phenolics, sterols [2], and alkaloids [3]. The root of "Bạch chỉ nam" originated from *M. pulchra* Kurz has frequently been used in the oriental medicine in An Giang province for the treatment of skin allergy, rheumatism bone pain, digestive disorder, abnormal pain, and diarrhoea [1]. Our previous research on the root of *M. pulchra* let to the isolation of four compounds [4]. In our continuing study, seven flavonoids were isolated from the ethyl acetate fraction from the root of *M. pulchra*. Their structures were elucidated by spectroscopic data and compared to those of reported in the literature. In the present paper daidzein (**2**), 7,5′-dihydroxy-3′-methoxyisoflavone (**4**), pseudobaptigenin (**5**), and 3-*O*-methylbutein (**6**) are first reported in *Millettia pulchra*.

2. Materials and methods

2.1. Materials

The fresh root of *Millettia pulchra* Kurz (15 kg) was bought in An Giang province in November, 2018. The voucher specimen (MP-112018) was identified by Dr. Vo Van Chi and

deposited at Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. The dried root (6.0 kg) was used for extraction and isolation of the pure compounds.

The solvents: ethanol 96%, *n*-hexane, ethyl acetate, chloroform, methanol, and *n*-butanol (Scharlau, Spain) were used for the isolation and extraction. Solvents for HPLC were purchased from the supplier of VWR Chemicals BDH (France).

2.2. General experimental procedures

Silica gel (40-63 μm, Merck) and Sephadex LH-20 (GE Healthcare, Sweden) were used for column chromatography (CC). TLC was performed using *Silica gel* 60 F_{254} plates (Merck). Spots were detected by UV and vanillin-sulfuric acid 5%/absolute ethanol solution. ESI-MS spectra were obtained from a Xevo TQ-S micro mass spectrometer (Waters, USA). An UPLC system (Waters, USA) with a DAD detector and an ACQUITY UPLC BEH C18 (130Å, 1.7 μm, 2.1 i.d. ×100 mm) column was used for the HPLC analysis. A Shimadzu SPD-20A pump and a LC-20AR UV/Vis detector with a Phenomenex Luna 100 RP-C18 (250 × 10 mm i.d., 5 μm) column were used for semi-preparative HPLC separation. NMR spectra were measured on a Bruker Avance Neo 400 using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA).

2.3. Extraction and isolation

The dried root of *M. pulchra* (6.0 kg) were ground and percolated with 95% ethanol (120 L). The solvent was evaporated under reduced pressure to obtain a concentrated ethanol extract (3 L). The ethanol extract was succesively partitioned with *n*-hexane (2 L × 4), ethyl acetate (2 L × 3) and saturated *n*-BuOH (2 L × 2), then the solvents were removed to obtain *n*-hexane (H, 50.0 g), ethyl acetate (E, 17.6 g) and *n*-BuOH (B, 147.0 g) fractions, respectively. The ethyl acetate fraction (E, 17.6 g) was filtrated to obtain a precipitation (EAt, 9.2 g) and a soluble fraction (EA, 8.4 g). The EA fraction (EA, 7.0 g) was separated by Sephadex LH-20 CC with dichlomethane-methanol (9:1) to obtain 10 subfractions (EA1-EA10). Compound **7** (4.6 mg) was isolated and further purified by Sephadex LH-20 CC with MeOH from the subfraction EA1 (320 mg). The subfraction EA4 (220 mg) was subjected to Sephadex LH-20 CC (MeOH) to give 4 fractions (EA4.1-EA4.4). After solvent evaporation, compound **1** (15.8 mg) was precipitated from fraction EA4.2. Fraction EA5 (1480 mg) was subjected to Sephadex LH-20 CC (MeOH) to give 2 fractions (EA5.1-EA5.2) and further purified by semi-preparative HPLC with isocratic condition of acetonitrile-water (35:65, v/v) to give compounds **5** (10 mg) and **6** (4.4 mg), respectively. The subfraction EA6 (2.16 g) was loaded onto Sephadex LH-20 CC (MeOH) to give 3 fractions (EA6.1-EA6.3) and semi-preparative HPLC separation (isocratic, acetonitrile-water, 35:65, v/v) afforded compounds **2** (15 mg), **3** (30 mg), and **4** (5.9 mg), respectively.

(6a*R,11a*R**)-Maackiain (1):** ivory-white crystals. ESI-MS: *m/z* = 285.0771 $[M+H]^+$, $C_{16}H_{12}O_5$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 7.39 (1H, d, *J*=8.4 Hz, H-1), 6.74 (1H, t, *J*=0.4 Hz, H-7), 6.58 (1H, dd, *J*=8.4, 2.8 Hz, H-2), 6.46 (1H, t, *J*=0.4 Hz, H-10), 6.44 (1H, d, *J*=2.8 Hz, H-4), 5.95 and 5.92 (2H, each d, *J*=1.6 Hz, -OCH_2O-), 5.49 (1H, d, *J*=6.8 Hz, H-11a), 4.24 (1H, ddd, *J*=11.2, 5.2, 0.8 Hz, H-6*eq*), 3.66 (1H, t, *J*=11.2 Hz, H-6*ax*), 3.49 (1H, dddt, *J*=11.2, 6.8, 5.2, 0.4 Hz, H-6a). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 157.0 (C-4a), 156.6 (C-3), 154.2 (C-10a), 148.1 (C-9), 141.7 (C-8), 132.1 (C-1), 117.9 (C-6b), 112.6 (C-11b), 109.7 (C-2), 104.8 (C-7), 103.6 (C-4), 101.3 (-OCH_2O-), 93.9 (C-10), 78.5 (C-11a), 66.4 (C-6), 40.1 (C-6a).

Daidzein (2): ivory-white crystals. ESI-MS: *m/z* = 255.37 $[M + H]^+$, $C_{15}H_{10}O_4$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.15 (1H, s, H-2), 8.07 (1H, d, *J*=8.8 Hz, H-5), 7.39 (2H, dt, *J*=8.8, 2.4 Hz, H-2′ and H-6′), 6.95 (1H, dd, *J*=8.8, 2.4 Hz, H-6), 6.87 (2H, dt, *J*=8.8, 2.4 Hz, H-3′ and H-5′), 6.86 (1H, d, *J*=2.4 Hz, H-8). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 176.8 (C-4), 163.9 (C-7), 158.5 (C-9), 157.3 (C-4′), 153.3 (C-2), 127.1 (C-5), 130.0 (C-3′ and C-5′), 124.6 (C-1′), 122.9 (C-3), 116.7 (C-10), 115.2 (C-6), 114.8 (C-2′ and C-6′), 101.9 (C-8).

Genistein (3): ivory-white powder. ESI-MS: *m/z* = 269.37 $[M-H]^-$, $C_{15}H_{10}O_5$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.07 (1H, s, H-2), 7.39 (2H, d, *J*=8.4 Hz, H-3′ and H-5′), 6.86 (2H, d, *J*=8.4 Hz, H-2′ and H-6′), 6.36 (1H, d, *J*=2.0 Hz, H-8), 6.24 (1H, d, *J*=2.0 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 180.9 (C-4), 164.8 (C-7), 162.5 (C-5), 158.4 (C-9), 157.5 (C-4′), 153.4 (C-2), 130.0 (C-3′ and C-5′), 123.4 (C-3), 121.9 (C-1′), 114.9 (C-2′ and C-6′), 104.9 (C-10), 98.8 (C-6), 93.4 (C-8).

7,5′-Dihydroxy-3′-methoxyisoflavone (4): yellowish powder. ESI-MS: *m/z* =283.38 $[M-H]^-$, $C_{16}H_{12}O_5$. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 8.12 (1H, s, H-2), 8.04 (1H, d, *J*=8.8 Hz, H-5), 7.03 (1H, dd, *J*= 1.6, 0.8 Hz, H-4′), 7.0 (2H, dd, *J*=1.6, 0.8 Hz, H-2′ and H-6′), 6.95 (1H, dd, *J*=8.8, 2.4 Hz, H-6), 6.85 (1H, d, *J*=2.4 Hz, H-8), 3.97 (1H, s, 5′-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 176.7 (C-4), 164.0 (C-7), 158.5 (C-9), 153.4 (C-2), 147.8 (C-5′), 146.1 (C-3′), 127.1 (C-5), 124.9 (C-3), 124.4 (C-1′), 120.2 (C-6′), 116.0 (C-10), 115.4 (C-4′), 114.8 (C-6), 111.2 (C-2′), 101.9 (C-8), 55.0 (5′-OCH_3).

Pseudobaptigenin (5): pale yellow crystals. ESI-MS: *m/z* = 283,27 $[M+H]^+$, $C_{16}H_{10}O_5$. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 8.34 (1H, s, H-2), 7.95 (1 H, d, *J*=8.8 Hz, H-5), 7.14 (1H, d, *J*=1.6 Hz, H-2′), 7.05 (1H, dd, *J*=8.6, 1.6 Hz, H-6′), 6.97 (1H, d, *J*=8.6 Hz, H-5′), 6.92 (1H, dd, *J*=8.8, 2.0 Hz, H-6), 6.85 (1H, d, *J*=2.0 Hz, H-8), 6.05 (2H, s, -OCH_2O-).^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 174.9 (C-4), 163.7 (C-7), 157.9 (C-9), 153.9 (C-2), 147.5 (C-3′), 147.4 (C-4′), 123.6 (C-3), 127.7 (C-5), 126.3 (C-1′), 122.9 (C-6′), 116.8 (C-10), 115.9 (C-6), 109.9 (C-2′), 108.6 (C-5′), 102.6 (C-8), 101.5 (-OCH_2O-).

3-*O*-Methylbutein (6): orange-yellow cubic crystals. ESI-MS: *m/z* = 285.30 $[M-H]^-$, $C_{16}H_{14}O_5$. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 8.04 (1H, d, *J*=8.8 Hz, H-6′), 7.80 (1H, d, *J*=15.2 Hz, H-8), 7.66 (1H, d, *J*=15.2 Hz, H-7), 7.39 (1H, d, *J*=1.6 Hz, H-2), 7.24 (1H, dd, *J*=8.0, 1.6 Hz, H-6), 6.87 (1H, d, *J*=8.0 Hz, H-5), 6.44 (1H, dd, *J*=8.8, 2.4 Hz, H-5′), 6.31 (1H, d, *J*=2.4 Hz, H-3′), 3.97 (3H, s, 3-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 192.1 (C-9), 166.2 (C-2′), 165.3 (C-4′), 149.6 (C-3), 148.1 (C-4), 144.6 (C-8), 132.1 (C-6′), 127.0 (C-1), 123.6 (C-6), 117.3 (C-7), 115.2 (C-5), 113.3 (C-1′), 110.8 (C-2), 107.9 (C-5′), 102.5 (C-3′), 55.2 (3-OCH_3).

Pongamol (7): colorless amorphous powder; ESI-MS: *m/z* = 295.0970 $[M+H]^+$, $C_{18}H_{14}O_4$. ^1H NMR (400 MHz, $CDCl_3$) δ_H: 8.02 (2H, m, H-2 and H-6), 7.89 (1H, dd, *J*=8.8, 0.4 Hz, H-6′), 7.66 (1H, dd, *J*=2.4, 0.4 Hz, H-2″), 7.57 (1H, dt, *J*=7.2, 1.2 Hz, H-4), 7.52 (2H, m, H-3 and H-5), 7.35 (1H, dd, *J*=8.8, 0.8 Hz, H-5′), 7.19 (1H, s, H-8), 7.02 (1H, dd, *J*=2.4, 0.8 Hz, H-3″), 4.17 (3H, s, 2′-OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 186.2 (C-9), 184.3 (C-7), 158.7 (C-4′), 153.7 (C-2′), 144.9 (C-2″), 135.6 (C-1), 132.2 (C-4), 128.7 (C-3 and C-5), 127.1 (C-2 and C-6), 126.5 (C-6′), 122.2 (C-1′), 119.6 (C-3′), 107.1 (C-5′), 105.3 (C-3″), 97.9 (C-8), 61.2 (2′-OCH_3).

3. Results and discussion

Compound **1** was isolated as ivory-white crystals. The ESI-MS showed the positive-ion peak with *m/z* 285.0771 $[M+H]^+$, calculated for the molecular formula of $C_{16}H_{12}O_5$ with 11 degrees of unsaturation. The ^{13}C NMR spectrum of the compound **1** showed 16 carbon signals in the region of δ_C 157-93, including 7 non-protonated carbons (δ_C 157.0 (C-4a), 156.6 (C-3), 154.2 (C-10a), 148.1 (C-9), 141.7 (C-8), 117.9 (C-6b), 112.6 (C-11b)), 7 methine carbons (δ_C 132.1 (C-1), 109.7 (C-2), 104.8 (C-7), 103.6 (C-4), 93.9 (C-10), 78.5 (C-11a), 40.1 (C-6a)), and 2 methylene carbons (one methylenedioxy and one oxymethylene groups). Those ^{13}C NMR data consisted with the typical structure of C_6-C_3-C_6 framework of a flavonoid, which was conferred to the pterocarpan scaffold with one sp^3 oxymethylene carbon C-6 and one sp^3 oxymethine carbon C-11a. The ^1H NMR spectrum of compound **1** showed the *ortho*-coupled proton signals of H-1 (δ_H 7.39, d, *J*=8.4 Hz) with H-2 (δ_H 6.58, dd, *J*=8.4, 2.8 Hz), and *meta*-coupled proton signals of H-2 and H-4 (δ_H 6.44, d, *J*=2.8 Hz) on the aromatic ring. The doublet proton signals were observed at 5.95 and 5.92 with a small coupling constant (*J*=1.6 Hz), which were assigned to the methylenedioxy group. In the COSY spectrum, the cross-peak correlations were observed between the protons H-1/H-2/H-4, H_2-6/H-6a/H-11a (δ_H 5.49, d, *J*=6.8 Hz) as depicted in Fig. 2, and the long-range correlations of $^4J_{H,H}$ and $^5J_{H,H}$ between H-1/H-11a and H-6a/H-7/H-10 [5]. The HMBC spectrum exhibited the correlations from H-1 to C-3/C-4a/C-11a, from H-2 to C-3/C-4/C-11b, from H_2-6 to H-4a/H-6a/H-6b/H-11a, from H-7 to C-6a/C-8/C-9/C-10a, and from H-10 to C-6b/C8/C-9/C10a (Fig. 2). The geometry of protons H-6a/H-11a and H-6/H-6*eq* were verified to be *cis* by small coupling constants of $J_{6a,11a}$=6.8 Hz and $J_{6a,6eq}$=5.2 Hz, meanwhile the orientations of H-6a/H-6*ax* were determined to be *trans* by large coupling constant of $J_{6a,6ax}$=11.2Hz. Thus, the relative configuration of protons H-6a and H-11a were determined to be *R**. Based on above NMR spectra data and in comparison with the spectroscopic data in the literature [6], compound **1** was identified as (6a*R**,11a*R**)-maackiain, a common flavonoid in the genus *Millettia*.

Compound **2** was isolated as ivory-white crystals. The ESI-MS spectral data showed the positive ion fragmentation at *m/z* 255.37 $[M+H]^+$. Therefore, the molecular weight of the compound **2** was 254 corresponding to the molecular formula of $C_{15}H_{10}O_4$ with 11 degrees of unsaturation. The ^{13}C NMR spectrum of compound **2** showed 15 carbon signals, including 8 methine carbons (δ_C 153.3 (C-2), 127.1 (C-5), 130.0 (C-3′ and C-5′), 115.2 (C-6), 114.8 (C-2′ and C-6′), and 101.9 (C-8)) and 7 non-protonated carbons (δ_C 176.8 (C-4), 163.9 (C-7), 158.5 (C-9), 157.3 (C-4′), 124.6 (C-1′), 122.9 (C-3), 116.7 (C-10)), which suggested that compound **2** was of C_6-C_3-C_6 flavonoid structure. The ^1H NMR spectrum of **2** showed seven aromatic methine groups. The proton at δ_H 8.15 (s) was connected to an oxygenated carbon of the pyran ring at δ_C 153.3, which was assigned to H-2. Thus, compound **2** was an isoflavonoid. The COSY spectrum revealed the correlations of *ortho*-coupled proton signals between H-5 (δ_H 8.07, d, *J*=8.8 Hz) and H-6 (δ_H 6.95, dd, *J*=8.8, 2.4 Hz),

between H-2′/H-6′ (δ_H 7.39, dd, *J*=8.8, 2.4 Hz) and H-3′/H-5′ (δ_H 6.87, dd, *J*=8.8, 2.4 Hz), and *meta*-coupled proton signals between H-6 and H-8 (δ_H 6.86, d, *J*= 2.4 Hz). The key HMBC correlations were observed from H-2 to C-3/C-4/C-9/C-1′, from H-5 to C-4/C-7/C-9, from H-6 to C-7/C-8/C-10, and from H-2′/H-6′ to C-1′/C-3′/C-4′/C-5′ (Fig. 2). Based on the spectral data in the literature [7], compound **2** was identified as daidzein, a common isoflavonoid found in Legume family (Fabaceae).

Compound **3** was isolated as an ivory-white powder. The ESI-MS spectrum presented the negative ion peak at *m/z* 269.37 $[M-H]^-$; the molecular weight of compound **3** is 270 corresponding to the molecular formula of $C_{15}H_{10}O_5$ and 11 degrees of unsaturation. The increase of 16 mass units of **3** compared to the molecular weight of **2** suggested the addition of a hydroxyl group in the structure of **3**. Fifteen carbon signals of a flavonoid structure including 7 methine carbons (δ_C 153.4 (C-2), 130.0 (C-3′ and 5′), 114.9 (C-2′ and 4′), 98.8 (C-6), 93.4 (C-8)) and 8 non-protonated carbons (δ_C 180.9 (C-4), 164.8 (C-7), 162.5 (C-5), 158.4 (C-9), 157.5 (C-4′), 123.4 (C-3), 121.9 (C-1′), 104.9 (C-10)) appeared in the downfield region from δ_C 180-93. The difference between **3** and **2** was the change of one aromatic methine to a non-protonated aromatic carbon in **3**. In addition, one pair of *meta*-coupled protons H-6 (δ_H 6.24, d, *J*=2.0 Hz) and H-8 (δ_H 6.36, d, *J*=2.0 Hz) together with two pairs of symmetric aromatic protons at δ_H 6.86 (2H, d, *J*=8.4 Hz, H-2′ and H-6′) and 7.39 (2H, d, *J*=8.4 Hz, H-3′ and H-5′) observed in the ^1H NMR spectrum of **3**, indicated that the hydroxyl group was introduced at C-5. The key HMBC and COSY correlations of **3** were shown in Fig. 2. Therefore, the structure of compound **3** was identified as genistein, which was isolated from *M. lasiantha* [8] and *M. pachyloba* [9].

Compound **4** was isolated as a yellowish powder. The ESI-MS spectrum data exhibited a negative-ion peak at *m/z* 283.38 $[M-H]^-$, which indicated the molecular formula of $C_{16}H_{12}O_5$, and implying 11 degrees of unsaturation. The ^{13}C NMR spectrum of compound **4** showed the presence of 16 carbons, including one methoxy group (δ_C 56.5, 5′-OCH_3), seven methines (δ_C 153.4 (C-2), 127.1 (C-5), 120.2 (C-6′), 116.0 (C-4′), 115.4 (C-6), 111.2 (C-2′), 101.9 (C-8)), three non-protonated carbons (δ_C 124.9 (C-3), 124.4 (C-1′), 116.0 (C-10)) and five oxygenated carbons (δ_C 176.7 (C-4), 164.0 (C-7), 158.5 (C-9), 147.82 (C-5′), 146.1 (C-3′)). The ^1H NMR spectrum of **4** showed seven methines and one methoxy group. The ^1H-^1H COSY spectrum presented the correlations between H-5 (δ_H 8.04, d, *J*=8.8 Hz), H-8 (δ_H 6.85, d, *J*=2.4 Hz), and H-6 (δ_H 6.95, dd, *J*=8.8, 2.4 Hz), and three *meta*-coupled proton signals at δ_H 6.97 (2H, dd, *J*=1.6, 0.8 Hz, H-2′ and H-6′), and δ_H 7.04 (1H, dd, *J*=1.6, 0.8 Hz, H-4′). The HMBC and ^1H-^1H COSY correlations of **4** were shown as in Fig. 2. Based on the NMR data and reference data in the literature [10], compound **4** was determined as 7,5′-dihydroxy-3′-methoxyisoflavone, which is first reported in the root of *Millettia pulchra.*

Compound **5** was obtained as a pale-yellow crystal. The ESI-MS spectral data showed a positive-ion peak at *m/z* 283.27 $[M+H]^+$. Therefore, the molecular formula of compound **5** was determined as $C_{16}H_{10}O_5$, consistent with 12 degrees of unsaturation. The ^{13}C NMR spectrum of compound **5** showed 16 carbon signals, including one carbonyl (δ_C 174.9), four oxygenated carbons (δ_C 163.7, 157.9, 147.5, and 147.4), three non-protonated carbons (δ_C 123.6, 126.3 and 116.8), seven methine carbons (δ_C 153.9, 127.7, 122.9, 115.9, 109.9, 108.6, and 102.6), and one methylenedioxy group (δ_C 101.5). The ^1H NMR spectrum of **5** exhibited seven protons (δ_H 8.34, 7.69, 7.14, 7.05, 6.97, 6.92, and 6.85) and one methylenedioxy group (δ_H 6.05). The ^1H-^1H COSY spectrum of **5** revealed two spin-coupling systems between H-5 (δ_H 7.95, d, *J*=8.8 Hz), H-6 (δ_H 6.92, dd, *J*=8.8, 2.0 Hz), and H-8 (δ_H 6.85, d, *J*=2.0 Hz) in A-ring, between H-5′ (δ_H 6.97, d, *J*=8.6 Hz), H-6′ (7.05, dd, *J*=8.6, 1.6 Hz), and H-2′ (δ_H 7.14, d, *J*=1.6 Hz) in B-ring of the isoflavonoid scaffold. The position of the methylenedioxy group was determined by the HMBC correlations from δ_H 6.05 to C-3′ and C-4′. The structure of compound **5** was deduced by HMBC and ^1H-^1H COSY spectra as shown in Fig 2. Based on the data from the reference [11], compound **5** was identified as 7-hydroxy-3',4'-methylenedioxyisoflavone or pseudobaptigenin, which were isolated from the root of *M. griffoniana* [12] and *M. speciosa* [13].

Compound **6** was isolated as orange-yellow cubic crystals. The negative-ion ESI-MS spectrum data showed an ion peak at *m/z* 285.30 $[M-H]^-$, corresponding to the molecular formula of $C_{16}H_{14}O_5$, implying 10 degrees of unsaturation. The ^{13}C NMR spectrum of compound **6** showed 16 carbon signals containing one carbonyl (δ_C 192.1), four oxygenated carbons (δ_C 166.2, 165.3, 149.6, and 148.1), six aromatic and two olefinic methine carbons (δ_C 144.6, 132.1, 123.6, 117.3, 115.2, 110.8, 107.9, and 102.5), two non-protonated carbons (δ_C 127.0 and 113.3), and one methoxy group (δ_C 55.2). All carbon signals appeared in

the down-field region from δ_C 192-102 of a C_6-C_3-C_6 flavonoid framework. The ^{1}H NMR spectrum displayed the signals of a *trans*-double bond (δ_H 7.80, d, *J*=15.2 Hz, H-8 and 7.66, d, *J*=15.2 Hz, H-7), six aromatic methines (δ_H 8.04, 7.39, 7.24, 6.87, 6.44, and 6.31), and one methoxy group (δ_H 3.97). Compound **6** possessed 10 degrees of unsaturation with one *trans*-double bond, which was suggested to be a chalcone. The ^{1}H-^{1}H COSY spectrum of **6** displayed three spin-coupling systems between H-5 (δ_H 6.87, d, *J*= 8.0 Hz), H-6 (δ_H 7.24, dd, *J*=8.0, 1.6 Hz), and H-2 (δ_H 7.39 (1H, d, *J*=1.6 Hz) in A-ring, between H-5′ (δ_H 6.44, dd, *J*=8.8, 2.4 Hz), H-6′ (8.04, d, *J*=8.8 Hz), and H-3′ (δ_H 6.31, d, *J*=2.4 Hz) in B-ring, and the *trans* coupling between H-7 and H-8. The planar structure of **6** was confirmed by the HMBC correlations from H-2 to C-1/C-3/C-4/C-6, from H-5 to C-1/C-3/C-4, from H-6 to C-2/C-4/C-7, from H-7 to C-1/C-8/C-9, from H-8 to C-1/C-7/C-9, from H-3′ to C-1′/C-2′/C-4′/C-5′, from H-5′ to C-1′/C-3′/C-4′, and from H-6′ to C-9/C-1′/C-2′/C-4′. Therefore, compound **6** was identified as 3-*O*-methylbutein [14].

Compound **7** was obtained as an amorphous colorless powder. The ESI-MS spectral data exhibited a positive-ion peak at *m/z* 295.0970 $[M+H]^+$, corresponding to the molecular formula of $C_{18}H_{14}O_4$ and 12 degrees of unsaturation. The ^{13}C NMR spectrum of compound **7** showed 18 carbon signals, containing 9 aromatic methine carbons (δ_C 144.9, 132.2, 128.7 (C-3 and C-5), 127.1 (C-2 and C-6), 126.5, 107.1, 105.3), one olefinic methine (δ_C 97.9), 7 non-protonated carbons (one carbonyl (δ_C 186.2), three oxygenated (δ_C 184.3, 158.7, and 153.7), and three non-oxygenated carbons (δ_C 135.6, 122.2, and 119.6)), and one methoxy group (δ_C 61.2). The ^{1}H NMR revealed the presence of five aromatic protons (δ_H 8.02-7.52), and a pair of *ortho*-coupled aromatic protons (δ_H 7.89, each 1H, d, *J*=8.8 Hz, and 7.35, d, *J*=8.8 Hz). In addition, the correlations between H-2″ (δ_H 7.66, d, *J*=2.4 Hz) and H-3″ (δ_H 7.02, d, *J*= 2.4 Hz) of the furan ring linked to carbon C-3′ (δ_C 119.6) and C-4′ (δ_C 158.7) in B-ring of a chalcone were observed. The structure of compound **7** was determined by the HMBC and ^{1}H-^{1}H COSY cross-peaks (Fig.2). On the basis of spectroscopic data in the literature, compound **7** was identified as pongamol, which was isolated from *M. pulchra* [15], *M. pachycarpa* [16], *M. brandisiana* [17], *M. erythrocalyx* [18], and *M. peguensis* [19].

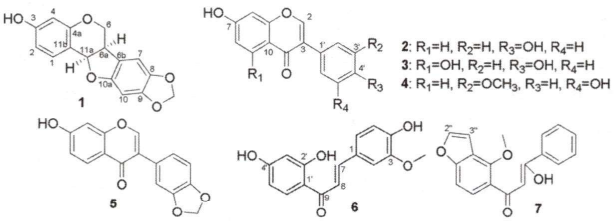

Fig.1. Chemical structures of compounds **1-7**

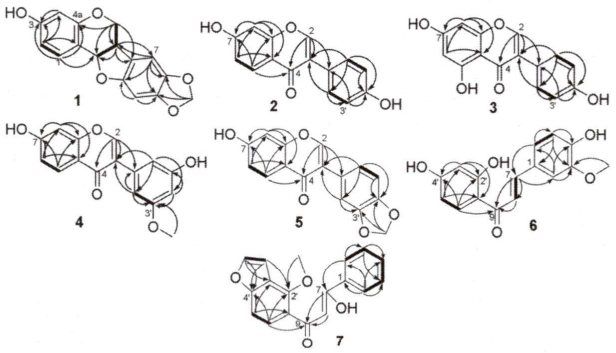

Fig.2. The key HMBC and COSY correlations of compounds **1-7**

4. Conclusion

In summary, seven compounds were isolated from ethyl acetate fraction of the root of *Millettia pulchra* Kurz. Their structures were identified as (6a*R**,11a*R**)-maackiain (**1**), daidzein (**2**), genistein (**3**), 7,5′-dihydroxy-3′-methoxyisoflavone (**4**), pseudobaptigenin (**5**), 3-*O*-methylbutein (**6**), and pongamol (**7**). Daidzein (**2**), 7,5′-dihydroxy-3′-methoxyisoflavone (**4**), pseudobaptigenin (**5**), and 3-*O*-methylbutein (**6**) are first reported in *M. pulchra* Kurz collected in An Giang province.

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References

1. Do Huy Bich, Dang Quang Chung, Bui Xuan Chuong, Nguyen Thuong Dong, Do Trung Dam, Pham Van Hien, Vu Ngoc Lo, Pham Duy Mai, Pham Kim Man, Doan Thi Nhu, Nguyen Tap, Tran Toan (2006), *Medicinal Plants and Animal in Vietnam* (in Vietnamese), Science and Technology Publisher, Vol. I, 131-133. **2.** Hui W. H., Chan W. S., Leung H. K. (1973), Triterpenoids and sterols from three *Millettia* species. *Phytochemistry*, 12(2), 474-475. **3.** Kamnaing P., Free S. N. Y. F., Fomum Z. T., Martin M. T., Kamnaing B. (1994), Millettonine, a guanidine alkaloid from *Millettia laurentii*. *Phytochemistry* 36(6), 1561-1562. **4.** Dinh T. B., Tran T. T. N., Ma C. T. (2020), Isolation of phenol derivatives from the radix of *Millettia pulchra* Kurz collected in An Giang province, *Ho Chi Minh City Journal of Medicine*, 24(3), 114-119. **5.** Ernö Pretsch, Philippe Bühlmann, Martin Badertscher (2020), *Structure Determination of Organic Compounds: Tables of Spectral Data* (5th Ed.), Springer, 187. **6.** Baruah P., Barua N. C.,

Sharma R. P., Baruah J. N., Kulanthaivel P., Herz W. (1984), Flavonoid from *Millettia pulchra*. *Phytochemistry*, 23(2), 443-447. **7.** Yasuda T., Kano Y., Saito K., Ohsawa K. (1994), Urinary and biliary metabolites of daidzin and daidzein in rats. *Biological and Pharmaceutical Bulletin*, 17(10), 1369-1374. **8.** Buyinza D., Derese S., Ndakala A., Heydenreich M., Yenesew A., Koch A., Oriko R. (2021), A coumestan and a coumaronochromone from *Millettia lasiantha*. *Biochemical Systematics and Ecology*, 97, 104277. **9.** Na Z., Fan Q. F., Song Q. S., Hu H. B. (2017), Three new flavonoids from *Millettia pachyloba*. *Phytochemistry Letters*, 19, 215-219. **10.** Li X., Li J., Wang D., Wang W., Cui Z. (2009), Chromone and flavonoids from *Maackia amurensis*. *Asian Journal of Traditional Medicines*, 4(3), 98-103. **11.** Yoon J. S., Sung S. H., Park J. H., Kim Y. C. (2004), Flavonoids from *Spatholobus suberectus*. *Archives of Pharmacal Research*, 27(6), 589-592. **12.** Ngamga D., Yankep E., Tane P., Bezabih M., Ngadjui B. T., Fomum Z. T., Abegaz B. M., Isoflavonoids from seeds of *Millettia griffoniana* (Bail). *Zeitschrift für Naturforschung B*, 60(9), 973-977. **13.** Ding P., Qiu J. Y., Ying G., Dai L. (2014), Chemical Constituents of *Millettia speciosa*. *Chinese Herbal Medicines*, 6(4), 332-334. **14.** Calanasan, C. A., MacLeod J. K. (1998), A diterpenoid sulphate and flavonoids from *Wedelia asperrima*. *Phytochemistry*, 47(6), 1093-1099. **15.** Wang W., Li N., Wang J., Chen G., Huang R., Zhao W., Li J., Si Y. (2016), Bioactive benzofuran-chalcones as potential NQO1 inducers from *Millettia pulchra* (Benth) kurzvar-laxior (Dunn) Z. Wei. *Phytochemistry*, 131, 107-114. **16.** Ashok K. Singhal Nabin C.^Barua Ram P. Sharma Jogendra N. Baruahet (1983), A chalcone and an isoflavone from *Millettia pachycarpa* seeds. *Phytochemistry*, 22(4), 1005-1006. **17.** Pailee P., Mahidol C., Ruchirawat S., Prachyawarakorn V. (2019), Diverse flavonoids from the roots of *Millettia brandisiana*. *Phytochemistry*, 162, 157-164. **18.** Sritularaka B., Likhitwitayawuid K., Conrad J., Kraus W. (2002), Flavonoids from the roots of *Millettia erythrocalyx*. *Phytochemistry*, 61(8), 943-947. **19.** Ganapaty S., Pushpalatha V., Babu G. J., Naidu K. C., Waterman P. G. (1998), Flavonoids from *Millettia peguensis* Ali (Fabaceae). *Biochemical Systematics and Ecology*, 26, 125-126.

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INVESTIGATION OF CHEMICAL CONSTITUENTS OF *CARDIOSPERMUM HALICACABUM* L. AND THEIR BIOLOGICAL ACTIVITIES

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Summary

Investigation of Chemical Constituents of *Cardiospermum halicacabum* L. and their Biological Activities

Four compounds were isolated from *n*-hexane and *n*-butanol extracts of *Cardiospermum halicacabum* growing in Vietnam by combined column chromatographic methods. Based on 1D-, 2D-NMR, ESI MS analyses, and comparisons with those published in work of literatures, the structures of these compounds were established as 3*β*-hydroxyglutin-5-ene (**1**), zerumbone (**2**), uridine (**3**), and daucosterol (**4**). It is intriguing to point out that, three out of them (**1-3**) were detected for the first time from the family Sapindaceae. Additionally, the cytotoxic activity of compound **3** was assessed against five human cancer cell lines (A549, T24, Huh-7, 8505, SNU-1) using the SRB method. Additionally, **3** was also evaluated for immunomodulatory capacity through stimulation of IL-2 expression in macrophages (RAW264.7). Compound **3** significantly stimulated IL-2 expression in RAW264.7 cells at a concentration of 100 µg/mL ($P<0.05$) while no affection was observed at all lower tested concentrations.

Keywords: *Cardiospermum halicacabum* L., *Uridine*, *Immunomodulatory effect, Cytotoxic activity.*

1. Introduction

Far from being a small family, Sapindaceae comprises about 144 genera and 1900 species, which are widely distributed in temperate to tropical regions [1]. Plants belonging to this family have been the topic of research in many phytochemical investigations and pharmacological activities due to their popular applications in traditionally medicinal treatments. One of the most studied species from the family, *Cardiospermum halicacabum* L. attracted the researcher's attention because of their containing structurally complex and biologically fascinating secondary metabolites such as alkaloids, carbohydrates, fatty acids, flavonoids, triterpenoids, and volatile esters [2],[3],[4].To now, many pharmacological investigations have been conducted on *C. halicacabum* extracts to