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Research Article

THE CHEMICAL CONSTITUENTS OF *Caesalpinia pulcherrima*

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ABSTRACT

Dried aerial parts of Caesalpinia pulcherrima collected in Tien Giang Province, Vietnam, were macerated and extracted with methanol at room temperature to prepare a crude extract. This crude was separated into n-hexane, ethyl acetate, and methanol extracts by a liquid-liquid partition method. Then phytochemical investigations of the ethyl acetate extract of the Caesalpinia pulcherrima by chromatography technique resulted in the isolation of six compounds. Their chemical structures were elucidated by NMR and MS spectroscopic data and confirmed through comparison with published literature data. The isolated compounds include two phenolics (umbelliferone ***1*** *and bergenin* ***2****), one lignan* ***3****, and three flavonoids (protosappanin A* ***4****, isoliquiritigenin* ***5,*** *and pulcherrimin* ***6****). Compounds* ***1-5*** *were isolated from this plant for the first time.*

Keywords**:** *Caesalpinia pulcherrima;* flavonoid; phenolic compounds

1. Introduction

Caesalpinia pulcherrima belongs to the Fabaceae family. The plant is native to Central America but widely grown in the tropical and subtropical regions such as Australia, Asia, and Africa. In traditional medicine, *Caesalpinia pulcherrima* has been used as a common remedy for the treatment of of disorders, including malaria infections, pyrexia, bronchitis, and epileptic seizures (Dela Torre et al., 2007; Li et al., 2020; Ogbeide et al., 2018). Different parts of *C. pulcherrima* have been reported to exhibit medicinal properties, such as anticancer, antioxidant, antimicrobial, and antimalarial activities (Deepika et al., 2020; Dela Torre et al., 2007; Moteriya & Chanda, 2020, Ogbeide et al., 2020). Previously, phytochemical studies on this species disclosed the presence of diterpenoids, flavonoids, steroids, phenols (Erharuyi et al., 2017; Li et al., 2020; Srinivas et al., 2003; Yodsaoue et al., 2011). This paper reported the isolation and structural elucidation of six compounds from the ethyl acetate extract of *Caesalpinia pulcherrima* collected at Cai Be district, Tien Giang Province[*], Vietnam.

2. Experiment

2.1. General experimental methods

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[*] The geographical names are presented using the administrative divisions at the time the study was conducted (before the merger of provinces and cities in Vietnam in July 2025).

The ESI-MS was recorded on a HR–ESI–MS MicrOTOF–Q mass spectrometer. The ^{1}H-NMR (600 MHz) and ^{13}C-NMR (125 MHz and 150 MHz) spectra were recorded on a Bruker Avance III spectrometer. Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} (Merck), and the isolated compounds were visualized by spraying with 10% sulfuric acid solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Merck).

2.2. Plant material

The aerial parts of *Caesalpinia pulcherrima* (L.) Sw. were collected at Cai Be District, Tien Giang Province, Vietnam in November 2020. The scientific name of this species was authenticated by Assoc. Prof. Dang Van Son, Institute of Tropical Biology, Vietnam Academy of Science and Technology.

2.3. Extraction and isolation

The dried powder of *Caesalpinia pulcherrima* (14.5 kg) was macerated in methanol at room temperature, then the filtrated solution was evaporated at reduced pressure to give a crude extract (980.5 g). The liquid–liquid partition method with *n*-hexane and ethyl acetate, in turn, was used to separate the crude extract into different fractions.

The ethyl acetate extract (350.8 g) was silica gel chromatographed and eluted by *n*-hexane – ethyl acetate (stepwise, 9:1, 4:1, 7:3, 1:1, 0:1, v/v) and ethyl acetate – methanol (stepwise, 19:1, 9:1, 4:1, 7:3, 1:1, 0:1, v/v) to give twelve fractions (EA1–EA12).

The fraction EA3 (45.8 g) was further fractionated by silica gel chromatography using n-hexane – ethyl acetate (20-100% ethyl acetate) and ethyl acetate – methanol (5-100% methanol) to afford eight subfractions (EA3.1-EA3.8). The subfraction EA3.4 (6.8 g) was chromatographed on a silica gel column, eluting with *n*-hexane–ethyl acetate (20-100% ethyl acetate), followed by Sephadex LH-20 chromatography column eluted with chloroform – methanol 1:1, *v/v*) to obtain compounds coded **1** (6.0 mg), **4** (4.2 mg), and **6** (4.8 mg).

The same procedure was applied to the fraction EA7 (42.7 g) eluted by *n*-hexane – ethyl acetate (20-100% ethyl acetate) and ethyl acetate – methanol (5-100% methanol) to give 10 subfractions (EA7.1-EA7.10). The subfraction EA7.4 (6.7 g) was subjected to further silica gel CC eluted with n-hexane–EtOAc (7:3, *v/v*), followed by RP_{18} silica gel chromatography column eluted with methanol-water (4:1, *v/v*) to give compounds: **2** (7.5 mg), **3** (10.8 mg), and **5** (4.5 mg).

2.4. Spectroscopic data

- **Umbelliferone (1)**. Colorless powder; ^{1}H-NMR (CD_3OD) (J in Hertz): δ_H 6.20 (d, 9.6, H-3), 7.86 (d, J = 9.6, H-4), 7.46 (d, J = 8.4, H-5), 6.81 (dd, J = 8.4, 2.4, H-6), and 6.73 (d, J = 2.4, H-8). ^{13}C-NMR (CD_3OD): δ_C 163.7 (C-2), 112.4 (C-3), 146.0 (C-4), 113.2 (C-4a), 130.7 (C-5), 114.5 (C-6), 163.1 (C-7), 103.4 (C-8), and 157.3 (C-8a).
- **Lyoniresinol (2).** White powder; ^{1}H-NMR (DMSO-d_6) (J in Hertz) δ_H 6.55 (s, H-2), 2.62 (dd, J = 15.0, 4.8, H-7a), 2.43 (dd, J = 14.4, 12.0, H-7b), 1.44 (m, H-8), 3.47 (dd, 10.8, 4.8, H-9a), 3.28 (m, H-9b), 6.29 (s, H-2'/H-6'), 4.24 (d, 4.8, H-7'), 1.85 (m, H-8'), 3.32

(Overlapped by signal of solvent) (H-9'a), 3.24 (dd, 10.2, 6.6, H-9'b), 3.77 (s, 3-OCH_3), 3.32 (s, 5-OCH_3), and 3.63 (s, 3'-OCH_3/5'-OCH_3). ^{13}C-NMR (DMSO-d_6): δ_C 128.6 (C-1), 106.7 (C-2), 146.9 (C-3), 137.2 (C-4), 146.4 (C-5), 125.0 (C-6), 32.2 (C-7), 40.1 (C-8), 64.6 (C-9), 137.7 (C-1'), 106.0 (C-2'/C-6'), 147.5 (C-3'/C-5'), 133.4 (C-4'), 39.0 (C-7'), 46.6 (C-8'), 62.3 (C-9'), 55.7 (3-OCH_3), 58.9 (5-OCH_3), and 56.1 (3'-OCH_3/5'-OCH_3).

- **Begenin (3).** Colorless needles; HR-ESI-MS: *m/z* 329.0860 $[M+H]^+$ (calcd. for $[C_{14}H_{16}O_9+H]^+$, 329.0872). ^{1}H-NMR data (CD_3OD) (J in Hertz): δ_H 3.70 (m, H-2), 3.43 (t, 8.4, H-3), 3.81 (t, J = 9.6, H-4), 4.04 (dd, J = 9.6, 9.0, H-4a), 7.09 (s, H-7), 4.95 (d, J = 10.8, H-10b), 4.02 (dd, J = 10.8, 1.0, H-11a), 3.66 (m, H-11b), and 3.91 (s, H-12). ^{13}C-NMR (CD_3OD): δ_C 83.1 (C-2), 71.9 (C-3), 75.6 (C-4), 81.4 (C-4a), 165.8 (C-6), 119.4 (C-6a), 111.1 (C-7), 152.4 (C-8), 142.3 (C-9), 149.4 (C-10), 117.3 (C-10a), 74.3 (C-10b), 62.7 (C-11) and 60.9 (C-12).
- **Isoliquiritigenin (4).** Yellow powder. ESI-MS: *m/z* 257.41. ^{1}H-NMR data (CD_3OD) (J in Hertz): δ_H 7.64 (d, J = 9.0, H-2/H-6), 6.87 (d, J = 9.0, H-3/H-5), 6.31 (d, J = 2.4, H-3'), 6.44 (dd, J = 9.0, 2.4, H-5'), 7.98 (d, J = 9.0, H-6'), 7.63 (d, J = 15.6, H-α), and 7.81 (d, J = 15.6, H-β). ^{13}C-NMR (CD_3OD): δ_C 127.9 (C-1), 131.8 (C-2/C-6), 116.9 (C-3/C-5), 161.6 (C-4), 114.7 (C-1'), 167.5 (C-2'), 103.9 (C-3'), 166.5 (C-4'), 109.2 (C-5'), 133.4 (C-6'), 118.4 (C-α), 145.6 (C-β), and 193.5 (C-β').
- **Protosappanin A (5).** White powder. ^{1}H-NMR data (DMSO-d_6) (J in Hertz): δ_H 7.07 (d, J = 8.4, H-1), 6.68 (dd, J = 8.4, 2.4, H-2), 6.65 (d, J = 2.4, H-4), 4.47 (s, H-6), 3.17 (s, H-8), 6.64 (s, H-9), and 6.63 (s, H-12). ^{13}C-NMR (DMSO-d_6): δ_C 129.4 (C-1), 112.2 (C-2), 158.2 (C-3), 107.9 (C-4), 77.5 (C-6), 205.4 (C-7), 47.9 (C-8), 116.7 (C-9), 144.5 (C-10), 144.2 (C-11), 116.5 (C-12), 125.0 (C-1a), 157.3 (C-4a), 122.8 (C-8a), and 129.5 (C-12a).
- **Pulcherimin (6**). Yellow powder. ^{1}H-NMR data ($CDCl_3$) (J in Hertz): δ_H 7.36 (d, J = 8.4, H-1), 6.88 (d, J = 8.4, H-2), 5.25 (s, H-5), 6.36 (d, J = 2.4, H-9), 6.45 (d, J = 2.4 H-11), 6.08 (s, -OCH_2O), 3.88 (s, 10-OCH_3), and 12.57 (s, 8-OH). ^{13}C-NMR ($CDCl_3$): δ_C 116.9 (C-1), 108.2 (C-2), 150.9 (C-3), 142.8 (C-4), 113.1 (C-4a), 62.8 (C-5), 134.5 (C-6a), 175.1 (C-7), 106.2 (C-7a), 162.3 (C-8), 97.8 (C-9), 165.3 (C-10), 92.4 (C-11), 156.2 (C-11a), 148.1 (C-12a), 118.9 (C-12b), 102.3 (-OCH_2O-), and 55.8 (10-OCH_3).

3. Results and discussion

Compound **1** was isolated as a colorless powder. The proton spectrum of **1** displayed three aromatic protons at δ_H 7.46 (1H, d, J = 8.4 Hz, H-5), 6.81 (1H, dd, J = 8.4, 2.4 Hz, H-6) and 6.73 (1H, d, J = 2.4 Hz, H-8) of a 1,2,4-trisubstituted benzene ring, two cis-configuration olefinic protons at δ_H 7.86 (1H, d, J = 9.6 Hz, H-4) and 6.20 (1H, d, J = 9.6 Hz, H-3). The ^{13}C NMR spectrum of 1 showed signals of 9 carbons, consisting of one ester carbon at δ_C 163.7 (C-2) and two olefinic carbons at δ_C 146.0 (C-4) and 112.4 (C-3) of a conjugated system. The HMBC spectrum also revealed cross-peaks of the aromatic proton at δ_H 7.46 with the olefinic carbon at δ_C 146.0 (C-4) and two olefinic protons at δ_H 7.86 and 6.20 with the ester carbon at δ_C 163.7 (C-2). This information suggested that **1** was a coumarin. Based on the compatibility of the NMR data of **1** with the ones in the literature (Meerungrueang et al. 2014), **1** was assigned to be umbelliferone (Figure 1).

Compound **2** was isolated as a white powder. The ^{1}H-NMR spectrum showed three aromatic protons [δ_H 6.55 (1H, s, H-2), 6.29 (2H, s, H-2'/H-6')], three methine signals [δ_H 4.24 (1H, d, J = 4.8 Hz, H-7'), 1.85 (1H, m, H-8'), 1.44 (m, H-8)], two non-equivalent methylene protons [δ_H 2.62 (1H, dd, J = 15.0, 4.8 Hz, H-7a), 2.43 (1H, dd, J = 14.4, 12.0 Hz, H-7b)]. The spectrum also showed two non-equivalent oxymethylene protons [δ_H 3.47 (1H, dd, J = 10.8, 4.8 Hz, H-9a), 3.28 (1H, m, H-9b), 3.32 (overlapped by signal of solvent, H-9'a), 3.24 1H, (dd, J = 10.2, 6.6 Hz, H-9'b)] and four methoxy signals [δ_H 3.77 (3H, s, 3-OCH_3), 3.32 3(3H, s, 5-OCH_3), 3.63 (6H, s, 3'-OCH_3/5'-OCH_3)].

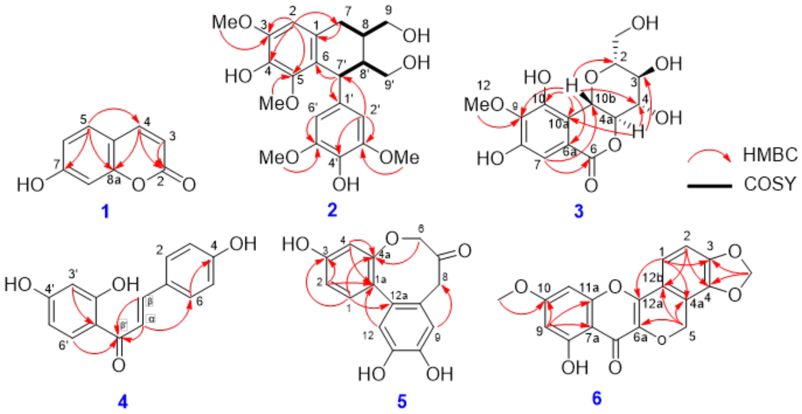

__Figure 1.__ Chemical structures and some key HMBC, COSY correlations of 1-6

The ^{13}C-NMR spectrum of 2 showed signals of 22 carbon, consisting of three methine groups (δ_C 46.6, 40.1, 39.0), one methylene group (δ_C 32.2), two oxymethylene groups (δ_C 64.6, 62.3), and four methoxy groups [δ_C 58.9, 56.1 (in double intensity), 55.7]. These data revealed that **2** was a 6,7'–cyclolignan. These proton assignments were supported by ^{1}H-^{1}H COSY correlations of H-7/H-8/H-9, H-7'/H-8'/H-9', and H-8/H-8'. The positions of the hydroxyl and methoxyl groups were supported by HMBC experiments as presented in Figure 1. The good compatibility of its NMR data with those in the literature (Cretin et al., 2015) proposed that **2** was lyoniresinol.

Compound **3** was isolated as colorless needles. The HR-ESI-MS of **3** displayed a quasimolecular ion peak at m/z 329.0860 [M+H]+ (calcd. for $[C_{14}H_{16}O_9+H]^+$, 329.0872). The proton spectrum of **3** displayed one singlet aromatic proton signal at δ_H 7.09 (1H, s, H-7), two non-equivalent methylene protons at δ_H 4.02 (1H, dd, J = 10.8, 1.0 Hz, H-11a), 3.66 (1H, m, H-11b), four oxymethine protons at δ_H 4.04, 3.81, 3.70, 3.43, and one singlet methoxyl group (δ_H 3.91, 3H, s, H3-12). The ^{13}C NMR and HSQC spectra of **3** showed 14 carbon signals consisting of one carboxyl carbon at δ_C 165.8 (C-6), a methoxyl group at δ_C 60.9 (C-12). The positions of the hydroxyl and methoxyl groups were supported by HMBC experiments as presented in Figure 1. Compound **3** was characterized as bergenin by comparing its spectral data with related literature (Nguyen et al., 2015).

Compound **4** was isolated as a yellow powder. The ^{1}H NMR spectrum of 4 showed signals for a pair of ortho coupled proton at δ_H 7.98 (1H, d, J = 9.0 Hz, H-6'), 6.44 (1H, dd, J = 9.0, 2.4 Hz, H-5') and a third signal at δ_H 6.31 (1H, d, J = 2.4 Hz, H-3') which was a meta coupled to H-5', assignable to the trisubstituted benzene ring. The signals at δ_H 7.64 (2H, d, J = 9.0 Hz, H-2/H-6), 6.87 (2H, d, J = 9.0 Hz, H-3/H-5) were assigned to the 1,4-disubstituted benzene ring. Two trans-configuration olefinic protons at δ_H 7.63 (1H, d, J = 15.6 Hz, H-α), and 7.81 (1H, d, J = 15.6 Hz, H-β) were also assigned. Among fifteen signals in the ^{13}C NMR spectrum, the ones at δ_C 193.5 (C=O), 145.6 (=CH-, C-β), and 118.4 (=CH, C-α) were typical characteristics of a chalcone skeleton. The positions of two hydroxyl groups were confirmed by the HMBC correlations, as shown in Figure 1. Since all of the above data were consistent with those of the reference (Udomputtimekakul et al., 2017), compound **4** was identified as isoliquiritigenin.

Compound **5** was isolated as a white powder. The ^{1}H NMR spectrum of **5** displayed two aromatic protons at δ_H 6.64 (1H, s, H-9), 6.63 (1H, s, H-12) of a 1,2,4,5-tetrasubstituted benzene ring, three aromatic protons at δ_H 7.07 (d, J = 8.4 Hz, H-1), 6.68 (dd, J = 8.4, 2.4 Hz, H-2), and 6.65 (d, J = 2.4 Hz, H-4) of a 1,2,4-trisubstituted benzene ring, and two singlet methylene protons at δ_H 4.47 (1H, s, H-6), 3.17 (1H, s, H-8). The ^{13}C NMR and HSQC spectra of **5** showed signals of 15 carbons, consisting of one ketone carbon at δ_C 205.4 (C-7), two benzene rings, and two methylene groups at δ_C 77.5 (C-6), 47.9 (C-8). The HMBC spectrum revealed cross-peaks of aromatic proton at δ_H 6.63 (H-12) and two aromatic protons of the rest ring at δ_H 6.68 and 6.65 with a quaternary aromatic carbon at δ_C 125.0 (C-1a). This information suggested that **5** was a biphenyl compound. The positions of two methylene groups were supported by HMBC experiments as presented in Figure 1. Compound **5** was determined as protosappanin A via the good compatibility of its NMR data with those published in the literature (Warinhomhaun et al., 2016, Liu et al., 2016).

Compound **6** was isolated as a yellow powder. The ^{1}H NMR spectrum of **6** showed the presence of two independent aromatic rings. The first one was comprised of a pair of meta coupled proton at δ_H 6.36 (1H, d, J = 2.4 Hz, H-9), 6.45 (1H, d, J = 2.4 Hz, H-11), a pair of an ortho coupled proton at δ_H 7.36 (1H, d, J = 8.4 Hz, H-1), 6.88 (1H, d, J = 8.4 Hz, H-2), and one chelated hydroxyl group at δ_H 12.57 (1H, s, 8-OH). The ^{13}C NMR spectrum showed 18 signals with one conjugated ketone carbon at δ_C 175.1 (C-7). Its NMR data also displayed one methoxyl group (δ_H 3.88, 3H, s; δ_C 55.8,10-OCH_3), one oxymethylene group (δ_H 5.25, 2H, s; δ_C 62.8, C-5), and one methylenedioxy group (δ_H 6.08, 2H, s; δ_C 102.3, -OCH_2O-). The positions of the methoxyl group and chelated hydroxyl group in the ring A of **6** were evidenced by HMBC cross-peaks of the aromatic proton at δ_H 6.36 with aromatic carbons at δ_C 106.2 (C-7a), 156.2 (C-11a), 165.3 (C-10), and of the methoxy proton at δ_H 3.88 with carbon signal at δ_C 165.3 (C-10). The presence of the oxymethylene group in **6** was proved by cross-peaks of δ_H 5.25 with carbons at δ_C 134.5 (C-6a), 118.9 (C-12b), 113.1 (C-4a). The spectrum also confirmed the position of the methylenedioxy group through cross-peaks of aromatic proton at δ_H 6.88 with carbons at δ_C 142.8 (C-4), 118.9 (C-12b), and of the methylenedioxy protons with aromatic carbons at δ_C 150.9 (C-3), 142.8 (C-4). Therefore, **6** was identified as pulcherrimin, as shown in the literature (McPherson et al., 1983).

4. Conclusions

From the ethyl acetate extract of *Caesalpinia pulcherrima*, six compounds were isolated, consisting of two phenolics (umbelliferone, **1** and bergenin, **3**), a lignan (lyoniresinol, **2**), and three flavonoids (isoliquiritigenin, **4**; protosappanin A, **5,** and pulcherimin, **6**). Their chemical structures were elucidated based on the NMR and HR-MS data analysis, as well as comparison to the published ones. To the best of our knowledge, except for **6**, the presence of the five remaining compounds, **1**–**5**, was reported for the first time in *C. pulcherrima*. Although **6** was a known compound, its full NMR data were reported for the first time.

- ❖ ***Conflicts of Interest:*** *Authors have no conflict of interest to declare.*

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MỘT SỐ HỢP CHẤT TỪ LOÀI *Caesalpinia pulcherrima*

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TÓM TẮT

Phần trên mặt đất của loài Caesalpinia pulcherrima được thu hái ở tỉnh Tiền Giang, Việt Nam được làm thành bột khô. Điều chế cao chiết methanol từ bột khô này bằng phương ngâm ở nhiệt độ phòng. Ba cao chiết n-hexane, ethyl acetate và methanol được điều chế từ cao methanol bằng phương pháp chiết lỏng – lỏng. Áp dụng các kĩ thuật sắc kí trên cao chiết ethyl acetate, sáu hợp chất (1-6) đã được phân lập. Sử dụng phương pháp phổ nghiệm NMR và MS, cấu trúc của những hợp chất này được xác định, bao gồm: hai hợp chất phenolic (umbelliferone ***1*** *và bergenin* ***2****), một lignan 3, và ba flavonoid (protosappanin A* ***4****, isoliquiritigenin* ***5*** *và pulcherrimin* ***6****). Lần đầu tiên, năm hợp chất* ***1-5*** *được xác định hiện diện trong loài này*

Từ khóa: *Caesalpinia pulcherrima*; flavonoids; phenolic compounds