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STILBENE AND FLAVONOID DERIVATIVES FROM *CAYRATIA CHENIANA* L. M. LU & J. WEN

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Summary

Stilbene and Flavonoid Derivatives from *Cayratia cheniana* L. M. Lu & J. Wen

Using chromatographic separation methods, seven compounds were isolated from the methanolic extract of the stems and leaves of *Cayratia cheniana.* Their structures were elucidated as pallidol (**1**), *trans*-resveratrol (**2**), piceid (**3**), apigenin (**4**), acacetin (**5**), apigenin 7-*O*-*β*-D-glucopyranoside (**6**), and luteolin 7-*O*-*β*-D-glucopyranoside (**7**) using 1D- and 2D-NMR experiments and comparison with those reported in the literature.

Keywords: *Cayratia cheniana, Flavonoid, Stilbene, Vitaceae.*

1. Introduction

The *Cayratia* genus (Vitaceae) contains over 60 species that are mostly distributed in tropical and subtropical regions of Asia, Africa, Australia, and the Pacific Islands [1]. To date, some secondary metabolites have been reported from *Cayratia* species, including stilbenes, flavonoids, alkaloids, saponins, proteins, and carbohydrates. *Cayratia* plants have also exhibited diverse pharmacological effects such as antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer, antioxidant, and inhibitory activity on fatty acid synthase [1],[2],[3]. *Cayratia cheniana* L. M. Lu & J. Wen is a new species, endemic to the limestone mountains of Ninh Thuan province of Vietnam, and was described by Lu et al. in 2016 [4]. In the current study, we report the isolation and structural elucidation of seven compounds from the stems and leaves of *C. cheniana.* To the best of our knowledge, this is the first report on the phytochemical constituents of *C. cheniana.*

2. Material and methods

2.1. General experimental procedures

The high-resolution mass spectra were recorded on an Agilent 6530 Accurate-Mass spectrometer (CA, USA). The NMR data were recorded on Bruker AVANCE NEO 600 FTNMR spectrometer. Column chromatography (CC) was carried out on *silica gel* (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck, Darmstadt, Germany), YMC*GEL (ODS-A, 12 nm S-150 μm, YMC, Japan, and Sephadex™ LH-20. Thin-layer chromatography (TLC) was performed on Merck precoated *silica gel* 60 F_{254} (1.05554.0001) and RP-C_{18} F_{254S} plates (1.15685.0001). Compounds on the plates were detected under UV light (254 and 365 nm) and visualized by spraying with 10% (v/v) H_2SO_4 aqueous solution and heating for 3-5 minutes.

2.2. Plant material

The stems and leaves of *Cayratia cheniana* L. M. Lu & J. Wen were collected at Ca Na, Ninh Thuan province, Vietnam, in 2020, and identified by Dr. Dang Viet Hung (Vietnam National University of Forestry, Dongnai Campus, Dongnai, Vietnam). A voucher specimen (No: CC2020) was deposited at the Institute of Marine Biochemistry (IMBC), VAST, Hanoi, Vietnam.

2.3. Extraction and isolation

The dried stems and leaves of *C. cheniana* (4 kg) were cut into pieces and extracted with MeOH (10 L × 3 times) at room temperature. The methanol solution was concentrated using a rotatory evaporator to give a MeOH extract (300 g), which was further suspended in H_2O and successively partitioned with *n*-hexane (H) and ethyl acetate (EtOAc) to afford H (100 g), EtOAc (15 g), and aqueous extracts (W), respectively. The EtOAc extract (15 g) was subjected to reversed-phase RP-C_{18} *silica gel* CC, eluting with gradient MeOH-H_2O (1:5 to 100% MeOH, v/v) to obtain nine fractions (E1-E9). Fraction E2 (150 mg) was subjected to a Sephadex™ LH-20 column using a mixture of MeOH-H_2O (1:1, v/v) and followed by *silica gel* CC eluting with

CH_2Cl_2-acetone (1:2, v/v) to yield compound **1** (4.5 mg). Fraction E4 (350 mg) was separated on *silica gel* CC and eluted with CH_2Cl_2-MeOH-H_2O (6:1:0.05, v/v) to obtain two subfractions E4A and E4B. Subfraction E4A (80 mg) was purified by RP-18 CC (acetone-H_2O 1:1.5, v/v) to afford compound **3** (20 mg). Fraction E5 (500 mg) was subjected to a Sephadex™ LH-20 column using a mixture of MeOH-H_2O (1:1, v/v) to afford four subfractions (E5A-E5D). Fraction E5D (100 mg) was separated on *silica gel* CC and eluted with CH_2Cl_2-acetone (1:2 v/v) and followed by RP-C_{18} CC with MeOH-H_2O (1:1, v/v), to yield compound **7** (5 mg). Fraction E6 (200 mg) was subjected to a Sephadex™ LH-20 column using a mixture of MeOH-H_2O (1:1, v/v) to afford four subfractions (E6A-E6D). Subfraction E6A (30 mg) was further subjected to fractionation over reversed-phase RP-C_{18} *silica gel* CC, eluting with acetone-H_2O (1:1.5, v/v) to obtain compound **6** (8 mg). Subfraction E6B (16 mg) was separated on *silica gel* CC and eluted with CH_2Cl_2-acetone (3:1, v/v) to yield compound **2** (4.5 mg). Fraction E7 (1 g) was subjected to a Sephadex™ LH-20 column using a mixture of MeOH-H_2O (1:1, v/v) to afford four subfractions (E7A-E7D). Sufraction E7D (600 mg) was isolated by RP-C_{18} CC with acetone-H_2O (1:3, v/v), as eluent to obtain compound **5** (4 mg) and two fractions, E7D1 and E7D2. Compound **4** (3 mg) was purified from fraction E7D2 (46 mg), by using an RP-C_{18} CC with acetone-H_2O (1:2, v/v).

Pallidol (**1**): amorphous powder, ^{1}H-NMR (600 MHz, methanol-d_4) and ^{13}C-NMR (150 MHz, methanol-d_4) data are given in Table 1; HR-QTOF-MS *m/z* 455.1472 (calcd. for $C_{28}H_{23}O_6^+$, 455.1489).

***trans*-Resveratrol** (**2**): colorless oil, ^{1}H NMR (methanol-d_4, 600 MHz): δ_H 6.47 (2H, d, J = 2.4 Hz, H-2, H-6), 6.18 (1H, t, J = 2.4 Hz, H-4), 7.36 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.78 (2H, d, J = 8.4 Hz, H-3', H-5'), 6.81 (1H, d, J = 16.8 Hz, H-α), 6.97 (1H, d, J = 16.8 Hz, H-β).

Piceid (**3**): colorless oil, ^{1}H-NMR (methanol-d_4, 600 MHz): δ_H 6.86 (1H, br s, H-2), 6.48 (1H, br s, H-4), 6.65 (1H, br s, H-6), 7.37 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.79 (2H, d, J = 8.4 Hz, H-3', H-5'), 6.68 (1H, d, J = 16.8 Hz, H-α), 7.09 (1H, d, J = 16.8 Hz, H-β), 4.92 (1H, d, J = 6.6 Hz, H-1''), 3.74 (1H, dd, J = 5.4, 12.0 Hz, H_a-6''), 3.95 (1H, dd, J = 2.4, 12.0 Hz, H_b-6''); ^{13}C-NMR (methanol-d_4, 150 MHz): δ_C 141.4 (C-1), 107.1 (C-2), 160.4 (C-3), 104.1 (C-4), 159.5 (C-5), 108.4 (C-6), 130.3 (C-1'), 128.9 (C-2', C-6'), 116.5 (C-3', C-5'), 158.4 (C-4'), 126.7 (C-α), 130.0 (C-β), 102.4 (C-1''), 74.9 (C-2''), 78.0 (C-3''), 71.5(C-4''), 78.2 (C-5''), 62.6 (C-6'').

Apigenin (**4**): yellow powder, ^{1}H NMR (DMSO-d_6, 600 MHz): δ_H 6.77 (1H, s, H-3), 6.19 (1H, d, J = 2.4 Hz, H-6), 6.48 (1H, d, J = 2.4 Hz, H-8), 7.92 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.92 (2H, d, J = 8.4 Hz, H-3', H-5'), 12.96 (1H, s, 5-OH).

Acacetin (**5**): yellow powder, ^{1}H-NMR (DMSO-d_6, 600 MHz): δ_H 6.86 (1H, s, H-3), 6.20 (1H, d, J = 1.8 Hz, H-6), 6.50 (1H, d, J = 1.8 Hz, H-8), 8.03 (2H, d, J = 9.0 Hz, H-2', H-6'), 7.11 (2H, d, J = 8.4 Hz, H-3', H-5'), 12.91 (1H, s, 5-OH), 3.86 (3H, s, 4'-OCH_3).

Apigenin 7-*O*-β-D-glucopyranoside (**6**): pale-yellow powder, ^{1}H NMR (DMSO-d_6, 600 MHz): δ_H 6.86 (1H, s, H-3), 6.45 (1H, d, J = 2.4 Hz, H-6), 6.82 (1H, d, J = 2.4 Hz, H-8), 7.95 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.93 (2H, d, J = 8.4 Hz, H-3', H-5'), 12.95 (1H, s, 5-OH), 5.06 (1H, d, J = 7.2 Hz, H-1''); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ_C 164.2 (C-2), 103.1 (C-3), 182.0 (C-4), 161.4 (C-5), 99.4 (C-6), 162.9 (C-7), 94.8 (C-8), 156.9 (C-9), 105.3 (C-10), 121.0 (C-1'), 128.6 (C-2', C-6'), 116.0 (C-3', C-5'), 161.1 (C-4'), 99.9 (C-1''), 73.1 (C-2''), 76.4 (C-3''), 69.5 (C-4''), 77.1 (C-5''), 60.6 (C-6'').

Luteolin 7-*O*-β-D-glucopyranoside (**7**): pale-yellow powder, ^{1}H NMR (DMSO-d_6, 600 MHz): δ_H 6.71 (1H, s, H-3), 6.43 (1H, d, J = 1.8 Hz, H-6), 6.78 (1H, d, J = 1.8 Hz, H-8), 7.40 (1H, d, J = 2.4 Hz, H-2'), 6.88 (1H, d, J = 8.4 Hz, H-5'), 7.43 (1H, dd, J = 2.4, 8.4 Hz, H-6'), 5.06 (1H, d, J = 7.2 Hz, H-1''); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ_C 164.2 (C-2), 103.1 (C-3), 182.0 (C-4), 161.1 (C-5), 99.5 (C-6), 162.9 (C-7), 94.7 (C-8), 156.9 (C-9), 105.3 (C-10), 121.0 (C-1'), 113.3 (C-2'), 145.9 (C-3'), 149.9 (C-4'), 116.0 (C-5'), 113.3 (C-6'), 99.9 (C-1''), 73.1 (C-2''), 76.4 (C-3''), 69.6 (C-4''), 77.1 (C-5''), 60.6 (C-6'').

3. Results and discussion

A MeOH extract of the stems and leaves of *C. chenania* was suspended in H_2O and successively partitioned with *n*-hexane and EtOAc. The EtOAc residue was fractionated by repeated column chromatography over *silica gel*, RP-C_{18}, and Sephadex LH-20, to afford seven compounds **1**–**7**.

Compound **1** was obtained as an amorphous powder. The ^{1}H-NMR spectrum of **1** exhibited signals for 6 aromatic protons including four in an A_2B_2 system at δ_H 6.94 (2H, d, J = 9.0 Hz), 6.67 (2H, d, J = 9.0 Hz) and two *meta*-coupled

protons at δ_H 6.13 (1H, d, J = 1.8 Hz), and 6.54 (1H, d, J = 1.8 Hz). In addition, two aliphatic CH protons were observed at δ_H 4.49 (1H, s) and 3.75 (1H, s). The ^{13}C-NMR spectrum of **1** presented 14 carbon signals, including 12 aromatic C-atoms in the range of δ_C 102.6-159.3 and two aliphatic CH C-atoms at δ_C 54.7 and 61.0.

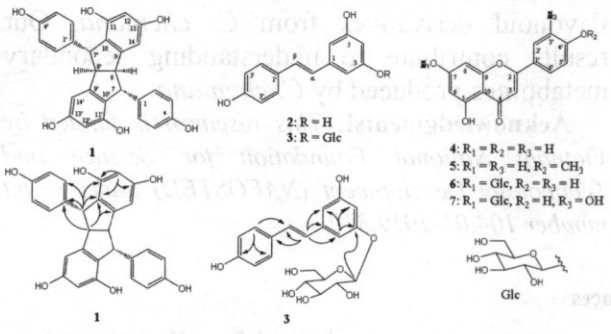

Fig. 1. Structures of compounds **1–7** and key HMBC correlations (→) of **1** and **3**

The HR-ESI-MS showed a protonated molecular ion at *m/z* 455.1472 $[M+H]^+$ (calcd. for $C_{28}H_{23}O_6^+$, 455.1489), suggesting that the molecular formula of **1** was $C_{28}H_{22}O_6$. Combined with ^{1}H, ^{13}C NMR spectra, it was possible to predict that **1** had a symmetrical structure, which was formed by the dimerization of two resveratrol units. By detailed analysis of key HMBC correlations (Fig.1), the structure of **1** was determined as a resveratrol dimer namely pallidol [5],[6]. The chemical shifts and coupling constants of two aliphatic protons of **1**: [H-7 δ_H 4.49 (1H, s), H-8 δ_H 3.75 (1H, s), compared to nepalensinol B [H-7 δ_H 4.29 (1H, s), H-8 δ_H 3.97 (1H, s)] indicated that the methine proton (H-8) and 4-hydroxyphenyl groups at C-7 were *cis*-orientated to each other [7]. Thus, the structure **1** was determined as shown in Fig. 1.

Compound **3** was obtained as a corless oil. The ^{1}H-NMR spectrum of **3** exhibited signals for 9 olefinic protons including three protons of a 1,3,5-trisubstitued aromatic ring at δ_H 6.81 (1H, br s, H-2), 6.48 (1H, br s, H-4), and 6.65 (1H, br s, H-6), four aromatic protons in an A_2B_2 system at δ_H 7.37 (2H, d, J = 8.4 Hz, H-2', H-6'), 6.79 (2H, d, J = 8.4 Hz, H-3', H-5'), and two *trans*-coupled olefinic protons at δ_H 6.68 (1H, d, J = 16.8 Hz, H-α) and 7.02 (1H, d, J = 16.8 Hz, H-β). In addition, the existence of a D-glucose moiety was indicated by an anomeric proton at δ_H 4.92 (1H, d, J = 6.6 Hz, H-1") and the ^{13}C NMR chemical shifts at δ_C 102.4 (C-1"), 74.9 (C-2"), 78.0 (C-3"), 71.5 (C-4"), 78.2 (C-5"), and 62.6 (C-6"). According to the large coupling constant, the configuration of glucose was β. From these spectral data, **3** was presumed to be a trihydroxystilbene glucoside. By detailed analysis of key HMBC correlations (Fig.1) and comparison with the reported spectroscopic data, the structure of **3** was determined as a *trans*-resveratrol 3-*O*-β-D-glucoside, namely piceid [8].

Compound **6** was obtained as a pale-yellow powder. The ^{1}H-NMR spectrum of **6** exhibited signals for an exchangeable proton at δ_H 12.95 (1H, s), an A_2B_2 system at δ_H 7.95 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.93 (2H, d, J = 8.4 Hz, H-3', H-5'), and an AB system at δ_H 6.45 (1H, d, J = 2.4 Hz, H-6), 6.82 (1H, d, J = 2.4 Hz, H-8). These two systems together with a singlet signal at δ_H 6.86 (1H, s, H-3) indicated the presence of an apigenin aglycone in compound **6** [9]. In addition, the existence of a D-glucose moiety was indicated by an anomeric proton at δ_H 5.06 (1H, d, J = 7.2 Hz, H-1") and the ^{13}C NMR chemical shifts at δ_C 99.9 (C-1"), 73.1 (C-2"), 76.4 (C-3"), 69.5 (C-4"), 77.1 (C-5"), and 60.6 (C-6"). Its β-configuration was evident from the coupling constant of anomeric proton. The upfield displacement of the C-7 (δ_C 162.9) in the ^{13}C NMR of **6** as compared to the signal of authentic apigenin [C-7 (δ_C 163.7)] [10] further supported the glycosylation of 7-hydroxyl group. Therefore, the structure of **6** was determined to be apigenin 7-*O*-β-D-glucopyranoside [10].

The other compounds were also identified on the basis of their NMR data and by comparison with literature data to be *trans*-resveratrol (**2**) [8], apigenin (**4**) [10], acacetin (**5**) [11], and luteolin 7-*O*-β-D-glucopyranoside (**7**) [10].

According to published literature, compounds **1**, and **2** showed strong fast-biding inhibitory activity on fatty acid synthase with IC_{50} values of 11.1 and 10.2 μM, respectively (epigallocatechin gallate was used as a positive control, IC_{50} = 49.0 μM), and they also exhibited antioxidant activities in the ORAC assay with vitamin C as a positive control [1]. Compounds **2** and **3** also displayed significant inhibition of the lipoxygenases (LOX) activity with the IC_{50} values of 12.3 and 55.4 μM, respectively, compared with baicalein (IC_{50} = 3.4 μM) and (+)-catechin (IC_{50} = 19.7 μM), used as positive control [8]. Whereas, compounds **4**, **6**, and **7** showed inhibitory effects against MAO (monoamine oxidase) activity with IC_{50} values of 6.5, 81.7, and 118.6 μM, respectively [10].

Table 1. The ^{1}H NMR (600 MHz) and ^{13}C NMR data (150 MHz) of compound **1** in methanol-d_4

Position	$^1\delta_C$	1	
		δ_C	δ_H (mult., J in Hz)
1, 1'	138.6	138.5	-
2, 2'	129.3	129.2	6.94 (d, 9.0)
3, 3'	116.1	116.0	6.67 (d, 9.0)
4, 4'	156.3	156.3	-
5, 5'	116.1	116.0	6.67 (d, 9.0)
6, 6'	129.3	129.2	6.94 (d, 9.0)
7, 7'	54.9	54.7	4.49 s
8, 8'	61.1	61.0	3.75 s
9, 9'	150.9	150.8	-
10, 10'	123.9	123.8	-
11, 11'	155.7	155.5	-
12, 12'	102.6	102.6	6.13 (d, 1.8)
13, 13'	159.5	159.3	-
14, 14'	103.1	103.4	6.54 (d, 1.8)

$^1\delta_C$ of pallidol measured in methanol-d_4 [6]

4. Conclusion

A chemical study of the methanol extract of *C. cheniana* stems and leaves obtained seven compounds, including pallidol (**1**), *trans*-resveratrol (**2**), piceid (**3**), apigenin (**4**), acacetin (**5**), apigenin 7-*O*-*β*-D-glucopyranoside (**6**), and luteolin 7-*O*-*β*-D-glucopyranoside (**7**). This is the first report on the presence of stilbene and flavonoid derivatives from *C. cheniana*. Our results contribute to understanding secondary metabolites produced by *C. cheniana*.

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CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF THE *n*-HEXANE EXTRACT FROM THE AERIAL PARTS OF *CURCUMA ZEDOAROIDES* CHAVEER. & TANEE

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Summary

Chemical Constituents and Cytotoxic Activity of the *n*-Hexane Extract from the Aerial Parts of *Curcuma zedoaroides* Chaveer. & Tanee

Curcuma zedoaroides Chaveer. & Tanee, belonging to the gennus *Curcuma* (Zingiberaceae), is a newly discovered species in Vietnam. In the present study, the chemical constituents and the *in vitro* cytotoxic activity of the *n*-hexane extract from the *C. zedoaroides* aerial parts were reported for the first time. The *n*-hexane extract showed the most potent cytotoxic effect among all tested extracts on seven human cancer cell lines (A549, MCF-7, HepG2, HT-29, HL-60, K562, and MDA-MB-231), with IC_{50} values ranging from 49.76 – 86.30 μg/mL using sulforhodamine B and MTT methods. Gas