



## Research article

# Isolation, structural determination of flavonoids and ellagic acid derivatives from the leaves of *Cleistanthus eberhardtii*

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## ABSTRACT

From the leaves of *Cleistanthus eberhardtii* (Gagnep.) Croizat, six known compounds were isolated, including four flavonoids, amentoflavone (**1**), isoquercetrin (**2**), astragalin (**3**), kaempferol (**4**) and two ellagic acid derivatives, 3,3'-O-dimethylellagic acid-4-O- $\alpha$ -L-rhamnopyranoside (**5**), and 3,3'-O-dimethylellagic acid-4-O- $\beta$ -D-glucopyranoside (**6**). The structures of these compounds were determined through MS and NMR spectra analyses, and comparison with literature data. To date, this is the first phytochemical investigation of the leaves of *C. eberhardtii*.

## INTRODUCTION

The genus *Cleistanthus* belongs to the Euphorbiaceae family, comprises about 140 species and is distributed in tropical regions [1]. Many species in this genus have attracted scientific interest due to their diverse bioactive compounds. For example, *C. collinus* contains

arylnaphthalide lignans such as diphyllin, cleistanthin A and B, which exhibit cytotoxic, anticancer, larvicidal, and antifungal activities [2, 3]. Similarly, lignans such as cleistanoxin from *C. tonkinensis* [4] and cleistanone from *C. indochinensis* have shown potent cytotoxic effects [5]. Furthermore, *Cleistanthus* species

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are known to contain flavonoids, tannins, saponins, triterpenoids, and polyphenols that exhibit antioxidant, antibacterial, anti-inflammatory, and immunosuppressive activities [6-9]. To the best of our knowledge, no study has been performed on the chemical constituents of the leaves of *C. eberhardtii*. Herein, we report the isolation and structural elucidation of six known compounds including four flavonoids: amentoflavone (**1**), isoquercetrin (**2**), astragalins (**3**), kaempferol (**4**), and two gallic acid derivatives: 3,3'-O-dimethylellagic acid-4-O- $\alpha$ -L-rhamnopyranoside (**5**), 3,3'-O-dimethylellagic acid-4-O- $\beta$ -D-glucopyranoside (**6**) from the leaves of *C. eberhardtii* (Figure 1).

## MATERIALS AND METHODS

### Plant materials

The leaves of *Cleistanthus eberhardtii* (Gagnep.) Croizat were collected in Phu Loc, Thua Thien Hue, Vietnam in August 2017 and identified by Dr. Nguyen The Cuong. A voucher specimen (VN-2151B) was deposited at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST) in Hanoi.

### General experiment procedures

NMR spectra were recorded on a Bruker 500 MHz spectrometer operating at 125 MHz for  $^{13}\text{C}$ -NMR, and at 500 MHz for  $^1\text{H}$ -NMR. The  $^1\text{H}$ -NMR chemical shift were referenced to  $\delta_{\text{H}}$  3.31 ppm ( $\text{CD}_3\text{OD}$ ) and 2.50 ppm ( $\text{DMSO}-d_6$ ), respectively. The  $^{13}\text{C}$ -NMR chemical shifts were referenced to the central peak of  $\text{CD}_3\text{OD}$  at  $\delta_{\text{C}}$  49.0 ppm and  $\text{DMSO } d_6$  at  $\delta_{\text{C}}$  39.5 ppm. ESI-MS spectra were obtained on an Agilent 1100 LC-MSD Trap spectrometer. TLC *silica gel* (Merck 60 F<sub>254</sub>) was used for thin layer chromatography. Column chromatography (CC) was carried out using *silica gel* 40 - 63  $\mu\text{m}$  (Merck 60

F<sub>254</sub>) and Sephadex LH-20 (Merck). All solvents were commercially obtained and redistilled prior to use.

### Extraction and isolation

Dried and ground leaves of *Cleistanthus eberhardtii* (2.2 kg) were extracted with MeOH (5 times  $\times$  5 L, 24 h) at room temperature. The methanol solutions were combined and concentrated under reduced pressure to obtain 180 g of a methanol residue. The methanol residue was suspended in  $\text{H}_2\text{O}$  (500 ml), and then partitioned successively with *n*-hexane (5 times  $\times$  1.5L), and EtOAc (5 times  $\times$  1.5 L). The extracts were concentrated under reduced pressure to give *n*-hexane (H-35 g), EtOAc (E-40g), and aqueous extracts (W-105 g), respectively.

The ethyl acetate extract (E-40 g) was further purified by gel filtration over a *silica gel* column chromatography (CC) using a mixture of  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (0% to 100% MeOH in  $\text{CH}_2\text{Cl}_2$ ) give 10 fractions E1-E10. Fraction E7 (5.01 g) was subjected to CC on *silica gel*, eluting with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (0% to 100% MeOH in  $\text{CH}_2\text{Cl}_2$ ) giving 6 subfractions E7.1-E7.6. Subfraction E7.4 (33 mg) was crystallized with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (9/1, v/v) to provide **5** (5.0 mg). Subfraction E7.5 (0.6 g) was subjected to CC on a Sephadex LH-20 (100% MeOH), giving 8 subfractions E7.5.1-E7.5.8. Subfraction E7.5.8 (26 mg) was crystallized with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (9/1, v/v) to provide **1** (3.6 mg). Fraction E8 (10.9 g) was subjected to CC on a Sephadex LH-20 (100% MeOH), giving 5 subfractions E8.1-E8.5. Subfraction E8.1 (66 mg) was separated on a Sephadex LH-20 column (100% MeOH) and followed by preparative TLC ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ , 95/5, v/v) to give **4** (5.5 mg). Subfraction E8.2 (4.17 g) was purified by CC on a Sephadex LH-20 (100% MeOH), followed by

recrystallization in CH<sub>2</sub>Cl<sub>2</sub>/MeOH (9/1, v/v) to provide **6** (3.9 mg). Subfraction E8.3 (1.06 g) was subjected to CC on a Sephadex LH-20 (100% MeOH), giving 7 subfractions E8.3.1-E8.3.7. Subfraction E8.3.1 (0.19 g) was separated by CC on *silica gel* using a mixture of CH<sub>2</sub>Cl<sub>2</sub>/MeOH (0% to 100% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to give 4 subfractions E8.3.1.1-E8.3.1.4. Subfraction E8.3.1.1 (20 mg) was further purified by preparative thin-layer chromatography (TLC) (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 95/5, v/v) to give **3** (3.7 mg) and **2** (6.1 mg).

**Amentoflavone (1)**: Yellow powder, ESI-MS: *m/z* 539 [M+H]<sup>+</sup>; <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta_{\text{H}}$  (ppm): 6.17 (1H, d, *J* = 2.0 Hz, H-6), 6.34 (1H, s, H-6''), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.55 (1H, s, H-3), 6.56 (1H, s, H-3''), 6.71 (2H, d, *J* = 8.5 Hz, H-3''', H-5'''), 7.08 (1H, d, *J* = 8.5 Hz, H-5'), 7.50 (2H, d, *J* = 8.5 Hz, H-2''', H-6'''), 7.83 (1H, dd, *J* = 2.0 Hz, 8.5 Hz, H-6'), 7.97 (1H, d, *J* = 2.0 Hz, H-2'). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD)  $\delta_{\text{C}}$  (ppm): 184.2 (C-4''), 183.8 (C-4), 166.2 (C-2), 165.9 (C-2''), 164.0 (C-7''), 163.1 (C-7), 163.1 (C-5), 162.5 (C-5''), 162.5 (C-4'''), 161.2 (C-4'), 159.3 (C-9), 156.5 (C-9''), 132.8 (C-6'), 129.3 (C-2'''), 129.3 (C-6'''), 128.8 (C-2'), 123.2 (C-1'), 123.1 (C-3'), 121.8 (C-1'''), 117.7 (C-5'), 115.8 (C-3'''), 115.8 (C-5'''), 105.6 (C-10''), 105.3 (C-10), 105.2 (C-8''), 103.9 (C-3), 103.3 (C-3'''), 100.3 (C-6''), 100.2 (C-6), 95.2 (C-8).

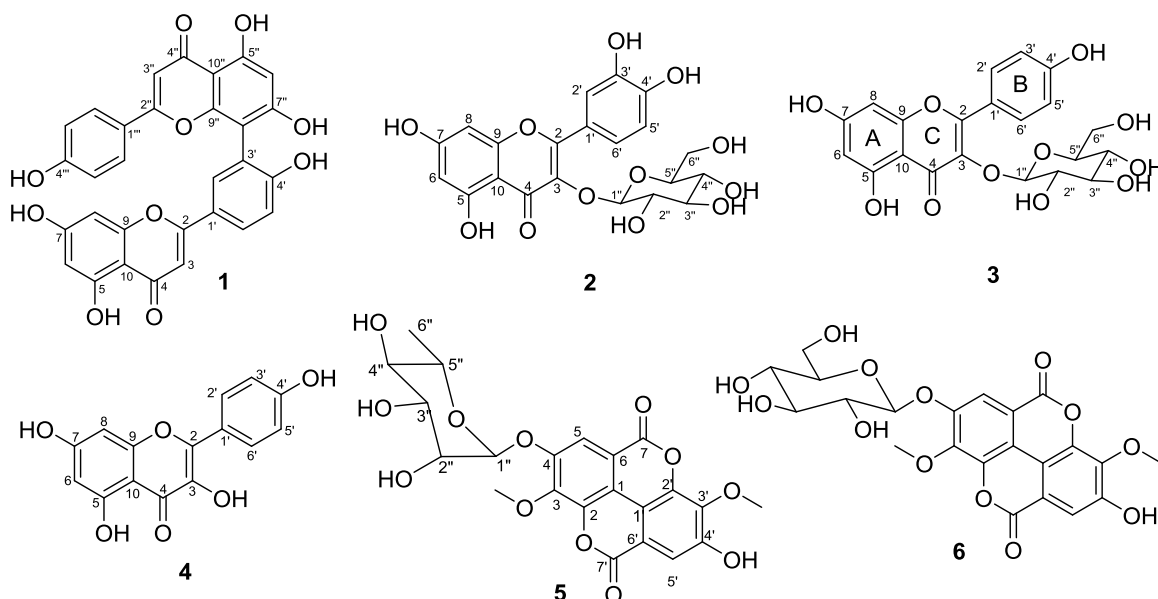
**Isoquercitrin (2)**: Yellow powder, <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta_{\text{H}}$  (ppm): 3.23 (1H, m, H-5''), 3.36 (1H, t, *J* = 9.5 Hz, H-3''), 3.44 (1H, t, *J* = 9.0 Hz, H-4''), 3.50 (1H, t, *J* = 9.0 Hz, H-2''), 3.60 (1H, dd, *J* = 5.5, 12.0 Hz, Hb-6''), 3.73 (1H, dd, *J* = 2.5, 12.0 Hz, Ha-6''), 5.26 (1H, d, *J* = 7.5 Hz, H-1''), 6.22 (1H, d, *J* = 2.0 Hz, H-6), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.89 (1H, d, *J* = 8.5 Hz, H-5'), 7.60 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 7.73 (1H, d, *J* = 2.0 Hz, H-2'). <sup>13</sup>C NMR (125 MHz,

CD<sub>3</sub>OD)  $\delta_{\text{C}}$  (ppm): 179.5 (C-4), 166.0 (C-7), 163.1 (C-5), 159.0 (C-9), 158.5 (C-2), 149.9 (C-4'), 145.9 (C-3'), 135.6 (C-3), 123.2 (C-6'), 123.1 (C-1'), 117.6 (C-5'), 116.0 (C-2'), 105.7 (C-10), 104.4 (C-1''), 99.9 (C-6), 94.7 (C-8), 78.4 (C-5''), 78.1 (C-3''), 75.7 (C-2''), 71.2 (C-4''), 62.6 (C-6'').

**Astragalin (3)**: Yellow powder, <sup>1</sup>H-NMR (500 MHz, CD<sub>3</sub>OD)  $\delta_{\text{H}}$  (ppm): 3.22 (1H, m, H-5''), 3.33 (1H, overlap, H-2''), 3.45 (2H, m, H-3'', H-4''), 3.55 (1H, dd, *J* = 5.5, 12.0 Hz, Hb-6''), 3.71 (1H, dd, *J* = 2.0, 12.0 Hz, Ha-6''), 5.21 (1H, d, *J* = 7.5 Hz, H-1''), 6.19 (1H, d, *J* = 1.5 Hz, H-6), 6.37 (1H, d, *J* = 1.5 Hz, H-8), 6.90 (2H, d, *J* = 8.5 Hz, H-5', H-3'), 8.07 (2H, d, *J* = 8.5 Hz, H-2', H-6').

**Kaempferol (4)**: White powder, <sup>1</sup>H-NMR: (500 MHz, CD<sub>3</sub>OD)  $\delta_{\text{H}}$  (ppm): 8.11 (2H, d, *J* = 8.0 Hz, H-2', 6'), 6.92 (2H, d, *J* = 8.0 Hz, H-3', H-5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6). <sup>13</sup>C-NMR (125 MHz, CD<sub>3</sub>OD)  $\delta_{\text{C}}$  (ppm): 174.4 (C-4), 165.6 (C-7), 162.5 (C-5), 160.6 (C-4'), 158.3 (C-9), 148.1 (C-2), 136.2 (C-3), 130.7 (C-2', C-6'), 123.8 (C-1'), 116.3 (C-3', C-5'), 104.6 (C-10), 99.3 (C-6), 94.5 (C-8).

**3,3'-O-Dimethyl ellagic acid-4-O- $\alpha$ -L-rhamnopyranoside (5)**: White powder; ESI-MS: *m/z* 475 [M-H]<sup>-</sup>; <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{H}}$  (ppm): 1.14 (3H, d, *J* = 6.0 Hz, H-6''), 3.35 (1H, m, H-4''), 3.52 (1H, m, H-5''), 3.72 (1H, m, H-3''), 3.96 (1H, m, H-2''), 4.05 (3H, s, OCH<sub>3</sub>-3'), 4.07 (3H, s, OCH<sub>3</sub>-3), 5.58 (1H, br s, H-1''), 7.52 (1H, s, H-5'), 7.78 (1H, s, H-5). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>, 125 MHz)  $\delta_{\text{C}}$  (ppm): 17.9 (CH<sub>3</sub>-6''), 60.9 (3'-OCH<sub>3</sub>), 61.6 (3-OCH<sub>3</sub>), 70.0 (C-2''), 70.3 (C-4''), 70.4 (C-5''), 71.5 (C-3''), 99.8 (C-1''), 110.9 (C-1'), 111.6 (C-5'), 111.7 (C-5), 111.9 (C-1), 112.6 (C-6'), 114.1 (C-6), 140.2 (C-3'), 141.0 (C-2), 141.5 (C-2'), 141.8 (C-3), 150.2 (C-4), 152.8 (C-4'), 158.2 (C-7'), 158.4 (C-7).



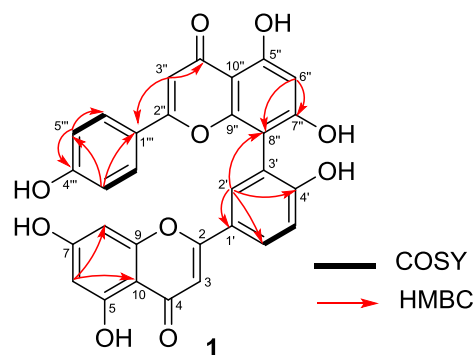
**Figure 1.** Structures of the isolated compounds 1-6

3,3'-*O*-Dimethylellagic acid 4-*O*- $\beta$ -D-glucopyranoside (**6**): Yellow powder, ESI-MS:  $m/z$  493 [M+H]<sup>+</sup>, <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_H$  (ppm): 3.28-3.53 (5H, overlap, Hb -6'', H-2'', H-3'', H-4'', H-5''), 3.70 (1H, br d,  $J$  = 12.0 Hz, Ha-6''), 4.05 (3H, s, OCH<sub>3</sub>-3'), 4.09 (3H, s, OCH<sub>3</sub>-3), 5.14 (1H, d,  $J$  = 7.5 Hz, H-1''), 7.54 (1H, s, H-5), 7.82 (1H, s, H-5').

### RESULTS AND DISCUSSION

Compound **1** was isolated as a yellow powder. The <sup>1</sup>H-NMR spectrum presented signals of an ABX system [ $\delta_H$  7.83 ( $J$  = 2.0, 8.5 Hz, H-6'), 7.08 ( $J$  = 8.5 Hz, H-5'), and 7.97 ( $J$  = 2.0 Hz, H-2')]; an A<sub>2</sub>B<sub>2</sub> system [ $\delta_H$  7.50 (2H, d,  $J$  = 8.5 Hz, H-2''', H-6'''), and 6.71 (2H, d,  $J$  = 8.5 Hz, H-3''', H-5''')], three singlets at  $\delta_H$  6.55 (H-3), 6.56 (H-3'') and 6.34 (H-6''), and two meta-coupling doublets at  $\delta_H$  6.17 ( $J$  = 2.0 Hz, H-6) and 6.39 ( $J$  = 2.0 Hz, H-8). The <sup>13</sup>C-NMR and DEPT spectra, with the aid of the HSQC spectrum revealed the presence of 30 carbon signals, including two carbonyl groups at  $\delta_C$  183.8 (C-4) and 184.2 (C-4''), twelve

aromatic methines and sixteen sp<sup>2</sup> quaternary carbons. This NMR data suggested that **1** was a biflavonoid. This was then confirmed by analyses of 2D NMR spectra, especially the HMBC spectrum. The linkage between the two flavone units was established by the HMBC cross-peaks of C-8'' with H-6'' ( $\delta_H$  6.34) and H-2' ( $\delta_H$  7.97) of the ABX system (Figure 2). Intensive analysis of the 2D-NMR spectra defined the structure of **1** as amentoflavone, which was previously reported [10, 11].



**Figure 2.** Key HMBC and COSY correlations of **1**

Compound **2** was isolated as a yellow powder. The  $^{13}\text{C}$ -NMR spectrum of compound **2** revealed 21 carbon signals, including 15 carbon signals characteristic of a flavonoid skeleton and six carbon signals of a  $\beta$ -D-glucopyranoside unit. The  $^1\text{H}$ -NMR spectrum of **2** exhibited signals of an anomeric proton at  $\delta_{\text{H}}$  5.26 (1H, d,  $J = 7.5$  Hz, H-1''), four oxymethines at  $\delta_{\text{H}}$  3.23-3.50, and one methylene at  $\delta_{\text{H}}$  3.73 (1H, dd,  $J = 2.5, 12.0$  Hz, H<sub>a</sub>-6''), 3.60 (1H, dd,  $J = 5.5, 12.0$  Hz, H<sub>b</sub>-6'') which were characteristic of a  $\beta$ -D-glucopyranoside. Additionally, the  $^1\text{H}$ -NMR spectrum of **2** showed the signals consistent with a quercetin unit at  $\delta_{\text{H}}$  6.41 (1H, d,  $J = 2.0$  Hz, H-8), 6.22 (1H, d,  $J = 2.0$  Hz, H-6), 7.73 (1H, d,  $J = 2.0$  Hz, H-2'), 7.60 (1H, dd,  $J = 2.0, 8.5$  Hz, H-6') and 6.89 (1H, d,  $J = 8.5$  Hz, H-5'). Through analyses of the 1D-NMR data of **2** and comparison with the reported data [12], compound **2** was identified as quercetin-3-*O*- $\beta$ -D-glucopyranoside, also known as isoquercitrin.

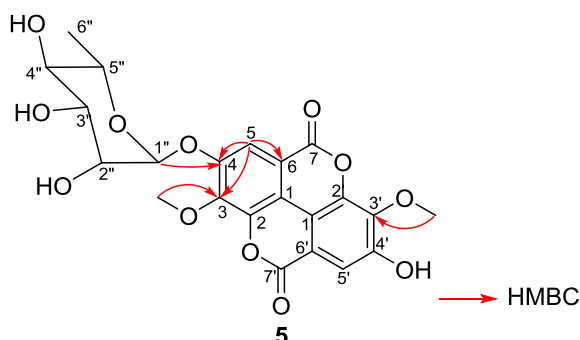
Compound **3** was isolated as yellow powder. The  $^1\text{H}$ -NMR spectrum of **3** revealed the proton signals of an A<sub>2</sub>B<sub>2</sub> aromatic ring system at  $\delta_{\text{H}}$  8.07 (2H, d,  $J = 8.5$  Hz, H-2', H-6'), 6.90 (2H, d,  $J = 8.5$  Hz, H-5', H-3'), and two meta-coupled aromatic protons at  $\delta_{\text{H}}$  6.38 (1H, d,  $J = 1.5$  Hz, H-8), 6.19 (1H, d,  $J = 1.5$  Hz, H-6). Additionally, the  $^1\text{H}$ -NMR spectrum of compound **3** exhibited the characteristic signals of a  $\beta$ -D-glucopyranosyl moiety, including an anomeric proton at  $\delta_{\text{H}}$  5.21 (1H, d,  $J = 7.5$  Hz, H-1''), four oxymethines at  $\delta_{\text{H}}$  3.22-3.45 ppm, and one methylene at  $\delta_{\text{H}}$  3.71 (1H, dd,  $J = 2.0, 12.0$  Hz, H<sub>a</sub>-6''), 3.55 (1H, dd,  $J = 5.5, 12.0$  Hz, H<sub>b</sub>-6''). These signals indicated the presence of a flavonol glycoside with kaempferol as the aglycone. The glycoside part was identified as  $\beta$ -D-glucopyranosyl

based on the anomeric proton signal in the NMR spectrum, which appeared as a doublet with a spin-coupling constant of  $J = 7.5$  Hz. Based on the above analyses and the comparison with literature data [13], the structure of compound **3** was determined to be kaempferol-3- $\beta$ -D-glucopyranoside, also known as astragalin.

Compound **4** was isolated as yellow powder. Comparison of the 1D NMR spectra of **4** with that of **3** revealed a structural similarity between the two compounds with the absence of a  $\beta$ -D-glucopyranoside unit in compound **4**. The  $^1\text{H}$ -NMR spectrum of **4** showed the signals consistent with a kaempferol unit at  $\delta_{\text{H}}$  6.42 (1H, d,  $J = 2.0$  Hz, H-8), 6.21 (1H, d,  $J = 2.0$  Hz, H-6), 8.11 (2H, d,  $J = 8.0$  Hz, H-2', H-6'), and 6.92 (2H, d,  $J = 8.0$  Hz, H-3', H-5'). The  $^{13}\text{C}$  NMR spectrum of compound **4** revealed 15 aromatic carbon signals including one carbonyl carbon signal at  $\delta_{\text{C}}$  174.4 (C-4), six aromatic methines and eight sp<sup>2</sup> quaternary carbons. Through analyses of the 1D NMR data of **4** and comparison with the reported data [14], compound **4** was identified as kaempferol.

Compound **5** was isolated as a white powder. Its ESI-MS showed the deprotonated molecule at  $m/z$  475 [M-H]<sup>-</sup>. The  $^1\text{H}$ -NMR spectrum of **5** exhibited the signals of two methoxy groups at  $\delta_{\text{H}}$  4.05 (3H, s, OCH<sub>3</sub>-3') and 4.07 (3H, s, OCH<sub>3</sub>-3), along with two singlet aromatic protons at  $\delta_{\text{H}}$  7.52 (1H, s, H-5'), and 7.78 (1H, s, H-5). Besides, the  $^1\text{H}$ -NMR spectrum of **5** also displayed the characteristic proton signals of an  $\alpha$ -rhamnopyranosyl moiety at  $\delta_{\text{H}}$  1.14 (3H, d,  $J = 6.0$  Hz, CH<sub>3</sub>-6''), 3.35 (1H, m, H-4''), 3.52 (1H, m, H-5''), 3.72 (1H, m, H-3''), 3.95 (1H, m, H-2'') and 5.56 (1H, br s, H-1''). Detailed analysis of  $^{13}\text{C}$ -NMR spectrum revealed the presence of 22 carbon signals, including two

carbonyl groups, two aromatic methines, two methoxy groups, one methyl group, five oxymethine groups and ten quaternary carbons. These NMR data analyses suggested that compound **5** was an ellagic acid derivative. The positions of the two methoxy groups located at C-3, and C-3' were revealed by the HMBC correlations of the carbons at C-3 ( $\delta_C$  141.8) and C-3' ( $\delta_C$  140.2) with the methoxy protons at  $\delta_H$  4.07, and 4.05, respectively. The rhamnopyranosyl moiety was attached to C-4 on the basis of the HMBC correlations from H-1'' ( $\delta_H$  5.56) to C-4 ( $\delta_C$  150.2) (Figure 3). Complete analysis of the 1D and 2D spectra of **5**, and comparison with the reported values indicated that compound **5** was 3,3'-di-O-methylellagic acid-4-O- $\alpha$ -L-rhamnopyranoside [15].



**Figure 3.** Key HMBC correlations of **5**

Compound **6** was isolated as a white powder. The  $^1\text{H-NMR}$  spectrum of **6** exhibited the signals of two methoxy groups at  $\delta_H$  4.05 (3H, s,  $\text{OCH}_3$ -3'), 4.09 (3H, s,  $\text{OCH}_3$ -3), two singlet aromatic protons at  $\delta_H$  7.54 (1H, s, H-5'), 7.82 (1H, s, H-5), one anomeric proton at 5.14 (1H, d,  $J = 7.5$  Hz, H-1'') and the remaining protons at  $\delta_H$  3.28 – 3.70 ppm. The  $^1\text{H-NMR}$  data of **6** were almost similar to those of **5** but differed in the sugar moiety. The data of five methines and

one methylene and the coupling constants of the sugar protons indicated the presence of a  $\beta$ -D-glucopyranosyl group in the structure of compound **6**. Based on the above evidence and by comparison with those reported in the reference [16], compound **6** was determined to be 3,3'-O-dimethylellagic acid 4-O- $\beta$ -D-glucopyranoside.

Except for kaempferol (**4**), all isolated compounds are reported here for the first time from the *Cleistanthus* genus. Their classification as flavonoids and ellagic acid derivatives aligns with the known chemotaxonomic profile of the genus.

## CONCLUSION

Investigation of the chemical constituents of the EtOAc extract from the leaves of *C. eberhardtii* resulted in the isolation and structural elucidation of four flavonoids (**1-4**) and two ellagic acid derivatives (**5-6**) on the basis of detailed analyses of their 1D and 2D NMR spectroscopic data and in comparison, with the literature values. Significantly, this is the first report of these compounds from *C. eberhardtii*, and this report represents the first phytochemical investigation from *C. eberhardtii* leaves. To further expand our understanding of the chemical and pharmacological properties of this species, future research should focus on analyzing the remaining leaf extracts for additional phytochemical constituents and evaluating their biological activities.

## ACKNOWLEDGMENTS

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## CONFLICTS OF INTEREST

None.

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