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Flavonoids from the stem bark of *Bauhinia semibifida* L.

Mulyadi Tanjung and Tjitjik Sri Tjahjandarie

Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

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ABSTRACT

*Two flavonoids, 6C-7O-dimethylaromadendrin **(1)**, and phlorizin (**2**) have been isolated from the stem bark of Bauhinia semibifida. The structures of both compounds have been elucidated based on UV, IR, HRESIMS, 1D and 2D NMR data. Compounds **1**–**2** were evaluated for their cytotoxic properties against P-388 cells, their IC_{50} values 3.98, and 25. 20 µg/mL, respectively.*

Keywords: Flavonoid, 6C-7O-dimethylaromadendrin, phlorizin, Bauhinia semibifida, Cytotoxic.

INTRODUCTION

Bauhinia is a large genus of Fabaceae family consisting of about 300 species and distributed in the tropical and subtropical region. The phytochemical studies of *Bauhinia* has known that this plants producing flavonoids [1,2], and stilbenoids [3,4,5]. This study is part of our research on the chemical constituents of *Bauhinia* species found in the Indonesia. In continuation of our research for phenolic compound in this medicinal plant, we report the isolation of flavonoids, 6C-7*O*-dimethylaromadendrin (**1**), and phlorizin (**2**) from the methanol extract of the stem bark of *Bauhinia semibifida*. This species has not been reported about phytochemical data. The cytotoxic activity against murine leukemia P-388 cells of the isolated compounds **1**–**2** are also briefly described.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were recorded on a JEOL ECA 400 spectrometer in DMSO *d6* at 400 (^{1}H) and 100 (^{13}C) MHz using TMS as the internal standard. The mass spectra was recorded using a Waters LCT Premier XE. UV and IR spectra were measured with a Shimadzu 1800 and Perkin Elmer Spectrum One FTIR spectrometer, respectively. Vacuum liquid chromatography (VLC) and radial chromatography were carried out using Si gel 60 GF_{254} and Si gel 60 PF_{254}, for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF_{254}, 0,25 mm thickness) were used. Solvents used for extraction and preparative chromatography were of technical grade and distilled before use.

Plant material

The stem bark of *B. semibifida* were collected from Bangkirai Hill, Samarinda, East Kalimantan, Indonesia. The species were identitified at the Herbarium Bogorienses, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia and a voucher specimen had been deposited at the Herbarium Bogorienses.

Extraction and isolation

The air-dried of stem bark of *B. semibifida* (2.5 kg) were macerated with MeOH two times at room temperature, and then concentrated under reduced pressure. The residue was suspended in water and partitioned sequentially with *n*-hexane (85 g) and EtOAc (105 g). The EtOAc extract was fractionated on silica gel by VLC eluting with mixtures *n*-hexane-EtOAc (9:1, 4:1, 7:3, and 1:1) to give two major fractions A-B. Fraction B (2.0 g), purified using radial chromatography eluted with chloroform and mixture chloroform-metanol (9:1, and 4:1) to give compounds **1** (4 mg) and **2** (40 mg).

6C-7*O*-dimethylaromadendrin (**1**), pale yellow solid, spectrum UV (MeOH) λ_{maks} nm (log ε): 213 (4.18), 293 (4.34) and 331sh; (MeOH+NaOH) 214 (4.24), 302 (4.39), 336 sh (4.19); (MeOH+$AlCl_3$) 214 (4.15), 316 (4.48), 417 sh (3.73); ($AlCl_3$+HCl) 211 (4.19), 316 (4.49), 410 sh (3.77). Spectrum IR (KBr) ν_{maks}: 3421 (OH), 2958, 2921 CH alkyl), 1639 (conj. C=O), 1588, 1490 (C=C aromatic), and 1261 (C-O-C ether) cm^{-1}. HR-ESI-MS *m/z* 217.1016 $[M+H]^+$ (calcd for $C_{17}H_{17}O_6$: 217.1025). ^{1}H NMR (400 MHz, DMSO *d6*) δ_H (ppm): 11.78 (1H, s, 5-OH), 9.52 (1H, s, 4'-OH), 7.28 (2H, d, *J* = 8.6 Hz, H-2'/6'), 6.74 (2H, d, *J* = 8.6 Hz, H-3'/5'), 6.17 (1H, s, H-8), 5.04 (1H, d, *J* = 11,6 Hz), 4.59 (1H, d, *J* = 11,6 Hz), 3.77 (s, 7-OCH_3), and 1.86 (s, 6-CH_3); ^{13}C NMR (100 MHz, DMSO *d6*) δ_c (ppm): 83.6 (C-2), 72.1 (C-3), 199.3 (C-4), 101.4 (C-4a), 159.7 (C-5), 104.8 (C-6), 165.8 (C-7), 91.7 (C-8), 161.4 (C-8a), 128.0 (C-1'), 130.2 (C-2'/6'), 115.4 (C-3'/5'), 158.3 (C-4'), 56.7 (7-OCH_3), and 7.5 (6-CH_3).

Phlorizin (**2**), white solid, spectrum UV (MeOH) λ_{maks} nm (log ε) : 215 (4.43), 260 (4.40), 299 (4.49); (MeOH+NaOH) 225 (4.42), 264 (4.42), and 324 (4.65); ($AlCl_3$+HCl) 216 (4.40), 262 (4.41), 307 (4.58), 355 sh (3.93); ($AlCl_3$+HCl) 214 (4.39), 264 (4.41), and 301 (4.49). IR (KBr) ν_{maks} (cm^{-1}): 3393 (OH), 2928, 2921 (CH alkyl), 1627 (conj. C=O), and 1518, 1375 C=C aromatic). HR-ESI-MS *m/z* 435.1293 $[M-H]^-$ (calcd for $C_{21}H_{23}O_{10}$: 435.1291). ^{1}H NMR (400 MHz, DMSO *d6*) δ_H (ppm): 6.99 (2H, d, *J* = 7.5 Hz, H-2/6), 6.59 (2H, d, *J* = 7.5 Hz, H-3/5), 6.08 (1H, d, *J* = 2,0 Hz, H-3'), 5.88 (1H, d, *J* = 2,0 Hz, H-5'), 4.89 (1H, d, *J* = 6.9 Hz, H-1''), 3.66 (1H, d, *J* = 11.6 Hz, H-6''$_{ax}$), 3.46 (1H, dd, *J* = 11.6; 4.6 Hz, H-6''$_{eq}$), 3.32 (4H, m, H-2''/3''/4''/5''), 3.25 (2H, t, *J* = 7.2 Hz, H_α), and 2.74 (2H, t, *J* = 7.2 Hz, H_β); ^{13}C NMR (100 MHz, DMSO *d6*) δ_c (ppm): 132.0 (C-1), 129.5 (C-2/6), 115.5 (C-3/5), 155.8 (C-4), 45.5 (C-α), 29.5 (C-β), 205.2 (C=O), 105.5 (C-1'), 161.4 (C-2'), 94.9 (C-3'), 165.2 (C-4'), 93.3 (C-5'), 166.0 (C-6'), 101.3 (C-1''), 73.7 (C-2''), 77.8 (C-3''), 69.9 (C-4''), 77.2 (C-5''), and 61.1 (C-6'').

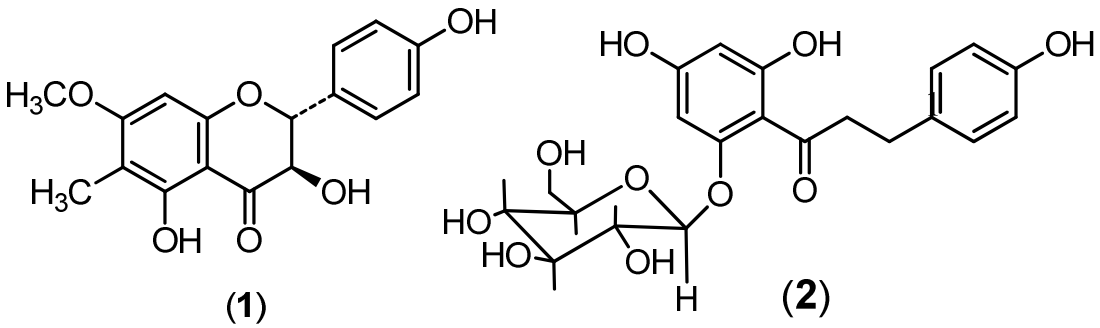

Fig. 1. Structures of flavonoid isolated

Cytotoxicity assay: Cytotoxic properties of the isolated compounds **1**–**2** against murine leukemia P-388 cells were evaluated according to the method of MTT assay as described previously [6,7]. The cytotoxicity assay was performed against murine leukemia P-388cells were grown in RPMI 1640 medium containing 10% fetal bovine serum, 2 mg mL^{-1} sodium carbonate, 100 μg mL^{-1} penicillin sodium salt, and 100 μg mL^{-1} penicillin streptomycin sulfate. The cells were harvested at the log phase of growth, and then seeded into 96-well plates (1×10^4 cells/well). After 24 h incubation at 37 ºC and 5% CO_2 to allow cell attachment, the cultures were exposed to the test compounds **1-5** were dissolved in DMSO at various concentrations and incubated for 48 h followed by MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay at 540 nm.

RESULTS AND DISCUSSION

Two flavonoids, namely 6C-7O-dimethylaromadendrin (**1**) and phlorizin (**2**) have been isolated from the stem bark of *Bauhinia semibifida*. Neither 6C-7O-dimethylaromadendrin (**1**) or phlorizin (**2**) were first time found in the genus *Bauhinia*.

6C-7O-dimethylaromadendrin (**1**) was isolated as an pale yellow solid, and its UV spectrum exhibited absorption maxima (213, 293, and 331sh nm) typical for a dihydroflavonol [8]. The IR spectrum indicated absorptions for hydroxyl (3393 cm^{-1}), conjugated carbonyl (1639 cm^{-1}), and aromatic (1588, 1490 cm^{-1}) groups. The HRESIMS spectrum showed a quasimolecular ion $[M+H]^+$ at *m/z* 217.1016 consistent to the molecular formula $C_{21}H_{23}O_{10}$, suggesting that **1** is a dihydroflavonol derivative containing an methoxyl and a methyl groups. In the ^1H NMR spectrum, the presence of two proton signals at δ_H 5.04, and 4.59 with multiplicities doublets (J = 11.6 Hz), respectively, confirmed for the dihydroflavonol skeleton in **1** [8]. The ^1H NMR spectrum of **1** also showed a proton singlet methoxyl signal at δ_H 3.77, and a methyl group (δ_H 1.86) and a proton singlet signal at δ_H 11.78 that is consistent with an OH-phenolic at C-5. Further analysis of the ^1H NMR spectrum in the aromatic region revealed the presence of a pair of doublets (J = 8.6 Hz) of two-proton signals (δ_H 7.28 and 6.74) and a singlet of one-proton signal (δ_H 6.17), suggesting that the methoxyl and methyl groups at ring A. This was substantiated by the presence of a conjugated carbonyl group (δ_C 199.3) and two methines of oxycarbons (δ_C 83.6 and 72.1). The presence of four signals of oxyaryl carbons (δ_C 165.8, 161.4, 159.7, and 158.3) suggested that 1 has the basic sructure of aromadendrin (= 5,7, 4'-trihydroxydihydroflavonol). [8]. By analysis of HMQC and HMBC spectra of **1**, the 5-OH phenolic signal (δ_H 11.78) exhibited ^1H-^{13}C long range correlations with the signals of three aromatic quarternary (δ_C 101.4, C-4a; 159.7, C-5; 104.8 C-6) carbon atoms, and correlation methyl proton singlet δ_H 1.86 with three quaternary carbon signal with the signals of two oxyaril at δ_C 159.7 (C-5), and 165.8 (C-7) and one quarternary carbon atom at δ_C 104.8 (C-6) consequently these correlations correspond to the methyl group at C-6. Furthermore, correlation methoxyl signal δ_H 3.77 with a oxyaril at δ_C 165.8 suggested that the methoxyl was unambiguously located at C-7. From these NMR data analysis, the dihydroflavonol isolated was assigned as 6C-7O-dimethyl-3,5,7,4'tetrahidroxyflavanone or known 6C-7O-dimethylaromadendrin [9]. Other HMQC and HMBC correlations, as well as ^{13}C NMR data assignment, that are consistent with the structure **1** are shown in Fig. 2. The absolute stereochemistry at C-2/C-3 was determined as shown in the structure **1**, based on the coupling constant (J = 11.6 Hz, *trans*) between H-2/H-3 [8].

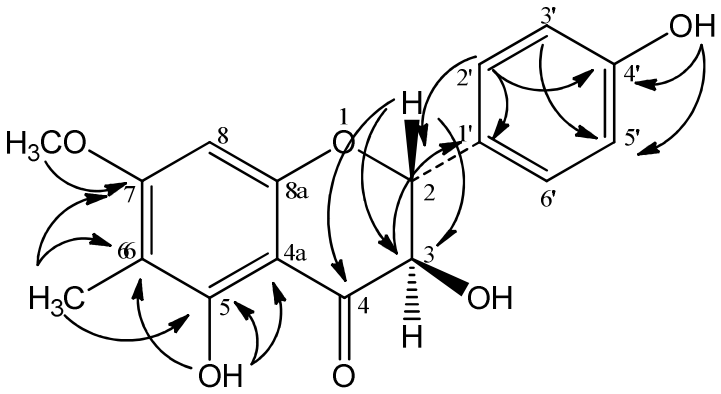

Fig. 2. Significant HMBC correlation for 1

Phlorizin (**2**) was isolated as an white solid, and its UV spectrum exhibited absorption maxima (215, 260, and 299 sh nm typical for a dihydrochalcone [10]. The IR spectrum indicated absorptions for hydroxyl (3393 cm^{-1}), conjugated carbonyl (1627 cm^{-1}), and aromatic (1518, 1375 cm^{-1}) groups. The HRESIMS spectrum showed a quasimolecular ion $[M-H]^-$ at *m/z* 435.1293 consistent to the molecular formula $C_{21}H_{23}O_{10}$, suggesting that **2** is a dihydrochalcone derivative containing an glucose group. In the ^1H NMR spectrum of **2** showed a pair of triplets (J = 7.2 Hz) of two-proton signals (δ_H 3.25 and 2.74) characteristic for H-α, and H-β a typical ABX system for a dihydrochalcone structure [10]. The presence of the proton signals of a pair of doublets (J = 2.0 Hz) in the aromatic region at δ_H 6.08 and 5.88 ppm, characteristic for H-3' and H-5' proton signals of the ring A. Furthermore, in the ^1H NMR spectrum, the appearance of a pair of doublets (J = 7.5 Hz) in the aromatic region at δ_H 6.99 and 6.59 ppm characteristic for a hydroxyl phenyl group of the ring B. Further analysis of the ^1H spectrum in the glucose region

revealed the presence of proton at δ_H 3.66 and 3.46 ppm, suggesting that the C anomeric from glucose skeleton. The placement of glucose skeleton in dihydrochalcone structure shown in HMQC and HMBC spectra. By analysis of HMBC spectra of **2**, the C-anomeric signal of glucose (δ_H 3.66 and 3.46) exhibited ^{1}H-^{13}C long range correlations with the signals of a oxyaril at δ_C 161.4 (C-2'), and correlations between δ_H 6.08 with two oxyaril signals at δ_C (161.2 (C-2'), and 165.2 (C-4'), one quaternary carbon signal at δ_C 105.5 (C-1'), and one methine carbon signal at δ_C 93.3 (C-5') suggested that glucose at 2'-*O*-glucose. From these NMR data analysis, the dihydrochalcone isolated was assigned as 2'-*O*-D-glucose-4,4',6'trihidroxydihydrochalcone or known phlorizin [11]. Other HMQC and HMBC correlations, as well as ^{13}C NMR data assignment, that are consistent with the structure **2** are shown in Fig. 3.

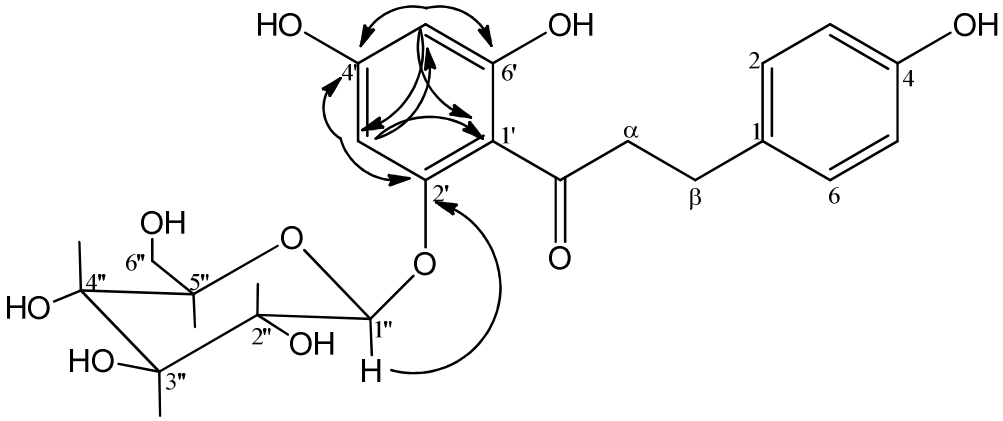

Fig.3. Significant HMBC correlation for 2

The results of cytotoxic activity of 6C-7O-dimethylaromadendrin (**1**) and phlorizin (**2**) against murine leukemia cells P-388 showed that showing their IC_{50} values were 3.98, and 25. 20 µg/mL, respectively. The results indicate that compounds 6C-7O-dimethylaromadendrin (**1**) showed moderate activity and phlorizin (**2**) was inactive [12].

CONCLUSION

Two flavonoids, 6C-7*O*-dimethylaromadendrin **(1)**, and phlorizin (**2**) were isolated from the stem bark of *Bauhinia semibifida*, a species has not been researched. The structures of both compounds were elucidated by extensive UV, IR, HRESIMS, 1D and 2D NMR data. 6C-7*O*-dimethylaromadendrin **(1)**, and phlorizin (**2**) were evaluated for their cytotoxic properties against P-388 cells, their IC_{50} values 3.98, and 25. 20 µg/mL, respectively.

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