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CHEMICAL CONSTITUENTS OF *BAUHINIA BRACTEATA* BENTH. LEAVES AND THEIR ANTIOXIDANT ACTIVITY

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Summary

Bauhinia bracteata Benth. (Fabaceae), commonly known as "Móng bò leo" in Vietnam, is a traditional medicinal plant widely used in several Asian countries, particularly in Vietnam, Myanmar, Cambodia, and Thailand. Six compounds were isolated and identified as quercetin (**1**), quercetin-3-*O*-*α*-L-arabinofuranoside (**2**), quercetin-3-*O*-*α*-L-rhamnopyranoside (**3**), 4'-*O*-methylmyricetin-3-*O*-*α*-L-rhamnopyranoside (**4**), 3-(*Z*)-hexenyl-1-*β*-D-glucopyranoside (**5**), and 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (**6**). Their structures were elucidated based on chromatographic techniques and spectroscopic analyses, including HR-ESI-MS and 1D and 2D NMR. Quercetin (**1**) exhibited the most potent antioxidant activities in scavenging DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, with IC_{50} values of 10.16 (95% CI: 8.74–11.85) and 6.36 (95% CI: 5.73–7.05) µM, respectively.

Keywords: *Bauhinia bracteata* Benth.; *Quercetin; 4'-O-methylmyricetin-3-O-α-L-rhamnopyranoside; Antioxidant.*

1. Introduction

Bauhinia bracteata Benth. (Fabaceae) is widely distributed throughout Vietnam, from the northern to the southern regions, and is also found in Myanmar, Cambodia, and Thailand. In Vietnam, the fresh leaves are traditionally used in folk medicine as a decoction for the treatment of diabetes. The bark is durable and used for tying ropes and braiding. In addition, a decoction of the stems is used to treat diarrhea [1]. In Thailand, the vines of *B. bracteata* are traditionally used in folk medicine for the treatment of skin irritation and as a remedy for rashes [2]. Phytochemical and pharmacological studies have demonstrated that *Bauhinia* species possess a wide range of biological activities, such as antimicrobial, antioxidant, nephroprotective, anticancer, hepatoprotective, antidiabetic, anti-inflammatory, and antidepressant effects both *in vitro* and *in vivo.* These activities are mainly attributed to the presence of flavonoids, steroidal saponins, bauhinoxepins, chromanones, and other phenolic compounds [3].

However, studies on the chemical constituents and biological activities of the leaves of *B. bracteata* in Vietnam remain limited. In the study by Udomputtimekakul et al., the chemical constituents of *B. bracteata* leaves were investigated. Three compounds, including friedelin, farresol, and isoliquiritigenin, were isolated [2]. Previous phytochemical studies on *B. bracteata* leaves have led to the isolation of several compounds, including ethyl gallate, gallic acid, hypericin, and myricitrin [4]. This study was conducted to identify the bioactive constituents responsible for antioxidant activity.

2. Materials and Methods

2.1. Plant material

The leaves of *Bauhinia bracteata* Benth. were collected from Ea Kiet Commune, Dak Lak Province, Vietnam, in July 2025. The plant material was taxonomically authenticated by MSc. Dao Viet Quoc, Center for Medicinal Resources, National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam. A voucher specimen (No. NIMM-0029306) has been deposited at the same institution.

2.2. General experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded in DMSO-d_6 on a Bruker AVANCE NEO 600 FT-NMR spectrometer (Bruker, Germany) using tetramethylsilane (TMS) as an internal standard. The high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) spectra were recorded using an AGILENT 6530 Q-TOF mass spectrometer (Agilent, USA). Column chromatography (CC) was performed using *silica gel* (63–200 µm particle size, Merck, Germany), YMC RP-18 *silica gel* (ODS-A, 12 nm S–150 µm particle size, Japan), and Sephadex LH-20 (GE Healthcare, Sweden). Thin-layer chromatography (TLC) was performed on *silica gel* 60 F_{254} plates (Merck, Germany) and RP-18 $F_{254}S$ plates (Merck,

Germany). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in ethanol, followed by heating.

2.3. Extraction and isolation

The dried leaves of *B. bracteata* (10.0 kg) were extracted with 96% ethanol (3 × 30 L) at room temperature for three days. The combined extracts were concentrated under reduced pressure using a rotary evaporator to yield a crude extract. The residue (2.0 kg) was suspended in water and further successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate. The ethyl acetate fraction (159.1 g) was subjected to *silica gel* CC using a stepwise gradient elution with CH_2Cl_2–MeOH (100:0–1:1, v/v) to give 10 sub-fractions (E1–E10) according to their TLC profiles. Sub-fraction E3 (6.1 g) was subjected to RP-18 CC and eluted with a gradient solvent system of MeOH–H_2O (1:1–15:1, v/v) to afford five sub-fractions (E3.1–E3.5). Sub-fraction E3.4 (300 mg) was purified by Sephadex LH-20 CC with elution solvent MeOH–H_2O (2:1–10:1, v/v) to yield compounds **1** (105.5 mg) and **6** (12.5 mg), respectively. Chromatography of sub-fraction E5 (7.2 g) on an RP-18 CC using a gradient solvent system of MeOH – H_2O (3:1–10:1, v/v) yielded five sub-fractions (E5.1–E5.5). Sub-fraction E5.3 (500 mg) was also subjected to a *silica gel* CC and eluted with a gradient solvent system of CH_2Cl_2 – MeOH (15:1–0:1, v/v) to give compounds **2** (50 mg) and **5** (30 mg), respectively. Sub-fraction E6 (5.7 g) was subjected to a *silica gel* CC and eluted with a gradient solvent system of CH_2Cl_2–MeOH (20:1–0:1, v/v) to afford five sub-fractions (E6.1–E6.5). Sub-fraction E6.5 (900 mg) was further subjected to the RP-18 CC and eluted with a gradient solvent system of MeOH–H_2O (2:1–15:1, v/v) to afford compounds **3** (15 mg) and **4** (20 mg), respectively.

Quercetin (1): Yellow powder, HR-ESI-MS *m/z* 303.0501 $[M+H]^+$ (calcd. for $C_{15}H_{11}O_7^+$, *m/z* 303.0505). ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 7.69 (1H, d, *J* = 2.4 Hz, H-2'), 7.55 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 6.90 (1H, d, *J* = 8.4 Hz, H-5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 175.9 (C-4), 163.9 (C-7), 160.8 (C-5), 156.2 (C-9), 147.7 (C-4'), 146.8 (C-2), 145.1 (C-3'), 135.8 (C-3), 122.0 (C-1'), 120.0 (C-6'), 115.6 (C-5'), 115.1 (C-2'), 103.0 (C-10), 98.2 (C-6), 93.4 (C-8).

Quercetin-3-*O*-*α*-L-arabinofuranoside (2): Yellow powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 7.56 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 7.49 (1H, d, *J* = 2.4 Hz, H-2'), 6.87 (1H, d, *J* = 8.4 Hz, H-5'), 6.42 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.59 (1H, d, *J* = 0.6 Hz, H-1"), 4.17 (1H, s, H-2"), 3.73 (1H, m, H-3"), 3.58 (1H, m, H-4"), 3.34 (1H, m, H-5" a), 3.30 (1H, m, H-5"b). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 177.8 (C-4), 164.3 (C-7), 161.3 (C-5), 157.0 (C-2), 156.4 (C-9), 148.5 (C-4'), 145.1 (C-3'), 133.4 (C-3), 121.8 (C-6'), 121.0 (C-1'), 115.6 (C-2'), 115.6 (C-5'), 107.9 (C-1"), 104.0 (C-10), 98.7 (C-6), 93.6 (C-8), 85.9 (C-4"), 82.2 (C-2"), 77.0 (C-3"), 60.7 (C-5").

Quercetin-3-*O*-*α*-L-rhamnopyranoside (3): Yellow powder. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 7.32 (1H, d, *J* = 2.4 Hz, H-2'), 7.27 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.39 (1H, d, *J* = 1.8 Hz, H-8), 6.21 (1H, d, *J* = 1.8 Hz, H-6), 5.29 (1H, d, *J* = 1.8 Hz, H-1"), 4.01 (1H, dd, *J* = 1.8, 3.6 Hz, H-2"), 3.54 (1H, dd, *J* = 3.6, 9.6 Hz, H-3"), 3.24 (1H, m, H-5"), 3.18 (1H, t, *J* = 9.6 Hz, H-4"), 0.84 (3H, d, *J* = 6.0 Hz, H-6"). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 177.9 (C-4), 164.4 (C-7), 161.5 (C-5), 156.6 (C-2), 157.5 (C-9), 148.6 (C-4'), 145.3 (C-3'), 134.4 (C-3), 121.3 (C-6'), 121.0 (C-1'), 115.9 (C-2'), 115.7 (C-5'), 104.3 (C-10), 102.0 (C-1"), 98.9 (C-6), 93.8 (C-8), 71.4 (C-4"), 70.7 (C-5"), 70.6 (C-3"), 70.2 (C-2"), 17.6 (C-6").

4'-*O*-Methylmyricetin-3-*O*-*α*-L-rhamnopyranoside (4): Yellow powder, HR-ESI-MS *m/z* 479.1194 $[M+H]^+$ (calcd. for $C_{22}H_{23}O_{12}^+$, *m/z* 479.1190). ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 6.81 (2H, s, H-2', H-6'), 6.28 (1H, d, *J* = 1.8 Hz, H-8), 6.13 (1H, d, *J* = 1.8 Hz, H-6), 5.15 (1H, d, *J* = 1.2 Hz, H-1"), 3.98 (1H, dd, *J* = 1.2, 3.0 Hz, H-2"), 3.74 (3H, s, 4'-OCH_3), 3.52 (1H, dd, *J* = 3.0, 9.0 Hz, H-3"), 3.18 (1H, m, H-5"), 3.15 (1H, m, H-4"), 0.82 (3H, d, *J* = 5.4 Hz, H-6"). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 177.5 (C-4), 166.9 (C-7), 161.3 (C-5), 156.8 (C-2), 156.8 (C-9), 150.7 (C-3', C-5'), 137.7 (C-4'), 134.7 (C-3), 125.0 (C-1'), 108.1 (C-2', C-6'), 103.3 (C-10), 102.2 (C-1"), 99.4 (C-6), 93.9 (C-8), 71.2 (C-4"), 70.5 (C-5"), 70.3 (C-3"), 70.1 (C-2"), 59.8 (4'-OCH_3), 17.5 (C-6").

3-(*Z*)-Hexenyl-1-*β*-D-glucopyranoside (5): Colorless gum. ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 5.41 (1H, m, H-4), 5.35 (1H, m, H-3), 4.13 (1H, d, *J* = 7.8 Hz, H-1'), 3.73 (1H, m, H-1a), 3.43 (1H, m, H-1b), 3.66 (1H, m, H-6'a), 3.43 (1H, m, H-6'b), 3.10 (1H, m, H-3'), 3.07 (1H, m, H-5'), 3.04 (1H, m, H-4'), 2.94 (1H, m, H-2'),

2.28 (2H, q, J = 7.2, 14.4 Hz, H-2), 2.02 (2H, q, J = 7.2, 15.0 Hz, H-5), 0.93 (3H, t, J = 7.2 Hz, H-6). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 132.8 (C-4), 125.1 (C-3), 102.7 (C-1'), 76.8 (C-3'), 76.7 (C-5'), 73.4 (C-2'), 70.1 (C-4'), 68.1 (C-1), 61.1 (C-6'), 27.4 (C-2), 20.0 (C-5), 14.0 (C-6).

2-(4-Hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (6): Amorphous powder. HR-ESI-MS *m/z* 331.1397 $[M+H]^+$ (calcd. for $C_{15}H_{23}O_8^+$, *m/z* 331.1393). ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H (ppm): 6.84 (1H, d, J = 1.8 Hz, H-2), 6.66 (1H, d, J = 8.0 Hz, H-5), 6.62 (1H, dd, J = 1.8, 8.0 Hz, H-6), 4.18 (1H, d, J = 7.8 Hz, H-1'), 3.90 (1H, m, H-8a), 3.60 (1H, m, H-8b), 3.74 (3H, s, 3-OCH_3), 3.67 (1H, m, H-6'a), 3.43 (1H, m, H-6'b), 3.14 (1H, m, H-3'), 3.09 (1H, m, H-5'), 3.04 (1H, m, H-4'), 2.96 (1H, m, H-2'), 2.74 (2H, m, H-7). ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C (ppm): 147.9 (C-3), 145.3 (C-4), 130.0 (C-1), 121.5 (C-6), 115.7 (C-5), 113.8 (C-2), 103.3 (C-1'), 77.4 (C-3'), 77.3 (C-5'), 74.0 (C-2'), 70.7 (C-4'), 70.3 (C-8), 61.7 (C-6'), 56.1 (3-OCH_3), 35.6 (C-7).

2.4. Antioxidant assay

2.4.1. DPPH assay

The capacity of six compounds to remove DPPH$^\bullet$ radical was determined according to a previously described procedure with slight modifications [5]. Various concentration samples (20 μL) in methanol were added to 180 μL of a 0.1 mM DPPH (Sigma-Aldrich, St. Louis, MO) in methanol. The mixture was incubated in the dark for 30 min at room temperature after vortex mixing, followed by measurement at 517 nm. Inhibition rate (I%) on DPPH$^\bullet$ radical was calculated in following formulation: I% = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$. Quercetin standard was used for comparison.

2.4.2. ABTS assay

The radical scavenging activity of six compounds for ABTS$^{\bullet+}$ radical was determined by a previously described procedure [5]. ABTS radical cation (ABTS$^{\bullet+}$) solution was produced by reacting ABTS (Sigma-Aldrich, St. Louis, MO) stock solution with 2.45 mM potassium persulfate in the dark for 16 h and adjusting the absorbance to 0.70 ± 0.02 at 734 nm. For the photometric assay, 180 μL of the ABTS$^{\bullet+}$ solution and 20 μL of the sample solution were mixed and measured immediately after 10 min at 734 nm. Inhibition rate (I%) on ABTS$^{\bullet+}$ radical was calculated by the following equation: I% = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$. Vitamin C standard was used for comparison.

3. Results and Discussion

Compound **1** was isolated as a yellow powder. The molecular formula of **1** was found to be $C_{15}H_{10}O_7$, based on the positive HR-ESI–MS ion at *m/z* 303.0501 ($[M+H]^+$, calcd. for 303.0505). The ^{1}H NMR spectrum of **1** showed a characteristic hydroxyl proton at δ_H 12.49 (1H, s, 5-OH), two *meta*-coupled aromatic protons at δ_H 6.42 (1H, d, J = 2.0 Hz, H-8) and 6.20 (1H, d, J = 2.0 Hz, H-6) attributable to the A-ring, and an ABX aromatic system at δ_H 7.69 (1H, d, J = 2.4 Hz, H-2'), 7.55 (1H, dd, J = 2.4, 8.4 Hz, H-6'), and 6.90 (1H, d, J = 8.4 Hz, H-5') corresponding to the B-ring. The ^{13}C NMR spectrum of **1** revealed fifteen carbon signals, including six carbons for the A-ring, six carbon signals for the B-ring, and three carbon signals for the C-ring of the flavonol. The structure of **1** was elucidated by detailed analysis of ^{1}H and ^{13}C NMR data in combination with 2D NMR experiments. Therefore, the structure of compound **1** was elucidated as quercetin, in agreement with previously reported data [6],[7].

Compound **2** was obtained as a yellow powder. Comparison of the ^{1}H and ^{13}C NMR data of compound **2** with those of **1** revealed that compound **2** was the glycosylated derivative of **1**. The ^{1}H NMR of compound **2** displayed an ABX aromatic system at δ_H 7.56 (1H, dd, J = 2.4, 8.4 Hz, H-6'), 7.49 (1H, d, J = 2.4 Hz, H-2') and 6.87 (1H, d, J = 8.4 Hz, H-5'). Additionally, an AX spin system was observed at δ_H 6.42 (1H, d, J = 2.0 Hz, H-8) and 6.21 (1H, d, J = 2.0 Hz, H-6). The coupling constant of anomeric proton (J = 0.6 Hz) indicated the *α*-configuration of arabinofuranosyl moiety [δ_H 5.59 (1H, d, J = 0.6 Hz, H-1"), 4.17 (1H, s, H-2"), 3.73 (1H, m, H-3"), 3.58 (1H, m, H-4"), 3.34 (1H, m, H-5"a), and 3.30 (1H, m, H-5"b)]. The ^{13}C NMR spectrum of **2** showed one carbonyl carbon signal at δ_C 177.8 (C-4) and nineteen carbon signals from δ_C 60.7 to 164.3, confirming quercetin as the aglycone and the arabinofuranosyl unit. In the HMBC spectrum, the correlation from H-1" (δ_H 5.59) to C-3 (δ_C 133.4) suggested that an arabinofuranosyl moiety was attached at C-3 (δ_C 133.4). Based on the data analysis and comparison with the reported values, compound **2** was identified as quercetin-3-*O*-*α*-L-arabinofuranoside [7],[8].

Compound **3** was obtained as a yellow powder. In the ^{1}H NMR spectrum of **3**, the presence of a hydroxyl group at δ_H 12.65 (1H, s, 5-OH), two doublets at δ_H 6.39 (1H, d, J = 1.8 Hz, H-8), 6.21

(1H, d, J = 1.8 Hz, H-6) on the A-ring, an aromatic ABX spin system at δ_H 7.32 (1H, d, J = 2.4 Hz, H-2'), 7.27 (1H, dd, J = 2.4, 8.4 Hz, H-6'), and 6.89 (1H, d, J = 8.4 Hz, H-5') on the B-ring. The signals for an anomeric proton at δ_H 5.29 (1H, d, J = 1.8 Hz, H-1") and one doublet signal at δ_H 0.84 (3H, d, J = 6.0 Hz, H-6"), indicated the presence of a rhamnose moiety in compound **3**. Based on the coupling constant of H-1"/H-2", the sugar anomeric configuration supported an α-configuration. The ^{13}C NMR spectrum of compound **3** showed twenty-one carbon resonances, including a carbonyl signal at δ_C 177.9 (C-4) and twenty carbon signals from δ_C 17.6 to 164.4. The HMBC correlation of H-1" (δ_H 5.29) to C-3 (δ_C 134.4) demonstrated that a rhamnose moiety was attached at C-3. Based on the NMR data and comparison with the references, compound **3** was determined as quercetin-3-*O*-α-L-rhamnopyranoside [6].

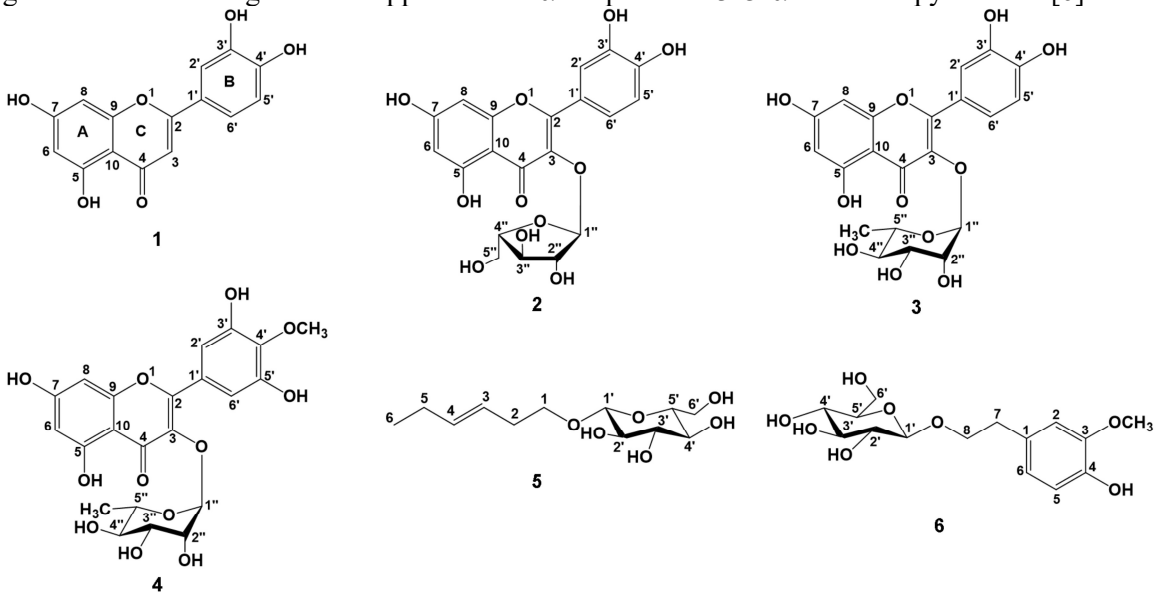


Fig. 1. Chemical structure of compounds **1–6** isolated from the leaves of *B. bracteata*

Compound **4** was obtained as a yellow powder. Its HR-ESI-MS spectrum exhibited a quasi-molecular ion peak at *m/z* 479.1194 $[M+H]^+$ (calcd. for 479.1190), corresponding to the molecular formula $C_{22}H_{22}O_{12}$. The ^{1}H NMR spectrum showed two *meta*-coupled protons at δ_H 6.28 (1H, d, J = 1.8 Hz, H-8) and 6.13 (1H, d, J = 1.8 Hz, H-6), along with a singlet at δ_H 6.81 (2H, s) integrating for two protons (H-2' and H-6'), indicating a symmetric B-ring characteristic of a myricetin-type skeleton. A methoxy signal at δ_H 3.74 (3H, s, -OCH_3) was also observed. Signals corresponding to an α-L-rhamnopyranosyl moiety were evident from the anomeric proton at δ_H 5.15 (1H, d, J = 1.2 Hz, H-1") and a methyl doublet at δ_H 0.82 (3H, d, J = 5.4 Hz, H-6"). The ^{13}C NMR spectrum further supported the structure, showing a carbonyl carbon at δ_H 177.5 and twenty-one carbon signals. The HMBC correlation of H-1" (δ_H 5.15) to C-3 (δ_C 134.7) demonstrated that a rhamnose moiety was attached at C-3. Further correlation was obtained from the methoxy signal of 4'-OCH_3 (δ_H 3.74) to C-4' (δ_C 137.7), and the methoxy group was deduced to be attached to C-4'. Based on these data, compound **4** was identified as 4'-*O*-methylmyricetin-3-*O*-α-L-rhamnopyranoside [9],[10].

Compound **5** was obtained as a colorless gum. The ^{1}H NMR spectrum of **5** showed the presence of a methyl group at δ_H 0.93 (3H, t, J = 7.2 Hz, H-6), two olefinic proton signals at δ_H 5.41 (1H, m, H-4) and 5.35 (1H, m, H-3), a signal at δ_H 3.73 (1H, m, H-1a), and two overlapped signals at δ_H 3.43 (2H, m, H-1b, H-6'b) indicative of methylene protons geminal with an oxygen atom. The signals at δ_H 2.28 (2H, q, J = 7.2, 14.4 Hz, H-2) and 2.02 (2H, q, J = 7.2, 15.0 Hz, H-5) showed the presence of two additional methylenes. The coupling constant of the anomeric proton (J = 7.8 Hz) indicated the β-configuration of glucose moiety [δ_H 4.13 (1H, d, J = 7.8 Hz, H-1'), 3.66 (1H, m, H-6'a), 3.43 (1H, m, H-6'b), 3.10 (1H, m,

H-3'), 3.07 (1H, m, H-5'), 3.04 (1H, m, H-4'), and 2.94 (1H, m, H-2')]. In the ^{13}C NMR spectrum, the signals for a *β*-D-glucopyranoside moiety were observed at δ_C 102.7 (C-1'), 76.8 (C-3'), 76.7 (C-5'), 73.4 (C-2'), 70.1 (C-4'), and 61.1 (C-6'). The HSQC, HMBC, and ^1H-^1H COSY correlations of **5** located the position of the double bond at the 3/4 position, while the absence of NOESY correlations between the signals at δ_H 5.41 (H-4) and 5.35 (H-3). Furthermore, the values of C-2 (δ_C 27.4) and C-5 (δ_C 20.0) are characteristic of the 3-(*Z*)-hexenyl moiety. Therefore, based on the analysis of the obtained data and comparison with the literature data, compound **5** was identified as 3-(*Z*)-hexenyl-1-*β*-D-glucopyranoside [11].

Compound **6** was isolated as an amorphous powder. The molecular formula of **6** was found to be $C_{15}H_{22}O_8$, based on HR-ESI-MS peak at *m/z* 331.1397 ($[M+H]^+$, calcd. for 331.1393). The ^1H NMR of compound **6** displayed signal for an ABX-type system at δ_H 6.84 (1H, d, *J* = 1.8 Hz, H-2), 6.66 (1H, d, *J* = 8.0 Hz, H-5), and 6.62 (1H, dd, *J* = 1.8, 8.0 Hz, H-6), the signals of an oxymethylene group at δ_H 3.90 (1H, m, H-8a) and 3.60 (1H, m, H-8b), a methylene proton at δ_H 2.74 (2H, m, H-7), and a methoxy group at δ_H 3.74 (3H, s, 3-OCH_3). The coupling constant of anomeric proton (*J* = 7.8 Hz) indicated the *β*-configuration of glucose moiety [δ_H 4.18 (1H, d, *J* = 7.8 Hz, H-1'), 3.67 (1H, m, H-6'a), 3.43 (1H, m, H-6'b), 3.14 (1H, m, H-3'), 3.09 (1H, m, H-5'), 3.04 (1H, m, H-4'), and 2.96 (1H, m, H-2')]. The ^{13}C NMR spectrum showed six carbon signals of an ABX spin system corresponding to a 1,3,4-trisubstituted aromatic ring at δ_C 147.9 (C-3), 145.3 (C-4), 130.0 (C-1), 121.5 (C-6), 115.7 (C-5), and 113.8 (C-2), six carbons of an *β*-glucose at δ_C 103.3 (C-1'), 77.4 (C-3'), 77.3 (C-5'), 74.0 (C-2'), 70.7 (C-4'), and 61.7 (C-6'). Besides, the signals of an oxymethylene carbon at δ_C 70.3 (C-8), a methylene carbon at δ_C 35.6 (C-7), and a methoxy group at δ_C 56.1 (3-OCH_3) were observed. The HMBC correlation of H-1' (δ_H 4.18) to C-8 (δ_C 70.3) demonstrated that a glucose moiety was attached at C-8. Further HMBC correlation from the methoxy protons (3-OCH_3, δ_H 3.74) to C-3 (δ_C 147.9) indicated that the methoxy group is attached to C-3. Based on the above spectroscopic evidence, the chemical structure of compound **6** was established as 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside [12],[13].

Table 1. Antioxidant activities of compounds from *B. bracteata* leaves

Compound	IC_{50} (μM)	
	$DPPH^{\bullet}$	$ABTS^{\bullet+}$
1	10.16 (8.74–11.85)	6.36 (5.73–7.05)
2	10.87 (9.24–12.79)	16.80 (14.77–19.17)
3	11.14 (9.74–12.77)	23.02 (21.10–25.21)
4	9.37 (8.30–10.67)	13.13 (11.32–15.35)
5	>50	>50
6	>50	>50
Quercetin[a]	13.61 (12.12–15.26)	–
Vitamin C[a]	–	8.23 (7.49–9.14)

IC_{50} was obtained with a confidence interval (n=3); C.I.: 95% confidence interval.

[a]positive control, (–) not tested.

The fractions included *n*-hexane, dichloromethane, ethyl acetate, and water. Among them, antioxidant activity testing revealed that the ethyl acetate fraction exhibited the strongest activity when evaluated on thin-layer chromatography plates. Consequently, the ethyl acetate fraction was selected for the isolation of compounds with potent antioxidant properties. The isolated compounds (**1**–**6**) were evaluated for their antioxidant activity (Table 1). Compounds **4**, **1**, **2**, and **3** exhibited potent activity against $DPPH^{\bullet}$ free radical with IC_{50} values of 9.37 (95% CI: 8.30–10.67), 10.16 (95% CI: 8.74–11.85), 10.87 (95% CI: 9.24–12.79), and 11.14 (95% CI: 9.74–12.77) μM, respectively. Quercetin was used as a positive control with an IC_{50} value of

13.61 (95% CI: 12.12–15.26) μM. Compound **1** exhibited potent activity against the $ABTS^{\bullet+}$ free radical with an IC_{50} value of 6.36 (95% CI: 5.73–7.05) μM. Compounds **4**, **2**, and **3** showed significant activity against the $ABTS^{\bullet+}$ free radical with IC_{50} values of 13.13 (95% CI: 11.32–15.35), 16.80 (95% CI: 14.77–19.17), and 23.02 (95% CI: 21.10–25.21) μM, respectively. Vitamin C was used as a positive control with an IC_{50} value of 8.23 (95% CI: 7.49–9.14) μM.

The ethyl acetate fraction contains flavonoids possessing a 3-OH phenolic group, a C2–C3 double bond, and a ketone group, which establish a conjugated system with both A and B rings. This structural configuration enables electron transfer to free radicals, resulting in the formation of stable radical intermediates. Furthermore, the C2–C3 double bond is suggested to enhance molecular planarity and increase the rigidity of the C ring. This structural constraint maintains the coplanarity of the A and C rings, bringing the 3-OH and 5-OH groups into closer proximity and thereby strengthening the interaction between the flavone skeleton and metal ions [14]. Based on the relationship between flavonoid chemical structures and their biological activities, the isolated flavonoid compounds exhibit potent scavenging effects against both DPPH and ABTS free radicals. These findings enrich the chemical diversity of the genus *Bauhinia* and suggest that *B. bracteata* is a promising source of bioactive flavonoid derivatives.

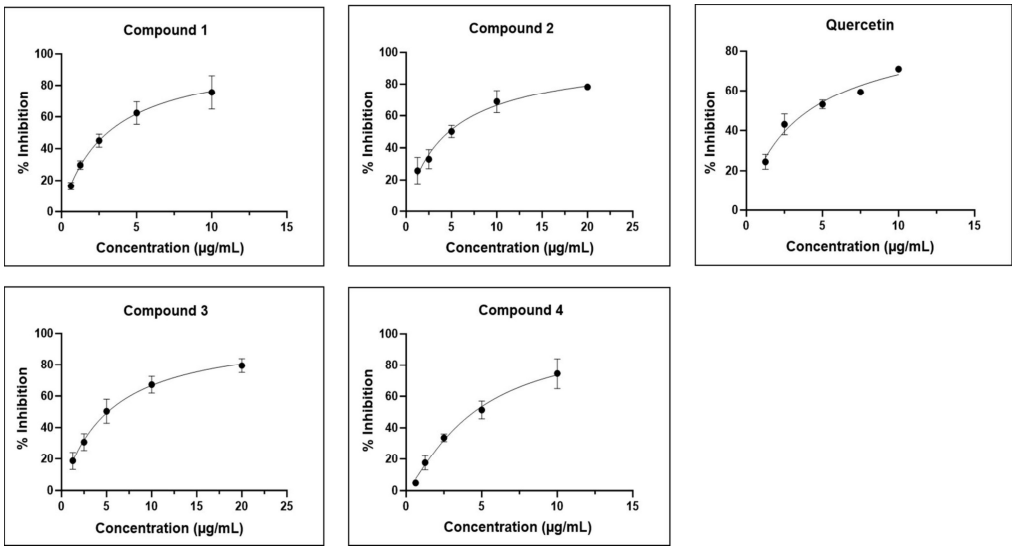


Fig. 2. DPPH radical scavenging activity (%) vs. concentration of the compounds and quercetin

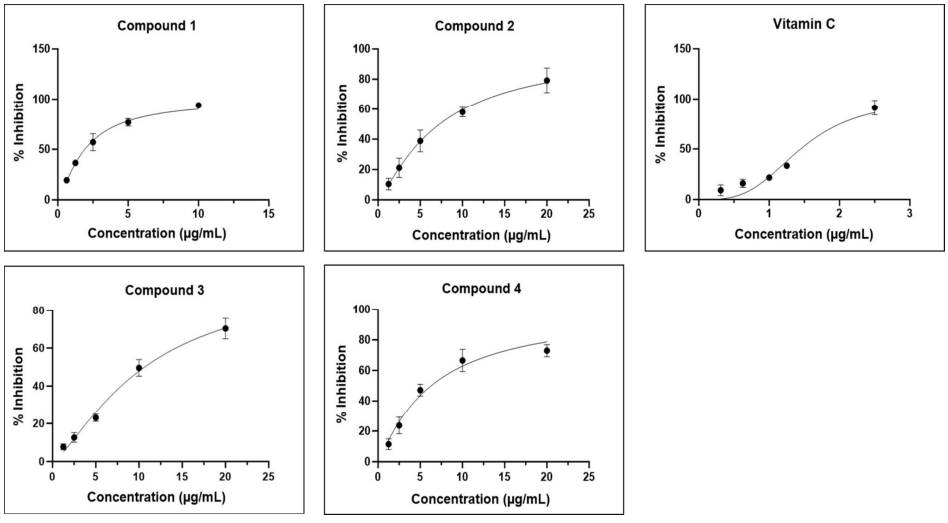


Fig. 3. ABTS radical scavenging activity (%) vs. concentration of the compounds and vitamin C

4. Conclusion

Six compounds including quercetin (**1**), quercetin-3-*O*-*α*-L-arabinofuranoside (**2**), quercetin-3-*O*-*α*-L-rhamnopyranoside (**3**), 4'-O-methylmyricetin-3-*O*-*α*-L-rhamnopyranoside (**4**), 3-(*Z*)-hexenyl-1-*β*-D-glucopyranoside (**5**), and 2-(4-hydroxy-3-methoxyphenyl)ethyl-*O*-*β*-D-glucopyranoside (**6**) were isolated from *B. bracteata* leaves. Compounds (**1**–**6**) were separated for the first time in this plant. Compounds **4**, **1**, **2**, and **3** exhibited potent activity against DPPH$^{\bullet}$ free radical with IC_{50} values of 9.37 (95% CI: 8.30–10.67), 10.16 (95% CI: 8.74–11.85), 10.87 (95% CI: 9.24–12.79), and 11.14 (95% CI: 9.74–12.77) μM, respectively. Compound **1** exhibited potent activity against the $ABTS^{\bullet+}$ free radical with an IC_{50} value of 6.36 (95% CI: 5.73–7.05) μM.

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