

CHEMICAL CONSTITUENTS FROM METHANOLIC EXTRACT OF *GARCINIA MACKEANIANA* LEAVES AND THEIR ANTIOXIDANT ACTIVITY

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Abstract. The phytochemical investigation of the methanolic extract of *Garcinia mackeaniana* Craib. leaves led to the isolation and determination of five known compounds, including one benzophenone 4,3',4'-trihydroxy-2,6-dimethoxybenzophenone (**1**), two flavone C-glucosides, vitexin (**2**) and 2''-O-acetylvitexin (**3**), one biflavone, amentoflavone (**4**), and one phenolic compound, methyl protocatechuate (**5**). The chemical structures of compounds **1-5** were characterized by the NMR-spectral methods. All isolated compounds were separated from *G. mackeaniana* for the first time. Benzophenone derivative **1** has shown the IC₅₀ value of 14.97 ± 0.8 µg/mL in the DPPH-antioxidant examination.

Keywords: *Garcinia mackeaniana*, leaves, phytochemistry, antioxidant activity.

Classification numbers: 1.1.1, 1.2.1.

1. INTRODUCTION

Genus *Garcinia* is a large genus of the flowering plants which belong to the family Clusiaceae. Plants of this genus with about 450 species are now native to Asia, Australia, America, and Southern Africa [1]. *Garcinia* species have always contributed valuable properties as traditional medicines to the food chemistry and pharmacology. For instance, the decoction of *G. cambogia* fruit rind was employed for rheumatism treatment, and bowel complaints [2]. The fruit of mangosteen (*G. mangostana*) is now well-known in food chemistry because of its distinctive and pleasant taste [3]. Polyisoprenylated benzophenone derived from some *Garcinia* species, namely garcinol, was recommended for the antioxidant therapeutic targets [4]. Phytochemical investigations of *Garcinia* plants indicated that xanthenes, flavonoids, and benzophenones were major components [1]. Among about 30 *Garcinia* species distributed in

Viet Nam, *G. mackeaniana* was selected for phytochemical investigation and antioxidant examination [5]. As part of phytochemical investigation [6], we now describe the isolation, structural elucidation of five known compounds from *G. mackeaniana* and their DPPH-radical quenching activity.

2. MATERIALS AND METHODS

2.1. General experimental procedures

ESI-MS spectra were recorded on Thermo Scientific LTQ Orbitrap XL spectrometer (USA). NMR spectra were obtained from Bruker 500 MHz spectrometer (125 MHz for ^{13}C and at 500 MHz for ^1H). Silica gel (40 - 63 μm), Sephadex LH-20 (25 - 100 μm), and RP-18 (150 μm , Kyoto-Japan) were applied for column chromatography (CC), while silica gel 60 F₂₅₄ (Merck) was used for TLC analysis. Compounds were detected by UV lamp (254 and 365 nm), and by spraying plates with indicators (10 % H_2SO_4 and vanillin).

2.2. Plant materials

The leaves of *Garcinia mackeaniana* Craib. were collected in Son La, Viet Nam in January 2018, and were identified by Dr. Nguyen Quoc Binh, Institute of Ecology and Biological Resources. A voucher specimen (VN-1641) was deposited in Department of Applied Biochemistry, Institute of Chemistry.

2.3. Extraction and isolation

The dried leaves powder of *G. mackeaniana* (1.3 kg) was immersed with MeOH (10 L \times 3 times) for 1 h at 50 $^\circ\text{C}$. The MeOH solution was then concentrated under decreased pressure to give a crude MeOH residue (89.1 g). This part was chromatographed on a silica gel column (10 \times 50 cm, 182.0 g), eluting with a stepwise gradient of *n*-hexane- CH_2Cl_2 (1:1 \rightarrow 0:1, v/v) and CH_2Cl_2 -MeOH (9:1 \rightarrow 0:1, v/v), to afford 15 fractions (MF.1-MF.15). Fraction MF.9 (0.9 g) was subjected to silica gel CC [CH_2Cl_2 -EtOAc (3:1, v/v)], to afford 4 fractions (MF.91-MF.94). Fraction Fr.91 (40 mg) was continued to separate by a RP-18 column [MeOH- H_2O (1:1, v/v)], to give compound **1** (8.0 mg). Compound **4** (7.0 mg) was separated from fraction MF.92 (0.3 g) by washing with MeOH. Fraction MF.11 (0.5 g) was further chromatographed on a silica gel column [CH_2Cl_2 - CH_3COCH_3 (1:1, v/v)], to give compounds **5** (2.5 mg) and **3** (15.0 mg). Finally, fraction MF.12 (0.7 g) was re-chromatographed on a silica gel column [CH_2Cl_2 -MeOH (30:1, v/v)], to obtain compound **2** (15.0 mg).

4,3',4'-Trihydroxy-2,6-dimethoxybenzophenone (1): Yellow amorphous powders; ESI-MS: m/z 291 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{15}\text{H}_{15}\text{O}_6$, 291); ^1H -NMR (500 MHz, CD_3OD , δ_{H} ppm): 6.17 (2H, s, H-3, H-5), 7.27 (1H, d, 2.5 Hz, H-2'), 7.17 (1H, dd, 2.5, 8.0 Hz, H-6'), 6.77 (1H, d, 8.0 Hz, H-5'), and 3.67 (6H, s, 2-OCH₃, 6-OCH₃); ^{13}C -NMR (125 MHz, CD_3OD , δ_{C} ppm): 197.0 (CO), 161.8 (C-4), 160.1 (C-2, C-6), 152.4 (C-4'), 146.2 (C-3'), 132.2 (C-1'), 124.7 (C-6'), 117.3 (C-2'), 115.7 (C-5'), 111.1 (C-1), 92.9 (C-3, C-5), and 56.11 (2-OCH₃, 6-OCH₃).

Vitexin (2): Yellow amorphous powders; ESI-MS: m/z 433 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{21}\text{O}_{10}$, 433); ^1H -NMR (DMSO- d_6 , 500 MHz, δ_{H} ppm): 13.14 (1H, s, 5-OH), 8.00 (2H, brd, 8.5 Hz, H-2', H-6'), 6.90 (2H, d, 8.5 Hz, H-3', H-5'), 6.74 (1H, s, H-3), 6.23 (1H, s, H-6), 4.72 (1H, d, 9.5 Hz, H-1''), 3.85 (1H, t, 9.0 Hz, H-2''), 3.76 (1H, brd, 11.0 Hz, H_a-6''), 3.52 (1H, dd, 6.0, 12.0 Hz, H_b-6''), and 3.25-3.36 (2H, m, H-4'', H-5''); ^{13}C -NMR (DMSO- d_6 , 125 MHz, δ_{C} ppm): 181.9 (C-4),

163.8 (C-2), 162.8 (C-7), 161.2 (C-4'), 160.4 (C-5), 156.0 (C-8a), 129.0 (C-2', C-6'), 121.6 (C-1'), 115.8 (C-3', C-5'), 104.6 (C-4a, C-8), 102.4 (C-3), 98.4 (C-6), 81.8 (C-5''), 73.5 (C-1''), 70.9 (C-2''), 78.7 (C-3''), 70.6 (C-4''), and 61.3 (C-6'').

2''-O-Acetylvitexin (3): Yellow amorphous powders; ESI-MS: m/z 475 [M+H]⁺ (calcd for C₂₃H₂₃O₁₁, 475); ¹H-NMR (500 MHz, CD₃OD, δ_H ppm): 8.05 (2H, d, 8.5 Hz, H-2', H-6'), 6.98 (1H, d, 8.5 Hz, H-3', H-5'), 6.62 (1H, s, H-3), 6.24 (1H, s, H-6), 5.56 (1H, m, H-2''), 5.10 (1H, d, 10.0 Hz, H-1''), 4.02 (1H, dd, 2.0, 12.0 Hz, H_a-6''), 3.83 (1H, dd, 5.5, 12.0 Hz, H_b-6''), 3.72 (1H, m, H-3''), 3.52 (1H, m, H-5''), and 1.80 (3H, s, CH₃CO); ¹³C-NMR (CD₃OD, 125 MHz, δ_C ppm): 184.1 (C-4), 172.0 (CH₃CO), 166.7 (C-2), 164.1 (C-7), 163.0 (C-5), 162.7 (C-4'), 158.6 (C-8a), 130.1 (C-2', C-6'), 123.7 (C-1'), 117.0 (C-3', C-5'), 105.7 (C-4a), 103.7 (C-3, C-8), 99.1 (C-6), 83.1 (C-5''), 77.8 (C-3''), 74.1 (C-2''), 73.0 (C-1''), 72.3 (C-4''), 63.0 (C-6''), and 20.5 (CH₃CO).

Amentoflavone (4): Yellow amorphous powders; ESI-MS: m/z 539 [M+H]⁺ (calcd for C₃₀H₁₉O₁₀, 539); ¹H-NMR (DMSO-*d*₆, 500 MHz, δ_H ppm): 13.10 (1H, s, 5''-OH), 12.98 (1H, s, 5-OH), 8.02 (1H, d, 2.5 Hz, H-2'), 7.99 (1H, d, 2.5, 9.0 Hz, H-6'), 7.58 (2H, d, 8.5 Hz, H-2''', H-6'''), 7.12 (1H, d, 9.0 Hz, H-5'), 6.82 (1H, s, H-3), 6.77 (1H, s, H-3''), 6.70 (2H, d, 8.5 Hz, H-3''', H-5'''), 6.44 (1H, d, 2.0 Hz, H-8), 6.37 (1H, s, H-6''), and 6.18 (1H, d, 2.0 Hz, H-6); ¹³C-NMR (DMSO-*d*₆, 125 MHz, δ_C ppm): 182.1 (C-4''), 181.8 (C-4), 164.2 (C-7), 164.0 (C-2), 163.7 (C-2''), 162.9 (C-7''), 161.5 (C-5), 161.0 (C-4'''), 160.6 (C-5'''), 160.1 (C-4'), 157.4 (C-8a), 154.6 (C-8''a), 131.5 (C-2'), 128.2 (C-2''', C-6'''), 127.7 (C-6'), 121.5 (C-1'''), 120.7 (C-1'), 120.4 (C-3'), 116.5 (C-5'), 115.8 (C-3''', C-5'''), 104.3 (C-8''), 103.8 (C-4a), 103.5 (C-4''a), 102.9 (C-3), 102.6 (C-3''), 99.0 (C-6''), 98.9 (C-6), and 94.1 (C-8).

Methyl protocatechuate (5): Yellow amorphous powder; ESI-MS: m/z 169 [M+H]⁺ (calcd for C₈H₉O₄, 169); ¹H-NMR (CD₃OD, 500 MHz, δ_H ppm): 7.43 (1H, dd, 2.0, 8.0 Hz, H-6), 7.42 (1H, d, 2.0 Hz, H-2), 6.82 (1H, d, 8.0 Hz, H-5), and 3.85 (3H, s, OCH₃); ¹³C-NMR (CD₃OD, 125 MHz, δ_C ppm): 167.5 (CO), 150.0 (C-4), 144.8 (C-3), 122.2 (C-1), 121.2 (C-6), 116.0 (C-5), 114.5 (C-2), and 50.8 (OCH₃).

2.4. DPPH-antioxidant assay

Free radical quenching assay of the isolated compounds **1-5** has been carried out by 1,1-diphenyl-2-picryl hydrazyl (DPPH) [7-9]. Briefly, DPPH (0.1 mM) was diluted in methanol. 200 μ L of this solution was added to 1.3 μ L of various concentrations of **1-5** in DMSO (128.0, 32.0, 8.0, and 2.0 μ g/mL). The mixture was performed by a 96-well plate at 25 °C in 30 min. Then, absorbance was determined by Biotek tool (at 517 nm). The percentage of DPPH quenching activity was computed by the following formula:

$$\text{Inhibitory percentage SC (\%)} = [(A_0 - A_1)/A_0] \times 100.$$

where A₀ was defined as the absorbance of control reaction, and A₁ represented for the absorbance in the presence of test or standard sample.

Each experiment was repeated three times, while resveratrol was used as a reference compound. The EC₅₀ value, also known as the concentration of tested samples that induced half maximal response has been calculated from linear regression of the serial SC values versus the concentrations by using Table Curve 2Dv4.

3. RESULTS AND DISCUSSION

3.1. The NMR-structural elucidation

Compound **1** was separated as yellow amorphous powders. The ^1H , and ^{13}C -NMR spectral data of **1** revealed a pattern of a benzophenone derivative. In detail, the ^1H -NMR spectrum was composed of two superimposed singlet proton signals H-3 and H-5 (δ_{H} 6.17), one ABX spin system of δ_{H} 6.77, d, 8.0 Hz, 7.17, dd, 2.5, 8.0 Hz, and 7.27, d, 2.5 Hz, and two superimposed singlet methoxy groups at δ_{H} 3.67. It suggested that the chemical structure **1** included a symmetrically 1,2,4,6-tetrasubstituted phenyl unit, and another 1,3,4-trisubstituted phenyl unit. The ^{13}C -NMR data contained two methoxy groups at δ_{C} 56.11, five aromatic methines at δ_{C} 92.9-124.7 ppm, six aromatic carbons at δ_{C} 146.2-160.1 ppm, and a carbonyl group at δ_{C} 197.0. The structure of **1** was supported by 2D-NMR evidence, in which the key HMBC correlations H-3 (δ_{H} 6.17)/C-1 (δ_{C} 111.1), C-2 (δ_{C} 160.1), and C-4 (δ_{C} 161.8), H-5 (δ_{H} 6.17)/C-1, C-4, and C-6 (δ_{C} 160.1), 2-OCH₃/C-2, 6-OCH₃/C-6 confirmed the appearance of 2,6-dimethoxy-4-hydroxyphenyl unit. Similarly, the remaining 1,3,4-trisubstituted phenyl moiety was highlighted with the key HMBC cross-peaks H-5'/C-1', and C-3', H-2' and H-6'/C-4'. The key HMBC correlations H-2' and H-6'/CO implied that two phenyl units were connected through the carbonyl group. From these findings and comparing with literature [10], compound **1** was determined to be 4,3',4'-trihydroxy-2,6-dimethoxybenzophenone.

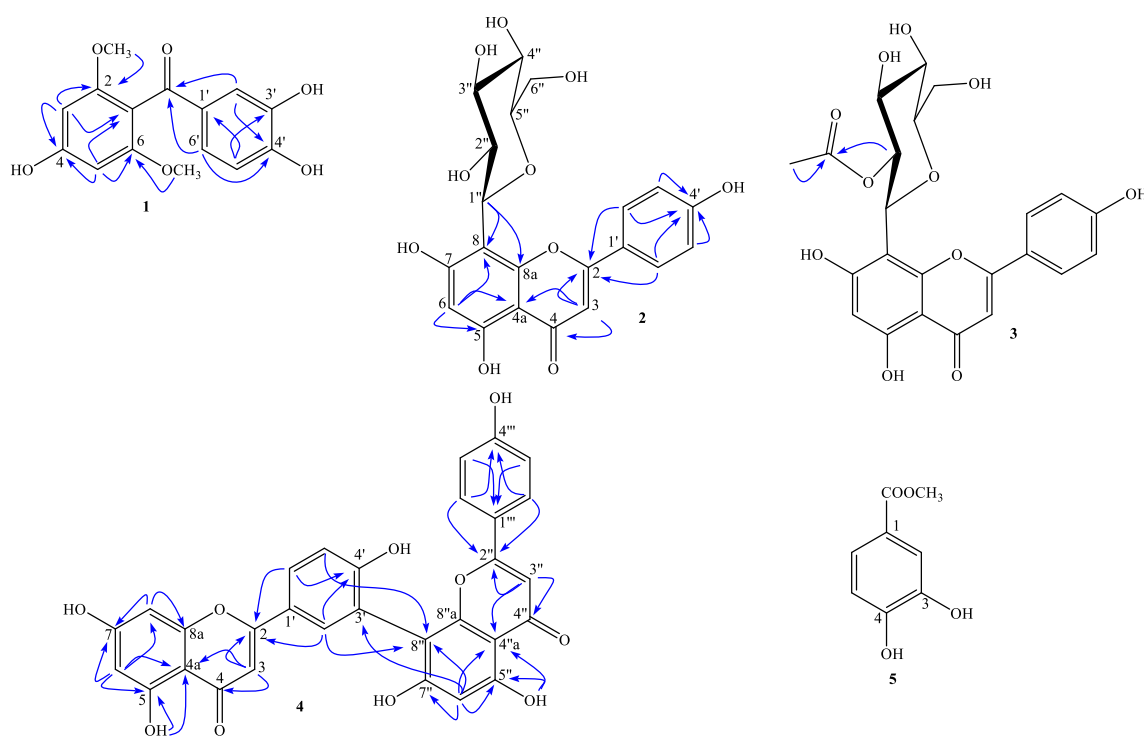


Figure 1. Isolated compounds from *G. mackeaniana* leaves and their key HMBC correlations.

Compound **2** was isolated as yellow amorphous powders. The positive ESI-MS spectrum showed the proton adduct ion at m/z 433 $[\text{M}+\text{H}]^+$, which alongside the ^{13}C -NMR data were consistent with the molecular formula of $\text{C}_{21}\text{H}_{20}\text{O}_{10}$. The ^1H -NMR spectral data of **2** were characteristic of a flavone C-glycoside, in which two singlet signals resonating at δ_{H} 6.74 and δ_{H} 6.23 were assigned to aromatic methine protons H-3 and H-6, respectively. A symmetric phenyl unit (B ring of flavone) was found to appear at δ_{H} 8.00 (2H, brd, 8.5 Hz, H-2', H-6'), and δ_{H} 6.90

(2H, d, 8.5 Hz, H-3', H-5'). The sugar unit of **2** with chemical shifts at δ_{H} 3.25-4.72 ppm, and coupling constant $J = 9.0$ Hz of the anomeric proton have demonstrated a β -D-glucopyranosyl unit [11-13]. The 2D-NMR spectroscopies were in agreement with the findings in the 1D-NMR (Figure 1). The chromene ring was formulated with HMBC correlations H-3 (δ_{H} 6.74)/C-2 (δ_{C} 163.8), C-4 (δ_{C} 181.9), and C-4a (δ_{C} 104.6), H-6 (δ_{H} 6.23)/C-4a, C-5 (δ_{C} 160.4), and C-8 (δ_{C} 104.6), whereas ring B was established and linked to carbon C-2 due to the key HMBC correlations between H-2' and H-6' (δ_{H} 8.00), H-3' and H-5' (δ_{H} 6.90)/C-4, and H-2' and H-6'/C-2. The important HBMC cross peaks H-1"/C-8, and C-8a confirmed that anomeric C-1" directly connected to C-8. Based on these findings and comparing with literature, compound **2** was elucidated as vitexin [14].

Compound **3** was isolated as yellow amorphous powders. The ^1H and ^{13}C -NMR spectral data of **3** were very similar to those of **2**, except for the presence of acetyl group at δ_{H} 1.80 (3H, s, CH_3CO) in the ^1H -NMR, and at δ_{C} 172.0 (CH_3CO) and δ_{C} 20.5 (CH_3CO) in the ^{13}C -NMR. The connectivity between the acetoxy group and carbon C-2" was determined by the HMBC correlation H-2"/CO (Figure 1). The chemical structure of **3** was further confirmed by the positive ESI-MS spectrum. The adduct ion at m/z 475 $[\text{M}+\text{H}]^+$ in the ESI-MS assigned to the molecular formula of **3** was to be $\text{C}_{23}\text{H}_{22}\text{O}_{11}$. Based on these findings, and comparing with literature data, compound **3** was identified to be 2"-O-acetylvitexin [15].

Compound **4** was separated as yellow amorphous powders, and had the molecular formula $\text{C}_{30}\text{H}_{18}\text{O}_{10}$ due to the observation of the proton adduct ion at m/z 539 $[\text{M}+\text{H}]^+$ in the positive ESI-MS spectrum. The ^1H -NMR data of **4** showed the characteristics of a biflavone. In comparison with compounds **2-3**, glycoside units of compounds **2-3** were replaced by a flavone unit [three aromatic protons at δ_{H} 6.82 (H-3), δ_{H} 6.18 (H-6), and 6.44 (H-8), and a ABX spin system at δ_{H} 7.12 (1H, d, 9.0 Hz, H-5'), δ_{H} 7.99 (1H, d, 2.5, 9.0 Hz, H-6'), and δ_{H} 8.02 (1H, d, 2.5 Hz, H-2')] in **4**. The ^{13}C -NMR data of **4** contained 30 carbon signals, which were assigned to twelve aromatic methine carbons, sixteen aromatic carbons, and two carbonyl carbons. The chemical structure of **4** was further confirmed by the 2D-NMR data (HSQC, and HMBC) (Figure 1). Especially, the connectivity between two monomeric flavone units was identified by the key HMBC J^3 -correlation from H-2' to C-8", as well as the key HMBC W-shape correlations from H-5' to C-8", and from H-6" to C-3'. In comparison with literature data, isolated compound **4** was determined to be a biflavone, which was trivially named amentoflavone [16]. Secondary metabolite **4** has ever been isolated from various *Garcinia* species, such as *G. brevipedicellata* stem heartwoods, or *G. livingstonei* leaves, however, it was now found in *G. mackeaniana* [17, 18] for the first time.

Compound **5** was obtained as yellow amorphous powders. The ^1H -NMR spectrum of **5** established an ABX spin system of H-5 (δ_{H} 6.82, d, 8.0 Hz), H-6 (δ_{H} 7.43, dd, 2.0, 8.0 Hz), and H-2 (δ_{H} 7.42, d, 2.0 Hz), and one methoxy singlet signal at δ_{H} 3.85. Therefore, it can be concluded that isolated compound **5** was to be a phenolic compound type of 1,3,4-trisubstituted benzene. Based on the ^{13}C -NMR/DEPT data [three methines at δ_{C} 114.5 (C-2), δ_{C} 116.0 (C-5), and δ_{C} 122.2 (C-6), four carbons at δ_{C} 121.2 (C-1), δ_{C} 144.8 (C-3), δ_{C} 150.0 (C-4), and δ_{C} 167.5 (CO), together with one methoxy group at δ_{C} 50.8 (OCH_3)], and comparison with literature compound [19], compound **5** was unambiguously determined to be methyl 3,4-dihydroxybenzoate, which was trivially named methyl protocatechuate. Despite its availability in nature, this is the first time this compound was found in genus *Garcinia*.

3.2. DPPH-antioxidant assay

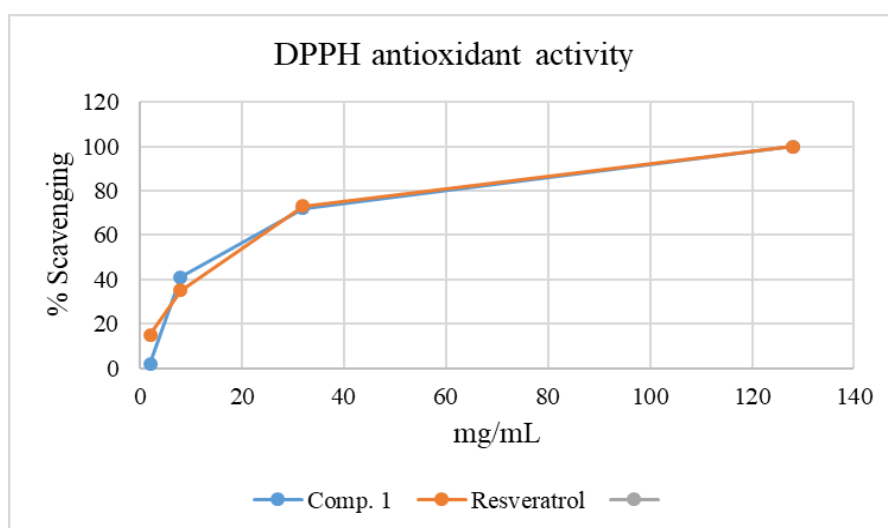


Figure 2. Radical quenching percent (% SC) of compound **1** and standard compound resveratrol. Data are described as Mean \pm SD (n = 3), $P < 0.05$.

All isolated compounds **1-5** were subjected to antioxidative examination with the target of DPPH-radical scavenging assessment. Compounds **2-5** failed to capture DPPH radicals at any concentrations (data not shown). In contrast, compound **1** showed the strong EC_{50} value of $14.97 \pm 0.8 \mu\text{g/mL}$, as compared with that of the positive control ($IC_{50} 11.61 \pm 0.09 \mu\text{g/mL}$). As shown in Figure 2, at the concentration of 128.0 mg/mL, benzophenone **1** completely controlled DPPH with SC = 100%. It is noticeable that benzophenones (compound **1**) derived from *Garcinia* plants are better than flavone glycosides and biflavones (compounds **2-4**), and phenols (compound **5**) in antioxidative treatments.

4. CONCLUSIONS

The present results provide information on the phytochemical investigation and DPPH-antioxidative assay relating to Vietnamese *Garcinia mackeaniana* species. From methanolic extract, five known compounds, comprising of one benzophenone 4,3',4'-trihydroxy-2,6-dimethoxybenzophenone (**1**), two flavone *C*-glucosides vitexin (**2**) and its 2''-*O*-acetyl derivative (**3**), one biflavone amentoflavone (**4**), and one *mono*-phenol methyl protocatechuate (**5**) were isolated. This is the first time we report the isolation of these compounds from *G. mackeaniana*. Given strong IC_{50} value in DPPH assay, benzophenone **1** and analogs derived *Garcinia* plants can become promising agents for antioxidative problems.

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