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Cover Page Footnote

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Antioxidant Constituents from the Bark of *Aglaia eximia* (Meliaceae)

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Abstract

The genus *Aglaia* is a a rich source of different compounds with interesting biological activities. A part of our continuing search for novel biologically active compounds from Indonesia *Aglaia* plants, the ethyl acetate extract of bark of *Aglaia eximia* showed significant antioxidant activity. Four antioxidant compounds, kaempferol (**1**), kaempferol-3-*O*-α-L-rhamnoside **(2),** kaempferol-3-*O*-β-D-glucoside **(3)** and kaempferol-3-*O*-β-D-glucosyl-(1→4)-α-L-rhamnoside **(4)** were isolated from the bark of *Aglaia eximia* (Meliaceae). The chemical structures of compounds **1**-**4** were identified on the basis of spectroscopic datas including UV, IR, NMR and MS along with by comparison with those spectra datas previously reported. All compounds showed DPPH radical-scavenging activity with IC_{50} values of 1.18, 6.34, 8.17, 10.63 μg/mL, respectively.

Abstrak

Kandungan Senyawa Antioksidan dari Kulit Batang *Aglaia eximia* (Meliaceae). Genus *Aglaia* adalah sumber yang kaya akan senyawa kimia yang bervariasi dengan aktivitas biologis yang menarik. Bagian dari penelitian kami untuk mencari senyawa aktif baru dari tumbuhan *Aglaia* Indonesia, ekstrak etil asetat kulit batang *Aglaia eximia* menunjukkan aktivitas antioksidan yang signifikan. Empat senyawa yang beraktivitas antioksidan, kaempferol (**1**), kaempferol-3-*O*-α-L-ramnosida (**2**), kaempferol-3-*O*-β-D-glukosida (**3**) dan kaempferol-3-*O*-β-D-glukosil-(1→4)-α-L-ramnosida (**4**) telah diisolasi dari batang tumbuhan *Aglaia eximia* (Meliaceae). Struktur kimia senyawa **1**-**4** telah diidentifikasikan berdasarkan interpretasi data spektroskopi meliputi UV, IR, NMR dan massa bersama dengan perbandingan data spektra yang dilaporkan sebelumnya. Semua senyawa menunjukkan aktivitas penghambatan radikal bebas DPPH dengan nilai IC_{50} berturut-turut 1,18; 6,34; 8,17 dan 10,63 μg/mL.

Keywords: Aglaia eximia, DPPH radical-scavenging activity, glycosides, kaempferol, Meliaceae

Introduction

Aglaia eximia (Meliaceae) is an ornamental tree that has long been recommended in Indonesian medicine for reducing fever, moisturizing the lungs, and for treating contused wound, coughs and skin diaseases [1-3]. Previous phytochemical studies of the species *A. eximia* reported some variety of compounds, including triterpenoids with cycloartane, dammarane, and cabraleahydroxylactone types [4-7], as well as stigmastane-types streoid [4,6] and flavonoids [8]. These metabolites have been described previously to exhibit anticancer, cytotoxic, insecticides, anti-inflammatory and antitumor activities [5,9,10].

The different parts of the genus *Aglaia* have been reported to contain biologically active classes of flavonoid compound [11]. It was suggested, for the same genus, that there are possibilities to generate the derivate compounds based on biosynthesis pathways of plants [12]. The flavonoids are a class of widely distributed phytochemicals, and scavenging of free radicals seems to play a considerable part in the antioxidant activity [13].

To the best of our knowledge, antioxidant activity of compounds or extracts from some members of *Aglaia* have been described previously [11,12], but no infor-

mation is available on the antioxidant activity of kaempferol and their glycosides from the species of *A. eximia*. In the further screening for antioxidant activity against DPPH radical-scavenging on polar fraction from *A. eximia*, we found that the ethyl acetate extract of the bark of *A. eximia* showed a DPPH radical-scavenging activity with an IC_{50} values of 20 μg/mL. We herein report the isolation and structure elucidation of kaempferol and their glycosides from the bark of *A. eximia* together with antioxidant activity against DPPH radical-scavenging.

Materials and Methods

General. Melting points were measured on an electro-thermal melting point apparatus and are uncorrected. Optical rotations on an ATAGO AP-300 automatic polarimeter. UV spectra were measured by using Shimazu UV-160A UV-Vis spectrophotometer. The IR spectra were measured on a Perkin–Elmer spectrum-100 FT-IR in KBr. Mass spectra with a Water Qtof HR-MS XEV^{otm} mass spectrometer; the- NMR spectra were measured with a JEOL JNM A-500 spectrometer using TMS as an internal standard. Chromatographic separations were carried out on silica gel 60 (70–230 mesh and 230–400 mesh, Merck). Preparative TLC glass and TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 5% $AlCl_3$ and 10% H_2SO_4 in ethanol, followed by heating on a hotplate at 100^oC for 2-5 minutes.

Plant material. The bark of *A. eximia* was collected in Bogor Botanical Garden, Bogor in June of 2011. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-1295315) was deposited at the herbarium.

Plant extraction. Dried ground bark (4.0 kg) of *A. eximia* was extracted successively with *n*-hexane, EtOAc, and MeOH for three days and then filtered, decanted, and evaporated under a vacuum. Evaporation resulted in the crude extracts of *n*-hexane (26.4 g), EtOAc (54.5 g), and MeOH (32.5 g), respectively. All of the extracts' antioxidant activity was evaluated against DPPH radical-scavenging and showed antioxidant activity with IC_{50} values of 59, 20, and 52 μg/mL, respectively. The EtOAc showed the strongest antioxidant activity. Subsequent phytochemical analysis was therefore focused on the EtOAc extract. The EtOAc extract was subjected to column chromatography over silica gel using mixture of $CHCl_3$/Me_2CO (10:0-0:10) as an eluen to afford 20 fractions (E01–E20). The E04–E05 fractions (13.9 mg) were combined and purified with preparative TLC on silica gel GF_{254} plates, eluted with $CHCl_3$:MeOH (9:1), to give **1** (7.0 mg), was identified based on spectroscopic methods. Fractions E11–E12 (112.4 mg) were combined and was chromatographed on a column of silica gel using mixtures of $CHCl_3$/MeOH (10:0-8:2) as an eluen to afford 22 fractions (F01-F22). Fractions F08-F21 (74.6 mg) were combined and was chromatographed on a column of silica gel using mixtures of $CHCl_3$/MeOH (10:0–9:1) to give **2** (18.6 mg), was identified based on spectroscopic methods.. Fractions E13-E15 (156.3 mg) were crystallized with MeOH to give **3** (88.0 mg), was identified based on spectroscopic methods. Fractions E16-E19 (296.6 mg) were combined and subjected to silica gel column chromatography using mixtures of $CHCl_3$/MeOH (10:0–0:10) as eluting solvents to afford 23 fractions (G01-G23). Fractions G16-G19 were combined (171.9 mg) and was chromatographed on a column of silica gel using mixtures of $CHCl_3$/MeOH (10:0–7:3) as eluting solvents to afford 20 fractions (H01-H19). Fraction E16-E19 were combined (56.4 mg) and was chromatographed on a column of silica gel using mixtures of $CHCl_3$/MeOH (10:0–7:3) as eluting solvents to give **4** (20.3 mg), was identified based on spectroscopic methods.

Kaempferol (1). Yellow powder, m.p. 276-278°C, UV (MeOH) λ_{max} nm (log ε) 367 (4.24); 257 (4.19); IR (KBr) ν_{max} 3433, 1641, 1478, 1164, 1051, 825 cm^{-1}. ^{1}H-NMR (CD_3OD, 500 MHz); ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1; HR- TOFMS (negative ion mode) *m/z* 285.3512 $[M-H]^-$, (calcd. $C_{15}H_{10}O_6$, *m/z* 286.3477).

Kaempferol-3-O-α-L-rhamnoside (2). Yellow powder, m.p. 183-185, UV (MeOH) λ_{max} nm (log ε) 349 (4.14), 268 (4.23); IR (KBr) ν_{max} 3421, 2922, 1676 and 1205 cm^{-1}; ^{1}H-NMR (CD_3OD, 500 MHz), see Table 1; ^{13}C-NMR (CD_3OD, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 431.7157 $[M-H]^-$ (calcd. $C_{21}H_{20}O_{10}$, *m/z* 432.7118).

Kaempferol-3-O-β-D-glucoside (3). Yellow powder, m.p. 201-203°C, UV (MeOH) λ_{max} nm (log ε) 351 (4.44), 266 (4.53); IR (KBr) ν_{max} 3401 1659, 1509, 1364, 1285, 1063 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C-NMR (DMSO-d_6, 125 MHz) see Table 1, HR-TOFMS (negative ion mode) *m/z* 447.8083 $[M-H]^-$ (calcd. $C_{21}H_{20}O_{11}$, *m/z* 448.3769).

Kaempferol-3-O-β-D-glucosyl-(1→4)-α-L-rhamnoside (4). Yellow powder, $[\alpha]^{20}{}_D$-20 (*c*, 0.5, MeOH), UV MeOH λ_{max} nm (log ε) 349 (4.17), 266 (4.25); IR (KBr) ν_{max} 3833, 1660, 1509, 1362, 1283, 1266, 1064 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C-NMR (DMSO-d_6, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) *m/z* 593.5167 $[M-H]^-$, (calcd. $C_{27}H_{30}O_{15}$, *m/z* 594.5181).

Identification of the presence of propose aglycone moiety in 2-4. Compounds **2-4** (2 mg) dissolved in MeOH were heated to reflux with 2 mL of sulphuric acid under stirring about six hours. The reaction mixture was concentrated under a vacuum and suspended in 20 mL water. The aqueous layer was extracted three times with ethyl acetate for 3x24 h; the organic layer was

Table 1. NMR Data of Compounds 1-4

Position Carbon	1		2		3		4	
	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (ΣH, mult *J* Hz)	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (ΣH, mult *J* Hz)	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (ΣH, mult *J* Hz)	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (ΣH, mult *J* Hz)
2	160.3 (s)	-	161.7 (s)	-	159.9 (s)	-	159.9 (s)	-
3	136.7 (s)	-	136.3 (s)	-	133.2 (s)	-	133.2 (s)	-
4	176.7 (s)	-	179.7 (s)	-	177.5 (s)	-	177.5 (s)	-
5	162.4 (s)	-	163.3 (s)	-	161.2 (s)	-	161.2 (s)	-
6	99.2 (d)	6.16 (IH, *d*, 2.0)	99.9 (d)	6.19 (1H, *d*, 2.0)	98.7 (d)	6.21 (1H, *d*, 2.0)	98.8 (d)	6.19 (1H, *d*, 2.0)
7	165.2 (s)	-	165.9 (s)	-	164.2 (s)	-	164.2 (s)	-
8	94.5 (d)	6.36 (IH, *d*, 2.0)	94.8 (d)	6.39 (1H, *d*, 2.0)	93.8 (d)	6.43 (1H, *d*, 2.0)	93.8 (d)	6.40 (1H, *d*, 2.0)
9	157.8 (s)	-	159.4 (s)	-	156.2 (s)	-	156.9 (s)	-
10	104.2 (s)	-	106.0 (s)	-	103.9 (s)	-	103.9 (s)	-
1′	123.3 (s)	-	122.7 (s)	-	120.9 (s)	-	120.9 (s)	-
2′	130.5 (d)	8.08 (IH, *d*, 9.1)	131.9 (d)	7.79 (1H, *d*, 9.1)	130.9 (d)	8.08 (1H, *d*, 9.1)	130.9 (d)	7.98 (1H, *d*, 9.1)
3′	116.4 (d)	6.90 (IH, *d*, 9.1)	116.6 (d)	6.92 (1H, *d*, 9.1)	115.1 (d)	6.88 (1H, *d*, 9.1)	115.2 (d)	6.88 (1H, *d*, 9.1)
4′	147.1 (s)	-	159.5 (s)	-	156.4 (s)	-	156.5 (s)	-
5′	116.4 (d)	6.90 (IH, *d*, 9.1)	116.6 (d)	6.92 (1H, *d*, 9.1)	115.1 (d)	6.88 (1H, *d*, 9.1)	115.2 (d)	6.88 (1H, *d*, 9.1)
6′	130.5 (d)	8.08 (IH, *d*, 9.1)	131.9 (d)	7.79 (1H, *d*, 9.1)	130.9 (d)	8.08 (1H, *d*, 9.1)	130.9 (d)	7.98 (1H, *d*, 9.1)
1″			103.6 (d)	5.4 (1H, *d*, 1.5)	100.9 (d)	5.45 (1H, *d*, 7.8)	101.4 (d)	5.31 (1H, *d*, 7.8)
2″			72.2 (d)	3.69 (1H, *dd*, 1.5, 3.4)	74.2 (d)	4.26 (1H, *dd*, 7.8, 9.0)	70.6 (d)	3.43 (1H, *dd*, 7.8, 10.4)
3″			72.1 (d)	3.31 (1H, *dd*, 3.4, 9.6)	76.4 (d)	3.37 (1H, *t*, 9.0)	74.2 (d)	3.38 (1H, *t*, 10.4)
4″			73.3 (d)	3.28 (1H, *t*, 9.6)	69.9 (d)	3.23 (1H, *t*, 9.0)	69.9 (d)	3.08 (1H, *t*, 10.4)
5″			72.0 (d)	4.18 (1H, *dd*, 5.9, 9.6)	77.5 (d)	3.17 (1H, *dd*, 5.4, 9.0)	76.4 (d)	3.26 (1H, *dd*, 2.2, 10.4)
6″			17.8 (q)	0.91 (3H, *d*, 5.9)	60.8 (t)	3.56 (1H, *dd*, 5.2, 10.1)	66.9 (t)	3.69 (1H, *dd*, 5.8, 11.5)
						3.83 (1H, *dd*, 2.3, 10.1)		3.22 (1H, *dd*, 2.2, 11.5)
1‴							100.8 (d)	4.37 (1H, *dd*, 1.4, 3.3)
2‴							71.8 (d)	3.27 (1H, *dd*, 3.3, 9.8)
3‴							70.4 (d)	3.24 (1H, *t*, 9.8)
4‴							75.7 (d)	4.42 (1H, *dq*, 6.5, 9.8)
5‴							68.3 (d)	3.26 (1H, *dd*, 2.2, 9.8)
6‴							17.3 (q)	0.98 (3H, *d*, 6.5)

washed with water and dried over anhydrous $MgSO_4$. The ethyl acetate layer was filtered over celite and concentrated under a vacuum to yield a compound which was identified as kaempferol (**1**) by comparison of its co-TLC with standard compound.

Determination of DPPH radical-scavenging Activity [14,15]. DPPH radical-scavenging assay. The antioxidant activity was conducted according to the method described in previous paper [16] using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging method together with L-ascorbic acid as a positive control. The samples test was dissolved in DMSO and mixed with a 20 μg/L of (DPPH) methanol solution, to give final concentrations of 10, 50, 100, 200, 400 and 800 μg/mL. After 30 minutes at room temperature, the absorbance values were measured at 517 nm and converted into percentage of antioxidant activity as follows:

$$\%inhibition = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance of\ control}$$

The IC_{50} value was determined by probit values and were plotted against the logarithmic values of concentrations of the test samples and a linear regression curve. It is the amount of sample necessary to decrease the absorbance of DPPH by 50%. All analyses were carried out in triplicate, and the results were expressed as the mean ± standard deviation (SD) and compared using Waller-Duncan test. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

The phytochemical test for the EtOAc extract showed the presence of flavonoids. By using DPPH radical-scavenging assay to guide separations, the EtOAc fraction was separated by column chromatography over silica gel by gradient elution. The fractions were repeatedly subjected to normal-phase column chromatography and preparative TLC on silica gel GF_{254} and yielded four antioxidant flavonoid compounds **1–4** (Figure 1).

Compound **1** was obtained as a yellow powder. The HR-TOFMS spectrum showed $[M-H]^-$ *m/z* 285.3912 (calcd. *m/z* 286.2487), which corresponded to the molecular formula of $C_{15}H_{10}O_6$ with 11 degrees of unsaturation. UV (MeOH) λ_{max} nm (log ε) 367 (4.24); 257 (4.19), suggested the presence of flavonoid compound. The IR spectra showed absorption peaks at 3433 cm^{-1} (OH),

1641 cm^{-1} (conjugated carbonyl), 1478 cm^{-1} (C=C olefinic), 1164 cm^{-1} (asymmetric C-O-C stretch) and 825 cm^{-1} (substituted benzene). The ^{1}H-NMR spectrum of compound **1** showed a characteristic AA´BB´ pattern of ring B at δ_H 8.08 (2H, *d*, *J*=9.1 Hz) and δ_H 6.90 (2H, *d*, *J*=9.1 Hz), corresponding to H-2´/H-6´ and H-3´/H-5´. The resonances of two *meta*-coupling protons of ring A were observed at δ_H 6.36 (1H, *d*, *J*=2.0 Hz) and δ 6.16 (1H, *d*, *J*=2.0 Hz). The addition of olefinic singlet signal in the deshielded region was assigned the presence hydroxyl substituen in ring C of **1**. The ^{13}C-NMR and DEPT 135° spectra showed the presence of six methines and nine quaternary carbons (14 sp^2 carbon) and a ketonic group, C-4, resonating at δ_C 176.7 ppm. In the HMBC spectrum, two *meta*-coupling protons of ring A (H-6 and H-8) exhibited long-range correlations13 to C-4 (176.7 ppm), C-7 (165.2 ppm), C-9 (157.8 ppm), C-5 (162.4 ppm) and C-10 (104.2 ppm), suggested that two hydroxyl group attached at C-5 and C-7, respectively. These functionalities accounted for eight of the total 11 degrees of unsaturation, and the remaining three degrees of unsaturation were consistent with the flavonoid structure. The full assignments of compound **1** are shown in Table 1. Comparison of the NMR data of **1** with kaempferol [16] showed high similarity, consequently compound **1** was identified as kaempferol.

Compound **2** was obtained as a yellow powder. The HR-TOFMS spectrum showed $[M-H]^-$ *m/z* 431.7157 $[M-H]^-$ (calcd. $C_{21}H_{20}O_{10}$, *m/z* 432.7118), the fragment ion peaks occurred at *m/z* 285.8202 $[M+H-146]^+$, indicating the loss of a terminal rhamnosyl unit (-142), which corresponded to the molecular formula of $C_{15}H_{10}O_6$ and thus required 12 degrees of unsaturation. The ^{1}H and ^{13}C-NMR spectrum of **2** resembled that of **1** except in the oxygenated region. The presence of methyl signal which occurred as a doublet at 0.91 (3H, *d*, *J*=5.9 Hz) together with anomeric signal proton at δ_H 5.40, (1H, *d*, *J*= 1.5 Hz) as well as with four oxygenated methines, resonating at δ_H 4.18 (1H, *dd*, *J*=5.9, 9.6 Hz), 3.69 (1H, *dd*, *J*=1.5, 3.4 Hz), 3,31 (1H, *dd*, *J*=3.4, 9.6 Hz), 3,28 (1H, *t*, 9.6 Hz), which is typical for a rhamnose moiety. An anomeric protons resonated at δ_H 5.40, (1H, *d*, *J*= 1.5 Hz), which were assigned to the one sugar units α-rhamnopyranosyl (Rha). The HMBC spectrum showed correlations between H-1″ at δ_H 5.4, (1H, *d*, *J*=1.5 Hz) with C-3 (δ_C 136.3 ppm) which further established the connectivity between rhamnopyranosyl units to the flavonoid on the C-3 (δ_C 136.3 ppm). In comparison the NMR data of **2** with the literature data [17, 18], compound **2** was identified as kaempferol-3-*O*-α-L-rhamnoside.

Compound **3** was obtained as a yellow powder. The HR-TOFMS spectrum showed $[M-H]^-$ *m/z* 447.4083, (calcd. *m/z* 448.3769), and a fragment ion $[kaempferol-2H]^-$ at *m/z* 284.0023 resulting from the loss of a glucose moiety $(M-H-162)^-$, which corresponded to the molecular formula

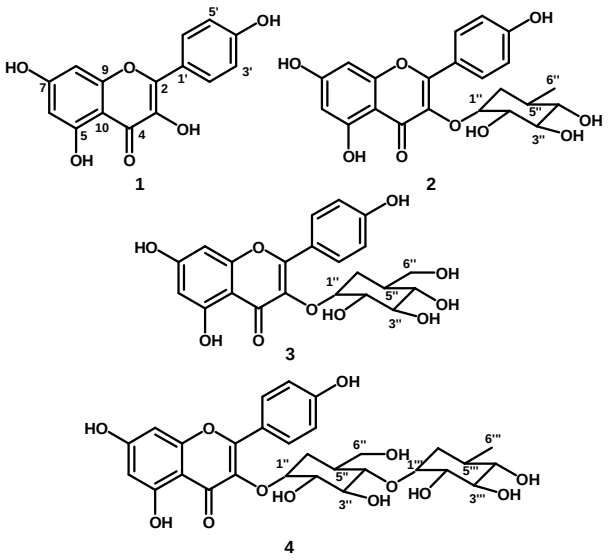

Figure 1. The Chemical Structure of Compounds 1-4

of $C_{21}H_{20}O_{10}$ and thus required 12 degrees of unsaturation. The ^{1}H and ^{13}C-NMR spectra of **3** resembled that of **2** except the presence of methine and methylene signals. The presence of methylene signal which occurred as a double doublet at 3.83 (1H, *dd*, *J*=2.3, 10.1 Hz) and 3.83 (1H, *dd*, *J*=5.2, 10.1 Hz) which is typical for a glucose moiety. An anomeric proton H-1″ resonating at δ_H 5.90, ^3J=4.5 Hz), the magnitude of the coupling constant ^3J=4.5 Hz revealed an axial-equatorial position which occurred as β-configuration [19]. In comparison the NMR data of **3** with the literature data [19, 20], and compound **3** was identified as kaempferol-3-*O*-α-D-glucoside.

Compound **4** was obtained as a yellow powder. The HR-TOFMS spectrum showed $[M-H]^-$ *m/z* 591.5877, (calcd. for *m/z* 592.5587). The fragment ion peaks occurred at *m/z* 448.9022 $[M+H-146]^-$, indicating the loss of a terminal rhamnosyl unit (*m/*z -142), 142 results and *m/z* 278.2 $[M+H-146-162]^-$, which was assigned to the additional loss of a primary hexosyl moiety (*m/z* -162), which corresponded to the molecular formula of $C_{28}H_{32}O_{14}$ and thus required 13 degrees of unsaturation. The ^{1}H and ^{13}C-NMR spectra of **4** resembled that of **3**, except for the presence of methine and methyl signals as well as an oxygenated region. The presence methyl signal resonating at 0.98 (3H, *d*, *J*=6.5 Hz) as a doublet, which is typical for a rhamnose moiety. Two anomeric protons resonating at δ_H 5.31 (1H, *d*, *J*=7.8 Hz) and at δ_H 5.35 (1H, *d*, *J*=1.4 Hz), suggested the presences two sugar units, β-glucopyranosyl (Glu), and α-rhamnopyranosyl (Rha) of **4**. The spin system of this sugar unit were confirmed by ^{1}H-^{1}H- COSY spectrum. The connectivity of the monosaccaride unit with kaempferol was established on the basis of HMBC correlation (Figure 2).

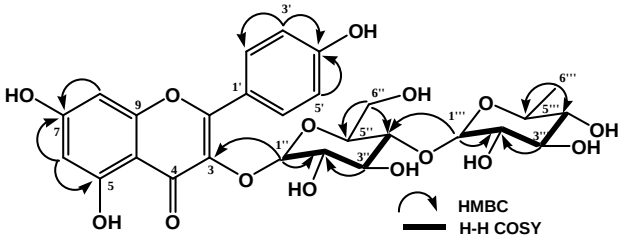

Figure 2. Selected HMBC and H-H COSY Correlations for 4

Table 2. Antioxidant Activity of Compounds 1-4 against DPPH Radical-scavenging

Compunds	IC_{50} (μg/mL)
1	1.18 ± 0.02
2	6.34 ± 0.05
3	8.17 ± 0.04
4	10.63 ± 0.11
Ascorbic acid*	0.15 ± 0.01

*positive control

The anomeric proton H-1″ at 5.31 (1H, *d*, *J*=7.8 Hz) was correlated to kaempferol C-3 (133.2 ppm), suggesting that sugar unit lies on C-3 of ring C of aglycone. The correlations between Glu-H-1″ (δ_H 5.31) and kaempferol C-3 (δ_C 133.2), and glu. H-4″ (δ_H 3.08) to rham. C-1‴ (δ_C 100.8), which further established the position of glucosyl-(1→4)-α-L-rhamnoside of the 2 sugar units to the kaempferol. The proton H-1″ at δ_H 5.31, ^3J=7.8 Hz) was assigned for axial-axial position whereas proton H-1‴ at δ_H 5.35, ^3J=1.4 Hz was assigned for axial-equatorial position which occurred to be the β- and α-configuration, respectively [18]. A comparison the carbon signals of **4** with the glucosyl moiety [19], the glucose C-2″, C-1″ experienced downfield and upfield as well as the C-5″; C-6″, shift due to the α- and β-effects of rhamnosylation. In comparison, the NMR data of **4** with the literature data [17, 18, 20], compound **4** was identified as kaempferol-3-*O*-β-D-glucosyl-(1→4)-α-L-rhamnoside.

Compounds **1–4** antioxidant activity were evaluated against DPPH radical-scavenging. The antioxidant activiy of compounds **1–4** are shown in Table 2. Compounds **1**-**4** showed weaker activity compared with standard compound, ascorbic acid. Among those isolated compounds, compound **1**, showed strongest activity with IC_{50} value of 1.18 ± 0.02 μg/mL. Previous structure-activity studies of flavonoids have pointed to the importance of the number and location of OH groups and could be more important for the antiradical efficacy. The effectivity of radical scavenging activity of **1**, is proposed by 4-hydroxy in the B ring as electron donating and being a radical target, together with 3-OH moiety in the C ring is also beneficial as antioxidant activity. A conjugated double bond between C2-C3 with 4-keto group further enhances the radical-scavenging capacity through electron delocalization from the B ring; the presence of hydroxyl group on C-3 and C-5 in combination with an α-β-unsaturated-4-carbonyl can improve the radical scavenging activity of **1** [19], whereas sugar moiety of compounds **2-4** were showed the steric effect. That could reduce the free radical scavenging activity as well as weaken the electronic distribution of flavonoid molecules. Dihedral angles of compounds **2** >**3** >**4** caused by the sugar unit in the C-ring, lead the conformation to reverse and make molecule lose coplanar varying degrees; this could reduce the electronic distribution of compounds **2-4**.

Based on frontier molecular orbital theory, the HOMO of flavonoid is mainly distributed in the B-ring, while the LUMO is distributed in the C-ring. The electron-donating capability of a molecule can be determined by the values of HOMO corresponds with a strong capability for donating electrons [19, 20]. It was suggested the higher of DPPH radical-scavenging was more focused on B-ring and the conjugate part than the C ring. It show us, that there is no significance to the IC_{50} vaules of compound **1-4** (Table 2).

Conclusions

Four antioxidant compounds, kaempferol (**1**), kaempferol-3-*O*-α-L-rhamnoside **(2),** kaempferol-3-*O*-β-D-glucoside **(3)** and kaempferol-3-*O*-β-D-glucosyl-(1→4)-α-L-rhamnoside **(4)** were isolated from the bark of *Aglaia eximia* (Meliaceae). Compounds **1**-**4** showed DPPH radical-scavenging activity with IC_{50} values of 1.18, 6.34, 8.17, 10.63 μg/mL, respectively.

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