Antioxidant Constituents from the Bark of Aglaia eximia (Meliaceae)

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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 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 data including UV, IR, NMR and MS along with those spectra data previously reported. All compounds showed DPPH radical-scavenging activity with IC<sub>50</sub> values of 1.18, 6.34, 8.17, 10.63 µg/mL, respectively.

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 diseases [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 steroid [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 spectrophotometer. Preparative TLC glass and TLC plates were precoated with silica gel GF$_254$ (Merck, 0.25 mm) and was chromatographed on a column of silica gel using mixtures of CHCl$_3$/MeOH (10:0–7:3) as eluting solvents to afford 22 fractions (E01–E20), 23 fractions (G01–G23). Fractions 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 afford 21 fractions (G01–G23). Fractions G16–G19 were prepared (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 4 (20.3 mg), was identified based on spectroscopic methods.

**Kaempferol (1).** Yellow powder, m.p. 276-278°C, UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 367 (4.24); 257 (4.19); IR (KBr) $\nu_{max}$ cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 500 MHz), see Table 1; HR-TOFMS (negative ion mode) $m/z$ 285.3512 [M-H], (calcd. C$_{13}$H$_{17}$O$_{6}$, $m/z$ 286.3477).

**Kaempferol-3-O-$\alpha$-l-rhamnoside (2).** Yellow powder, m.p. 183-185, UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 349 (4.14), 268 (4.23); IR (KBr) $\nu_{max}$ cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 500 MHz), see Table 1; HR-TOFMS (negative ion mode) $m/z$ 431.7157 [M-H], (calcd. C$_{23}$H$_{29}$O$_{16}$, $m/z$ 432.7118).

**Kaempferol-3-O-$\beta$-D-glucoside (3).** Yellow powder, m.p. 201-203°C, UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 351 (4.44), 266 (4.53); IR (KBr) $\nu_{max}$ cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 500 MHz) see Table 1; HR-TOFMS (negative ion mode) $m/z$ 447.8083 [M-H], (calcd. C$_{23}$H$_{29}$O$_{18}$, $m/z$ 448.3769).

**Kaempferol-3-O-$\beta$-D-glucosyl-(1→4)-$\alpha$-L-rhamnoside (4).** Yellow powder, [$\alpha$]$^{20}_{D}$-20 (c, 0.5, MeOH), UV (MeOH) $\lambda_{max}$ nm (log $\varepsilon$) 349 (4.17), 266 (4.25); IR (KBr) $\nu_{max}$ cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$, 500 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

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The DPPH radical-scavenging activity was determined using the method described in a previous paper [16] using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging method together with L-ascorbic acid as a positive control. The antioxidant activity was conducted according to the method described in the 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 were 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:

\[
\%\text{inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}
\]

The IC\text{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 EtoOAc extract showed the presence of flavonoids. By using DPPH radical-scavenging assay to guide separations, the EtoOAc 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\text{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 \(C_{13}H_{13}O_4\) with 11 degrees of unsaturation. UV (MeOH) \(\lambda_{\text{max}}\) 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), 1767 cm\(^{-1}\) (C=O), 1627 cm\(^{-1}\) (C=C), and 1521 cm\(^{-1}\) (C=C). The NMR data of compounds 1–4 are listed in Table 1.

### Table 1. NMR Data of Compounds 1-4

<table>
<thead>
<tr>
<th>Position</th>
<th>(^{13}\text{C}-\text{NMR} \delta_{c}(\text{mult.}))</th>
<th>(^{1}H\text{-NMR} \delta_{\text{H}}(2\text{H}, \text{mult. }J \text{ Hz}))</th>
<th>(^{13}\text{C}-\text{NMR} \delta_{c}(\text{mult.}))</th>
<th>(^{1}H\text{-NMR} \delta_{\text{H}}(2\text{H}, \text{mult. }J \text{ Hz}))</th>
<th>(^{13}\text{C}-\text{NMR} \delta_{c}(\text{mult.}))</th>
<th>(^{1}H\text{-NMR} \delta_{\text{H}}(2\text{H}, \text{mult. }J \text{ Hz}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.3 (s)</td>
<td>-</td>
<td>161.7 (s)</td>
<td>-</td>
<td>159.9 (s)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>135.7 (s)</td>
<td>-</td>
<td>153.6 (s)</td>
<td>-</td>
<td>133.2 (s)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>176.7 (s)</td>
<td>-</td>
<td>177.5 (s)</td>
<td>-</td>
<td>177.5 (s)</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>162.4 (s)</td>
<td>-</td>
<td>161.2 (s)</td>
<td>-</td>
<td>161.2 (s)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>99.2 (d)</td>
<td>6.16 (1H, d, 2.0)</td>
<td>99.9 (d)</td>
<td>6.19 (1H, d, 2.0)</td>
<td>98.7 (d)</td>
<td>6.21 (1H, d, 2.0)</td>
</tr>
<tr>
<td>6</td>
<td>165.2 (s)</td>
<td>-</td>
<td>164.2 (s)</td>
<td>-</td>
<td>164.2 (s)</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>94.5 (d)</td>
<td>6.36 (1H, d, 2.0)</td>
<td>94.8 (d)</td>
<td>6.39 (1H, d, 2.0)</td>
<td>93.8 (d)</td>
<td>6.43 (1H, d, 2.0)</td>
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<tr>
<td>8</td>
<td>157.8 (s)</td>
<td>-</td>
<td>156.2 (s)</td>
<td>-</td>
<td>156.9 (s)</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>104.2 (s)</td>
<td>-</td>
<td>103.9 (s)</td>
<td>-</td>
<td>103.9 (s)</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>123.3 (s)</td>
<td>-</td>
<td>120.9 (s)</td>
<td>-</td>
<td>120.9 (s)</td>
<td>-</td>
</tr>
<tr>
<td>1’</td>
<td>130.5 (d)</td>
<td>8.08 (1H, d, 9.1)</td>
<td>131.9 (d)</td>
<td>7.79 (1H, d, 9.1)</td>
<td>130.9 (d)</td>
<td>8.08 (1H, d, 9.1)</td>
</tr>
<tr>
<td>2’</td>
<td>136.4 (d)</td>
<td>6.90 (1H, d, 9.1)</td>
<td>135.5 (d)</td>
<td>6.92 (1H, d, 9.1)</td>
<td>115.1 (d)</td>
<td>6.88 (1H, d, 9.1)</td>
</tr>
<tr>
<td>3’</td>
<td>147.1 (s)</td>
<td>-</td>
<td>156.4 (s)</td>
<td>-</td>
<td>156.4 (s)</td>
<td>-</td>
</tr>
<tr>
<td>4’</td>
<td>116.4 (d)</td>
<td>6.90 (1H, d, 9.1)</td>
<td>116.6 (d)</td>
<td>6.92 (1H, d, 9.1)</td>
<td>115.1 (d)</td>
<td>6.88 (1H, d, 9.1)</td>
</tr>
<tr>
<td>5’</td>
<td>135.0 (d)</td>
<td>8.08 (1H, d, 9.1)</td>
<td>131.9 (d)</td>
<td>7.79 (1H, d, 9.1)</td>
<td>130.9 (d)</td>
<td>8.08 (1H, d, 9.1)</td>
</tr>
<tr>
<td>6’</td>
<td>103.6 (d)</td>
<td>5.4 (1H, d, 1.5)</td>
<td>100.9 (d)</td>
<td>5.45 (1H, d, 7.8)</td>
<td>101.4 (d)</td>
<td>5.31 (1H, d, 7.8)</td>
</tr>
<tr>
<td>1’’</td>
<td>72.2 (d)</td>
<td>3.69 (1H, d, 1.5, 3.4)</td>
<td>74.2 (d)</td>
<td>4.26 (1H, d, 7.8, 9.0)</td>
<td>70.6 (d)</td>
<td>3.43 (1H, d, 7.8, 10.4)</td>
</tr>
<tr>
<td>2’’</td>
<td>72.1 (d)</td>
<td>3.31 (1H, d, 3.4, 9.6)</td>
<td>76.4 (d)</td>
<td>3.37 (1H, t, 9.0)</td>
<td>74.2 (d)</td>
<td>3.38 (1H, t, 10.4)</td>
</tr>
<tr>
<td>3’’</td>
<td>72.3 (d)</td>
<td>3.28 (1H, t, 9.6)</td>
<td>69.9 (d)</td>
<td>3.23 (1H, t, 9.0)</td>
<td>69.9 (d)</td>
<td>3.08 (1H, t, 10.4)</td>
</tr>
<tr>
<td>4’’</td>
<td>72.0 (d)</td>
<td>4.18 (1H, dd, 5.9, 9.6)</td>
<td>77.5 (d)</td>
<td>3.17 (1H, dd, 5.4, 9.0)</td>
<td>76.4 (d)</td>
<td>3.26 (1H, dd, 2.2, 10.4)</td>
</tr>
<tr>
<td>5’’</td>
<td>17.8 (q)</td>
<td>0.91 (3H, d, 5.9)</td>
<td>60.8 (t)</td>
<td>3.56 (1H, dd, 5.2, 10.1)</td>
<td>66.9 (t)</td>
<td>3.69 (1H, dd, 5.8, 11.5)</td>
</tr>
<tr>
<td>6’’</td>
<td>100.8 (d)</td>
<td>4.37 (1H, dd, 1.4, 3.3)</td>
<td>71.8 (d)</td>
<td>3.27 (1H, dd, 3.3, 9.8)</td>
<td>70.4 (d)</td>
<td>3.24 (1H, t, 9.8)</td>
</tr>
</tbody>
</table>

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Compound 2 was obtained as a yellow powder. The HR-TOFMS spectrum showed [M-H] m/z 431.7157 [M-H]+ (calcd. C_{20}H_{24}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_{16}H_{13}O_{8} 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 anomic signal proton at δ_{H} 5.40 (1H, d, J=1.5 Hz) as well as with four oxygenated methines, resonating at δ_{H} 5.40 (1H, d, J=1.5 Hz), 3.69 (1H, dd, J=5.9, 9.6 Hz), 3.31 (1H, dd, J=3.4, 9.6 Hz), 3.28 (1H, J=9.6 Hz), which is typical for a rhamnose moiety. An anomic 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-rhamnopyranoside.

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 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 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 anomic proton H-1″ resonate at δ_{H} 5.90, J=4.5 Hz, the magnitude of the coupling constant J=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_{19}H_{14}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 anomic 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 presence 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).
The anomic 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” (δH 5.31) and kaempferol C-3 (δC 133.2), and glu. H-4” (δH 3.08) to rham. C-1” (δC 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 δH 5.31, J=7.8 Hz) was assigned for axial-axial position whereas proton H-1” at δH 5.35, J=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 activity 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 IC₅₀ 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-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 IC₅₀ values 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  IC₅₀ values of 1.18, 6.34, 8.17, 10.63 μg/mL, respectively.

Acknowledgement

This study was financially supported by Third World Academic Sciences (TWAS) for research grant No. 12-006 RG/CHE/AS G-UNESCO FR: 3240271335, 2013-2014 by US). We thank Dr. Ahmad Darmawan and Sofia Fajriah, M.Si at the Research Center for Chemistry, Indonesian Science Institute, for performing the NMR measurements. We are grateful to Uji Pratomo, M.Si., at the Center Laboratory of Universitas Padjadjaran for performing the HR-TOFMS measurements.

References


