3-20-2015

Flavonoid Compounds from the Bark of Aglaia eximia (Meliaceae)

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Sianturi, Julinton; Purnamasari, Mayshah; Mayanti, Tri; Harneti, Desi; Supratman, Unang; Awang, Khalijah; and Hayashi, Hideo (2015) "Flavonoid Compounds from the Bark of Aglaia eximia (Meliaceae)," *Makara Journal of Science*: Vol. 19 : Iss. 1 , Article 2.

DOI: 10.7454/mss.v19i1.4476

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This study was financially supported by the Directorate General of Higher Education (International Collaboration and International Scientific publication Grant, 2012–2013, by US and Third World Academic Sciences, 2014-2015, by US). We thank Dr. Ahmad Darmawan, and Sofa Fajriah, M.Sc., at the Research Center for Chemistry, Indonesian Science Institute, for performing the NMR measurements. We grateful to Mr. Uji Pratomo, M.Sc., at the Center Laboratory of Universitas Padjadjaran for performing the HR-TOFMS measurements.

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This article is available in Makara Journal of Science: https://scholarhub.ui.ac.id/science/vol19/iss1/2
Flavonoid Compounds from the Bark of *Aglaia eximia* (Meliaceae)

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**Abstract**

Three flavonoid compounds, kaempferol (1), kaempferol-3-O-α-L-rhamnoside (2), and kaempferol-3-O-β-D-glucosyl-α-L-rhamnoside (3), were isolated from the bark of *Aglaia eximia* (Meliaceae). The chemical structures of compounds 1–3 were identified with spectroscopic data, including UV, IR, NMR (¹H, ¹³C, DEPT 135°, HMQC, HMBC, ¹H-¹H-COSY NMR), and MS, as well as a compared with previously reported spectra data. All compounds were evaluated for their cytotoxic effects against P-388 murine leukemia cells. Compounds 1–3 showed cytotoxicity against P-388 murine leukemia cells with IC₅₀ values of 1.22, 42.92, and >100 mg/mL, respectively.

**Keywords:** *Aglaia eximia*, cytotoxic activity, flavonoid compound, Meliaceae

**Introduction**

The genus *Aglaia* is the largest genus of the family Meliaceae and comprises more than 105 species mainly distributed in the tropical rain forests of Southeast Asia [1]. Previous phytochemical studies on this genus have revealed the presence of various compounds with interesting biological activities, including flavaglines (cyclopenta[bc]benzofuran, cyclopenta[bh]benzopyrans, and benzo[b]oxepines) [2], bisamides [2,3], limonoids [4], sesquiterpenes [5], lignans [6], flavonoids [7], triterpenoids (baccharane, cycloartane, dammarane, glabratel, lupane, and tirucallane types) [8-10], and steroids (cholestanate, ergostanate, pregnane, and stigmastane types) [9-11]. Some of these compounds were shown to exhibit interesting pharmacological properties, including insecticidal [7], cytotoxic [8-9], antiviral [12], anticancer [13], antifungal [14], and anti-inflammatory [15].

*Aglaia eximia* is a higher plant and mainly distributed in the southern region of the island of Sumatera in Indonesia [16]. The plant’s bark is used in Indonesian folk medicine to reduce fever, moisturize the lungs, treat contusions, coughs, and skin diseases [16-17]. During the course of our continuing search for bioactive compounds in Indonesian medicinal plants, we isolated and described three flavonoid derivates, kaempferol (1),
kaempferol-3-O-a-L-rhamnoside (2), and kaempferol-3-O-beta-D-glucosyl-a-L-rhamnoside (3), from the ethyl acetate extract of the bark of A. eximia (Meliaceae).

Materials and Methods

Equipments. UV spectra were measured using a Shimadzu UV-160A ultraviolet-visible spectrometer, with MeOH. IR spectra were recorded with Perkin-Elmer 1760X FT-IR in KBr. 1H, 13C, DEPT 135°, HMQC, HMBC, and H-H-COSY NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as the internal standard. The mass spectra were obtained with a JEOL JNM-A-500 spectrometer using 1% DMSO as the internal standard. The mass spectra were measured by using a JEOL JNM-A-500 spectrometer using TMS as the internal standard. The mass spectra were measured by using a JEOL JNM-A-500 spectrometer using TMS as the internal standard.

Plant material. A. eximia (Meliaceae) bark was collected in the Bogor Botanical Garden, Bogor, West Java Province, Indonesia, in June 2011. The plant was identified by the staff of the Bogor Botanical Garden, Bogor, Indonesia, and a voucher specimen (No. Bogor Botanical Garden) was deposited at the herbarium.

Plant extraction. The bark (4 kg) was ground with a wood-grinding machine into powder (3.8 kg). n-hexane, EtOAc, and MeOH (distillation repeated) were extracted successively from the powdered bark at room temperature for 3 days and then filtered, decanted, and evaporated under vacuum. Evaporation resulted in crude extracts of n-hexane (26.4 g), EtOAc (54.5 g), and MeOH (32.5 g). The n-hexane, ethyl acetate, and methanol extracts exhibited cytotoxic activity against P-388 murine leukemia cells with IC50 values of 58, 23, and 40 mg/mL, respectively. The phytochemical test for the EtOAc extract showed the presence of flavonoids. A portion of the EtOAc extract (2.4 g) was subjected to column chromatography over silica gel using gradient 5% mixture of CHCl3 and Me2CO as the eluent (10:0–10:0) to provide 20 fractions (E01–E20). The E04–E05 fractions (13.9 mg) were combined and purified with preparative TLC on silica gel GF254 plates, eluted with CHCl3:MeOH (9:1), to give 1 (7.0 mg). The E11–E12 fractions (112.4 mg) were combined and subjected to silica gel column chromatography using gradient 1% mixture of CHCl3 and MeOH as the eluent (10:0–8:2) to afford 22 fractions (F01–F22). The F8–F21 fractions (74.6 mg) were combined and subjected to silica gel column chromatography using gradient 0.1% mixture of CHCl3 and MeOH as the eluent (10:0–9:1) to give 2 (18.6 mg). The F16–F19 fractions (296.6 mg) were combined and subjected to silica gel column chromatography using several gradient 10%, 5%, and 2.5% mixtures of CHCl3 and MeOH as eluent (10:0–0:10) to provide 23 fractions, G01–G23. The G6–G8 fractions (171.9 mg) were combined and subjected to silica gel column chromatography using several gradient 5%, 1%, and 0.5% mixtures of CHCl3 and MeOH as the eluent (10:0–7:3) to provide 20 fractions (H01–H20). The H6–H8 fractions (50 mg) were separated with preparative TLC on silica gel using an eluent of CHCl3:MeOH (9:1) to give 3 (20.3 mg).

Determination of cytotoxic activity. The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3 × 104 cells/cm². After 24 h of incubation for cell attachment and growth, various sample concentrations were added. The compounds added were first dissolved in DMSO at the required concentration. Then six concentrations were prepared using phosphate buffer solution (PBS, pH = 7.30–7.65). The control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding the MTT reagent [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazole blue], and the incubation was continued for another 4 h, during which the MTT-stop solution containing sodium dodecyl sulfate (SDS) was added and another 24 h incubation period was conducted. Optical density was read by using a microplate reader at 550 nm. The IC50 values were taken from the plotted graph of the percentage of live cells compared to control (%), which received only PBS and DMSO, versus the tested compound concentrations (mg/mL). The IC50 value is the concentration required for 50% growth inhibition. All assays and analyses were each run in triplicate and averaged.

Results and Discussion

The bark of A. eximia was ground, and then n-hexane, EtOAc, and MeOH were extracted. The EtOAc extract showed flavonoid compounds. Therefore, the phytochemical analysis focused on the ethyl acetate extract. The EtOAc extract was subjected to column chromatography over silica gel with a gradient elution of n-hexane-EtOAc-MeOH. The fractions were repeatedly subjected to column chromatography on silica gel (70–230 and 200–400 mesh) and preparative TLC on silica gel GF254 to provide three flavonoid compounds 1–3 (Figure 1).

Kaempferol (1), yellow powder. UV MeOH λmax nm (log ε) 367 (4.24), 257 (4.19); NaOH 398.4, 326.8; NaOAc 355.4, 302.2; NaOAc/H3BO3 353, 257; AlCl3 392.8, 350; AlCl3/HCl 391.6, 348.8; IR (KBr) νmax/cm3433.2 (O-H stretch), 1640.8 (Ring C=O stretch), 1478 (C=C ring stretch), 1163.7 (asymmetric C-O-C stretch), 1051.3 (symmetric C-O-C stretch), 825.1 (substituted benzene ring); 1H-NMR (CD3OD, 500 MHz), see Table 1; 13C-NMR (CD3OD, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) m/z 285.3912 [M-H], (calcd. for C15H10O6, m/z 286. 2487).
Kaempferol-3-O-α-L-rhamnose (2), yellow powder. UV MeOH λ_{max} nm (log ε) 349.4 (4.14), 268 (4.23); (intensity not decreased); NaOH 405.4, 324.5; NaOAc 362.4, 306.2; NaOAc/H_{2}BO_{3} 306.2, 268; AlCl_{3} 396.8, 353; AlCl_{3}/HCl 396.6, 348.4; IR (KBr) ν_{max} /cm^{-1} 3421 (O-H stretch), 2922 (C-H sp^{2} stretch), 1676 (Ring C=O stretch), 1205 (asymmetric C-O-C stretch) dan 823 (Benzene substituted); ^{1}H-NMR (CD_{3}OD, 500 MHz), see Table 1; ^{13}C-NMR (CD_{3}OD, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) m/z 431.5157 [M-H]^{-} (calculated for C_{21}H_{20}O_{10}, m/z 432.7118).

Kaempferol-3-O-β-D-glucosyl-α-L-rhamnose (3), yellow powder. UV MeOH λ_{max} nm (log ε) 348.8 (4.17), 266 (4.25); (intensity not decreased); NaOH 399.4, 326.4; NaOAc 359.8, 306.2; NaOAc/H_{2}BO_{3} 350.8, 266.2; AlCl_{3} 397.2, 350.8; AlCl_{3}/HCl 395.6, 347.2; IR (KBr) ν_{max}/cm^{-1} 3383.6 (O-H stretch), 2981.8 (C-H sp^{2} stretch), 1659.9 (Ring C=O stretch). 1509.2(C=O ring stretch), 1282.6 (asymmetric C-O-C stretch), 1266.3 (symmetric C-O), 1182.5 (C-H stretch), 1064.4 (symmetric C-O-C stretch), 838.1 (substituted benzene); ^{1}H-NMR (DMSO, 500 MHz); see Table 1; ^{13}C-NMR (DMSO, 125 MHz), see Table 1; HR-TOFMS (negative ion mode) m/z 594.1877 [M-H]^{-}, (calculated for C_{22}H_{22}O_{15}, m/z 594.5587).

Compound 1 was obtained as a yellow powder. The HR-TOFMS spectrum showed [M-H]^{-} m/z 285.3912 (calcd m/z 286.2847), which corresponded to the molecular formula of C_{13}H_{12}O_{6} and thus required 11 degrees of unsaturation, originating from seven C sp^{2}, one C=O, and the remaining tricyclic flavonoids. UV spectra using shift reagents showed the presence of 6-OH, 4′-OH, 3-OH, 7-OH, and 5-OH. The IR spectra showed absorption peaks at 3343.2, 1640.8, 1478, 1163.7, 1051.3, and 825.1/cm suggesting the presence of hydroxyl groups, carbonyl groups, C=C olefin rings, symmetric and asymmetric C-O-C as well as substituted benzene, respectively. The ^{1}H-NMR (CD_{3}OD 500 MHz) spectrum showed the presence of four methine groups, resonating at δ_{H} 8.08 (2H, d, J=9.1, H-2′; H-6′), 6.90 (2H, d, J=9.1, H-3′; H-5′), 6.36 (1H, d, J=2.0; H-8), 6.16 (1H, d, J=2.0; H-6). The characteristic AA′BB′ pattern of ring B was observed at δ_{H} 8.08 (d, 9.1) and 6.90 (d, 9.1) for H-2′/H-6′ and H-3′/H-5′, respectively. Two meta-protons of ring A resonated at δ_{H} 6.36 (d, 2.0), 6.16 (d, 2.0), for H-8 and H-6, respectively. Proton pairing was also confirmed with the ^{1}H-1H-COSY spectrum. The ^{13}C-NMR (CD_{3}OD 125 MHz) 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. 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.

A comparison of the NMR data of 1 with the data for kaempferol [19] revealed that the structures of the two compounds are very similar; compound 1 was identified as kaempferol.

Compound 2 was obtained as a yellow powder. The HR-TOFMS spectrum showed [M-H]^{-} m/z 431.5157, (calcd m/z 432.7118), which corresponded to the molecular formula of C_{21}H_{20}O_{10} and thus required 12 degrees of unsaturation, originating from seven C sp^{2}, one C=O, and the remaining tricyclic flavonoids and the rhamnoside ring. The ^{1}H-NMR spectrum showed the presence of six methines sp^{2}, resonating at δ_{H} 7.79 (2H, d, J=9.1, H-2′; H-6′), 6.92 (2H, d, J=9.1 H-3′; H-5′), 6.39 (1H, d, J=2.0, H-8), 6.19 (1H, d, J=2.0, H-6), one proton anomic, resonating at δ_{H} 5.4, (1H, d, J=1.5, H-1″), four methines oxygenated, resonating at δ_{H} 4.18 (1H, dd, J=5.9; 9.6, H-5″), 3.69 (1H, dd, J=1.5; 3.4, H-2″), 3.31 (1H, dd, J=3.4; 9.6, H-3″), 3.28 (1H, t, J=9.6, H-4″) and one methyl, resonating at δ_{H} 0.90 (3H, d, 5.9, H-6″) indicated the presence of rhamnose ring formed by substitution reaction of UDP-Rhamnose, catalyzed by enzyme of UDP-Rhamnose: kaempferol; 3-rhamnosyltransferase [20]. The ^{13}C-NMR and DEPT 135° spectra showed the presence of six methines sp^{2}, four oxygenated methines, nine quaternary carbons, one methyl, and one methine anomic at δ_{C} 103.6. Determination of stereochemistry 2 was established with the proton pairing coupling constant. The anomic proton, resonating at δ_{H} 5.4, “J=1.5 had an axial-equatorial position in H-1″ and H-2″ suggesting a α-l-rhamnose. The position of the rhamnose ring was confirmed with the HMBC spectrum, whereas the pairing of the rhamnose protons was confirmed with the ^{1}H-1H-COSY spectrum. The position of C-3 (136.3) has a correlation with the proton of H-1″. A comparison of the NMR data of 2 with the data for kaempferol-3-O-rhamnoside [19] revealed that the structures of the two compounds are very similar; therefore, 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 591.1877, (calculated for m/z 592.5587), which corresponded to the molecular formula of C_{28}H_{32}O_{14} and thus required 13 degrees of unsaturation, originating from seven C sp^2, one C=O, and the remaining tricyclic flavonoids, glucose, and the rhamnoside ring. The ^1H-NMR spectrum in DMSO of 3 showed signals for kaempferol, resonating at δ_H 6.19 (1H, d, 2.0), 6.40 (1H, d, 2.0), 7.98 (2H, d, 9.1), 6.88 (2H, d, 9.1) and anomic protons at 5.31 (1H, d, 7.8) and 5.34 (1H, d, 1.4) characteristic of glucose and rhamnose (Table 1), formed by oxidation reaction at methyl moiety of 2, continued with substitution reaction of UDPRhamnose, catalyzed by enzyme of UDPRhamnose; kaempferol; 3-rhamnosyltransferase [20]. From the coupling constants of the anomic protons on 3, the configuration at C-1'' (glucose ring) and C-1''' (rhamnose ring) was determined to be the β- and α-configuration, respectively. The ^13C-NMR spectra for 2 and 3 were similar, except for a signal corresponding to the ring of glucose and rhamnose (Table 1). Two characteristics of anomic carbons were observed at δ_C 101.4 and 100.8. When compared to the carbon signals of the glucosyl moiety [22], the glucose C-2", C-1" experienced lowfield and upfield as well as the C-5"; C-6", shift due to the α- and β-effects of rhamnosylation.

A comparison of the NMR data of 3 with those for kaempferol-3-O-β-d-glucosyl-α-L-rhamnose [19] revealed that the structures of the two compounds are very similar; therefore, compound 3 was identified as a kaempferol-3-O-β-d-glucosyl-α-L-rhamnose. Based on the comparison with the chemical shift of the corresponding carbons in kaempferol and their derivatives [19-20], the structures of 1, 2, and 3 were determined to be kaempferol (1), Kaempferol-3-O-α-L-rhamnoside (2), and kaempferol-3-O-β-d-glucosyl-α-L-rhamnose (3). This is the first report of flavonoid derivatives from various Aglaia species (Meliaceae). The cytotoxicity effects of the three isolated compounds 1–3 against P-388 murine leukemia cells were investigated according to the method described in previous papers [18,21], and artonin E (IC_{50} 0.3 mg/mL) was used as a positive control [23].

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Table 1. NMR Data for Compounds 1 (CD_{3}OD), 2 (CD_{3}OD), and 3 (DMSO)
The cytotoxic activity of isolated compounds 1–3 are shown in Table 2. Activity of kaempferol (1) was influenced by the hydroxyl group in rings A, B, and C that have a role as hydrogen bond donors that can improve the reactivity of 1. The hydroxyl group in aromatic ring can support their reactivity and corrosiveness. These results suggest that total sugar moieties in flavonoid skeleton can decrease cytotoxic activity.

Three known flavonoid compounds (1–3) have been isolated from the bark of Aglaia eximia (Meliaceae) and was found in this plant for the first time. Compounds 1–3, were evaluated for their cytotoxic activity against P-388 murine leukemia cells, in vitro. As a results indicated that the presence of a sugar moiety in flavonoid skeleton can decrease cytotoxic activity.

Acknowledgements

This study was financially supported by the Directorate General of Higher Education (International Collaboration and International Scientific publication Grant, 2012–2013, by US and Third World Academic Sciences, 2014-2015, by US). We thank Dr. Ahmad Darmawan, and Sofia Fajriah, M.Sc., at the Research Center for Chemistry, Indonesian Science Institute, for performing the NMR measurements. We grateful to Mr. Uji Pratomo, M.Sc., at the Center Laboratory of Universitas Padjadjaran for performing the HR-TOFMS measurements.

References


