Makara Journal of Science

Volume 19
Issue 1 *March*

Article 2

3-20-2015

Flavonoid Compounds from the Bark of Aglaia eximia (Meliaceae)

Julinton Sianturi
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia

Mayshah Purnamasari
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia

Tri Mayanti
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia

Desi Harneti
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia

Unang Supratman
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia, u_supratman@unpad.ac.id

See next page for additional authors

Flavonoid Compounds from the Bark of Aglaia eximia (Meliaceae)

Cover Page Footnote

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.

Authors

Julinton Sianturi, Mayshah Purnamasari, Tri Mayanti, Desi Harneti, Unang Supratman, Khalijah Awang, and Hideo Hayashi

Makara J. Sci. 19/1 (2015), 7-12
doi: 10.7454/mss.v19i1.4476

Flavonoid Compounds from the Bark of *Aglaia eximia* (Meliaceae)

Julinton Sianturi[1], Mayshah Purnamasari[1], Tri Mayanti[1], Desi Harneti[1], Unang Supratman[1*], Khalijah Awang[2], and Hideo Hayashi[3]

1. Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Sumedang, Indonesia
2. Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 59100, Malaysia
3. Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Gakuen-cho, Sakai, Osaka 599-8531, Japan

[*]*E-mail: u_supratman@unpad.ac.id*

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 (^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, ^{1}H-^{1}H-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 IC_{50} values of 1.22, 42.92, and >100 mg/mL, respectively.

Abstrak

Senyawa Flavonoid dari Kulit Batang *Aglaia eximia* (Meliaceae). Tiga senyawa flavonoid, kaempferol (**1**), kaempferol-3-*O*-α-L-ramnosida (**2**), dan kaempferol-3-*O*-β-D-glukosil-α-L-ramnosida (**3**), diisolasi dari batang *Aglaia eximia* (Meliaceae). Struktur kimia senyawa **1–3** diidentifikasi berdasarkan data spektroskopi, meliputi UV, IR, NMR (^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, ^{1}H-^{1}H-COSY NMR), dan MS, serta perbandingan dengan data spektra yang diperoleh sebelumnya. Seluruh senyawa dievaluasi pengaruh sitotoksiknya terhadap sel murine leukimia P-388. Senyawa **1-3** menunjukkan aktivitas sitotoksik terhadap sel murine leukimia P-388 dengan nilai IC_{50} berturut-turut 1,22; 42,92, dan >100 μg/mL.

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[*b*]benzofuran, cyclopenta[*bc*]benzopyrans, and benzo[*b*]oxepines) [2], bisamides [2,3], limonoids [4], sesquiterpenes [5], lignans [6], flavonoids [7], triterpenoids (baccharane, cycloartane, dammarane, glabretal, lupane, and tirucallane types) [8-10], and steroids (cholestane, ergostane, 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*-α-L-rhamnoside **(2**), and kaempferol-3-*O*-β-D-glucosyl-α-L-rhamnoside **(3)**, from the ethyl acetate extract of the bark of *A. eximia* (Meliaceae).

Materials and Methods

Equipments. UV spectra were measured by using a Shimadzu UV-160A ultraviolet-visible spectrometer, with MeOH. IR spectra were recorded with Perkin-Elmer 1760X FT-IR in KBr. ^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, and ^{1}H-^{1}H-COSY NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as the internal standard. The mass spectra were recorded with HR-ESI-TOFMS. Chromatographic separations were carried out on silica gel 60 (70–230 mesh and 230–400 mesh), TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm), and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating.

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 Bogoriense Herbarium, Bogor, Indonesia, and a voucher specimen (No. Bo-1295315) 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 IC_{50} 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 $CHCl_3$ and Me_2CO as the eluent (10:0–0:10) to provide 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). The E11–E12 fractions (112.4 mg) were combined and subjected to silica gel column chromatography using gradient 1% mixture of $CHCl_3$ and MeOH as the eluent (10:0–8:2) to afford 22 fractions (F01–F22). The F8–21 fractions (74.6 mg) were combined and subjected to silica gel column chromatography using gradient 0.1% mixture of $CHCl_3$ 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 $CHCl_3$ 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 $CHCl_3$ 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 $CHCl_3$: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 3×10^4 cells/cm^3. 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 IC_{50} 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 IC_{50} 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 GF_{254} to provide three flavonoid compounds **1–3** (Figure 1).

Kaempferol (1), yellow powder. UV MeOH λ_{max} nm (log ε) 367 (4.24), 257 (4.19); NaOH 398.4, 326.8; NaOAc 355.4, 302.2; NaOAc/H_3BO_3 353, 257; $AlCl_3$ 392.8, 350; $AlCl_3$/HCl 391.6, 348.8; IR (KBr) ν_{max}/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); ^{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* 285.3912 [M-H], (calcld. for $C_{15}H_{10}O_6$, *m/z* 286. 2487).

Kaempferol-3-*O*-α-L-rhamnoside (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_3BO_3 306.2, 268; $AlCl_3$ 396.8, 353; $AlCl_3$/HCl 396.6, 348.4; IR (KBr) ν_{max} /cm 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_3OD, 500 MHz), see Table 1; ^{13}C-NMR (CD_3OD, 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-rhamnoside (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_3BO_3 350.8, 266.2; $AlCl_3$ 397.2, 350.8; $AlCl_3$/HCl 395.6, 347.2; IR (KBr) ν_{max}/cm 3383.6 (O-H stretch), 2981.8 (C-H sp^2 stretch), 1659.9 (Ring C=O stretch). 1509.2(C=C 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 $C_{27}H_{30}O_{15}$, *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* 286.2487), which corresponded to the molecular formula of $C_{15}H_{10}O_6$ 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 3433.2, 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_3OD 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-^{1}H-COSY spectrum. The ^{13}C-NMR (CD_3OD 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.

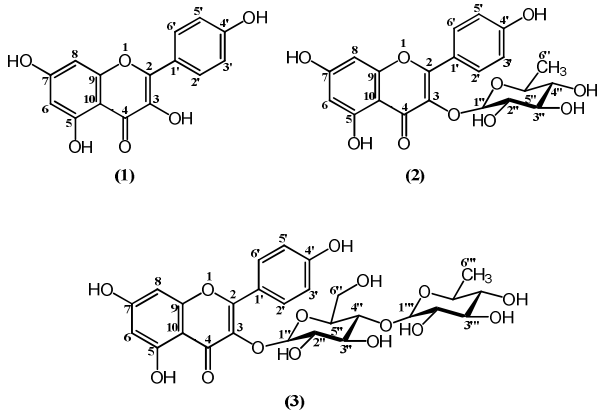

Figure 1 The Structure of Compounds 1–3

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 anomeric, 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*, 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 UDPRhamnose, catalyzed by enzyme of UDPRhamnose; 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 anomeric at δ_C 103.6. Determination of stereochemistry **2** was established with the proton pairing coupling constant. The anomeric proton, resonating at δ_H 5.4; ^3J=1.5 had an axial-equatorial position in H-1″ and H-2″ suggesting a α-L-rhamnoside. The position of the rhamnoside ring was confirmed with the HMBC spectrum, whereas the pairing of the rhamnose protons was confirmed with the ^{1}H-^{1}H-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 ^{1}H-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 anomeric protons at 5.31 (1H, *d*, 7.8) and 5.34 (1H, *d*, 1.4) characteristic of glucose and rhamnoside (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 anomeric 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 ^{13}C-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 anomeric 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-rhamnoside [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-rhamnoside. 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-rhamnoside **(3)**. This is the first report of flavonoid derivates 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].

Table 1. NMR Data for Compounds 1 (CD_3OD), 2 (CD_3OD), and 3 (DMSO)

Position	**1**		**2**		**3**	
	^{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)	-
3	136.7 (s)	-	136.3 (s)	-	133.2 (s)	-
4	176.7 (s)	-	179.7 (s)	-	177.5 (s)	-
5	162.4 (s)	-	163.3 (s)	-	161.2 (s)	-
6	99.2 (d)	6.16 (IH, *d*, 2.0)	99.9 (d)	6.19 (1H, *d*, 2.0)	98.8 (d)	6.19 (1H, *d*, 2.0)
7	165.2 (s)	-	165.9 (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)	6.40 (1H, *d*, 2.0)
9	157.8 (s)	-	159.4 (s)	-	156.9 (s)	-
10	104.2 (s)	-	106.0 (s)	-	103.9 (s)	-
1′	123.3 (s)	-	122.7 (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)	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)	115.2 (d)	6.88 (1H, *d*, 9.1)
4′	147.1 (s)	-	159.5 (s)	-	156.5 (s)	-
5′	116.4 (d)	6.90 (IH, *d*, 9.1)	116.6 (d)	6.92 (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)	7.98 (1H, *d*, 9.1)
1″			103.6 (d)	5.4 (1H, *d*, 1.5)	101.4 (d)	5.31 (1H, *d*, 7.8)
2″			72.2 (d)	3.69 (1H, *dd*, 1.5, 3.4)	70.6 (d)	3.43 (1H, *dd*, 7.8, 10.4)
3″			72.1 (d)	3.31 (1H, *dd*, 3.4, 9.6)	74.2 (d)	3.38 (1H, *t*, 10.4)
4″			73.3 (d)	3.28 (1H, *t*, 9.6)	69.9 (d)	3.08 (1H, *t*, 10.4)
5″			72.0 (d)	4.18 (1H, *dd*, 5.9, 9.6)	76.4 (d)	3.26 (1H, *ddd* 2.2, 5.8, 10.4)
6″			17.8 (q)	0.91 (3H, *d*, 5.9)	66.9 (t)	3.22 (1H, *dd*, 2.2, 11.5) 3.69 (1H, *dd*, 5.8, 11.5)
1‴					100.8 (d)	5.35 (1H, *d*, 1.4)
2‴					71.8 (d)	4.37 (1H, *dd*, 1.4, 3.3)
3‴					70.4 (d)	3.27 (1H, *dd*, 3.3, 9.8)
4‴					75.7 (d)	3.24 (1H, *t*, 9.8)
5‴					68.3 (d)	4.42 (1H, *dq*, 6.5, 9.8)
6‴					17.3 (q)	0.98 (3H, *d*, 6.5)

Mult: Multiplicity

Table 2. Cytotoxicity Activity of Compounds 1–3 Against P-388 Murine Leukemia Cells

Compounds	IC_{50} (mg/mL)
Kaempferol **(1)**	1.22 ± 0.02
Kaempferol-3-*O*-α-L-rhamnoside **(2)**	42,92 ± 0.12
Kaempferol-3-*O*-β-D-glucosyl-α-L-Rhamnoside **(3)**	>100 ± 0.01

The cytotoxicity 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 properties can improve the denaturating effect on membrane protein-forming pores that may lead to cell death, whereas the hydroxyl in the sugar ring for **2** and **3**, has a many chiral center can decrease of hydrogen bond donors [24] that affects their reactivity and corrosiveness. These results suggest that total sugar rings may lead to a decrease in the cytotoxic activity of the compound.

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 leukimia cells, *in vitro*. As a results indicated that the presence of a sugar moeity 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 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.

References

[1] Pannel, C.M. 1992. A taxonomic monograph of the genus *Aglaia* Lour. (Meliaceae). Kew Bulletin Additional Series XVI, Royal Botanic Gardens, Kew, London. pp. 359-362.

[2] Brader, G., Vajrodaya, S., Greger, H., Bacher, M., Kalchhauser, H., Hofer, O. 1998. Bisamides, lignans, triterpenes, and insecticidal cyclopenta[*b*]benzofurans from Aglaia species. J. Nat. Prod. 61(12): 1482-1490, http://dx.doi.org/10.1021/NP9801965.

[3] Chaidir., Lin, W.H., Ebel, R., Edrada, R., Way, V., Nimtz, M., Sumaryono, W., Proksch, P. 2001. Rocaglamides, glycosides, and putrescine bisamides from Aglaia dasyclada. J. Nat. Prod. 64 (9): 1216-1220, http://dx.doi.org/10.1021/ np0102354.

[4] Fuzzati, N., Dyatmiko, W., Rahman, A., Achmad, F., Hostettmann, K. 1996. Triterpenoids, lignans and a benzofuran derivative from the bark of Aglaia elaeagnoidea. Phytochemistry. 42(5): 1395-1398, http://dx.doi.org/10.1016/0031-9422(96)00130-6.

[5] Joycharat, N., Greger, H., Hofer, O., Saifah, E. 2008. Flavaglines and triterpenoids from the leaves of Aglaia forbesii. Phytochemistry. 69(1): 206-211, http://dx.doi.org/10.1016/j.phytochem. 2007.06.016.

[6] Wang, B., Ebel, R., Wang, C., Wray, V., Proksch, P. 2002. New methoxylated aryltetrahydro-naphtalene lignans and a norlignan from Aglaia cordata. Tetrahedron Lett 43(33): 5783-5787, http://dx.doi.org/10.1016/S0040-4039(02)01180-2.

[7] Greger, H., Pache, T., Brem, B., Bacher, M., Hofer, O. 2001. Insecticidal flavaglines and other compounds from Fijian Aglaia species. Phyto-chemistry 57(1): 57-64, http://dx.doi.org/10.1016/ S0031-9422(00)00471-4.

[8] Awang, K., Loong, X., Leong, K.H., Supratman, U., Litaudon, M., Mukhtar, M.R., Mohamad, K. 2012. Triterpenes and steroids from the leaves of *Aglaia exima* (Meliaceae). Fitoterapia 83(8): 1391-1395, http://dx.doi.org/10.1016/j.fitote.2012. 10.004.

[9] Harneti, D., Supriadin, A., Ulfah, M., Safari, A., Supratman, S., Awang, K., Hayashi, H. 2014. Cytotoxic constituents from the bark of Aglaia eximia (Meliaceae). Phytochemistry Lett. 8 pp. 28-31, http://dx.doi.org/10.1016/j.phytol.2014.01-005.

[10] Mohamad, K., Sèvenet, T., Dumontet, V., Pas, M., Tri, M.V., Hadi, H., Awang, K., Martin, M. 1999. Dammarane triterpenes and pregnane steroids from *Aglaia lawii* and *A. tomentosa*. Phytochemistry. 51(8): 1031-1037, http://dx. doi.org/10.1016/S0031-9422(99)00053-9.

[11] Rivero-Cruz, J.F., Chai, H.B., Kardono, L.B., Setyowati, F.M., Afriatini, J.J., Riswan, S., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Swanson, S.M., Kinghorn, A.D. 2004. Cytotoxic constituents of the twigs and leaves of *Aglaia rubiginosa*. J. Nat. Prod. 67(3): 343-347, http://dx.doi.org/10.1021/np0304417.

[12] Esimone, C.O., Eck, G., Nworu, C.S., Hoffmann, D., Uberla, K., Proksch, P. 2010. Dammarenolic acid, a secodammarane triterpenoid from Aglaia sp. shows potent anti-retroviral activity in vitro. Phytomedicine. 17(7): 540-547, http://dx.doi.org/ 10.1016/j.phymed.2009.10.015.

[13] Hwang, B.Y., Su, B.N., Chai, H., Mi, Q., Kardono, L.B., Afriastini, J.J., Riswan, S., Santarsiero, B.D., Mesecar, A.D., Wild, R., Fairchild, C.R., Vite, G.D., Rose, W.C., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M., Swanson, S.M., Kinghorn,

A.D. 2004. Silvestrol and episilvestrol, potential anticancer rocaglate derivatives from *Aglaia silvestris*. J. Org. Chem. 69(10): 3350-3358.

[14] Engelmeier, D., Hadacek, F., Pacher, T., Vajrodaya, S., Greger, H. 2000. Cyclopenta[*b*]benzofurans from *Aglaia* species with pronounced antifungal activity against rice blast fungus (*Pyricularia grisea*). J. Agric. Food. Chem. 48(4): 1400-1404, http://dx.doi.org/10.1021/jf990509h.

[15] Proksch, P., Giaisi, M., Treiber, M.K., Palfi, K., Merling, A., Spring, H., Krammer, P.H., Li-Weber, M. 2005. Rocaglamide derivatives are immuno-suppressive phytochemicals that target NF-AT activity in T cells. J. Immunol. 174: 7075-7084, http://dx.doi.org/10.4049/jimmunol.174.11.7075.

[16] Inada, A., Murata, H., Inatomi, Y., Darnaedi, D., Nakanishi, T. 1997. Pregnanes and triterpenoid hydroperoxides from the leaves of *Aglaia grandis*. Phytochemistry 45(6): 1225-1228, http://dx.doi.org/ 10.1002/chin.199744210.

[17] Mabberley, D. J., Pannel, C.M., Sing, A. M. 1995. Meliceae Flora Malesiana, vol 12. pp.1-407.

[18] Harneti, D., Tjokronegoro, R., Safari, A., Supratman, U., Loong, X.M., Mukhtar, M. R., Mohamad, K., Awang, K., Hayashi, H. 2012. Cytotoxic triterpenoids from the bark of *Aglaia smithii* (Meliaceae). Phytochemistry Lett. 5(3): 496-499. http://dx.doi.org/10.1016/j.phytol.2012-.04.013.

[19] Chaturvedula, V.S.P., Prakash, I. 2011. Kaempferol glycosides from *Siraitia grosvenorii*. J Chem Pharm Res. 3(6): 799-804.

[20] Dewick, P.M. 2009. Medicinal natural products a biosynthetic approach, 3rd ed. Jhon Wiley & Sons. Nottingham. pp. 170-175.

[21] Kazuma, K., Noda, N., Suzuki, M. 2003. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry. 62(2): 229-237. http://dx.doi.org/10.1016/S0031-9422(02) 00486-7

[22] Liu, Y., Peterson, D.A., Kimura, H., Schubert, D. 1997. Mechanism of cellular 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. J. Neurochem. 69(2): 581-593. http://dx. doi.org/10.1046/j.1471-4159.1997.69020581.x.

[23] Hakim, E.H., Achmad, S.A., Juliawaty, L.D., Makmur, L., Syah, Y.M., Aimi, A., Kitajima, M., Takayama, H., Ghisalberti, E.L. 2007. Prenylated flavonoids and related compounds of the Indonesian Artocarpus (Moraceae). J. Nat. Med. 61(2): 229-236.

[24] Struck, S., Scihmidt, U., Gruening, B., Jeger, I.S., Hossbach, J., Preissner, R. 2008. Toxicity versus potency: Elucidation of toxicity properties discriminating between toxins, drugs, and natural compounds. Genome Inform. International Conference on Genome Informatics. 20, 231-242.