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Cytotoxic Activity of Flavonols from *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg.

Aktivitas Sitotoksik Flavonol yang Diisolasi dari *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg.

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ABSTRACT

Two flavonols, glyasperin A (**1**), and meliternatin (**2**) has been isolated from the leaves of *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg. Extraction and isolation of flavonols were used methanol with the maceration method. The process of fractionation and purification used column chromatography and radial chromatography. The structure of both flavonols was determined by spectroscopic methods, including UV-Vis, IR, HRESIMS, 1D NMR (^{1}H, and ^{13}C-NMR) and 2D NMR (HMBC and HMQC). The cytotoxic activity of glyasperin A (**1**), and meliternatin (**2**) toward P-388 leukemia murine cells by MTT method, showing IC_{50} values 3.44 and 30.04 µg/mL, respectively**.**

Keywords: cytotoxic, flavonol, glyasperin A, meliternatin, *Macaranga gigantea*.

Introduction

Macaranga (Euphorbiaceae) is one of the pioneer plants that are found in secondary forests, especially those that get lots of suns. The genus *Macaranga* consists of 310 species, and in Indonesia, around 140 species are found. The spread of this plant is quite extensive, covering Africa to the tropical regions of Asia to the Pacific region (Blattner et al., 2001). This plant is widely used by the community as traditional medicines, among others, as medicine for wounds, infections, diarrhea, and coughs (Heyne, 1987). *Macaranga* produces phenolic compounds, especially flavonoids (Agustina et al., 2012, Tanjung et al., 2018, 2014) and stilbenoids (Aldin et al., 2019; Beutler et al., 1998; Tjahjandarie et al., 2019). Flavonoids and stilbenoids of *Macaranga* have terpenyl side chains

such as isoprenyl (C_5), geranyl (C_{10}), and farnesyl (C_{15}), which are attached to the aromatic nucleus. Flavonoids and stilbenoids of *Macaranga* show bioactivity as antimalarial, antioxidant, antimicrobial, anti-inflammatory, and anticancer (Pailee et al., 2015; Peresse et al., 2017; Magadula et al., 2014). On this occasion, two flavonols will be reported, namely glyasperin A **(1)**, and meliternatin **(2)** from the ethyl acetate extract of *Macaranga gigantea* (Rchb.f. & Zoll.) Müll.Arg. leaves. Phytochemical data on this plant is still very limited. It will also be reported to test the cytotoxic activity of the two flavonols toward P-388 murine leukemia cells using the MTT method.

Material and Methods

General procedure

Cerium sulfate reagent is used as a stain to show flavonoids compounds. Silica gel is used as a stationary phase in gravity column chromatography and radial chromatography. Thin layer chromatography analysis (TLC) using T25 silica gel 60 GF_{254} 0.25 mm (Merck) TLC plates. The UV spectrum was determined with a Shimadzu 1800 UV-Vis spectrophotometer. The IR spectrum was determined with the Shimadzu IR spectrophotometer. The mass spectrum was determined with the HRESIMS Merck Waters LCT XE ESI-TOF spectrometer, the NMR spectrum was determined by the NMR JEOL ECA 400 spectrophotometer operating at 400 MHz (^{1}H- NMR) and 100 MHz (^{13}C-NMR). Cytotoxic activity test against P-388 murine leukemia cells was determined using the MTT method.

Plant materials

Plant samples used in the study were *M. gigantea* leaves. Plant samples were obtained from the forest area of Jalan Samarinda-Sanga-Sanga, Palaran District, Samarinda, East Kalimantan. The identification of plant samples was carried out at the Bogoriensis Herbarium.

Experiments

1. Extraction and isolation

   Extraction of *M. gigantea* (2.5 kg) leaves using methanol at room temperature for 24 hours three times. Evaporation of the solvent using a low-pressure device produces crude methanol extract. The crude methanol extract, partitioned with n-hexane to remove chlorophyll and nonpolar compounds. The methanol extract was added with 10% v/v H_2O and partitioned with ethyl acetate. Evaporation of the solvent using a low-pressure device produces a crude EtOAc extract of 70 g. Separation of EtOAc extracts using gravity column chromatography using *n*-hexane: EtOAc (9: 1 to 3: 7) produces three main fractions, namely the A-C fraction. Fractions A and C show the presence of flavonoid compounds with cerium sulfate reagents characterized by brownish-yellow spots. The separation of fraction C by gravity column chromatography using a mixture of *n*-hexane: EtOAc (8: 2 and 1: 1) produced three subfractions, C_1-C_3. Separation of C_2

subfraction from the Sephadex column using methanol results in C_{21}-C_{23} subfraction. Purification of the C_{21} subfraction by radial chromatography with a mixture of *n*-hexane: acetone (9: 1 to 7: 3) resulted in 20 mg of meliternatin (**2**). Separation of fraction A by gravity column chromatography using hexane: EtOAc (9: 1 and 7: 3) produces three subfractions, namely A_1-A_3. Purification of the A_1 subfraction by radial chromatography using *n*-hexane: $CHCl_3$ (7: 3 to 100% $CHCl_3$) produced 45 mg glyasperin A (**1**) compounds.

2. Cytotoxic activity

Determination of the anticancer activity test of the flavonols (**1-2**) was determined using the MTT method *in vitro.* Cytotoxic activity of the isolated compounds **1**-**2** to P-388 murine leukemia cells was determined according to the MTT assay, as previously reported (Tanjung et al., 2018; Tjahjandarie et al., 2020).

Results and Discussion

The glyasperin A (**1**) compound is the result of isolation in the form of a yellow solid with a melting point of 164–166ºC, showing a quasi-molecular ion peak at $[M+H]^+$ *m/z* 421.6510 consistent to a chemical formulation of $C_{25}H_{27}O_6$ by high-resolution ESIMS spectrum. Glyasperin A (**1**) in MeOH, showing three maxima absorptions at λ_{max} nm (log ε) 253 (3.52); 270 (3.51), and 336 (3.58) possess of flavonol moiety (Tanjung et al., 2009). The IR spectrum of glyasperin A (**1**) in KBr, showing the functional group of conjugated carbonyl at 1649 cm^{-1}, hydroxy at 3350 cm^{-1}, and aromatic at1446 to 14502 cm^{-1}, respectively. The ^{1}H-NMR spectrum of glyasperin A (Table 1, $CDCl_3$) consists of two units of aromatic, a set of isoprenyl protons, and four protons of hydroxy. One aromatic proton signal in ring A showed at δ_H 6.47 (1H, *s,* H-8) and three aromatic proton signals of the ABX system in ring B showed at δ_H 6.93 (IH, *d*, *J* = 8.4 Hz, H-5′), δ_H 7.99 (1H, *dd*, *J* = 8.4 and 2.2 Hz, H-6′) and δ_H 8.00 (1H, *d*, *J* = 2.2 Hz, H-2′). The glyasperin A (**1**) compound also showed the presence of two proton units of isoprenyl consisting of two vinylic proton signals [δ_H 5.29 (1H, *t*, *J* = 7.2 Hz, H-2′′) and δ_H 5.36 (1H, *t*, *J* = 7.2 Hz, H-2′′′)], two methylenes [δ_H 3.45 (2H, *d*, *J* = 7.8 Hz, H-1′′′) and δ_H 3.47 (2H, *d*, *J* = 7.8 Hz, H-1′′), and four methyl protons [δ_H 1.78 (3H, *s,* H-4′′); δ_H 1.80 (3H, *s,* H-4′′′); δ_H 1.82 (3H, *s,* H-5′′), and δ_H 1.85 (3H, *s,* H-5′′′)]. Four hydroxy proton showed at δ_H 5.58 (1H, *s,* 4′-OH); δ_H 6.22 (1H, *s,* 7-OH); δ_H 6.57(1H, *s,* 3-OH), δ_H 12.12 (1H, *s,* 5-OH). The ^{13}C-NMR spectrum of glyasperin A (Table 1, $CDCl_3$), showing 25 carbon peaks that are completely separated. One carbonyl carbon (δ_C 175.3) and six oxycarbon signals (δ_C 135.5; δ_C 145.8; δ_C 155.1, δ_C 156.4; δ_C 157.8; δ_C 161.6) indicate compound **1** is a kaempferol derivative. The HMBC spectrum determined the location of the four hydroxy groups and two isoprenyl side chains in the kaempferol skeleton. The HMBC spectrum showed related to a hydroxy

proton at δ_H 12.12 (5-OH) to C-4a (δ_C 103.6), C-5 (δ_C 157.8), and C-6 (δ_C 109.3). The methylene of isoprenyl at δ_H 3.47 (H-1˝) related to C-5, C-6, C-7 (δ_C 161.6), C-2˝ (δ_C 121.1), and C-3˝ (δ_C 136.3), indicating the isoprenyl side chain was attached to C-6. A hydroxy proton at δ_H 6.22 (7-OH) showed correlations to C-6, C-7, and C-8 (δ_C 94.3), supporting an isoprenyl at C-6. An aromatic proton at δ_H 7.99 (H-6′) related to C-3′ (δ_C 127.1), C-4′ (δ_C 156.4), and C-1˝˝ (δ_C 30.2), and a methylene proton at δ_H 3.45 (H-1˝˝) related to C-2′ (δ_C 127.7), C-3′, C-4′, C-2˝˝ (δ_C 121.3), and C-3˝˝ (δ_C 135.8), indicating the others isoprenyl side chain was attached to C-3′. Based on the above spectroscopic data, the chemical structure of the isolated compound is glyasperin A (Tanjung et al., 2009). The relation between the proton signal and the carbon signal, supporting the structure of the glyasperin A compound, can be seen in Figure 1 and Table 1.

Table 1. NMR spectrum of glyasperin A in $CDCl_3$

No. C	δ_H (mult, *J* in Hz)	δ_C	HMBC
2	-	145.8	-
3	-	135.5	-
4	-	175.3	-
4a	-	103.6	-
5	-	157.8	-
6	-	109.3	-
7	-	161.6	-
8	6.47 (*s*)	94.3	C-4a, C-6, C-7, C-8a
8a	-	155.1	-
1'	-	123.4	-
2'	8.00 (*d*, 2.2)	127.7	C-4', C-6'
3'	-	127.1	-
4'	-	156.4	-
5'	6.93 (*d*, 8.4)	116.1	C-1', C-3'
6'	7.99 (*dd*, 8.4; 2.2)	129.8	C-3', C-4'
1''	3.47 (*d*, 7.8)	21.5	C-5, C-6, C-7, C-2'', C-3''
2''	5.29 (*t*, 7.2)	121.1	C-1'', C-4'', C-5''
3''	-	136.3	-
4''	1.78 (*s*)	26.0	C-2'', C-3'', C-5''
5''	1.82 (*s*)	18.1	C-2'', C-3'', C-4''
1'''	3.45 (*d*, 7.8)	30.2	C-2', C-3', C-4', C-2''', C-3'''
2'''	5.36 (*t*, 7.2)	121.3	C-1''', C-4''', C-5'''
3'''	-	135.8	-
4'''	1.80 (*s*)	25.9	C-2''', C-3''', C-5'''
5'''	1.82 (*s*)	18.0	C-2''', C-3''', C-4'''
3-OH	6.57 (*s*)	-	C-2, C-3, C-4
5-OH	12.12 (*s*)	-	C-4a, C-5, C-6
7-OH	6.22 (*s*)	-	C-6. C-7, C-8
4'-OH	5.58 (*s*)	-	C-3', C-4', C-5'

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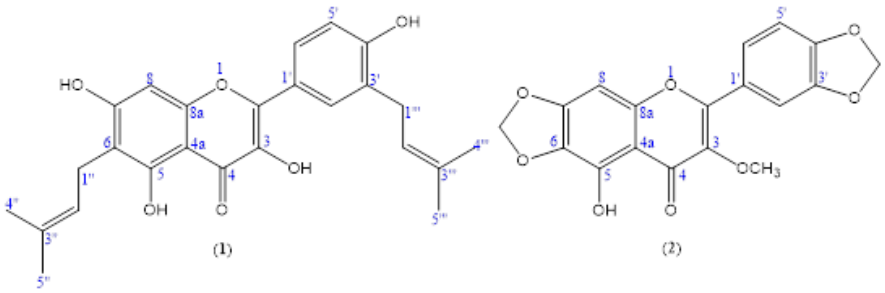

Figure 1. Structures of glyasperin A and meliternatin

The meliternatin (**2**) compound is the result of isolation in the form of a yellow solid with a melting point of 196–197ºC, showing a quasi-molecular ion peak at $[M+H]^+$ *m/z* 371.0769 consistent to a chemical formulation of $C_{19}H_{14}O_8$ by high-resolution ESIMS spectrum. The UV spectrum (λ_{max} nm) (log ε) 247 (4.22); 270 (4.08) and 336 (4.34), and IR spectrum (ν (cm^{-1}): 1641, 1502) alike to **1**. The ^{1}H-NMR spectrum of meliternatin (Table 2, $CDCl_3$) consists of two units of aromatic, a set of methylenedioxy, and two protons of methoxy. An aromatic proton in ring A, showing at δ_H 6.65 (1H, *s,* H-8), and the protons of ABX system in ring B, showing at δ_H 6.91 (IH, *d*, *J* = 8.4 Hz, H-5′), δ_H 7.63 (1H, *dd*, *J* = 8.4 and 1.8 Hz, H-6′) and δ_H 7.56 (1H, *d*, *J* = 1.8 Hz, H-2′). The meliternatin (**2**) compound also showed the presence of a set of methylenedioxy [δ_H 6.04 (2H, *s*, 6-O-CH_2-O-7) and δ_H 6.05 (2H, *s*, 3′-O-CH_2-O-4′)], and two proton of methoxy [δ_H 3.86 (3H, *s*, 3-OCH_3) and δ_H 4.12 (3H, *s*, 5-OCH_3)]. The ^{13}C-NMR spectrum of meliternatin (Table 2, $CDCl_3$), showing 19 carbon peaks that are completely separated. One carbonyl carbon (δ_C 174.0) and seven oxycarbon signals (δ_C 134.8, δ_C 140.8; δ_C 141.1; δ_C 147.9; δ_C 149.4; δ_C 152.6; δ_C 153.0;) indicate compound **2** is a quercetin derivative (Saputri et al., 2018). The HMBC spectrum determined the location of the two methoxy groups and two methylenedioxy in the quercetin skeleton. The HMBC spectrum showed related to an aromatic at δ_H 6.65 (H-8) to C-4a (δ_C 113.1), C-6 (δ_C 134.8), C-7 (δ_C 153.0), and C-8a (δ_C 153.7). The proton of methylenedioxy at δ_H 6.04 (6-O-CH_2-O-7) related to C-6, and C-7, indicating the methylenedioxy was fused at C-6, and C-7. An aromatic proton at δ_H 7.63 (H-6′) related to C-2 (δ_C 152.6), C-3′ (δ_C 147.9), C-4′ (δ_C 149.4), and C-6′ (δ_C 123.1), and methylenedioxy proton at δ_H 6.05 (3′-O-CH_2-O-4′) related to C-3′, and C-4′, indicating the others methylenedioxy was fused to C-3′, and C-4′. Based on the above spectroscopic data, the chemical structure of the isolated compound is meliternatin (Saputri et al., 2018). The relation between the proton signal and the carbon signal, supporting the structure of the meliternatin compound, can be seen in Figure 2 and Table 2.

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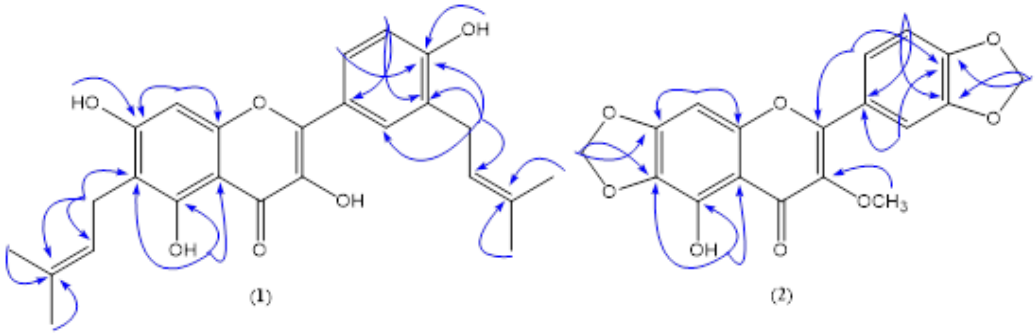

Figure 2. HMBC selected of glyasperin A and meliternatin

Table 2. NMR spectrum of meliternatin in $CDCl_3$

No. C	δ_H (mult, *J* in Hz)	δ_C	HMBC
2	-	152.6	-
3	-	140.8	-
4	-	174.0	-
4a	-	113.1	-
5	-	141.1	-
6	-	134.8	-
7	-	153.0	-
8	6.65 (*s*)	93.0	C-4a, C-6, C-7, C-8a
8a	-	153.7	-
1'	-	124.5	-
2'	7.56 (*d*, 1.8)	108.4	C-1', C-3', C-4', C-6'
3'	-	147.9	-
4'	-	149.4	-
5'	6.91 (*d*, 8.4)	108.5	C-1', C-3'
6'	7.63 (*dd*, 8.4; 1.8)	123.1	C-2, C-3', C-4', C-5'
3-OCH_3	3.86 (*s*)	59.9	C-3
5-OCH_3	4.12 (*s*)	61.3	C-5
6,7-OCH_2-O	6.04 (*s*)	101.7	C-6, C-7
3',4'-OCH_2-O	6.05 (*s*)	102.2	C-3', C-4'

The cytotoxic activity of glyasperin A (**1**) to P-388 leukemia murine cells by MTT method, showing moderate activity with IC_{50} values 3.44 µg/mL. The meliternatin (**2**), showing IC_{50} values 30.04 µg/mL, and inactive activity.

Conclusion

Two flavonol derivates, glyasperin A and meliternatin, were isolated from the leaves of *M. gigantea.* Glyasperin showed moderate activity to P-388 cells, meliternatin was inactive.

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