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 (¹H, and ¹³C-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 IC₅₀ 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 (¹H-NMR) and 100 MHz (¹³C-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 H₂O 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, C₁-C₃. Separation of C₂
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 melaternatin (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 (Tanjug 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 164–166°C, showing a quasi-molecular ion peak at [M+H]^+ m/z 421.6510 consistent to a chemical formulation of C_{25}H_{22}O_{8} by high-resolution ESIMS spectrum. Glyasperin A (1) in MeOH, showing three maxima absorptions at \( \lambda_{\text{max}} \) nm (log \( \varepsilon \)) 253 (3.52); 270 (3.51), and 336 (3.58) possess of flavonol moiety (Tanjug 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 at 1446 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 \( \delta_H \) 6.47 (1H, s, H-8) and three aromatic proton signals of the ABX system in ring B showed at \( \delta_H \) 6.93 (1H, d, \( J = 8.4 \) Hz, H-5’), \( \delta_H \) 7.99 (1H, dd, \( J = 8.4 \) and 2.2 Hz, H-6’), and \( \delta_H \) 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 [\( \delta_H \) 5.29 (1H, t, \( J = 7.2 \) Hz, H-2‘')] and \( \delta_H \) 5.36 (1H, t, \( J = 7.2 \) Hz, H-2‘‘)]. Two methylenes [\( \delta_H \) 3.45 (2H, d, \( J = 7.8 \) Hz, H-1‘‘‘')] and \( \delta_H \) 3.47 (2H, \( J = 7.8 \) Hz, H-1‘‘‘‘), and four methyl protons [\( \delta_H \) 1.78 (3H, s, H-4‘‘‘‘); \( \delta_H \) 1.80 (3H, s, H-4‘‘’‘); \( \delta_H \) 1.82 (3H, s, H-5‘‘‘‘), and \( \delta_H \) 1.85 (3H, s, H-5‘‘’‘)]. Four hydroxy proton showed at \( \delta_H \) 5.58 (1H, s, 4‘-OH); \( \delta_H \) 6.22 (1H, s, 7-OH); \( \delta_H \) 6.57(1H, s, 3-OH), \( \delta_H \) 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 (\( \delta_C \) 175.3) and six oxycarbon signals (\( \delta_C \) 135.5; \( \delta_C \) 145.8; \( \delta_C \) 155.1, \( \delta_C \) 156.4; \( \delta_C \) 157.8; \( \delta_C \) 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 δH 12.12 (5-OH) to C-4a (δC 103.6), C-5 (δC 157.8), and C-6 (δC 109.3). The methylene of isoprenyl at δH 3.47 (H-1′′) related to C-5, C-6, C-7 (δC 161.6), C-2′ (δC 121.1), and C-3′ (δC 136.3), indicating the isoprenyl side chain was attached to C-6. A hydroxy proton at δH 6.22 (7-OH) showed correlations to C-6, C-7, and C-8 (δC 94.3), supporting an isoprenyl at C-6. An aromatic proton at δH 7.99 (H-6′) related to C-3′ (δC 127.1), C-4′ (δC 156.4), and C-1′′′ (δC 30.2), and a methylene proton at δH 3.45 (H-1′′′) related to C-2′ (δC 127.7), C-3′, C-4′, C-2′′ (δC 121.3), and C-3′′′ (δC 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 CDCl3

| No. | C      | δH (mult, J in Hz) | δC   | 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′  |
The melternatin (2) compound is the result of isolation in the form of a yellow solid with a melting point of 196–197°C, showing a quasi-molecular ion peak at [M+H]+ m/z 371.0769 consistent to a chemical formulation of C_{30}H_{34}O_{8} by high-resolution ESI MS 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 showed related to an aromatic at δ_{H} 6.65 (H-8) to C-4a (δ_{C} 134.8), C-6 (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 melternatin (Saputri et al., 2018). The relation between the proton signal and the carbon signal, supporting the structure of the melternatin compound, can be seen in Figure 2 and Table 2.

Figure 1. Structures of glyasperin A and melternatin
Figure 2. HMBC selected of glyasperin A and meliternatin

Table 2. NMR spectrum of meliternatin in CDCl₃

| No. | C  | δₙ (mult, J in Hz) | δₖ | 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-OCH₃ |    | 3.86 (s)         | 59.9  | C-3  |
| 5-OCH₃ |    | 4.12 (s)         | 61.3  | C-5  |
| 6,7-OCH₂-O |    | 6.04 (s)         | 101.7 | C-6, C-7 |
| 3',4'-OCH₂-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 IC₅₀ values 3.44 µg/mL. The meliternatin (2), showing IC₅₀ 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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