Meliglabrin, A New Flavonol Derivative from the leaves of Melicope glabra (Blume) T.G. Hartley

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Abstract – A new flavonol derivative, meliglabrin (1) along with three known flavonols, ternatin (2), meliternatin (3), and 5,4′-dihydroxy-3,7,3′-trimethoxyflavon (4) were isolated from the leaves of Melicope glabra (Blume) T.G. Hartley. Their structures were determined using extensive spectroscopic methods, including UV, IR, HRESIMS, 1D and 2D NMR. Compounds 1 - 4 were evaluated for their cytotoxicity against murine leukemia P-388 cells, compound 4 showed moderate activity.

Keywords – Meliglabrin; flavonol, Melicope glabra, P-388 cells

Introduction

Melicope glabra (Blume) T.G. Hartley locally known as ‘Ki Sampang’ belongs to the Rutaceae family found in all of Indonesia Island. The aqueous decoction of leaves of M. glabra are used in Indonesia as traditional medicine for the treatment of fever, infections, and cough. The Melicope genus has been shown to be prolific a number of secondary metabolites, particularly alkaloids, flavonoids, coumarins and showed biological activities such as anticancer, antifungal and antioxidant.

The phytochemical survey from the bark of M. glabra was isolated coumarins and lignan but the leaves until now has not been reported. In this paper, we wish to report the isolation and structural elucidation of a new flavonol, meliglabrin (1) along with three known compounds, ternatin (2), meliternatin (3), and 5,4′-dihydroxy-3,7,3′-trimethoxyflavon (4) from the leaves of M. glabra. The cytotoxic activity of compounds 1 - 4 against murine leukemia P-388 cells from this plant are also reported.

Experimental

General experimental procedures – UV spectra were measured with a Shimadzu 1800 spectrometer, FTIR spectrum One Perkin-Elmer instrument, respectively. 1H and 13C NMR spectra were recorded with a JEOL ECA 400 spectrometer operating at 400 (1H) and 100 (13C) MHz in CDCl3. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, [M-H]− negative ion mode. Vacuum liquid chromatography (VLC) and planar radial chromatography were carried out using Si gel 60 GF254 and Si gel 60 PF254, for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF254, 0.25 mm thickness) were used.

Plant materials – The leaves of M. glabra were collected in March 2017 from Gunung Salak, Bogor, West Java, Indonesia. The plant material was identified by Mr. Ismail Rachman from the Herbarium Bogoriense, Bogor. A voucher specimen (PL 60325) was deposited in Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and isolation – The powdered and dried leaves of M. glabra (1.7 kg) were macerated in methanol at room temperature two times and, after evaporation of the methanol extract, gave a dark residue (210 g). The extract was redissolved in MeOH-water (9:1) and partitioned with n-hexane (95 g) and ethyl acetate (30 g) fractions. The ethylacetate extract (29 g) was further fractionated by vacuum liquid chromatography on silica gel (150 g) eluted with n-hexane-ethyl acetate of increasing polarity (9:1, 4:1, 7:3, 1:1, and 1:4) to give three major fractions A-C. Fraction A (4.68 g) was separated by column chromatography eluted with n-hexane-ethyl acetate (9:1 to 7:3) to produce subfractions A1-A3. Subfraction A1 was...
was purified by planar radial chromatography using n-hexane-CHCl₃ (from 4:1 to 1:4) to yield compound 1 (20 mg), 3 (15 mg), and 4 (23 mg). Fraction B (13 g) was refractionated using column chromatography and eluted n-hexane-ethyl acetate (from 8:2 to 3:7) to produce subfractions B₁-B₃. Subfraction B₁ was purified by planar radial chromatography using n-hexane-acetone (from 9:1 to 1:1) to yield compound 2 (9 mg).

**Meliglabin (1)** – Yellow solid, mp. 119 - 121 °C, UV (MeOH) \( \lambda_{\text{max}} \) nm (log e) : 245 (4.20), 255 (3.99), 296 (4.01) and 344 (4.20). IR (KBr) \( \nu_{\text{max}} \) cm⁻¹: 3421, 1645, 1560, 1481 and 1132. \(^1\)H and \(^13\)C NMR see Table 1. HRESIMS: \( m/z \) [M-H] calcd. for C₁₅H₁₈O₃ 357.0610, found 357.0613.

**Ternatin (2)** – Yellow solid. \(^1\)H NMR (CDCl₃, 400 MHz): \( \delta_H \) 12.44 (1H, s, 5-OH), 7.79 (1H, dd, \( J = 9.1, 2.0 \) Hz, H-6'), 7.78 (1H, d, \( J = 2.0 \) Hz, H-2'), 7.06 (1H, d, \( J = 9.1 \) Hz, H-5'), 6.42 (1H, s, H-6), 6.01 (1H, s, 4'-OH), 3.98 (3H, s, 3'-OCH₃), 3.94 (3H, s, 7-OCH₃), 3.92 (3H, s, 8-OCH₃), 3.87 (3H, s, 3-OCH₃). \(^13\)C NMR (CDCl₃, 100 MHz): \( \delta_C \) 179.1 (C-4), 158.4 (C-7), 157.4 (C-5), 155.8 (C-2), 148.5 (C-8a/4'), 146.4 (C-3'), 139.4 (C-3), 122.9 (C-6'), 128.8 (C-8), 122.7 (C-1'), 114.8 (C-5'), 110.8 (C-2'), 105.4 (C-4a), 95.6 (C-6), 61.7 (8-OCH₃), 60.2 (3-OCH₃), 56.5 (7-OCH₃), 56.1 (3'-OCH₃). HRESIMS: \( m/z \) [M-H] calcd. for C₁₅H₁₈O₈ 373.1916, found 373.1912.

The \(^1\)H and \(^13\)C NMR spectral data are consistent with published data.\(^8\)

**Meliternatin (3)** – Pale white solid, mp. 167 - 169 °C. UV (MeOH) \( \lambda_{\text{max}} \) nm (log e) : 247 (4.22), 270 (4.08) and 336 (4.34). IR (KBr) \( \nu_{\text{max}} \) cm⁻¹: 1641, 1524, 1479 and 1114. \(^1\)H NMR (CDCl₃, 400 MHz): \( \delta_H \) 7.63 (1H, dd, \( J = 8.4, 1.8 \) Hz, H-6'), 7.56 (1H, d, \( J = 1.8 \) Hz, H-2'), 6.91 (1H, d, \( J = 8.4 \) Hz, H-5'), 6.65 (1H, s, H-8), 6.05 (2H, s, 3',4'-OCH₃-O), 6.04 (2H, s, 6,7-OCH₂-O), 4.12 (3H, s, 5-OCH₃), 3.86 (3H, s, 3-OCH₃). \(^13\)C NMR (CDCl₃, 100 MHz): \( \delta_C \) 174.0 (C-4), 153.7 (C-7), 153.7 (C-8a), 153.0 (C-4'), 152.6 (C-2'), 149.4 (C-3'), 141.1 (C-5), 140.8 (C-3), 134.8 (C-6), 124.5 (C-1'), 123.1 (C-6'), 113.1 (C-4a), 108.5 (C-5'; 3',4'-OCH₂-O), 108.4 (C-2'; 6,7-OCH₂-O), 93.0 (C-8), 61.3 (5-OCH₃), 59.9 (3-OCH₃). HRESIMS: \( m/z \) [M-H] calcd. for C₁₅H₁₈O₈ 373.0613, found 373.0613. The \(^1\)H and \(^13\)C NMR spectral data are consistent with published data.\(^9\)

**5,4'-Dihydroxy-3,7,3'-trimethoxyflavon (4)** – Yellow solid, \(^1\)H NMR (acetone-\( d₆\), 400 MHz): \( \delta_H \) 12.72 (1H, s, 5-OH), 8.64 (1H, s, 4'-OH), 7.75 (1H, d, \( J = 2.0 \) Hz, H-2'), 7.67 (1H, dd, \( J = 8.4, 2.0 \) Hz, H-6'), 6.97 (1H, d, \( J = 8.4 \) Hz, H-5'), 6.62 (1H, d, \( J = 2.4 \) Hz, H-8), 6.27 (1H, d, \( J = 2.4 \) Hz, H-6), 3.91 (3H, s, 3'-OCH₃), 3.87 (3H, s, 7-OCH₃), 3.86 (3H, s, 3-OCH₃). \(^13\)C NMR (acetone-\( d₆\), 100 MHz): \( \delta_C \) 179.5 (C-4), 166.5 (C-7), 162.7 (C-5'), 157.6 (C-6), 153.1 (C-3), 137.1 (C-3'), 117.8 (C-5'), 116.8 (C-4'), 105.4 (C-4a), 95.5 (C-6), 61.3 (8-OCH₃), 60.1 (3-OCH₃), 56.3 (7-OCH₃), 56.1 (3'-OCH₃). HRESIMS: \( m/z \) [M-H] calcd. for C₁₅H₁₈O₈ 373.0613, found 373.0613. The \(^1\)H and \(^13\)C NMR spectral data are consistent with published data.\(^9\)

| No.C | \( \delta_H \) (mult, \( J \) in Hz) | \( \delta_C \) | HMBC |
|------|----------------------------------|---------|------|
| 2    | -                                | 155.8   | -    |
| 3    | -                                | 138.2   | -    |
| 4    | -                                | 179.3   | -    |
| 4a   | -                                | 106.3   | -    |
| 5    | -                                | 151.8   | -    |
| 6    | -                                | 130.1   | -    |
| 7    | -                                | 155.1   | -    |
| 8    | 6.54 (s, 1H)                     | 93.2    | C-4a, C-6, C-7, C-8a |
| 8a   | -                                | 153.9   | -    |
| 1'   | -                                | 124.2   | -    |
| 2'   | 7.59 (d, 1.8, 1H)                | 108.7   | C-2, C-4', C-6' |
| 4'   | -                                | 149.7   | -    |
| 5'   | 6.95 (d, 8.4, 1H)                | 108.6   | C-1', C-3' |
| 6'   | 7.68 (dd, 8.4; 1.8, 1H)          | 123.8   | C-2, C-2', C-4' |
| 5-OH | 12.88 (s, 1H)                    | -       | C-4a, C-5, C-6 |
| 7-OH | 6.50 (s, 1H)                     | -       | C-7, C-8 |
| 3-OCH₃| 3.85 (s, 3H)                    | 61.0    | C-3 |
| 6-OCH₃| 4.04 (s, 3H)                    | 60.3    | C-6 |
| 3',4'-OCH₂-O-| 6.08 (s, 2H) | 101.8  | C-3', C-4' |
Cytotoxic activity – All isolated compounds (1-4) were subjected to cytotoxic evaluation against murine leukemia P-388 cells according to the MTT method with artonin E as the positive control.11-12 The P-388 cells were seeded into each 96-well cell culture plate at a density of 3 x 10^4 cells/well and incubated at 37 °C for 48 h. The number of cells that inhibited by each of compounds 1-4 were measured using microplate reader spectrometer at λ 540 nm after incubation for 24 hours in CO_2 incubator at 37 ºC. All of isolated compounds by variations in concentration of 1000; 100; 30; 10; 3; 1; 0.3 and 0.1 μg/mL with triplicate treatment tested on cell cultures murine leukemia P-388. The IC_{50} value can be calculated through extrapolation 50% absorption lines to various concentrations of each compound using regression analysis.

Result and Discussion

Compound (1) was isolated as yellow solid, mp. 119 - 121 ºC. The HRESIMS displayed a negative molecular ion peak [M-H]^{−} at m/z 357.0613 (calcd. 357.0610) indicating a molecular formula of C_{18}H_{14}O_{8}. The UV maximum absorption at λ_{max} 245 (4.20), 255 (3.99), 296 (4.01) and 344 (4.20) nm typical for a flavonol chromophore. The IR spectrum indicated absorptions for hydroxyl (3421 cm^{−1}), conjugated carbonyl (1645 cm^{−1}), aromatic (1560 - 1481 cm^{−1}) and ether (1132 cm^{−1}) groups, respectively.13 The 1H NMR (Table 1) spectrum of 1 showed an ABX system at δ_{H} 7.68 (1H, dd, J = 8.4; 1.8 Hz, H-6′), 7.59 (1H, d, J = 1.8 Hz, H-2′), 6.95 (1H, d, J = 8.4 Hz, H-5′), and a singlet at δ_{H} 6.54 (1H, s, H-8) in the aromatic region. The 1H NMR spectrum of 1 also showed a chelated hydroxyl group at δ_{H} 12.88 (1H, s, 5-OH), a hydroxyl signals at δ_{H} 6.50 (1H, s, 7-OH), two methoxyls at δ_{H} 4.04 (3H, s, 6-OCH_{3}), 3.85 (3H, s, 3-OCH_{3}), and a methylenedioxy at δ_{H} 6.08 (2H, s, 3′,4′-OCH_{2}-O). Eighteen carbon signals were observed by 13C NMR spectrum. Two of them signals at δ_{C} 138.2 and δ_{C} 179.3 are characteristic for C-3 and C-4 of a flavonol structure.12 The placement of hydroxyl, methoxyl and methylenedioxy groups in flavonol structure was established by HMBC spectra (Fig. 2). The proton signal of a chelated hydroxyl group (δ_{H} 12.33, 5-OH) correlated with three quaternary carbons [δ_{C} 151.8 (C-5); 130.1 (C-6); 106.3 (C-4a)]. The proton signal of methoxyl group at δ_{H} 4.04 correlated to δ_{C} 130.1 (C-6) showing that a methoxyl group was placed at C-6. A hydroxyl proton signal at δ_{H} 6.50 (7-OH) correlated with one quaternary carbon signal δ_{C} 155.1 (C-7), and one methine carbon signal δ_{C} 93.2 (C-8) indicating that a hydroxyl group was placed at C-7. The aromatic proton signal (δ_{H} 6.54, H-8) showed long-range correlations with four quaternary carbons [δ_{C} 155.1 (C-7), 153.9 (C-8a)], 130.1 (C-6); 106.3 (C-4a)]. In the 1H NMR spectrum, proton signal of an ABX system in the aromatic region at ring B indicated a methylenedioxy group fused at C-3′ and C-4′. Therefore, another methoxyl group was placed at C-3. The proton signal of methoxyl group at δ_{H} 3.85 correlated to δ_{C} 138.2 showing that a methoxyl group was placed at C-3. The proton signal of a methylenedioxy group (δ_{H} 6.08, 3′,4′-OCH_{2}-O-) showed long-range correlations with two

Fig. 1. Flavonols 1 - 4 isolated from the leaves of M. glabra.
quaternary carbons at \( \delta_C 149.7 \) (C-3') and \( \delta_C 150.0 \) (C-4'). One of aromatic proton signal of ABX system (\( \delta_H 7.59 \), H-2') showed correlations with two quaternary carbons \( \delta_C 155.8 \) (C-2), 150.0 (C-4'), and one methine carbon signal \( \delta_C 123.8 \) (C-6'). The aromatic proton signal (\( \delta_H 6.95 \), H-5') showed correlations with two quaternary carbons \( \delta_C 124.2 \) (C-1'), and 149.7 (C-3'). Furthermore, the aromatic proton signal (\( \delta_H 7.68 \), H-6') showed correlations with two quaternary carbons \( \delta_C 155.8 \) (C-2), 150.0 (C-4'), and one methine carbon signal \( \delta_C 108.7 \) (C-2'). From these NMR data analysis, meliglabrin (1) was assigned as 5,7-dihydroxy-3,6-dimethoxy-3',4'-methylenedioxyflavone. Other HMBC correlations consistent with the structure 1 are shown in Table 1 and Fig. 2.

The isolated compounds 1 - 4 were assessed for their anticancer activity against murine leukemia P-388 cells. The result of anticancer activity are presented in Table 2, showing their IC\(_{50}\) were 48.30, 15.98, 30.04, and 5.02 µg/mL, respectively (artonin E as a positive control, IC\(_{50}\) 1.33 µg/mL). These anticancer activity data suggested that the compounds 1 - 3 were inactive and compound 4 showed moderate activity. The hydroxy group at C-4' and methoxy group at C-3' (compounds 2 and 4) enhances activity than methylenedioxy group at C-3' and C-4' (compounds 1 and 3). The same thing, the structure-activity relationship of flavonol from *M. triphylla*, the presence of hydroxy group at C-4' and methoxy group at C-3' showed moderate activity against murine leukemia P-388 cells. The presence of methoxy group at C-8 in compound 2 decreases anticancer activity compared to compound 4.

### Acknowledgments

This research was supported by Universitas Airlangga, Ministry of Research, Technology and Higher Education, Republic of Indonesia (Penelitian Hibah Mandat, Universitas Airlangga, 2018).

### References

(1) Hartley, T. *Sandakanian*. 1994, 4, 47-74.
(2) Nakashima, K.; Oyama, M.; Ito, T.; Akao, Y.; Witono, J. R.; Darnaedi, D.; Tanaka, T.; Murata, J.; Iinuma, M. *Tetrahedron*. 2012, 68, 2421-2428.
(3) Tanjung, M.; Saputri, R. D.; Wahjoedi, R. A.; Tjahjandarie, T. S. *Molbank*. 2017, M939, 1-5.
(4) Simonsen, H. T.; Adsersen, A.; Brenners, P.; Heinrich, M.; Wagner, Smitt, U.; Jaroszewski, J. *W. Phytother. Res*. 2004, 18, 542-545.
(5) Chung, L. Y.; Yap, K. F.; Goh, S. H.; Mustaфа, M. R.; Imiyabir, Z. *Phytochemistry*. 2008, 69, 1548-1554.
(6) Kassim, N. K.; Rahmani, M.; Ismail, A.; Sukari, M. A.; Ee, G. C.; Nasir, N. M.; Awang, K. *Food. Chem. 2013*, 139, 87-92.
(7) Oyama, M.; Nakashima, K.; Kamiya, T.; Haba, M.; Ito, T.; Murata, H.; Tanaka, T.; Adachi, T.; Iinuma, M.; Kinoshita, T. *Pytochem. Lett*. 2013, 6, 215-218.
(8) Cambie, R. C.; Pan, Y. J.; Bowden, B. F. *Biochem. Syst. Ecol*. 1996, 24, 461-462.
(9) Higa, M.; Imamura, M.; Ogihara, K.; Suzuka, T. *Chem. Pharm. Bull*. 2013, 61, 384-389.
(10) Tanjung, M.; Hakim, E. H.; Syah, Y. *M. Chem. Nat. Comp*. 2017, 53, 215-218.
(11) Tanjung, M.; Hakim, E. H.; Syah, Y. M. *Chem. Nat. Prod. Commun*. 2012, 10, 1309-1310.
(12) Tjahjandarie, T. S.; Padijustutti, P.; Saputri, R. D.; Tanjung, M. J. *Chem. Pharm. Res*. 2014, 6, 786-790.
(13) Hou, R. S.; Duh, C. Y.; Wang, S. K.; Chang, T. T. *Phytochemistry*. 1994, 35, 271-272.