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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.[1] The *Melicope* genus has been shown to be prolific a number of secondary metabolites, particularly alkaloids,[2-3] flavonoids,[4-5] coumarins[6-7] and showed biological activities such as anticancer, antifungal and antioxidant.

The phytochemical survey from the bark of *M. glabra* were isolated coumarins and lignan but the leaves until now has not been reported.[6] 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. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECA 400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz in $CDCl_3$. 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 GF_{254} and Si gel 60 PF_{254}, for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF_{254}, 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 A_1-A_3. Subfraction A_1

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was purified by planar radial chromatography using *n*-hexane-$CHCl_3$ (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 B_1-B_2. Subfraction B_1 was purified by planar radial chromatography using *n*-hexane-acetone (from 9:1 to 1:1) to yield compound **2** (9 mg).

Meliglabrin (**1**) – Yellow solid, mp. 119 - 121 ºC, UV (MeOH) λ_{max} nm (log ε) : 245 (4.20), 255 (3.99), 296 (4.01) and 344 (4.20). IR (KBr) ν_{max} cm^{-1}: 3421, 1645, 1560, 1481 and 1132. ^1H and ^{13}C NMR see Table 1. HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{18}H_{13}O_8$ 357.0610, found 357.0613.

Ternatin (**2**) – Yellow solid. ^1H NMR ($CDCl_3$ 400 MHz): δ_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'-OCH_3), 3.94 (3H, s, 7-OCH_3), 3.92 (3H, s, 8-OCH_3), 3.87 (3H, s, 3-OCH_3). ^{13}C NMR ($CDCl_3$ 100 MHz): δ_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), 95.5 (C-6), 61.7 (8-OCH_3), 60.2 (3-OCH_3), 56.5 (7-OCH_3), 56.1 (3'-OCH_3). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{19}H_{17}O_8$ 373.1916, found 373.1912. The ^1H and ^{13}C NMR spectral data are consistent with published data.[8]

Meliternatin (**3**) – Pale white solid, mp. 167 - 169 ºC. UV (MeOH) λ_{max} nm (log ε) : 247 (4.22), 270 (4.08) and 336 (4.34). IR (KBr) ν_{max} cm^{-1}: 1641, 1524, 1479 and 1114. ^1H NMR ($CDCl_3$ 400 MHz): δ_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'-OCH_2-O), 6.04 (2H, s, 6,7-OCH_2-O), 4.12 (3H, s, 5-OCH_3), 3.86 (3H, s, 3-OCH_3). ^{13}C NMR ($CDCl_3$ 100 MHz): δ_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'-OCH_2-O), 108.4 (C-2'; 6,7-OCH_2-O), 93.0 (C-8), 61.3 (5-OCH_3), 59.9 (3-OCH_3).). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{18}H_{13}O_8$ 357.0610, found 357.0613. The ^1H and ^{13}C NMR spectral data are consistent with published data.[9]

5,4'-Dihydroxy-3,7,3'-trimethoxyflavon (**4**) – Yellow solid, ^1H NMR (acetone-d_6, 400 MHz): δ_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'-OCH_3), 3.87 (3H, s, 7-OCH_3), 3.86 (3H, s, 3-OCH_3). ^{13}C NMR (acetone-d_6, 100 MHz): δ_C 179.5 (C-4), 166.5 (C-7), 162.7 (C-5), 157.6

Table 1. NMR Spectroscopic data (400 MHz in $CDCl_3$) for meliglabrin (**1**)

No.C	δ_H (mult, *J* in Hz)	δ_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'
3'	-	149.7	-
4'	-	150.0	-
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-OCH_3	3.85 (*s*, 3H)	61.0	C-3
6-OCH_3	4.04 (*s,* 3H)	60.3	C-6
3',4'-OCH_2-O-	6.08 (*s*, 2H)	101.8	C-3', C-4'

(C-8a), 156.8 (C-2), 150.5 (C-4'), 148.2 (C-3'), 139.3 (C-3), 123.3 (C-6'), 122.6 (C-1'), 116.1 (C-5'), 112.5 (C-2'), 106.4 (C-4a), 98.4 (C-6), 92.8 (C-8), 61.3 (3'-OCH_3), 56.4 (7-OCH_3), 56.5 (3-OCH_3). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{18}H_{17}O_7$ 344.0896, found 344.0900. The ^1H and ^{13}C NMR spectral data are consistent with published data.[10]

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 3×10^4 cells/well and incubated at 37 ºC 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$_{50}$ 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.[12] 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 ^{13}C 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 HMQC and 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

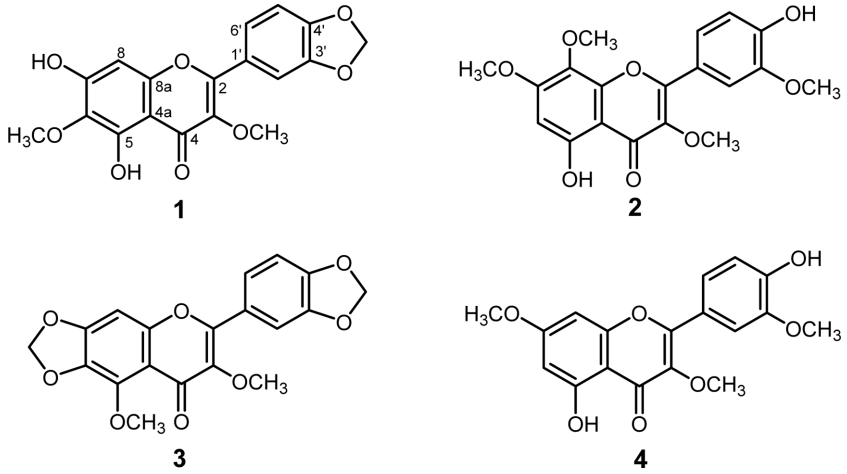

Fig. 1. Flavonols **1 - 4** isolated from the leaves of *M. glabra*.

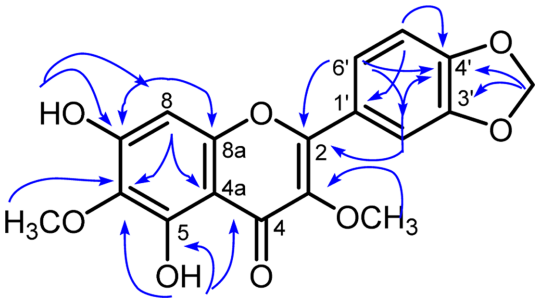

Fig. 2. Selected HMBC correlations for compound **1**.

Table 2. Cytotoxicity activity of compounds **1** - **4** against murine leukemia P-388 cells

Compounds	IC_{50} (μg/mL)
Meliglabrin (**1**)	48.30 ± 1.65
Ternatin (**2**)	15.98 ± 1.05
Meliternatin (**3**)	30.04 ± 1.78
5,4'-Dihydroxy-3,7,3'-trimethoxyflavon (**4**)	5.02 ± 0.45

quaternary carbons at δ_C 149.7 (C-3′) and δ_C 150.0 (C-4′). One of aromatic proton signal of ABX system (δ_H 7.59, H-2′) showed correlations with two quaternary carbons δ_C 155.8 (C-2), 150.0 (C-4′), and one methine carbon signal δ_C 123.8 (C-6′). The aromatic proton signal (δ_H 6.95, H-5′) showed correlations with two quaternary carbons δ_C 124.2 (C-1′), and 149.7 (C-3′). Furthermore, the aromatic proton signal (δ_H 7.68, H-6′) showed correlations with two quaternary carbons δ_C 155.8 (C-2), 150.0 (C-4′), and one methine carbon signal δ_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.[14] The presence of methoxy group at C-8 in compound **2** decreases anticancer activity compared to compound **4**.

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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.; Bremner, 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.; Mustafa, 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. *Phytochem. Lett.* **2013**, *6*, 215-218.
(8) Cambie, R. C.; Pan, Y. J.; Bowden, B. F. *Biochem. Syst. Ecol*. **1996**, *24*, 461-462.
(9) Chung, L. Y.; Yap, K. F.; Goh, S. H.; Mustafa, M. R.; Imiyabir, Z. *Phytochemistry* **2008**, *69*, 1548-1554.
(10) Higa, M.; Imamura, M.; Ogihara, K.; Suzuka, T. *Chem. Pharm. Bull*. **2013**, *61*, 384-389.
(11) Tanjung, M.; Hakim, E. H.; Syah, Y. M. *Chem. Nat. Comp.* **2017**, *53*, 215-218.
(12) Tanjung, M.; Hakim, E. H.; Elfahmi, Latip, J.; Syah, Y. M. *Nat. Prod. Commun.* **2012**, *10*, 1309-1310.
(13) Tjahjandarie, T. S.; Pudjiastuti, P.; Saputri, R. D.; Tanjung, M. *J. Chem. Pharm. Res*. **2014**, *6*, 786-790.
(14) Hou, R. S.; Duh, C. Y.; Wang, S. K.; Chang, T. T. *Phytochemistry* **1994**, *35*, 271-272.

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