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Cytotoxic and Antioxidant Activities of Flavonols from *Melicope quercifolia*

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ABSTRACT

Melicope quercifolia, a plant from the Rutaceae family, is known for its medicinal properties, especially in traditional herbal medicine. Flavonoids, bioactive compounds widely found in plants, are recognized for their antioxidant and cytotoxic effects, making them promising candidates for cancer therapy and combating oxidative stress. Two flavonols, pachypodol (**1**) and ternatin (**2**), were isolated from the stem bark of *M, quercifolia*. Compounds **1-2** were isolated and purified with column and planar radial chromatography. The structures of **1-2** were determined based on HRESI-MS data, UV, 1D, and 2D NMR spectra. Compound **1** showed high activity against HeLa cells (IC_{50} values 0.65 µg/mL), moderate against P-388 cells (IC_{50} values 2.45 µg/mL), and weak activity against (DPPH radical (IC_{50} values 254 µg/mL.

Keywords: *Melicope quercifolia*, Flavonol, pachipodol, Ternatin, Cytotoxic effect, Antioxidant

Introduction

Melicope quercifolia is a Rutaceae family member and is endemic to Indonesia.[1] The decoction of the leaves of *M. quercifolia* is used to treat skin diseases and fevers and is anti-inflammatory.[1] In previous phytochemical investigations, the *Melicope* genus produced phenolic compounds such as alkaloids, coumarins, acetophenones, and flavonoids that exhibited antioxidant, anti-cancer, anti-virus, anti-inflammatory, and anti-malaria activities.[2-8] The phytochemical report previously showed that two coumarins, meliquercifolins A and B, from the leaves of *M. quercifolia* showed high activity against HeLa cells[1]. Four flavonol derivatives from *M. glabra* leaves showed moderate activity against P-388 cells.[5] Flavonols and coumarins from the leaves of *M. glabra* showed oxygen radical antioxidant capacity and breast cancer (MDA-MB-231 and HCC1937).[6]

The present research isolated two quercetin derivatives, pachypodol (1) and ternatin (2), from *M. quercifolia* stem bark. The cytotoxic activity of flavonols 1-2 was assayed using cervical cancer (HeLa cells), breast cancer (MCF-7 cells), leukemia cancer (P-388 cells) by MTT assay, antioxidant activity against DPPH radical scavenging.

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Materials and Methods

General experimental procedures

A UV-1800 Shimadzu spectrophotometer measured the λ_{max} of flavonols **1-2** in MeOH. The NMR of the isolated compounds was recorded using a JEOL JNM FTNMR spectrometer, operating at 400 MHz using acetone-*d6* as solvent and tetramethylsilane as reference standards. The chemical formula of flavonols **1-2** was measured using a high-resolution mass spectrometer (LCT Premier™ XE Waters). Silica gel was used as the stationary phase in the gravity column and radial planar chromatography.

Plant material

The stem bark of M. quercifolia was collected from Cigudek District, Bogor (Latitude 6^0 43' 27" S: Longitude 106^0 36' 6" E), Indonesia, on Nov. 15. 2017. The voucher specimens (no. 20171511) were deposited as a reference in the Herbarium Bogoriense, National Research and Innovation Agency, Bogor, Indonesia.

Extraction and isolation

The stem bark of *M. quercifolia* (1.9 kg) was extracted with 96% MeOH by maceration for three days, twice at 30° C. Methanol solvent was evaporated with a vacuum rotary evaporator (Rotavapor R-300, Switzerland) to produce a dark brown concentrated methanol extract (105 g). The MeOH extract (500 mL) was partitioned with *n*-hexane (500 mL, three times) to give 15.6 g *n*-hexane extract. Further, the remaining MeOH extract was partitioned with EtOAc by the same condition as *n*-hexane to yield 7.1 g EtOAc extract. The EtOAc extract (7.0 g) was fractionationated by silica gel column gravity CC, eluted with *n*-hexane-EtOAc (from 9:1; 4:1; 7:3, and 2:3 v/v), providing three fractions, A (1.2 g), B (900 mg), and C (1.5 g). Sephadex LH-20 CC separated fraction C eluted with methanol to give two subfractions, C_1 (120 mg), and C_2 (350 mg). The subfraction C_2 by planar radial chromatography using a mixture of *n*-hexane: diisoprophyl ether (from 1:1 to 3:7 v/v) yielded pachypodol, **1** (17.0 mg) and ternatin **2**, (23 mg).

Cytotoxic activity

The cytotoxic effect of compounds **1-2** against cervical cancer (HeLa cells), breast cancer (MCF-7 cells), and leukemia cancer (P-388 cells)

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was evaluated by the colorimetric method using MTT assay.[9-10] The HeLa, MCF-7, and P-388 cells, respectively, were cultivated in the RPMI 1640 medium, containing 1 mL fetal bovine serum, antibiotic (penicillin and streptomycin, each 100 µg/mL) in a 5% CO_2 incubator (Thermo Fisher Scientific, USA) at room temperature for 48 hours.[11-13] Compounds 1-2 in the concentration of (0.1, 1, 10, 50, and 100 µg/mL) were added to the HeLa, MCF-7, and P-388 cells, respectively, and then incubated at room temperature for 24 hours. Determination of an inhibitory concentration of compounds 1 2 using a microplate reader at λ = 590 nm (Biobase BK-EL10C, China). Doxorubicin was used as a positive control for the cytotoxic assay.[14-15]

Antioxidant activity

The antioxidant activity of compounds 1-2 against DPPH radical scavenging was evaluated by colorimetric method using a UV-Vis spectrophotometer at λ = 517 nm (UV-1800 Shimadzu, Japan). Ascorbic acid was used as a positive control for the antioxidant assay. The sample solution in methanol (compounds 1-2) was prepared in the concentration of 10, 100, 500, 1000, and 2500 µg/mL. The preparation of 1000 mg/ml of sample solution by adding 100 µL of sample solution (2500 µg/mL) to 100 µL of acetate buffer (pH 5.5) and 50 µL of DPPH 5.10^{-4} mol/L, then incubated at 20^0 C for 30 min. The equation calculated flavonols 1-2 inhibition percentage against DPPH radical scavenging: % Inhibition = $(A_o - A_s/A_o) \times 100$. Whereas A_o = the absorbance of DPPH solution without sample solution. A_o = the absorbance of the mixture of DPPH solution and sample solution. [16-18]

Result and Discussion

Two flavonol derivatives, pachypodol (1) and ternatin (2), pachypodol (**1**) and ternatin (**2**), were isolated from *M. quercifolia* stem barks., and their structures were established by HR-ESI-MS, UV, IR, and NMR spectra.

Pachypodol (**1**) was obtained as a yellow solid, melting point 167-169^o C, and exhibits the molecular ion $[M+H]^+$ at *m/z* 345.0923, and the chemical formula $C_{18}H_{17}O_7$ by HRESI-MS spectrum. The UV spectrum of 1 showed the λ_{max} (log ε): 254 (4.18), 268 (4.11), and 354 (4.12) nm and displayed the wave number (cm^{-1}): 3425, 1636, 1515, 1448, and 1170 in the IR spectrum. Pachypodol (1) showed five protons of two aromatic units at δ_H 6.27 (1H, *d*, *J* = 2.2 Hz, H-6), δ_H 6.62 (1H, *d*, *J* = 2.2 Hz, H-8)], δ_H 7.75 (1H, *d*, *J* = 2.0 Hz, H-2'), δ_H 6.97 (1H, *d*, *J* = 8.4 Hz, H-5'), and δ_H 7.67 (1H, *dd*, *J* = 8.4 & 2.0 Hz, H-6')]. Compound 1 also showed two hydroxy groups at δ_H 12.72 (1H, *s*, 5-OH), δ_H 8.64 (1H, *s*, 4'-OH)], and three methoxy groups at δ_H 3.86 (3H, *s*, 3-OCH_3), δ_H 3.87 (3H, *s*, 7-OCH_3), and δ_H 3.91 (3H, *s*, 3'-OCH_3). The location of two hydroxy and three methoxy groups (Table 1) was confirmed by HMQC and HMBC spectra. Compound 1 exhibits 18 perfectly separated carbon signals in the ^{13}C NMR spectrum (Table 1). The HMBC spectrum confirmed the position of 18 carbon signals. Based on the correlation of two and three bonds in the HMBC spectrum (Figure 1), the structure of compound **1** is 5,4'-dihydroxy-3,7.3'-trimethoxyflavone and is known as pachipodol.[5]

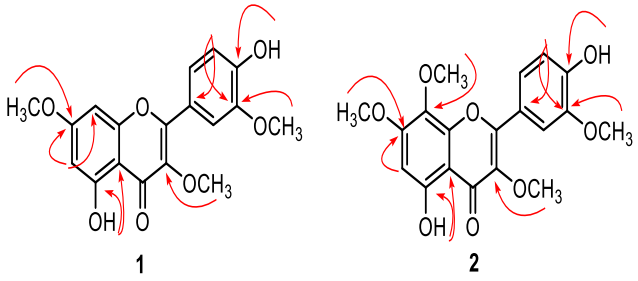

Figure 1: HMBC correlations of flavonols **1-2**

Ternatin (**2**) was isolated as a yellow powder, m.p 212-214^o C, showing the molecular ion $[M+H]^+$ at *m/z* 375.1120 and the molecular formula $C_{19}H_{19}O_8$ by mass spectrum. The UV and IR spectra of 2 showed the λ_{max} (log ε): 267 (4.15), 273 (4.12), 307 (4.17), and 365 (4.01) nm], and IR spectra *ν* (cm^{-1}): 3410, 1627, 1515, 1448, and 1161 very similar to compound **1**. The ^1H and ^{13}C NMR of 2 showed the same pattern in the B and C rings with compound 1 (Table 1). The significant difference in ternatin (2) shows the addition of one methoxy group at δ_H 3.92 (3H, *s*, 8-OCH_3). The long-range correlation between the proton and carbon signals in the HMBC spectrum (Figure 1) is 5,4'-dihydroxy-3,7,8,3'-tetramethoxyflavon, known as ternatin.[5]

The cytotoxic activity of pachypodol (**1**) against HeLa, MCF-7, and P-388 cells showed an IC_{50} value of 0.65, 9.30, and 2.45 µg/mL, respectively (Table 2). Pachypodol (**1**) showed high activity in HeLa cells, moderate against P-388 cells, and was inactive toward MCF-7.[11,15] Ternatin (**2**) was inactive against HeLa, MCF-7, and P-388 cells. A methoxy group at C-8 on compound **2** reduces the cytotoxic activity. The antioxidants of **1-2** showed an IC_{50} value of 254 and 658 µg/mL, respectively. Pachypodol (**1**) showed weak activity, and ternatin (**2**) was inactive against DPPH radical scavenging.[16-18] A methoxy group in compound **2** also reduces antioxidant activity.

Table 1: ^1H and ^{13}C NMR data of flavonols **1-2**

	1		2	
No	δ_H (multiplicity, *J* in Hz)	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C
1	-		-	
2	-	156.8	-	155.8
3	-	139.3	-	139.4
4	-	179.5	-	179.1
4a	-	106.4	-	105.4
5	-	162.7	-	157.4
6	6.27 (*d*, 2.2)	98.4	6.42 (*s*)	95.5
7	-	166.5	-	158.4
8	6.62 (*d*, 2.2)	92.8	-	128.8
8a	-	157.6	-	148.5
1'	-	122.6	-	122.7
2'	7.75 (*d*, 2.0)	112.5	7.78 (*d*, 2.0)	110.8
3'	-	148.2	-	146.4
4'	-	150.5	-	148.5
5'	6.97 (*d*, 8.4)	116.1	7.06 (*d*, 9.1)	114.8
6'	7.67 (*dd*, 8.4; 2.0)	123.3	7.79 (*dd*, 9.1; 2.0)	122.9
5-OH	12.72 (*s*)	-	12.44 (*s*)	-
4'-OH	8.64 (*s*)	-	6.01 (*s*)	-
3-OCH_3	3.86 (*s*)	56.3	3.,87 (*s*)	60.2
7-OCH_3	3.87 (*s*)	56.4	3.94 (*s*)	56.5
8-OCH_3	-	-	3.92 (*s*)	61.7

3'-OCH_3	3.91 (*s*)	61.3	3.98 (*s*)	56.1

Table 2: Cytotoxic and antioxidant activities of flavonols 1-2

Compound	HeLa (µg/mL)	MCF-7 (µg/mL)	P-388 (µg/mL)	DPPH (µg/mL)
Pachypodol (1)	0.65	9.30	2.45	254
Ternatin (2)	>100	>100	16.90	658
Doxorubicin	0.08	0.09	0.08	-
Ascorbic acid	-	-	-	1.89

Conclusion

Two flavonol derivatives, pachypodol (**1**) and ternatin (**2**), were isolated from *M. quercifolia* stem barks. Pachypodol (**1**) showed high activity against HeLa cells, moderate activity against P-388 cells, and weak activity against DPPH radical scavenging.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors declare that the articles displayed are original, and all claims related to the content of this article are the authors' responsibility.

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