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# Dihydroflavonols from the leaves of *Macaranga recurvata* and their cytotoxic and antioxidant activities

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**ABSTRACT**

*Three dihydroflavonol derivatives, macarecurvatin A (1), macarecurvatin B (2), and 6,8-diisoprenylaromadendrin (3) have been isolated from the leaves of Macaranga recurvata. The structures of these compounds were determined based on UV, IR, HRESIMS, 1D and 2D NMR data. All of compound isolated were evaluated for their inhibitory effect on scavenging DPPH radical and their growth inhibition on MCF7, and tamoxifen-resistant MCF7 (MCF7/TAMR) breast cancer cell showed high antioxidant and high cytotoxic activities.*

**Keywords:** Macarecurvatin A and B, 6,8-Diisoprenylaromadendrin, Dihyroflavonol, *Macaranga recurvata*, Antioxidant, Cytotoxic

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## INTRODUCTION

*Macaranga* is a large genus of Euphorbiaceae consisting of about 280 species and distributed in the tropical region of the world, including Indonesia [1,2]. *Macaranga* known local name as "Mahang". The phytochemical studies has knowed that this plants producing phenolic compounds, particularly the isoprenylated, geranylated, and farnesylated flavonoids and stilbenes [3,4]. The phytochemical study on Indonesian *Macaranga*, recently we reported the isolation of isoprenylated flavanols from the leaves of *Macaranga gigantea* [5,6], isoprenylated, and geranylated flavonols from *Macaranga rhizinoides* [7]. Flavonoids and stilbenoids of this plant showed that various activities including antioxidant [8], cyclooxygenase inhibitory [9], antibacterial [10], and anticancer [11]. In this paper, we report the isolation of dihydroflavonol compounds, 6,8-diisoprenylaromadendrin (**1**), macarecurvatin A (**2**), and macarecurvatin B (**3**) from the methanol extract of the leaves of *Macaranga recurvata*. The antioxidant activity of compounds **1**– **3** against DPPH and the cytotoxic activity of compounds **1**– **3** against breast cancer cells are also briefly described.

## EXPERIMENTAL SECTION

The leaves of *Macaranga recurvata* were collected in July 2012 from the conserved forest of Rimba Panti, Pasaman, West Sumatera, Indonesia. The plant was identified by Mr Ismail Rachman, Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia, and the voucher specimen was deposited in the herbarium. The dried and powdered leaves of *Macaranga recurvata* (1.3 kg) were macerated in methanol at room temprature two times, and the methanol extract was evaporated under reduced

pressure to give a dark brown residue (85 g). Furthermore, the methanol extract were dissolved with methanol-water 10% and partition with n hexane and ethyl acetate. The ethyl acetate extract (17 g) was separated by vacuum liquid chromatography on silica gel eluted with *n* hexane-ethyl acetate mixture containing increasing amount of ethyl acetate (4:1, 7:3; and 1:1) and to give four major fraction A-D. Fraction B (376 mg), purified using planar radial chromatography eluted with n hexane-$CHCl_3$ = 2:3, and 1:4 to yielded 6,8-diisoprenylaromadendrin (26.9 mg). The separation of fraction C (630 mg) by radial chromatography with n hexane-$CHCl_3$ = 2:3, 1:4 and $CHCl_3$ to give macarecurvatin B (41.1 mg). Furthermore, separation of fraction D (1.5 g) with same methods by with n hexane-ethyl acetate = 4:1, and 7:3 to give two subfraction $D_1$-$D_2$. Purification of subfraction $D_2$ using radial chromatography eluted n hexane-$CHCl_3$ = 1:4, $CHCl_3$ and $CHCl_3$-EtOAc = 9:1 yielded macarecurvatin A (34.7 mg).

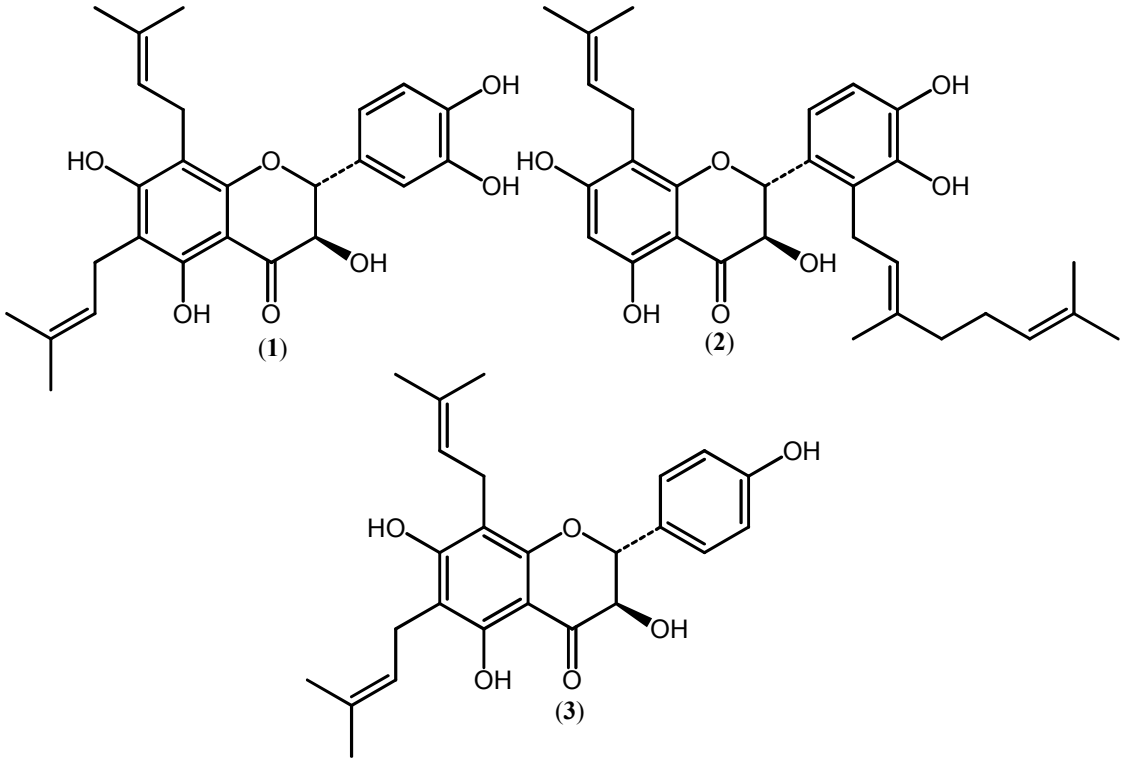


**Figure 1. Dihydroflavonols isolated from *M. recurvata***

Macarecurvatin A (**1**), pale yellow solid, UV (MeOH) $\lambda_{maks}$ nm (log ε): 204 (4.84); 225 sh (4.69) dan 291 (4.35); (MeOH + NaOH) 211 (4.98); 288 (4.18); and 344 (4.36); (MeOH + $AlCl_3$) 206 (4.90); and 308 (4.39); ($AlCl_3$+ HCl) 204 (4.87), 308 (4.39); and 368 (3.74); (NaOAc): 207 (4.94); and 293 (4.34). HRESIMS: *m/z* $[M+H]^+$ 441.1902 (calcd for $C_{25}H_{29}O_7$: 441.1908). $^1$H NMR (400 MHz, acetone-*d6*): see Table 1. $^{13}$C NMR (100 MHz, acetone-*d6*): see Table 1

Macarecurvatin B (**2**), pale yellow solid, UV (MeOH) $\lambda_{maks}$ nm (log ε): 207 (4.91); 229 sh (4.63); and 295 (4.36); (MeOH + NaOH) 208 (4.98), 248 (4.39); and 333 (4.44); (MeOH + $AlCl_3$) 210 (4.95); 315 (4.41); and 404 (3.75); ($AlCl_3$+ HCl) 207 (4.96); 314 (4.40); and 368 (3.77); (NaOAc): 207 (4.94); and 297 (4.38). HRESIMS: *m/z* $[M+H]^+$ 509.2534 (calcd for $C_{25}H_{29}O_7$: 509.2461). $^1$H NMR (400 MHz, acetone-*d6*): see Table 2. $^{13}$C NMR (100 MHz, acetone-*d6*): see Table 2.

6,8-diisoprenylaromadendrin (**3**), pale yellow solid, UV (MeOH) $\lambda_{maks}$ nm (log ε): 203 (4.35); 226 sh (4.20); and 298 (3.91); (MeOH + NaOH) 211 (4.98); 245 (4.13); and 342 (4.06); (MeOH + $AlCl_3$) 203 (4.63); and 316 (4.08); ($AlCl_3$+ HCl) 203 (4.64); and 311 (4.09); (NaOAc): 206 (4.92); and 298 (3.85). LCESIMS: *m/z* 424 ($M^+$). $^1$H NMR (400 MHz, acetone *d*-6) $\delta_H$ (ppm): 11.96 (1H, *s*, 5-OH); 8.51 (1H, *s*, 7-OH); 7.42 (2H, *d*, *J* = 8.4 Hz; H-2'/6'); 6.90 (2H, *d*, *J* = 8.4 Hz; H-3'/5'); 5.18 (1H, *tm*, *J* = 6.8 Hz; H-2''); 5.13 (1H, *tm*, *J* = 6.8 Hz; H-2'''); 5.03 (1H, *d*, *J* = 11.7

Hz; H-2); 4.61 (1H, *d*, *J* = 11.7 Hz; H-3); 3.32 (2H, *d*, *J* = 7.3 Hz, H-1''); 3.24 (2H, *d*, *J* = 7.0 Hz, H-1'''); 1.75 (3H, *s*, H-5''); 1.64 (3H, *s*, H-4''); 1.60 (3H, *s*, H-4'''); and 1,53 (3H, *s*, H-5'''). $^{13}$C-NMR (100 MHz, acetone *d*-6) $\delta_C$ (ppm): 198.9 (C-4); 162.7 (C-7); 159.7 (C-5); 158.7 (C-8a); 158.6 (C-4'); 134.4 (C-3'''); 132.3 (C-3''); 130.1 (C-2'/6'); 129.4 (C-1'); 123.1 (C-2'''); 123.0 (C-2''); 115,8 (C-3'/5'); 109,1 (C-6); 108.1 (C-8); 101.6 (C-4a); 84.2 (C-2); 73.3 (C-3); 25.8 (C-4''/4'''); 22.2 (C-1'''), 21.8 (C-1''), 17.9 (C-5'') and 17.8 (C-5''').

DPPH scavenging activity test: Determination of the antioxidant activity of the isolated performed using reagent DPPH (2,2-diphenyl-1-pikrihidrazil) was measured by UV spectrometer at λ 517 nm [12,13,14]. Determination of antioxidant activity done by the dissolving a compounds assay with methanol, then added solution of 0.1 M buffer acetate (pH 5.5) and added DPPH radical solution of $5.10^{-4}$ M. Determination of the inhibition of isolated compounds against DPPH radical was observed using a spectrometer at λ 517 nm after incubation for 30 min at 20°C.

MTT assay: The *in vitro* cytotoxic activity was determined by the MTT method [15,16,17]. The breast cancer cells (MCF7, and MCF7/TAMR) were grown in RPMI 1640 medium containing 10% fetal bovine serum, 2 mg $mL^{-1}$ sodium carbonate, 100 μg $mL^{-1}$ penicillin sodium salt, and 100 μg $mL^{-1}$ streptomycin sulfate. The cells were harvested at the log phase of growth, and then seeded into 96-well plates ($1 \times 10^4$ cells/well). After 24 h incubation at 37 ºC and 5% $CO_2$ to allow cell attachment, the cultures were exposed to the test compounds **1-3** were dissolved in DMSO at various concentrations and incubated for 48 h followed by MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay at 540 nm.

## RESULTS AND DISCUSSION

Three dihydroflavonols, macarecurvatin A (**1**), macarecurvatin B (**2**), and 6,8-diisoprenylaromadendrin (**3**) have been isolated from the methanol extract of the leaves of *Macaranga recurvata*. The structures of these compounds were determined based on UV, IR, HRESIMS, 1D and 2D NMR data.

Macarecurvatin A (**1**) was isolated as pale yellow powder and its UV spectrum showed absorption maxima (204, 225 sh, and 291 nm) typical for a dihydroflavonol and showed a bathocromic shift on addition of $AlCl_3$, $AlCl_3$+ HCl, and NaOAc [18]. The HR-ESI-MS spectrum (positive mode) of **1** showed a quasimolecular $[M+H]^+$ ion (*m*/*z* 441.1902) consistent with a molecular formula $C_{25}H_{29}O_7$ (calculated $[M+H]^+$ 441.1908), suggesting that **1** is a dihydroflavonol derivative containing two isoprenyl groups. In the $^1$H NMR spectrum (Table 1.) the presence of a pair of of doublets (*J* = 11.7 Hz) at $\delta_H$ 4.97 and 4.56 confirmed for the dihydroflavonol skeleton. The $^1$H-NMR spectrum of compound **1** showed three aromatic proton signals for ABX system at $\delta_H$ 7.07 (1H, *d*, *J* = 1.8 Hz); 6.91 (1H, *d*, *J* = 8.1 Hz); and 6.85 ppm (1H, *dd*, *J* = 8.1, 1.8 Hz) corresponding to the group substitutent at C-3'and C-4' in ring B dihydroflavonol. There is no aromatic proton signal in ring A suggested that isoprenyl groups at C-6 and C-8. The existence of two isoprenyl chain of compound **1** showed the presence of four methyl groups ($\delta_H$ 1.75, 1.64, 1.60, and 1.55 ppm), two methylene groups ($\delta_H$ 3.32 and 3.24 ppm), two vinyl groups ($\delta_H$ 5.17, and 5.12 ppm). The presence of five signals of oxyaryl ($\delta_C$ 162.7, 159.7, 158.7, 146.4, and 145.7) in the $^{13}$C NMR (APT experiment, Table 1) suggested that **1** has the basic structure of taxifolin (= 3,5,7,3',4'-pentahydroxy flavanone). The correlation of the one bond and the two/three bond $^1$H-$^{13}$C compound **1** can be seen in the HMQC and HMBC spectra (Table 1). Key $^1$H-$^{13}$C long range correlations found in the HMBC spectrum, particularly from the chelated –OH ($\delta_H$ 11.97) and the methylene (δH 3.32 and 3.24) signals confirmed the assignment of structure **1** for macarecurvatin A or known as 6,8-diisoprenyl taxifolin [19]. Other HMQC and HMBC correlations, as well as $^{13}$C NMR data assignment, that are consistent with the structure **1** are shown in Table 1.

Macarecurvatin B (**2**) was also obtained as pale yellow powder. The ion peak at *m/z* 509.2534 $[M+H]^+$ (calcd 509.2461, Δ 1,4 ppm) in the HRESIMS spectrum gave the molecular $C_{30}H_{36}O_7$ suggesting that **2** is a dihydroflavonol derivative containing an geranyl and isoprenyl groups. Based on UV, $^1$H and $^{13}$C NMR spectrum (Table 2), the compound **2** also showed characteristics of the taxifolin structure. The $^1$H-NMR spectrum of compound **2** a pair of doublets (*J* = 8.4 Hz) at $\delta_H$ 7.04 and 6.82 corresponding to the group substitutent at H-5'and H-6' in ring B dihydroflavonol. The presence of a singlet ($\delta_H$ 6.06) of aromatic signal, suggesting that one of the side chain groups must be located either at C-6 or C-8. The presence of geranyl and isoprenyl groups in compound **2** was showed by the $^1$H NMR signals of five methyl singlets ($\delta_H$ 1.58, 1.54 (6H), 1.51, and 1.51), three methine vinyl ($\delta_H$ 5.20, 5.12, and 5.02), and four methylene ($\delta_H$ 3.58, 3.16, 1.98 and 1.95) signals. The placement of geranyl and isoprenyl groups at C-2' and C-6 or C-8 were determined based on HMQC and HMBC spectrum. In the HMBC

spectrum, the long-range correlation between a proton signal chelate-OH group at δH 11.97 ppm with an oxyaril ($\delta_C$ 162.6), a quarternary ($\delta_C$ 101.4), and a methine ($\delta_C$ 96.6) carbon signals, showing that C-6 is unsubstituted. The placement of geranyl group at C-2' in the HMBC spectrum, the long-range correlation between a proton signal from methylene proton signals at $\delta_H$ 3.58 (geranyl) with an oxyaril carbon [$\delta_C$ 145.7 (C-2'], three quarternary carbons [$\delta_C$ 135.2 (C-3'''), 129.2 (C-2'), 128.4 (C-1')] and a methine carbon [$\delta_C$ 124.3 (C-2''')]. From the long-range correlation between a methylene proton signals at $\delta_H$ 3.16 (isoprenyl) with two oxyaril carbons [$\delta_C$ 165.4 (C-7), 161.1 (C-8a)], two quarternary carbons [$\delta_C$ 131.3 (C-3''), 108.5 (C-8)] and a methine carbon [$\delta_C$ 123.4 (C-2'')] confirmed the placement of isoprenyl group at C-8. Based on data from 1D and 2D NMR of compound **2** is 8-isoprenyl-2'-geranyltaxifolin or known as macarecurvatin B [19]. Complete HMBC correlations in support of structure **2** are shown in Table 2.

**Table 1. NMR spectroscopic data of macarecurvatin A (1)**

| No.C | $\delta_H$ (mult, *J* Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| 2 | 4.97 (*d*, 11.7) | 84.3 | C-3, C-4, C-2', C-6' |
| 3 | 4.56 (*d*, 11.7) | 73.3 | C-2, C-4, C-1' |
| 4 | - | 198.8 | - |
| 4a | - | 101.6 | - |
| 5 | - | 159.7 | - |
| 6 | - | 109.0 | - |
| 7 | - | 162.7 | - |
| 8 | - | 108.2 | - |
| 8a | - | 158.7 | - |
| 1' | - | 130.1 | - |
| 2' | 7.07 (*d*, 1.8) | 115/8 | C-2, C-4', C-6' |
| 3' | - | 145.7 | - |
| 4' | - | 146.4 | - |
| 5' | 6.91 (*d*, 8.1) | 120.6 | C-1', C-3' |
| 6' | 6.85 (*dd*, 8.1; 1.8) | 115.7 | C-2, C-2', C-4' |
| 1" | 3.32 (*d*, 7.0) | 21.8 | C-5, C-6, C-7, C-2', C-3' |
| 2"' | 5.17 (*tm*, 6.7) | 123.1 | C-3', C-4', C-5' |
| 3" | - | 132.2 | - |
| 4" | 1.64 (*s*) | 25.9 | C-2', C-3', C-5'' |
| 5" | 1.75 (*s*) | 17.9 | C-2', C-3', C-4'' |
| 1"' | 3.24 (*d*, 7.0) | 22.2 | C-7, C-8, C-8a, C-2''', C-3''' |
| 2"' | 5.12 (*tm*, 6.7) | 123.0 | C-3''', C-4''', C-5''' |
| 3"' | - | 131.1 | - |
| 4"' | 1.60 (*s*) | 25.8 | C-2''', C-3''', C-5''' |
| 5"' | 1.55 (*s*) | 17.9 | C-2''', C-3''', C-4''' |
| 5-OH | 11.97 (*br*, *s*) | - | C-4a, C-5, C-6 |

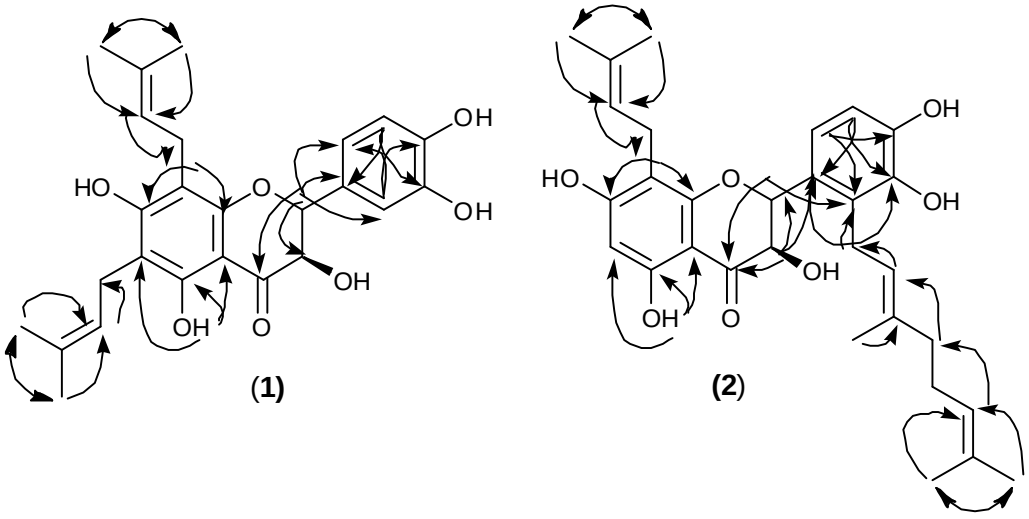


**Figure 2. The significant HMBC spectrum for compound 1 and 2**

**Table 2. NMR spectroscopic data of macarecurvatin B (2)**

| No.C | $\delta_H$ (mult, *J* Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| 2 | 5.33 (*d*, 11.7) | 80.8 | C-3, C-4, C-2', C-6' |
| 3 | 4.71 (*d*, 11.7) | 73.0 | C-2, C-4, C-1' |
| 4 | - | 198.8 | - |
| 4a | - | 101.4 | - |
| 5 | - | 162.6 | - |
| 6 | 6.06 (*s*) | 96.6 | - |
| 7 | - | 165.4 | - |
| 8 | - | 108.5 | - |
| 8a | - | 161.1 | - |
| 1' | - | 128.4 | - |
| 2' | - | 129.2 | C-2, C-4', C-6' |
| 3' | - | 145.7 | - |
| 4' | - | 146.4 | - |
| 5' | 6.82 (*d*, 8.4) | 113.3 | C-1', C-3' |
| 6' | 7.04 (*d*, 8.4) | 119.6 | C-2, C-2', C-4' |
| 1" | 3.16 (*d*, 6.7) | 22.0 | C-5, C-6, C-7, C-2', C-3' |
| 2'' | 5.12 (*tm*, 6.8) | 123.4 | C-3', C-4', C-5' |
| 3" | - | 131.3 | - |
| 4" | 1.51 (*s*) | 25.7 | C-2', C-3', C-5'' |
| 5" | 1.54 (*s*) | 17.7 | C-2', C-3', C-4'' |
| 1"' | 3.58 (*d*, 6.7) | 25.1 | C-7, C-8, C-8a, C-2''', C-3''' |
| 2"' | 5.20 (*tm*, 7.3) | 124.3 | C-3''', C-4''', C-5''' |
| 3"' | - | 135.2 | - |
| 4"' | 1.95 (*t*, 7.0) | 40.4 | C-2''', C-3''', C-5''' |
| 5"' | 1.98 (*t*, 7.8) | 27.5 | C-2''', C-3''', C-4''' |
| 6"' | 5.02 (*tm*, 7.3) | 125.0 | C-7, C-8, C-8a, C-2''', C-3''' |
| 7"' | - | 131.1 | C-3''', C-4''', C-5''' |
| 8"' | 1.51 (*s*) | 17.8 | - |
| 9"' | 1.54 (*s*) | 25.8 | C-2''', C-3''', C-5''' |
| 10"' | 1.58 (*s*) | 16.4 | C-2''', C-3''', C-4''' |
| 5-OH | 11.97 (*br*, *s*) | - | C-4a, C-5, C-6 |

6,8-diisoprenylaromadendrin (**3**) was obtained as yellow powder and the pattern of UV spectrum of **3** is very similar with compound **1** and **2**. Based on $^1$H and $^{13}$C NMR spectrum, the compound **3** also showed characteristics of the aromadendrin structure. The $^1$H-NMR spectrum of compound **3** showed a pair of doublets ($J$ = 8.4 Hz) in the aromatic region at $\delta_H$ 7.42 and 6.90 (each 2H), suggested the signal of a *p*-hydroxyphenyl at B ring. There is no aromatic proton signal in ring A suggested that isoprenyl groups at C-6 and C-8. Based on data from LCESIMS, 1D and 2D NMR of compound **3** is 6,8-diisoprenylaromadendrin [20].

Activity of radical scavenging against DPPH, and cytotoxic properties against breast cancer cells (MCF7, and MCF7/TAMR) were evaluated according to the method of MTT assay of macarecurvatin A (**1**), macarecurvatin B (**2**), and 6,8-diisoprenylaromadendrin (**3**) are shown in Table 3.

**Table 3. Antioxidant and cytotoxic activities of dihydroflavonol isolated**

| Compound | DPPH (μM) | MCF7 (μM) | MCF7/TAMR (μM) |
|---|---|---|---|
| Macarecurvatin A (**1**) | 130 | 5.26 | 5.66 |
| Macarecurvatin B (**2**) | 277 | 0.96 | 1.25 |
| 6,8-diisoprenylaromadendrin (**3**) | 1,936 | 5.03 | 5.83 |
| Ascorbic acid | 329 | - | - |

The results of antioxidant activity showed macarecurvatin A (**1**) and B (**2**) has very high activity and 6,8-diisoprenylaromadendrin (**3**) was inactive. The hydroxyl group at C-3' in ring B in compound **1** and **2** increases the antioxidant activity compared to compound **3**. The results of growth inhibition on MCF7, and tamoxifen-resistant MCF7/TAMR showed macarecurvatin B (**2**) have very high activity and macarecurvatin A (**1**) and 6,8-diisoprenylaromadendrin (**3**) have moderate activity.

## CONCLUSION

Three dihydroflavonol, macarecurvatin A (**1**), macarecurvatin B (**2**), and 6,8-diisoprenylaromadendrin (**3**) have been isolated from the leaves of *Macaranga recurvata*. The antioxidant activity of compounds **1**-**3** were evaluated

by measuring their ability to scanvenge the DPPH radical showed macarecurvatin A > macarecurvatin B > 6,8-diprenylaromadendrin. The result indicate that compound **1** and **2** to give very high activity than ascorbic acid as positive control. The structure-activity relationship of compounds **1**–**3** against DPPH radical scavenging suggested that the presence of hydroxyl group at C-3' on macarecurvatin A and B more active than 6,8-diprenylaromadendrin. The growth inhibition of macarecurvatin B (**2**) on MCF7, and MCF7/TAMR showed very high activity. Macarecurvatin A (**1**), and 6,8-diisoprenylaromadendrin (**3**) showed moderate activity.

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