Journal of Applied Pharmaceutical Science Vol. 13(06), pp 087-092, June, 2023
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2023.101645
ISSN 2231-3354

Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill

Alifia Muharram[1], Diah Ayu Rachmawati[1], Shola Mardhiyyah[1], Tjitjik Srie Tjahjandarie[1], Ratih Dewi Saputri[2], Norizan Ahmat[3], Mulyadi Tanjung[1*]

[1]Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
[2]Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia.
[3]Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia.

ARTICLE INFO

Received on: 13/01/2023
Accepted on: 21/04/2023
Available Online: 04/06/2023

Key words:
Macaranga involucrata, flavonoid, diterpenoid, cytotoxic, antioxidant.

ABSTRACT

Two flavonols, macagigantin (**1**) and brussoflavonol E (**2**), and two diterpenoids, deheiculatin C (**3**) and poilaneic acid (**4**), were obtained from *Macaranga involucrata* (Roxb.) Baill. leaves. Their structures were fully detailed on high-resolution electrospray mass spectra, 1D NMR (^{1}H, ^{13}C), and 2D NMR (heteronuclear multiple quantum coherence, heteronuclear multiple bond coherence) spectra. The cytotoxic and antioxidant activities of compounds **1–4** were evaluated in murine leukemia cancer cells (P-388) and DPPH radical assays, respectively. Compounds **1–3** showed moderate activity against P-388 cells with IC_{50} values of 26.3, 12.8, and 21.2 µM, respectively. Compounds **1–2** showed high activity against DPPH radical with IC_{50} values of 332.1 and 125.6 µM, respectively.

INTRODUCTION

Macaranga involucrata (Roxb.) Baill. (Euphorbiaceae) is one of the pioneer plants mainly found in the secondary forest regions in Kalimantan Island, Indonesia. Several plants of *Macaranga* have been used as herbal medicines (Qi *et al.*, 2017). The decoction of the leaves of *Macaranga recurvata* has been used to treat cancer by Dayak people (Tjahjandarie *et al.*, 2019). Previous reports have shown that *Macaranga* plants produce terpenoids (Qi *et al.*, 2017), flavonoids (Le *et al.*, 2021; Marliana *et al.*, 2018; Tanjung *et al.*, 2010), and stilbenoids (Aldin *et al.*, 2021; Pailee *et al.*, 2015; Tanjung *et al.*, 2018; Yang *et al.*, 2015). Five diterpenoids (cembranoids type), deheiculatins C, and G-J from *Macaranga deheiculata* showed moderate activity against human 11β-hydroxydehydrogenase type 1 (Qi *et al.*, 2017). Schweinfurthin A, a stilbenoid (piceatannol derivatives) from *Macaranga schweinfurthii*, exhibited potent cytotoxicity against lung cells (A549) and leukemia cells (NCI 60) (Klausmeyer *et al.*, 2010; Yoder *et al.*, 2007). Macarecurvatin B, a geranylated dihydroflavonol from *M. recurvata*, displayed potent activity against murine leukemia cancer cells (P-388) (Tanjung *et al.*, 2012). Nymphaeols A-C, three flavanones from *Macaranga tanarius*, showed high antioxidant activity against DPPH radical scavenging (Phommart *et al.*, 2005).

Macaranga involucrata are indigenous plants from Kalimantan Island, Indonesia. The flavonol and diterpenoid from *M. involucrata* leaves have not been reported for cytotoxic and radical scavenging activities. Furthermore, the isolation of **1–4** from *M. involucrata* leaves, the cytotoxic activity against P-388 cells, and the antioxidant activity against DPPH radical were also reported.

**Corresponding Author*
Mulyadi Tanjung, Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.
E-mail: mulyadi-t @ fst.unair.ac.id

MATERIALS AND METHODS

General experimental procedures

The UV-1,800 Shimadzu and the FTIR Shimadzu Tracer-100 spectrophotometer measured the maximum absorption (λ_{max}) and the isolated functional groups. The NMR spectra of flavonols and diterpenoids in acetone-d_6 were measured on a JEOL FTNMR ECA 400 spectrometer. The high-resolution electrospray mass spectra (HR-ESI-MS) of isolates were measured by an LCT Premier™ XE (Waters) mass spectrometer. Column chromatography (CC) used polyamide and Sephadex LH-20. The isolated spot in TLC used a cerium sulfate reagent and a UV lamp.

Plant materials

The leaves of *M. involucrata* (Fig. 1) were gathered from Gamsungi Village, Tobelo, North Maluku, Indonesia, in December 2017. The plant material, encoded specimens (MI-GTMU-IS3), was identified at Herbarium Bogoriense, Bogor, Indonesia.

Extraction and isolation

Extraction of secondary metabolites contained in a dry powder of *M. involucrata* leaves (1.1 kg) using maceration with methanol for 4 days was applied at room temperature. The methanol extract obtained was filtered, and solvent evaporation was carried out using a rotavapor to produce a thick methanol extract. The thick methanol extract was partitioned with hexane and ethyl acetate to produce hexane extracts (25 g) and ethyl acetate extracts (62 g).

The separation of EtOAc extract (60 g) by polyamide CC, eluting by a mobile phase (hexane-EtOAc 7:3 *v/v*), afforded three fractions (A-C). The separation of fraction B (1.65 g) by Sephadex LH-20 CC with methanol as a mobile phase afforded subfractions B_1-B_4. The purification of fraction B_3 (735 mg) by silica gel planar radial chromatography, eluting by a mobile phase (hexane-chloroform 3:7 to 1:1 *v/v*), afforded **1** (7 mg). The separation of fraction C (1.5 g) using the same method as fraction B resulted in two subfractions, C_1-C_2. Purification of subfraction C_2 with the same method as in subfraction B_3 using hexane-ethyl acetate as a mobile phase (19:1 to 7:3 *v/v*) resulted in compound **2** (21 mg).

Figure 1. *Macaranga involucrata*.

The separation of hexane extract (23 g) by CC, using polyamide as a stationary phase, eluting by a mobile phase (hexane-EtOAc 4:1 *v/v*), afforded two fractions (D-E). Sephadex LH-20 separated fraction E (3.15 g) with methanol to produce subfractions E_1-E_2. Fraction E_2 (815 mg) was purified by radial chromatography, eluting by hexane-diisopropyl ether 19:1 to 9:1 *v/v*), affording **3** (23 mg) and **4** (30 mg).

Identification of the isolated compounds

Compounds **1-4** (Fig. 1) were determined by UV, IR, HR-ESI-MS, 1D NMR (^{1}H, ^{13}C), and 2D NMR [heteronuclear multiple quantum coherence (HMQC) heteronuclear multiple quantum coherence (HMBC)] spectra.

Macagigantin (1): yellow solid, UV (MeOH) λ_{max} nm (log ε): 232 (4.08), 254 (4.03), 271 (4.13), and 368 nm (4.22). IR (KBr, cm^{-1}): 3,353, 1,648, 1,602, and 1,446. HR-ESI-MS: *m/z* $[M]^+$ calculated for $C_{30}H_{34}O_6$ 490.2356, found 490.2355. ESI-MS (*m/z*, % relative abundance): 490 (M^+, 17), 421 ($[M\text{-}C_5H_9]^+$, 24), 353 ($[M\text{-}C_{10}H_{17}]^+$, 56), and 299 ($[M\text{-}C_{14}H_{23}]^+$, 100). ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 1.51 (3H, *s*, H-13″), 1.54 (3H, *s*, H-14″), 1.57 (3H, *s*, H-12″), 1.79 (3H, *s*, H-15″), 1.85 (2H, *t*, *J* = 7.9 Hz, H-8″), 1,91 (2H, *t*, *J* = 8,5 Hz, H-9″), 1.97 (2H, *t*, *J* = 7,5 Hz, H-4″), 2.06 (2H, *q*, *J* = 7.0 Hz, H-5″), 3.36 (2H, *d*, *J* = 7.2 Hz, H-1″), 5.00 (1H, *t*, *J* = 6.8 Hz, H-10″), 5.05 (1H, *t*, *J* = 6.9 Hz, H-6″), 5.29 (1H, *t*, *J* = 7.3 Hz, H-2″), 6.58 (1H, *s*, H-8), 7.00 (2H, *d*, *J* = 9.0 Hz, H-3′/5′), 8.13 (2H, *d*, *J* = 9.0 Hz, H-2′/6′), and 12.41 (1H, *s,* 5-OH). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 146.6 (C-2), 136.6 (C-3), 176.5 (C-4), 104.0 (C-4a), 158.9 (C-5), 111.7 (C-6), 162.6 (C-7), 93.8 (C-8), 155.5 (C-8a), 123.4 (C-1′), 130.3 (C-2′/6′), 116.2 (C-3′/5′), 160.0 (C-4′), 21.9 (C-1″), 123.2 (C-2″), 135.1 (C-3″), 40.4 (C-4″/8″), 27.0 (C-5″), 124.8 (C-6″), 135.4 (C-7″), 27.3 (C-9″), 125.0 (C-10″), 131.5 (C-11″), 25.8 (C-12″), 17.6 (C-13″), 16.2 (C-14″), and 16.1 (C-15″).

Brussoflavonol E (2): yellow solid, UV (MeOH) λ_{max} nm (log ε): 239 (4.10), 258 (4.04), 276 (4.16), and 373 nm (4.25). IR (KBr, cm^{-1}): 3,363, 1,649, 1,620, and 1,440. HR-ESI-MS: *m/z* $[M+H]^+$ calculated for $C_{25}H_{27}O_7^+$ 439.1680, found 439.1679. ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 1.63 (3H, *s,* H-4″), 1.73 (3H, *s,* H-4‴), 1.75 (3H, *s,* H-5″), 1.77 (3H, *s,* H-5‴), 3.34 (2H, *d*, *J* = 7.2 Hz, H-1″), 3.40 (2H, *d*, *J* = 7.2 Hz, H-1‴), 5.26 (1H, *t*, *J* = 7.3 Hz, H-2″), 5.37 (1H, *t*, *J* = 7.4 Hz, H-2‴), 6.54 (1H, *s*, H-8), 7.70 (1H, *d*, *J* = 2.0 Hz, H-2′), 7.60 (1H, *d*, *J* = 2.0 Hz, H-6′), 7.93 (1H, *s*, 3-OH), 8.79 (1H, *s*, 3′-OH), 9.67 (1H, *s*, 7-OH), and 12.42 (1H, *s*, 5-OH). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 146.8 (C-2), 136.6 (C-3), 176.4 (C-4), 103.8 (C-4a), 158.9 (C-5), 111.6 (C-6), 162.6 (C-7), 93.7 (C-8), 155.5 (C-8a), 129.0 (C-1′), 122.8 (C-2′), 145.0 (C-3′), 146.3 (C-4′), 113.2 (C-5′), 123.1 (C-6′), 21.9 (C-1″), 123.1 (C-2″), 131.6 (C-3″), 25.8 (C-4″), 17.8 (C-5″), 29.0 (C-1‴), 123.3 (C-2‴), 132.8 (C-3‴), 25.9 (C-4‴), and 17.9 (C-5‴).

Deheiculatin C (3): light yellow oil, UV (MeOH) λ_{max} nm (log ε): 220 (4.50), and 274 nm (3.89). HR-ESI-MS: *m/z* $[M+H]^+$ calculated for $C_{20}H_{31}O_3^+$ 317.2120, found 319.2122. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H ppm: 0.82 (3H, *d*, *J* = 8.4 Hz, H-16), 0.85 (3H, *d*, *J* = 8.4 Hz, H-15), 1.27 (1H, *m*, H-15), 1.61

(3H, *s*, H-19), 1.79 (1H, *s*, H-1), 1.86 (2H, *m*, H-14), 2.14 (2H, *m*, H-9), 2.29 (2H, *m*, H-13), 2.57 (2H, *m*, H-6), 2.72 (2H, *m*, H-10), 4.42 (1H, *dd*, *J* = 9.7; 4.7 Hz, H-5), 5.09 (1H, *t*, *J* = 15.7 Hz, H-11), 5.15 (1H, *s*, H-18b), 5.19 (1H, *s*, H-18a), 5.55 (1H, *dd*, *J* = 15.7; 9.6 Hz, H-2), 5.88 (1H, *t*, *J* = 6.6 Hz, H-7), and 5.93 (1H, *d*, *J* = 15.7 Hz, H-3). ^{13}C-NMR (100 MHz, $CDCl_3$), δ_C ppm: 50.3 (C-1), 132.9 (C-2), 130.7 (C-3), 149.6 (C-4), 71.2 (C-5), 36.5 (C-6), 146.9 (C-7), 135.6 (C-8), 26.2 (C-9), 38.6 (C-10), 120.8 (C-11), 130.5 (C-12), 32.4 (C-13), 30.2 (C-14), 32.9 (C-15), 20.6 (C-16), 19.7 (C-17), 112.5 (C-18), 15.7 (C-19), and 173.2 (C-20).

Poilaneic acid (4): light yellow oil, UV (MeOH) λ_{max} nm (log ε): 226 (4.53), and 280 nm (4.00). HR-ESI-MS: *m/z* $[M+H]^+$ calculated for $C_{20}H_{31}O_2^+$ 303.2221, found 303.2223. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H ppm: 0.80 (3H, *d*, *J* = 6.8 Hz, H-16), 0.82 (3H, *d*, *J* = 6.8 Hz, H-15), 1.35 (2H, *m*, H-14), 1.49 (1H, *m*, H-15), 1.50 (2H, *m*, H-13), 1.64 (3H, *s*, H-19), 1.73 (1H, *s*, H-1), 1.81 (3H, *s*, H-18), 2.00 (2H, *m*, H-9), 2.93 (2H, *m*, H-10), 3.08 (2H, *m*, H-6), 5.18 (1H, *t*, *J* = 9.9 Hz, H-7), 5.21 (1H, *dd*, *J* = 15.6; 9.8 Hz, H-2), 5.57 (1H, *t*, *J* = 7.5 Hz, H-5), 6.03 (1H, *d*, *J* = 8.2 Hz, H-11), and 6.06 (1H, *d*, *J* = 15.6 Hz, H-3). ^{13}C-NMR (100 MHz, $CDCl_3$), δ_C ppm: 47.9 (C-1), 131.4 (C-2), 130.5 (C-3), 135.2 (C-4), 125.8 (C-5), 26.0 (C-6), 128.1 (C-7), 131.1 (C-8), 38.6 (C-9), 26.3 (C-10), 148.0 (C-11), 128.8 (C-12), 32.2 (C-13), 29.6 (C-14), 32.8 (C-15), 21.1 (C-16), 19.4 (C-17), 20.1 (C-18), 14.6 (C-19), and 173.5 (C-20).

Cytotoxic activity

According to the previous work, the MTT assay was used to assess the cytotoxic activity of **1–4** against murine leukemia cancer cells (P-388), cells for 48 hours in RPMI-1640 media with 10% FBS at 37°C and 5% CO_2. In the 96-well plate, P-388 cells were given compounds **1–4** and incubated for 24 hours at 37°C with 5% CO_2. The microplate reader spectrometer measured the active compound's capacity to kill cancer cells at λ = 590 nm. Artonin E was used as the cytotoxic assay's positive control (Saputri *et al.*, 2021; Tanjung *et al.*, 2012).

Antioxidant activity

The antioxidant activity of **1–4** was carried out against DPPH radicals using the UV-Vis spectrophotometer. The test solution was prepared in triplicate at concentrations of 100, 50, 25, 10, and 1 µM. The measurement of the antioxidant activity of **1–4** at a concentration of 100 µM was carried out through the mixture of 200 µl of **1–4** in 250 µM, 200 µl of acetate buffer (pH 5.5) was added, and 100 µl of 5×10^{-4} M DPPH radical solution was added. Compounds **1–4** were incubated for 30 minutes at room temperature. The determination of the inhibition of antioxidant activity against DPPH radical was at λ_{max} 517 nm. Ascorbic acid was used as an antioxidant assay's positive control (Aminah *et al.*, 2014; Phommart *et al.*, 2005).

RESULT AND DISCUSSION

Macagigantin (**1**), brussoflavonol E (**2**), deheiculatin C (**3**), and poilaneic acid (**4**) were isolated from the leaves of *M. involucrata*. Their structures were determined using HR-ESI-MS, 1D, and 2D NMR spectra (Supplementary Figs. S1–S13).

Compound **1** (macagigantin) was isolated as a yellow solid, having the chemical formula $C_{30}H_{34}O_6$ by HR-ESI-MS at ion peak $[M]^+$ at *m/z* 490.2355 (calcd 490.2356). The ^{1}H NMR exhibited the proton signals of two protons, a farnesyl side chain and a chelate of hydroxy. A pair of ortho-coupled (*J* = 9.0 Hz) at δ_H 8.13 (H-2′/6′) and 7.00 (H-3′/5′) at ring B, and a singled at δ_H 6.58 (H-8) at ring A. A signal of the farnesyl side chain consists of four methyls [δ_H 1.51 (H-13″), 1.54 (H-14″), 1.57 (H-12″), 1.79 (H-15″)], five methylenes [δ_H 1.85 (H-8″), 1,91 (H-9″), 1.97 (H-4″), 2.06 (H-5″), 3.36 (H-1″)], and three vinylic [δ_H 5.00 (H-10″), 5.05 (H-6″), 5.29 (H-2″)]. Compound **1** also showed a hydroxy proton at δ_H 12.41 (5-OH). The ^{13}C NMR of **1**, showing four oxyaryl carbons [δ_C 155.5 (C-8a),158.9 (C-5), 160.0 (C-4′), 162.6 (C-7)], two oxy-carbons [136.6 (C-3), δ_C 146.6 (C-2)], three quaternary carbons [δ_C 104.0 (C-4a), 111.7 (C-6), 123.4 (C-1′)], three methine carbons [δ_C 93.8 (C-8), 116.2 (C-3′/5′), 130.3 (C-2′/6′)], and a carbonyl carbon [176.5 (C-4)] recommended a kaempferol derivative. The HMBC described the farnesyl chain location in the kaempferol skeleton (Fig. 2). The HMBC spectrum, correlations of a hydroxy at δ_H 12.41 (5-OH) to an oxyaryl carbon at δ_C 158.9 (C-5), and two quaternary carbons [δ_C 104.0 (C-4a), 111.7 (C-6)] indicated a farnesyl chain at C-6. The methylene at δ_H 3.36 (H-1″), correlations to δ_C 162.6 (C-7), 123.2 (C-2″), 135.1 (C-3″), C-5, and C-6, supporting the farnesyl chain bounded at C-6 (Fig. 3). Based on the NMR data, compound **1** was identified as macagigantin (Aminah *et al.*, 2014; Tanjung et al., 2009).

Compound **2** (brussoflavonol E) showed the chemical formula $C_{25}H_{27}O_7^+$ at ion peak $[M+H]^+$ at *m/z* 439.1679 (calcd 439.1680) based on HR-ESI-MS data. The ^{1}H NMR exhibited a set of meta-coupled (*J* = 2.0 Hz) at δ_H 7.70 (H-2′) and 7.60 (H-6′) at ring B, and a singled at δ_H 6.54 (H-8) at ring A. Compound **2** also exhibited two isoprenyls consists of four methyls [δ_H 1.63 (H-4″), 1.73 (H-4‴), 1.75 (H-5″), 1.77 (H-5‴)], two methylenes [δ_H 3.34 (H-1″), 3.40 (H-1‴)], and two vinylic [δ_H 5.26 (H-2″), 5.37 (H-2‴)]. Compound **1** also showed four hydroxy protons at δ_H 7.93 (3-OH), 8.79 (3′-OH), 9.67 (7-OH), and 12.42 (5-OH). One hydroxy proton at C-4′ was not detected in the ^{1}H NMR. Five oxyaryls [δ_C 145.0 (C-3′), 146.3 (C-4′), 155.5 (C-8a), 158.9 (C-5), 162.6 (C-7)], two oxy-carbons [136.6 (C-3), δ_C 146.8 (C-2)], and a carbonyl carbon [176.4 (C-4)] recommended a quercetin skeleton in the ^{13}C NMR. The location of two isoprenyl chains in the quercetin skeleton (Fig. 2) was described with the HMBC spectra. The HMBC spectrum (Fig. 2), long-range correlations of a hydroxy at δ_H 12.41 (5-OH) to C-5, C-4a, C-6, and a methylene proton at δ_H 3.36 (H-1″), correlations to C-7, C-2″, C-3″, C-5, and C-6, indicating the isoprenyl chain bounded at C-6. Another methylene proton at δ_H 3.40 (H-1‴) correlated to C-2′, C-5′, C-2‴, and C-3‴ indicated that the isoprenyl chain bounded at C-3′. Furthermore, the structure of compound **2** was identified as brussoflavonol E (Son *et al.*, 2001).

Compound **3** (deheiculatin C) has the chemical formula $C_{20}H_{31}O_3^+$ based on ion peak $[M+H]^+$ at *m/z* 319.2122 (calcd 319.2120) from the HR-ESI-MS data, indicating six degrees of unsaturation. The 1D NMR (^{1}H, ^{13}C) of **3** consists of three methyls [δ_H 0.82 (H-16), 0.85 (H-17), 1.61 (H-19), δ_C 15.7 (C-19), 19.7 (C-17), 20.6 (C-16)], six methylenes [δ_H 1.86 (H-14), 2.14 (H-9), 2.29 (H-13), 2.57 (H-6), 2.72 (H-10), 5.15 (H-18b), 5.19 (H-18a), δ_C 26.2 (C-9), 30.2 (C-14), 32.4 (C-13), 36.5 (C-6), 38.6 (C-10), 112.5 (C-18)], seven methines [δ_H 1.27 (H-15), 1.79 (H-1), 4.42 (H-5), 5.09 (H-11), 5.55 (H-2), 5.88 (H-7), 5.93 (H-3), δ_C 26.2

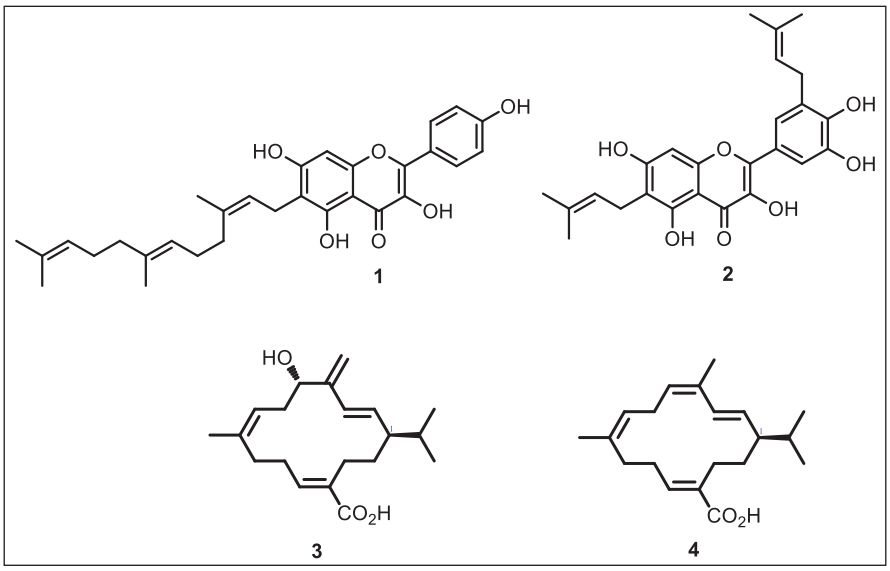

Figure 2. Isolated flavonols and diterpenoids of *M. involucrata*.

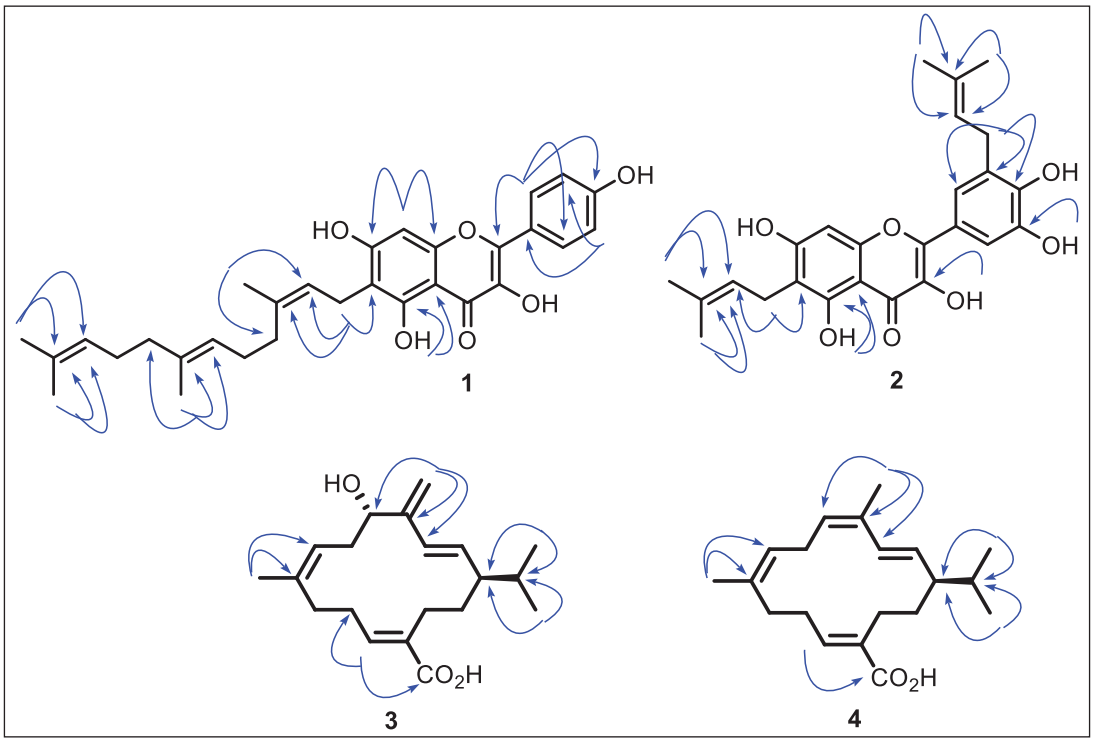

Figure 3. HMBC correlations of **1–4**.

(C-9), 32.9 (C-15), 50.3 (C-1), 71.2 (C-5), 120.8 (C-11), 130.7 (C-3), 132.9 (C-2)], three quaternary carbons [δ_C 130.5 (C-12), 135.6 (C-8), 149.6 (C-4)], and a carbonyl (δ_C 173.2 (C-20), indicating that **3** is a cembrane diterpenoid (Qi *et al.*, 2017). The location of proton and carbon signals on the structure of **3**, using the HMBC spectrum (Fig. 2). Two methyl protons (δ_H 0.82 (H-16), 0.85 (H-17) correlated to C-1, C-15, indicating isopropyl bounded at C-1. H-1 (δ_H 1.79) correlations with C-2, and a methylene terminal at H-18 (δ_H 5.15, and 5.19) to C-3, C-4, C-5, indicating a diene and hydroxy group at C-5 on cembrane skeleton. The methylene terminal (C-18) supports the correlations of a methine proton (H-5, δ_H 4.42) to C-2, C-3, C-18, C-6, also indicating methylene at C-6. A methyl proton (δ_H 1.61, H-18), showing correlations with C-7 and C-8. A methylene proton (δ_H 2.72. H-10) correlated to C-8, and a double bond (C-11, C-12), showing a carboxyl acid at C-12. Therefore, the structure of **3** was described as deheiculatin C (Qi *et al.*, 2017).

Compound **4** (poilaneic acid) had the chemical formula $C_{20}H_{31}O_2^+$ from the HR-ESI-MS data with ion peak $[M+H]^+$ at *m/z* 303.2223 (calcd 303.2221). The ^{1}H NMR of **4** consists of four methyls [δ_H 0.80 (H-16), 0.82 (H-15), 1.64 (H-19), 1.81 (H-18)], five methylenes [δ_H 1.35 (H-14), 1.50 (H-13), 2.00 (H-9),

Table 1. Cytotoxic and antioxidant data of compounds **1–4**.

Compounds	IC_{50} (μM)	
	P-388	DPPH
Macagigantin (1)	26.3 ± 0.32	332.1 ± 0.50
Brussoflavonol E (2)	12.8 ± 0.20	125.6 ± 1.02
Deheiculatin C (3)	21.2 ± 0.15	780.8 ± 1.35
Poilaneic acid (4)	>100	>1,000
Artonin E	1.33 ± 0.07	—
Ascorbic acid	—	329.0

2.93 (H-10), 3.08 (H-6)], and seven methines [δ_H 1.49 (H-15), 1.73 (H-1), 5.18 (H-7), 5.21 (H-2), 5.57 (H-5), 6.03 (H-11), 6.06 (H-3)]. The ^{13}C NMR of 4 consists of 20 separate carbon signals. The long-range correlations of **4** were similar to deheiculatin C (**3**), except at H-5 and H-18 in the HMBC spectra. A methyl proton at δ_H 1.81 (H-18)] correlated to C-3, C-4, C-5, and δ_H 5.57 (H-5), correlations to C-5, C-7, and C-18. Based on the NMR spectrum, the structure of **4** was described as poilaneic acid (Le *et al.*, 2021).

The MTT assay was used to assess the cytotoxic activity of compounds **1–4** against P-388 cells (Saputri *et al.*, 2021; Tanjung et al., 2021, 2012). Compounds **1–3** (Table 1) against P-388 cells showed moderate activity, and compound **4** was inactive. Brussoflavonol E (**2**) is more active than macagigantin (**1**). The presence of two hydroxy groups at C-3′ and C-4′ and an isoprenyl chain at C-5′ in compound **2** increased the cytotoxic activity compared to the hydroxy at C-4′ in macagigantin (**1**). Compounds **3** and **4** are diterpenoids from cembranoid derivatives. The hydroxy (C-5) methylene terminal (C-18) in compound **3** is more active than the methyl at C-18, as well as a double bond at C-4 and C-5 (Qi *et al.*, 2017).

Compounds **1–2** showed high activity against DPPH radical scavenging; compound **2** was more active than ascorbic acid. Further, macagigantin (**1**) exhibited antioxidant activity equivalent to ascorbic acid. Compounds **3–4** were inactive against DPPH radical scavenging (Tanjung *et al.*, 2013).

CONCLUSION

Macagigantin (**1**), brussoflavonol E (**2**), deheiculatin (**3**), and poilaneic acid (**4**) were isolated from *M. involucrata* leaves. Compounds **1–3** showed moderate activity against P-388 cells. Compounds **1–2** exhibited high activity against DPPH radicals.

ACKNOWLEDGMENTS

This research was supported through Hibah Riset Mandat Kolaborasi Mitra Luar Negeri, Universitas Airlangga, 2020, No. 782/UN3.15/PT/2021.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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How to cite this article:
Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J Appl Pharm Sci, 2023; 13(06):087–092.

SUPPLEMENTARY MATERIAL

Supplementary data can be downloaded from the link (https://japsonline.com/admin/php/uploadss/3905_pdf.pdf)