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

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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 (¹H, ¹³C), 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 IC₅₀ values of 26.3, 12.8, and 21.2 μM, respectively. Compounds 1–2 showed high activity against DPPH radical with IC₅₀ 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.

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MATERIALS AND METHODS

General experimental procedures

The UV-1,800 Shimadzu and the FTIR Shimadzu Tracer-100 spectrophotometer measured the maximum absorption ($\lambda_{\text{max}}$) and the isolated functional groups. The NMR spectra of flavonoids 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 two fractions, C$_1$ and C$_2$. Purification of fraction C$_1$ with the same method as in subfraction B$_2$ using hexane-ethyl acetate as a mobile phase (19:1 to 7:3 v/v) resulted in compound 2 (21 mg).

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$_3$. Fraction E$_1$ (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 (HMBQC)] spectra.

Macagigantin (1): yellow solid, UV (MeOH) $\lambda_{\text{max}}$ nm (log e): 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+H]$^+$ calculated for C$_{25}$H$_{34}$O$_{12}$ 490.2356, found 490.2355. ESI-MS: m/z 490.[M-H].

Brussoflavonol E (2): yellow solid, UV (MeOH) $\lambda_{\text{max}}$ nm (log e): 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$_{34}$O$_{12}$ 439.1680, found 439.1679.

Deheucilacin C (3): light yellow oil, UV (MeOH) $\lambda_{\text{max}}$ nm (log e): 220 (4.50), and 274 nm (3.89). HR-ESI-MS: $m/z$ [M+H]$^+$ calculated for C$_{20}$H$_{23}$O$_{7}$ 317.2120, found 319.2122.

General experimental procedures
ion peak [M]+ at m/z 490.2355 (calcd 490.2356). The 1H 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 8.13 (H-2/6′) and 7.00 (H-3/5′) at ring B, and a singlet at δ 6.58 (H-8) at ring A. A signal of the farnesyl side chain consists of four methylenes [δ 1.51 (H-13′), 1.54 (H-14′), 1.57 (H-12′), 1.79 (H-15′)], five methylenes [δ 1.85 (H-8′), 1.91 (H-9′), 1.97 (H-4′), 2.06 (H-5′), 3.36 (H-1′)], and three vinylc [δ 5.00 (H-10), 5.05 (H-6′), 5.29 (H-2′)]. Compound 1 also showed a hydrogen proton at δ 12.41 (5-OH). The 13C NMR of 1, showing four oxaryl carbons [δ 155.5 (C-8a), 158.9 (C-5), 160.0 (C-4′), 162.6 (C-7)], two oxycarbons [136.6 (C-3), δ 146.6 (C-2)], three quaternary carbons [δ 104.0 (C-4a), 111.7 (C-6), 123.4 (C-1′)], three methine carbons [δ 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 7.93 (3-OH) to an oxaryl carbon at δ 158.9 (C-5), and two quaternary carbons [δ 104.0 (C-4a), 111.7 (C-6)] indicated a farnesyl chain at C-6. The methylene at δ 3.66 (H-1′), correlations to δ 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 (brusslovafonol E) showed the chemical formula C26H32O13 at ion peak [M+H]+ at m/z 439.1679 (calcd 439.1680) based on HR-ESI-MS data. The 1H NMR exhibited a set of meta-coupled (J = 2.0 Hz) at δ 7.70 (H-2′) and 7.60 (H-6′) at ring B, and a singlet at δ 6.54 (H-8) at ring A. Compound 2 also exhibited two isoprenyls consists of four methylenes [δ 1.63 (H-4′), 1.73 (H-4″), 1.75 (H-5′), 1.77 (H-5′′)], two methylenes [δ 3.34 (H-1′), 3.40 (H-1″)], and two vinylc [δ 5.26 (H-2′), 5.37 (H-2″)]. Compound 1 also showed four hydroxy protons at δ 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 1H NMR. Five oxylaryls [δ 145.0 (C-3′), 146.3 (C-4′), 155.5 (C-8a), 158.9 (C-5), 162.6 (C-7)], two oxycarbons [136.6 (C-3), δ 146.8 (C-2)], and a carbonyl carbon [176.4 (C-4)] recommended a quercetin skeleton in the 13C 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 7.93 (3-OH) to C-5, C-4a, C-6, and a methylene proton at δ 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 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 brusslovafonol E (Son et al., 2001).

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% CO2. In the 96-well plate, P-388 cells were given compounds 1–4 and incubated for 24 hours at 37°C with 5% CO2. The microscope reader spectrophotometer 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 µL of acetate buffer (pH 5.5) was added, and 100 µl of 5 × 10−3 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 λmax 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), brusslovafonol E (2), deheiculatin C (3), and polianicic 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 C26H32O13 by HR-ESI-MS at
(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\textsubscript{C} 130.5 (C-12), 135.6 (C-8), 149.6 (C-4)], and a carbonyl (δ\textsubscript{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\textsubscript{H} 0.82 (H-16), 0.85 (H-17) correlated to C-1, C-15, indicating isopropyl bounded at C-1, H-1 (δ\textsubscript{H} 1.79) correlations with C-2, and a methylene terminal at H-18 (δ\textsubscript{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\textsubscript{H} 4.42) to C-2, C-3, C-18, C-6, also indicating methylene at C-6. A methyl proton (δ\textsubscript{H} 1.61, H-18), showing correlations with C-7 and C-8. A methylene proton (δ\textsubscript{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\textsubscript{20}H\textsubscript{31}O\textsubscript{2}\textsuperscript{+} from the HR-ESI-MS data with ion peak [M+H]\textsuperscript{+} at m/z 303.2223 (calcd 303.2221). The 'H NMR of 4 consists of four methyls [δ\textsubscript{H} 0.80 (H-16), 0.82 (H-15), 1.64 (H-19), 1.81 (H-18)], five methylenes [δ\textsubscript{H} 1.35 (H-14), 1.50 (H-13), 2.00 (H-9),

![Figure 2. Isolated flavonols and diterpenoids of M. involucrata.](image1)

![Figure 3. HMBC correlations of 1–4.](image2)
2.93 (H-10), 3.08 (H-6), and seven methines [δ₁ 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 ¹³C 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 1.81 (H-18) correlated to C-3, C-4, C-5, and δ 5.75 (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 emicranbiol 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) showed 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.

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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.

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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM) P-388</th>
<th>IC₅₀ (µM) DPPH</th>
</tr>
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<tbody>
<tr>
<td>Macagigantin (1)</td>
<td>26.3 ± 0.32</td>
<td>332.1 ± 0.50</td>
</tr>
<tr>
<td>Brussoflavonol E (2)</td>
<td>12.8 ± 0.20</td>
<td>125.6 ± 1.02</td>
</tr>
<tr>
<td>Deheiculatin C (3)</td>
<td>21.2 ± 0.15</td>
<td>780.8 ± 1.35</td>
</tr>
<tr>
<td>Poilaneic acid (4)</td>
<td>&gt;100</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Artonin E</td>
<td>1.33 ± 0.07</td>
<td>—</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>—</td>
<td>329.0</td>
</tr>
</tbody>
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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.

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REFERENCES


**SUPPLEMENTARY MATERIAL**

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

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