An anthraquinone derivative from Coptospella tomentosa (Blume) root (Merung)

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Abstract
Coptospella tomentosa (Blume) (Merung) is a type of tropical plants traditionally being used as a medicine by the Dayak tribes in Indonesia. This experiment aims to identify compound in the class of anthraquinone derivative from the ethyl acetate fraction of Coptospella tomentosa (Blume) root. In this study, an anthraquinone derivative was isolated from the ethyl acetate fraction of Coptospella tomentosa root using flash column chromatography. The compound was identified as 1-hydroxy-2-(hydroxymethyl) anthracene-9,10-dione (digiferruginol) (1). The structure of 1 was established based on Ultraviolet Visible, Fourier Transformed Infra-Red (FTIR), Nuclear Magnetic Resonance (NMR), and Quadrupole Time-of-Flight Mass Spectrometry (UPLC/QToF-MS) spectroscopic data. The antitumor and antioxidant activities of this compound were investigated by MTT assay against murine leukemia P-388 cells and DPPH free radical scavenging method, respectively. Antitumor and antioxidant activity test results show that compound 1 may have potent antitumor property but with moderate antioxidant activity.

Keywords: antitumor, antioxidant, Coptospella tomentosa, DPPH, traditionally

INTRODUCTION
Coptospella tomentosa (Blume), known locally as Merung or Manuran, is one of the tropical plants found in East Kalimantan, Indonesia. The local community of Dayak Kenyah uses the root of Coptospella tomentosa to treat malaria (Ardina et al. 2017; Oyediran, et al. 2015). In addition, a decoction of the Coptospella tomentosa roots is also used to treat parasitic worm infections (Lin 2005). The previous studies indicate that the extract of Coptospella tomentosa has several biological activities, i.e., inhibits hem polymerization, shows antilasmodial activity (Ardina et al. 2017; Ardina et al. 2019), has toxicity to Artemia salina (Karolina et al. 2018; Supriningrum et al. 2016), and shows antioxidant activity against DPPH free radical scavenging (Bohari et al. 2019).

This experiment aims to identify compounds in the class of anthraquinone derivative from the ethyl acetate fraction of the Coptospella tomentosa (Blume) root.

MATERIAL AND METHODS
Isolation and Purification
The crude extract of Merung (164.67 g) obtained from the maceration of 6 kg dried powdered roots of Coptospella tomentosa (Merung), then partitioned with n-hexane and ethyl acetate to produce fractions of n-hexane (5.33 g), ethyl acetate (61.13 g) and methanol (71.13 g) (Bohari et al. 2019).

The ethyl acetate fraction (61.13 g) was subjected to a flash column chromatography using a silica gel 60 (70-230 mesh ASTM) eluted with n-hexane-EtOAc in a polarity gradient method to give six fractions (E1 = 162.9 mg, E2 = 227.4 mg, E3 = 703.1 mg, E4 = 1,898.7 mg, E5 = 2,476.3 mg and E6 = 24,083.3 mg). E1 fraction further fractionated by the same chromatographic method and eluent system, resulting in four derived fractions (E11 = 45 mg, E12 = 149.3 mg, E13 = 200.8 mg, and E14 = 695.3 mg). The E2 fraction was purified by recrystallization with n-hexane: ethyl acetate (3:7). About 33 grams of compound 1 were obtained.

Antitumor Assay
In vitro MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl) cytotoxic assay against murine P-388 leukemia cells measured the antitumor activity of the...
Table 1. 1H- and 13C-NMR Spectroscopic data of 1-hydroxy-2-(hydroxymethyl)anthracene-9,10-dione (digiferruginol) (1).

<table>
<thead>
<tr>
<th>No</th>
<th>13C-NMR (Kuo et al. 1995)</th>
<th>1H-NMR (Kuo et al. 1995)</th>
<th>HMBC 1H→13C</th>
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<tr>
<td>1-OH</td>
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<td>156.64</td>
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<td>2</td>
<td>131.24</td>
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<tr>
<td>4</td>
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<tr>
<td>4a</td>
<td>138.25</td>
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<td>126.82</td>
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<td>6</td>
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<tr>
<td>11</td>
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<td>57.69</td>
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</table>

The presence of the –CH2OH group indicated when the proton signaled at 4.64 ppm (d, J = 12 Hz, 2H) (H-11) coupled with a signal at 5.44 ppm (t, J = 11.0 Hz, OH), and attached to C-11 (57.39 ppm). HMBC spectrum data shows that H-11 has a long-distance correlation with carbon at C-1, C-2, C-3, and C-4, so that the -CH2OH group attached to C-2. The doublet signaled at 7.89 ppm (d, J = 15.6 Hz) (H-3) was coupled to 7.74 ppm (d, J = 17.9 Hz) (4). The H-NMR spectrum of compound 1 also showed peaks at 8.17 ppm (d, J = 7.0 Hz, 1H) (5), 7.93 ppm (t, J = 6.2; 7.4 Hz, 2H) (5 and 6) and 8.22 ppm (d, J = 6.8 Hz, 1H) (Table 1). These peaks implied the existence of unsubstituted C rings. The absorption pattern in the H and C-NMR spectrum of the ring C of compound 1 is similar to the ring C of 2-ethoxy-1-hydroxyanthraquinone (Ee et al. 2009). Based on UV, IR, 1D-2D-NMR, QToF MS data, and compared with the NMR literature data (Kuo et al. 1995), it was concluded that compound 1 was 1-hydroxy-2-(hydroxymethyl)anthracene-9,10-dione (digiferruginol) (Fig. 1).

The 1-hydroxy-2-(hydroxymethyl)anthracene-9,10-dione (digiferruginol) has an IC50 value of 6.87 μg/mL in


Antioxidant assay with scavenging DPPH Radicals

Compound (1) was dissolved in methanol and made in several concentrations (20, 40, 80, 100 ppm), each solution was put 4 ml into a cuvette, and then added 1 mL of 0.024 μg/mL DPPH solution, homogenized and incubated in a dark room for 30 minutes. Then absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength (508-520 nm). The blanks were made without adding samples. All treatment was performed three times. IC50 determination was conducted using linear regression of %inhibition versus concentrations (Erwin et al. 2019; Supomo et al. 2019).

**RESULT AND DISCUSSION**

Compound (1) was obtained as an orange powder with a melting point of 205-208 °C. UPLC/QToF MS spectrum data shows [M-OH]+ = 237.0547, according to the molecular formula C11H10O4. The UV-Vis spectrum of compound 1 shows characteristic absorption at λ 253, 279, and 325 nm for the anthraquinone skeleton. UV spectrum data obtained absorption peaks at λmax at 402, 325, 279, and 253 nm. IR absorption peaks were 3263.56, 3076.46, 2927.94, 2854.65, 1635.64 and 1670.35 cm⁻¹. These peaks identified as OH, –C=O–H, aliphatic CH, chelated C=O, and unchelated C=O, respectively. The presence of an OH group in position 1 was shown by absorption in UV spectrum at λmax of 402 nm. The peak supported the evidence at 3263.56 cm⁻¹ in the FT-IR spectrum and 12.74 ppm (s, 1H) in 1H-NMR. The absorption of 1635.64 and 1670.35 cm⁻¹ in the FTIR spectrum shows the presence of chelated and unchelated quinone carbonyls (Ee et al. 2009). The absorption bands indicated the presence of aromatic and aliphatic CH group in the FTIR spectrum at 3076.46 (aromatic CH), 2927.94, and 2854 cm⁻¹ (aliphatic CH), respectively.
the MTT assay against murine leukemia P-388 cells and IC₅₀ value of the antioxidant activity of 26.30 μg/mL against DPPH free radical.

CONCLUSION

The 1-hydroxy-2-(hydroxymethyl) anthracene-9,10-dione (digifuranogin) has been isolated from the ethyl acetate fraction of the Coptosapelta tomentosa root. Antitumor and antioxidant activity test results showed that 1-hydroxy-2-(hydroxymethyl) anthracene-9,10-dione (1) has significant antitumor activity potential, but its antioxidant activity was moderate with IC₅₀ values of 6.67 and 26.30 μg/mL, respectively.

ACKNOWLEDGEMENTS

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