



## Chemical Constituents from the Leaves of *Dischidia nummularia* (Asclepiadaceae)

Sonni Maurit Benu<sup>1</sup>, Riga Riga<sup>2</sup>, and Lia Dewi Juliawaty<sup>1,\*</sup>

<sup>1</sup>Natural Products Research Group, Organic Chemistry Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia

<sup>2</sup>Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Jalan Prof. Dr. Hamka, Air Tawar Barat, Padang 25132, Indonesia

**Abstract** – The investigation of chemical constituents from *Dischidia nummularia* leaves, which belongs to the Asclepiadaceae family, resulted in five known compounds. Three compounds were suggested as triterpenoid derivatives, namely glutinol (**1**),  $\alpha$ -amyrin (**2**) and friedelin (**3**). In addition, two other compounds were a steroid derivative (**4**), i.e., stigmast-5-ene-3 $\beta$ -ol (**4**) and a xanthone derivative, i.e.,  $\alpha$ -mangostin (**5**). The chemical structures of **1–5** were elucidated based on detailed analysis of NMR (1D and 2D) spectroscopic data and compared with literature data. Interestingly, compounds **1** and **5** were first obtained from the Asclepiadaceae family, while compound **2** was isolated for the first time from the *Dischidia* genus. The acetone crude extract and compounds **1–3** showed weak cytotoxicity against murine leukemia P-388 cells. After all, the current study is the first report of a phytochemical study of *D. nummularia*.

**Keywords** – *Dischidia nummularia*, Triterpenoid, Steroid, Xanthone, Cytotoxicity

### Introduction

*Dischidia* plant, a type of epiphytic living abundantly in other plants as the host, is one of the genera of the milkweed family group (Asclepiadaceae), which consists of 120 species.<sup>1,2</sup> Generally, *Dischidia* are herbs and small shrubs as well. They are native to the tropics and subtropics regions<sup>2,3</sup> and are widely distributed in China, Taiwan, India, Bangladesh, and Southeast Asia with three non-endemic species occurring in the East Coast tropics of Australia.<sup>4</sup> For decades, organic extracts from *Dischidia* have been reported to have biological activities such as anticancer,<sup>5</sup> antiproliferative,<sup>6</sup> anti-inflammatory<sup>3,7,8</sup> and antioxidant.<sup>5,10</sup> Furthermore, previous phytochemistry research on this genus revealed the presence of various compounds, including triterpenes (friedelin (**4**), friedelinol, dischidiol, glutinone,  $\beta$ -amyrin acetate, taraxerol, 3 $\beta$ ,6 $\beta$ , 19 $\alpha$ ,23-tetrahydroxyurs-12-en-28-oic acid, 3 $\beta$ ,6 $\alpha$ ,19 $\alpha$ ,23-tetrahydroxyurs-12-en-28-oic acid and 6 $\beta$ ,19 $\alpha$ ,22 $\alpha$ -trihydroxyurs-12-en-3-oxo-28-oic acid), steroids (disformone and sitosteryl-3-*O*- $\beta$  glucopyranoside), and flavonoids

(kaempferol, isorhamnetin and quercetin).<sup>3,9</sup> One species of *Dischidia* is *D. nummularia*, which is known as a herb epiphytic (length of 10–50 cm) with a divided part and a smooth stem. It is distributed in Srilanka, India, Bangladesh, Australia, and Southeast Asia,<sup>11</sup> including Indonesia (throughout). In Kupang, Indonesia, the boiled water of the leaves of the plant are used as folk medicine to treat cysts, cervical cancer, heart disease, gonorrhea, *apthae tropicae* (tropical sores) and to relieve the pain of stings from *Ikan Sembilang* (a spiny catfish type). Thus far, based on our best knowledge, the chemical constituents of *D. nummularia* have not yet been reported. It is the important point of this research to evaluate the chemical constituents of this plant and its bioactivity. Therefore, the isolation of the compounds as well as cytotoxic evaluation of the crude extract and isolated compounds (**1–5**) from *D. nummularia* leaves are described herein.

### Experimental

**General experimental procedures** – <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a spectrometer of Agilent DD2 system, operating at 500 (<sup>1</sup>H) and 125 (<sup>13</sup>C) MHz, using CDCl<sub>3</sub> as the solvent and tetramethylsilane (TMS) as the internal standard. Optical rotations were measured using an Autopol IV polarimeter (Rudolph Research Analytical,

\*Author for correspondence

Lia Dewi Juliawaty, Natural Products Research Group, Organic Chemistry Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia  
Tel: +62-812-215-9124; E-mail: liadewi@chem.itb.ac.id

New Jersey). Melting points were measured on an Electrothermal melting point 110 V. Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were carried out using Merk Si gel 60 GF<sub>254</sub> art.7731 and Si gel PF<sub>254</sub> art.7749, respectively. Gravitational column chromatography (GCC) was carried out using Merk Si gel G60 35–70 mesh (art.7734) and Sephadex LH-20 (Amersham Pharmacia Biotech). Thin layer chromatography (TLC) analyses used precoated Si gel plates (Merk Kieselgel 60 GF<sub>254</sub>, 0.25 mm thickness, art.5554).

**Plant materials** – The leaves of *D. nummularia* were collected from Kupang, Timor Island, East Nusa Tenggara Province, Indonesia, in March 2016. The plant was determined and deposit at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences with voucher specimen BO-1274278.

**Extraction and isolation** – The dried leaves powdered of *D. nummularia* (1 kg) were extracted at room temperature with acetone three times (each 24 h) to give a dark yellow crude extract (200 g). Part of the crude extract (40 g) was fractionated using VLC and eluted with the gradient polarity solvent system, i.e., *n*-hexane 100%, *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub> (9:1 and 1:1), CH<sub>2</sub>Cl<sub>2</sub> 100%, CH<sub>2</sub>Cl<sub>2</sub>-EtOAc (1:1 and 1:4), EtOAc 100%, acetone 100% and MeOH 100% to afford 9 fractions (F1–F9). Fraction F3 (1.1 g) was refractionated using the same method and eluted with *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub> (7:3, 6:4, 4:6, and 2:8), CH<sub>2</sub>Cl<sub>2</sub> 100%, acetone 100% and MeOH 100% to yield 5 subfractions (F3.1–F3.5). Subfraction F3.1 (389 mg) was subjected to CPC eluted with *n*-hexane-EtOAc (12:1) to give 4 fractions (F3.1.1–F3.1.4). Fraction F3.1.3 (147 mg) was purified by GCC using Sephadex LH-20 (MeOH) to afford compound **1** (15 mg). Next, fraction F3.2 (100 mg) was separated by CPC eluted with *n*-hexane-EtOAc (15:1) to obtain compound **2** (5 mg). Meanwhile, fraction F4 (2.0 g) was purified by Si gel GCC (*n*-hexane-CHCl<sub>3</sub>, 9:1) to afford compound **3** (15 mg). Fraction F7 (10 g) was also separated by VLC eluted with *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub> (1:1, 3:7, and 1:9), CH<sub>2</sub>Cl<sub>2</sub> 100%, CH<sub>2</sub>Cl<sub>2</sub>-acetone (9:1), acetone 100% and MeOH 100% into 8 fractions (F7.1–F7.8). Subfraction F7.4 (148 mg) was also subjected to a GCC using Sephadex LH-20 (MeOH) to give compound **4** (22 mg). Furthermore, subfraction F7.6 (1.2 g) was further purified by CPC (*n*-hexane-EtOAc, 8:2) to obtain compound **5** (15 mg).

**Glutinol (1)** – Colorless crystals; mp 208–210°C;  $[\alpha]_D^{22}$ : +58.5 (*c* 9 × 10<sup>-4</sup>, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 3.46 (1H, t, H-3), 5.62 (1H, m, H-6), 1.04 (3H, s, H-23), 1.14 (3H, s, H-24), 0.85 (3H, s, H-25), 1.01 (3H, s, H-26),

1.10 (3H, s, H-27), 1.16 (3H, s, H-28), 0.99 (3H, s, H-29), 0.95 (3H, s, H-30); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 18.2 (C-1), 27.8 (C-2), 76.3 (C-3), 40.8 (C-4), 141.4 (C-5), 122.0 (C-6), 23.7 (C-7), 47.4 (C-8), 34.8 (C-9), 49.7 (C-10), 34.5 (C-11), 30.2 (C-12), 39.3 (C-13), 37.8 (C-14), 32.1 (C-15), 36.0 (C-16), 30.1 (C-17), 43.1 (C-18), 35.1 (C-19), 28.2 (C-20), 33.1 (C-21), 39.0 (C-22), 25.5 (C-23), 29.0 (C-24), 16.2 (C-25), 19.6 (C-26), 18.4 (C-27), 32.03 (C-28), 32.4 (C-29), 34.5 (C-30).

**α-Amyrin (2)** – White solid; mp 183–185°C;  $[\alpha]_D^{22}$ : +54.1 (*c* 8 × 10<sup>-4</sup>, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 3.15 (1H, H-3), 0.67 (1H, H-5), 5.12 (1H, H-12), 0.94 (3H, s, H-23), 0.76 (3H, s, H-24), 0.73 (3H, s, H-25), 0.89 (3H, s, H-26), 1.01 (3H, s, H-27), 0.95 (3H, s, H-28), 0.85 (3H, d, *J* = 6.0 Hz, H-29), 0.75 (3H, d, *J* = 7.0 Hz, H-30); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 38.7 (C-1), 28.7 (C-2), 79.0 (C-3), 38.5 (C-4), 55.1 (C-5), 18.3 (C-6), 32.8 (C-7), 40.0 (C-8), 47.6 (C-9), 36.8 (C-10), 23.3 (C-11), 124.3 (C-12), 139.5 (C-13), 42.0 (C-14), 27.2 (C-15), 26.5 (C-16), 33.7 (C-17), 58.9 (C-18), 39.6 (C-19), 39.5 (C-20), 31.2 (C-21), 41.4 (C-22), 28.0 (C-23), 15.5 (C-24), 15.6 (C-25), 16.8 (C-26), 23.2 (C-27), 28.0 (C-28), 17.4 (C-29), 21.3 (C-30).

**Friedeline (3)** – Colorless crystal; mp 259–263°C;  $[\alpha]_D^{22}$ : –21.0 (*c* 5.1 × 10<sup>-1</sup>, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 1.67 (1H, m, H-1a), 1.96 (1H, m, H-1b), 2.23 (1H, m, H-2a), 2.39 (1H, m, H-2b), 2.23 (1H, m, H-4), 1.27 (1H, m, H-6a), 1.74 (1H, m, H-6b), 1.33 (1H, m, H-7a), 1.45 (1H, m, H-7b), 1.37 (1H, m, H-8), 1.50 (1H, m, H-10), 1.53 (1H, m, H-18), 0.94 (1H, m, H-22a), 1.48 (1H, m, H-22b), 0.86 (3H, d, *J* = 6.0 Hz, H-23), 0.70 (3H, s, H-24), 0.85 (3H, s, H-25), 0.98 (3H, s, H-26), 1.03 (3H, s, H-27), 1.16 (3H, s, H-28), 0.93 (3H, s, H-29), 0.98 (3H, s, H-30); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 22.3 (C-1), 41.5 (C-2), 213.3 (C-3), 58.2 (C-4), 42.2 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.4 (C-10), 35.6 (C-11), 30.5 (C-12), 38.3 (C-13), 39.7 (C-14), 32.4 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.7 (C-21), 39.2 (C-22), 6.8 (C-23), 14.7 (C-24), 17.9 (C-25), 20.3 (C-26), 18.7 (C-27), 32.1 (C-28), 31.8 (C-29), 35.0 (C-30).

**Stigmast-5-ene-3β-ol (4)** – White solid; mp 135–138°C;  $[\alpha]_D^{22}$ : –20.0 (*c* 25 × 10<sup>-4</sup>, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 3.51 (1H, m, H-3), 2.21 (2H, H-4), 2.26 (2H, H-4), 5.33 (1H, dd, *J* = 1.8; 4.8 Hz, H-6), 0.66 (3H, s, H-18), 0.99 (3H, s, H-19), 0.90 (3H, d, *J* = 6.6 Hz, H-21), 0.79 (3H, d, *J* = 6.6 Hz, H-26), 0.82 (3H, d, *J* = 6.6 Hz, H-27), 0.86 (3H, t, *J* = 7.2 Hz, H-29); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 37.38 (C-1), 32.03 (C-2), 71.96 (C-3), 42.45 (C-4), 140.87 (C-5), 121.87 (C-6), 31.78 (C-7),

32.05 (C-8), 50.25 (C-9), 36.29 (C-10), 21.22 (C-11), 39.90 (C-12), 42.41 (C-13), 56.89 (C-14), 24.45 (C-15), 28.40 (C-16), 56.17 (C-17), 12.12 (C-18), 19.16 (C-19), 36.64 (C-20), 18.92 (C-21), 34.07 (C-22), 26.16 (C-23), 45.95 (C-24), 29.26 (C-25), 19.97 (C-26), 19.55 (C-27), 23.19 (C-28), 12.00 (C-29).

**$\alpha$ -Mangostin (5)** – Yellow oil;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz):  $\delta$  13.76 (1H, s, 1-OH), 9.50 (1H, s, 3-OH), 6.30 (1H, s, H-4), 6.82 (1H, s, H-5), 9.45 (1H, s, H-6), 3.43 (2H, d,  $J = 6.0$  Hz, H-11), 5.26 (1H, m,  $J = 6.0$  Hz, H-12), 1.83 (3H, s, H-14), 1.69 (3H, s, H-15), 4.08 (2H, d,  $J = 6.0$  Hz, H-16), 5.26 (1H, m,  $J = 6.0$  Hz, H-17), 1.76 (3H, s, H-19), 1.83 (3H, s, H-20), 3.80 (3H, s, 7-OCH<sub>3</sub>);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz):  $\delta$  160.7 (C-1), 108.7 (C-2), 161.7 (C-3), 93.3 (C-4), 155.1 (C-4a), 101.7 (C-5), 155.8 (C-6), 142.6 (C-7), 137.1 (C-8), 112.3 (C-8a), 182.1 (C-9), 103.7 (C-9a), 154.7 (C-10a), 21.5 (C-11), 121.6 (C-12), 132.2 (C-13), 18.0 (C-14), 25.6 (C-15), 26.9 (C-16), 123.2 (C-17), 135.6 (C-18), 25.9 (C-19), 18.3 (C-20), 62.1 (O-CH<sub>3</sub>).

**Cytotoxicity assay** – The crude extract and compounds **1–3** were evaluated for their cytotoxicity against murine leukemia P-388 cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] assay as previously reported.<sup>12–14</sup> The cells were seeded in 96-well plates (cell density of  $3 \times 10^4$  cells/cm<sup>3</sup>). The crude extract and each compound (**1–3**) were added in various concentrations and incubated for 48 hours, where the crude extract and compounds were dissolved in dimethyl sulfoxide (DMSO). After 48 hours of incubation, 10  $\mu\text{L}$  MTT reagent was added to each sample and then incu-

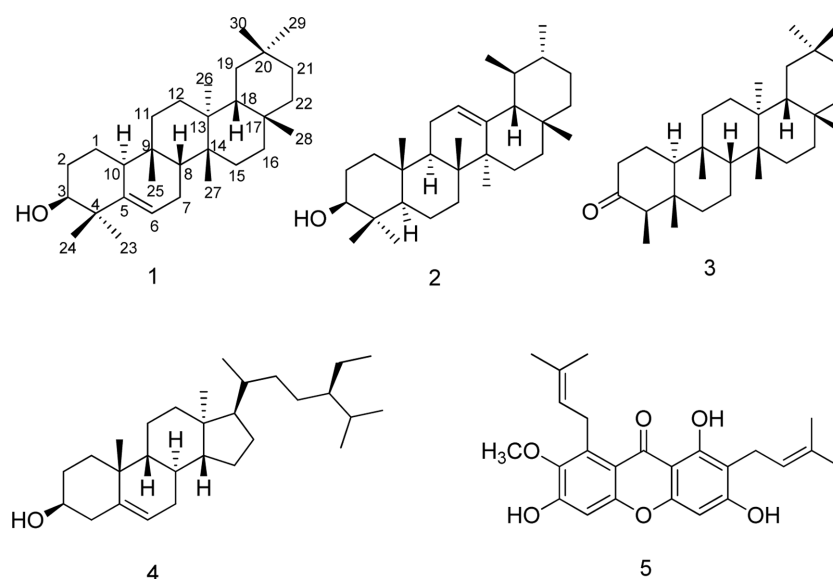
bated for 4 hours. The MTT-stop solution containing sodium dodecyl sulfate (SDS) was added and the incubation continued for 24 hours. Optical density was read with a microplate reader at 550 nm. IC<sub>50</sub> values were taken from the plotted graph of the percentage of live cells compared with control. MTT solution and DMSO (without cells and media) were taken as negative controls, while artonin E was used as a positive control.<sup>14,15</sup>

## Results and Discussion

The leaves of *D. nummularia* were collected from Timor Island, East Nusa Tenggara, Indonesia. Then, the dry leaves powdered were extracted with acetone. Separation and purification of acetone extract using several chromatography techniques, including vacuum liquid chromatography (VLC), gravitational column chromatography (GCC) and centrifugal planar chromatography (CPC), yielded compounds **1–5** (Fig. 1).

All chemical structures of isolated compounds were determined by an extensive analysis of NMR (1D and 2D) spectroscopic data and by comparison with previously reported data. Compounds **1–5** were identified as glutinol (**1**),<sup>16</sup>  $\alpha$ -amyrin (**2**),<sup>17</sup> friedelin (**3**),<sup>18</sup> stigmast-5-ene-3 $\beta$ -ol or  $\beta$ -sitosterol (**4**)<sup>19</sup> and  $\alpha$ -mangostin (**5**).<sup>20</sup> The isolation of all compounds (**1–5**) from *D. nummularia* was investigated for the first time in this study. Moreover, to our knowledge, compounds **1** and **5** were first isolated from the Asclepiadaceae family. In addition, the finding of compound **2** was first reported in the *Dischidia* genus.

Compound **1** was isolated as colorless crystals with



**Fig. 1.** Chemical structures of compounds **1–5** isolated from the leaves of *D. nummularia*.

$[\alpha]_D^{22}$ :  $+58.5$  ( $c$   $9 \times 10^{-4}$ ,  $\text{CHCl}_3$ ), and melting point of 208–210°C, which was identified as glutinol, a triterpenoid. The  $^1\text{H}$  NMR spectrum of **1** exhibited a total of eight methyl protons signals at  $\delta_{\text{H}}$  0.85 (3H, s, H-25), 0.95 (3H, s, H-30), 0.99 (3H, s, H-29), 1.01 (3H, s, H-26), 1.04 (3H, s, H-23), 1.10 (3H, s, H-27), 1.14 (3H, s, H-24) and 1.16 (3H, s, H-28). Moreover, also showed the deshielded proton signal at  $\delta_{\text{H}}$  3.46 (1H, t, H-3) was assigned to oxygenated methine proton, which was directly attached to a hydroxyl group and one olefin methine proton at  $\delta_{\text{H}}$  5.26 (1H, m, H-6). The  $^{13}\text{C}$  NMR spectrum indicated a total of 30 carbon signals containing an oxygenated methine carbon which is hydroxyl group at  $\delta_{\text{C}}$  76.3 (C-3), an olefin quaternary carbon at  $\delta_{\text{C}}$  141.4 (C-5) and olefin methine carbon at  $\delta_{\text{C}}$  122.0 (C-6), eight methyl carbons at  $\delta_{\text{C}}$  25.5 (C-23), 29.0 (C-24), 16.2 (C-25), 19.6 (C-26), 18.4 (C-27), 32.03 (C-28), 32.4 (C-29), 34.5 (C-30); ten methylene carbons at  $\delta_{\text{C}}$  18.2 (C-1), 27.8 (C-2), 23.7 (C-7), 34.5 (C-11), 30.2 (C-12), 32.1 (C-15), 36.0 (C-16), 35.1 (C-19), 33.1 (C-21), 39.0 (C-22); three methine carbons at  $\delta_{\text{C}}$  47.4 (C-8), 49.7 (C-10) and 43.1 (C-18); and six quaternary carbons at  $\delta_{\text{C}}$  40.8 (C-4), 34.8 (C-9), 39.3 (C-13), 37.8 (C-14), 30.1 (C-17) and 28.2 (C-20).

Careful investigation by the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectroscopic data established that compound **1** was glutinol, an triterpenoid type with C-3 oxidized to a hydroxyl and double bond at C-5 and C-6 that was confirmed by data comparison with those in the literatures<sup>16</sup> and selected HMBC correlation (Fig. 2) of methyl proton at  $\delta_{\text{H}}$  1.04 (3H, s, H-23) and 1.14 (3H, s, H-24) with oxymethine carbon (C-3). Another correlation showed by olefin methine proton at  $\delta_{\text{H}}$  5.26 (1H, m, H-6) with quaternary carbon (C-4) and two units of methine carbon (C-8 and C-10).

Additionally, all isolated compounds have been reported as having various biological activities. Compound **1** showed strong cytotoxic toward human ovarian cancer cell (OVACAR3 cell) with  $\text{IC}_{50}$  value of  $6 \mu\text{M}$ <sup>21</sup> and anti-inflammatory activity with active  $\text{IC}_{50}$  value of  $2.86 \mu\text{M}$ .<sup>22</sup> Compound **2** was have significant anticancer activity on four cancer cell lines (MCF-7, BEL-7402, SPC-A-1 and SGC-7901) with  $\text{IC}_{50}$  values of  $7.2 \pm 0.12$ ,  $8.2 \pm 0.29$ ,  $7.6 \pm 0.06$  and  $5.0 \pm 0.12 \mu\text{M}$ <sup>23</sup> and showed antimicrobial activity against *S. aureus*, *E. Coli*, *S. pneumoniae*, *S. typhi* and *C. albicans* with the same MICs and MBCs at 0.37 and  $0.75 \mu\text{g/mL}$ , respectively.<sup>24</sup> Compound **3** revealed significant inhibition (89.64%) of castor oil-induced diarrhea<sup>25</sup> and also identified as the best possible inhibitor against prostate cancer CYP17A1 which was tested on hormone-sensitive (22Rv1) and insensitive (DU145)

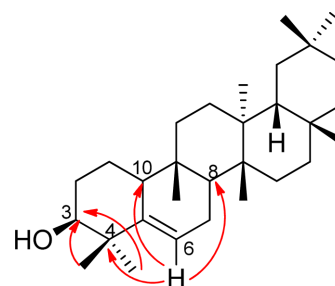


Fig. 2. Selected HMBC correlations of compound **1**.

cell lines with the  $\text{IC}_{50}$  values were found to be 72.025 and  $81.766 \mu\text{g/mL}$ , respectively.<sup>26</sup> Compound **4** was shown to inhibit cells growth toward T47D, MCF-7, HeLa and WiDr cell lines with  $\text{IC}_{50}$  values of 0.55, 0.87, 0.76 and  $0.99 \mu\text{M}$ , respectively<sup>27</sup> and exhibited significantly anti-diabetic activity at all glucose concentrations with minimum increase 66.48% at  $10 \mu\text{g/mL}$  and maximum increase 74.46% at  $80 \mu\text{g/mL}$  glucose concentration.<sup>28</sup> Then compound **5** showed antibacterial activity against *P. acnes* and *S. aureus* with the same MICs and MBCs at 6.25 and  $50 \mu\text{g/mL}$ , respectively;<sup>29</sup> strong antimalarial activity with cytotoxic effect against the resistant plasmodium falciparum with  $\text{IC}_{50}$  value of  $0.2 \pm 0.01 \mu\text{M}$ ,<sup>30</sup> promising antiviral activity where exposure of compound **5** caused 55%, 48%, 50% and 47% reduction of infected cells when HepG2 cells were infected with DENV-1, DENV-2, DENV-3 and DENV-4, respectively;<sup>30</sup> anti-inflammatory activity by diminished the LPS expressions in ocostatin M signaling through the mitogen-activated protein kinase (MAPK) pathways<sup>30</sup> and potential anticancer activity by the cell growth inhibition of human leukemia cell line HL60 at  $\text{IC}_{50}$  value of  $10 \mu\text{M}$ .<sup>31</sup>

Nonetheless, the present study was the first time to investigate the cytotoxic activity of acetone crude extract and isolates (**1–3**) of *D. nummularia* leaves against murine leukemia P-388 cells according to the method of the MTT assay as described earlier.<sup>12,14</sup> Weak cytotoxicity was exhibited by the acetone crude extract with  $\text{IC}_{50}$  value of  $58.33 \mu\text{g/mL}$  and compounds **1–3** with  $\text{IC}_{50}$  values of 69.88,  $>234.74$  and  $216.17 \mu\text{M}$ , respectively (Table 1). Interestingly, compounds **4** and **5** were previously reported as active with  $\text{IC}_{50}$  values of 0.57 and  $7.80 \mu\text{M}$ .<sup>32,33</sup> Although the extract as well as compounds **1–3** were not active to the murine leukemia P-388 cells, but compounds **4** and **5** have been reported to be active against murine leukemia P-388 cells. In addition, compounds **1–3** that were not active in the murine leukemia P-388 cells have also reported to have potential activity against other various bioactivities as described before. Furthermore, more studies

**Table 1.** Cytotoxicity of extract and compounds 1–5 against murine leukemia P-388 cells

Samples	IC <sub>50</sub>
Acetone crude extract	58.33 µg/mL
Glutanol (1)	69.88 µM
α-Amyrin (2)	>234.74 µM
Friedelin (3)	216.17 µM
Stigmast-5-ene-3β-ol (4)	0.57 µM
α-Mangostin (5)	7.80 µM
Artonin E*	0.68 µM

\*Positive control

regarding the other bioactivities profile of the extract as well as chemical constituents of this plant are required.

### Acknowledgments

We would like to thank the Indonesia Endowment Fund for Education (LPDP) scholarship of the Ministry of Finance, Indonesia for giving financial support to the first author. We are grateful to Prof. Dr. Yana Maolana Syah and Dr. Elvira Hermawati at Institut Teknologi Bandung for helping with NMR measurements.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

### References

- Rahman, M. A.; Wilcock, C. C. *J. Biogeography* **1991**, *18*, 51–58.
- Hajari, Y.; Sulistijorini.; Ariyanti, N. S. *J. Trop. Life Sci.* **2018**, *8*, 269–278.
- Chen, Z. S.; Lee, G. H.; Kuo, Y. H. *Phytochemistry* **1993**, *34*, 783–786.
- Forster, P. I.; Liddle, D. J. *Austrobaileya* **1988**, *2*, 507–514.
- Nurrani, L.; Ady, S.; Sugio, K. Antioxidant Activities of Four Medicinal Plants Utilization by Tribes in North Sulawesi; International Seminar Forest and Medicinal Plants of Manado: Indonesia, **2013**.
- Khalil-ur-Rehman, M.; Wenzig, E. M. P.; Kretschmer, N.; Kunert, O.; Hofer, F.; Bauer, R. *Planta Med.* **2019**, *85*, 1525–1526.
- Wuyang, H. Traditional Chinese Medicinal Plants and Their Endophytic Fungi: Isolation, identification, and bioassay; The University of Hong Kong: China, **2008**.
- Yang, L.; Wang, X. M.; Yu, B. L.; Yao, G. W.; Li, X. L.; Chen, J. B.; Xie, R. *China Trop. Med.* **2015**, *15*, 1302–1305.
- Ma, X.; Yang, C.; Zhang, Y. *Magn. Reson. Chem.* **2008**, *46*, 571–575.
- Tari, Y.; Cunha, T. D.; Lulan, T. Y.; Buang, Y. *J. App. Chem. Sci.* **2012**, *1*, 39–46.
- Silalahi, M.; Nisyawati.; Walujo, E. B.; Supariatna, J.; Mangunwardoyo, W. *J. Ethnopharmacol.* **2015**, *175*, 432–443.
- Sahidin.; Hakim, E. H.; Juliawaty, L. D.; Syah, Y. M.; Din, L. B.; Ghisalberti, E. L.; Latip, J.; Said, I. M.; Achmad, S. A. *Z. Naturforsch. C. J. Biosci.* **2005**, *60*, 723–727.
- Yang, R. L.; Yan, Z. H.; Lu, Y. *J. Agric. Food Chem.* **2008**, *56*, 3024–3027.
- Riga, R.; Happyana, N.; Hakim, E. H. *J. Appl. Pharm. Sci.* **2019**, *9*, 108–124.
- Hakim, E. H.; Achmad, S. A.; Juliawaty, L. D.; Makmur, L.; Syah, Y. M.; Aimi, N.; Kitajima, M.; Takayama, H.; Ghisalberti, E. L. *J. Nat. Med.* **2007**, *61*, 229.
- Rosandy, A. R.; Din, L. B.; Yaacob, W. A.; Yusoff, N. I.; Sahidin, I.; Latip, J.; Nataqain, S.; Noor, N. M. *Malaysian J. Anal. Sci.* **2013**, *17*, 50–58.
- Vazquez, L. H.; Palazon, J.; Navarro-Ocaña, A. *Phytochemicals* **2012**, *23*, 487–502.
- Rodrigues, P. M.; Gomes, J. V. D.; Jamal, C. M.; Neto, A. C.; Santos, M. L.; Fagg, C. W.; Fonseca-Bazzo, Y. M.; Magalhaes, P. O.; de Sales, P. M.; Silveira, D. *Food Chem. Toxicol.* **2017**, *109*, 1063–1068.
- Katiyar, D.; Singh, V.; Gilani, S. J.; Goel, R.; Vats, A.; Bajaj, U. *Int. J. Drug Dev. Res.* **2014**, *6*, 92–98.
- Ha, L. D.; Hansen, P. E.; Vang, O.; Duus, F.; Pham, H. D.; Nguyen, L. H. D. *Chem. Pharm. Bull (Tokyo)*. **2009**, *57*, 830–834.
- Chen, Y.; Li, J. *Trop. J. Pharm. Res.* **2021**, *20*, 1331–1335.
- Adebayo, S. A.; Shai, L. J.; Eloff, J. N. *Asian Pac. J. Trop. Med.* **2017**, *10*, 42–46.
- Abu-Lafi, S.; Rayan, B.; Kadan, S.; Abu-Lafi, M.; Rayan, A. *Oncol. Lett.* **2019**, *17*, 713–717.
- Ekalu, A.; Ayo, R. G. O.; Habila, J. D.; Hamisu, I. *JOTCSA* **2019**, *6*, 411–418.
- Antoniamy, P.; Duraipandiyam, V.; Ignacimuthu, S.; Kim, J. H. *South Indian J. Bio. Sci.* **2015**, *1*, 34–37.
- Joshi, B. P.; Bhandare, V. V.; Vankawala, M.; Patel, P.; Patel, R.; Vyas, B.; Krishnamurty, R. *J. Biomol. Struct. Dyn.* **2022**, *14*, 1–26.
- Hasibuan, P. A. Z.; Sitorus, P.; Satria, D. *Asian J. Pharm. Clin. Res.* **2017**, *10*, 306–308.
- Zeb, M. A.; Khan, S. U.; Rahman, T. U.; Sajid, M.; Seloni, S. *Pharm. Pharmacol. Int. J.* **2017**, *5*, 204–207.
- Tanngoen, P.; Lamlertthon, S.; Tiyafoonchai, W. *Int. J. Pharm. Pharma. Sci.* **2019**, *11*, 45–49.
- Chen, G.; Li, Y.; Wang, W.; Deng, L. *Expert Opin. Ther. Pat.* **2018**, *28*, 415–427.
- Muchtaridi, M.; Wijaya, C. A. *Asian J. Pharm. Clin. Res.* **2017**, *10*, 440–445.
- Nurhamidah, N. Characterization of Secondary Metabolites and Cytotoxic and Antibacterial Activities from Fruit of *Ficus aurata* (Miq.); Universitas Andalas Padang: Indonesia, **2016**.
- Hermawati, E. Xanthone from Fruit Skin of *Garcinia mangostana* (Guttiferae) and Its Bioactivity as Anti-tumor: Master Thesis; Institut Teknologi Bandung; Indonesia, **2011**.

Received September 25, 2022

Revised March 14, 2023

Accepted March 14, 2023