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Chemical Constituents from *Macaranga lowii* King ex Hook.f. (Euphorbiaceae) and Their Effects on Antioxidant Activity

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The genus *Macaranga* comprises about 300 species worldwide and is commonly distributed in the tropical region of Africa, Southeast Asia, China and India. The plant of this genus was reported to possess various medicinal properties which have been traditionally used to treat various illnesses such as fungal infection, malaria, stomachache, and diarrhoea. This genus also contains many phenolic compounds, particularly prenylated flavonoids and stilbenoids, followed by terpenoids and tannins. The phytochemical study on the crude acetone extract from the stem bark of *Macaranga lowii* was carried out to isolate chemical constituents using several chromatographic techniques and characterise the structure of the isolated compounds using various spectroscopic methods. The powdered stem bark of *M. lowii* (2.05 kg) was macerated in acetone for 24 hours at room temperature and repeated three times. The crude extract (38.8 g) was dissolved in methanol (200 mL) and fractionated with diethyl ether to reduce tannin and yield a crude extract with less tannin (10.9 g). The crude extract was subjected to vacuum liquid chromatography (VLC) to give nine semi-purified fractions (ML1-9). Fractionation and purification of fraction ML2 (448 mg) yielded compound **1** (6.2 mg) and **3** (0.3 mg). Fraction ML7 (220 mg) was subjected to radial chromatography (RC) to give a pure compound **2** (2.1 mg). The compounds were elucidated using Nuclear Magnetic Resonance (NMR), Ultraviolet-Visible (UV-Vis), Infrared (IR) and Mass spectrometry (MS), as well as comparison with the previous literature. Two phenolic compounds namely pentadecyl ferulate (**1**) and scopoletin (**2**) together with a sterol known as stigmasterol (**3**) were purified successfully from the stem bark of *M. lowii*. Scopoletin (**2**) exhibited moderate activity against DPPH radical scavenging with the IC_{50} value of 34.82 μM.

Keywords: *Macaranga lowii*; pentadecyl ferulate; scopoletin; stigmasterol; DPPH radical scavenging

I. INTRODUCTION

Macaranga is a large genus in the family of Euphorbiaceae which comprises approximately 300 species worldwide (Lim *et al.,* 2009). This genus is commonly known by the locals in Malay Peninsular as "Mahang" while in Borneo it is called as "Benuah" and "Merkabong" (Wong, 2002). The members of

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this genus are commonly found in the forests as well as in disturbed areas (Zakaria *et al.,* 2008). The species from *Macaranga* is widely distributed in New Guinea, Borneo and from West Africa to the south Pacific islands (Whitmore, 1967). Traditionally, *Macaranga* species were used in folklore medicine to treat various illnesses. For instance, *M. conifera* was used to treat malaria and dropsy, *M. hypoleuca* was utilised as febrifuge, expectorant and anti-spasmodic (Eswani *et al.,* 2010) while *M. tanarius* was useful for the itchy skin (Kandowangko *et al.,* 2018). Due to the medicinal properties exhibited in *Macaranga* species, researchers have shown their interest in investigating the chemical constituents that are accountable to heal various illnesses. Previous chemical investigations on *Macaranga* species revealed that this genus contains a large number of prenylated flavonoids and stilbenoids (Johari *et. al.,* 2019; Kamarozaman *et. al.,* 2018a, 2019; Peresse *et. al.,* 2017; Segun *et al.,* 2019), terpenoids (Luo *et. al.,* 2018; Qi *et al.,* 2017) as well as tannins (Gunawan-Puteri & Kawabata, 2010). These classes of secondary metabolites demonstrated a broad spectrum of biological activities including α-glucosidase (Gunawan-Puteri & Kawabata, 2010) and acetylcholinesterase inhibitory (Kamarozaman *et al.,* 2019), antiplasmodial (Zakaria *et al.,* 2012), cytotoxicity (Kamarozaman *et. al.,* 2018b, Segun *et. al.,* 2019; Tanjung *et al.,* 2018) as well as antioxidant (Sutthivaiyakit *et al.,* 2002) properties.

In our search for bioactive constituents from this genus, *M. lowii* was selected to be phytochemically studied. The previous study on the leaves of this species has reported the presence of three flavonoids namely macalowiinin, 4'-*O*-methyl-8-isoprenylnaringenin and 4'-*O*-methyl-5,7,4'-trihydroxyflavone (acasetin). Acasetin demonstrated moderate activity against murine leukaemia (P-388) cells with the IC_{50} value of 58.7 μM (Agustina *et al.,* 2012). We herein report the isolation of two phenolics and a sterol from the crude acetone extract of the stem bark of *M. lowii* as well as the biological activities of these isolates.

II. MATERIALS AND METHODS

A. List of Instruments and Chemicals

The UV spectra of the isolated compounds were recorded by using Perkin-Elmer Lambda 35 UV-Vis while the IR absorption was obtained from the Perkin-Elmer FT-IR spectrometer. The NMR spectra (1D and 2D) were measured on Bruker 500 Ultrashield NMR spectrometer at 500 MHz (1H at 500 MHz and 13C-APT at 125MHz). The assays of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and cytotoxicity were analysed on Gen-5 Microplate Reader (Synergy HT). Silica gel 60 PF254 (Merck 1.07747) was used for vacuum liquid chromatography (VLC) while silica gel Merck 60 (0.040-0.063 mm, 230-400 mesh ASTM, Merck 1.09385) was prepared for column chromatography. The radial chromatography plate was prepared with silica gel 60 PF254 containing gypsum (1.07749) and the aluminium supported silica gel 60 F254 0.25 mm, 20x20 cm (1.05554) was used for thin layer chromatography (TLC) analysis. The 2,2-diphenyl-1-picrylhydrazyl was purchased from Sigma Chemical Co. (St Louis, Missouri, USA).

B. Plant Material

The stem bark of *M. lowii* was collected from Taman Negara Kuala Keniam, Pahang, Malaysia. This species was identified by Dr. Shamsul Khamis, a botanist from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (FSG8) was deposited at the herbarium of Forest Research Institute Malaysia (FRIM).

1. Extraction

The stem bark of *M. lowii* (4 kg) was air-dried and ground to powder form. The granules (2.05 kg) were extracted by acetone (5 L) at room temperature for 24 hours and repeated three times. The extract was filtered and concentrated under reduced pressure by using a rotary evaporator to obtain crude acetone extract (38.8 g). The crude acetone extract was further dissolved in methanol (200 mL) and mixed with diethyl ether to reduce the tannin. The mixture was filtered and concentrated to remove diethyl ether and methanol to give crude acetone extract with less tannin (10.9 g).

2. Fractionation and isolation

The crude extract (10.9 g) was subjected to vacuum liquid chromatography (VLC) with the solvent system *n*-hexane:ethyl acetate (hex:EtOAc) in increasing polarity to give nine semi-purified fractions, ML1 (hex:EtOAc, 9 (135 mL):1 (15 mLl)), ML2 (hex:EtOAc, 8 (120 ml):2 (30 Ml)), ML3 (hex:EtOAc, 7 (105 mL):3 (45 mL)), ML4 (hex:EtOAc, 5 (75 mL):5 (75 mL)), ML5 (hex:EtOAc, 6 (90 mL):4 (60 mL)), ML6 (hex:EtOAc, 3 (45 mL):7 (105 mL)), ML7 (hex:EtOAc, 1 (15 mL):9 (135 mL)), ML8 (EtOAc:MeOH, 9 (135 mL):1 (15 mL)), ML9 (MeOH, 100% (150 mL)).

Fraction ML2 (448 mg) was chromatographed twice using 2 cm diameter column chromatography (CC) (hex:EtOAc from 10:0 to 1:1, v/v) to give a mixture of two compounds. After recrystallisation using *n*-hexane, the mixture was separated and yielded compounds **1** (6.2 mg) and **3** (0.3 mg). Fraction ML7 (220 mg) was subjected to radial chromatography (RC) (1 mm plate, CHCl3:MeOH from 10:0 to 4:1, v/v) to afford a pure compound **2** (2.1 mg).

C. Bioassay

Compounds **1** and **2** were tested on DPPH radical scavenging and cytotoxicity against HT-29 and CCD-18Co cell lines. Due to inadequate yield, compound **3** was not able to be tested for both assays.

1. DPPH radical scavenging

The radical scavenging activity of isolated compounds was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Vaidyaratnam, 2002) with slight modification. Trolox was used as the standard for this assay. The isolated compounds and Trolox were dissolved in ethanol to the concentration of 1 mg/mL. Then, the stock solutions were diluted to 200 μg/mL and pipetted into the 96-well plate in descending concentrations of 100, 80, 60, 40, 20, 10 and 5 μL. A specific amount of ethanol was added to each plate to make the total volume of 195 μL. Finally, 5 μL of DPPH solution was added to each well and incubated for 30 min in dark condition at room temperature. The absorbance of the solution was measured using a spectrophotometer at 517 nm.

The radical scavenging activity was expressed as percent inhibition and the IC_{50} value was determined from the graph of % inhibition versus concentration. The calculation was expressed as follows:

$$\% \text{ scavenging activity} = \frac{\text{Abs control} - (\text{Abs sample} - \text{Abs blank}) \times 100\%}{\text{Abs control}}$$

2. Cytotoxicity (SRB Assay)

The HT-29 human colorectal cancer cells and CCD-18Co human fibroblast cells were obtained from American Tissue Culture Collection (ATCC, USA). The cells were cultured in RPMI-1640 containing 10% FBS and 1% antibiotic-antimycotic.

Sulforhodamine B (SRB) assay (Anaya-Eugenio *et al.,* 2019) was used to assess the cytotoxicity of the isolated compounds. A 100 μL cell suspension was seeded into a 96-well plate with a concentration of 1-5x10^4 and incubated at 37°C and 5% CO_2 for 24 hours. Then, 100 μL of six concentrations (0.94-30 μg/mL) of serially diluted compounds were added to the respective wells. Hydrogen peroxide and H_2O_2 were used as the positive control, enriched media as the negative control, and enriched media added with DMSO was used as the vehicle control. The plate was then incubated for 48 hours. After that, the cells were fixed with 50 μL of 50% cold (4°C) trichloroacetic acid (TCA) and incubated at 4°C for 1 hour. The plate was washed with running tap water five times and left to dry overnight. SRB stain (100 μL, 0.4%) dissolved in 1% acetic acid was added to each well and incubated at room temperature for 30 minutes. The plate was then washed with 1% acetic acid four times to remove excess unbounded stain and was left to dry overnight. Tris base (pH ~ 10.5) (200 μL of 10 mM) was added to each well and the plate was shaken for 15 minutes. The plate was read with a maximum sensitivity OD of 564 nm. The graph of viability against concentration was plotted and IC_{50} values were determined.

C. Spectral Data of Isolated Compounds

*1. Pentadecyl ferulate (**1**)*

White amorphous powder (6.2 mg). ESI-MS m/z: 405.1 $[M+H]^+$. UV (MeOH) λ_{max} 205, 233, 294, 324 nm. IR (KBr) ν_{max} 3300 (OH), 1462 (C=C), 1711 (C=O), 1015 (C-O) cm^{-1}. NMR (500 MHz): ^1H NMR data ($CDCl_3$, 500 MHz): δ_H 7.64 (1H, *d*, J = 16.0 Hz, H-7), 7.10 (1H, *d*, J=1.5 Hz, H-2), 7.06 (1H, *dd*, J= 8.0, 1.5 Hz, H-6), 6.94 (1H, *d*, J=8.0 Hz, H-5), 6.33 (1H, *d*, J= 16.0 Hz, H-8), 4.22 (2H, t, J=6.5 Hz, H-10), 1.73 (2H, *m*, H-11), 1.41 (2H, *m*, H-12), 1.27 (2H, *m*, H-13 – H-23), 0.91 (3H, *t*, J= 7.0 Hz, H-24), 1.82 (3H, *s*, 3-OCH_3). ^{13}C-APT NMR data ($CDCl_3$, 125 MHz): δ_C 167.4 (C-9), 147.8 (C-4), 146.7 (C-3), 144.6 (C-7), 127.0 (C-1), 123.0 (C-2), 115.7 (C-8), 114.7 (C-5), 109.3 (C-6), 64.6 (C-10), 55.9 (C-OCH_3), 28.7 (C-11), 26.0 (C-12), 22.7-31.9 (C-13 – C-23), 14.1 (C-24).

*2. Scopoletin (**2**)*

Yellowish crystal (6.0 mg). EI-MS m/z: 192.0 $[M^+]$. UV (MeOH) λ_{max} nm: 231, 348, 400. IR (KBr) ν_{max} 3331 (OH), 1668 (C=O), 1448 (C=C), 1019 (C-O) cm^{-1}. ^1H-NMR (Acetone-d_6, 600 MHz) δ_H ppm: 7.61 (1H, *d*, *J*= 9.5 Hz, H-3), 6.91 (1H, *s*, H-5), 6.86 (1H, *s*, H-8), 6.26 (1H, *d*, *J*= 9.5 Hz, H-2), 3.95 (3H, *s*, OCH_3-6); ^{13}C-APT NMR (Acetone-d_6, 150 MHz) δ ppm: 161.3 (C-1), 150.3 (C-7), 149.9 (C-9), 144.2 (C-6), 143.4 (C-3), 113.3 (C-2), 111.4 (C-4), 107.7 (C-5), 103.2 (C-8), 56.4 (C-OCH_3)

*3. Stigmasterol (**3**)*

White powder. NMR (500 MHz): ^1H NMR data ($CDCl_3$, 500 MHz): δ_H 5.48 (1H, *t*, H-6), 5.36 (1H, *m*, H-21), 4.84 (1H, *m*, H-20), 3.55 (1H, *tdd*, H-3), 1.03 (3H, *s*, H-29), 0.94 (3H, *d*, H-19), 0.85 (3H, *t*, H-24), 0.84 (3H, *d*, H-26, 0.83 (3H, *d*, H-27), 0.71 (3H, *s*, H-28).

III. RESULTS AND DISCUSSION

Compound **1** which was obtained as pale yellowish semisolid has a molecular formula of $C_{25}H_{40}O_4$ corresponding to the pseudo-molecular ion peak at *m/z* 405.1 $[M+H]^+$ in the ESI-MS spectrum. The UV absorption ni MeOH at λ_{max} 205, 233, 294 and 324 nm indicated the presence of a substituted aromatic system in the structure. The presence of the aromatic system was supported by the IR absorption at 3300 (OH), 1462 (C=C), 1711 (C=O) and 1015 (C-O) cm^{-1}. The presence of *meta*-coupled and *ortho*-coupled aromatic protons at δ_H 7.10 (1H, *J*= 1.5 Hz) and δ_H 6.94 (1H, *J*= 8.0 Hz), and a doublet of a doublet at δ_H 7.06 (1H, *J*= 8.0, 1.5 Hz) in the ^1H NMR spectrum of **1** illustrated the presence of ferulic acid moiety in the molecule. A pair of doublet signals at δ_H 7.64 (1H) and 6.33 (1H), a triplet at δ_H 4.22 (2H), methylene at δ_H 1.73 (2H), 1.41 (2H) and 1.27 (22H), as well as methyl signal at δH 0.91 (3H) ppm, indicated the existence of a long alkyl side chain in the compound containing 15 carbons. The ^{13}C-APT NMR spectrum demonstrated two oxyaril carbons at δ_C 146.7 (C-3) and 147.8 (C-4), three methine aromatic carbons at δ_C 123.0 (C-2), 114.7 (C-5), and 109.3 (C-6), two olefinic carbons at δ_C 144.6 (C-7) and 115.7 (C-8), and a methyl carbon for methoxy resonated at δ_C 55.9 ppm. The methylene carbons at δ_C 28.7 (C-11), 26.0 (C-12) and 22.7-31.9 (C-13 – C-23) as well as methyl carbon at δ_C 14.1 (C24) supported that compound **1** consists of the long alkyl side chain. The ^1H-^{13}C HMBC spectrum displayed a long-range correlation between olefinic proton at δ_H 7.64 (1 H) and carbons at δ_C 127.0 (C-1), 123.0 (C-2), 114.7 (C-5), 109.3 (C-6), and 167.4 (C-9) thus confirming the position of the long alkyl ester side chain at the ferulic acid moiety (Figure 1). In addition, the correlation between olefinic proton at δ_H 6.33 (1 H) and carbon at δ_C 127.0 (C-1) further confirm the attachment of the long side chain at C-1 of the moiety. Meanwhile, the presence of a methoxy group at C-3 was deduced by the correlation of methyl proton of methoxy at δ_H 3.95 (3 H) and carbon at δ_C 146.7 (C-3). Another significant correlation displayed was between methylene proton at δ_H 4.22 (2 H) and carbons at δ_C 167.4 (C-9), 28.7 (C-11), and 26.0 (C-12) as well as the correlation between methyl proton at δ_H 0.91 (3 H) with C-22 and C-23 located around δ_C 22.7-31.9 ppm thus confirming the long alkyl side chain. Based on the comparison of the ^1H, ^{13}C-APT, and HMBC NMR spectra with the literature (Wang *et al.,* 2013), compound **1** was elucidated as pentadecyl ferulate (Figure 3).

Table 1. NMR spectroscopic data of compound **1** [δ (ppm), *J* (Hz)]

No.	Compound 1		
	δ_H	δ_C	HMBC ($^1H \rightarrow {}^{13}C$)
1	-	127.0	-
2	7.10 (d, *J*=1.5)	123.0	-
3	-	146.7	-
4	-	147.8	-
5	6.94 (d, *J*=8.0)	114.7	-
6	7.06 (dd, *J*=8.0, 1.5)	109.3	C-7
7	7.64 (d, *J*=16.0)	144.6	C-1, C-2, C-5, C-6
8	6.33 (d, *J*=16.0)	115.7	C-1
9	-	167.4	-
10	4.22 (t, *J*=6.5)	64.6	C-9, C-11, C-12
11	1.73 (m)	28.7	-
12	1.41 (m)	26.0	-
13 – 23	1.27 (m)	22.7-31.9	-
24	0.91 (t, *J*=7.0)	14.1	C-22, C-23
3-OCH_3	1.82 (s)	55.9	C-3

NMR spectra recorded at 500 MHz (^1H) and 125 MHz (^{13}C-APT) in $CDCl_3$-d_1

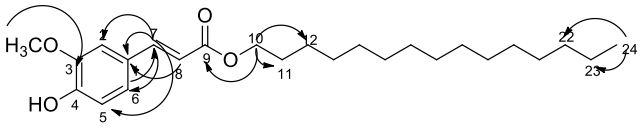


Figure 1 Selected HMBC correlation of **1**

Compound **2** was isolated as a yellowish crystal. The EI-MS spectrum exhibited a pseudo-molecular ion peak at *m/z* 192 [M^+] corresponding to the molecular formula of $C_{10}H_8O_4$. The UV absorption in MeOH at λ_{max} 231, 348 and 400 nm showed the characteristic of coumarin. It is supported by the IR absorptions of the hydroxyl group at 3430 (O-H) and 1019 (C-O bend); aromatic ring at 2944, 2832 (Csp^3- H stretch); and 1448 (C=C Ar) cm^{-1}. The ^1H NMR spectrum of **2** displayed the presence of two olefinic protons at δ_H 7.61 (1H, *d*, *J*= 9.5 Hz, H-3) and 6.26 (1H, *d*, *J*= 9.5 Hz, H-2). A pair of one proton singlet resonated at δ_H 6.91 (1H, *s*) and 6.86 (1H, *s*) belongs to H-5 and H-8, while one methoxy group appeared at δ_H 3.95 ppm. There are 10 carbon atoms represented by 10 carbon signals in the ^{13}C-APT NMR spectrum. One carbonyl ester appeared in the downfield region at δ_C 206.9 (C-1), three oxyaryl carbons at δ_C 144.2 (C-6), 149.9 (C-9), and 150.3 (C-7), two methine olefinic carbons at δ_C 113.3 (C-2) and 143.4 (C-3), two aromatic methine carbons at δ_C 107.7 (C-5) and 103.2 (C-8), one aromatic quaternary carbon at δ_C 111.4 (C-4) and one methyl carbon for methoxy appeared at the upfield region of δ_C 56.4. The ^1H-^{13}C HMBC spectrum exhibited a long-range correlation between aromatic methine at δ_H 6.91 (H-5) and carbons at δ_C 143.4 (C-3), and 150.3 (C-7), while another methine at δ_H 6.86 (H-8) also showed correlation with carbon at δ_C 150.3 (C-7) (Figure 2). The correlation between olefinic proton at δ_H 7.61 (H-3) with carbons at δ_C 107.7 (C-5) and 161.3 (C-1) also can be observed from the spectrum. In addition, the position of methoxy at C-6 was further confirmed by the correlation of methyl of a methoxy group at δ_H 3.95 and C-6 which is located at δ_C 144.2 ppm. Based on the comparison of ^1H and ^{13}C-APT NMR spectra with the literature (Darmawan *et al.,* 2012), compound **2** was identified as scopoletin (Figure 3).

Table 2. NMR spectroscopic data of compound **2** [δ (ppm), *J* (Hz)]

No.	Compound 2		
	δ_H	δ_C	HMBC ($^1H \rightarrow {}^{13}C$)
1	-	-	-
2	6.26 (d, *J*=9.5)	113.3	-
3	7.61 (d, *J*=9.5)	143.4	C-1, C-5
4	-	111.4	-
5	6.91 (s)	107.7	C-3, C-7
6	-	144.2	-
7	-	150.3	-
8	6.86 (s)	103.2	C-7
9	-	149.9	-
10-OCH_3	3.95 (s)	56.4	C-6

NMR spectra recorded at 600 MHz (^1H) and 150 MHz (^{13}C-APT) in Acetone-d_6

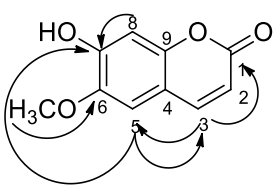


Figure 2. Selected HMBC correlation of **2**

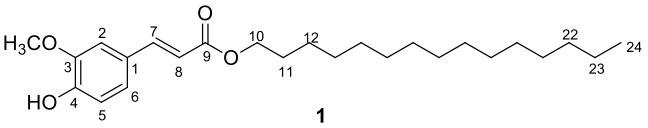
Compound **3** was purified as a white powder. From the ^1H NMR spectrum, compound **2** showed the presence of three olefinic protons, one at sterol moiety and two at the side chain

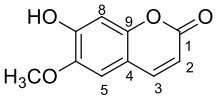
which resonated at δ_H 5.48 (t, 1H, H-6), 5.36 (m, 1H, H-21), and 4.84 (m, 1H, H-20), as well as one oxygenated proton at δ_H 3.55 (H-3) which located at C-3. Other signals in the ^{1}H NMR spectrum displayed the characteristic of sterols by the presence of three methyl doublets at δ_H 0.94 (H-19), 0.84 (H-26), and 0.83 (H-27), and one methyl triplet that appeared at δ_H 0.85 (H-240, and two methyl singlets at δ_H 0.71 (H-28) and 1.03 (H-29), corresponding to six different methyl groups. Based on the comparison with the literature data (Chaturvedula & Prakash, 2012), compound **3** was characterised as stigmasterol (Figure 3).

Table 3. Highlighted NMR spectroscopic data of compound **3** [δ (ppm), *J* (Hz)]

No	δ_H
3	3.55 (1H, tdd)
6	5.48 (1H, t)
19	0.94 (3H, d)
20	4.84 (1H, m)
21	5.36 (1H, m)
24	0.85 (3H, t)
26	0.84 (3H, d)
27	0.83 (3H, d)
28	0.71 (3H, s)
29	1.03 (3H, s)

NMR spectra recorded at 500 MHz (^{1}H) in $CDCl_3$-d_1





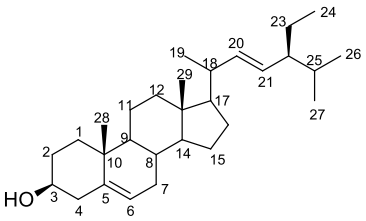


Figure 3. Structure of compounds **1-3**

To the best of our knowledge, this is the first report on the isolation of compound **1** from the family of Euphorbiaceae. This ferulate ester was synthesised by Anselmi and co-workers in 2004 via a reaction of ferulic acid with the corresponding alkanol. Later in 2013, Wang and co-researchers reported its first occurrence in nature through the isolation of *Salicornia herbacea*. The NMR spectral data of compound **1** obtained exhibited similar features to the studies reported by Anselmi *et al.* (2004) and Wang *et al.* (2013) in which the ferulate ester contains a 15-carbon chain. In addition, compounds **2** and **3** were the first report from the isolation of this species.

Compounds **1** and **2** were assessed for DPPH radical scavenging and cytotoxicity of HT-29 (human colorectal adenocarcinoma) and CCD-18Co (human colon fibroblast) cell lines. Nevertheless, compound **3** could not be tested due to the insufficient amount. In DPPH radical scavenging assay, compound **2** exhibited moderate activity against DPPH radical with the IC_{50} value of 34.82 μM compared to the positive control, Trolox with the IC_{50} value of 15.57 μM. However, the IC_{50} value of compound **1** was not detected. Furthermore, compound **1** was also found to be inactive against HT-29 and CCD-18Co cell lines.

The previous study by Wang and co-researchers reported that compound **1** which consists of 15-carbon as its side chain demonstrated potent antiproliferative activities against HepG2 and A549 cell lines with the IC_{50} values of 56 ± 2.32 and 48 ± 1.89 μM, respectively. It was also reported that this compound demonstrated potent antioxidant activity and was comparable to ascorbic acid in FRAP, DPPH and superoxide radical scavenging assays. The IC_{50} values in DPPH and superoxide radical scavenging assays are 27.6 ± 1.89 and 38.6 ± 2.23 μM, respectively which were lower than the IC_{50} values of ascorbic acid (Wang *et al.,* 2013). Nevertheless, our data is not in good agreement with the previous report.

Compound **2** was previously isolated from the leaves of *M. denticulata* and tested for DPPH radical scavenging activity with BHT used as the positive control. This compound showed moderate activity with the IC_{50} value of 0.342 ± 0.026 mM while BHT exhibited the IC_{50} value of 0.031 ± 0.001 mM (Sutthivaiyakit *et al.,* 2002). Another report in 2013 by Mogana and co-researchers on the same assay revealed the EC_{50} value of 191.51±0.01 μM for compound **2**. These studies supported our findings on the moderate activity of compound **2** against DPPH radical scavenging with the IC_{50} value of 34.82 μM.

Compound **3** was first isolated from the genus *Macaranga* and has been studied a lot by other researchers. Previously, compound **3** was isolated from the leaves of *Clerodendrum inerme* Linn and investigated for DPPH radical scavenging activity with quercetin used as a positive control. This compound displayed significant activity with the IC_{50} value of 220 μg/mL which was higher than quercetin with the IC_{50} value of 135 μg/mL (Shanthakumar *et al.,* 2013).

IV. CONCLUSION

Phytochemical study on the stem bark of *M. lowii* has successfully purified two phenolic compounds namely pentadecyl ferulate (**1**) and scopoletin (**2**) as well as a sterol known as stigmasterol (**3**). To the best of our knowledge, this is the first report of the occurrence of pentadecyl ferulate (**1**) in the family of Euphorbiaceae. In addition, the isolation of scopoletin (**2**) and stigmasterol (**3**) is the first report in this species. Scopoletin (**3**) exhibited moderate activity against DPPH radical scavenging with the IC_{50} value of 34.82 μM. Meanwhile, pentadecyl ferulate (**1**) was not active against DPPH radical scavenging and cytotoxicity against HT-29 and CCD-18Co cell lines. The phytochemical investigation of *M. lowii* should be conducted on different parts of the species to increase the chemical profiling data and search for potent bioactive compounds.

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VI. REFERENCES

Agustina, W, Juliawaty, LD, Hakim, EH & Syah, YM 2012, 'Flavonoids from *Macaranga lowii*', ITB J. Sci., vol. 44, pp. 13–18.

Anaya-Eugenio, GD, Addo, EM, Ezzone, N, Henkin, JM, Ninh, TN, Ren, Y, Soejarto, DD, Kinghorn, AD & Carcache de Blanco, EJ 2019, 'Caspase-dependent apoptosis in prostate cancer cells and zebrafish by corchorusoside C from *Streptocaulon juventas*', J. Nat. Prod., vol. 82, no. 6, pp. 1645–1655.

Anselmi, C, Centini, M, Granata, P, Sega, A, Buonocore, A, Bernini, A & Facino, RM 2004, 'Antioxidant Activity of Ferulic Acid Alkyl Esters in a Heterophasic System: A Mechanistic Insight', Journal of Agricultural and Food Chemistry, vol. 52, pp. 6425-6432.

Chaturvedula, VSP & Prakash, I 2012, 'Isolation of Stigmasterol and ß-Sitosterol from the dichloromethane extract of *Rubus suavissimus*', International Current Pharmaceutical Journal, vol. 1, no. 9, pp. 239-242.

Darmawan, A, Kosela, S, Kardono, L & Syah, M 2012, 'Scopoletin, a coumarin derivative compound isolated from *Macaranga gigantifolia* Merr.', Journal of Applied Pharmaceutical Science, vol. 2, no. 12, pp. 175–177.

Eswani, N, Kudus, AK, Nazre, M & Noor, AAG 2010, 'Medicinal Plant Diversity and Vegetation Analysis of Logged over Hill Forest of Tekai Tembeling Forest Reserve, Jerantut, Pahang', Journal of Agricultural Science, vol. 2, no. 3.

Gunawan-puteri, MDPT & Kawabata, J 2010, 'Novel a - glucosidase inhibitors from *Macaranga tanarius* leaves', Food Chemistry, vol. 123, pp. 384-389. doi: 10.1016/j.foodchem.2010.04.050

Johari, MSM, Ahmat, N, Kamarozaman, AS & Isa, MHM 2019, 'Prenylated Flavonols from the Leaves of *Macaranga gigantea* (Rchb.f. & Zoll.)', Scientific Research Journal, vol. 16, no. 1, pp. 29-40. doi: 10.24191/srj.v16i1.5534

Kamarozaman, AS, Ahmat, N, Rahman, NFA & Yen, KH 2018a, 'Prenylated dihydrostilbenes from *Macaranga heynei* (Euphorbiaceae)', Malaysian Journal Analytical Sciences, vol. 22, pp. 258-263.

Kamarozaman, AS, Ahmat, N, Low, ALM, Rajab, NF, Zamkhuri, Z, Shaameri, Z, Sidik, NJ & Kamaruzzaman, NDA 2018b, 'Isolation of dihydrostilbenes from *Macaranga heynei* and their bioactivities', International Journal of Engineering & Technology, vol. 7(4.14), pp. 133-136.

Kamarozaman, AS, Ahmat, N, Isa, SNM, Hafiz, ZZ, Adenan, MI, Yusof, MIM, Azmin, NFN & Latip, J 2019, 'New dihydrostilbenes from *Macaranga heynei* I.M. Johnson, biological activities and structure-activity relationship', Phytochemistry Letters, vol. 30, pp. 174-180.

Kandowangko, NY, Latief, M & Yusuf, R 2018, 'Short Communication: Inventory of traditional medicinal plants and their uses from Atinggola, North Gorontalo District, Gorontalo Province, Indonesia', Biodiversity, vol. 19, no. 6, pp. 2294-2301.

Lim, TY, Lim, YY & Yule, CM 2009, 'Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species', Food Chemistry, vol. 114, no. 2, pp. 594–599.

Luo, P, Yin, ZY, Sun, ZJ, Chen, H, Li, CJ, Zhou, HH, Gu, Q & Xu, J 2018, 'Cembrane-type diterpenoids from *Macaranga pustulata*', Fitoterapia, vol. 129, pp. 162-166.

Mogana, R, Teng-Jin, K & Wiart, C 2013, 'Anti-Inflammatory, Anticholinesterase, and Antioxidant Potential of Scopoletin Isolated from *Canarium patentinervium* Miq. (Burseraceae Kunth)', Evidence-Based Complementary and Alternative Medicine, Volume 2013.

Peresse, T, Jezequel, G, Allard, PM, Pham, VC, Huong, DTM, Blanchard, F & Roussi, F 2017, 'Cytotoxic Prenylated Stilbenes Isolated from *Macaranga tanarius*', Journal of Natural Products, vol. 80, no. 10, pp. 2684–2691.

Qi, W, Shen, Y, Wu, Y, Leng, Y, Gao, K & Yue, J 2017, 'Deheiculatins A-L, 20-oxygenated cembranoids from *Macaranga deheiculata*', Phytochemistry, vol. 136, pp. 101–107.

Segun, PA, Ogbole, OO, Ismail, FMD, Nahar, L, Evans, AR, Ajaiyeoba, EO & Sarker, SD 2019, 'Bioassay-guided isolation and structure elucidation of cytotoxic stilbenes and flavonols from the leaves of *Macaranga barteri*', Fitoterapia, vol. 134, pp. 151-157.

Skehan, P, Storeng, R, Scudiero, D, Monks, A, McMahon, J, Vistica, D & Boyd, M 1990, 'New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening', Journal of National Cancer Institute, vol. 82, no. 13, pp. 1107–1112.

Sutthivaiyakit, S & Unganont, S 2002, 'Diterpenylated and prenylated flavonoids from *Macaranga denticulata*', Tetrahedron, vol. 58, pp. 3619–3622.

Tanjung, M, Juliawaty, LD, Hakim, EH & Syah, YM 2018, 'Flavonoid and stilbene derivatives from *Macaranga trichocarpa*', Fitoterapia, vol. 126, pp. 74–77.

Vaidyaratnam, Varier, PS 2002, 'Indian Medicinal Plants-A Compendium of 500 species, I', Orient Longman Publishing House, Kottakkal-India, 146.

Wang, X, Zhang, M, Zhao, Y, Wang, H, Liu, T & Xin, Z 2013, 'Pentadecyl ferulate, a potent antioxidant and antiproliferative agent from the halophyte *Salicornia herbacea*', Food Chemistry, vol. 141, pp. 2066-2074.

Wong, TM 2002, A Dictionary of Malaysian Timbers. Malayan Forest Records No. 30. Forest Research Institute Malaysia.

Whitmore, TC 1967, 'Studies in *Macaranga*, an easy genus of Malayan wayside trees', Malayan Nature Journal, vol. 20, no. 3, pp. 89–99.

Zakaria, R, Fadzly, N, Rosely, N, Mansor, M & Zakaria, M 2008, 'The distribution of *macaranga*, Genus (Family Euphorbiaceae) in Penang Island, Peninsular Malaysia', Journal of Bioscience, vol. 19, no. 2, pp. 91–99.

Zakaria, I, Ahmat, N, Jaafar, FM & Widyawaruyanti, A 2012, 'Fitoterapia Flavonoids with antiplasmodial and cytotoxic activities of *Macaranga triloba*', Fitoterapia, vol. 83, no. 5, pp. 968–972.