Jurnal Sains Kesihatan Malaysia **14**(1) 2016: 23-29

Kertas Asli/*Original Article*

Compounds from the Antioxidant Active Fraction of M. Platytyrea
(Sebatian daripada Pecahan Antioksidan Aktif M. Platytyrea)

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ABSTRACT

This article discusses on the natural compounds from the ant plant (Myrmecodia species, family: Rubiaceae). The ethyl acetate (EtOAc) extract from the tuber of M. platytyrea was fractionated by using medium pressure liquid chromatography, giving eight fractions (F1-F8). Those fractions were evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Fraction F5 was recorded as potent (EC_{50} = 21.57 ± 1.40 µg/mL). Then, it was purified by using column chromatography (CC) (mobile phase = chloroform: EtOAc). From the CC, ten fractions (F5F1-F5F10) were obtained and compound (1) was isolated from F5F3 via preparative thin layer chromatography (TLC). After spraying with anisaldehyde-sulphuric reagent, compound (1) gave a green TLC spot (R_f = 0.65, 100% $CHCl_3$, multiple development). The ^{1}H-Nuclear Magnetic Resonance (NMR) spectroscopy (500 MHz, $CDCl_3$) was performed to determine the chemical framework of (1). This compound was identified as morindolide, having an iridoid structure. Meanwhile, the mass spectra for compounds (2) and (3) were analysed. The data presented the molecular ion at m/z 375 $[M-H]^-$ and 255, suggesting the formulation of 2-(2-methylbutyryl)phloroglucinol glucoside and a flavanone, respectively. From the literature, compound (1) was firstly isolated from a Chinese natural medicine, the dried root of Morinda officinalis (family: Rubiaceae). The flavonoids are also included as the biologically active compounds from Myrmecodia. In short, this is the first occurrence of morindolide from the ant plant.

Keywords: Phytochemistry; flavonoid; morindolide; myrmecodia; spectra

ABSTRAK

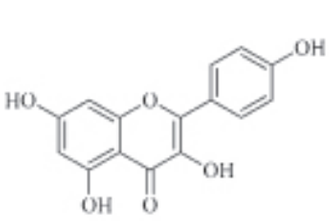
Artikel ini membincangkan sebatian semulajadi dari pokok sarang semut (spesies Myrmecodia, famili: Rubiaceae). Extrak etil asetat (EtOAc) dari tuber M. platytyrea telah difraksikan melalui kromatografi cecair bertekanan sederhana, memberikan lapan fraksi (F1-F8). Fraksi tersebut telah dinilai melalui esei 2, 2-difenil-1-pikrilhidrazil (DPPH). Fraksi F5 telah direkodkan sebagai poten (EC_{50} = 21.57 ± 1.40 µg/mL). Kemudian, ia telah ditulenkan melalui kromatografi kolum (KK) (fasa bergerak = klorofom: EtOAc). Dari KK, sepuluh fraksi (F5F1-F5F10) telah diperolehi dan sebatian (1) diasingkan dari F5F3 melalui kromatografi lapisan nipis (KLN) secara preparatif. Selepas semburan anisaldehid-sulfurik, sebatian (1) memberikan satu titik KLN berwarna hijau (R_f = 0.65, 100% $CHCl_3$, perkembangan KLN secara berulang kali). Spektroskopi Resonans Magnet ^{1}H-Nuklear (RMN) (500 MHz, $CDCl_3$) dilakukan untuk menentukan rangka kimia sebatian (1). Sebatian ini dikenalpasti sebagai later morindolid, yang mengandungi struktur iridoid. Sementara itu, spektrum jisim untuk sebatian (2) dan (3) turut dianalisa. Data yang menunjukkan ion molekul pada m/z 375 $[M-H]^-$ dan 255, mencadangkan formulasi, masing-masing untuk 2-(2-metilbutiril)phloroglusinol glukosida dan satu flavanon. Dari literatur, sebatian (1) julung kali diasingkan dari satu ubatan semulajadi dari China, iaitu akar Morinda officinalis (famili: Rubiaceae). Flavonoid juga turut dikenali sebagai sebatian yang aktif secara biologi dari Myrmecodia. Ringkasnya, pembentangan ini merupakan laporan buat pertama kali mengenai morindolid dari pokok sarang semut.

Kata kunci: Fitokimia; flavonoid; morindolid; myrmecodia; spektrum

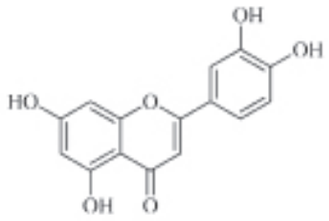
INTRODUCTION

This paper discusses about the pharmacochemical investigation of the ant plant (*Myrmecodia* species). It is a herbaceous with the potential as an alternative therapy in treating cancer (Hamsar et al. 2012; Achmad et al. 2014). *Myrmecodia* has the second-widest distribution among the five known genera of the Rubiaceae family, with its species are found in Malaysia, throughout the entire Indonesian Archipelago, the Philippines, Papuasia and its associated islands, the Solomon Islands, and the Cape York Peninsula in Australia (Huxley & Jebb 1993; Lok et al. 2009).

Other *Myrmecodia* species, such as *M. tuberosa* (Setyani et al. 2012; Sujono et al. 2014) and M. pendens (Saptarini et al. 2014) received scientific investigations. From the literature, the extract of *M. pendens* was analyzed by using high-performance liquid chromatography (HPLC) and five flavonoids were identified and quantified.



Kaempferol



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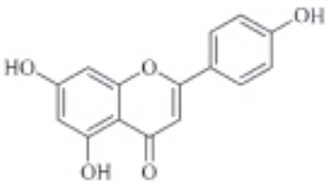
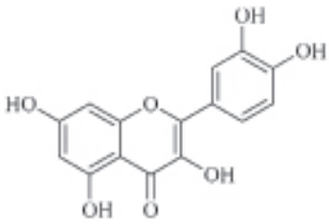
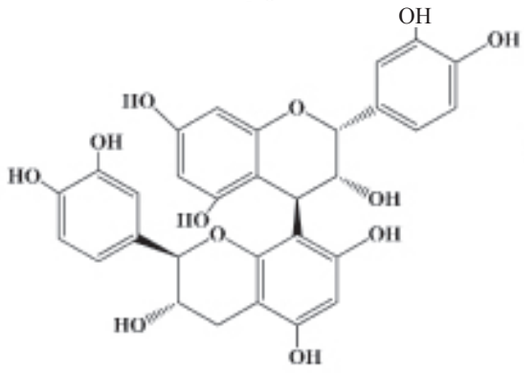


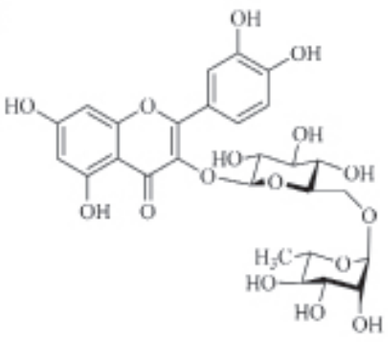
Apigenin



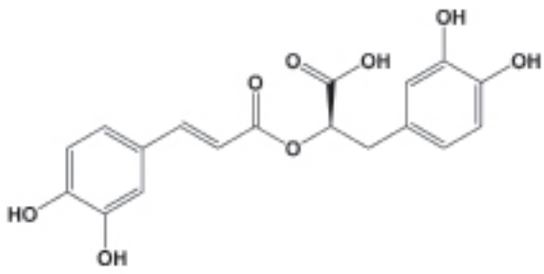
Quercetin



Procyanidin B1

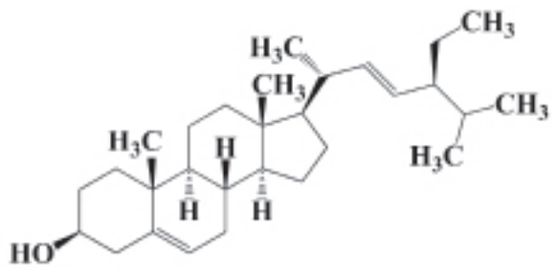


Rutine

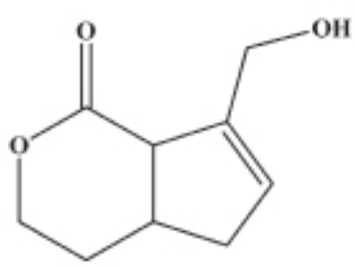


Rosmarinic acid

FIGURE 1. The chemical compounds from *M. pendens* (Engida et al. 2013)



Stigmasterol



Morindolide

FIGURE 2. The chemical compounds from *M. platytyrea* (Mohamad Haris 2014)

The compounds include kaempferol, luteoline, rutine, quercetin and apigenin (Figure 1; Engida et al. 2013). Other research discovered that the ant plants contained active compounds which inhibited xanthine oxidase activity (Hari Susanti 2014) and are possible to be developed as immunomodulatory (Hertiani et al. 2010; Sumardi et al. 2013) and antimicrobial (Efendi et al. 2013) agents. The bio-synthesis of silver nanoparticles by using the water extract of *M. pendan* was also documented (Zuas et al. 2014). Modern techniques that consisted of UV/visible, liquid chromatography/electrospray ionization/tandem mass spectrometry and HPLC were employed for identifying the antioxidative compounds in the ethyl acetate (EtOAc) fraction of *M. pendan*. Three phenolics (rosmarinic acid, procyanidin B1, and polymer of procyanidin B1) were identified (Engida et al. 2014). In addition, the EtOAc extract from *M. platytyrea* tuber revealed its potency in the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) modified method (EC_{50} = 32.91 ± 2.23 µg/mL) (Mohamad Haris, 2014).

The literature search revealed that other genus in Rubiaceae, called *Hydnophytum* (Abdullah *et al*., 2010; Darwis et al. 2014), contains biologically active moiety, as well (e.g. 7,3′,5′-trihydroxyflavanone). In order to investigate the chemical compositions, the ant plants were subjected to extraction techniques (Engida et al. 2014). Supercritical carbon dioxide extraction was utilized for extracting polyphenols such as gallic acid, catechin, ferulic acid, caffeic acid, p-coumaric acid, quercetin, luteolin and kaempferol in the *M. pendans* (Sanjaya et al. 2014). Meanwhile, the pharmacochemical study of *M. platytyrea* revealed the isolation of stigmasterol and compound (1), known as morindolide (Figure 2; Mohamad Haris, 2014).

MATERIALS AND METHODS

The tubers of *M. platytyrea* subsp. *Antoinii* (Becc.) Huxley & Jebb were collected in Indonesia in June 2009. The plant was identified by Prof. Dr. Eko Baroto Walujo, Faculty of Botany, Research Centre for Biology, Indonesian Institute of Science, Bogor.

The procedures for the fractionation of the EtOAc extract and DPPH assay were mentioned elsewhere (Mohamad Haris 2014). The purification step was performed by using column chromatography (CC) (mobile phase = chloroform: EtOAc). From the CC, ten fractions (F5F1-F5F10) were obtained and compound (1) (9.9 mg) was isolated from F5F3 via preparative thin layer chromatography (TLC). The ^{1}H-Nuclear Magnetic Resonance (NMR) spectroscopy (500 MHz, $CDCl_3$) was performed to determine the chemical framework of (1). In addition, liquid chromatography-mass spectrometry (LC-MS) analysis (both in positive and negative modes) was performed. The Quadrupole-Time-Of-Flight (QTOF, ion source = Dual ESI) mass spectrometer was utilized to analyze the mass of compounds 2 and 3.

RESULTS AND DISCUSSION

After spraying the TLC plate with anisaldehyde-sulphuric reagent, compound (1) gave a green spot (R_f = 0.65, 100% $CHCl_3$, multiple development). From the ^{1}H-Nuclear Magnetic Resonance (NMR) spectra (Table 1; 500 MHz, $CDCl_3$) this compound was identified as morindolide, having an iridoid structure. The iridoid carbon skeleton with hydroxymethyl group of C-3 position with a double bond at C-2/C-4 and ester group at C-9 was supported by the data from the Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy (Figure 3). Compound (1) showed the retention time at 0.3 minute from the LCMS. The data presented the molecular ion at m/z 191 $[M+Na]^+$, in support for the formulation of morindolide ($C_9H_{12}O_3$) (Figure 4). Meanwhile, from another LC-MS analysis (in negative mode), the Total Ion Current (TIC) chromatogram (Figure 5) showed the peaks' intensities were highly recorded at the retention time = 34 min for compounds (**2**) and (**3**). The data presented the molecular ion at m/z 375

TABLE 1. The correlation between ^{1}H and ^{13}C - NMR data of compound (1), determined by COSY, HMQC and HMBC

Carbon Position	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
1	3.79 (1H, d)	50.36
2	-	140.17
3	4.29 (2H)	60.53
4	5.80 (1H)	129.03
5	2.15, 2.81 (2H, m, J = 10 Hz, 15 Hz)	38.49
6	2.92 (1H)	34.58
7	2.09 (2H, m, J = 10 Hz, 15 Hz)	128.96
8	4.28, 4.42 (2H, m, J = 5 Hz, 10 Hz)	67.43
9	-	172.93

[M-H]- and 255 (Figure 6), in suggesting the formulation of an acylated flavanone and a flavanone (liquiritigenin), respectively. Another moiety, 2-(2-methylbutyryl) phloroglucinol glucoside, might be identified, as well.

From the Total Ion Current (TIC) chromatogram (Figure 5), an ion (compound 4) with higher abundance could be witnessed at retention time = 38.28 minute, $[M-H]^-$ = 403 m/z. Also seen at the same retention time, is a base peak of $[M-H]^-$ = 283 m/z. The ion of $[M-H]^-$ = 283 m/z could be suggested for calycosin, an *O*-methylated isoflavone ($C_{16}H_{12}O_5$) (Chun-qing et al. 1997). On the other hand, 283 m/z could also be assigned to the loss of [M - H - 120]-, a possible diagnostic ion for *C*-glycoside. It was documented that for *C*-glycoside, major fragmentation pathway would involve the cross-link cleavage of the saccharidic residue (Ye et al. 2012). More efforts in the extract's purification is in demand, to characterise the chemical structure of compound (5) (Figure 8). Meanwhile, from Figure 8, the ^{1}H-NMR spectrum (300 MHz, D_2O) of compound (5) suggested compound 5 as 2-(2-methylbutyryl)phloroglucinol glucoside with a chemical formula of $C_{17}H_{24}O_9$, and the exact mass of 372. In conclusion, the occurrence of an acylated flavanone (**2**), $[M-H]^-$ = 375 m/z), liquiritigenin or isoliquiritigenin (compound 3, m/z = 255 = $[M-H]^-$, Ye, M. et al. 2012) and the rest of the ions would be confirmed in future, once the NMR data interpretations are completed.

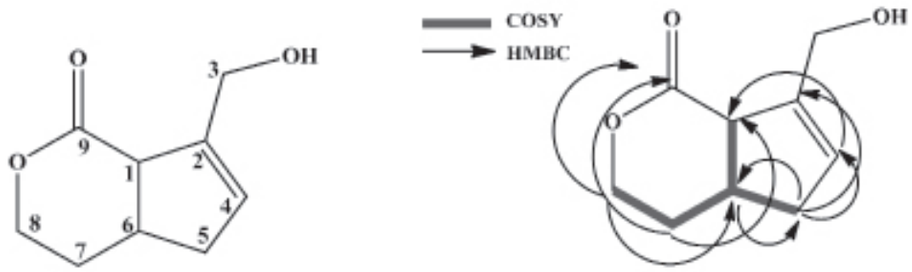


FIGURE 3. The carbons in (1) (left) and its COSY and HMBC correlations (right)

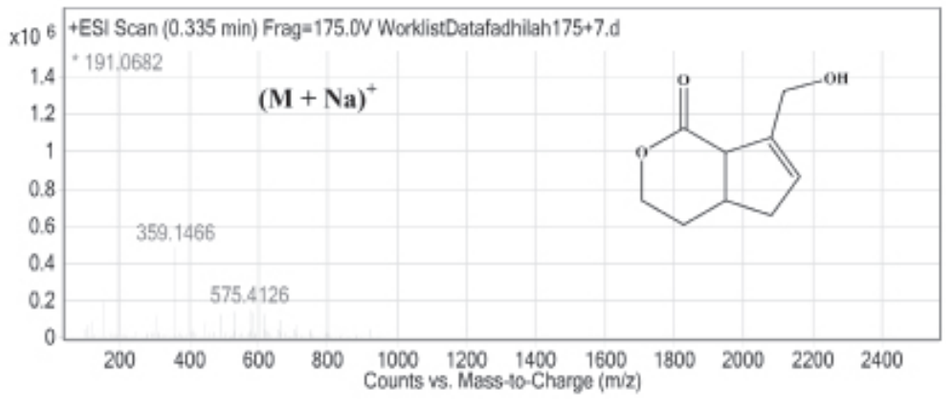


FIGURE 4. The spectra of morindolide showed the relative formula mass (168)

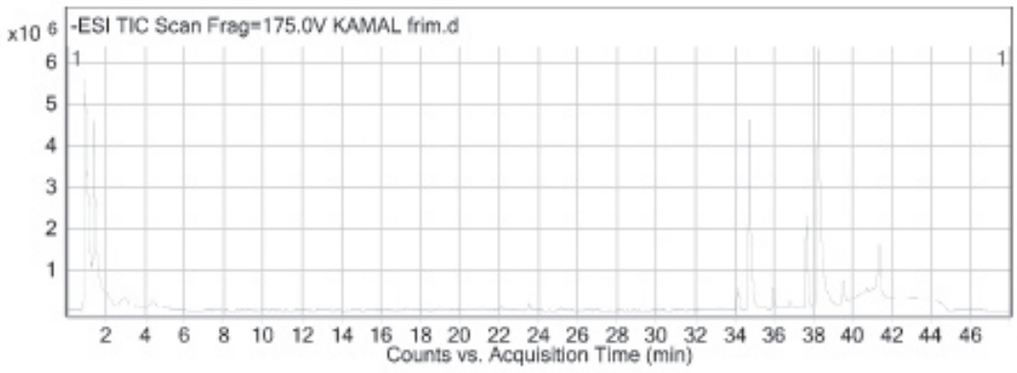
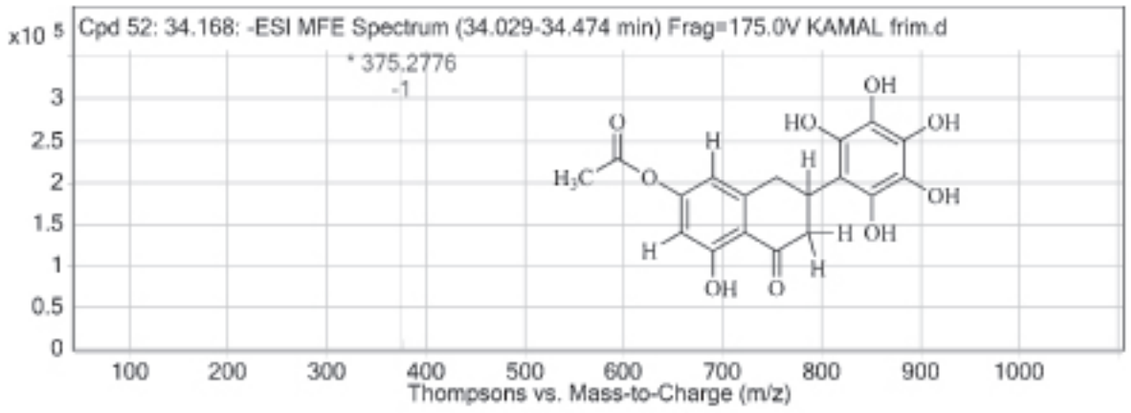
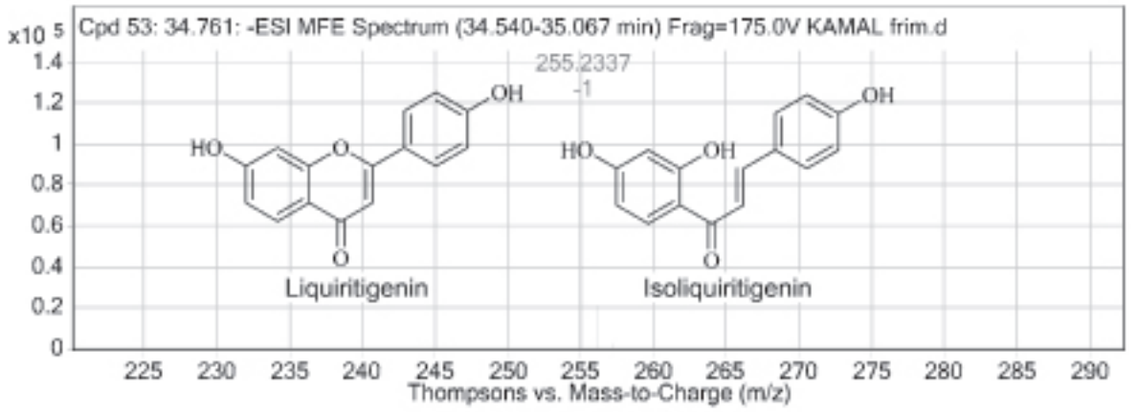


FIGURE 5. The Total Ion Current (TIC) chromatogram of Compound 2 and 3



(a)



(b)

FIGURE 6. The mass data suggests that two compounds (2) (a) and (3) (b) were eluted in retention time, t_R = 34 – 35 minutes

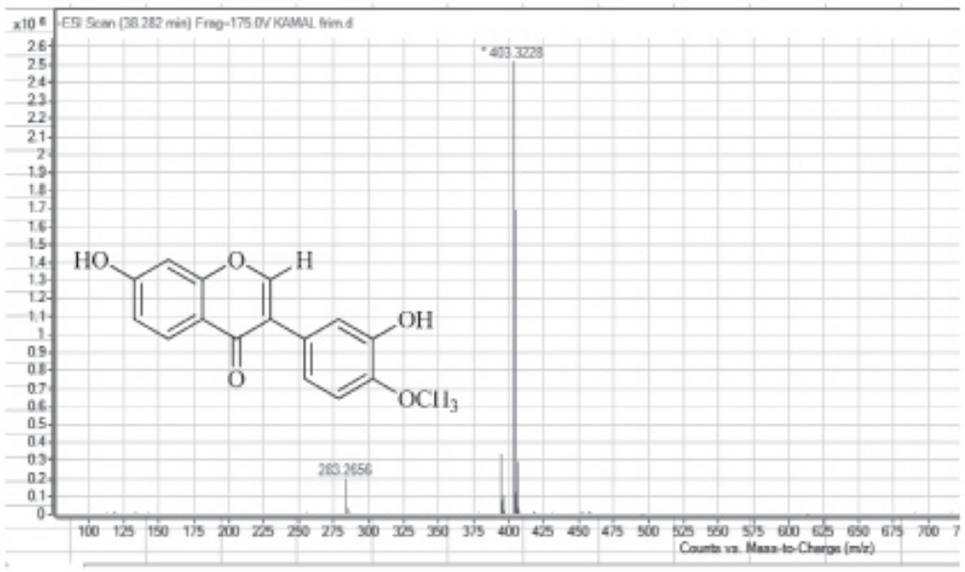


FIGURE 7. The mass data suggests the base peak of $[M-H]^-$ = 283 m/z could indicate the occurrence of compound 4 as a derivative of calycosin, an *O*-methylated isoflavone, eluted in retention time, t_R = 38 minutes

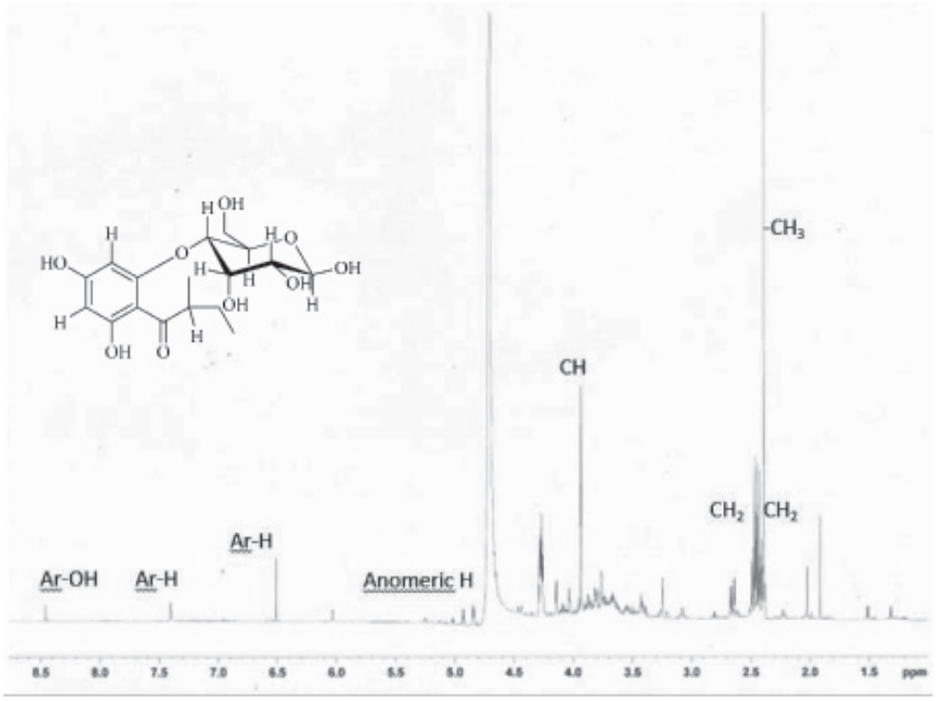


FIGURE 8. The ^{1}H-NMR spectrum (300 MHz, D_2O) of compound (5)

CONCLUSION

From the literature review, compound (1) was firstly isolated from a Chinese natural medicine, the dried root of *Morinda officinalis* (family: Rubiaceae) (Yoshikawa *et al*., 1995). The flavonoids are also regarded as the biologically active compounds from this species. In short, this is the first occurrence of morindolide from *Myrmecodia*.

ACKNOWLEDGMENT

This work is supported by Ministry of Higher Education, UiTM and Forest Research Institute of Malaysia. The authors wish to thank Mr. Mohd Izwan Mohamad Yusof, Integrative Pharmacogenomics Institute (iPROMISE) for the technical support.

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Received: July 2015
Accepted for publication: October 2015