



## C<sub>23</sub>-carbazole alkaloids from malayan *Murraya koenigii* (L.) spreng

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### ABSTRACT

*Murraya koenigii* (Curry leaf tree), from the Rutaceae family, is a medicinally important herb of Indian origin and now were widely distributed throughout southern Asia. The bark of Malayan *Murraya koenigii* was selected for phytochemical investigation. The isolation of chemical constituents from its hexane and dichloromethane extract was carried out by using different chromatographic techniques. Six carbazoles with C<sub>23</sub>-framework was isolated and identified as mahanimbine, murrayamine-J, murrayazolinol, mahanimbinol, murrayakoeninol and bicyclomahanimbine. The structural characterization of the isolated compounds was supported by spectroscopic methods, including NMR, IR, UV, MS spectra data.

**Keywords:** *Murraya koenigii*, Rutaceae, C<sub>23</sub>-framework, carbazole alkaloid, mahanimbine.

### INTRODUCTION

Among the 14 species under the genus *Murraya*, *Murraya koenigii* (L.) Spreng and *Murraya paniculata* (L.) Jack, belonging to the Rutaceae family (citrus family), are the most commonly found in Malaysia [1,2].

The plant *M. koenigii* (curry leaf tree) is native to India and is now distributed in southern Asia [3]. It is widely cultivated for its aromatic leaves. Its leaves are used as traditional vegetable or locally known as ulam, especially used as Indian culinary practices [4]. Traditionally, it used to treat on rheumatism, traumatic injury, dysentery, diarrhea and snake bite [5,6]. The plant is also reported to have antioxidant, antidiabetic, anticarcinogenic, anti-dysenteric, stimulant, hypoglycaemic and antimicrobial activities [7,8,9,10].

Phytochemistry screening on *M. koenigii* shows the presence of alkaloids, carbohydrates, protein, amino acids, saponins, flavonoids and coumarins by various researchers. [11,12]. Carbazole alkaloids are the major constituents of the plant are known to possess cytotoxic, antioxidative, antimutagenic and anti-inflammatory activities [3,13,14].

In continuation of our studies on carbazole alkaloids of *M. koenigii*, we report the isolation of six C<sub>23</sub>-carbazole alkaloids from the bark of *M. koenigii* collected from Pahang, Malaysia, which have been identified as mahanimbine **1**, murrayamine-J **2**, murrayazolinol **3**, mahanimbinol **4**, murrayakoeninol **5** and bicyclomahanimbine **6**. Their structures were elucidated by combination of various spectroscopic methods such as 1D and 2D NMR, IR, UV and MS.

### EXPERIMENTAL SECTION

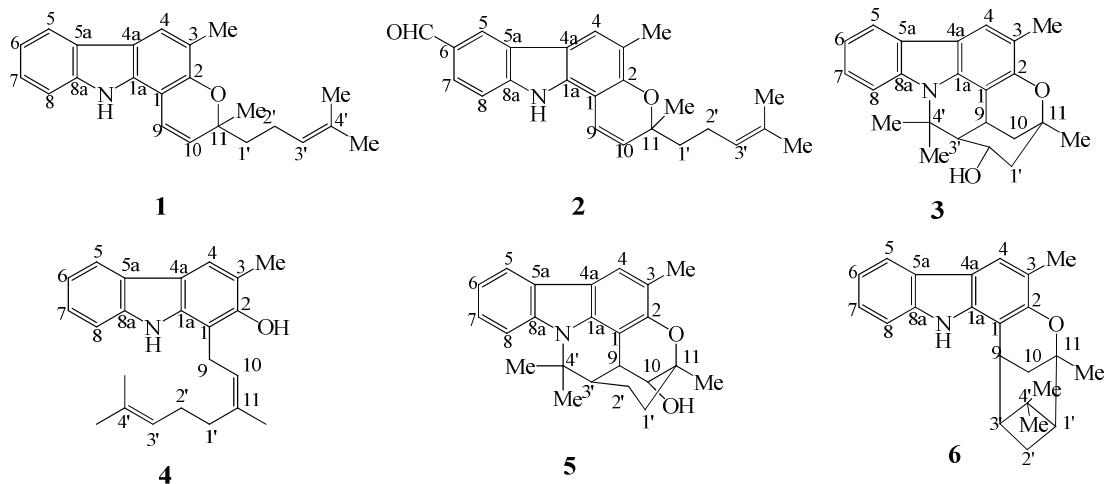
#### General Experimental Procedures

Nuclear Magnetic Resonance spectra (NMR) were recorded using Joel (500 MHz). Deuterated chloroform (CDCl<sub>3</sub>) was used as the NMR solvent. Chemical shifts ( $\delta$ ) were reported in ppm and coupling constants (*J*) in Hz. Mass spectra (MS) were determined by using the LC-mass spectrometry. The ultra violet spectrum (UV) was obtained in

methanol on a Perkin Elmer UV-visible spectrophotometer and the wavelength of the spectrum was determined in the range of 200 to 1000 nm. The infrared spectrums (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with methanol as dilution solvent of the sample. Column chromatography were prepared by using Silica Gel 60F<sub>254</sub>, 70-230 mesh ASTM and 230-400 mesh ASTM as stationary phase. Silica Gel 60F<sub>254</sub> containing Gypsum was used as stationary phase for the preparative thin layer chromatography. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel 60F<sub>254</sub> TLC sheets.

### Plant Material

The species selected for the current study is *Murraya koenigii* (L.) Spreng (TM1006) which was collected from Jerantut, Pahang, Malaysia in January 2012. The specimen was identified by the phytochemical group, Chemistry Department, Faculty of Science and Mathematics, University Pendidikan Sultan Idris, Malaysia.



### Extraction and Isolation

The dried barks (3.0 kg) of *M. koenigii* were extracted with hexane and dichloromethane (three times each) continuously at room temperature. The extracts were combined and were concentrated under reduced pressure to yield brown syrup of hexane crude (106.0 g) and CH<sub>2</sub>Cl<sub>2</sub> crude (32.0 g). After evaporation of the solvent, 30.0 g of the hexane crude extract was subjected to column chromatography over silica gel (gradient solvent system: hexane, CH<sub>2</sub>Cl<sub>2</sub> and Methanol) yielded three known compounds: mahanimbine [**1**, R<sub>f</sub> = 4.4 cm in Hex:CH<sub>2</sub>Cl<sub>2</sub> (1:1)], murrayamine-J [**2**, R<sub>f</sub> = 4.1 cm in CH<sub>2</sub>Cl<sub>2</sub>:MeOH (99:1)], murrayazolinol [**3**, R<sub>f</sub> = 3.4 cm in CH<sub>2</sub>Cl<sub>2</sub>:MeOH (99:1)]. While 30.0 g of CH<sub>2</sub>Cl<sub>2</sub> crude extract was subjected to column chromatography over silica gel (gradient solvent system: hexane, CH<sub>2</sub>Cl<sub>2</sub> and Methanol) yielded another three known compounds: mahanimbilol [**4**, R<sub>f</sub> = 3.2 cm in Hex:CH<sub>2</sub>Cl<sub>2</sub> (1:1)] and murrayakoeninol [**5**, R<sub>f</sub> = 3.3 cm in CH<sub>2</sub>Cl<sub>2</sub>: MeOH (99:1)], bicyclomahanimbine [**6**, R<sub>f</sub> = 5.1 cm in Hex:CH<sub>2</sub>Cl<sub>2</sub> (1:1)].

### Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of isolated compounds. Among the spectroscopic techniques UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and LC-MS were carried out.

**Mahanimbine** (C<sub>23</sub>H<sub>25</sub>NO), **1**. Colourless crystal, mp 94-95 °C (ethyl acetate). UV λ<sub>max</sub> (MeOH) nm (log ε): 238 (4.39), 288 (3.83). IR ν<sub>max</sub> cm<sup>-1</sup>: 3338, 1646, 1610, 1444, 1156, 1121, 782. LC-MS, m/z: 332.2002 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively.

**Murrayamine-J** (C<sub>23</sub>H<sub>23</sub>NO<sub>2</sub>), **2**. Pale yellowish oil. UV λ<sub>max</sub> (MeOH) nm (log ε): 215 (4.25), 248 (4.35), 265 (4.25), 290 (4.33), 310 (4.45). IR ν<sub>max</sub> cm<sup>-1</sup>: 3332, 2855, 1675, 1615, 1575, 1156, 782. LC-MS, m/z: 346.1783 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively.

**Murrayazolinol** (C<sub>23</sub>H<sub>25</sub>NO<sub>2</sub>), **3**. Brown solid. UV λ<sub>max</sub> (MeOH) nm (log ε): 248 (4.84), 265 (4.82), 305 (4.40). IR ν<sub>max</sub> cm<sup>-1</sup>: 3360, 1650, 1620, 1380. LC-MS, m/z: 348.1957 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively.

**Mahanimbilol** (C<sub>23</sub>H<sub>27</sub>NO), **4**. Yellowish oil. UV λ<sub>max</sub> (MeOH) nm (log ε): 213 (4.74), 238 (4.35), 285 (4.15), 305 (4.20). IR ν<sub>max</sub> cm<sup>-1</sup>: 3455, 3400, 3030, 1625, 1605, 1160. LC-MS, m/z: 334.2151 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively.

**Murrayakoeninol** (C<sub>23</sub>H<sub>25</sub>NO<sub>2</sub>), **5**. Brown solid. UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 214 (4.50), 275 (4.15), 308 (4.10). IR  $\nu_{\max}$  cm<sup>-1</sup>: 3405, 1635, 1455, 1330, 1160. LC-MS, m/z: 348.1943 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively

**Bicyclomahanimbine** (C<sub>23</sub>H<sub>25</sub>NO), **6**. Brown solid. UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 242 (4.20), 255 (4.35), 265 (4.15), 305 (4.40). IR  $\nu_{\max}$  cm<sup>-1</sup>: 3455, 2950, 2920, 2840, 1625, 1605, 1350, 1156. LC-MS, m/z: 332.2008 [M+H]<sup>+</sup>. <sup>1</sup>H and <sup>13</sup>C NMR see Table 1 and Table 2, respectively.

## RESULTS AND DISCUSSION

The bark of *M. koenigii* collected from Pahang, Malaysia gave six C<sub>23</sub>-carbazole alkaloids. The fractionation of the hexane extract of the bark by column chromatography and TLC afforded mahanimbine **1**, murrayamine-J **2** and murrayazolinol **3**. The fractionation of the CH<sub>2</sub>Cl<sub>2</sub> extract of the bark by column chromatography and TLC afforded another three compounds: mahanimbilol **4**, murrayakoeninol **5**, and bicyclomahanimbine **6**. All compounds gave blue to purple colour on spraying with 10% sulphuric acid at 105 °C. They also show the similar spectroscopic features with the carbazole alkaloids published in the literature.

**Table 1:** <sup>1</sup>H NMR [500 MHz,  $\delta_{\text{H}}$  (J, Hz)] of Compound **1**, **2**, **3**, **4**, **5** and **6** in CDCl<sub>3</sub>

Position	$\delta_{\text{H}}$ , J Hz					
	<b>1</b> [16]	<b>2</b> [17]	<b>3</b> [18]	<b>4</b> [19]	<b>5</b> [20]	<b>6</b> [21]
NH	7.81 (br s)	8.35 (br s)		7.85 (br s)		7.42 (br s)
1						
1a						
2						
3		6.81 (d, 8.6 Hz)				
4	7.69 (s)	7.83 (d, 8.6 Hz)	7.49 (s)	7.68 (s)	7.50 (s)	7.67 (s)
4a						
5	7.94 (d, 8.1 Hz)	8.46 (d, 1.7 Hz)	7.90 (d, 7.5 Hz)	7.93 (d, 8.0 Hz)	7.89 (d, 8.0 Hz)	7.92 (d, 8.0 Hz)
5a						
6	7.21 (td, 7.5, 1.2 Hz)		7.16 (td, 6.9, 1.2 Hz)	7.17 (td, 8.0, 1.2 Hz)	7.16 (td, 8.1, 1.2 Hz)	7.16 (td, 8.0, 1.2 Hz)
7	7.33 (td, 6.9, 1.2 Hz)	7.89 (dd, 8.6, 1.7 Hz)	7.25 (td, 7.0, 1.2 Hz)	7.30 (td, 7.5, 1.2 Hz)	7.26 (td, 7.0, 1.2 Hz)	7.28 (td, 8.0, 1.2 Hz)
8	7.35 (d, 7.5 Hz)	7.47 (d, 8.6 Hz)	7.47 (d, 8.0 Hz)	7.37 (d, 8.6 Hz)	7.47 (d, 7.8 Hz)	7.37 (d, 7.5 Hz)
8a						
9	6.60 (d, 9.8 Hz)	6.66 (d, 9.7 Hz)	3.32 (m)	3.62 (d, 6.9 Hz)	3.32 (m)	3.29 (d, 9.2 Hz)
10	5.65 (d, 9.7 Hz)	5.71 (d, 9.7 Hz)	1.22 (m)	5.38 (m)	3.82 (d, 8.3 Hz)	2.06 (m)
11						
2-OH				5.21 (br s)		
3-Me	2.37 (s)		2.36 (s)	2.40 (s)	2.23 (s)	2.34 (s)
6-CHO		10.07 (s)				
11-Me	1.48 (s)	1.47 (s)	1.50 (s)	1.90 (s)	1.49 (s)	1.44 (s)
1'	1.79 (t, 8.1 Hz)	1.74 (m)	1.76 (dd, 16.1, 6.9 Hz)	2.11 (m)	1.53 (m)	2.50 (t, 7.4 Hz)
2'	2.20 (m)	2.16 (m)	3.83 (m)	2.11 (m)	1.74 (dd, 16.1, 7.5 Hz)	1.65 (m)
3'	5.15 (tt, 7.5, 1.2 Hz)	5.10 (t, 6.3 Hz)	2.17 (m)	5.06 (m)	2.18 (m)	2.70 (t, 7.5 Hz)
4'						
4'-Me'	1.62 (s)	1.58 (s)	1.29 (s)	1.66 (s)	1.30 (s)	0.74 (s)
4'-Me''	1.72 (s)	1.66 (s)	1.93 (s)	1.59 (s)	1.92 (s)	1.56 (s)

Compound **1** was obtained as colourless crystal (mp 94-95°C) [15]. The IR spectrum of compound **1** indicating the presence of N-H (3338 cm<sup>-1</sup>), C-O and C-H stretching (1121 cm<sup>-1</sup> and 782 cm<sup>-1</sup>, respectively). It was readily recognized as C<sub>23</sub>-pyranocarbazole derivative from its preliminary spectral data. Its molecular weight was deduced as C<sub>23</sub>H<sub>25</sub>NO by LC-MS at m/z 332.2002 [M+H]<sup>+</sup>. Its UV spectrum showed characteristic absorbance for pyranocarbazole moiety with  $\lambda_{\max}$  238 and 288 nm [15]. The <sup>1</sup>H NMR spectrum (Table 1) of compound **1** was showed a broad downfield signal at  $\delta$  7.81 assignable to the present of N-H in the carbazole nucleus. The signals at  $\delta$  7.94 (d, J = 8.1, H-5),  $\delta$  7.35 (d, J = 7.5, H-8),  $\delta$  7.33 (td, J = 6.9, 1.2, H-7) and  $\delta$  7.21 (td, J = 7.5, 1.2, H-6) of **1** was deduced for an unsubstituted carbazole ring-C (Table 1). Two olefinic protons at  $\delta$  6.60 (d, J = 9.8, H-9) and  $\delta$  5.65

(*d*,  $J = 9.7$ , H-10) due to a pyran ring annulated to a carbazole moiety. Finally, the  $^1\text{H}$  NMR spectrum contained signals for an aromatic methyl at  $\delta$  2.37 (s), a methyl group at  $\delta$  1.48 (s) and one prenyl group (2-methylpent-2-en-5yl substituent) adjacent to an oxygen in pyran ring of the pyranocarbazole at C-11 with 5.15 (*tt*,  $J = 7.5$ , 1.2 Hz, H-3'), 2.20 (*m*, H-2'), 1.79 (*t*,  $J = 8.1$ , H-3'), 1.72 (s, 4''-Me) and 1.62 (s, 4'-Me). The  $^{13}\text{C}$  NMR spectrum (Table 2) indicated the present of 23 carbons resonances and proven to be a  $\text{C}_{23}$ -carbazole. The complete assignments of carbon signals and location of **1** was achieved on the basis of DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra. The structure of compound **1** was identified as mahanimbine [16].

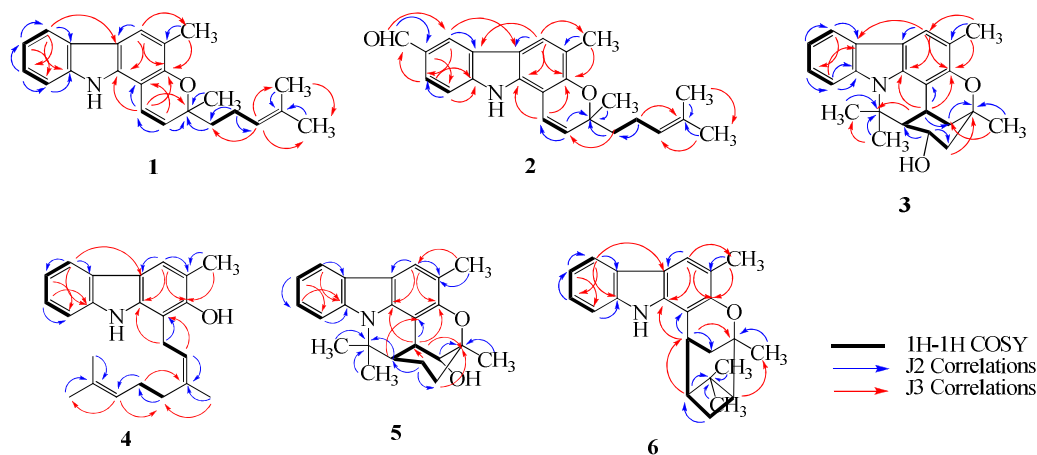
Table 2:  $^{13}\text{C}$  NMR [125 MHz,  $\delta_{\text{C}}$ ] of Compound **1**, **2**, **3**, **4**, **5** and **6** in  $\text{CDCl}_3$

Position	$\delta_{\text{C}}, J \text{ Hz}$					
	<b>1</b> [16]	<b>2</b> [17]	<b>3</b> [18]	<b>4</b> [19]	<b>5</b> [20]	<b>6</b> [21]
1	104.2	105.1	105.3	107.9	105.4	106.4
1a	139.4	136.8	142.4	138.5	142.4	137.8
2	149.9	152.7	153.3	151.3	153.3	153.3
3	123.9	111.0	118.5	116.7	118.5	120.1
4	121.2	121.0	119.7	119.4	119.7	119.4
4a	116.6	117.2	115.1	124.0	115.1	124.2
5	119.3	122.9	120.1	119.5	120.1	119.3
5a	118.4	124.3	127.2	117.1	127.2	115.8
6	119.4	129.5	119.5	119.5	119.5	119.3
7	124.2	126.5	123.1	124.3	123.1	124.0
8	110.4	110.8	113.6	110.4	113.6	110.4
8a	134.8	143.4	140.7	139.5	140.7	139.3
9	117.5	116.8	36.9	24.8	36.9	37.5
10	128.4	129.6	21.5	121.4	72.2	38.4
11	78.1	78.8	79.5	139.2	79.5	83.6
3-Me	16.1		15.5	16.7	15.5	16.8
6-CHO		192.1				
11-Me	25.8	26.2	25.1	16.5	25.2	27.5
1'	40.7	41.0	36.0	39.7	21.5	46.5
2'	22.7	22.8	72.2	26.5	36.0	25.7
3'	118.4	124.0	49.5	123.7	49.5	37.9
4'	131.6	132.0	60.6	132.2	60.6	39.5
4'-Me'	17.6	17.7	23.1	25.8	23.1	18.7
4'-Me''	25.7	25.8	30.2	17.8	30.2	35.2

Compound **2** was obtained as pale yellowish oil. This compound was determined to have molecular formula  $\text{C}_{23}\text{H}_{23}\text{NO}_2$  by LC-MS. The UV spectrum at 215, 248, 265, 290, 310 nm suggested that this compound was thought to be a 6-oxygenated pyranocarbazole derivatives [17]. Examination of the aromatic region of the  $^1\text{H}$  NMR spectrum suggested that compound **2** was also a similar to that of **1**. The  $^1\text{H}$  NMR spectrum of compound **2** was showed a broad downfield signal at  $\delta$  8.35 deduced to the present of N-H in the carbazole nucleus. A characteristic aldehydic singlet at  $\delta$  10.07 in  $^1\text{H}$  NMR spectrum together with a strong carbonyl band at  $1675 \text{ cm}^{-1}$  revealed a formyl group attached to C-6. In the aromatic region of the  $^1\text{H}$  NMR spectrum, one set of ABX mutually-coupled proton system at  $\delta$  8.46 (1H, *d*,  $J = 1.7$ , H-5),  $\delta$  7.89 (1H, *dd*,  $J = 8.6$ , 1.7, H-7), and  $\delta$  7.47 (1H, *d*,  $J = 8.6$ , H-8), which were deshielded by an aldehyde substituent at C-6 in the C-ring. An AB type spin system at  $\delta$  6.66 (*d*,  $J = 9.7$  Hz), 5.71 (*d*,  $J = 9.7$  Hz) for H-9 and H-10, respectively, was affecting by an oxygenated substituent at C-2. This gave further evidence for the characteristic of pyranocarbazole skeleton [17]. Conversely, the signals in the high field region contained signals for a methyl group at  $\delta$  1.47 (s) and one prenyl group (2-methylpent-2-en-5yl substituent) adjacent to an oxygen in pyran ring of the pyranocarbazole at C-11 with  $\delta$  5.10 (*t*,  $J = 6.3$  Hz, H-3'), 2.16 (*m*, H-2'), 1.80 (*m*, H-1'), and a long-range coupling with geminal dimethyls at 1.68 (s, 4'-Me) and 1.58 (s, 4'-Me). The  $^{13}\text{C}$  NMR spectrum (Table 2) indicated the present of 23 carbons resonances, a  $\text{C}_{23}$ -carbazole. The complete assignments of carbon signals and location of substituent on the pyranocarbazole skeleton of **2** was achieved on the basis of DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra. The structure of compound **2** was identified as murrayamine-J [17].

Compound **3** was obtained as brown solid. Its molecular formula was determined to be  $\text{C}_{23}\text{H}_{25}\text{NO}_2$  by LC-MS. The UV spectrum at 245, 265 and 305 nm and was readily recognized as  $\text{C}_{23}$ -pyranocarbazole derivative from its preliminary spectral data [18]. The IR spectrum showed absorption peaks at  $3360 \text{ cm}^{-1}$  confirmed the pyranocarbazole framework with an additional OH group. The  $^1\text{H}$  NMR spectrum of murrayazolinol exhibited signals for an aromatic methyl group at  $\delta$  2.36 (3- $\text{CH}_3$ ), and confirmed an aromatic substitution pattern similar to mahanimbine **1**. The differences between compound **1** and **3** were the additional substitution of a monoterpene moiety and the absence of N-H signal in compound **3**. The  $^1\text{H}$  NMR spectrum of the monoterpene moiety of **3** showed a signal for the carbonyl hydrogen of a secondary alcohol, which appeared as a multiplet at  $\delta$  3.83. The  $^{13}\text{C}$  NMR spectrum (Table 2) indicated the present of 23 carbons resonances and determined to be a  $\text{C}_{23}$ -carbazole. The

complete assignments of carbon signals and location of substituent on the skeleton of **3** was supported on the basis of DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra. Based on the spectroscopic data, structure **3** was assigned to murrayazolinol [18].



**Figure 1: Selected COSY and HMBC correlations in Compound 1, 2, 3, 4, 5 and 6**

Compound **4** was obtained as yellowish oil. This compound showed by mass spectral LC-MS at  $m/z$  334.2151  $[\text{M}+\text{H}]^+$  and determined to be  $\text{C}_{23}\text{H}_{27}\text{NO}$ . The IR spectrum showed absorptions at  $\nu_{\text{max}}$  3455 and  $3400\text{ cm}^{-1}$  revealed the presence of an OH group in addition to the N-H group. This was confirmed by the presence in the  $^1\text{H}$  NMR spectrum of a signal at  $\delta$  5.21 for the OH group, in addition to the carbazole NH signal at  $\delta$  7.85. The UV spectrum,  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **4** (Table 1 and 2) were similar to those of mahanimbine **1** indicating them to be 2-oxygenated-3-methylcarbazole alkaloids [19]. The  $^1\text{H}$  NMR spectrum of **4** contained signals for three methyl at  $\delta$  1.59, 1.66 and 1.90, a four proton multiplet at  $\delta$  2.11, the benzylic  $\text{CH}_2$  doublet at  $\delta$  3.62 and two vinyl proton multiplets at  $\delta$  5.06 and 5.38 indicating the presence of a geranyl substituent. The presence of a singlet at  $\delta$  7.68 and typical unsubstituted carbazole ring (H-5, H-6, H-7 and H-8) indicated that all three substituents were on the same ring of the carbazole. The OH substituent was proven to be at C-2 when the chemical shift of OH signal was not chelated to the carbazole nitrogen, which carbon signal (C-OH) shifted to a lower field at  $\delta$  151.3 when compared to the C-OH at C-1 which would appear at a higher field at about  $\delta$  145.0. The long range coupling between the aromatic methyl protons and H-4 indicated the methyl group to be at C-3, suggesting that **4** was a 2-hydroxy-1-(3-methyl-2-butenyl)carbazole, also known as mahanimbilol **4** [19]. Further correlating information was confirmed by the experiment of DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, HMQC and HMBC spectra.

Compound **5** with molecular formula  $\text{C}_{23}\text{H}_{25}\text{NO}_2$  by LC-MS, displayed absorption bands at  $\lambda_{\text{max}}$  214, 275 and 308 nm indicating the presence of an oxygenated carbazole chromophore. The  $^{13}\text{C}$  NMR spectrum of **5** (Table 2) displayed signals for 23 carbons which proven to be a  $\text{C}_{23}$ -skeleton of carbazole alkaloid. The  $^1\text{H}$  NMR spectrum (Table 1) exhibited signals for five aromatic methine protons, of which one appeared as singlet, two as doublets and two as triplet doublets. The HMBC spectrum of **5** suggested the presence of an unsubstituted carbazole ring by showing correlation of the four aromatic methine protons with neighboring carbons resemblance those in **1**. Their exact positions were confirmed by  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC spectra. The  $^1\text{H}$  NMR spectrum also showed for one aromatic methyl at  $\delta$  2.23, one methyl attached to a tetra-substituted oxygen bearing group at  $\delta$  1.49 and one gem-dimethyl group at  $\delta$  1.30 and 1.92. The position of OH substituent was further confirmed by the HMBC spectrum with the correlations of the aliphatic methyl protons with mutually coupled methylene protons and the OH bearing carbon. It also showed the correlation between H-10 ( $\delta$  3.82 (*d*),  $J = 8.3$ ) and C-1 ( $\delta$  105.4) to give further evidence to confirmed the OH group position. Compound **5** was closely resemblance to compound **3** except for the position of OH substituent. Based on the spectroscopic data, structure **5** was assigned to murrayakoeninol [20].

Compound **6** had the molecular formula  $\text{C}_{23}\text{H}_{25}\text{NO}$  by LC-MS. The UV spectrum showed characteristic absorbance at 242, 255, 260, and 305 nm for the typical absorption of a 2-hydroxy-3-methylcarbazole chromophore. The IR spectrum of compound **6** indicating the presence of N-H ( $3455\text{ cm}^{-1}$ ), C-CH<sub>3</sub> ( $2850\text{ cm}^{-1}$ ) and C-O ( $1156\text{ cm}^{-1}$ ). The aromatic region of the  $^1\text{H}$  NMR spectrum (Table 1) resembled that of mahanimbine **1**, indicating a similar aromatic substitution pattern on the carbazole skeleton. However, the monoterpene moiety fused at C-1 and C-2 was different. In consideration of the molecular formula, and the absence of olefinic protons present in mahanimbine **1**, bicyclomahanimbine **6** was proposed as a hexacyclic base with a cyclobutane system. The presence of a cyclobutane

ring was supported by the downfield shift of the methyl group at 4'-CH<sub>3</sub> to  $\delta$  0.74 in bicyclomahanimbine when compared to the methyl group of mahanimbine at  $\delta$  1.62. The <sup>13</sup>C NMR spectrum (Table 2) also shows the present of 23 carbons resonances. The complete assignments of carbon signals and location of substituent on the skeleton of **6** was deduced on the basis of DEPT, <sup>1</sup>H-<sup>1</sup>H COSY, HMQC and HMBC spectra. Based on these spectroscopic data, the structure **6** was assigned to bicyclomahanimbine [21].

### CONCLUSION

The present study was aimed to investigate the chemical constituents of *Murraya koenigii* (TM 1006) collected from Pahang, Malaysia. Six C<sub>23</sub>-carbazole alkaloids was isolated and identified as mahanimbine **1**, murrayamine-J **2**, murrayazolinol **3**, mahanimbilol **4**, murrayakoeninol **5** and bicyclomahanimbine **6**. The spectral data of the compound was also compared with those in the literature.

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