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Polysubstituted Flavonoids from the Leaves of *Murraya paniculata* (Rutaceae)

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Abstract – Chemical studies on the constituents of the leaves of *Murraya paniculata* (Lynn) Jack have furnished three highly-oxygenated flavonoids; gardenin E (1), gardenin A (2), and gardenin C (3). Structures of the compounds were elucidated based on NMR, MS, UV, IR data and also by comparison with the previous works. The antimicrobial activities of these compounds and the crude extracts were also evaluated.
Keywords – *Murraya paniculata*, flavonoids, antimicrobial activity

Introduction

Murraya paniculata (Lynn) Jack or locally known as kemuning is a Rutaceous shrub found mostly in warm countries. According to Huxley (1992), *Murraya paniculata* is the only species from Rutaceae family that have aromatic flowers. This plant is widely used in the treatment of a variety of diseases. In Philiphines the leaves are used in the treatment of diarrhea and dysentery. The ground bark is used as a tonic and also rubbed on body to cure bodyache (Burkill, 1996). Previous investigations have revealed the presence of flavonoids and coumarins from the roots and leaves of *Murraya paniculata* collected in Sri Lanka and Indonesia (De Silva *et al.*, 1980; Kinoshita and Firman, 1996). We now report the isolation and structural elucidation of three polysubstituted flavonoids from the crude chloroform extract of the leaves of the plant species and their antibacterial properties. Phytochemical studies on *M. paniculata* originated from Malaysia has never been reported previously.

Experimental

General – Melting points (uncorrected) were determined on Kohfler melting points apparatus. Infrared spectra were recorded using KBr disc on Perkin Elmer FTIR spectrophotometer model 1650. Ultraviolet spectra were obtained in ethanol on a Shidmazu UV-2100 spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on JEOL Spectrometer at 500 and 125 MHz, respectively with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an AEI-MS 12 spectrometer. Column chromatography was carried using silica gel (Merck 9385) and Merck silica gel 60 PF_{254} was used for TLC analysis.

Plant Material – The plant material was collected from Kampung Gelok, Lenggong Perak, Malaysia in 1995. The plant was identified by Mr. Anthonysamy Savarimuthu (formerly of UPM), and the voucher specimen was deposited in the herbarium of Department of Biology, University Putra Malaysia. The leaves were air dried for 2 weeks at room temperature before being used.

Extraction and Isolation – Ground dried leaves of *Murraya paniculata* (2.2 kg) was defatted by soakings with petroleum ether. The residue was extracted three times with chloroform at room temperature. The solvent was removed under reduced pressure to give 244 g of dark gummy solid. A portion of the chloroform extract (20 g) was subjected to flash column chromatography separation and eluted stepwise with 100% petroleum ether, mixtures of petroleum ether/chloroform, 100% chloroform, mixture of chloroform/ methanol and 100% methanol to give 35 fractions.

Fraction 14 obtained from column chromatography separation was evaporated and the residue was rechromatographed on a mini column to give 18 subfractions of 100 ml each. Subfractions 8-10 were combined and the solvent was removed. The solid product obtained was recrystallized in methanol to give 3',5',5'-trihydroxy-4',6,7,8-tetramethoxyflavone (gardenin E) (**1**) (18 mg), appeared

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as yellow needles, m.p 229-230°C (Literature, Gupta *et al.* 1975, m.p 234°C). UV λmax (Ethanol) nm (log ∈): 328 (1.7), 282 (1.8). IR ν max (cm^{-1}, KBr disc) 3458 (OH), 2996, 2950, 2849, 1656, 1520, 1596, 718, 697. MS *m/z*, (% intensity); 390 (M^+, 72), 375 (100), 360 (33), 330 (15), 211 (8), 183 (10), 167 (5), 164 (5), 149 (9), 121 (11), 69 (27), 43 (8), 28 (10), 15 (29). ^{13}C NMR, ppm (125 MHz, $CDCl_3$); 182.6, 163.9, 152.6, 151.3, 148.5, 145.3, 139.2, 135.9, 132.7, 125.5, 106.3, 105.8, 103.9, 62.0, 61.5, 60.5, 59.1.

Work-up procedure on subfractions 19-20 of flash column chromatography separation furnished yellow amorphous solid of 5-hydroxy-3',4',5',6,7,8-hexamethoxyflavone (gardenin A) (**2**) (31 mg), m.p 163-164°C (Literature, Quijano *et al.* 1980, m.p 164-165°C). UV λ_{max} (Ethanol) nm (log ∈): 330 (0.9), 284 (1.0). IR ν_{max} (cm^{-1} KBr disc) 3446 (OH), 2930, 1745, 1658, 1502, 1426, 1375, 1285, 1126, 1024, 728, 676. MS *m/z*, (% intensity); 418 (M^+, 73), 403 (100), 211 (10), 192 (4), 183 (11), 127 (5), 69 (14). ^{13}C NMR, (125 MHz, $CDCl_3$), δ_{ppm}; 182.9, 163.6, 153.6, 153.1, 149.5, 145.8. 141.5, 136.6, 132.9, 126.3, 107.0, 104.8, 103.6, 62.0, 61.7, 61.1, 56.3.

Fraction 15 from column chromatography was rotary-evaporated and the solid obtained was washed with ether and recrystallized with methanol to give yellow crystals of 5',5-dihydroxy-3',4',6,7,8-trimethoxyflavone (gardenin C) (**3**) (64 mg), m.p 175-177°C (Literature, Gupta *et al*, m.p 179-180°C). IR ν_{max} (cm^{-1}, KBr disc) 3446 (OH), 2940, 1652, 1456, 1422, 1376, 1278, 1116, 1042, 920, 838, 736. MS *m/z*, (% intensity); 404 (M^+, 75), 389 (100), 359 (10), 211 (9), 183 (8), 181 (5), 178 (5). ^{13}C NMR, (125 MHz, $CDCl_3$), δ_{ppm}; 183.0, 163.6, 153.1, 152.6, 149.8, 149.5, 146.0, 138.8, 136.6, 133.0, 126.8, 107.0, 106.7, 104.9, 102.5, 62.1, 61.7, 61.2, 61.1, 56.1.

Bioassay – Anti-microbial activity investigation on the extracts of leaves of *Murraya paniculata* and three isolated flavonoids were carried out. The petroleum ether, chloroform and methanol extracts and three flavonoids were subjected to the test toward one species of bacteria (*Bacillus cereus*, UI 1447) and four species of fungi (*Aspergillus ochaceous*, NRRL 398), (*Saccharomyces lipolytica*, ATTC 16617), (*Saccharomyces cerevisiae*, ATCC 20341), and (*Candida lipolytica*, ATCC 2075). The tests were carried out according to the methods described previously (Mackeen *et al.* 1997).

Results and Discussions

Three highly-oxygenated flavonoids were isolated from chloroform extract of the leaves of the plant species. The compounds were characterized using spectroscopic methods and by comparison with the literature. The high-field NMR study including HMQC and HMBC correlation techniques was therefore undertaken to complement the published NMR data. Repeated chromatographic separation of the chloroform crude extract of the leaves yielded 18 mg of yellow substance of compound **1**, which was recrystallized from methanol and had a melting point of 229-230°C (Gupta *et al.* 1975, m.p 234°C). The infrared spectrum indicated the presence of a carbonyl group with strong absorption at 1650 cm^{-1} and a strong and broad band at 3458 cm^{-1} due to the presence of hydroxyl groups. The aromatic ring was represented by signal at 718 and 1520 cm^{-1}. The ultraviolet spectrum exhibited maximum absorptions at 328 and 282 nm characteristic of a flavonoid nucleus. On addition of aluminium chloride to the ethanolic solution of compound **1**, no bathochromic shift was observed, indicating that there was no OH group at C-3. The mass spectrum gave a molecular ion peak at m/z 390 which correspond to the molecular formula $C_{19}H_{18}O_9$. The ^1H NMR spectrum of compound **1** showed two singlets at the aromatic region. A singlet at δ6.71 was assigned to H-3 proton , while another two proton singlets at δ7.03 were for protons at H-2' and H-6' position. The presence of hydroxyl group signal occurred at δ12.66 due to the OH group chelated to the carbonyl functionality. The OMe groups resonate as singlets at δ3.75, δ3.80, δ3.91 and δ4.01. Two other equivalent OH groups attached to C-3' and C-5' occur as a broad singlet at δ9.67 (Table 1). The ^{13}C NMR spectrum indicates the presence of 19 carbon atoms, including twelve quartenary carbons which resonate at 182.6 (C-4), 163.9 (C-2), 152.6 (C-8), 151.3 (C-3'/C-5'), 148.5 (C-9), 145.3 (C-5), 139.2 (C-4'), 135.9 (C-7), 132.7 (C-6), 125.5 (C-1') and 103.9 ppm (C-10). Three methine carbons resonated at 106.3 (C-2'/C-6') and 105.8 ppm (C-3), while four methyl carbons appeared at 62.0, 61.5, 60.5 and 59.1 ppm. The assignments of ^1H and ^{13}C chemical shifts were substantiated by HMQC and HMBC correlation spectra. The HMQC spectrum indicated that proton signals at 6.71 ppm (H-3) and 7.03 ppm (H-2'/C-6') showed correlation with carbon signals at 105.8 ppm (C-3) and 106.3 ppm (C-2'/C-6'), respectively. The HMBC spectrum showed that peak at 6.71 ppm (H-3) correlated with the carbon signals at 163.9 (C-2), 182.6 (C-4), 103.9 (C-10), 125.5 (C-1') and 106.3 ppm (C-2'/C-6'). Similarly, proton peaks at 7.03 ppm (H-2'/H-6') also exhibited correlations with carbon signals at 163.9 (C-2), 105.8 (C-3), 125.5 (C-1') and 139.2 ppm (C-4'). The complete assignments are summarized in Table 2. The compound **1** could therefore be assigned as 3',5',5-trihydroxy-4',6,7,8-tetramethoxyflavone (gardenin E), which was isolated earlier from *Gardenia lucida*

and *G. turgida* (Gupta *et al.*, 1975). The mass fragmentation patterns supported the elucidation of the structure. The fragment ions at m/z 375 and 360 were due to the subsequent loss of two methyl groups from the molecule. The peaks at m/z 211 and 164 were both formed through the *retro*- Diels-Alder pathways.

Compound **2** was obtained as yellow powder (30 mg) and was recrystallized from methanol. The compound decomposed at 163-164°C (Quijano *et al.* 1980, m.p 164-165°C). Its infrared spectrum exhibited strong absorption bands for chelated hydroxyl group (3446 cm^{-1}) and a carbonyl group at ν_{max} 1658 cm^{-1}. The mass spectrum data indicated a molecular ion peak at 418 corresponding to the molecular formula $C_{21}H_{22}O_9$. Compound **2** was also shown to have flavone nucleus with typical characteristic ultraviolet spectrum which showed the strong absorption at 330 and 284 nm.

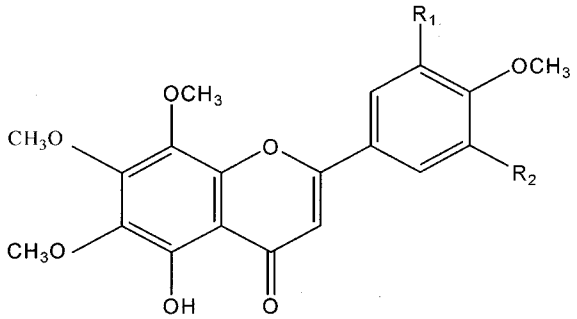
The ^1H NMR spectrum indicated the presence of 22 protons with the aromatic region showing the presence of one proton singlet (δ6.63) which correspond to H-3 and another two proton singlets (δ7.18) was assigned to H-2 and H-6 (Table 1). This suggested that the molecule has symmetrical trisubstituted B-ring. The presence of a low field broad resonance at δ12.46 was attributed to the OH group at C-5. The ^{13}C NMR data showed the presence of 21 carbon atoms, which supported the molecule structure including twelve quartenary carbon atoms. The carbonyl carbon atom gave the chemical shift value at δ182.9 whereas C-2 was slightly shifted to the higher field at δ163.6. The C-6, C-7 and C-8 exhibited peaks at δ132.9, 136.6, and 153.1, respectively, because of the attachment to the methoxyl groups. The remaining carbon resonances at 104.8, 145.8, 107.0, 149.5, 126.3, 103.6, 153.6, 141.5 ppm can be assigned to C-3, C-5, C-9, C-10, C-1', C-2'/C-6', C-3'/C-5' and C-4', respectively. The signals for methyl carbon were at 62.0, 61.7, 61.1 and 56.3 ppm. All these assignments were further supported by HMQC and HMBC correlation spectra shown in Table 2. The HMQC correlation revealed that the proton signal at 6.63 ppm (H-3) showed a crosspeak with carbon signal at 104.8 ppm (C-3), whereas proton signals at 7.18 ppm due to equivalent H-2' and H-6' protons correlated to carbon signal at 103.6 ppm (C-2'/C-6'). Among the long range heteronuclear interactions, signal at 6.63 ppm (H-3') were shown to correlate to carbon signals at 163.6 (C-2), 182.9 (C-4), 107.0 (C-9) and 126.3 ppm (C-1). Signals at 7.18 ppm (H-2'/H-6') also showed interactions with corresponding peaks of C-2, C-3, C-1', C-3', C-4' and C-5'. All the spectral evidences suggested that compound **2** was 5-hydroxy-3',4',5',6,7,8-hexamethoxyflavone (gardenin A), previously isolated from roots of *Gardenia turgida* and buds of *G. lucida* (Quijano *et al.* 1980). The assignment was in agreement with its mass fragmentation patterns, which was very similar to those of 3',5',5-trihydroxy-4',6,7,8-tetramethoxyflavone (gardenin E). The base peak at m/z 403 was formed through the loss of a methyl group from the molecular ion. The fragments produced from the *retro*-Diels- Alder pathways appeared at m/z 211 and 192. An ion peak at m/z 211 subsequently lost one CO group to give a peak at m/z 183.

Compound **3** was isolated as yellow crystals after recrystallization from methanol to give melting point of 175-177°C (Gupta *et al* 1975, m.p 179-180°C). The IR spectrum showed the presence of carbonyl group at 1652 cm^{-1} and hydroxyl group at 3446 cm^{-1}. A molecular ion peak was observed at m/z in the MS suggesting a molecular formula of $C_{20}H_{20}O_9$. The ^1H NMR spectrum of **3** integrated for the presence of 20 protons. The spectrum also showed the presence of five methoxyl groups with resonances at δ 3.96, δ3.97, δ3.98, δ3.99 and δ4.11. The two doublets at δ7.05 (J = 1.98 Hz) were for the meta-coupled protons assigned to H-2' and H-6' suggesting that the B-ring was not symmetrical. The singlet at δ6.60 was allocated to H-3 and

Table 1. ^1H NMR spectral data for compounds **1**, **2** and **3** in ppm (500 MHz, in $CDCl_3$)

H	1	2	3
3	6.71 (s)	6.63 (s)	6.60 (s)
2'	7.03 (s)	7.18 (s)	7.05 (*d*, 1.98 Hz)
6'	7.03 (s)	7.18 (s)	7.22 (*d*, 1.98 Hz)
5-OH	12.66	12.46	12.50
3-OH	9.67		
5-OH	9.67		6.01
6-OCH_3	3.91	3.98	3.99*
7-OCH_3	3.75	3.96	3.98*
8-OCH_3	4.01	4.12	4.11
3'-OCH_3		3.97	3.97*
4'-OCH_3	3.80	3.94	3.96*
5'-OCH_3		3.94	

*may interchangable



Gardenin E (1) : R1=R2=OH
Gardenin E (2) : R1=R2=OCH_3
Gardenin E (3) : R1=OCH_3, R2=OH

Table 2: HMQC and HMBC correlations of compounds **1** and **2**

	Gardenin E (1)				Gardenin A(2)			
Carbon number	δ^1H (ppm)	δ^{13}C (ppm)	HMQC correlations	HMBC correlations	δ^1H (ppm)	δ^{13}C (ppm)	HMQC correlations	HMBC correlations
2		163.9	–	H-2', H-6', H-3	–	163.6	–	H-2', H-6', H-3
3	6.71 (s)	105.8	H-3	H-2', H-6',	6.63 (s)	104.8	H-3	H-2', H-6',
4	–	182.6	–	H-3	–	182.9	–	H-3
5	–	145.3	–	–	–	145.8	–	–
6	–	132.7	–	OCH_3 (C-6)	–	132.9	–	OCH_3 (C-6)
7	–	135.9	–	OCH_3 (C-7)	–	136.6	–	OCH_3 (C-7)
8	–	152.6	–	OCH_3 (C-8)	–	153.1	–	OCH_3 (C-8)
9	–	148.5	–	–	–	107.0	–	H-3
10	–	103.9	–	H-3	–	149.5	–	–
1'	–	125.5	–	H-2', H-6', H-3	–	126.3	–	H-2', H-6', H-3
2'		106.3	H-2	H-3	7.18 (s)	103.6	H-2	–
3'	–	151.3	–	–	–	153.6	–	H-2', H-6', OCH_3(C-3')
4'	–	139.2	–	H-2', H-6', OCH_3(C-4')	–	141.5	–	H-2', H-6', OCH_3(C-4')
5'	–	151.3	–	–	–	153.6	–	H-2', H-6', OCH_3(C-5')
6'	7.03(s)	106.3	H-6'	H-3	7.18(s)	103.6	H-6	–
6-OCH_3	3.91	61.5	6-OCH_3	–	3.98	62.0	6-OCH_3	–
7-OCH_3	3.75	59.1	7-OCH_3	–	3.96	61.7	7-OCH_3	–
8-OCH_3	4.01	62.0	8-OCH_3	–	4.12	61.1	8-OCH_3	–
3'-OCH_3	4.01		-	–	3.97	56.3	3'-OCH_3	–
4'-OCH_3	3.80	60.5	4'-OCH_3	–	3.94	61.1	4'-OCH_3	–
5'-OCH_3	–	–	–	-	3.94	56.3	5'-OCH_3	–

another singlet which appeared at very low field at δ12.50 was assigned to chelated hydroxy at C-5 (Table 1). The ^{13}C NMR data showed the signals appropriate to two protonated and ten unprotonated aromatic carbons of flavone skeleton. The signals at δ163.6 and 104.9 were due to C-2 and C-3 atoms, respectively. The signal at the lower field at δ183.0 could be assigned to carbonyl group (C-4). The compound **3** was identified as 5',5-dihydroxy-3',4',6, 7,8-trimethoxyflavone (gardenin C), which was previously isolated from roots of *Gardenia lucida* (Gupta *et al.*, 1975). The mass spectrum data confirmed the assignment of the structure. Its mass fragmentation pattern was similar to the fragmentation pathway of compounds **1** and **2**, with the presence of peaks at m/z 389, 211, 183, 181 and 178.

As for bioassay investigation, the crude chloroform extract of leaves of *Murraya paniculata* showed moderate activity against *Bacillus cereus* and *Saccharomyces cerevisiae*. However, gardenin E (**1**), gardenin A (**2**) and gardenin C (**3**) were inactive against all the pathogenic microbes used in the tests.

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