

COUMARINS FROM *MURRAYA PANICULATA* (RUTACEAE)

(Koumarin daripada *Murraya Paniculata* (Rutaceae))

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Abstract

Phytochemical study on leaves of *Murraya paniculata* have yielded four coumarins of auraptene (**1**), *trans*-gleinadiene (**2**), 5,7-dimethoxy-8-(3-methyl-2-oxo-butyl)coumarin (**3**) and toddalene (**4**). The compounds were isolated using chromatographic methods and identified using spectroscopic techniques. Antimicrobial activity evaluation on the crude extracts and pure compounds indicated chloroform extracts of the leaves exhibited moderate activity and only gleinadiene (**2**) showed moderate activity against *Bacillus cereus*. *trans*- Isomer of Gleinadiene (**2**) has never been reported from this plant previously.

Keywords : *Murraya paniculata*; auraptene; gleinadiene; antimicrobial

Abstrak

Kajian fitokimia terhadap daun *Murraya paniculata* telah menemui empat koumarin; auraptena (**1**), *trans*-gleinadiena (**2**), 5,7-dimetoksi-8-(3-metil-2-oks-butil)koumarin (**3**) dan toddalena (**4**). Semua sebatian ini telah dipencilkan melalui kaedah kromatografi dan dikenalpasti melalui teknik spektroskopi. Penilaian aktiviti anti-mikrob terhadap ekstrak dan sebatian tulen menunjukkan bahawa ekstrak kloroform memperlihatkan aktiviti yang rendah dan hanya gleinadiena (**2**) menunjukkan aktiviti yang lemah terhadap *Bacillus cereus*. Isomer-*trans* bagi gleinadiena masih belum pernah dilaporkan daripada tumbuhan ini sebelum ini.

Katakunci : *Murraya paniculata*; auraptena; gleinadiena; antimikrob

Introduction

Rutaceae is a large family of trees, shrubs and climbers recognized easily from aromatic or lime-like smell of the broken twigs or fruits or of the crushed leaves. Some constituents of essential oils, such as citronella and bergamot, are obtained by distillation from plants of this family, and many species are used in native medicine. There are 16 from 161 genera and 60 from 1700 species occur in Malaysia, mostly found in lowland areas. *Murraya paniculata* which belong to Rutaceae family is one of the two genus species that can be found in West Malaysia. The plant which also well known as “kemuning” or orange jasmine also known as Chinese box in America and Canada [1]. The leaves are rather lather and dark shiny green. Their root bark is used as an anodyne or local anesthetic for the treatment of gout, contusion and bone ache [2].

The ground bark of *Murraya paniculata* is used in mixture of a drink and as antidote in snake bites and rubbed on the bitten limb. The ground bark of the root is eaten and rubbed on body to cure body ache. The powdered leaves is used as an application to fresh cuts, and decoction of the leaves is drunk in dropsy. They possess antibiotic activity against *Mycococcus pyogenes* and *Escherichia coli* [3, 4]. Both leaves and roots of the plant are used in folk medicine for the treatment of stomachache, toothache and gout [5], and treatment of diarrhea, dysentery and useful against rheumatism, cough and hysteria [3, 6, 7]. It is also reported that it is used to treat cuts, joint pain, body aches [8], and venereal disease [9]. Previous studies have reported several flavonoids and coumarins from the leaves and roots of *M. paniculata* [9, 10, 11]. Here we report the isolation and structural determination of coumarins from the leaf extracts of *M. paniculata* and their antimicrobial properties. The isolation of *trans*-isomer of gleinadiene from *M. paniculata* has never been reported previously.

Experimental

General procedure

Melting points were determined with Kofhler hot stage apparatus and were uncorrected. Infrared spectra were recorded with Perkin Elmer FTIR model 1725X spectrophotometer using KBr discs. Mass spectra were recorded on a Finnigen Mat SSQ710 spectrometer. ¹H-NMR spectra were obtained using 500 MHz JEOL spectrophotometer in CDCl₃. ¹³C-NMR spectra were determined in CDCl₃ operating at 125 MHz. TMS was used for internal standard while chemical shifts were reported in ppm. Vacuum column chromatography was employed by using silica gel Merck Kieselgel PF₂₅₄. Preparative thin layer chromatography utilized Merck silica gel PF₂₅₄. Analytical TLC was performed on commercially available TLC plastic sheets precoated with Kieselgel 60 F₂₅₄ (0.2 mm thickness).

Plant material

The plant sample was collected from Ipoh, Perak (Malaysia). A voucher specimen (No. RK 2954-96) was deposited in the Herbarium of the Department of Biology, Universiti Putra Malaysia.

Extraction and isolation of compounds

The leaves of *Murraya paniculata* were air-dried, ground to powder (1.5 kg) and extracted successively with petroleum ether, chloroform and methanol. The extracts were concentrated to produce semi solids weighed 21.6, 64.2 and 20.5 g of extracts respectively. The petroleum ether extract (19.6 g) was fractionated on a vacuum column chromatography of silica gel eluted with mixtures of petroleum ether, petroleum ether/chloroform and chloroform/methanol. A total of 50 fractions of 250 ml each were collected. Fraction 36 was rotary-evaporated, and the remaining solid was washed using ether and recrystallised with petroleum ether to yield auraptene (**1**); colourless crystals (65.2 mg), C₁₉H₂₂O₃, m.p. 64-65°C (Lit. [12] m.p. 67-68°C). MS m/z (% intensity): 298 (M⁺, 1), 163 (47), 162 (75), 136 (40), 93 (28), 81 (56), 69 (100), 41 (68). ¹H- and ¹³C-NMR data are in good agreement with the published data [12].

The chloroform extract (30.0 g) was chromatographed over a vacuum column chromatography on silica gel. The column was eluted with solvent mixtures of increasingly polarity (petroleum ether, petroleum ether/chloroform, chloroform, chloroform/methanol) and 89 fractions of 250 ml each were collected. Solid obtained from fraction 29 upon washing with ether was recrystallised with methanol and gave gleinadiene (5,7-dimethoxy-8-[(*E*)-3'-methylbuta-1',3'-dienyl]coumarin (**2**); yellow needles (261.3 mg), C₁₆H₁₆O₄, m.p. 138-141°C (Lit. [13] m.p. 120-121°C). IR ν_{max} (cm⁻¹, KBr disc); 2948, 1640, 1618, 1456, 1328, 1266, 1206, 1144, 1060, 992, 888, 746, 656. MS m/z, (% intensity); 272 (M⁺, 100), 257 (14), 241 (95), 226 (16), 213 (60), 198 (22), 182 (21), 155 (13), 141 (21), 128 (25), 115 (70), 91 (19); 77 (25), 63 (22), 51 (18). The ¹H-NMR and ¹³C-NMR data are summarized in Table 1. Solid product of fractions 34-38 from chloroform extract was washed with ether and recrystallised with methanol to give 5,7-dimethoxy-8-(3-methyl-2-oxo-butyl)coumarin (**3**); colourless needles (32.5 mg), C₁₆H₁₈O₅, m.p. 124-126°C (Lit. [14] m.p.121-122°C). MS m/z (% intensity) 290 (M⁺, 12), 272 (1), 219 (100), 205 (2), 176 (3), 161 (30), 133 (6), 118 (6), 89 (4), 77 (5), 51 (3). ¹H- and ¹³C-NMR data are in good agreement with the published data [14].

Fractions 58-59 from chloroform extract were rotary evaporated to give solid which was washed with ether and recrystallised with ethanol to give toddalenone (**4**); yellow needles (22.4 mg), C₁₅H₁₄O₅, m.p. 225-229°C (Lit.[12], m.p. 244-246°C). MS m/z (% intensity): 274 (M⁺, 34), 259 (52), 243 (100), 231 (31), 216 (35), 215 (9), 188 (29), 173 (43), 160 (6), 145 (18), 117 (10), 115 (21), 89 (18), 77 (12), 63 (21), 51 (12). ¹H- and ¹³C-NMR are in good agreement with the published data [12].

Antimicrobial assay

Crude extracts and isolated compounds from *Murraya paniculata* were tested against one gram positive bacteria (*Bacillus cereus*) and 4 fungi (*Saccharomyces cerevisiae*, *Candida lypolytica*, *Saccharomyces lypolytica* and *Aspergillus ochraceous*) using the disc diffusion assay as described previously [15].

Result and Discussion

Extraction followed by chromatographic fractionation on the extracts of leaves of *Murraya paniculata* have yielded four coumarins; auraptene (**1**), gleinadiene (**2**), 5,7-dimethoxy-8-(3-methyl-2-oxo-butyl)coumarin (**3**) and toddalenone (**4**). The structures of the compounds were elucidated based on similarities of their spectral and physical data with the literature values. Out of four coumarins isolated, only compound (**2**) has not been

reported previously from this plant species. The characterization of compound (2) are described here besides the antimicrobial activity of the plant extracts.

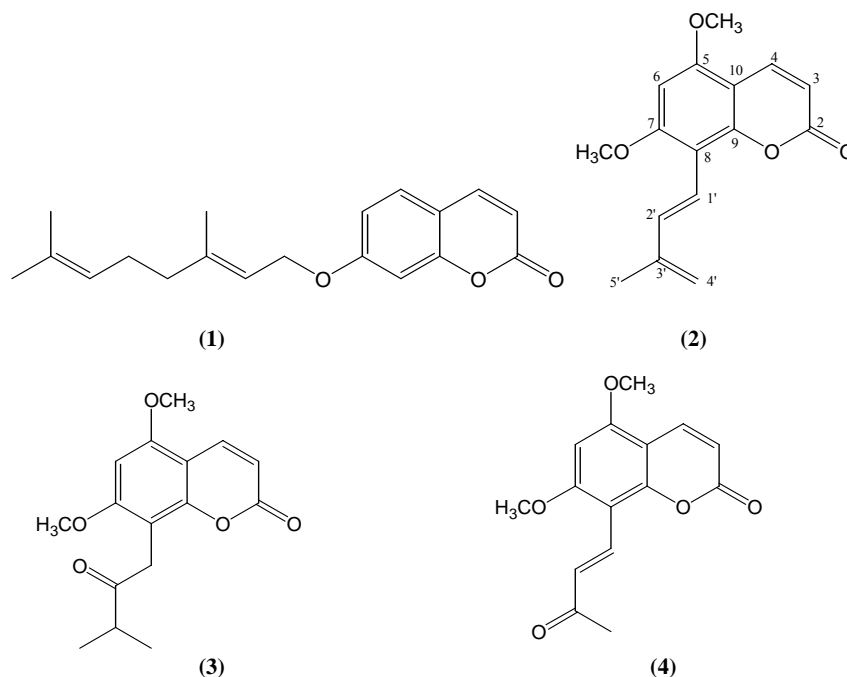


Figure 1. Structure of coumarins isolated from *Murraya paniculata*

The IR spectrum of (2) showed peak at 1728 cm^{-1} assigned as C=O group, while signal at 1618 cm^{-1} was due to the presence of double bonds. The MS showed the presence of a molecular ion at m/z 272 corresponding to the molecular formula $\text{C}_{16}\text{H}_{16}\text{O}_4$.

The integration of the ^1H NMR spectrum of (2) indicated the presence of 16 protons. A pair of doublets at δ 7.98 ($J = 9.7\text{ Hz}$) and 6.16 ($J = 9.7\text{ Hz}$) were characteristic of H-4 and H-3 in a coumarin nucleus, while another pair of doublets at δ 6.81 ($J = 16.3\text{ Hz}$) and 7.37 ($J = 16.3\text{ Hz}$) were assigned to H-2' and H-1', respectively. Coupling constant 16.3 Hz indicates that the structure has *trans* configuration. A methyl group signal at δ 2.02 and a two doublets due to two methylene protons at δ 5.16 and 5.07 (H-4') indicated that the coumarin contained a 3'-methylbuta-1',3'-dienyl side chain. The aromatic proton at H-6 occurs as a singlet at δ 6.32 to the upfield because of shielding effect of two methoxyl groups. The singlets at δ 3.96 and 3.94 were due to the presence of two methoxy groups at C-5 and C-7 in the aromatic ring respectively.

The assignments of ^1H - and ^{13}C -NMR data were confirmed by HMQC spectrum. The HMBC spectrum revealed H-4 at δ 7.98 correlated with carbon signal for C-2 (161.2 ppm) and C-5 (153.5 ppm). The proton signal at δ 7.37 (H-1') showed cross-peak with carbon signals at 103.8 ppm (C-8), 117.1 ppm (C-2') and 143.3 ppm (C-3'). H-2' at δ 6.81 found to correlate with C-1' (135.7 ppm) and C-3' (143.3 ppm). The proton signal at δ 6.32 (H-6) showed cross-peak with carbon signals at 161.1 ppm (C-7), 103.8 ppm (C-8) and 107.2 ppm (C-10). In addition, proton signal at δ 6.16 (H-3) correlated with carbon signal at 155.6 ppm (C-9) and 107.2 ppm (C-10). The complete NMR data of the compound (2) are displayed in Table 1. The structure of this compound was established based on the similarity of its spectral and physical data to those of gleinadiene previously isolated from *M. gleinei* root as reported by Kumar *et al.* [13]. However, there were inexplicable differences in the melting points for both compounds. The *cis* isomer of gleinadiene, (5,7-dimethoxy-8-[(*Z*)-3'-methylbutan-1',3'-dienyl]coumarin) which had been isolated from *Murraya paniculata* by Kinoshita and Firman [16] had coupling constant of 12.1 Hz. Our data demonstrated a larger value (16.3 Hz) which we suggest corresponds to *trans*-isomer of the compound. The melting point of toddalene (4) in the present report was also found to be rather

far from the reported value [14]. The difference in melting points of several diastereoisomers of coumarins isolated from *Murraya* species are rather common phenomena [13].

Table 1: ^1H - and ^{13}C -NMR Chemical Shifts (δ) and Coupling Patterns of the Protons and Correlations in HMBC Techniques of Compound (2)

Carbon no.	^{13}C (ppm) (CDCl_3 , 67.9 MHz)	^1H (ppm) (CDCl_3 , 500 MHz)	HMBC Correlation
2	161.2	-	-
3	110.9	6.16, <i>d</i>	C-9, C-10
4	138.7	7.98, <i>d</i>	C-5, C-2
5	153.5	-	-
6	90.3	6.32, <i>s</i>	C-7, C-8, C-10
7	161.1	-	-
8	103.8	-	-
9	155.6	-	-
10	107.2	-	-
1'	135.7	7.37, <i>d</i>	C-8, C-2', C-3'
2'	117.1	6.81, <i>d</i>	C-1', C-3'
3'	143.3	-	-
4'	117.0	5.16, <i>d</i> 5.07, <i>d</i>	-
5'	18.3	2.02, <i>s</i>	-
5-OCH ₃	56.0	3.96, <i>s</i>	-
7-OCH ₃	55.9	3.94, <i>s</i>	-

All of the extracts and pure compounds were tested against gram positive bacteria and fungi. The chloroform extract of the leaves of *Murraya paniculata* demonstrated weak activity against *Bacillus cereus* and *Saccharomyces cerevisiae* with inhibition zone of 9 and 8 mm. respectively. No activity was observed against other microbes. In addition, all other extracts of the plant were found to be non active against all the microbes used in the test. Of all the isolated compounds, only compound (2) exhibited weak antimicrobial activity against *Bacillus cereus* (8 mm inhibition zone). Thus, the antibacterial activity of the chloroform extract may be due to the synergistic effect of compound (2) since compounds (1), (2) and (3) were isolated from chloroform whereas (1) was obtained from petroleum ether extract.

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