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Phytochemical Studies of *Mussaenda hainanensis* Merr.

KE YUAN*, WEN-TAO QIAO† and MING-WEN YIN‡
Research and Development Center of Natural Medicine,
Zhejiang Forestry University, Lin'an, P.R. China
Tel: (86)(571)63743607; E-mail: yuan_ke001@yahoo.com.cn

Present study reports the chemical constituents of *Mussaenda hainanensis* Merr. The compounds were isolated by silica gel column chromatography and Sephadex LH-20 methods. Their structures were identified by physicochemical properties and spectroscopic analysis. Twelve compounds were elucidated as xanthone (**1**), eugenol (**2**), 3,5-dimethoxy-4-hydroxy-benzaldehyde (**3**), hydroquinone (**4**), cinnamaldehyde (**5**), *trans*-phytol (**6**), β-sitosterol (**7**), stigmasterol (**8**), ursolic acid (**9**), oleanolic acid (**10**), 2α,3β,19α,23-hydroxytormentic acid (**11**) and rutundic acid (**12**).

Key Words: *Mussaenda hainanensis* Merr., Chemical constituents.

INTRODUCTION

Mussaenda hainanensis Merr. (Rubiaceae) is a kind of climbing shrub with hairy branches that has attractive flowers[1], distributed in shady hillside, valley and shrub jungle. It grows in Hainan Province of China only.

In the present investigation, Ren-sheng XU and other people have reported the isolation and structural determination of several saponins and iridoids[2-9] from *Mussaenda pubescens* Ait.f (Rubiaceae). *Mussaenda pubescens* Ait.f is a liana-like shrub, distributed in east, south and southwest China, such as Fujian, Guangdong, Guangxi, Yunnan, Sichuan, Guizhou and other provinces. *Mussaenda pubescens* Ait. f. is a Chinese folk medicine commonly used in diuretic, antiphlogistic and antipyretic treatments[10]. It is also used to detoxify mushroom poisoning and to terminate early pregnancy in some parts of southeast China[11,12].

No report is available on the chemical constituents of *Mussaenda hainanensis* Merr. Twelve compounds were obtained from petroleum ether- and EtOAc-soluble extracts of this plant.

EXPERIMENTAL

Melting points were determined with a WRS-1B micro-melting point apparatus and uncorrected. ^{1}H, ^{13}C NMR spectra were recorded on a Bruker DPX 400 NMR spectrometer with TMS used as internal standard. ESI-MS was recorded on a Bruker Daltonics mass spectrometer. The silica gel for TLC and column chromatography

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†College of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou, P.R. China.
‡Department of Chemistry, Zhengzhou University, Zhengzhou, P.R. China.

were obtained from Qingdao Marine Chemical Inc., China. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz.

Plant material: The aerial parts of *Mussaenda hainanensis* Merr. were collected in September, 2007, at Sanya City, Hainan Province, People's Republic of China. A voucher specimen was identified by Prof. Shi-man HUANG of Hainan University.

Extraction and isolation: Dried aerial parts of the plant (9.0 kg) were extracted three times with 70 % EtOH at room temperature for 5 d. After evaporation of EtOH at 50 ºC *in vacuo*, the residual aqueous solution was extracted with petroleum ether (60-90 ºC), EtOAc and *n*-BuOH to yield 30, 80 and 110 g residues of each fraction, respectively.

The petroleum ether extract (30 g) was subjected to silica gel column chromatography using petroleum ether-EtOAc (100:0→0:100 gradient mixture), EtOAc-MeOH (100:0→10:1 gradient mixture) as solvents. Twenty fractions were collected (I-XX) according to the TLC control. Fraction X was further submitted to silica gel column chromatography using petroleum ether-EtOAc (40:1) to give compound **1** (55 mg), fraction IX was further submitted to silica gel column chromatography using petroleum ether-EtOAc (20:1) to give compound **2** (22 mg), fraction XII was further submitted to silica gel column chromatography using petroleum ether-CH_2Cl_2 (2:1) to give compound **6** (55 mg), fraction XIII was further submitted to silica gel column chromatography using petroleum ether-EtOAc (10:1) to give compounds **7** and **8** (22 mg).

A portion of the EtOAc extract (80 g) was subjected to silica gel column chromatography eluted with petroleum ether-EtOAc-MeOH (10:1:0→0:1:100, gradient mixtures). Twenty-one fractions were collected (I-XXI). Fraction III was further submitted to silica gel column chromatography using $CHCl_3$-MeOH (30:1) to give compound **4** (10 mg). Fraction V was further submitted to silica gel column chromatography using petroleum ether-acetone (5:1→2:1) to give compounds **5** (9 mg) and **3** (59 mg). Fraction VIII was further submitted to silica gel column chromatography and Sephadex LH-20 using $CHCl_3$-MeOH to give compounds **9** (68 mg), **10** (52 mg), **11** (12 mg) and **12** (36 mg).

Xanthone (1): Compound **1** (55 mg) was obtained as white amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (2H, d, *J* = 8.0 Hz, H-4,5), 7.76 (2H, d, *J* = 8.0 Hz, H-1,8), 7.46 (2H, dd, *J* = 8.0, 2.0 Hz, H-3,6), 7.35 (2H, dd, *J* = 8.0, 2.0 Hz, H-2,7); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8 (=CO), 154.5 (C-10,12), 136.1 (C-9, 11), 126.6 (C-4,5), 125.3 (C-1,8), 122.7 (C-3,6), 121.3 (C-2,7). The spectral data showed complete agreementwith the literature[13].

Eugenol (2): Compound **2** (22 mg) was obtained as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.84 (1H, d, *J* = 8.8 Hz, H-2), 6.68 (2H, d, *J* = 8.6 Hz, H-1), 5.95 (1H, d, *J* = 8.8 Hz, H-6'), 5.48 (1H, s, OH-4'), 5.08 (1H, s, H-2'), 5.05 (1H, d, *J* = 8.8 Hz, H-5'), 3.87 (3H, s, -OCH_3), 3.31 (2H, d, *J* = 6.8 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 146.4 (C-3'), 143.9 (C-4'), 137.8 (C-2), 131.9 (C-1'), 121.2 (C-1), 115.5 (C-2'), 114.2 (C-5'), 111.1 (C-6'), 55.9 (C- 3'-OH), 39.9 (C-3). The spectral data resembled the reported values[14].

3,5-Dimethyoxy-4-hydroxy-benzaldehyde (3): Compound **3** (59 mg) was obtained as white amorphous powder; ^{1}H NMR (400 MHz, MeOD) δ: 7.22 (2H, s, H-2,6), 9.78 (1H, s, -CHO), 3.89 (6H, s, $-OCH_3$); ^{13}C NMR (100 MHz, MeOD). δ: 191.5 (-CHO), 56.5 ($-OCH_3$), 129.4 (C-1), 107.7 (C-2,6), 149.0 (C-3,5), 143.1 (C-4). The spectral data showed complete agreement with the literature[15].

Hydroquinone (4): Compound **4** (10 mg) white amorphous powder, m.p. 172 ºC; ^{1}H NMR (400 MHz, MeOD) δ: 6.61 (H-2,3,5,6); ^{13}C NMR (100 MHz, MeOD) δ: 150.1 (C-1,4), 115.7 (C-2,3,5,6). The melting point of the mixture (the sample and the standard substance) does not drop. The sample and the standard substance have thesame R_f value.

Cinnamaldehyde (5): Compound **5** (9 mg) was obtained as light yellow oil; ^{1}H NMR (400 MHz, acetone-d_6) δ: 9.63 (1H, d, *J* = 7.7 Hz, H-1), 7.57 (1H, d, *J* = 16 Hz, H-3), 6.65 (1H, d, *J* = 16 Hz, H-2), 7.38 (2H, dd, *J* = 8.2, 2.0 Hz, H-2',6'), 7.20 (2H, dd, *J* = 8.2, 2.0 Hz, H-3',5'), 6.91 (1H, d, *J* = 8.2 Hz, H-4'); ^{13}C NMR (100 MHz, acetone-d_6) δ: 193.7 (C-1), 127.4 (C-2), 153.8 (C-3), 127.4 (C-1'), 111.5 (C-2', 6'), 124.6 (C-3',5'), 116.1 (C-4'). The R_f value of the sample is the same with the standard substance and the physical data showed agreement with cinnamaldehyde.

***trans*-Phytol (6):** Compound **6** (55 mg) was obtained as light yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.13 (2H, d, *J* = 7.6 Hz, H-1), 5.39 (1H, m, H-2), 1.98 (2H, d, *J* = 6.6 Hz, H-4), 0.87-1.98 (12H, brs). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 59.3 (C-1), 123.1 (C-2), 140.1 (C-3), 39.9 (C-4), 25.1 (C-5), 36.7 (C-6), 32.7 (C-7), 37.4 (1C-8), 24.4 (C-9), 37.3 (C-10), 32.8 (C-11), 37.2 (C-12), 24.8 (C-13), 39.4 (C-14), 27.9 (C-15), 22.7 (C-16), 16.1, 19.7, 19.7, 22.7 ($4\times CH_3$). The spectral data showed complete agreement with the literature[16].

β-Sitosterol (7): Compound **7** (125 mg) was obtained as a crystalline solid, m.p. 137-139 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.35 (1H, dd, *J* = 5.2 Hz, H-6), 3.50 (1H, t, *J* = 4.08 Hz, H-3), 2.27 (1H, m, H-4b), 2.22 (1H, m, H-4a), 2.01 (1H, m, H-12b), 1.95 (1H, m, H-7b), 1.86 (1H, m, H-23b), 1.85 (1H, m, H-1b), 1.82 (1H, m, H-16b), 1.00 (3H, s, 18-Me), 0.92 (3H, d, *J* = 6.6 Hz, 21-Me), 0.85 (3H, t, *J* = 7.9 Hz, 29-Me), 0.81 (6H, 26-Me, 27-Me), 0.65 (3H, s, 19-Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.3 (C-1), 26.1 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.7 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 29.2 (C-16), 56.1 (C-17), 19.4 (C-18), 11.9 (C-19), 36.1 (C-20), 18.8 (C-21), 31.9 (C-22), 28.2 (C-23), 45.8 (C-24), 31.6 (C-25), 12.0 (C-26), 19.8 (C- 27), 23.1 (C-28), 19.0 (C-29). The physical and spectral data showed complete agreement with the literature[17].

Stigmasterol (8): Compound **8** (25 mg) was obtained as a crystalline solid, m.p. 162-163 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.52 (1H, m, H-3), 5.36 (1H, m, H-6), 5.02 (1H, dd, *J* = 8.0, 16.0 Hz, H-23), 5.15 (1H, dd, *J* = 8.8, 16.0 Hz, H-22), 0.70 (3H, s, CH_3-18), 1.09 (3H, s, CH_3-19), 1.02 (3H, d, *J* = 6.4 Hz, CH_3-21); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.9 (C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.7 (C-12), 42.3 (C-13), 56.9 (C-14), 24.3 (C-15), 28.9 (C-16), 56.0 (C-17), 12.1 (C-18), 19.4

(C-19), 40.5 (C-20), 21.1 (C-21), 138.3 (C-22), 129.3 (C-23), 51.2 (C-24), 31.9 (C-25), 19.0 (C-26), 21.2 (C-27), 25.4 (C-28), 12.2 (C-29). The physical and spectral data showed complete agreement with the literature[18].

Ursolic acid (9): Compound **9** (62 mg) was obtained as white amorphous powder, m.p. 277-278 °C; $[\alpha]^{20}_{D}$ + 59° (c = 0.3, pyridine); ^{13}C NMR (100 MHz, CDCl$_3$) δ: 40.6 (C-1), 28.8 (C-2), 79.7 (C-3), 40.4 (C-4), 56.7 (C-5), 19.5 (C-6), 34.3 (C-7), 40.8 (C-8), 49.0 (C-9), 38.1 (C-10), 24.5 (C-11), 126.9 (C-12), 139.6 (C-13), 43.3 (C-14), 29.2 (C-15), 25.3 (C-16), 49.0 (C-17), 54.4 (C-18), 40.8 (C-19), 40.0 (C-20), 31.8 (C-21), 38.2 (C-22), 29.2 (C-23), 19.6 (C-24), 16.3 (C-25), 17.7 (C-26), 24.4 (C-27), 181.6 (C-28), 17.8 (C-29), 21.5 (C-30). The physical and spectral data showed complete agreement with the literature[19].

Oleanolic acid (10): Compound **10** (58 mg) was obtained as white amorphous powder, m.p. 307-309 °C; $[\alpha]^{20}_{D}$ + 73.3° (c = 0.15, CHCl$_3$). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 40.5 (C-1), 28.8 (C-2), 79.7 (C-3), 40.0 (C-4), 56.7 (C-5), 19.5 (C-6), 33.8 (C-7), 40.6 (C-8), 47.6 (C-9), 39.8 (C-10), 24.5 (C-11), 123.6 (C-12), 145.2 (C-13), 43.2 (C-14), 29.2 (C-15), 24.1 (C-16), 49.6 (C-17), 42.7 (C-18), 48.4 (C-19), 31.6 (C-20), 34.9 (C-21), 33.8 (C-22), 29.2 (C-23), 17.7 (C-24), 17.6 (C-25), 17.8 (C-26), 26.4 (C-27), 181.8 (C-28), 33.6 (C-29), 23.9 (C-30). The physical and spectral data showed complete agreement withthe literature[20].

2α,3β,19α,23-Hydroxytormentic acid (11): Compound **11** (12 mg) was obtained as white amorphous powder; ESI-MS: m/z 503 [M-H] ; ^{13}C NMR (100 MHz, CDCl$_3$) δ: 48.6 (C-1), 71.9 (C-2), 78.4 (C-3), 44.7 (C-4), 48.4 (C-5), 19.9 (C-6), 36.2 (C-7), 41.4 (C-8), 49.4 (C-9), 38.9 (C-10), 24.9 (C-11), 129.7 (C-12), 139.2 (C -13), 43.1 (C-14), 29.5 (C-15), 27.2 (C-16), 49.6 (C-17), 55.1 (C-18), 73.6 (C-19), 48.8 (C-20), 37.5 (C-21), 17.4 (C-22), 69.2 (C-23), 14.5 (C-24), 18.3 (C-25), 16.6 (C-26), 24.9 (C-27), 182.2 (C-28), 27.3 (C-29), 18.4 (C-30). The physical and spectral data showed complete agreement with the literature[21].

Rutundic acid (12): Compound **12** (36 mg) was obtained as white amorphous powder, m.p. 255-258 °C; $[\alpha]^{20}_{D}$ + 34.2° (c = 0.3, MeOH); IR (film) ν_{max} 3724, 2886, 2345, 1734, 1661, 1552, 1498, 1438, 1367, 1053 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ: 5.30 (1H, m, H-12), 0.70, 0.76, 0.92, 0.95, 1.29, 1.33 (each 3H, s, 6×CH$_3$); ^{13}C NMR (100 MHz, CD$_3$OD) δ: 41.0 (C-1), 27.3 (C-2), 74.1 (C-3), 40.7 (C-4), 48.8 (C-5), 19.3 (C-6), 33.7 (C-7), 39.5 (C-8), 48.3 (C-9), 37.8 (C-10), 24.7 (C-11), 129.4 (C-12), 140.0 (C-13), 42.6 (C-14), 29.6 (C-15), 26.6 (C-16), 43.2 (C-17), 55.1 (C-18), 73.6 (C-19), 43.1 (C-20), 28.6 (C-21), 38.1 (C-22), 67.5 (C-23), 12.7 (C-24), 17.7 (C-25), 17.5 (C-26), 25.1 (C-27), 182.3 (C-28), 27.4 (C-29), 16.6 (C-30). The physical and spectral data showed complete agreement with the literature[22].

RESULTS AND DISCUSSION

The 70 % EtOH extract of the aerial parts of *Mussaenda hainanensis* Merr. was partitioned between water and petroleum ether, between water and ethyl acetate and between water and *n*-butanol successively. The petroleum ether fraction was subjected to silica gel column chromatography, eluted with petroleum ether-EtOAc (100:0→0:100, gradient mixture), EtOAc-MeOH (100:0→10:1, gradient mixture). The ethyl acetate fraction was subjected to silica gel column chromatography eluted with petroleum ether-EtOAc-MeOH (10:1:0→0:1:100, gradient mixtures). In summary, 12 compounds were isolated from the petroleum ether and acetyl acetate fractions of the 70 % EtOH extract of this plant, including xanthone (**1**), eugenol (**2**), 3,5-dimethoxy-4-hydroxy-benzaldehyde (**3**), hydroquinone (**4**), cinnamaldehyde (**5**), *trans*-phytol (**6**), β-sitosterol (**7**), stigmasterol (**8**), ursolic acid (**9**), oleanolic acid (**10**), 2α,3β,19α,23-hydroxytormentic acid (**11**), rutundic acid (**12**). The compounds **1-6** and **11** were isolated from *Mussaenda hainanensis* Merr. for the first time.

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