Phenylpropanoids and flavones from *Asystasia gangetica* (L) T. Anderson var. *micrantha* (Acanthaceae)

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Abstract

*Asystasia gangetica* (L) T. Anderson var. *micrantha* (Acanthaceae), commonly known as ‘Chinese violet’ or ‘rumput Israel’, is a straggling herb usually found among short grasses and along pathways. This plant is used traditionally to treat diabetes mellitus, ear disease and gonorrhea, while its anthelmintic activity helps to treat swelling and rheumatism. The present study was designed to isolate and elucidate bioactive compounds from this plant. Methanolic extract of the plant leaves was fractionated by using PLC. Selected fractions were subjected to preparative HPLC and recycling HPLC for further purification.

All the compounds were monitored by using UHPLC. The structures of isolated compounds were characterized by using spectroscopic method including NMR, IR and UV data. Four constituents, namely ferulic acid, methyl caffeate, chrysoeriol and chrysoeriol-4′-glycoside were isolated. All the compounds were identified in the genus *Asystasia.*

Keywords: *Asystasia gangetica,* flavone, phenylpropanoids, HPLC, NMR.

Introduction

*Asystasia gangetica* (L) T. Anderson var. *micrantha* (Acanthaceae), commonly known as Chinese violet or *rumput Israel* in Malaysia, is a straggling herb usually found among short grasses and along pathways. This plant is used traditionally to treat diabetes mellitus, ear disease and gonorrhea; while its anthelmintic activity helps to treat swelling and rheumatism.

Pharmacologically it has been proven that this plant possesses a wide range of biological activity. This includes antioxidant, antibacterial, antidiabetic and antiasthmatic and many more. Previous phytochemical study of this plants has resulted to the discovery of iridoids, flavonoids and some other compounds. In this study, we report the isolation of four compounds namely ferulic acid, methyl caffeate, chrysoeriol and chrysoeriol-4′-glycoside.

Material and Methods

General methods: 1D (1H NMR, 13C NMR) and 2D (HMBC, HMQC, NOESY, COSY) NMR spectra were recorded on Bruker 600 MHz ultrashield NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Deuterated methanol was used as solvent and TMS as internal reference standard (0.00 ppm). Chemical shifts were reported in ppm and δ scale and the coupling constants were given in Hz.

Extraction and isolation of chemical constituents: Fresh *A. gangetica* leaves (2.3 kg) were macerated in 10 L of MeOH at room temperature for 24 hours and repeated three times. Solvent was removed at reduced pressure to give 294.2 g sticky crude extract. The crude extract was dissolved in H2O and MeOH and subjected to liquid-liquid partition by using *n*-hexane and ethyl acetate to give hexane (89 g, F1), ethyl acetate (62 g, F2) and residual aqueous (102 g, F3) fractions. The fraction F2 was further fractionated with PLC on reverse phase column eluted with ACN and H2O to yield nine sub-fractions (F2.1-2.9).

The sub-fractions F2.5, F2.6 and F2.8 were purified by recycling HPLC eluted with ACN and H2O on reverse phase column to give compounds 1-3 (10, 8.9 and 14.6 mg respectively). Subsequently, fraction F3 was subjected to PLC to afford 9 sub-fractions (F3.1-3.9). The sub-fraction F3.5 was determined as pure and identified as compound 4 (23.3 mg).

Ferulic acid: Yellow solid. 1H NMR (600 MHz, Methanol-d4) δH 7.57 (d, J = 15.9 Hz, 1H, H-7), 7.09 (d, J = 2.1 Hz, 1H, H-2), 7.07 (dd, J = 8.3, 2.1 Hz, 1H, H-6), 6.97 (d, J = 8.3 Hz, 1H, H-5), 6.30 (d, J = 15.9 Hz, 1H, H-8), 3.91 (s, 3H, -OCH3). 13C NMR (151 MHz, Methanol-d4) δC 169.74 (C=O), 149.98 (C-3), 146.60 (C-4), 144.99 (C-7), 127.65 (C-1), 121.23 (C-6), 115.56 (C-2), 113.35 (C-5), 111.19 (C-8), 55.02 (-OCH3).

Methyl caffeate: White needles. 1H NMR (500 MHz, Methanol-d4) δH 7.57 (d, J = 15.9 Hz, 1H, H-7), 7.06 (d, J = 2.0 Hz, 1H, H-2), 6.96 (dd, J = 8.2, 2.0 Hz, 1H, H-6), 6.80 (d, J = 8.2 Hz, 1H, H-5), 6.28 (d, J = 15.9 Hz, 1H, H-8), 3.78
Chrysoeriol: Yellow powder. $^1$H NMR (600 MHz, Methanol-d$_4$) δ$_H$ 7.53 (dd, J = 8.6, 2.0 Hz, 1H, H-6'), 7.46 (d, J = 2.0 Hz, 1H, H-2'), 7.27 (d, J = 8.6 Hz, 1H, H-5'), 6.63 (s, 1H, H-3), 6.44 (d, J = 2.0 Hz, 1H, H-8), 6.21 (d, J = 2.0 Hz, 1H, H-6), 5.07 (d, J = 7.4 Hz, 1H, H-1''), 3.95 (s, 1H, -OCH$_3$), 3.94 – 3.89 (m, 1H, H-6''a), 3.78 – 3.73 (m, 1H, H-6''b), 3.62 – 3.57 (m, 1H, H-2'''), 3.55 (t, J = 6.9 Hz, 1H, H-3''), 3.52 (dd, J = 5.8, 2.0 Hz, 1H, H-5''), 3.48 – 3.43 (m, 1H, H-4''''). $^{13}$C NMR (126 MHz, Methanol-d$_4$) δ$_C$ 182.38 (C-4), 164.68 (C-7), 163.88 (C-2'), 161.69 (C-5), 157.92 (C-9), 149.84 (C-4''), 149.54 (C-3''), 125.10 (C-1''), 119.90 (C-6'), 115.79 (C-5'), 109.74 (C-2'), 104.01 (C-10), 103.72 (C-3), 100.58 (C-1''), 98.93 (C-6), 93.83 (C-8), 76.92 (C-5'''), 76.43 (C-2'''), 73.38 (C-3'''), 69.90 (C-4'''), 61.11 (C-6'''), 55.56 (-OCH$_3$).

Chrysoeriol-4'-glucoside: Orange sticky solid. $^1$H NMR (500 MHz, Methanol-d$_4$) δ$_H$ 7.53 (dd, J = 8.6, 2.0 Hz, 1H, H-6'), 7.46 (d, J = 2.0 Hz, 1H, H-2'), 7.27 (d, J = 8.6 Hz, 1H, H-5'), 6.63 (s, 1H, H-3), 6.44 (d, J = 2.0 Hz, 1H, H-8), 6.21 (d, J = 2.0 Hz, 1H, H-6), 5.07 (d, J = 7.4 Hz, 1H, H-1''), 3.95 (s, 1H, -OCH$_3$), 3.94 – 3.89 (m, 1H, H-6''a), 3.78 – 3.73 (m, 1H, H-6''b), 3.62 – 3.57 (m, 1H, H-2'''), 3.55 (t, J = 6.9 Hz, 1H, H-3''), 3.52 (dd, J = 5.8, 2.0 Hz, 1H, H-5''), 3.48 – 3.43 (m, 1H, H-4''''). $^{13}$C NMR (126 MHz, Methanol-d$_4$) δ$_C$ 182.38 (C-4), 164.68 (C-7), 163.88 (C-2'), 161.69 (C-5), 157.92 (C-9), 149.84 (C-4''), 149.54 (C-3''), 125.10 (C-1''), 119.90 (C-6'), 115.79 (C-5'), 109.74 (C-2'), 104.01 (C-10), 103.72 (C-3), 100.58 (C-1''), 98.93 (C-6), 93.83 (C-8), 76.92 (C-5'''), 76.43 (C-2'''), 73.38 (C-3'''), 69.90 (C-4'''), 61.11 (C-6'''), 55.56 (-OCH$_3$).

Results and Discussion

Compounds 1 and 2 are phenolic compounds with one methoxy substituent. The first compound was isolated as yellow solids while the latter was purified as white needles. Both compounds showed very similar $^1$H NMR pattern. For compound 1, the splitting pattern at aromatic region showed two doublets at 7.09 ppm (d, J = 2.1 Hz, 1H), 6.97 ppm (d, J = 8.3 Hz, 1H) and one doublet doublet at 7.07 ppm (dd, J = 8.3, 2.1 Hz, 1H). The splitting pattern of these protons showed a typical ABX spin system.

The presence of one sharp peak at 3.99 ppm belongs to a methoxy group and the position of this substituent was determined by the HMBC experiment where a correlation between this proton signal with the carbon signal at 149.98 ppm (C-3) was observed. Two doublets at 7.57 (d, J = 15.9 Hz, 1H) and δ$_H$ 6.30 (d, J = 15.9 Hz, 1H) showed the presence of a trans olefinic double bond based on the coupling constant value. The presence of a conjugated carboxylic acid was shown in $^{13}$C NMR at 169.74 ppm. Based on the spectroscopic evidence, it is included that compound 1 is trans-ferlicic acid.

For compound 2, one ABX spin system was also observed, but at more shielded chemical shifts, at δ$_H$ 7.06 (d, J = 2.0 Hz, 1H), 6.96 (dd, J = 8.2, 2.0 Hz, 1H) and 6.80 (d, J = 8.2 Hz, 1H). One methoxy group at δ$_H$ 3.78 (s, 3H) was also observed and showed a correlation with the signal of Conjugated C=O at δ$_C$ 168.41 ppm. The olefinic proton signals at 6.30 (d, J = 15.9 Hz, 1H) and 7.57 (d, J = 15.9 Hz, 1H) confirmed the structure of compound 2 as methyl caffeate.

Compound 3 was isolated as orange solid. Based on $^1$H NMR, the compound was a flavone derivative based on the presence of the typical flavone proton signal at 6.67 ppm (s, 1H) which belongs to H-3. Two meta-coupled doublets were observed at 6.51 (d, J = 2.2 Hz, 1H) and 6.24 (d, J = 2.1 Hz, 1H) assignable to H-6 and H-8 in the ring A. Moreover, an ABX system of aromatic signals at δ$_H$ 7.27 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H) and 7.53 (dd, J = 8.6, 2.0 Hz, 1H) ppm, together with two carbon signal at δ$_C$ 149.54 and 149.84 ppm indicated that C-3' and C-4' were oxygenated. A methoxy signal at δ$_H$ 3.99 (s, 3H) ppm which showed an HMBC correlation with carbon signal at δ$_C$ 148.13 ppm, positioned the methoxy group at C-3' on the ring B. The structure of compound 3, therefore, was concluded as chrysoeriol.

Compound 4 was purified as an orange sticky solid. $^1$H NMR signals in the aromatic region were very similar to those of compound 3, but with the presence of signals for one sugar moiety which were observed by the presence of an anomeric proton signal at δ$_H$ 5.07 (d, J = 7.4 Hz, 1H) ppm and the anomeric carbon signal at δ$_C$ 100 ppm. Early prediction indicated that the attachment of glucoside moiety is at C-4' which was later confirmed by the HMBC correlation between the anomeric proton in glucoside and C-4'. Thus, it was confirmed that the structure of compound 4 is chrysoeriol-4'-O-glucoside.
Pharmacologically it is reported that ferulic acid possessed various biological activities such as antioxidant, anti-inflammatory and can reduce the risk for coronary heart diseases.\textsuperscript{17-20} It is also reported that this compound possessed anti-diabetic effect which could be ascribed to its potent antioxidant capacity.\textsuperscript{21} Some naturally occurring caffeates exhibit bioactivities such as antibacterial, antiviral, anti-inflammatory, antiatherosclerotic, anti-HIV and many more.\textsuperscript{22}

As for chrysoeriol, it is reported that this compound is a good lipase inhibitory activity, better than luteolin due to the presence of methoxy group. It also showed a very good cytotoxic activity\textsuperscript{23} while chrysoeriol-4'-glucoside shows selectivity towards HeLa.\textsuperscript{16} Thus, the isolation of these compounds indirectly supports the medicinal uses of this plants especially as anti-inflammatory, anti-diabetic and anticancer remedy.

**Conclusion**

Four phytochemical constituents namely ferulic acid (1), methyl caffeate (2), chrysoeriol (3) and chrysoeriol-4'-glucoside (4) have been isolated from *A. gangetica*.

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