Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

IRIDOID GLYCOSIDES AND PHENYLPROPANOIDS FROM *Asystasia gangetica* (L) T. ANDERSON VAR. *Micrantha* (ACANTHACEAE)

(Iridoid Glikosida dan Fenilpropanoid daripada *Asystasia gangetica* (L) T. Anderson Var. *Micrantha* (Acanthaceae))

Isna Athirah Othman[1, 2], Norizan Ahmat[1, 2]*, Amjad Ayad Qatran Al-Khdhairawi[2]

[1]Faculty of Applied Sciences,
Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[2]Atta-ur-Rahman Institute for Natural Product Discovery,
Universiti Teknologi MARA, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia

**Corresponding author: noriz118@uitm.edu.my*

Received: 20 November 2019; Accepted: 29 June 2020; Published: August 2020

Abstract

Asystasia gangetica (L) T. Anderson var. *micrantha* (Acanthaceae) or commonly known as "Chinese Violet" or "*rumput Israel"* in Malaysia is a rapidly growing herb usually found among short grasses and along pathways. This plant is used traditionally to treat diabetes mellitus, ear disease and gonorrhea. Its anthelmintic activity helps to treat swelling and rheumatism. The present study was designed to isolate and elucidate chemical constituents from *A. gangetica*. The methanolic extract of the *A. gangetica* leaves was fractionated by using vacuum liquid chromatography (VLC) and preparative high-performance liquid chromatography (HPLC). Selected fractions were purified by recycling HPLC and monitored by using ultra high-performance liquid chromatography (UHPLC). The structures of isolated compounds were characterized by using various spectroscopic methods, mainly nuclear magnetic resonance (NMR) and comparisons with previously reported data. Five phytochemical constituents namely, salidroside **(1)**, verbascoside **(2)**, forsythiaside **(3)**, 3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(4)**, and 4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(5)** were purified using recycling HPLC. Compounds **2**, **3** and **5** were identified for the first time from *A. gangetica*.

Keywords: acanthaceae**,** *Asystasia gangetica*, iridoid glycosides, phenylpropanoid, high-performance liquid chromatography, nuclear magnetic resonance

Abstrak

Asystasia gangetica (L) T. Anderson var. *micrantha* (Acanthaceae) yang juga dikenali sebagai "Chinese Violet" atau "rumput Israel" di Malaysia adalah herba yang mudah membiak, biasanya ditemui di kalangan rumput pendek dan sepanjang jalan. Tumbuhan ini secara traditionalnya digunakan untuk merawat diabetes mellitus, sakit telinga dan gonorea. Keupayaan antihemintiknya membantu merawat bengkak dan reumatik. Kajian ini diolah untuk memencilkan dan mengenalpasti sebatian aktif daripada *A. Gangetica.* Ekstrak metanol daripada daun *A. Gangetica* difraksi menggunakan kromatografi cecair vakum (VLC) dan penyediaan kromatografi cecair bertekanan tinggi (HPLC). Fraksi terpilih telah ditulenkan menggunakan HPLC kitar semula dan dipantau menggunakan kromatografi cecair bertekanan tinggi ultra (UHPLC). Struktur sebatian yang dipencilkan dikenalpasti menggunakan pelbagai teknik spektroskopi terutamanya resonan magnetik nuklear (NMR) dan perbandingan dengan data yang

telah dilaporkan sebelumnya. Lima sebation kimia iaitu salidrosida **(1)**, verbascosida **(2)**, forsithiasida **(3)**, 3''-*O*-kafeoil-6-*O*-rhamnopiranosil katalpol **(4)** dan 4''-*O*-kafeoil-6-*O*-rhamnopiranosil katalpol **(5)** telah dipencilkan menggunakan HPLC pengitaran semula. Sebatian **2**, **3** dan **5** adalah dilaporkan buat pertama kali dari *A. gangetica*.

Kata kunci: acanthaceae**,** *Asystasia gangetica*, iridoid glikosida, fenilpropanoid, kromatografi cecair bertekanan tinggi, resonan magnetik nuklear

Introduction

In the present study, the leaves of *A. gangetica* were investigated for compounds in tropical plants of Malaysia. The preliminary phytochemical screening of this plant showed the presence of flavonoids, alkaloids, glycosides, coumarins, tannins, steroids, terpenoids and saponins [1]. Previous studies on this plant have led to the isolation of some iridoid glycosides and flavonoids [1-3]. Several studies have reported the bioactivities of this plant such as antidiabetic, antiasthmatic, anti-inflammatory activity and antioxidant [4]. In this study, our research group attempt to isolated five compounds, namely salidroside **(1)**, verbascoside **(2)**, forsythiaside **(3)**, 3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(4)**, and 4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(5)** from the methanol extract of the leaves of *A. gangetica*. The structures of the isolated compounds were characterized by using several spectroscopic methods including NMR and comparisons with previously reported data.

Materials and Methods

Chemicals and raw materials

All the chemicals used were of analytical and HPLC grade and purchased from Sigma Chemical Co. (St Louis, Missouri). The leaves of *A. gangetica* were collected in Kapar, Klang, Selangor and were identified by Dr Shamsul Khamis, a botanist from Universiti Kebangsaan Malaysia (UKM), and the voucher specimens were deposited in the Herbarium, School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor with the voucher number UKMB40431.

Extraction and isolation

Standard procedures for extraction and isolation of pure compounds from plant materials were used in this research [2]. The leaves were air-dried and ground to obtain a powdered sample (2.3 kg) and were macerated in methanol for 24 h to extract the constituents. The solvent was filtered and concentrated under reduced pressure to obtain crude extract (294.2 g). The crude extract was dissolved in 90% aqueous methanol and subjected to liquid-liquid partition by using hexane and ethyl acetate (EA) to obtain three major fractions. The EA partition (62 g) and aqueous residual partition (83 g) were further fractionated by preparative liquid chromatography (PLC) to obtain nine smaller fraction each. Fraction 2 of EA partition was subjected to recycling HPLC to obtain compounds **1** (3.6 mg) and **2** (126.3 mg). Purification on fraction 3 of the residual aqueous partition produced compounds **3**, **4** and **5** (1.7, 10.2 and 6.7 mg respectively).

Purification and structure elucidation

The structural elucidation of the isolated compounds was done by utilizing several spectroscopic methods. IR spectra were performed on Bruker Tensor II FT-IR, while UV spectra were recorded on Gen-5 Microplate Reader (Synergy HT). The ^{1}H NMR and ^{13}C NMR were recorded in methanol-d_4 on Bruker 600 Ultrashield NMR spectrometer measured at 600 MHz for ^{1}H NMR and 151 MHz for ^{13}C NMR. Peak multiplicities were presented in *H*z and chemical values were shown in ppm (δ). Various chromatographic techniques were used to purify the chemical constituents. For fractionation, liquid chromatography (VLC) was applied by using silica gel 60, 70 – 230 mesh ASTM (Merck 1.07747). Further fractionation was done by using semi-preparative HPLC, while recycling HPLC was applied for the final purification process.

Results and Discussion

Five constituents, namely, salidroside **(1)**, verbascoside **(2)**, forsythiaside **(3)**, 3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(4)**, and 4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(5)** were successfully isolated from the leaves of *A. gangetica*. Compounds were elucidated based on ^{1}H and ^{13}C NMR spectroscopy as well as comparison with the spectroscopic data with values obtained from related literature. The ^{13}C and ^{1}H NMR data are presented in Table 1 and 2 respectively. Salidroside (**1**) [5], a naturally occurring tyroside, was obtained as a white amorphous powder. The ^{1}H NMR showed the presence of an AA''XX'' aromatic ring system, where two pairs of symmetrical protons overlapped at 7.09 (*d*, *J*=8.4, 2H, H-2, 4) and 6.72 (*d*, *J*=8.4, 2H, H-1, 5), followed by the signals at δ_H 4.06 (*td*, *J*=8.9, 6.7, 1H, H-7), 3.75 – 3.71 (*m*, 1H, H-7) and δ_H 2.90 – 2.82 (*m*, 1H, H-8) which corresponded to the 5-hydroxyphenylethyl moiety. The spectrum also displayed signals of a typical glycoside moiety at δ_H 4.31 (*d*, *J*=7.8, 1H, H-1''), 3.20 (*dd*, *J*=9.1, 7.9, 1H, H-2''), 3.37 (*t*, *J*=8.6, 1H, H-3''), 3.32 – 3.26 (*m*, 2H, H-4'',5''), 3.88 (*dd*, *J*=11.9, 2.0, 1H, H-6'') and 3.69 (*dd*, *J*=11.9, 5.2, 1H, H-6''). Salidroside was previously purified from the heartwood of *Acer tegmentosum* and it was shown to possess analgesic and anti-gastropathy properties [6].

Verbascoside **(2)** [7] was isolated as an amorphous powder. ^{1}H NMR showed resonances at δ_H 6.72 (*d*, *J*=2.1, 1H, H-2), 6.70 (*d*, *J*=8.0, 1H, H-5), 6.59 (*dd*, *J* = 8.0, 2.0 Hz, 1H, H-6), 3.77 – 3.71 (*m*, 1H, H-7), 4.10 – 4.03 (*m*, 1H, H-7) and 2.87 – 2.75 (*m*, 2H, H-8) which corresponded to the 3,4-dihydroxyphenylethyl moiety. The signals of two trans-olefinic protons at δ_H 6.30 (*d*, *J* = 15.8 Hz, 1H, H-7') and 7.62 (*d*, *J* = 15.9 Hz, 1H, H-8') as well as the ABX system signals at 7.09 (*d*, *J* = 2.0 Hz, 1H, H-2'), 6.81 (*d*, *J* =8.1, 1H, H-5'), 6.98 (*dd*, *J* = 8.2, 2.1 Hz, 1H, H-6') clearly showed the presence of a *trans*-caffeoyl unit. A glucose unit was observed at δ_H 4.40 (*d*, *J* = 7.9 Hz, 1H, H-1''), 4.95 (*m*, 1H, H-2''), 3.84 (*t*, *J* = 9.2 Hz, 1H, H-3''), 4.95 (*m*, 1H, H-4''), 3.60 – 3.52 (*m*, 2H, H-5'', 6'') and 3.67 – 3.60 (*m*, 1H, H-6''), while the signals at 5.21 (*d*, *J* = 1.8 Hz, 1H, H-1'''), 3.96 – 3.94 (*m*, 1H, H-2'''), 3.60 – 3.52 (*m*, 1H, H-3'''), 3.36 – 2.32 (*m*, 1H, H-4'''), 3.67 – 3.60 (*m*, 1H, H-5''') and 1.11 (*d*, 6.1, 3H, H-6''') belonged to rhamnose. The site of acylation was determined by HMBC correlation at between C=O/ H-4'', which indicated that the caffeoyl unit was attached to C-4'' of the glucose, while a correlation of C-1''/H-8 confirmed the attachment of 3,4-dihydroxyphenylethyl moiety at the anomeric carbon. As for the rhamnose, the correlation found at C-3''/H-1''' confirmed the attachment at the C-3'' of the glucose. This compound was previously isolated from *Buddleja globosa* leaves and was reported to possess antimicrobial properties [8].

Forsythiaside (**3**) [9-10] was obtained as an orange sticky solid. The ^{1}H NMR signals were comparable to those of **2**. The signals for the 3,4-dihydroxyphenylethyl were exhibited at δ_H 6.70 (*d*, *J* = 2.1 Hz, 1H, H-2), 6.79 (*d*, *J* = 8.2 Hz, 1H, H-5), 6.56 (*dd*, *J* = 8.1, 2.1 Hz, 1H, H-6) and 2.83 – 2.78 (*m*, 2H, H-7). The presence of *trans*-caffeoyl moiety was observed at 7.06 (*d*, *J* = 2.1 Hz, 1H, H-2'), 6.66 (*d*, *J* = 8.0 Hz, 1H, H-5'), 6.92 (*dd*, *J* = 8.2, 2.0 Hz, 1H, H-6'), 6.32 (*d*, *J* = 15.9 Hz, 1H, H-7') and 7.59 (*d*, *J* = 15.8 Hz, 1H, H-8'). The signals at δ_H 4.36 (*d*, *J* = 7.8 Hz, 1H, H-1''), 3.36 (*d*, *J* = 11.3 Hz, 1H, H-2''), 3.57 – 3.53 (*m*, 1H, H-3''), 4.03 (*dd*, *J* = 9.6, 6.3 Hz, 1H, H-4''), 3.60 – 3.57 (*m*, 1H, H-5''), 4.40 – 4.37 (*m*, 1H, H-6'') and 4.52 (*dd*, *J* = 11.8, 2.1 Hz, 1H, H-6'') belonged to the glucose moiety, while the signals at 5.20 (*d*, *J* = 1.8 Hz, 1H, H-1'''), 3.97 (*dd*, *J* = 3.4, 1.9 Hz, 1H, H-2'''), 3.75 – 3.72 (*m*, 1H, H-3'''), 3.46 – 3.43 (*m*, 1H, H-4'''), 3.43 – 3.40 (*m*, 1H, H-5'''), 1.27 (*d*, *J* = 6.2 Hz, 3H, H-6''') indicated the presence of rhamnose. By comparing the ^{1}H NMR data to those of compound **2**, compound **3,** however, showed a significant change at H-4'' and H-6'' signals which shifted downfield by 0.92 and 0.87 ppm respectively. The signal for C-3'' also became more shielded by 6.27 ppm which indicated the loss of a glycosyl bond at this site. The long-range correlation observed at C-5''/H-1''' confirmedd the attachment of rhamnose at C-6'' position of glucose. This compound was first isolated from *Forsythia suspensa* [9] and was found to possess strong antioxidant and antibacterial activity [11].

3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol (**4**) [12] was isolated as orange sticky solid. The signals observed at δ_H 5.11 (*d, J* = 9.6, 1H, H-1), 6.40 (*dd, J* = 6.0, 1.9 Hz, 1H, H-3), 5.10 (*d, J* = 10.5, 1H, H-4), 2.47 – 2.39 (*m*, 1H, H-5), 4.05 (*dd, J* = 8.2, 1.1 Hz, 1H, H-6), 3.67 (*s*, 1H, H-7), 2.59 (*dd, J* = 9.7, 7.6 Hz, 1H, H-9), 3.82 (*d, J* = 13.1 Hz, 1H, H-10) and 4.18 (*d, J* = 13.2 Hz, 1H, H-10) indicated the presence of cyclopentan-pyran structure ring system with a double bond between C-3 and C-4. The ^{1}H NMR resonance at δ_H 4.80 (*d, J* = 7.9 Hz, 1H, H-1'') was assigned to the anomeric proton and their *J* coupling indicated β–glucopyranose structure as a sugar unit. The complete assignment for glucose and rhamnose moiety is presented in Table 2. Additionally, long-range correlations observed at C-1''/H-1 and C-1'''/H-6 showed the attachment site for the sugars. After the complete interpretation of the NMR data and comparison of the data with those reported in the literature were carried out, the core skeleton of **4** was determined to be 6-*O*-rhamnopyranosyl catalpol (**4a**) [11]. The presence of caffeoyl moiety was determined from the presence of the signals at δ_H 7.09 (*t, J* = 2.0 Hz, 1H, H-2'), 6.81 (*d, J* = 8.2 Hz, 1H, H-5'), 6.99 (*dd, J* = 8.2, 2.1 Hz, 1H, H-6'), 7.62 (*d, J* = 15.8 Hz, 1H, H-7') and 6.36 (*d, J* = 15.8 Hz, 1H, H-8'). ^{1}H NMR chemical shifts of the rhamnose moiety of **4** were compared to those of **4a** where the H-3" signal was seen to significantly shift downfield by 1.49 ppm. The other signals were comparable to those in literature. This pattern established that the position of the caffeoyl ester of compound **4** is at C-3" of rhamnose. Table 2 shows the spectroscopic data (^{1}H NMR) for compounds **4** – **5**. This compound was previously isolated from *Veronica hookeri* [12] and was shown to exhibit antioxidant properties [2].

4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol (**5**) [15] was isolated as orange sticky solid. The NMR signals showed a very close similarity to those of **4** [16]. However, in compound **5**, a significant downfield shift by 1.79 ppm was observed at H-4", followed by upfield shifts for H-3" and H-5", which established that caffeoyl esterification happened at C-4". Signals for catalpol iridoid was shown at δ_H 5.13 (*d, J* = 9.8, 1H, H-1), 6.41 (*dd, J* = 6.0, 1.8, 1H, H-3), 5.17 – 5.09 (*m*, 1H, H-4), 2.51 – 2.45 (*m*, 1H, H-5), 4.07 (*dd, J* = 8.2, 1.1 Hz, 1H, H-6), 3.68 (*s*, 1H, H-7), 2.60 (*dd, J* = 9.7, 7.6, 1H, H-9), 3.84 (*d, J* = 13.0 Hz, 1H, H-10) and 4.18 (*d, J* = 13.2 Hz, 1H, H-10). Signals for the caffeoyl moieties roused at δ_H 7.09 (*d, J* = 2.1, 1H, H-2'), 6.81 (*d, J* = 8.2, 1H, H-5'), 6.99 (*dd, J* = 8.2, 2.2, 1H, H-6'), 7.66 (*d, J* = 15.8, 1H, H-7') and 6.38 (*d, J* = 15.9, 1H, H-8'). The signals for glucose and rhamnose moiety as well as the comparison of ^{1}H NMR data for compounds **4** – **5** are presented in Table 2. This compound has been previously purified from *Verbascum thapsus* [15]. To date, no literature has been found to describe the bioactivity of the compound, however, catalpol derivatives have been proven to possess laxative, diuretic, hypoglycemic, antihyperglycemic and immunomodulatory properties [13, 14]. The comparison of the ^{13}C NMR for compounds **1** – **5** is tabulated in Table 1. Figure 1 shows the structure of the compounds.

Table 1. Spectroscopic data (^{13}C NMR) for compounds **1** – **5**

No. of Carbon	**Compound**				
	1 δ_c	**2** δ_c	**3** δ_c	**4** δ_c	**5** δ_c
Aglycone					
1	114.7	130.14	130.02	93.80	93.80
2	129.5	115.78	115.69		
3	155.4	144.72	144.74	140.89	140.85
4	129.5	143.27	143.26	102.05	102.19
5	114.7	114.98	114.98	35.88	35.88
6	129.4	119.93	119.87	82.98	82.40

Table 1 (cont'd). Spectroscopic data (^{13}C NMR) for compounds **1** – **5**

No. of Carbon	Compound				
	1 δ_c	**2** δ_c	**3** δ_c	**4** δ_c	**5** δ_c
7	70.7	70.87	71.02	58.13	57.92
8	35.0	35.17	35.28	65.16	65.18
9				41.91	41.89
10				60.09	60.08
Caffeoyl					
1'		126.30	126.30	126.36	126.47
2'		113.92	113.71	115.15	115.12
3'		145.42	145.39	148.31	148.17
4'		148.38	148.24	145.43	145.76
5'		115.19	115.16	113.87	113.95
6'		121.86	121.75	121.71	121.54
7'		113.35	145.86	146.21	145.43
8'		146.66	113.45	113.46	113.79
C=O		166.96	167.75	167.33	167.51
Glucose					
1''	103.0	102.80	103.00	98.34	98.86
2''	73.7	74.80	74.29	73.45	73.90
3''	76.7	80.30	74.03	76.31	76.30
4''	76.6	69.23	69.01	70.39	70.40
5''	70.3	74.62	74.02	77.23	77.24
6''	61.4	63.05	63.24	61.54	61.55
Rhamnose					
1'''		101.64	101.32	96.38	98.33
2'''		70.96	70.95	69.13	69.96
3'''		70.69	68.65	68.91	69.86
4'''		72.42	72.61	72.81	73.45
5'''		69.04	70.87	68.90	68.97
6'''		17.08	16.47	16.63	16.60

Table 2. Comparison of ^{1}H NMR data for compounds **4** – **5**

No. of proton	Compound		
	4	**4a**	**5**
Aglycone			
1	5.11 (*d*, 9.6, 1H)	5.07 (*d*, 10, 1H)	5.13 (*d*, 9.8, 1H)
3	6.40 (*dd*, 6.0, 1.9, 1H)	6.35 (*dd*, 6, 2, 1H)	6.41 (*dd*, 6.0, 1.8, 1H)
4	5.10 (*d*, 10.5, 1H)	5.05 (*dd*, 6, 5, 1H)	5.17 – 5.09 (*m*, overlapped)
5	2.47 – 2.39 (*m*, 1H)	2.38 (*m*, 1H)	2.51 – 2.45 (*m*, 1H)

Table 2 (cont'd). Comparison of ^{1}H NMR data for compounds **4** – **5**

No. of proton	Compound		
	4	**4a**	**5**
6	4.05 (*d*, 8.2, 1H)	3.99 (*dd*, 8, 2, 1H)	4.07 (*dd*, 8.2, 1.1, 1H)
7	3.67 (*s*, 1H)	3.62 (d, 2, 1H)	3.68 (*s*, 1H)
8			
9	2.59 (*dd*, 9.7, 7.6, 1H)	2.54 (*dd*, 10, 8, 1H)	2.60 (*dd*, 9.7, 7.6, 1H)
10	3.82 (*d*, 13.1, 1H) 4.18 (*d*, 13.2, 1H)	3.81 (*d*, 13, 1H) 4.13 (*d*, 13, 1H)	3.84 (*d*, 13.0, 1H) 4.18 (*d*, 13.2, 1H)
Caffeoyl			
1'			
2'	7.09 (*d*, 2.0, 1H)		7.09 (*d*, 2.1, 1H)
3'			
4'			
5'	6.81 (*d*, 8.2, 1H)		6.81 (*d*, 8.2, 1H)
6'	6.99 (*dd*, 8.2, 2.1, 1H)		6.99 (*dd*, 8.2, 2.2, 1H)
7'	7.62 (*d*, 15.8, 1H)		7.66 (*d*, 15.8, 1H)
8'	6.36 (*d*, 15.8, 1H)		6.38 (*d*, 15.9, 1H)
C=O			
Glucose			
1''	4.80 (*d*, 7.9, 1H)	4.77 (*d*, 8, 1H)	4.80 (*d*, 7.9, 1H)
2''	3.32 – 3.21 (*m*, overlapped)	3.25 (*dd*, 8, 9, 1H)	3.32 – 3.22 (*m*, overlapped)
3''	3.42 (*t*, 9.0, 1H)	3.38 (*t*, 9, 1H)	3.43 (*t*, 9.1, 1H)
4''	3.32 – 3.21 (*m*, overlapped)	3.24 (*dd*, 8, 10, 1H)	3.32 – 3.22 (*m*, overlapped)
5''	3.32 – 3.21 (*m*, overlapped)	3.30 (*m*, 1H)	3.32 – 3.22 (*m*, overlapped)
6''	3.95 (*dd*, *J* = 8.9, 2.7 Hz, 1H) 3.64 (*dd*, *J* = 12.0, 6.7 Hz, 1H)	3.61 (*dd*, 12, 6, 1H) 3.91 (*dd*, 12, 2, 1H)	3.65 (*dd*, 12.0, 6.7, 1H) 3.95 (*dd*, 12.0, 2.1, 1H)
Rhamnose			
1'''	5.04 (*d*, 1.8, 1H)	4.92 (*d*, 2, 1H)	4.99 (*d*, 1.8, 1H)
2'''	3.93 – 3.91 (*m*, 1H)	3.84 (*dd*, 2, 3, 1H)	3.74 – 3.68 (*m*, overlapped)
3'''	5.16 (*dd*, 3.5, 1.7, 1H)	3.67 (*dd*, 9, 3, 1H)	3.74 – 3.68 (*m*, overlapped)
4'''	3.51 (*t*, 9.5, 1H)	3.38 (*t*, 9, 1H)	5.17 – 5.09 (*m*, overlapped)
5'''	3.79 – 3.75 (*m*, 1H)	3.63-3.69 (*m*, 1H)	3.87 – 3.81 (*m*, overlapped)
6'''	1.33 (*d*, 6.3, 3H)	1.25 (*d*, 6, 3H)	1.33 (*d*, 6.2, 3H)

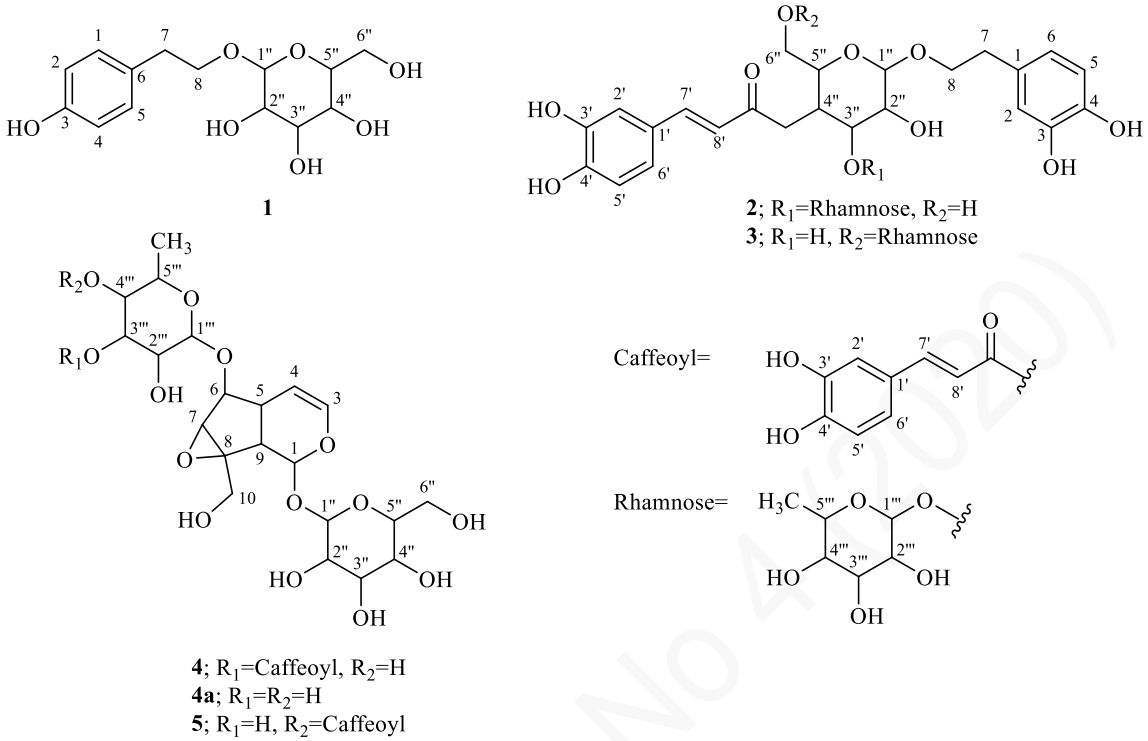

Figure 1. Structure of compounds **1** – **5**

Conclusion

Five known compounds, including a tyrosol derivative, two iridoid glycosides and two phenylpropanoids have been isolated and characterized from the methanol extract of the leaves of *A. gangetica*. These compounds include salidroside **(1)**, verbascoside **(2)**, forsythiaside **(3)**, 3''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(4)**, and 4''-*O*-caffeoyl-6-*O*-rhamnopyranosyl catalpol **(5)**. Compounds **2**, **3**, and **5** were identified for the first time in *A. gangetica*. As previous reports found that these types of phenolic compounds displayed diverse pharmacological properties, further detailed investigation of their biological activities is recommended.

Acknowledgements

The authors would like to thank the Ministry of Higher Education, Malaysia for the financial support under MyPhD Scholarship and the Fundamental Research Grant Scheme [600-RMIFRGS 5/3 (0064/2016)].

References

1. Gopal, T., K., Megha, G., Chamundeeswari, D. and Reddy, C., U. (2013). Phytochemical and pharmacological studies on the whole plant of *Asystasia gangetica*. *Indian Journal of Research in Pharmacy and Biotechnology*, 1(3): 365-370.
2. Worawittayanon, P., Ruadreo, J., Disadee, W., Sahakitpichan, P., Sitthimonchai, S., Thasana, N., Ruchirawat, S. and Kanchanapoom, T. (2012). Iridoid and flavone glycosides from *Asystasia gangetica* subsp. *micrantha* and *Asystasia salicifolia* and their antioxidant activities. *Biochemical Systematics and Ecology*, 40:38-42.
3. Senthamilselvi, M. M., Kesavan, D. and Sulochana, N. (2011). A new biflavone glycoside from flowers of *Asystasia gangetica*. *Chemistry of Natural Compounds*, 47(3):360-362.
4. Tilloo, S., K., Pande, V. B., Rasala, T., M. and Kale V., V. (2012). *Asystasia gangetica*: Review on multipotential application. *International Research Journal of Pharmacy*, 3(4):18-20.

5. Mastihubová, M. and Poláková, M. (2016). A selective and mild glycosylation method of natural phenolic alcohols. *Beilstein Journal of Organic Chemistry*, 12: 524-530.
6. Yoo, Y. M., Nam, J. H., Kim, M. Y., Choi, J., Lee, K. T. and Park, H. J., 2009. Analgesic and anti-gastropathic effects of salidroside isolated from *Acer tegmentosum* heartwood. *The Open Bioactive Compounds Journal*, 2: 1-7.
7. López-Rodríguez, R., Herrera-Ruiz M., Trejo-Tapia, G., Domínguez-Mendoza, B. E., González-Cortazar M. and Zamilpa, A. (2019). *In vivo* gastroprotective and antidepressant effects of iridoids, verbascoside and tenuifloroside from *Castilleja tenuiflora* Benth. *Molecules*, 24: 1292.
8. Pardo, F., Perich, F., Villarroel, L. and Torres, R., 1993. Isolation of verbascoside, an antimicrobial constituent of *Buddleja globosa leaves*. *Journal of Ethnopharmacology*, 39(3): 221-222.
9. Nishibe, S., Okabe, K., Tsukamoto H., Sakushima A. and Hisada S. (1982). The structure of forsythiaside isolated from *Forsythia suspensa. Chemical and Pharmaceutical Bulletin*, 30(3): 1048-1050.
10. Jia, J., Zhang, F., Li, Z., Qin, X. and Zhang, L. (2015). Comparison of fruit of *Forsythia suspensa* at two different maturation stages by NMR-based metabolomics. *Molecules*, 20: 10065-10081.
11. Qu, H., Zhang, Y., Wang, Y., Li, B. and Sun, W., 2008. Antioxidant and antibacterial activity of two compounds (forsythiaside and forsythin) isolated from *Forsythia suspensa*. *Journal of Pharmacy and Pharmacology*, 60(2): 261-266.
12. Kroll-Møller, P., Pedersen, K. D., Gousiadou C., Kokubun, T., Albach D., Taskova, R., Garnock-Jones. P. J., Gotfredsen C. H. and Jensen S. R. (2017). Iridoid glucosides in the genus *Veronica* (Plantaginaceae) from New Zealand. *Phytochemistry*, 140: 174-180.
13. Kupeli, E., Harput, U. S., Varel, M., Yesilada, E., Saracoglu, I. (2005). Bioassay-guided isolation of iridoid glucosides with antinociceptive and anti-inflammatory activities from *Veronica anagallis-aquatica* L. *Journal of Ethnopharmacology*, 102: 170-176.
14. Oh, H., Pae, H. O., Oh, G. S., Lee, S. Y., Chai, K. Y., Song, C. E., Kwon, T. O., Chung, H. T., Lee, H. S. (2002). Inhibition of inducible nitric oxide synthesis by catalposide from *Catalpa ovata. Planta Medica*, 68: 685-689.
15. Warashina, T., Miyase, T. and Ueno, A. (1991). Iridoid glycosides from *Verbascum thapsus* L. *Chemical and Pharmaceutical Bulletin*, 39(12): 3261-3264.
16. Helfrich, E. and Rimpler, H., 1999. Iridoid glycosides and phenolic glycosides from *Holmskioldia sanguinea*. *Phytochemistry*, 50(4): 619-627.