



Phenolic Compounds from the Aerial Parts of *Adenophora triphylla* (Thunb.) A. DC. var. *triphylla* and their Free Radical Scavenging Activity

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Abstract

Adenophora triphylla (Thunb.) A. DC. var. *triphylla* (Family: Campanulaceae) is distributed in Japan, Korea, and China. It is locally known as “Saiyousyajin” in Japan and the roots are used in traditional medicine to treat chronic bronchitis and whooping cough, and also as anti-inflammatory and antitussive agents. Till now, there is no report on the chemical constituents of aerial parts. Thus, the main aim of this study was to isolate and identify major chemical constituents of aerial parts of *A. triphylla* var. *triphylla*, and to evaluate their free radical scavenging activity. The 70% methanol extract of the aerial parts was subjected to repeated column chromatography using MCI gel CHP-20P, Sephadex LH-20, ODS and silica gel columns to isolate the five phenolic components (1-5). Free radical scavenging activity of the extract and compounds was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity method. The structures of the isolated compounds were elucidated as luteolin (1), luteolin 4'-O- β -glucopyranoside (2), luteolin 7-O- β -glucopyranoside (3), luteolin 7-O-neohesperidoside (4) and chlorogenic acid (5) based on their nuclear magnetic resonance (NMR) spectral data and comparison with literature values. All these compounds were isolated for the first time from *A. triphylla* var. *triphylla*. The extract showed weak free radical scavenging activity. Among isolated compounds, luteolin (1), luteolin 7-O- β -glucopyranoside (3), luteolin 7-O-neohesperidoside (4) and chlorogenic acid (5) showed potent free radical scavenging activity. Results from this study suggest that the aerial parts of *A. triphylla* var. *triphylla* might be a potential plant source for the development of functional foods, however further detailed research is necessary.

Keywords: *Adenophora triphylla*; Saiyousyajin; Phenolic compounds; Free radical scavenging

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Introduction

Medicinal plants and their phytochemicals have played a vital role in human healthcare as an important source of traditional medicines, drug discovery and development of nutritional and functional foods [1-3]. However, many plant species are yet to be explored for their chemical constituents and potential biological activities and health-promoting effects.

The genus *Adenophora* belonging to Campanulaceae family consists of about 62 species distributed in East Asia and Europe, among which, 12 species are distributed in Japan [4]. *Adenophora triphylla* (Thunb.) A. DC. var. *triphylla*

(“Saiyousyajin” in Japanese) and *A. triphylla* (Thunb.) A. DC. var. *japonica* (Regel) H. Hara (“Tsuriganeninjin” in Japanese) are among many varieties of plant *A. triphylla* (Thunb.) A. DC. and are distributed in Japan, Korea, and China [4, 5]. Roots of both of these plants are used in traditional medicine to treat chronic bronchitis and whooping cough and as an anti-inflammatory and antitussive agents [5-8]. There have been many studies on the chemical constituents/biological activities of roots and leaves of *A. triphylla* var. *japonica* [5, 7, 9, 10]. Kim et al. [5] reported the high phenolic and flavonoid contents and potent free radical scavenging activities of leaves of *A. triphylla* var. *japonica*, however, no



such studies are reported on the aerial parts of *A. triphylla* var. *triphylla* for the best of our knowledge. Thus, in this study, the main aim was to isolate and identify major chemical constituents of aerial parts of *A. triphylla* var. *triphylla* and to evaluate their free radical scavenging activity.

Materials and Methods

General Experimental Procedure

¹H- and ¹³C- NMR spectra were measured on BRUKER AVANCE 600 NMR Spectrometer (Bruker, Billerica, MA, USA) (¹H-NMR: 600 Hz and ¹³C-NMR: 150 Hz). Chemical shift values (δ_H and δ_C) are given in ppm with reference to tetramethyl silane (TMS). Column chromatography (CC) was carried out with MCI gel CHP20P (75 ~ 150 μ m, Mitsubishi Chemical Industries Co. Ltd., Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan) and silica gel 60 (0.040-0.063 mm, Merck KGaA, Darmstadt, Germany). Thin layer chromatography (TLC) was performed on a pre-coated silica gel 60 F₂₅₄ (Aluminum sheet, Merck KGaA, Darmstadt, Germany).

Plant Material

The aerial parts (stems and leaves) of *A. triphylla* var. *triphylla* were collected from Mt. Tawarayama Kumamoto, Japan in August 2015 and shade dried for two weeks. Plant material was identified by Mr.

Masato Watanabe, Technical Officer, School of Pharmacy, Kumamoto University.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich, Co. (Tokyo, Japan). 6-Hydroxy- 2, 5, 7, 8- tetramethylchroman-2-carboxylic acid (Trolox) was from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and 2-morpholinoethanesulfonic acid monohydrate (MES) was purchased from Dojindo Chemical Research (Kumamoto, Japan).

Extraction and Isolation

The dried aerial parts (2600 g) were then extracted twice with 70% MeOH (18 L). The combined extract was evaporated under reduced pressure to give 414.0 g of extract. The extract was suspended in water and subjected on MCI gel CHP20P column chromatography (CC) and eluted successively with water, 40%, 70% and 100% MeOH to give eight fractions (1~8). Fraction 2 (109.4 g, H₂O eluate) was subjected on Sephadex LH-20 CC (H₂O) to obtain four subfractions (2-1~2-4). Subfraction 2-3 was subjected on MCI gel CC (H₂O) to afford compound **5** (153.8 mg). Fraction 7 (3.05 g, 80% MeOH eluate) was subjected on Sephadex LH-20 CC (MeOH) to afford compound **1** (174.4 mg). Fraction 6 (4.0 g, 60% MeOH eluate) was subjected to Sephadex LH-20 CC (H₂O-MeOH; 1:1) to afford compound **2** (211.4 mg).

Table 1. Proton NMR spectroscopic data of compound **1 - 4** (δ_H , mult. (J in Hz))

Position	1 ^a	2 ^b	3 ^a	4 ^b
3	6.65, s	6.56, s	6.74, s	6.57, s
6	6.17, d (2.1)	6.18, d (2.0)	6.43, d (2.1)	6.38, d (2.1)
8	6.43, d (2.1)	6.41, d (2.0)	6.78, d (2.1)	6.73, d (2.1)
2'	7.40, d (2.3)	7.41, d (2.3)	7.40, d (2.1)	7.38, d (2.5)
5'	6.87, d (8.2)	7.28, d (8.5)	6.89, d (8.4)	6.89, d (8.5)
6'	7.38, dd (8.2, 2.3)	7.39, dd (8.5, 2.3)	7.43, dd (8.4, 2.1)	7.40, dd (8.5, 2.5)
Glc-1		4.93, d (7.5)	5.05, d (7.5)	5.18, d (6.7)
Glc-2		3.40-4.00	3.40-4.00	3.40-4.00
Glc-3		3.40-4.00	3.40-4.00	3.40-4.00
Glc-4		3.40-4.00	3.40-4.00	3.40-4.00
Glc-5		3.40-4.00	3.40-4.00	3.40-4.00
Glc-6		3.40-4.00	3.40-4.00	3.40-4.00
Rha-1				5.28, d (1.2)
Rha-2				3.40-4.00
Rha-3				3.40-4.00
Rha-4				3.40-4.00
Rha-5				3.40-4.00
Rha-6				1.33, d (6.2)

^a in DMSO-*d*₆, ^b in CD₃OD



Table 2. ¹³C NMR spectroscopic data of compound 1 - 4

Position	1 ^a	2 ^b	3 ^a	4 ^b
2	146.8	165.4	164.5	166.9
3	135.7	105.1	103.2	105.6
4	175.8	183.8	182.0	184.0
5	160.7	163.2	161.1	163.0
6	98.1	100.3	99.6	102.5
7	163.8	166.1	163.0	164.4
8	93.3	95.1	94.8	97.3
9	156.1	159.4	156.7	159.0
10	102.9	105.5	105.4	107.1
1'	121.9	127.2	121.4	123.5
2'	115.4	118.0	113.6	114.3
3'	145.0	148.6	145.8	147.1
4'	147.6	150.0	150.1	151.2
5'	115.5	114.9	116.0	117.0
6'	119.9	120.0	119.2	120.6
Glc-1		103.3	99.9	99.6
Glc-2		74.8	73.1	78.3
Glc-3		77.5	76.4	79.0
Glc-4		71.3	69.6	71.4
Glc-5		78.5	77.2	79.1
Glc-6		62.3	60.7	62.5
Rha-1				101.0
Rha-2				72.2
Rha-3				72.2
Rha-4				74.0
Rha-5				70.0
Rha-6				18.3

^a in DMSO-*d*₆, ^b in CD₃OD.

Fraction 3 (14.3 g) and fraction 4 (2.5 g) were combined and subjected to Sephadex LH-20 CC (50% MeOH) followed by ODS CC (20%, 30%, 40% MeOH) and silica gel CC (CH₂Cl₂: MeOH: H₂O =8:2:0.1) to afford compound 3 (1610.7 mg) and 4 (7.0 mg).

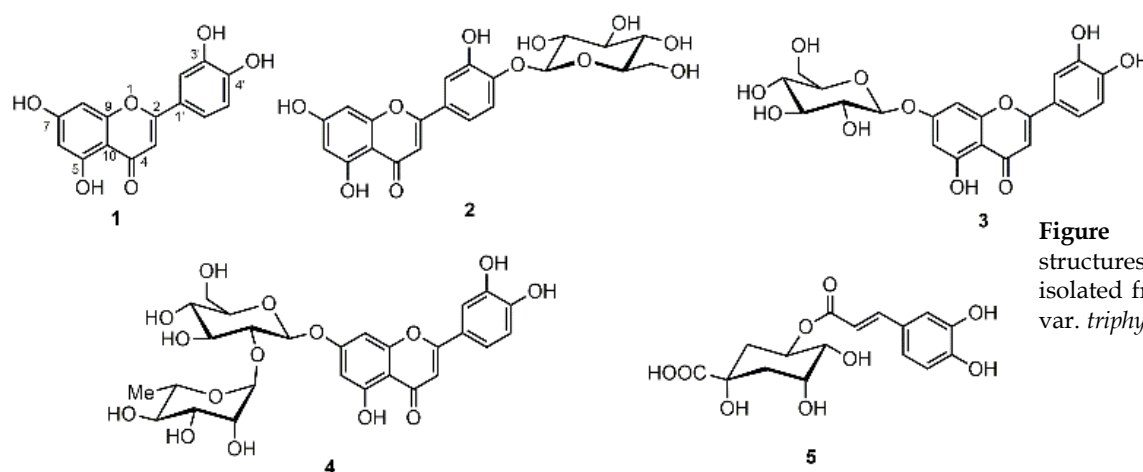


Figure 1. Chemical structures of compounds isolated from *A. triphylla* var. *triphylla*

Measurement of DPPH Free Radical Scavenging Activity

The antioxidant potential was determined using the DPPH free radical scavenging method as described previously [3].

Results and Discussion

The 70% methanol extract of aerial parts of *A. triphylla* var. *triphylla* was subjected to various column chromatographic methods including MCI gel CHP20P, Sephadex LH-20, ODS and silica gel to afford five compounds. The structures of these compounds were elucidated as luteolin (1) [11], luteolin 4'-O-β -glucopyranoside (2) [12], luteolin 7-O-β -glucopyranoside (3) [11, 12], luteolin 7-O-neohesperidoside (4) [13] and chlorogenic acid (5) [14] (**Figure 1**) based on their NMR spectral data and comparison with literature values. Proton and ¹³C NMR data for compounds 1-4 are provided in **Table 1** and **Table 2**, respectively. All of these compounds were isolated for the first time from *A. triphylla* var. *triphylla*. Previously, Hashiba et al. [10] had reported flavonoids including luteolin (1), luteolin 7-O-β -glucopyranoside (3), luteolin 4'7-di-O-β-glucopyranoside, quercetin, quercetin 3-O-β-glucopyranoside from the leaves of *A. triphylla* var. *japonica*. Characterization of similar flavonoids in the aerial parts of *A. triphylla* var. *triphylla* in this study suggests their chemotaxonomic similarity and these compounds can be used as the chemotaxonomic markers for these varieties. Further studies on other varieties of *A. triphylla* or other species of *Adenophora* may help explore their further similarity.

The 70% extract and all isolated compounds were evaluated for their DPPH free radical scavenging activity (**Table 3**). The extract showed weak free

radical scavenging activity with IC₅₀ value of 248.3 µg/mL. Compounds **1**, **3**, **4**, and **5** showed potent free radical scavenging activity with IC₅₀ values of 10.6, 14.3, 20.4 and 19.3 µg/mL, respectively as compared to positive control Trolox (IC₅₀=12.8 µg/mL).

Table 3. IC₅₀ (µg/mL) values of extract and isolated compounds of *A. triphylla* var. *triphylla* for DPPH free radical scavenging activity

Compounds	IC ₅₀ value (µg/mL)
70% Methanol Extract	248.3±2.4
Luteolin (1)	10.6±0.7
Luteolin 4'-O-β-glucopyranoside (2)	62.9±0.9
Luteolin 7-O-β-glucopyranoside (3)	14.3±1.5
Luteolin 7-O-neohesperidoside (4)	20.4±2.4
Chlorogenic acid (5)	19.3±2.1
Trolox (positive control)	12.8± 1.1

In recent years, there is growing attention on the plant-based functional foods. Previously, Kim et al. [5] reported the high phenolic and flavonoid contents and free radical scavenging activities of leaves of *A. triphylla* var. *japonica* but the active compounds were not reported. In our study, the extract of aerial parts of *A. triphylla* var. *triphylla* showed weak activity but the isolated compounds showed strong free radical scavenging activity. Hashiba et al. [10] had also reported similar flavonoids from *A. triphylla* var. *japonica*, hence, luteolin derivatives can be regarded as the active antioxidant compounds in both of these varieties. Flavonoids including luteolin derivatives are well reported as strong antioxidant phytochemicals with various health-promoting and disease prevention activities [15–20]. Further detailed research on these plants may help in the development of functional foods.

Conclusion

Five bioactive phenolic compounds; luteolin (**1**), luteolin 4'-O-β-glucopyranoside (**2**), luteolin 7-O-β-glucopyranoside (**3**), luteolin 7-O-neohesperidoside (**4**) and chlorogenic acid (**5**) were isolated for the first time from the leaves *A. triphylla* var. *triphylla*. Some of the isolated compounds showed potent free radical scavenging activity. The aerial parts of *A. triphylla* var. *triphylla* might be an important plant

source for the development of functional foods. However, detailed research related to pharmacological activity and safety are necessary in future.

Author's Contribution

HPD conceived the idea and designed the study. Both authors contributed to experiments and analysis, and wrote the manuscript.

Competing Interest

No competing interests were disclosed.

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Ethical Approval and Consent

Not applicable.

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