Nepal J Biotechnol. 2020 July;8(1):12-16 Research article DOI: https://doi.org/10.3126/njb.v8i1.30205

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal
Journal Homepage: www.nepjol.info/index.php/njb
ISSN (Online): 2467-9313
ISSN (Print): 2091-1130

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

Kengo Hori[1], Hari Prasad Devkota [1,2]

[1]Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

[2]Program for Leading Graduate Schools, Health life Sciences: Interdisciplinary and Glocal Oriented (HIGO) Program, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

Article history:-Received: 17 May 2020; Revised: 19 Jun 2020; Accepted: 25 Jun 2020; Published online: 31 Jul 2020

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

Corresponding author, email: devkotah@kumamoto-u.ac.jp

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 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

^{1}H- and ^{13}C- NMR spectra were measured on BRUKER AVANCE 600 NMR Spectrometer (Bruker, Billerica, MA, USA) (^{1}H-NMR: 600 Hz and ^{13}C-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 μm, 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 F_{254} (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, H_2O eluate) was subjected on Sephadex LH-20 CC (H_2O) to obtain four subfractions (2-1~2-4). Subfraction 2-3 was subjected on MCI gel CC (H_2O) 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 (H_2O-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 CD_3OD

Table 2. ^{13}C 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 CD_3OD.

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 (CH_2Cl_2: MeOH: H_2O =8:2:0.1) to afford compound **3** (1610.7 mg) and **4** (7.0 mg).

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 ^{13}C 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

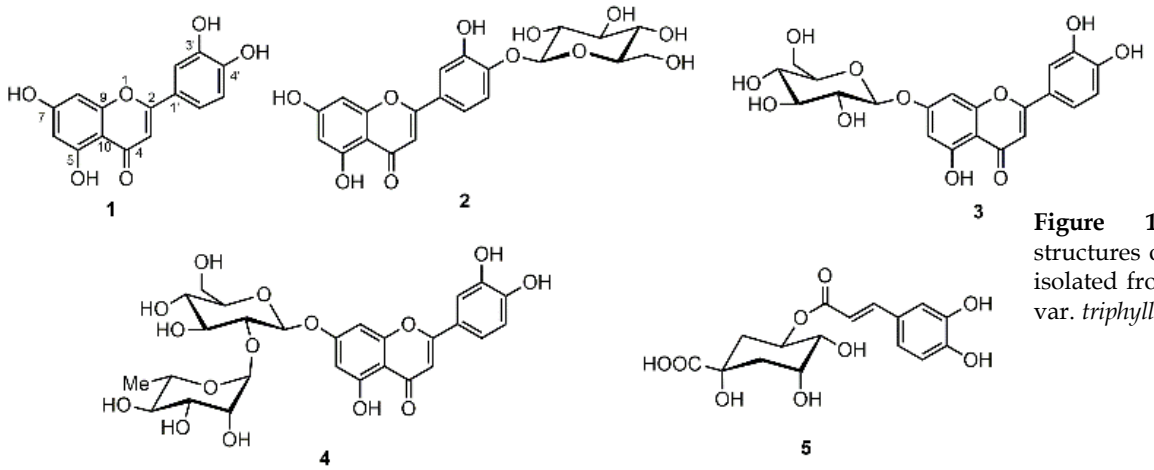

Figure 1. Chemical structures of compounds isolated from *A. triphylla* var. *triphylla*

radical scavenging activity with IC_{50} value of 248.3 µg/mL. Compounds **1**, **3**, **4**, and **5** showed potent free radical scavenging activity with IC_{50} values of 10.6, 14.3, 20.4 and 19.3 µg/mL, respectively as compared to positive control Trolox (IC_{50}=12.8 µg/mL).

Table 3. IC_{50} (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 verities. 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.

Funding

This work was supported partially by Program for Leading Graduate Schools, Health life Sciences: Interdisciplinary and Glocal Oriented (HIGO) Program, MEXT, Japan

Acknowledgments

We are grateful to Ms. Teruo Tanaka of Institute of Resource Development and Analysis, Kumamoto University for the measurement of NMR.

Ethical Approval and Consent

Not applicable.

References

1. Belwal T, Devkota HP, Hassan HA, Ahluwalia S, Ramadan MF, Mocan A, Atanasov AG. Phytopharmacology of Acerola (*Malpighia* spp.) and its potential as functional food. Trends in Food Science & Technology. 2018 Apr 1;74:99-106. https://doi.org/10.1016/j.tifs.2018.01.014
2. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnology advances. 2015 Dec 1;33(8):1582-614. https://doi.org /10.1016/j.biotechadv.2015.08.001
3. Dirar AI, Alsaadi DH, Wada M, Mohamed MA, Watanabe T, Devkota HP. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. South African Journal of Botany. 2019 Jan 1;120:261-7. https://doi.org/ 10.1016/j.sajb.2018.07.003
4. Ohashi H, Monda Y, Murata J, Yonekura K, Kihara H. Wild Flowers of Japan. Tokyo: Heibonsha. 2017.
5. Kim JH, Hong JY, Shin SR, Yoon KY. Comparison of antioxidant activity in wild plant (*Adenophora triphylla*) leaves and roots as a potential source of functional foods. International journal of food sciences and nutrition. 2009 Jan 1;60(sup2):150-61. https://doi.org/10.1080/09637480902956594
6. Suzuki H. Encyclopedia of Traditional Oriental Medicine. Ishiyaku Publishers. 2005.
7. Ahn EK, Oh JS. Lupenone isolated from *Adenophora triphylla* var. japonica extract inhibits adipogenic differentiation through the downregulation of PPARγ in 3T3- L1 cells. Phytotherapy Research. 2013 May;2 7(5):7 61-6. https:// doi.org/10.1002/ptr.4779

8. Watanabe M, Devkota HP, Sugimura K, Watanabe T. A Guidebook of Medicinal Plant park. Kumamoto:School of Pharmacy, Kumamoto University. 2018.
9. Konno C, Saito T, Oshima Y, Hikino H, Kabuto C. Structure of methyl adenophorate and triphyllol, triterpenoids of *Adenophora triphylla* var. *japonica* roots. Planta medica. 1981 Jul;42(07):268-74. https://doi.org/10.1055/s-2007-971639
10. Hashiba K, Iwashina T, Matsumoto S. Variation in the quality and quantity of flavonoids in the leaves of coastal and inland *Campanula punctata*. Biochemical systematics and ecology. 2006 Dec 1;34(12):854-61. https://doi.org/10.1016/j.bse.2006.04.012
11. Harborne JB, Mabry TJ. The flavonoids. Boston, MA: Springer US. 1982. https://doi.org/10.1007/978-1-4899-2915-0
12. Lee MH, Son YK, Han YN. Tissue factor inhibitory flavonoids from the fruits of *Chaenomeles sinensis*. Archives of pharmacal research. 2002 Dec 1;25(6):842. https://doi.org/10.1007/BF02977002
13. Li YL, Li J, Wang NL, Yao XS. Flavonoids and a new polyacetylene from *Bidens parviflora* Willd. Molecules. 2008 Aug;13(8):1931-41. https://doi.org/10.3390/molecules13081931
14. Devkota HP, Joshi KR, Ozasa T, Fukunaga S, Yoshida N, Yahara S. Steroidal glycosides from the fruits, aerial parts and tubers of potato (*Solanum tuberosum*). J. Pharmacognosy & Phytochemistry. 2015;3:252-5.
15. Khan H, Sureda A, Belwal T, Çetinkaya S, Süntar İ, Tejada S, Devkota HP, Ullah H, Aschner M. Polyphenols in the treatment of autoimmune diseases. Autoimmunity reviews. 2019 Jul 1;18(7):647-57. https://doi.org/10.1016/j.autrev.2019.05.001
16. Rice-Evans C. Flavonoids and isoflavones: absorption, metabolism, and bioactivity. Free Radical Biology and Medicine. 2004;7(36):827-8. https://doi.org/10.1016/j.freeradbiomed.2003.12.012
17. Raffa D, Maggio B, Raimondi MV, Plescia F, Daidone G. Recent discoveries of anticancer flavonoids. European journal of medicinal chemistry. 2017 Dec 15 ; 142 :2 13-28. https://doi.org /10.1016/j.ejmech.2017.07.034
18. Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L, Mena P, Del Rio D, Crozier A. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Archives of toxicology. 2014 Oct 1;88(10):1803-53. https://doi.org /10.1007/s00204-014-1330-7
19. López-Lázaro M. Distribution and biological activities of the flavonoid luteolin. Mini reviews in medicinal chemistry. 2009 Jan 1;9(1):31-59. https://doi.org/10.2174/138955709787001712
20. Lin Y, Shi R, Wang X, Shen HM. Luteolin, a flavonoid with potential for cancer prevention and therapy. Current cancer drug targets. 2008 Nov 1;8(7):634-46. https://doi.org/ 10.2174/156800908786241050