



New Flavone Glycoside from *Stellaria pallida* (Dumort.) and Biological Activities

Rasha R. Abd El-Latif*, Ragaa M. A. Mansour

Phytochemistry and Plant Systematics Department, National Research Center, Cairo, Egypt.

*Corresponding author's E-mail: rasharefaat38@yahoo.com

Accepted on: 23-12-2015; Finalized on: 31-01-2016.

ABSTRACT

Aim of this study is isolation and identification of flavonoid compounds from *Stellaria pallida* (Dumort.). A new flavonoid compound was isolated from the 70% methanolic extract of *S. pallida* and identified as apigenin 6-C-(2''-O- α -L-rhamnopyranosyl)- β -D-glucopyranoside-7-O- α -L-rhamnopyranoside (1). In addition, isovitexin 7-O- α -L-rhamnopyranoside (2), apigenin 6,8-di-C- β -D-glucopyranoside (3) and isovitexin (4) were also isolated and identified for the first time from *S. pallida*. The structure elucidation of the isolated compounds were performed by chromatographic, chemical and spectroscopic methods. Antimicrobial activity using agar diffusion assay and antioxidant activity using DPPH assay were also determined for three different extracts of the plant.

Keywords: *Stellaria pallida*, Caryophyllaceae, flavonoids, antimicrobial activity, antioxidant activity.

INTRODUCTION

Plants have always played an important role as a source of medicines. Increased efforts to survey plants as sources of new drugs in recent years have stepped up the pace of the discovery of new bioactive compounds. New trends in natural products research have great importance for the discovery of novel bioactive compounds from plants¹. Flavonoids consist of a large group of polyphenolic compounds having a benzo- γ -pyrone structure and are ubiquitously present in plants². Flavonoids possess diverse biological activities, for instance, antiulcer and anti-inflammatory³, antidibatic⁴, antiviral, antioxidant⁵, cytotoxic and antitumor⁶. Family Caryophyllaceae (pink family or flowering plant family) contains approximately 125 to 306 species. *Stellaria* is a genus of about 120 species of flowering plants of the family Caryophyllaceae. *Stellaria* species were reported to include Phenolic acids, flavonoids, C-glycosyl flavones, terpenoid, saponin, lipids, amino acids, alkaloids and essential oils⁷. A few species of this genus have medicinal values as antiviral, antilipemic, antitumour, antiallergic and antioxidant along with their use in folk medicine for the treatment of inflammations of digestive and respiratory tracts, malaria and cancer⁸. Among these, *S. pallida*, commonly known as lesser chickweed, and it is usually found on coastal sand dunes, in sandy waste places, or on cultivated sandy soils.

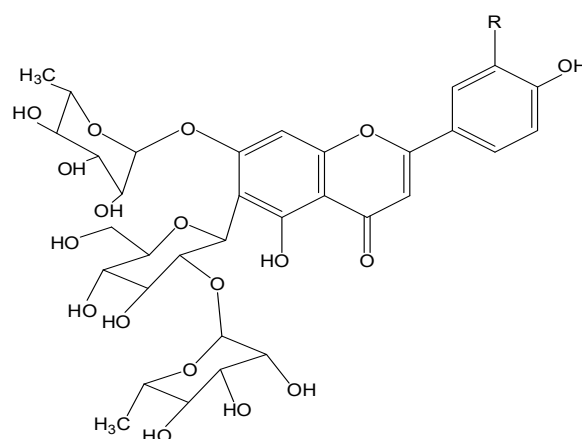
It is reported to be useful in inflammation of the digestive, renal, respiratory and reproductive tracts. This plant is also possesses diuretic, expectorant and anti-asthmatic properties and due to the high nutritional value, it is used as a leaf vegetable, often raw in salads⁹.

The present study of the 70% methanol extract from whole plant of *S. pallida* led to the isolation and structure determination of a new flavone glycoside (1), in addition, three known compounds were identified (Figure 1).

Figure 1 shows the structures of compounds 1 isolated from *Stellaria pallida*



Stellaria pallida



R: H apigenin 6-C-(2''-O- α -L-rhamnopyranosyl)- β -D-glucopyranoside-7-O- α -L-rhamnopyranoside (1)

Figure 1: Structures of compound 1 from *Stellaria pallida*.

MATERIALS AND METHODS

General experimental procedures

UV spectra were recorded on a Shimadzu UV-vis 240 spectrophotometer (Shimadzu, Kyoto, Japan). The 1D NMR data (^1H , ^{13}C) were acquired in DMSO- d_6 on a Jeol EX 270 MHz and 500 MHz spectrometers. ^1H chemical shifts (δ_H) were measured in ppm, relative to TMS and ^{13}C NMR chemical shifts (δ_C) to DMSO- d_6 and converted to TMS scale by adding 39.5. HRESIMS were obtained on a JEOL JMS HX 110 at the Center for Instrumental Analysis, Hokkaido University, Japan. For column chromatography (CC), polyamide S6 (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (Pharmazia, Uppsala, Sweden) were used. Paper chromatographic analysis was carried out on Whatmann No.1 and 3 MM (Whatmann Ltd. Maidstone, Kent, England) using solvent systems (1) H_2O ; (2) 15% AcOH; (3) BAW (n-BuOH: AcOH: H_2O 4:1:5, upper layer); (4) C_6H_6 : n-BuOH: H_2O : pyridine (1: 5: 3: 3, upper layer).

Plant material

Whole plant of *S. pallida* was collected from Abo youssef, Alexandria in May 2013 and authenticated by Prof. Dr. Salwa A. Kawshty, Phytochemical and Plant Systematics Department, NRCA Voucher specimen (no.1105) was deposited in the Herbarium of NRC (CAIRC).

Extraction and isolation

The air-dried powdered whole plant (800 g) of *S. pallida* was exhaustively extracted with 70% aq. MeOH at room temperature. The extracts were concentrated under reduced pressure giving a residue (20 g). A portion of the extract (15 g) was subjected to preparative paper chromatography (PPC) on Whatmann 3MM using H_2O as eluent giving five main fractions (1-5). Paper chromatography was carried out on Whatmann paper No.1. Fractions 3 and 4 were added and subjected to PPC using H_2O as eluent yielding three sub-fractions (A-C). Fraction C was subjected to Sephadex LH-20 CC (MeOH: H_2O 80:10) to yield fraction (C1), which subjected to PPC using H_2O followed by Sephadex column using (MeOH: H_2O 80:10) to yield compound 1. The migration of pure flavonoid was detected in UV light.

Sugar analysis of 1

Compound 1 was subjected to acid hydrolysis (2N HCl, 2h, 100°C) and mild acid hydrolysis (0.1N HCl, 1h, 100°C) using the method of reference^{10,11}. The products being chromatographed with authentic sugar samples and available compounds.

Antimicrobial activity

Antimicrobial efficiency of (ME), (AME) and (EAE) of *S. pallida* were examined against five pathogenic organisms using agar well diffusion method¹². Gram-positive bacteria comprised *Bacillus subtilis* (NCTC 1040) and *Staphylococcus aureus* (NCTC 7447); Gram-negative bacteria comprised *Escherichia coli* (NCTC 10416) and

Pseudomonas aeruginosa (ATCC 10145); and the yeast was *Candida albicans* (IMRU 3669). All the tested organisms were supplied by the Biotechnological Research Center, Al-Azhar University (Cairo, Egypt). 200 μg of plant extracts were applied on 6.0 mm filter paper discs and the discs were placed on the agar surface containing the test organisms. The previously mentioned bacteria were incubated overnight at 37°C for 24 hours in the nutrient media, while the yeast was cultured at 28°C for 4 days on potato dextrose agar (PDA) medium for observation of antimicrobial activity of the plant extracts. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone around the discs in mm against various pathogens and the results (zone of inhibition) were compared with the activity of the standards, viz., Ampicillin (AM), Amoxicillin (AX), Erythromycin (E).

Antioxidant activity

Radical scavenging activity of different extracts of *S. pallida* against the stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep-violet to light-yellow) were measured at 517 nm¹³⁻¹⁵. Radical scavenging activity of *S. pallida* extracts under investigation was measured according to the method of^{16,17}.

RESULTS AND DISCUSSION

Compound 1 was isolated as yellow powder and showed chromatographic properties similar to those reported for flavonoid glycosides¹⁸. The molecular formula of 1, determined as $\text{C}_{33}\text{H}_{40}\text{O}_{21}$, was deduced from the observed $[\text{M}+\text{H}]^+$ at m/z 725.19523 by its HR-SI mass spectroscopy. UV spectral data with diagnostic shift reagents indicated a flavone with substituted – OH group at C-7, while those at C-5 and C-4' are free¹¹. ^1H -NMR spectrum of 1 (Table 1) showed resonances characteristic for apigenin aglycon moiety with downfield-shifted ~ 0.5 nm of H-8, compared to that of apigenin, suggesting 7-O-glycosylation¹⁰, in addition to three anomeric proton signals suggested a triglycoside moiety. The first anomeric proton at δ 4.82, $J=10.0$ Hz was assigned to H-1'' of β -glucopyranosyl linked to apigenin aglycone through C-C bond and its downfield-shifted ~ 0.2 nm suggested the presence of glycosylation on the C-2 hydroxyl of glucose moiety¹⁰. The second anomeric proton at δ 4.99 was assigned to H-1''' of terminal α -rhamnopyranosyl unit attached to C-glycoside¹⁰. The last anomeric proton at δ 5.18 assigned to α -rhamnopyranosyl unit located at C-7, as well as the two doublets at δ 0.90 and δ 1.1 each with $J=6.0$ Hz assigned to CH_3 of terminal α -rhamnopyranosyl unit and α -rhamnopyranosyl unit at position 7, respectively¹⁰. The ^{13}C -NMR spectrum confirmed the presence of 15 carbon signals assigned for the apigenin and 18 signals for the sugar units. The received spectrum also revealed the presence of C-substitution at C-6 of apigenin confirmed



by its downfield-shifted ~ 9.9 ppm with a consequent upfield shifts of related *ortho* and *para* carbons (C-5, C-7 and C-9) by (1.7, 1.4 and 0.5), respectively compared with free apigenin¹⁹.

Table 1: ¹H and ¹³C NMR spectral data for compound **1**

Atom		1	
		$\delta^1\text{H}$ (J in Hz)	$\delta^{13}\text{C}$
Aglycone	2		164.8
	3	6.69 (s)	103.5
	4		181.8
	5		159.8
	6		108.7
	7		162.3
	8	7.03 (s)	94.5
	9		156.8
	10		105.9
	1'		121.4
6-C-Glc	2'	7.92 d (8.9)	129.1
	3'	6.97 d (8.2)	116.6
	4'		162
	5'	6.97 d (8.2)	116.6
	6'	7.92 d (8.9)	129.1
	1''	4.82 d (10.0)	71.9
2''-O-Rha	2''		79.9
	3''		78.8
	4''		70.5
	5''		80.8
	6''		60.4
	1'''	4.99 d (1.5)	100.3
7-O-Rha	2'''		70.5
	3'''		70.8
	4'''		71.9
	5'''		68.1
	6'''	0.90 d (6.0)	17.6
	1''''	5.18 (br.s)	98.9
7-O-Rha	2''''		70.3
	3''''		70.6
	4''''		71.8
	5''''		69.8
	6''''	1.1 d (6.0)	17.5

Note: Measured in DMSO-*d*₆

Additionally, the anomeric carbon signal of glucose shifted upfield to 71.9 confirmed the C-glycosylation of the flavonoid aglycone while the C-2'' of glucose was

downfield-shifted ~ 9 ppm at δ 79.9 due to rhamnosylation of glucose at C-2''^{20,21} with a consequent upfield shift of the two adjacent carbons C-1'' (~ 1.5 ppm) and C-3'' (~ 0.2 ppm).

This large rhamnosylation shift at C-2'' of glucose confirmed the presence of additional 7-O-glycosylation²¹.

The upfield-shifted of C-7 signal by ~ 1.4 ppm compared to that of apigenin with downfield shifted of C-10 ~ 2.2 ppm confirmed the glycosylation on the 7-OH²¹.

The anomeric carbon atom of rhamnose unit at position 7 of the aglycone was resonated at δ 98.9^{19,22}.

The HMBC experiment showed long-range correlations between the following proton and carbon pairs: H-1'' (δ 4.82) and C-6 (108.7), H-1''' (δ 4.99) and C-2'' (79.9), H-1'''' (δ 5.18) and C-7 (162.3) which confirmed this structure.

Complete acid hydrolysis of **1** yielded rhamnose as the sugar moiety and an intermediate which has CO-PC properties similar to compound **4** (isovitexin).

On mild acid hydrolysis, isovitexin 7-O-rhamnopyranoside was identified as an intermediate by direct PC comparison with compound **2**.

On the basis of the foregoing discussion the structure of **1** has been established as apigenin 6-C-(2''-O- α -L-rhamnopyranosyl)- β -D-glucopyranoside-7-O- α -L-rhamnopyranoside.

Table 1. ¹H and ¹³C NMR spectral data of compound **1**.

Spectral data

Apigenin 6-C-(2''-O- α -L-rhamnopyranosyl)- β -D-glucopyranoside-7-O- α -L-rhamnopyranoside (**1**).

Yellow powder; *R_f* values: 0.25 (BAW), 0.68 (15% AcOH), 0.85 (H₂O); ¹H NMR (500 MHz, DMSO-*d*₆) δ : 6.69 (1H, s, H-3), 7.03 (1H, s, H-8), 7.92 (2H, d, *J* = 8.9 Hz, H-2', H-6') 6.97 (2H, d, *J* = 8.2 Hz, H-3', H-5'), 4.82 (1H, d, *J* = 10.0 Hz, H-1''), 4.99 (1H, d, *J* = 1.5 Hz, H-1'''), 0.90 (3H, d, *J* = 6.0 CH₃ Hz, H-6'''), 5.18 (1H, br.s, H-1''''), 1.1 (3H, d, *J* = 6.0 Hz, CH₃, H-6''''), ¹³C NMR (125 MHz, DMSO-*d*₆) δ : 164.8 (C-2), 103.5 (C-3), 181.8 (C-4), 159.8 (C-5), 108.7 (C-6), 162.3 (C-7), 94.5 (C-8), 156.8 (C-9), 105.9 (C-10), 121.4 (C-1'), 129.1 (C-2'), 116.6 (C-3'), 162.0 (C-4'), 116.6 (C-5'), 129.1 (C-6'), 71.9 (C-1''), 79.9 (C-2''), 78.8 (C-3''), 70.5 (C-4''), 80.8 (C-5''), 60.4 (C-6''), 100.3 (C-1'''), 70.5 (C-2'''), 70.8 (C-3'''), 71.9 (C-4'''), 68.1 (C-5'''), 17.6 (C-6'''), 98.9 (C-1''''), 70.3 (C-2''''), 70.6 (C-3''''), 71.8 (C-4''''), 69.8 (C-5''''), 17.5 (C-6''''), HR-ESIMS (+) *m/z* 725.19523 [M+H]⁺ (calculated for C₃₃H₄₀O₂₁, 725.19345).



Biological activity

Antimicrobial activity

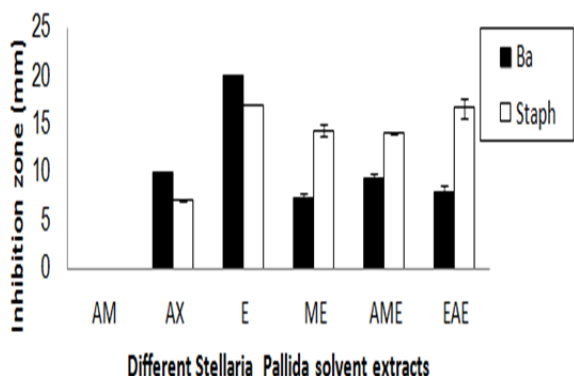


Figure 2(a): Antimicrobial activity of *Stellaria pallida* extracts on *Bacillus subtilis* and *Staphylococcus aureus*

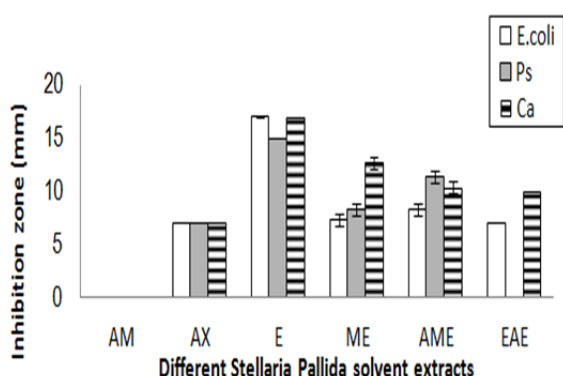


Figure 2(b): Antimicrobial activity of *Stellaria pallida* extracts on *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*

The antimicrobial activity of tested extracts of *S. pallida* (Table 2; Figure 2a and Figure 2b) showed that Erythromycin (E) was highly active against all tested pathogens while Amoxicillin showed a weak activity to *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and a moderate activity to *Bacillus subtilis*. It was evident that all pathogens were exhibited a highly resistance against Ampicillin. Also, the results showed that, EAE exhibited higher antimicrobial activity against the multidrug resistant *Staphylococcus aureus* (16.0±1.0 mm) compared to the standard antibiotic Amoxicillin (7.0 ± 0.0) while it was inactive against *Pseudomonas aeruginosa*. AME showed more antimicrobial activity than ME against all the tested bacteria except *Staphylococcus aureus*. On the other hand, ME showed high activity against *Candida albicans* (12.7±0.58) compared to the standard antibiotic Amoxicillin (7.0 ± 0.0) but moderate activities were obtained by AME and EAE (10.3±0.58 & 10.0±0.0), respectively.

Antioxidant activity

As shown in (Table 3) tested extracts of *S. pallida* exhibited weak antioxidant activities on scavenging DPPH free radicals at different concentration.

Table 2: The antimicrobial screening of different extracts of *Stellaria pallida* (200 µg/disc) using disc diffusion assay.

Test organism		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Bacterial code						
Standard antibiotics	AM	NI	NI	NI	NI	NI
	AX	10.0±0	7.0±0	7.0±0	7.0±0	7.0±0
	E	20.0±0	17.0±0	17.0±0	15.0±0	17.0±0
ME		7.3±0.58	14.3±0.58	7.3±0.58	8.3±0.58	12.7±0.58
AME		9.3±0.58	14±0.0	8.3±0.58	11.3±0.58	10.3±0.58
EAE		8.3±0.58	16.0±1.0	7.0±0.0	NI	10.0±0.0

AM, Ampicillin; AX, Amoxicillin and E, Erythromycin NI, no inhibition; ME, Methanolic extract; AME Aqueous methanol extract; EAE, Ethyl acetate extract.

Table 3: Antioxidant activity of different extracts of *Stellaria pallida*

µl of the sample added to 3ml DPPH	% Inhibition				
	Conc. in µg/ml	Ascorbic acid	ME	AME	EAE
19	15.8	98.1	20.0	16.0	21.1
38	31.6	98.2	24.2	23.7	22.0
77	64.1	98.3	26.1	26.3	22.7

(ME), methanolic extract; (AME), 70% aqueous methanolic extract; (EAE) ethyl acetate extract.

CONCLUSION

In the present study, to the best of our knowledge, compounds **1** had not been isolated from plants before. In addition three known compounds were isolated for the first time from *S. pallida*. Biological investigation of different extracts of *S. pallida* promising significant antimicrobial activity and weak antioxidant activity.

Acknowledgement: This research was funded by National Research Center, Egypt. We are grateful for the Center for Instrumental Analysis, Hokkaido University, Japan for HR-ESIMS measurement.

REFERENCES

1. Miller JS, Yuan L. Drug Discovery and Traditional Chinese Medicine, edited by Yuan, L., Science, Regulation and Globalization, Kluwer Academic Publishers, Norwell, MA. 2001, 33-42.
2. Buer CS, Imin N, Djordjevic MA. Flavonoids: New roles for old molecules. *Integr J. Plant Biol.* 52, 2010, 98–111.
3. Saxena M, Saxena J, Pradhan A. Flavonoids and phenolic acids as antioxidants in plants and human health, *Int. J. Pharm. Sci. Rev. Res.* 16(2), 2012, 28, 130-134.
4. Vessal M, Hemmati M, Vasei M. Antidiabetic effects of quercetin in streptozotocin induced diabetic rats, *Comp Biochem Physiol.* 135, 2003, 357-364.
5. Ghasemzadeh A, Jaafar HZE. Antioxidant potential and anticancer activity of young ginger (*Zingiber officinale* Roscoe) grown under different CO₂ concentration, *Jour Med Plant Res.* 5, 2011, 3247-3255.
6. Paliwal S, Sundaram J, Mitragotri S. Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound. *Br Jour Cancer.* 92, 2005, 499-502.
7. Anupam S, Disha A. Phytochemical and Pharmacological Potential of Genus *Stellaria*. *J Pharm Res.* 5(7), 2012, 3591-3596.
8. Calzada F. Additional antiprotozoal constituents from *Cuphea pinetorum*, a plant used in Mayan traditional medicine to treat diarrhoea. *Phytother Res.* 19, 2005, 725–727.
9. Kirtikar KR, Basu BD. *Indian Medicinal Plants, Volume II.* International Book Distributors, Dehradun, India. 2006, 939.
10. Markham KR, Geiger H. ¹H NMR spectroscopy of flavonoids and their glycosides in DMSO-d₆. In: Harborne JB. editor. *The flavonoids, Advances in Research Since 1986.* London: Chapman & Hall. 444, 1994, 464–469.
11. Mabry TJ, Markham KR, Thomas MB. *The systematic identification of flavonoids.* New York, NY: Springer Verlag. 1970, 35-109.
12. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well method. *Acta Biologiae et Medecine Experimentalis.* 15, 1990, 113–115.
13. Blois MS. Antioxidant determination by the use of stable free radical, *Nature.* 181, 1958, 1119-1120.
14. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 85, 2004, 231–237.
15. Ratty AK, Sunamoto J, Das NP. Interaction of flavonoids with 2,2-diphenyl-1-picrylhydrazyl free radical, liposomal membranes and soybean lipoxygenase-1, *BiochemPharmacol.* 37, 1988, 989-995.
16. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *SJST.* 26(2), 2004, 211-219.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und –Technologie.* 28, 1995, 25–30.
18. Harborne JB. *Phytochemical methods.* London: Chapman & Hall, 1984.
19. Harborne JB, Mabry TJ. *The flavonoids - Advances in research.* London: Chapman & Hall, 1982.
20. Agrawal PK, Bansal MC. Flavonoid glycosides. In: Agrawal PK., editor. *Carbon-13 NMR of flavonoids,* New York: Elsevier. 1989, 283-363.
21. Markham KR, Chari VM. Carbon-13 NMR spectroscopy of flavonoids. In: Harborne JB, Mabry TJ, Mabry H. editors. *The flavonoids.* London: Chapman & Hall. 39, 43, 45, 50, 1975, 866-915.
22. Mabry TJ, Markham KR, Chari VM. Carbon-13 NMR spectroscopy of the flavonoids. In: Harborne JB, Mabry TJ. editors. *The flavonoids: advances in Research,* London: Chapman & Hall. 1982, 19-132.

Source of Support: Nil, **Conflict of Interest:** None.

