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Antibacterial and Antioxidant Activities of Two New Kaempferol Glycosides Isolated from *Solenostemma argel* Stem Extract

R.E. Shafek, N.H. Shafik and H.N. Michael
Department of Chemistry of Tanning Materials and Leather Technology,
National Research Center, Dokki, Cairo, Egypt

Abstract: Chemical constituents, antibacterial and antioxidant activities of the 70% aqueous ethanolic extract of *Solenostemma argel* stems (AE) were investigated. Its chromatographic, chemical and spectral analysis revealed the presence of two new natural kaempferol glycosides namely: kaempferol 3-O-α-D-glucopyranosyl (1→2) β-D-xylopyranoside and kaempferol 3-O-α-L-arabinopyranosyl (1→2) β-D-galactopyranoside, together with the following known compounds:- kaempferol 3-O-α-L-arabinoside; 3-O-β-D-xyloside; 7-O-α-L-rhamnoside; 7-O- α-L-arabinoside; 3,7-di-O- β-D-glucoside; 3,7-di-O- α-L-rhamnoside and kaempferol. The two new natural kaempferol glycosides and its extract (AE) were active against both Gram-positive and Gram-negative bacteria to some extent. The results presented here for the antioxidant activity, using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free-radical assay, indicated a high radical scavenging activity of (AE) and a moderate activity for the isolated new compounds, so consequently we can say that they might be a valuable natural antioxidant source.

Key words: *Solenostemma argel*, Apocynaceae, new kaempferol glycosides, antibacterial, antioxidant activities

INTRODUCTION

The family Asclepiadaceae treated as a subfamily (Asclepiadoideae) in the Apocynaceae (Endress and Bruyns, 2000) includes 348 genera, with about 2,900 species. They are mainly located in the tropics to subtropics, especially in Africa and South America. This family is a rich source of indoline, alkaloids, steroidal alkaloids, pregnanes and their glycosides which have been shown to possess antitumour and anticancer activities and they are closely resemble cardiac glycosides (Si-Qi *et al*., 1993; Deepak *et al*., 1989; Srivastava *et al*., 1993). Its other chemical constituents are the cyanogenetic glycosides, saponins, tannins, coumarins, flavonoids, phenolic acids and triterpenoids (Aeri, 2007). *Solenostemma argel* (Del.) Hayne (Apocynaceae) is a desert plant widely distributed in Egypt (Wadi Allaqui) with the common name hargel (El-Hadidi and Fayed, 1995) and in Sudan which is its richest source (El-Ghazali, 1997). It is the most important one from the many Egyptian plants which are known to be of potential medicinal value in herbal medicine. *S. argel* is used for the treatment of diabetes and jaundice, its leaves possess purgative properties which may be due to the latex present in the stem parts (El-Kamali and Khalid, 1996). Also, an extract from the leaves of this plant showed fungitoxic activity (Abd El-Hady and Ouf, 1993). It is used for the treatment of some diseases of liver and kidney and for allergies and as incense in the treatment of measles and sometimes crushed and used as remedy for suppurating wounds. It is an effective remedy for bronchitis and is used to treat neuralgia and sciatica. Its leaves are infused to treat gastro-intestinal cramps, stomach-ache, colic, cold and urinary tract infections and is effective as an anti-syphilitic if used for prolonged periods of 40-80 days (Boulos, 1983; Hammiche and Maiza, 2006). The native Sudanese have commonly used *S. argel* to suppress stomach pain, pains due to childbirth and loss of appetite. It proved that its crude aqueous extracts possessed larvicidal activity against mosquito larvae (El-Kamali, 2001).

From the previous phytochemical studies, it was found that its leaves were characterized by high carbohydrates, low crude fibre, protein, crude oil, ash and high potassium, calcium, magnesium, sodium and low copper, ferrous, manganese, lead and contained phytic acid and tannin (Murwan *et al*., 2010). Kamel (2003) proved that it contained acylated phenolic glycosides. Another study in its chloroform extract showed that it had an anti-inflammatory activity and it contained a new pregnene glycoside (solenoside A) and a known one besides kaempferol 3-O-glucoside and 3-O-rutinoside

Corresponding Author: R.E. Shafek, Department of Chemistry of Tanning Materials and Leather Technology, National Research Center, Dokki, Cairo, Egypt

(Innocenti *et al*., 2005). Also it was found that its aerial parts contained two monoterpene glucosides, a pregnane glucoside, benzyl alcohol O-β-apiofuranosyl (1→6) β-glucopyranoside, 2-phenyl-ethyl O-α-arabinopyranosyl (1→6) β-glucopyranoside, astragalin and kaempferol 3-O-neohesperidose (Kamel *et al*., 2000). In a previous studies, (Tharib *et al*., 1986; Abd El-Hady *et al*., 1994) four compounds have been isolated from its stem but not identified and have been subjected to a preliminary screen for potential antimicrobial activity. So, in this study we attempt to identify the chemical structures of the natural polyphenolic compounds present in the 70% aqueous ethanolic extract of *S. argel* stems (AE), where two new natural kaempferol glycosides namely:- kaempferol 3-O-β-D-glucopyranosyl (1→2) β-D-xylopyranoside (S_1) and kaempferol 3-O-α-L-arabinopyranosyl (1→2) β-D-galactopyranoside (S_3), were isolated together with the following known compounds:- kaempferol 3,7-di-O-α-L-rhamnoside (S_2), 3,7-di-O-β-D-glucoside (S_4), 3-O-α-L-arabinoside (S_5); 3-O-β-D-xyloside (S)$_6$ 7-O-α-L-rhamnoside (S_7); 7-O-α-L-arabinoside (S_8) and kaempferol (S_9). All these pure natural compounds were isolated and purified through many chromatographic techniques and their chemical structures were identified by chemical analysis and spectroscopic methods. In addition, the antimicrobial effect of its extract (AE) and the two new natural kaempferol glycosides (S_1) and (S_3) besides kaempferol (S_9) have been studied on six different microorganisms (bacteria). Also, their antioxidant activity was investigated by DPPH, as stable radicals, method.

MATERIALS AND METHODS

Plant material: *Solenostemma argel* were collected from Aswan at March 2009. Authentication was performed by Dr. M. El-Gebali, former researcher of botany at the National Research Centre. A voucher specimen is deposited in the National Research Centre Herbarium (CAIRC) for future references.

Chemicals and instruments: ^{1}H (200 MHz) and ^{13}C (50 MHz)-NMR spectra were recorded on Varian GEMINT-200 spectrometer; the chemical shifts were recorded in DMSOd_6 and are given in ppm values. UV spectra were measured on Shimadzu spectrophotometer model UV-240; column chromatography (CC): was performed using polyamide 6S (Riedel, De Haën, Germany) and Sephadex LH-20 (Pharmacia); paper chromatography (PC) and preparative paper chromatography (PPC): were carried out on Whatman No.1 and 3MM, respectively, using solvent systems (1) BAW (n-BuOH: AcOH: H_2O, 6: 1: 2); (2) H_2O; (3) 15 % AcOH (AcOH: H_2O, 15: 85) and (4) Forestal (AcOH: conc. HCl: H_2O; 30:3:10) and were visualized under UV light using aluminium chloride ($AlCl_3$) and Naturstoff reagent A (Diphenyl boric acid- β-amino ethyl ester (NA) as spraying reagents. Aniline hydrogen phthalate was used as specific reagent for sugar analysis.

Extraction, fractionation and isolation: The powdered air-dried stems of *S. argel* (500 g) were exhaustively extracted with 70% ethanol at room temperature. The two dimensional paper chromatography (TDPC) of (AE) using the solvent systems (1) and (3), respectively, revealed the presence of many components of flavonoid and phenolic nature.

(AE) was concentrated under vacuum then chromatographed on a polyamide 6S column; elution being performed with water followed by water-ethanol mixtures to give six fractions (F_{1-6}). The two new compounds (S_1, 83 mg) and (S_3, 76 mg) were isolated from F_2 (2.8 g) (eluted with 20% ethanol) and F_3 (2.3 g) (eluted with 40% ethanol), respectively, as well as (S_2, 54 mg) from F_2 and (S_4, 34 mg) from F_3, by PPC using Whatman 3MM papers and H_2O for irrigation and were purified applying Sephadex LH-20 column eluted with aqueous ethanol. While F_4 (1.7 g) (eluted with 60% ethanol) and F_5 (1.2 g) (eluted with 80% ethanol) were further fractionated using Sephadex LH-20 column and aqueous methanol as developing system to afford (S_5, 43 mg) and (S_6, 37 mg) from F_4, (S_7, 29 mg) and (S_8, 31 mg) from F_5. The last compound (S_9, 47 mg) was isolated from F_6 (0.84 mg) by PPC with BAW as eluent.

Antibacterial activity: The antibacterial effect of the extract (AE) and the two new natural kaempferol glycosides (S_1 and S_3) besides kaempferol (S_9) was studied by using the agar diffusion method (Maruzzella and Freundlich, 1959) on six different strains of bacteria i.e., two gram-ve namely *Escherichia coli* and *Brodetella brochiseptica*, four gram+ve namely *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus pumilus* and *Bacillus subtilis*. Bacterial test organism were cultured on nutrient agar slant media (Jacobs and Gerstein, 1960) and incubated at 37°C for 24 h. The antimicrobial assay has been carried out by cup-plate diffusion technique. The clear zone of inhibition around the cup was measured in millimeters (diameter of cup 10 mm). Ten mg of each tested sample were dissolved in 2 mL diethyl sulfoxide and 8 mL distilled water to obtain 10 mL solvent, 0.1 mL of each tested against the above test organism. The plates were incubated at 37°C for 24 h. The antibacterial activity was measured as growth zone inhibition of microorganism. All the tests run in triplicate for each sample and the mean inhibition zones were given to assess the activity.

Evaluation of antioxidant activity: The potential antioxidant activity of the extract (AE), (S_1), (S_3) and (S_9) was assessed on the basis of the scavenging activity of the stable (DPPH) free radical (Gamez *et al*., 1998). Weighed samples were dissolved in distilled DMSO and 10 μL of each or an ascorbic acid aqueous standard (from 0-100 μg mL^{-1}), was added to 90 μL of 100 μM DPPH (Sigma, St. Louis, MO) in ethanol solution in a 96-well micro-titer plate. After incubation in the dark at 37°C for 30 min, the decrease in absorbance of each solution was measured at 515 nm using an ELISA micro-plate reader (Blo Rad, model 550). Absorbance of a blank containing an equal volume of DMSO and DPPH solution was prepared and measured as well. Percentage DPPH radical scavenging activity = $1 - [A_{sample}/A_{control}] \times 100$, where A_{sample} and $A_{control}$ are absorbance of sample and control, respectively. The concentration of sample required to scavenge 50% of DPPH (IC_{50}) was determined. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. The experiment was carried out in triplicate.

Kaempferol 3-O-β-D–glucopyranosyl (1→2) β-D-xylopyranoside (S_1): R_f-values x (100): 58 (BAW), 78 (15% AcOH), 45 (H_2O) ; UV spectral data (λ_{max} nm) MeOH : 262, 320sh, 344 +NaOMe: 272, 322, 398; +NaOAc (b): 274, 302sh, 363; NaOAc/H_3BO_3: 265, 300sh, 348; +$AlCl_3$: 276, 302sh, 344sh, 399; $AlCl_3$/HCl: 277, 304sh, 343, 397; ^{1}H NMR (DMSO-d_6): aglycone moiety: δ (ppm) 8.05 (d, J = 8.0 Hz, H-2`, 6`); 6.91 (d, J = 8.0 Hz, H-3`,5`); 6.44 (d, J = 2.5 Hz, H-8); 6.20 (d, J = 2.5 Hz, H-6). Sugar moiety: δ (ppm) 5.37 (d, J = 8.0 Hz, H-1`` of xylose); 5.70 (d, J = 7.5 Hz, H-1``` of glucose); 3.0-3.88 (m, rest of sugar protons). ^{13}C NMR (DMSO-d_6); aglycone moiety: δ (ppm) 156.4 (C-2); 133.2 (C-3); 177.6 (C-4); 161.2 (C-5); 98.7 (C-6); 164.1 (C-7); 93.5 (C-8); 156.3 (C-9); 104.1 (C-10); 121.2 (C-1`); 130.5 (C-2`); 115.5 (C-3`); 159.8 (C-4`); 115.2 (C-5`); 130.8 (C-6`). Sugar moieties: δ (ppm) 99.6 (C-1``); 76.5 (C-2``); 75.9 (C-3``); 69.2 (C-4``); 65.6 (C-5``); 103.5 (C-1```); 74.2 (C-2```); 76.5 (C-3```); 69.7 (C-4```); 76.5 (C-5```); 60.7 (C-6```).

Kaempferol 3-O-α-L-arabinopyranosyl (1→2) β-D-galactopyranoside (S_3): R_f-values×(100): 56 (BAW), 74 (15% AcOH), 43 (H_2O) ; UV spectral data (λ_{max} nm) MeOH : 265, 325sh, 347 +NaOMe: 270, 324, 397; +NaOAc (b): 275, 306sh, 365; NaOAc/H_3BO_3: 265, 302sh, 350; +$AlCl_3$: 277, 305sh, 345sh, 399; $AlCl_3$/HCl: 275, 308sh, 340, 399; ^{1}H NMR (DMSO-d_6): aglycone moiety: δ (ppm) 8.1 (d, J = 8.0 Hz, H-2`, 6`); 6.92 (d, J = 8.0 Hz, H-3`,5`); 6.46 (d, J = 2.5 Hz, H-8); 6.23 (d, J = 2.5 Hz, H-6). Sugar moiety: δ (ppm) 5.70 (d, J = 7.5 Hz, H-1`` of galactose); 5.33 (d, J = 4.0 Hz, H-1``` of arabinose); 3.2-4.1 (m, rest of sugar protons). ^{13}C NMR (DMSO-d_6); aglycone moiety: δ (ppm) 156.3(C-2); 133.1 (C-3); 177.3 (C-4); 161.0 (C-5); 98.7 (C-6); 164.3 (C-7); 93.6 (C-8); 156.3 (C-9); 103.8 (C-10); 120.7 (C-1`); 130.8 (C-2`); 115.0 (C-3`); 159.9 (C-4`); 115.0 (C-5`); 130.8 (C-6`). Sugar moieties: δ (ppm) 100.3 (C-1``); 78.7 (C-2``); 74.1 (C-3``); 69.2 (C-4``); 74.5 C-5``); 61.3 (C-6``); 102.6 (C-1```); 71.5 (C-2```); 70.3 (C-3```); 66.8 (C-4```); 64.8 (C-5```).

RESULTS AND DISCUSSION

The present study deals with the investigation of the polyphenolic constituents of the 70% aqueous ethanolic extract of *Solenostemma argel* stem (AE), where nine flavonoid compounds (S_1-S_9) including two new ones (S_1) and (S_3), were isolated in pure form after successive chromatographic separations using polyamide 6S column for fractionation and PPC or Sephadex LH-20 column for isolation or purification.

The glycoside (S_1) appeared on paper chromatograms as brown spot under UV light changed to yellow colour when exposed to ammonia vapours. R_f-values and colour reactions of (S_1) and its UV spectral data showed its accordance with 3-substituted kaempferol (Markham, 1982) where the addition of NaOMe led to a bathochromic shift (54 nm) in band I without decrease in intensity with the appearance of a shoulder at (320 nm) i.e., position 3 was occupied while position 7 was free. This was confirmed by the bathochromic shift (12 nm) produced on the addition of NaOAc. The presence of a free 5 hydroxyl group and a substituted one at position 3 was evidenced by the bathochromic shift (52 nm) produced in band I by the addition of $AlCl_3$/HCl. Complete acid hydrolysis of (S_1) gave rise to the aglycone kaempferol and the sugars xylose and glucose which were identified by Co-PC with authentic markers. Its partial acid hydrolysis gave rise to an intermediate which was identified as kaempferol 3-O-β-D-xylopyranoside through complete acid hydrolysis and Co-PC. Further confirmation of its structure was achieved through ^{1}H-NMR spectroscopy which ensured the structure of (S_1) to be kaempferol 3-O-β-D-glucopyranosyl(1→2) β-D-xylopyranoside through the appearance of the chemical shifts δ (ppm) at 8.05 (d, J = 8.0 Hz, H-2`, 6`); 6.91 (d, J = 8.0 Hz, H-3`,5`); 6.44 (d, J = 2.5 Hz, H-8); 6.20 (d, J = 2.5 Hz, H-6) and the presence of a doublet signal at δ 5.37 ppm with coupling constant 8.0 Hz which is of the anomeric proton of β-D-xylose attached directly to position -3 while the presence of another doublet signal at δ 4.70 ppm with coupling constant 7.5 Hz which of the anomeric proton of the terminal sugar β-D-glucose attached to position 2 of xylose. Finally the structure of (S_1) was confirmed through ^{13}C NMR spectrum, where the recorded

C-signals of the aglycone possess chemical shifts similar to those reported for kaempferol (Agrawal, 1989; Harborne, 1993) besides the appearance of signals of the C-1``` of β-D-glucose at 103.5 ppm and C-2`` of β-D-xylose at 76.5 ppm downfield than the unsubstituted one at 100.2 and 75.2, respectively.

The isolated, purified compound (S_3) was found to possess R_f-values, colour reaction and UV spectral data similar to those of kaempferol 3-O-diglycoside (Markham, 1982). Complete acid hydrolysis of (S_3) yielded the aglycone moiety kaempferol and the two sugars arabinose and galactose which were identified by Co-PC with authentic markers. Partial acid hydrolysis of the component (S_3) gave an intermediate which was identified as kaempferol 3-O-β-D-galactopyranoside through Co-PC and UV spectral data. Further confirmation of (S_3) as kaempferol 3-O-α-L-arabinopyranosyl(1→2) β-D-galactopyranoside was achieved through ^{1}H-NMR spectroscopy which gave data identical to those of kaempferol with a substitution at position 3 with a sugar where a doublet signal appears at δ 5.70 ppm (J = 7.5 Hz) of the β-D-galactose anomeric proton attached directly to position 3 of kaempferol while that appeared at δ 4.63 ppm (J = 4.0 Hz) of the anomeric proton of the terminal sugar α-L-arabinose. This proposed structure was confirmed by ^{13}C NMR spectrum, where the recorded C-signals of the aglycone possess chemical shifts similar to those reported for kaempferol (Agrawal, 1989; Harborne, 1993) besides the appearance of signals of the C-2`` of β-D-galactose at δ 78.7 ppm and C-1``` of α-L-arabinose at 102.6 ppm downfield than the unsubstituted one at 72.4 and 99.2, respectively. So from the above data the structure of (S_3) was finally confirmed as kaempferol 3-O-α-L-arabinopyranosyl(1→2) β-D-galactopyranoside.

Antibacterial activity: Data of the antibacterial tested compounds are shown in Table 1 which revealed that the aglycone kaempferol (S_9) was more effective as antibacterial substance than the extract (AE) to some extent, (S_1) and (S_3) had almost the same moderate activity.

DPPH radical scavenging activity: The antioxidant activity of flavonoids, i.e., their ability to scavenge reactive oxygen species (ROS) like hydroxyl radicals ($OH^•$), superoxide anions ($O_2^{•-}$) and lipid peroxy radicals may be the most important function of flavonoids in the body because oxidative stress in biological systems have been proven to be responsible for several damages and diseases. Thus, the data of the antioxidant activity of the aqueous ethanolic extract (AE) of *Solenostemma argel* stem and the two new natural kaempferol glycosides (S_1) and (S_3), in addition to kaempferol (S_9) were recorded in Table 2.

From the data of Table 2 we can concluded that compounds (S_1) and (S_3) exhibited moderate activities as free-radical scavengers in the DPPH assay while (S_9) and

Table 1: Data of the antibacterial activity of tested samples

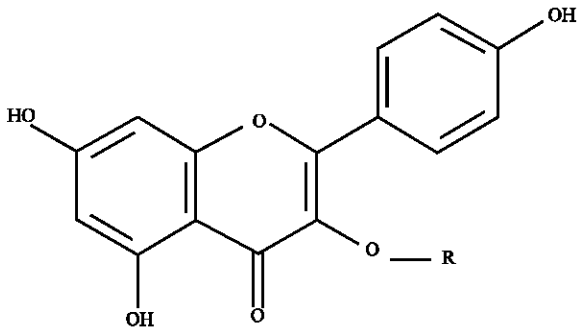
	Tested bacteria					
	Gram-negative bacteria		Gram-positive bacteria			
Samples	Es.	Br.	St.	Sa.	Ba.P.	Ba.S.
S_1	++	+	++	++	++	++
S_3	+	+	+	++	++	++
S_9	++	+++	+++	++	+++	+++
AE	+++	++	+	++	+++	++

Es.: *Escherichia coli,* Br.: *Brodetella brochiseptica,* St.: *Staphylococcus aureus*, Sa.: *Sarcina lutea,* Ba.P.: *Bacillus pumilus,* Ba.S.: *Bacillus subtilis*, +: Active with diameter of inhibition zone of 5-12 mm, ++: Inhibition zone 13-20 mm, +++: Inhibition zone more than 20 mm

Table 2: Data of the antioxidant activity of tested samples

Tested samples	S_1	S_3	S_9	AE	L-ascorbic acid
DPPH (IC_{50}) μg mL^{-1}	78.60±1.52	74.32±1.65	37.08±0.67	52.13±0.75	13.76±0.32

Values represent the Mean±SD and mean of three replicates



(S_1) R =β-D-glucopyranosyl (1→2) β-D-xylopyranoside, (S_3) R = α-L-arabinopyranosyl (1→2) β-D-galactopyranoside, (S_9) R = H

(AE) had strong activity but still less than that of the positive control (L-ascorbic acid).

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