Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(6): 860-865, Dez. 2010

Received 27 Oct 2009; Accepted 29 Apr 2010; Available online 26 Nov 2010.

Article

Chemical constituents from *Astragalus annularis* Forssk. and *A. trimestris* L., Fabaceae

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RESUMO: "Constituintes químicos de *Astragalus annularis* Forssk. and *A. trimestris* L., Fabaceae." A investigação química das partes aéreas de *Astragalus annularis* Forssk. e *A. trimestris* L., Fabaceae, do Egito, resultou no isolamento de oito compostos, pela primeira vez nestas duas espécies. Estes compostos foram identificados por métodos químicos, espectroscopia de RMN, espectroscopia UV, bem como análise de EM. A atividade antimicrobiana dos compostos isolados foi testada contra *Staphylococcus aureus, Escherichia coli* e *Candida albicans.*

Unitermos: Fabaceae, *Astragalus,* saponinas, flavonoids, atividade antimicrobiana.

ABSTRACT: The phytochemical investigation of the aerial parts of *Astragalus annularis* Forssk. and *A. trimestris* L., Fabaceae, growing in Egypt, resulted in the isolation of eight compounds, for the first time from these two species. These compounds were identified by chemical methods, NMR spectroscopy, UV spectroscopy as well as MS analysis. The antimicrobial activity of the isolated compounds were tested against *Staphylococcus aureus, Esherichia coli* and *Candida albicans.*

Keywords: Fabaceae, *Astragalus,* oleanane saponins, flavonoids, antimicrobial activity.

INTRODUCTION

Astragalus species represent very old and well known remedy in the traditional medicine of China, Bulgaria and Russia. They are used mainly as superior tonics, for shortness of breath, general weakness, lack of appetite and to invigorate vital energy (Ionkova, 1995). Some *Astragalus* species are also used as diuretics and for treatment of stomach ulcers, diabetes, nephritis, chronic bronchitis, postpartum urine retention and leprosy (Foster, 1998). In the course of our phytochemical investigation of the secondary metabolites from Astragalus species endemic to Egypt, a number of cycloartane saponins, oleanane saponins, flavonoids, isoflavans and spinocoumarins were isolated (El-Sebakhy et al., 2000; Gabriboldi et al., 1995; Orsini et al., 1994; Radwan et al., 2007; Verotta et al., 2001).

The present paper deals with the phytochemical investigation and the antimicrobial activity screening of the aerial parts of two Egyptian *Astragalus* species namely *A. annularis* Forssk. and *A. trimestris* L. Thus, eight compounds were isolated and identified. From *A. annularis* , soyasaponin III (**1**), isorhamnetin-3-*O*-glucoside (**2**), isorhamnetin-3-*O*-rutinoside (**3**) and sorbifolin (**4**) were isolated. Whereas from *A.trimestris*, soyasaponin I (**5**), apigenin-7-*O*-β-D-glucopyranose-(1'''-3'')β-D-glucopyranoside (**6**), sorbifolin (**4**), 8-methoxyvestitol (**7**) and stigmasterol (**8**) were isolated. None of the mentioned compounds have been previously isolated from these two species of *Astragalus.* It is worth mentioning that this is the first phytochemical investigation of *A. annularis* Forssk.

MATERIAL AND METHODS

Melting points were determined by saturat SMP heating stage microscope. UV spectra were obtained on Perkin-Elmer®, Lambada 3B UV/VIS spectrophotometer. 1D and 2D NMR spectra were measured at 400 MHz and 100 MHz on Varian® and Burker® Spectrometers. The HR-FAB-MS were recorded on Bruker Biopex instrument. Silica gel (70-230 mesh, Merck®) was used for column chromatography and silica gel G (Merck®) for TLC.

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ISSN 0102-695X
DOI: 10.1590/S0102-695X2010005000047

Plant material

The aerial parts of *Astragalus annularis* Forssk. and *A. trimestris* L., Fabaceae, were collected from Rosetta East of Alexandria, Egypt, in March 2003. The identity of the plant was kindly identified by Prof. Dr. Rafeek Mohamed (Faculty of Science, Alexandria University). Voucher specimens are deposited in the herbarium of Faculty of Science, University of Alexandria, Egypt.

Antimicrobial testing

One ml of 24 h broth culture of each of the tested organisms was separately inoculated into 100 mL of sterile molten nutrient agar maintained at 45 ºC. Stock solutions of the isolates in DMSO were diluted to give serial dilutions resulting in concentrations ranging from 1000 to 8 µg/mL. After the tested compounds have been diluted, a volume of the standardized *inoculum* equal to the volume of the diluted tested isolate is added to each dilution vessel. The inoculated, serially diluted isolates were incubated at 37 ºC for 24 h. Ampecillin and clotrimazole were used as positive controls. Minimal inhibitory concentration (MIC) is expressed as the lowest concentration which inhibited growth judged by lack of turbidity in the tube (Jian & Kar, 1971).

Extraction and isolation

The dried aerial parts of *A. annularis* (5 kg) and *A. trimestris* (2.5 kg) were separately extracted with 90% EtOH. The alcoholic extracts were concentrated *in vacuo* and dissolved in H_2O and then partitioned with light peteroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The different extracts were concentrated and screened using TLC.

The *n*-butanol soluble extract of *A. annularis* (16.3 g) was chromatographed on silica gel column using EtOAc and the polarity was increased with MeOH. The fractions eluted with 40% MeOH in EtOAc afforded 40 mg of (**1**). The ethyl acetate soluble extract (6.5 g) was chromatographed on silica gel column using $CHCl_3$, and the polarity was increased with MeOH. The fractions eluted with 15% MeOH in $CHCl_3$ yielded 25 mg of (**2**), while those fractions eluted with 22% MeOH in $CHCl_3$ were subjected to pTLC using $CHCL_3$-MeOH (9:1) as a mobile phase, to afford 8 mg of (**3**). The chloroform soluble extract (8.5 g) was chromatographed on silica gel column using $CHCl_3$, and the polarity was increased with MeOH. The fractions eluted with 5% MeOH in $CHCl_3$ yielded 12 mg of (**4**).

Soyasaponin III (**1**): White amorphous powder, $[\alpha]_D$ +20° (MeOH; *c* 0.42). FAB-MS *m/z* 819 [M + Na], 797 [M+H], 796 [M], m.p. 216-217 °C (MeOH). ^{1}H-NMR (CD_3OD) δ 0.87, 1.04, 1.06 , 1.15, 1.25, 1.28, 1.49 (each 3H, s, *tert*-Me x 7), δ 4.88 (1H, d, *J*=8.0 Hz, glu A H-1), 5.15 (1H, d, *J*=7.6 Hz, gal H-1) , δ 5.38 (1H, br s, H-12) .The ^{13}C-NMR: Table 1.

Isorhamnetin-3-O-glucoside (**2**): yellow crystals, m.p. 180-181 ºC. UV λ_{max} nm (abs.) MeOH: 355, 255, MeOH /NaOMe 413, 273, MeOH/$AlCl_3$ 402, 265, MeOH/$AlCl_3$/ HCl 399, 267 , MeOH/NaOAc, 400,274. EI-MS (rel. abund %) 316(100), 315(27), 288(5.5), 287(22), 273(4), 222(20), 153(10). ^{1}H-NMR: Table 2 and ^{13}C- NMR: Table 3.

Isorhamnetin-3-O-rutinoside (**3**): yellow crystals, m.p. 214-216 ºC. UV λ_{max} nm (abs.) MeOH: 357, 255, MeOH /NaOMe 416, 272, MeOH/$AlCl_3$ 407, 267, MeOH/$AlCl_3$/ HCl 404, 265 , MeOH/NaOAc, 418,275. EI-MS (rel. abund %) 316(100), 315(25), 288(5), 287(22), 273(4), 222(21), 153(12). ^{1}H-NMR: Table 2 and ^{13}C- NMR: Table 3.

Sorbifolin (**4**): yellow crystals, m.p. 246-248 ºC. UV λ_{max} nm (abs.) MeOH: 334, 275, MeOH/NaOMe 370, 276, MeOH/$AlCl_3$ 365, 302, MeOH/$AlCl_3$/HCl 354, 301, MeOH/NaOAc, 388, 276. EI-MS (rel. abund %) 301(16), 300(33), 285(18), 257(22), 182(3), 167(5), 118(15). ^{1}H-NMR: Table 2 and ^{13}C- NMR: Table 3.

The *n*-butanol soluble extract of *A. trimestris* (18 g) was chromatographed on silica gel column using EtOAc followed by EtOAc-MeOH mixtures with gradual increase of MeOH contents. The fractions eluted with 15% MeOH in EtOAc were further purified on silica gel CC to give 12 mg of (**6**), while those fractions eluted with 50% MeOH in EtOAc afforded 24 mg of (**5**). The chloroform soluble extract (7 g) was chromatographed on silica gel column using $CHCl_3$-MeOH mixtures. The fractions eluted with 100% $CHCl_3$ yielded 28 mg of (**8**) which was identified as stigmasterol by direct comparison with authentic sample [m.p., m.m.p. and TLC]. Whereas the fractions eluted with 2% and 5% MeOH in $CHCl_3$ yielded 8 mg and 40 mg of (**4**) and (**7**), respectively.

Soyasaponin I (**5**): White amorphous powder, [α]D -5.9° (MeOH; *c* 0.51). FAB-MS *m/z*: 965 [M + Na], 943 [M +H], 942 [M], m.p. 255-257 °C (MeOH). ^{1}H-NMR (CD_3OD) δ 0.78, 0.82, 0.91, 0.99, 1.08, 1.16 (each 3H, s, *tert*-Me x 7), 1.76 (3H, *d , J*=5.9 Hz, Rha H-6), δ 4.78 (1H, *d, J*=8.0 Hz, glu A H-1), 5.15 (1H, *d, J*=7.6 Hz, gal H-1), δ 5.22 (1H, br s, H-12) and 5.76 (1H, *b s*, Rha H-1). ^{13}C-NMR: Table 1.

Apigenin-7-O-β-D-glucopyranose-(1'"-3")β-D-glucopyranoside (**6**): yellow crystals, m.p. 214-216 ºC. UV λ_{max} nm (abs.) MeOH: 348, 267, MeOH/NaOMe 400, 265, MeOH/$AlCl_3$ 367, 274, MeOH/$AlCl_3$/HCl 365, 273

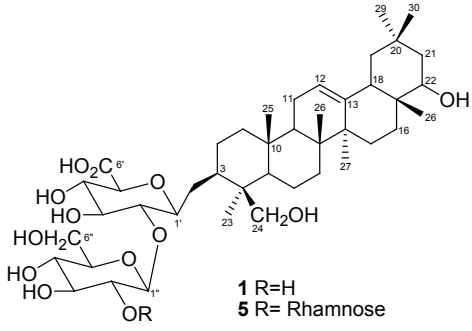
, MeOH/NaOAc, 400, 270. ^{1}H-NMR: Table 2 and ^{13}C-NMR: Table 3.

8-Methoxyvestitol (**7**): yellow flakes, m.p. 135-136 ºC. UV λ_{max} nm (abs.) MeOH 212, 279, NaOH 214, 290, AlCl3 214, 279. 1H-NMR 3.84 (1H, s, H-2eq), 4.25 (1H, d, *J*=10.3 Hz, H-2_{ax}), 3.44 (1H, m, H-3ax), 2.76 (1H, dd, *J*=5.4, 15.1 Hz, H-4_eq), 2.90 (^{1}H, *dd, J*=10.2, 15.1 Hz, H-4_{ax}), 6.82 (1H, d, *J*=8.2 Hz, H-5), 6.66 (1H, d, *J*=8.2 Hz, H-6), 6.24 (1H, d, *J*=1.9 Hz, H-3'), 6.30 (1H, d, *J*=1.9, 7.9 Hz, H-5'), 6.34 (1H, d, *J*=7.9 Hz, H-6'), 3.75 (3H, s, 8- OCH_3), 3.84 (3H, s, 4'- OCH_3).

RESULTS AND DISCUSSION

Compound **1** showed a characteristic molecular ion peak at *m/z* 819.4387 $[M + Na]^+$ in the positive FAB mass spectrum, corresponding to the molecular formula $C_{42}H_{68}O_{14}$. The occurence of seven tertiary methyl signals and an olefinic proton signal in the ^{1}H-NMR spectrum suggested **1** to be an oleanane derivative. The sapogenol obtained by acid hydrolysis of **1** was identified as soyasapogenol B by TLC comparison with a reference sample. The ^{13}C-NMR spectrum (Table 1) showed the presence of two sugar moieties, and also showed the presence of a hydroxymethyl group at C-24 and a hydroxyl group at C-22. The ^{13}C-NMR signals of the sugar part of 1 were identical to those reported for β-D-galactopyranosyl-(1"-2')-β-glucuronopyranosyl (Agrawal, 1992; Shujiro et al., 1978), while the signals due to the aglycone part (Table 1) were in a good agreement with those reported for soyasapogenol B except for C-3, which was shifted downfield indicating glycosylation (Fukunaga et al., 1987). Therefore, the structure of compound 1 was elucidated to be 3-*O*-[β-D-galactopyranosyl-(1"-2')-β-D-glucuronopyranosyl]-soyasapogenol B, which is known as soyasaponin III (Kitagawa et al., 1985; Kitagawa et al., 1988).

Compound 5 gave a molecular ion peak at *m/z* 965.5167 $[M + Na]^+$ on positive FAB mass spectral analysis. Thus the molecular formula of 5 was suggested to be $C_{48}H_{78}O_{18}$. The ^{1}H-NMR and ^{13}C-NMR spectra of 5 (Table 1), after acid hydrolysis pointed to the same aglycone, soyasapogenol B, as in 1, and established the presence of three sugars. The FAB mass spectrum, ^{1}H-NMR and ^{13}C-NMR spectral data of **5** were in a good agreement to those reported for soyasaponin I (Kitagawa et al., 1983; Wang et al., 1990), so the structure of **5** was suggested to be soyasaponin I which is 3-*O*-[α-L-rhamnopyranosyl-(1'"-2")-β-D-galactopyranosyl-(1"-2')-glucuronopyranosyl] soyasapogenol B.



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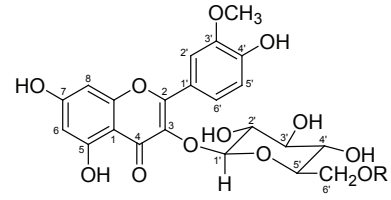
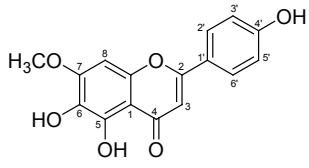
5 R= Rhamnose



2 R=H
3 R= Rhamnose



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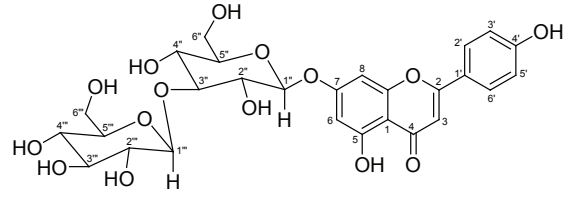

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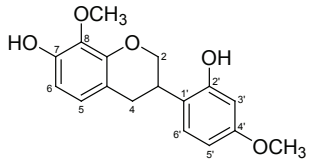

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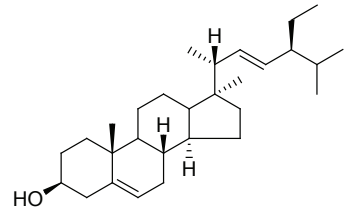

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Table 1. ^{13}C-NMR spectral data of (**1**), (**5**) at 100 MHz in CD_3OD.

Carbon atom	Soyasaponin III (**1**)	Soyasaponin I (**5**)
1	38.1	38.6
2	27.0	25.9
3	89.9	90.5
4	43.8	43.4
5	55.7	55.6
6	19.2	18.3
7	33.7	33.1
8	40.3	39.7
9	47.9	47.3
10	36.3	36.3
11	23.8	23.6
12	122.7	122.0
13	144.0	144.5
14	42.0	42.1
15	27.0	25.9
16	28.2	28.7
17	37.3	37.4
18	45.1	45.0
19	46.1	46.5
20	31.3	30.6
21	42.2	42.1
22	76.0	75.5
23	22.4	22.8
24	61.7	62.8
25	15.3	15.9
26	17.0	17.0
27	24.6	25.4
28	29.1	28.7
29	33.7	33.1
30	21.3	20.8
Glu 1'	103.2	104.4
2'	77.2	77.4
3'	76.6	76.2
4'	72.5	74.5
5'	77.2	77.4
6'	175.0	174.0
Gal 1''	100.4	100.7
2''	72.3	76.3
3''	74.5	75.5
4''	70.5	71.1
5''	76.4	77.4
6''	61.7	60.8
Rhm 1'''		102.4
2'''		72.8
3'''		73.0
4'''		74.5
5'''		69.7
6'''		19.1

Different qualitative chemical tests and spectral analyses indicated that compounds (**2**), (**3**) and (**6**) were flavonoidal glycosides (Mabry et al., 1970). UV spectrum of **2** in MeOH and different shift reagents revealed the presence of free OH at positions 5, 7 and 4' (Mabry et al., 1970), while UV spectrum in methanol, after acid hydrolysis, suggested 3-*O*-glycosylation. 1H-NMR data of **2** (Table 2) showed two meta coupled aromatic protons at δ_H 5.58 and δH 5.73 (1H, d, *J*=2.0 Hz, 1H each) assigned for H-6 and H-8, respectively, confirming a 5,7-disubstituted ring A. An ABX system was observed at δH 6.43 (1H, d, *J*=8.8 Hz), δ_H 7.40 (1H, dd, *J*=8.8, 2.0 Hz) and δ_H 7.74(1 H, d, *J*=2.0 Hz) in accordance to 3',4' disubstituted ring B. The site of glycosylation was further confirmed by ^{13}C-NMR spectrum of **2** (Table 3), which showed upfield shift of C-3 (δ 135.1) and downfield shift of C-2 (δ 157.9), compared with the aglycone (Shujiro et al., 1978). Moreover, NMR spectra indicated the presence of one methoxyl group as concluded from the signals observed at δ_H 3.70 (3 H, s) and δc 55.3 ppm. The UV spectra of **2** in $AlCl_3$ and $AlCl_3$/HCl shift reagents suggested the location of the methoxyl group to be at C-3' (Mabry et al., 1970). Furthermore the sugar moiety was confirmed to be glucose by co-chromatography, after acid hydrolysis, with reference sample. The EI-MS showed a diagnostic ion peak at *m/z* 316 characteristics to the aglycone isorhamnetin. Therefore, the structure of compound 2 was concluded to be isorhamnetin-3-*O*-β-D-glucopyranoside (Ceska & Derek, 1984).

Table 2. ^{1}H-NMR spectral data of **2, 3, 4** and **6** at 400 MHz in DMSO (**2, 3** and **4**) and CD_3OD (**6**).

#	6	4	3	2
3			6.53 (1H, s)	6.15 (1H, S)
6	5.58(1H, d, *J* =2.0 Hz)	5.91 (1H, d, *J* =2.0 Hz)		6.86 (1H, d, *J* =2.0 Hz)
8	5.73 (1H, d, *J* =2.0 Hz)	6.06 (1H, d, *J* =2.0 Hz)	6.58 (1H, s)	7.51 (1H, d, *J* =2.0 Hz)
2'	7.74(1 H, d, *J* =2.0 Hz)	7.92 (1 H, d, *J* =2.0 Hz)	7.85 (1 H, d, *J* =7.2 Hz)	8.02 (1 H, d, *J* =8.5 Hz)
3'			6.92 (1 H, d, *J* =7.2 Hz)	6.86 (1H, d, *J* = 9 Hz)
5'	6.43(1H, d, *J* = 8.8 Hz)	6.62 (1H, d, *J* = 8.4 Hz)	6.92 (1 H, d, *J* =7.2 Hz)	6.86 (1H, d, *J* = 9 Hz)
6'	7.40(1H, dd, *J*=8.8,2.0 Hz)	7.63 (1H, dd, *J*=8.4, 2.0 Hz)	7.85 (1 H, d, *J* =7.2 Hz)	8.02 (1 H, d, *J* =8.5 Hz)
1"	5.25(1H, d, *J* = 6.5 Hz)	4.98 (1H, d, *J* = 7.2 Hz)		5.51 (1H, d, *J* = 8 Hz)
1'"		4.65 (1H, d, *J* = 1.6 Hz)		4.92 (1H, d, *J* = 8 Hz)
6'"		1.21 (3H, d, *J* = 5.6 Hz)		
OCH_3	3.70 (3 H, s)	3.71 (3 H,s)	3.86 (3H, s)	

Table 3: ^{13}C-NMR spectral data of **2, 3, 4** and **6** in DMSO (**2, 3** and **4**) and CD_3OD (**6**).

#	2	3	4	6
2	157.9 (s)	157.9(s)	164.9(s)	164.0 (s)
3	135.1(s)	136.9(s)	103.6(d)	102.2 (s)
4	177.9(s)	178.1(s)	182.8(s)	181.9 (s)
5	165.2(s)	166.2(s)	153.2(s)	160.9(s)
6	99.2(d)	99.7(d)	131.4(s)	99.4 (d)
7	162.4(s)	163.1(s)	157.4(s)	163.1 (s)
8	95.9(d)	94.1(d)	93.9(d)	94.2 (d)
9	156.9(s)	157.8(s)	152.6(s)	157.1 (s)
10	105.8(s)	105.4(s)	104.3(s)	106.0 (s)
1'	122.4(s)	122.4(s)	121.8(s)	121.8 (s)
2'	115.8(d)	113.8(d)	128.0(d)	130.9 (d)
3'	149.4(s)	150.1(s)	115.6(d)	115.4 (d)
4'	148.0(s)	148.2(s)	161.3(s)	160.1 (s)
5'	113.2(d)	114.1(d)	115.6(d)	115.4 (d)
6'	123.3(d)	123.8(d)	128.0(d)	130.9 (d)
OCH_3	55.3(q)	54.8(q)	59.51(q)	
Sugar A				
1"	103.1(d)	104.2(d)		98.3 (d)
2"	74.4(d)	75.7(d)		74.61 (d)
3"	77.3(d)	77.0(d)		82.29 (d)
4"	76.0(d)	70.7(d)		70.10 (d)
5"	70.1(d)	77.0(d)		75.64 (d)
6"	61.1(t)	68.6(t)		61.25 (t)
Sugar B				
1"'		101.7(d)		104.3 (d)
2"'		70.7(d)		76.33 (d)
3"'		72.6(d)		76.81 (d)
4"'		74.6(d)		70.6 (d)
5"'		68.6(d)		75.1 (d)
6"'		17.1(s)		61.38 (t)

Compound **3** showed spectroscopic data similar to those of compound **2** except in the presence of an additional sugar moiety. The presence of two sugar units was further supported by the appearance of two anomeric protons in ^{1}H-NMR (Table 2) at δ_H 4.98 (^{1}H, d, *J*=7.2 Hz) and 4.65 (1H, d, *J*=1.6 Hz) with their corresponding carbon signals at δ_C 104.2, and δ_C 101.7, respectively in the HMQC spectrum, assignable for H-1" and H-1"' of glucose and rhamnose. The presence of a doublet at δ 1.21 integrated for three protons in 1H-NMR and a quartet at 17.1 in ^{13}C-NMR were typical for the rhamnose methyl group. The interglycosidic linkage was confirmed as glucose (6"-1"') rhamnose by the HMBC spectrum and the downfield shift of C-6" of glucose (δ 66.8) characterisrtic for rutinoside (Markham et al.,1978; Markham & Terani, 1976). The identity of the sugars were further confirmed by acid hydrolysis and co-chromatography with authentic samples. Accordingly, compound 3 was proved to be isorhamnetin-3-*O*-rutinoside (Sen et al., 1992).

The UV spectra of compound **6** in different shift reagents indicated the presence of free 5 and 4' dihydroxyl groups. On the other hand, the UV spectra of its aglycone indicated that it is 5,7 and 4' trihydroxy flavones (Mabry et al., 1970) suggesting 7-*O*-glycosylation. The ^{1}H-NMR (Table 2) and ^{13}C- NMR data of compound 6 (Table 3) were in complete agreement with this suggestion as they showed the characteristic pattern of 5,7-disubstituted ring A and 4'-monosubstituted ring B. The diglucosidic nature of compound 6 was revealed by the appearance of two anomeric protons at δ_H 5.51 (1H, d, *J*=8 Hz) and δH 4.92 (1H, d, *J*=8 Hz) in ^{1}H-NMR, with their corresponding carbon signals at δ 98.3, 104.3, respectively. The 7-*O*-glycosylation of compound 6 was confirmed by the long range correlation between the carbon at δ 163.1 (C-7) and the proton at δ 5.51 (H-1") in the HMBC spectrum (Figure 1). In addition, the correlation between the carbon at δ 82.29 (C-3") and the proton at δ 4.92 (H-1"') verified the interglycosidic linkage to be (1"'→3") thus the structure of compound **6** was suggested to be apigenin-7-*O*-β-D-glucopyranose-(1"'-3") β-D-glucopyranoside (Veitch et al., 1998) which is the first report for its isolation from genus *Astragalus*.

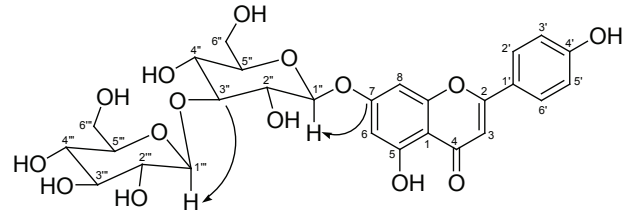

Figure 1. Key HMBC correlations of *apigenin*-7-O-*β-D-glucopyranose-(1"'-3")β-D-glucopyranoside* (**6**).

Different colour reactions indicated the flavonoidal nature of compound **4** (Mabry et al., 1970). Both EI-MS and ^{13}C-NMR (Table 3) suggested the molecular formula $C_{16}H_{12}O_6$ indicating a flavone with one methoxyl and three hydroxyl groups. The two ^{1}H-NMR singlets (Table 2) at δ 6.53, 6.58 assigned for H-3 and H-8 (Feranandez et al., 1989; Gonzalez et al., 1985), respectively, the UV spectra of **4** with different shift reagents (Mears & Mabry, 1972), as well as the MS fragment at *m\z* 182 indicated free hydroxyl groups at C-5 and C-6 and a methoxyl group at C-7. The UV spectra indicated a free hydroxyl group at C-4' (Mabry et al., 1970; Asaad, 2002) which was further supported by the presence of two doublets at δ_H 6.92 and δ_H 7.85 (*J*=7.2 Hz, 2H each). The ^{13}C-NMR data supported the above substitution patterns (Agrawal, 1989). The data of compound **4** were in a good agreement with those reported for sorbifolin (Amer et al., 2001). It is worth mentioning that this is the first report for its isolation from genus *Astragalus*.

The ^{1}H-NMR, UV spectral data as well as m.p. of compound **7** were in full agreement with those reported for 8-methoxyvestitol (Assad, 2002).

Antibacterial and antifungal assays for some of the isolated compounds were carried out against a Gram-positive bacterium, *Staphylococcus aureus*, Gram-negative bacteria, *Esherichia coli* and the fungus *Candida albicans*. The results were shown in Table 4, where compound **5** showed a moderate activity against *E. coli* and compounds **5** & **6** showed a moderate activity against *C. albicans*, and none of the tested compounds showed any activity against the *S .aureus*.

Table 4. Minimum inhibitory concentration (MIC) of some isolated compounds from *Astragalus* species.

COMPOUND	MIC (µg /mL)		
	Esherichia coli	*Staphylococcus aureus*	*Candida albicans*
1	156.25	-	-
2	-	-	187.5
3	-	-	-
4	125	-	125
5	62.5	-	31.25
6	-	-	62.5
Ampicillin	8	0.25	-
Clotrimazole	-	-	2

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