SUPLPLEMENTARY MATERIAL TO

Chemical composition and antioxidant activity of *Astragalus monspessulanus* L. growing in semiarid areas of Algeria

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ISOLATION OF THE FRACTIONS

7 g of ethyl acetate extract were subjected to vacuum liquid chromatography VLC (50 mm × 50 mm; fractions of 100 ml) on RP-18 using a gradient system of H_2O/MeOH (80/20 to 0/100) to afford 9 fractions (Fr_1-Fr_9). Subfraction Fr_6 (695 mg) was separated into 9 subfractions ($Fr_{6.1}$-$Fr_{6.9}$) by chromatography over silica gel column with a gradient system of $CHCl_3$/MeOH (100/0 to 70/30). 10 mg of compound **12** were obtained by precipitation of $Fr_{6.9}$ in MeOH. Fraction Fr_7 (780 mg) was subjected to CC over silica gel and eluted with petroleum ether/EtOAc (100/0 to 0/100) producing 10 subfractions ($Fr_{7.1}$-$Fr_{7.10}$). $Fr_{7.4}$ (39 mg) was chromatographed on a silica gel CC eluting with petroleum ether/$CHCl_3$ (100/0 to 15/85) to yield 4.3 mg of compound **13**. Fraction Fr_9 (232 mg) was further chromatographed on a silica gel CC eluting with petroleum ether/$CHCl_3$ (100/0 to 70/30) to yield 15 mg of pure compound **11**.

The *n*-butanol extract (7 g) was submitted to vacuum liquid chromatography VLC (50 mm × 50 mm; fractions of 100 ml) on RP-18 using H_2O/MeOH (80:20 to 0:100) to obtain 15 fractions (Fr_1–Fr_{15}). Fr_1 (5.53 g) was subjected to polyamide CC eluted with a gradient of H_2O/MeOH (100:0 to 0:100) to obtain 16 subfractions ($Fr_{1.1}$–$Fr_{1.16}$). $Fr_{1.5}$ (333 mg) was subjected to polyamide CC eluted with a gradient of toluene/MeOH to get 12 subfractions ($Fr_{1.5.1}$–$Fr_{1.5.12}$). Purification of $Fr_{1.5.11}$ (57 mg) by HPLC column lead two compounds **3 (**3.4 mg) and **6** (2 mg). Further purification of $Fr_{1.5.4}$ (36.1 mg) by TLC (SiO_2) using $CHCl_3$/MeOH/H_2O (8:2:0.2) gave compound **8** (3.8 mg). $Fr_{1.5.8}$ (26 mg) was chromatographed over polyamide CC using a gradient of toluene/MeOH (20:80 to 0:100) to yield four subfractions ($Fr_{1.5.8.1}$–$Fr_{1.5.8.4}$). $Fr_{1.5.9}$ (32 mg) was also chromatographed over polyamide CC using a gradient of toluene/MeOH (10:90 to 0:100) as eluent to yield five subfractions ($Fr_{1.5.9.1}$–$Fr_{1.5.9.5}$). The mixed subfractions $Fr_{1.5.9.3}$, $Fr_{1.5.9.4}$ and $Fr_{1.5.8.2}$ (41.6 mg) were chromatographed over SiO_2 CC using $CHCl_3$/MeOH (5:95 to 0:100) as eluent, to obtain six subfractions. The fifth subfraction (15 mg) was purified by HPLC to yield compounds **1 (**4.2 mg) and **5 (**2 mg). The mixed subfractions $Fr_{1.7}$, $Fr_{1.8}$, $Fr_{1.9}$ and $Fr_{1.10}$ (134.5 mg) were subjected to CC over silica gel eluting with CH_2Cl_2/acetone (100:0 to 0:100) to obtain 8 subfractions ($Fr_{1.7.1}$–$Fr_{1.7.8}$). $Fr_{1.7.5}$ was chromatographed on preparative TLC (RP-18) using MeOH/H_2O (3:7) as eluent to give compound **7** (5 mg). Subfraction $Fr_{1.12}$ (117 mg) was submitted to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (10%) to get 4 subfractions ($Fr_{1.12.1}$–$Fr_{1.12.4}$). $Fr_{1.12.1}$ was purified by HPLC column to produce compounds **2** (3.8 mg) and **4** (2.4 mg). Compound **9** (15 mg) was obtained by precipitation of Fr_4 (60 mg) in MeOH. The residue of this fraction (Fr_4) was subjected to CC over silica gel and eluted with gradient system $CHCl_3$/MeOH (100:0 to 80:20), to give compound **10** (7 mg).

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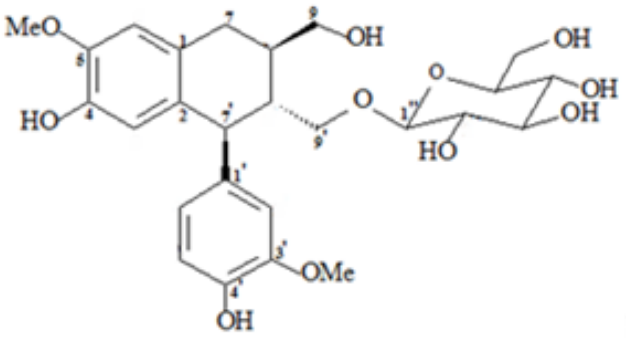
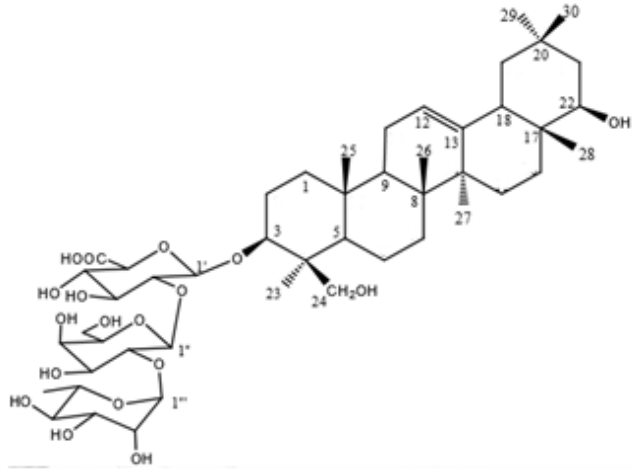
CHARACTERIZATION DATA FOR COMPOUNDS **8** AND **9** IN DMSO-d_6



Structure of Isolariciresinol 9'-O-β-D-glucopyranoside (**8**)

*Isolariciresinol 9'-O-β-D-glucopyranoside (**8**)*. White amorphous powder. $[\alpha]_D$ = + 16 (*c* = 0.9 g mL^{-1}, MeOH /CH_2Cl_2 (1/0.5)). ^{1}H NMR (500 MHz, DMSO-d_6, δ / ppm): 1.72 (1H, *m*, H-8'), 1,91 (1H, *m*, H-8), 2.72 (2H, *d*, *J*= 8.0 Hz, H-7), 2.96 (1H, *m*, H_a-9'), 2.97 (1H, *t*, *J*= 7.8 Hz, H-2''), 3.01 (1H, *ddd*, J_1 = 9.3, J_2 = 4.7, J_3 = 2.6 Hz, H-5''), 3.03 (1H, *dd*, J_1 = 9.3, J_2 = 7.8 Hz, H-4''), 3.13 (1H, *t*, *J* = 7.8 Hz, H-3''), 3.41 (1H, *dd*, *J*= 11.7; 2.6 Hz, H_a-6''), 3.45 (1H, *m*, H_a-9); 3.57 (1H, *m*, H_b-9), 3.63 (1H, *dd*, J_1 = 11.7, J_2 = 4.7 Hz, H_b-6''), 3.71 (6H, *s*, 5-OMe/3'-OMe), 3.90 (1H, *dd*, J_1 = 9.8, J_2 = 1.9 Hz, H_b-9'), 3.95 (1H, *d*, *J* = 7.8 Hz, H-1''), 4.03 (1H, *d*, *J* = 10.7 Hz, H-7'), 6.08 (1H, *sl*, H-3), 6.50 (1H, *dd*, *J* = 8.2; 1.8 Hz, H-6'), 6.61 (1H, *sl*, H-6), 6.68 (1H, *d*, *J* = 8.2 Hz, H-5'), 6.80 (1H, *d*, *J* = 1.8 Hz, H-2'); ^{13}C NMR (125 MHz, DMSO-d_6, δ / ppm): 32.5 (CH_2, C-7), 37.5 (CH, C-8), 44.1 (CH, C-8'), 45.5 (CH, C-7'), 55.5 (5-OMe), 55.6 (3'-OMe), 61.0 (CH_2, C-6''), 62.8 (CH_2, C-9), 67.6 (CH_2, C-9'), 70.0 (CH, C-4''), 73.3 (CH, C-2''), 76.7 (CH, C-5''), 76.8 (CH, C-3''),104.1 (CH, C-1''), 111.8 (CH, C-6), 113.9 (CH, C-2'),115.5 (CH, C-5'), 116.2 (CH, C-3),121.1 (CH, C-6'), 127.0 (C, C-1), 132.7 (C, C-2), 136.9 (C, C-1'), 144.0 (C, C-4), 144.5 (C, C-5, C-4'), 147.1 (C, C-3'). ESI-MS (*m/z*, (relative abundance, %)): 545 ($(C_{26}H_{34}O_{11}+Na)^+$, 100).



Structure of Soyasaponin I (**9**)

*Soyasaponin I (**9**)*. White amorphous solid. $[\alpha]^{20}_D$ = - 12 (*c* = 0.9 g mL^{-1}, MeOH). ^{1}H NMR (500 MHz, DMSO-d_6, δ / ppm): 0.83 (3H, *s*, H-28), 0.92 (3H, *s*, H-30), 0.93 (1H, *m*, H-5), 0.95 (1H, *m*, H-19a), 0.98 (3H, *s*, H-26), 1.01 (1H, *m*, H-1a), 1.03 (3H, *s*, H-29), 1.04 (2H, *m*, H-2a), 1.13 (3H, *s*, H-27), 1.24 (3H, *s*, H-23), 1.27 (3H, *d*, *J*= 8.6 Hz, H-6'''), 1.29 (2H, *m*, H-16a, H-16b), 1.32 (1H, *m*, H-21b), 1.36 (2H, *m*, H-6a), 1.44 (1H, *m*, H-21a), 1.42 (1H, *m*, H-7a), 1.54 (1H, *m*, H-7b), 1.57 (1H, *m*, H-9), 1.63 (2H, *m*, H-6b), 1.65 (1H, *m*, H-1b), 1.76 (2H, *m*, H-2b), 1.75 (1H, *m*, H-19b), 1.86 (2H, *m*, H-15a, H-15b), 1.87 (2H, *m*, H-11a, H-11b), 2.07 (1H, *d*, *J* = 14.7 Hz, H-18), 3.22 (1H, *d*, *J* = 11.3 Hz, H-24a), 3.37 (1H, *dd*, J_1= 5.1, J_2 = 3.5 Hz, H-22), 3.40 (1H, *dd*, J_1 = 10.3, J_2 = 3.5 Hz, H-3), 3.42 (1H, *t*, *J* = 9.6 Hz, H-4'''), 3.46 (1H, *t*, *J* = 9.4 Hz, H-4'), 3.48 (1H, *m*, H-5''), 3.54 (1H, *dd*, J_1 = 9.5; J_2 = 3.1 Hz, H-3''),

3.61 (1H, *d*, *J* = 7.9 Hz, H-5'), 3.62 (1H, *dd*, J_1 = 9.5; J_2 = 7.5 Hz, H-2"), 3.64 (1H, *dd*, J_1 = 9.4, J_2 = 7.9 Hz, H-3'), 3.72 (1H, *dd*, J_1 = 9.6, J_2 = 3.5 Hz, H-3'"), 3.72 (1H, *m*, H-6"a/H-6"b), 3.74 (1H, *dl*, *J* = 3.1 Hz, H-4"), 3.76 (1H, *d*, *J* = 7.9 Hz, H-2'), 3.92 (1H, *dd*, J_1 = 3.5; J_2 = 1.9 Hz, H-2'"), 4.12 (1H, *m*, H-5'"), 4.13 (1H, *d*, *J* = 11.3 Hz, H-24b), 4.45 (1H, *d*, *J* = 7.9 Hz, H-1'), 4.87 (1H, *d*, *J* = 7.5 Hz, H-1"), 5.14 (1H, *d*, *J* = 1.9 Hz, H-1'"), 5.25 (2H, *t*, *J* = 3.3 Hz, H-12). ^{13}C NMR (125 MHz, DMSO-d_6, δ / ppm): 16.6 (CH_3, C-25), 17.7 (CH_3, C-26), 18.5 (CH_3, C-6'"), 19.5 (CH_2, C-6), 20.6 (CH_3, C-28), 23.6 (CH_3, C-23), 25.0 (CH_2, C-11), 25.6 (CH_3, C-27), 27.0 (CH_2, C-2), 27.3 (CH_2, C-15), 29.2 (CH_3, C-29), 30.0 (CH_2, C-16), 31.5 (C, C-20), 32.7 (CH_3, C-30), 34.5 (CH_2, C-7), 37.6 (C, C-10), 38.7 (C, C-17), 39.8 (CH_2, C-1), 40.9 (C, C-8), 42.3 (CH_2, C-21), 43.5 (C, C-14),44.9 (C, C-4), 46.9 (CH, C-18), 47.6 (CH_2, C-19), 47.9 (CH, C-9), 57.5(CH, C-5), 62.3 (CH_2, C-6"), 64.5 (CH_2, C-24), 69.6 (CH, C-5'"), 71.7 (CH, C-3'"), 72.3 (CH, C-4", C-2'"), 74.3 (CH, C-4'), 74.4 (CH, C-4'"), 76.4 (CH, C-3"), 76.5 (CH, C-5"), 77.1 (CH, C-22), 77.3 (CH, C-2', C-5'), 78.2 (CH, C-3'), 78.5 (CH, C2"), 92.7 (CH, C-3), 102.4 (CH, C-1"), 102.5 (CH, C-1'"), 105.7 (CH, C-1'), 123.8 (CH, C-12), 145.4 (C, C13), 175.6 (C, COOH). ESI-MS (*m/z*, (relative abundance, %)): 965 ($(C_{48}H_{78}O_{18}+Na)^+$, 100).

DPPH RADICAL SCAVENGING ACTIVITY ASSAY

The free radical scavenging activity of *n*-butanol extract of *Astragalus mospessulanus* L. was measured *in vitro* by 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) according to the procedure described by (Saeed *et al.* 2012). The stock solution was prepared by dissolving 2.5 mg DPPH with 100 ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations. The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm. Ascorbic acid was used as reference compound. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging activity (\%)} = \left[\frac{Acontrol - A\ sample}{A\ control}\right] \times 100 \qquad (1)$$

The antiradical activity of tested extract is expressed as a relative or absolute decrease of concentration of DPPH or as IC_{50} (concentration of extract decreasing the absorbance of the DPPH solution by 50 %).

REFERENCES

N. Saeed, M. R. Khan, M. Shabbir, *The official Journal of the International Society for Complementary Medicine Research (ISCMR).* **12** (2012) 221

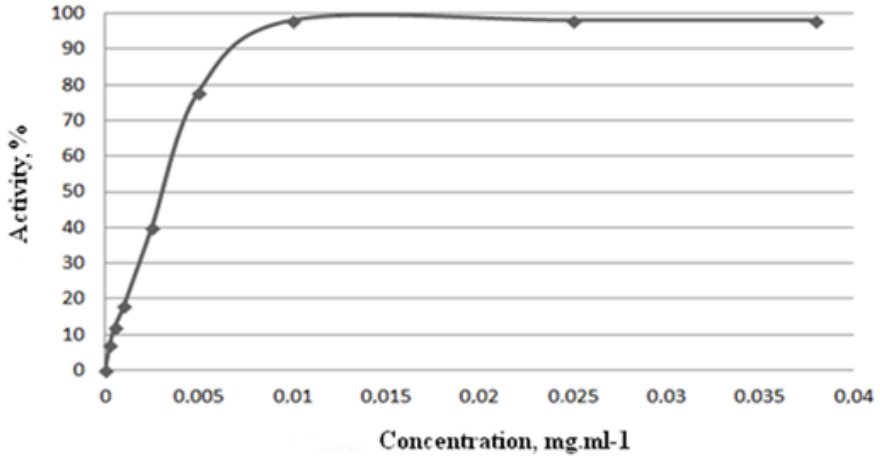


Fig. S1. Evolution of DPPH radical scavenging activity with concentration of ascorbic acid

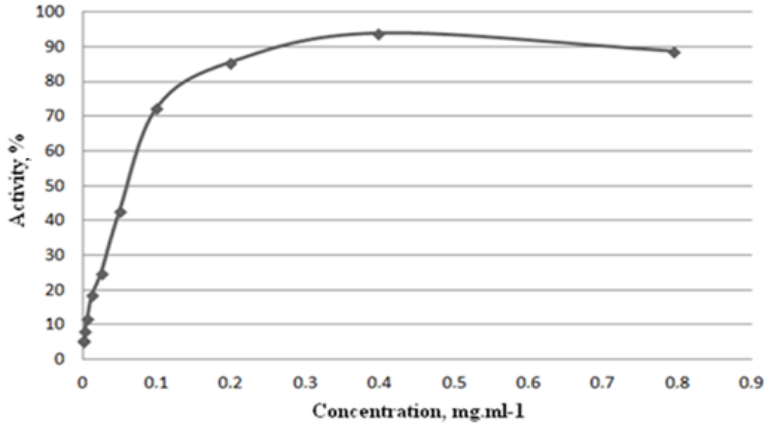


Fig. S2. Evolution of DPPH radical scavenging activity with *n*-BuOH extract concentration of *Astragalus monspessulanus*. The Data was represented as Mean (n=3)