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SUPPLEMENTARY 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

The air-dried and powdered material of *Astragalus monspessulanus* L. (1000 g) was extracted three times with EtOH/H_2O (70/30, 10 L, each time for 3 days) at room temperature. After filtration and evaporation, crude extract suspended in H_2O was partitioned successively with petroleum ether (b.p. 40–65 °C), ethyl acetate and *n*-butanol to give three different polar parts. The fractions were evaporated in vacuo to give the following extracts: petroleum ether (8.3 g), ethyl acetate (12.1 g), and *n*-butanol (15.33 g).

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 ($Fr_{1.1}$–$Fr_{1.16}$). Subfraction $Fr_{1.12}$ (117 mg) was submitted to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (90:10) to obtain 4 subfractions ($Fr_{1.12.1}$–$Fr_{1.12.4}$). $Fr_{1.12.1}$ (32 mg) was purified by HPLC to isolate compounds **1** (3.8 mg) and **2** (2.4 mg). $Fr_{1.5}$ (333 mg) was subjected to polyamide CC eluted with a gradient of toluene/MeOH to obtain 12 subfractions ($Fr_{1.5.1}$–$Fr_{1.5.12}$). $Fr_{1.5.8}$ (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) to afford five subfractions ($Fr_{1.5.9.1}$––$Fr_{1.5.9.5}$). The combined subfractions $Fr_{1.5.8.2}$, $Fr_{1.5.9.3}$ and $Fr_{1.5.9.4}$ (41.6 mg) were chromatographed over SiO_2 CC using $CHCl_3$/MeOH (5:95 to 0:100) as eluent, to obtain six subfractions ($Fr_{1.5.9.A}$–$Fr_{1.5.9.F}$). Subfraction $Fr_{1.5.9.E}$ (15 mg) was purified by HPLC to yield compounds **3** (4.2 mg) and **4** (2 mg). Purification of $Fr_{1.5.11}$ (57 mg), by HPLC column, led to compounds **5** (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 **7** (3.8 mg). The combined 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 TLC (RP-18) using MeOH/H_2O (3:7) as eluent to give compound **8** (5 mg) as a

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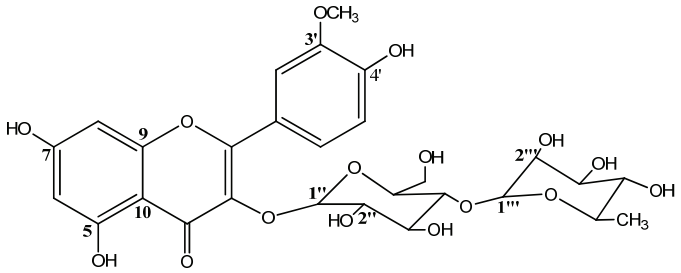
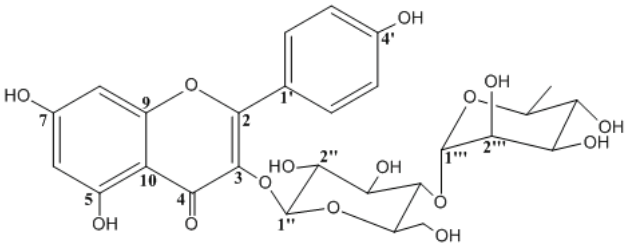
mixture of 2α-OH and 2β-OH epimers in a ratio of 3:2 (estimated from the relative intensities of the H-5 and H-1″ ^{1}H-NMR signals). Compound **9** (15 mg) was obtained by precipitation of Fr_4 (60 mg) in MeOH. The residue of this fraction (Fr_4) was subjected to CC over silica gel and eluted with the gradient system $CHCl_3$/MeOH (100/0 to 80/20), to give compound **10** (7 mg).

The ethyl acetate extract (7 g) was 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). Fraction Fr_9 (232 mg) was chromatographed on silica gel CC eluting with petroleum ether/$CHCl_3$ (100:0 to 70:30) to yield 15 mg of pure compound **11**. Fraction Fr_6 (695 mg) was further separated into 9 subfractions ($Fr_{6.1}$–$Fr_{6.9}$) by chromatography over a 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 ($Fr_{7.1}$–$Fr_{7\cdot10}$). $Fr_{7.4}$ (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**.

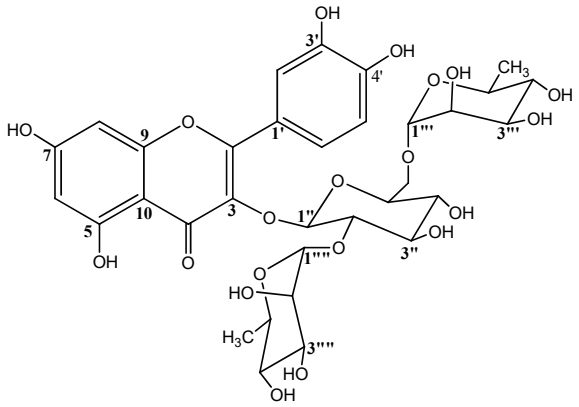
CHARACTERIZATION DATA FOR COMPOUNDS **2** AND **4**–**10**



Isorhamnetin 3-O-*(4-O*-α-*L-rhamnopyranosyl*-β-*D-glucopyranoside) (calendoside III) (**2**).* ^{1}H-NMR (500 MHz, MeOD, δ / ppm): 1.09 (3H, *d*, J = 6.2 Hz, H-6‴), 3.22 (1H, *t*, J = 9.8 Hz, H-4‴), 3.27 (1H, *dd*, J_1 = 9.3, J_2 = 7.3 Hz, H-4″), 3.40 (1H, *t*, J = 7.3 Hz, H-3″), 3.41 (1H, *ddd*, J_1 = 9.3, J_2 = 4.7, J_3 = 1.3 Hz, H-5″), 3.42 (1H, *m*, H-5‴), 3.46 (1H, *t*, J = 7.3 Hz, H-2″), 3,48 (1H, *dd*, J_1 = 9.8, J_2 = 3.4 Hz, H-3‴), 3.61 (1H, *dd*, J_1 = 3.4, J_2 =1.5 Hz, H-2‴), 3.80 (1H, *dd*, J_1 = = 10.8, J_2 =1.3 Hz, H-6″a), 3.92 (1H, *dd*, J_1 = 10.8, J_2 = 4.7 Hz, H-6″b), 3.95 (3H, *s*, 3′-OMe), 4.52 (1H, *d*, J = 1.5 Hz, H-1‴), 5.25 (1H, *d*, J = 7.3 Hz, H-1″), 6.20 (1H, *d*, J = 2.1 Hz, H-6), 6.42 (1H, *d*, J = 2.1 Hz, H-8), 6.92 (1H, *d*, J = 8.6 Hz, H-5′), 7.64 (1H, *dd*, J_1 = 8.6, J_2 = 2.1 Hz, H-6′), 7.95 (1H, *d*, J = 2.1 Hz, H-2′); ^{13}C-NMR (125 MHz, MeOD, δ / ppm): 18.4 (CH_3, C-6‴), 57.3 (CH_3, 3′-OMe), 69.0 (CH_2, C-6″), 70.3 (CH, C-5‴), 72.2 (CH, C-4″), 72.6 (CH, C-3‴), 72.8 (CH, C-2‴), 74.3 (CH, C-4‴), 76.4 (CH, C-2″), 78.7 (CH, C-3″), 95.4 (CH, C-8), 100.5 (CH, C-6), 103.1 (CH, C-1‴), 104.9 (CH, C-1″), 105.6 (C, C-10), 115.1 (CH, C-2′), 116.7 (CH, C-5′), 123.0 (C, C-1′), 124.5 (C, C-6′), 135.6 (C, C-3), 145.9 (C, C-3′), 148.9 (C, C-4′), 158.5 (C, C-9), 159.0 (C, C-2), 163.0 (C, C-5), 166.1 (C, C-7), 179.5 (C, C-4); ESI-MS (*m*/*z*, (relative abundance, %)): 779 ($(C_{28}H_{32}O_{16}+Na)^+$, 100).

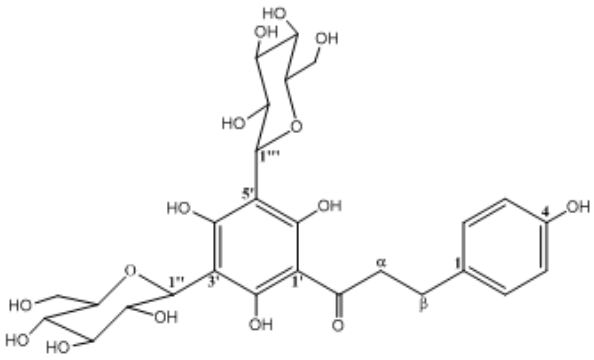


Kaempferol 3-O-*(4-O*-α-*L-rhamnopyranosyl*-β-*D-glucopyranoside) (**4**).* Yellow amorphous solid. ^{1}H-NMR (500 MHz, MeOD, δ / ppm): 0.95 (3H, *d*, *J*= 6.4 Hz, H 6‴), 3.20 (1H, *ddd*, J_1 = 9.1, J_2 = 6.2, J_3 = 2.2 Hz, H-5″), 3.28 (1H, *t*, *J* = 9.1 Hz, H 4″), 3.33 (1H, *t*, *J* = 9.2 Hz, H-4‴), 3.50 (1H, *dd*, J_1 = 12.0, J_2 = 6.2 Hz, H-6″a), 3.55 (1H, *t*, *J* = 9.1 Hz, H-3″), 3.61 (1H, *dd*, J_1 = 9.1, J_2 = 7.7 Hz, H-2″), 3.72 (1H, *dd*, J_1 = 12.0, J_2 = 2.2 Hz, H-6″b), 3.77 (1H, *dd*, J_1 = 9.2, J_2 = 3.4 Hz, H-3‴), 3.99 (1H, *dd*, J_1 = 3.4, J_2 = 1.6 Hz, H-2‴), 4,02 (1H, *dq*, J_1 = 9.2, J_2 = 6.4 Hz, H-5‴), 5.23 (1H, *d*, *J* = 1.6 Hz, H-1‴), 5.73 (1H, *d*, *J* = 7.7 Hz, H-1″), 6.15 (1H, *brs*, H-6), 6.34 (1H, *brs*, H-8), 6.88 (2H, *d*, *J* = 9.1 Hz, H-3′, H-5′), 8.04 (2H, *d*, *J* = 9.1 Hz, H-2′, H-6′); ^{13}C-NMR (125 MHz, MeOD, δ / ppm): 17.7 (CH_3, C-6‴), 62.8 (CH_2, C-6″), 70.1 (CH, C-5‴), 71.9 (CH, C-4″), 72.4 (CH, C-3‴), 72.6 (CH, C-2‴), 74.2 (CH, C-4‴), 79.2 (CH, C-3″), 80.3 (CH, C-2″), 94.6 (CH, C-8), 100.3 (CH, C-1″), 101.0 (CH, C-6), 102.8 (CH, C-1‴), 105.9 (C, C-10), 116.3 (CH, C-3′, C-5′), 123.1 (C, C-1′), 132.2 (CH, C-2′, C-6′), 134.4 (C, C-3), 158.4 (C, C-9), 158.5 (C, C-2), 161.6 (C, C-4′), 163.2 (C, C-5), 165.6 (C, C-7), 179.4 (C, C-4). ESI-MS (*m/z*, (relative abundance, %)): 617 ($(C_{27}H_{30}O_{15}+Na)^+$, 100).

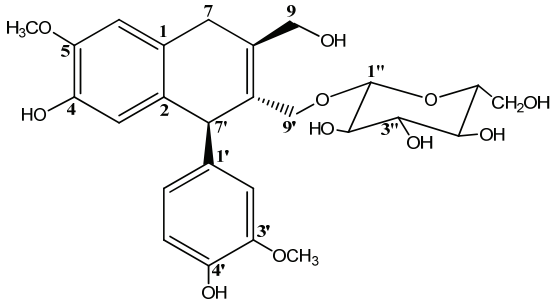


Quercetin 3-O-*(2,6*-α-*L-dirhamnopyranosyl*-β-*D-glucopyranoside) (**5**).* ^{1}H-NMR (500 MHz, MeOD, δ / ppm): 1.00 (3H, *d*, *J* = 5.6 Hz, H-6‴), 1.08 (3H, *d*, *J* = 5.9 Hz, H-6⁗), 3.24 (1H, *t*, *J* = 9.4 Hz, H-4⁗), 3.28 (1H, *m*, H-4″), 3.32 (1H, *m*, H-4‴), 3.33 (1H, *m*, H-5″), 3.38 (1H, *m*, H-6″a), 3.43 (1H, *m*, H-5‴), 3.50 (1H, *dd*, J_1 = 9.4, J_2 = 3.4 Hz, H-3⁗), 3.54 (1H, *t*, *J* = 8.8 Hz, H-3″), 3.58 (1H,

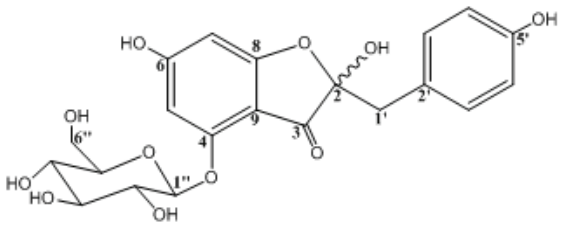
dd, J_1 = 3.4, J_2 =1.6 Hz, H-2‴), 3.63 (1H, *dd*, J_1 = 8.8, J_2 = 7.8 Hz, H-2″), 3.81 (1H, *dd,* J_1 = 9.8, J_2 = 3.8 Hz, H-3‴), 3.83 (1H, *dd*, J_1 = 11.5, J_2 = 1.6 Hz, H-6″b), 4,01 (1H, *dd*, J_1 = 3.8, J_2 = 1.6 Hz, H-2‴), 4.07 (1H, *m*, H-5⁗), 4.50 (1H, *d*, *J* = 1.6 Hz, H-1⁗), 5.22 (1H, *d*, *J* = 1.6 Hz, H-1‴), 5.59 (1H, *d*, *J* = 7.8 Hz, H-1″), 6.19 (1H, *d*, *J* = 2.1 Hz, H-6), 6.37 (1H, *d*, *J* = 2.1 Hz, H-8), 6.87 (1H, *d*, *J* = 8.1 Hz, H-5′), 7.60 (1H, *dd*, J_1 = 8.1, J_2 = 2.1 Hz, H-6′), 7.62 (1H, *d*, *J* = = 2.1 Hz, H-2′); ^{13}C-NMR (125 MHz, MeOD, δ / ppm): 17.6 (CH_3, C-6‴), 17.9 (CH3, C-6⁗), 68.4 (CH2, C-6″), 69.9 (CH, C-5⁗), 70.1 (CH, C-5‴), 72.0 (CH, C-4″), 72.3 (CH, C-3‴), 72.4 (CH, C-2‴, C-3⁗), 72.5 (CH, C-2⁗), 74.0 (CH, C-4⁗), 74.2 (CH, C-4‴), 77.3 (CH, C-5″), 79.1 (CH, C-3″), 80.2 (CH, C-2″), 94.8 (CH, C-8), 99.9 (CH, C-6), 100.6 (CH, C-1″), 102.4 (CH, C-1⁗), 102.8 (CH, C-1‴), 106.1 (C, C-10), 116.2 (CH, C-5′), 117.5 (CH, C-2′), 123.6 (C, C-1′), 123.7 (C, C-7′), 134.6 (C, C-3), 146.1 (C, C-3′), 149.7 (C, C-4′), 158.6 (C, C-9), 159.1 (C, C-2), 163.3 (C, C-5),165.8 (C, C-7), 179.5 (C, C-4). ESI-MS (*m/z*, (relative abundance, %)): 779 (($C_{33}H_{40}O_{20}$+Na)$^+$, 100).



3′,5′-di-C-β-*D-glucopyranosylphloretin* (***6***). Yellow amorphous powder. $[\alpha]_D$ = +83.6 (*c* = 1.0 g mL^{-1}, MeOH). ^{1}H-NMR (500 MHz, MeOD, δ / ppm): 2.86 (2H, *m*, H-β), 3.34 (2H, *m*, H-α), 3.42 (2H, *m*, H-5″, H-5‴), 3.51 (2H, *t*, *J* = 9.4 Hz, H-3″, H-3‴), 3.53 (2H, *t*, *J* = 9.4 Hz, H-4″, H-4‴), 3.62 (2H, *t*, *J* = = 9.4 Hz, H-2″, H-2‴), 3.82 (2H, *dd*, J_1 = 12.5, J_2 = 2.0 Hz, H-6″a, H-6‴a), 3.86 (2H, *dd*, J_1 = 12.5, J_2 = 2.0 Hz, H-6″b, H-6‴b), 4.94 (2H, *d*, *J* = 9.4 Hz, H-1″, H-1‴), 6.67 (2H, *d*, *J* = 8.5 Hz, H-3, H-5), 7.04 (1H, *d*, *J* = 8.5 Hz, H-2, H-6); ^{13}C-NMR (125 MHz, MeOD, δ / ppm): 31.3 (CH_2, C-β), 48.0 (CH_2, C-α), 62.1 (CH_2, C-6″, C-6‴), 71.2 (CH, C-4″, C-4‴), 74.3 (CH, C-2″, C-2‴), 76.9 (CH, C-1″, C-1‴), 79.3 (CH, C-3″, C-3‴), 82.9 (CH, C-5″, C-5‴), 104.6 (C, C-3′, C-5′), 106.2 (C, C-1′), 116.3 (CH, C-3, C-5), 130.6 (CH, C-2, C-6), 134.1 (C, C-1), 156.6 (C, C-4), 162.2 (C, C-2′, C-6′), 163.1 (C, C-4′), 207.2 (C, C=O). ESI-MS (*m/z*, (relative abundance, %)): 597 (($C_{27}H_{34}O_{15}$–H)$^-$, 100).

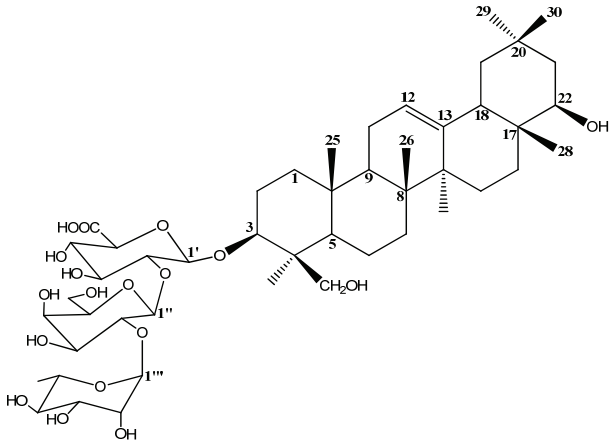


Isolariciresinol 9′-O-β-*D-glucopyranoside (**7**).* White amorphous powder. $[\alpha]_D$ = +16 (*c* = 0.9 g mL^{-1}, MeOH /CH_2Cl_2 (1/0.5)). ^{1}H-NMR (500 MHz, DMSO-d_6, δ / ppm): 1.72 (1H, *m*, H-8′), 1,91 (1H, *m*, H-8), 2.72 (2H, *d*, *J* = = 8.0 Hz, H-7), 2.96 (1H, *m*, H-9′a), 2.97 (1H, *t*, *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_1 = 11.7, J_2 = 2.6 Hz, H-6″a), 3.45 (1H, *m*, H-9a); 3.57 (1H, *m*, H-9b), 3.63 (1H, *dd*, J_1 = 11.7,J_2 = 4.7 Hz, H-6″b), 3.71 (6H, *s*, 5-OMe/3′-OMe), 3.90 (1H, *dd*, J_1 = 9.8, J_2 = 1.9 Hz, H-9′b), 3.95 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.03 (1H, *d*, *J* = 10.7 Hz, H-7′), 6.08 (1H, *brs*, H-3), 6.50 (1H, *dd*, J_1 = 8.2, J_2 = 1.8 Hz, H-6′), 6.61 (1H, *brs*, 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).



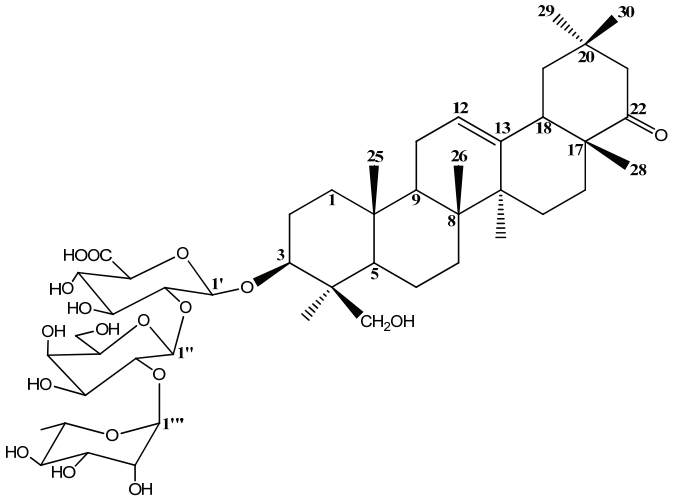
*Hovetrichoside C (**8**).* Amorphous powder. $[\alpha]^{25}_D$ = –54.1 (*c* = 1.9 g mL^{-1}, MeOH). (Major): ^{1}H-NMR (500 MHz, DMSO-d_6, δ / ppm): 2.90 (2H, *m*, H-1′), 3.17 (1H, *m*, H-4″), 3.19 (1H, *m*, H-3″), 3.24 (1H, *m*, H-2″), 3.25 (1H, *m*, H-5″), 3.47 (1H, *m*, H-6″a), 3.61 (1H, *m*, H-6″b), 4.90 (1H, *d*, *J* = 8.2 Hz, H-1″), 5.93 (1H, *d*, *J* = 1.8 Hz, H-7), 6.00 (1H, *d*, *J* = 1.8 Hz, H-5), 6.55 (2H, *d*, *J* = 7.1 Hz, H-4′, H-6′), 6.92 (2H, *d*, *J* = 7.1 Hz, H-3′, H-7′);^{13}C-NMR (125 MHz, DMSO-d_6, δ / ppm): 40.4 (CH_2, C-1′), 60.4 (CH_2, C-6″), 69.3 (CH, C-4″), 72.9 (CH, C-2″),

76.7 (CH, C-3″), 77.2 (CH, C-5″), 91.5 (CH, C-7), 95.2 (CH, C-5), 99.4 (CH, C-1″), 101.9 (C, C-9), 105.5 (C, C-2), 114.7 (CH, C-4′, C-6′), 124.1 (C, C-2′), 131.3 (CH, C-3′, C-7′), 155.9 (C, C-5′), 156.7 (C, C-4), 168.4 (C, C-6), 171.9 (C, C-8), 192.4 (C, C-3). ESI-MS (*m/z*, (relative abundance, %)): 449 ($(C_{21}H_{22}O_{11}–H)^-$, 100). (Minor): ^{1}H-NMR (500 MHz, DMSO-d_6, δ / ppm): 2.90 (2H, *m*, H-1′), 3.17 (1H, *m*, H-4″), 3.19 (1H, *m*, H-3″), 3.24 (1H, *m*, H-2″), 3.25 (1H, *m*, H-5″), 3.47 (1H, *m*, H-6″a), 3.61 (1H, *m*, H-6″b), 4.98 (1H, *d*, J = 8.2 Hz, H-1″), 5.93 (1H, *d*, J = 1.8 Hz, H-7), 6.05 (1H, *d*, J = 1.8 Hz, H-5), 6.55 (2H, *d*, J = 7.1 Hz, H-4′, H-6′), 6.92 (2H, *d*, J = 7.1 Hz, H-3′, H-7′);^{13}C-NMR (125 MHz, DMSO-d_6, δ / ppm): 40.4 (CH_2, C-1′), 60.3 (CH_2, C-6″), 69.2 (CH, C-4″), 73.0 (CH, C-2″), 76.7 (CH, C-3″), 77.1 (CH, C-5″), 91.7 (CH, C-7), 95.7 (CH, C-5), 99.2 (CH, C-1″), 101.9 (C, C-9), 105.5 (C, C-2), 114.7 (CH, C-4′, C-6′), 124.1 (C, C-2′), 131.3 (CH, C-3′, C-7′), 155.9 (C, C-5′), 156.7 (C, C-4), 168.4 (C, C-6), 171.9 (C, C-8), 192.7 (C, C-3).



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, *d*, *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).



Dehydrosoyasaponin I ***(10)***. White amorphous solid. $[\alpha]_D$ = –15.2 (*c* = 0.23 g mL^{-1}, MeOH). ^{1}H-NMR (600 MHz, CD_3OD, δ / ppm): 0.87 (3H, *s*, H-30), 0.92 (3H, *s*, H-25), 0.96 (1H, *m*, H-5), 1.00 (3H, *s*, H-26), 1.01 (3H, *s*, H-28), 1.03-1.66 (1H, *m*, H-1), 1.03 (3H, *s*, H-29), 1.12-2.17 (2H, *m*, H-16), 1.29 (3H, *s*, H-23), 1.30 (3H, *s*, H-27), 1.30 (3H, *d*, *J* = 8.6 Hz, H-6‴), 1.33-1.85 (2H, *m*, H-15), 1.34 (1H, *m*, H-19$_a$), 1.39–1.66 (2H, *m*, H-6), 1.40–1.68 (1H, *m*, H-7), 1.64 (1H, *m*, H-9), 1.13 (1H, *m*, H-2), 1.8 (1H, *m*, H-2), 1.92 (2H, *m*, H-11a, H--11b), 1.99 (1H, *m*, H-21), 2.59 (1H, *m*, H-21), 2.23 (1H, *t,* *J* = 13,8 Hz, H-19b), 2.37 (1H, *dd*, J_1 = 13.8, J_2 = 3.8 Hz, H-18), 3.23 (1H, *d*, *J* = 11.5 Hz, H-24a),

3.42 (1H, *dd*, J_1 = 10.3, J_2 = 4.5 Hz, H-3), 3.42 (1H, *t*, J = 9.6 Hz, H-4‴), 3.46 (1H, *t,* J = 9.2 Hz, H-4′), 3.51 (1H, *m*, H-5″), 3.56 (1H, *dd*, J_1 = 9.6; J_2 = 3.5 Hz, H-3″), 3.61 (1H, *d*, J = 9.2 Hz, H-5′), 3.62 (1H, *dd*, J_1 = 9.2, J_2 = 8.3 Hz, H-3′), 3.66 (1H, *dd*, J_1 = 9.6, J_2 = 7.5 Hz, H-2″), 3.72 (1H, *m*, H-6″b), 3.73 (1H, *nd*, H--3‴), 3.74 (1H, *nd*, H-4″), 3.76 (1H, *m*, H-6″a), 3.78 (1H, *d*, J = 8.3 Hz, H-2′), 3.94 (1H, *dd*, J_1 = = 3.3; J_2 = 1.6 Hz, H-2‴), 4.12 (1H, *m*, H-5‴), 4.16 (1H, *d*, J = 11.5 Hz, H-24b), 4.48 (1H, *d*, J = 8.3 Hz, H-1′), 4.90 (1H, *d*, J = 7.5 Hz, H--1″), 5.15 (1H, *d*, J = 1.6 Hz, H-1‴), 5.35 (2H, *t*, J = 3.5 Hz, H-12);^{13}C-NMR (150 MHz, CD_3OD, δ / ppm): 14.9 (CH_3, C-25), 15.9 (CH_3, C-26), 16.9 (CH_3, C-27, C-6‴), 18.0 (CH_2, C-6), 19.7 (CH_3, C-28), 22.0 (CH_3, C-23), 23.5 (CH_2, C-11), 24.2 (CH_3, C-30), 24.8 (CH_2, C-2), 25.7 (CH_2, C-15), 27.0 (CH_2, C-16), 30.8 (CH_3, C-29), 32.6 (CH_2, C-7), 33.7 (C, C-20), 36.1 (C, C-10), 38.3 (CH_2, C--1), 39.4 (C, C-8), 41.6 (C, C-14), 43.3 (C, C-4), 46.2 (CH_2, C-19), 47.6 (CH, C--18), 47.3 (CH, C-9), 48.0 (C, C-17), 50.3 (CH_2, C-21), 55.9 (CH, C-5), 60.8 (CH_2, C-6″), 62.9 (CH_2, C-24), 68.1 (CH, C-5‴), 70.3 (CH, C-3‴), 70.8 (CH, C--4″, C-2‴), 72.8 (CH, C-4′, C-4‴), 74.9 (CH, C-3″), 75.7 (CH, C-5″), 75.8 (CH, C-2′, C-5′), 76.9 (CH, C-3′), 77.8 (CH, C2″), 91.0 (CH, C-3), 100.7 (CH, C-1″), 100.9 (CH, C-1‴), 104.1 (CH, C-1′), 123.7 (CH, C-12), 141.4 (C, C13), 175.6 (C, COOH), 218.3 (CH, C-22). ESI-MS (*m/z*, (relative abundance, %)): 963 ($(C_{48}H_{76}O_{18}+Na)^+$, 100).

SPECTRUM DATA FOR COMPOUNDS **6** AND **8**

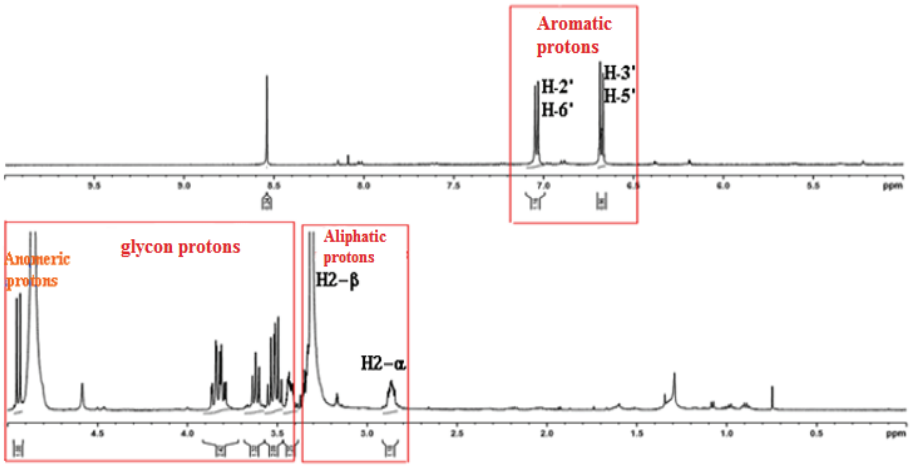


Fig. S-1. ^{1}H-NMR spectrum for compound **6**.

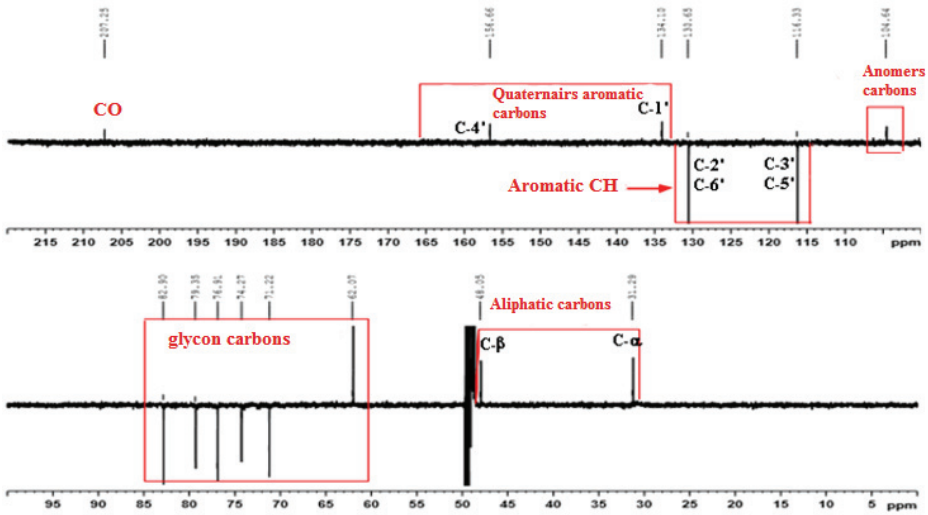


Fig. S-2. ^{13}C-NMR spectrum for compound **6**.

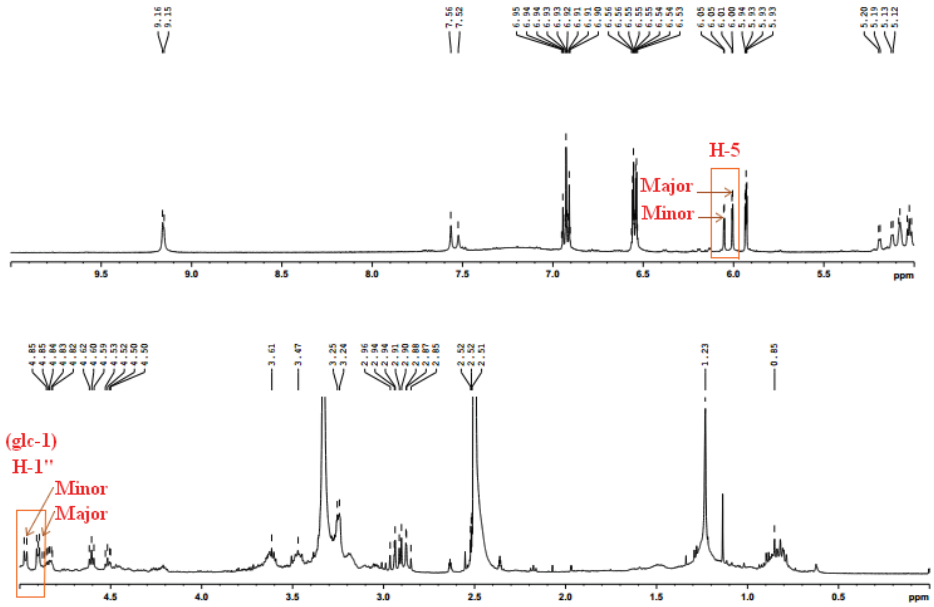


Fig. S-3. ^{1}H-NMR spectrum for compound **8**

DPPH RADICAL SCAVENGING ASSAY

The free radical scavenging activity of *n*-butanol extract of *Astragalus monspessulanus* L. was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to the procedure described by Saeed *et al.* 2012.[1] The stock solution was prepared by dissolving 2.5 mg DPPH in 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 a 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 the reference compound. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging activity, \%} = 100[(A_{\text{control}}–A_{\text{sample}})/A_{\text{control}}] \quad (1)$$

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

FERROUS IRON-CHELATING ASSAY

The ferrous iron-chelating (FIC) activity of *n*-butanol extract of *A. monspessulanus* L. was performed to determine the inhibition of the formation of iron(II)–ferrozine complex after treatment of test material with Fe^{2+}, according to the procedure described by Decker and Welch, 1990.[2] The reaction mixture (1.50 mL) contained 500 µL test material (*n*-butanol extract (0–35 mg) or $Na_2EDTA.\ 2H_2O$ (0–25 µg)), 100 µl $FeCl_2$ (0.6 mM in water) and 900 µL methanol. The control contained all the reaction reagents except the extract and EDTA. The mixture was incubated at room temperature for 5 min. Next, 100 µL of ferrozine (5 mM in methanol) was added, mixed thoroughly and left in the dark for a further 10 min to complex the residual Fe^{2+} ions. The absorbance of the solution was measured spectrophotometrically at $\lambda_{562\ \text{nm}}$ against a methanol blank. The percentage inhibition of ferrozine–Fe^{2+} formation was calculated, using Eq. (2):

$$\text{Chelating effect , \%} = \text{jsc}\ [1–(A_{\text{sample}}/A_{\text{control}})] \times 100 \quad (2)$$

The concentration of the extract/standard that chelated 50 % of the ferrous ion (EC_{50} / µg mL^{-1}) was calculated through linear interpolation between the values above and below 50 % activity.

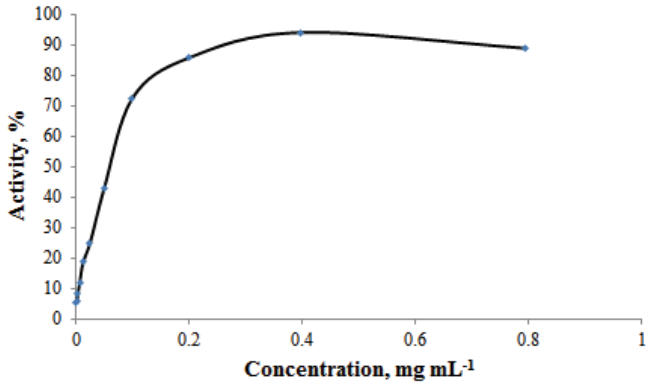


Fig. S-4. DPPH radical scavenging activity of *Astragalus monspessulanus* *n*-BuOH extract. The data is represented as the mean (n=3).

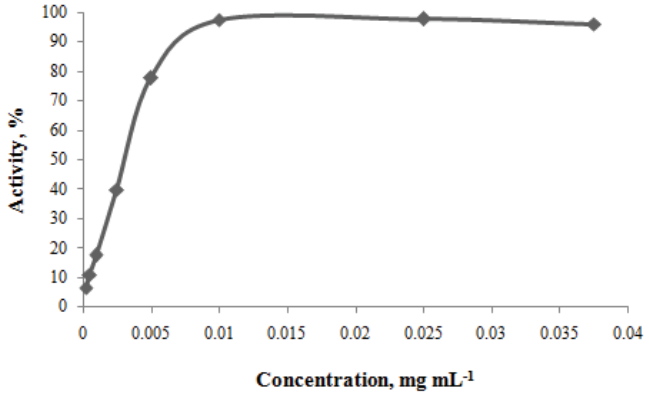


Fig. S-5. DPPH radical scavenging activity of ascorbic acid.

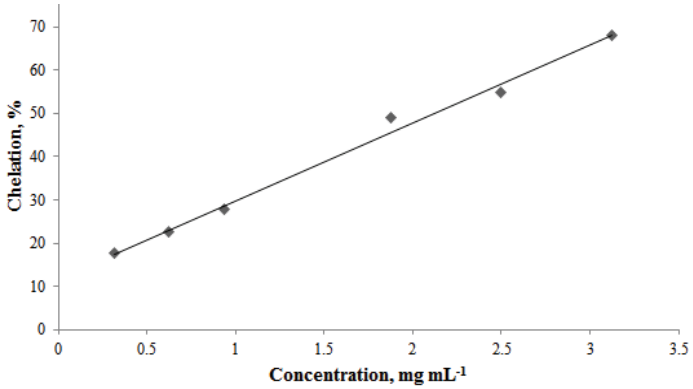


Fig. S-6. Ferrous iron-chelating activity of *Astragalus monspessulanus n*-BuOH extract. The data was represented as the mean (n=3).

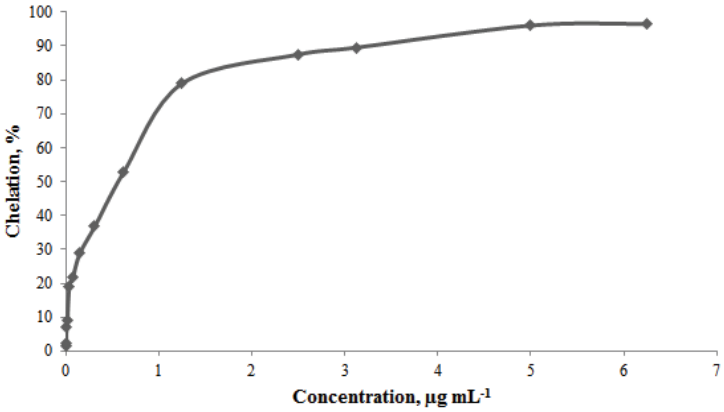


Fig. S-7. Ferrous iron-chelating activity of EDTA.

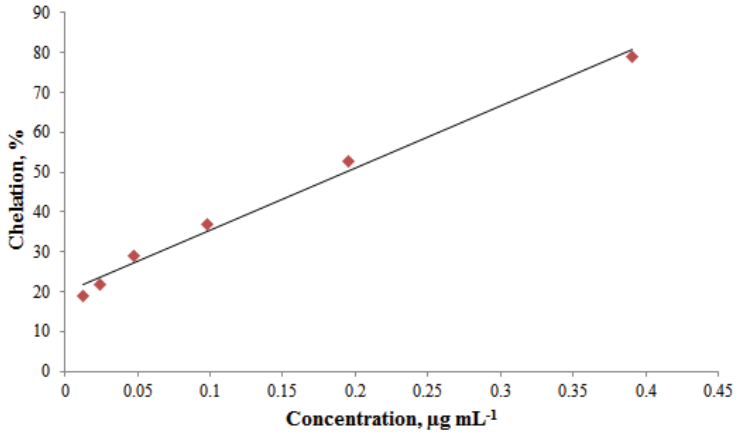


Fig. S8. Ferrous iron-chelating activity of EDTA – linear part.

REFERENCES

1. N. Saeed, M. R. Khan, M. Shabbir, *Off. J. Int. Soc. Comp. Med. Res. (ISCMR)* **12** (2012) 221
2. E. A. Decker, B. Welch, *J. Agric. Food Chem.* **36** (1990) 674.