

Supporting Information

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Astragaloside IV and Cycloastragenol Production Capacity of *Astragalus trojanus* Calli

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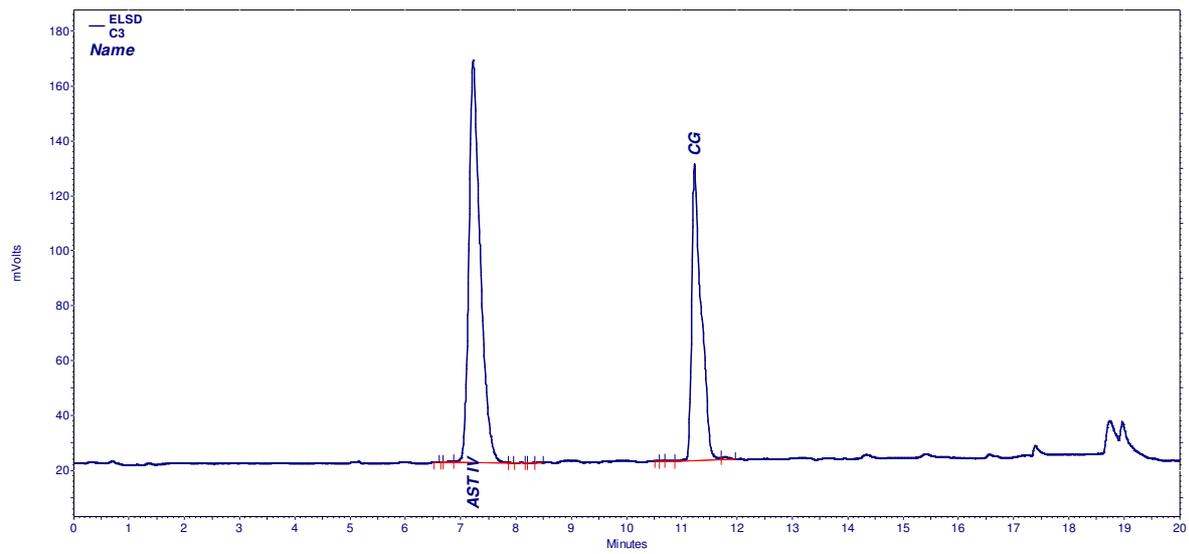
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S1. Sample Preparation and HPLC-ELSD Method

All the samples were extracted for 15 minutes with 5 mL HPLC grade methanol for 3 times. After sonication, samples were centrifuged until clear extracts were obtained. All of the clear extracts were combined and evaporated under vacuum. Evaporated samples were lyophilised to get dry extracts. Dried extracts were dissolved with HPLC grade methanol to obtain concentration of 5 mg/mL. All the sample solutions were passed through 0.45 nylon membrane filters prior to injections.

HPLC-ELSD analyses were performed on a Thermo Surveyor Plus instrument, equipped with quaternary pump, autosampler, column oven, diode array (Thermo Fisher Scientific, MA, USA) and Softa 300S ELSD detector (SoftA Corporation, CO, USA). For all separations a Thermo Hypersil GOLD RP (100x4.6 mm, 5 µm particle size; Thermo Fisher Scientific, MA, USA) HPLC column was used. LC separations were carried out using following solvents: water (A) and acetonitrile (B) and gradient elution was performed as: 0 min 72A/28B, in 5 min to 70A/30B, in 4 min to 38A/62B, in 3 min to 30A/70B hold for 3 min. Additionally, column was washed with 5A/95B for 2.5 min and prior to the next injection the column was equilibrated for 2.5 min with the beginning conditions. Detection was performed with ELSD detector with the settings as: 40°C at spray chamber, 70°C at operating chamber, 105°C at drift tub and N₂ pressure 50 psi. Flow rate was 2 mL/min, column temperature was 30°C and injection volume was 10 µL.

Two main compounds of *Astragalus* species, astragaloside IV (AST IV) and cycloastragenol (CA), were calibrated for quantitative analysis of samples. Standard stock solutions were prepared with methanol (2000 µg/mL) and additional six levels were prepared by dilution of stock solutions (1000 µg/mL, 500 µg/mL, 250 µg/mL, 100 µg/mL, 62.5 µg/mL, 25 µg/mL) with methanol (S2). Retention times for AST IV was 7.22 min and CA was 11.23 min. Regression coefficient for AST IV was 0.9958 and for CA was 0.9973.



S2. HPLC-ELSD chromatogram of AST IV and CA.

S3. ¹³C- ve ¹H-NMR spectral data of CA*

C/H	δ _C (ppm)	δ _H (ppm), J (Hz)
1	33.82 t	1.68 m, 1.73 m
2	30.85 t	1.65 m, 1.75 m
3	78.96 d	3.25 dd(11.2, 4.5)
4	42.77 s	
5	54.02 d	1.37 m
6	69.39 d	3.40 ddd (10.0, 10.0, 4.5)
7	38.57 t	1.40 m, 1.50 m
8	48.35 d	1.84 m
9	22.00 s	
10	30.00 s	
11	27.80 t	1.32 m, 1.55 m
12	32.98 t	1.61 m, 1.28 m
13	46.34 s	
14	47.00 s	
15	46.58 t	1.45 m, 2.00 m
16	74.26 d	4.69 ddd (8.0, 8.0, 5.2)
17	58.70 d	2.39 d (8.0)
18	21.57 q	1.30 s
19	31.98 t	0.42, 0.59 d (4.5)
20	88.10 s	
21	28.08 q	1.25 s
22	35.17 t	1.67 m, 2.65 m
23	26.13 t	2.06 m
24	82.52 d	3.79 dd (8.0, 7.0)
25	72.90 s	
26	26.32 q	1.16 s
27	27.24 q	1.26 s
28	28.37 q	1.30 s
29	15.61 q	0.93 s
30	20.36 q	1.03 s

*¹H-NMR: 600 MHz; ¹³C-NMR: 150 MHz, CD₃OD

S4. ¹³C- ve ¹H-NMR spectral data of AST IV*

C/H	δ_C (ppm)	δ_H (ppm), J (Hz)
1	32.66 t	1.29 m, 1.57 m
2	30.07 t	1.69 m, 1.96 m
3	89.77 d	3.21 dd (11.1, 4.5)
4	43.12 s	
5	52.93 d	1.64 m
6	79.86 d	3.57 ddd (10.0, 10.0, 4.5)
7	34.80 t	1.62 m, 1.93 m
8	46.21 d	1.89 m
9	22.11 s	
10	30.08 s	
11	26.63 t	1.37 m, 1.95 m
12	33.82 t	1.62 m, 1.70 m
13	46.45 s	
14	46.80 s	
15	45.87 t	1.42 m, 2.07 m
16	74.37 d	4.69 ddd (8.0, 8.0, 5.2)
17	58.82 d	2.40 d (8.0)
18	21.00 q	1.27 s
19	29.16 t	0.28 d, 0.62 d (4.5)
20	88.46 s	
21	28.19 q	1.23 s
22	35.19 t	1.66 dd (12.0, 1.5) 2.64 dd (12.0, 6.0)
23	26.44 t	2.06 m, 2.09 m
24	82.51 d	3.79 dd (8.0, 5.0)
25	72.50 s	
26	27.50 q	1.29 s
27	26.00 q	1.16 s
28	28.19 q	1.31 s
29	16.34 q	1.04 s
30	19.77 q	1.05 s
1'	107.00 d	4.31 d (7.6)
2'	75.50 d	3.21 dd (7.6, 8.5)
3'	77.71 d	3.32 t (8.5)
4'	70.96 d	3.49 m
5'	66.31 t	3.20 t (11.0) 3.85 dd (11.0, 4.0)
1'	104.79 d	4.36 d (7.8)
2'	75.32 d	3.26 dd (9.0, 7.8)
3'	78.43 d	3.35 t (9.0)
4'	71.50 d	3.29 t (9.0)
5'	77.53 d	3.26 ddd (9.0, 9.0, 4.5)
6'	62.60 t	3.67 dd (12.0, 4.5) 3.87 dd (12.0, 3.5)

*¹H-NMR: 600 MHz; ¹³C-NMR: 150 MHz, CD₃OD.