Supplementary Data

Oligosaccharides from *Asparagus cochinchinensis* for ameliorating LPS-induced acute lung injury in mice

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Table S1. Raw samples of Asparagi Radix investigated in this work.

Sample No.	Source	Sample no	Source
S1	Tongren, Guizhou	S11	Linghai, Liaoning
S2	Kaili, Guizhou	S12	Guangzhou, Guangdong
S3	Dafang, Guizhou	S13	Guizhou
S4	Sansui, Guizhou	S14	Emei, Sichuan
S5	Xingyi, Guizhou	S15	Chengdu, Sichuan
S6	Guizhou	S16	Hezhou, Guangxi
S7	Beijing	S17	Nanning, Guangxi
S8	Ganzhou, Jiangxi	S18	Chongqing
S9	Nanchang, Jiangxi	S19	Anguo, Hebei
S10	Changsha, Hunan	S20	Ezhou, Hubei

Table S2. Method validation data: precision, stability, repeatability.

Peak No.	Precision (n=6)		Stability (n=6)		Repeatability (n=6)	
	RSD of RT (%)	RSD of PA (%)	RSD of RT (%)	RSD of PA (%)	RSD of RT (%)	RSD of PA (%)
1	0.246	0.850	0.124	0.916	0.199	0.759
2	0.083	1.521	0.074	1.066	0.084	0.551
3	0.016	1.982	0.024	2.041	0.030	1.742
4	0.032	0.377	0.027	1.083	0.035	1.870
5	0.007	0.677	0.014	1.116	0.015	3.908
6	0.004	0.950	0.020	1.236	0.020	3.764
7	0.005	3.254	0.013	0.233	0.027	3.407
8	0.019	3.327	0.020	0.766	0.079	3.263
9	0.014	2.733	0.023	0.836	0.013	3.945
10	0.007	2.704	0.016	1.010	0.018	4.630
11	0.013	1.947	0.021	1.679	0.013	4.351
12	0.008	1.054	0.018	2.981	0.032	4.614
13	0.023	0.542	0.036	3.340	0.024	4.695
14	0.010	0.727	0.034	4.699	0.018	4.778
15	0.025	0.796	0.033	4.717	0.017	4.212
16	0.009	1.158	0.034	4.025	0.023	4.758
17	0.011	1.258	0.044	4.412	0.017	4.745
18	0.023	1.325	0.032	4.878	0.022	4.020

Table S3. Similarity evaluation of precision test, stability test and repeatability test.

Precision test	1	2	3	4	5	6	Reference fingerprint
1	1.000	0.972	0.926	0.989	0.968	0.983	0.989
2	0.972	1.000	0.972	0.969	0.985	0.974	0.993
3	0.926	0.972	1.000	0.931	0.938	0.954	0.967
4	0.989	0.969	0.931	1.000	0.956	0.982	0.987
5	0.968	0.985	0.938	0.956	1.000	0.954	0.982
6	0.983	0.974	0.954	0.982	0.954	1.000	0.990
Reference fingerprint	0.989	0.993	0.967	0.987	0.982	0.990	1.000
Stability test	1	2	3	4	5	6	Reference fingerprint
1	1.000	0.983	0.985	0.957	0.974	0.986	0.991
2	0.983	1.000	0.987	0.955	0.986	0.993	0.994
3	0.985	0.987	1.000	0.958	0.974	0.981	0.991
4	0.957	0.995	0.958	1.000	0.967	0.970	0.977
5	0.974	0.986	0.974	0.967	1.000	0.991	0.992
6	0.986	0.993	0.981	0.970	0.991	1.000	0.997
Reference fingerprint	0.991	0.994	0.991	0.977	0.992	0.997	1.000
Repeatability test	1	2	3	4	5	6	Reference fingerprint
1	1.000	0.960	0.908	0.896	0.910	0.964	0.980
2	0.960	1.000	0.850	0.872	0.848	0.994	0.959
3	0.908	0.850	1.000	0.896	0.988	0.842	0.955
4	0.896	0.872	0.896	1.000	0.895	0.873	0.944
5	0.910	0.848	0.998	0.895	1.000	0.844	0.955
6	0.94	0.994	0.842	0.873	0.844	1.000	0.958
Reference fingerprint	0.980	0.959	0.955	0.944	0.955	0.958	1.000

Table S4. The similarities of Asparagi Radix from different batches.

Sample No.	Similarity	Sample No.	Similarity
S1	0.733	S11	0.813
S2	0.940	S12	0.925
S3	0.969	S13	0.928
S4	0.940	S14	0.813
S5	0.969	S15	0.837
S6	0.930	S16	0.960
S7	0.984	S17	0.995
S8	0.981	S18	0.913
S9	0.978	S19	0.983
S10	0.979	S20	0.983

Table S5. Eigenvalue and variance contribution rate of three main component factors.

	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
PC1	8.688	48.269	48.269
PC2	6.166	34.255	82.524
PC3	1.563	8.682	91.206

Table S6. Loadings of three principal component factors.

Peak No.	Score of PC1	Score of PC2	Score of PC3
1	-2.40638	-2.02306	2.36401
2	-4.43693	-0.42716	2.11400
3	-5.74931	1.49778	0.97175
4	-5.91597	2.77622	0.25125
5	-5.11347	2.94140	-0.08369
6	-5.04143	3.30692	0.47276
7	-4.29186	4.21033	-0.18952
8	-3.46337	4.67543	-0.26659
9	-1.70097	5.47911	-0.18944
10	0.45089	5.58178	-0.14695
11	2.82241	4.84210	0.07483
12	4.09941	4.52194	0.13808
13	5.21291	3.74843	0.35271
14	5.93734	2.86956	0.54230
15	6.44892	1.91407	0.64679
16	6.65567	1.10405	0.67453
17	6.65800	0.45176	0.66899
18	6.51737	-0.06614	0.48394

Table S7. Scores of three principal component factors.

Sample No.	Score of PC1	Score of PC2	Score of PC3
S1	-1.98908	-5.58178	-0.10784
S2	-0.50510	-2.62799	0.37208
S3	-0.83796	-1.10223	-0.98648
S4	-1.97857	-1.8676	-1.32539
S5	-1.58307	-0.11836	2.36401
S6	2.85206	3.80159	-0.37817
S7	0.53024	0.34670	1.68751
S8	2.94687	4.07526	2.12938
S9	-0.14242	0.82057	-0.47822
S10	-0.46916	3.23603	-0.09876
S11	3.35429	-1.66584	-1.47932
S12	-3.83931	1.02353	-0.74628
S13	4.61327	-2.59128	-0.48261
S14	2.41966	-1.69342	1.22619
S15	6.65800	-1.38776	-0.01741
S16	-1.92181	2.83458	-1.59688
S17	-1.42082	1.49828	-0.50314
S18	-5.21163	-1.61433	2.15512
S19	-1.98908	-5.58178	-0.10784
S20	-0.50510	-2.62799	0.37208

Fig.S1. Twenty batches of Asparagi Radix raw material samples (S1–S20).

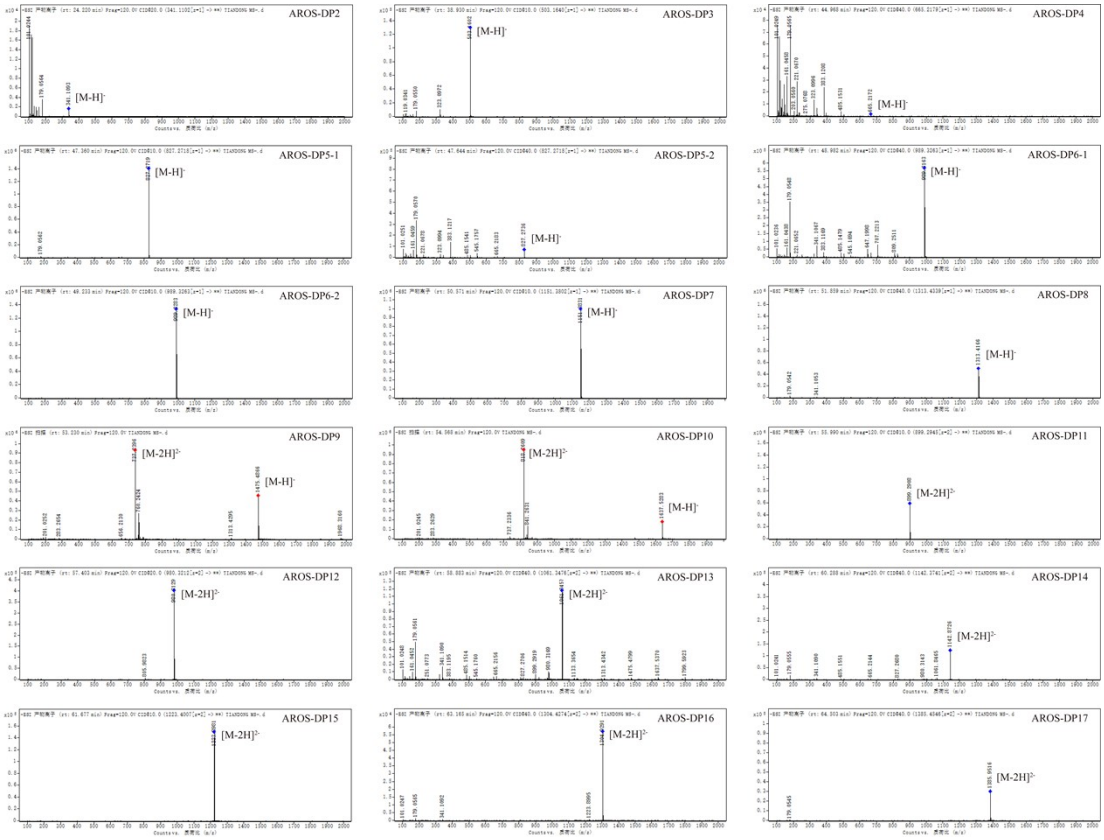

Fig.S2. HPLC-QTOF-MS analysis of AROS.

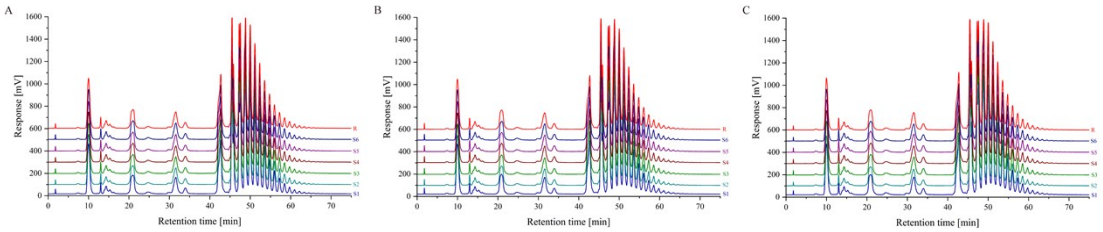

Fig.S3. Method validation of HPLC-ELSD chromatogram analysis. HPLC-ELSD chromatograms of precision test (A), stability test (B), and repeatability test (C).

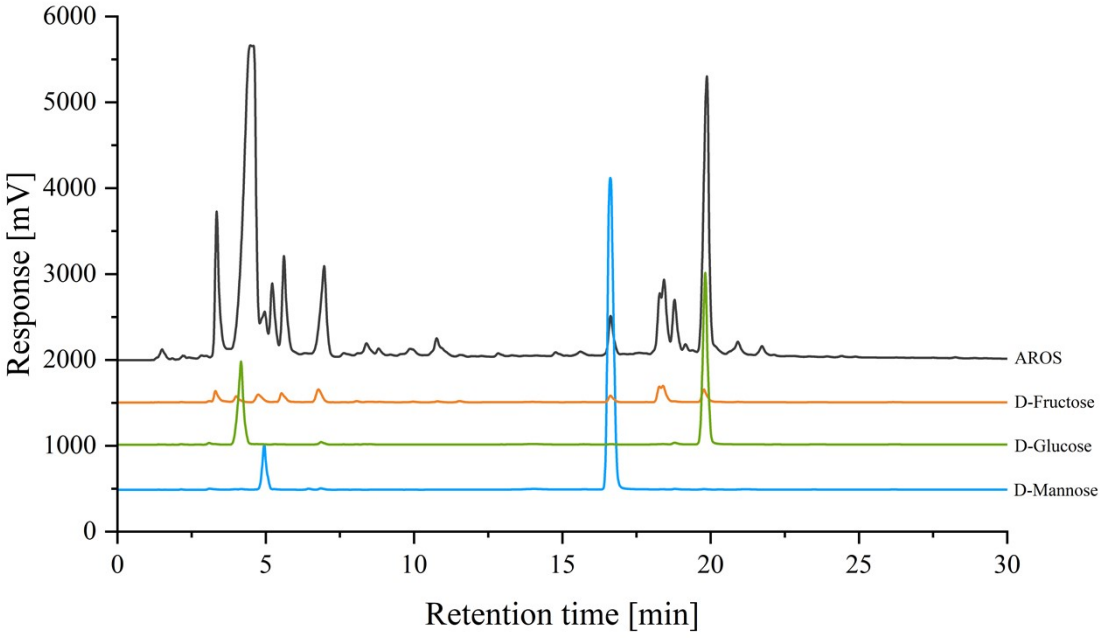

Fig.S4. Chromatogram of PMP derivatives of trifluoroacetic acid hydrolysates of AROS.

In this study, the monosaccharide composition in AROS was determined by HPLC using pre-column derivatization with PMP. The chromatography was analyzed using an Agilent 1260 Infinity II LC system (California, United States) equipped with an Agilent ZORBAX Extend-C18 column (4.6 × 150 mm，5 μm) with a flow rate of 1.0 mL/min. The mobile phase was composed of 20 mM ammonium acetate in deionized water as phase A and acetonitrile as phase B. The column temperature was set at 30 ˚C. The gradient elution program was set as follows: 0-30 min, 5 % B - 95 % B. The DAD detector was set at 254 nm.

Oligosaccharide samples were mixed with 2.5 mL trifluoroacetic acid (TFA) solution (2 M) and hydrolyzed for 4 h at 80 ˚C in an oven. Then, the mixed solution was cooled to room temperature, a small amount of methanol was added several times, and dried at 55 ˚C to remove excess TFA. The resulting monosaccharides were dissolved in deionized water.

Then, 400 μL of hydrolysis was mixed with an isometric ammonia solution and PMP-methanol (0.5 M) solution. The mixture was incubated at 70 ˚C for 2h in a water bath, cooled to room temperature and neutralized with 400 μL of glacial acetic acid. Chloroform was added and mixed vigorously, and the chloroform layer was carefully removed. The extraction process was repeated three times to remove the PMP residues. Finally, the aqueous layer was filtered through a 0.22 μm filter before further analysis.