

SUPPLEMENTARY MATERIAL

Screening of anti-fatigue active ingredients of *Eleutherococcus senticosus* via spectrum-effect relationship based on factor analysis and LC-MS/MS

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Abstract

ES contains compounds known to have significant anti-fatigue activity. In recent years, it has received extensive attention because it's efficient. However, its active ingredients on antifatigue are still unclear. This study attempts to establish the spectrum-effect relationship of *ES* antifatigue to screen the effective components. The results showed that the similarity of 15 *ES* fingerprints obtained by LC-MS/MS was 0.533~0.992, and the chemical structures of 22 common peaks were identified. The anti-fatigue activity of 15 batches of *ES* was characterized by forced swimming test of mice and quantified by CAFI, among which S4, S1 and S5 had better activity. 9 components (caffeic acid, 5-(4-*O*- β -D-glucosylferoyl)-quinic acid, (+/-)13-HODE, isofraxidin, eleutheroside E, syringin, pinoresinol diglucoside or its isomer, 7,8-dihydrodehydrocarbinol alcohol-4-*O*- β -D-glucoside, secoisolariciresinol-4-*O*- β -D-glucoside) highly related to anti-fatigue activity may be the effective components of *ES*.

Keywords: *Eleutherococcus senticosus*; spectrum-effect relationship; anti-fatigue; factor analysis; forced swimming test of mice; LC-MS/MS

Table S1 Similarity calculation results of HPLC fingerprint of *ES*

No.	Similarity	No.	Similarity	No.	Similarity
S1	1	S6	0.533	S11	0.835
S2	0.918	S7	0.981	S12	0.980
S3	0.992	S8	0.655	S13	0.992
S4	0.977	S9	0.923	S14	0.951
S5	0.584	S10	0.984	S15	0.990

Note: S1 was taken as the reference chromatogram

1 **Table S2** Characterization results of common peaks in the fingerprint of *ES*

RT No. (min)	Adductor ion (<i>m/z</i>)	Neutral mass (<i>m/z</i>)	Error (ppm)	Molecular formula	MS ² Fragment ion	Compound	Chemical class
P1 2.96	203.0371[M+Na] ⁺	203.0526	7.8	C ₆ H ₁₂ O ₆	-	D-(+)-Glucose	Monosaccharide
P2 3.30 7	341.1098[M-H] ⁻	341.1089	-3.8	C ₁₂ H ₂₂ O ₁₁	341.1159 ;281.095; 179.0592 ;119.0367	<i>ES</i> disaccharide	Disaccharide
P3 4.46 2	191.0187[M-H] ⁻	191.0197	-5.23	C ₆ H ₈ O ₇	191.0253 ;129.0224	Citric acid	Organic acid
P4 6.1	128.0343[M-H] ⁻	128.0353	-7.8	C ₅ H ₇ NO ₃	128.0393	DL-Pyroglutamic Acid	Phenolic acid
P5 13.1	153.0183[M-H] ⁻	153.0193	-6.5	C ₇ H ₆ O ₄	153.0224	Protocatechuic acid	Phenolic acid
P6 18.1	529.1562[M-H] ⁻	529.1563	-0.19	C ₂₃ H ₃₀ O ₁₄	367.1164 ;191.949;173.049 0	5-(4- <i>O</i> -β-D-glucosylferoyl)-quinic acid	Organic acid
P7 21.0	417.1401[M+COOH] ⁻	417.140	-0.24	C ₁₇ H ₂₄ O ₉	371.1425 ;208.0855;	Syringin	Phenylpropanoi

			2		129.9780		d	
P8	23.9	353.0871[M-H] ⁻	353.087 8	-1.98	C ₁₆ H ₁₈ O ₉	191.0601 ;179.0384 ; 135.0468	Chlorogenic acid	Phenolic acid
P9	26.6	179.0337[M-H] ⁻	179.035 0	-4.7	C ₉ H ₈ O ₄	179.0388 ;135.0474	Caffeic acid	Phenolic acid
P1 0	29.9	353.0871[M-H] ⁻	353.087 8	-2.0	C ₁₆ H ₁₈ O ₉	191.0598 ;135.0494;	Chlorogenic acid isomer	Phenolic acid
P1 1	36.3	727.2454[M+COOH] ⁻	727.245 5	-0.1	C ₃₂ H ₄₂ O ₁ 6	-	Pinoresinol diglucoside or its isomer	Lignans
P1 2	36.5	757.2567[M+COOH] ⁻	757.256 1	-0.8	C ₃₃ H ₄₄ O ₁ 7	-	Mediresinol Di- <i>O</i> -β-D-glucopyranoside	Lignans
P1 3	36.8	787.2656[M+COOH] ⁻	787.266 6	-1.3	C ₃₄ H ₄₆ O ₁ 8	741.2791 ;579.2220 ; 417.1657	Eleutheroside E	Lignans
P1 4	38.7	523.2185[M-H] ⁻	523.218 5	0	C ₂₆ H ₃₆ O ₁ 1	361.1744	Secoisolariciresinol-4- <i>O</i> -β-D-glucoside	Lignans
P1 5	39.3	221.0451[M-H] ⁻	221.045 5	-1.8	C ₁₁ H ₁₀ O ₅	206.0261 ;191.0024	Isofraxidin	Coumarin

P1 6	39.5	567.2079[M+COOH] ⁻	567.208 3	-0.7	C ₂₆ H ₃₄ O ₁ 1	567.2217 ;359.1598 ; 329.1463	7,8-dihydrodehydrocarbinol alcohol-4- <i>O</i> -β-D-glucoside	Phenylpropanoi d
P1 7	40.2	515.1195[M-H] ⁻	515.119 5	0	C ₂₅ H ₂₄ O ₁ 2	471.1137 ;353.0962 ; 335.0846 ;191.0600 ; 179.0391	Isochlorogenic acid B	Phenolic acid
P1 8	41.2	723.5019~723.5094 [M+COOH] ⁻ 679.5217[M+H] ⁺	-	-	-	677.5134	Not identified	-
P1 9	41.8	445.0777[M-H] ⁻	445.077 6	0.2	C ₂₁ H ₁₈ O ₁ 1	269.0526 ;175.0286	Apigenin-7- <i>O</i> -β-D glucuronide	Flavone
P2 0	52.5	647.3806[M-H] ⁻	647.380 1	-0.7	C ₃₆ H ₅₆ O ₁ 0	-	Hederagenin-3- <i>O</i> -β-D-glucuronopyranosi de	Coumarin
P2 1	53.4	763.4270[M-H] ⁻	763.427 4	-0.5	C ₄₁ H ₆₄ O ₁ 3	-	Momordin Ic	Saponin
P2 2	55.6	-	-	-	-	-	Not identified	-

P2 3	61.9	295.2275[M-H] ⁻	295.227 9	-1.4	C ₁₈ H ₃₂ O ₃	295.2351 ;277.2239 ; 195.1427 ;123.1216	(+/-)13-HODE	Organic acid
P2 4	64.8	347.1691~347.1706[M] ⁻	-	-	-	301.1744	Not identified	-
P2 5	71.8	271.2267[M-H] ⁻	271.227 9	-4.4	C ₁₆ H ₃₂ O ₃	225.2275	3-hydroxyhexadecanoic acid	Organic acid

Table S3 Composition score coefficient matrix

Index	Common factor 1	Common factor 2
Exhaustion time	0.330	-0.094
BUN	-0.185	0.392
LA	0.136	0.193
LG	0.233	0.058
MG	0.347	-0.216
ATP	0.068	0.203
SOD	-0.092	0.255
MDA	0.055	0.234

Table S4 Correlation degree and ranking of GRA

Peaks	Correlation degree	Ranking
P1	0.907	14
P2	0.751	24
P3	0.905	16
P4	0.925	8
P5	0.941	2
P6	0.928	6
P7	0.927	7
P8	0.911	12
P9	0.907	13
P10	0.923	9
P11	0.944	1
P12	0.827	21
P13	0.932	5
P14	0.907	15
P15	0.939	4
P16	0.941	3
P17	0.898	17
P18	0.889	18

P19	0.818	22
P20	0.714	25
P21	0.918	10
P22	0.834	20
P23	0.917	11
P24	0.857	19
P25	0.804	23

Table S5 Information of 15 batches of *ES* from different regions

No.	Regions	Harvest time	No.	Regions	Harvest time
S1	Hulun Buir, Inner Mongolia	2020.11	S9	Tonghua, Jilin	2020.09
S2	Xiaoxing'an Mountain, Heilongjiang	2020.12	S10	Shenyang, Liaoning	2020.10
S3	Yichun, Heilongjiang	2020.12	S11	Benxi, Liaoning	2020.11
S4	Harbin, Heilongjiang	2020.12	S12	Lvliang, Shanxi	2021.03
S5	Mudanjiang, Heilongjiang	2020.11	S13	Jiaozuo, Henan	2021.03
S6	Jilin, Jilin	2020.11	S14	Bozhou, Anhui	2021.04
S7	Antu, Jilin	2020.11	S15	Wuxue, Hubei	2021.03
S8	Jingyu, Jilin	2020.10	-	-	-

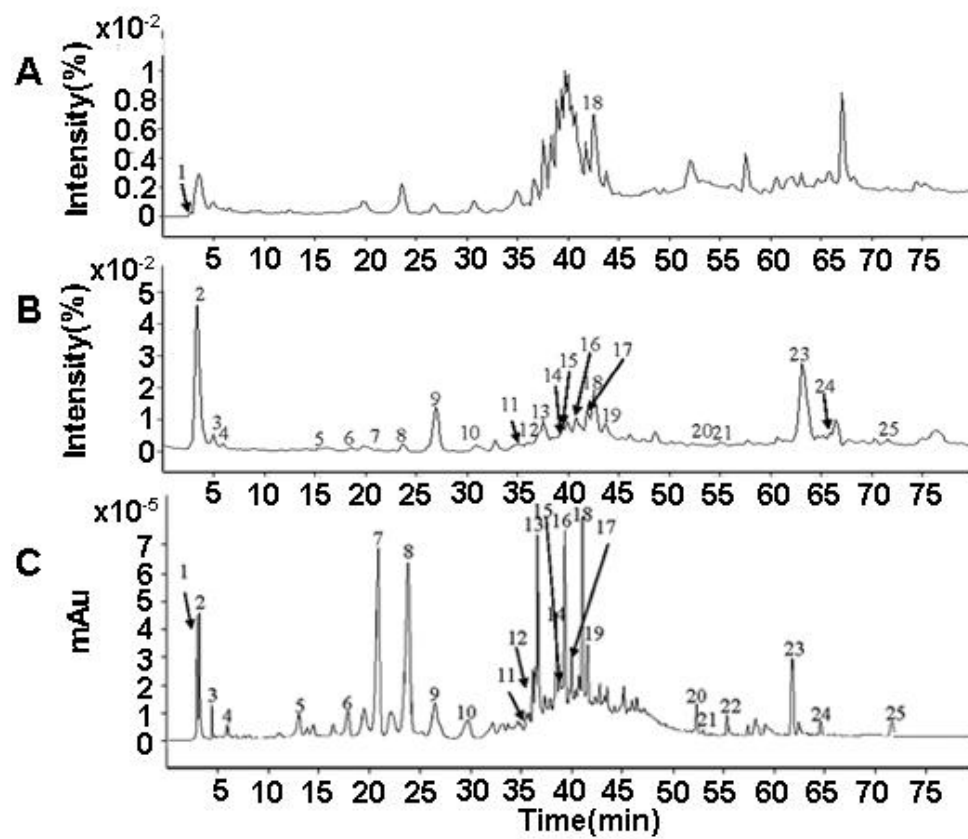


Fig. S1. TIC chromatogram of *ES* in positive ion mode (A), TIC chromatogram of *ES* in negative ion mode (B) and HPLC chromatogram of *ES* (C)

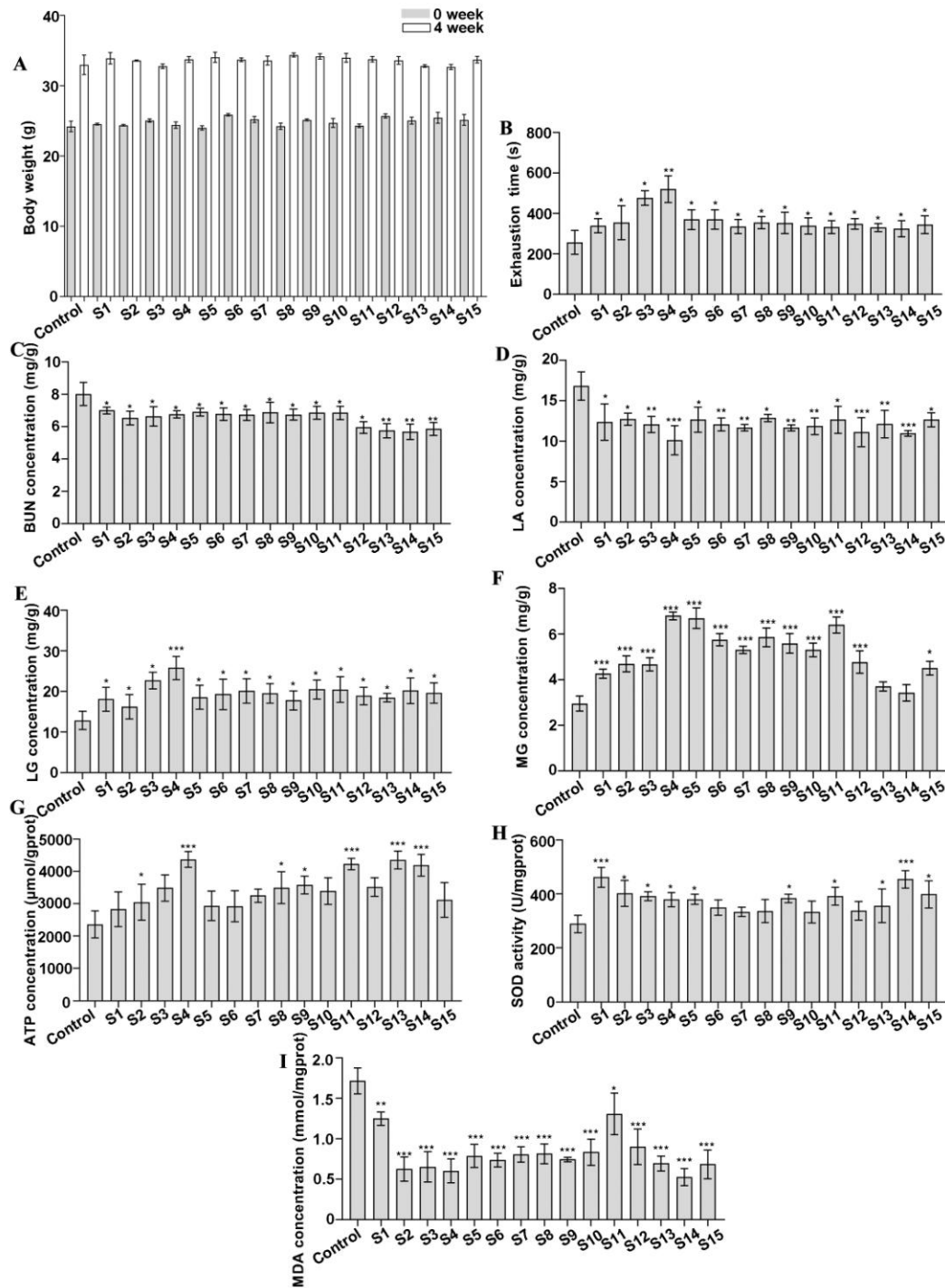


Fig. S2 Effects of *ES* from different regions on body weight (A), exhaustion time (B), BUN (C), LA (D), LG (E), MG (F), ATP (G), SOD (H) and MDA (I) in mice. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs Control group)

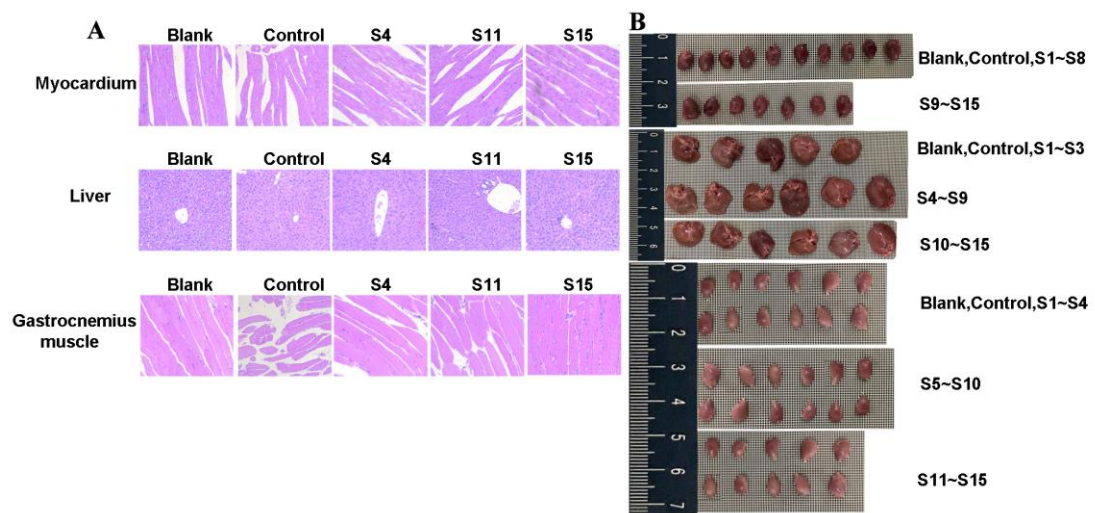


Fig. S3. H&E staining of myocardium, liver and gastrocnemius muscle in mice ($\times 200$) (A), macro anatomy diagram of mice (B).