Supporting information

Anti-Aging Potential of Caffeoyl-Spermidine Derivatives from *Lycium ruthenicum* Murr.: Insights from UPLC-QTOF-MS/MS, *Caenorhabditis elegans*, and Mechanistic Studies

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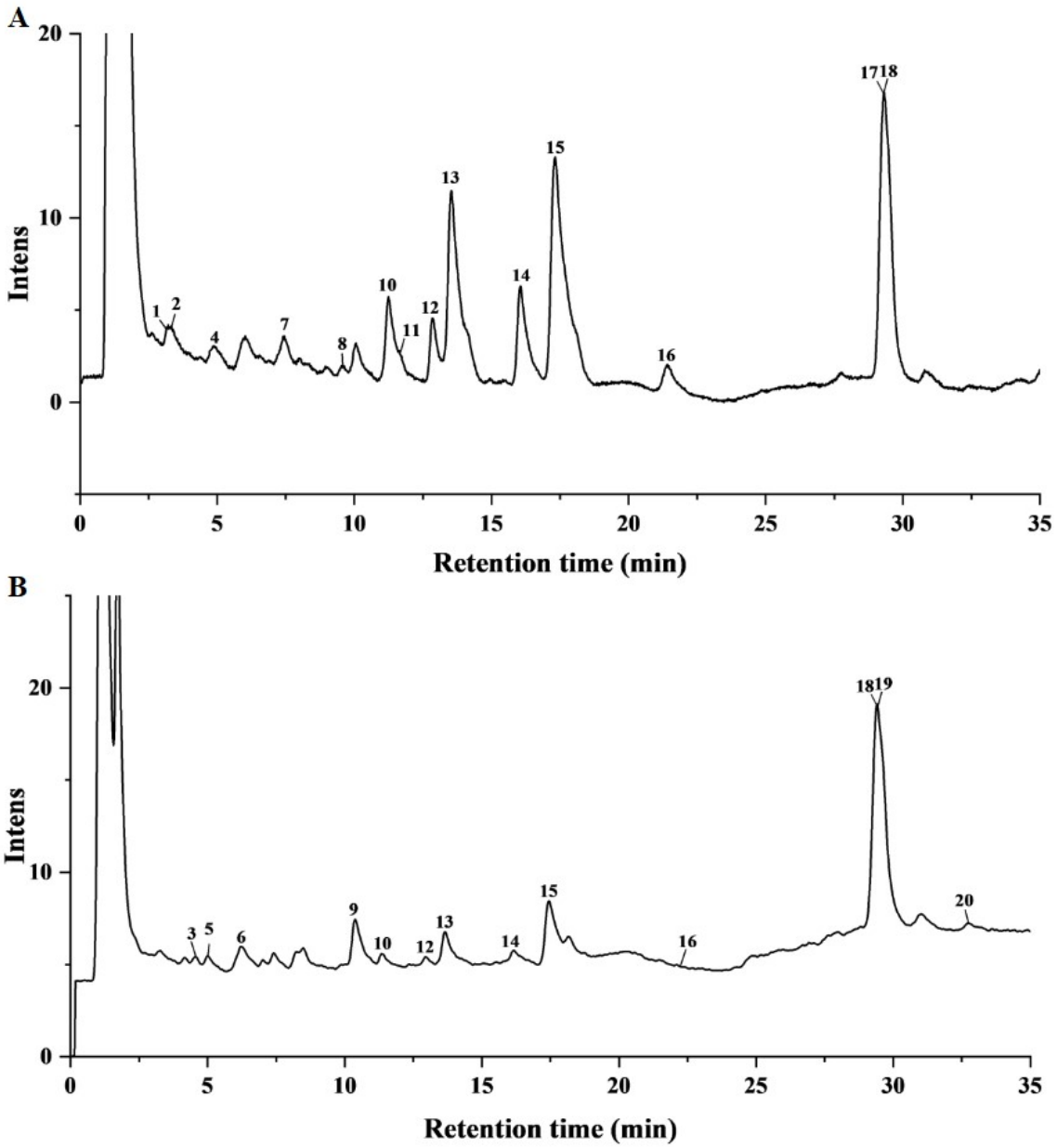


Figure S1. The total ion chromatogram (TIC) of *Lycium ruthenicum* Murr. extract obtained by UPLC-QTOF-MS/MS: (A) Positive ion mode; (B) Negative ion mode.

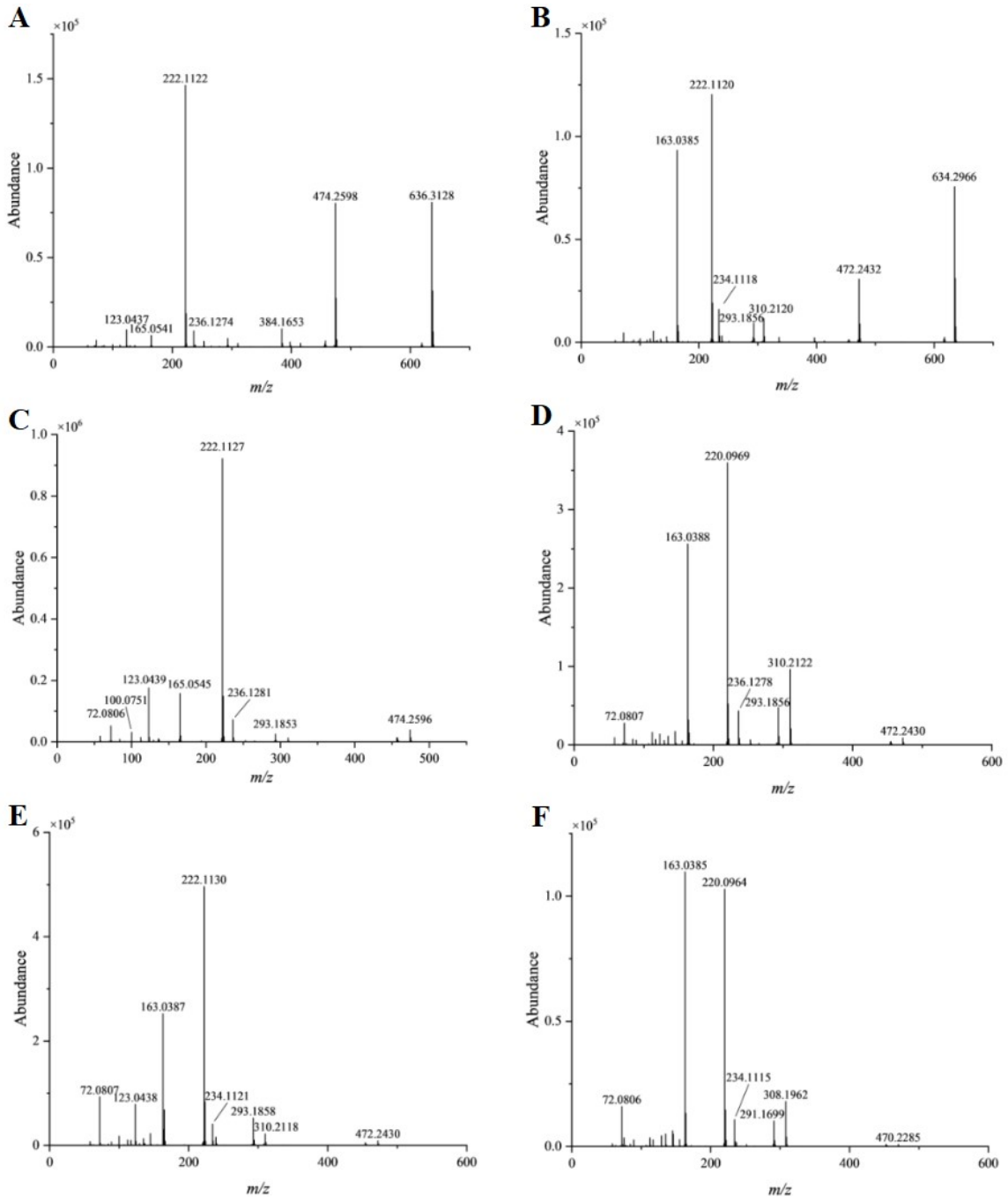


Figure S2. The MS/MS spectra of six main caffeoyl-spermidine derivatives from *Lycium ruthenicum* Murr. extract obtained by UPLC-QTOF-MS/MS analysis in positive ion mode: (A) C10 (*m/z* 636, N1,N10-bis- (dihydrocaffeoyl) spermidine hexoside), (B) C12 (*m/z* 634, N1-dihydrocaffeoyl-N10-caffeoyl-spermidine-hexoside), (C) C13 (*m/z* 474, N1,N10-bis-(dihydrocaffeoyl) spermidine), (D) C14 (*m/z* 472, N1-caffeoyl-N10-dihydrocaffeoyl spermidine), (E) C15 (*m/z* 472, N1-dihydrocaffeoyl-N10-caffeoyl-spermidine), and (F) C16 (*m/z* 470, N1,N10-dicaffeoyl-spermidine).

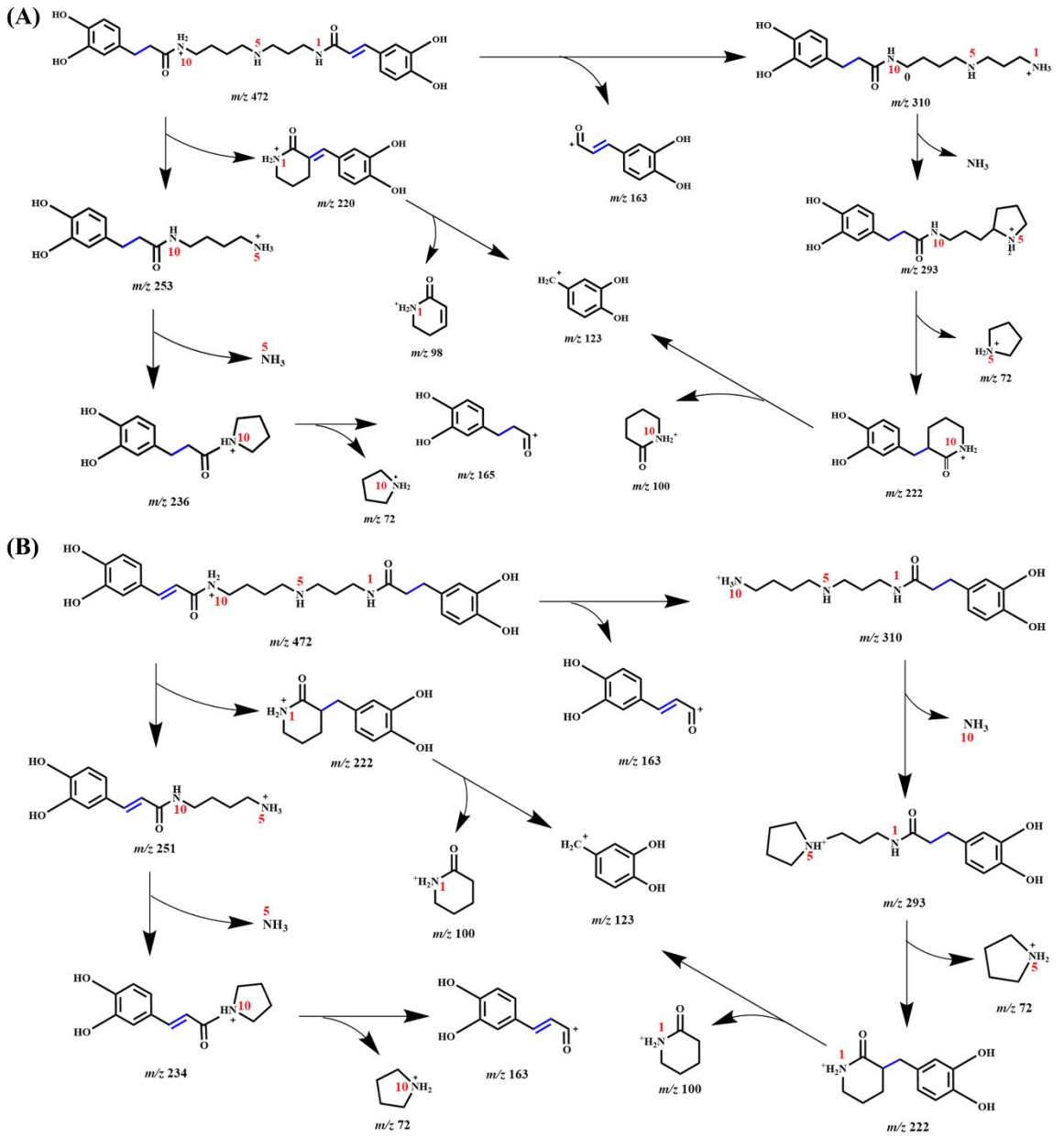


Figure S3. Proposed mass spectrometric fragmentation pathways for (A) compound **C14** (N1-caffeoyl, N10-dihydrocaffeoyl spermidine, *m/z* 472 $[M+H]^+$) and (B) compound **C15** (N1-dihydrocaffeoyl, N10-caffeoyl spermidine, *m/z* 472 $[M+H]^+$), derived from UPLC-QTOF-MS/MS in positive ion mode.

NMR data of N1,N10-dicaffeoyl spermidine standard (C16)

^{1}H NMR δ_H (600 MHz; D_2O): 1.57-1.61 (2 H, m, -CH_2-), 1.65-1.69 (2 H, m, -CH_2-), 1.86-1.91 (2 H, m, -CH_2-), 3.00-3.04 (4 H, m, -CH_2-), 3.26 (2 H, t, *J* 6.7 Hz, -CH_2-), 3.34 (2 H, t, *J* 6.5 Hz, -CH_2-), 6.26-6.32 (2 H, m, =CH-), 6.79-6.82 (2 H, m, =CH-), 6.94 (2 H, d, *J* 8.2 Hz, =CH-), 7.00-7.01 (2 H, m, =CH-), 7.24-7.29 (2 H, m, =CH-).

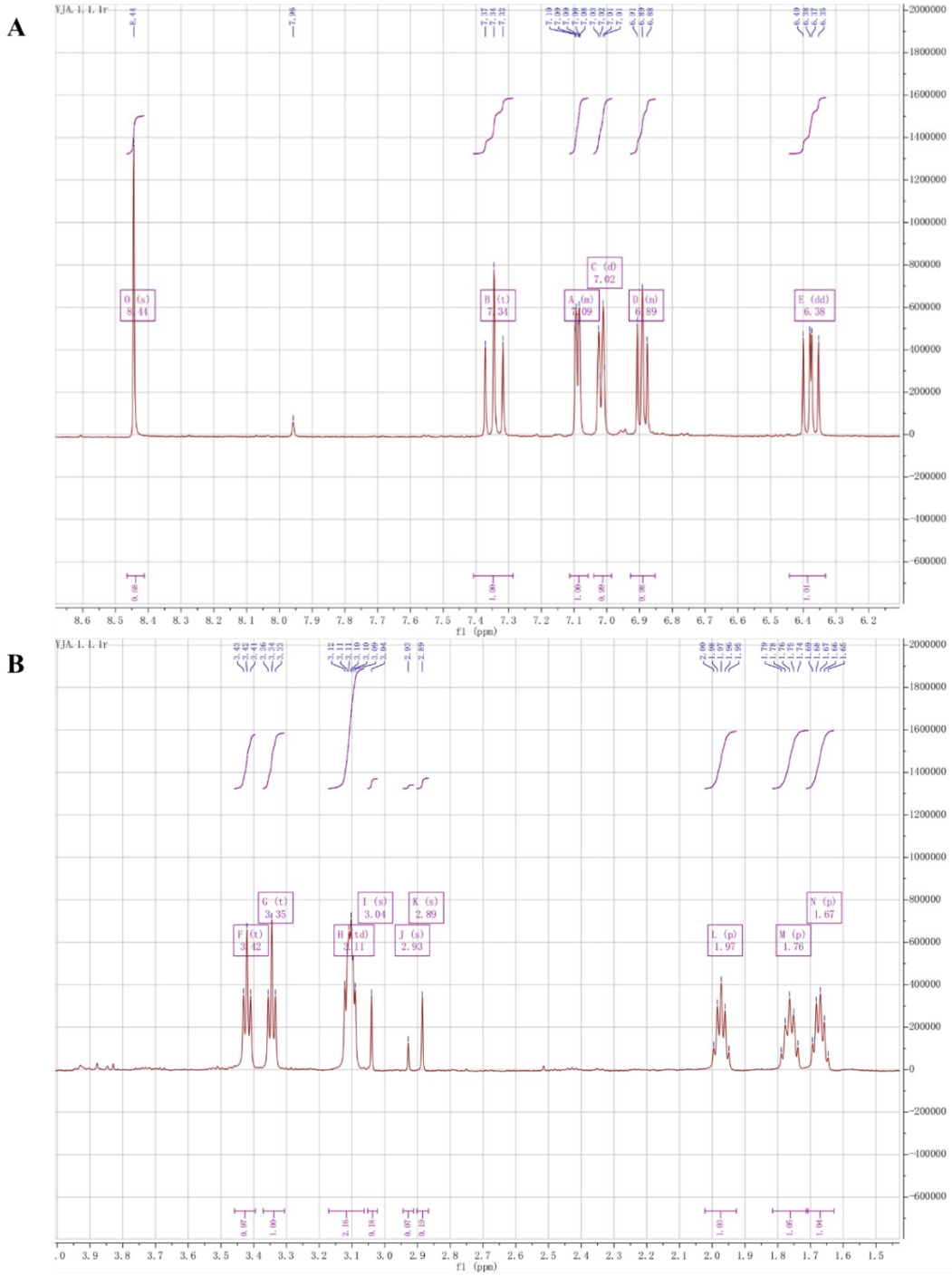


Figure S4. The ^{1}H NMR spectrum of the standard compound **C16** (N1,N10-dicaffeoyl-spermidine), with (A) region from 6.0 to 8.6 ppm and (B) from 1.4 to 4.0 ppm.

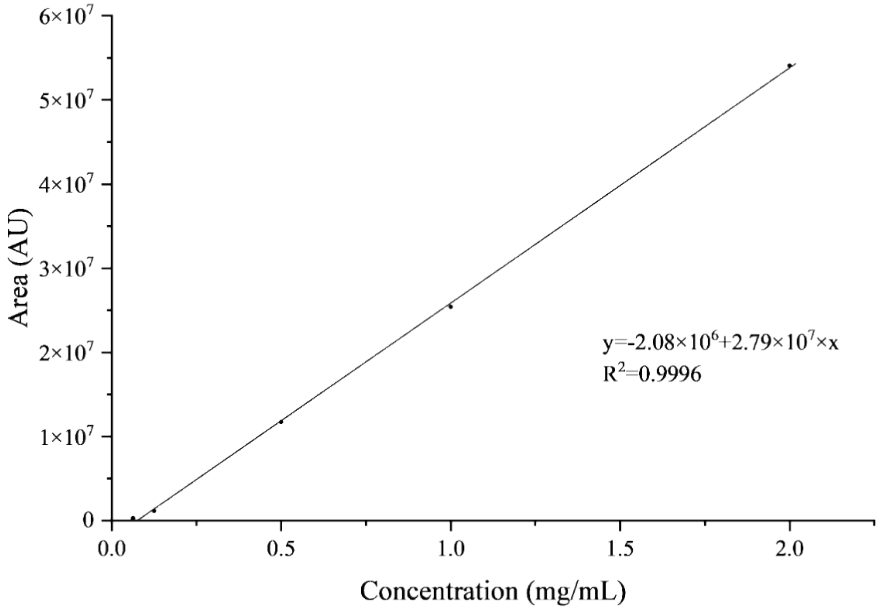


Figure S5. Linear standard curve of the standard compound **C16** (N1,N10-dicaffeoyl-spermidine)

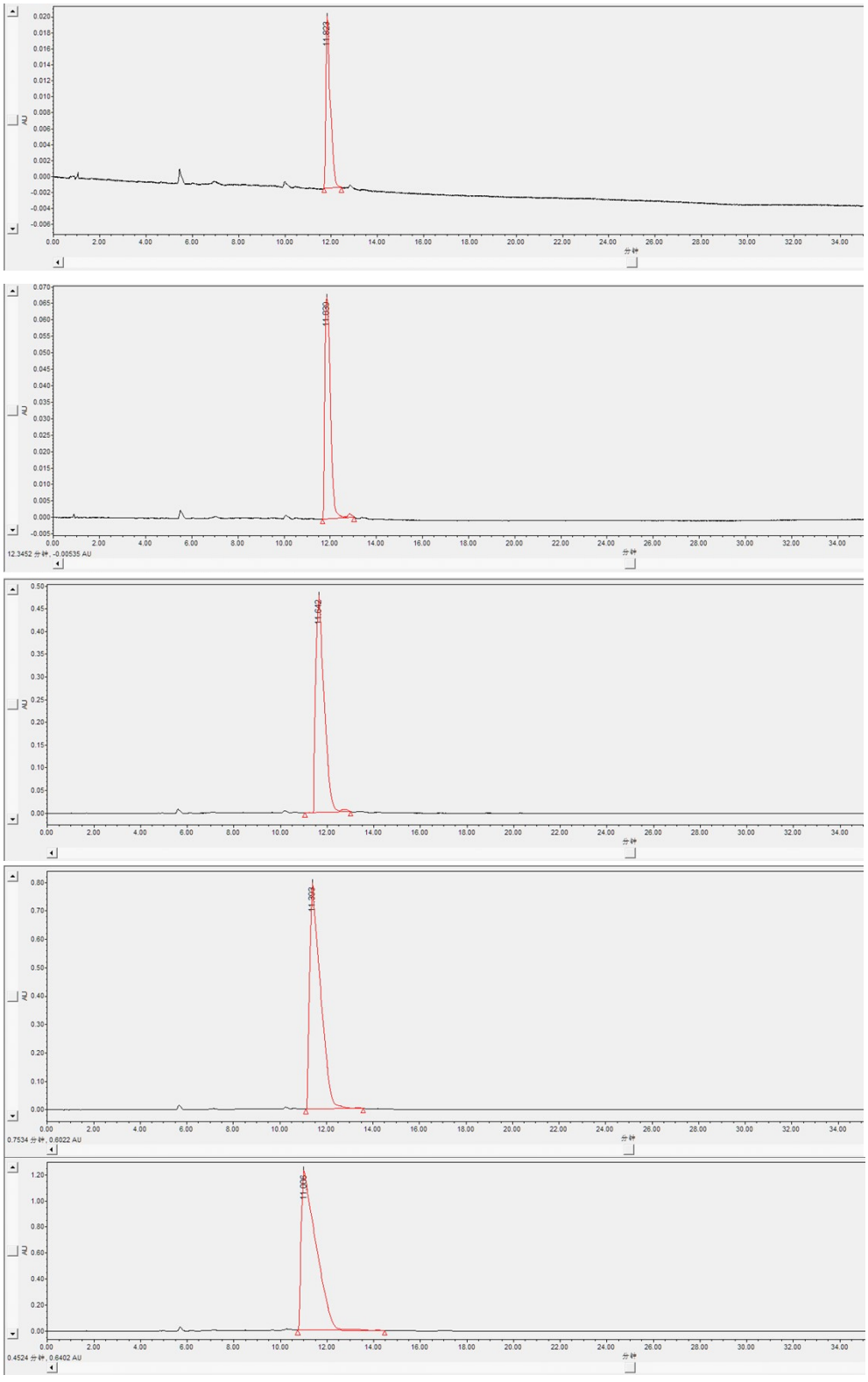


Figure S6. Raw HPLC chromatograms used to construct the calibration curve for compound C16. From top to bottom, the chromatograms correspond to concentrations of 0.0625 mg/mL, 0.125 mg/mL, 0.5 mg/mL, 1 mg/mL, and 2 mg/mL, respectively. The detection wavelength was 280 nm.

Figure S7. The *Lycium ruthenicum* Murr. extract powder after being freeze-dried.

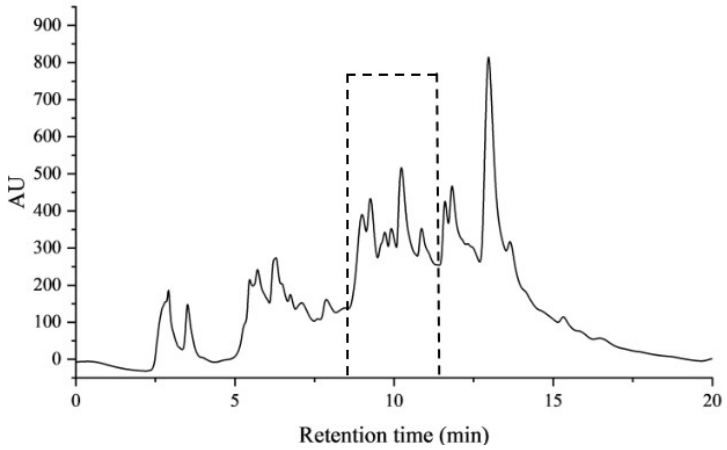


Figure S8. The enriched caffeoyl-spermidine derivatives eluting between 7.9 and 11.6 minutes in the semi-preparative HPLC process from *Lycium ruthenicum* Murr. extracts.

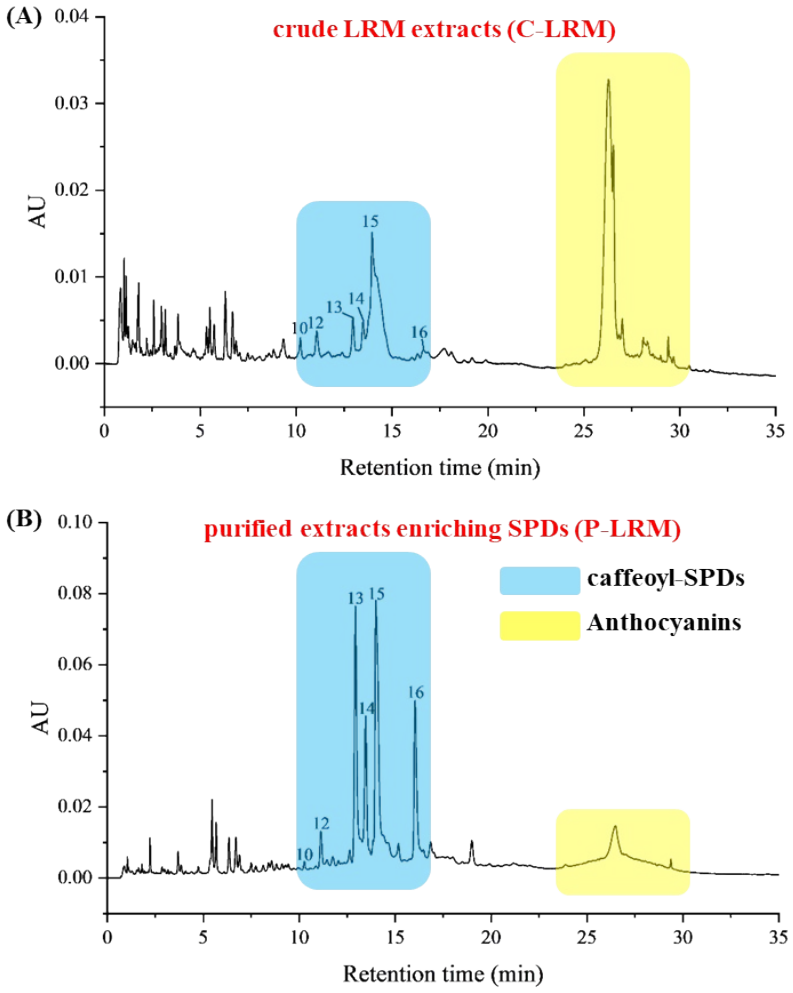
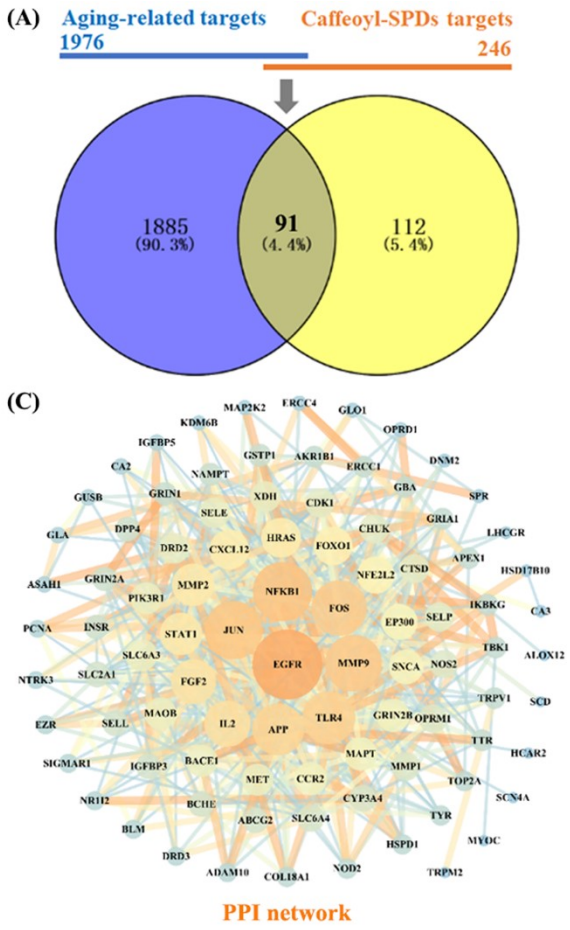


Figure S9. UPLC chromatograms of (A) crude *Lycium ruthenicum* Murr. (LRM) extracts (C-LRM) and (B) purified extracts enriched in caffeoyl-spermidine derivatives (P-LRM). The blue fraction corresponds to caffeoyl-spermidine derivatives, while the yellow fraction is enriched in anthocyanins.



(B) Top 20 targets ranked by degree value

Gene	Protein name	Uniprot ID	Degree
EGFR	Epidermal growth factor receptor	P00533	46
NFKB1	Nuclear factor NF-kappa-B p105 subunit	P19838	38
JUN	Transcription factor Jun	P05412	37
MMP9	Matrix metalloproteinase-9	P14780	36
FOS	Protein c-Fos	P01100	35
TLR4	Toll-like receptor 4	O00206	33
APP	Amyloid-beta precursor protein	P05067	32
IL2	Interleukin-2	P60568	28
FGF2	Fibroblast growth factor 2	P09038	27
MMP2	72 kDa type IV collagenase	P08253	25
HRAS	GTPase HRas	P01112	23
CXCL12	Stromal cell-derived factor 1	P48061	23
FOXO1	Forkhead box protein O1	Q12778	22
NFE2L2	Nuclear factor erythroid 2-related factor 2	Q16236	21
EP300	Histone acetyltransferase p300	Q09472	20
SNCA	Alpha-synuclein	P37840	19
MAPT	Microtubule-associated protein tau	P10636	18
GRIN2B	Glutamate receptor ionotropic, NMDA 2B	Q01097	18
CCR2	C-C chemokine receptor type 2	P41597	18

Figure S10. (A) A total of 91 intersection targets were obtained through Venn diagram. (B) The list of top 20 targets ranked by degree value. (C) The protein-protein interaction (PPI) network of 91 targets.

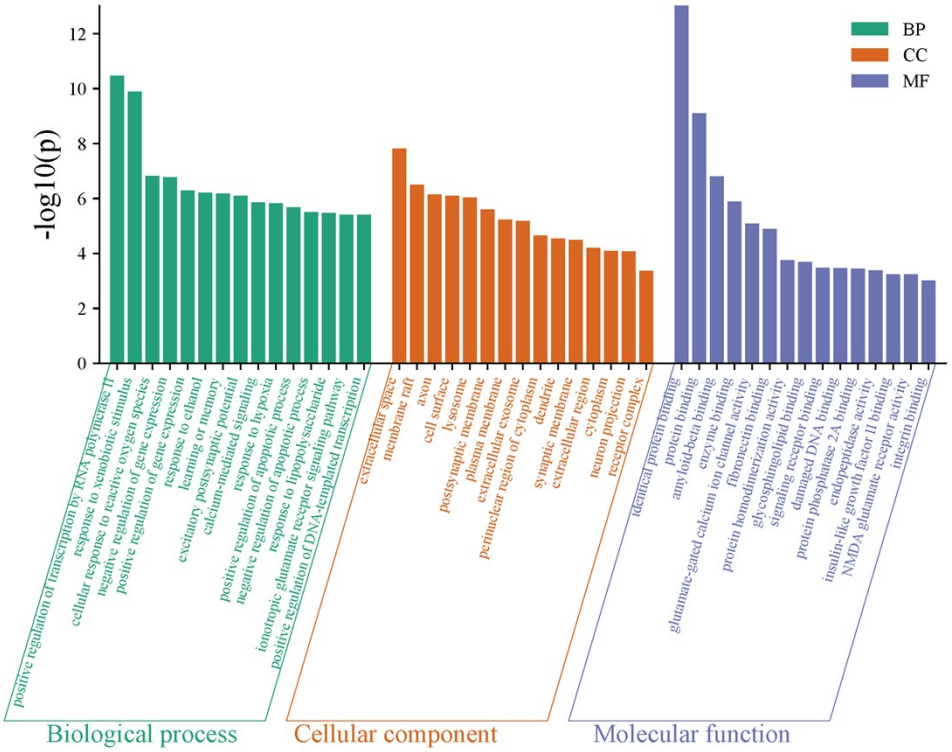


Figure S11. Gene Ontology (GO) functional analysis of the 91 target genes illustrating enriched biological processes (BP), molecular functions (MF), and cellular components (CC).

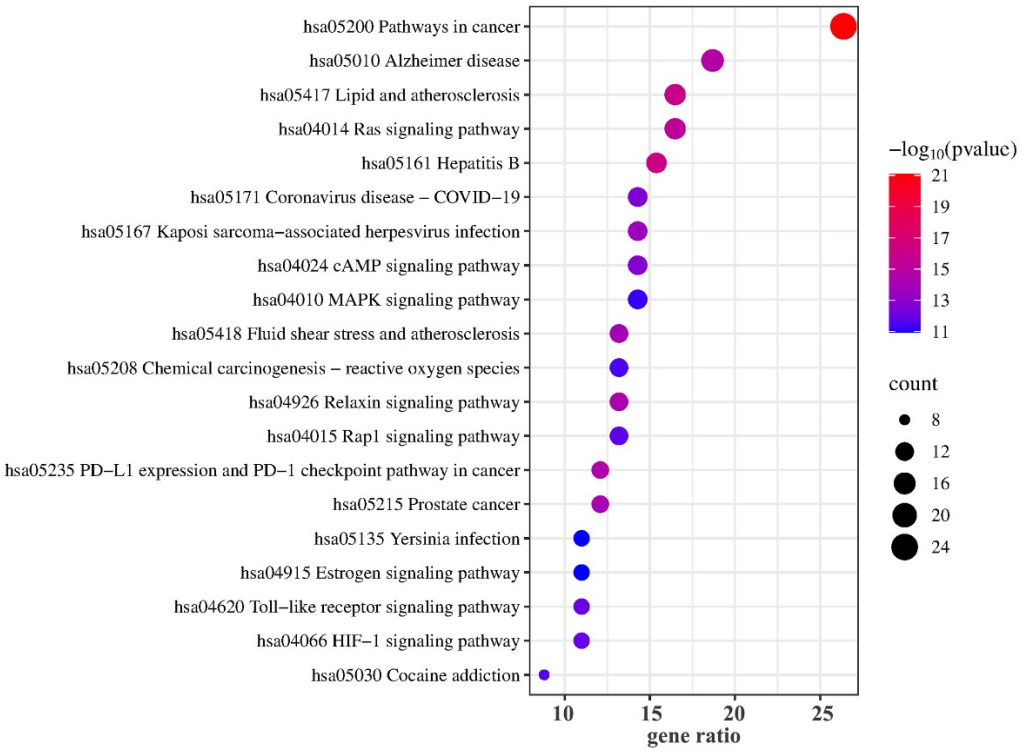


Figure S12. KEGG pathway enrichment analysis of the top 20 enriched pathways.

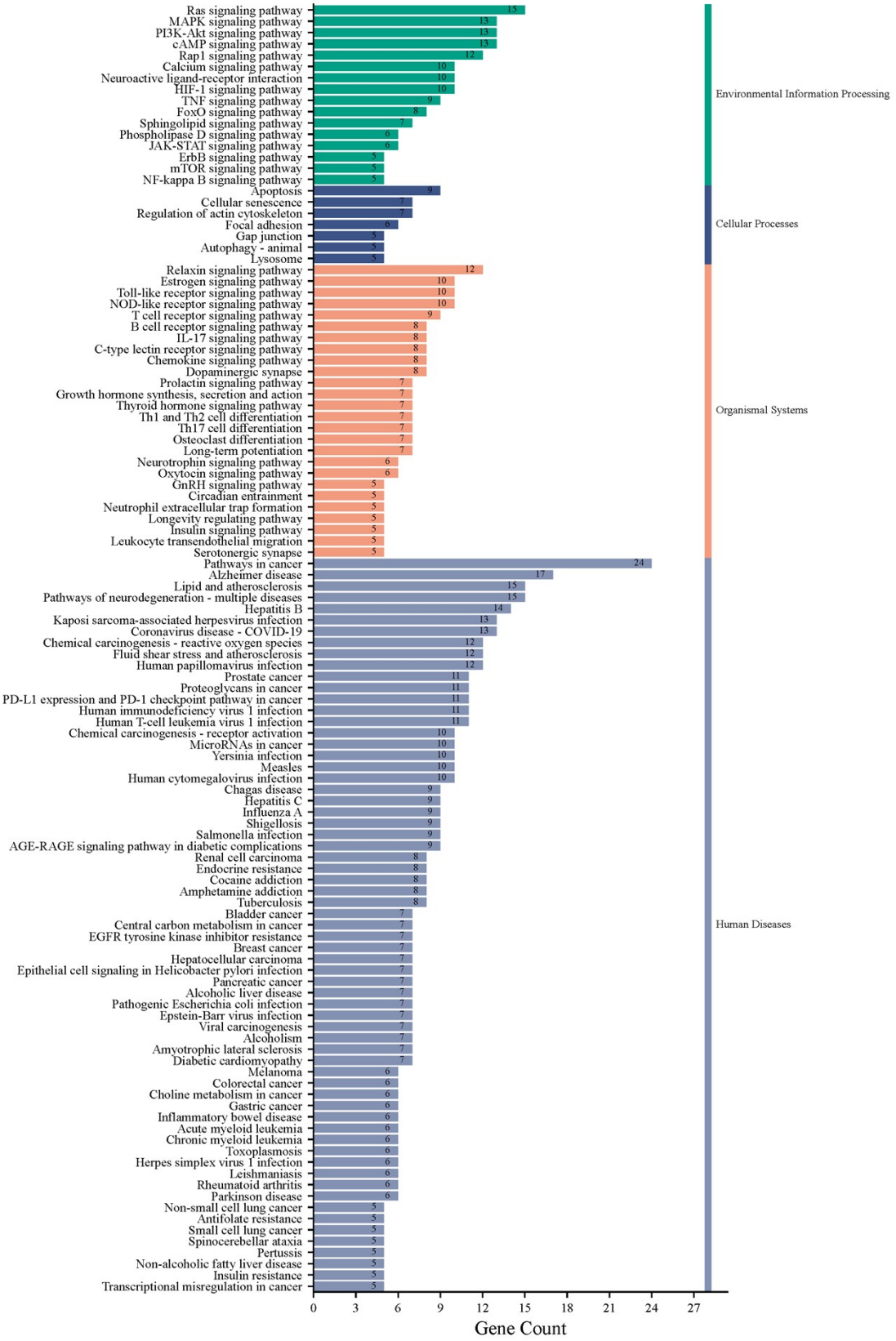


Figure S13. KEGG enrichment analysis of 114 pathways that containing ≥ 5 targets.

Table S1. Primers used for quantitative RT-PCR Analysis.

Gene	Human gene	Frontier primer	Reverse primer
daf-2	INSR	GGTGGAAGGTTCGCTGACAATC	CGTCTTGGGAATATCTTGGCTGTAG
daf-16	FOXO1	GAGTTGAACAGTGTCCGTGGATC	TAGTGGCATTGGCTTGAAGTTAGTG
alp-1	APP	CACACTTTGAACAGATACCGTCA	GTTGATGCGAAGATCGATGTAG
ptl-1	MAPT	GTAAATGTGAAGAGCAAAGTGGGAAG	TTTGGATTGGGCGTTGTAGAGTC
let-60	HRAS	ATCAGCACTCACCATTCAACTCATC	AGGCATGTCTCACCGTCTATCAC
mek-2	MAPK2P2	AGATTGAACTGGCTGATAGTCTTGAAG	AGATTGAACTGGCTGATAGTCTTGAAG
actin-1	Target gene	CCACGAGACTTCTTACAACTCCATC	CTTCATGGTTGATGGGGCAAGAG

Table S2. Six selected pathways and their associated genes identified through network pharmacology analysis of caffeoyl-spermidine derivatives from *Lycium ruthenicum* Murr.

Pathway Name	Associated Genes
Alzheimer's Disease	ADAM10, APP, CHUK, GRIN1, GRIN2A, GRIN2B, HSD17B10, HRAS, INSR, MAPT, NFKB1, NOS2, PIK3R1, MAP2K2, SNCA, IKBKG, BACE1
MAPK Signaling Pathway	CHUK, EGFR, FGF2, FOS, HRAS, INSR, JUN, MAPT, MET, NFKB1, MAP2K2, VEGFA, IKBKG
PI3K-Akt Signaling Pathway	CHUK, EGFR, FGF2, HRAS, IL2, INSR, MET, NFKB1, PIK3R1, MAP2K2, TLR4, VEGFA, IKBKG
TNF Signaling Pathway	CHUK, FOS, JUN, MMP9, NFKB1, PIK3R1, SELE, IKBKG, NOD2
Insulin Signaling Pathway	FOXO1, HRAS, INSR, PIK3R1, MAP2K2
Apoptosis	CHUK, CTSD, FOS, HRAS, JUN, NFKB1, PIK3R1, MAP2K2, IKBKG

Table S3. Top 10 genes most frequently shared across pathways identified in the network pharmacology analysis of caffeoyl-spermidine derivatives from *Lycium Ruthenicum* Murr., with their respective pathway involvement.

Rank	Gene	Pathways	Pathways Involved
1	CHUK	5	Alzheimer's, MAPK, PI3K-Akt, TNF, Apoptosis
2	HRAS	5	Alzheimer's, MAPK, PI3K-Akt, Insulin, Apoptosis
3	IKBKG	5	Alzheimer's, MAPK, PI3K-Akt, TNF, Apoptosis
4	MAP2K2	5	Alzheimer's, MAPK, PI3K-Akt, Insulin, Apoptosis
5	NFKB1	5	Alzheimer's, MAPK, PI3K-Akt, TNF, Apoptosis
6	PIK3R1	5	Alzheimer's, PI3K-Akt, TNF, Insulin, Apoptosis
7	FOS	4	MAPK, PI3K-Akt, TNF, Apoptosis
8	INSR	4	Alzheimer's, MAPK, PI3K-Akt, Insulin
9	JUN	4	MAPK, PI3K-Akt, TNF, Apoptosis
10	EGFR	2	MAPK, PI3K-Akt