Systematical studies on the in vivo substance basis and the pharmacology mechanism of Acanthopanax Senticocus Harms leaves by a PLC-Q-TOF-MS coupled with a target-network method

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Description S1: 20kg dried ASL was extracted 2 times, for the first time, it was extracted under reflux in 240 L of 60% ethanol for 2 h, and second times, it was extracted under reflux in 200 L of 60% ethanol for 2 h. The decoctions were combined, and then concentrated in vacuum to 20 L at 60 ℃ and the final concentration of the obtained stock solution of ASL was 1 g / mL. 10L of the stock solution was separated and purified by the DM-130 macroporous resin, which was firstly eluted with 4BW distilled water, and then the active ASL sites was eluted with 4BW 40% ethanol at a speed of 2 BV/h, the eluent was collected and freeze-dried.

Table S1 Metabolic pathway settings

Mass	Formular	Description
+2.0157	+H2	Reduction
+15.9949	+O	Hydroxylation
+18.0106	+H2O	Hydration
+14.0157	+CH2	Methylation
-2.0157	-H2	Desaturation
+1.9793	-CH2+O	Demethylation+hydroxylation
+13.9793	+O-H2	Hydroxylation+desaturation
-4.0313	-H4	Two sequential desaturation
-12.0364	-C2H4+O	Ethyl to alcohol
-14.0157	-CH2	Demethylation
-27.9949	-CO	Decarbonylation
-29.9742	+O2+H2	Nitro reduction
-30.0106	-CH2O	Hydroxymethylene loss
-44.9977	-COOH	Decarbonylation
+176.0321	+C6H8O6	Glucuronide conjugation
+192.027	+C6H8O7	Hydroxylation+glucuronide conjugation
+289.0732	+C10H15N3O5S	Glutathione conjugation
-0.0364	-CH4+O	2-Ethoxyl to acid
+17.9742	-CH2+O2	Demethylation+2*hydroxylation
+15.9585	-CH4+O2	Ethyl to carboxylic acid
+29.9742	-H2+O2	Quinone formation

Table S2 10 drug prototype components detected in brain tissue after oral administration

NO.	Component Name	Observed m/z	Formula	Observed t_R (min)	PPM	MS^2 (m/z) (neg)
P1	Chlorogenic acid ismer1	353.0844	C16H18O9	3.12	-9.6	191.0561,179.0351,135.0446
P2	Chlorogenic acid	353.0844	C16H18O10	4.23	-8.1	191.0561
P9	3-O-Feruloylquinic Acid	367.1025	C17H20O9	6.11	-1.2	353.0876,179.0351,191.0561,335.0760
P20	Rutin isomer	609.1413	C27H30O16	7.78	-7	301.0333,300.0334,343.2118,151.0034
P10	Rutin	609.14	C27H30O16	8.04	-9.2	301.0333,300.0334,151.0034,431.0974
P11	Hyperoside	463.0878	C21H20O12	8.22	0.3	300.0334,301.0333,271.0248,178.0272
P13	Isoquercitrin	463.0863	C21H20O12	8.62	-3	300.0334,301.0333,271.0248,255.0299,178.0272
P16	3,5-diCQA	515.1188	C25H24O12	10.58	-0.3	191.0561,353.0876,335.0677,179.0351,135.0446
P17	Quercitrin	447.0916	C21H20O11	10.91	-2.6	301.0333,300.0334
P19	4,5-diCQA	515.1175	C25H24O12	13.1	-2.9	191.0561,353.0876,335.0677,179.0351

Table S3 7 metabolites detected in brain tissue after oral administration

NO.	Metabolite Name	Formula	m/z Found	PPM	Observed t_R (min)	MS^2 (m/z) (neg)	Prptotypes
M1	Alcohols dehydration	C16H16O8	335.077	0.8	5.56	179.0351,161.0458	Chlorogenic acid ismer1 Chlorogenic acid
M2	Alcohols dehydration	C16H16O8	335.0767	-0.1	5.84	179.0351,161.0458	
M15	Deglucosylation	C15H10O7	301.0334	-4.8	7.70	271.0248,255.0299	Hyperoside Isoquercitrin
M16	Deglucosylation	C15H10O8	301.0336	-4.1	8.18	271.0248,255.0299	
M17	Glucuronide conjugation	C27H28O18	639.1188	-1.5	5.94	300.0334,301.0333,271.0248	
M19	Deglucosylation	C15H10O6	285.0388	-4	11.22	285.0392,191.0561	Quercitrin
M20	Demethylation	C20H18O11	433.0766	-1.2	9.22	300.0334,301.0333,271.0248	

Table S4 Compound targets related to ischemic stroke disease

metabolism type	compound name	target symbol
prototype	hyperoside	NOS1
prototype	hyperoside	PTGS1
prototype	hyperoside	PTGS2
prototype	Rutin	ALOX5
prototype	Rutin	INS
prototype	Rutin	IL6
prototype	Rutin	IL1B
prototype	Rutin	PRKCH
prototype	Rutin	TNF
prototype	quercitrin	PTGS2
prototype	Trifolin	CAMKK2
prototype	Trifolin	F7
prototype	Trifolin	NOS1
prototype	Trifolin	PTGS2
prototype	Scopolin	PTGS1
prototype	Leucopelargonidin	ESR1
prototype	Leucopelargonidin	PTGS1
prototype	Leucopelargonidin	PTGS2
prototype	Cassiaside	CAMKK2
prototype	Cassiaside	PTGS2
prototype	Eupatin	CAMKK2
prototype	Eupatin	F7
prototype	Eupatin	ESR2
prototype	Eupatin	NOS1
prototype	Eupatin	PTGS2
prototype	Eupatin	VEGFA
prototype	Skimmin	PTGS2
prototype	Genistin	CAMKK2
prototype	Genistin	F7
prototype	Genistin	PTGS2
prototype	Cinchonain Ⅰb	PTGS2
prototype	Liriodendrin	CAMKK2
prototype	Liriodendrin	PTGS2
metabolite	quercetin	ALOX5
metabolite	quercetin	ABCG2
metabolite	quercetin	BIRC5
metabolite	quercetin	BCL2A1
metabolite	quercetin	CD40LG
metabolite	quercetin	CCL2
metabolite	quercetin	CCR2
metabolite	quercetin	F7

metabolism type	compound name	target symbol
metabolite	quercetin	DUOX2
metabolite	quercetin	HSPA1A
metabolite	quercetin	HSPA1B
metabolite	quercetin	ICAM1
metabolite	quercetin	IL1A
metabolite	quercetin	IL1B
metabolite	quercetin	IL10
metabolite	quercetin	IL6
metabolite	quercetin	MMP9
metabolite	quercetin	NF-κB
metabolite	quercetin	MAPK1
metabolite	quercetin	NQO1
metabolite	quercetin	NOS1
metabolite	quercetin	PTGS1
metabolite	quercetin	PTGS2
metabolite	quercetin	PRKCH
metabolite	quercetin	SELE
metabolite	quercetin	THBD
metabolite	quercetin	TGFB1
metabolite	quercetin	TNF
metabolite	quercetin	VCAM1
metabolite	quercetin	VEGFA
metabolite	caffeic acid	PTGS1
metabolite	caffeic acid	PTGS2

Table S5 Traced involved metabolic pathways and co-related targets

Target Symbol	Gene Name	Pathways
ALOX5	arachidonate 5-lipoxygenase	Arachidonic acid metabolism
ABCG2	ATP-binding cassette, sub-family G, member 2	ABC transporters
BIRC5	baculoviral IAP repeat containing 5	NF-κB signaling pathway
BIRC5	baculoviral IAP repeat containing 5	Apoptosis
BIRC5	baculoviral IAP repeat containing 5	TNF signaling pathway
BCL2A1	BCL2-related protein A1	NF-κB signaling pathway
BCL2A1	BCL2-related protein A1	Apoptosis
CAMKK2	calcium/calmodulin-dependent protein kinase 2, beta	AMPK signaling pathway
CCL2	chemokine (C-C motif) ligand 2	TNF signaling pathway
CCL2	chemokine (C-C motif) ligand 2	Chemokine signaling pathway
CCR2	chemokine (C-C motif) receptor 2	Chemokine signaling pathway
F7	coagulation factor VII	
DUOX2	dual oxidase 2	Thyroid hormone synthesis
ESR1	estrogen receptor 1	Estrogen signaling pathway
ESR1	estrogen receptor 1	Thyroid hormone signaling pathway
ESR2	estrogen receptor 2 (ER beta)	

Target Symbol	Gene Name	Pathways
HSPA1A	heat shock 70kDa protein 1A	Estrogen signaling pathway
HSPA1B	heat shock 70kDa protein 1B	Estrogen signaling pathway
INS	insulin	Insulin signaling pathway
ICAM1	intercellular adhesion molecule 1	NF-κB signaling pathway
ICAM1	intercellular adhesion molecule 1	TNF signaling pathway
IL1A	interleukin 1, alpha	Necroptosis
IL1B	interleukin 1, beta	NF-κB signaling pathway
IL1B	interleukin 1, beta	Necroptosis
IL1B	interleukin 1, beta	Toll-like receptor signaling pathway
IL1B	interleukin 1, beta	IL-17 signaling pathway
IL1B	interleukin 1, beta	TNF signaling pathway
IL1B	interleukin 1, beta	Inflammatory mediator regulation of TRP channels
IL10	interleukin 10	Jak-STAT signaling pathway
IL6	interleukin 6	Toll-like receptor signaling pathway
IL6	interleukin 6	HIF-1 signaling pathway
IL6	interleukin 6	Jak-STAT signaling pathway
IL6	interleukin 6	TNF signaling pathway
MMP9	matrix metallopeptidase 9	TNF signaling pathway
MMP9	matrix metallopeptidase 9	IL-17 signaling pathway
MAPK1	mitogen-activated protein kinase 1	MAPK signaling pathway
NF-κB	nuclear factor-kappa B	MAPK signaling pathway
NF-κB	nuclear factor-kappa B	IL-17 signaling pathway
NF-κB	nuclear factor-kappa B	TNF signaling pathway
NF-κB	nuclear factor-kappa B	NF-κB signaling pathway
NQO1	NAD(P)H dehydrogenase, quinone 1	
NOS1	nitric oxide synthase 1 (neuronal)	Arginine and proline metabolism
PTGS1	prostaglandin-endoperoxide synthase 1	Arachidonic acid metabolism
PTGS2	prostaglandin-endoperoxide synthase 2	Arachidonic acid metabolism
PRKCH	protein kinase C, beta	MAPK signaling pathway
PRKCH	protein kinase C, beta	Calcium signaling pathway
PRKCH	protein kinase C, beta	T cell receptor signaling pathway
PRKCH	protein kinase C, beta	mTOR signaling signaling pathway
PRKCH	protein kinase C, beta	PI3K-Akt signaling pathway
PRKCH	protein kinase C, beta	Wnt signaling pathway
PRKCH	protein kinase C, beta	VEGF signaling pathway
PRKCH	protein kinase C, beta	Phosphatidylinositol signaling system
PRKCH	protein kinase C, beta	Arachidonic acid metabolism
PRKCH	protein kinase C, beta	Arachidonic acid metabolism
PRKCH	protein kinase C, beta	Arachidonic acid metabolism
SELE	selectin E	
THBD	thrombomodulin	MAPK signaling pathway
THBD	thrombomodulin	PI3K-Akt signaling pathway

Target Symbol	Gene Name	Pathways
TGFB1	transforming growth factor, beta 1	MAPK signaling pathway
TGFB1	transforming growth factor, beta 1	Cytokine-cytokine receptor interaction
TGFB1	transforming growth factor, beta 1	TGF-beta signaling pathway
TGFB1	transforming growth factor, beta 1	PI3K-Akt signaling pathway
TGFB1	transforming growth factor, beta 1	NF-κB signaling pathway
TGFB1	transforming growth factor, beta 1	toll-like receptor signaling pathway
TNF	tumor necrosis factor	MAPK signaling pathway
TNF	tumor necrosis factor	NF-κB signaling pathway
TNF	tumor necrosis factor	Cytokine-cytokine receptor interaction
TNF	tumor necrosis factor	AMPK signaling pathway
TNF	tumor necrosis factor	mTOR signaling pathway
TNF	tumor necrosis factor	TNF signaling pathway
TNF	tumor necrosis factor	PI3K-Akt signaling pathway
TNF	tumor necrosis factor	TGF-beta signaling pathway
TNF	tumor necrosis factor	MHC Ⅰ pathway
TNF	tumor necrosis factor	toll-like receptor signaling pathway
VCAM1	vascular cell adhesion molecule 1	NF-κB signaling pathway
VCAM1	vascular cell adhesion molecule 1	MAPK signaling pathway
VEGFA	vascular endothelial growth factor A	RAS signaling pathway
VEGFA	vascular endothelial growth factor A	VEGF signaling pathway
VEGFA	vascular endothelial growth factor A	MAPK signaling pathway

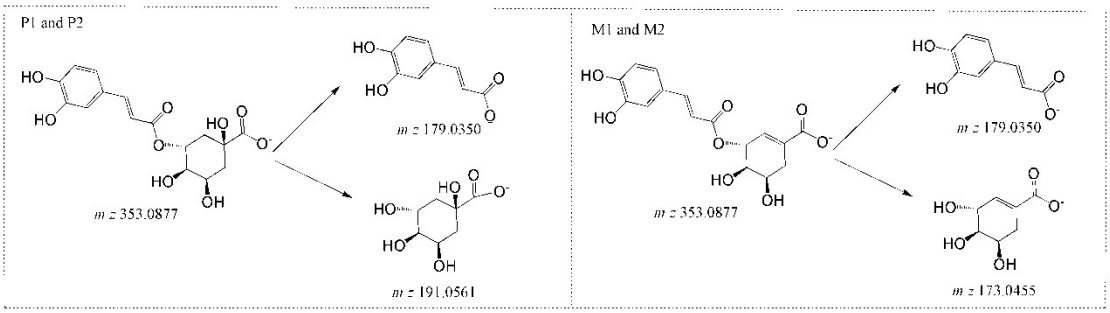

Figure S1 The proposed fragmentation pathways of Chlorogenic acid isomers and their metabolites

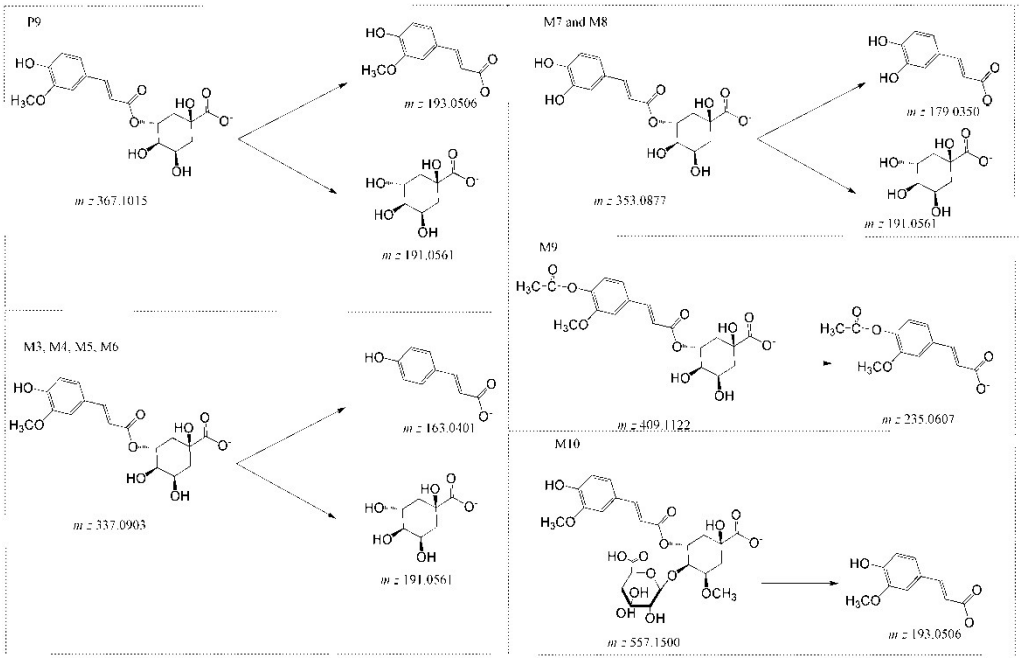

Figure S2 The proposed fragmentation pathways of 3-O-Feruloylquinic Acid (P 9) and its metabolites

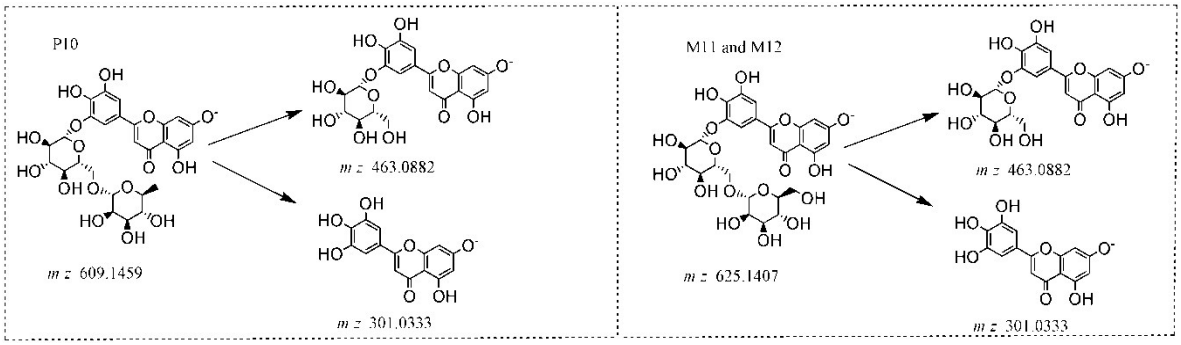

Figure S3 The proposed fragmentation pathways of Rutin (P10) and its metablites

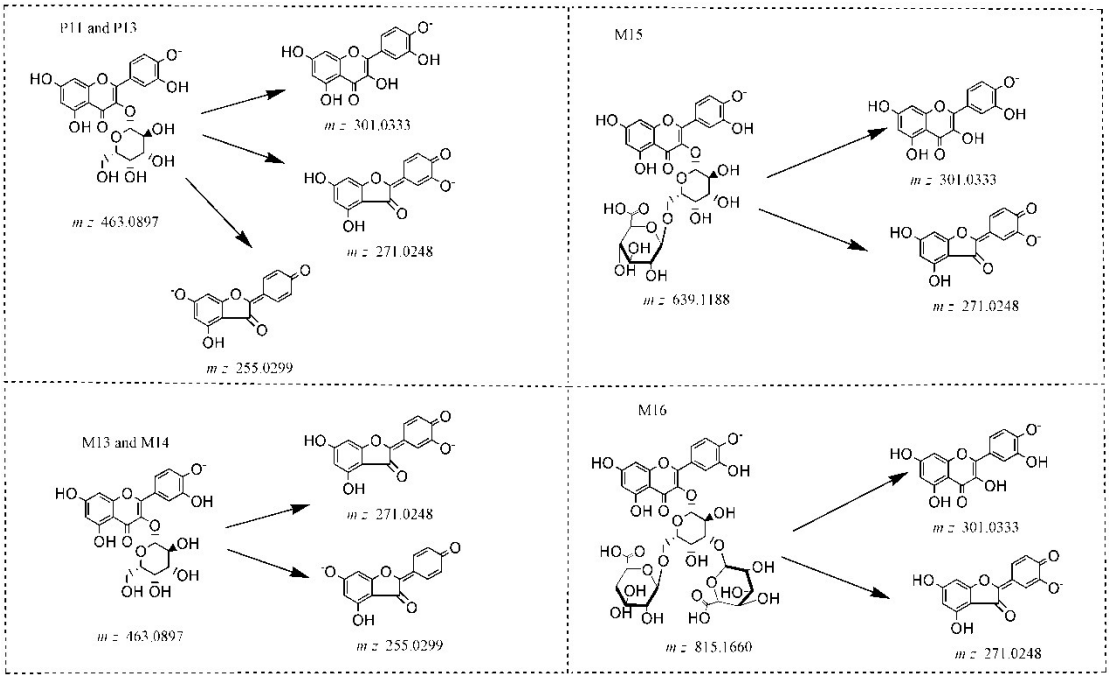

Figure S4 The proposed fragmentation pathways of Hyperoside (P11) and its metabolites

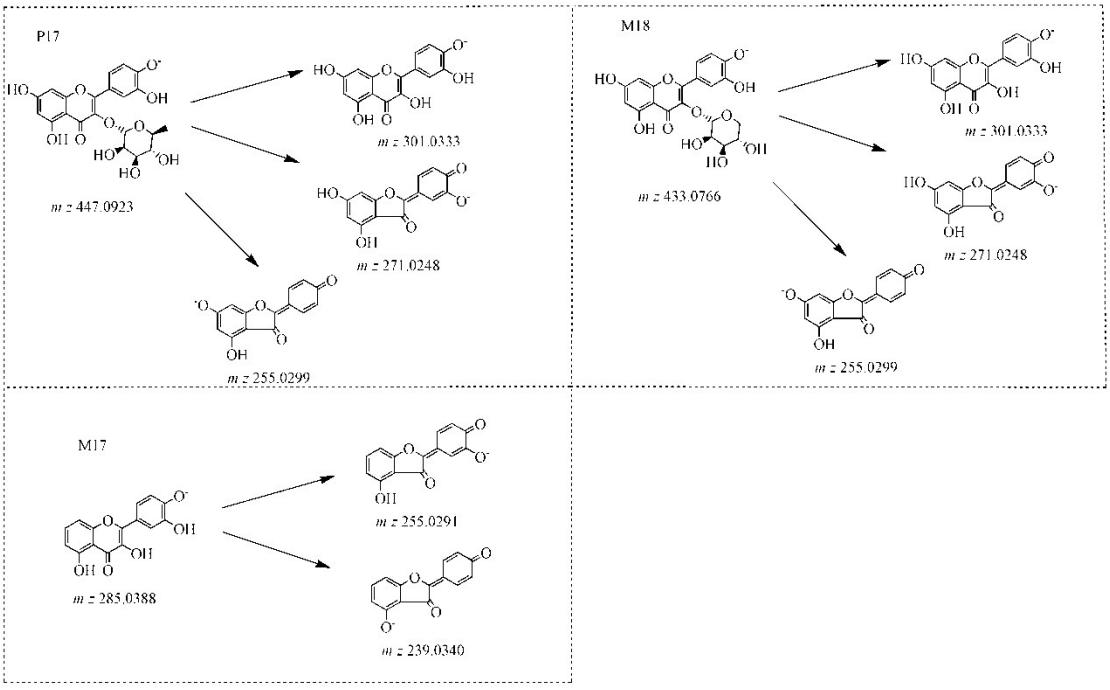

Figure S5 The proposed fragmentation pathways of Quercitrin (P17) and its me tabolites