

# Network pharmacology study on the active components of *Pterocypsela elata* and the mechanism of their effect against cerebral ischemia

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**Objective:** The aim of this study was to identify the active anti-ischemic components of *Pterocypsela elata* (*P. elata*) using a network pharmacology approach to construct an effective component anti-cerebral ischemic target network and systematically analyze this medicinal material.

**Methods:** Pharmacological studies have shown that *P. elata* has an obvious effect against cerebral ischemia. To identify the potential targets, 14 components of *P. elata* were docked to each structural element of the targets in the DRAR-CPI database by reverse docking technology. We then compared the identified potential targets with FDA-approved targets for stroke/cerebral infarction treatment in the DrugBank database and identified the active components of *P. elata* and their potential targets for stroke/cerebral infarction treatment. The active component-target networks were constructed using Cytoscape 3.5.1 software. The target protein-protein interactions were analyzed using the STRING database. KEGG pathway analysis and gene ontology (GO) enrichment analysis were performed through the Database for Annotation, Visualization and Integrated Discovery (DAVID).

**Results:** There were 14 active components identified from *P. elata* and 21 potential targets identified for cerebral ischemia treatment, including carbonic anhydrase 2, ribosyldihydro-nicotinamide dehydrogenase, cholinesterase, and glutathione S-transferase P. The main involved pathways include metabolic pathways, complement and coagulation cascades and steroid hormone biosynthesis.

**Conclusion:** Through a network pharmacology approach, we predicted the active components of *P. elata* and their potential targets for cerebral ischemia treatment. Our results provide new perspectives and clues for further studies on the anti-cerebral ischemia mechanism of *P. elata*.

**Keywords:** network pharmacology, *Pterocypsela elata*, cerebral ischemia, molecular docking

## Introduction

Traditional Chinese medicine (TCM) originated in China and has evolved over thousands of years. To counteract diseases, Chinese people have gradually accumulated a profound knowledge of medicine and rich experience through thousands of years of clinical practice, medical development and innovation, and have formed a comprehensive and systematic TCM theory and unique medical system. Due to the synergistic effect of multi-component, multi-pathway and multi-target systems, the

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material basis, mechanisms and preparation of TCM active compounds remain unclear, and the quality of TCM is difficult to control. Additionally, TCM lacks scientific, logical and effective evaluation systems for assessing efficacy and safety, which makes it difficult to conduct comprehensive and systematic research at the tissue, organ, cell and molecular levels.<sup>1</sup> Therefore, to eliminate obstacles to the modernization of TCM, it is important to clarify the material basis and mechanisms of TCM active compounds.

Network pharmacology approaches are based on the new “multi-target, multidrug, multi-pathway” concept. This approach can integrate the various databases of Chinese medicines, proteins, genes and others for analysis to construct a drug-target-disease network using bioinformatics to predict the mechanism of a medicine. The holistic and systematic features of the network pharmacology approach are consistent with the synergistic effect of multiple components in TCM. Through network pharmacology analysis, we can investigate TCM systematically, identify the active components, clarify the potential targets and mechanisms, and contribute to the modernization of TCM.<sup>2-5</sup>

With the aging of the population and the development of the economy, ischemic stroke has become a disease with a high morbidity and mortality that seriously affects health. Ischemia/reperfusion injury following ischemic stroke is a complex pathophysiological process. Therefore, the treatment and prevention of cerebral ischemia has long been the focus of medical research. *Pterocypsela elata* (*P. elata*), also known as Indian lettuce or bitter lettuce, is an annual or biennial herb belonging to the genus *Pterocypsela Shih* of the Compositae family, which is distributed throughout China. It has a long history of being used as a vegetable, animal feed and medicine. Both the whole herb and the root can be used in TCM, and it has the functions of clearing away heat and toxic materials, promoting blood circulation, and is an anti-oxidant and anti-diabetic.<sup>6</sup> Studies have shown that it also has anti-ischemic effects. As a potential anti-ischemic drug, it deserves to be further studied. In this study, we screened the active anti-ischemic components of *P. elata* using a network pharmacology approach and constructed an active component anti-ischemic target network for analysis.

## Materials and methods

### Compound information

Based on previous studies in our group,<sup>7-10</sup> a total of 14 molecular entities in *P. elata* were obtained: lactuside B (C21H32O9), 11 $\beta$ ,13-dihydrolactucin acetate (C17H20O6),

11 $\beta$ , 13-dihydrolactucin (C15H18O5), 9 $\alpha$ -hydroxyleucodin-9-O- $\beta$ -xylopyranoside (C20H26O8), Guaianolide I (C23H30O11), Guaianolide II (xFF08;C15H12O3), (24R)-5 $\alpha$ -stigmast-7, 22(E)-dien-3 $\alpha$ -ol (C29H48O), oleanolic acid (C30H48O3),  $\beta$ -amyrin (C30H50O),  $\beta$ -sitosterol (C29H50O), daucosterol (C35H60O6), 3, 3', 4-trimethoxyellagic acid (C17 H12O8), stearic acid (C18H36O2).

### Software and network database

ChemDraw 15.0 software, Open Babel GUI 2.4.1 software, Cytoscape 3.5 software, Batman-TCM database (<http://bionet.ncpsb.org/batman-tcm/index.php/Home/Index/index>), <http://cpi.bio-x.cn/drar/DrugBank> 5.1.0 software (<https://www.drugbank.ca/>, version 5.1.1, released 2018-07-03), String 10.5 software (<http://www.string-db.org/>), KEGG software (<http://www.kegg.jp/>).

### Experimental methods

The chemical structures of 14 compounds in *P. elata* were drawn using ChemDraw software and then converted to files in sdf-MDL format using Open Babel GUI 2.4.1 software. All files of chemical structures were saved and uploaded to the Drug Repositioning and Adverse Reaction via Chemical-Protein Interactome (DRAR-CPI) server.

The FDA-approved targets for stroke/cerebral infarction treatment were identified using keywords such as “stroke”, “cerebral infarction” and “cerebral ischemia” to search the DrugBank database. Comparing the targets identified by the 14 compounds in *P. elata* with the FDA-approved targets, we found potential active components in *P. elata* and their targets for stroke/cerebral infarction treatment.

The potential targets were then analyzed via the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database using single-target and multi-target analysis. For each target, gene ontology (GO) enrichment analysis was performed for the aspects of biological process, molecular function, cellular component and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to explore the relationships between potential targets.

Finally, the active component-target-pathway network was visualized and analyzed using the Cytoscape software. Bioinformatic tools were employed to study the “multi-component, multi-target, and multi-pathway” mechanism.

## Cell experiments

### Oxygen-glucose deprivation (OGD) and recovery (OGDR)<sup>11</sup>

OGD model: Glucose-containing complete medium was replaced by Hank's balanced salt solution (glucose-free; Invitrogen, 88284). Human neuroblastoma cell (SH-SY5Y cells) were incubated in hypoxic conditions (5% CO<sub>2</sub>, 0.3% O<sub>2</sub> and 94.7% N<sub>2</sub>) for 4 hrs. Brain injury following cerebral ischemia was modeled in vitro.

OGDR: Four hours after OGD, the glucose-free medium was replaced by complete medium containing glucose and 10% (v/v) fetal bovine serum. Cells were placed in an incubator containing 95% air and 5% CO<sub>2</sub> at 37 °C for 48 hrs. Cerebral ischemia/reperfusion injury was modeled in vitro. The cells of the control group were always incubated in complete medium and placed in an incubator containing 95% air and 5% CO<sub>2</sub> at 37 °C.

Intervention group: Sixty minutes before OGD and 24 h after OGD, a sterilized water extraction of *P. elata* (ie, the crude drug) at concentrations of 1 mg/mL, 2 mg/mL, and 4 mg/mL was added into the medium. For the control group, the same volume of sterile distilled water was added.

### MTT assay

Cell viability was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay. Cells were seeded into 96-well culture plates at a density of 2×10<sup>3</sup> cells/well. After OGDR, 20 µL of MTT (5 g/L) was added to each well, and the cells were incubated for 4 h. After discarding the supernatant, 150 µL of dimethyl sulfoxide (DMSO) was added to each well, and the plates were placed in an incubation shaker for 10 min. The absorbance at 490 nm was measured using a spectrophotometer.

## Animal experiments

### Experimental animals

A total of sixty male Sprague-Dawley rats (weight: 260 to 300 g) were obtained from the Experimental Animal Center, the Academy of Medical Sciences of Zhengzhou University. The animal certificate number is SYXK (Yu) 2005-0012.

### Rat model of cerebral ischemia

The rats were randomly divided into 5 groups, 12 rats per group, named as the sham surgery group, model group, low-concentration group (10 g/kg), moderate concentration group (20 g/kg), and high concentration group (40 g/kg). Each group was pre-administered with a water extraction

of *P. elata* (ie, the crude drug) for two weeks, twice per day, at concentrations of 10, 20, and 40 g/kg. Arterial occlusion lines were prepared using a No. 1.5 fishing line (produced by DaDong Yang). Each line was cut to a length of 5–6 cm. One end of the line was heated to form a spherical shape, and then the line was soaked in a 1% heparin solution for storage. The rats were anesthetized using 10% chloral hydrate. The rat model for cerebral ischemia and reperfusion injury was prepared using Longa's suture method.<sup>13</sup> It was established by modified middle cerebral artery occlusion (MCAO). The supine position was fixed on the hand table, and the common carotid artery (CCA) was separated from the median incision of the neck. The internal carotid artery (ICA) and external carotid artery (ECA) were separated from the right side in turn. The branches of ECA were electrocoagulated with a coagulator, and the distal part of the main trunk of ECA was separated and ligated. After clipping CCA and ICA with non-invasive artery, a loose joint was made and ECA was cut off near the bifurcation of CCA. The prepared occlusion line was inserted from the broken end of ECA. After the bifurcation of CCA, ICA non-invasive artery clamp was loosened. The occlusion line was inserted into the middle cerebral artery through ICA. When resistance was felt, the occlusion line was stopped, fixed, the non-invasive artery clamp on CCA was loosened, and the depth of occlusion line was inserted. 1.8–2.0 cm. All animals were occluded for 2 hrs and then pulled the occluded spherical end back to ECA for reperfusion. Neurological function assessment scores were derived 48 h after ischemia-reperfusion. The active animals were sacrificed, and specimens were obtained. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006), and were approved by the ethics committee of Xinxiang Medical University.

### Rat neurological deficit scores

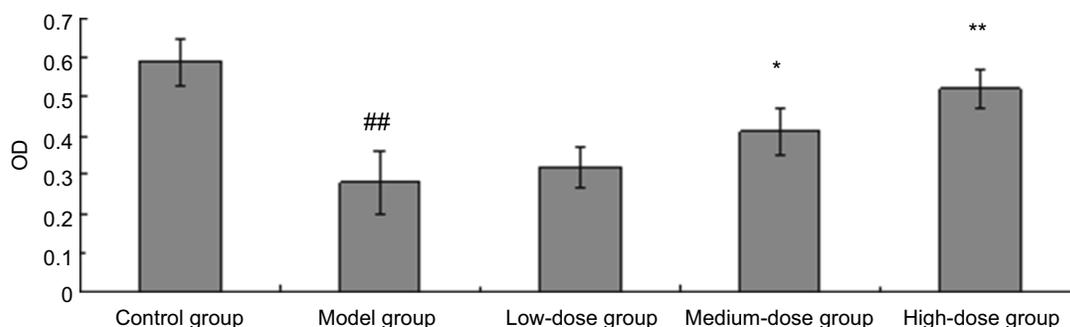
The neurological status was assessed after the animal was awake and 24 h after the injury according to the Zea-longa scoring system.<sup>13</sup> A score of 0 was given to normal animals with no symptoms and no signs of neurological deficit. A score of 1 was given to the animals with mild neurological deficit, including the adduction and flexion of the left anterior limb after lifted by the tail. A score of 2 was given to animals whose body turned to the left while crawling (tail-catching phenomenon). A score of 3 was

given to animals that fell to the left side while standing. A score of 4 was given to animals that could not walk on their own and had lost consciousness. Animals with scores of 1–3 were included in the experiment; those with a score of 0 or 4 were excluded.

#### Measurement of cerebral infarction volume

After neurological function assessment, 5 rats were randomly selected from each group. The rats were quickly decapitated on ice and placed at  $-20^{\circ}\text{C}$  for 10 min. The cerebellum, lower brain stem, olfactory bulb and frontal poles were excised. A series of 2 mm-thick continuous coronal slices, from anterior to posterior, were cut from the rest of the brain and incubated in 2% triphenyl tetrazolium chloride (TTC) solution for 30 min at  $37^{\circ}\text{C}$ . The operation process should be protected

from light, and the solution should cover the brain tissue. The principle of TTC staining relies on the oxidation of TTC by dehydrogenase. Tissue with normal levels of dehydrogenase is stained red, whereas the ischemic infarction tissue lacks dehydrogenase and is white. After staining, the brain slices were fixed with paraformaldehyde and stored at  $4^{\circ}\text{C}$ . The infarction volume of each slice was calculated by image analysis software according to the following formula: total infarction volume = sum of infarction areas  $\times$  slice thickness. To compensate for brain tissue swelling, the estimated infarction volume was corrected using the following formula: percentage of infarction volume in total volume = (measured infarction volume  $\times$  (1-(ipsilateral hemisphere volume-contralateral hemisphere volume)/ipsilateral hemisphere volume)/total brain volume  $\times$  100%.



**Figure 1** The effect of *Pterocypsel elata* (*P. elata*) on cell viability after oxygen-glucose deprivation/recovery ( $\bar{x} \pm s$ ,  $n=3$ ).

**Notes:** ### $P<0.01$  vs sham surgery group; \* $P<0.05$ , \*\* $P<0.01$  vs model group.



**Figure 2** Photomicrographs of each group (100 $\times$ ).

## Results

### The effect of *P. elata* on cell viability

As shown in Figure 1, cell viability was significantly decreased in the model group with oxygen-glucose deprivation/recovery ( $P<0.01$ ). Different concentrations of *P. elata* could increase the cell viability to various degrees. Compared with the model group, medium and high concentrations of *P. elata* showed stronger effects ( $P<0.05-0.01$ ). Photomicrographs of each group are shown in Figure 2.

### The effect of *P. elata* on neurological deficit scores of rats

As shown in Table 1, twenty-four hours after ischemia-reperfusion injury, the score of the model group was significantly higher than that of the sham surgery group ( $P<0.01$ ). The groups with different concentrations of *P.*

*elata* had lower behavioral scores in the injured rats, and the differences were significant between the behavioral scores of these groups and that of the model group ( $P<0.01$  or  $P<0.05$ ). Among them, the medium- and high-concentration groups showed stronger effects ( $P<0.01$ ).

### Effect of *P. elata* on infarction volume after ischemia-reperfusion injury

Cerebral infarction was assessed by TTC staining 48 h after reperfusion, and the white part was the infarction area. In the model group, the infarction area was large. In the sham surgery group, no infarction area was found. After *P. elata* treatment, the infarction area of the model group was reduced, and the reduction was most pronounced in the high-concentration group (Figure 3).

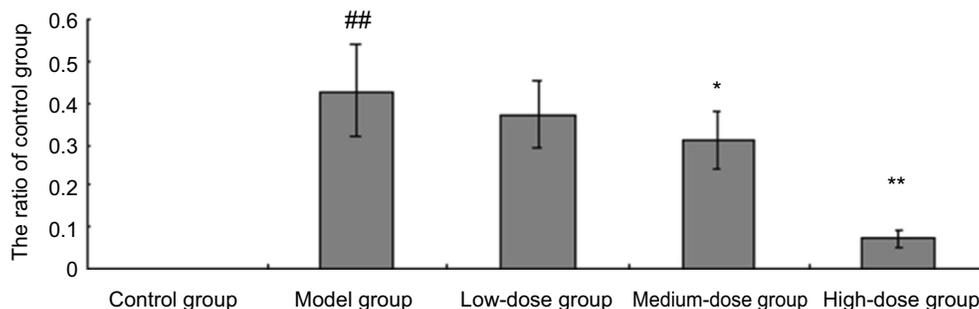
### Main active components and their protein target information

The interactions between potential drug molecules and proteins were analyzed by reverse docking based on their three-dimensional structures via the DRAR-CPI server. A scoring function was employed to score and rank the binding affinities between protein and ligand, and potential targets were predicted. The Z-score represents the strength of the interaction between a drug molecule and its protein target. A target with a Z-score value less than  $-0.5$  is generally considered to be a potential target. The 14

**Table 1** The effect of *Pterocypsel elata* (*P. elata*) on rat neurological deficit scores ( $\bar{x} \pm s$ ,  $n=8$ )

Group	Awake after anesthesia	48 h after ischemia-reperfusion
Sham surgery	0.00±0.00	0.00±0.00
Model	3.50±0.55	3.83±0.41 <sup>#</sup>
Low dose	3.63±0.41	3.02±0.63*
Medium dose	3.50±0.55	2.74±0.63**
High dose	3.67±0.52	2.27±0.75**

Notes: <sup>#</sup> $P<0.05$  vs sham surgery group; \* $P<0.05$ , \*\* $P<0.01$  vs model group.



**Figure 3** Effect of *Pterocypsel elata* (*P. elata*) on infarction volume and the ratio of control group after ischemia-reperfusion injury ( $\bar{x} \pm s$ ,  $n=8$ ).

Notes: <sup>###</sup> $P<0.01$  vs sham surgery group; \* $P<0.05$ , \*\* $P<0.01$  vs model group.

**Table 2** *Pterocypselata elata* active components and Z-scores

No. component	Protein target (Z-score)
1. Lactuside B	<i>HMGR</i> (-0.603), <i>ACE</i> (-0.591), <i>CAI</i> (-1.884), <i>CA2</i> (-1.721), <i>PDE5A</i> (-0.502), <i>BCHE</i> (-0.619), <i>HSD11B1</i> (-1.087), <i>DPP4</i> (-0.919), <i>NR3C1</i> (-0.816), <i>GSTP1</i> (-0.887), <i>NR1I2</i> (-1.693), <i>SERPINE1</i> (-0.624), <i>NQO2</i> (-2.390)
2. 11 $\beta$ , 13-dihydroxylactucin acetate	<i>ACE</i> (-0.635), <i>CA2</i> (-0.752), <i>PDE5A</i> (-0.989), <i>BCHE</i> (-1.595), <i>HSD11B1</i> (-1.018), <i>NR3C1</i> (-1.259), <i>NR1I2</i> (-1.491), <i>SERPINE1</i> (-0.528), <i>NQO2</i> (-0.786)
3. 11 $\beta$ , 13-dihydroxylactucin	<i>HMGR</i> (-0.577), <i>ACE</i> (-0.660), <i>CAI</i> (-0.505), <i>CA2</i> (-0.838), <i>PDE5A</i> (-0.555), <i>BCHE</i> (-0.803), <i>HSD11B1</i> (-0.734), <i>NR3C1</i> (-1.256), <i>ITGAL</i> (-1.309), <i>NR1I2</i> (-1.030), <i>SERPINE1</i> (-1.207), <i>F2</i> (-0.894), <i>NQO2</i> (-1.080)
4. 9 $\alpha$ -hydroxyleucodin-9-O- $\beta$ -xylopyranoside	<i>HMGR</i> (-0.577), <i>ACE</i> (-0.660), <i>CAI</i> (-0.505), <i>CA2</i> (-0.838), <i>PDE5A</i> (-0.555), <i>BCHE</i> (-0.803), <i>HSD11B1</i> (-0.734), <i>NR3C1</i> (-1.256), <i>ITGAL</i> (-1.309), <i>NR1I2</i> (-1.030), <i>SERPINE1</i> (-1.207), <i>F2</i> (-0.894), <i>NQO2</i> (-1.080)
5. Guaianolide I	<i>NOS2</i> (-1.073), <i>ACE</i> (-0.893), <i>CA2</i> (-0.714), <i>NQO2</i> (-1.805), <i>HSD11B1</i> (-1.655), <i>NR3C1</i> (-1.379), <i>GSTP1</i> (-1.147), <i>ITGAL</i> (-1.592), <i>HMGR</i> (-0.702), <i>NR1I2</i> (-1.654)
6. Guaianolide II	<i>NAT2</i> (-1.349), <i>CA2</i> (-2.386), <i>NR3C1</i> (-1.218), <i>ALB</i> (-1.269), <i>ITGAL</i> (-1.385), <i>NOS2</i> (-0.632), <i>SERPINE1</i> (-1.620)
7. Guaianolide III	<i>NAT2</i> (-0.932), <i>CA2</i> (-0.991), <i>ALB</i> (-1.731), <i>BCHE</i> (-0.581), <i>AKR1C1</i> (-0.602), <i>ITGAL</i> (-0.995), <i>SERPINE1</i> (-1.579)
8. (24R)-5 $\alpha$ -stigmast-7, 22 (E)-dien-3 $\alpha$ -ol	<i>HMGR</i> (-0.589), <i>CAI</i> (-1.478), <i>CA2</i> (-0.810), <i>BCHE</i> (-1.526), <i>HSD11B1</i> (-1.226), <i>GSTP1</i> (-1.317), <i>NR1I2</i> (-1.617), <i>NQO2</i> (-2.048)
9. Oleanolic acid	<i>HMGR</i> (-1.989), <i>CA2</i> (-0.909), <i>BCHE</i> (-1.089), <i>DPP4</i> (-1.163), <i>GSTP1</i> (-3.065), <i>NOS2</i> (-1.296), <i>PLG</i> (-1.920), <i>NQO2</i> (-1.870), <i>ALB</i> (-1.498)
10. $\beta$ -Amyrin	<i>HMGR</i> (-1.390), <i>CALM1</i> (-1.147), <i>CA2</i> (-1.101), <i>BCHE</i> (-2.478), <i>DPP4</i> (-1.709), <i>GSTP1</i> (-1.338), <i>NOS2</i> (-1.930), <i>NQO2</i> (-0.649)
11. $\beta$ -Sitosterol	<i>PDE5A</i> (-0.654), <i>HSD11B1</i> (-1.967), <i>DPP4</i> (-0.538), <i>GSTP1</i> (-1.717), <i>NR1I2</i> (-2.381), <i>NQO2</i> (-0.600)
12. Daucosterol	<i>GSTP1</i> (-2.541), <i>CA2</i> (-1.923), <i>BCHE</i> (-0.933), <i>NQO2</i> (-1.433), <i>HSD11B1</i> (-2.310), <i>ITGAL</i> (-1.116), <i>HMGR</i> (-2.110)
13. 3, 3', 4-Trimethoxylellagic acid	<i>AKR1C1</i> (-1.399), <i>NAT2</i> (-1.445), <i>CA2</i> (-1.536), <i>NR3C1</i> (-0.682), <i>GSTP1</i> (-0.630), <i>ITGAL</i> (-2.171), <i>SERPINE1</i> (-1.881), <i>NQO2</i> (-0.686), <i>ALB</i> (-1.714)
14. Steric acid	<i>PLG</i> (-0.534)

components from *P. elata* resulted in 1,747 targets. After analyzing the target information, 312 non-repetitive potential targets were identified.

The abovementioned targets were compared with the FDA-approved targets for stroke/cerebral infarction treatment in the DrugBank database. A total of 14 potential active

**Table 3** Potential targets of *Pterocypsela elata* (*P. elata*)

Uniprot ID	Protein target	Target gene	Frequency
P00918	Carbonic anhydrase 2	CA2	12
P16083	Ribosylidihyronicotinamide dehydrogenase [quinone]	NQO2	11
P06276	Cholinesterase	BCHE	9
P04035	3-hydroxy-3-methylglutaryl-coenzyme A reductase	HMGCR	8
P28845	Corticosteroid 11-beta-dehydrogenase isozyme 1	HSD11B1	8
P09211	Glutathione S-transferase P	GSTP1	8
P04150	Glucocorticoid receptor	NR3C1	7
P20701	Integrin alpha-L	ITGAL	7
O75469	Nuclear receptor subfamily 1 group 1 member 2	NR1I2	7
P05121	Plasminogen activator inhibitor 1	SERPINE1	7
P12821	Angiotensin-converting enzyme	ACE	5
O76074	cGMP-specific 3',5'-cyclic phosphodiesterase	PDE5A	5
P00915	Carbonic anhydrase 1	CA1	4
P27487	Dipeptidyl peptidase 4	DPP4	4
P35228	Nitric oxide synthase, inducible	NOS2	4
P02768	Serum albumin	ALB	4
P11245	Arylamine N-acetyltransferase 2	NAT2	3
Q04828	Aldo-keto reductase family 1 member C1	AKR1C1	2
P00747	Plasminogen	PLG	2
P00734	Prothrombin	F2	2
P0DP23	Calmodulin	CALM1	1

components in *P. elata* (Table 2) and 21 potential targets for stroke/cerebral infarction treatment were identified (Table 3).

## GO enrichment analysis of targets

The abovementioned potential targets of *P. elata* were assessed for single-target or multi-target GO enrichment analysis in the STRING database. There were 798 genes related to biological processes, and these appeared 1,372 times. The 26 biological processes with an occurrence frequency greater than or equal to 5 are shown in Figure 4A. There were 149 genes related to molecular functions that appeared 221 times. Sixteen molecular functions with an occurrence frequency greater than or equal to 3 are shown in Figure 4B. There were 86 genes related to cellular components that appeared 164 times. Sixteen cellular components with an occurrence frequency greater than or equal to 3 are shown in Figure 4C.

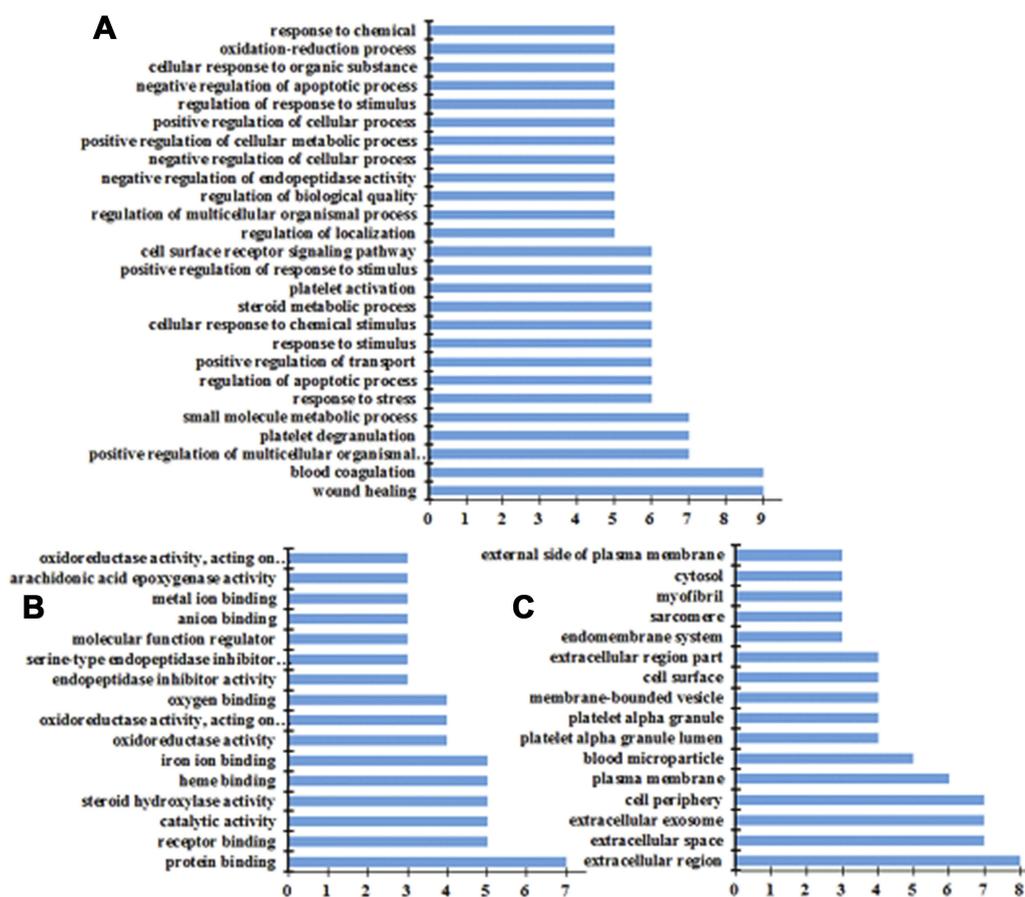
## Pathway annotation and construction of a component-target-pathway network

The potential targets were analyzed using KEGG pathways. The results showed that targets were involved in 133 annotated KEGG pathways and appeared 264 times. Thirty-five pathways with an occurrence frequency greater than 3 are listed in Figure 5, including metabolic pathways,

complement and coagulation cascades, steroid hormone biosynthesis, metabolism of xenobiotics by cytochrome P450, drug metabolism-cytochrome P450, and the oxytocin signaling pathway. The active component-target-metabolic pathway network is shown in Figure 6.

## Discussion

Cell experiments showed that different concentrations of *P. elata* could increase cell viability to various degrees after oxygen-glucose deprivation/recovery. The groups with medium and high concentrations of *P. elata* showed stronger effects than the model group ( $P < 0.05-0.01$ ). A rat model for cerebral ischemia and reperfusion injury was prepared using Longa's suture method.<sup>12</sup> Through the neurological deficit score of the rats awake after anesthesia and at 48 h after drug administration, we learned that the three concentrations of *P. elata* (low, medium and high) could all reduce the neurological deficit score of the rats ( $P < 0.05-0.01$ ). TTC staining also showed that the low, medium and high concentrations of *P. elata* could all reduce the infarction volume at 48 hrs after reperfusion compared to the model group. The high-concentration group had the most obvious effect. The results demonstrated that *P. elata* has anti-ischemic effects.



**Figure 4** Results of GO enrichment analysis of potential targets. (A) Biological processes, (B) cellular components, (C) molecular functions.

Pharmacological experiments showed that *P. elata* has obvious anti-ischemic effects. Subsequently, the potential targets of each active compound in *P. elata* were obtained via the DRAR-CPI database by using reverse docking technology. Through a comparison to FDA-approved targets for stroke/cerebral infarction treatment in the DrugBank database, a total of 14 active components with potential activity against stroke/cerebral infarction and 21 potential targets were identified. Active components include lactuside B, 11 $\beta$ , 13-dihydrolactucin acetate, 11 $\beta$ , 13-dihydrolactucin, 9 $\alpha$ -hydroxyleucodin-9-O- $\beta$ -xylopyranoside, guaianolide I, guaianolide II, guaianolide III, (24R)-5 $\alpha$ -stigmast-7, 22 (E)-dien-3 $\alpha$ -ol, oleanolic acid,  $\beta$ -amyryn,  $\beta$ -sitosterol, daucosterol, 3, 3', 4-trimethoxylellagic acid, and steric acid. Among these, lactuside B has been reported to have anti-cerebral ischemia effects.<sup>7</sup> The genes of 14 potential targets were *CA2*, *NQO2*, *BCHE*, *HMGCN*, *HSD11B1*, *GSTP1*, *NR3C1*, and *ITGAL*.

STRING is a database used for analyzing protein-protein associations. GO enrichment analysis can elucidate

which GO terms are over-represented or under-represented in the aspect of biological processes, molecular functions, and cellular components.<sup>13,14</sup> The obtained potential targets of *P. elata* were analyzed via GO enrichment analysis, and target-pathway networks were constructed via the STRING database. There were 26 biological processes with an occurrence frequency greater than or equal to 5. Among them, wound healing and blood coagulation appeared most frequently, followed by the positive regulation of multicellular organismal and platelet degradation. There were 16 molecular functions with an occurrence frequency greater than or equal to 3. Among them, protein binding occurred most frequently, followed by receptor binding, catalytic activity, and steroid hydroxylase activity. There were 16 cellular components with an occurrence frequency greater than or equal to 3. Among them, extracellular region was the most frequent, followed by extracellular space, extracellular exosome, and cell periphery.

KEGG pathway enrichment analysis was performed in the KEGG pathway database to generate hypotheses about

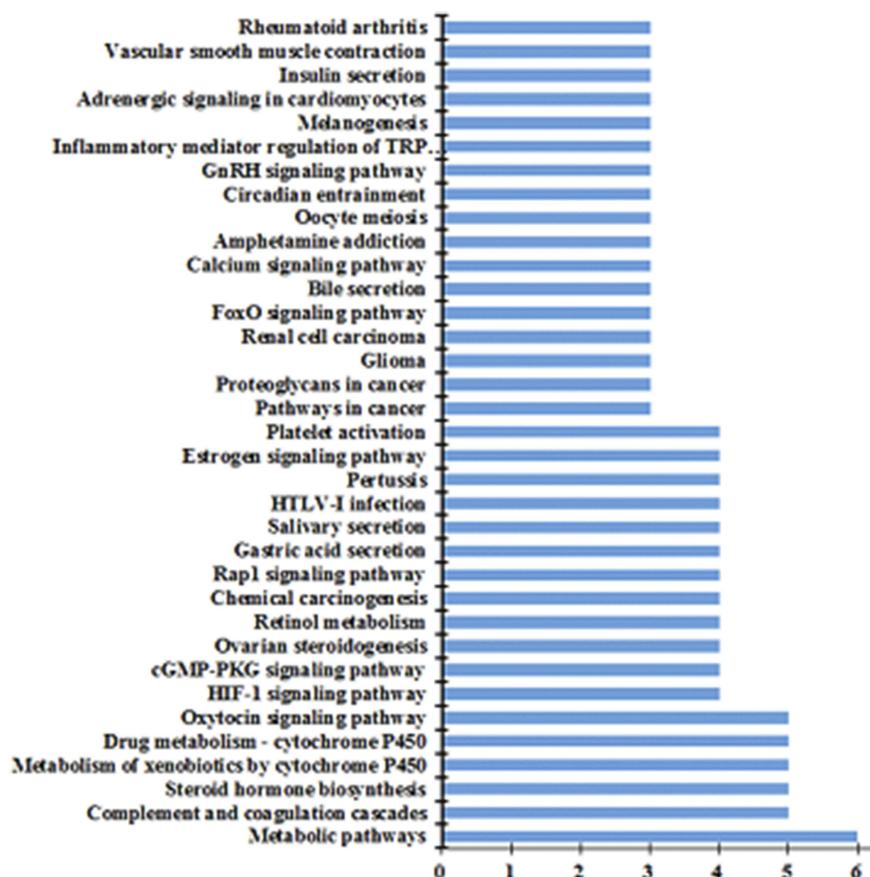


Figure 5 KEGG pathway analysis for *Pterocypsel elata* (*P. elata*).

potential targets and relevant pathways. The results showed 133 pathways associated with potential targets, and these appeared 264 times. Thirty-five pathways with an occurrence frequency greater than or equal to 3 are listed in Figure 3, including metabolic pathways, complement and coagulation cascades, steroid hormone biosynthesis, metabolism of xenobiotics by cytochrome P450, drug metabolism-cytochrome P450, and the oxytocin signaling pathway. Among them, amino acid metabolism, the coagulation cascade pathway, steroid hormone biosynthesis, and neuroactive ligand-receptor interaction are the pathways that are involved in cerebral ischemia-reperfusion injury, and these pathways are associated and interact with each other. Upon cerebral ischemia, a large amount of excitatory amino acids, including amino acids and aspartic acid, accumulate in the synaptic cleft, which can activate AMPA and NMDA receptors (glutamate receptors). Furthermore, cerebral ischemia induces significant  $\text{Ca}^{2+}$  influx and activates downstream  $\text{Ca}^{2+}$ -dependent cell death pathways, which causes necrosis or apoptosis of neuronal cells and glial cells<sup>15</sup> and further aggravates the

cerebral ischemic injury. Steroid hormones can protect against cerebral ischemia injury by reducing oxidative stress and inhibiting the NMDA receptor.<sup>16,17</sup>

An active component-target-metabolic pathway network was constructed using the merge function of Cytoscape software. In the network diagram, “nodes” represent active components, potential targets and pathways; “edges” represent the interactions of the component-target and target-pathway. An analysis of the target protein-protein interaction (PPI) networks found that *ACE*, *NOS2*, *NR3C1*, *CAI*, *PLG*, *ALB*, *CALMI*, *AKRIC1*, *HMGCR*, *NQO2* were the main target proteins in the interaction network and have anti-ischemic effects. Among them, ACE (angiotensin-converting enzyme) is a key component of the classical renin-angiotensin system (RAS), which can produce a series of angiotensins, such as angiotensin-I (Ang I), Ang II, and Ang-(1–7), and further plays an important role in the regulation of blood pressure, fluid-electrolyte balance, and protection against cerebral ischemia-reperfusion injury.<sup>18</sup> Nitric oxide (NO) is the

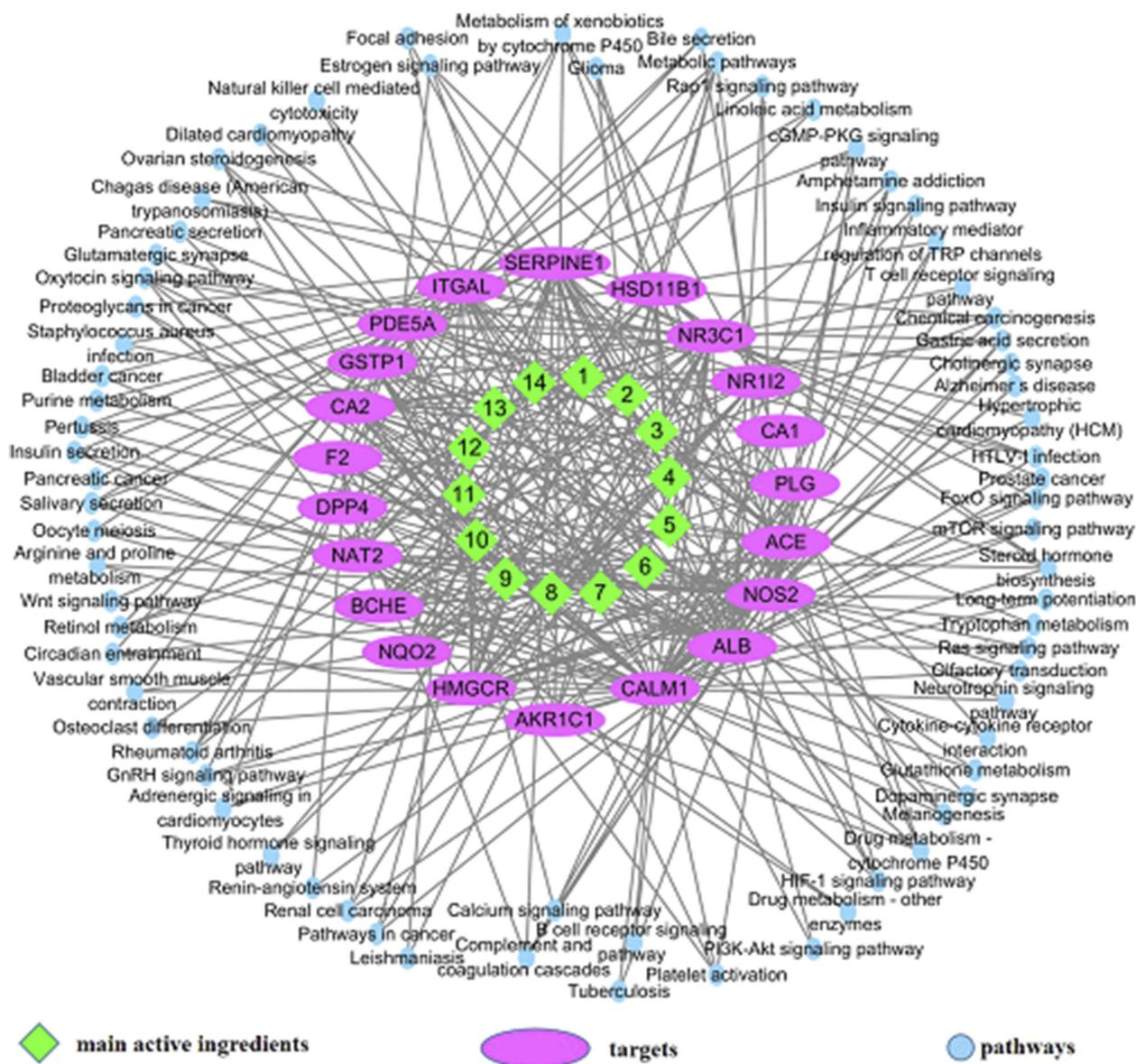


Figure 6 Active component-target-pathway network for *Pterocypselo elata* (*P. elata*).

product of L-arginine catalyzed with nitric oxide synthase (NOS). NOS is mainly distributed in macrophages, inflammatory neutrophils, microglia and astrocytes and induces NOS mRNA expression under ischemic and hypoxic conditions.<sup>19</sup> The large amount of NO produced by NOS during cerebral ischemia and reperfusion is toxic to nerve cells.<sup>20</sup>

In summary, our studies proved that *P. elata* has good anti-ischemic effects. Further analysis by network pharmacology showed that the main active components of *P. elata* include lactuside B, 11 $\beta$ , 13-dihydrolactucin acetate, and

11 $\beta$ , 13-dihydrolactucin, which regulate potential targets (*NR3C1*, *CA1*, *PLG*, *ACE*, *NOS2*, *ALB*, *CALM1*, *AKR1C1*, *HMGCR*, and *NQO2*) and intervene in metabolic pathways, complement and coagulation cascades, steroid hormone biosynthesis and other pathways to achieve a synergistic protection against cerebral ischemic injury. However, the main active components of *P. elata* and their regulation mechanisms require further verification. Our study provides a new perspective and clues for further studies on the anti-ischemic mechanism of the main active components of *P. elata*.

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## Disclosure

The authors confirm that there are no conflicts of interest in this work.

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