Using Network Pharmacology to Explore Therapeutic Effect of *Polygonum capitatum* on Renal Calculus in Rats

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Abstract [**Objectives**] To explore the therapeutic effect of *Polygonum capitatum* on renal calculus in rats based on network pharmacology. [**Methods**] Through the preliminary construction of *P. capitatum*-urolithiasis disease target network, explore the active components, action pathway and action target of *P. capitatum*-urolithiasis treatment, and use 1% ethylene glycol + 2% ammonium chloride to induce SD rat kidney calcium oxalate stone model to verify the efficacy of *P. capitatum*-urolithiasis treatment. [**Results**] Through the network pharmacological prediction, it is found that the important active components in *P. capitatum* were quercetin, gallic acid, rutin, silybin, catechin, kaempferol and so on; potential active targets include INS, CAT, IL-6, MOCOS, *etc.* The results also suggest that forkhead transcription factor signaling pathway (FoxO signaling pathway), tumor necrosis factor signaling pathway (TNF signaling pathway) and hypoxia-inducible factor signaling pathway (HIF-1 signaling pathway) are the core pathways. The results of biochemical indicators in animal experiment showed that the contents of serum urea nitrogen (BUN), creatinine (Cr) and malondialdehyde (MDA) in renal tissue in the treatment group (200, 500 mg/kg) were significantly lower than those in the model group, while the content of superoxide dismutase (SOD) was significantly higher than that in the model group. In addition, the kidney tissue H&E staining sections showed that *P. capitatum* alcohol extract administration group rats kidney calcium oxalate crystals were significantly reduced compared with the model group, the degree of renal tubular lumen expansion was lighter than the model group, suggesting that *P. capitatum* alcohol extract has the effect of improving renal calculus in rats. [**Conclusions**] This study provides a theoretical reference for the deep development of *P. capitatum* in the treatment of renal calculus.

Key words *Polygonum capitatum*, Urolithiasis, Network pharmacology

1 Introduction

The causes of urolithiasis are complex and diverse, and it is a common disease in the urinary system, which may be mainly related to environmental, dietary, living habits, immune response, microbial and genetic factors[1]. Clinically, it mostly manifests as low back pain, renal colic, hematuria and other symptoms. In addition, studies have found that persistent urolithiasis may lead to the occurrence of pyelonephritis, which in turn leads to diseases such as papillary necrosis and perirenal abscess. At present, the treatment of urolithiasis is relatively simple, mainly including two options: *in vivo* drug lithotripsy and *in vivo* and *in vitro* surgical lithotripsy. Literature research found that urolithiasis was a common disease in Guizhou Province. Traditional Chinese medicine has the characteristics of less side effects, safety and effectiveness, and shows good clinical value in urolithiasis.

The special product *Polygonum capitatum* in Guizhou Province is a dried whole herb of *P. capitatum*, which has the effects of diuretic drenching, heat clearing and detoxification, blood circulation and pain relief. In long-term clinical practice, it is often used to treat urolithiasis, urinary tract infections, pyelonephritis, cystitis and other urinary tract diseases[2]. At present, the main active components of *P. capitatum* are flavonoids and phenolic acids[3]. Besides, *P. capitatum* extract has significant anti-inflammatory, diuretic, antibacterial, antioxidant and other pharmacological activities[4]. It is preliminarily speculated that the mechanism of *P. capitatum* on urolithiasis may be related to its anti-inflammatory activity. However, the active components and targets of *P. capitatum* in the treatment of urolithiasis are still unclear, neither its mechanism of action, which seriously hinders the deep development of *P. capitatum* in the treatment of pyelonephritis.

Network pharmacology is a research concept and model that keeps pace with the times. By revealing the complex network relationship between "drug-gene-target-disease", it is helpful for understanding the molecular basis of disease from multiple dimensions and perspectives. Based on network analysis, this method can comprehensively and deeply study the interaction between drugs and targets and predict the pharmacological mechanism of drugs, which is similar to the principle of multi-component and multi-way treatment of diseases in traditional Chinese medicine[5]. Based on this, this study explored the main components, key targets and action pathways of *P. capitatum* in the treatment of urolithiasis through network pharmacology, and established a rat renal calcium oxalate stone model *in vivo* to preliminarily verify its efficacy.

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2 Materials and methods

2.1 Experimental animals Thirty-five SPF SD male rats (200 – 220 g) were provided by the Animal Experimental Center of Guizhou Medical University (Animal License No. SYXK (Gui) 2023-0002). The animals were adaptively fed in the animal room for one week before the start of the experiment.

2.2 Drugs and reagents *P. capitatum* was purchased from Duyun City, Qiannan, Guizhou Province, and was identified as the dried whole plant of *Polygonum capitatum* of Polygonaceae by Lin Yan, Associate Professor, School of Pharmaceutical Sciences, Guizhou Medical University. The identification results are shown in the published paper of our research team[6]. Potassium citrate, ethylene glycol, ammonium chloride, 0.9% sodium chloride and 10% formalin solution were all analytically pure domestic reagents purchased from Guizhou Simianti Reagent Co., Ltd. Superoxide dismutase (SOD) detection kit, malondialdehyde (MDA) detection kit, creatinine (Cr) kit and urea nitrogen (BUN) kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.3 Instruments Electronic balance (Sartorius Scientific Instrument Co., Ltd., model BSA223S), LC-04B medical refrigerated centrifuge (Jiangsu Zhengji Instrument Co., Ltd.), vacuum rotary evaporator (Tokyo Physical and Chemical Instrument Co., Ltd.), electric thermostatic water bath (Ningbo Qun'an Experimental Instrument Co., Ltd.), Laboratory lyophilizer (Beijing Litaide Technology Co., Ltd.), Multiskan microplate reader (Thermo Fisher Labsystems, Finland), *etc.*

2.4 Preparation of ethanol extract of *P. capitatum* The dried whole herb of the *P. capitatum* was pulverized and reflux extraction with 95% ethanol was performed for 3 times, 2 h each time. The extract were concentrated and combined with the filtrate, and freeze drying treatment was carried out to obtain a powder sample of the *P. capitatum* ethanol extract.

2.5 Network pharmacology analysis

2.5.1 Collection and screening of active compounds and target proteins from *P. capitatum*. After our research team carried out the separation of *P. capitatum* and literature search, we obtained 30 flavonoids and 27 phenolic acids. Because the traditional Chinese medicine of the *P. capitatum* is not include in the TCMSP database at present, we used 57 compounds from *P. capitatum* to query related target genes in the form of single compounds through TCMSP database (http://tcmspw.com/tcmsp.php) and PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Then the compounds without target information were removed, and the obtained related gene targets were converted into human genes through a String database.

2.5.2 Collection of urolithiasis target and construction of PPI network. With the keyword "urolithiasis" in DisGeNET database (http://www.disgenet.org/), GeneCards database (https://www.genecards.org), through searching and analyzing the OMIM database (https://omim.org/) separately, and then removing the duplicate, we obtained the urolithiasis related targets. Then, we plotted a Venn diagram through a Draw Venn Diagram website (http://bioinformatics.psb.ugent.be/webtools/Venn/) for the collected target proteins and disease targets related to the *P. capitatum* active compound, and obtained that gene data of the intersection of the compound and the disease target. Next, the obtained component and disease intersection targets were imported into a String database, a Multiple proteins "item was selected with a Homo sapiens" as the keyword, and an intersection gene was imported. The minimum required interaction score was set as high confidance $\geqslant 0.7$ to obtain the PPI network of drug and disease targets, and the node attribute table was imported into Cytoscape 3.6.0 software to construct the target-target network. Then the active compounds and urolithiasis targets were also imported into Cytoscape 3.6.0 software to construct the drug active component-disease network.

2.5.3 GO and KEGG pathway network. The obtained intersection genes were imported into DAVID database to obtain the enrichment analysis results of intersection gene GO and KEGG pathways, and the high-level bubble map function of Oimicshare software was used to draw (http://www.omicshare.com). The top 20 analysis data of GO enrichment pathway and pathway enrichment pathway were plotted with P as the parameter to obtain the corresponding results. The top 20 analysis data of GO enrichment pathway and KEGG enrichment pathway were plotted with *P* value as the parameter to obtain the corresponding results.

2.6 Animal pharmacological experiments Control group: feed + free drinking water + 2 mL distilled water + 2 mL 0.3% CMC-Na; model group: feed + 1% ethylene glycol free drinking water + 2 mL 2% ammonium chloride solution per day; positive drug group: feed + 1% ethylene glycol + 2 mL 2% ammonium chloride solution + 2 mL 25% potassium citrate solution; high dose group: feed + 1% ethylene glycol free drinking water + 2 mL 2% ammonium chloride solution gavage in the morning + low dose alcohol extract of *P. capitatum* (200 mg/kg) gavage in the afternoon; low dose group: feed + 1% ethylene glycol free drinking water + 2 mL 2% ammonium chloride solution gavage in the morning + high dose alcohol extract of *P. capitatum* (500 mg/kg) gavage in the afternoon.

After the last administration, the rats were anesthetized and sacrificed. About 8 mL blood was collected from the abdominal aorta and centrifuged at 3 500 r/min for 10 min. The upper serum was used to detect the contents of urea nitrogen and creatinine. The bilateral kidneys of rats were removed at −20 ℃, and the residual vascular pedicles and adipose tissues around the kidneys were removed with normal saline and weighed on an electronic balance. After the right kidney (stored in a refrigerator at −20 ℃) was rinsed with sterile saline, the kidney tissue was cut with tissue scissors, and the weight of the right kidney tissue of rats in each group was accurately weighed and homogenized. After thorough homogenization, it was centrifuged at 4 500 r/min for 10 min, and 10% of the homogenized supernatant was taken for testing, subpackaged and frozen for use. The kidney on the other side was cut

longitudinally, put into a centrifuge tube, fixed in 10% formalin solution for 20 h, numbered, and sent to Seville Biological Co., Ltd. for H&E staining. The serum of rats was taken and the contents of SOD, MDA, Cr and BUN in the serum of each rat were detected by superoxide dismutase detection kit, malondialdehyde detection kit, creatinine kit and urea nitrogen kit. The data were analyzed using GraphPad Prism 6.01, which was expressed as mean ± standard deviation, and one-way ANOVA was used for comparison between groups, and $P < 0.05$ was considered statistically significant.

3 Results and analysis

3.1 Network pharmacology prediction result

3.1.1 Screening of active compounds and target proteins from *P. capitatum*. After removing the compounds without targets through screening in TCMSP database and PubChem database, 28 components with potential activity were obtained, of which the top ten components are shown in Table 1. A total of 665 target genes were obtained after String database conversion and deleting the duplicates.

Table 1 Potential active components from *Polygonum capitatum*

No.	Chemical components	*OB*//%	*DL*
1	Quercetin	46.43	0.28
2	Ellagic acid	43.06	0.43
3	Kaempferol	41.88	0.24
4	Gallic acid	31.69	0.04
5	Rutin	3.20	0.68
6	Silybin	0.93	0.93
7	Kaempferol-3-O-glucopyranoside	14.03	0.74
8	Epicatechin-3-O-gallate	17.89	0.75
9	Catechin	54.83	0.24
10	Taxifolin	57.84	0.27

3.1.2 Screening of protein targets. A total of 72, 357 and 25 genes for urolithiasis were screened from DisGeNET, GeneCards and OMIM databases, respectively. A total of 388 genes related to urolithiasis were obtained after mass removal. The obtained urolithiasis targets and the active compound targets are intersected through a Draw Venn Diagram website to obtain 60 intersected gene targets.

3.1.3 Construction of PPI network. The 60 common targets were imported into the String database, and the compound-target relationship table and the attribute node table were made, and the protein interaction result map was imported into Cytoscape software. The nodes changed from small to large, and that color changed from light to dark, which indicate that the Degree value of the target point is larger, indicating that the biological activity of the node in the PPI network is higher. Topological analysis showed that the top ten protein targets were ALB (46), INS (40), IL6 (37), CAT (37), VEGFA (31), MMP9 (29), IL1B (29), CCL2 (27), ESR1 (26) and NOS3 (26). These results preliminarily suggest that these proteins play an important role in the treatment of urolithiasis with *P. capitatum*.

3.1.4 GO functional enrichment analysis and KEGG pathway enrichment analysis. Cellular Component (CC), Biological Process (BP) and Molecular Function (MF) are the main general analysis in the field of biology. GO enrichment analysis was performed on the common target of *P. capitatum* for urolithiasis treatment from these three aspects, and the results were as follows (Fig. 1A). It can be seen from the figure that it mainly involves the membrane system and cytoplasm of cells; the biological processes involved mainly include metabolic regulation, cell stress response, organic metabolism, biological regulation, *etc.*; the molecular functions involved include protein binding, catalytic activity, ion binding, anion coordination, *etc.* In addition, KEGG enrichment analysis was performed on these 60 common targets, and the top 20 important pathways were selected for bubble map visualization (Fig. 1B). The larger the bubble in the figure, the larger the *P* value, indicating that this pathway plays a more important role in the mechanism of *P. capitatum* in the treatment of urolithiasis. As a result, these pathways mainly include cancer pathway, forkhead transcription factor signaling pathway (FoxO signaling pathway), tumor necrosis factor signaling pathway (TNF signaling pathway), Hypoxia-inducible factor signaling pathway (HIF-1 signaling pathway) and Drug metabolism-cytochrome P450.

3.1.5 *P. capitatum*-compound-target network. The 29 monomeric compounds of *P. capitatum* were intersected with urolithiasis targets separately, and the summarized common target genes were introduced into Cytoscape 3.6.0 software to obtain the compound-target-pathway network (Fig. 2). The top six compounds were quercetin (Degree 27), gallic acid (Degree 23), rutin (Degree 20), silybin (Degree 20), catechin (Degree 18) and kaempferol (Degree 18); the top ten targets were INS (Degree 25), CAT (Degree 25), IL6 (Degree 25), MOCOS (Degree 24), ALB (Degree 22), ALPL (Degree 20), VEGFA (Degree 16), CCL2 (Degree 14), GUSB (Degree 14), and NOS3 (Degree 13). These results indicate that quercetin, gallic acid, rutin, silybin and other compounds are the main components of *P. capitatum* for the treatment of urolithiasis.

3.2 Effect of *P. capitatum* extract on renal calculus in rats

3.2.1 Anatomical results of rat kidney. The kidney dissection results of rats in the control group, the model group, the positive drug group, the low dose of *P. capitatum* alcohol extract group, and the high dose of *P. capitatum* alcohol extract group are shown in Fig. 3. It can be seen from the figure that there was no significant pathological damage in the kidneys of rats in each group, and the color and shape of the normal kidneys were maintained. However, the kidneys of individual mice in the low-dose group and the high-dose group were slightly swollen, and the surface of the kidneys was not smooth and frosted when touched. The results of H&E staining showed that the structure of renal tubules and glomeruli in the control group was normal and clear, and no obvious calcium oxalate crystals were found. In the model group, calcium oxalate crystallization was obvious, the renal tubular lumen was swollen and dilated, there were some necrotic and exfoliated epithelial cells in the lumen, and the renal cystic cavity adhered to the surrounding tissues with unclear boundaries. The results

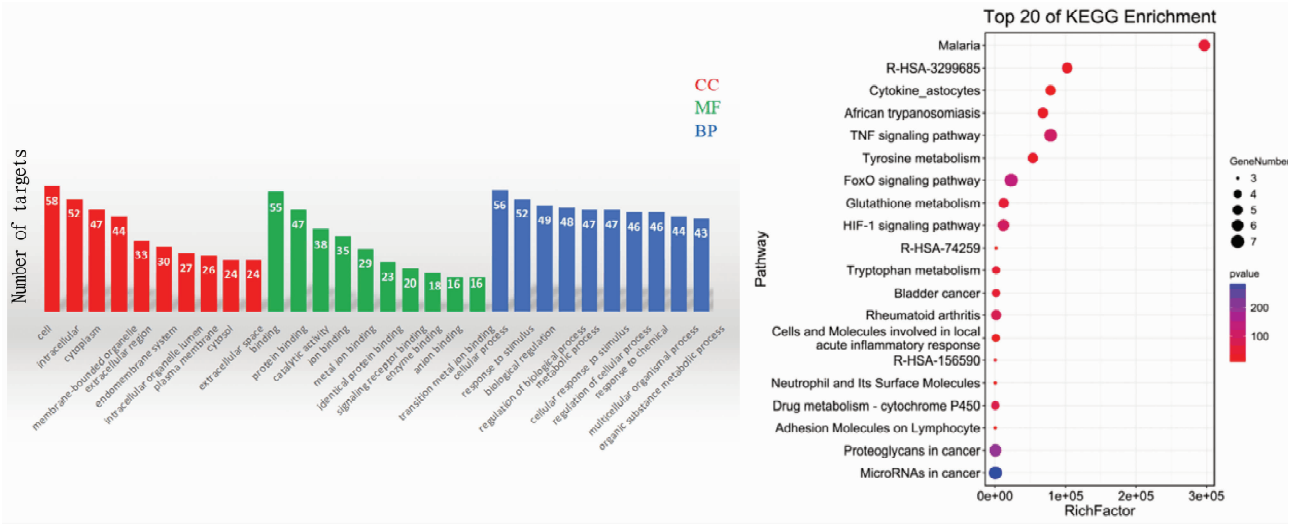
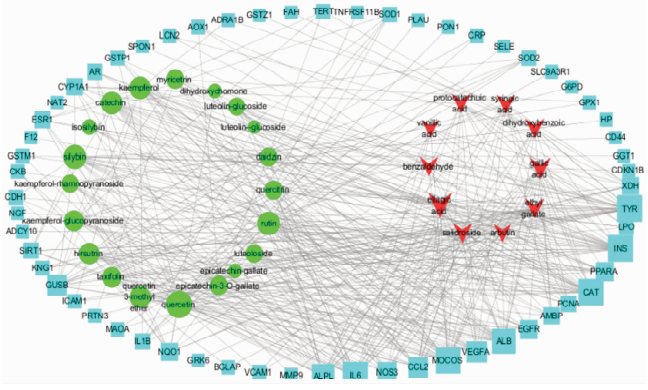


Fig. 1 GO enrichment analysis (A) and KEGG pathway enrichment analysis (B)

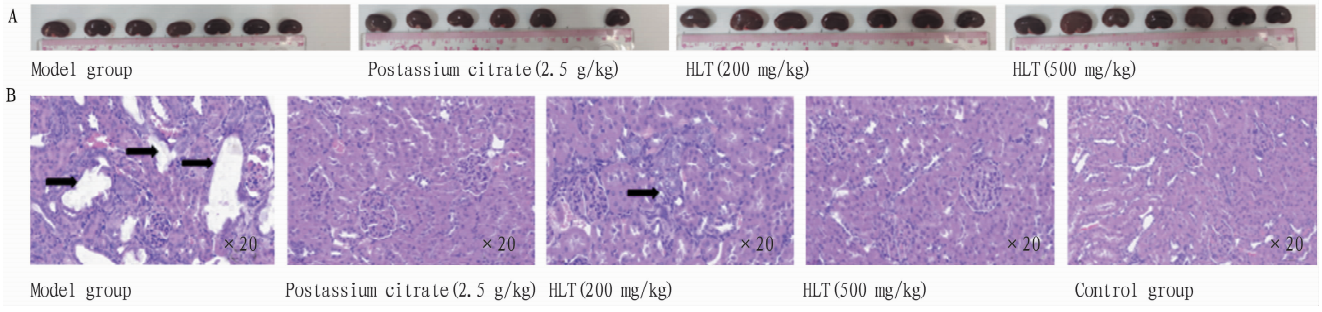


NOTE Green O denotes flavonoids, red V denotes phenolic acids, and blue boxes denote urolithiasis disease targets.

Fig. 2 Compound-target network diagram

showed that the lumen dilation of the positive drug group and the administration group was significantly lower than that of the model group, which preliminarily indicated that the extract of *P. capitatum* (500 mg/kg) had the effect of improving the renal calculus in rats.

3.2.2 Detection results of biochemical indicators in renal tissue and serum of rats. As shown in the blood biochemical results of rats in each experimental group (Fig. 4), compared with the model group, the levels of serum urea nitrogen (BUN), serum creatinine (Cr) and malondialdehyde (MDA) in kidney tissue in the *P. capitatum* alcohol extract groups (200, 500 mg/kg) were lower than those in the model group; the content of superoxide dismutase (SOD) was higher than that in the model group, which further indicated that the alcohol extract of *P. capitatum* could improve the renal calculus in rats.

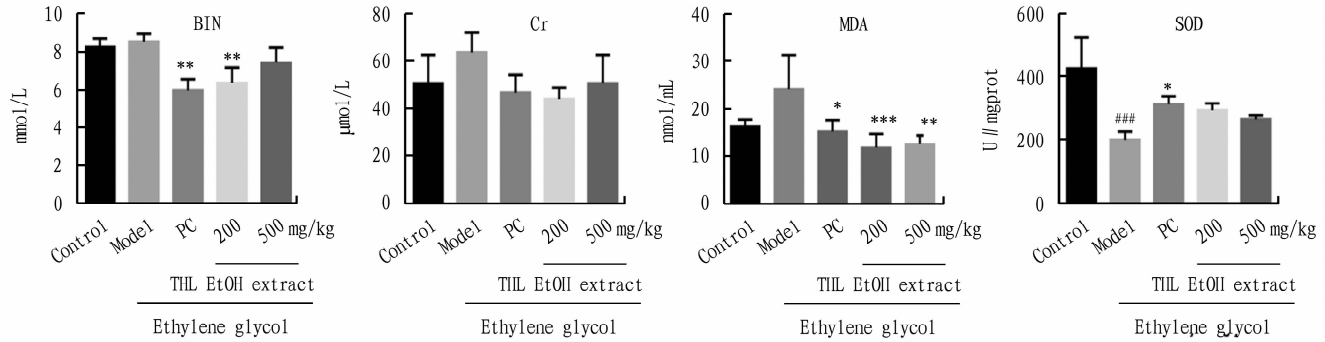


NOTE A. Kidney of each group, THL: *P. capitatum*; B. H&E staining of kidney tissue; the black arrow indicates calcium oxalate crystals; positive drug, potassium citrate.

Fig. 3 Treatment of kidney stones with the alcohol extract of *Polygonum capitatum*

4 Discussion

Modern studies have shown that urolithiasis may cause pyelonephritis, the mechanism of which may be that urolithiasis hinders the smooth flow of the urinary tract, hinders the free outflow of urine, causes the accumulation of urine, leads to bacterial growth, and stones damage the urinary tract mucosa, making the urinary system more vulnerable to bacterial infection[7]. In addition, urolithiasis is closely related to metabolic disorders, such as abnormal uric acid metabolism, abnormal lipid metabolism, abnormal blood sugar metabolism, *etc.*[8-9]. The results of network pharmacological prediction showed that the main active components of *P. capitatum*, such as quercetin, rutin and silybin, were common fla-



NOTE PC denotes positive control; THL EtOH extract denotes *P. capitatum* alcohol extract; $^{\#\#\#}P < 0.001$, compared with control; $^{**}P < 0.01$, and $^{***}P < 0.001$, compared with the model group.

Fig. 4 Contents of BUN, Cr in serum and MDA, SOD in renal tissue of rats

vonoids. Forkhead transcription factor signaling pathway, tumor necrosis factor signaling pathway and hypoxia-inducible factor signaling pathway play an important role in the mechanism of *P. capitatum* flavonoids and phenolic acids in the treatment of urolithiasis. Furthermore, gallic acid, quercetin, rutin and silibinin are compounds with high nodal value, which are the key active ingredients for the treatment of urolithiasis. In terms of targets, the enrichment results showed that insulin, catalase and other targets were the main targets. FoxO is an important transcription factor involved in cell regulation and oxidative stress, which can protect kidney cells by reducing the release of oxidative stress substances. It has been found that FoxO is closely related to the metabolism of blood lipids and blood glucose. When the human body is severely deficient in oxygen molecules, the body will secrete a large number of HIF-1 cytokines, which will further induce the expression of HIF-1 factors, such as elevated blood glucose, hypoxia and other factors[10-11]. In addition, in this study, calcium oxalate-induced renal calculus rats were preliminarily explored, and it was found that oxidative stress may play a positive role in the production of nitric oxide, thus increasing the concentration of malondialdehyde in rat serum. Therefore, MDA and SOD are often used to assess antioxidant and oxidative capacity in the body[12]. MDA can attack the biomembrane containing polyunsaturated fatty acids, and can be used as an indicator to evaluate the oxidation level of the body, and can induce the production of lipid peroxidation products. SOD is an enzyme from cells, which has certain enzymatic activity and can help dismutate superoxide anion, while its dismutation product hydrogen peroxide can be completely removed by catalase and glutathione peroxidase[13]. The results showed that the alcohol extract of *P. capitatum* could effectively reduce the content of MDA and increase the content of SOD in the extract of renal tissue, suggesting that *P. capitatum* may achieve the effect of treating renal calculus by alleviating oxidative damage and reducing calcium oxalate crystallization, thereby reducing the risk of pyelonephritis.

In summary, we preliminarily predicted the active ingredients, targets and key pathways of *P. capitatum* in the treatment of urolithiasis by using network pharmacological methods. In addition, we established, the calcium oxalate renal calculus model of rats *in vivo* to preliminarily verify the efficacy of *P. capitatum* alcohol extract in the treatment of urolithiasis, but the related in-depth mechanism of action needs to be further explored.

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