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Uric Acid-lowering Effects of *Clerodendranthus spicatus*

Demei HU[△], Wenyu MA[△], Jianlian SHI, Leizhuang YANG, Yan SHEN, Jia LIU[*]

Kunming Medical University Haiyuan College, Kunming 650106, China

Abstract [**Objectives**] To investigate the uric acid-lowering effects of *Clerodendranthus spicatus* (Thunb.) C. Y. Wu ex H. W. Li. [**Methods**] After the optimization and scale-up of *C. spicatus* extraction, HP-20 macroporous resin was employed for fractionation using four eluents with ethanol concentrations of 10%, 40%, 70%, and 95%, yielding fractions designated as Fr. 1 to Fr. 4. The pharmacological effects of these fractions (Fr. 1-Fr. 4) were subsequently investigated in hyperuricemic model animals. [**Results**] Fr. 1-Fr. 4 reduced the serum levels of uric acid (UA), xanthine oxidase (XOD), blood urea nitrogen (BUN), and creatinine (CRE) in hyperuricemic rats, with Fr. 4 demonstrating the optimal effect. All fractions increased body weight, decreased renal index, and exhibited certain protective effects against renal injury. [**Conclusions**] *C. spicatus* exhibits anti-hyperuricemic activity by reducing serum uric acid levels and also displays obvious renal protective effects.

Key words *Clerodendranthus spicatus*, Fractionation, Uric acid-lowering, Hyperuricemia

1 Introduction

Clerodendranthus spicatus (Thunb.) C. Y. Wu ex H. W. Li (Lamiaceae), also known as cat's whiskers, is the dried aerial part of the plant. This species is distributed across multiple provinces in China, including Guangxi, Guangdong, Yunnan, Fujian, Hainan, and Taiwan[1-2]. It possesses a sweet and slightly bitter taste, exhibits a cooling nature, and primarily influences the kidney meridian. The plant is known for its effects in clearing heat, inducing diuresis and expelling stones, and is commonly used in the treatment of diabetes, nephritis, urinary calculi, rheumatism, and other diseases[3]. According to Dai medicine, this herb is recognized for its abilities to clear heat and detoxify, induce diuresis and expel stones, cool the blood, and stop bleeding, thereby serving commonly as a detoxifying agent[4]. The chemical constituents of *C. spicatus* include flavonoids, phenols[5], terpenoids[6-7], volatile oils[8], lignans[9-10], alkyl glycosides[11], coumarins[12], and sterols[13]. It has been reported that *C. spicatus* significantly improves hyperuricemia and gouty nephropathy, but its specific mechanism of action remains unclear[14]. In this study, the extraction process of *C. spicatus* was optimized, and the resulting extract was separated into four fractions by column chromatography. Each fraction was enriched with specific components, which can reduce the dosage of active compounds in products and mitigate side effects associated with other inactive components.

A fraction exhibiting significant uric acid-lowering activity was screened out, which may offer valuable guidance for the clinical application of its pharmacological properties and therapeutic efficacy. Additionally, this finding contributes to a more scientific and comprehensive understanding of *C. spicatus*.

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[△] These authors contributed equally to this work.

Demei HU, master's degree, lecturer; Wenyu MA, master's degree.

[*] Corresponding author. Jia LIU, master's degree, professor.

2 Materials

2.1 Test materials *C. spicatus* was obtained from Kunming Changchen Technology Co., Ltd. Male Sprague-Dawley (SD) rats, weighing (200 ± 20) g, were used in the study.

2.2 Instruments The instruments employed were electric heating mantle (Jiangsu Jinyi Instrument Technology Co., Ltd.), chromatography column (Kunming Changchen Technology Co., Ltd.), rotary evaporator (Gongyi Huayi Co., Ltd.), and HP-20 macroporous adsorption resin (Tianyun Yunkai Resin Technology Co., Ltd.).

2.3 Reagents The reagents utilized in this study included 95% ethanol (Shandian Pharmaceutical Co., Ltd., Yanglin Industrial Development Zone, Yunnan), methanol (Guangdong Guanghua Technology Co., Ltd.), silica gel G plate (Qingdao Ocean Chemical Factory), rosmarinic acid (Beijing North Weiye Metrology Institute), caffeic acid (North Weiye Metrology Group Co., Ltd.), and benzoic acid (North Weiye Metrology Group Co., Ltd.). Additionally, adenine, ethambutol hydrochloride, and 0.9% sodium chloride solution were employed. Kits for blood urea nitrogen (BUN; serial No.: 10125102215), creatinine (CRE; serial No.: 10125112420), uric acid (UA; serial No.: 1012512138), and xanthine oxidase (XOD; serial No.: ATYL12101) were procured from Beijing Boxbio Science & Technology Co., Ltd.

3 Methods and results

3.1 Orthogonal test An orthogonal test design table comprising four factors at three levels was generated using SPSS AU. Nine experiments were carried out according to the design table. The extraction was performed based on the four factors in the orthogonal design: ethanol concentration, solid-liquid ratio, extraction duration, and number of extraction cycles. Each extraction was conducted in duplicate, and the average extraction rate of the two parallel samples was calculated to represent the extraction rate under the corresponding conditions. The results are shown in Table 1.

Table 1 Results of the orthogonal test

No.	Factor 1 (ethanol concentration)	Factor 2 (solid-liquid ratio)	Factor 3 (extraction duration)	Factor 4 (number of extraction cycles)	Extraction rate//%
1	1 (60%)	1 (1∶15)	1 (1 h)	1	22.93
2	1 (60%)	2 (1∶20)	3 (2 h)	2	28.45
3	1 (60%)	3 (1∶25)	2 (1.5 h)	3	30.88
4	2 (70%)	1 (1∶15)	3 (2 h)	3	27.35
5	2 (70%)	2 (1∶20)	2 (1.5 h)	1	17.92
6	2 (70%)	3 (1∶25)	1 (1 h)	2	20.01
7	3 (80%)	1 (1∶15)	2 (1.5 h)	2	23.68
8	3 (80%)	2 (1∶20)	1 (1 h)	3	25.56
9	3 (80%)	3 (1∶25)	3 (2 h)	1	24.57
k_1	27.42	24.65	22.83	21.81	
k_2	21.76	24.16	24.16	24.05	
k_3	24.60	25.15	26.79	27.93	
Range	5.66	0.99	3.96	6.12	

According to the analysis of the orthogonal test results, the factors influencing the extraction rate were ranked as follows: number of extraction cycles > ethanol concentration > extraction duration > solid-liquid ratio.

Based on the orthogonal test, both the extraction rate and the spot characteristics observed in thin-layer chromatography (TLC) were investigated. The effects of various extraction methods on the enrichment of components were analyzed by TLC. A mixed standard solution containing rosmarinic acid, caffeic acid, and benzoic acid was prepared, and the extracted substances from each group were dissolved in methanol. A solvent mixture composed of cyclohexane, ethyl acetate, isopropanol, and formic acid in a ratio of 10∶3∶2∶1 was used as the developing solvent. The developing chamber was saturated for 15 min prior to deveopment. After development, the samples were observed under ultraviolet light at a wavelength of 365 nm. The results are presented in Fig. 1. According to the spot analysis of TLC, the number of spots was consistent across all extraction methods.

3.2 Scale-up extraction Based on the orthogonal test results and TLC analysis, the conditions selected for scale-up extraction were as follows: number of extraction cycles, 3; ethanol concentration, 60%; extraction duration, 2 h; and solid-liquid ratio, 1∶25. A total of 7 kg of *C. spicatus* was extracted using this method. After concentration, 2.177 kg of extract was obtained.

3.3 Fractionation of the extract Of the 2.177 kg extract, 0.268 kg was reserved as a reference sample. The remaining 1.909 kg was subjected to fractionation using HP-20 macroporous resin and eluted with ethanol solutions of decreasing polarity. Four fractions were obtained by elution using ethanol concentrations of 10%, 40%, 70%, and 95%, designated as Fr. 1 to Fr. 4, respectively. TLC was employed throughout the elution process to monitor the separation of components at each polarity level. Each fraction was concentrated under reduced pressure, yielding the following: 10% ethanol fraction (Fr. 1), 1 313.2 g; 40% ethanol fraction (Fr. 2), 69.9 g; 70% ethanol fraction (Fr. 3), 70.6 g; and 95% ethanol fraction (Fr. 4), 41.2 g.

3.4 Pharmacological tests

3.4.1 Experimental methods. (i) Drug dosage. The human clinical dose of raw *C. spicatus* is 20 g per day. According to the body surface area conversion between rats and humans, the equivalent dose (5.2 g crude drug/kg) was set for Fr. 1-Fr. 4. The dosage design was as follows: blank group, administered the same volume of distilled water; model group, administered the same volume of distilled water; positive drug group, administered 5.2 mg/kg; Fr. 1-Fr. 4 groups, each administered 5.2 g crude drug/kg, respectively.

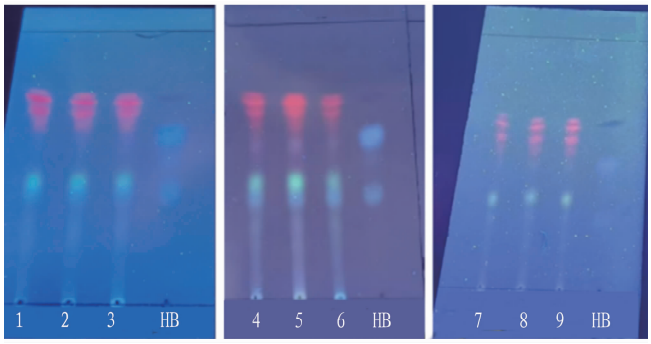
(ii) Drug preparation. 100 mg of adenine and 250 mg of ethambutol hydrochloride powder were accurately weighed, sterilized at high temperature, and subsequently dissolved in 10 mL of 0.9% sodium chloride solution to prepare a suspension of adenine and ethambutol hydrochloride. The suspension was subjected to ultrasonication for 10 min and then stored at 4 ℃ in a refrigerator. Prior to each intragastric administration, the suspension was thoroughly shaken. The volume for intragastric administration was 10 mL/kg.

(iii) Grouping, model establishment and administration. Fifty-six healthy adult SD rats were randomly divided into 7 groups ($n=8$ each): blank control group, model control group, positive drug group, Fr. 1 group, Fr. 2 group, Fr. 3 group, and Fr. 4 group. Rats in each group were administered intragastrically at the corresponding dosages, while the blank control and model control groups were administered an equivalent volume of distilled water daily for 28 consecutive days. With the exception of the blank control group, all other groups were subjected to intragastric administration of adenine and ethambutol hydrochloride to induce hyperuricemia (HUA).

(iv) Effects of *C. spicatus* on serum levels of UA, XOD, CRE and BUN in hyperuricemic rats. Blood samples were collected 24 h after the final administration in HUA rats. Serum was separated, and the levels of UA, XOD, CRE, and BUN were determined and calculated according to the kit instructions.

(v) Effects of *C. spicatus* on renal index in hyperuricemic rats. The renal index was calculated according to the following formula based on body weight and total weight of bilateral kidneys. Renal index = (Weight of bilateral kidneys/Rat body weight) × 100%.

(vi) Effects of *C. spicatus* on renal histopathology in hyperuricemic rats. Fixation: fresh renal tissues were immersed in 4% paraformaldehyde fixative at a ratio of fixative volume to tissue volume of 10∶1. Washing and dehydration: the fixed renal tissues were dehydrated using a graded ethanol series to remove the fixative and crystalline precipitates. The tissues were sequentially dehydrated in 50%, 70%, 85%, 95%, and finally absolute ethanol, with each step lasting 1 h. Clearing: xylene was used as a clearing agent for 1 h to displace ethanol in the tissue samples.



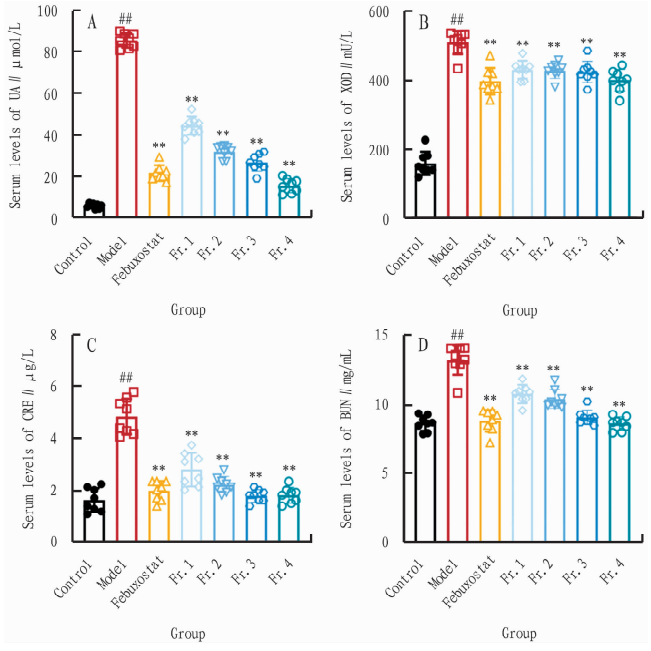
NOTE The digits on the TLC plate correspond to those presented in Table 1. HB represents the mixed standard.

Fig. 1 Thin-layer chromatogram

Paraffin infiltration and embedding: renal tissues were immersed in a 1∶1 mixture of melted paraffin and xylene for 1 h, followed by immersion in melted paraffin alone for 3 h. Sectioning: the embedded paraffin block was trimmed into a regular quadrangular frustum, fixed in the specimen clamp of a rotary microtome with the section surface parallel to the knife edge, and then sectioned. Mounting and drying: the paraffin ribbon was segmented into individual sections, flattened in warm water, mounted onto glass slides, gently heated to ensure complete spreading, and subsequently dried in an incubator. Dewaxing and hydration: sections were sequentially immersed in xylene I for 20 min, absolute ethanol II for 5 min, and 75% ethanol for 5 min, followed by rinsing with tap water. Eosin staining: sections were dehydrated in 85% and 95% graded ethanol, then stained with eosin solution for 5 min. Dehydration and mounting: sections were sequentially treated with absolute ethanol I for 5 min, absolute ethanol II for 5 min, absolute ethanol III for 5 min, xylene I for 5 min, and xylene II for 5 min for clearing, followed by mounting with neutral balsam. Microscopic examination: images were captured and analyzed.

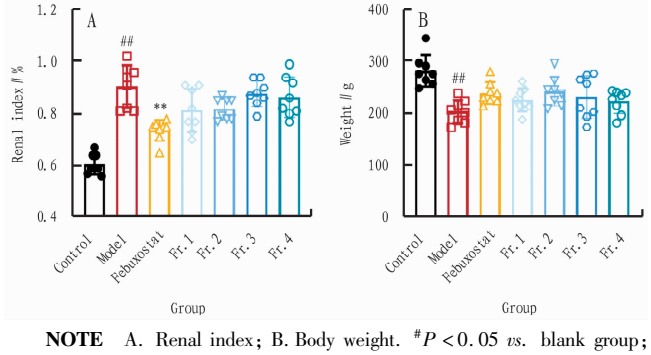
(vii) Statistical analysis. SPSS 25.0 software was used for statistical analysis. Data from each group were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among multiple groups were performed using one-way ANOVA. The *LSD* test was used when the variances were homogeneous, whereas the Dunnett's T3 test was applied in cases of heterogeneous variances. A P value of less than 0.05 was considered statistically significant.

3.4.2 Experimental results. (i) Effects of *C. spicatus* on serum levels of UA, XOD, CRE and BUN in hyperuricemic rats. Compared to the blank group, the serum levels of UA, XOD, CRE and BUN in the model group were significantly increased, with a statistically significant difference ($P < 0.01$). Compared to the model group, the levels of UA, XOD, CRE and BUN in the Fr. 1-Fr. 4 groups were significantly decreased ($P < 0.01$). The results indicated that all fractions of *C. spicatus* (Fr. 1-Fr. 4) reduced the serum levels of UA, XOD, CRE and BUN in hyperuricemic rats, with the 95% ethanol fraction (Fr. 4) exhibiting the most pronounced effect. The results are shown in Fig. 2.



NOTE A. UA; B. XOD; C. CRE; D. BUN. $^{\#}P < 0.05$ *vs.* blank group; $^{*}P < 0.05$ *vs.* model group.

Fig. 2 Serum levels of UA, XOD, CRE and BUN in rats



NOTE A. Renal index; B. Body weight. $^{\#}P < 0.05$ *vs.* blank group; $^{*}P < 0.05$ *vs.* model group.

Fig. 3 Effects of DTX on renal index and body weight in model rats ($\bar{x} \pm s$, $n = 8$)

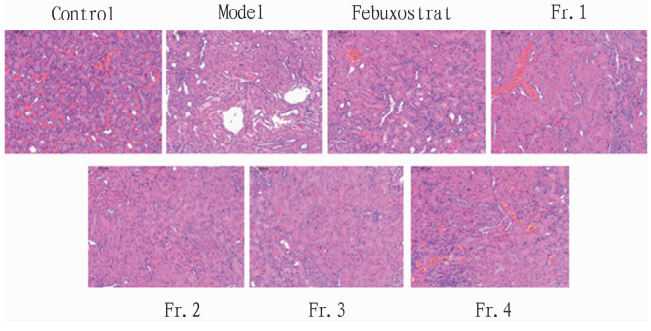


Fig. 4 Effects of *Clerodendranthus spicatus* on renal histopathology in hyperuricemic rats (×100)

(ii) Effects of *C. spicatus* on renal index in hyperuricemic rats. Compared to the blank group, the body weight of the model group was significantly decreased, with a statistically significant difference ($P < 0.05$). In comparison to the model group, the

body weight of the Fr. 1-Fr. 4 groups showed an increasing trend, although this difference was not statistically significant ($P > 0.05$). Compared to the blank group, the renal index of the model group was significantly increased ($P < 0.05$). Compared to the model group, the renal index of the Fr. 1-Fr. 4 groups showed a decreasing trend, but this difference was not statistically significant ($P > 0.05$). The results indicated that the Fr. 1-Fr. 4 groups increased the body weight and reduced the renal index of rats. The results are shown in Fig. 3.

(iii) Effects on renal histopathology in hyperuricemic rats. The results showed that after HE staining, renal cells in the blank group exhibited normal morphology with a clear glomerular structure, no hyperplasia or atrophy, intact capillaries, no inflammatory cell infiltration in the renal interstitium, and no obvious pathological changes. Compared to the blank group, the model group showed obvious edema in the glomeruli and surrounding renal tubular epithelial cells within the renal cortex, accompanied by cytoplasmic changes and a significant increase in cell volume, presenting marked pathological alterations. Compared to the model group, the Fr. 1 group showed swelling of renal tubular epithelial cells with increased volume, but no inflammatory cell infiltration was observed around these cells. Additionaly, the glomerular structure remained clear, showing no obvious pathological changes. The Fr. 2 and Fr. 3 groups exhibited slight swelling of renal tubular epithelial cells in the absence of inflammatory cell infiltration, and the glomerular structure remained intact with no obvious pathological changes.

In the Fr. 4 group, no glomerular hyperplasia or atrophy was observed; the capillaries remained intact with normal wall morphology, and no inflammatory cell infiltration appeared in the renal interstitium. These results indicate that *C. spicatus* exerts a certain protective effect against renal injury. The results are shown in Fig. 4.

4 Discussion

In this study, the extraction process of *C. spicatus* was optimized. According to the extraction rate and spot characteristics observed via TLC, a scale-up extraction was performed. The concentrated extract was fractionated using HP-20 macroporous resin, yielding four fractions designated Fr. 1 to Fr. 4. This method reduces the dosage of inactive components in product applications and provides a theoretical basis for product development. Pharmacological tests showed that *C. spicatus* significantly improved renal morphology, renal index, and serum levels of UA, XOD, CRE, and BUN in a rat model of HUA. Notably, the 95% ethanol elution fraction exhibited the most pronounced therapeutic effects. HE staining results demonstrated that *C. spicatus* alleviated renal injury in HUA rats and exerted a renoprotective effect. Collectively, these findings indicate that *C. spicatus* possesses anti-hyperuricemic activity, effectively reduces serum uric acid levels, and displays a distinct renoprotective effect.

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