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Short communication

Anti-Hyperuricemic and Xanthine Oxidase Inhibitory Effects of *Pogostemon cablin* (Blanco) Benth.

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Abstract

Background and objectives: Hyperuricemia is one of the major causes of gout and other oxidative stress-related diseases. Inhibition of xanthine oxidase activity, an enzyme that converts hypoxanthine to uric acid, has been considered as one of the therapeutic methods for hyperuricemia. *Pogostemon cablin* (Blanco) Benth. has been widely used in traditional medicine in Asia. This study aimed to evaluate the anti-hyperuricemic and xanthine oxidase inhibitory effect of extracts and fractions of *Pogostemon cablin*. **Methods:** The dried aerial parts of *P. cablin* were soaked in 70% ethanol at room temperature for 72 h and were successively fractionated with n-hexane, ethyl acetate and n-butanol. Xanthine oxidase inhibitory activity of the samples was determined spectrophotometrically at 290 nm. The hypouricemic effect of extract was investigated in normal and hyperuricemic mice induced by potassium oxonate. **Results:** Our results showed that the ethyl acetate fraction of *P. cablin* was able to inhibit xanthine oxidase with IC_{50} value of 96.39 ± 2.56 µg/mL. Moreover, *P. cablin* ethanol extract was found to reduce blood uric acid in Swiss albino mice at doses of 100 and 300 mg/kg and increased renal excretion of uric acid. **Conclusion:** *Pogostemon cablin* and extracts and fractions may have the potential to prevent hyperuricemia and require further clinical research.

Keywords: hyperuricemia; medicinal plant extract; *Pogostemon cablin*; uric acid; xanthine oxidase

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Introduction

Uric acid is the end product of purine metabolism in the human body, synthesized from external food or endogenously [1]. Hyperuricemia is a condition in which blood uric acid levels are abnormally high and can lead to arthritis, kidney stones, especially gout, a common rheumatic disease, and acute arthritis [2]. Xanthine oxidase is a homodimer with a molecular weight of 290 kDa, abundant in the liver and intestines [3]. It affects purine metabolism, and oxidizes hypoxanthine to xanthine and then to uric acid. Finally, uric acid is converted to superoxide ($O^{2\bullet-}$) and hydroperoxide (H_2O_2) [4]. The inhibition of xanthine oxidase activity has been widely suggested as a therapeutic strategy for managing hyperuricemia by reducing the concentration of uric acid. allopurinol has gained extensive utilization as a pharmacotherapeutic agent for the management of hyperuricemia due to its proficiency in inhibiting xanthine oxidase activity. Nevertheless, the administration of allopurinol is associated with potential adverse effects, including allergies, hypersensitivity reactions, and nephropathy.

Pogostemon cablin (Blanco) Benth., is distributed and mainly used in Asian countries. It is an aromatic damp-resolving drug in traditional Chinese medicine, commonly used to treat

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digestive disorders, vomiting, chest tightness, fatigue, abdominal pain, diarrhea, and headaches [5]. Pharmacological investigations have revealed numerous significant effects of *P. cablin*, including antioxidant, analgesic, anti-inflammatory, anti-aggregation, anti-thrombotic, anti-depressant, anti-emetic, hemostatic, and cytotoxic activities [6]. However, at present, *P. cablin*'s inhibitory effect on xanthine oxidase enzyme, lowering blood uric acid has not been studied and published. Therefore, in this study, we evaluated the effect of *P. cablin* extract and fractions to inhibit xanthine oxidase in vitro and its anti-hyperuricemic in vivo, to provide information which support the gout treatment.

Materials and Methods

Ethical considerations

All animals were maintained according to a protocol approved by the Ethical Committee of the Vietnam National University, Hanoi (code DHYD.CS.22.01, 05/December/2022), following the international rules for animal research.

Chemicals

Xanthine oxidase, xanthin, allopurinol, potassium oxonate (Sigma-Aldrich, Singapore); ethanol, *n*-hexane, ethyl acetate, *n*-butanol, dimethyl sulfoxide (DMSO) (Longxing Chemical, China) Uric acid assay kit (MAK077, Sigma-Aldrich, Singapore) and creatinine assay kit (MAK080, Sigma-Aldrich, Singapore) were used in this study.

Plant material

The dried aerial parts of *Pogostemon cablin* (Blanco) Benth. was purchased in December 2022 from Hanoi, Vietnam. The plant samples were authenticated by Department of Pharmacognosy and Traditional Medicine, University of Medicine and Pharmacy, Vietnam National University, Hanoi, and a voucher specimen (No: DLPC22UMP-VNU) was deposited at the Department of Pharmacology, University of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam.

The dried aerial parts of *Pogostemon cablin* (Blanco) Benth. (1 kg) were soaked in 70% ethanol at room temperature for 72 hours (3 L× 3 times). The combined extracts were filtered and evaporated under reduced pressure to yield a green residue (97.93 g), which was suspended in water and successively partitioned with organic solvents, then concentrated to yield three extracts of *n*-hexane (4.1 g), ethyl acetate (EtOAc 25.4 g), *n*-butanol (37.5g), and a water-soluble (5.6 g).

Xanthine oxidase inhibitory assay

The xanthine oxidase inhibitory activity of the samples was determined using the protocol described previously [7].

Animals

Fifty Swiss albino mice were randomly divided into 5 groups, 10 mice each. The hyperuricemia was induced by single intraperitoneal injection of potassium oxonate suspension [8]. Group I served as the physiological control and was given distilled water continuously for four days, followed by an intraperitoneal injection of 0.9% NaCl. Group II served as the pathological control and was also given distilled water continuously for four days, followed by an intraperitoneal injection of potassium oxonate (300 mg/kg b.w.); one hour later mice were given water. Group III served as the control drug group and was given distilled water continuously for four days, followed by an intraperitoneal injection of potassium oxonate (300 mg/kg b.w.) and one hour later allopurinol (10 mg/kg b.w.). Group IV received oral EtOH extract at a dosage of 100 mg/kg body weight once in the morning for four consecutive days, followed by an intraperitoneal injection of potassium oxonate (300 mg/kg b.w.) on the fifth day, and one hour later, the mice were given another dose of EtOH extract. Group V received oral EtOH extract at a dosage of 300 mg/kg b.w. once in the morning for four consecutive days, followed by an intraperitoneal injection of potassium oxonate (300 mg/kg b.w.) on the fifth day, and one hour later, the mice were given another dose of EtOH extract. Blood samples were collected from the tail of the mice one hour after injection and water/extract administration. Urine was collected from each mouse for five hours after the last drug dose, and the uric acid and urinary creatinine levels were quantified. At the end of the experiment, blood tail was taken from all mice in each group, and serum was analyzed for uric acid and creatinine levels by commercial kits.

Statistical analysis

The data were expressed as mean±SEM(standard error of the mean) and statistical processing based on One–Way ANOVA or T-test (SigmaStat-3.5 software). The test results reached

statistical significance with 95% confidence when $p<0.05$.

Results and Discussion

The results of this study (Table 1) demonstrated that the EtOAc fraction had the ability to inhibit xanthine oxidase with an IC_{50} values of 96.39±2.56 µg/mL, while other fractions showed weak inhibitory activity. The positive control, allopurinol, showed strong xanthine oxidase inhibitory effect with IC_{50} values of 1.08 ± 0.09.
The anti-hyperuricemic effect of *Pogostemon cablin* extract is shown in Table 2.

Table 1. The xanthine oxidase inhibitory effect of *Pogostemon cablin* extract and fractions

Sample	IC_{50} (µg/mL)
EtOH extract	>100
EtOAc fraction	96.39 ± 2.56
n-Hexan fraction	>100
BuOH fraction	>100
H_2O fraction	>100
Allopurinol	1.08 ± 0.09

Our results showed that blood uric acid and urinary uric acid of mice increased after injection of potassium oxonate in the acute model. This results are consistent with the mechanism of hyperuricemia in rodents as well as the results of previous studies [9]. The results of Table 2 also show that *P. cablin* EtOH extract at both doses and allopurinol, did not change the serum creatinine concentration compared with group I and group II ($p>0.05$).
The group of mice injected intraperitoneally with potassium oxonate and treated with *Pogostemon cablin* EtOH extract at both doses of 100 mg/kg b.w. and 300 mg/kg b.w. showed reduced uric acid levels in serum and in urine, statistically significant compared with the group II ($p<0.05$). The results showed that in a mouse model of acute uric acid elevation, *P. cablin* EtOH extract at the doses of 100 mg/kg and 300 mg/kg was able to prevent the increase in blood uric acid levels and at the same time increase creatinine excretion through urine.
Uric acid, a metabolic byproduct arising from the breakdown of dietary proteins, particularly purine metabolism, is normally eliminated through renal excretion. However, in cases where renal function is compromised, the complete clearance of uric acid through the urinary system is impeded, leading to its excessive accumulation in the joints and kidneys [10]. Hyperuricemia is a pathological state characterized by elevated levels of uric acid, primarily attributed to the excessive production of uric acid by xanthine oxidase [11]. The dysregulation of purine metabolism is recognized as a significant contributor to the development of hyperuricemia. Notably, xanthine oxidase inhibitors, such as allopurinol and febuxostat, have been specifically designed and formulated for the long-term treatment of gout [12]. Given their capacity to effectively lower uric acid levels, xanthine oxidase inhibitors might be good candidates for the management of gout.
In this study, we presented pharmacological evidence supporting the potential use of *P. cablin* in the treatment of gout. This effect can be attributed to the presence of various bioactive compounds, including terpenoids, phytosterols, flavonoids, organic acids, lignins, glycosides, alcohols, pyrone, and aldehydes [13].

Conclusion

Pogostemon cablin demonstrates the ability to inhibit xanthine oxidase activity in vitro. Furthermore, *P. cablin* extract effectively reduces the levels of serum uric acid in hyperuricemic mice induced by potassium oxonate, indicating its potential in attenuating hyperuricemia through xanthine oxidase inhibition.

Table 2. Anti-hyperuricemic effect of *Pogostemon cablin* EtOH extract

	Uric acid (mg/dL) in serum	Uric acid (mg/dL) in urine	Serum creatinine (mmol/L)	Urine creatinine (mmol/L)
Group I	1.15 ± 0.17	2.25 ± 0.27	0.52 ± 0.07	6.12 ± 1.21
Group II	1.62 ± 0.24*	3.72 ± 0.25*	0.56 ± 0.08	6.45 ± 1.15
Group III	1.04 ± 0.12$^{\#}$	1.83 ± 0.26$^{\#}$	0.53±0.06	6.67 ± 1.82
Group IV	1.18± 0.27$^{\#}$	2.54 ± 0.31$^{\#}$	0.57 ± 0.09	6.73 ± 1.34
Group V	1.12 ± 0.25$^{\#}$	2.36 ± 0.22$^{\#}$	0.58 ± 0.05	7.05 ± 2.31

Data represent the mean±SEM (n= 10); $^{*}p<0.05$ compared to the group I: $^{\#}p<0.05$ compared to the group II; group I: control, distilled water; group II: pathological control, potassium oxonate; group III: control drug, allopurinol; group IV: EtOH extract at with the dose of 100 mg/kg; group V: EtOH extract with the dose of 300 mg/kg

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Author contributions
Bui Thanh Tung contributed in supervision, concept and designing of the study; Do Thi Hong Khanh contributed in designing of the study; Nguyen Thi Thuy, Trinh Mai Phuong, Nguyen Thi Minh Anh contributed in resources; literature search and writing were done by Do Thi Hong Khanh, Nguyen Thi Thuy, Trinh Mai Phuong, Nguyen Thi Minh Anh; Bui Thanh Tung criticaly reviewed the manuscript

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations
EtOH: ethanol; n-Hex: n-hexan; EtOAc: ethyl acetate; BuOH: butanol