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Xanthin Oxidase Activity of Acetone Extract from *Cinnamomum burmannii* (Nees & T. Nees) Blume. as an Antigout

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Abstract

Background: *Cinnamomum burmannii commonly referred to as Indonesian cinnamon, belongs to the Lauraceae family and is recognized for its substantial economic and pharmacological significance. The bark is predominantly utilized as it is enriched with bioactive constituents, including cinnamaldehyde, eugenol, and coumarin, which are known to exhibit antioxidant, antibacterial, antifungal, antidiabetic, and anti-inflammatory properties. In purine metabolism, xanthine oxidase (XO) functions as a key enzyme by facilitating the oxidation of hypoxanthine into xanthine and subsequently into uric acid. Elevated XO activity has been associated with increased uric acid levels, leading to conditions such as hyperuricemia and gout. Accordingly, this study was conducted to assess the inhibitory potential of the acetone extract of C. burmannii against xanthine oxidase activity*. **Methodology:** *Dried and authenticated bark samples were macerated using acetone as the extraction solvent. Xanthine oxidase inhibition was evaluated invitro using a UV–Vis spectrophotometric assay at 295 nm. Various extract concentrations (0.370–23.684 µg/mL) were tested under controlled conditions (pH 7.6, 25 °C, xanthine as the substrate). The IC_{50} values were determined by linear regression analysis, and the relative inhibitory concentration (RIC_{50}) was calculated in comparison with allopurinol.* **Findings:** *The acetone extract of C. burmannii exhibited potent xanthine oxidase inhibitory activity with an IC_{50} of 21.029 ppm (compared to allopurinol, IC_{50} 2.7 ppm), demonstrating the potential of acetone as a solvent for extracting bioactive compounds, with a RIC_{50} value of 0.13 indicating higher activity than previously reported ethanol extracts.* **Contribution:** *These findings indicate that acetone provides better selectivity in extracting compounds from C. burmannii, resulting in stronger xanthine oxidase inhibition. The acetone extract may serve as a promising natural alternative source for the development of antigout agents.*

Keywords: *Acetone extract; Antigout; Cinnamomum burmannii; IC_{50}; Xanthine oxidase*

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INTRODUCTION

Gout referred to as hyperuricemia, is classified as a metabolic condition in which monosodium urate crystals are deposited within body tissues as a consequence of elevated uric acid concentrations in the bloodstream (Li et al., 2025). The prevalence of hyperuricemia has been reported to vary widely across countries, with global estimates ranging from 2.6% to 36% among adult populations in various epidemiological studies, and recent data indicate an increasing trend in some populations. In the United States, the NHANES survey reported that approximately 21% of adults, representing over 40 million individuals, have hyperuricemia. In Europe, prevalence rates can reach several tens of percent, such as 48% in Finland and 24.5% in Ireland. In Asia, increasing prevalence has also been documented, including 17.7% in China and 11.4% in South Korea, while in the Middle East, the prevalence in Qatar is reported at 21,2%. Data from Africa show variability across populations, although some local reports indicate relatively high prevalence rates. Factors such as urbanization, dietary patterns high in purines, obesity, and population aging are believed to contribute to this rising prevalence, highlighting the urgent need for strategies aimed at early detection, management, and prevention of hyperuricemia on a broader scale (Du et al., 2024).

In the human body, nearly 90% of uric acid is generated from purine catabolism, a biochemical process predominantly mediated by the enzymes guanase and xanthine oxidase (XO) (Li et al., 2025). Within the purine degradation pathway, xanthine oxidase serves a crucial function by catalyzing the sequential conversion of hypoxanthine into xanthine and subsequently into uric acid. When XO activity becomes excessive, uric acid synthesis is markedly increased, thereby contributing to the development of hyperuricemia, gout, and various metabolic disorders. Given its pivotal involvement in uric acid biosynthesis, XO has been widely recognized as a major molecular target in the design of antihyperuricemic and antigout therapeutics (Furuhashi, 2025). Several clinically applied agents, including allopurinol, febuxostat, and topiroxostat, exert their pharmacological effects through the inhibition of XO. Although allopurinol is well established as an effective uric acid–lowering drug, its administration necessitates dosage adjustments in individuals with renal dysfunction and is associated with a potential risk of hypersensitivity reactions. Febuxostat demonstrates greater efficacy in reducing serum urate compared to allopurinol, with a similar safety profile, although cardiovascular risk should be carefully considered (Xie et al., 2023). In contrast, topiroxostat exhibits comparable efficacy to febuxostat, is well tolerated, and may provide additional benefits on blood pressure and urinary albumin excretion (Jenkins et al., 2022). Although these medications are proven to be effective, long-term use is often associated with various adverse effects. Mild to moderate effects include gastrointestinal disturbances and skin reactions, whereas serious reactions, such as hypersensitivity syndrome, hepatotoxicity, and polyarthritis, have also been reported (Cicero & Ivan, 2021). This encourages the search for new XO inhibitors from natural products, which are natural compounds usually through extraction and purification, that offer high efficacy, structural diversity, broad biological activity, safety, and more affordable prices with minimal side effects (Ullah et al., 2024; Zeng et al., 2025). A natural therapeutic option with considerable

potential for managing hyperuricemia is represented by *Salvia plebeia* extract, which has been demonstrated, through both in vitro and in vivo investigations, to effectively lower uric acid concentrations (Kim et al., 2017).

One potential natural source is *Cinnamomum burmannii* (Indonesian cinnamon), a plant native to Indonesia that is widely distributed in the provinces of Jambi and West Sumatra (Menggala & Damme, 2018). In the food industry, cinnamon is commonly used in bread, cakes, and beverages as an additive (Ilmi et al., 2022). Phytochemical analysis of *C. burmannii* extract shows the presence of various phenolic compounds such as pyrocatechol, catechol, guaiacol, and hydroquinone, which are the result of the degradation of complex polyphenolic compounds. Not only that, alkaloids, tannins, saponins, and triterpenoids are also present in *C. burmannii* extract (Lusiana et al., 2022). *C. burmanni* extract contains other active compounds such as cinnamaldehyde, cinnamic acid, catechin, epicatechin, and coumarin (Handayani et al., 2024). The secondary metabolites in *C. burmannii* have many biological activities, including antioxidant, antiinflammatory, antidyslipidemia, antidiabetic, antibacterial, antihyperuricemic, and antifungal properties (Mohamed et al., 2020; Balijepalli et al., 2017; Djarot et al., 2023; Dwitiyanti et al., 2019; Emilda, 2018; Forestryana & Arnida, 2020; Sankaranarayanan et al., 2024).

The selection of extraction solvents has a significant impact on the profile of bioactive compounds, extract yield, and ultimately biological activity, including enzyme inhibition activity (Lee et al., 2024). Solvents with different polarities will extract different groups of compounds, affecting the solubility of target compounds and their stability during the extraction process. Extraction using acetone has advantages because it yields higher total phenolic and total flavonoid contents compared to ethanol and methanol as solvents (El Mannoubi, 2023). Solvents such as water or ethanol are often used because of their safety and ability to dissolve polar compounds. However, the bioactive compounds in *C. burmannii* consist not only of polar compounds, but also semi-polar compounds such as flavonoids and terpenoids, which are more soluble in solvents such as acetone (Ervina et al., 2023). Therefore, the selection of the appropriate solvent is a crucial factor in determining the selectivity of the extraction process and the quality of the bioactive compounds produced. To the best of our knowledge, the extraction of *C. burmannii* bark using acetone as a solvent has not yet been reported. Therefore, this study aims to evaluate the extraction efficiency of acetone in isolating bioactive compounds from *C. burmannii* bark and to assess their inhibitory activity against xanthine oxidase. The findings contribute to the development of alternative natural-based antigout agents and support further pharmacological exploration of *C. burmannii* extracts.

METHOD

Plant Material and Authentication

Cinnamon bark (*Cinnamomum burmannii*) used in this study was collected from the vicinity of Lake Talang, Nagari Kampung Batu Dalam, Danau Kembar District, Solok Regency, West Sumatra, Indonesia. The plant material was taxonomically

authenticated at the Herbarium of Andalas University (ANDA) as *Cinnamomum burmannii* (Ness & T. Nees) Blume of the Lauraceae family.

Preparation of Acetone Extract

After collection, the bark was carefully cleaned to remove extraneous matter, air-dried, and ground into a fine powder. A portion of the powdered material (50 g) was macerated with acetone at a ratio of 1:10 (w/v) for three consecutive cycles at room temperature, each lasting three days with occasional stirring. After each cycle, the macerate was filtered, and the combined filtrates were concentrated to obtain a viscous crude extract. The extract yield was subsequently calculated and recorded for further phytochemical and biological analyses.

Xanthine Oxidase Inhibitory Assay

The extract and allopurinol (as a positive control) are tested for xanthine oxidase enzyme inhibition in vitro using UV spectrophotometry with a modified method. Xanthine oxidase inhibition activity is measured based on the decrease in uric acid formation determined at a wavelength of 295 nm. Twenty microliters of sample were pipetted into a 96-well microplate in triplicate and 1 well was added for control 1 (as Negative control) of each sample without enzyme addition. Then, 90 µl of pH 7.6 phosphate buffer was added, followed by 30 µl of enzyme solution (0.1 U/ml in buffer). A second control without sample addition was also prepared to determine the absorbance of the enzyme. After enzyme addition, the plate was incubated for 15 minutes at 25°C. After incubation, add 60 µl of substrate (150 µM in buffer) and incubate for 30 minutes. Then measure the absorbance with a Microplate Reader at a wavelength of 295 nm (Abdulhafiz et al., 2020).

Determination of IC_{50} and RIC_{50} Values

IC_{50} value (the concentration of inhibitor that produces 50% inhibition of xanthine oxidase activity) can be determined by linear regression analysis between the concentration of the test compound and the percentage inhibition of xanthine oxidase activity using the equation y = a + bx. The IC_{50} value is obtained from the x value after substituting y = 50 (Rishikesan et al., 2021). To obtain the inhibition percentage, the following formula is used,

$$\text{Inhibition (\%)} = 1 - \frac{B}{A} \text{ x } 100\ \%$$

Where, A : blank solution absorban – blank control
B : test solution absorban – test solution control

The RIC_{50} value is also calculated, which is the relative IC_{50} ratio of the reference compound to the test sample, expressed in µg of equivalent reference compound per µg of test sample (Cooper et al., 2022).

$$RIC_{50} = \frac{IC_{50} \text{ Standard compound}}{IC_{50} \text{ test sample}}$$

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). The IC_{50} values were determined by linear regression analysis of extract concentration versus percentage inhibition using the equation $y = a + bx$.

RESULT AND DISCUSSION

This study used crushed *Cinnamomum burmannii* as a sample. A total of 50 grams of sample was extracted using the maceration method with acetone as a solvent and occasional stirring, then filtered. The maceration method was chosen because it is simple, does not involve heating, and thus minimizes damage to the bioactive components contained in natural materials (Putri & Mentari, 2022). The process of soaking in this extraction liquid allows the liquid to penetrate the cell walls and enter the cells (Cao et al., 2025). From this extraction, 4.9442 grams of extract was obtained with a yield of 9.88%.

Xanthine oxidase plays an important role in the purine degradation pathway by converting hypoxanthine to xanthine, which is further converted to uric acid. When XO activity is excessive, uric acid production increases, which can lead to hyperuricemia, gout, and other metabolic complications (Ullah et al., 2024). Xanthine oxidase enzymes can only work with the appropriate substrate, namely xanthine, and catalyze the conversion of xanthine by adding water and oxygen, producing uric acid and hydrogen peroxide (Korsmo, 2024). The wade formed has the ability to diffuse and penetrate cell membranes, which can cause damage to cells far from the site of its formation (Orrico et al., 2025). The xanthine oxidase inhibition activity test (in vitro) was performed using spectrophotometry at a wavelength of 295 nm, using xanthine substrate and xanthine oxidase enzyme. During testing, the pH was set at 7.6, which is the optimal pH for xanthine oxidase enzyme activity (Rahminiwati et al., 2023). A decrease in pH to a more acidic or alkaline level canTo obtain the IC_{50} value, a linearity curve was created showing the relationship between the percentage of inhibition and the concentration of the extract (as shown on Figure 1.).

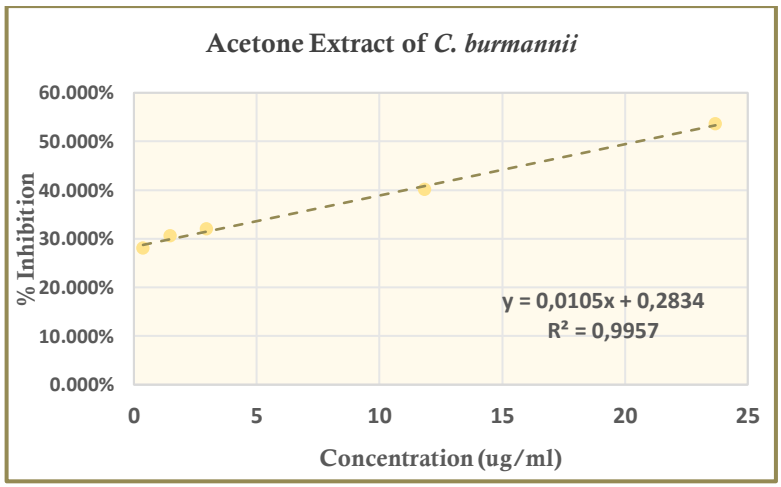

Figure 1. Percentage inhibition linearity curve with extract concentration

From the linearity curve, a regression equation was obtained and used to calculate the IC_{50} value as shown in Table 1. The purpose of this test was to determine whether *C. burmannii* acetone extract could inhibit the formation of urate crystals and hydrogen peroxide. Previously, research on the inhibition of xanthine oxidase by *C. burmannii* extract was also conducted by Wahyuni et al., (2016) using 80% ethanol extract and reported an IC_{50} value of 1294.58 ppm. This IC_{50} value indicates that ethanol extract has relatively low xanthine oxidase inhibition, which limits its therapeutic potential as a natural alternative for gout treatment and its effectiveness is still lacking when compared to more conventional pharmaceutical drugs, such as allopurinol, which has a much lower IC_{50} in XO inhibition.

The results of the xanthine oxidase inhibition activity test showed that the acetone extract of *C. burmannii* had an IC_{50} value of 21.029 ppm and allopurinol had an IC_{50} value of 2.7 ppm. The RIC_{50} value of the acetone extract was 0.13, which means that 0.13 mg of allopurinol is equivalent to 1 mg of acetone extract in inhibiting 50% of xanthine oxidase enzyme activity. The xanthine oxidase inhibitory activity obtained was significantly weaker than that of the 80% ethanol extract (IC_{50} = 1294.58 ppm) and the reference drug allopurinol, which exhibited potent inhibition with an IC_{50} value of 6.75 ppm and an RIC_{50} value of 0.005 (Wahyuni et al., 2016). RIC_{50} 80% ethanol extract is 0.005, which means that 0.005 mg of allopurinol is equivalent to 1 mg of 80% ethanol extract in inhibiting 50% of xanthine oxidase enzyme activity. This shows that acetone extract is significantly more active than 80% ethanol extract. Several active compounds contained in *Cinnamomum burmannii* are thought to play an important role in the mechanism of xanthine oxidase enzyme inhibition. These compounds include cinnamaldehyde, coumarin, catechin, and epicatechin, each of which has strong biological activity as antioxidants and enzyme inhibitors (Fais et al., 2023; Rahmi et al., 2021; Xue et al., 2023).

Tabel 1. Percentage Inhibition and IC_{50} of *C. burmannii* Acetone Extract

Extract concentration (ug/ml)	Extract absorbance (n = 1)	Uric acid absorbance	Inhibition (%)	IC_{50} (ug/ml)	RIC_{50}
23.684	0.850 ± 0.137	1.833	53.610	21.029	0,13
11.842	1.099 ± 0.014		40.062		
2.961	1.247 ± 0.019		31.951		
1.480	1.272 ± 0.017		30.587		
0.370	1.319 ± 0.012		28.041		

The IC_{50} value was determined from the linear regression equation (y = a + bx) at 50% inhibition.

One of the reasons for this difference in bioactivity is the type of solvent used in the extraction, which affects the chemical compounds that are extracted (Hua et al., 2024). Acetone is a solvent with a low boiling point (56 °C), which allows efficient solvent evaporation without excessive heating, maintaining the stability of bioactive compounds such as flavonoids, terpenoids, phenolics, and semi-polar alkaloids that are usually easily degraded by heat (Hauenstein et al., 2020; Krivošija et al., 2025). With this high volatility, extraction can be carried out more

quickly and efficiently, thereby not only saving energy but also optimizing the quality of the extract produced. This shows that the acetone extract is more effective in inhibiting xanthine oxidase activity than ethanol extract. Acetone is not only a more efficient solvent, but it can also produce extracts with higher and more stable active compound content. This opens up the potential for the development of more effective natural-based gout treatments with minimal side effects compared to synthetic drugs.

CONCLUSION

The acetone extract of *Cinnamomum burmannii* bark exhibited notable *in vitro* xanthine oxidase inhibitory activity, with an IC_{50} value of 21.029 ppm and a RIC_{50} of 0.13 relative to allopurinol. These findings indicate that acetone provides superior selectivity for extracting bioactive constituents associated with xanthine oxidase inhibition compared with the previously reported 80% ethanol extract. Despite showing lower potency than the reference drug allopurinol, the acetone extract demonstrated substantially enhanced activity relative to ethanol-based extracts, highlighting its potential as a natural source of xanthine oxidase inhibitors. However, the present study is limited by its *in vitro* experimental design and the absence of active compound isolation or *in vivo* validation. Overall, this study offers novel evidence supporting the use of acetone as an effective extraction solvent for *C. burmannii* and provides a scientific foundation for future investigations focusing on compound characterization, mechanism-of-action studies, and pharmacological evaluation to substantiate its potential application in hyperuricemia and gout management.

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