Extract Ethanol of Tempuyung (Sonchus arvensis) Leaves as Anti-Hyperuricemia: In Vitro Studies

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Abstract: Hyperuricemia is a condition where there is an increase in blood uric acid levels above 7 mg/dL in men and 6 mg/dl in women. One of the enzymes that plays a role in hyperuricemia is xanthine oxidase which can inhibit uric acid synthesis. Tempuyung leaves (Sonchus arvensis) are one of the plants that have anti-hyperuricemia properties. This study aims to determine the effect of ethanol extract of tempuyung leaves on xanthine oxidase in vitro. Tempuyung leaves come from Palembang, South Sumatra, Indonesia, and are macerated with 96% ethanol until a thick extract is obtained. The xanthine oxidase inhibition test was carried out on tempuyung leaf extract and Allopurinol with respective concentrations of 6.25; 12.5; 25; and 50 ppm and followed by absorption measurements using UV-V spectrophotometry at a wavelength of 293 nm and determining the IC₅₀ value. The research results showed that the ethanol extract of daun tempuyung leaves had an IC₅₀ value of 23.37 ppm, higher than allopurinol of 17.16 ppm which was used as a reference. These results classify the ethanol extract of daun tempuyung leaves as a strong inhibitor of the xanthin oxidase enzyme. Therefore, the ethanol extract of daun tempuyung leaves has the potential to be anti-hyperuricemic.

Keywords: Hyperuricemia; Sonchus arvensis; xanthine oxidase inhibitor.

INTRODUCTION

Hyperuricemia is a body condition with serum uric acid levels exceeding 7 mg/dL and can occur in both men and women (Martinon & Glimcher, 2006; So & Martinon, 2017). Consumption of added sugar and artificial sweeteners in food and drinks has been associated with an increased risk of hyperuricemia (Dalbeth et al, 2021). Additionally, excessive consumption of alcohol and red meat is also associated with hyperuricemia, which contributes to the formation of monosodium urate (MSU) crystals. However, only 10% of hyperuricemia sufferers experience gouty arthritis (Busso & So, 2010; Ghaemi-Oskouie & Shi, 2011). Uric acid is influenced by genetic factors, metabolism, and certain comorbidities such as metabolic syndrome, heart disease, and kidney disease (Zhao et al., 2022). Research related to gout found that hyperuricemia is a risk factor for the pathogenesis of vascular endothelial dysfunction (Manampiring, 2011). In general, the prevalence of hyperuricemia affects 3.9% of people in the United States (George, Leslie, & Minter, 2023). According to the 2007–2016 National Health and Nutrition Examination Survey (NHANES) data, the prevalence rate of hyperuricemia in the United States was 20.2% for men and 20.0% for women between 2015 and 2016 (Li, Zhang, & Zeng, 2020). The prevalence of hyperuricemia varies in Asian countries, with a prevalence in China of 6%--to 25%, a
prevalence in Taiwan from 10%–52%, and a prevalence in Indonesia of 18% (Raja et al., 2019).

Uric acid is the result of the breakdown of purines in the body. Protein sources originating from the internal organs of animals and seafood can increase uric acid levels in the blood (Cicerello, 2018; Hainer, Matheson, & Wilkes, 2014). At the body's normal pH of around pH 7.4, uric acid circulates in the form of ionized urate. Purine metabolism mainly occurs in the liver, but can also occur in other tissues that contain the xanthine oxidase enzyme. This xanthine oxidase converts urate into alantoin form which is more easily soluble in water. Under normal conditions, the body can regulate uric acid levels well. However, disorders of purine metabolism or kidney function can cause excessive accumulation of uric acid in the blood, which can cause hyperuricemia or even gout (Chen et al., 2024).

Endogenous purine production can be accelerated by the activity of phosphoribosyl-pyrophosphate synthetase (PRPP) and defects in the regulatory enzyme hypoxanthine phosphoribosyl transferase (HPRT). Conditions like this occur in rhabdomyolysis, hemolysis, and tumor lysis. Urate excretion occurs primarily in the kidney and is responsible for hyperuricemia in 90% of individuals with decreased glomerular filtration, decreased tubular secretion, and increased tubular reabsorption all contributing to decreased excretion. Hyperuricemia can be caused by a short-term or long-term reduction in glomerular filtration (George et al., 2023).

Most people with hyperuricemia have no symptoms and do not need treatment unless they have gout, arthritis, or nephrolithiasis. To prevent lysis syndrome in malignancies in patients receiving cytolytic therapy who are asymptomatic, uric acid-lowering drugs are recommended (Engel, Just, Bleckwenn, & Weckbecker, 2017). Some uric acid-lowering drugs are used as arthritis prophylaxis for gout, nephrolithiasis, and chemotherapy-related hyperuricemia, such as the xanthine oxidase inhibitor allopurinol. Probenecid is used as second-line therapy in gout patients because this drug works as an inhibitor of URAT1 (Uric Acid Transporter 1) which increases uric acid production. Recombinant uricase (Rasburicase) converts uric acid to allantoin, which is rapidly eliminated by the kidneys and is used as a preventive measure for chemotherapy-induced hyperuricemia (George et al., 2023). URAT1 (Uric Acid Transporter 1) is a transporter protein that plays a role in regulating uric acid levels in the body. This protein is mainly found in the kidneys and intestines. Its function is to regulate the reabsorption of uric acid back into the blood from urine produced by the kidneys. URAT1 inhibitors such as probenecid can interfere with this process, resulting in increased excretion of uric acid through urine and decreased uric acid levels in the blood (Panche, Diwan, & Chandra, 2016).

However, some experts advise against using ULT (Uric Acid Lowering Therapy) in acute gout and base its use on adequate anti-inflammatory therapy due to the prolonged duration of inflammation and the risk of recurrent gout attacks (Jia et al., 2022) ULT (Uric Acid -Lowering Therapy) is a type of therapy used to reduce uric acid levels in the body, usually using drugs such as allopurinol or febuxostat. ULT aims to prevent repeated gout attacks and reduce the risk of long-term complications due to high uric acid levels, such as kidney stones or joint damage. In contrast to preventing gout symptoms in asymptomatic patients, ULT does not worsen kidney problems in asymptomatic patients. The use of ULT lowers blood pressure in hyperuricemia patients with hypertension and reduces the risk of hypertension and cardiovascular disease in hyperuricemia patients because diseases associated with hyperuricemia can increase the risk of hypertension (Zhang et al., 2021).
Allopurinol has several negative side effects and may also increase the likelihood of an acute episode, especially in the early stages of treatment when uric acid deposits are being mobilized out of the tissues (Engel et al., 2017; Pillinger & Mandell, 2020). Another alternative for lowering uric acid is tempuyung (S.arvensis). Residents use tempuyung to treat kidney stones, but further research shows that tempuyung can also be used to treat gout. Tempuyung has the same effectiveness as anti-nephrolithiasis and anti-gout, according to a study comparing its efficacy with colchicine. Tempuyung leaf extract can increase immunomodulatory action against inflammation (Jayani, Krisnawan, Oktaviyanti, & Kartini, 2020). Therefore, tempuyung (S. Arvensis) has the potential as an alternative solution in the treatment of uric acid reduction therapy and considering the side effects of using allopurinol (Engel et al., 2017).

Flavonoids from tempuyung leaves can reduce blood uric acid levels by inhibiting xanthine oxidase. The enzymes xanthine oxidase and guanase catalyze the conversion of hypoxanthine to xanthine, which then produces uric acid. Therefore, xanthine oxidase inhibition is very important as a pharmaceutical treatment for hyperuricemia and gout (Hendriani, Nursamsiar, & Tjitraresmi, 2017).

Previous research such as that carried out by (Suwartiny, Rafi, & Rohaeti, 2022) and (Parisa et al., 2023) only highlighted the utilization, compound content, and biological activity of the medicinal plant S. arvensis. Research still needs to be carried out to determine the effect of tempuyung leaves as anti-hyperuricemia, especially its inhibitory activity on xanthine oxidase, so this research aims to analyze the xanthine oxidase inhibitory activity of ethanol extract of S. arvensis leaves as anti hyperuricemia in vitro.

MATERIALS AND METHODS
This in vitro experimental research was carried out at the Basic Medical Chemistry Laboratory, Faculty of Medicine, Sriwijaya University, Indonesia. Research began in January–December 2021.

Research Procedure
Plant Extraction
The maceration method used for extraction in this research uses 96% ethanol as the extraction solvent for dried and ground plant material. This plant material comes from the tempuyung (Sonchus arvensis L.) plant which grows in Palembang City, South Sumatra, Indonesia. Five hundred grams of ground tempuyung (Sonchus Arvensis) leaves were macerated with ethanol for 72 hours and evaporated at 40°C with the help of a Rotary Evaporator (Heidolph) vacuum pump to extract the thick active components.

Xanthine Oxidase Inhibition Assay
The 99.5% xanthine substrate solution has a molecular weight of 152.11 grams/mol. In this investigation, 2.293 mg of xanthine substrate was required to make 100 mL of 0.15 mM xanthine substrate. Where the xanthine substrate solution is made and then put into a 10 ml measuring flask. After that, dissolution was carried out with 5 drops of 1M NaOH, and the xanthine substrate solution was added with sodium phosphate buffer pH 7.5 until it reached a volume of 100 ml. The tempuyung leaf sample solution was made with 5 mg of tempuyung leaf extract, a few drops of DMSO, and 10 mL of phosphate buffer to reach a stock solution concentration of 100 ppm. The sample solution was then diluted with distilled water to produce different concentrations, including 6.25; 12.5; 25; and 50 ppm. Allopurinol at a concentration of 6.25; 12.5; 25 and 50 ppm were used as positive controls. The allopurinol solution is
made by weighing 10 ml of the solution into a 5 ml volumetric flask and adding CO2-free distilled water to a certain extent (Tariza, 2021). This solution was then further diluted with distilled water to obtain concentrations of 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL (Zhang et al., 2021). Control blank testing (B0) was carried out by mixing 300 µL sodium phosphate buffer pH 7.5, 100 µL DMSO, and 100 µL distilled water, then incubating at 37°C for 5 minutes. After that, 200 µL of xanthine oxidase substrate solution was added and incubated again for 30 minutes at the same temperature. The reaction was stopped with 200 µL 0.5 M HCl and absorbance was measured at 293 nm using a UV-Vis Spectrophotometer (Shimadzu UV-1800). Blank testing (B1) uses a mixture of DMSO, sodium phosphate buffer, xanthine oxidase enzyme solution, and distilled water, with the same procedure. The control sample test (S0) uses sodium phosphate buffer, cherry leaf ethyl acetate extract, and distilled water, while the test sample (S1) adds an enzyme solution to the mixture. The allopurinol control test (A0) and allopurinol test (A1) were carried out with sodium phosphate buffer, allopurinol solution, and distilled water, with the same procedure, but the enzyme solution was also added to the allopurinol test. All reactions were stopped with 0.5 M HCl and absorbance was measured at 293 nm (Suwandi & Perdana, 2017).

The xanthine oxidase enzyme inhibition test was carried out by measuring the sample absorption value using a UV-1800 UV Spectrophotometer with a wavelength of 293 nm with three repetitions. The absorption results of samples, blanks, and positive controls measured using a UV-Vis spectrophotometer measured the percent inhibition using the formula (Putri et al., 2016):

\[
\%\text{Inhibisi} = \frac{\text{Absorbansi kontrol} - \text{absorbansi sampel}}{\text{Absorbansi kontrol}} \times 100\%
\]

Percent results inhibition from sample, blank and control positive plotted on the x and y axes with use linear regression \(y = a + bx\), then count sign IC\(_{50}\) For get big concentration required solution in hinder enzyme xanthine oxidase by 50%. An IC value of 50 (50% inhibitor concentration) was found use equality linear regression with the y value is 50 so obtained equality (Tariza, 2021):

\[
\text{IC}_{50} = \frac{50 - a}{b}
\]

Information:
- x: sample concentration
- y: percentage of inhibition of enzyme inhibitory activity
- a: intercept, namely the intersection point between a line and the y-axis on the diagram or the Cartesian axis if the value x = 0
- b: slope, which is a measure of the slope of a line.

**Phytochemical Test**
Tests were carried out on the content of alkaloids, flavonoids, triterpenoids, steroids, saponins, tannins, and quinones.

**Alkaloid Test**
A total of 3 mL of tempuyung leaf extract solution was reacted with 5 mL of 2% HCl and stirred, then the mixture was separated into three test tubes. Add three drops of Mayer's reagent to the first test tube and add water until the volume is 100 mL. A positive result is obtained if there is a white or yellow precipitate. Two drops of Dragendorff's reagent added to the second test tube give a positive result if there is a
red, brick, or orange-colored precipitate. Three drops of Wagner’s reagent were added to the third test tube, and a positive result was obtained if a brown precipitate was present.

**Saponin Test**
Tempuyung leaf extract was put into a test tube and 10 mL of distilled water was added. Let cool, then shake for ten seconds. The result is positive if foam forms in less than ten minutes.

**Flavonoid Test**
Tempuyung leaf extract was dissolved in methanol and 0.5 grams of magnesium metal plus five drops of concentrated HCl. The solution is then heated. The result is positive if a red or yellow color forms.

**Tannin Test**
One mL of tempuyung leaf extract is reacted with 2 mL of 1% FeCl3, and the result is positive if the solution changes color to blue or dark blue.

**Triterpenoid Test**
One mL of tempuyung leaf extract was dissolved in 0.5 mL of chloroform and 0.5 mL of anhydrous acetic acid was added. Then 1-2 mL of concentrated sulfuric acid was added through the wall of the test tube. The result is positive if a brownish or purple ring forms.

**Quinones Test**
By adding NaOH to one mL of tempuyung leaf extract, a quinone test was carried out and a red hue appeared successful (Malik et al, 2022; Setyawaty, B, & Dewanto, 2020; Wulandari et al, 2021).

**Data Analysis**
The data obtained were analyzed using linear regression in Microsoft Excel to calculate IC50 and determine the relationship between the concentration of tempuyung leaf extract and its inhibitory effect.

**RESULTS AND DISCUSSION**
After obtaining a thick extract, a phytochemical test was carried out. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Test</th>
<th>Appearance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids: Dragendorff</td>
<td>Orange sediment</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids: Mayer</td>
<td>Yellow solution</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids: Wagner</td>
<td>The sediment is brown</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Red</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Brownish ring</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>Yellow sediment</td>
<td>-</td>
</tr>
<tr>
<td>Quinone</td>
<td>Red</td>
<td>+</td>
</tr>
</tbody>
</table>

From phytochemical tests carried out on tempuyung leaves, it was found that they are rich in flavonoids, alkaloids and triterpenoids which have strong antioxidant abilities and ward off free radicals (Khan, 2012; Owona, Abia, & Moundipa, 2020; Tungmunnithum et al, 2018). Results show that the ethanol extract of tempuyung
leaves and allopurinol are effective as inhibitors of the xanthine oxidase enzyme as shown in Figure 1 and Figure 2.

According to the graph, an intercept (a) of 7.519 indicates that the percentage inhibition of the xanthine oxidase enzyme is 7.519% when the extract concentration is 0 ppm. A slope (b) of 1.1818 indicates that for every one-unit increase in extract concentration (e.g., 1 ppm), the percentage inhibition of the enzyme increases by 1.1818%. The IC$_{50}$ value of approximately 35.94 ppm means that the concentration of extract required to achieve 50% inhibition of xanthine oxidase enzyme is around 35.94 ppm.

For every one-unit increase in the concentration of tempuyung leaf ethanol extract, it is estimated that there will be an increase in the inhibitory power of Xanthine oxidase by 1.1818%. R$^2$ The value of 0.9606 indicates that 96.06% of the variability in Xanthine oxidase inhibition can be explained by the concentration of tempuyung leaf ethanol extract, indicating a strong correlation. Between the concentration of tempuyung leaf ethanol extract and the inhibitory power of silent enzymes.

In the graph, the intercept (a) of 27.634 indicates that without any inhibitor present, the enzyme's activity is already reduced by 27.634%. The slope (b) of 1.3036 shows that for each unit increase in inhibitor concentration, the enzyme inhibition percentage increases by approximately 1.3036%. The IC$_{50}$ value, calculated as approximately 17.15 ppm, represents the concentration of the inhibitor needed to inhibit 50% of the enzyme's activity.

Every one-unit increase in allopurinol concentration (ppm) is estimated to increase the inhibition of Xanthine oxidase by 1.3036%. R$^2$ of 0.8889 indicates that 88.89% of the variability in Xanthine oxidase inhibition can be explained by Allopurinol.
indicating a strong correlation between allopurinol concentration and the observed enzyme inhibition.

Figure 2. Inhibition of Xanthine Oxidase by Allopurinol

The results showed that the ethanol extract had the potential for high inhibitory activity and the extraction of large amounts of bioactive components. The flavonoid content in tempuyung leaf extract, which is known for its strong reactive oxygen removal activity, has been linked to various biological activities (Hendriani et al., 2017).

The lower the IC$_{50}$ value, the stronger the compound's inhibitory ability against the xanthine oxidase enzyme. This has significant biological implications because compounds with low IC$_{50}$ can be more effectively used as drugs to control uric acid levels and treat related conditions (Chen et al., 2024).

Based on the linear regression graph, allopurinol as a positive control showed very potent inhibitory activity against xanthine oxidase with an IC$_{50}$ of 17.16 ppm; Thus, it is certain that the method used in this experiment is correct. The ethanol extract of tempuyung leaves in this experiment showed an IC$_{50}$ of 23.37 ppm which was considered to still have strong inhibitory activity against the xanthine oxidase enzyme (IC$_{50}<50$) (Priska, Peni, & Carvallo, 2019). Tempuyung leaf extract is confirmed to contain flavonoids which have the potential to act as antihyperuricemia and can be used as an alternative to allopurinol.

Flavonoids in tempuyung leaves can inhibit xanthine oxidase through several potential mechanisms, such as (1) Antioxidant properties, which enable it to neutralize free radicals and fight oxidative stress. Xanthine oxidase is an enzyme that contributes to the formation of free radicals, including reactive oxygen species (Lobo, Patil, Phatak, & Chandra, 2010), (2) Direct interaction with the enzyme, certain flavonoids can interact directly with the xanthine oxidase enzyme, changing its structure or inhibiting active site, thereby inhibiting enzyme activity and reducing conversion. Xanthine into uric acid (Panche et al., 2016), (3) Anti-inflammatory effect, flavonoids have anti-inflammatory properties which can reduce inflammation. This reduction in
inflammation can lead to a decrease in xanthine oxidase gene expression and activity (Al-Khayri et al., 2022) (4) Regulation of signaling pathways, flavonoids can also influence various signaling pathways in cells, including pathways involved in the regulation of gene expression. Flavonoids can reduce xanthine oxidase production by affecting its signaling pathway (Tungmunnithum et al., 2018).

The flavonoids found in tempuyung leaves have the potential to inhibit xanthine oxidase so they are useful in reducing uric acid production in the body and controlling medical conditions such as hyperuricemia or gout. However, it should be noted that the exact mechanism and influence of tempuyung leaf flavonoids on xanthine oxidase requires further research for a better understanding.

CONCLUSION
This study tested the potential of tempuyung leaf ethanol extract in reducing hyperuricemia. The results showed strong inhibition of the xanthine oxidase enzyme, indicating its effectiveness in reducing hyperuricemia. Phytochemical analysis revealed the presence of various compounds such as alkaloids, flavonoids, and triterpenoids in the extract. We conclude that further research is needed regarding the active compounds of tempuyung leaf extract which can specifically reduce hyperuricemia. This research should be continued in vivo using experimental animals.

CONFLICT OF INTEREST
Not applicable.

REFERENCES
Hendriani, R., Nursamsiar, N., & Tjitraresmi, A. (2017). In vitro and in silico evaluation


