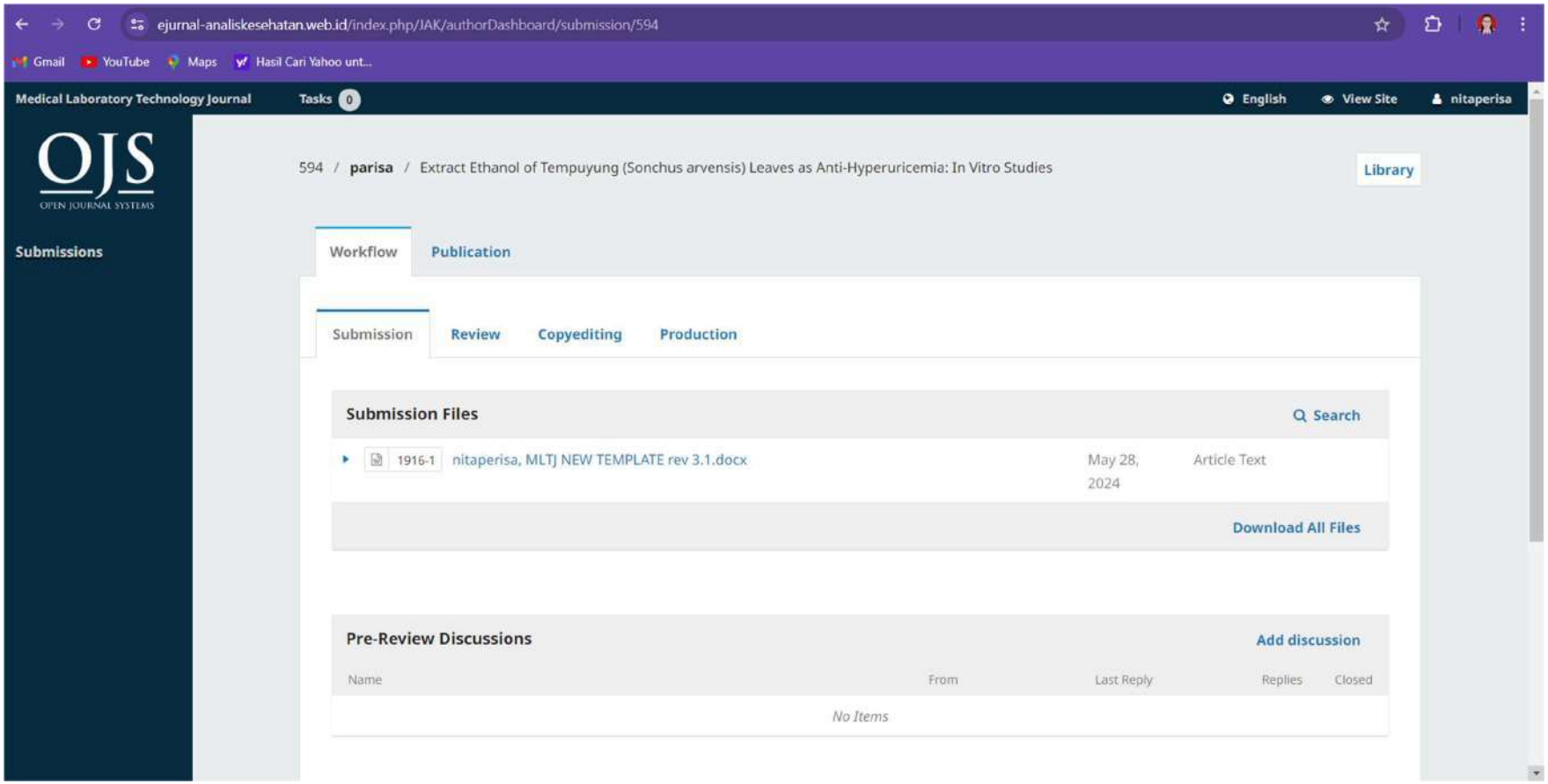
Judul : Extract Ethanol of Tempuyung (Sonchus arvensis) Leaves as Anti-Hyperuricemia: In Vitro Studies
Penulis : Nita Parisa, Rachmat Hidayat, Fatmawati
Jurnal : Medical Laboratory Technology Journal
SINTA : 2

1. Submit artikel (28 May 2024)
2. Reviewer pertama (7 Juni 2024)
3. Reviewer kedua (19 Juni 2024)
4. Revisi artikel (20 Juni 2024)
5. Letter of Acceptance-LOA (29 Juni 2024)
6. Publikasi artikel di website jurnal (29 Juni 2024)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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 for men and 6 mg/dl for women. One of the enzymes that play a role in the occurrence of hyperuricemia is xanthine oxidase, where inhibition of the enzyme may lead to prevent the synthesis of uric acid. Tempuyung (Sonchus arvensis) is a plant that has anti-hyperuricemia properties. The study aimed to determine the effect of the ethanol extract of tempuyung leaves as an anti-hyperuricemic in vitro. This study tested four extract concentrations, namely 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm, followed by absorbance measurements with UV-V is spectrophotometry at a wavelength of 293 nm and determination of the IC50 value. The study showed that the ethanol extract of tempuyung leaves had an IC50 value of 23.37 ppm higher than allopurinol of 17.16 ppm used as reference. These results classified ethanol extract of tempuyung leaves as a strong inhibitor of the xanthin oxidase enzyme. Hence, the ethanol extract of tempuyung leaves has the potential to be an anti-hyperuricemic.*

Keyword: *Sonchus arvensis, Xanthine Oxidase Inhibitor, Hyperuricem*

INTRODUCTION

Hyperuricemia is a condition where the serum urate level exceeds 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 beverages has been linked to an increased risk of hyperuricemia (Dalbeth, Gosling, Gaffo, & Abishek, 2021). Moreover, excess consumption of alcohol and red meat is also linked to hyperuricemia, which contributes to the formation of monosodium urate (MSU) crystals. However, only 10% of individuals with hyperuricemia develop gout arthritis (Busso & So, 2010; Ghaemi-Oskouie & Shi, 2011). Gout is influenced by genetics, 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). Gout is the most typical side effect of hyperuricemia and affects 3.9% of Americans (George, Leslie, & Minter, 2023). According to data from the National Health and Nutrition Examination Survey (NHANES) 2007–2016, the prevalence rates of hyperuricemia in the United States were 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 China having a prevalence of 6%–25%, Taiwan's prevalence ranging from 10%–52%, and Indonesia's prevalence at 18% (Raja et al., 2019).

Uric acid is the result of purine breakdown. Animal internal organs and seafood are sources of proteins that can raise uric acid levels in the blood (Cicerello, 2018; Hainer, Matheson, & Wilkes, 2014). At the normal physiological pH of 7.4, uric acid circulates in the ionized form of urate. Purine metabolism mainly occurs in the liver, but it can also be produced in any other tissue that contains xanthine oxidase. This enzyme converts urate to the more water-soluble form of allantoin. Endogenous purine production can be accelerated by phospho-ribosyl-pyrophosphate (PRPP) synthetase activity and a defect in the regulatory enzyme hypoxanthine phosphoribosyl transferase (HPRT). Such conditions happen in rhabdomyolysis, haemolysis, and tumour lysis. Urate excretion occurs primarily in the kidneys and is responsible for hyperuricemia in 90% of individuals with reduced glomerular filtration, reduced tubular secretion, and increased tubular reabsorption all appear to contribute to

underexcretion. 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 develop gout, arthritis, or nephrolithiasis. To prevent lysis syndrome in malignancies in patients receiving cytolytic therapy who are asymptomatic, uric acid-lowering medications are recommended (Engel, Just, Bleckwenn, & Weckbecker, 2017). Some uric acid-lowering medications are used as arthritis prophylaxis for gout, nephrolithiasis, and chemotherapy-related hyperuricemia, like the xanthine oxidase inhibitor allopurinol. Probenecid is used as a second-line therapy for gout patients because it operates as a URAT1 inhibitor, increasing uric acid output. Recombinant uricase (Rasburicase) transforms uric acid into allantoin, which is quickly eliminated by the kidneys and utilized as a preventative measure for hyperuricemia brought on by chemotherapy (George et al., 2023).

However, some experts advise against using ULT in acute gout and instead base its use on adequate anti-inflammatory therapy due to its protracted duration of inflammation and risk of recurrent gout attacks (Jia et al., 2022). 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 lowers 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).

The use of allopurinol for asymptomatic patients and the failure to monitor urate-lowering therapy (ULT) are bad for the patient. Allopurinol has several negative side effects and may also increase the likelihood of acute episodes, particularly in the early stages of treatment when uric acid deposits are being mobilized out of the tissues (Engel et al., 2017; Pillinger & Mandell, 2020). Tempuyung (*S. arvensis*) is a plant that has a long history of being used as medicine. The locals use tempuyung to treat kidney stones, but more research indicates that it may also be used to treat gout. Tempuyung has the same effectiveness as anti-nephrolithiasis and anti-gout, according to a study that compared its efficacy to that of colchicine. Tempuyung leaves extract can boost immunomodulatory action in inflammation (Jayani, Krisnawan, Oktaviyanti, & Kartini, 2020).

The aim of the current study was to find potential treatments for hyperuricemia by examining the xanthine oxidase inhibitory activity of *S. arvensis* leaves'-ethanol extract. Through the inhibition of xanthine oxidase, flavonoids are known to lower blood uric acid levels. The xanthine oxidase enzyme and guanase catalyze the conversion of hypoxanthine into xanthine, which is followed by the oxidation of xanthine into uric acid, which is also mediated by xanthine oxidase, to produce uric acid. Thus, xanthine oxidase inhibition is crucial as a pharmaceutical treatment for hyperuricemia and gout (Hendriani, Nursamsiar, & Tjitraresmi, 2017). Hence, this study aims to determine the effect of an ethanol extract of tempuyung leaves as an anti-hyperuricemic in vitro.

MATERIALS AND METHOD

This in vitro experimental study was carried out at the Basic Medical Chemistry Laboratory, Faculty of Medicine, Sriwijaya University. Research begins in January–December 2021.

1. Research Procedure

1.1. Plant Extraction

The maceration method used for extraction in this study utilized 96% ethanol as the extracting solvent for the dried and ground plant materials. Five hundred grams of grounded tempuyung leaves were macerated with ethanol for 72 hours and evaporated at 40 °C with the help of a vacuum pump to extract their thick active component.

1.2. Xanthine oxidase inhibitory assay

The 99.5% xanthine substrate solution has a molecular weight of 152.11 gram/mol. In this investigation, 2.293 mg of xanthine substrate were needed to make 100 mL of 0.15 mM xanthine substrate. A tempuyung leaves sample solution was made with 5 mg of tempuyung leaves extract, a few drops of DMSO, and 10 mL of phosphate buffer to achieve a stock solution concentration of 100 ppm. The sample solution was then diluted with distilled water to produce different concentrations. Allopurinol at concentrations of 6.25, 12.5, 25, and 50 ppm was used as a positive control. The xanthine oxidase enzyme inhibition test was carried out by measuring the absorbance value of the sample using a spectrophotometer with a wavelength of 293 nm with three repetitions.

1.3. Phytochemical Test

Testing was done for alkaloids, flavonoids, triterpenoids, steroids, saponins, tannins, and quinones contents.

a. Alkaloid test

About 3 mL of tempuyung leaves 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 when there is a white or yellow precipitate. Two drops of Dragendroff's reagent added to the second test tube produce positive result when there is a red, brick, or orange precipitate. Three drops of Wagner's reagents were added into the third test tube, and a positive result occurs when there is brown precipitate.

b. Saponin test

Tempuyung leaves extract was put into the test tube and added to 10 mL of distilled water. Let it cool, then shake for ten seconds. The result is positive when the foam has formed in less than ten minutes.

c. Flavonoid test

Tempuyung leaves extract was dissolved with methanol and 0,5 gram of magnesium metal added with five drops of concentrated HCl. That solution was then heated. The result was positive when a red or yellow color was formed.

d. Tannin test

One mL of tempuyung leaves extract reacted with 2 mL of 1% FeCl3, and the result was positive when the solution changed color to blue or dark blue.

e. Triterpenoid test

One mL of tempuyung leaves extract was solved into 0,5 mL of chloroform and added to 0,5 mL of anhydrous acetic acid. Later, 1-2 mL of concentrated sulfate acid were added through the test tube wall. The result was positive when a brownish or violet ring was formed.

f. Quinones test

By adding NaOH to one mL of tempuyung leaves extract, the quinone test was conducted, and it was successful when a red hue emerged (Malik, Auliya, & Iqbal, 2022; Setyawaty, B, & Dewanto, 2020; Wulandari, Chandra, Zulharmita, & Rivai, 2021).

2. Data Analysis

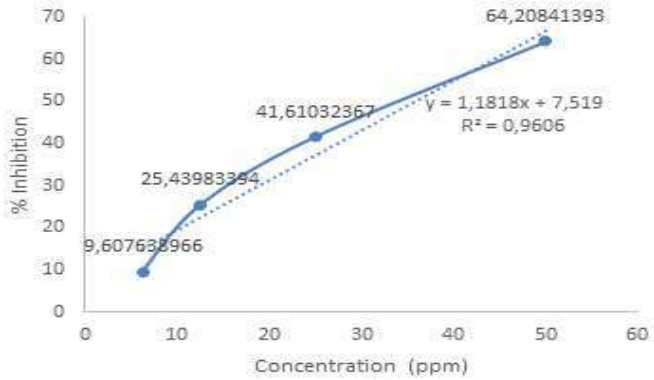
The obtained data were analyzed by using linear regression in Microsoft Excel to calculate the IC50 and determine the relationship between the tempuyung leaves extract concentration and its inhibition effect.

Ethical statement

The authors would like to declare that there is no ethical clearance in this research.

RESULTS AND DISCUSSION

The selected plant and allopurinol xanthine oxidase inhibition assay results are shown in Figure 1. and Figure 2.



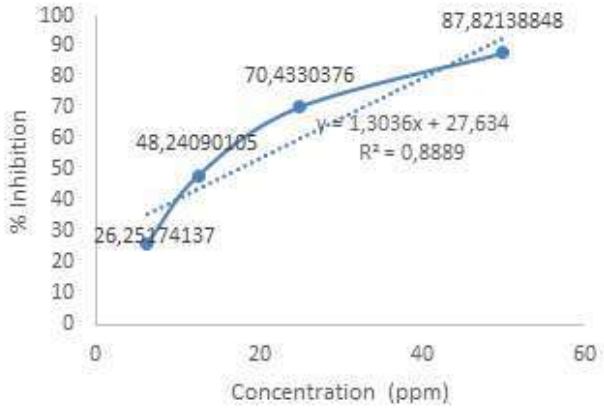
IC50: 23.37 ppm

Figure 1. Xanthine oxidase inhibition by Ethanol extract from tempuyung leaves

For every one-unit increase in the concentration of ethanol extract from tempuyung leaves, there is an expected increase of 1.1818% in Xanthine oxidase inhibition. The R2 value of 0.9606 suggests that 96.06% of the variability in Xanthine oxidase inhibition can be explained by the concentration of the ethanol extract from tempuyung leaves, demonstrating a strong correlation between the ethanol extract from tempuyung leaves concentration and the observed enzyme inhibition.

IC50: 17.16 ppm

Figure 2. Xanthine oxidase inhibition of Allopurinol



For every one-unit increase in the concentration of allopurinol (ppm), there is an expected increase of 1.3036% in Xanthine oxidase inhibition. R2 of 0.8889 suggests that 88.89% of the variability in Xanthine oxidase inhibition can be explained by the Allopurinol, indicating a strong correlation between the allopurinol concentration and the observed enzyme inhibition. These Results show that the ethanol extract of tempuyung leaves asts as a strong inhibitor for the xanthine oxidase enzyme. Thus, the ethanol extact of tempuyung leaves has the potential to be an anti-hyperuricemia agent. After a thick extract was obtained, the phytochemical test was done. The result shown innthe table 1 below :

Table 1. Phytochemical Test

Test	Appearance	Result
Alkaloid: Dragendroff	Orange sediment	+
Alkaloid: Mayer	Yellow solution	+
Alkaloid: Wagner	Brown sediment	+
Flavonoid	Red color	+
Triterpenoid	Brownish ring	+
Steroid	-	-
Saponin	Foam	-
Tannin	Yellow sediment	-
Quinone	Red	+

From the phytochemical test done for tempuyung leaves, it was found that it is rich in flavonoid, alkaloid, and triterpenoid compounds, which have strong antioxidant and free radical scavenger capabilities (Khan, 2012; Owona, Abia, & Moundipa, 2020; Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018).

Results showed that the ethanol extract had high potential inhibitory activity, especially since it came from direct extraction. This finding indicated that ethanol direct extraction produced a great extraction of bioactive components. The flavonoid contents of tempuyung leaves extract,

which is well-known for its potent reactive oxygen-scavenging activity, have been linked to a variety of biological activities (Hendriani et al., 2017). If a compound has a lower IC50 against xanthine oxidase, it indicates that the compound is more effective at inhibiting uric acid production. This finding could have significant biological implications, especially in the treatment of conditions such as hyperuricemia and gout. It is showed that the lower the IC50 value, the better the inhibitory activity towards the xanthine oxidase enzyme (Chen et al., 2024). Based on the liner regression graph, allopurinol as a positive control showed very potent inhibitory activity toward xanthine oxidase with an IC50 17.16 ppm; thus, it is confirmed that the methods used in this experiment were true. Ethanol extract of tempuyung leaves in this experiment showed an IC50 23.37 ppm, which is still considered to have strong inhibitory activity towards the xanthine oxidase enzyme (IC50 <50) (Priska, Peni, & Carvallo, 2019). It is confirmed that tempuyung leaves extract has flavonoid contents, which have potential as anti-hyperuricemia agents and used as an alternative for allopurinol.

The flavonoids in tempuyung leaves may inhibit xanthine oxidase through several potential mechanisms, such as:

1. Antioxidant properties, which allow them to neutralize free radicals and combat oxidative stress. Xanthine oxidase is an enzyme that contributes to the formation of free radicals, including reactive oxygen species. Flavonoids can help reduce the production of free radicals and relieve oxidative stress by inhibiting xanthine oxidase activity.
2. Direct interactions with enzymes, certain flavonoids can interact directly with the xanthine oxidase enzyme, changing its structure or inhibiting its active site, resulting in the inhibition of these enzymes' activity and reducing the conversion of xanthine to uric acid.
3. Anti-inflammatory effects, flavonoids possess anti-inflammatory properties that can reduce inflammation. This reduction in inflammation can lead to a decrease in the expression and activity of the xanthine oxidase gene.
4. Regulation of signaling pathways, flavonoids can also influence various signaling pathways in cells, including those involved in gene expression regulation. Flavonoids can reduce the production of xanthine oxidase by affecting it signaling pathways.

The flavonoids present in tempuyung leaves have the potential to inhibit xanthine oxidase, which can be useful in reducing uric acid production in the body and controlling medical conditions such as hyperuricemia or gout. However, it is important to note that the precise mechanism and effects of flavonoids in tempuyung leaves on xanthine oxidase require further research for a better understanding**.**

CONCLUSION

The study examined the potential of the ethanol extract of Tempuyung leaves to reduce hyperuricemia. The results showed a strong inhibition of the xanthine oxidase enzyme, indicating its effectiveness in lowering hyperuricemia. Phytochemical analysis revealed the presence of various compounds such as alkaloids, flavonoids, and triterpenoids in the extract. Tempuyung leaves under investigation demonstrates xanthine oxidase inhibitory activity. The current study’s findings indicate that tempuyung leaves ethanol extract a had strong inhibitory effect on xanthine oxidase, suggesting its potential to be developed as an agent for treating hyperuricemia. We conclude that it is necessary to do further research regarding the tempuyung leaves extract-active compound, which specifically can lower hyperuricemia. This research should be continued in vivo by using experimental animals. It might indicate that further study regarding the tempuyung leaves extract potential to be an anti-hyperuricemia agent is highly needed.

ACKNOWLEDGEMENT

I would like to thank those who have helped compile the article. and hopefully it can be useful for readers

CONFLICT OF INTEREST

Not Applicable

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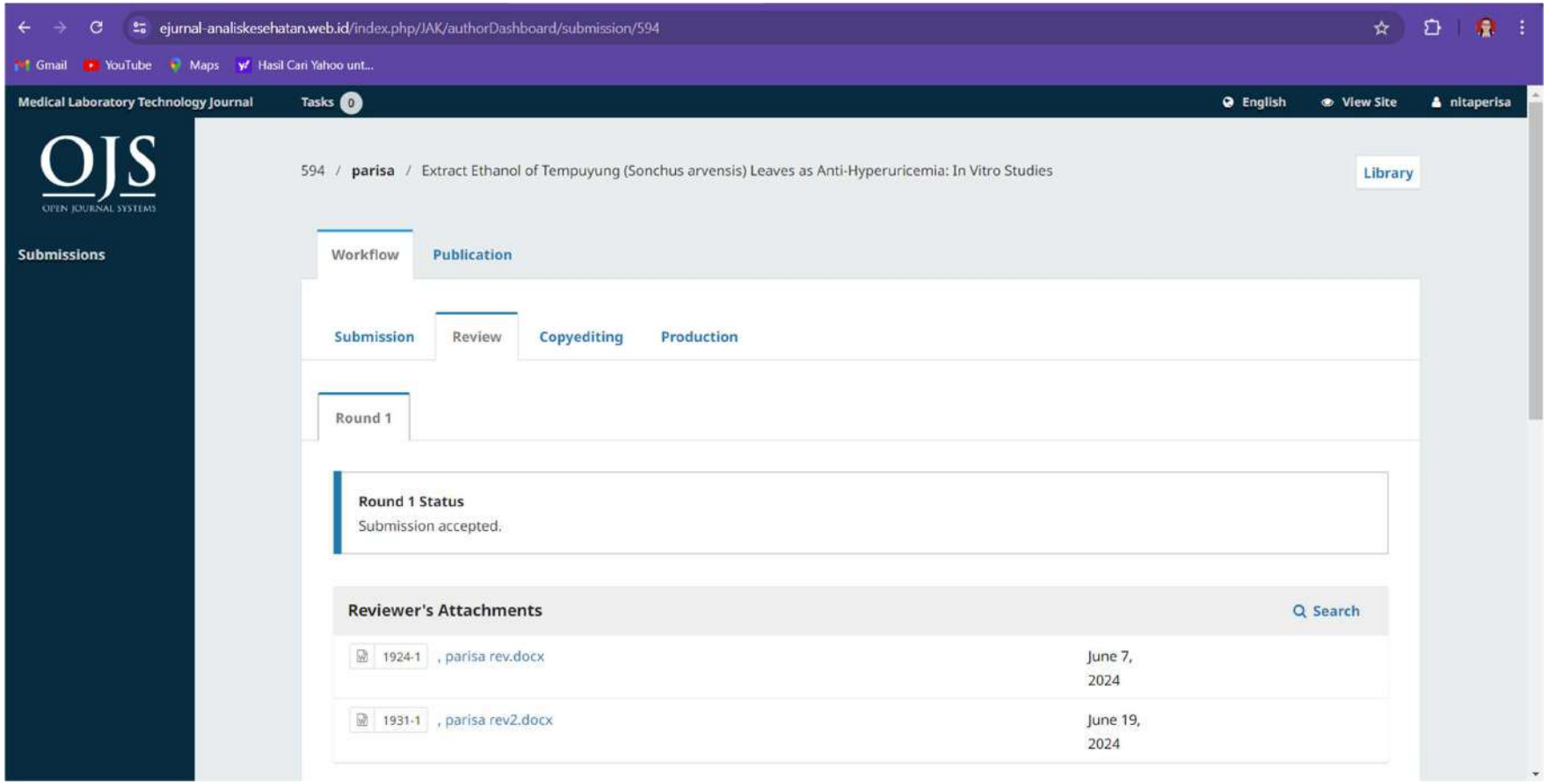
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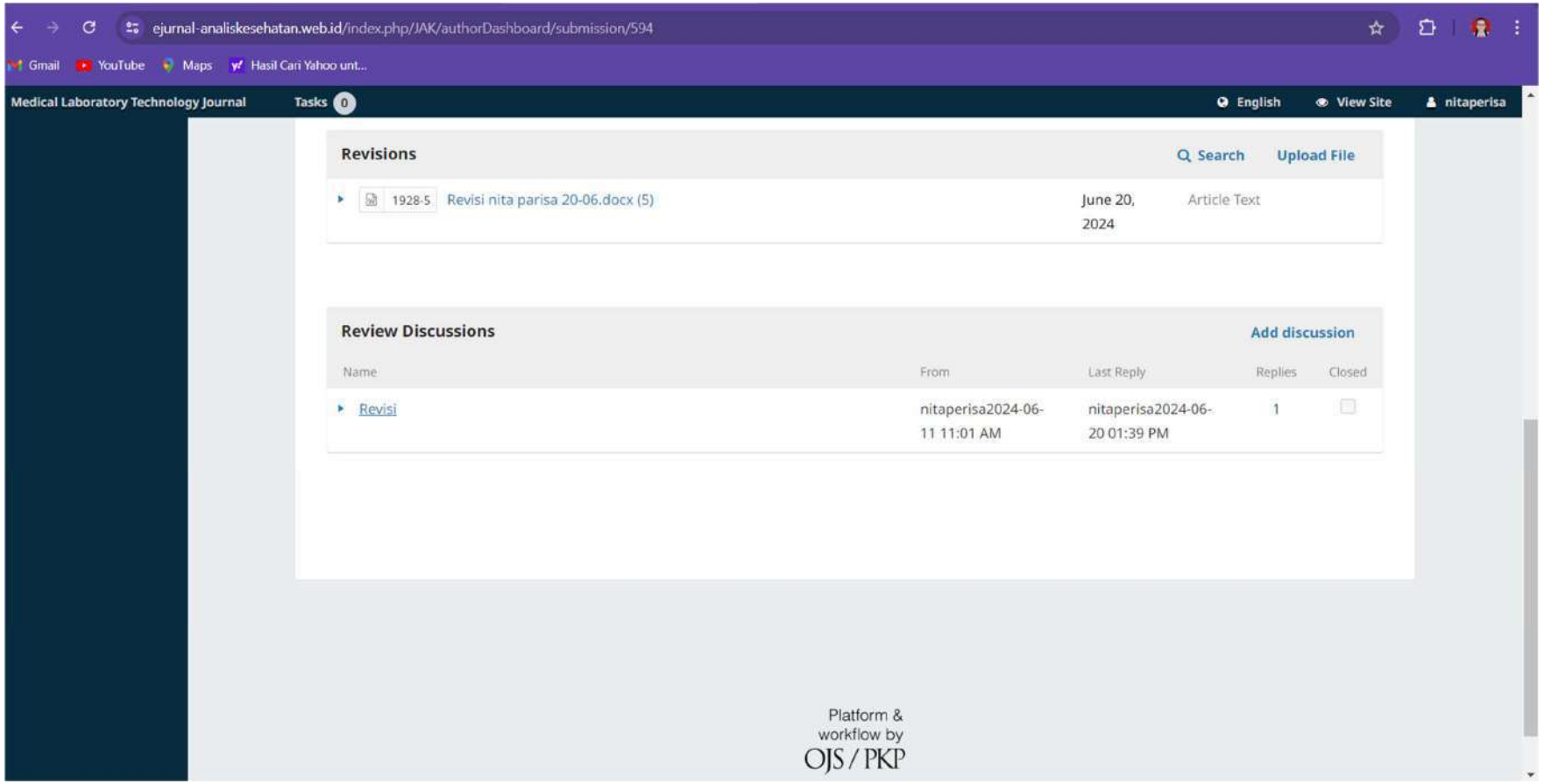
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Keyword: *Sonchus arvensis, Xanthine Oxidase Inhibitor, Hyperuricem*

INTRODUCTION

Hyperuricemia is a condition where the serum urate level exceeds 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 beverages has been linked to an increased risk of hyperuricemia (Dalbeth, Gosling, Gaffo, & Abishek, 2021). Moreover, excess consumption of alcohol and red meat is also linked to hyperuricemia, which contributes to the formation of monosodium urate (MSU) crystals. However, only 10% of individuals with hyperuricemia develop gout arthritis (Busso & So, 2010; Ghaemi-Oskouie & Shi, 2011). Gout is influenced by genetics, 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). Gout is the most typical side effect of hyperuricemia and affects 3.9% of Americans (George, Leslie, & Minter, 2023). According to data from the National Health and Nutrition Examination Survey (NHANES) 2007–2016, the prevalence rates of hyperuricemia in the United States were 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 China having a prevalence of 6%–25%, Taiwan's prevalence ranging from 10%–52%, and Indonesia's prevalence at 18% (Raja et al., 2019).

Uric acid is the result of purine breakdown. Animal internal organs and seafood are sources of proteins that can raise uric acid levels in the blood (Cicerello, 2018; Hainer, Matheson, & Wilkes, 2014). At the normal physiological pH of 7.4, uric acid circulates in the ionized form of urate. Purine metabolism mainly occurs in the liver, but it can also be produced in any other tissue that contains xanthine oxidase. This enzyme converts urate to the more water-soluble form of allantoin. Endogenous purine production can be accelerated by phospho-ribosyl-pyrophosphate (PRPP) synthetase activity and a defect in the regulatory enzyme hypoxanthine phosphoribosyl transferase (HPRT). Such conditions happen in rhabdomyolysis, haemolysis, and tumour lysis. Urate excretion occurs primarily in the kidneys and is responsible for hyperuricemia in 90% of individuals with reduced glomerular filtration, reduced tubular secretion, and increased tubular reabsorption all appear to contribute to underexcretion. 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 develop gout, arthritis, or nephrolithiasis. To prevent lysis syndrome in malignancies in patients receiving cytolytic therapy who are asymptomatic, uric acid-lowering medications are recommended (Engel, Just, Bleckwenn, & Weckbecker, 2017). Some uric acid-lowering medications are used as arthritis prophylaxis for gout, nephrolithiasis, and chemotherapy-related hyperuricemia, like the xanthine oxidase inhibitor allopurinol. Probenecid is used as a second-line therapy for gout patients because it operates as a URAT1 inhibitor, increasing uric acid output. Recombinant uricase (Rasburicase) transforms uric acid into allantoin, which is quickly eliminated by the kidneys and utilized as a preventative measure for hyperuricemia brought on by chemotherapy (George et al., 2023).

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Commented [rr4]: Clarified sentence: Hyperuricemia is a condition of the body with serum uric acid levels exceeding 7 mg/dL

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However, some experts advise against using ULT in acute gout and instead base its use on adequate anti-inflammatory therapy due to its protracted duration of inflammation and risk of recurrent gout attacks (Jia et al., 2022). 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 lowers 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).

The use of allopurinol for asymptomatic patients and the failure to monitor urate-lowering therapy (ULT) are bad for the patient. Allopurinol has several negative side effects and may also increase the likelihood of acute episodes, particularly in the early stages of treatment when uric acid deposits are being mobilized out of the tissues (Engel et al., 2017; Pillinger & Mandell, 2020). Tempuyung (*S. arvensis*) is a plant that has a long history of being used as medicine. The locals use tempuyung to treat kidney stones, but more research indicates that it may also be used to treat gout. Tempuyung has the same effectiveness as anti-nephrolithiasis and anti-gout, according to a study that compared its efficacy to that of colchicine. Tempuyung leaves extract can boost immunomodulatory action in inflammation (Jayani, Krisnawan, Oktaviyanti, & Kartini, 2020).

The aim of the current study was to find potential treatments for hyperuricemia by examining the xanthine oxidase inhibitory activity of *S. arvensis* leaves'-ethanol extract. Through the inhibition of xanthine oxidase, flavonoids are known to lower blood uric acid levels. The xanthine oxidase enzyme and guanase catalyze the conversion of hypoxanthine into xanthine, which is followed by the oxidation of xanthine into uric acid, which is also mediated by xanthine oxidase, to produce uric acid. Thus, xanthine oxidase inhibition is crucial as a pharmaceutical treatment for hyperuricemia and gout (Hendriani, Nursamsiar, & Tjitraresmi, 2017).

.....Hence, this study aims to determine the effect of an ethanol extract of tempuyung leaves as an anti-hyperuricemic in vitro.

MATERIALS AND METHOD

This in vitro experimental study was carried out at the Basic Medical Chemistry Laboratory, Faculty of Medicine, Sriwijaya University. Research begins in January–December 2021.

1. Research Procedure

1.1. Plant Extraction

The maceration method used for extraction in this study utilized 96% ethanol as the extracting solvent for the dried and ground plant materials. Five hundred grams of grounded tempuyung leaves were macerated with ethanol for 72 hours and evaporated at 40 °C with the help of a vacuum pump to extract their thick active component.

1.2. Xanthine oxidase inhibitory assay

The 99.5% xanthine substrate solution has a molecular weight of 152.11 gram/mol. In this investigation, 2.293 mg of xanthine substrate were needed to make 100 mL of 0.15 mM xanthine substrate. A tempuyung leaves sample solution was made with 5 mg of tempuyung leaves extract, a few drops of DMSO, and 10 mL of phosphate buffer to achieve a stock solution concentration of 100 ppm. The sample solution was then diluted with distilled water to produce different concentrations. Allopurinol at concentrations of 6.25, 12.5, 25, and 50 ppm was used as a positive control..... The xanthine oxidase enzyme inhibition test was carried out by measuring the absorbance value of the sample using a spectrophotometer with a wavelength of 293 nm with three repetitions.

.....

1.3. Phytochemical Test

Testing was done for alkaloids, flavonoids, triterpenoids, steroids, saponins, tannins, and quinones contents.

a. Alkaloid test

About 3 mL of tempuyung leaves 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 when there is a white or yellow precipitate. Two drops of Dragendroff's reagent added to the second test tube produce positive result when there is a red, brick, or orange precipitate. Three drops

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of Wagner's reagents were added into the third test tube, and a positive result occurs when there is brown precipitate.

b. Saponin test
Tempuyung leaves extract was put into the test tube and added to 10 mL of distilled water. Let it cool, then shake for ten seconds. The result is positive when the foam has formed in less than ten minutes.

c. Flavonoid test
Tempuyung leaves extract was dissolved with methanol and 0,5 gram of magnesium metal added with five drops of concentrated HCl. That solution was then heated. The result was positive when a red or yellow color was formed.

d. Tannin test
One mL of tempuyung leaves extract reacted with 2 mL of 1% FeCl3, and the result was positive when the solution changed color to blue or dark blue.

e. Triterpenoid test
One mL of tempuyung leaves extract was solved into 0,5 mL of chloroform and added to 0,5 mL of anhydrous acetic acid. Later, 1-2 mL of concentrated sulfate acid were added through the test tube wall. The result was positive when a brownish or violet ring was formed.

f. Quinones test
By adding NaOH to one mL of tempuyung leaves extract, the quinone test was conducted, and it was successful when a red hue emerged (Malik, Auliya, & Iqbal, 2022; Setyawaty, B, & Dewanto, 2020; Wulandari, Chandra, Zulharmita, & Rivai, 2021).

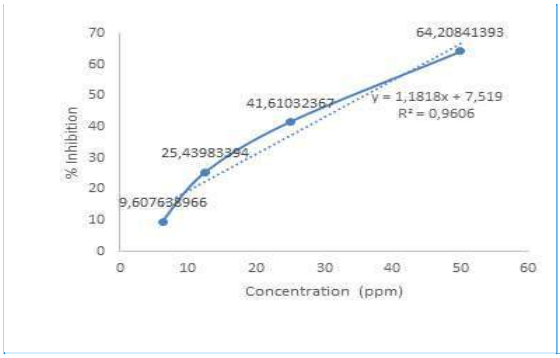
2. Data Analysis
The obtained data were analyzed by using linear regression in Microsoft Excel to calculate the IC50 and determine the relationship between the tempuyung leaves extract concentration and its inhibition effect.

Ethical statement

The authors would like to declare that there is no ethical clearance in this research.

RESULTS AND DISCUSSION

The selected plant and allopurinol xanthine oxidase inhibition assay results are shown in Figure 1. and Figure 2.



IC50: 23.37 ppm

Figure 1. Xanthine oxidase inhibition by Ethanol extract from tempuyung leaves

For every one-unit increase in the concentration of ethanol extract from tempuyung leaves, there is an expected increase of 1.1818% in Xanthine oxidase inhibition. The R2 value of 0.9606 suggests that 96.06% of the variability in Xanthine oxidase inhibition can be explained by the concentration of the ethanol extract from tempuyung leaves, demonstrating a strong correlation

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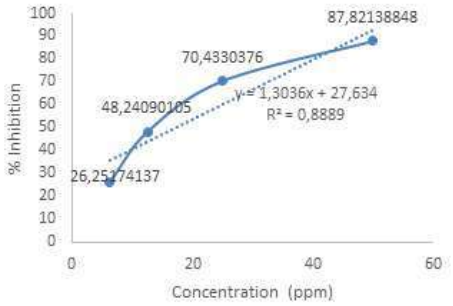
between the ethanol extract from tempuyung leaves concentration and the observed enzyme inhibition.

IC50: 17.16 ppm

Figure 2. Xanthine oxidase inhibition of Allopurinol



For every one-unit increase in the concentration of allopurinol (ppm), there is an expected increase of 1.3036% in Xanthine oxidase inhibition. R2 of 0.8889 suggests that 88.89% of the variability in Xanthine oxidase inhibition can be explained by the Allopurinol, indicating a strong correlation between the allopurinol concentration and the observed enzyme inhibition. These Results show that the ethanol extract of tempuyung leaves asts as a strong inhibitor for the xanthine oxidase enzyme. Thus, the ethanol extact of tempuyung leaves has the potential to be an anti-hyperuricemia agent. After a thick extract was obtained, the phytochemical test was done. The result shown innthe table 1 below :

Table 1. Phytochemical Test

Test	Appearance	Result
Alkaloid: Dragendroff	Orange sediment	+
Alkaloid: Mayer	Yellow solution	+
Alkaloid: Wagner	Brown sediment	+
Flavonoid	Red color	+
Triterpenoid	Brownish ring	+
Steroid	-	-
Saponin	Foam	-
Tannin	Yellow sediment	-
Quinone	Red	+

From the phytochemical test done for tempuyung leaves, it was found that it is rich in flavonoid, alkaloid, and triterpenoid compounds, which have strong antioxidant and free radical scavenger capabilities (Khan, 2012; Owona, Abia, & Moundipa, 2020; Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018).

Results showed that the ethanol extract had high potential inhibitory activity, especially since it came from direct extraction. This finding indicated that ethanol direct extraction produced a great extraction of bioactive components. The flavonoid contents of tempuyung leaves extract, which is well-known for its potent reactive oxygen-scavenging activity, have been linked to a variety of biological activities (Hendriani et al., 2017). If a compound has a lower IC50 against xanthine oxidase, it indicates that the compound is more effective at inhibiting uric acid production. This finding could have significant biological implications, especially in the treatment of conditions such as hyperuricemia and gout. It is showed that the lower the IC50 value, the better the inhibitory activity towards the xanthine oxidase enzyme (Chen et al., 2024). Based on

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the liner regression graph, allopurinol as a positive control showed very potent inhibitory activity toward xanthine oxidase with an IC50 17.16 ppm; thus, it is confirmed that the methods used in this experiment were true. Ethanol extract of tempuyung leaves in this experiment showed an IC50 23.37 ppm, which is still considered to have strong inhibitory activity towards the xanthine oxidase enzyme (IC50 <50) (Priska, Peni, & Carvallo, 2019). It is confirmed that tempuyung leaves extract has flavonoid contents, which have potential as anti-hyperuricemia agents and used as an alternative for allopurinol.

The flavonoids in tempuyung leaves may inhibit xanthine oxidase through several potential mechanisms, such as:

1. Antioxidant properties, which allow them to neutralize free radicals and combat oxidative stress. Xanthine oxidase is an enzyme that contributes to the formation of free radicals, including reactive oxygen species. Flavonoids can help reduce the production of free radicals and relieve oxidative stress by inhibiting xanthine oxidase activity.
2. Direct interactions with enzymes, certain flavonoids can interact directly with the xanthine oxidase enzyme, changing its structure or inhibiting its active site, resulting in the inhibition of these enzymes' activity and reducing the conversion of xanthine to uric acid.
3. Anti-inflammatory effects, flavonoids possess anti-inflammatory properties that can reduce inflammation. This reduction in inflammation can lead to a decrease in the expression and activity of the xanthine oxidase gene.
4. Regulation of signaling pathways, flavonoids can also influence various signaling pathways in cells, including those involved in gene expression regulation. Flavonoids can reduce the production of xanthine oxidase by affecting it signaling pathways.

The flavonoids present in tempuyung leaves have the potential to inhibit xanthine oxidase, which can be useful in reducing uric acid production in the body and controlling medical conditions such as hyperuricemia or gout. However, it is important to note that the precise mechanism and effects of flavonoids in tempuyung leaves on xanthine oxidase require further research for a better understanding**.**

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CONCLUSION

The study examined the potential of the ethanol extract of Tempuyung leaves to reduce hyperuricemia. The results showed a strong inhibition of the xanthine oxidase enzyme, indicating its effectiveness in lowering hyperuricemia. Phytochemical analysis revealed the presence of various compounds such as alkaloids, flavonoids, and triterpenoids in the extract. Tempuyung leaves under investigation demonstrates xanthine oxidase inhibitory activity. The current study's findings indicate that tempuyung leaves ethanol extract a had strong inhibitory effect on xanthine oxidase, suggesting its potential to be developed as an agent for treating hyperuricemia. We conclude that it is necessary to do further research regarding the tempuyung leaves extract-active compound, which specifically can lower hyperuricemia. This research should be continued in vivo by using experimental animals. It might indicate that further study regarding the tempuyung leaves extract potential to be an anti-hyperuricemia agent is highly needed.

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ACKNOWLEDGEMENT

I would like to thank those who have helped compile the article. and hopefully it can be useful for readers

CONFLICT OF INTEREST

Not Applicable

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Medical Laboratory Technology Journal
Volume(No), Year, Page
Received YYYY-DD-MM; Revised YYYY-DD-MM; Accepted YYYY-DD-MM
Available online at : http://ejurnal-analiskesehatan.web.id

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

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….. The research aims to determine the effect of ethanol extract of tempuyung leaves on xanthine oxidase in vitro, where tempuyung leaves (Sonchus arvenis) is a plant that has antihyperuricemia properties. This research tested four extract concentrations, namely 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm, followed by absorbance 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 tempuyung leaves with allopurinol as an effective inhibitor 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 tempuyung leaves as a strong inhibitor of the xanthin oxidase enzyme. Therefore, the ethanol extract of tempuyung leaves has the potential to be antihyperuricemic.*

Keywords: *Sonchus arvensis, Xanthine Oxidase Inhibitor, Hyperuricemia*

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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 beverages has been associated with an increased risk of hyperuricemia (Dalbeth, Gosling, Gaffo, & Abishek, 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 people with hyperuricemia develop 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 data from the 2007–2016 National Health and Nutrition Examination Survey (NHANES), 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 prevalence in China of 6%–25%, prevalence in Taiwan ranging from 10%–52%, and prevalence in Indonesia of 18% (Raja et al., 2019) .

Uric acid is the result of the breakdown of purines in the body. Protein sources from 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 7.4 pH, 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 a form of allantonin which is more easily soluble in water. Under normal conditions, the body can regulate uric acid levels well. However, disturbances in 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 phospho-ribosyl-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 apparently 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 for 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 which is being 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 caused by 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) .

The use of allopurinol in asymptomatic patients and failure to monitor uric acid-lowering therapy (ULT) has negative consequences for patients. 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). Meanwhile, tempuyung (*S.arvensis*) is a plant that has a long history of use as medicine. Local 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 on inflammation (Jayani, Krisnawan, Oktaviyanti, & Kartini, 2020). Therefore, tempuyung (*S. Arvensis*) has the potential as an alternative solution in the treatment of acid reduction therapy and considering the side effects of using allopurinol (Engel Pilinger).

The aim of this study was to find a potential treatment for hyperuricemia by testing the xanthine oxidase inhibitory activity of ethanol extract of *S. arvensis leaves*. By inhibiting xanthine oxidase, flavonoids are known to reduce blood uric acid levels. The enzymes xanthine oxidase and guanase catalyze the conversion of hypoxanthine to xanthine, which is followed by the oxidation of xanthine to uric acid, which is also mediated by xanthine oxidase, thus producing uric acid. Therefore, inhibition of xanthine oxidase is very important as a pharmaceutical treatment for hyperuricemia and gout (Hendriani, Nursamsiar, & Tjitraresmi, 2017) .

This research found gaps from previous research such as research conducted by (Suwartiny, Rafi, & Rohaeti, 2022) and (Parisa et al., 2023) which only highlighted the utilization, compound content and biological activity of the medicinal plant S. arvensis. Therefore, this study aims to determine the effect of ethanol extract of tempuyung leaves as antihyperuricemia in vitro using phytochemical testing.

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Commented [rr6]: This sentence is replaced with: another alternative for lowering uric acid is tempuyung (S.arvensis)

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Commented [rr8]: This sentence has been changed to: 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

Commented [rr9]: This sentence should be replaced with: Previous research such as that conducted 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 antihyperuricemia, especially the inhibitory activity against xanthine oxidase, so this research aims to analyze the xanthine oxidase inhibitory activity by ethanol extract of S. arvensis leaves as antihyperuricemia in vitro.

MATERIALS AND METHODS

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

Research procedure

1.1. 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 Kersen plant which grows in Palembang City, South Sumatra, Indonesia. Five hundred grams of ground tempuyung leaves were macerated with ethanol for 72 hours and evaporated at 40°C with the help of a Rotary Evaporator vacuum pump to extract the thick active components.

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1.2. 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 to reach 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 was made by weighing 10 ml of the solution into a 5 ml measuring flask and adding CO2-free distilled water to the limit (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 (M & MA, 2021) . Blank control 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. 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) involved sodium phosphate buffer, cherry leaf ethyl acetate extract, and distilled water, while the sample test (S1) added 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 allopurinol test also added an enzyme solution. All reactions were stopped with 0.5 M HCl and the absorbance was measured at 293 nm (DW & F, 2017) .

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The xanthine oxidase enzyme inhibition test was carried out by measuring the absorption value of the sample using a UV Spectrophotometer UV-1800 with a wavelength of 293 nm with three repetitions. The absorbance results of the sample, blank and positive control which were measured using a UV-Vis spectrophotometer measured the percent inhibition using the formula (NE, R, & MG, 2016) :

$$\%\text{Inhibisi} = \frac{\text{Absorbansi kontrol} - \text{absorbansi sampel}}{\text{Absorbansi kontrol}} \text{ x } 100\%$$

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

$$IC_{50} = \frac{50 - a}{b}$$

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

1.3. Phytochemical Test

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

a. 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 Dragendroff's reagent added to the second test tube give a positive result if there is a red, brick or orange precipitate. Three drops of Wagner's reagent were added to the third test tube, and a positive result was obtained when a brown precipitate was present.
b. T es saponins
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 has formed in less than ten minutes.
c. Flavonoid Test
Tempuyung leaf extract is 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 is formed.
d. Tannin Test
One mL of tempuyung leaf extract was reacted with 2 mL of 1% FeCl3, and the result was positive if the solution changed color to blue or dark blue.
e. 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. Positive results if a brownish or purple ring forms.

f. Quinone Test
By adding NaOH to one mL of tempuyung leaf extract, a quinone test was carried out and a red hue appeared successful (Malik, Auliya, & Iqbal, 2022; Setyawaty, B, & Dewanto, 2020; Wulandari, Chandra, Zulharmita, & Rivai, 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.

Ethical statement

The authors would like to state that there was no ethical clearance in this study.

RESULTS AND DISCUSSION

test results , ethanol extract of tempuyung leaves and allopurinol as effective inhibitors of the xanthine oxidase enzyme are shown in Figure 1 and Figure 2.

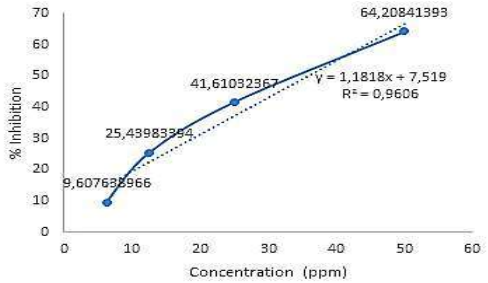

Figure 1 . Inhibition of xanthine oxidase with ethanol extract of tempuyung leaves was 1.1818 % with IC50: 23.37 ppm

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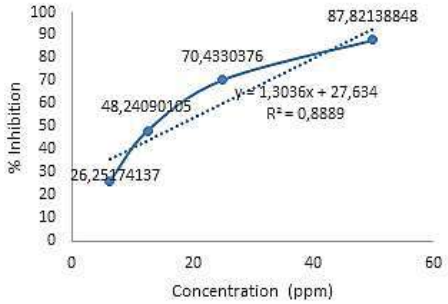
Commented [rr15]: preferably: Figure 1 . Inhibition of xanthine oxidase by ethanol extract of tempuyung leaves

For every one unit increase in the concentration of tempuyung leaf ethanol extract, it is estimated that there will be an increase in the inhibition of Xanthine oxidase by 1.1818%. R2 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 inhibition of silent enzymes



IC50: 17.16 ppm

Figure 2. Inhibition of Xanthine oxidase against Allopurinol by 1.3036% with IC50: 23.37

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Commented [rr17]: preferably: Figure 2 . Inhibition of xanthine oxidase by Allopurinol

For every one unit increase in allopurinol concentration (ppm), it is estimated that there is an increase in Xanthine oxidase inhibition of 1.3036%. R2 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. These results indicate that the ethanol extract of tempuyung asts leaves has an effect as a strong inhibitor of the xanthine oxidase enzyme. Thus, the ethanol extract of tempuyung leaves has the potential to be an antihyperuricemic agent.

After obtaining a thick extract, a phytochemical test was carried out. The results are shown in table 1 below :

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Table 1. Qualitative tests of phytochemical types in plant samples

Test	Appearance	Results
Alkaloids: Dragendroff	Orange sediment	+
Alkaloids: Mayer	Yellow solution	+
Alkaloids: Wagner	The sediment is brown	+
Flavonoids	red	+
Triterpenoids	Brownish ring	+
Steroids	-	-
Saponin	Foam	-
Tannin	Yellow sediment	-
quinone	Red	+

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 scavenge free radicals (Khan, 2012; Owona, Abia, & Moundipa, 2020; Tungmunnithum, Thongboonyou, Pholboon, & Yangsabai, 2018) .

The results showed that the ethanol extract had the potential for high inhibitory activity, especially because the extract came from direct extraction. These findings indicate that direct extraction of ethanol results in the extraction of large bioactive components. The flavonoid content in tempuyung leaf extract, which is known for its strong reactive oxygen removal activity, has been associated with various biological activities (Hendriani et al., 2017) .

If a compound has a lower IC50 value against xanthine oxidase, it means that the compound is more effective in inhibiting the activity of the xanthine oxidase enzyme. The xanthine oxidase enzyme plays a role in the production of uric acid. Therefore, inhibiting this enzyme can help reduce uric acid production in the body, which is very important in the treatment of things like hyperuricemia (high uric acid levels in the blood) and gout (gout). The lower the IC50 value, the stronger the compound's inhibitory ability against the xanthine oxidase enzyme. This has significant biological implications, as compounds with a low IC50 can be more effectively used as drugs to control uric acid levels and treat related conditions (Chen et al., 2024) .

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Based on the liner regression graph, allopurinol as a positive control showed very potent inhibitory activity against xanthine oxidase with an IC50 of 17.16 ppm; Thus, it is confirmed that the method used in this experiment is correct. The ethanol extract of tempuyung leaves in this experiment showed an IC50 of 23.37 ppm which was considered to still have strong inhibitory activity against the xanthine oxidase enzyme (IC50<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) . Flavonoids can help reduce the production of free radicals and relieve oxidative stress by inhibiting xanthine oxidase activity (2) Direct interactions with enzymes, certain flavonoids can interact directly with the xanthine oxidase enzyme, changing its structure or inhibiting its active site, resulting in inhibiting the enzyme's activity and reducing conversion. xanthine into uric acid (Panche et al., 2016) (3) Anti-inflammatory effects, 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) .

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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 effect of tempuyung leaf flavonoids on xanthine oxidase requires further research for a better understanding.

CONCLUSION

This research 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. This may indicate that further research regarding the potential of tempuyung leaf extract as an antihyperuricemic agent is needed.

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CONFESSION

I would like to thank those who have helped compile this article. and hopefully it can be useful for readers

CONFLICT OF INTEREST

Not applicable

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Volume(No), Year, Page
Received YYYY-DD-MM; Revised YYYY-DD-MM; Accepted YYYY-DD-MM

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

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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 avernis) are one of the plants that have antihyperuricemia 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, macerated with 96% ethanol until a thick extract is obtained. The xanthine oxidase inhibition test was carried out on tempuyung leaf extract and Allo[purinol with respective concentrations of 6.25; 12.5; 25; and 50 ppm. 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 antihyperuricemic.*

Keywords: *Sonchus arvensis, Xanthine Oxidase Inhibitor, Hyperuricemia*

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, Gosling, Gaffo, & Abishek, 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 prevalence in China of 6%–25%, prevalence in Taiwan ranging from 10%–52%, and 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 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 alantonin 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 phospho-ribosyl-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 apparently 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) .

The use of allopurinol in asymptomatic patients has negative consequences for the patient. 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) . Local 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 acid reduction therapy and considering the side effects of using allopurinol (Engel Pilinger).

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 anihyperuricemia, 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 antihyperuricemia in vitro.

MATERIALS AND METHODS

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

Research procedure

1.1. 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 2015 Heidolph Brand Rotary Evaporator vacuum pump to extract the thick active components.

1.2. 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 (M & MA, 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 Shimadzu Brand UV-1800 UV Spectrophotometer . 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 (DW & F, 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 (NE, R, & MG, 2016) :

$$\%\text{Inhibisi} = \frac{\text{Absorbansi kontrol} - \text{absorbansi sampel}}{\text{Absorbansi kontrol}} \text{ x } 100\%$$

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):

$$IC_{50} = \frac{50 - a}{b}$$

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.

1.3. Phytochemical Test

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

a. 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 Dragendroff'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.

b. This is saponin

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.

c. 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.
d. 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.
e. 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.
f. 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, Auliya, & Iqbal, 2022; Setyawaty, B, & Dewanto, 2020; Wulandari, Chandra, Zulharmita, & Rivai, 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.

Ethical statement

The authors would like to state that there was no ethical clearance in this study.

RESULTS AND DISCUSSION

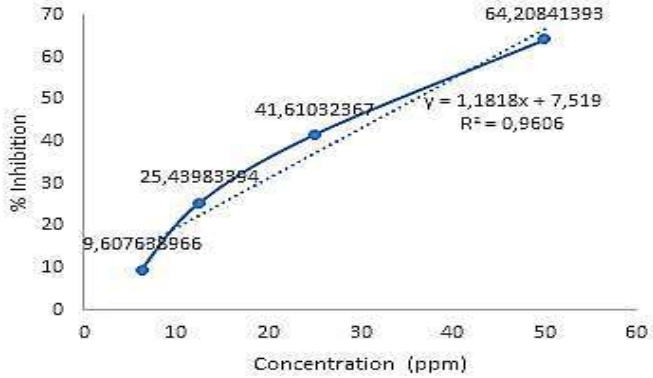
After obtaining a thick extract, a phytochemical test was carried out. The results are shown in table 1 below:

Table 1. Qualitative tests of phytochemical types in plant samples

Test	Appearance	Results
Alkaloids: Dragendroff	Orange sediment	+
Alkaloids: Mayer	Yellow solution	+
Alkaloids: Wagner	The sediment is brown	+
Flavonoids	red	+
Triterpenoids	Brownish ring	+
Steroids	-	-
Saponins	Foam	-
Tannin	Yellow sediment	-
quinone	Red	+

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, Thongboonyou, Pholboon, & Yangsabai, 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.

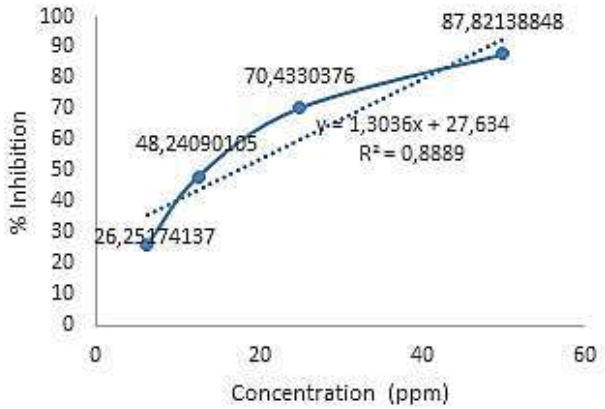


IC50: 35.94 ppm

Figure 1. **Inhibition of Xanthine Oxidase By Ethanol Extract Of Tempuyung Leaves**

According to the graph, an intercept (a) of 7.519 indicates that the percentage inhibition of 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 IC50 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%. R2 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.



IC50: 17.15 ppm

Figure 2. Inhibition of Xanthine Oxidase by Allopurinol

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 IC50 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%. R2 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. These results indicate that the ethanol extract of tempuyung ast leaves has an effect as a strong inhibitor of the xanthine oxidase enzyme. Thus, the ethanol extract of tempuyung leaves has the potential to be an antihyperuricemia drug.

The results showed that the ethanol extract had the potential for high inhibitory activity, especially because the extract came from direct extraction. These findings indicate that direct extraction of ethanol results in 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 IC50 value, the stronger the compound's inhibitory ability against the xanthine oxidase enzyme. This has significant biological implications, because compounds with low IC50 can be more effectively used as drugs to control uric acid levels and treat related conditions (Chen et al., 2024) .

Based on the liner regression graph, allopurinol as a positive control showed very potent inhibitory activity against xanthine oxidase with an IC50 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 IC50 of 23.37 ppm which was considered to still have strong inhibitory activity against the xanthine oxidase enzyme (IC50<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.

CONFESSION

I would like to thank those who have helped compile this article. and hopefully it can be useful for readers

CONFLICT OF INTEREST

Not applicable

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ARTICLE ACCEPTANCE LETTER

To
Nita Parisa
Department of Pharmocacology, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia

Dear author/s
I have pleasure to inform you that your following Original Article has been accepted for publication in Medical Laboratory Technology Journal

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

***Nita Parisa[1], Rachmat Hidayah[2], Fatmawati[3]**

[1]Department of Pharmocacology, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia, [2]Department Program in Biomedical Science, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia, [3]Department of Biochemical, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia.
*Email: nitaparisaa@gmail.com.

It will be published in Volume 10, No.1, June 2024 issue. It is further mentioned for your information that our journal is a double blind peer reviewed indexed international journal.

With regards
Yours sincerelv

Leka Lutpiatina
Editor

Medical Laboratory Technology Journal

Received 2023-28-05; Revised 2023-20-06; Accepted 2023-25-06

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[1]Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia, [2]Department of Medical Biology, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia, [3]Department of Biochemical, Faculty of Medicine, Universitas Sriwijaya Palembang, Indonesia.
*Email: nitaparisa@unsri.ac.id. DOI: 10.31964/mltj.v10i1.xxx

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INTRODUCTION

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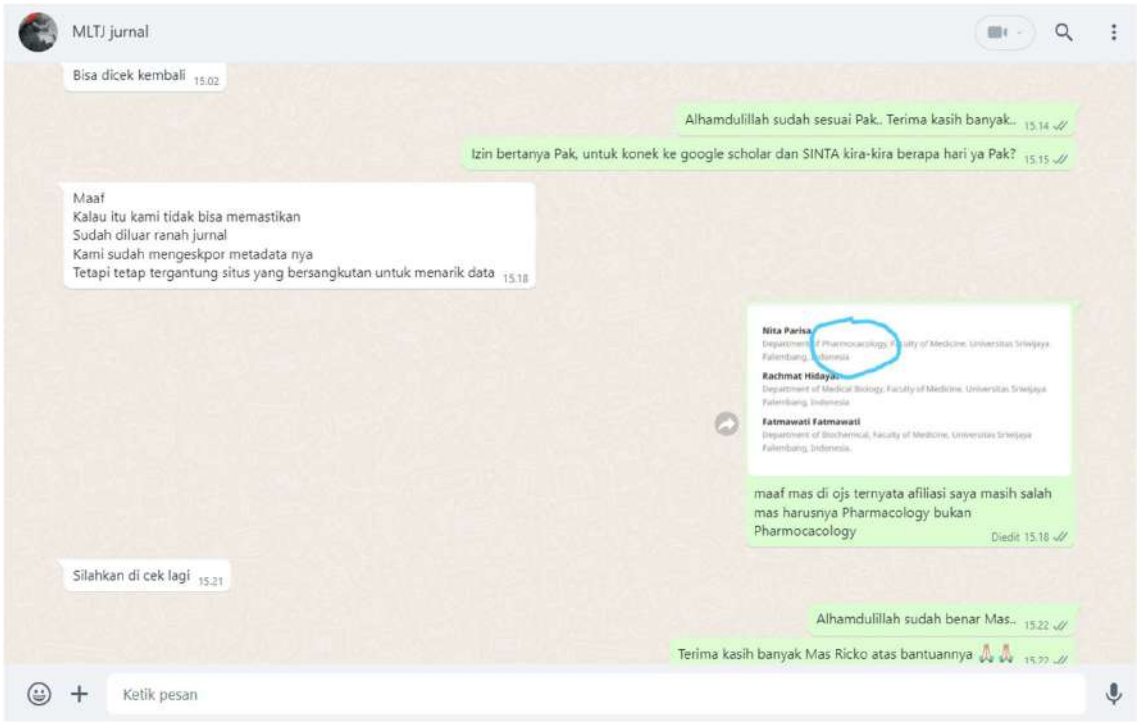
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DOI: https://doi.org/10.31964/mltj.v10i1.594
Keywords: Hyperuricemia, Sonchus arvensis, xanthine oxidase inhibitor
Abstract
PDF
Published
2024-06-29
How to Cite
Parisa, N., Hidayat, R., & Fatmawati, F. (2024). Extract Ethanol of Tempuyung (Sonchus arvensis) Leaves as Anti-Hyperuricemia: In Vitro Studies. Medical Laboratory Technology Journal, 10(1), 63–72. https://doi.org/10.31964/mltj.v10i1.594
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Medical Laboratory Technology Journal
10(1), 2024, 63-72
Received 2023-28-05; Revised 2023-20-06; Accepted 2023-25-06
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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 alantonin 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 phospho-ribosyl-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):

$$\%\text{Inhibisi} = \frac{\text{Absorbansi kontrol} - \text{absorbansi sampel}}{\text{Absorbansi kontrol}} \text{ x } 100\%$$

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 lC value of 50 (50% inhibitor concentration) was found use equality linear regression with the y value is 50 so obtained equality (Tariza, 2021):

$$IC_{50} = \frac{50 - a}{b}$$

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 Dragendroff'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 IC_{50} and determine the relationship between the concentration of tempuyung leaf extract and its inhibitory effect.

RESULTS AND DISCUSSION

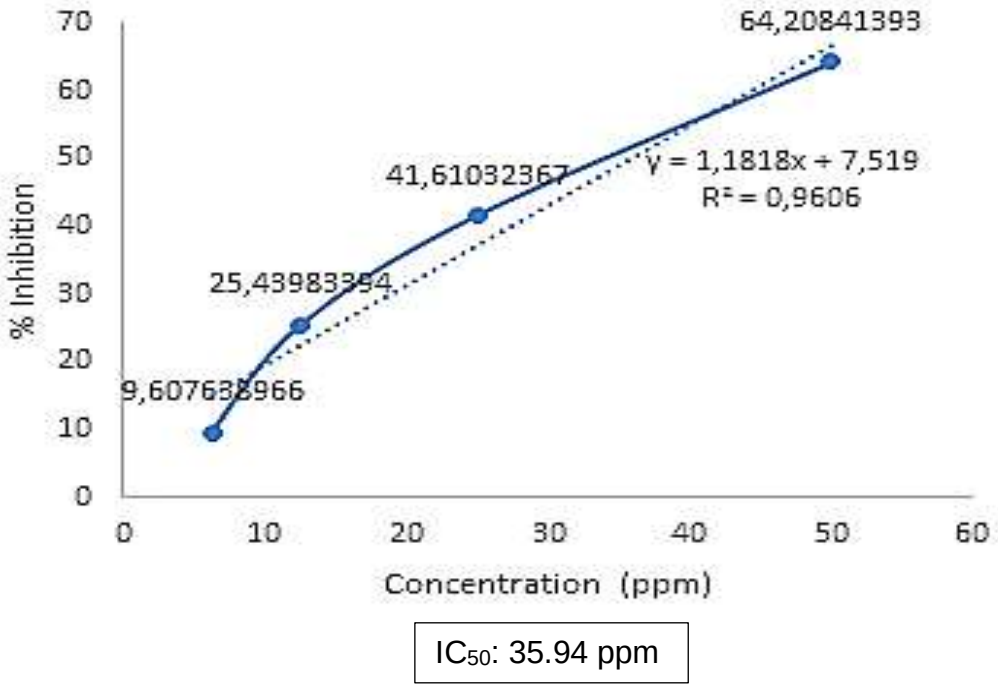
After obtaining a thick extract, a phytochemical test was carried out. The results are shown in Table 1.

Table 1. Qualitative Tests of Phytochemical Types in Plant Samples

Test	Appearance	Results
Alkaloids: Dragendroff	Orange sediment	+
Alkaloids: Mayer	Yellow solution	+
Alkaloids: Wagner	The sediment is brown	+
Flavonoids	Red	+
Triterpenoids	Brownish ring	+
Steroids	-	-
Saponins	Foam	-
Tannin	Yellow sediment	-
Quinone	Red	+

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.



IC_{50}: 35.94 ppm

Figure 1**.** Inhibition of Xanthine Oxidase By Ethanol Extract of Tempuyung Leaves

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%. R2 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%. R2 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..

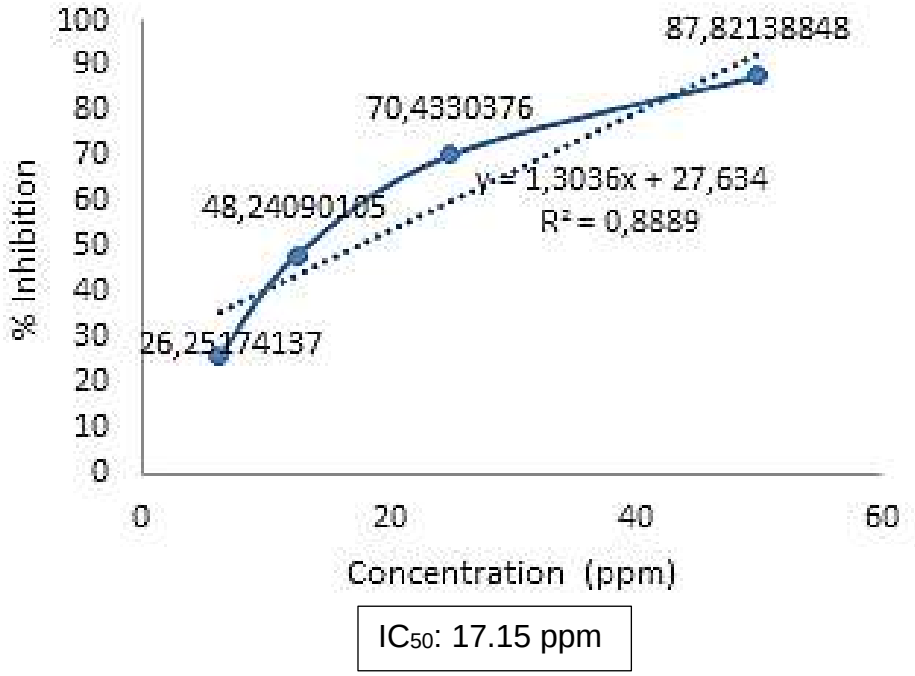


Figure 2. Inhibition of Xanthine Oxidase by Allopurinol

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.

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