



Inhibition Effect of N-Hexane Extract of Cherry Mistletoe Leaves (*Dendrophthoe pentandra* (L) Miq.) on Xanthine Oxidase

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ABSTRACT

Introduction. Cherry leaf extract has been found to have the potentiality to impede the xanthine oxidase enzyme. It is believed that cherry mistletoe leaves also possess similar properties as they are hemiparasites of their host. This study aims to determine the inhibition effect of N-Hexane extract of mistletoe cherry leaves on the xanthine oxidase. **Methods.** This research was conducted as an experimental in-vitro study. Cherry mistletoe leaves were extracted by N-Hexane solvent. The extract was divided into five doses, i.e. 40 mg/L, 20 mg/L, 10 mg/L, 5 mg/L, and 2.5 mg/L. Phytochemical screening was conducted to identify secondary metabolites. The impact of xanthine oxidase inhibition was quantified using a UV-vis spectrophotometer at wavelength of 400 nm. **Results.** N-Hexane extract of cherry mistletoe leaves contains alkaloids and triterpenoids. N-Hexane extract of mistletoe cherry leaves at a dose of 40 mg/L, 20 mg/L, 10 mg/L, 5 mg/L, and 2.5 mg/L was able to inhibit the xanthine oxidase enzyme with the line equation $y = -0.2543x + 91.305$. The IC₅₀ value for the N-Hexane extract of cherry mistletoe leaves is 162 mg/L. **Conclusion.** N-Hexane extract of cherry mistletoe leaves has the potency to inhibit the xanthine oxidase enzyme in the medium category.

1. Introduction

Xanthine oxidase is an oxidoreductase enzyme which has a role as a catalyst for the oxidation of hypoxanthine to xanthine, and the oxidation of xanthine to uric acid. Xanthine oxidase is an independent catalytic homodimer subunit, this enzyme has a role in the purine degradation pathway. Increased activity of this enzyme causes increased levels of uric acid in the blood or hyperuricemia.¹

Hyperuricemia is a condition where blood uric acid levels increase above normal limits.² Hyperuricemia occurs if blood uric acid levels are above 7.0 mg/dL for men and above 6.0 mg/dL for women.³ Hyperuricemia can occur due to increased uric acid production or decreased uric acid excretion or it can also be a combination of these two processes.⁴

Based on previous research, it is estimated that as many as 21% of the general population and as many as 25% of hospitalized patients experience asymptomatic hyperuricemia. The National Health and Nutrition Examination Survey (NHANES) states that in 2007-2016 in the United States, hyperuricemia was found in 20.2% of men and 20.0% of women. The prevalence of hyperuricemia increased in men from

19.7% to 25% and in women from 20.5% to 24.1% from 2006 to 2014. Most studies state that the prevalence of hyperuricemia is generally lower in developing countries than in developed countries.⁴

Flavonoid compounds have the function of inhibiting xanthine oxidase and can capture superoxide free radicals, so they have the function of reducing uric acid levels.⁵

Cherry mistletoe (*Dendrophthoe pentandra*) is a hemiparasitic plant that obtains its food source from its host. The content of active compounds in cherry mistletoe is almost the same as its host plant, such as polyphenols, flavonoids and saponins. Therefore, this plant is considered to have the same potential as cherry as an anti-hyperuricemia.^{6,7}

Based on research conducted by Sinulingga in 2023, cherry mistletoe contains secondary metabolite compounds of steroids, triterpenoids, tannins, alkaloids, and flavonoids in the non-polar fraction which are very active in inhibiting the xanthine oxidase enzyme.⁸ Then, research conducted by Nirwana showed that cherry mistletoe contains flavonoid compounds, alkaloids, terpenoids, tannins, and saponins.⁹

Based on in vitro research conducted by Ikadah

and Ukrida, ethanol extract of cherry leaves contains secondary metabolites which can inhibit the action of the xanthine oxidase enzyme.¹⁰ In research conducted in 2022, talok (cherry) leaves contain active compounds that can act as xanthine oxidase inhibitors in silico and in vitro, 60% ethanol extract showed an inhibitory effect on the xanthine oxidase enzyme.¹¹

There have been studies that have tested the inhibitory effect of cherry leaves on the xanthine oxidase enzyme. However, there has been no research on the inhibitory effect of N-Hexane extract of cherry mistletoe leaves on xanthine oxidase. It is necessary to research the inhibitory effect of N-Hexane extract of cherry mistletoe leaves on xanthine oxidase.

2. Methods

This study was in vitro experimental research at the Medical Basic Chemistry Laboratory, Faculty of Medicine, Universitas Sriwijaya. This study aims to assess the ability of the phytochemical content of ethyl extract of cherry mistletoe leaves (*Dendrophthoe pentandra* (L) Miq.) in inhibiting the action of the xanthine oxidase enzyme. The samples used were cherry mistletoe leaves (*Dendrophthoe pentandra* (L) Miq.) which have the characteristics of leaves that look green, fresh, have a perfect shape, are clean, not moldy, and not rotten. The Ethics Committee of the Faculty of Medicine, Sriwijaya Universitas Sriwijaya has approved this research (Ethic Certificate No. 388-2023).

Extraction

Cherry mistletoe leaves with an initial weight of 900 grams were washed with running water until clean. Then the cherry mistletoe leaves were dried without direct sunlight until dry. Dry cherry mistletoe leaves could be seen from the color change on the leaves which turn brownish.

Cherry mistletoe leaves were made into simplicia by blending the dried cherry mistletoe leaves until they became simplicia or powder. Total simplicia of the cherry mistletoe leaves was 475 grams.

Simplicia 475 grams were taken into 1 large dark glass bottle and inserted using a funnel. Simplicia was macerated using N-Hexane solvent at room temperature for 3x24 hours. The extract was filtered using filter paper and a funnel inserted into an Enlenmeyer flask. This maceration process yielded 14.08 grams of N-Hexane extract of cherry mistletoe

leaves.

Phytochemical Test

Phytochemical test was conducted to identify alkaloid, flavonoid, triterpenoid, steroid, saponin, and tannin in N-Hexane extract of cherry mistletoe leaves. Alkaloid was identified by adding 5 mL of 2% HCl to extract of cherry mistletoe leaves based on was Dragendroff's test, Mayer's test, and Wegner's test. Flavonoid was identified by dissolving methanol, and 0.5 grams of magnesium, and 5 drops of HCl into N-Hexane extract of cherry mistletoe leaves.⁸

Triterpenoid and steroid were identified by adding 0.5 mL of anhydrous acetic acid, 0.5 mL of chloroform, and 2 mL of concentrated sulfuric acid into N-Hexane extract of cherry mistletoe leaves. Saponin was identified by adding 10 mL of hot water into N-Hexane extract of cherry mistletoe leaves and shaking vigorously for 10 seconds. The last, tannis was identified by added with 2 mL of 1% FeCl₃ into N-Hexane extract of cherry mistletoe leaves.⁸

Inhibitory Test of Xanthine Oxidase

N-Hexane extract of mistletoe cherry leaves was diluted to five concentrations. Allopurinol was used as a positive control. The concentrations used in dilution for N-Hexane extract of mistletoe cherry leaves were 40 mg/L, 20 mg/L, 10 mg/L, 5 mg/L, and 2.5 mg/L. The concentrations in dilution using allopurinol were 600 mg/L, 450 mg/L, 300 mg/L, 150 mg/L, and 75 mg/L. Each sample will be reacted and the absorbance value will be measured using an UV-Vis spectrophotometer at 400 nm. The inhibition value was calculated by using absorbance value. The percentage of inhibition for each concentration was used to create a linear regression graph. The IC₅₀ of N-Hexane extract of mistletoe cherry leaves was determined by a linear regression graph.

3. Result

Secondary Metabolites

The phytochemical screening test was a method to identify the secondary metabolite compounds in the N-Hexane extract of cherry mistletoe leaves. Phytochemical screening tests are carried out to determine alkaloids, flavonoids, saponins, triterpenoids, and tannins. The results of the phytochemical test are presented in Table 1.

Table 1. Secondary metabolites of N-Hexane extract of cherry mistletoe leaves

No	Metabolites	Color Deposition	Results
1.	Alkaloid	Orange red/ Yellowish white/ Yellowish	Positive
2.	Flavonoid	Greenish yellow	Negative
3.	Triterpenoid	Brownish Ring	Positive
4.	Saponin	Clear	Negative
5.	Tannin	Cloudy	Negative

Table 2. IC₅₀ of N-Hexane extract of mistletoe cherry leaves

Sample	Linear Line Equations	IC ₅₀
Cherry mistletoe leaves	$y = -0.2543x + 91.305$	162 mg/L
Allopurinol	$y = 0.0527x + 48.525$	27.98 mg/L

From the results of the phytochemical screening that have been carried out, cherry mistletoe leaves contain alkaloids indicated by positive results in the Dragendroff test with orange-red sediment and positive results in the Mayer test with yellowish-white sediment. However, the Wegner test showed negative results with a yellowish precipitate. Then, in the N-Hexane phytochemical test, cherry mistletoe leaves do not contain flavonoids, saponins and tannins as indicated by negative test results and brownish yellow sediment results in the flavonoid test, clear in the saponin test and cloudy in the tannin test.

Xanthine Oxidase Inhibition

N-Hexane extract of mistletoe cherry leaves at a dose of 40 mg/L, 20 mg/L, 10 mg/L, 5 mg/L, and 2.5 mg/L was able to inhibit the xanthine oxidase enzyme with the line equation $y = -0.2543x + 91.305$. Allopurinol, a positive control, at a dose of 600 mg/L, 450 mg/L, 300 mg/L, 150 mg/L, and 75 mg/L was able to impede the xanthine oxidase enzyme with the line equation $y = 0.0527x + 48.525$ (Table 2).

It was known that the N-Hexane activity of towards xanthine oxidase was the lowest is at 10 mg/L and highest at a concentration of 20 mg/L. It showed that minimum inhibitory is at concentrations of 75 mg/L and 300 mg/L, namely 46.15% and the maximum inhibitory activity found in allopurinol at concentrations of 150 and 600 mg/L, namely 84.62%.

Based on line equation on Table 2, IC₅₀ of N-Hexane extract of cherry mistletoe leaves against xanthine oxidase was 162 mg/L, while IC₅₀ of allopurinol against xanthine oxidase was 27.98 mg/L. To inhibit 50% of the xanthine oxidase enzyme, only 27.98 mg/L of allopurinol was needed, but 162 mg/L of N-Hexane extract from cherry mistletoe leaves was needed. Allopurinol is better at inhibiting the xanthine oxidase enzyme than N-Hexane extract of cherry mistletoe leaves.

4. Discussion

According to study conducted by Sinulingga, et al. in 2023, the results of research on cherry mistletoe leaves using ethyl acetate solution obtained positive results on alkaloids, flavonoids, tannins and terpenoids.⁸ Then, research conducted by Tioline et al., in 2021 using an infusion solvent showed that cherry mistletoe leaves contain alkaloids, flavonoids, tannins and terpenoids. This is different from the results of research carried out by the author which shows that the results of N-Hexane inhibition of cherry mistletoe leaves only contain alkaloids as indicated by positive results in the Dragendroff and

Mayer tests and positive results in the triterpenoid test. Differences in solvent polarity influence the types of secondary metabolites identified. N-Hexane is a non-polar solvent, so it attracts non-polar secondary metabolites such as alkaloids and triterpenoids.^{8,13}

Alkaloids are nitrogen-containing compounds that can be found in various plants and have various biological activities. A positive alkaloid test indicates that there are alkaloids in a substance or plant. The formation of a yellow or brownish-yellow precipitate, which usually occurs when the test substance reacts with certain reagents, is part of the interpretation of a positive alkaloid test.^{12,14}

Alkaloids are natural organic compounds that have varied mechanisms of action. In the context of neurodegenerative disorders, alkaloids have been found to help inhibit the progression of such disorders by reducing acetylcholinesterase (AChE) activity and through other pharmacological implications.¹⁵ In addition, alkaloids also have antibacterial activity by inhibiting the formation of bacterial nucleic acids and proteins, affecting cell membrane permeability, and disrupting bacterial metabolism, along with other mechanisms.¹⁶ The mechanism of action of alkaloids also involves calcium transport and inhibition of acetylcholinesterase, which occurs at nanomolar concentration levels. This variety of mechanisms contributes to the wide range of effects exhibited by alkaloids in different biological processes.¹⁶

The presence of terpenoids in a sample can cause positive results in certain tests, such as the Salkowski test. Terpenoids are a broad and diverse group of secondary metabolites produced by various plants, especially conifers. Identification of terpenoids is often done through chemical methods, including the Salkowski test. This test produces a reddish-brown color change when the test solution is mixed with concentrated sulfuric acid and chloroform, indicating the presence of terpenoids. Various studies have shown that terpenoids have various biological activities, such as anti-inflammatory and antioxidant properties. So, positive results on the terpenoid test can indicate the potential presence of these bioactive compounds in the sample examined.^{18,19}

The results of research carried out by Sinulingga, et al. in 2022 showed the results of the IC₅₀ extract with a value of 9.86 mg/dL using ethyl acetate solution. Then, research conducted by Tioline et al in 2021 using an infusion solution showed an IC₅₀ value of 81.27 mg/dL. Apart from that, study reported by Syarif et al. in 2020, The IC₅₀ of the ethanol extract of

cherry leaves is 34.197 mg/dL and is in the very active category as an inhibitor of the α -glucosidase enzyme.²⁰ The strength level of xanthine oxidase was tested at an IC₅₀ value with the sample being classified as very active if the value was <50 mg/L, active if the value was in the range of 50-100 mg/L, medium if the value was in the range of 101-250 mg/L and very weak if the value is in the range of 250-500 mg/L. From the two studies carried out, when using ethyl acetate solution, the extract inhibition results were very active because they were below the value range of <50 mg/L. Meanwhile, if you use an infusion solution, the active extract will be inhibited because it is in the range of 50-100 mg/L.²¹

In contrast to study conducted by the author using N-Hexane solvent, the results obtained were that the N-Hexane extract of cherry mistletoe leaves was moderate against xanthine oxidase. Meanwhile, in the research, the inhibitory effect of allopurinol was 27,98 mg/L, which means that the allopurinol inhibition test was very active against xanthine oxidase. This difference can occur due to differences in the solvent used. Previous research used polar or semipolar solvents. Secondary metabolites attracted by polar solvents such as flavonoids, saponins and tannins have a better potentiality to impede the xanthine oxidase enzyme. Meanwhile, secondary metabolites attracted by nonpolar solvents such as alkaloids and triterpenoids have a low potentiality to impede the xanthine oxidase enzyme.¹⁸⁻²¹ Thenmozhi et al.'s research revealed that flavonoids had a greater potentiality to impede the xanthine oxidase enzyme than alkaloids did. The capacity of secondary metabolites to impede the xanthine oxidase enzyme may be influenced by high quercetin levels in flavonoids.²²

In cherry mistletoe, identified Quercetin is a structured flavonoid compound like allopurinol and xanthine. Quercetin, allopurinol, and xanthine have hydroxyl groups as acceptors enzyme as well as two aromatic rings. Quercetin and xanthine oxidase bonds can occur due to van der Waals interactions, specific hydrogen bond interactions of the exocyclic quercetin hydroxyl groups (C-3, C-4, and C-5) with enzyme residues Glu 802 and Arg 880, hydrophobic residues from the site enzyme activity, and the existence of steric complementarity between the conjugated tricyclic structures of quercetin due to the aromaticity of the ring. Interaction hydrogen bonds with Mo-OH, Glu 802, Thr 1010, and Arg 880 controls the overall orientation of quercetin, especially the interaction of Thr 1010 and Arg 880 with the 7-OH group of quercetin. In addition to the 3-OH group, the 5-OH group and 7-OH group also has an impact on the bond between quercetin and xanthine oxidase enzyme. The hydrogen bond interaction between the Mo-OH group and the 5-OH group helps position quercetin but does not have a significant effect on the bond closure. The present crystal structure shows that the 3-OH and C-

4 carbonyl groups of quercetin are involved in hydrogen bonding interactions with the carboxylate groups of the chain addition to Glu 802 enzyme, despite several kinetic studies concluded that 3 -OH groups do not give significant contribution to binding affinity.^{8,21}

5. Conclusion

N-Hexane extract of cherry mistletoe leaves contains alkaloids and triterpenoids and has the effect of inhibiting xanthine oxidase in the medium category.

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