ANTIHYPERURESEMIA ACTIVITY OF ETHANOL EXTRACTS AND FRACTIONS OF NIPAH LEAF FRONDS (*NYPA FRUTICANS*. WURMB)

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ABSTRACT

Hyperuresemia is a metabolite syndrome characterised by high levels of uric acid in the blood. Hyperuresmia in advanced stages can cause gout or pyrexia (a disease that affects the joints and causes joint damage), kidney stones, kidney damage, and hypertension. The nipah plant (Nypa fruticans. Wurmb) is traditionally used in the treatment of stomach pain, diabetes, fever, toothache, uric acid reduction and headache medicine. In general, the content of secondary metabolite compounds from nipah plants includes flavonoids, phenols, tannins, terpenois, steroids, alkaloids and saponins. The purpose of this study was to determine the xanthine oxidase inhibitory activity of ethanol extracts and fractions of nipah leaves (Nypa fruticans. Wurmb). Extraction process using maceration method with 96% ethanol solvent, fractionation using liquid-liquid partition method with separating funnel. . Testing the inhibition of xanthine oxidase enzyme activity in vitro using UV/VIS spectrophotometer. The results showed that ethanol extract, water fraction, ethyl acetate fraction and n-hexane fraction showed inhibitory activity of xanthine oxidase enzyme with IC_{50} values of 47.558 ± 2.862; 18.755 ± 3.821; 3.543 ± 1.440 and 110.213 ± 1.577 ppm, respectively. From the results of the study it can be concluded that ethanol extracts and fractions of nipah leaf fronds (Nypa fruticans.Wurmb) can inhibit the activity of xanthine oxidase enzyme with ethyl acetate fraction giving the highest inhibitory activity with IC_{50} value of 3,543±1,440 ppm.

KEYWORDS Nypa fruticans.Wurmb; Xanthine oxidase; Antihyperuresaemia; Fractions.

INTRODUCTION

Hyperuresemia is a metabolite syndrome characterized by high levels of uric acid in the blood (more than 7.0 mg/dL in men and more than 6.0 mg/dL in women) (Juwita *et*

al., 2017). Uric acid is produced from the process of purine catabolism assisted by the enzymes guanase and xanthine oxidase. The enzyme xanthine oxidase plays a role in catalyzing the oxidation process of hypoxanthine into xanthine which will then be converted into uric acid. Under normal circumstances, uric acid is carried to the kidneys by the bloodstream and excreted from the body through feces and urine, but for some reason the kidneys are unable to remove existing uric acid, resulting in an increase in uric acid levels in the blood, this condition is called hyperuricemia (Ghanbari *et al*., 2022). In the advanced stages, hyperuricemia causes joint damage (gout) and arthritis. Clinical manifestations of urate deposition include gouty arthritis, crystal accumulation in bone-damaging tissues (tofus), urate stones, and gouty nephropathy (Tripolino *et al*., 2021). The prevalence of hyperuricemia in Indonesia is estimated between 2.3-17.6% while the prevalence of gout varies between 1.6-13.6 per thousand population (Badri *et al*., 2020).

Inhibition of xanthine oxidase enzyme activity is one of the therapeutic approaches in the treatment of hyperuresemia. By inhibiting the activity of the xanthine oxidase enzyme, it can prevent the oxidation of hypoxanthine to uric acid (Liu *et al*., 2021). Allopurinol is an antihyperuresemia drug that works by inhibiting the activity of the xanthine oxidase enzyme. The mechanism of alopurinol is to inhibit the synthesis of purines which are xanthine precursors. However, long-term consumption of allopurinol causes bone marrow depression, Stevens Johnson, liver damage and kidney toxicity (Djakad, 2020). Therefore, it is important to find alternatives to allopurinol as a xanthine oxidase inhibitor, one of which is by administering antioxidants. Flavonoids as strong antioxidants can inhibit the activity of the xanthine oxidase enzyme by binding to the active side of the enzyme so that xanthine metabolism into uric acid can be inhibited (Zhao *et al*., 2020). Nipah (*Nypa fruticans*. Wurmb) is one type of mangrove plant with high flavonoid content.

The nipah plant (*Nypa fruticans*. Wurmb) is one of the endemic mangrove plants in Indonesia. Nipah plants (*Nypa fruticans*. Wurmb) are traditionally used in the treatment of stomach pain, diabetes, fever, toothache, uric acid reduction and headache medicine (Khairi et al., 2020). In general, the content of secondary metabolite compounds from nipah plants includes flavonoids, phenols, tannins, terpenois, steroids, alkaloids and saponins. In addition, nipah plants also contain protocatechuic acid compounds, catechins, chlorogenic acid, epicatechin, kaemferol, and pengsianancin (Astuti et al., 2020). Phenolic compounds identified in nipah fruit include chlorogenic acid, protocatechuic acid, kaempferol, gallic acid, hydroxybenzoic acid, rutin, quercetin, cinnamic acid, and anthocyanins (Prasad *et al*., 2013). Moonrungsee *et al*., 2022 in their research reported that the administration of ethanol extract and water extract of nipah fruit can inhibit xanthine oxidase enzyme activity (Moonrungsee *et al*., 2022). In addition, giving syrup from ethanol extract of nipah leaves can reduce uric acid levels from hyperuresemia rats (Juliana, 2022).

Based on the above explanation, it encourages researchers to further examine the potential of ethanol extracts and fractions of nipah leaf fronds as antihyperuresemia. This study includes phytochemical screening, total flavonoids and antihyperuresemia test. Antihyperuresemia test was conducted in-vitro using the method of inhibiting xanthine oxidase enzyme activity with allopurinol as a positive control. Data were obtained by measuring the total flavonoid content of the extract as well as the IC_{50} value of the ethanol extract and fraction of nipah leaf midrib in inhibiting xanthine oxidase enzyme activity.

RESEARCH METHOD

This in vitro laboratory experimental study tested the antihyperuresemia activity by the method of inhibiting the xanthine oxidase enzyme activity of the ethanol extract and fraction of nipah leaf fronds (*Nypa fruticans*. Wurmb).

(A) Tools and materials. UV/VIS spectrophotometer (genesys 10s), maceration vessel, rotary evaporator (DLab 100 RE), separatory funnel (pyrex), glassware, nipah leaf fronds powder, 96% ethanol, distilled water, ethyl acetate, hexane, phosphate buffer pH 7.5 0.05M, HCl 1N, xanthine substrate (Sigma), xanthine oxidase enzyme (5 units, Sigma), PBS pH 7.5 0.05 M (Sigma), DMSO 1%.

(B) Extraction. A total of 300 grams of nipah leaf fronds powder was extracted by maceration using 96% ethanol solvent for 3 x @ 24 hours at room temperature with occasional stirring. The macerate was concentrated with a rotary evaporator at a temperature of 40 – 60^{O}C and thickened over a water bath.

(C) Fractionation. Fractionation was carried out by liquid-liquid extraction method as much as 20 g of extract dissolved with distilled water as much as 150 mL, then put in a separating funnel. Fractionation was carried out sequentially using n-hexane and ethyl acetate solvents as much as 150 mL with 3 replications. The fractions obtained were then concentrated with a rotary evaporator and concentrated on a water bath.

(D) Phytochemical Screening. The phytochemical screening procedure was modified from Sithara *et al*., 2016. Phytochemical screening of ethanol extract of nipah leaf midrib was carried out on phenolic compounds, alkaloids, flavonoids, terpenoids, saponins and tannins.

(E) Xanthine oxidase enzyme inhibitory activity. The inhibitory activity of xanthine oxidase enzyme was evaluated using UV-Vis spectrophotometer, the measured uric acid resulted from the reaction of xanthine substrate and xanthine oxidase enzyme. This method was modified from Owen and Havlik et al., 2011, as a comparison allopurinol was used. A total of 1 mL of sample solution added 2.9 mL of PBS pH 7.5 0.05 M, then added 2 mL of xanthine substrate solution 0.15 mM, preincubated at 25^{O}C for 15 minutes, then added 0.1 mL of xanthine oxidase enzyme solution (0.1 units/mL) and incubated at 25^{O}C for 30 minutes and added 1 mL of 1 N HCl to stop the reaction. The absorbance was measured at a wavelength of 290 nm. The xanthine oxidase enzyme inhibitory activity was expressed as percent xanthine oxidase enzyme inhibition, calculated by the following equation:

$$\%\ Inhibition = \frac{abs\ control - abs\ sampel}{abs\ sampel} x\ 100$$

The IC_{50} value was calculated using the linear regression equation formula between concentration versus % inhibition. Inhibition activity is expressed as Inhibition Concentration 50% (IC_{50}), which is the concentration of the sample that can inhibit the work of the enzyme canthine oxidase by 50%. The IC_{50} value is obtained from the x value after replacing y = 50 (Raharjo & Pambudi, 2021).

Table 1. Assay procedure of xanthine oxidase activity inhibition

Sample	Volum (mL)			
	Sample	Control Samle	Blanko	Control blanko
Etanol extract (300, 200, 100, 50, dan 5 ppm)	1	1	-	-
Fraction (300, 200, 100, 50, dan 5 ppm)	1	1	-	-
Allopurinol (0,625, 1,25, 2,5, 5, dan 10 ppm)	1	1	-	-
PBS pH 7,5 0,05 M	2,9	2,9	3,9	3,9
Substrat xantin 0,15 mM	2	2	2	2
Incubate for 15 minutes at 25^{O}C				
Xanthine oxidase solution	0,1	-	0,1	-
PBS pH 7,5 0.05M	-	0,1	-	0,1
Incubate for 30 minutes at 25^{O}C				

HCl 1N	1	1	1	1

RESULT AND DISCUSSION

The utilization of natural materials and herbal products in medicine has become an alternative by people in developing countries including Indonesia. About 60% of the world's population uses herbal medicine and 80% of the population in developing countries make herbs a primary health need (WHO, 2013). Nipah (*Nypa fruticans*. Wurmb) is a palm-like mangrove plant that belongs to the Arecaceae family which is spread on the coast throughout Indonesia.

In this study the samples used were nipah leaf fronds (*Nypa fruticans*. Wurmb) obtained from Jatimalang Beach, Purworejo Regency, Central Java. The determination process was carried out at the Ahmad Dahlan University Biology Learning Laboratory from the results of determination confirmed the sample used *Nypa fruticans*. Wurmb. The extraction process was carried out by maceration. The selection of maceration method is based on maceration is a simple method and suitable for compounds that are not heat resistant. While the solvent used is 96% ethanol which is a universal solvent. From the extraction results, the extract yield was 9.8%.

Phytochemical screening is one way to determine the content of secondary metabolites in a sample (Harborne, 1987). This analysis is very useful to determine the main class of active compounds from ethanol extracts and fractions of nipah leaves. Phytochemical screening in this study includes tests of alkaloids, flavonoids, phenolics, saponins, triterpenoids and steroids. From the results of phytochemical screening that has been done, the ethanol extract of nipah leaf fronds contains compounds of terpenoids, steroids, flavonoids, phenolics, and saponins. The water fraction contains compounds of terpenoids, steroids, flavonoids, phenolics and saponins. The ethyl acetate fraction contains compounds of flavonoids, phenolics and saponins. While the n-hexane fraction only contains steroid and flavonoid compounds. The results of phytochemical screening of extracts and fractions of nipah leaves can be seen in table 2.

Antihyperuresemia activity testing is based on the inhibition of xanthine oxidase enzyme activity in the substrate xanthine to uric acid. The principle of this test is to measure the uptake of uric acid as the end product of the catalysis reaction between xanthine oxidase and xanthine. The assay is performed using spectrophotemetry at the maximum wavelength. Determination of the maximum wavelength was carried out before testing with the aim of determining the optimum conditions, from the optimization obtained a maximum wavelength of 290 nm. This wavelength is the maximum wavelength of uric acid produced from the catalysis reaction between xanthine oxidase and xanthine subtrate xanthine. Tests were carried out on blank solution, blank control, sample and sempel control. Samples consisted of ethanol extract, nipah leaf fraction and allopurinol. The use of allopurinol as a comparator is because allopurinol is a competitive inhibitor of the enzyme xanthine oxidase in converting xanthine into uric acid. The similar chemical structure of allopurinol with xanthine causes competition with xanthine in binding the active side of the enzyme (Xue *et al*., 2023). The blank and blank control tests were conducted to determine the enzyme activity in converting xanthine substrate into uric acid without any addition. Tests on allopurinol and samples were carried out to determine how much inhibition ability against xanthine oxidase enzyme activity was given. Testing on sample control and blank control with the aim as a correction factor if the sample gives absorbance.

Table 2 Phytochemical screening assay of extracts and fractions of *Nypa fruticans*. Wurmb

Secondary metabolite	Hasil				Result
	Ekstrak	Water	Etil asetat	n-Heksane	
Alkaloid					
a. Mayer	-	-	-	-	-
b. Dragendorf	-	-	-	-	-
c. Wagner	-	-	-	-	-
Steroid	+	+	-	+	Dark green
Terpenoid	+	+	-	+	Brown
Flavonoid	+	+	+	-	Orange
Fenolik	+	+	+	-	Dark green
Saponin	+	+	+	-	Foam
Tanin	+	+	+	-	Dark

Note : + : Detected, - : Not Detected

The results of testing the inhibition of xanthine oxidase enzyme activity against allopurinol as a comparison standard showed that alopurinol had an inhibitory effect on xanthine oxidase activity with an IC50 value of 1.28 ± 1.12 ppm. Allopurinol acts as a competitive inhibitor that has a structure resembling xanthine substrate. Allopurinol is also known to have tens of times stronger affinity for the xanthine oxidase enzyme than the xanthine substrate so that if in the environment there is allopurinol together with xanthine, then allopurinol will react more with the xanthine oxidase enzyme to form a product (oxypurinol) than with its own substrate so that the activity of the xanthine oxidase enzyme will decrease and less uric acid is formed (Raharjo, 2022). The results of measuring the inhibition of xanthine oxidase enzyme activity in nipah leaf midrib samples showed the following results. Ethanol extract obtained IC_{50} value of 47,558 ± 2,862 ppm, water fraction obtained IC_{50} value of 18,755 ± 3,821 ppm, ethyl acetate fraction obtained IC_{50} value of 3,543 ± 1,440 ppm and n-hexane fraction obtained IC_{50} value of 110,213 ± 1,577 ppm. The measurement results of xanthine oxidase enzyme inhibitory activity can be seen in table 3.

Based on the table above, the ethyl acetate fraction has the highest activity with an IC_{50} value of 3,543 ± 1,440 ppm, this is related to the presence of phenolic compounds and flavonoids. Polyphenolic compounds, especially tannins, will react with proteins resulting in the formation of tannin-protein complexes that reduce the activity of xanthine oxidase catalysts (Widuri *et al*., 2016), while the inhibitory activity by flavonoids is due to their structural framework geometric positions that are almost similar to xanthine and the presence of hydroxyl groups at positions C5 and C7 which allow them to be oxidized by xanthine oxidase (Malik *et al*., 2018).

Table 3. Measurement results of xanthine oxidase enzyme inhibitory activity

Sample	IC_{50} result (ppm)
Allopurinol	1,28±1,12
Ekstrak Etanol	47,558±2,862
Fraksi Etanol	18,755±3,821
Fraksi Etil asetat	3,543±1,440
Fraksi Heksane	110,213 ± 1,577

CONCLUSION

Based on the research that has been done, it can be concluded that the ethanol extract of nipah leaf midrib (*Nypa fruticans*.Wurmb) contains bioactive compounds of steroids, terpenoids, phenolic flavonoids, saponins and tannins. In addition, the ethyl acetate fraction showed the highest xanthine oxidase enzyme inhibitory activity. The content of bioactive compounds that dominantly affect the inhibitory activity of xanthine oxidase enzyme are

flavonoids, phenolics and tannins.

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