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Original Article

Xanthine Oxidase Inhibitory Potential of *Ficus nekbudu* Warb.: A Phytochemical and Bioactivity Study

Feronia Reni Cyrena Santoso[1]*, Tagor M. Siregar[2], Harti Fornita Zendrato[1]
*Corresponding author: *feronia.santoso@uph.edu*
[1]Department of Pharmacy, Faculty of Health Science, Pelita Harapan University, Banten, 15811, Indonesia
[2]Faculty of Science and Technology, Pelita Harapan University, Banten, 15811, Indonesia

Abstract
Hyperuricemia resulting from excessive xanthine oxidase (XO) activity can lead to gout arthritis. Synthetic compounds such as allopurinol are effective but associated with adverse effects, driving interest in plant-derived alternatives. *Ficus nekbudu* Warb. (*Moraceae*) is an underexplored species in a genus with documented pharmacological potential; however, its XO inhibitory activity has not previously been investigated. This research aimed to evaluate the XO inhibitory activity of *Ficus nekbudu* Warb. leaf extracts. Methods: Leaves were extracted sequentially by reflux using n-hexane, ethyl acetate, and 96% ethanol. Total phenolic content (TPC) and total flavonoid content (TFC) were determined then XO inhibitory activity was measured spectrophotometrically at 290 nm using allopurinol as a positive control, and Pearson correlation analyses were performed. The 96% ethanol extract yields the highest TPC (89.20 ± 3.49 mg GAE/g), while the ethyl acetate extract contains the greatest TFC (137.04 ± 7.19 mg QE/g). All three extracts exhibit XO inhibition, with the n-hexane extract showing the strongest activity (IC_{50} = 20.58 ± 6.51 µg/mL), followed by ethyl acetate (24.05 ± 5.38 µg/mL) and ethanol (26.30 ± 0.70 µg/mL), compared with allopurinol (3.57 ± 0.06 µg/mL). *Ficus nekbudu* Warb. leaf extracts demonstrated xanthine oxidase inhibitory activity across different polarity fractions. These findings suggest that phenolic and flavonoid compounds may contribute to the observed inhibitory activity, although further studies are required to clarify the specific active compounds and inhibition mechanism. Overall, Ficus nekbudu Warb. shows potential as a natural candidate for hyperuricemia management.

Keywords: *Ficus necbudu*, flavonoids, phenolic, uric acid, xanthine oxidase.

INTRODUCTION

Ficus is a genus of plants from the *Moraceae* family which are generally distributed in tropical or subtropical climates countries, including Indonesia. Common ficus species include the fig tree (*Ficus carica* L.) and *Ficus benjamina,* known in Indonesia as banyan. Several other species of the ficus genus, such as *Ficus deltoidea* J., or tabat barito, which can be found in the forests of Kalimantan, *Ficus sycomorus*, *Ficus Lutea*, and *Ficus saligna* are commonly known and have been researched for their potential. The Ficus genus includes various species reported to have antioxidant capacity, including *Ficus carica* L., with an IC_{50} of 659.97 ± 0.92 mg/mL, and antimicrobial activity against bacteria *Bacillus cereus* and *Staphylococcus aureus* (Mahmoudi et al., 2016). Other research also reported that the fruit of this species could inhibit xanthine oxidase, with an IC_{50} of 27.5 µg/mL (Irum et al., 2023). *Ficus deltoidea* J. has antioxidant capacity (IC50 34.473 ± 0.037 ppm) (Manurung et al., 2017). Other research states that one species of the Ficus genus, namely *Ficus saligna*, has anti-inflammatory properties, namely inhibition of 15-LOX (IC_{50} = 40 ± 3 µg/mL) and

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strong inhibition of xanthine oxidase (IC_{50} = 46.8 ± 1.5 µg/mL) (Lawal et al., 2019). Extracts from *Ficus lutea* were reported to have the ability to inhibit α-glucosidase at a concentration of 0.5 mg/mol (64.3 ± 3.6%) (Olaokun et al., 2013). Another study stated that *Ficus nota* diffraction of ethyl acetate and chloroform extract into several fractions and obtained the highest xanthine oxidase inhibition value, namely 348.4 µg/mL (Mancia et al., 2019).

The data above show that the Ficus genus has potential antioxidant, antimicrobial, antidiabetic, and anti-inflammatory activities. One of the capabilities of the inflammation-causing enzymes mentioned above is the inhibition of xanthine oxidase (XO), which can reduce inflammation. This inflammation is better known as gout or hyperuricemia. An inflammation of the joints caused by a continuous increase in the amount of uric acid in the body can lead to the formation of monosodium urate crystals, which deposit around the joints and result in arthritis (Kuo et al., 2015). Flavonoids, as secondary metabolites of plants, have been reported to have the ability to inhibit xanthine oxidase. Research by Lin et al in 2015, chrysin, a bioactive flavonoid compound, has XO inhibitory activity with IC_{50} (1.26 ± 0.04) × 10-6 mol L^{-1}. Another reported flavonoid bioactive compound is luteolin, with a Ki value of (2.38 ± 0.05) × 10^-6 mol L^{-1} (Yan et al., 2013), while other research reports an IC_{50} of 0.16 µM (Mohos et al., 2020). Several ficus species that have high flavonoid content include *Ficus carica* L with a total flavonoid of 16.211 ± 0.156 mg QE/g DE (Mahmoudi et al., 2016), supported by other research with a total flavonoid of 858.43 ± 10.76 mg QE/ 100 g DW (Amessis-Ouchemoukh et al., 2017). Another study reported total flavonoids from *Ficus deltoidea* J. (369.05 ± 0.052 µg CE/mg extract) (Manurung et al., 2017).

The high amount of flavonoids in two ficus species, namely *Ficus carica* L. and *Ficus deltoidea* J., as well as some previous data that mentions the activity of the ficus genus, can indicate that there is a possibility that another ficus species, namely *Ficus necbudu* Warb., has the potential for large amounts of flavonoids and has the ability to inhibition of xanthine oxidase as in Ficus saligna which has been reported previously (Lawal et al., 2019). There are very few studies on the *Ficus necbudu* Warb species, and none on total flavonoids or xanthine oxidase inhibition. Therefore, the ability of the flavonoid compounds possessed by the *Ficus necbudu* Warb. A plant with xanthine oxidase inhibitory activity can be identified by testing its inhibitory activity against xanthine oxidase. Based on the description above, the potential of the *Ficus necbudu* Warb. species to contain flavonoids as one of the potentially very large secondary metabolites, in line with this, its ability to inhibit xanthine oxidase activity also has great potential. Therefore, this study aims to determine the total flavonoid content of *Ficus necbudu* Warb. and to evaluate its inhibitory activity against xanthine oxidase, thereby addressing the existing research gap and assessing its potential effect.

METHODS

Materials

Equipment used included a reflux apparatus (Iwaki, Indonesia), moisture balance (Ohaus, USA), analytical balance (Ohaus, USA), rotary evaporator with Rotachill and vacuum pumping unit (Hei-VAP Value, Germany), water bath (Memmert, Germany), oven (Memmert UF

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55, Memmert, Germany), micropipette (Thermo Fisher Scientific, USA), and UV-Vis spectrophotometer (Shimadzu UV-1800, Japan).

Chemicals and reagents included simplicia powder and leaf extracts of *Ficus nekbudu* Warb. n-hexane, ethyl acetate, and ethanol 96% (Brataco, Indonesia); chloroform-saturated water, toluene, glacial acetic acid, magnesium powder, hydrochloric acid, ferric chloride ($FeCl_3$), chloroform, acetic anhydride, concentrated H_2SO_4, methanol, Na_2CO_3, aluminum chloride ($AlCl_3$), and sodium hydroxide (NaOH) (Merck, Germany); Dragendorff reagent, Folin–Ciocalteu reagent, and gallic acid (Merck, Darmstadt, Germany); quercetin 99% and sodium acetate (Merck, Darmstadt, Germany); allopurinol, xanthine oxidase (0.1 unit/mL), xanthine substrate (0.15 mM), and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA); phosphate buffer (0.05 M, pH 7.5) and HCl 1 N (prepared in-house); and CO_2-free distilled water and distilled water (prepared in-house).

Standardization of simplicia

Water content was analyzed by weighing the simplicia on a moisture balance device with a precision of 1 g. The temperature is set to 105°C, and the device is operated (Ningtyas & Erwiyani, 2022). Water and ethanol soluble content *were analyzed by weighing f*ive grams of simplicia and placed into a container, followed by the addition of 100 mL of chloroform-saturated water. The mixture is shaken for 6 hours, then left to stand for 18 hours. Filtration is performed, and 20 mL of the filtrate is transferred to a porcelain cup and evaporated at 105°C. The sample is weighed until a constant weight is achieved, and the percentage of water-soluble content is calculated. For the ethanol-soluble content, five grams of simplicia are weighed and placed into a container (bottle) with 100 mL of ethanol added. The mixture is shaken for 6 hours, then left to stand for 18 hours. Filtration is performed, and 20 mL of the filtrate is transferred to a porcelain cup and evaporated at 105°C. The sample is weighed until a constant weight, and the percentage of ethanol-soluble content is calculated (Kesehatan, 2017).

Simplicia extraction

Extraction was carried out using a multistage method with three solvents of different polarities: n-hexane, ethyl acetate, and 96% ethanol. A total of 50 grams of simplicia were weighed and put into the extraction flask, and 100 mL of n-hexane solvent was added. The flask is paired with a condenser and heated on a hot plate to 60 °C. Extraction was carried out 3 hours after the first drop (Kurniawati et al., 2022). The extract was filtered through filter paper, and the filtrate was evaporated in a rotary evaporator at 40°C until a dry extract was obtained. The residue obtained from filtering the n-hexane extract was used for extraction with ethyl acetate. The extraction procedure was carried out in the same way as the previous n-hexane extraction. The ethyl acetate extract was filtered, and the filtrate was evaporated using the same method as before. The residue obtained after filtering the ethyl acetate extract was then reused for extraction with 96% ethanol; the procedure was the same as before. The 96% ethanol extraction results were filtered and evaporated as before.

Characterization of the extract

Characterization was evaluated by thin-layer chromatography (TLC) and

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phytochemical screening. In the TLC test, the mobile phase was prepared in 10 mL of toluene: ethyl acetate: glacial acetic acid (7:2:0.5, Akhtar et al., 2022). The mobile phase is inserted into the TLC chamber and saturated. TLC silica gel is given a distance of 2 cm from the top and bottom edges, and the bottom mark is smeared with the extract dissolved in each solvent. Place the silica gel in the chamber and observe until the mobile phase reaches the upper limit mark. The plate is removed and dried. Observations were carried out under UV light at wavelengths of 254 nm and 366 nm (Kesehatan, 2017)

Phytochemical screening was analyzed using various methods, for alkaloids the sample is dissolved in ethanol until fully dissolved, then filtered. The filtrate is divided into three tubes, to which Mayer's, Dragendorff's, and Bouchardat's reagents are added. A positive result is indicated by the formation of a white precipitate with Mayer's reagent, an orange-brown precipitate with Dragendorff's reagent, and a brown to black precipitate with Bouchardat's reagent. The presence of alkaloids is confirmed if two out of three reactions are positive (Astarina et al., 2013). For flavonoids, a 0.5 mL sample is dissolved in ethanol, followed by the addition of 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M sodium acetate. A yellow color indicates a positive result for flavonoids (Sembiring et al., 2018). Phenolic compound was analyzed by taking 1 milliliter of the sample, and dissolved in methanol, followed by the addition of 5 mL of 10% Folin-Ciocalteu reagent and 4 mL of 1% NaOH. The color change from blue to blackish blue indicates the presence of phenols in the sample (Bayani, 2021). Saponin test was performed by preparing 1 gram of the sample with 100 mL of hot water, heated to boiling for 15 minutes, and filtered. The filtrate (solution A) is used to test for saponins, tannins, and quinones. Ten milliliters of solution A are placed in a test tube, and the tube is vigorously shaken vertically for 10 seconds until a foam 1 to 10 cm high forms. If the foam does not disappear after the addition of 2 N HCl, the sample is considered positive for saponins. Tannin content was tested by placing five milliliters of solution A in a test tube, and gelatin was added. The formation of a white precipitate indicates the presence of tannins in the sample. Quinones was analyzed by placing 5 milliliters of solution A in a test tube, and one drop of 1 N NaOH was added. The formation of a red color indicates the presence of quinones in the sample (Farnsworth, 1966). Steroids, triterpenoids: The sample is dissolved in a solvent and evaporated until thickened. The residue is then dissolved in 0.5 mL of chloroform and mixed with 0.5 mL of anhydrous acetic acid. Two milliliters of concentrated sulfuric acid are dripped along the inside of the tube. The formation of a greenish-blue ring indicates the presence of steroids, while the formation of a brownish or violet ring indicates the presence of triterpenoids (Sahriawati et al., 2020).

Total phenolic content

A total of 1 mL of the standard solution (gallic acid) was added to a test tube, then 5 mL of Folin-Ciocalteu 10% was added, and let stand for 8 minutes. Add 4 mL of 1% NaOH, incubate for 1 hour. All procedures were carried out in the dark. Absorption was measured at 740 nm using a UV-Vis spectrophotometer. The absorption results are used to create a standard curve. The test solution was treated the same as the standard solution, without variation in concentration. The

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absorption obtained is then entered into the line equation derived from the standard solution to obtain the phenolic content expressed as mg Gallic Acid Equivalent/gram sample (mg GAE/g).

Total Flavonoids content

A total of 0.5 mL of each standard solution series was pipetted into a test tube, then 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added to each concentration series, and incubated for 30 minutes. Absorption was measured at 435 nm using a UV-Vis spectrophotometer. The test solution is treated the same as the standard solution, with no variation in concentration. The absorption obtained was then entered into the line equation obtained from measuring the standard solution, and the flavonoid content was expressed in mg Quercetin Equivalent/gram sample (mg QE/g).

Xanthine oxidase inhibitory activity

A total of 1 mL of the standard solution or extract from each concentration series was added to 2.9 mL of 0.05 M phosphate buffer solution (pH 7.5), 2 mL of 0.15 mM xanthine substrate, and 0.1 mL of 0.1 unit/mL xanthine oxidase. The mixture is then incubated at room temperature for 30 minutes. After incubation, 1 mL of 1 N HCl is added to terminate the reaction. Absorbance is measured at a wavelength of 290 nm. A similar procedure is applied to control samples and standard controls, except that the 0.1 unit/mL xanthine oxidase solution is omitted (Lestari et al., 2014). A blank test is performed under the same conditions, but without adding the test solution (allopurinol and extract). Additionally, a blank control is conducted without the test solution and without xanthine oxidase (0.1 unit/mL) (**Table 1**).

Statistical Analysis

Data were analyzed using one-way ANOVA, presented as mean ± standard deviation (Olaokun et al., 2013) and the Pearson correlation analysis is presented with correlation coefficient values (Gogtay & Thatte, 2017)

Table 1. Test procedure for inhibition of xanthine oxidase activity.

Material	Volume (mL)					
	Blank	Blank Control	Allopurinol	Allopurinol Control	Sample	Sample Control
Extract	-	-	-	-	1.0	1.0
Allopurinol	-	-	1.0	1.0	-	-
Phosphate buffer	3.9	4.0	2.9	3.0	2.9	3.0
Xanthine substrate	2.0	2.0	2.0	2.0	2.0	2.0
XO	0.1	-	0.1	-	0.1	-
Incubated at 25 °C for 30 minutes						
HCl	1.0	1.0	1.0	1.0	1.0	1.0
Absorbance is measured at the maximum wavelength (290 nm)						

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RESULTS

Standardization of simplicia

Based on the results of simplicia standardization (**Table 2**), the water content of the *Ficus necbudu* Warb. Simplicia showed a result of 3.36%. The results of the soluble content test showed that simplicia was more soluble in water, as indicated by the water-soluble essence content of 7.44%.

Table 2. Standardization of simplicia.

Test	Percentage (%)
Water content	3.36
Water soluble content	7.44
Ethanol soluble content	6.17

Simplicia extraction

Based on the type of solvent and each repetition, the extract yield with the largest percentage was 96% ethanol extract (4.45%), followed by n-hexane, and finally, the smallest yield was ethyl acetate. (**Table 3**). All extracts have the same organoleptic with blackish green, dry, and specific odour (**Figure 1**).

Table 3. Extraction yield

Name of Sample	Extract (g)	Yield (%)
N-hexane Extract	0.60	0.60
Ethyl acetate Extract	0.36	0.36
Ethanol 96% Extract	3.02	3.01

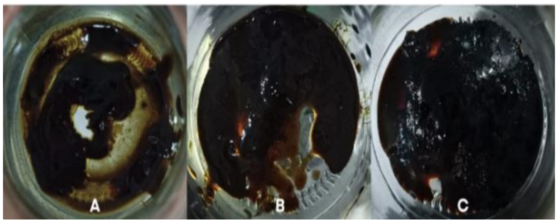

Figure 1. *Ficus necbudu* Warb. extract (A) N-Hexane extract, (B) Ethyl acetate extract, (C) Ethanol 96% extract.

Characterization of the extract

Qualitative identification of flavonoids was performed based on color separation using the TLC method, assisted by the appearance of citroborate spots. Another spotting agent, NH_3, was used, allowing for more specific identification of flavonoid groups. Based on the results obtained in **Figure 2**, isoflavones without free 5-OH and flavonoids of the anthocyanidin 3,5-diglycoside type were identified in the extract. The phytochemical screening for both the simplicia and the extract in various solvents is provided in **Table 4**.

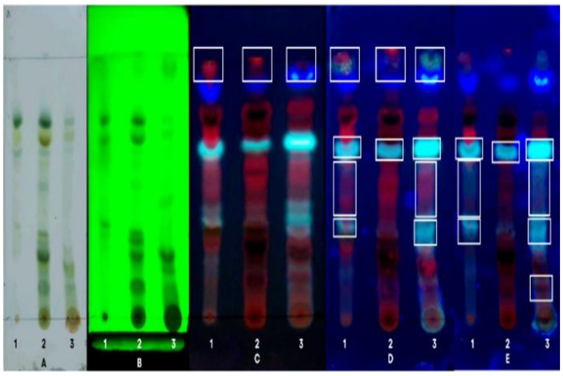
Figure 2. TLC result of (1) n-hexane extract, (2) ethyl acetate extract, (3) 96% ethanol extract; observed under (A) visible light, (B) UV 254 nm, (C) UV 366 nm, (D) UV 366 nm after citroborate spraying, and (E) UV 366 nm after NH_3 spraying.

Table 4. Phytochemical screening

	Simplicia	N-Hexane	Ethyl Acetate	Ethanol 96%
Alkaloids	+	+	-	-
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Tannin	+	-	-	+
Quinones	-	-	-	-
Saponins	+	-	-	+
Steroids	+	+	-	-
Triterpenoids	-	-	-	-

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Total phenolic content

Quantitative testing of phenolic compounds was performed using the Folin-Ciocalteu method, a colorimetric test that measures the absorbance of the complex formed during the reaction. Based on the results from the three extracts, the 96% ethanol extract had the highest total phenolic content at 89.20 mg GAE/g, followed by ethyl acetate (67.00 mg GAE/g) and n-hexane (32.12 mg GAE/g) (**Figure 3**).

Total Flavonoids content

The flavonoids test is based on colorimetric analysis, which relies on color changes for detection, with measurements performed at 435 nm. Based on the total flavonoids test, the results indicated that the ethyl acetate extract exhibited the highest flavonoid content compared to other solvents, as shown in **Figure 3**.

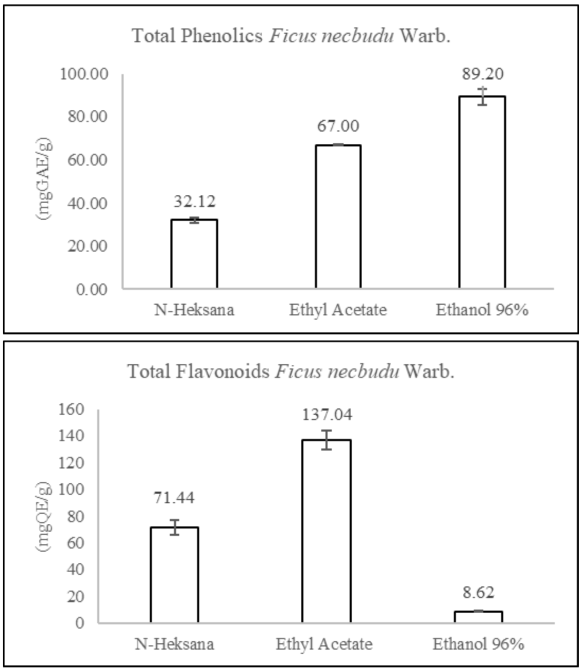

Figure 3. Total phenolic (top) and total flavonoid (bottom) of the extract.

Xanthine oxidase inhibitory activity

The principle of this test is to measure the amount of uric acid formed from the reaction catalyzed by xanthine oxidase in the presence of an inhibitory compound, in this case allopurinol, as the standard or positive control, and the extract as the test. The sample was measured at 290 nm (Lestari et al., 2014). Based on the tests performed as shown in **Table 5**, the three *Ficus necbudu Warb.* leaf extracts were classified as very strong, as their IC_{50} values were below 50 ppm.

Table 5. IC_{50} Value

Sample	IC_{50} (µg/mL)
Allopurinol	3.57 ± 0.06
N-hexane extract	20.58 ± 6.51
Ethyl acetate extract	24.05 ± 5.38
Ethanol 96% extract	26.30 ± 0.70

Statistical Analysis

The correlation between the phenolic value and the IC_{50} of the n-hexane extract (-0.738) was strong and negative (**Table 6**). The ethyl acetate extract showed a very strong positive correlation ($r = 0.982$) between phenolic content and IC_{50}. Meanwhile, the relationship between the phenolic content and IC_{50} value of the 96% ethanol extract was very weak and negative (-0.075). Correlation tests were also performed between flavonoid content and IC_{50} values for xanthine oxidase inhibition. The relationship between flavonoid content and IC_{50} in the n-hexane extract was moderate, with a positive correlation ($r = 0.663$). The ethyl acetate extract exhibited a weak positive correlation (0.488), whereas the 96% ethanol extract showed a strong positive correlation (0.736).

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Table 6. Correlation value

Extract	Correlation	Pearson Correlation
N-Hexane	Phenolic – IC_{50}	-0.738
	Flavonoid – IC_{50}	0.663
Ethyl acetate	Phenolic – IC_{50}	0.982
	Flavonoid – IC_{50}	0.488
Ethanol 96%	Phenolic – IC_{50}	-0.075
	Flavonoid – IC_{50}	0.736

DISCUSSION

Standardization of simplicia

Low water content improves simplicia quality by inhibiting microbial growth and reducing hydrolytic degradation of active compounds, thereby ensuring greater stability during long-term storage (Anggarani et al., 2019). The water content of 3.36% obtained for *Ficus nekbudu* Warb. simplicia meets the requirement of ≤ 10% stipulated in PerBPOM No. 32 of 2019 for traditional herbal materials. Water and ethanol are universal solvents; ethanol shows greater selectivity for several secondary metabolites and is more effective than water (Kumar et al., 2023). Ethanol can generally dissolve phytochemical compounds such as flavonoids, phenolics, terpenoids, and glycosides (Mróz et al., 2023; Teixeira et al., 2022). Thus, the properties of ethanol are more favorable than water, and the difference in essence content between the two is not too large, so the use of ethanol as a solvent is considered more profitable in the extraction of target compounds.

Simplicia extraction

The higher 96% ethanol shows that it can extract more compounds than the other two solvents. The amount of yield obtained is influenced by the choice of solvent with a polarity that is close to the compound to be separated (Zhang et al., 2018). The nature of 96% ethanol, which tends to be polar, shows that the extracted compound has a polarity that is close to the polarity of ethanol (Azzahra & Budiati, 2022). Apart from that, ethanol, which is also a universal solvent, makes nonpolar and semipolar compounds that have not previously been extracted in n-hexane and ethyl acetate solvents, extracted with ethanol (Kumar et al., 2023).

Characterization of the extract

In the TLC characterization, citroborate is a specific spotting agent used to identify flavonoids with ortho-dihydroxy groups. The extract may contain flavonoids that can be detected by the color change resulting from spraying with citroborate spots. The appearance of citroborate spots was observed to change to greenish blue under 366 nm UV light. Another spotting agent, NH_3, was used, allowing for more specific identification of flavonoid groups (Andersen & Markham, 2005).

Detection of the presence of alkaloids by phytochemical screening showed that the simplicia and n-hexane extracts were positive for alkaloids. The principle of alkaloid screening is a precipitation reaction caused by ligand replacement. Ligands are ions or atoms that have one or more free electrons that can share their excess electrons with the central ion or atom, where the bond formed between them is covalent. The nitrogen atom in the alkaloid, which has free electrons, will replace the iodine ion possessed by the Dragendroff and Mayer reagents. As a result of this ion replacement, a white precipitate will form after reacting with Mayer, and a brownish-orange precipitate if it reacts with Dragendroff (Astarina et al., 2013).

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Phenolic screening using the Folin-Ciocalteu method yields a blue color change when phenol is present. Compounds containing phenol will form a yellow color when reacted with Folin-Ciocalteu, and, in alkaline conditions (e.g., by adding 1% NaOH), a blue color will form (Bayani, 2021). It was found that all samples tested showed a color change from blue to blackish blue, indicating that simplicia and the three extracts contained phenolics. The results show that phenolics exhibit polarities ranging from nonpolar to polar, as evidenced by the positive results in the three solvents.

The reaction of flavonoids with aluminum chloride ($AlCl_3$) forms a yellow complex (Suwartini et al., 2021); adding sodium acetate maintains the previously formed yellow color (Sembiring et al., 2018). Based on the screening results, it was found that simplicia and the three extracts (n-hexane, ethyl acetate, and ethanol 96%) contained flavonoids, as evidenced by the yellow color change observed in all samples. This shows that there are various types of flavonoids, with flavonoids in the glycoside form having good solubility in ethanol solvents such as 96% ethanol, while flavonoids in the aglycone form are soluble in low-polarity solvents such as n-hexane (Nabavi et al., 2020).

Phytochemical screening for tannin testing was carried out using three treatments, including Stiasny's reagent. The addition of Stiasny's reagent aims to detect the presence of catechol tannins (Farnsworth, 1966). Stiasny's reagent contains formaldehyde and concentrated HCl. When tannin is reacted with this reagent, it reacts with formaldehyde in an acidic environment, with heating, to form a pink precipitate (Ogawa & Yazaki, 2018). Catechate tannins or hydrolyzed tannins are polymers of flavonoid compounds that are bound to carbon, so that tannins will be found more often in extracts with high polarity. This is because the hydroxyl groups that bond to the atoms in the tannin structure tend to be more attracted by solvents with high polarity, such as 96% ethanol (Hidayah, 2016).

Saponin was identified by a foam test and the addition of HCl; the foam results from the ability of the glycoside compounds in saponin to form foam in water (Agustina et al., 2017). The addition of HCl increases the polarity of the saponin, allowing it to form a micellar structure. The polar nature of saponin means that this compound is only attracted to solvents with close polarity, namely, 96% ethanol (Putri & Lubis, 2020). Therefore, based on the results, the simplicia and 96% ethanol extracts were found to contain saponins by the foam test.

Apart from alkaloids, phenolics, flavonoids, tannins, and saponins, positive results for steroid compounds were also obtained in samples of simplicia and n-hexane extract. The screening method is called the Lieberman Burchard method. The reagent used is a mixture of anhydrous acetic acid and concentrated sulfuric acid. The sample was dissolved with chloroform because of the close polarity of chloroform to steroid compounds, namely, nonpolar. The steroid compounds present in the sample are extracted with the help of the addition of anhydrous acetic acid. This acid will also ensure that the medium is free of water and that acetyl derivatives form, which are steroid compounds. Concentrated sulfuric acid is then dripped through the wall of the test tube, and with the addition of concentrated sulfuric acid, a

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brownish-blue color will form, which indicates that there are steroids in the sample. The qualitative detection of steroid compounds in the n-hexane extract reflects their nonpolar nature, as evident from their chemical structures. Steroids are dominated by long hydrocarbon chains, so their solubility in polar compounds is very poor (Sahriawati et al., 2020).

Total phenolic content

The Folin-Ciocalteu reagent can reduce phenol groups contained in the sample. A reduction and oxidation reaction occurs between the reagent and the phenol group; the phenol group will be oxidized by the reagent, while the reagent itself will be reduced during this process. The reaction between the phenol group and the reagent is optimal in alkaline conditions, so adding NaOH will create them. The formation of a complex compound from the reaction between the reagent and the phenol group will change the solution to a blue color, with the higher the concentration of phenol in the sample, the bluer the solution. The blue color formed will be measured by a spectrophotometer at the maximum wavelength (Bayani, 2021). The higher levels observed in ethanol compared to other solvents can be attributed to phenolic compounds having a polarity close to that of ethanol. The principle of like dissolves like states that if a compound dissolves in a solvent with the same or similar polarity, phenolic compounds will dissolve very easily in ethanol (Julianti et al., 2019).

Total Flavonoids content

Flavonoids, when combined with the $AlCl_3$ reagent, form a stable acid complex. This complex forms between $AlCl_3$ and the C-4 ketone group, the C-3 hydroxyl group, or the C-5 group. Additionally, the addition of $AlCl_3$ results in the formation of another stable acid complex with the ortho-hydroxyl group in flavonoids, specifically in ring A or B (Makuasa & Ningsih, 2020). Sodium acetate is added to detect the 7-OH group, which may be present in the sample, and to maintain the wavelength in the visible range (Suwartini et al., 2021). Ethyl acetate, a semipolar solvent, is highly effective for extracting compounds ranging from nonpolar to polar (Lawal et al., 2019). Flavonoids, with varying polarities from nonpolar to polar, are attracted by solvents such as ethyl acetate, which enhances their solubility in this solvent (Nabavi et al., 2020).

Xanthine oxidase inhibitory activity

During enzyme testing, environmental conditioning is one factor that supports enzymatic reactions. The addition of 0.05 M potassium phosphate buffer, pH 7.5, will provide appropriate conditions for enzyme activity. The use of pH 7.5 is based on optimization testing by Lestari (2014). This is in line with Bisswanger (2014), who states that the optimal pH range for most enzymes is 7.5 or 7-8. Xanthine is the substrate used in this assay. Xanthine is a purine compound that, if catalyzed by xanthine oxidase, will form uric acid. It is very important to monitor the substrate concentration because, at a certain concentration, the substrate will be saturated, leading to a decrease in the reaction rate. In addition, substrate concentration greatly influences the rate of the enzyme reaction (Bisswanger, 2014). In this test, the substrate concentration was 0.15 mM, based on optimization by Lestari (Lestari et al., 2014). Incubation was carried out for 30 minutes at 25°C; this time was determined based on previous research

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that optimized incubation time for the enzymatic reaction. Temperature also plays a role in creating good environmental conditions for enzymes to react. The use of temperature for most protocols again considers the conditions of the laboratory where the test is conducted.

The enzymatic reaction was stopped by adding HCl. Under ideal pH conditions, enzymes function optimally in producing products, in this case, uric acid. Changes in pH, particularly extreme pH changes, can affect the bond between the substrate and the enzyme. The active site of the enzyme bound to the substrate will change with alterations in pH (Vitolo, 2020). Allopurinol, a positive control or standard, has been proven to competitively inhibit xanthine oxidase, thus preventing the catalysis of uric acid formation (Sekine et al., 2023). The IC_{50} value was categorized into several strength levels based on the obtained concentrations. An $IC_{50} < 50$ ppm is classified as very strong, 50–100 ppm as strong, 100–150 ppm as moderate, 150–200 ppm as weak, and more than 200 ppm as very weak (Fauziah et al., 2021). All three *Ficus nekbudu* Warb. leaf extracts demonstrated very strong xanthine oxidase inhibitory activity, each yielding IC_{50} values below 50 μg/mL.

Statistical Analysis

Flavonoids are a type of phenolic compound with significant potential to inhibit xanthine oxidase, and were analyzed (Lin et al., 2015). To assess the relationship between flavonoids and xanthine oxidase inhibition, a correlation analysis was performed. The analysis was conducted to determine the relationship between the phenolic and flavonoid content of each extract and the IC_{50} value. The correlation test yields a value between -1 and +1. Positive or negative results indicate the direction of the relationship between the variables under analysis. A positive relationship implies that as the x value increases, the y value also increases, while a negative relationship indicates that as the x value increases, the y value decreases. The closer the correlation value is to ±1, the stronger the correlation strength (Gogtay & Thatte, 2017).

The correlation analysis revealed that phenolic content has the strongest association with IC_{50} in the ethyl acetate extract (r=0.982), while flavonoid content shows the strongest correlation with IC50 in the 96% ethanol extract (r=0.736). These results indicate that the XO inhibitory activity of *Ficus nekbudu* Warb. is mediated by different compound classes depending on solvent polarity: flavonoids dominate in the ethanol fraction, whereas phenolic compounds are the primary contributors in the ethyl acetate fraction. (Lin et al., 2015). Isoflavones that were identified by TLC may play a key role, as this subclass has demonstrated direct XO inhibition through substrate competition

CONCLUSION

Ficus nekbudu Warb. leaf extracts contain substantial amounts of phenolic and flavonoid compounds across all three polarity fractions. The 96% ethanol extract yielded the highest total phenolic content (89.20 ± 3.49 mg GAE/g), while the ethyl acetate extract contained the greatest total flavonoid content (137.04 ± 7.19 mg QE/g), reflecting the selectivity of each solvent for compounds of matching polarity. All three extracts demonstrated very strong xanthine oxidase inhibitory activity ($IC_{50} < 50$ μg/mL), with the n-hexane extract exhibiting the most potent inhibition, followed by ethyl acetate and 96% ethanol.

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Pearson correlation analysis indicated that the relationship between phytochemical content and XO inhibitory activity varied among solvent fractions. Phenolic content showed a strong positive correlation with XO inhibition in the ethyl acetate extract (r = 0.982), whereas flavonoid content showed a stronger correlation with inhibition in the 96% ethanol extract (r = 0.736). These results indicate that XO inhibitory activity in *F. nekbudu* Warb. is not attributable to a single compound class, but is instead modulated by the interplay between compound polarity and solvent selectivity. Collectively, these findings establish *F. nekbudu* Warb. as a promising natural candidate for the development of plant-based XO inhibitors for hyperuricemia management. Future work should prioritise isolation and structural characterisation of the active constituents, particularly the isoflavone subclass identified by TLC, along with in vivo validation of the observed inhibitory activity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' DECLARATION

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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