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RESEARCH ARTICLE

Anti-hyperuricemia Potential of Ethyl Acetate Fraction from Ethanolic Stem Extract of *Arcangelisia flava*

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Abstract

Arcangelisia flava, with its secondary metabolites in flavonoids, has shown promising potential as an alternative treatment for hyperuricemia. The study aimed to determine the effectiveness of the ethyl acetate fraction from the ethanolic stem extract of *Arcangelisia flava* in inhibiting xanthine oxidase. This research, conducted at the Medical Basic Chemistry Laboratory Universitas Sriwijaya in September–December 2022, used in vitro study methods. The stem of *Arcangelisia flava* was extracted by maceration using ethanol. The ethanolic stem extract of *Arcangelisia flava* was then fractionated using n-hexane and ethyl acetate. The ethyl acetate fraction and ethanolic extract of *Arcangelisia flava* were measured to inhibit the xanthine oxidase using UV-vis spectrophotometry with allopurinol as a comparison. The IC_{50} was calculated by linear regression. The ethanol extract and ethyl acetate fraction have flavonoids, alkaloids, terpenoids, and quinones. The IC_{50} value of the ethanol extract was 30.04 ppm, the ethyl acetate fraction was 23.99 ppm, and the allopurinol was 17.16 ppm. The ethyl acetate fraction inhibited xanthine oxidase better than the ethanol extract. The study's significant finding is that the ethyl acetate fraction of the ethanolic stem extract of *Arcangelisia flava* strongly inhibits xanthine oxidase, offering a potential new avenue for treating hyperuricemia.

Keywords: *Arcangelisia flava*, ethyl acetate fraction, xanthine oxidase inhibitory

Introduction

One of the common diseases in society is hyperuricemia, or what is commonly called gout arthritis. Hyperuricemia is generally when uric acid levels are >7 mg/dl in the blood. Uric acid levels >6.8 mg/dl significantly increase the risk of uric acid crystallization.[1] Crystallization of uric acid occurs in soft tissues and joints through monosodium urate crystals, which appear as needle-shaped crystals when viewed under a microscope. Uric acid deposition, such as uric acid stones, can also occur in the kidneys, usually mixed with calcium oxalate crystals.[2]

The first-line treatment of hyperuricemia is a xanthine oxidase inhibitor, namely allopurinol.[3] This drug blocks the transformation of uric acid precursors into uric acid.[4] The xanthine oxidase functions in the production of uric acid by destroying purine nucleotide.[5] Allopurinol could be a xanthine oxidase inhibitor broadly used to treat gout. Allopurinol is additionally an uncommon but well-known cause of possibly extremely intense liver damage that habitually presents with noticeable extreme touchiness highlights such as those seen with sedate response with eosinophilic and systemic side effects. This incorporates fever, broad hasty, eosinophilic, a typical lymphocytosis, lymphadenopathy, and other organ-inclusion with ordinary idleness of 2–6 weeks.[6]

Natural xanthine oxidase inhibitors using phytomedicines (herbal medicines) are increasing.[7] One of them is yellow wood (*Arcangelisia flava*).[1,8–11] *Arcangelisia flava* in Figure was found in Sumatra, Java, Kalimantan,

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Sulawesi, Nusa Tenggara, Halmahera, Papua, Thailand, Philippines, Indochina, and Malaya.[12,13] The part of *Arcangelisia flava* was generally used as a traditional medicine by Southeast Asians as an antibacterial, antidiarrhea, antiasthma, antimalarial, antifungal, and antitumor for its stem. However, other plant parts, such as the roots, are occasionally used.[1,8–11]

The secondary metabolite compounds of *Arcangelisia flava* were alkaloids, saponins, tannins, and flavonoids.[1,11] Quercetin, which is one of the flavonoids, has been clinically proven to be able to treat gout through inhibition of xanthine oxidoreductase, which is the last step in the production of intracellular uric acid, by administering tablets containing 500 mg of quercetin for four weeks, can significantly reduce plasma uric acid concentration by 26.5 μmol/l.[14] Methanol extract of *Arcangelisia flava* has a phenolic content of 6.274 mg GAE/g and a total flavonoid content of 1.66%.[8] The xanthine oxidase inhibitory of ethanol extract of *Arcangelisia flava* showed that the stem (IC_{50}=30.44 ppm) has the activity of lowering uric acid levels better than the leaves (IC_{50}=174.62 ppm).[15] Ethanol is a universal solvent, so ethanol extracts can provide large yields and high phytochemical results. Because it is not yet known which fraction is active from this ethanol extract against the inhibition reaction, it is necessary to fractionate using solvents with different polarity, especially semi-polar solvents such as ethyl acetate, where ethyl acetate solvents can dissolve certain flavonoid compounds. The study aimed to determine the effectiveness of the ethyl acetate fraction from the ethanolic stem extract of *Arcangelisia flava* in inhibiting xanthine oxidase.

Methods

All protocols were based on the certificate of ethical approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Sriwijaya No. 104-2022. The stem of *Arcangelisia flava* was obtained from Musi Rawas district, South Sumatera, Indonesia, and determined in the Biosystematics Laboratory, Department of Biology, by the Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, Palembang, in September–December 2022 used in vitro study method.

The extraction method used in this study was a meticulous cold extraction with maceration. A 750 g stem of *Arcangelisia flava* simplicia was soaked in 96% ethanol for 3×24 hours and filtered. This process involves maceration three times with 96% ethanol, ensuring a comprehensive extraction. The extract filtrate was dried using a rotary evaporator. The ethyl acetate fraction was prepared from the ethanolic stem extract of *Arcangelisia flava* by putting 23 g of ethanol extract into a separatory funnel, adding 250 ml of n-hexane, shaking it for 30 minutes, and then letting it stand. Separate the filtrate and residue. This liquid-liquid extraction was repeated three times, ensuring the thoroughness of the process. The remaining extract obtained from the first extraction was again reacted with 250 ml of ethyl acetate, shaken for 30 minutes, and allowed to stand again to form the remaining extract and ethyl acetate filtrate. The ethyl acetate filtrate was evaporated to obtain the ethyl acetate fraction from the ethanolic stem extract of *Arcangelisia flava*. A comprehensive approach was used to identify second metabolites in simplicial

Figure Yellow Wood (*Arcangelisia flava*)

with qualitative analysis called phytochemical screening,[16,17] ensuring the accuracy and reliability of our results.

The solution was prepared by incorporating 1 mg of the sample into a beaker glass and adding a few drops of dimethyl sulfoxide (DMSO) until dissolved. Dissolve sample with sodium phosphate buffer pH 7.5 to 10 ml. The test stock solution with a concentration of 100 ppm was then diluted, and four kinds of concentrations were obtained: 50 ppm, 25 ppm, 12.5 ppm, and 6.25 ppm. Allopurinol solution was prepared by weighing 10.225 mg of allopurinol powder, adding four drops of 1 M NaOH, then putting it into a 5 ml measuring flask and adding CO_2-free aqua dest to the limit.

The xanthine substrate solution was prepared using the 99.5% xanthine substrate (Sigma-Aldrich, X0626), which has a mass weight of 152.11 g/mol. In this study, 100 ml of 0.15 mM xanthine substrate was used 0.15 mM xanthine substrate solution was prepared by incorporating 2.293 mg of xanthine substrate into a glass beaker, which was then dissolved with five drops of 1 M NaOH. This solution was added with sodium phosphate buffer pH 7.5 to reach a volume of 100 ml. The xanthine oxidase enzyme solution was prepared by adding 12.5 mg of xanthine oxidase from bovine milk lyophilized powder (Sigma-Aldrich, X4376) to a 5 ml measuring flask and dissolving it with phosphate buffer to a volume of 5 ml.

The xanthine oxidase inhibition assay procedure was performed using a UV-vis spectrophotometer using the method shown in Table 1.[17] The inhibition activity was calculated using the formula:

$$\%\text{inhibition}=((A-B)-(C-D))/((A-B))\times 100\%$$

Based on the results obtained after using this formula, the sample concentration and percentage inhibition of the xanthine oxidase enzyme were plotted using the linear regression equation (x, y): y=a+bx; with x: sample concentration, and y: enzyme inhibition presentation.

The calculation is continued by assessing the inhibition concentration of 50% (IC_{50}). IC_{50} shows the sample concentration that can inhibit the activity of the xanthine oxidase enzyme by 50%. After obtaining a linear equation (x, y), IC_{50} can be calculated by entering a value of 50 in the variable y so that the equation is obtained: $IC_{50}=(50-a)/b$; with a: intercept and b: slope.

Results

A 750 mg of *Arcangelisia flava* stem simplicia was macerated with 96% ethanol solvent to obtain 23.54 grams of ethanol-concentrated extract. From 23 grams of ethanol-concentrated extract, liquid-liquid fractionation was obtained so that the ethyl acetate fraction was 1.52 grams. The results of this yield can be seen in Table 2.

The ethanol extract and ethyl acetate fraction obtained were tested for secondary metabolite content using phytochemical tests, showing flavonoids, alkaloids, terpenoids, and quinone in

Table 1 Inhibition Xanthine Oxidase Enzyme Procedure

Substances	Volume (µL)					
	Blanco		Sample		Allopurinol	
	A	B	C	D	C	D
Phosphate buffer pH 7.5	300	300	300	300	300	300
DMSO	100	100	-	-	-	-
Sample	-	-	100	100	-	-
Allopurinol	-	-	-	-	100	100
Xanthine oxidase enzyme	-	100	-	100	-	100
Aqua dest	100	100	100	100	100	100
Incubated for 5 minutes at 37°C						
Xanthine substrate	200	200	200	200	200	200
Incubated for 30 minutes at 37°C						
HCl 0.5 M	200	200	200	200	200	200
Measure the absorbance at a wavelength of 293 nm						

Note: A: absorbance of blanco (without sample), B: absorbance control of blank (without sample and enzyme), C: absorbance of sample/allopurinol, D: absorbance control of sample/allopurinol (without enzyme)

Table 2 ***Arcangelisia flava*** **Stem Extraction Results**

Sample	Weight	Yield	
Ethanol extract	750 g	23.54 g	3.14%
Ethyl acetate fraction	23 g	1.52 g	6.61%

ethanol extract and ethyl acetate fraction (Table 3).

The inhibition of the ethyl acetate fraction compared to the ethanol extract was higher, especially at doses of 25 ppm, with inhibition differences of 30.25%. On the contrary, the inhibition of ethyl acetate fraction compared to allopurinol was only higher at doses of 50 ppm, with a difference in inhibition of 4.53%, and a difference in IC_{50} value of 6.83 ppm (Table 4).

The result shows that the ethyl acetate fraction has an IC_{50} value lower than ethanol extract and a higher IC_{50} than allopurinol. If the IC_{50} value is <25 ppm, the inhibitory ability of the substance is classified as very strong, the IC_{50}=25–50 ppm is strong, IC_{50}=50–100 ppm is less strong, and IC_{50}>100 ppm is weak. Allopurinol and the ethyl acetate fraction were classified as very strong inhibitors of the xanthine oxidase enzyme.

Table 3 Results of Phytochemical Screening of ***Arcangelisia flava***

Chemical Groups	Results	
	Ethanol Extract	Ethyl Acetate Fraction
Flavonoids	+	+
Alkaloid: Dragendroff	+	+
Alkaloid: Mayer	+	+
Alkaloid: Wagner	+	+
Tannins	–	–
Terpenoids	+	+
Steroids	–	–
Saponins	–	–
Quinones	+	+

Note: +: presence, –: absence

Discussion

Oxygen can be reduced by xanthine oxidase to superoxide anion, which will turn into hydrogen peroxide, a reactive oxygen species (ROS) toxic to cells in the body. In the presence of xanthine oxidase enzyme, xanthine/hypo-xanthine will be converted into uric acid. Uric acid is a pro-oxidant and can induce the formation of other radicals that can oxidize lipid membranes. Excessive xanthine oxidase activity can damage cells due to these free radicals.[17–19] To counteract ROS, antioxidants are needed. A large number of natural and synthetic products have been discovered.[17–22]

Several studies have shown that some plant extracts have antioxidant activity, such as the gooseberry plant, which can inhibit the cyclooxygenase enzyme, thus inhibiting the formation of prostaglandins.[20] Dry fruit extract of *Phaleria macrocarpa* (Scheff.) Boerl has an antioxidant effect at a dose of 62.5 mg.[21] Some plants that have essential oils also have potent antioxidant activity, such as clove extract,

Table 4 Xanthine Oxidase Inhibition and IC_{50}

Sample	Concentration (ppm)	Inhibition Percent	Linear Regression Equation	IC_{50} (ppm)	Inhibition Effect
Ethanol extract	50	79.16			
	25	42.69	y=1.53x+4.12	30.04	Strong
	12.5	31.32			
	6.25	6.49			
Ethyl acetate fraction	50	83.29			
	25	72.94	y=1.65x+10.37	23.99	Very strong
	12.5	34.98			
	6.25	5.14			
Allopurinol	50	87.82			
	25	70.43	y=1.30x+27.63	17.16	Very strong
	12.5	48.24			
	6.25	26.25			

while the nanoemulsion form with a combined formulation of clove extract and grape seed oil, it was found that the less the concentration of clove extract, the smaller the antioxidant activity.[22]

Based on previous literature studies, some secondary metabolites that have been investigated can inhibit the enzyme xanthine oxidase, including flavonoids, alkaloids, saponins, tannins, and terpenoids.[1,11,15,18,19] In the results of the phytochemical test, the ethanol extract and ethyl acetate fraction of the ethanolic stem extract of *Arcangelisia flava* contains secondary metabolites such as flavonoids, alkaloids, and terpenoids, which are bioactive compounds in plants that can inhibit xanthine oxidase enzyme.

In another study, the ethyl acetate extract of the flesh of *Salacca edulis* Reinw. was more active in inhibiting xanthine oxidase with an IC_{50} value of 24.75 ppm. In contrast, ethanol extract has an IC_{50} value of 44.95 ppm.[23] Phytochemical testing of *Arcangelisia flava* (L.) Merr. contained tannins, triterpenoids, and alkaloids. From analyzing the chemical compounds are known as 9-octadecenoic acid (Z)-(CAS) oleic acid (40.42%); 3,6-octadecadienoic acid methyl ester (CAS) methyl 3,6-octadecadienoate (24.88%); hexadecanoic acid (CAS) palmitic acid (6.54%); 9-octadecenoic acid (Z)-, methyl ester (CAS) methyloleate (4.56%); and phenol, 2-methoxy-(CAS) guaiacol (3.19%).[24]

The roots of this plant contain glycosides and alkaloids, particularly the isoquinoline group, specifically berberine, jatrorizin, and palmatine. There are minor alkaloids such as columbamine, dehydrocoridalmin, homoaromolin, talifendin, and diterpene fibraleusin. From phytochemical study on the *Arcangelisia flava* have alkaloid group (berberine, thalifendine, jatrorrhizine, palmatine, columbamine, dehydrocorydalmine, dihydro berberine, 8-hydroxy-berberine, homoaromoline, limacine, pycnarrhine), terpenoids group (fibleucin, 6-hydroxyfibleucin, fibraurin, 6-hydroxyfibraurin, tinophyllol, 2-dehydroarcangelisinol, 6-hydroxyarcangelisin, 2a,3a-epoxy-2,3-dihydropenianthic acid methyl ester, 2a,3a-epoxy-2,3,7,8a-tetrahydropenianthic acid methyl ester, 2b, 3a-dihydroxy-2,3,7,8a-tetrahydropenianthic acid-2,17-lactone), others group (p-hydroxybenzaldehyde, vanillin, 20-hydroxyecdysone, pachybasin).[11]

Arcangelisia flava stem extract indicates the content of berberine compounds in large quantities. Berberine had a bright yellow color at TLC and appeared in the 420 nm area in the UV-vis spectrum.[25] Berberine has anti-inflammatory and antiangiogenic effects in a rheumatoid arthritis rat model by decreasing inflammatory factors and suppressing p-ERK, p-p38, and p-JNK activation. Moreover, it is detailed that doxanthine oxidaserubicin-induced vascular congestion and inflammatory cell penetration within the liver were vastly weakened by berberine pretreatment.[26]

Conclusion

The ethyl acetate fraction of the ethanolic stem extract of *Arcangelisia flava* has antihyperuricemia potential with better xanthine oxidase inhibition than the ethanol extract.

Conflict of Interest

All of the authors have declared that they have no conflicts of interest.

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