

Xanthine oxidase inhibitory activity of *Arcangelisia flava*

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ABSTRACT

Background: Hyperuricemia is characterized by elevated levels of uric acid in the blood, produced from its precursor's xanthine and hypoxanthine via xanthine oxidase. *Arcangelisia flava*, an herbal medicine containing flavonoids, may decrease uric acid levels by inhibiting the xanthine oxidase.

Objective: This study was to determine the effectiveness of ethanol extracts of *A. flava* leaves and stems in inhibiting xanthine oxidase.

Methods: The leaves and stems of *A. flava* were extracted using ethanol 96%. Xanthine oxidase activity was measured using UV Vis spectrophotometry and represented as IC₅₀ value. Allopurinol was used as a positive control.

Results: The IC₅₀ value of xanthine oxidase inhibitory of ethanol extract of *A. flava* leaves and stems as well as allopurinol was 174.62, 30.44, 24.03 ppm.

Conclusion: The stems of *A. flava* have the activity of lowering uric acid levels better than the leaves.

Keywords: *Arcangelisia flava*, hyperuricemia, uric acid, xanthine oxidase

Introduction

Hyperuricemia occurs when the uric acid level exceeds the limit of uric acid solubility in the tissue or serum uric acid (>6.0 mg/dL in females or >7.0 mg/dL in males). Hyperuricemia has close associations with gout, obesity, diabetes, hypertension, and cardiovascular disease [1]. Uric acid is the final product of xanthine oxidase (XO)-catalyzed purine conversion in the liver. The main endogenous source of purines is the nucleic acids breakdown, whereas the primary exogenous source is the catabolism of dietary proteins and fructose [2]. Allopurinol is a xanthine oxidase inhibitor that decreases the production of uric acid [3].

Xanthine oxidase (XO) is the main generator of reactive oxygen species (ROS) in human body. XO may function as a dehydrogenase that transfers electron to nicotinamide adenine dinucleotide (NAD⁺), while oxidizing hypoxanthine to xanthine and producing uric acid. A high level of uric acid

can crystallize and lead to kidney stones and gouty arthritis. XO inhibitors are used to treat gout by reducing uric acid production [1].

Numerous natural products contain bioactive compound such as alkaloids, polyphenols, terpenoids, saponins, and phenylethanoid glucosides. Polyphenols have biological activities such as antioxidant, hypoglycemic, anti-inflammatory, antitumor, antiviral, and antihypertensive [2]. According to their chemical structures, polyphenol are divided into several types, such as flavonoid, stilbenoid, and lignan [3]. Flavonoids (quercetin, apigenin, and scutellarein), tannins (agrimoniin and ellagitannin), chalcones (melanoxethine), triterpenes (ginsenoside Rd and ursolic acid), stilbenoids (resveratrol and piceatannol), alkaloids (berberin and palmatin), have found to be XO inhibitor agent [1]. Flavonoid inhibits xanthine oxidase activity and may play a role in the inhibition of superoxide activity in human tissue.

Flavonoids contain double bonds on the C2 and C3 atoms and typically function as inhibitors. The presence of hydroxyl at C5 and C7 and carbonyl groups at C4 can form hydrogen bonds and contribute to inhibitor interactions at the active site of the xanthine oxidase [4-5]. Flavones and flavonols have higher potential as competitive inhibitors than other flavonoid groups because hydroxyl group is easier to capture electrons from the active site of the xanthine oxidase [6]. The bioactive plant components as luteolin, quercetin, isorhamnein, chrysin, galangin, cajanin stilbene acid, and prosapogenin inhibit xanthine oxidase more effectively than allopurinol [7].

Widespread use is made of the bioactive substances found in plants to preserve the health of the body. People prepared medicinal plants by boiling, mashing, and extracting plant juices. The utilized plant parts included leaves (34%), roots (25%), bark (13%), sap (13%), shoots (9%) and fruit (6%). Data showed that leaves were used more frequently than roots, stems, and others. This is because people are more knowledgeable about the health benefits of leaves than stems and roots and because leaves are easier to consume [8].

A. flava, also known as “yellow wood”, has demonstrated pharmacological activity. The *Arcangelisia* genus contains alkaloids, diterpenoids, and other chemical compounds with antibabesial, antimicrobial, antioxidant, anti-inflammatory, antimalarial, antidiabetic, antitumor, cardiogenic, and antihypertensive activities [9]. The leaves of *A. flava* contain berberine, flavonoid, and saponin, while the stems contain flavonoid, alkaloid, triterpenoid, and saponin [10-11]. *A. flava* has been used empirically as a herbal medicine, and it is known that *A. flava* contains active compounds that have the potential to inhibit xanthine oxidase, but no study has been conducted on this. Therefore, it is necessary to evaluate the anti-hyperuricemic potential of *A. flava* based on its xanthine oxidase inhibitory activity.

Methods

Extraction process

Simplicia was prepared from dried leaves and stems of *A. flava*, by mashing 1000 grams of dried *A. flava*. Simplicia was then extracted by maceration technique for 3x24 hours with 96% ethanol. The maceration was repeated three times, then the extracts were oven-dried at temperature a 40°C to obtain a thick extract. Phytochemical screening in the extract leaves and stems were also performed.

Xanthine oxidase inhibition assay

The 0.15 mM xanthine solution was prepared by dissolving xanthine (Sigma X0626) in NaOH solution and adjusting the pH to 7.5. The xanthine oxidase (Sigma X4376) was diluted to a final concentration of 0.2 U/mL using a potassium phosphate buffer (pH 7.5). The concentration of ethanol extract from the leaves and stems of *A. flava* was used at 6.25 ppm, 12.5 ppm, 25 ppm, and 50 ppm. Allopurinol was used as a positive control for the xanthine oxidase inhibition test.

The inhibitory xanthine oxidase assay consisted of 300 µL of potassium phosphate buffer (pH 7.5), 100 µL of the sample, 100 µL of solution enzyme, and 100 µL aquadest. The test solution was incubated at 37°C for 15 minutes. Next, 200 µL of 0.15 mM xanthine substrate solution was added to the test solution and incubated at 37°C for 30 minutes. The reaction was terminated by adding 200 µL 0.5 M HCl. The percent inhibition was measured using a Perkin Elmer Lambda 25 UV-Vis spectrophotometer at a wavelength of 293 nm. The inhibition activity of xanthine oxidase was determined by comparing the absorbance of uric acid formed in the test solution with the absorbances of the negative and positive controls [12].

Data analysis

Percent inhibition of xanthine oxidase was calculated using the formula: [(absorbance of control – absorbance of sample)/ absorbance of

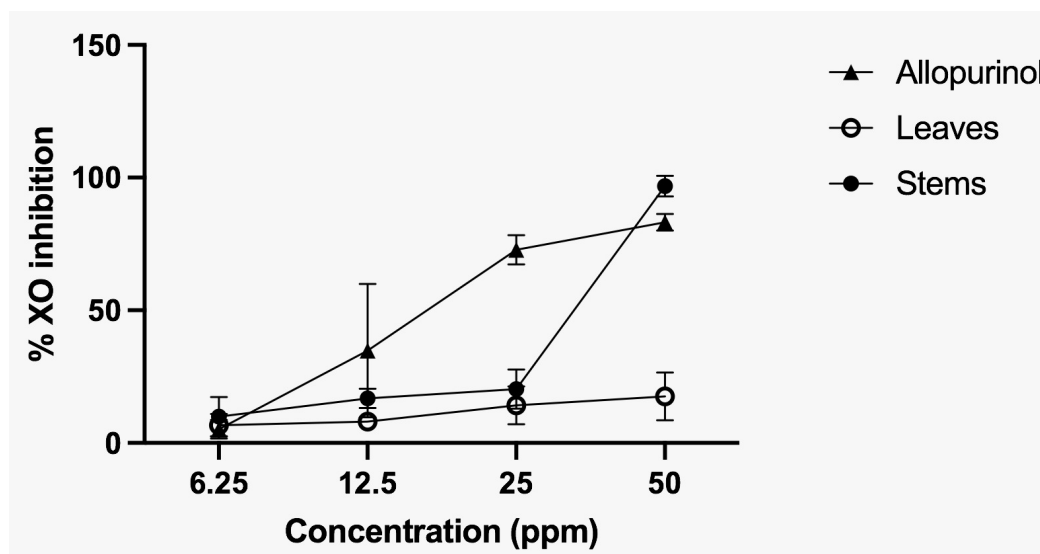


Figure 1. Comparison of the percentage of xanthine oxidase inhibition of the sample and allopurinol

Table 1. The IC_{50} value of leaf extract, stem extract, and allopurinol

Extract	IC_{50} (ppm)
Ethanol leaves	174.62
Ethanol stems	30.44
Allopurinol	24.03

control] $\times 100$. After obtaining the percent inhibition, linear regression was performed with the x-axis representing the concentration and the y-axis representing the percent inhibition. The equation of the line $y = ax + b$ was then derived. The IC_{50} value was derived from this linear regression, indicating the inhibitory activity of XO.

Results

The dry powder form of the leaf and stem of *A. flava* was extracted using ethanol 96%. This process produced a thick extract of *A. flava* leaves 43 grams and a thick extract of *A. flava* stems 42 grams. Extraction yields were 4.4% and 4.2%. Phytochemical screening in the extract leaves and stems of *A. flava* proved that the leaves contain alkaloid, flavonoids, saponin, triterpenoid, and tannin. Stems contained alkaloid, flavonoid, triterpenoid, and tannin.

The ethanol extract of leaves, stems, and allopurinol had IC_{50} values of 174.62, 30.44, and 24.03 ppm, respectively (Figure 1, Table 1).

From this value, the extract of *A. flava* stems had a more significant xanthine oxidase inhibition than the extract of *A. flava* leaves, and the value was comparable to the IC_{50} value of allopurinol. In addition, the percentage of XO inhibition with 50 ppm leaf extract, stem extract, and allopurinol treatment was 17.56, 96.8, and 83.2%, respectively, indicating that stem extract and allopurinol produced a greater degree of inhibition than leaf extract.

Discussion

The phytochemical analysis of *A. flava* leaf extract revealed the presence of alkaloid, flavonoid, saponin, triterpene, and tannin. The stem extract of *A. flava* includes alkaloid, flavonoid, triterpene, and tannin. Chemical constituents isolated and identified from *A. flava* in previous studies included alkaloids (berberine, thalifendine, jatrorrhizine, palmatine, columbamine, dehydrocorydalmine, dihydroberberine, 8-hydroxyberberine, homoaromoline, limacine, pycnarrhine), terpenoids (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, and fibaruretin) and the simple phenolic compounds (p-hydroxybenzaldehyde, vanillin, 20-hydroxyecdysone, pachybasin) [9] [13].

Previous studies concluded that flavonoid compounds contributed to xanthine oxidase inhibition. The flavone and flavonol groups are more effective than other flavonoid groups at inhibiting xanthine oxidase because it is easier for the hydroxyl group to capture electrons from the active site of the xanthine oxidase enzyme. Chrysin, apigenin, luteolin, and scutellarein are flavones. The flavonol groups including galangin, kaempferol, quercetin, 3-O-methylquercetin, and quercetin-3-O-glucoside, as well as the flavonoid class of polyphenolic compounds, have xanthine oxidase inhibitory activity, with luteolin having the strongest inhibitory activity [14-16]. Anthocyanin is a flavonoid derivative with a high antioxidant capacity ($IC_{50} = 0.64$ ppm) and the ability to inhibit xanthine oxidase [17]. Tannins such as agrimoniin and ellagitannin, chalcones (melanoxethin), triterpenes (ginsenoside Rd and ursolic acid), stilbenes (resveratrol and piceatannol), and alkaloid compounds (berberine and palmatine) have a high potential as xanthine oxidase inhibitors [12].

Other studies revealed that the total flavonoid content in the methanol extract of *A. flava* stems was 16.7 ppm, and that the methanol extract of *A. flava* leaves had the most potent ability to scavenge DPPH radicals ($79.68 \pm 0.17\%$) and hydroxyl radicals ($90.51 \pm 0.08\%$). The high concentration of flavonoids in the methanol leaf extract may account for its radical-scavenging properties. The methanol extract contains more phenol than the hexane, ethyl acetate, and flavonoid extracts [18-19].

Flavonoids can also reduce uric acid levels by inhibiting xanthine oxidase activity in purine bases, while aqueous and methanol extracts of

A. flava stems have antioxidant activity against DPPH with EC_{50} values of 68.3 0.8 and 25.7 1.7 ppm, respectively [20]. Secondary metabolites in plants are produced by the biosynthesis of phenolic compounds that are transported from the leaves to other plant storage tissues via phloem or xylem tissue. This may explain why *A. flava* stems contain more active compounds than the leaves.

Conclusion

The stems of *A. flava* have greater xanthine oxidase inhibitory activity than the leaves, so they can be used as an antihyperuricemia. The following research is required to identify the compounds in *A. flava* that inhibit xanthine oxidase.

Author contributions

F, SU, FR, AF conducted data collection and wrote the manuscript. F obtained funding, provided guidance to study design, supervised study, reviewed and finalised the manuscript. SA and MA contributed to the completion of the manuscript.

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