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Research Article

IN VITRO EVALUATION OF XANTHINE OXIDASE INHIBITORY ACTIVITY OF *SONCHUS ARVENSIS* LEAVES

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ABSTRACT

Objective: This studyexaminedthe inhibiting effects of different extracts of *S.arvensis* leaves onthe activity of xanthine oxidase, an essential enzyme for uric acid synthesis.

Methods: Activity test was conducted *in vitro* by measuring the activity of xanthine oxidase using UV spectrophotometry.

Results: The IC_{50} of ethanolic extract of *S. arvensis* leaves was 23.64 µg/mL. Meanwhile, the n-hexane, ethylacetateandwater fraction of the extract had respective IC_{50} of 263.19, 16.20 and 141.80 µg/mL. When the leaves were extracted with ethyl acetate, the extract thus obtained showed an $IC_{50}$of 15.29 µg/mL.Allopurinol, as reference drug, had IC_{50} of 4.84 µg/mL.

Conclusion: Ethyl acetate extract of *S. arvensis* leaves potential to be developed as agent to treat hyperuricemia and gout.

Keywords: S*onchusarvensis,* Gout, Xanthine oxidase, Inhibitory effect, *In vitro.*

INTRODUCTION

Gout is a painful inflammatory arthritis which can lead to decreasein quality of life [1]. The disease occurs when body fluids are saturated due to high levelof uric acid that eventually settles in the joints. Gout could be overcome by lowering the levels of uric acid in the blood to normal limits. Uricosurics and uricostatics are drugs commonly used for this purpose.Uricosuricdrugsworkby inhibiting thereabsorption ofuricacidinthe kidneytubulesin a competitivefashion, so that theremoval of uric acidthrough the kidneyscan be improved. Uricostatic drug inhibits the work xanthine oxidase enzyme that convertshypoxanthine into xanthine and xanthine into uric acid,thus reducing uric acid production. Uric acid is the final product of purine metabolism in human body, that exits the body through urinary excretion. This substance has very low solubility and tend to form crystals. Uric acidaccumulated in the jointsis commonlyfoundinthe form of monosodiumuriccrystals,thatinducesinflammatoty reactions[2,3].A representative drug widely used to treat hyperuricemia and gout is allopurinol. Allopurinol, which works by preventing the formation of uric acid through the inhibition of the enzyme xanthine oxidase, is particularly useful in dealing with chronic gout characterized by tophi formed by deposits of uric acid crystals in the joints, kidneys, and soft tissue. The tophaceous bodies will eventually impaired renal function [4]. Apart from its efficacy, allopurinol can cause hazardous side effects such as nephropathy, allergic reactions and increase in toxicity of 6-mercaptopurine [5]. Furthermore, the drug could also inducehepatitisand allergic reactions [6]. This fact warrantsthe search for new alternatives to lower uric acid with minimal side effects. Many has now turned to plant-derived substances as the source of medication. This research was conducted in an attempt to develop substances with xanthine oxidase inhibiting activity derived from the plant *Sonchus arvensis*. This plant has been traditionally used in Indonesia as gout remedy [7].In the previous studies the species of sonchus has been known for its diverse activities and has been used in relieving disorders such as hepatotoxicty [8] nephrotoxicity [9], cardiotoxicity [10], asthma [11], and oxidative stress [12]. As to its effect on xanthine oxidase, however, no in depth studieshas been conducted.

MATERIALS AND METHODS

Plant materials

*S. arvensis*leaves, was collectedduring the period of December 2012throughMarch 2013from thebotanicalgarden of Manoko in Lembang, West Java, Indonesia.The plant materials were authenticated atHerbarium Bandungense theSchool of Life Sciencesand Technology, Bandung Institute of Technology.

Plant Extraction

This study used the cold maceration method for extraction, with96% ethanol and ethyl acetateas extracting solvents for the driedand groud plant materials. The macerate was collected once everyday, followed by soaking the residue with the same solvent system. This procedure was repeated for three consecutive days. The macerate thus accumulated was concentrated using rotary evaporator under reduced pressure. This was followed by fractionation of the ethanolic extract using n-hexane, ethylacetateandwater.

Xanthine oxidase inhibitory assay

Activity test was conducted *in vitro* by measuring the activity of the enzyme xanthine oxidase using UV spectrophotometry [6,13,14,15,16,17] with the slight modification. Xanthine oxidase enzyme from bovine milk was prepared by dilution of the enzyme to a final concentration 2 Units/mL. 1 mM xanthine substrate solution was made by adding 5 drops of 1.0 M NaOH to increase the solubility of xanthine. Ethanolic and ethyl acetate extracts and also fractions of ethanol extract were dissolved in 1% dimethyl sulfoxide (DMSO) and made the test concentration at 50, 100 and 200 mg/mL. Allopurinolis usedas apositivecontrol.

Total volume of the assay mixture was 3.2 mLconsisting of 1 mL sample test plant studied at various concentration, 1mL of 0.15 M phosphate buffer (pH 7.8), 100 µL solution of the enzyme xanthine oxidase. After preincubation of the test solution at 37 °C for 15 min, the reaction was initiated by addition of 100 µL of xanthine substrate solution and incubated at 37 °C for 30 min.

The reaction was stopped by adding 1 mL of 1N HCl. Spectrophotometer absorbance at 295 nm, suggesting the formation of uric acid. Percent of inhibition of xanthine oxidase activity of the test sample was determined by measuring the absorbance of uric acid from the mixture without test extracts (blank samples) compared with the absorbance of a mixture of test extracts. IC50 values were obtained by linear regression analysis of a plot a series of different sample concentrations against percent inhibition.

RESULTS

As shown in Table 1, Results of inhibitory test on xanthine oxidase activity showed that the ethanolic extrac of *S. arvensis*leaves had IC_{50} of 23.64 µg/mL, and the ethyl acetate extract gave an IC_{50} value of

15,29 µg/mL Meanwhile, the fractions of n-hexane, ethylacetateandwater of ethanolic extract had the respective IC_{50} of 263.19, 16.20 and 141.80 µg/mL. Table 1 further shows that, used as reference drug, allopurinol had IC_{50} value of 4.84 µg/mL.

Table 1: It shows that xanthine oxidase inhibitory ictivity of *Sonchus Arvensis*leaves. The leaves were firstly extracted with ethanol and ethyl acetate. The ethnolic extract was further fractionated with n-hexane, ethyl acetate and water. All extracts and fractions were tested for their xanthine oxidase inhibitory activity using UV spectrophotometry

Plant material	Concentration (µg/mL)			IC_{50}	p
	50	100	200		
Ethyl acetate extract	54.07 ± 14.65	72.25 ± 17.43	86.75 ± 18.51	15.29	0.855
Ethanolic extract	47.37 ± 7.08	82.18 ± 8.86	88.45 ± 14.65	23.64	0.932
Ethyl acetat fraction of Ethanolic extract	52.06 ± 14.79	72.80 ± 17.82	83.16 ± 17.91	16.20	0.778
n-Hexane fraction of Ethanolic extract	8.19 ± 1.71	15.46 ± 5.61	37.84 ± 10.97	263.19	0.007
Water fraction of Ethanolic extract	17.82 ± 7.59	28.20 ± 8.10	74.55 ± 12.64	141.80	0.061
Allopurinol	59.14 ± 3.26	71.38 ± 7.01	91.75 ± 11.99	4.84	-

p values were obtained from comparison of treatment groups to Allopurinol.

Results of phytochemical screening, as shown in Table 2, demonstrated that the drug contained flavonoid and steroids/triterpenoids.

Table 2: It showsresults of phytochemical screening of *Sonchus arvensis* leaves and its ethanolic and ethyl acetate extracts.

Chemical Class	Ground Leaves	Ethanolic extract	Ethyl acetate extract
Alkaloids	-	-	-
Flavonoids	+	+	+
Steroids/Triterpenoids	+	+	+
Saponins	-	-	-

DISCUSSION

The present study was carried out to investigate the xathine oxidase inhibitory activity of *S. arvensis* leaves extracts and fractions in search for substances that might have potential as alternatives to treat hyperucemia and gout.

Extraction was done on ground leaves of *S. arvensis* with cold maceration followed by low pressure evaporation at mininal heating to preserve active substances contained, which are mostly phenolic and flavonoid such as kaemferol, quercetin, orientin, rutin, hyperoside, catechin and myricetin [18]. Flavonoids have been known to have blood uric acid lowering activity through inhibition of xanthine oxidase. Kaemferol is a xanthine oxidase inhibitor without any additional superoxide scavenging activity, while quercetin and myricetin are inhibitors with additional superoxide scavenging activity [19].

The synthesis of uric acid in the oxidative pathway occurs via the conversion of hypoxanthine into xanthine, catalyzed by the eznymes xanthine oxidase and guanase. This is followed by oxidation of xanthine into uric acid, which is also catalyzed by xanthine oxidase. Inhibition of xnthine oxidase is thus essential as pharmacological intervention for hyperuricemia and gout [2].

Results showed that the ethyl acetate fraction gave the highest inhibition among the fractions tested. However, ethyl acetate extract obtained from direct extraction of the leaves was shown to be superior in terms of activity.These findings indicated that more bioactive components were extracted with direct extraction with ethyl acetate. Many biological activites exerted by plant extracts have been associated with flavonoid content, known for effective reactive oxygen-scavenging activity [18]. As shown by the result of phytochemical screening, the crude drug, ethanolic as well as ethyl acetate extract showed positive results for flavonoid. Further study is needed to investigate whether flavonoid is the one responsible for the xanthine oxidase-inhibiting activity.

CONCLUSION

S. arvensis under investigation exhibit xanthine oxidase inhibitory activity. Results of present study suggest that ethyl acetate extract of *S. arvensis* leaves has the most potent activity in inhibiting xanthine oxidase activity, indicating its potential to be developed as agent for treating hyperucemia and gout.

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