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Research Article

XANTHINE OXIDASE INHIBITORY ACTIVITY OF SOME INDONESIAN MEDICINAL PLANTS AND ACTIVE FRACTION OF SELECTED PLANTS

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ABSTRACT

Objective: xanthine oxidase inhibitory activity was assayed on 13 traditional plants. The selection of the plants was based on literatures and has been traditionally used in Indonesia. Those selected plants have been used for gout treatment.

Methods: xanthine oxidase inhibition activity was observed through UV absorbance of uric acid reduction. IC_{50} of the plant extract, which had the greatest activity of xanthine oxidase inhibition, was obtained from various extract concentrations.

Results: Ethyl acetate fraction of *Woodfordia floribunda* (sidawayah) has the greatest xanthine oxidase inhibitory activity (IC_{50}= 38.92 ppm) among thirty-two other extracts that were used in this research.

Conclusion: sidawayah plants used traditionally shown to have inhibitory activity xanthine oxidase.

Keywords: Xanthine oxidase Inhibitor; Gout; Sidawayah; *Woodfordia floribunda* Salisb

INTRODUCTION

Gout is a metabolic disease. It occurs due to increasing deposit of urate in the body accompaning with hyperuricemia[1]. Gout has heterogeneous clinical symptoms that which are increasing in levels of serum acid, recurrent attacks of acute inflammation in the joints associated with the accumulation of monosodiumurate (MSU) crystals in synovial fluid. MSU crystal is built up on the tissue or around the joints, kidney disorders and kidney stone formation cause by the buildup of uric acid in kidney tissue[2]. The catalysis of xanthine by xanthine oxidase enzyme (XO) can lead to the accumulation of uric acid and ultimately cause gout.

$$xanthine + O_2 + H_2O \rightarrow urate + H_2O_2$$

Sidawayah (*Woodfordia floribunda* Salisb) is used traditionally in some countries such as India, Nepal and Indonesia for alternative treatments to reduce uric acid production. Moreover, the *Woodfordia floribunda* Salisb has been reported to have anti-inflammation, anti-oxidant, anti-hyperglicemic, anti-asthmatic and anti-inflammation[3,4,5]. The aim of this research is to verify xanthine oxidase inhibiting activity from some plant extracts and that fraction.

MATERIALS AND METHODS

Plant collection

Plants that were used this research were selective based on ethnobotanical literature[6,7,8,9].

Table 1: List of families and species of plant for treatment of gout

Family/Species	Plant part
Zingiberaceae	
Alpinia galanga Linn	Rhizome
Kaempferia galanga Linn	Rhizome
Poaceae	
Cymbopogon nardus Linn	Petiolum, folium
Oxalidaceae	
Averrhoa bilimbi Linn	folium
Acanthaceae	
Barleria prionitis Linn	folium
Justicia gendarussa Burm	folium
Lauraceae	
Cinnamomum burmanii (C.Nees&T.Nees) C. Nees ex Blume	cortex
Cucurbitaceae	
Coccinia grandis Linn Voight	folium
Verbenaceae	
Lantana camara Linn	folium
Moringaceae	
Moringa oleifera Lam	radix
Plantaginaceae	
Plantago major Linn	Radix, folium
Fabaceae	
Tamarindus indica Linn	Pulp, lignum
Lythraceae	
Woodfordia floribunda Salisb	Flos

Some plants were taken from Manoko's Herbarium, Bandung and the others were from central Java.

Preparation of crude extracts

Materials were divided into two groups, dried and fresh samples. Samples were extracted using reflux (ethanol) and decoc (water) for 2 hours. Ethanol extracts were dried using rotavapor, while water extracts were dried using freeze dry.

Fractionation

Liquid-liquid extraction using three different kind of solvent which

were n-hexane, ethyl acetate and n-butanol.

Assay of xanthine oxidase activity

The xanthine oxidase activity with xanthine as the substrate was assayed spectrophotometrically (EC 1.1.3.22)[10]. Mixture consisted of 1 mL plant extract solution (100 μg/mL), 2.9 mL 50 mM potassium phosphate buffer (pH 7.5 at 25°C) that were initiated by adding to 2 mL of the substrate solution (xanthine 0.15 mM). Xanthine 0.15 mM was prepared by dissolving it in 100 μL NaOH and the pH was adjusted to 7.5. The mixture was incubated at 25°C for 15 min. After preincubation, the reaction was initiated by the addition of 0.1 mL (0.1 units/mL in phosphate buffer, pH 7.5 at 25°C) xanthine oxidase enzyme (from bovine milk, Sigma X1875). Xanthine oxidase was prepared in cold potassium phosphate buffer immediately before used. The mixture was incubated at 25°C for 30 min, for stopping reaction 1 mL HCl 1 N was added. The absorbance was recorded at 290 nm using an UV spectrophotometer. Allopurinol (100μg/mL) was used as positive control[11,12].

One unit will convert 1.0 μmol of xanthine to uric acid per minute. Xanthine oxidase activity was expressed as the percentage inhibition of XO, which was calculated as,

$$\%inhibition = \frac{x - y}{x}.100$$

x is the activity of the enzyme without plant extract (Δabs. with enzyme - Δabs. without enzyme), and *y* is the activity of the enzyme with plant extract (Δabs. with enzyme - Δabs. without enzyme).

This research had been comparing xanthine oxidase inhibitory from plant extracts to first line drug for gout treatment, allopurinol. The xanthine oxidase inhibitor allopurinol is by far the most commonly used hypouricemic agent and lower serum urate in overproducers, urate stone formers, and patients with renal disease.

IC_{50} was determined after xanthine oxidase activity assay on 100 μg/mL, while samples had more than 50% activity then calculated IC_{50} concentrations ranging 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL, 100 μg/mL and 120 μg/mL . All tests were performed in triplicate.

RESULTS

The best xanthine oxidase inhibitory were shown *Alpinia galanga* Linn (57.99%) and *Woodfordia floribunda* Salisb (55.33%). Both of them were dried sample and extracted by reflux. *Alpinia galanga* Linn extract had IC_{50} value at 65.36 μg/mL and *Woodfordia floribunda* Salisb extract which showed IC_{50} value at 94.79 μg/mL inhibition of xanthine oxidase enzyme activity. These results were compared with the positive control allopurinol (2.49 μg/mL). The percentage inhibition of *Alpinia galanga* Linn fraction that assayed at 100 μg/mL is 16.11% for n-butanol fraction, 20.11 % for ethyl acetate fraction and 80.96% for n-hexane fraction. While *Woodfordia floribunda* Salisb had percentage inhibition is 37.81% for n-hexane fraction, 64.92% for ethyl acetate fraction and 47.28% for n-butanol fraction.

Table 2: Xanthine oxidase inhibitory activity assay of all extracts

Sample	%Inhibition
Sample (Ethanol extracts)	
Fresh *Tamarindus indica* Linn's pulp	16.49 ± 28.18
Dried *Tamarindus indica* Linn's pulp	21.40 ± 6.87
Fresh *Tamarindus indica* Linn's lignum	44.90 ± 1.25
Fresh *Plantago major* Linn's folium	17.35 ± 1.11
Dried *Plantago major* Linn's folium	21.70 ± 2.59
Dried *Plantago major* Linn's Radix	3.66 ± 10.22
Fresh *Justicia gendarussa* Burm's folium	21.16 ± 11.25
Dried *Justicia gendarussa* Burm's folium	18.48 ± 1.01
Fresh *Moringa oleifera* Lam's radix	18.24 ± 1.55
Dried *Moringa oleifera* Lam's radix	16.45 ± 1.23
Fresh *Lantana camara* Linn's folium	17.17 ± 1.17
Dried *Lantana camara* Linn's folium	-0.09 ± 4.01
Fresh *Cinnamomum burmanii(C.Nees & T.Nees)C. Nees ex* Blume's cortex	15.26 ± 2.01
Dried *Cinnamomum burmanii(C.Nees & T.Nees)C. Nees ex* Blume's cortex	15.08 ± 5.58
Fresh *Averrhoa bilimbi* Linn's folium	25.76 ± 1.39
Dried *Averrhoa bilimbi* Linn's folium	38.23 ± 0.17
Fresh *Alpinia galanga* (L.) Willd's rhizome	-7.28 ± 0.82
Dried *Alpinia galanga* (L.) Willd's rhizome	57.99 ± 12.2
Fresh *Kaempferia galanga* Linn's rhizome	14.60 ± 4.78
Dried *Kaempferia galanga* Linn's rhizome	28.86 ± 3.76
Fresh *Barleria prionitis* Linn's folium	1.73 ± 0.98
Dried *Barleria prionitis* Linn's folium	-3.21 ± 0.95
Fresh *Cymbopogon nardus* Linn's petiolum	17.76 ± 0.62
Dried *Cymbopogon nardus* Linn's petiolum	18.12 ± 1.79
Fresh *Cymbopogon nardus* Linn's folium	18.12 ± 0.51
Dried *Cymbopogon nardus* Linn's folium	20.33 ± 3.41
Dried *Coccinia grandis* Linn Voight's folium	-4.01 ± 3.28
Dried *Woodfordia floribunda* Salisb's flos	55.33 ± 1.91
Sample (Water extracts)	
Fresh *Cymbopogon nardus* Linn's petiolum	0.74 ± 3.42
Dried *Cymbopogon nardus* Linn's folium	5.20 ± 9.82
Fresh *Moringa oleifera* Lam's radix	2.08 ± 7.97
Dried *Lantana camara* Linn's folium	12.18 ± 23.02
Allopurinol	98.06 ± 0.82

Woodfordia floribunda Salisb IC_{50} value's was exhibited 38.92 μg/mL for ethyl acetate fraction, 137.45 μg/mL for n-hexane fraction, and 79.10 μg/mL for n-butanol fraction. These results were compared with the standard drug allopurinol showed (IC_{50} = 2.49 μg/mL).

Table 3: Xanthine oxidase inhibitory activity assay of *Woodfordia floribunda* Salisb fractions

Concentration (100 μg / mL)	XO Inhibition (%)
n-hexane fraction	37.81
Ethyl acetate fraction	64.92
n-butanol fraction	47.28

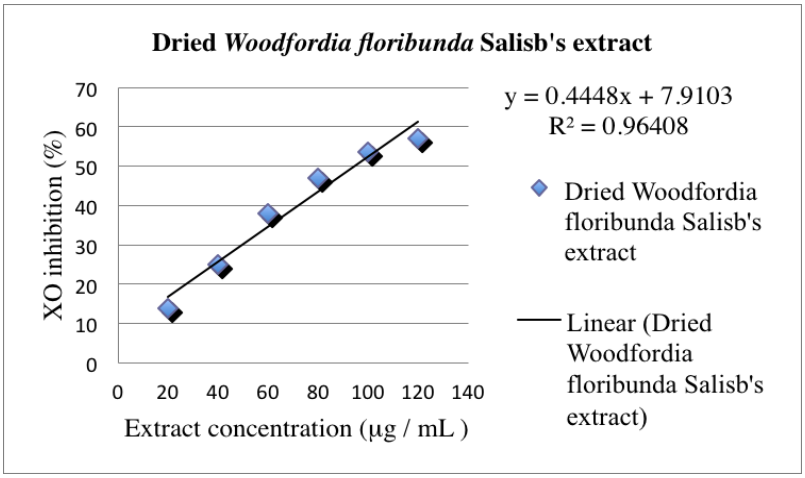

Fig. 1: Lineweaver-Burk plots of *Woodfordia floribunda* Salisb extract (IC_{50})

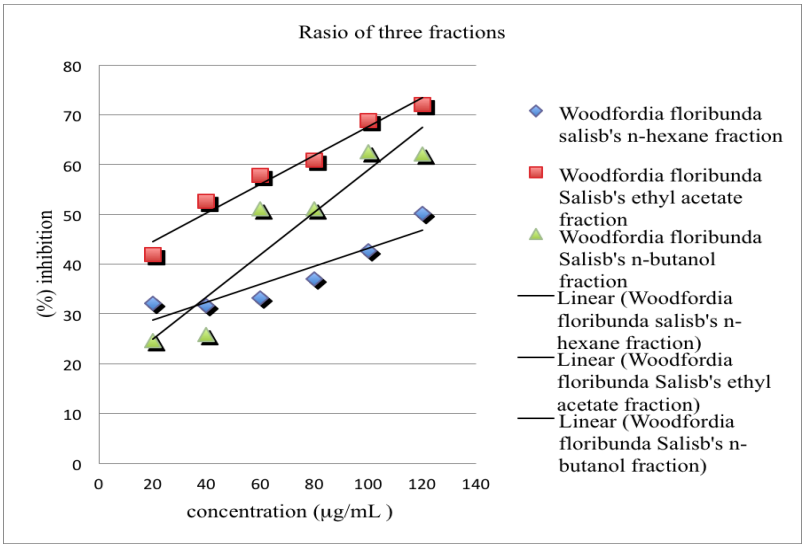

Fig. 2: Comparison of three fractions *Woodfordia floribunda* Salisb

DISCUSSION

Phytochemical screening was indicated *Woodfordia floribunda* Salisb contains powerful flavonoid, which had seen accord to previous research which are conducted by Finose (2011) and Pratap (2007) sidawayah contains phenols, flavonoids, tannins and anthraquinones. Purwantiningsih (2010) concluded that the inhibition of xanthine oxidase activity of ethanol extract was contributed by flavonoid compound.

Alpinia galanga Linn had the best xanthine oxidase inhibitory activity at concentration 100 µg/mL, 57.99% inhibition, followed by 80.96% inhibition of the n-hexane's fraction. Research conducted Tadataka (1988) that chloroform extract had inhibitory activity of xanthine oxidase active. This research had been comparing to earlier research that showed that the best xanthine oxidase inhibitor was from chloroform's extract, while from our research the best xanthine oxidase inhibitor is n-hexane fraction. *Woodfordia floribunda* Salisb had second best activity xanthine oxidase inhibitor, previous research showing that *Woodfordia floribunda* Salisb had topoisomerase II inhibitor activity (Kuramochi-Motegi et al., 1992). This plant was reported to have anti-viral (anti-EV71) (Choi et al., 2010) immunostimulatory activity and had inhibit cell cancer HEP-2 and SK-N-MC activity (Shah, 2010; Vikas, 2011), however previous reports on the xanthine oxidase inhibitor activity hadn't been done.

There is correlative information with our result. According to research Kumaraswamy (2008) and Ghante (2012), *Woodfordia floribunda* Salisb have anti-inflammatory activity. Overall, the best activity IC_{50}=38.92 µg/mL between all fractions is ethyl acetate **(Figure 2).**

CONCLUSION

Thirty-two extracts have been tested for xanthine oxidase inhibitory activity. Ethanol extract showed the best activity by dried *Woodfordia floribunda* Salisb at concentration 100 µg/mL because inhibit 55.33% of the activity, After fractionation shown the highest ethyl acetate fractions 64.92% at 100 µg/mL, with IC_{50} is 38.92 µg/mL. Allopurinol as a comparison has xanthine oxidase inhibitory activity was assayed at 100 µg/mL, 98.06 % with IC_{50} is 2.49 µg/mL.

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