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Research Article

In Vitro Evaluation of Xanthine Oxidase Inhibitory Activity of Selected Medicinal Plants

Rini Hendriani[1,2*], Elin Yulinah Sukandar[1], KusnandarAnggadiredja[1], Sukrasno[1]

[1]*School of Pharmacy, Bandung Institute of Technology, Jalan Ganeca no. 10 Bandung;*
[2]*Faculty of Pharmacy, Universitas Padjadjaran, Jalan Ry Bandung-Sumedang, Jatinangor*

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ABSTRACT

The objective of this study was to investigate natural herbals that can be developed to help treat gout. Gout is a painful joint inflammatory disease caused by a buildup of uric acid in the joint tissues whose production is catalyzed by xanthine oxidase. The study began with the screening of several plants which are used empirically to treat gout in Indonesia. The screened plant parts were *Mimosa pudica* Linn. herbs, *Orthosiphon stamineus* Benth leaves, *Sonchus arvensis* L. leaves, *Andrographis paniculata* (Burn.f.) Nees leaves, *Centella asiatica* (L) Urban herbs, *Catharanthus roseus* leaves, *Sida rhombifolia* L. stems, *Physalis peruviana* leaves, *Tinospora crispa* (L.) Miers stems, *Anredera cordifolia* (Ten.) Steenis. herbs, *Annona muricata* L. leaves and *Imperata cyllindrica* stems. Activity tests were conducted *in vitro* by measuring the activity of the enzyme xanthine oxidase using UV spectrophotometry. Results showed the ethanol extracts *S. rhombifolia* stems showed the highest inhibition on xanthine oxidase activity with IC_{50} of 21.43 µg/mL, followed by *S. Arvensis* leaves extract with IC_{50} of 23.64 µg/mL. The xanthine oxidase inhibitory potential and IC_{50} values of the other extracts are also reported. Results of the present study suggest the potential use of *S. rhombifolia* and *S. Arvensis* extracts in the therapy of hyperucemia and gout.

Keywords: gout, medicinal plants, xanthine oxidase inhibitor.

INTRODUCTION

Gout is a painful inflammatory arthritis which can eventually lead to the decrease in quality of life[1]. It is also a risk factor for mortality and cardiovascular morbidity[2]. The prevalence is reported to be increasing in many countries. In the USA the figure reached as much as 3.9 % (8.3 million inhabitants) during 2007-2008[3]. In Taiwan the results of Nutrition and Health Surveys in Taiwan (NAHSIT) in the period of 2005-2008 showed that the prevalence of gout reported increased by 3.47 % from the previous survey[4]. In the UK, based on IMS survey disease analyzer, an increase of 1.4 % has been observed during 2000-2005[2]. In Indonesia, in particular, the recorded prevalence of gout in Java Island was 1.7 %[4]. Gout is characterized by hyperuricemia[5], which is considered a risk factor for serious diseases such as tophaceous gout, gouty nephropathy, and nephrolithiasis. Hyperuricemia occurs due to excessive production of uric acid or low excretion of the acid. The production of uric acid is catalyzed by xanthine oxidase in the liver[6]. Inhibition of xanthine oxidase can, therefore, be one of the therapeutic avenues for the treatment of gout. Allopurinol is used clinically in the treatment of gout. Currently, allopurinol is one of the most widely used modern medicines to inhibit the synthesis of uric acid[1]. Allopurinol works by inhibiting xanthine oxidase, an enzyme that converts hypoxanthine into xanthine, and then xanthine to uric acid. Thus, the drug can reduce the concentration of blood uric acid. However, it is not uncommon that the use of allopurinol is accompanied by side effects such as nephropathy, allergic reactions and increased toxicity of 6-mercaptopurine[7], as well as hepatitis, nephropathy, and allergic reactions[6]. Besides treatment with synthetic medicines, alternative measures using agents derived from plants may also be considered in helping to lower body uric acid levels. This can be done while putting an effort to reduce detrimental effects of synthetic medicines. The objective of the present study was to find and develop alternative treatment for hyperuricemia in the form of materials derived from plants of Indonesian origin which have been used empirically as remedies to treat gout.

MATERIALS AND METHODS

Plant Materials

The plant materials were collected during the period of December 2012 through March 2013 from the botanical garden medicinal plants of Manoko in Lembang area of West Java, Indonesia. These materials of plant were *Mimosa pudica* Linn. herbs, *Orthosiphon stamineus* Benth leaves, *Sonchus arvensis* L. leaves, *Andrographis paniculata* (Burn.f.) Nees leaves, *Centella asiatica*(L) Urban herbs, *Catharanthus roseus* leaves, *Sida rhombifolia* L. stems, *Physalis peruviana* leaves, *Tinospora crispa* (L.) Miers stems, *Anredera cordifolia* (Ten.) Steenis. herbs, *Annona muricata* L. leaves and *Imperata cyllindrica*) stems. The plants were authenticated at Herbarium Bandungense, the School of Life Sciences and Technology, Bandung Institute of Technology.

**Author for Correspondence*

Table 1: Xanthine oxidase inhibitory activity of twelve medicinal plants which are often used as remedies for gout in Indonesia. The parts plant tested were firstly extracted with ethanol. The ethanolic extract, were tested for their xanthine oxidase inhibitory activity using UV spectrophotometry.

Plant extracts studied	Percent of Inhibition (%)			IC_{50} (µg/mL)
	50 µg/mL	100 µg/mL	200 µg/mL	
Mimosa pudica Linn. herbs	33.98± 9.78	62.26± 6.26	89.87± 8.87	83.12
Orthosiphon stamineus Benth leaves	24.94± 8.43	68.59± 5.55	81.70± 7.91	92.14
Sonchus arvensis L. leaves	47.37± 7.09	82.18± 8.86	88.45± 14.65	23.64
Andrographis paniculata (Burn.f.) Nees leaves	31.19± 4.79	54.13± 5.13	85.57± 5.57	97.09
Centella asiatica (L) Urban herbs	30.02± 8.02	56.27± 6,27	87.68± 5,55	95.33
Catharanthus roseus leaves	30.26± 7.72	50.27± 5.27	76.90± 7.32	108.55
Sida rhombifolia L. stems	50.85± 5.85	80.59± 8.59	93.52± 6.62	21.43
Physalis peruviana leaves	40.35± 4.08	72.02± 7.02	90.10± 5.58	60.28
Tinospora crispa (L.) Miers stems	37.33± 4.05	43.32± 4.32	78.41± 7.41	106.07
Anredera cordifolia (Ten.) Steenis. herbs	49.71± 12.01	53.24± 8.24	66.20± 9.69	66.20
Annona muricata L. leaves	40.06± 9.57	47.96± 7.96	71.29± 7.36	102.02
*Imperata cyllindrica*stems	36.52± 7.34	60.54± 6.54	86.53± 8.53	81.97
Allopurinol	59.14± 3.26	71.38± 7.01	91.75± 11.99	4.84

Plant Extraction

This study used the cold maceration extraction method for all medicinal plants tested, with 96% ethanol as extracting solvent for the dried and ground plants 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.

Xanthine Oxidase Inhibitory Assay

The activity test for xanthine oxidase inhibition was conducted *in vitro* by measuring the enzyme's activity using UV spectrophotometry as described in previous studies[6,8-13] with several modifications. Xanthine oxidase enzyme from bovine milk was purchased from Sigma, prepared by dilution of the enzyme to a solution to obtain a concentration of 2 Units/mL. Xanthine substrate solution was prepared by adding 5 drops of 1.0 M NaOH to increase the solubility, followed by the preparation of 1 mM xanthine solution. The plants extracts were dissolved in 1% dimethylsulfoxide (DMSO) and made into a series of dilution to obtain final concentrations of 50, 100 and 200 µg/mL. The reference drug allopurinol was used as positive control. The total volume of the assay mixture was 3.2 mL and consisting of 1 mL plant extracts solution studied, 1 mL 0.15 M phosphate buffer (pH 7.8), 100 µL of the enzyme xanthine oxidase solution. After preincubation of the test solution at 37°C for 15 min, the reaction was initiated by addition 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. The absorption was measured at 295 nm to indicate the formation of uric acid. The percent of xanthine oxidase inhibitory activity of the assayed samples was determined by measuring absorbance of uric acid from assay mixture without test extract (blank sample) and with test extract. IC_{50} values were obtained by linear regression analysis of the plot several different sample concentrations against percent inhibition.

RESULTS

As shown in Table 1, results of inhibitory test on xanthine oxidase activity of twelve medicinal plants which are often used as remedies for gout in Indonesia indicate that *S. rhombifolia* had the highest percent inhibition, with an IC_{50} of 21.43µg/mL, followed by *S. arvensis* that had IC_{50} of 23.64 µg/mL. The IC_{50} value of *M. pudica, O. stamineus, A. paniculata, C. asiatica, P. peruviana, A. cordifolia,* and *I. cyllindrica* were less than 100 µg/mL. Meanwhile, the IC_{50} value of *C. roseus, T. crispa,* and *A. Muricata* were greater than100 µg/mL. Table 1 further shows that allopurinol, used as reference drug, had IC_{50} value of 4.84 µg/mL.

DISCUSSION

The present study was carried out to investigate the xanthine oxidase inhibitory activity of twelve medicinal plants empirically used as remedies for gout in Indonesia, in search for substances that might have potential as alternatives to treat hyperucemia and gout. The first step of extraction of ground dried herbs selected was done by cold maceration and vacuum evaporation so that the process was done at minimal heating exposure to preserve the active substances. The 96% ethanol was considered as the solvent of choice because of its distinct polarity. The hydroxyl group in ethyl alcohol is polar, while the other group in the molecule is the nonpolar alkyl[14]. This characteristic of ethanol enables the solvent to dissolve more active secondary metabolites contained in the plants. The formation of uric acid occurs through the formation of xanthine from hipoxanthine and guanine, catalyzed by the enzymes xanthine oxidase and guanase. The oxidized xanthine is then converted into uric acid by xanthine oxidase. It is thus clear that the enzyme xanthine oxidase plays an essential role in the pathophysiolgy of hyperurisemia and gout[15]. Results showed that all plant species under investigation exhibit xanthine oxidase inhibitory activity. However, the highest percent of inhibition was shown by *S. rhombifolia* with IC_{50} of 21.43µg/mL, Previous studies demonstrated that the active

steroid compound ($C_{29}H_{49}O_9$) in the plant reduced uric acid[16]. In other studies the plant has been shown to have hipouricemic effect in vivo[17] and it has been further used as an anti-inflammatory[18], anti-arthritis[19], and antioxidant[20]. *S. arvensis*, with IC_{50} of 23.64 µg/mL, has been used traditionally in Indonesia as gout remedy[21]. Indeed, 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[22], nephrotoxicity[23], cardiotoxicity[24], asthma[25], and oxidative stress[26]. *M. pudica, O. stamineus, A. paniculata, C. asiatica, P. peruviana, A. cordifolia*, and *I. cyllindrica* are still considered potential to be developed as remedies for gout because each of them had IC_{50} of less than 100 µg/ml. Meanwhile, *C. roseus, T. crispa, A. Muricata*, with each IC_{50} of greater than 100 µg/mL are considered less potential for development, albeit their traditional use.

CONCLUSIONS

The extract of all plant species under investigation exhibited xanthine oxidase inhibitory activity, however the two with highest activity were *S. rhombifolia* stems extract with IC_{50} of 21.43 µg/mL, and *S. arvensis* leaves with IC_{50} of 23.64 µg/mL. This result further suggests the potential of these two species for development as alternative means for combating hyperuricemia and gout.

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