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DETERMINATION OF ANTI-HYPERURICEMIC EXTRACT OF CELERY, *SIDAGURI*, AND *TEMPUYUNG* BY *IN VITRO* METHOD

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Abstract

Gout is certain a consequence indicated by accumulation of monosodium urate in body causing gout arthritis. *Celery* (*Apium graveolens*), *sidaguri* (*Sida rhombifolia*), and *tempuyung* (*Sonchus arvensis*) are Indonesian native medicinal plants to reduce uric acid contents in blood. Based on their active compounds, they may inhibit xanthine oxidase while the enzyme itself could convert xanthine into uric acid in blood. The objective of this study was to determine the inhibition of single extract and mixture of extracts of *celery, sidaguri,* and *tempuyung* toward xanthine oxidase by *in vitro* method Inhibition of xanthine oxidase by *in vitro* was examined at the optimum condition (20 ^{o}C of incubation temperature, pH 7.5, 0.1 unit/ml of xanthine oxidase concentration, and 0.7 mM of xanthine concentration) was compared with allopurinol as positive control. The results indicated that single extract of 300 ppm of *sidaguri*, 1200 ppm of *celery*, and 300 ppm of *tempuyung* that have greatest inhibition of 42.41%, 67.23 %, and 47.51%, respectively. In addition, the combined extract of *sidaguri, celery*, and *tempuyung* with certain ratio showed *in vitro* inhibition of 71.62 %.

*Keywords***:** Ethanol extract, *Celery* (*Apium graveolens*), *sidaguri* (*Sida rhombifolia*), *tempuyung* (*Sonchus arvensis*), inhibition, xanthine oxidase, *in vitro*.

INTRODUCTION

Generating added value of bioresources is an activity closely related to biopharmaceutical industry. *Sidaguri* (*Sida rhombifolia* L) is a commonly found wild plant that has not been widely cultivated, while *Seledri*/ celery is a plant which is usually used as vegetables. Both herbs are believed to be potential cures for and prevention of gout. Gout is a metabolic disorder due to the accumulation of uric salt crystals in the joints that causes an acute inflammatory response. In people with gout, uric acid levels in the blood exceeds normal limits (hiperuricemia) so often referred to as uric acid diseases.

As a joint disease, gout is categorized into a degenerative disease and has been commonly found among males of over 40 years old. The recently increasing life expectation has made it important to conduct analysis on this disease as a generative one either from the aspect of the disease itself, its prevention or its remedy.

The use of medicines with the feel *back to nature* in Indonesia is very positive. Medicinal plants often used to treat gout are cat's whiskers, *gandarusa*, spoon leaf, celery, *sidaguri*, and *tempuyung* (Dalimartha 2006). Research on the efficacy of medicinal plants as antigout can be approximated by the mechanism of inhibition of xanthine oxidase (XO) both *in vitro* and *in vivo* and through a diuretic effect that can decrease the concentration of uric acid in the blood and through anti-inflammatory effects. Combination of *sidaguri* and celery extract can inhibit xanthine oxidase lower than allopurinol *in vitro* and can decrease the concentration of uric acid in rats with lower potency compared with allopurinol (Iswantini

et al. 2004). Therefore, it need to be added *tempuyung* extract that is known to have diuretic effects and can assist in lowering the concentration of uric acid and have a higher potency compared with allopurinol. The objective of this study was to determine the inhibition of a single extract and extract mixtures of celery, *sidaguri*, and *tempuyung* toward XO activity by *in vitro* method.

METHOD

This research was conducted in the following stages of sample preparation, extraction, and determination of inhibition of xanthine oxidase activity by *in vitro* method to determine the best extract.

Sample Preparation

Raw materials of *sidaguri*, celery, and *tempuyung* obtained from kebun percobaan Pusat Studi Biofarmaka, Bogor Agricultural University. All materials were separated from dirt or other foreign materials then washed and chopped. Samples were dried in the open air until the moisture content was less than 10%.

Extraction

The powder samples were extracted with 30% ethanol using a maceration method with a ratio of 1:10. The sample and solvent were shaked for 6 hours using a shaker, then allowed to stand for 24 hours. The filtrate was separated and the process repeated 3 times with the same type and amount of the solvent. All of the filtrates were collected and evaporated with rotary evaporator apparatus to obtain viscous extract, then dried, weighed and counted yield

In vitro Assay of Xanthine Oxidase Activity Inhibition (Tamta *et al.* 2006)

In vitro assay of XO activity inhibition was performed on each single extract with various concentrations and combination of plant extracts from celery, *sidaguri*, and *tempuyung*. *In vitro* assay of XO activity inhibition used spectrophotometric method at optimum conditions. The optimum conditions of assay refered to the Iswantini and Darusman (2003).

RESULTS AND DISCUSSION

Single Extract inhibition on Xanthine Oxidase Activity by *in Vitro* method

Inhibition of XO assay was performed on all the single extracts with the variation of concentration and combination extract with a variation of the concentration ratio obtained from each single extract with the greatest inhibition percentage. Variations of extract concentration used were around the extract concentration that had the greatest percent inhibition in previous studies (Iswantini and Darusman 2003), and (Wardani 2008). It also made observations of enzyme activity without addition of extracts (blank) to determine the effect of the enzyme activity inhibition by the extract.

Results showed that all tested extracts had a lower activity compared with the blank. This indicated that the single extract of *sidaguri*, celery, and *tempuyung* potentially inhibited the activity of xanthine oxidase. Most, inhibition of xanthine oxidase enzyme activity percentage of *sidaguri*, celery, and tempuyung single extract increased with the increasing of extract concentration. Inhibition of crude extract are shown in Figure 1.

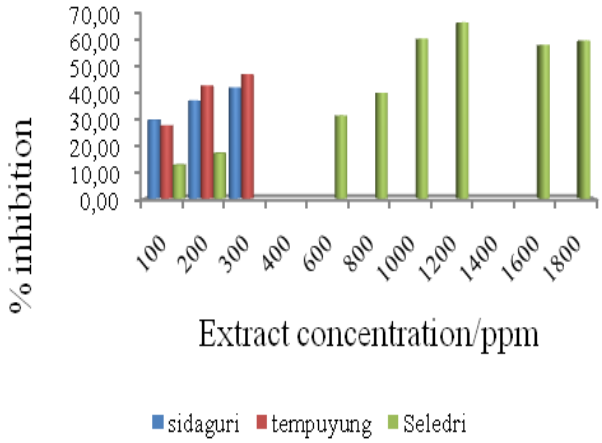

Figure 1 Percentage of sidaguri, celery, and tempuyung ethanol extract inhibition against the xanthine oxidase in various concentrations.

The result explained that the single extract ethanol of *sidaguri* 300 ppm, celery 1200 ppm, and *tempuyung* 300 ppm had the greatest inhibition respectively at 42.41%, 67.23 %, and 47.51%.

Inhibition of Combined Extract on The Activity of Xanthine Oxidase *in Vitro*

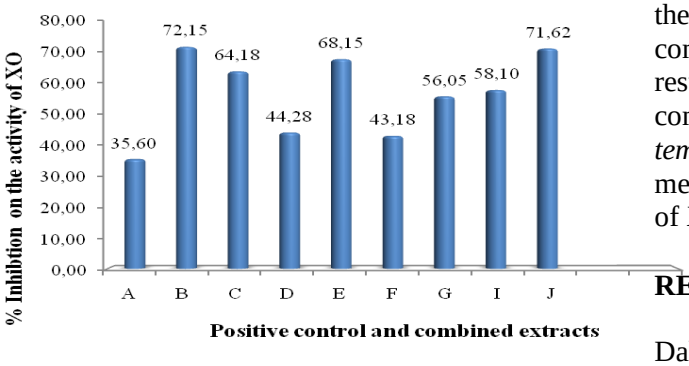

Figure 2 Inhibition percentage of positive control and combined extract (*sidaguri*:celery:*tempuyung*) against xanthine oxidase enzyme.

Figure 2 shows the inhibition activity of positive control (allopurinol) (A and B) and the combined extracts of *sidaguri*, celery, and *tempuyung* with varying ratio (C-J). The result explains that the combination of ethanol extract of *sidaguri*, celery, and *tempuyung* with certain ratio had the greatest inhibition, amounting to 71.62 %. This value was also larger than the inhibition activity of their single extracts and larger than positive control (allopurinol 50 ppm, Fig. 2 A) which only amounted to 35.60 %, and and the same as allopurinol 100 ppm 0f 72.15% (Fig.2 B).

These combined extracts were expected to improve the results of previous study, ie formula of combined *sidaguri*-celery which could decrease rats' blood uric acid level lower than allopurinol (Iswantini *et al.* 2004). Therefore, with the addition of *tempuyumg* ethanol extract into combined ethanol extract of *sidaguri* and celery, it was expected to decrease the rats' blood uric acid level exposed to hyperuricemia, with higher potency than allopurinol by *in vivo*, so that could prevent and or treat gout disease trough the inhibition of xanthine oxidse enzyme or through its diuretic effect.

CONCLUSION

Single extract of 300 ppm of *sidaguri*, 1200 ppm of *celery*, and 300 ppm of *tempuyung* that have greatest inhibition of 42.41%, 67.23 %, and 47.51%, respectively. In addition, the combined extract of *sidaguri, celery*, and *tempuyung* with certain ratio showed *in vitro* inhibition of 71.62 %, so it could be said that there was synergistic interaction between the components of the extract. Based on the results of *in vitro*, It was indicated that the combined extract *sidaguri*, celery, and *tempuyung* has a potential as a antigout medicine through the mechanism of inhibition of XO.

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