# Activities of The Combined Extracts of Tempuyung (*Sonchus arvensis*) and Black Cumin (*Nigella sativa*) Against Xanthine Oxidase Inhibition on Hyperuricemic Mice

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***Abstract*—The combination extracts of tempuyung (*Sonchus arvensis*) and black cumin (*Nigella sativa*) can decrease uric acid levels on the previous result research. However, the mechanism of decreasing uric acid levels was unknown certainly. This study aimed to determine the inhibitory effect of xanthine oxidase by the combination extracts of black cumin and tempuyung on hyperuricemic mice. Hyperuricemic mice were induced by 250 mg/kgBW potassium oxonate was given one hour before treatment. The mice were divided into 3 groups, group I was given 10 mg/kgBW allopurinol (positive control), group II was given 0.5 mL/20gBW aquadest (negative control) and group III was given the combination extracts of black cumin-tempuyung with dose 200 mg/kgBW during 4 days administration. The supernatant of liver was taken and measured of xanthine oxidase levels by spectrophotometer UV at lamda 290 nm. The data of xanthine oxidase activity were analyzed by Kruskal-Wallis and Mann-Whitney method. Xanthine oxidase activity of the combination extracts of blackcumin-tempuyung was 4.54±0.9 U/mg, very significantly than control negative was 8.00±0.22 U/mg ($p<0.05$). Inhibition xanthine oxidase by the combination extracts of black cumin-tempuyung was 43.26±11.29%, lower than allopurinol was 90.19±0.36%.**

***Keywords—Hyperuricemic; xanthine oxidase inhibition; Sonchus arvensis; Nigella sativa***

## I. INTRODUCTION

Uric acid is the end product of purine degradation that comes from within the body or diet that is considered as waste that must be disposed. Increased levels of uric acid in the blood >7.0 mg/dL causes hyperuricemia [1] may lead to gout arthritis, gout nephropathy, or kidney stones. Hyperuricemia prevalence ranged from 2.6 to 47.2%, which varies in different populations [2].

Hyperuricemia treatment is done by lowering uric acid levels in the blood, grouped in two mechanisms that prevent the formation of uric acid (uricostatic) and accelerate the elimination of uric acid (uricosuric). Uricostatic works by binding xanthine oxidase enzyme in two stages. The first stage is the inhibition the formation of xanthine from hypoxanthine, the next stage inhibit the formation of uric acid from xanthine [3]. While the elimination of uric acid in the urine is accelerated by inhibition of tubular reabsorption [4].

Allopurinol is the one uricostatic drug proven effective in treating hyperuricemia. However it has some side effects such as skin allergies, fever, chills, leukopenia, eosinophilia, arthralgia and pruritis [3]. It required another potent alternative medicine to treat hyperuricemia with low side effects.

Tempuyung can be used to cure gout [5]. Flavonoid compounds contained in extracts of tempuyung leaves allegedly able to control excess uric acid concentration [6]. Tempuyung extracts could inhibit the work of xanthine oxidase in the amount of 14.76% at a concentration of 125 µg/mL [7]. The one of the flavonoid compound from tempuyung extract is 7,4'-hydroxiflavone [8]. Besides tempuyung, black cumin also able to treat gout by increasing the overall metabolism [9].

Chemical constituents of black cumin are flavonoids, tannins, and alkaloids [10]. In vivo tempuyung water extract and black cumin in a dose of 200 mg / kg is able to have a uric acid levels respectively by 0.72 mg / dL and 1.2 mg / dL. The combination of the two extracts with their respective levels of 100 mg / kg body weight of uric acid containing 0.38 mg / dL significantly smaller compared to the negative control at 3.1 mg / dL [11].

## II. MATERIALS AND METHODS

### A. *Materials*

The samples of tempuyung and black cumin were bought from Pasar Gede, Surakarta. Herbarium voucher specimen was prepared and deposited in the Herbarium of Pharmacy Biology at Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia. The samples were dried, and then powdered with a blender. Dried powder is then weighed and ready to be extracted.

Animals used for testing were male Swiss rats, which obtained from Laboratory of Pharmacology of Universitas Muhammadiyah Surakarta. The age of Swiss rats were approximately 2–3 months and weighted 20–30 g.

### B. *Determination of Doses*

Dose of allopurinol and potassium oxonate was used 10 mg/kg [11] and 250 mg/kg [12], whereas the combine extract of tempuyung and black cumin with dose 200 mg/kgBW [11].

### C. *Making The Stock Preparation Testing and Potassium Oxonate*

The combined extract of tempuyung and black cumin each dose of 100 mg/kg was made by weighing each extract 20 mg, mixed and then dissolved in distilled water ad 10.0 ml.Potassium oxonate always made freshly each will be used. Potassium oxonate has a solubility of 5 mg/mL. A dose of 250 mg/kg made stock as much as 10.0 mL, by weighing the potassium oxonate 50.0 mg on an analytical balance, then diluted with 10 mL of distilled water ad in the flask.

### D. *Conditioning of Test Animals and The Hyperuricemia Mice*

The testing animals adapted to the research environment at a temperature of the cage for one week prior to treatment. Test animals were fasted 1 hour before the study began [12].

The testing animals were treated for 4 days. Making the hyperuricemia condition were induced with potassium oxonate by intraperitoneal with dose 250 mg/kg BW at 07.00 am. Test animals were treated for 4 days. Making the potassium induce hyperuricemia with oksonat by intraperitoneal 250 mg / kg at 07.00 am. The next hour was given treatment negative control, positive control, and extract [12].

### E. *Treatment of Testing Preparations*

In the treatment testing has been used 15 rats were weighed. Then the rats were divided into 3 groups of testing animals, each group of 5 animals:

1) Group 1 (positive control): allopurinol po 10 mg/kgBW.
2) Group 2 (negative control): distilled water po 0.5 mL/ 20gBW.
3) Group 3: The combination of tempuyung and black cumin exracts with dose 200 mg/kgBW.

### F. *Xanthine Oxidase Assay*

Making the standard curve

Nine series xanthine oxidase concentration has been made (2; 4; 6; 8; 10; 12; 14; 16; 18) x 10-3 U/mL of stock xanthine oxidase 0.1 U/mL, then added xanthine 50 μM and phosphate buffers 50 mM ad 5 mL. The mixture was incubated at a temperature of 37oC for 15 minutes, then the reaction was stopped by addition of 0.5 M HCl 0.58 mL. Readout wavelength UV spectrophotometry at λ 290 nm. The results of standard curve linear equation y = 720.9x + 0.021 with r = 0.997.

*Measurement of the xanthine oxidase activity*

The mouse were drawn heart on the fourth day after 1 hour treatment. Liver washed with 0.9% NaCl and placed in a mixture of 1.15% w / v KCl and 0.1 mMol EDTA, then weighed as much as 1 g after the liver was cut and during the preparation temperature was maintained at a cold temperature (4 °C ). Hearts was homogenized in 4 mL of 50 mM phosphate buffer (pH 7.4). Then do the homogenate centrifuged at 3000 rpm for 10 minutes. Taken as much as 100 mL of the supernatant is then added xanthine 50 μM and phosphate buffer 50 mM ad 5 mL. The mixture was incubated at 37 °C for 15 minutes, then the reaction was stopped by addition of 0.5 M HCl 0.58 mL. measurement with UV spectrophotometry at λ 290 nm.

### G. *Determinationof Protein Content By Lowry Method*

*Making the standard curve*

0.05% of the stock BSA made into 9 series of concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5) x 10-3% by taking stock solution (100; 200; 300; 400; 500; 600; 700; 800; 900) mL, respectively distilled water to 1000 mL. 8 mL of Lowry reagents B wait up to 10 minutes and then added 1 mL of Lowry reagent A wait until OT 20 minutes. Absorbance measurements carried out on a

maximum wavelength of 741 nm. Standard curve y = 15,14x + 0.012 with r = 0.9986.

*H. Measurement Of Protein Content*

Liver washed with NaCl 0.9%, weighed as much as 1 g, was placed in KCl 1.15%. Then cutted and homogenized with 4 mL phosphate buffer (pH 7.4) 50 mM. Then the mixture was precipitated by the addition of 200 mg of ammonium sulfate crystals and centrifuged at a speed of 11000 rpm for 10 minutes, taken 100 mg of sediment. The precipitate was dissolved in acetate buffer (pH 5) ad 10 mL, 400 mL taken plus 8 mL Lowry B, allow 10 minutes. Then add 1 mL Lowry A, OT let stand for 20 minutes. Measurements by UV-Vis Spectrophotometer at λ 741 nm [13].

*I. DataAnalysis Technique*

The activity of the enzyme xanthine oxidase (XO) was expressed as U/mg protein. One Unit xanthine oxidase was 1 mol xanthine converted into uric acid per minute at a temperature of 37°C at pH 7.4. The protein content determined by Lowry method using BSA as standard. Data obtained from studies that form xanthine oxidase enzyme activity was not homogeneous though transformed, so as analyzed by nonparametric methods Kruskal-Wallis and Mann-Whitney with a 95% confidence level. Activity of xanthine oxidase inhibition percentage was calculated by dividing the difference between the average negative control and treatment with an average of negative controls multiplied by 100%. Schematically can be seen in the following formula:

$$\text{Percent of XO inhibition activity} = \frac{A - B}{A} \times 100\%$$

Annotation :

A = the average of XO activity of negative control

B = the XO activity of treatment group

Data of xanthine oxidase inhibition percentage were analyzed by independent t-test.

## III. RESULT AND DISCUSSION

Measurement data of xanthine oxidase and protein levels are used to calculate the xanthine activity of each protein and power oxidase inhibition (Table 1). The value of xanthine oxidase activity of the extract was significantly lower than the negative control (p=0.009). But, when compared with the positive control, the value of the xanthine oxidase activity of the extract was higher (p = 0.009).

Allopurinol had a stronger activity in inhibiting the xanthine oxidase than the combination of tempuyung-black cumin extract (p=0.000). The combination of tempuyung and black cumin extracts was able to inhibit xanthine oxidase with the value of 43.26±11.29%. Although not as powerful as allopurinol inhibition with the value of 90.19±0.36%, but it had great potential to be developed as an alternative medicine for lowering uric acid levels. The lower value of the effect of the combination of black cumin-tempuyung extract than allopurinol caused the combined extracts contained compounds that were very complex.

The combination of black cumin and tempuyung extract was expected to give a synergistic effect in inhibiting xanthine oxidase enzyme, but it was not reached. Inhibition values of the combination of black cumin-tempuyung extracts with dose of 200 mg/kg body weight was 43.26%, lower than the extract single of tempuyung with dose of 200 mg/kgBW was 70.30% [14] and black cumin was 51.01% [15]. The compounds that acted as inhibitors of the xanthine oxidase enzyme that produces uric acid was a flavonoid [16]. Research by reference [11] estimated luteolin was contained in tempuyung extract. Levels of total flavonoids contained in extracts tempuyung was 0.086%, while the water extract of black cumin was 0.4%.

Flavonoids had the three benzene rings in its structure with a combination of hydroxyl groups, sugars, oxygen and methyl alleged to have different activities. The presence of the double bond at the C2 and C3 which maked the rings A and C planar, while ring B was able to inhibit xanthine oxidase in mice hyperuricemic [17]. In vitro luteolin had a flavone skeleton with hydroxyl group on the number 5 and 7 of C ring had a strong potential for inhibiting xanthine oxidase with $IC_{50}$ values of 0.96.

Table 1. Activities of xanthine oxidase (xo) and inhibition percentage

| Treatment | XO levels (U/g liver) | Protein levels (mg/g liver) | XO activities (U/mg) | % inhibition |
|---|---|---|---|---|
| PO + Aquades (negative control) | 2.96 ± 0.08 | 0.02 ± 0.00 | 8.00 ± 0.21 | |
| PO + allopurinol (positive control) | 2.96 ± 0.08 | 0.12 ± 0.00 | 0.78 ± 0.03* | 90.19 ± 0.36 |
| PO + the combined extract | 1.95 ± 0.13 | 0.03 ± 0.01 | 4.54 ± 0.90* | 43.26 ± 11.29 |

Description :

(*) : Significantly different with the negative control (p<0.05)

(#) : Significantly different with the positive control (p<0.05)

n= 5

Quersetin and kaempferol with the flavonol skeleton also had a hydroxyl group on the number 5 and 7 of C ring had an $IC_{50}$ value of 0.44 and 0.67. The relationship between xanthine oxidase inhibition with the compounds contained structure indicates that the hydroxyl group on the number 5 and 7 of C ring was essential to inhibit xanthine oxidase [18].

The protein content in the delivery of allopurinol is higher than the combined extract and negative control that needed to be tested to determine the statistical significance of the differences. The protein content in the combined extract and negative control group did not differ significantly ($p=0.110$). Allopurinol protein levels significantly higher than the tempuyung-black cumin extract ($p = 0.009$). When compared to negative controls, the levels of allopurinol was also significantly higher ($p=0.008$). The cause of high levels of protein in the administration of allopurinol was still unknown. Research on hamster cells showed that the cells were given allopurinol contains 12.82 mg protein/mL enzyme, whereas cells containing the protein hyperuricemia 9.45 mg/mL enzyme [19]. Avoid combining SI and CGS units, such as current in amperes and magnetic field in oersteds. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity that you use in an equation.

## IV. CONCLUSION

The combination extract of black cumin and tempuyung with dose of 200 mg/kgBW was able to inhibit of the activity of the xanthine oxidase enzyme of 43.26±11.29% lower than allopurinol.

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