

Original Article

ANTIHYPERURICEMIA ACTIVITY OF THE ETHANOL EXTRACT OF *ROSELLE* CALYX AND ITS FRACTION (*HIBISCUS SABDARIFFA* LINN) ON MALE WISTAR RATS

SRI WAHYUNINGSIH^{a, b} ELIN YULINAH SUKANDAR^a, SUKRASNO^a, DEBBY NADYA LOFIKA^b

^aSchool of Pharmacy, Bandung Institute of Technology, Jalan Ganeca no. 10 Bandung, Indonesia, ^bFaculty of Pharmacy, Universitas Jenderal Achmad Yani, Jalan Terusan Jenderal Sudirman Po Box 148 Cimahi, Indonesia
Email: sri_wahyuningsih40@yahoo.co.id

Received: 21 Nov 2015 Revised and Accepted: 03 Feb 2016

ABSTRACT

Objective: The objective of this study was to determine anti-hyperuricemia activity of ethanol extract of *roselle* calyx (*Hibiscus sabdariffa* L) and its fraction on male Wistar rats.

Methods: Two different methods were used. First method induction was done by giving potassium oxonate and diet high purine whereas allopurinol as a comparison. The parameter was level uric acid in serum (uricostatic activity). Second methods induction was done by giving potassium oxonate intraperitoneally and sodium urate orally; with probenecid as comparison. Parameters used were uric acid level in serum and urine (uricosuric activity).

Results: Ethanol extract *roselle* calyx at a dose of 40 mg/kg bw, water fraction at a dose of 37.5 mg/kg bw and ethyl acetate fraction at a dose of 6.25 mg/kg bw at day 14 showed a significant reduction in serum uric acid level compared to control group ($p < 0.05$). Besides that ethanol extract of *Roselle* calyx at dose 80 mg/kg bw, water fraction at doses 37.5 and 75 mg/kg bw also ethyl acetate at doses 6.25 and 12.5 mg/kg bw had uricosuric effect that increased the excretion of uric acid in urine significantly compared to control group ($p < 0.05$).

Conclusion: The water fraction of *roselle* calyx at doses 37.5 and 75 mg/kg bw and ethyl acetate at dose 6.25 and 12.5 mg/kg bw was effective as an anti-hyperuricemic agent. The ethanol extract of *roselle* calyx and its fraction had a uricosuric effect similar to probenecid. The best uricosuric effect was showed by ethyl acetate fraction at dose 6.25 mg/kg bw.

Keywords: *Hibiscus sabdariffa* Linn, Water fraction, Ethyl acetate fraction, Calyx, Uric acid, Antihyperuricemia

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INTRODUCTION

The prevalence of hyperuricemia has increased in the last five years in the world [1-3]. Uric acid is the end product of purine degradation. A Higher level of blood uric acid is called hyperuricemia and can cause the precipitation of uric acid in the joint inducing pain sensory and the symptom called Gout. Hyperuricemia could be due to the increase of metabolism (overproduction) of uric acid, or reduction secretion in the urine (underexpression) or a combination of both [4]. Hyperuricemia may be an asymptomatic condition with an increased serum uric acid level, if uric acid concentration higher than 7.0 mg/dl, it is associated with an increased risk for Gout [4]. Allopurinol is the only drug approved for use in inhibiting uric acid synthesis through the inhibition of xanthine oxidase. The major side effects of allopurinol are skin rash, leukopenia, and occasional gastrointestinal toxicity [4,5]. Because of that, many types of research are developed for medicinal plants having the effect of lowering the uric acid level that can be therapeutic options for hyperuricemia. Medicinal plants are believed to be important sources of new substances with potential therapeutic effect. The *Roselle* calyx (*Hibiscus sabdariffa* Linn) have been used in Indonesian traditional medicine for the hyperuricemia treatment [6]. However data as the hypouricemic agent has not been reported, so this experiment was conducted to find out the activity of *roselle* calyx as anti hyperuricemic. The aims of this study were to determine the anti hyperuricemic activity of extract ethanol of *roselle* calyx (*Hibiscus sabdariffa*) and its fractions on male Wistar rats.

MATERIALS AND METHODS

Material

Oxonic acid potassium salt/Potassium oxonate (Sigma), uric acid (Sigma), Probenecid (Sigma), Allopurinol (Sigma), Uric acid kit reagent (Reagent Human), *Gnetum genom* seeds.

***Roselle* calyx collection and determination**

Roselle calyx was collected from Manoko Lembang Indonesia. It was determined in The Herbarium Bandungense School of Life Science Biosciences ITB. *Roselle* calyx was then dried and ground to powder.

***Roselle* calyx extraction**

Roselle powder (250 g) was extracted using Soxhlet apparatus with ethanol 96 % as solvent until clear filtrat was obtained. The extract was evaporated until a viscous extract was obtained (yield: 35.8%).

***Roselle* calyx extracts characterization**

Specific parameters such as organoleptic, water soluble extractive and ethanol soluble extractive, nonspecific parameters were included loss on drying, total ash, and acid insoluble ash, phytochemical screening was measured.

***Roselle* calyx extracts fractionation**

Fractionation of the extract was performed by dispersing extract in ethanol-water (1:1) followed by liquid extraction with hexane. The aqueous fraction was further extracted with ethyl acetate. Hexane, ethyl acetate, and the water fractions obtained were evaporated under reduced pressure to dryness. Yield of n-hexane, ethyl acetate, and water fractions were 46.27%, 15.28 %, and 8.68%.

Antihyperuricemic assay

The animals that used were male Wistar rats aged 8-10 w, 200-250 gram weight, from Biosciences and Biotechnology Research Center, ITB. Antihyperuricemic assay was performed in two methods. The first methods were done in order to evaluate uricostatic activity. The second method was employed to evaluate the uricosuric activity. This experiments design was approved by the Animal Research Ethics Committee of ITB (Reg. No. 03/KEPHB-ITB/11-2014).

The first method of the antihyperuricemic assay was performed using ten groups of male Wistar rats and each group consist of five rats. Group I (control) was given orally with Na CMC suspension; group II with allopurinol 9 mg/kg bw; group III, IV were administered with ethanol extract of *roselle* calyx at dose 40 and 80 mg/kg bw; Group V, VI, VII were administered with water fraction at dose 18.75, 37.5 and 75 mg/kg bw and group VIII, IX, X were administered with ethyl acetate fraction at dose 6.25, 12.5 and 25 mg/kg bw. To induce hyperuricemia, a daily high purine diet comprised of 10% *Gnetum gnetum* L seeds was also given to rats for 14 d. At day 7 and 14, initial uric acid level (t_0) was taken, and 1 hour later, potassium oxonate was given at 250 mg/kg bw intraperitoneally. One hour after induction with potassium oxonate, the drug was administered. Then the blood of each group was collected from tail vein after 1 hour. The day before the experiment, animals fasted for 12 h. The blood was centrifuged at 10.000 rpm for 10 minutes. The quantity of uric acid was measured using kit reagent that contained uricase and peroxidase by spectrophotometer (Microlab 300®).

In the second method (uricosuric assay), we modified Yonetani method [7-9]. We used eleven groups male Wistar rats and each group consist of five rats. Group I(control) was given orally with Na CMC suspension; group II with Probenecid at dose of 27 mg/kg bw; group III, IV, V was given ethanol extract doses 40, 80 and 120 mg/kg bw; Group VI, VII, VIII was administered with water fraction at doses 18.75, 37.5 and 75 mg/kg bw and group IX, X, XI was administered with ethyl acetate fraction at dose 6.25, 12.5 and 25 mg/kg bw. At the beginning of the experiment, uric acid level was measured. The animals received the tested material for three days. In the day 3, potassium oxonate was given twice with interval 1 hour. After 15 min, the animal was given oral dosage in accordance to the groups, the immediately rats were kept in the metabolism cages. Thirty minutes later sodium urate was given at dose 15 mg/kg bw [9], and then at minute 20, 40, and 60, blood and urine 24 h were collected to determine the levels of plasma uric acid and level uric acid excreted during 24 h [10]. The determination of uric acid was performed as described in the previous paragraph.

Statistical analysis

The result was statistically analyzed by using analysis of variance (ANOVA) and T student using SPSS 16.

RESULTS AND DISCUSSION

The characteristic of extract used in these experiments was shown in table 1. It showed that ethanol extract of *Roselle* calyx contained flavonoid, saponin, polyphenol, and quinone.

Table 1: Characteristic of *roselle* calyx ethanol extract

| Parameter | Result |
|--------------------------------|---|
| Organoleptic | Thick extract, brownish-black, sour |
| Water soluble extractive (%) | 42.09±2.01 |
| Ethanol soluble extractive (%) | 28.56±0.97 |
| Loss on drying (%) | 7.79±0.99 |
| Total ash (%) | 7.16±0.29 |
| Acid insoluble ash (%) | 0.46±0.03 |
| Phytochemical screening | flavonoid, saponin, polyphenol, quinone |

Data were given in mean±SD.

In this research, hyperuricemia was induced by giving high purine foods for 14 d. In this study, the best dose of ethanol extract that could reduce the level of uric acid were 40 and 80 mg/kg bw, and this result was used to calculate the dose of the fraction from ethanol extract.

In the method I, in order to increase the level of uric acid in rats, we used the food containing high purines by adding 10% *Gnetum gnetum* seeds. The food was given for 14 d. It is based on research that has been done by Ni Luh that in men who consume *Gnetum gnetum* seeds much as 100 g can increase uric acid levels [11].

The uric acid level of each group with method I (uricostatic effect) is shown in fig. 1.

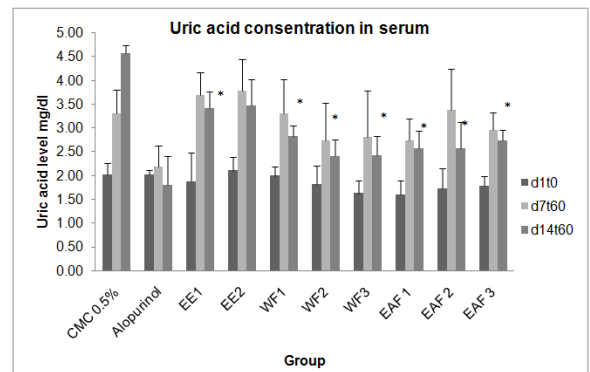


Fig. 1: It shows Uric acid mean concentration in serum of each group at day 1, 7 and 14. Group I: CMC 0.5%, Allopurinol 9 mg/kg bw, EE: ethanol extract dose 40, 80 and 120 mg/kg bw, WF: Water Fraction dose 18.75, 37.5, and 75 mg/kg bw, EAF: ethyl acetate fraction dose 6.25, 12.5 and 25 mg/kg bw at day 14, n=5

*p<0.05, showed statistically significant reduction in serum uric acid level compared to control group

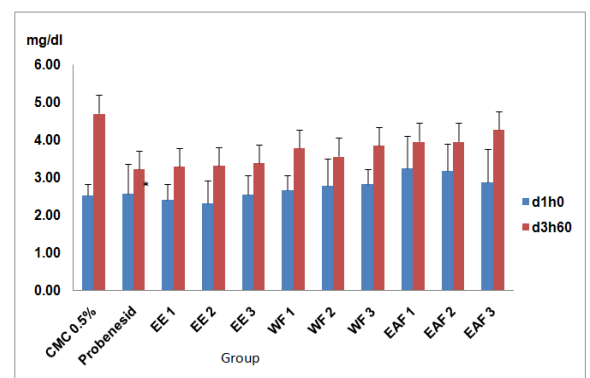


Fig. 2: Uric acid mean concentration in serum of each group at 3rd days experiment with probenecid as comparation, EE: ethanol extract dose 40, 80 and 120 mg/kg bw, WF: Water Fraction dose 18.75, 37.5, and 75 mg/kg bw, EAF: ethyl acetate fraction dose 6.25, 12.5 and 25 mg/kg bw, n= 5

*p<0.05, significant reduction in serum uric acid level compared to control group

In fig. 1, showed that the serum uric acid levels in each group at day 7 showed an increase, but at day 14 decreased levels of uric acid except in the control group continued to show improvement. Ethanol extract of 40 mg/kg bw showed decreased levels of uric acid was significantly different when compared with controls (p<0.05), as well as water fraction dose of 3.5 mg/kg bw and a dose of 75 mg/kg bw, while the ethyl acetate fraction of all doses significantly different when compared with the control group (p<0.05). This shows that the extract or fractions *roselle* flower petals have the potential to reduced uric acid levels in serum

The second method was employed to evaluate the uricosuric activity. In this experiment using probenecid was used as the positive control. The uricosuric effect is shown by the excretion of uric acid in the kidney that can be seen by an increase in the level of uric acid in the urine. Probenecid acts by inhibiting the reabsorption of tubules proximal and consequently reduces the level of uric acid in the blood [10, 12]. Comparison with probenecid was used to evaluate the ability of extract the lower level uric acid reabsorption

in the kidney that can be seen by the increase of uric acid level in the urine [13]. Potassium oxonate was given twice. The first administration to increase plasma uric acid and urine volume, while the second treatment was to maintain the high plasma uric acid for two hours or more [7].

The present study showed that ethanol extract of *roselle* calyx and its fraction had a uricosuric effect because the concentration of uric acid in urine 24 h in the third day was higher than the control group and statistically significant ($p < 0.05$). This suggests that ethanol extract *roselle* calyx and its fraction are also as uricosuric. Ethyl acetate fraction dose 6.5 mg/kg bw showed better than extract or water fraction of *roselle*.

The second method showed that the uricosuric effect of ethyl acetate fraction was higher compared to water fraction or the ethanol extract. The effect of water fraction is increased by increasing the dose while ethyl acetate fraction the smallest dose (6.25 mg/kg bw) gave better effect compared to the higher dose.

The uric acid levels of each group with the second method II (uricosuric activity) are shown in fig. 2 and 3.

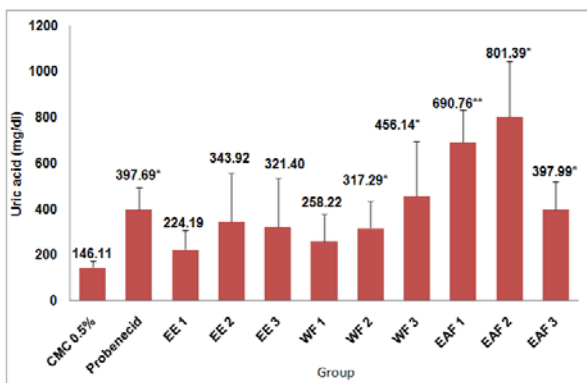


Fig. 3: Average uric acid mean concentration in 24 h urine of each group at 3rd days experiment. EE: ethanol extract dose 40, 80 and 120 mg/kg bw, WF: Water Fraction dose 18.75, 37.5, and 75 mg/kg bw, EAF: ethyl acetate fraction dose 6.25, 12.5 and 25 mg/kg bw, n = 5

* $p < 0.05$, compared to control group, ** $p < 0.01$, compared to control group

Fig. 3 showed that the levels of uric acid in the urine in the group probenecid significantly higher compared to control, so with the as w water and ethyl acetate fraction.

Ethanol extract at dose 40, 80, 120 mg/kg bw gave comparable uricosuric activity with probenecid. Water fraction showed dose-dependent activity in the range dose 18.75 mg/kg bw to 75 mg/kg bw, and the activity at dose 75 mg/kg bw was significantly higher than probenecid ($p < 0.01$). Ethyl acetate fraction at dose 6.25 and 12.5 mg/kg bw gave much higher uricosuric activity than probenecid, however, the effect decreased at higher doses. The uric acid in urine of rat treated with ethyl acetate at 6.25 mg/kg bw was highest approximately 2 x than probenecid, indicate that the uricosuric compounds of *roselle* present in the ethyl acetate fraction

CONCLUSION

The ethanol extract of *roselle* calyx and its fraction had a uricosuric effect similar with probenecid. The best uricosuric effect was showed by ethyl acetate fraction at dose 6.25 mg/kg bw. The water fraction of *roselle* calyx at dose 37.5 and 75 mg/kg bw and ethyl acetate at dose 6.25 and 12.5 mg/kg bw were effective as an anti-hyperuricemia agent.

ACKNOWLEDGMENT

This research was funded by Research and Community Service Universitas Jenderal Ahmad Yani.

CONFLICT OF INTERESTS

Declared none

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