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Antihyperuricemic Effect of Ethanol Extract of Snake Fruit (*Salacca edulis* Reinw.) var. Bongkok on Wistar Male Rat

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Abstract: The aim of the study was to investigate antihyperuricemic effect of snake fruit *(Salacca edulis* Reinw.) var. Bongkok Wistar male rates. Antihyperuricemic investigation on Wistar male rats showed that administration of ethanol extract at doses of 200 mg/kg bw decreased serum uric acid level significantly compared to control group at hour 6 and 7 ($P < 0.05$) after inducing with potassium oxonate intraperitoneally simultaneously with uric acid orally. Whereas, administration of ethanol extract at doses of 100 mg/kg bw did not decrease serum uric acid level significantly different compared to control group at hour 6 and 7 ($P < 0.05$). Determination of uric acid level in urine, administration of ethanol extract at a dose of 200 mg/kg bw, or probenecid as a standard drug, at a dose of 45 mg/kg bw increased excretion of urine uric acid level significantly different compared to control group in day of 7 ($P < 0.05$) after inducing with potassium oxonate intraperitoneally simultaneously with uric acid orally. However, increase of uric acid excretion by ethanol extract was lower compared to that of probenecid at a dose of 45 mg/kg bw. Mechanism of action of the ethanol extract as an antihyperuricemia has been proposed by inhibition of xanthine oxidase and finally decreased the synthesis of uric acid and increased the excretion of urine uric acid level.

Key words: Snake fruit var., Bongkok, ethanol extract, antihyperuricemic, probenecid, Wistar male rat.

1. Introduction

Snake fruit (*Salacca edulis* Reinw.) belongs to the class of *Salacca* originated from Southeast Asia. The fruit was named snake fruit because skin of the fruit is brown and looks like a snake skin. Form of fruit is egglike in shape, it contains three pieces of seeds covered with white flesh. In Indonesia there are many snake fruit cultivars, which is known in Java, Sumatera and other island. There are some varieties of snake fruit such as Manonjaya, Bongkok, Banjarnegara, Condet, Pondoh, Bali, Enrengkang, and Sidempuan. Most of snake fruit have an astringent taste and are not sweet. Snake fruit var. Bongkok from Conggeang, a sub district of Sumedang West Java, is more sour, bitter, stringent and not sweet than the other snake fruit.

Snake fruit (*Salacca edulis* Reinw.) var. Bongkok, which grows in Sumedang Regency, West Java, contained flavonoid, alkaloid, terpenoid, tannin, and quinon compound groups, whereas saponin was not found [1]. Two compounds, i.e., 3-hydroxystigmastan-5(6)-en (β-sitosterol) and pyrolle-2.4-dicarboxylic acid-methylester isolated of snake fruit ethyl acetate extract. The pyrolle-2.4-dicarboxylic acid-methylester isolated from snake fruit var. Bongkok is a new compound [2]. Snake fruit var. Pondoh contains sucrose, glucose, fructose and volatile compounds as methyl esters of

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butanoic acids, 2-methylbutanoic acid, hexanoic acid, pentanoic acid and carboxylic acids [3].

Compounds of β-sitosterol and pyrolle-2.4-dicarboxylic acid-methylester can be seen in Figs. 1 and 2.

Snake fruit var. Bongkok becomes an unfavorable fruit and wasted product. In 2003, the harvested snake fruit var. Bongkok decreased by 24%, leading to the extinction. In order to overcome this problem, it is needed to gain the additional economic values of the snake fruit var. Bongkok by studying pharmacological effects *in vivo* test using experimental animal to become medicine or functional food.

Xanthine oxidase (XO) is a flavin enzyme that catalyzes the oxidation of both hypoxanthine and xanthine to uric acid [4]. During the process of purin oxidation by xanthine oxidase, reactive oxygen species such as peroxides generated Xanthine oxidase participates in purine degradation using molecular oxygen as the electron acceptor thereby resulting in production of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) [5]. Therefore, XO inhibitors have been proposed as potential therapeutic agents for treating hyperuricemia as they could be used to block the biosynthesis of uric acid [6]. The aim of the study is to determine antihyperuricemia, as well as to predict mechanism of action of snake fruit *(Salacca edulis* Reinw.) var. Bongkok.

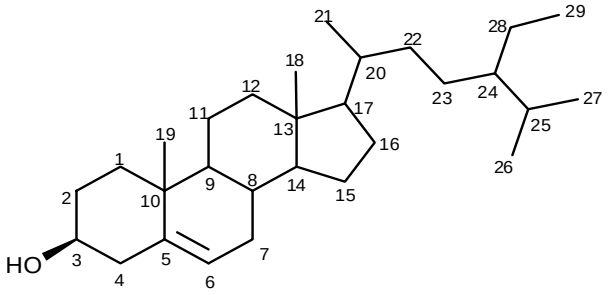

Fig. 1 3-Hydroxystigmastan-5(6)-en (β-sitosterol).

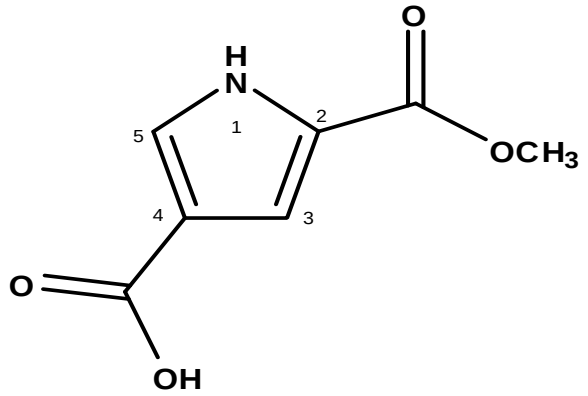

Fig. 2 Pyrolle-2.4-dicarboxylic acid-methylester.

The composition of the species is very attracted to study, its compound has not been known up to now. High uric acid level in blood known as gout can enhance cardiovascular disorder. Allopurinol is used commercially as anti gout with mechanism of action of xanthine oxidase inhibitors. Allopurinol is indicated when uricosuric drugs fail to reduce serum urate lower than 7.0 mg per dL. Xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid. Xanthine oxidase is a complex *metalloflavoprotein* [7].

The ethanol extract at concentrations of 0.01, 0.02, 0.2, 2, and 2,000 μg/mL showed xanthin oxidase inhibition by 20.89%, 32.78%, 44.96%, 50.30%, and 50.25%, respectively, with IC_{50} of 44.95 μg/mL. At the same concentrations, the pyrolle-2.4-dicarboxylic acid-methylester showed *xanthin oxidase* inhibition by 27.7%, 30.5%, 37.3%, 50.27% and 50.55% respectively, with IC_{50} of 48.86 μg/mL. Allopurinol as a standard drug showed IC_{50} of 0.92 μg/mL [2]. Moreover, during the oxidation, free radicals are also generated. Allopurinol (4-hydrxipirazolo [3,4-d] pyrimidin), an analog hypoxanthine, is a specific potent inhibitor for xanthine oxidase, hence decreases blood uric acid level [5]. In this research, it was found that the ethanol extract of snake fruits var. Bongkok showed antioxidant activity decreased serum uric acid level with its mechanism of action similar to which showed xanthin oxidase inhibition *in vitro* [2].

This paper describes mechanism of action of the ethanol extract of snake fruit var. Bongkok as an antihyperuricemia which has been proposed by inhibition of xanthine oxidase and finally decreased the synthesis of uric acid and increased the excretion of urine uric acid level. This study revealed the existence of antihyperuricemia of snake fruit var. Bongkok.

2. Materials and Methods

2.1 Plant Material

The snake fruit (*Salacca edulis* Reinw.) var. Bongkok was collected from Conggeang, a sub district of Sumedang West Java, Indonesia, and it was weighed, peeled, fractionated into little pieces, and dried at 40 °C in tunnel drier to constant weight. The dried samples were ground to fine powder by using a grinder. The dried powdered snake fruit (*Salacca edulis* Reinw.) var. Bongkok (10 g) was macerated in ethanol (100 mL) and kept in container overnight, then filtered (Whatman No.1 filter paper). The filtrates were evaporated at 40 °C.

2.2 Animal Model of Hyperuricemia on Male Rat (in vivo)

Experimetal animal model of hyperuricemia induced by uricase inhibitor potassium oxonate has been used to study [8]. Briefly, male rats were injected intraperitoneally with potassium oxonate (200 mg/kg) and natrium urate (15 mg/kg) orally 1 h before the final drug administration to increase the serum urate level. Whole blood samples were collected from rat by tail vein bleeding. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuge at 12,000 rpm min to obtain the serum. The serum was stored at -20 °C until assayed. Serum uric acid was determined by the phosphotungstic acid methods [9].

2.3 Drug Administration

Snake fruit var. Bongkok extract and probenecid at various concentrations were dissolved in CMC-Na 0.5%. Suspension volume per dose administered based on body weight of rats.

The extract and positive control (drug) orally for five consecutive days performed on twenty-four male Wistar rats randomly divided into four groups, as the following: control group (CMC 0.5%); ethanol group (dose 100 mg/kg bw); ethanol group (dose 200 mg/kg bw); probenecid group (45 mg/kg bw).

Whole blood samples were collected from rat by tail vein bleeding. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 12,000 rpm for 5 min to obtain the serum. The serum was stored at -20 °C until assayed. Dilute urine 1 + 10 with dist. Water, determinate uric acid by the phosphotungstic acid method.

3. Result and Discussion

Fig. 3, antihyperuricemic investigation to Wistar male rats showed by administration of ethanol extract at doses 200 mg/kg bw, decreased serum uric acid level significantly different compared to control group at hour 6 and 7 ($P < 0.05$) after induced with potassium oxonate intraperitoneally simultaneously with uric acid orally. Whereas, administration of ethanol extract at dose of 100 mg/kg bw did not decreased serum uric acid level significantly different compared to control group at hour 6 and 7 ($P < 0.05$). But, probenecid did not decrease serum uric acid level significantly, it is to accelerate the excretion of urine uric acid.

Nguyen and the others [10] have reported inhibitory effect on xanthine oxidase some of medicinal plants in the South of Vietnam such as *Artemisia vulgaris* (leaf), *caesalpinia sappan* (wood), *Blumea blasamifera* (aerial parts) and *T. scandens* (stem).

The efficacy of *Biota orientalis* extract and its main flavonoids consist of quercetin and rutin in reducing serum urate levels in mouse model of hyperuricemia induced by the uricase inhibitor potassium oxonate and *in vivo* inhibiting xanthine oxidase activities in mouse liver [9]. This study is the first to reveal that flavonoids, including Genistein, Apigenin, Quercetin, Rutin and Astilbin, did not show any significant effect on xanthine oxidase activity *in vitro*, but did have a significant effect on xanthine oxidase activities *in vivo*. Moreover, serum xanthine oxidase activity was correlated with serum uric acid levels, while no correlation was observed for liver xanthine oxidase activity [11].

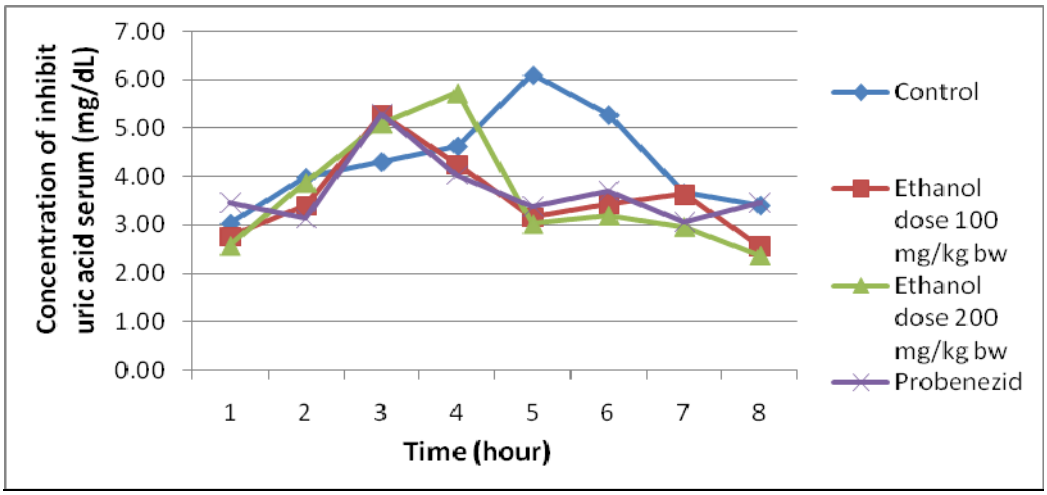

Fig. 3 Antihyperuricemic investigation to Wistar male rats showed by administration of ethanol extract of snake fruit var. Bongkok at doses 100, 200 mg/kg Bw.

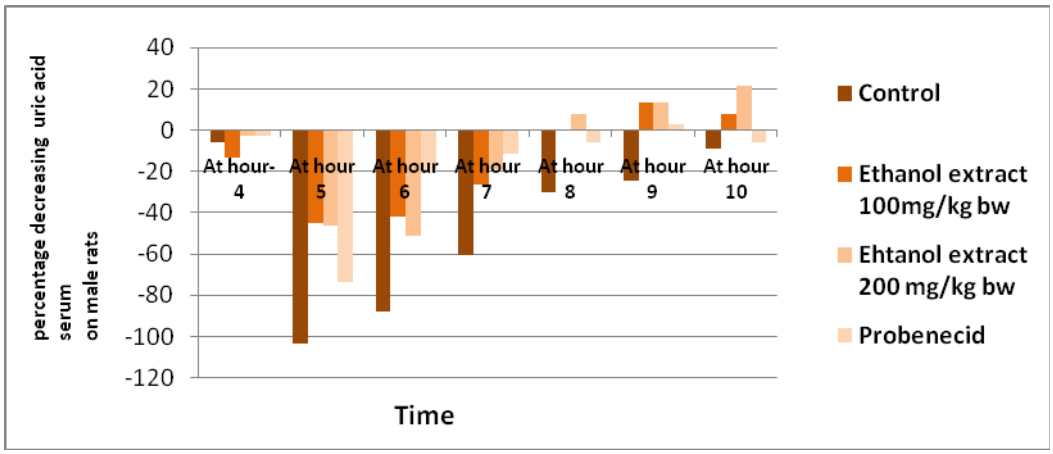

Fig. 4 Percentage of decreasing serum uric acid in male rats.

Fig. 4 shows that, determination of uric acid level in urine, administration of ethanol extract at a dose of 200 mg/kg bw, or probenecid as a standard drug, at a dose of 45 mg/kg bw increased excretion of urine uric acid level significantly different compared to control group in day of 7 ($P < 0.05$) after induced with potassium oxonate intraperitoneally simultaneously with uric acid orally. However, increase of uric acid excretion by ethanol extract was lower compared to that of probenecid at a dose of 45 mg/kg bw. Probenecid is used as anti gout with mechanism of action to accelerate the excretion of urine uric acid (as uricosuric), it has no effect to decrease uric acid serum.

Fig. 5 shows that determination of uric acid levels in urine indicate that administration of ethanol extract at doses of 100 and 200 mg/kg bw showed increased excretion of uric acid in urine is lower than the standard drug. Probenecid at a dose of 45 mg/kg bw increased excretion of urine uric acid level significantly different compared to control group in day of 7 ($P < 0.05$) after induced with potassium oxonate intraperitoneally simultaneously with uric acid orally.

Mechanism of drug action antihyperuricemia consisted of two types that were inhibit the activity of xanthine oxidase and accelerate the excretion of urine uric acid [11]. Moreover, probenecid increases urine uric acid excretion by inhibition of proximal tubulus reabsorption, hence it reduces serum uric acid level

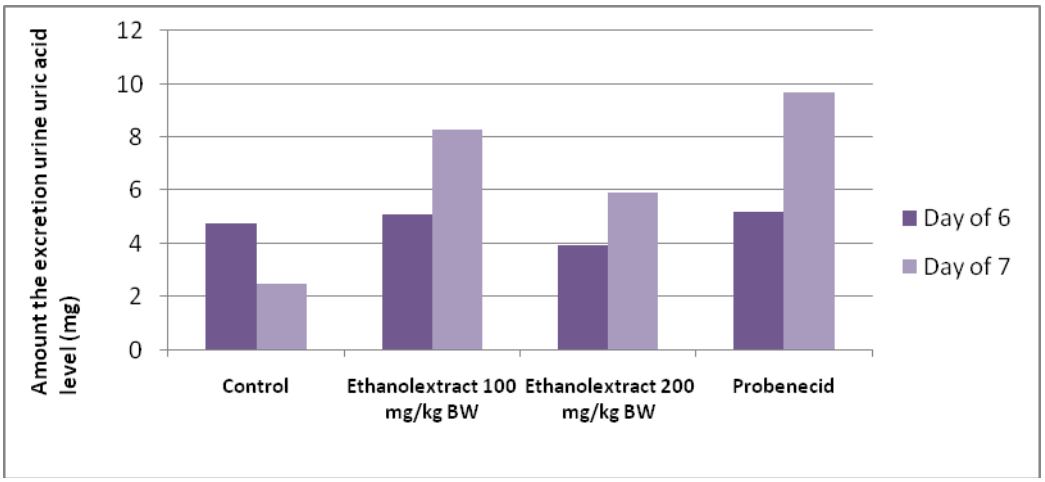

Fig. 5 The excretion of urine uric acid level.

[7]. In this research, it was found that ethanol extract of snake fruit var. Bongkok increased the excretion of urine uric acid level.

This research has discovered the existence of antihyperuricemia in ethanol extract of snake fruit var. Bongkok. Mechanism of action of the ethanol extract as an antihyperuricemia has been proposed to decrease the synthesis of uric acid. Antihyperuricemia mechanism of action of ethanol extract of the uricosuric causes an increase in urinary excretion of uric acid.

The existence of pharmacological active compound from extract of snake fruit (*Salacca edulis* Reinw.) var. Bongkok can be used as a potential lead compound to develop the extract of snake fruit var. Bongkok as medicine and functional food.

4. Conclusion

Mechanism of action of the ethanol extract of snake fruit var.. Bongkok as an antihyperuricemia has been proposed to decrease the synthesis of uric acid and as an *uricosuric* because of increasing the excretion of urine uric acid.

Further researches can be conducted to develop of antihyperuricemic medicine as well as functional food from extract of snake fruit var.. Bongkok are safety tests and formulation development of the extracts and its active compound.

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