

## In Vivo Uricosuric Effect of Red Betel Leaves (*Piper crocatum* RUIZ & PAV) Ethanol Extract

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### Abstract

Hyperuricemia a condition where a person has high levels of uric acid in the blood. Red betel (*Piper crocatum* RUIZ & PAV) is a plant empirically used to reduce uric acid levels. This study aimed to determine the effective dose of red betel plant in reducing urinary uric acid levels in male Wistar rats with hyperuricemia. Hyperuricemia condition was obtained by giving chicken liver juice 25 mL/kg orally and potassium oxonate 250 mg/kg intraperitoneally. Red betel extract (RBE) is made by maceration using 50% ethanol. Male Wistar rats were divided into five groups: negative control (CMC-Na 0.5%), Positive control (Probenecid 4, 5 mg/kg), and extract at doses 11.5, 23 and 46 mg/kg. The results showed that the RBE increased uric acid secretion through urine (uricosuric) compared to controls. The optimal dose for the uricosuric effect of the extract is 46 mg/kg, although this effect is lower than probenecid.

**Keywords:** Red betel, uric acid, potassium oxonate, uricosuric

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## 1 Introduction

Uric acid is produced by the breakdown of purine molecules in the body, which are normally eliminated by the kidneys through urine. Excess uric acid can induce an accumulation of crystals (tofus) in the tissues and connective tissue throughout the body, initiating an inflammatory reaction that can cause pain (Gout Arthritis), but only when the kidneys are no longer capable of producing the components of uric acid. Which is comparable in the body, it can cause excessive uric acid to accumulate like crystals (tofus) in the body and connective tissue throughout the body, thereby triggering an inflammatory reaction that can cause pain (Gout Arthritis) [1].

Based on Riskesdas for gout sufferers in Indonesia in 2018, the results were 11.9% and 24.7% based on diagnosis or symptoms [2]. In Indonesia, gout is second only to osteoarthritis. The prevalence of gout is highest in coastal residents due to the habit or pattern of eating fish and consuming alcohol. In Indonesia, gout is estimated to occur in 840 people every 100,000 [3].

Treatment of gout with synthetic drugs has many side effects, so many people in Indonesia turn to traditional medicine, one of the traditional plants that has the effect of reducing uric acid is red betel. Red betel (*Piper crocatum* RUIZ & PAV) belongs to the Piperaceae family and lives in tropical areas. Indonesia is a country that is crossed by the equator so the climate in Indonesia is a tropical climate, this causes the biodiversity found in Indonesia's tropical forests because plants can grow and develop well. Indonesia's biodiversity must continue to be explored and explored because it has the potential to become a plant that is beneficial for most people, especially in terms of medicine. One of the plants that lives in tropical climates and has potential as a medicine is red betel from the Piperaceae. Red betel is antiseptic, for example it can be used as a mouthwash, to clean feminine areas, and as a medicine for eye inflammation, while parts of red betel leaves can be used as an anti-diabetic, anti-cancer, inflammatory drug, hypertension, hepatitis, Ambien [4],[5].

The benefits of red betel are due to its presence of several active compounds, including flavonoids, alkaloids, polyphenols, tannins and essential oils. Red betel leaf extract contains active compounds, especially alkaloids, flavonoids, steroids, saponins, tannins, polyphenols, quinones, and essential oil groups [6],[7].

Research regarding red betel as a uricosuric has not yet been widely studied. Based on this, research was conducted to evaluate the potential of red betel extract (RBE) as a uricosuric agent in reducing urinary uric acid in rats induced with chicken liver juice.

## 2 Methods

### 2.1 Extraction

Red betel leaves were sourced from Lembang, West Java, and an ethanol extract was prepared using the maceration method with a 50% ethanol solvent. The red betel leaves that have been collected are sorted wet and washed under running water. After the washing stage, chopping and drying are carried out using a drying cabinet with a temperature of 40-60°C. Extraction was done by cold extraction by macerating 500 grams using a macerator and 2 liters of 50% ethanol as a solvent. Weigh a certain amount of sumplicia powder, put it in a macerator, add 50% ethanol solvent, then macerate for 24 hours for three days. The filtrate is collected, then the filtrate resulting from maceration is evaporated using a rotary evaporator until a thick extract is formed, then the extract is dried using 50°C until a thick extract of red betel leaves is obtained.

### 2.2 Phytochemical screening

Phytochemical screening for red betel leaf includes an examination of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, polyphenols, monoterpenes, and sesquiterpenoids.

### 2.3 Animals and grouping

Healthy male Wistar rats, with weights ranging from 150 to 200 grams, were acquired from the Bioscience and Biotechnology Research Center Laboratory at Bandung Institute of Technology. They were acclimatized

for seven days. Test animals were grouped into six groups (4 animals/group) consisting of the negative control (0.5% sodium carboxymethyl cellulose); positive control (probenecid 4.5 mg/200 g); The test group received doses of RBE at 11.5, 23, and 46 mg/kg.

#### 2.4 Chicken Liver Juice Induction

Wash 300 grams of fresh chicken liver, cut it into small pieces, then put it into a blender, then add 75 mL of distilled water. Once smooth, put the chicken liver into a container. Chicken liver juice is made fresh every day. The dose of chicken liver juice induced in test animals was 5 ml/200g BW given two times/day adjusted to the maximum capacity of the volume of fluid that mice can drink, namely 10 ml/200g BW[8].

#### 2.5 Preparation of Potassium Oxonate

To create hyperuricemia conditions in test animals, the dose of potassium oxonate given is 250 mg/kg (50 mg/200 g). A total of 625 mg of potassium oxonate was weighed and dissolved in 0.9% NaCl solution to a volume of 25 mL. The concentration of potassium oxonate suspension obtained was 25 mg/mL.

#### 2.6 Uricosuric activity test

On Day 0, the urine uric acid levels of all test animals in each group were assessed, after which they were administered chicken liver juice for three consecutive days. All groups received an intraperitoneal induction of 250 mg/kg of potassium oxonate on the fourth day. All groups received their respective test preparations orally one hour after the potassium oxonate induction. Urine collection was carried out at 60, 90, and 120 minutes and 24 hours after administration of the test preparation. A total of 1 mL of rat urine sample was put into an Eppendorf tube, then 9 ml of distilled water was added and then homogenized. 20µl of urine was taken, and 1000µl of uric acid reagent was added. After that, it was incubated for 10 minutes at room temperature (37 °C). Uric acid levels were measured using a micro-lab instrument at a wavelength of 546 nm.

#### 2.7 Statistic analysis

Data were analyzed using GraphPad Prism 8.3. Data displayed are the mean  $\pm$  SD.  $P < 0.05$  is significantly different.

### 3 Results and Discussions

The red betel leaves used in the research were in the form of dried simplicia, which then carried out characterization of the simplicia such as determining water-soluble and ethanol-soluble content, determining total ash content, determining water-soluble ash content, determining acid-insoluble soluble ash content, determining water content, and simplicia phytochemical screening tests were carried out including tests for polyphenols, quinones, tannins, alkaloids, flavonoids, saponins, monoterpenoids & sesquiterpenoids, as well as steroids & triterpenoids. Testing the characteristics of simplicia ensures that red betel leaf simplicia meets the requirements to be used in research. The results of the simplicia characteristics are shown in table 1. The phytochemical screening test for simplicia aims to see the red betel leaf simplicia content.

Table 1 Characteristics of Simplicia

Characteristics of Simplicia	Results	References [2]
Water content	2,8% w/v $\pm$ 0,28	<10%
Total Ash Content	18,1% w/w $\pm$ 0,20	<12,1%
Water Soluble Ash Content	4,05% w/w $\pm$ 0,2	-
Acid Insoluble Ash Content	10,25% w/w $\pm$ 0,15	<2,3%
Water Soluble Essence Content	36,30% w/w $\pm$ 0,29	>13,9%
Ethanol Soluble Essence Content	31,24% w/w $\pm$ 0,23	>8,9%

This research used the maceration method to extract red betel leaves. This method was chosen so that the metabolite compounds do not decompose/damage due to heating, therefore, cold extraction is used. The solvent used to extract red betel leaf simplicia is 50% ethanol. 50% ethanol was chosen because the ethanol solvent is universal, more non-toxic, and more polar, so the metabolite compounds in red betel leaves are easier to extract with polar solvents. After the extraction process is complete, the filtrate resulting from maceration is evaporated using a rotary evaporator until a thick extract is formed. Then phytochemical screening is carried out again to ensure that the active compounds remain in the extract. The results of the phytochemical screening can be seen in Table 2.

Table 2 Phytochemical Screening of Simplicia &amp; Extracts

Compound	Simplicia	Extract
Alkaloids	+	+
Alkaloids	+	+
Saponin	+	+
Tannin	+	+
Polyphenols	+	+
Monoterpenoids & Sesquiterpenoids	+	+
Steroids	+	+
Quinone	-	-

Note : + = detected, - = not detected

Phytochemical screening results align with research conducted by Jayalakshmia in 2015, which showed positive results for phenolic compounds, tannins, glycosides and steroids. Then according to research conducted by Rukmini, the results of the phytochemical test were positive for alkaloid and flavonoid compounds [9],[10].

The inducers used were potassium oxonate and chicken liver juice to test the uricosuric activity. Chicken liver juice was chosen because chicken liver juice is a food source of purine which contains 150 -1000 mg/100 grams of purine. Purine is a component of nucleic acid found in the nucleus of the body's cells and uric acid itself is the final product of purine metabolism in the body, so if purine levels increase in the body, hyperuricemia will occur. Potassium oxonate was chosen as the inducer because it has a fast onset inhibiting mice's uricase enzyme. The uricase enzyme in mice is an enzyme that plays a role in converting uric acid into the compound allantoin, which is more soluble in water, so it is easily excreted. The effective dose to inhibit uricase enzyme activity is 250 mg/Kg BW. This dose is usually used to induce gout in mice, which are used as animal models of hyperuricemia [11],[12]. Probenecid in this study was used as a standard because it has a uricosuric effect in reducing uric acid levels in the blood. Probenecid will increase the excretion of uric acid through urine so that less uric acid is contained in the blood.

The results of the uricosuric activity test are shown in Figure 1. Based on our results, the probenecid and all doses RBE groups exhibited elevated uric acid excretion in the urine, significantly higher than the control group ( $P<0.05$ ). The higher uric acid increase in the urine compared to the control group indicates that red betel has uricosuric capabilities. In the case of probenecid, uric acid excretion in the

urine was notably higher and significantly different with all doses RBE ( $P<0.05$ ). The uricosuric activity of RBE is dose-dependent. The RBE group exhibited the highest uric acid excretion in urine at a dosage of 46 mg/kg, although this effect was less pronounced than that of probenecid.

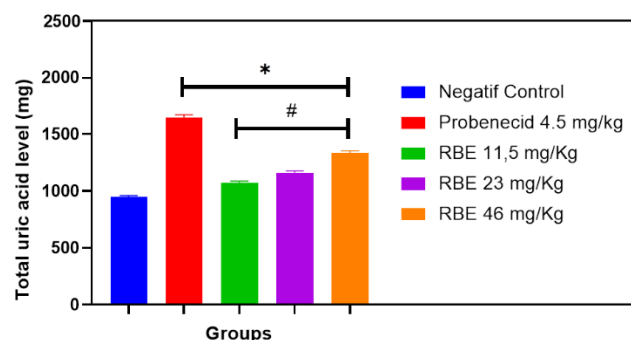


Figure 1. RBE uricosuric activity on day 4. Data presented are the mean  $\pm$  SD, N=4, \* = statistically significant compared to the control group ( $P<0.05$ ), # = statistically significant compared to probenecid ( $P<0.05$ ), RBE = red betel extract.

The flavonoid compounds influence red betel leaf extract's anti-hyperuricemia ability in increasing uric acid excretion in rat urine [13]. The flavonoid compounds present in red betel extract can also enhance urine production in male Wistar rats. This mechanism operates by elevating the glomerular filtration rate within the body [14]. Research conducted by Li reported a relationship between dietary intake of flavonoids (anthocyanins and flavanones) and hyperuricemia, significantly reducing serum uric acid levels and hyperuricemia [15]. The ability of red betel as an anti-hyperuricemic is strengthened by research conducted by Wahyuningsih et al. that ethanol extract of red betel can decrease blood uric acid levels [16].

#### 4 Conclusions

All doses of RBE exhibit uricosuric activity, with the most effective dose being 46 mg/kg. Red betel shows promise as a potential anti-hyperuricemic agent, but further research is required for development.

## 5 Declarations

### 5.1 Acknowledgments

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### 5.3 Author contribution

All authors contributed equally to this research

### 5.4 Ethic

This research has received approval from the Preclinical Ethics Committee, Faculty of Pharmacy, General Achmad Yani University, with no 8008.2/KEP-UNJANI/I/2021.

### 5.5 Conflict of Interest

The author declares that there are no conflicts of interest in this research

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