

ACTIVITY OF ETHANOLIC EXTRACT FROM *Justicia gendarussa* Burm. LEAVES ON DECREASING THE URIC ACID PLASMA

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Abstract

The effect of *Justicia gendarussa* leaves extract has been investigated using oxonate-induced hyperuricemic male albino rats. In this experiment thirty five male albino rats were divided randomly into seven groups. There were three group doses variations of *J. gendarussa* extract that were 1.3 g/kg bw, 2.6 g/kg bw and 5.2 g/kg bw; two comparison groups that were allopurinol 0.68 g/kg bw and herbal "X" 0.85 g/kg bw; and two control groups that were induced control potassium oxonate 0.25 mg/kg bw and normal control CMC 0,5 %. Intraperitoneal administration of potassium oxonate 0.25 mg/kg bw was given one hour before administration of test drugs in eight day and plasma uric acid was measured in rats after two hours. Plasma uric acid was measured by spectrophotometric on 520 nm with enzymatic method. The results showed that all extracts could reduced uric acid level of rats. The decreasing potency of uric acid level was equal to doses increase, so the best result that can reduce uric acid level was *J. gendarussa* extract at a dose 5.2 g/ kg bw. This results indicated that *J. gendarussa* leaves extract may be effective for the prevention and the treatment of hyperuricemia.

Keywords: enzymatics, hyperuricemia, Justicia gendarussa Burm, spectrophotometric

1. Introduction

Hiperuricemia is the condition that is shown by uric acid level in blood which higher than normal and it can cause by the increasing of production or decreasing of uric acid excretion. Hyperuricemia action can accumulate uric acid crystal in bowl, so it can cause a pain which is known as Gout [1]. Clinically reported, the key factor uric acid is related not only to an increased risk of gout, but also to an increased risk of cardiovascular disorder, nephrolithiasis and diabetes [2].

Allopurinol remains to be a dominant urate-lowering agent, however, adverse effects limit its therapy [3]. Allopurinol can cause the side effect, such as nephrolithiasis, allergic reaction and increase the toxicity of 6-merkaptopurin [4,5], so its important to perform the experiment to evaluate hypouricemic drug by using medicinal plants. Medicinal Plants are believed to be an important sources of new chemical substances with potential therapeutic effect.

The leaves of *Justicia gendarussa* have been used in Indonesian traditional medicine for treatments of rheumatoid gout, and dyssuria [6,7], but the data as hypouricemic agent has not found, so this experiment was conducted to find out the activity of *J. gendarussa*

leaves ethanolic extract on oxonate-induced hyperuricemic rats [6,8]. The measurement of uric acid concentration in blood plasma rats is performed by using enzymatic method [9]. The result enzymatic process was measured by spectrophotometric on 520 nm.

2. Methods

Plant materials. *J. gendarussa* Burm (Acanthaceae) leaves were collected from Pharmacy Department of Indonesian University Garden in Depok Indonesia. The identification of the plant was authenticated by Eko Baroto Waluyo the expert at Research Center for Biology, Indonesian Institute of Sciences, Bogor. *Gendarussa* leaves were washed with water, dried on air at room temperatur, and then in oven at 40 °C. The dried leaves crushed and expressed trough the sieved to get powders mesh 50.

Reagents and Chemicals. Potasium oxonate (Aldrich Chemical), ether (Merck), Radox reagent (Radox Laboratories), allopurinol (Nanjing Pharma Chemical Plant), heparin, and CMC.

Animals. Albino male *Rattus novergicus* strains Sprague-Dawley rats (200-300 g) were purchased from Animal Veteranery of Bogor Agricultural university.

They were allowed one week to adapt to their environment before used for experiments.

Preparation of extract. The powders of *Gendarussa* leaves (200 g) were macerated with 60% ethanol at room temperature, shake frequently for 6 hours, then allowed to stand for 24 hours. The liquid extract filtered 2 times through cotton sieve. The residue was extracted for 4 times as similar with those above procedure [10]. All filtrates were mixed and evaporated by rotary vacuum evaporator and then dried on waterbath until a thick extract was obtained.

The extract was characterized by this parameters [10]: 1) Specific parameters. Organoleptics: form, color, odour and taste; water soluble extractive: acid insoluble ash, 2) Nonspecific parameters included: loss on drying; total ash; acid insoluble ash, 3) Phytochemical screening: the extract was screened for the presence of secondary metabolites such as alkaloids, flavonoids, sterols/terpens, tannins and saponins. After all parameters were fulfilled as general standard requirement, then the extract was used for pharmacology assay.

Assay of gendarussa extract as hypouricemic agent.

This experiment has been performed by using seven groups of Albino male rats (*Rattus norvegicus*) strain *Sprague-Dawley*, and each group consists of five rats. The samples were orally administered with 3 varieties of doses. The doses was equal calculated to dried leaves dosage (10,3 g) for adult which is commonly use as traditional drug for treatment gout. The extract doses for rats were: Group I (doses 1) 1.3 g/kg bw; Group II (doses 2) 2.6 g/kg bw; and Group III (doses 3) 5.2 g/kg bw.

Two comparison groups that were allopurinol 0.18 g/kg bw and Herbal "X" 0.85 g/kg bw; and two control groups that were induced control potassium oxonate 0.25 mg/kg bw and normal control carboxy methylcellulose 0.5%. All groups were treated for 7 days by giving each treatment according the group one time a day. At the 8th day, intraperitoneal administration of potassium oxonate 0.25 mg/kg bw was given one hour before administration of test drugs and plasma uric acid was measured in rats after two hours.

Preparation of blood and plasma. Fresh blood was collected by haematocrit through sinus orbital, then was put into microtube which contained anticoagulant heparin. The blood samples were then centrifuged at 7000 rpm for 5 min to separate the plasma. Plasma samples which contained uric acid, were reacted with Kit reagent and then incubated for 15 min at 25 °C.

Measurement of uric acid plasma. Two hours after induction, the measurement of uric acid level was performed by using enzymatic method. The quantity of uric acid was measured by spectrophotometric-

enzymatic using Kit reagent (Randox) which contained uricase and peroxide. The result of enzymatic process was measured by spectrophotometric on 520 nm.

Statistical analysis. The results were statistically analyzed by Kolmogorov-Smirnov and one way ANOVA Test. The results of homogeneity test were analyzed by Lavene and normality test by Kolmogorov-Smirnov.

3. Results and Discussion

The results of extract characteristic were given in Table 1. Specific parameters such as organoleptics, water soluble extractive and diluted ethanol soluble extractive, nonspecific parameters were included loss on drying, total ash and acid insoluble ash; phytochemical screening.

Pharmacological assay is performed to investigate the decrease of uric acid concentration in blood plasma hyperuricemia. The result showed that extract groups and standard groups could decrease uric acid level. The uric acid concentration (mg/dL) of each group is given in Fig 1.

Table 1. Characteritic of Extract

Parameter	Result
Specific parameters:	
Organoleptics	Thick extract, brownish-black, slightly bitter taste.
Water soluble extractive	66.46%
Diluted ethanol soluble extractive	62.68%
Nonspecific parameters:	
Loss on drying	21.25%
Total ash	7.52%
Acid insoluble ash	5.13%
Phytochemical screening:	Alkaloid, flavonoid, terpen/sterol and saponin

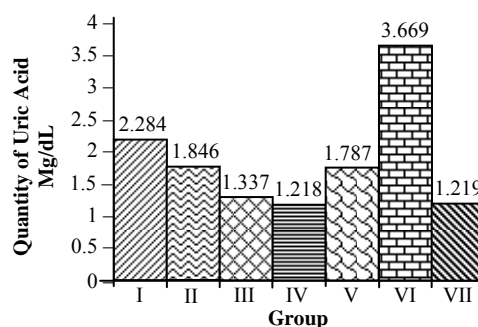


Figure 1. Uric Acid Average Concentration of Each Group After 8 Days Experiment. Group I = Extract 1.3 g/kg bw, Group II = Extract 2.6 g/kg bw, Group III = Extract 5.2 g/kg bw, Group IV = Allopurinol 0.18 g/kg bw, Group V = Herbal "X" 0.85 g/kg bw, Group VI = Induction Control Potassium Oxonate 0.25 g/kg bw, Group VII = Normal Control CMC 0.5% Dose 3 mL/200 g bw

The characterization of extract was performed to get the extract quality according to standard requirement of medicinal plants extract. After all parameters were fulfilled as general standard requirement, then the extract could be used for pharmacological assay.

Pharmacological assay has been performed by using seven groups of albino male rats (*Rattus norvegicus*) strain *Sprague-Dawley*, and each group consists of five rats.

Group I, II, III were orally administered with extract samples because the samples were usually give orally for humans. For decreasing individual variation of rats, the samples were given 3 mL/200 g body weight, the same volume for all groups to decrease individual variation. Each sample was suspended in 0,5% CMC solution.

Group IV was orally administered with allopurinol as standard because allopurinol was sintetic drug which is commonly used to be a dominant urate-lowering agent. Allopurinol dose was 200 mg for people, so the rats dosage were equal to 0.18g/kg bw.

Group V was orally administered with Herbal "X" as standard of herbal medicine, because it has been used and marketed in large area in Indonesia. Dosage of herbal for adults were two capsules a day, which are equal to 940 mg a day. Dosages for rat was equal to 0.85 mg/kg bw.

Group VI was intraperitoneally administered with potassium oxonate as control induction. The increasing of uric acid in plasma rats could be induced by caffeine or potassium oxonate. This research used potassium oxonate, because it was a potent inhibitor uricase or uric oxidase and could be used with animal model such as rat, mice, rabbit, dog and pig. To make hyperuricemia in plasma rats, potassium oxonate was given by i.p. with a dose 0.25 g/kg bw [3,4]. The highest uric acid level could reach 2 hours after potassium oxonate were given intraperitoneally.

Group VII was normal group. This group was only orally administered with 0.5% CMC solution. All groups were treated for 7 days by giving each treatment one time a day. At the 8th day, all groups were induced by potassium oxonate intraperitoneal with a dose of 0.25 g/kg bw of rat except group VII (normal control) which was only given 0.5% CMC solution. One hour after the given sample, the blood of each group was taken through sinus orbital of rat. This method was chosen because the procedure was fast, the volume of blood was much enough and fluently, and did not made the animal stressed, so the risk of lisis was decreased significantly.

Before taking blood, the rat must be anesthesia to make unconsciousness. The blood was collected by haematocrit through sinus orbital, then put into microtube which has been filled with heparin to prevent the coagulation of blood. The blood was centrifuged at 7000 rpm for 5 minutes to separate the plasma. The plasma is commonly used to measure the quantity of uric acid. The quantity of uric acid was measured by spectrophotometric-enzymatic by using Kit reagent (Randox) which contained uricase and peroxide. This method was chosen because it was simple, selective and specific to determine the quantity of uric acid. Uric acid could be measured by spectrophotometer because uric acid oxidation that is produced with uricase could produce hydrogen peroxide. Then, the hydrogen peroxide will react with dichlorohydroxybenzenuisulphonate (DCHBS) and p-aminophenazon (PAP) produces quinonimin as chromophor. This reaction was catalized by peroxidase. Plasma sample which contained uric acid were reacted with Kit reagent and then incubated for 15 min at 25 °C. This incubation was intended to get optimum absorption and quinonimine was relatively stable. The measurement were performed at λ 520 nm to avoid the interferences of hemolysis hemoglobin and turbidimetric reaction. The true λ maximum was at 512 nm.

The results was statistically analyzed by Kolmogorov-Smirnov and one way ANOVA Test. Test showed that the data of uric acid level from all groups had significant different to induction group ($\alpha < 0,05$). The result showed that extract groups and standard groups could decrease uric acid level. The result from Minimal Significant Different Test, is showing that uric acid concentration after providing extract at the dose 3 (5.2 g/kg bw), was not significantly different with allopurinol standard and normal control ($\alpha > 0,05$). The effectivity of extract at the dose 3 was not significantly different with allopurinol on decreasing uric acid concentration.

Uric acid quantity after providing with doses 1 and 2 were significantly different with allopurinol standard, but not significantly different with Herbal "X". The lowering effectiveness of uric acid concentration provided with extract at doses 1.3 g /kg bw, extract 2.6 g/kg bw, extract 5.2 g/kg bw, allopurinol 0.18 g/kg bw and herbal "X" 0.85 g/kg bw were 56.53%, 74.41 %, 95.14%, 100.04%, and 76.82%. The data showed that the effectiveness of *J. gendarussa* leaves ethanolic extract were increased equally to dosages level. The extract with the dose 5.2 g/kg bw showed the greatest effectiveness and significantly different with Herbal "X", but not with allopurinol. The effectiveness data of each group was shown in Fig 2.

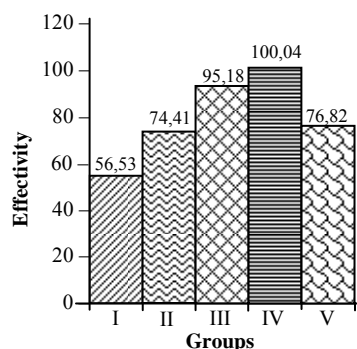


Figure 2. The Effectiveness Extract Groups and Standard Toward Normal Control. Group I = Extract 1.3 g/kg bw, Group II = Extract 2.6 g/kg bw, Group III = Extract 5.2 g/kg bw, Group IV = Allopurinol 0.18g/kg bw, Group V = Herbal "X", 0.85 g/kg bw

4. Conclusion

From the data presented in this paper we can conclude that the chemical constituents of ethanolic extract are flavonoids, terpenes/sterols, and saponins. We have also found that all of ethanolic extract from *J. gendarussa* leaves at doses 1.3 g/kg bw, 2.6 g/kg bw, 5.2 g/kg bw, can decrease serum uric acid levels in hyperuricemic rats. The effectiveness of *J. gendarussa* leaves ethanolic extract increased as a function of doses level. The extract at the dose of 5.2 g/kg bw showed the greatest effectiveness and significantly different with Herbal "X", but not with allopurinol.

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