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# Activity of Ethanol Extract of Homalomena Occulta Rhizome in Inhibiting Kidney Stone Formation in Wistar Rats

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**Abstract.**
*Homalomena occulta* is a plant that grows in Madiun, especially Caruban. Patients can overcome kidney stone disease by boiling the *H. occulta* (nampu) rhizome with water. The present study aimed to evaluate the antilithiatic effect of the ethanol extract of the *H. occulta* rhizome in preventing nephrolithiasis in rats. Ethylene glycol was used to induce the urolithiasis in the Wistar rats. The rats were divided into six groups, namely I (normal), II (ethylene glycol induced), III (commercial drug treated, Cystone® 750 mg/kg BW), IV (*H. occulta* rhizome extract treated, dose 250 mg/kg BW), V (*H. occulta* rhizome extract treated, dose 500 mg/kg BW), and VI (*H. occulta* rhizome extract treated, dose 1000 mg/kg BW).

**Keywords:** kidney stone, ethylene glycol, calcium level, *Homalomena occulta*

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## 1. Introduction

Kidney stone disease including kidney disease is quite high in prevalence. The prevalence of kidney stone disease in Indonesia is 0.6 percent. The highest prevalence was in DIY (1.2%), followed by Aceh (0.9%), West Java, Central Java, and Central Sulawesi each with 0.8 %.

The prevalence of the disease is estimated at 13% in adult males and 7% in adult females, with peaks in the third and fourth decades of life. The incidence of kidney stones based on data collected from hospitals throughout Indonesia in 2002 was 37,636 new cases, with 58,959 visits. In addition, the number of patients treated reached 19,018 people, with a mortality of 378 people [1].

Kidney stones are small stones that form in the kidneys due to deposition that occurs in the urine moving down the urinary pipe (ureters). This stone can block the urinary tract (urethra) and when urinating causes pain and difficulty getting out. Kidney stones

are likely to form when one or more calcium crystal-forming factors are found and cause aggregation of stone formation [2].

Generally, kidney stones occur because the body lacks fluids so that cloudiness or urine becomes concentrated. As a result, there is a blockage in the channel from the kidneys to the bladder. Stones in the kidneys are formed from chemicals that are usually found in urine such as calcium, uric acid, phosphate, and usually there are other chemicals. There are four types of stones that are often found in the kidneys, namely calcium oxalate stone (80%), struvite stones (10%), uric acid stones (9%), cystine stones (1%) [3].

The risk factors for the formation of kidney or urinary tract stones are closely related to metabolic abnormalities in each person, the type of food consumed, the volume of fluid, gender, and genetics. Of these factors, the most influential are the consumption of food and water or water that is drunk, age, and especially those with high calcium levels are at risk of increasing calcium levels in urine so that it has an impact on decreasing urine acidity. This is one of the originators of stone formation. Likewise, if the water you drink is very a little occurThe imbalance between the amount of salt and the volume of water in the kidneys causes high levels of saturation and consequently crystallization occurs. The results of monitoring at several hospitals in Jakarta show that patients with kidney stones who are treated in hospitals generally drink less than one liter of water per day.

Kidney stones are formed due to the presence of calculi in the urinary tract due to two basic phenomena. The first phenomenon is the supersaturation of urine by stone-forming constituents, including calcium, oxalate, and uric acid. Crystals or foreign bodies can act as a calculus matrix, where ions of the supersaturated crystal form form microscopic crystal structures. The calculi that are formed cause symptoms when they hit the ureters when they go to the urinary bladder [4].

Phenomenon second, which The most likely role in the formation of calcium oxalate calculi is the deposition of calcium matrix calculi material in the renal papilla, which is usually Randall's plaque (which is always composed of calcium phosphate). Calcium phosphate precipitates on the basement membrane of the thin Loop of Henle, erodes into the interstitium, and then accumulates in the subepithelial space of the renal papilla. The subepithelial deposits, long known as Randall plaques, are eventually eroded through the papillary urothelium. The rock matrix, calcium phosphate, and calcium oxalate are gradually deposited on the substrate to form calculus in the urinary tract [5].

Treatment of kidney stones includes emergency management of renal (ureteral) colic, including if there is an indication for surgical intervention, and medical therapy

for the calculi.The treatment includes chemical drugs such as diuretic drugs, xanthine oxidase inhibitors, potassium citrate, traditional medicine, as well as by performing a surgery/surgery .

Nampu or occulta is a plant that based on the empirical experience of the Madiun people can shed kidney stones so that the plant is used as an alternative treatment for kidney stones. Homalomena occulta or known by the local name but contains saponins, flavonoids, tannins, and polyphenols [6]. The content of chemical compounds from Nampu is sesquiterpenoids, monoterpenoids, triterpenoids, phenolic acids, and essential oils . One of the Nampu content that has activity as a Xanthine Oxidase Inhibitor is apigenin. To determine the activity of these plants, an in vivo study was conducted.Homalomena.

## 2. Research Methods

This study used a post test only control group design research method using 24 Rattus norvegicus divided into 6 groups consisting of a normal group, a negative control group, a positive control group and a test group of Homalomena occulta rhizome ethanol extract at a dose of 250 mg/kg BW, a dose of 500 mg/kg BW, and a dose of 1000 mg/kg BW. The extraction process was carried out in the integrated chemical laboratory of the University of Muhammadiyah Malang. Prior to the plant extraction process, a determination was made at the UPT. Purwodadi Botanical Garden Plant Conservation Center-LIPI. After the extraction process, the activity test was carried out by observing the kidney characteristics based on the shape, color, kidney weight, and kidney ratio. Measurement of calcium levels was carried out using the instrument AAS (*Atomic Absorption Spectrophotometry*) conducted at the State University of Malang. The results obtained from this method are data on kidney ratios and kidney calcium levels which are compared between each group to determine the magnitude of the decrease in the kidney ratio and the percentage of inhibition of kidney stones. Then the results were analyzed using the one way ANOVA method using the SPSS 18.0 edition to find out whether there were significant differences between each treatment group.

## 3. Results And Discussion

### 3.1. Plant Determination

The results of the determination showed that the plant we used in this study was a nampu plant of the Homalomena occulta species from the Arecaceae family.

### 3.2. Plant Extraction

The extraction process was carried out by maceration method using 96% ethanol as solvent. Maceration was carried out by soaking 1 kg of dry simplicia powder with a solvent ratio of 1:10. After obtaining the filtrate, it was then concentrated using a rotary evaporator to obtain a thick extract. The extract obtained as much as 133.2 grams with a weight yield of 13.3% extract.

### 3.3. Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolites in the form of a group of compounds found in the ethanolic extract of nampu rhizome (Homalomena occulta). The phytochemical screening carried out included the identification of saponin, triterpenoid and steroid glycoside compounds and the identification of flavonoid compounds because it was based on the journal Daimartha (2003) and Zeng, et al,. (2011), the rhizome can contain compounds from this group. Phytochemical screening was carried out by color testing and Thin Layer Chromatography (TLC). In the ethanol extract of Homalomena occulta rhizome, positive results were obtained containing flavonoids, sapogenin triterpenoids and free steroids/terpenoids.

### 3.4. Activity Test

The activity test was carried out by observing the characteristics of the rat kidney including the shape, color, and ratio of the kidney, as well as analyzing kidney calcium levels using the AAS (Atomic Absorption Spectrophotometry) method. Prior to the activity test, the experimental animals were selected with a weight between 150 g - 200 g, and adapted for approximately 1 week. After that, the experimental animals were selected randomly and divided into 6 treatment groups. Group 1 is a normal control, the rats were fed and drank moderately. Group 2 is a negative control group, rats were given 1% ethylene glycol induction by oral route. Group 3 is the positive control group or the

comparison, the rats were given Cystone® at a dose of 750mg/KgBW which previously was given 1% ethylene glycol by the oral route.

250 mg/kg BW where previously given ethylene glycol 1% by oral route. Group 5 was a test dose of 500 mg/kg BW rats were given an ethanol extract of the rhizome of Homalomena occulta at a dose of 500 mg/kg BW where previously given ethylene glycol 1% by oral route. Group 6 was the test group at a dose of 1000 mg/Kg BW rats were given an ethanol extract of the rhizome of Homalomena occulta at a dose of 1000 mg/kg BW where previously 1% ethylene glycol was given by oral route.

The treatment for each group was carried out for 17 days, then on the 18th day the animals were killed by being anesthetized using chloroform. The rats that had been sacrificed were dissected, then their kidneys were taken and analyzed.

## 3.5. Kidney Characteristics Analysis

Analysis of kidney characteristics was carried out on each kidney of rats including kidney color, kidney shape, kidney weight, and the calculation of the ratio of kidney weight using the formula:

$$= \frac{100\ (g)}{\text{Berat badan tikus (g)}} \text{ x Berat Ginjal (g)}$$

The results of kidney characteristics can be seen in table 1. Based on the results of kidney characteristics, there was an increase in kidney weight of rats induced by ethylene glycol, ethylene glycol can increase oxalate levels in the kidneys, this is because ethylene glycol in the body is metabolized into oxalic acid. Rate High oxalate levels are known to increase the bond between calcium and oxalate to form crystals. The hyperoxaluria condition causes an increase in the deposition of calcium oxalate crystals in the kidneys, thereby increasing the weight of the rat kidney.

Observations based on kidney weight and weight ratio showed that the negative control group had a greater kidney weight and weight ratio than the other groups, namely the normal group, the positive control group and the dose test group, this is because the administration of ethylene glycol causes changes in kidney structure in terms of shape, color and size. as well as kidney weight due to nephrotoxicity due to too high calcium levels in the kidneys.

Based on Table 1, all groups had no different shapes and colors, but the highest kidney weight ratio occurred in the test group at a dose of 500 mg, followed by a test group at a dose of 1000 mg. This is not in accordance with what was predicted that when experimental animals were given kidney stone induction treatment and then given the

Table 1: Average analysis of rat kidney characteristics.

| Experimental Group | Color | Shape | Flat Rat weight | Kidney Weight | Kidney Ratio |
|---|---|---|---|---|---|
| Normal Group (KN) | Red tanned | Peanut Red | 191 g | 1.4258 g | 0.7453 |
| Negative Control Group (KNf) | Red tanned | Peanut Red | 174 g | 1.3660 g | 0.7845 |
| Positive Control Group (KP) | Red tanned | Peanut Red | 152 g | 1.1028 g | 0.7272 |
| EERN test group dose 250 mg/kg BW | Red tanned | Peanut Red | 163 g | 1.0599 g | 0.6458 |
| EERN test group dose 500 mg/kg BW | Red tanned | Peanut Red | 145 g | 1.1422 g | 0.8190 |
| EERN test group dose 1000 mg/kg BW | Red tanned | Peanut Red | 150 g | 1.1979 g | 0.8051 |

test solution, the extract dose was expected to decrease the kidney ratio significantly, but this was the opposite. This may be due to oxidative stress in experimental animals because in the administration of a sufficiently thick test solution which will trigger edema and can make experimental animals in a poor pathophysiological state.

Based on previous studies, the highest kidney ratio was in the negative group, this was caused by chronic or acute accumulation of calcium oxalate causing lipid peroxidation, which in turn caused damage to renal epithelial cells, where swelling occurred which led to an increase in kidney weight (Ghodasara, et al. , 2010). Calcium crystals contained in the kidneys are also the cause of the increase in kidney weight (Hadjadeh, et al., 2008). The results of statistical tests on the ratio of kidney weight in each group and several variations in the dose increase of EERN 250 mg/kg BW, 500 mg/kg BW and 1000 mg/kg BW did not give a significant difference between each group.

## 3.6. Analysis of Kidney Calcium Levels

After analyzing the ratio of the rat kidney, then measuring the calcium level in the kidney using the Atomic Absorption Spectrophotometry (AAS) instrument. The rat kidney was stored in an evaporating dish and placed in an oven at 100oC for 24 hours. After that, the dried kidneys were ground in a mortar and then put into a volumetric flask. Add 10 ml of concentrated nitric acid then heated on a water bath, stop heating then add Hydrogen Peroxide (H2O2) until the solution is clear while heating. After the solution is clear, then cool and do the dilution, take 5 ml and add aquadesate to 50 ml then filter with whatman paper.

Then the solution was measured using AAS. Rat kidney calcium is calculated by the formula [7]:

$$\text{kadar kalsium} = \frac{\text{x}\left(\frac{\mu g}{mL}\right).\text{V(mL)}}{\text{Vs (mL)}} \text{ x Fp}$$

**Fp : Dilution factor of the result of destruction**

The results of the measurement of calcium levels in the kidneys can be shown in table 2. Based on the results of measurements of calcium levels in the kidneys of rats, it was found that the calcium levels in the negative group were higher than the calcium levels in the normal group. This is due to the presence of 1% ethyleneglycol which causes the accumulation of calcium oxalate crystals where the more acidic the pH of the urine is, the more likely it is to form calcium bonds. The lowest calcium levels were found in the normal group where the rats were allowed to eat and drink as usual. The highest calcium levels were found in the positive control group, which was not in line with predictions. This can be caused by the preparation of the comparison solution (positive control) at the beginning of the study which was not in accordance with the solubility of the cysteine

Description :

more oxalate in the kidney. Other factors could caused by gift

X : Concentration analyte in sample solution.

V : Total volume of the examined solution Vs : Sample volume.

solution to experimental animals where conditions of experimental animals such as stress will also affect the body's metabolism so that metabolic processes in the body do not run normally.

TABLE 2: Average Calcium Levels in the Kidneys ± RSD.

| Treatment Group | Average calcium level (µg/ml)) |
|---|---|
| Normal Group (KN) | 3.4103 ± 0.12 |
| Negative Control Group (KNf) | 3.9757 ± 0.02 |
| Positive Control Group (KP) | 6.9577 ± 0.17 |
| EERN test group Dosage 250 mg/kg BW | 4.2880±0.06 |
| EERN test group Dosage 500 mg/kg BW | 4.8037 ± 0.04 |
| EERN test group Dosage 1000 mg/kg BW | 5.7450 ± 0.13 |

Calcium levels in the dose test group obtained results that were not in accordance with the initial prediction because when compared with the negative group the calcium levels in the test group were higher than the negative control. Normally the highest calcium levels will be obtained by the negative control group because at In the negative control, there was an induction of kidney stones, namely 1% ethyleneglycol which would

further trigger the accumulation of calcium in the kidneys. In the treatment of the test group, the results of calcium levels were below negative control because there was already administration of a test solution that could inhibit the formation of kidney stones so that levels of calcium will decrease. In this study, the higher the test dose, the higher the calcium level. This is not as predicted, because the Homalomena occulta extract according to the phytochemical screening carried out positively contains flavonoids, which flavonoids will inhibit the formation of kidney stones (Bambang S, 2016).

### 3.7. Statistic test

Statistical analysis in this study used one way ANOVA test, homogeneity test and Post Hoc LSD test managed using SPSS 18.0. The ANOVA test was carried out to find out how much influence the ethanol extract of Homalomena rhizome had *occulta* toinhibition of kidney stone formation in wistar rats induced with ethylene glycol.

In the statistical test the ratio of the rat kidney showed a significance value in the homogeneity test of 0.045 where $p<0.05$. So from these results it is stated that the data is not homogeneous. After that, the LSD post hoc test was carried out and the results in each group did not have a significant difference with a significant value ($p> 0.05$).

In the statistical test of renal calcium levels, the significance value in the homogeneity test was 0.013 where $p <0.05$. So from these results it is stated that the data is not homogeneous. One way ANOVA analysis obtained a significance value of 0.000 where the p value $<0.05$. So from these results indicate that there are significant differences between treatments/groups. After that, a post hoc LSD test was performed. There were significant differences in the normal group with a dose of 250 mg, normal with a dose of 500 mg, negative with 500 mg, and 1000 mg with all groups. While the results that showed no significant difference were the normal group and the negative group, the negative group with a dose of 250 mg, and the test group with a dose of 250 mg with a dose of 500 mg.

## 4. Conclusion

In the study of the activity of inhibiting kidney stone formation from the ethanolic extract of nampu rhizome (Homalomena occulta), induced by ethylene glycol with parameters of kidney ratio and calcium content using the AAS (Absorbtion Atomic Spectroscopy) method, it can be concluded that: 1000 mg could not inhibit the formation of kidney stones in ethyleneglycol-induced wistar rats.

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