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Evaluation of Polyalthia Longifolia Leaf Extract Diuretics using Urine Volume Analysis and Electrolyte Excretion Potential

Natasha Hana Julia Monim[1], Irene Sondang Lingga[2], Mustika Endah Pratiwi[3], Krisna Dewi[4]

[1,2,3,4]Pharmacy Study Program. Cenderawasih University

ABSTRACT

Background: Diuretics are an essential class of drugs in the management of hypertension and edema. Glodokan Tiang leaves (Polyalthia longifolia). traditionally used in Indian medicine. are thought to have diuretic effects. This study aimed to evaluate the diuretic activity of ethanol extract of Glodokan Tiang leaves in male mice (Mus musculus) and compare its potency with the diuretic Furosemide. as well as identify the underlying phytochemical content.

Methods: Ethanol extract was obtained through maceration and tested for phytochemical content. A total of 25 male mice (Mus musculus) were divided into five treatment groups: negative control (Na-CMC 0.5%). positive control (Furosemide 0.104 mg/20gBW). and three extract doses (125. 250. and 500 mg/70KgBW). Cumulative urine volume was measured for 6 hours post-treatment. and the Diuretic Index and Urine Excretion Percentage were calculated. Data were analyzed using one-way ANOVA followed by Duncan's post hoc test.

Results: Phytochemical screening showed that the positive extract contained secondary metabolites of flavonoids. alkaloids. tannins. and saponins. Diuretic activity test results showed significant differences between groups ($p<0.05$). The highest extract dose (500 mg/70KgBW) produced the highest Diuretic Index of 1.81. classified as a potential diuretic (> 1.50). Duncan's test confirmed that the diuretic activity at a dose of 500 mg/70KgBW was not statistically significantly different from the positive control (Furosemide 0.104 mg/20gBW).

Conclusion: Ethanol extract of Glodokan tiang leaves has strong diuretic potential. with the 500 mg/70 kg body weight dose proven to be the most effective.

KEYWORDS: Polyalthia longifolia. Glodokan Tiang. Diuretic. Furosemide. Flavonoids.

ARTICLE DETAILS

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INTRODUCTION

Diuretics are a class of pharmacological substances that are essential in clinical practice. functioning to increase the excretion of water. sodium (Na+). and chloride (Cl−) (Katzung. 2001). This increased excretion is crucial for balancing extracellular fluid and reducing blood volume. making it the primary therapy for conditions such as congestive heart failure. hypertension. edema. and nephrotic syndrome (Susilowati & Nur'aini. 2022). Furosemide (as a potent loop diuretic) is effective. but its use is often limited by various adverse side effects. including hypokalemia (K+ deficiency). hyperuricemia. hypercalcemia. and impaired glucose tolerance (Warouw et al.. 2020). These limitations have prompted global efforts to explore and validate natural resources as alternative diuretic agents with better safety profiles.

Indonesia. as a tropical country with abundant biodiversity. has great potential for the development of herbal medicines. One plant species that has attracted attention is Glodokan Tiang (Polyalthia longifolia). a member of the Annonaceae family. Empirically. various parts of this plant have been used in traditional medicine. for example in India to treat thrush and fever (Kastanja & Patty. 2022).

Phytochemical analysis of Glodokan Tiang leaves has identified the presence of pharmacologically relevant secondary metabolites. including flavonoids. alkaloids. tannins. and saponins. In particular. flavonoid and alkaloid compounds are known to have strong diuretic potential. The mechanism of action of flavonoids as diuretics involves

inhibiting the reabsorption of Na+. K+. and Cl− in the renal tubules. while alkaloids work by increasing the excretion of Na+ and Cl−. The synergistic mechanism of these two groups of compounds is believed to play a role in triggering an increase in urine volume (diuresis) (Nurihardiyanti et al.. 2015). Although chemical content data is available. there has been no adequate scientific validation of the specific diuretic activity of ethanol extracts of Glodokan Tiang leaves to support their traditional use. Therefore. this study was designed to: Identify the groups of secondary metabolite compounds in the ethanol extract of Glodokan Tiang leaves and identify the diuretic activity of the extract in male mice (Mus musculus) through urine volume measurements. and determine the most effective extract dose and compare its potency with Furosemide as a positive control. This study is expected to provide a strong scientific basis for the development of Glodokan Tiang as a candidate for an effective natural diuretic agent.

MATERIALS AND METHODS

2.1. Preparation of Materials

Glodokan tiang leaves were collected. botanically identified. and dried. Extraction was performed using the maceration method with 70% ethanol solvent for 3×24 hours. The concentrated filtrate was evaporated to obtain Ethanol Concentrate Extract. Furosemide (6.5 mg/kgBW) was used as a standard positive control. dissolved in 0.5% CMC-Na (as a negative control).

2.2. Test Animals

Twenty-five (25) male mice (Mus musculus. BW 20–30 g) were used. The animals were maintained under standard conditions (temperature 25±2°C. 12-hour light/dark cycle) and acclimatized for seven days. The animals were fasted for 18 hours before testing but were given water ad libitum.

2.3. In vivo Diuretic Test Procedure

The mice were randomly divided into five groups (n=5):

Group	Oral Administration (Volume 0.5 mL)	Dosage
I	Suspensi CMC-Na 0.5%	-
II	Furosemid	6.5 mg/kgBB
III	P1 Glodokan tiang	100 mg/kgBB
IV	P2 Glodokan tiang	250 mg/kgBB
V	P3 Glodokan tiang	500 mg/kgBB

Each mouse was induced with 10 mL/kgBW of sterile distilled water orally. After 30 minutes. oral treatment was administered according to the group. Urine was collected using individual metabolic cages. The cumulative urine volume was recorded every hour for 6 hours. The parameters calculated included: total urine volume and Diuretic Index (DI).

DI=Urine Volume of Negative Control Group (mL)Urine Volume of Test Group (mL)

2.4. Data Analysis

Data were presented as Mean±Standard Deviation (SD). Analysis of differences between groups was performed using one-way ANOVA. followed by Tukey HSD (or LSD) post-hoc test at a significance level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis of Polyalthia longifolia Leaf Ethanol Extract

Qualitative phytochemical screening was performed to identify groups of secondary metabolites dissolved in P. longifolia leaf ethanol extract. which have potential biological activity. particularly diuretic activity.

Golongan Senyawa	Test Reagent	Qualitative Result	Description
Alkaloids	Dragendorff. Mayer. Wagner	Brick red precipitate (Dragendorff).Orange precipitate (Wagner)	Detected
Flavonoids	Mg powder. concentrated HCl. Amyl Alcohol	Orange or yellow color	Detected
Saponins	Foam/Bubbles(Vertical Shaking)	Stable foam formed (1-10 cm high)	Detected
Tannin	FeCl3 1%	Black color formed	Detected
Steroid/Terpenoid	Salkowski (H2SO4 /Anhydrous acetate)	No brownish/blue-green ring	Not detected

The phytochemical screening results confirmed the presence of alkaloids. flavonoids. saponins. and tannins in the ethanol extract of Glodokan tiang leaves. Flavonoids and alkaloids are specifically hypothesized to be the compounds responsible for diuretic activity (Latuconsina & Citraningtyas. 2014).

Flavonoids were detected positively through screening tests. producing an orange/yellow color. indicating that these compounds were successfully extracted using ethanol. which is a polar solvent. The positive reaction of alkaloids. producing a brick red and orange precipitate. occurred due to the presence of nitrogen atoms with free electron pairs that were able to react with complex ions in the Dragendorff and Wagner reagents (Sangi et al.. 2008). Meanwhile. steroids and terpenoids can be explained by the principle of like dissolves like. Because steroids and terpenoids tend to be nonpolar. the 96% ethanol solvent used (which is polar-semipolar) may not be efficient in optimally extracting these compounds. or their concentration may be too low to be detected in qualitative tests (Larasati and Putri. 2023).

3.2. Evaluation of Diuretic Activity in vivo

Diuretic activity testing was conducted on male mice (Mus musculus) by measuring cumulative urine volume over 6 hours and calculating the Diuretic Index (DI) for three different extract doses: P1 (125 mg/70KgBW). P2 (250 mg/70KgBW). and P3 (500 mg/70KgBW). compared to Negative Control (Na-CMC 0.5%) and Positive Control (Furosemide 0.104mg/20gBW).

3.2.1. Urine Volume and Diuretic Index Results

Treatment Group. Total Urine Volume (mean ± SD. mL/6 hours). Percentage Urine Excretion. Diuretic Index (DI). Diuretic Category

Treatment Group	Total Urine Volume (mean ± SD. mL/6 hours)	Percentage Urine Excretion	Diuretic Index (DI)	Diuretic Category
Negative Control (NC)	1.51 ± 0.19	45.80%	1.00	Not Potent
Positive Control (PC)	2.92±0.23	87.81%	1.93	Potent
Dose D1 (125 mg)	2.16±0.24	61.92%	1.36	Moderately Potent
Dose D2 (250 mg)	2.25±0.11	66.45%	1.46	Moderately Potent
Dose D3(500 mg)	2.95±0.14	81.97%	1.81	Potent

The cumulative urine volume results showed a clear increase in the extract treatment group compared to the Negative Control (Na-CMC). This increase was dose-dependent. with the highest dose (D3: 500 mg/70 kg BW) producing the highest total urine volume. namely 2.95±0.14 mL. which was almost equivalent to the positive control Furosemide (2.92±0.23 mL).

3.2.2. Statistical Test (ANOVA and Duncan)

Analysis using One-way ANOVA showed a significance value (Sig.) of 0.000. which is less than 0.05 ($p<0.05$). This statistically proves that there is a significant difference in diuretic activity between treatment groups.

The Duncan post-hoc test provided specific details:

• The D3 dose extract group (500 mg/70 kg BW) showed a Diuretic Index (DI) of 1.81. Based on the criteria of Séverin et al. (2023). a DI >1.50 is classified as a potential (strong) diuretic.

• Dose 3 was not statistically significantly different from the Positive Control (Furosemide. DI=1.93). This confirms that the highest dose extract has diuretic efficacy comparable to a standard strong diuretic drug.

• Doses 1 (DI=1.36) and D2 (DI=1.46) fall within the moderate potency category (1.0<DI<1.50) and are not significantly different from each other. but both are markedly different from the Negative Control.

3.2.3. Diuretic Action Mechanism

The strong diuretic activity of P. longifolia leaf extract at a dose of 500 mg/70 kg BW is associated with the presence of flavonoids and alkaloids. Flavonoid Mechanism: Flavonoid compounds (as indicated by screening results) are thought to act as diuretic agents by inhibiting the reabsorption of Na+. K+. and Cl− ions in the renal tubules (Lingga et al.. 2014). thereby increasing electrolyte levels in the tubule lumen and drawing more water (diuresis). Alkaloid Mechanism: Alkaloids work synergistically with flavonoids by increasing the excretion of Na+ and Cl− in the renal tubules. which ultimately increases urine volume (Septiana et al.. 2021). Higher secondary metabolite content. which is a function of the extract dose administered. directly correlates with a greater diuretic response.

CONCLUSION

1st. The ethanol extract of Polyalthia longifolia leaves contains secondary metabolites such as alkaloids. flavonoids. tannins. and saponins.

2nd. The ethanol extract of P. longifolia leaves exhibits significant diuretic activity in male mice (Mus musculus). with dose-dependent efficacy.

3rd. The D3 extract dose (500 mg/70 kg body weight) was the most effective dose. providing high diuretic potential (ID=1.81) and showing no significant difference in effectiveness compared to the positive control Furosemide.

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