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Acute Toxicity Test of Cassia alata Leaves Extract on Rats (*Rattus norvegicus*)

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ABSTRACT

The leaves of *Cassia alata* exhibit potential properties as antioxidants, immunostimulants, antimicrobials, anti-dengue agents, and laxatives. This study aimed to determine the LD_{50} value of the administration of *Cassia alata* leaves extract using the Thompson-Weil method. A total of 25 rats (*Rattus norvegicus*) were utilized in the study, divided into five dose groups. One group served as the control (P0), receiving only distilled water, while the four treatment groups were administered doses of 500 (P1), 1000 (P2), 1500 (P3), and 2000 (P4) mg/kg BW. Toxic symptoms and changes in body weight were observed for 14 days. The results showed that there was one death at a dose of 1500 mg/kg BW and two deaths at a dose of 2000 mg/kg BW. The administration of *Cassia alata* leaf extract resulted in toxic symptoms, including reduced motor activity, respiratory difficulties, and weight loss. The LD_{50} value obtained was 1.9 g/kg BW. The extract of *Cassia alata* leaves exhibit a toxic effect on rats.

Keywords: *Cassia alata*, thompson-weil, toxicity, mild toxicity

ABSTRAK

Daun candle bush (*Cassia alata*) berpotensi sebagai antioksidan, imunostimulan, antimikroba, anti dengue dan sebagai laksatif. Penelitian ini bertujuan untuk menentukan nilai LD_{50} pada pemberian ekstrak daun candle bush menggunakan metode thompson-weil. Hewan uji yang digunakan yaitu tikus (*Rattus norvegicus*) sebanyak 25 ekor dan terbagi menjadi 5 kelompok dosis, satu kelompok kontrol (P0) yang hanya diberikan aquades, dan empat kelompok perlakuan dengan tingkatan dosis 500 (P1), 1000 (P2), 2000 (P3) dan 4000 (P4) mg/kg BB. Gejala toksik dan perubahan berat badan diamati selama 14 hari. Hasil penelitian menunjukkan adanya kematian pada dosis 2000 mg/kg BB sebanyak 1 ekor dan pada dosis 4000 mg/kg BB sebanyak 2 ekor. Pemberian bahan uji ekstrak daun candle bush (*Cassia alata*) menimbulkan gejala toksik berupa penurunan aktivitas gerak, kesulitan bernafas, tidur dan penurunan bobot badan. Nilai LD_{50} yang diperoleh adalah 1,9 g/kg BB, ekstrak daun candle bush (*Cassia alata*) memiliki efek toksik sedang pada tikus.

Kata kunci: *Cassia alata*, thompson-weil, toksisitas, toksik sedang

INTRODUCTION

Traditional medicine consists of substances or mixtures derived from plants, animals, minerals, liquid preparations, or combinations utilized by a community (Ekor, 2014). Most medicinal use in Indonesia is still processed using traditional methods. The scientific testing of herbal products remains insufficient, leading to uncertainties regarding their dosage, efficacy, identification, toxicity, standardization, and regulation. Herbal medicines have garnered interest from diverse groups, including academics, researchers, and healthcare professionals, who aim to comprehend the safety and benefits of plants with medicinal properties (Elfahmi et al., 2014; Fadholly et al., 2025).

Candle bush (*Cassia alata*) is among the most commonly utilized medicinal plants. This plant is commonly used empirically as an antiparasitic, laxative, and treatment for skin and respiratory diseases (Dewi *et al*., 2019). According to reported studies, candle bush leaf extract has potential as an antioxidant and exhibits immunostimulatory and antimicrobial activity. Other studies have also noted that this plant has antiviral activity against dengue (Chew *et al*., 2022). Some of the benefits of this plant are reported from its chemical content, such as glycosides, flavonoids, tannins, saponins, and anthraquinone derivatives like chrysophanol, chrysophanic acid, and aloe-emodin (Fatmawati et al., 2020).

Although candle bush has many benefits for the body, toxicity tests are necessary to measure the toxic effects of a compound. Acute, subacute, chronic, and subchronic toxicity tests utilizing test animals serve as effective models for examining biochemical, physiological, and pathological responses in humans to a test preparation (Erhirhie et al., 2018). Acute toxicity tests are defined as the toxic effects that manifest following a single oral dose within 24 hours. Acute toxicity test results, represented as LD_{50}, cannot be definitively used to establish the safety of a substance or preparation in humans. However, they can provide an indication of relative toxicity and help identify toxic effects in the event of human exposure (BPOM RI, 2014). The median lethal dose or LD_{50} is a statistical measure after a single dose is administered, often used to express toxic dose levels as quantitative data. Clinical symptoms, physiological symptoms, and toxic mechanisms serve as qualitative data (Hamm et al., 2017). The Thompson-Weil method can be used to calculate LD_{50}. This method has a high level of reliability, accurate results, and does not require a large number of test animals (Fadila et al., 2023). Therefore, this study aimed to determine the LD_{50} value of candle bush leaf extract (*Cassia alata*) using the Thompson-Weil method and its effect on animal behavior.

MATERIALS AND METHOD

Time and Place of Research

This study was conducted from February to April 2024 at the Laboratory Animal Management Unit, School of Veterinary Medicine and Biomedical Sciences, IPB University.

Preparation of candle bush leaf extract (Cassia alata)

Candle bush leaves were obtained from the Center for Bioactive Compounds, IPB University, in dried form (*simplisia*). The *simplisia* was then ground using a blender. The extraction method used was maceration. Four hundred grams of candle bush leaf *simplisia* powder (*Cassia alata*) was placed in an inert container or glass jar, then 1000 mL of ethanol solvent was added. The mixture was stirred using a stirrer, occasionally shaken, and left to stand for 1–2 days. The residue and filtrate were then separated using filter paper. The filtrate obtained was collected and concentrated. Phytochemical screening was then conducted to identify flavonoids, alkaloids, tannins, phenols, saponins, and steroids/triterpenoids.

Preparation of Test Animals

The use of mice as test animals in this study has been approved by the Animal Ethics Committee (AEC), School of Veterinary Medicine and Biomedical Sciences, IPB University, with approval number 035/KEH/SKE/IXI/2023. The experimental animals used were 25 male Wistar rats (*Rattus norvegicus*) weighing 200–300 grams. The rats were divided into five groups, each consisting of five male rats selected at random. Group P0 served as the negative control and was only given distilled water without the candle bush leaf extract (EDKC). At the same time, the treatment groups were divided into four: P1 received EDKC at 500 mg/kg body weight, P2 received EDKC at 1000 mg/kg body weight, P3 received EDKC at 2000 mg/kg body weight, and P4 received EDKC at 4000 mg/kg body weight.

Acute Toxicity Test LD_{50}

In the acute toxicity test LD_{50}, each treatment group received 1 mL of candle bush leaf extract dissolved in distilled water orally via a tube, comprising one control group and four groups with varying doses. The mice were observed for 1–4 hours to detect any visible toxic symptoms such as tremors, convulsions, salivation, diarrhea, allergies, weakness, sleepiness, and coma. Observations were repeated until day 14

after dose administration by counting the number of dead mice in the experimental groups. Body weight measurements were also conducted twice weekly.

Data Analysis

The data collected in this study were primary data from observations of test animals, both control and treatment groups. The data obtained were quantitative and qualitative. The quantitative data obtained were the number of test animals that died, while the qualitative data obtained were symptoms of toxic effects. Changes in body weight of the test animals were analyzed statistically using a one-way ANOVA test, and the data were processed using SPSS 26 software. The LD_{50} value was obtained from the number of mice that died and those that survived in each group. The LD_{50} value was then calculated using the Thomson-Weil method.

RESULTS

Phytochemical Screening Test

Table 1 shows the results of phytochemical screening of the candle bush (*Cassia alata*) leaf extract.

Table 1 Phytochemical screening test of candle bush leaf extract (*Cassia alata*)

Secondary metabolites	Test Results
Flavonoids	+
Alkaloids	+
Tannins	+
Phenol	+
Saponins	+
Steroids / Triterpenoids	-

Note: A (+) sign indicates the presence of metabolite compounds, and a (-) sign indicates the absence of metabolite compounds.

Observations of Animal Test

Table 2 and Figure 1 presents the results of administering the candle bush leaf extract to all test animal groups. The test animals' body weight was measured before and after treatment. Data on changes in the body weight of the test animals are presented in Table 3 and Figure 2. Table 4 presents data on mouse mortality during 14 days of treatment with candle bush leaf extract (*Cassia alata*).

Table 4 Data on the mortality of mice given candle bush leaf extract (*Cassia alata*) for 14 days

Group	Number of Rats	Rat Death Toll
P0	5	0
P1	5	0
P2	5	0
P3	5	1
P4	5	2

DISCUSSION

Acute toxicity testing is conducted to examine toxic effects that occur shortly after the administration of a substance (Chinedu *et al*., 2013). Generally, acute toxicity testing is conducted over a minimum period of 24 hours. The lethal or toxic dose range is expressed as LD_{50}. The LD_{50} value calculated using the Thompson-Weil method is commonly used to determine the toxicity level of a compound. If a suspension or preparation does not cause death within 14 days post-treatment, the suspension can be categorized as practically non-toxic (Zhang et al., 2022).

Observations were conducted post-treatment for each rat in every group to assess changes that occurred after treatment in comparison to pre-treatment conditions. Observations included toxic symptoms,

Table 2 Observation of Toxicity Signs in Mice Administered Candle Bush Leaf Extract

Symptom	P0					P1					P2					P3					P4				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Allergies	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weakness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+
Difficulty Breathing	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+
Sleep	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+

Note: A (+) sign indicates that something occurred, while a (-) sign indicates that something did not occur.

(A) (B)

Figure 1 Clinical symptoms in mice given treatment. A: mouse experiencing breathing difficulties in group P4; B: mouse experiencing clinical symptoms of weakness and sleepiness in group P3.

Table 3 Body weight of mice before and after treatment with candle bush leaf extract (*Cassia alata*) for 14 days.

	Average body weight (g)				
	P0	P1	P2	P3	P4
Before Treatment (Day 1)	210 ± 0.12	208 ± 0.34	210 ± 0.11	210 ± 0.87	206 ± 0.16
After Treatment (Day 14)	227 ± 0.78*	219± 0.56*	220± 0.33*	198± 0.15*	186± 0.15*

Note: The asterisk (*) indicates a significant difference between before and after treatment.

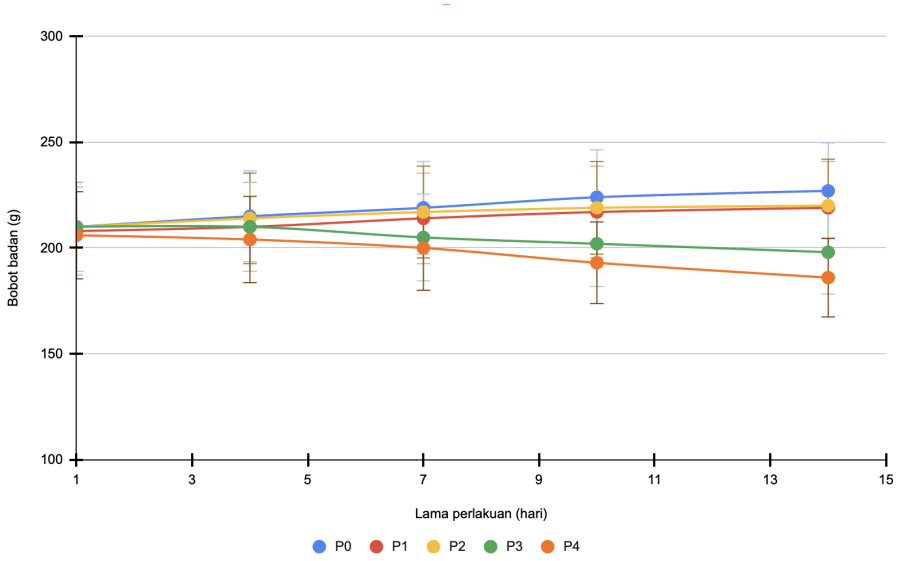

Figure 2 Changes in the average body weight of mice given candle bush leaf extract (*Cassia alata*) for 14 days.

changes in body weight, and mortality (Murwanti et al., 2023). This study identified several toxic symptoms, including convulsions, salivation, tremors, diarrhea, allergies, weakness, difficulty breathing, and sleeping difficulties. Based on Table 2, the toxic symptoms exhibited by mice in groups P3 and P4 were weakness, difficulty breathing, and sleeping difficulties. Active compounds found in plants are almost always toxic

when administered in high doses. The presence of excessive secondary metabolites, such as saponins and alkaloids, is suspected to cause cellular damage in organs. This occurs because these compounds exhibit a longer excretion time during metabolism than other compounds, resulting in extended exposure to liver cells and the potential for liver cell damage.

Saponins and alkaloids present in normal physiological amounts confer various benefits. Saponins serve as antibacterial, antifungal, antiviral, and antioxidant agents, whereas alkaloids function as antidiarrheal, antibacterial, and antimalarial agents (Müller et al., 2013; Timilsena et al., 2023). Excessive alkaloid content in mice causes rapid damage to the cerebellum. The damage is intensified by clinical symptoms noted in mice, including a reduction in locomotor activity. The cerebellum is a brain region responsible for regulating balance, orientation, body position, muscle tone, and coordination (Malekmohammad et al., 2021; Akinboye et al., 2023). Saponins are believed to damage red blood cells by increasing the permeability of their lipid bilayer, resulting in hemolysis. Saponin-induced hemolysis may lead to kidney damage (Cao et al., 2024).

Body weight measurements were taken before and after treatment, and the average body weight of each group was calculated before and after treatment. Based on Table 3 and Figure 1, the P0, P1, and P2 groups showed an increase in body weight, while the P3 and P4 groups showed a decrease in body weight. Statistical analysis showed a significant difference in the average body weight of mice before and after treatment with candle bush leaf extract (*Cassia alata*), which could be an initial indicator of its toxic effects (Macko et al., 2021). Body weight in animal toxicity studies with high doses generally results in weight loss due to reduced appetite (Lee et al., 2012). Reduced appetite can be caused by compounds found in red grapes, such as alkaloids, steroids, and flavonoids. These compounds may function as stomach poisons or toxins; if ingested by rats, they can interfere with the digestive process and lead to a refusal to eat (Panche et al., 2016).

The rat mortality data from the acute toxicity test indicated a mortality order of 0, 0, 1, 2. In the Weil table, the R-values of 0, 0, 1, and 2 have an f (factor) value of 1. The mortality data were analyzed using the Thomson and Weil methods, resulting in an LD_{50} value of 1.9 g/kg body weight, with the toxicity level classified as moderate toxicity (BPOM, 2014). This study excluded microscopic observation of organs, which may have facilitated the identification of significant microscopic lesions present in the organs. Further research should be conducted, including microscopic observation of organs, such as histopathology, to determine whether the extract of candle bush leaves (*Cassia alata*) can affect organs at the microscopic level.

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