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The Potential of *Acalypha indica L.* Leaf Extract as a Repellent Against *Aedes aegypti* Mosquitoes

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Abstract: Number one of the issues in controlling *Aedes aegypti* mosquitoes is the limitations of using chemical repellents, which often have harmful side effects for humans and the environment. The solution to this problem is using natural materials, such as *Acalypha indica L*. This study aimed to inform the results of analyzing the potential of leaf extract *Acalypha indica* in repelling *Aedes aegypti* mosquitoes. This study is experimental, using a post-test-only control group design. The subjects of the study were leaf extracts of *Acalypha indica* at concentrations of 10%, 15%, and 20%. The object was 2-5 days old female *Aedes aegypti* mosquitoes, totaling 675 individuals with 9 replications. The research variables include the number of mosquitoes landing and the protective efficacy. Data were collected through measurements and observations and analyzed using the *Kruskal-Wallis* test. At the 6th hour of testing, the protective efficacy for concentrations of 10%, 15%, and 20% was 75%, 70%, and 90%, respectively. Statistical analysis revealed significant differences in protective efficacy among the treatment groups ($p<0.05$). The concentration of 20% has proven potential in repelling *Aedes aegypti* mosquitoes, with 90% protective efficacy until the 6th hour of testing, according to the standards set by the Indonesian Pesticide Commission.
Keywords: Acalypha indica, Aedes aegypti, repellent, protective efficacy

1. Introduction

Dengue Hemorrhagic Fever (DHF) is a mosquito-borne viral infection caused by the dengue virus. The disease manifests with symptoms such as high fever, severe headaches, joint and muscle pain, skin rashes, and, in severe cases, bleeding, organ failure, and even death. DHF remains a significant global health concern, particularly in tropical and subtropical regions. According to a report from the World Institute "WHO," DHF cases declined during the COVID-19 pandemic from 2020-2022; however, following the easing of the pandemic, the number of cases has drastically increased worldwide. This rise is not only in the number of cases but also in the geographical spread of the disease. Indonesia is among the 30 countries with the highest endemic rates of DHF [1]. In 2022, Indonesia recorded 143,266 cases of DHF with 1,237 fatalities, an increase compared to 2021, which had 73,518 cases and 705 deaths [2].

The *Aedes* mosquito carries the transmission of DHF in Indonesia, specifically, the *aegypti* and *albopictus* species, which have the *dengue* virus. These mosquitoes spread the virus from one person to another, resulting in the virus incubating within the human body [3], [4]. This rapid transmission must be addressed seriously to prevent more significant health issues. Avoiding mosquito bites is the easiest preventive, particularly from the species that are the primary vectors of this disease [5], [6].

One method to avoid bites from *Aedes aegypti* mosquitoes is using repellents [7]. Repellents are substances applied to the skin to protect against mosquito bites [8]. DEET (*N, N-Diethyl-meta-toluamide*) is a chemical-repellent ingredient popularly used by the general public. Continuous use of these repellents may lead to mosquito resistance [9] and negatively impact human health [10]–[12]. Therefore, seeking safer alternatives, such as repellents made from natural plant-based materials, is important [13], [14].

Research on natural repellents has garnered attention in recent years. Several plants, such as *Cosmos caudatus, Tagetes erecta*, and *Tithonia diversifolia*, have been studied and shown effectiveness in repelling mosquitoes. Extracts from *Cosmos caudatus* leaves can repel *Culex quinquefasciatus* mosquitoes by up to 92.7% at a concentration of 11% [15]. Additionally, *Tagetes erecta* flowers and *Tithonia diversifolia* leaves exhibit high protective efficacy against *Aedes aegypti* mosquitoes [16], [17]. These plants contain natural active compounds such as alkaloids, flavonoids, and tannins that repel mosquitoes. Indonesia's biodiversity can further develop the potential of plants with similar active compounds as natural repellents.

One of the plants that is interesting to study is *Acalypha indica L.* This weed is known for its herbal medicinal properties [18], [19]. It has also been studied

as a natural larvicide for *Culex quinquefasciatus* and *Anopheles sp.* [20], [21]. The active compounds from phytochemical analysis at the Herbal Materia Medica Laboratory in Batu Malang include steroids, alkaloids, flavonoids, tannins, and saponins, which are known to have bioactive properties, including insecticidal and repellent effects. However, despite its potential, studies on this plant as a mosquito repellent remain unexplored.

Therefore, this study aims to fill this gap by analyzing the potential of *Acalypha indica* leaf extract as a repellent against *Aedes aegypti* mosquitoes. Based on preliminary experiments, the research will examine three different concentrations (10%, 15%, and 20%), identifying 15% as the most effective concentration. By comparing its efficacy with lower (10%) and higher (20%) concentrations, this study seeks to provide scientific evidence supporting *Acalypha indica* as a natural, sustainable, and environmentally safe alternative for *Aedes aegypti* mosquito control. The findings from this study are expected to contribute to the development of plant-based repellents, particularly utilizing Indonesia's rich biodiversity, and offer safer alternatives to synthetic chemical repellents.

2. Material and Methods

This study is purely experimental and employs a post-test-only control group design for research. This research was conducted at the Entomology Laboratory of Health Polytechnic of the Ministry of Health Surabaya from January to June 2024.

2.1. Material

The materials used include 500 grams of *Acalypha indica* leaf powder; 675 *Aedes aegypti* females from the F3 generation, aged 2–5 days (selected for their peak blood-seeking activity to support egg production); test cages measuring 40 cm on each side; a counter; a stopwatch; an aspirator; latex gloves; distilled water; 96% ethanol; paper cups; sugar water; beaker glass; an oven; and a rotary evaporator.

2.2. Methods

2.2.1. Extraction of 50% Acalypha indica Leaves

The preparation of the extract was conducted using the maceration method. The process began with oven-drying the Acalypha indica leaves at 40–60°C, which were then ground and sifted into a fine powder. 500 grams of the powder was soaked in 96% ethanol at a ratio of 1:2 for 24 hours while being stirred occasionally and then filtered to obtain the first filtrate. The residue was washed again with 96% ethanol for 24 hours, and this process was repeated three times to ensure that the active compounds were extracted maximally and efficiently. Finally, the rotary evaporation process was performed to produce a concentrated extract.

2.2.2. Repellent Formulation

The concentrated extract of *Acalypha indica* leaves was diluted with distilled water to 10%, 15%, and 20% in 50 ml for this study. The concentrations were calculated using the following dilution formula:

$$C1 \times V1 = C2 \times V2 \quad (1)$$

Description:
C : concentration of solution
V : volume of solution

2.2.3. Repellent Efficacy Test

The repellent efficacy procedure adheres to WHO standards [22]. Before testing, mosquitoes should remain unfed and fast for 24 hours. Volunteers are required to wash their hands with unscented soap, rinse them with water, and wear gloves up to the fingertips.

The repellent test begins by inserting the left arm, without any application, into a test cage containing 25 *Aedes aegypti* mosquitoes for 5 minutes, during which the total mosquito landings are recorded. Subsequently, the right arm, coated with *Acalypha indica* leaf extract from wrist to elbow, is inserted into the same cage for 5 minutes, and the total mosquito landings are recorded.

Observations were performed every 5 minutes during the first hour and repeated every hour for a total duration of 6 hours, starting from 08:00 to 13:00. The test was conducted on both control and treatment groups (with concentrations of 10%, 15%, and 20%), with each group undergoing nine replications. New mosquitoes were used for each replication to ensure reliability. During testing, the room temperature and humidity were controlled to maintain optimal conditions for mosquito growth (temperature 26–30°C and humidity 60–80%).

2.3. Data Analysis

The formula used to calculate the protective efficacy value from the test results is as follows:

$$Protective\ Efficacy = \frac{C - T}{C} \times 100\% \quad (2)$$

Description:
C : total landings of mosquitoes on the control arm
T : total landings of mosquitoes on the treatment arm

According to the Indonesian Pesticide Commission, a repellent is effective if it provides 90% protection up to the 6th hour [23]. To analyze the protective efficacy of *Acalypha indica* leaf extract, the data was initially tested for normality and homogeneity. If the data followed a normal distribution, a *One-Way ANOVA* test was conducted. Otherwise, the *Kruskal-Wallis* test was applied. Significant differences identified through *the Kruskal-Wallis* test were further analyzed using the *Mann-Whitney* test to determine specific group differences.

2.4. *Ethical Clearance*

The Health Research Ethics Committee of the Health Polytechnic of the Ministry of Health Surabaya has approved this study, assigning it the approval number EA/2403/KEPK-Poltekkes_Sby/V/2024.

3. Results and Discussion

Results of the mosquito repellent efficacy test indicate that in the first hour, the highest frequency of mosquito landings at a 10% concentration was 2 mosquitoes, while at 15% and 20% concentrations, it was 1 mosquito each. The number of mosquito landings increased up to the sixth hour of testing, with 5 mosquitoes landing at 10% and 15% concentrations and 2 mosquitoes at 20% concentration.

The table above indicates that a 20% concentration exhibits the highest protective efficacy, with a value of 90% at the 6th hour of testing. In contrast, the protective efficacy values for 10% and 15% concentrations remain below 90%, specifically 75% and 70%, respectively. According to the standards set by the Indonesian Pesticide Commission, a repellent is considered effective if its protective efficacy remains above 90% up to the 6th hour of testing. Therefore, the 20% concentration can be deemed to have met the established standards, whereas the 10% and 15% concentrations have not.

The *Shapiro-Wilk* test indicated that the protective efficacy percentages of *Acalypha indica* leaf extract were not normally distributed ($p \leq 0.05$). Consequently, the *Kruskal-Wallis* test was used for variance analysis, revealing significant differences in efficacy across 10%, 15%, and 20% concentrations ($p \leq 0.05$). *Post Hoc* analysis with the *Mann-Whitney* test identified significant differences between treatment groups, as detailed in the table 2.

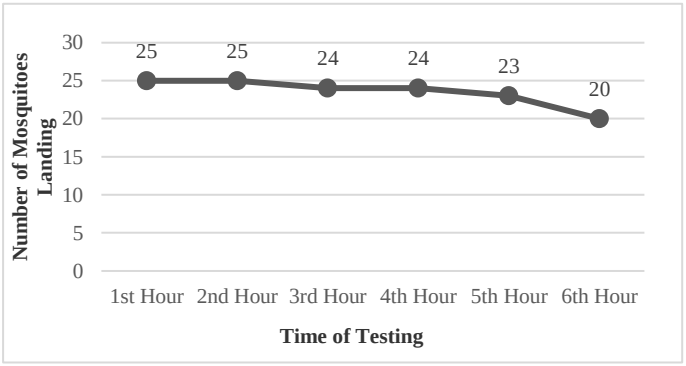

Figure 1. Highest Frequency of *Aedes aegypti* Mosquito Landings in the Control Group

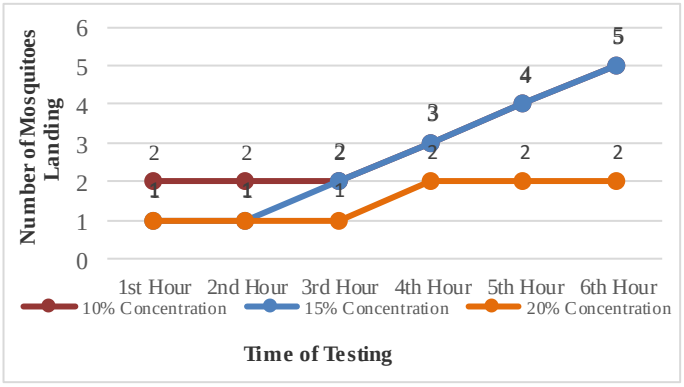

Figure 2. Highest Frequency of *Aedes aegypti* Mosquito Landings in the Treatment Group

Table 1. Protective Efficacy Percentage of *Acalypha indica* Leaf Extract in the Treatment Group

Testing Hour	Median (Min-Max)			*p-value**
	10% Concentration	15% Concentration	20% Concentration	
1st Hour	92 (92-96)	96 (92-100)	96 (96-100)	0.007
2nd Hour	92 (92-96)	96 (92-96)	96 (96-100)	0.003
3rd Hour	91.6 (86.9-92)	91.6 (88-95.8)	95.6 (91.6-96)	0.004
4th Hour	87.5 (83.3-91.6)	87.5 (83.3-91.6)	91.6 (91.6-95.8)	0.000
5th Hour	82.6 (75-86.9)	82.6 (75-87.5)	91.3 (85-95.8)	0.001
6th Hour	75 (70-80)	70 (70-80)	90 (85-100)	0.000
*p-value***	0.000	0.000	0.000	

* *Kruskal-Wallis Test*
** *Friedman Test*

.Table 2. Results of the *Mann-Whitney* Test

Concentration	*p-value*					
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
10% - 15%	0.119	0.065	0.139	0.289	0.345	0.564
10% - 20%	0.003	0.002	0.002	0.001	0.001	0.000
15% - 20%	0.059	0.029	0.033	0.001	0.002	0.000

The table above clearly shows that the protective efficacy values of *Acalypha indica* leaf extract at concentration pairs of 10% with 20% and 15% with 20% show p-values less than 0.05 ($p<0.05$). Significant differences in the protective efficacy values between these two pairs of concentration groups are consistently observed from the first to the 6th hour of testing. Conversely, the concentration pair of 10% with 15%

shows no significant difference in protective efficacy values.

Based on the research findings, the number of mosquitoes landing increased, and the protective efficacy decreased throughout the testing period. This phenomenon was caused by the evaporation of *Acalypha indica* leaf extract from the skin due to human body heat, causing the aroma of the extract to dissipate and its repellent effectiveness to diminish. The decrease in protective efficacy correlates directly with reducing the repellent potency of the plant extract used. Chemical compounds from natural extracts can repel mosquito bites effectively because they produce an odor [15], [24].

Testing the protective efficacy of extracting *Acalypha indica* leaf as a repellent for *Aedes aegypti* mosquitoes showed significant differences in results between the various concentrations examined. Of the three concentrations tested, the concentration with potential as a repellent is 20%. This is because the 20% concentration still protects 90% up to the 6th hour of testing, while the 10% and 15% concentrations exhibit less than 90% protection. According to the standard established by the Indonesian Pesticide Commission, a repellent is considered effective if it can provide protection more significant than 90% until the 6th hour of testing [25].

Repellents efficacy tested from the natural ingredient of *Acalypha indica* leaf extract showed their effect in repelling mosquitoes. *Aedes aegypti* mosquitoes tended to avoid areas treated with the repellent, indicating its potential efficacy [25]. The results of phytochemical testing at the Herbal Materia Medica Laboratory in Batu Malang showed that *Acalypha indica* leaves contain flavonoids, alkaloids, tannins, saponins, and steroids. During testing, these active compounds influenced the repellent effect of *Aedes aegypti* mosquitoes on human volunteers.

The extraction of *Acalypha indica* leaves using the maceration method with 96% ethanol contains 5.0084% flavonoids [26]. Flavonoids, the most extensive class of phenolic compounds, are highly effective at inhibiting fungi, bacteria, and viruses growth [27]. Flavonoid's mechanism as repellents is through their function as a toxin or inhibitor of respiration. Flavonoids enter the body of *Aedes aegypti* mosquitoes and the air they inhale, causing respiratory disturbances that ultimately lead to the death of the mosquito [28]–[30]. This highlights the potential of flavonoids derived from *Acalypha indica* as a natural and effective mosquito repellent.

While flavonoids have primary toxicity to the respiratory system, alkaloids and steroids tend to impact the neural system more. Alkaloids are chemical compounds that inhibit the enzyme acetylcholinesterase, causing excessive accumulation of acetylcholine at synapses [31]. In mosquitoes, this results in uncontrolled muscle contractions, paralysis, and often death [30], [32]. Meanwhile, steroids act as neurotoxins that affect neurotransmission and inhibit ion transport. Steroids entering the mosquito's body disrupt its nervous system, causing impairments in neurotransmission. This disruption leads to weakness in the mosquito and can hinder its development [17]. While these compounds are effective, the respiratory toxicity of flavonoids remains the primary mechanism, emphasizing their role as the main active compound in *Acalypha indica's* repellent properties.

The presence of tannins in *Acalypha indica* leaf extract also caused a significant difference in mosquitoes landing between the treatment and control arms. Tannins are chemical compounds with astringent properties that cause a bitter taste sensation on the tongue, mouth dryness, and throat constriction [33]. The astringent effect of these tannins interferes with normal mosquito feeding behavior, making it uncomfortable for them and reducing their ability to bite, causing mosquitoes to avoid areas containing tannins generally [16].

Another active compound involved in repelling mosquitoes is saponin. Saponins are chemical compounds with various toxic effects on insects, including mosquitoes. They act as stomach toxins that disrupt insect digestion, inhibit nutrient absorption, and lead to death [34]. In addition, saponins remove the lipid layer coating on the surface of insects, leading to dehydration and damage to cell membranes and metabolism through respiratory systems, ultimately resulting in mosquito death [35], [36].

4. Conclusion

The best concentration of leaves extract *Acalypha indica* is 20% to repel *Aedes aegypti* mosquitoes; this concentration has potential, with 90% protective efficacy until the 6th hour of testing, according to the standards set by the Indonesian Pesticide Commission.

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