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RESEARCH ARTICLE

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The Effectiveness Of Patchouli Leaf Decoction *(Pogostemon cablin Benth)* as a Bioinsecticide Against *Aedes sp*

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Abstract

Background: *Aedes* sp. is a primary vector responsible for transmitting several diseases such as dengue hemorrhagic fever, filariasis, chikungunya, leptospirosis, and bubonic plague. The expansion of its population from urban to rural areas necessitates new control measures. Patchouli leaves (*Pogostemon cablin* Benth) contain bioactive compounds such as flavonoids, tannins, and phenolic hydroquinone with potential mosquito-killing properties. **Objective:** To determine the effectiveness of boiled water extract of patchouli (*Pogostemon cablin* Benth) leaves against the mortality of *Aedes* sp. mosquitoes. **Methods:** An experimental design was used with three treatment groups receiving patchouli leaf extract concentrations of 80%, 90%, and 100%, along with a negative control (aquadest) and a positive control. Each treatment involved 25 mosquitoes with three replications. Observations were made over a 24 hour period (1440 minutes). Data were analyzed using a one-way ANOVA test. **Results:** The average mosquito mortality was 6.6 mosquitoes (27%) at 80% concentration, 11.6 mosquitoes (48%) at 90% concentration, and 16 mosquitoes (64%) at 100% concentration. **Conclusion:** The boiled water extract of patchouli leaves (*Pogostemon cablin* Benth) demonstrated effectiveness in increasing *Aedes* sp. mortality, with higher concentrations showing greater mosquito-killing activity.

Keywords: *Pogostemon cablin*, Aedes sp., mosquito mortality, phytochemical, patchouli leaf extract

Introduction

Aedes spp. are the primary vectors responsible for the transmission of dengue hemorrhagic fever (DHF). The population of these mosquitoes has become widespread, not only in densely populated urban areas but also in rural regions. The main challenge in controlling the disease lies in the uncontrolled breeding and spread of *Aedes* spp., which significantly contributes to the persistence and escalation of transmission (1). Diseases transmitted by *Aedes* spp. as vectors include dengue hemorrhagic fever, filariasis, chikungunya, leptospirosis, and plague. These diseases have become significant public health concerns in Indonesia, with high morbidity and mortality rates, and the potential to trigger outbreaks (2).

The prevention of Dengue Hemorrhagic Fever (DHF), which is transmitted by *Aedes* spp. mosquitoes, can be achieved through the use of natural insecticides. Natural insecticides are derived from plants containing bioactive chemical compounds that are toxic to insects but are environmentally friendly due to their biodegradability and minimal risk to human health. One such plant with promising insecticidal potential is patchouli (*Pogostemon cablin* Benth.) leaves (3). These leaves contain various secondary metabolites with flavonoids, tannins, and phenolic hydroquinones are dominated in aqueous extracts. *Pogostemon cablin* is a well-known plant widely cultivated for its essential oil, which is extracted through distillation. The leaves of patchouli are known to contain essential oils as well as a range of bioactive compounds, including flavonoids, saponins, tannins, glycosides, terpenoids and steroid (4).

A previous study by Diana Pramifta et al. (2011) demonstrated that the essential oils extracted from the leaves (A), stems (B), and a leaf-stem mixture (C) of patchouli (*Pogostemon cablin* Benth.) yielded different extraction percentages: 2.97% for leaf oil (A), 0.15% for stem oil (B), and 2% for the mixed oil (C). The primary constituent in all three samples was patchouli alcohol, with the highest concentration found in the stem oil (62.45%), compared to the leaf oil (46.52%) and the mixed oil (46.92%). Among the three, stem essential oil (B) exhibited the highest toxicity, with an LC_{50} value of 19.16 ppm, followed by the leaf (A) and mixed oils (C), with LC_{50} values of 22.09 ppm and 22.10 ppm, respectively. Insecticidal activity assays were conducted on third instar larvae of *Aedes aegypti*. The results indicated that all three essential oils (A, B, and C) were active as natural insecticides, with LC_{50} values of 46.40 ppm, 31.04 ppm, and 47.59 ppm, respectively(5).

In a previous study conducted by Aning Ayucitra (2014), the repellent potential of several essential oil–containing plants against *Aedes aegypti* mosquitoes was evaluated. Among the tested plants, patchouli leaves (*Pogostemon cablin* Benth) were examined at concentrations of 45%, 55%, 65%, and 75%. The essential oil extracted from patchouli leaves demonstrated significant repellent activity, particularly at a concentration of 55% v/v. At this concentration, the repellent efficacy remained remarkably high—recorded at 97.6%—even after three hours of application. This indicates that topical application of 0.1 mL of 55% v/v patchouli leaf essential oil per 30 cm^2 of skin surface can effectively repel mosquito bites by 97.6% for up to three hours. However, the r9epellent efficacy decreased over time, with observed activities of 91.2%, 88.4%, and 79.2% at the fourth, fifth, and sixth hours, respectively (6).

Methods

This study was experimental research aimed at evaluating the larvicidal effectiveness of aqueous boiled extract of patchouli leaves (*Pogostemon cablin* Benth) against *Aedes* spp. mosquitoes. A Completely Randomized Design (CRD) was employed, in which all treatment concentrations were applied to each test group. The study was conducted from April to May 2021 at the Parasitology Laboratory of Politeknik Bina Husada Kendari. Fresh patchouli leaves, selectively harvested based on maturity, were used as the primary material. Leaves from the fourth and fifth nodes of the main stem were selected. The plant population was sourced from patchouli grown in Andoolo Village, Andoolo Subdistrict, South Konawe Regency. The samples used were aqueous extracts of patchouli leaves at concentrations of 80%, 90%, and 100%. Other materials included distilled water (aquadest), clean water, label paper, filter paper, flannel cloth, and *Aedes* spp. mosquitoes as test organisms. An electric heating device was used to apply the extract in vapor form. Laboratory equipment used in the study included an analytical balance, measuring cylinders, beakers, funnels, glass stir rods, Pasteur pipettes, Petri dishes, 100 mL volumetric flasks, thermometers, a hot plate, an aspirator, a manual counter, and mosquito cages made of plastic and wood. Additional tools such as paper cups and jars were also utilized as containers during testing.

Preparation of Patchouli Leaf Extract

The selected patchouli leaves were manually harvested and washed with running water to remove impurities. After cleaning, the leaves were drained and allowed to dry before being stored in a beaker for the extraction process.

Extraction Process

One hundred grams of patchouli leaves were placed into a beaker and mixed with 100 mL of distilled water. The mixture was boiled at approximately 60°C until the volume reduced to about 60 mL. After cooling to room temperature, the solution was filtered through filter paper to obtain a clear extract. This extract was then used to prepare test solutions at various concentrations.

Preparation of Test Solutions

Three concentrations of patchouli leaf extract were prepared in 100 mL volumetric flasks. For the 80% concentration, 80 mL of the extract was mixed with 20 mL of distilled water. The 90% concentration was made by combining 90 mL of extract with 10 mL of distilled water. For the 100% concentration, 100 mL of pure extract was used without any added distilled water. Each solution was homogenized and labeled according to its concentration (Hasyim et al., 2019).

Effectiveness Testing

A total of 25 Aedes sp. mosquitoes were placed in a test cage for each treatment group. An electric device, filled with the test solution at the appropriate concentration, was placed in the cage and activated with electricity. Observations were made over a 24-hour period to record the number of mosquitoes that died in each group. Each treatment was repeated to ensure the consistency of the results (Hasyim et al., 2019).

Data Analysis

The mosquito mortality data for each concentration were analyzed by calculating the mortality percentage using the following formula:

$$Effectiveness\ (\%) = \frac{Number\ of\ Dead\ Mosquitos}{Total\ of\ Number\ Mosquitos} x100\%$$

This formula was used to compare the effectiveness of each concentration of patchouli leaf extract in killing the test mosquitoes. (Pratiwi et al., 2014).

Results

The study was conducted from April to May 2021 at the Parasitology Laboratory of the Health Analyst Program, Politeknik Bina Husada Kendari. The results of the study on the Effectiveness of Patchouli Leaf *(Pogostemon cablin Benth)* Decoction Extract as a Bioinsecticide Against Aedes sp., using distilled water as a diluent for each concentration, are presented in Table 2.

Table 1. Observation results of Aedes sp. mortality after exposure to patchouli leaf decoction extract over a period of 1,440 minutes (24 hours)

Sample	Total Mosquito Mortality	Average Mosquito Mortality	Mosquito Mortality Effectiveness
Patchouli Leaf Decoction 80%	20	6,6	27 %
Patchouli Leaf Decoction 90 %	35	11,6	48 %
Patchouli Leaf Decoction 100 %	48	16	64 %
Positive Control	75	25	100 %
Negative Control	0	0	0 %

Based on the data in Table 2, it can be observed that each concentration of patchouli leaf decoction extract exhibited a similar effect on Aedes sp. mortality, with total mosquito deaths for each concentration and three repetitions as follows: 20 mosquitoes at 80% concentration, 35 mosquitoes at 90% concentration, and 48 mosquitoes at 100% concentration. The positive control, using an electric mosquito killer (Hit-electric), resulted in 25 mosquito deaths within 24 hours, while the negative control (distilled water) showed no mosquito mortality. These observations indicate that patchouli leaf decoction extract is effective in killing Aedes sp., as evidenced by the survival of mosquitoes in the control group (Aedes sp. not treated). The effects of the patchouli leaf decoction extract are shown in Figure 1

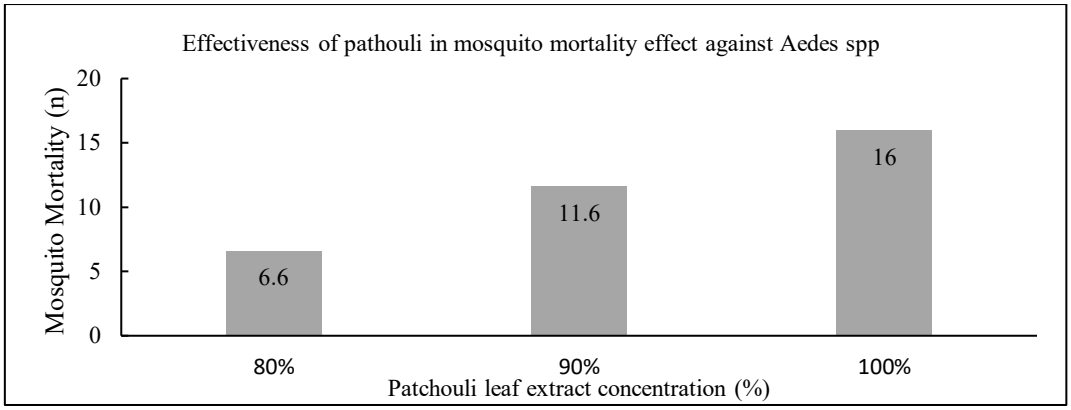

Figure 1. The Effectiveness of patchouli leaf extract at different concentrations on mosquito mortality against *Aedes* sp.

Figure 1 shows the graph of the percentage of Aedes sp. mortality, illustrating that patchouli leaf decoction extract can cause mortality in Aedes sp. starting from the lowest concentration of 80% to the highest concentration of 100%. At 80% concentration, the average mosquito mortality was 27%, at 90% concentration, the average mortality was 35%, and at 100% concentration, the average mortality was 64%. This indicates that the concentration with the highest mosquito mortality effect is 100%, which caused the most mosquito deaths compared to the other concentrations.

Tabel 2. One way anova analysis

Sample	n	Average Mosquito Mortality	*p**
Patchouli Leaf Decoction 80%	3	6,6	
Patchouli Leaf Decoction 90 %	3	11,6	<0.001
Patchouli Leaf Decoction 100 %	3	16	

**One way anova*

The results in table 3 show a significant value of <0.001 (< 0.05), indicating that the treatment with patchouli leaf decoction extract concentrations has an effect on Aedes sp. mortality

Table 3. LC_{50} values of *Aedes* sp. at different observation times

Sample Code	1	2	3	4	5	6	7	8	9
Time (minute)	5	10	20	40	60	120	240	480	1440
LC_{50} Value (%)	-	-	1,576	1,450	2,087	2,354	2,176	1,899	1,032

Based on Table 3, the LC_{50} value obtained from the probit analysis shows a decrease in value from the initial minutes to the final minutes.

Table 4. LT_{50} value of patchouli leaf extract against *Aedes* sp. at a specific concentration

Sample Code	1	2	3
Concentration (%)	80	90	100
LT_{50} Value (minute)	3.382	3.125	3.114

Based on Table 4, the LT_{50} value indicates that as the concentration increases, the time required to kill the mosquitoes decreases.

Discussion

A study was conducted to test the effectiveness of patchouli leaf decoction extract (Pogostemon cablin Benth) as a bioinsecticide against Aedes sp. In this study, patchouli leaves were selected as the sample for preparing the decoction extract.

The effectiveness test of patchouli leaf extract against Aedes sp. involved first preparing the extract solutions by diluting the concentrated decoction with distilled water (aquadest) according to the dilution formula. The solution was then transferred into a 100 mL volumetric flask, adjusted to the required volume, and homogenized. The test was carried out with three treatment groups at different concentrations: 80%, 90%, and 100%, with a negative control (aquadest) and a positive control (Hit-electric). Aquadest was used as the negative control because it is a neutral solvent that does not affect Aedes sp. growth. The aim of using different concentrations was to determine the most effective concentration for killing Aedes sp. A total of 25 mosquitoes were used in each treatment group, and each concentration was repeated three times to assess the precision and consistency of the results. The observation period lasted 1,440 minutes (24 hours).

After 24 hours of observation, the results were found to be less efficient, as the outcomes differed from the first experiment and the number of mosquitoes killed was very low. At 80% concentration, 7 mosquitoes were killed in the first trial, 6 in the second trial, and 7 in the third trial, resulting in a 27% mortality rate. At 90% concentration, 13 mosquitoes were killed in the first trial, 12 in the second, and 10 in the third, yielding a 48% mortality rate. At 100% concentration, 18 mosquitoes were killed in the first trial, 16 in the second, and 14 in the third, leading to a 64% mortality rate. The percentage of mosquito mortality observed from the three tested concentrations revealed that the lowest mortality occurred at the 80% concentration, while the highest was observed at the 100% concentration. In the positive control group (Hit-electric), mosquito mortality reached 100%, whereas in the negative control group (aquadest), no mortality was observed (0%). Several factors may have influenced the variability in results among concentrations, including insufficient homogenization of the extract with the diluent (aquadest), inadequately dried patchouli leaves, or prolonged storage of the decoction extract, potentially leading to the degradation of active compounds.

The lowest mosquito mortality was observed at the 80% concentration, with 7 mosquitoes dead in the first trial, 6 in the second, and 7 in the third, totaling 20 dead mosquitoes and an average mortality of 6.6, or 27%. The highest mortality was recorded at the 100% concentration, with 18, 16, and 14 mosquitoes dead in the first, second, and third trials respectively, totaling 48 dead mosquitoes, with an average of 16 and a mortality rate of 64%. These results indicate that the mortality rate of Aedes sp. increases with higher concentrations of the extract.

Statistical analysis using one-way ANOVA in yielded a p-value of 0.000 (< 0.05), indicating a statistically significant difference in mosquito mortality across the different extract concentrations and control groups. Lethal Concentration 50 (LC_{50}) refers to the concentration required to kill 50% of the test mosquito population. Based on the probit analysis, the LC_{50} values decreased over time, indicating increasing effectiveness of the extract as exposure duration progressed. Lethal Time 50 (LT_{50}) refers to the time required to kill 50% of the test mosquito population at a given concentration. LT_{50} was determined using probit analysis. Based on the results of the study, it was found that the higher the concentration of the extract, the shorter the time required to achieve 50% mortality. This indicates that increased extract concentrations enhance the speed of insecticidal action against *Aedes* sp.

The results of the study revealed that decoction extract of patchouli leaves (*Pogostemon cablin* Benth) has potential as an insecticide attributed to the presence of bioactive chemical compounds in the leaves, such as flavonoids, saponins, and essential oils, which can act as toxic agents against *Aedes* sp., either through contact or ingestion. Saponins may disrupt cellular interactions and ultimately lead to the death of *Aedes* sp., while flavonoids are known to interfere with the mosquito's nervous system, causing paralysis and subsequent mortality (7). Essential oils contain active compounds that have the ability to repel insects, including mosquitoes, from approaching humans. These compounds help prevent direct contact between mosquitoes and humans, thereby reducing the risk of mosquito bites and the transmission of mosquito-borne diseases (8). The results of this study differ from those reported

by (6), in which a 55% v/v concentration demonstrated high repellent activity, maintaining an effectiveness of 97.6% even after three hours of exposure. In contrast, the present study observed lower insecticidal effectiveness at higher concentrations (up to 100%) over a 24-hour period. This discrepancy may be due to differences in the mode of action assessed—repellency versus lethality—as well as variations in formulation, application method, or mosquito behavior in response to the extract. Further investigation is needed to compare the repellent and lethal properties of *Pogostemon cablin* under standardized conditions. At a concentration of 55% v/v, patchouli leaf essential oil demonstrated excellent repellent activity, maintaining a repellent efficacy of 97.6% even after three hours of application. This finding indicates that applying 0.1 mL of patchouli essential oil at a 55% v/v concentration to every 30 cm^2 of skin surface can effectively repel 97.6% of mosquito bites within the first three hours. However, the repellent effectiveness gradually declined in subsequent hours recorded at 91.2% in the fourth hour, 88.4% in the fifth, and 79.2% by the sixth hour. Additionally, larval mortality has been attributed to the presence of secondary metabolites such as flavonoids, saponins, and tannins, which exert toxic effects on mosquito larvae and contribute to the insecticidal potential of the extract.

Conclusion

The results of the study concluded that the extract exhibits insecticidal activity with increasing effectiveness at higher concentrations. At 80% concentration, the extract resulted in the death of 20 mosquitoes with a mortality rate of 27%; at 90%, it caused 35 deaths with a mortality rate of 48%; and at 100%, it was most effective, killing 48 mosquitoes with a mortality rate of 64%. These findings suggest that patchouli leaf decoction extract has potential as a natural bioinsecticide, with its efficacy increasing proportionally to the concentration used.

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Conflict of Interest

The authors declare that there's no conflict of interest regarding this article.

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