



## Potential of Noni Leaf Extract (*Morinda citrifolia L.*) As *Aedes aegypti* Mosquito Repellent

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### ARTICLE INFO

#### Article History:

Received Oct, 18<sup>th</sup>, 2023

Accepted Feb, 21<sup>st</sup>, 2024

Published online Mar, 31<sup>st</sup>, 2024

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#### Keywords:

Repellent;

Noni leaf extract;

*Aedes aegypti*;

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### ABSTRACT

Dengue fever is transmitted every year by the *Aedes aegypti* mosquitoes in Indonesia. One way to prevent this is by using repellents, such as the noni leaf extract (*Morinda citrifolia L.*). The objective of this study was to analyze the potential of the noni fruit (*Morinda citrifolia L.*) leaf extract as a repellent against *Aedes aegypti*. The study employed a post-test research design with only a control group, using concentrations of 12.5%, 25%, and 37.5%, and 25 mosquitoes with 6 repetitions. The technique for data collection involved counting mosquitoes that perched on hands. The study was conducted from November 2022 to May 2023. Data analysis was performed using SPSS Anova One Way Post Hoc and the power protection formula. The research on *Aedes aegypti* mosquitoes that perched for 6 hours showed that the noni leaf extract concentration of 37.5% had an average of 2.5 tails perched. The concentration of the noni leaf extract is 37.5%, according to the Pesticide Committee standard (1995). The average protection power for 6 hours is 90.86%. The study also suggests investigating the side effects of use and the addition of an HPMC gelling agent. However, for concentrations of 12.5% and 25%, the protective power is less than 90%. This study shows that concentrations of 37.5% are effective for repelling *Aedes aegypti* mosquitoes. Further research can be conducted using old or yellowed leaves, as well as for repelling culex and *Anopheles* mosquitoes.

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## INTRODUCTION

According to data from the Ministry of Health of the Republic of Indonesia, the frequency of dengue occurrences has fluctuated significantly. The Case Fatality Rate (CFR) increased from 0.70% in 2020 to 0.95% in 2021.<sup>1,2</sup> In 2022, the percentage decreased from 0.90% to 0.86%.<sup>3</sup> Dengue fever is transmitted by the *Aedes aegypti* or *Aedes albopictus* mosquito vector.<sup>4</sup> DHF is influenced by various factors, including the host, pathogen, and environment. It is a concept known as the Epidemiological Triangle.<sup>5,6</sup>

The high number of dengue cases in Indonesia necessitates a dengue eradication program that prioritizes the suppression of dengue vector populations through environmental.<sup>7</sup> A poor environment can lead to the formation of puddles, which can serve as breeding improvements grounds for *Aedes aegypti* larvae. The presence of *Aedes aegypti* larvae is strongly associated with the incidence of dengue fever in the region.<sup>8</sup> The use of insecticides to control *Aedes aegypti* mosquitoes may lead to the mosquitoes developing resistance to them.<sup>9,10</sup> The most effective way to achieve this is through the mosquito nests eradication movement. To prevent dengue fever, it is recommended to apply safe lotions and use repellents.<sup>11,12</sup>

Repellents are substances used to repel insects and other animals and prevent their bites and stings on humans.<sup>13</sup> The noni plant (*Morinda citrifolia* L.) can be used as a repellent. Its leaves contain alkaloids, saponins, flavonoids, tannins, polyphenols, ethanol and glycosides, which can be used as a repellent. Furthermore, noni is one of the plants that can produce essential oils.<sup>14,15</sup> Flavonoids are polyphenolic compounds that occur naturally in fruits, vegetables, and cereals. They possess antioxidant, antimicrobial, and anti-inflammatory properties. Flavonoids are odorous and can cause nerve atrophy.<sup>16</sup> Saponins can cause hemolytic reactions that destroy red blood cells, resulting in membrane damage.<sup>17</sup> Alkaloids are compounds with an alkaline nature. They increase the demand for oxygen and cause paralysis in insects, which makes them effective as insect repellents.<sup>18</sup>

Previous research has shown that the noni fruit (*Morinda citrifolia* L.) can act as a repellent for *Aedes aegypti* mosquitoes, which are vectors of dengue fever, chikungunya, and zika. The study also found that noni leaves were effective

against malaria, filariasis, and encephalitis mosquitoes.<sup>19,20</sup> The noni fruit contains saponins and flavonoids, which have an insecticidal effect while the noni leaves contain alkaloid chemicals, saponins, flavonoids, tannins, polyphenols, ethanol and glycosides. These compounds have the potential to act as antibacterial agents against *Escherichia coli* and *Salmonella typhimurium*. The betel plant extract (*Piper betle*, L.) contains chemical compounds such as saponins, flavonoids, tannins, eugenol, cavicol, and alkaloids that have been proven to repel the *Aedes aegypti* mosquito.<sup>21,22</sup> The objective of this study was to analyze the protective effect of the noni leaf extract as a repellent against *Aedes aegypti*.

## MATERIAL AND METHOD

This study employed a purely experimental approach, specifically a true experiment, and utilized a simple design with a posttest-only control group. The study population consisted of 25 *Aedes aegypti* mosquitoes. Samples of 25 randomly selected female mosquitoes aged 3-5 days were used. The methodology employed was based on the efficacy test of repellents on human skin, following the WHO 2009 standards.<sup>23</sup>

The study involved observing the number of mosquitoes perched for 5 minutes every hour over a period of 6 hours in both the control and treatment groups. The Federer formula was used for the calculation.<sup>24</sup> Each treatment was replicated six times.

$$(t - 1)(n - 1) > 15$$

Description:

t = number of groups

n = number of subjects per group

## Ingredients

Noni leaf extract was used, extracted by maceration. A total of 25 *Aedes aegypti* mosquitoes were used in the test, which took place in a cage. The necessary equipment included a plastic cup, a counter, a stopwatch, an aspirator, cotton, latex gloves, Aquades, ethanol 96%, a wet towel, a paper cup, and sugar water.

## Noni extract process

The noni leaf extract was created with a concentration of 50% (weight/volume). To dry the noni leaves, place them in an oven at 105°C.

Once dry, crush and sift the leaves into a powder. Weigh 500 grams of the noni leaf powder and place it in a maceration container. Soak the powder in 1000 ml of 96% ethanol solvent (the ratio of noni leaf powder to ethanol is 1:2) until it is completely submerged. Let the maceration vessel containing Simplisa stand for 24 hours, stirring occasionally. After 24 hours, the extract is filtered to obtain the filtrate. In the final step, the remaining filtrate is filtered again and 96% 1:2 ethanol solvent is added. The mixture is left for 24 hours and the noni leaf extract is then diluted to 50% (w/v).

### Dilution

The concentrations used in this experiment were 12.5% (volume/volume), 25% (v/v) and 37.5% (v/v). The following dilution formula was used to calculate the concentration of the noni leaf extract.<sup>25</sup>

$$C1 \times V1 = C2 \times V2$$

Description:

C = The Product of Concentration

V = Volume

### Experimental procedure

This experimental procedure is sourced from the World Health Organization (WHO), 2009. To begin, prepare a mosquito cage measuring 35-40 cm on each side. Each cage should contain 25 female *Aedes aegypti* mosquitoes that have not been fed for 24 hours prior to testing. One cage should be used for testing repellents, while the other will serve as a positive control. Before testing, it is important to wash your hands with unscented soap and rinse them thoroughly with water. Finally, rinse your hands with a 70% alcohol solution. During testing, the hands are protected by gloves made of materials that cannot be penetrated by mosquito bites. Latex gloves are commonly used for this purpose. The next steps involve using the left hand as a control, which is not given any treatment, and placing it in the cage for 15, 30, 45, and 60 minutes at 15-minute intervals. The number of mosquitoes perching on the hand is then counted. The right arm is then smeared with noni leaf extract at concentrations of 12%, 25%, and 37.5%.

The following steps were taken to conduct the experiment: First, the right arm was entered as a control and given noni leaf extract. The extract

was then inserted into the cage and the number of mosquitoes was counted. This process was repeated six times. Next, each replication was performed by replacing the mosquitoes with new ones. At the end of the experiment, the number of mosquitoes collected during each observation period was averaged for each repetition. Finally, the temperature and humidity were measured using a hygrometer and thermometer.

### Data Analysis

The study was analyzed using SPSS 16.0 software. The level of significance was set at 0.05 ( $p=0.05$ ) with a confidence level of 95% ( $\alpha=0.05$ ). The noni leaf extract concentrations were tested for their ability to repel *Aedes aegypti* mosquitoes using the one-way analysis of variance (ANOVA) followed by post-hoc tests to determine significantly different groups.<sup>26</sup> The level of protection was calculated by using the following formula.<sup>27</sup>

$$DP = \frac{K - P}{K} \times 100\%$$

Description:

DP : Repellent

K : The number of mosquitoes perched in the therapeutic arm

P : number of mosquitoes perched on the negative control

According to the Pesticide Commission (1995), power protection is considered effective if it can provide 90% protection for up to 6 hours.<sup>28</sup> The research conducted was approved by the Surabaya Health Polytechnic Ethics Committee under the number EA/1427/KEPK - Poltekkes\_Sby/V/2023.

## RESULTS

Noni (*Morinda citrifolia* L.) exhibits bioactive properties. Table 1 demonstrates that the noni leaf extract offers protection against *Aedes aegypti* mosquito bites. The results suggest the potential use of noni leaf extract as an effective mosquito repellent agent, which could have a positive impact on controlling diseases spread by this vector.

The study has demonstrated that the noni leaf extract lotion has protective properties against *Aedes aegypti* mosquitoes for up to 6 hours. The lotion was found to repel mosquitoes with an

average of 74.85% at a concentration of 12.5%, 84.86% at 25%, and 90.86% at 37.5% (Table 1).

The one-way analysis of variance (ANOVA) test results at concentration variations of 12.5%, 25%, and 37.5% indicate a significant difference in protective power between treatment groups ( $p < 0.05$ ). A post hoc test is required to determine which pairs of groups are specifically different from each other. Table 2 displays the results of post hoc tests on concentration variations.

The post hoc test revealed significant differences in protective power among the treatment groups of 12.5%, 25%, and 37.5% ( $p < 0.05$ ). The table indicates that the greater the concentration of noni leaf extract, the greater the protection against the *Aedes aegypti* mosquito.

**DISCUSSION**

The control group had a higher number of mosquitoes landing on them than the other treatments due to the lack of repellent. In contrast, the noni leaf extract treatment group had fewer mosquitoes perching. This suggests that the active compounds in noni leaves affect the perching behavior of *Aedes aegypti* mosquitoes. The noni leaf extract contains active ingredients, including flavonoids, tannins, quinines, saponins, alkaloids and triterpenoids, which have mosquito-repellent properties.

**Table 1. Average Protection Power of Noni Leaf Extract as *Aedes aegypti* Mosquito Repellent**

Hours	Protection Power Percentage (%)			
	Control	12.5%	25%	37.5%
1	0	74.6	86	93.3
2	0	76.6	84.6	90.6
3	0	77.3	86	92
4	0	76	84	90
5	0	73.3	84.6	90
6	0	71.3	84	89.3
Amount	0	449.1	509.2	545.2
Average	0	74.85	84.86	90.86

Source: Primary Data, 2023

**Table 2. Post Hoc Test Results in the Treatment Group**

Repellent	Probability		
	12,5%	25%	37,5%
12,5%		0.030	0.029
25%	0.030		0.045
37,5%	0.029	0.045	

Source: Primary Data, 2023

The large number of mosquitoes perched on them contributes to the high humidity, as evaporation from the mosquito's body accelerates air loss.<sup>29</sup> The humidity in this study was around 66%, which is within the range of 60-80%. Optimal humidity (60-80%) can facilitate the reproductive activity and development of mosquito eggs. Therefore, these humidity conditions can increase the population of *Aedes aegypti* mosquitoes which, in turn, can increase the risk of disease transmission by these mosquitoes. As well as humidity, temperature is an important factor. Mosquitoes prefer warm environments and body temperature can affect the number of active mosquitoes. Mosquitoes are more active at higher temperatures.<sup>30</sup>

The feeding behavior of mosquitoes is influenced by several factors, including heat, humidity, odor, CO2 concentration and visual stimuli. Dark or light colors can also affect the visual stimulation of mosquitoes. Mosquitoes prefer dark colors, so the presence of dark-colored objects or people may be more attractive to them.<sup>29</sup>

Several studies show that flavonoids, tannins, quinines, saponins, alkaloids and triterpenoids have mosquito-repellent properties. Flavonoids belong to the phenolic compounds. Phenolics are compounds with hydroxyl groups in the form of aromatic rings and are very important for use as insecticides and repellents.<sup>31</sup> Phenolic compounds are most abundant in upland plants.<sup>32</sup> The use of noni leaves (*Morinda citrifolia L.*) as an *Aedes aegypti* repellent has never been studied, but other parts of the noni plant have been studied. Previous research concluded that noni fruit (*Morinda citrifolia L.*) has a protective effect of 93.33%, which can be considered effective because the protective effect exceeds 90%.<sup>21,33</sup>

The repellent properties of the ethanolic extract of the noni fruit are probably due to the active compounds it contains, such as flavonoids, tannins, saponins, alkaloids and glycosides. Flavonoid compounds, tannins, alkaloids and saponins can control the *Aedes aegypti* mosquitoes and the most effective in preventing mosquitoes from landing are alkaloids.<sup>34</sup>

This difference is due to the amount of active chemicals contained in each concentration of the

noni leaf extract. The higher the concentration of active ingredients in the noni leaf extract, the greater its effectiveness in protecting the skin from mosquito bites.<sup>35</sup>

Alkaloid compounds are derivatives of aldehydes; aldehydes and ketones have a distinctive odor that is thought to stimulate the nerves of mosquitoes, causing them to leave the sample.<sup>36</sup>

The dominant characteristic odor of alkaloids is detected by mosquito antennae with sensilla, which *Aedes aegypti* mosquitoes possess. Sensillum has a complex of olfactory receptor nerves called ORNs (Olfactory Receptor Neurons). Then, alkaloids from noni leaves are translated by the mosquitoes' brains (lobe antennae) into non-attracting molecules so that the mosquitoes are not attracted and avoid them. After this process, the OBP is triggered to provide odor molecules from typical alkaloids to reduce olfactory sensitivity to the attractant molecules. This OBP complex travels through the lymph fluid to the dendrites and binds to the or (olfactory receptor), which is then delivered to the brain center. Finally, it produces behavioral responses that change to keep away from the odor.<sup>36</sup>

Power Protection Repellent Noni leaf extract at a concentration of 37.5% provided an average protection of over 90% for 6 hours. Noni leaf extract at concentrations of 12.5% and 25% gave an average protection of less than 90% for 6 hours. The noni leaf extract at 37.5% is worthy of being called repellent and more effective in the range of power protection because the concentration of 37.5% has a range of 89.3-93.3%, which is higher than the concentration of 12.5% at 71.3%-77.3% and the concentration of 25% at 84%-86%. According to the Pesticide Commission (1995), a repellent can be said to be effective if the lowest level of protection can reach 90% for 6 hours.<sup>37</sup>

Concentrations of 12.5% and 25% in the first hour to six hours are not optimal for repelling the *Aedes aegypti* mosquito, providing less than 90% protection, because high dispersibility also promotes a rapid release of the active ingredient. The evaporation of chemical compounds contained in the noni leaf extract increases over time, causing the odor of the noni extract to

disappear, leading to a decrease in the repellent potential.<sup>38</sup>

The protective power of the noni leaf extract will decrease over time in its ability to repel mosquitoes. This is due to the resistance of the aroma extract to evaporation. The lower the protective power, the lower the repellent power of the noni leaf extract.<sup>39</sup>

The flavonoids, tannins, saponins, alkaloids and glycosides contained in the noni leaf extract (*Morinda citrifolia L.*) are effective in repelling mosquitoes. The odor emitted by plant extracts, fruits and seeds is a terpenoid compound that is thought to act as a mosquito repellent. Flavonoid compounds have the ability to pass through the cuticle lining of the mosquito's body, so they can damage the mosquito's respiratory system.<sup>40</sup>

The active ingredient in noni leaves can prevent mosquitoes from landing on arms. The most effective concentration is 37.5% noni leaf extract (*Morinda citrifolia L.*) with a proven protection of 93.33%. It also suggests that the higher the concentration, the higher the active ingredient in the noni leaf, which may discourage *Aedes aegypti* from colonizing. This is supported by previous researchers who suggested that the more active ingredient added, the greater the protective effect.<sup>38</sup> The higher the concentration of the extract used, the lower the average number of mosquito habitats.<sup>13</sup>

With concentrations ranging from 12.5% to 25%, research shows that the noni leaf extract has promising potential as a natural repellent. The optimal concentration of 37.5% indicates higher efficacy, making it an attractive alternative to protect against insect bites. The noni leaf extract repellent can be used as a replacement for synthetic repellents, which can have negative long-term effects on human health. As a natural ingredient, the noni leaf extract is expected to provide a safe and effective solution to insect pests. In addition to the health benefits, the use of natural ingredients also supports environmental conservation efforts. The fact that the noni leaf extract repellent can be stored for up to a month makes it a practical and effective option. Choosing this solution not only supports human health, but also maintains harmony with the environment.

The addition of a gelling agent offers an alter-

native to increase protection for 6 hours. The gelling agent is a hydrocolloid substance that can increase the viscosity and stabilize the gel preparation.<sup>41</sup> Gel preparations in future studies can use HPMC (Hydroxypropyl Methyl Cellulose) as a gel base. The physical stability of antiseptic hand sanitizer gel using preservatives in the form of methylparaben base gel in the form of HPMC can provide good standardization of stability requirements in accordance with the specifications set by Indonesia (SNI 06-2588-1992).

## CONCLUSION AND RECOMMENDATION

The noni leaf extract (*Morinda citrifolia L.*) has been shown to be an effective repellent against *Aedes aegypti* mosquitoes. Furthermore, the 37.5% concentration was found to be most effective in providing protection against *Aedes aegypti* mosquitoes for up to 6 hours, with an average protection of 90.86% and a range of 89.3-93.3%.

The recommendations are for the public to consider using noni leaves in vector control efforts as noni leaves contain caproic acid, octanoic acid and decanoic acid, which can be extracted using ethanol or citric acid as a solvent. Although noni leaves have an unpleasant ammonia-like odor, this can be removed during the extraction process.

More research is needed on the effectiveness of the noni leaf extract as a repellent for mosquitoes such as *Culex quinquefasciatus* and *Anopheles*. In addition, the long-term side effects of using noni leaves need to be studied. Another area for further research is the addition of a gelling agent to the noni leaf extract to increase its energy-protecting properties for up to 6 hours.

## ACKNOWLEDGMENTS

The author expresses gratitude to the Director of the Department of Environmental Health, Institute of Technology, Ministry of Health, Surabaya, for their support during this study, as well as to their researcher colleagues, laboratory teachers who assisted in carrying out the study, and the Unair drug testing service. The text also mentions the use of noni leaves extracted from the Surabaya Environmental Health Entomology Laboratory.

## AUTHOR CONTRIBUTIONS

DN and LF organized and planned the research; LF and MS conducted the research in this area; LF, N, and M analyzed the data; LF, MS and DN discussed the results; LF, SW and N wrote the paper; SW, IS, and DN composed the article. DN = Demes Nurmayanti; LF = Lailatul Fithriyah; MS = Mahawiraja Setiawan; N = Ngadino; SW = Slamet Wardoyo; M = Marlik; IS = Irwan Sulistio.

## CONFLICTS OF INTEREST

The authors state that no conflict of interest exists.

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