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Efficacy of *Toona sureni* Merr. leaf extract against *Aedes* spp. and *Culex* spp. by electric diffusion

Sulasmi, Budirman, Haderiah, La Taha, Nurhaidah
Department of Environmental Health, Poltekkes Kemenkes Makassar, Makassar, Indonesia

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ABSTRACT

Aedes spp. and *Culex* spp. mosquitoes are major vectors of dengue fever and filariasis. The extensive use of chemical insecticides has contributed to resistance and environmental concerns, underscoring need for eco-friendly alternatives. Suren leaf extract (*Toona sureni* Merr.) contains bioactive compounds with potential as a botanical insecticide. This study evaluates suren leaf extract delivered via an electric diffusion device for indoor adult mosquito control at concentrations of 45%, 55%, and 65% against *Aedes* spp. and *Culex* spp. A post-test-only control group design was applied with three replications, and outcomes were recorded over a 60 minutes exposure period. Mortality increased with both concentration and exposure time. At 60 minutes, mortality increased from 80% to 100% in *Aedes* spp. and from 64% to 96% in *Culex* spp. when the concentration increased from 45% to 65%. Two-way analysis of variance (ANOVA) confirmed significant effects of concentration and exposure time ($p < 0.001$). These findings indicate that electric diffusion delivery of suren leaf extract is effective and supports its potential use for indoor vector control in endemic areas.

Corresponding Author:

Sulasmi
Department of Environmental Health, Poltekkes Kemenkes Makassar
Wijaya Kusuma street 1 no. 2, Banta-bantaeng, Rappocini, Makassar 90222, Indonesia
Email: sulasmi@poltekkes-mks.ac.id

1. INTRODUCTION

Mosquito-borne diseases remain a growing global public health threat [1]. *Aedes* spp. and *Culex* spp. mosquitoes are primary vectors of dengue hemorrhagic fever (DHF), chikungunya, Zika virus, and lymphatic filariasis diseases which collectively affect millions of people worldwide [2]. The incidence and geographic spread of these mosquito borne infections have increased in many regions over the past decades, highlighting the need for effective and sustainable vector control strategies. In Indonesia, the situation is increasingly alarming. Recent surveillance data indicate a sharp rise in dengue cases from 140,000 cases with 894 deaths in 2023 to 210,644 cases with 1,239 deaths by the 43rd week of 2024 spanning 259 districts across 32 provinces [3]. This surge reflects the limitations of current vector control strategies and underscores the urgent need for more effective, sustainable alternatives. Conventional vector control relies heavily on synthetic insecticides such as pyrethroids, organophosphates, and organochlorines. However, widespread resistance in *Aedes* spp. and *Anopheles* spp. populations has significantly compromised their efficacy [4]. Moreover, the persistence of chemical residues poses risks to human health and ecosystems [5], contradicting the principles of the sustainable development goals and the one health approach. These challenges necessitate a shift toward safer, eco-friendly vector control tools.

Botanical insecticides derived from plant secondary metabolites offer a promising alternative. Previous studies have demonstrated the efficacy of various plant extracts: neem (*Azadirachta indica*) disrupts larval development in *Aedes* spp. [6], clove oil exhibits spatial repellency [7], and soursop leaf extract reduces adult mosquito populations [8]. However, most of these applications rely on manual spraying or direct contact methods, which suffer from poor dispersion, uneven coverage, and limited indoor compatibility [9].

Suren leaves (*Toona sureni* Merr.) contain bioactive compounds including flavonoids, alkaloids, and saponins known to interfere with insect metabolic pathways, act as neurotoxins, and inhibit feeding behavior. Although Sidabutar *et al.* [10] isolated flavonoid constituents from suren leaves, supporting its bioactivity, its application has remained confined to crude or topical formulations. Critically, no prior study has evaluated its adulticidal efficacy against both *Aedes* spp. and *Culex* spp. using electric diffusion a method that enables controlled, residue free release of volatile compounds in enclosed spaces.

This study addresses a critical gap in vector control strategies by evaluating the efficacy of *Toona sureni* Merr. leaf extract at concentrations of 45%, 55%, and 65% against adult *Aedes* spp. and *Culex* spp. mosquitoes via electric diffusion. The novelty of this research lies in the synergistic combination of a botanical insecticide with an advanced application technology. The findings demonstrate the significant potential of suren leaf extract as an environmentally benign alternative for vector control and confirm that the electric diffusion method substantially enhances the efficacy of botanical formulations.

2. METHOD

2.1. Research design

This study used a pure experimental design with a post-test-only control group. This design was chosen because no baseline data were required and aimed to minimize bias in measuring mosquito mortality caused by external factors. The post-test-only model is also commonly used in bioactivity testing of botanical insecticide and has been recommended in several previous studies [11], [12]. This design allows observation of the effect of various concentrations of suren leaf extract (45%, 55%, and 65%) on *Aedes* spp. and *Culex* spp. mortality. The mosquitoes were compared to an untreated control group. Using a control group allows for a valid comparison so that observed changes can be truly attributed to the treatment rather than to other external factors [13]. This study was designed to ensure internal validity by avoiding bias due to baseline measurements. A total of 625 mosquitoes were used, consisting of 25 mosquitoes per treatment group with three repetitions. The independent variable in this study was the concentration of suren leaf extract, the dependent variable was the mosquito mortality rate in a predetermined time interval of 10 minutes for 60 minutes. The overall experimental workflow is illustrated in Figure 1.

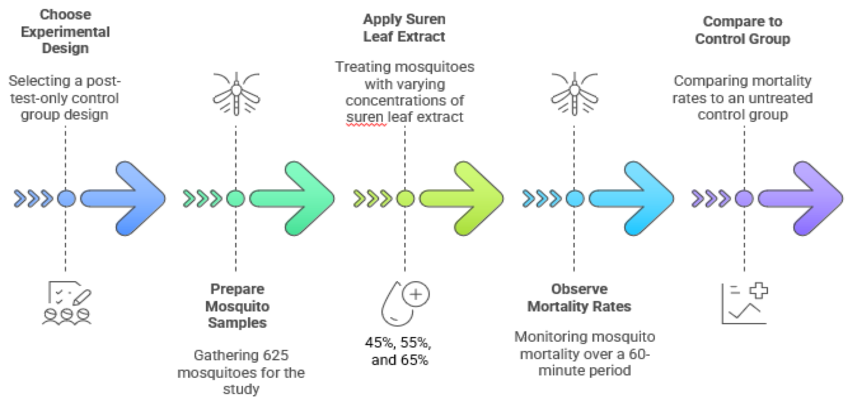

Figure 1. Experimental design for mosquito mortality study

2.2. Materials and tools

The main materials were fresh *Toona sureni* Merr. leaves collected from South Sulawesi, Indonesia. The leaves were shade-dried for 72 hours and ground into a fine 500-gram powder, which was processed into an extract using a 70% ethanol solvent. The 70% ethanol solvent was chosen because it can dissolve bioactive compounds in suren leaves efficiently without damaging the active components [14]. The test subjects consisted of 3- to 5-day-old female *Aedes* spp. and *Culex* spp. mosquitoes reared in the laboratory to maintain the uniformity of mosquito age. A commercial electric vaporizer (220 V, 5 W) was used as the diffusion device. Testing was conducted in a 1×1×1 m³ cubic chamber with transparent paper/plastic walls. Supporting equipment included a rotary evaporator (50 °C), a blender, a sieve, an analytical balance, a stopwatch, and an observation sheet.

2.3. Research procedure

Preparation of suren leaf extract: fresh *Toona sureni* Merr. leaves were washed and shade-dried for three days (approximately 72 hours) before being ground into a fine powder. The extraction was carried out using the maceration method. A total of 500 g of the dried leaf powder was soaked in 1.5 L of 70% ethanol. After several days, the mixture was filtered, and the residue was re-macerated with 750 mL of fresh 70% ethanol. The combined filtrates were then concentrated using a rotary evaporator at 50 °C to obtain a concentrated extract. Mosquito mortality testing: a total of 25 female mosquitoes (3- to 5-day-old) per replicate were introduced into a 1×1×1 m^3 testing chamber. For each test, 5 mL of the suren leaf extract at a specified concentration (45%, 55%, or 65%) was loaded into the electric vaporizer, which was then activated inside the sealed chamber. Mortality was observed at 10, 30, 45, and 60-minute intervals. A mosquito was recorded as dead if it showed no movement upon gentle prodding with a fine brush. The control group was exposed to an empty, activated vaporizer under identical conditions. Each concentration, including the control, was tested with three replicates for each mosquito species.

2.4. Data analysis

Mortality data were tested for normality (Shapiro-Wilk) and homogeneity of variance (Levene's test). A two-way analysis of variance (ANOVA) (SPSS v26, α =0.05) was conducted to assess the main effects of concentration and exposure time, as well as their interaction, on mosquito mortality. Post-hoc comparisons were performed using Tukey's honestly significant difference (HSD) test where applicable. Mortality rates were expressed as percentages and presented in tabular form.

3. RESULTS AND DISCUSSION

The integration of *Toona sureni* leaf extract with electric diffusion technology presents a compelling alternative to synthetic insecticides. A key advantage of this approach is its potential for residue-free vector control, making it suitable for indoor applications. Unlike conventional sprays that leave persistent chemical residues on surfaces, the electric diffusion system disperses bioactive compounds as a vapor that degrades rapidly in the air. This characteristic, coupled with the biodegradable nature of the plant-based extract, minimizes the ecological impacts associated with synthetic pyrethroids or organophosphates.

While the insecticidal potential of various botanicals such as garlic, cloves, and soursop leaves has been established [15], existing research has predominantly focused on larvicidal effects or manual application methods. Notably, the efficacy of *Toona sureni* Merr. leaf extracts, specifically when delivered via electric diffusion against adult *Aedes* spp. and *Culex* spp. mosquitoes, remains insufficiently explored [10]. Furthermore, a comparative analysis of the electric diffusion method against other application techniques for adult mosquito control is absent from the literature. This study therefore aims to address these critical gaps by evaluating the efficacy of suren leaf extract administered through electric diffusion, assessing its potential as a novel tool for household vector control. In support of this hypothesis, the results showed that mosquito mortality increased with increasing concentration of suren leaf extract and duration of exposure. Specifically, Table 1 illustrates the gradual increase in the mortality rate of *Culex* spp. mosquitoes exposed to suren leaf extract at 45% concentration. Initial mortality was only 4% at 10 minutes but increased to 64% at 60 minutes. This confirms that even at low concentrations, the duration of exposure plays an important role in increasing insecticide effectiveness.

Table 1. Mortality rate of *Culex spp.* mosquitoes on suren leaf extract with a concentration of 45%

Length of observation time	Number of dead *Culex* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	1	1	1	1	4
20 minutes	0	3	5	4	4	16
30 minutes	0	8	6	6	7	28
40 minutes	0	12	10	10	11	44
50 minutes	0	14	14	14	14	56
60 minutes	0	15	16	17	16	64

A more striking change was seen at the 55% extract concentration (Table 2), where the mortality rate of *Culex* spp. rose from 8% at 10 minutes to 72% at 60 minutes. This increase highlights that the extract's effectiveness becomes more apparent with longer exposure times, although higher concentrations are still required for faster and more effective population control. The maximum effect was achieved at the highest concentration of 65% (Table 3). At the 10 minutes, the average mortality rate was 8% but increased sharply to 96% at the 60 minutes. This significant spike in mortality, especially after 40 minutes, indicates that the high concentration is highly effective and capable of almost totally eradicating mosquitoes in a relatively short period. The effectiveness of suren leaf extract was not only limited to *Culex* spp., but was also evident in *Aedes* spp., as shown in Table 4; the mortality rate of *Aedes* spp. mosquitoes at 45% extract concentration increased from 8% at the 10 minutes to 80% at the 60 minutes. These results indicate that *Aedes* spp. tends to be more sensitive to exposure to suren leaf extract, especially at longer exposure durations, so significant population control can be achieved even at low concentrations.

Table 2. Mortality rate of *Culex* spp. mosquitoes on suren leaf extract with a concentration of 55%

Length of observation time	Number of dead *Culex* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	1	2	2	2	8
20 minutes	0	4	6	5	5	20
30 minutes	0	7	8	7	7	28
40 minutes	0	13	13	11	12	48
50 minutes	0	16	16	15	16	64
60 minutes	0	17	19	19	18	72

Table 3. Mortality rate of *Culex* spp. mosquitoes on suren leaf extract with a concentration of 65%

Length of observation time	Number of dead *Culex* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	2	2	3	2	8
20 minutes	0	5	6	7	6	27
30 minutes	0	10	9	10	10	40
40 minutes	0	16	14	16	15	60
50 minutes	0	20	19	20	20	80
60 minutes	0	23	25	24	24	96

Table 4. Mortality rate of *Aedes* spp. mosquitoes on suren leaf extract with a concentration of 45%

Length of observation time	Number of dead *Aedes* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	1	2	2	2	8
20 minutes	0	4	4	4	4	16
30 minutes	0	8	6	8	7	28
40 minutes	0	11	10	12	11	44
50 minutes	0	16	14	15	15	60
60 minutes	0	21	19	20	20	80

A similar pattern of increased mortality is also seen in Table 5, where *Aedes* spp. exposed to 55% extract concentration experienced an increase in mortality from 8% at the 10 minutes to 92% at 60 minutes. This sharp increase, especially after 30 minutes, reinforces that *Aedes* spp. is more sensitive to active compounds in suren leaf extract than *Culex* spp. At the highest extract concentration of 65% (Table 6), the mortality rate of *Aedes* spp. started from 12% in the first 10 minutes and reached 100% by the 60 minutes. This substantial increase confirms that high concentrations effectively eradicate adult mosquitoes. These findings directly support the research hypothesis that the electric diffusion method of suren leaf extract is effective as an environmentally friendly and practical botanical insecticide for disease vector control.

Table 5. Mortality rate of *Aedes* spp. mosquitoes on suren leaf extract with a concentration of 55%

Length of observation time	Number of dead *Aedes* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	2	2	3	2	8
20 minutes	0	4	5	6	5	20
30 minutes	0	8	7	12	9	36
40 minutes	0	13	11	15	13	52
50 minutes	0	18	16	20	18	72
60 minutes	0	24	21	25	23	92

Table 6. Mortality rate of *Aedes* spp. mosquitoes on suren leaf extract with a concentration of 65%

Length of observation time	Number of dead *Aedes* spp. mosquitoes Replication				Average mortality	Average mortality (%)
	Control	I	II	III		
10 minutes	0	3	2	3	3	12
20 minutes	0	5	5	7	6	24
30 minutes	0	10	9	13	11	44
40 minutes	0	15	14	19	16	64
50 minutes	0	24	19	24	22	88
60 minutes	0	25	24	25	25	100

This study also compared the findings with previous studies to strengthen the interpretation of the results. The trend that the effectiveness of botanical insecticides increases proportionally with concentration was also observed by Pereira *et al.* [16], who explained that flavonoids and other bioactive compounds in plant extracts work optimally at high doses because they can disrupt the metabolism and nervous system of insects more intensively. Cui *et al.* [12] also found that saponins from other plants can trigger death in target insects through a similar mechanism [12], [17]. Further support is provided by Maris *et al.* [18], who reported toxicity of *Toona sureni* leaf hydrosol and aqueous extract against *Aedes aegypti*, reinforcing the potential of suren leaves as a source of bioinsecticidal agents.

The results of this study are also in line with Jung *et al.* [19], who conducted a comparative test of liquid-type electric vaporizers showing a knock-down of up to 72.8% against *Aedes albopictus* in 3 hours of operation. This mechanism allows the controlled release of bioactive substances, maximizing vapor contact with mosquitoes in enclosed spaces. However, several other studies have reported that the effectiveness of natural insecticides is not always maximized in adult populations or specific species. For example, Falkowski *et al*. [11] found that some active compounds were only effective in the larval phase, while the effect on adult mosquitoes tended to be lower. In other words, the effectiveness of the results highly depends on the type of extract, the application method, and the biological susceptibility of the target mosquitoes. The use of electric diffusion in this study was able to distribute the active compounds more evenly so that adult mosquitoes were optimally exposed. In addition, Balabanidou *et al.* [20] found that mosquitoes with thinner cuticles or faster metabolism tend to be more susceptible to botanical insecticide exposure. This high sensitivity of *Aedes* spp. is in line with the report of Youssefi *et al*. [21], which states that monoterpenoids such as carvacrol and thymol are also effective in eradicating *Aedes* spp. eggs and larvae with vapor/diffusion-based applications [21], [22]. However, some studies mention that the effectiveness of botanical insecticides may decrease if mosquitoes have adapted or developed resistance, so repeated applications in real environments need to be considered to minimize the risk of resistance [23].

Statistical analysis through two-way ANOVA also clarified the significant effects of concentration and exposure time. For *Culex* spp., both concentration ($F = 104.293$, $p < 0.001$) and exposure duration ($F = 558.580$, $p < 0.001$) significantly affected mortality rates, with a significant interaction between the two factors ($F = 6.741$, $p < 0.001$). Meanwhile, in *Aedes* spp., concentration ($F = 25.326$, $p < 0.001$) and exposure time ($F = 215.020$, $p < 0.001$) also had a significant effect, although without a significant interaction ($F = 1.701$, $p = 0.119$). These data corroborate that increasing concentration and exposure duration are key factors in the effectiveness of botanical insecticides, with *Aedes* spp. showing higher sensitivity.

The results of this study are also in line with the research of Liang *et al.* [13], which confirmed that botanical insecticides are more effective when used at high doses and with adequate contact. In contrast, Ochieng *et al.* [24] cautioned that environmental factors such as temperature and humidity can affect the volatility of active compounds in the field. So laboratory effectiveness is not necessarily the same as field results [24], [25]. However, this result is slightly different from that of Falkowski *et al.* [11], who showed that the effectiveness of botanical insecticides in the larval phase was more dominant than in adult mosquitoes. This difference could be attributed to the application method; the use of electric diffusion in this study was able to distribute the active compounds more evenly, resulting in optimal exposure of adult mosquitoes. In addition, *Aedes* spp. appeared more sensitive than *Culex* spp., which may be due to differences in cuticle structure and metabolism between species [20].

These findings open opportunities to develop more environmentally friendly mosquito control strategies based on natural materials [23], [26] in areas prone to diseases such as dengue fever and filariasis without causing adverse side effects to human health or the environment. With the increasing problem of resistance to chemical insecticides, using suren leaf extract as a botanical insecticide has excellent potential to be applied on a broader scale. The diversity of plant-based active compounds, including terpenoids, phenols, and flavonoids, can reduce likelihood of resistance due to their complex mechanisms of action [27]. This approach is in line with the global trend to develop biodegradable alternative insecticides that have low toxicity to non-target organisms [28], [29]. Nevertheless, this study has some limitations that need to be considered. One major limitation is that the experiments were conducted under laboratory conditions, which do not reflect the complexity of the actual environment. Additionally, the concentration range tested, while demonstrating clear efficacy, was not designed for precise determination of LC_{50} and LT_{50} values through probit analysis. Furthermore, this study did not include a solvent-only control (e.g., 70% ethanol without extract), which limits the ability to fully isolate the contribution of the solvent to observed mortality. However, given the low volume used (5 mL in a 27 L chamber) and prior evidence that 70% ethanol vapor is non-lethal under similar conditions [22], the solvent effect is considered minimal. Further research is urgently needed to test the effectiveness of suren leaf extract under field conditions [24], evaluate its impact on non-target organisms, and develop more stable and applicable formulations [30]. Comparative studies with other botanical insecticides or combinations of multiple bioactive compounds may also provide additional insights into the synergies and relative effectiveness of suren leaf extracts.

Overall, the results indicate that suren leaf extract (*Toona sureni* Merr.) delivered via electric diffusion significantly increases mortality of *Aedes* spp. and *Culex* spp. mosquitoes. The effect is strongest at high concentrations and sufficient exposure times. With increasing resistance to chemical insecticides, natural-based approaches offer innovative and sustainable solutions for future disease vector control.

4. CONCLUSION

This study investigated the adulticidal effectiveness of suren leaf extract (*Toona sureni* Merr.) delivered using an electric diffusion device against *Aedes* spp. and *Culex* spp. The results showed that the extract was effective across the tested concentrations, with the 65% concentration producing the highest mortality, reaching 100% in *Aedes* spp. and 96% in *Culex* spp. within 60 minutes. These findings indicate that suren leaf extract, when applied via electric diffusion, has potential as a botanical adulticide for indoor mosquito control. Further research is recommended to evaluate performance under field conditions and to optimize formulation stability and diffusion efficiency before broader application.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Sulasmi	✓	✓		✓		✓	✓	✓		✓		✓	✓	✓
Budirman			✓			✓			✓	✓	✓			
Haderiah	✓				✓			✓		✓				✓
La Taha	✓	✓						✓		✓				✓
Nurhaidah	✓		✓		✓			✓		✓				✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

ETHICAL APPROVAL

This study used only adult mosquitoes (arthropods) and did not involve vertebrate animals or human subjects. All procedures followed standard biosafety guidelines for arthropod handling. Ethical clearance for the study was obtained from the Health Research Ethics Committee of Poltekkes Kemenkes Makassar (No. 1271/M/KEPK-PTKMS/VIII/2024).

DATA AVAILABILITY

All authors confirm that the data supporting the findings of this study are available in the article [and/or supplementary materials].

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BIOGRAPHIES OF AUTHORS

Sulasmi is a lecturer at Poltekkes Kemenkes Makassar, majoring in environmental health. She is interested in the field of environmental health. She has published several times and conducted research on environmental health issues. She can be contacted at email: sulasmi@poltekkes-mks.ac.id.

Budirman is a lecturer at Poltekkes Kemenkes Makassar, majoring in Environmental Health. He is interested in environmental health and environmental management. He has published several times and conducted research on environmental health and environmental issues. He can be contacted at email: budirman@poltekkes-mks.ac.id.

Haderiah is a lecturer at Poltekkes Kemenkes Makassar, majoring in environmental health. She is interested in the field of environmental health. She has published several times and conducted research on environmental health issues. She can be contacted at email: haderiah.nuru64@gmail.com.

La Taha is a lecturer at Poltekkes Kemenkes Makassar, majoring in environmental health. He is interested in the field of environmental health. He has published several times and conducted research on environmental health issues. He can be contacted at email: lataha1962.kesling@gmail.com.

Nurhaidah is a lecturer at Poltekkes Kemenkes Makassar, majoring in environmental health. She is interested in the field of environmental health. She has published several times and conducted research on environmental health issues. She can be contacted at email: nurhaidah@poltekkesdepkes-sby.ac.id.