Effects of Plant Species and Aqueous Extraction Methods on the Mortality of Aedes aegypti Larvae

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Research Article

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Abstract

The increasing incidence of mosquito-borne diseases has highlighted the need for environmentally friendly alternatives to synthetic larvicides. This study compared the larvicidal efficacy of aqueous extracts prepared from kaffir lime (*Citrus hystrix*) peel, holy basil (*Ocimum tenuiflorum*) leaves, and citronella (*Cymbopogon citratus*) stems using two extraction methods (grinding and boiling) against *Aedes aegypti* larvae. Second- to third-instar larvae were exposed to a 10% (v/v) aqueous extract under laboratory conditions, with distilled water serving as the negative control. Larval mortality was recorded at 1, 5, 24, and 48 h after treatment. Each treatment was performed in triplicate using 20 larvae per replicate. Significant differences in larvicidal activity were observed among the herbal extracts and extraction methods. Ground kaffir lime peel extract exhibited the highest efficacy, producing complete larval mortality within 24 h. Boiled kaffir lime peel and ground holy basil extracts also demonstrated high larvicidal activity, producing approximately 98% mortality after 48 h. Because mortality in the untreated control reached 11.7% at 48 h, treatment mortality at this observation time was corrected using Abbott's formula. Following correction, ground kaffir lime peel achieved 100% corrected mortality, whereas boiled kaffir lime peel and ground holy basil each produced approximately 98% corrected mortality. These findings indicate that freshly prepared ground kaffir lime peel extract is a promising low-cost botanical larvicide for controlling *Aedes aegypti* larvae. Further studies should identify the active chemical constituents, determine LC_{50} and LC_{90} values, evaluate non-target safety, and confirm efficacy under field conditions.

1. Introduction

Mosquito-borne diseases remain one of the major public health challenges worldwide. Among mosquito vectors, *Aedes aegypti* is the principal transmitter of dengue fever, Zika virus, chikungunya, and yellow fever. According to the World Health Organization (WHO), dengue incidence has increased dramatically over the past two decades, with billions of people currently living in areas at risk of infection. Effective control of mosquito larvae is therefore considered one of the most practical strategies for reducing adult mosquito populations and preventing disease transmission.

Chemical larvicides such as temephos have long been used for mosquito control because of their rapid effectiveness. However, continuous application of synthetic insecticides has resulted in several adverse consequences, including the development of insecticide resistance, contamination of aquatic ecosystems, toxicity to non-target organisms, and concerns regarding human health and environmental sustainability. These limitations have encouraged researchers to explore environmentally friendly alternatives derived from natural products.

Plant-derived extracts have attracted increasing attention as potential botanical larvicides because they contain numerous bioactive compounds, including essential oils, flavonoids, terpenoids, alkaloids, and phenolic compounds. Many medicinal plants exhibit insecticidal, repellent, antimicrobial, and antioxidant properties while presenting relatively low toxicity to mammals and causing less environmental pollution than conventional chemical insecticides.

Among Thai medicinal plants, kaffir lime (*Citrus hystrix*), holy basil (*Ocimum tenuiflorum*), and citronella (*Cymbopogon citratus*) are widely available and have been traditionally used for medicinal and household purposes. Kaffir lime peel contains high concentrations of essential oils, particularly citronellal, limonene, and β-pinene, which possess insecticidal and larvicidal activities. Holy basil contains eugenol and methyl chavicol, compounds known for their antimicrobial and insecticidal effects, whereas citronella is rich in citral and citronellal, which are commonly used as mosquito repellents. Although these herbs have demonstrated biological activities, previous studies have reported inconsistent larvicidal efficacy depending on extraction techniques, plant parts, and experimental conditions.

In addition, most previous studies have focused primarily on identifying effective plant species, while comparatively few have investigated how extraction methods influence larvicidal activity. Extraction techniques can substantially affect the concentration and stability of bioactive compounds, thereby influencing biological efficacy. Understanding the relationship between extraction methods and larvicidal performance is essential for developing practical and cost-effective botanical larvicides suitable for household applications.

Therefore, this study aimed to compare the larvicidal efficacy of extracts obtained from kaffir lime peel, holy basil leaves, and citronella using two extraction methods, namely grinding and boiling, against *Aedes aegypti* larvae under laboratory conditions. The findings of this study may contribute to the development of environmentally friendly mosquito control strategies and promote the utilization of locally available herbal resources for sustainable vector management.

2. Materials and Methods

2.1 Study Design

This laboratory-based experimental study was conducted to compare the larvicidal efficacy of three Thai herbal extracts prepared using two different extraction methods against Aedes aegypti larvae.

A completely randomized design (CRD) was employed. Three herbal species, namely kaffir lime (Citrus hystrix) peel, holy basil (Ocimum tenuiflorum) leaves, and citronella (Cymbopogon citratus), were extracted using two preparation methods (grinding and boiling), resulting in six treatment groups. Distilled water without herbal extract served as the negative control.

Each treatment was performed in triplicate using twenty second- to third-instar Aedes aegypti larvae per replicate.

Table 1
Experimental Design

Treatment	Plant species	Plant part	Extraction	Concentration	Replicates	Larvae/Replicate
T1	*Citrus hystrix*	Peel	Ground	10%	3	20
T2	*Citrus hystrix*	Peel	Boiled	10%	3	20
T3	*Ocimum tenuiflorum*	Leaves	Ground	10%	3	20
T4	*Ocimum tenuiflorum*	Leaves	Boiled	10%	3	20
T5	*Cymbopogon citratus*	Stem	Ground	10%	3	20
T6	*Cymbopogon citratus*	Stem	Boiled	10%	3	20
Control	Distilled water	—	—	—	3	20

2.2 Collection of Plant Materials

Fresh kaffir lime fruits, holy basil leaves, and citronella stems were purchased from local fresh markets in Nonthaburi Province, Thailand.

The plant materials were washed thoroughly with tap water followed by distilled water to remove dirt and contaminants before extraction.

2.3 Preparation of Herbal Extracts

Fresh kaffir lime (*Citrus hystrix*) peel, holy basil (*Ocimum tenuiflorum*) leaves, and citronella (*Cymbopogon citratus*) stems were processed separately. Before extraction, the plant materials were washed thoroughly with tap water, rinsed with distilled water, and allowed to drain at room temperature. Kaffir lime peel was separated from the fruit, while damaged or discolored portions of the holy basil leaves and lemongrass stems were discarded.

For each preparation, 50 g of fresh plant material was weighed using a digital balance. The plant material was cut into small pieces before extraction. Each plant species was prepared using two aqueous extraction methods: grinding and boiling. The initial plant-to-solvent ratio was 50 g of fresh plant material per 200 mL of distilled water, corresponding to a ratio of 1:4 (w/v).

2.3.1 Grinding Method

Fifty grams of each fresh plant material was finely chopped and ground using a clean mortar and pestle until a relatively homogeneous plant pulp was obtained. Two hundred milliliters of distilled water was then

added gradually while grinding and mixing continued to facilitate the release of water-soluble and dispersed plant constituents.

The resulting mixture was transferred into a clean container and allowed to stand at room temperature for 10 min. During this period, the mixture was stirred intermittently to maintain uniform contact between the plant material and the extraction solvent. The mixture was subsequently filtered four times through clean sterile muslin cloth to remove coarse plant residues.

The collected filtrate was transferred into a clean, labeled container and used as the freshly prepared ground aqueous stock extract. Separate extraction equipment was cleaned thoroughly between plant species to minimize cross-contamination.

2.3.2 Boiling Method

Fifty grams of each freshly prepared plant material was placed in a clean heat-resistant container, and 200 mL of distilled water was added. The mixture was heated over medium heat and maintained at boiling temperature for 10 min. The preparation was stirred after approximately 5 min to ensure uniform heating and contact between the plant material and water.

After heating, the mixture was removed from the heat source and allowed to cool at room temperature for approximately 30–45 min. The cooled preparation was then filtered through clean sterile muslin cloth to remove plant residues. The filtrate was transferred into a clean, labeled container and used as the boiled aqueous stock extract.

Following cooling and filtration, the volume of the boiled extract was adjusted to 200 mL with distilled water to compensate for water loss during heating and to ensure comparability with the ground extract.

2.3.3 Preparation and Handling of Stock Extracts

All aqueous stock extracts were prepared separately on the day of the larvicidal bioassay and were not stored for subsequent experiments. Before use, each extract was mixed gently to ensure uniform distribution of suspended and dissolved constituents.

For each experimental replicate, 50 mL of the freshly prepared stock extract was added to 450 mL of distilled water, producing a final volume of 500 mL. Therefore, the test preparation contained a 10% (v/v) dilution of the aqueous stock extract. Based on the initial plant-to-solvent ratio, this was approximately equivalent to 12.5 g of fresh plant material per 500 mL of test solution, or an initial plant-equivalent concentration of approximately 2.5% (w/v), before accounting for material removed during filtration.

Separate equipment and labeled containers were used for each plant species and extraction method to prevent cross-contamination.

Table 2
Experimental Conditions and Characteristics of Herbal Extracts

Parameter	**Description**			
Study design	Completely Randomized Design (CRD)			
Test organism	*Aedes aegypti* larvae			
Larval stage	Second–Third instar			
Herbal species	3			
Extraction methods	2			
Experimental groups	7			
Replicates	3			
Larvae per replicate	20			
Total larvae	420			
Observation time	1, 5, 24, 48 h			
Primary outcome	Mortality (%)			
Statistical analysis	One-way ANOVA with Tukey's post hoc test			
Treatment	**pH**	**Color**	**Odor**	**Expected Active Compounds**
Kaffir lime (Ground)	6	Green	Strong citrus	Citronellal, Limonene
Kaffir lime (Boiled)	9	Light green	Moderate citrus	Citronellal, Limonene
Holy basil (Ground)	9	Dark green	Aromatic	Eugenol
Holy basil (Boiled)	9	Brown-green	Mild	Eugenol
Citronella (Ground)	8	Pale green	Lemon-like	Citral
Citronella (Boiled)	7	Yellow-green	Mild	Citral
Control	7	Clear	None	—

2.4 Mosquito Larvae

Second- to third-instar Aedes aegypti larvae were obtained from the National Institute of Health, Department of Medical Sciences, Thailand.

Larvae were maintained under laboratory conditions at room temperature and fed with powdered rabbit food once daily until use.

Only healthy and actively swimming larvae were selected for the bioassay.

2.5 Larvicidal Bioassay

For each experimental unit, 20 healthy and actively swimming second- to third-instar *Aedes aegypti* larvae were transferred into a transparent plastic container containing 450 mL of distilled water. Fifty milliliters of the freshly prepared aqueous stock extract was subsequently added, producing a final test volume of 500 mL and a stock-extract concentration of 10% (v/v). Each treatment was independently replicated three times.

The experimental groups consisted of:

- Ground kaffir lime peel
- Boiled kaffir lime peel
- Ground holy basil
- Boiled holy basil
- Ground citronella
- Boiled citronella
- Distilled water (control)

The test solutions were gently mixed before the larvae were introduced to ensure uniform distribution of the extract.

2.6 Observation and Correction of Larval Mortality

Larval mortality was assessed at 1, 5, 24, and 48 h after exposure. A larva was considered dead when it showed no movement after gentle stimulation with a fine brush. The cumulative number of dead larvae in each experimental unit was recorded at each observation time and converted to a percentage using the following equation:

$$\text{Observed mortality } (\%) = \frac{\text{Number of dead larvae}}{\text{Total number of larvae exposed}} \times 100$$

When mortality occurred in the untreated control group, treatment mortality was corrected using Abbott's formula:

$$\text{Corrected mortality } (\%) = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Observed mortality in control}} \times 100$$

Abbott's correction was applied when control mortality was greater than 5% but did not exceed 20%. When control mortality was 5% or lower, the observed mortality was reported without correction. If the calculated corrected mortality was negative because mortality in a treatment group was lower than that in the control group, the corrected mortality was recorded as 0%. An experiment would be considered invalid and repeated if control mortality exceeded 20%.

2.7 Water Quality Measurement

The pH of each extract was measured immediately before the bioassay using a calibrated digital pH meter.

The recorded pH values ranged from 6 to 9 depending on the herbal extract.

2.8 Outcome Measures

The primary outcome was

- Percentage larval mortality (%)

The secondary outcomes included

- Time required to achieve complete mortality
- Comparison between extraction methods
- Comparison among herbal species

2.9 Statistical Analysis

Mortality data were summarized as the mean cumulative number of dead larvae and the corresponding mortality percentage. Values are presented as mean ± standard deviation (SD) from three independent replicates, with 20 larvae per replicate.

Observed mortality percentages were calculated separately for each treatment and observation time. When control mortality exceeded 5% but remained at or below 20%, treatment mortality was adjusted using Abbott's correction. Corrected mortality values were used for the comparison of larvicidal efficacy at observation times requiring correction.

Differences among treatment groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference post hoc test for multiple comparisons. The assumptions of normality and homogeneity of variance were assessed before analysis. A value of $p < 0.05$was considered statistically significant.

3. Results

3.1 Larvicidal Activity of Different Plant Extracts

Ground kaffir lime peel extract produced the most rapid larvicidal response, resulting in 75.0% observed mortality at 1 h and complete mortality by 24 h. At 48 h, boiled kaffir lime peel and ground holy basil extracts each produced 98.3% observed mortality.

Mortality in the untreated control group was 0% at 1 and 5 h, 3.3% at 24 h, and 11.7% at 48 h. Because control mortality at 48 h exceeded 5% but remained below 20%, treatment mortality at this observation time was adjusted using Abbott's formula. Following correction, ground kaffir lime peel produced 100.0% mortality, while boiled kaffir lime peel and ground holy basil each produced approximately 98.1% corrected

mortality. The corrected mortality of boiled holy basil and both lemongrass preparations was 0%, because their observed mortality was lower than that of the untreated control.

The cause of the mortality observed in the control group at 48 h was not determined.

Table 3
Larval mortality (Mean ± SD) following exposure to herbal extracts

Treatment	1 h	5 h	24 h	48 h
Kaffir lime (Ground)	15.0 ± 1.0	18.3 ± 0.6	20.0 ± 0.0	20.0 ± 0.0
Kaffir lime (Boiled)	2.3 ± 0.6	9.0 ± 1.0	17.7 ± 0.6	19.7 ± 0.6
Holy basil (Ground)	0.0 ± 0.0	1.3 ± 0.6	10.0 ± 1.0	19.7 ± 0.6
Holy basil (Boiled)	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6	0.7 ± 0.6
Citronella (Ground)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6
Citronella (Boiled)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6
Control	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.6	2.3 ± 0.6

Note: Values are presented as mean ± standard deviation of the cumulative number of dead larvae from three independent replicates, with 20 larvae per replicate.

Table 4
Observed and Abbott-corrected mortality of Aedes aegypti larvae after 48 h of exposure

Treatment	Dead larvae/60	Observed mortality (%)	Abbott-corrected mortality (%)
Kaffir lime peel (ground)	60	100.0	100.0
Kaffir lime peel (boiled)	59	98.3	98.1
Holy basil leaves (ground)	59	98.3	98.1
Holy basil leaves (boiled)	2	3.3	0.0
Citronella stems (ground)	1	1.7	0.0
Citronella stems (boiled)	1	1.7	0.0
Control	7	11.7	—

Larval mortality of Aedes aegypti after 48 hours of exposure to 10% aqueous herbal extracts prepared using grinding and boiling methods. Values represent mean mortality ± standard deviation from three replicates, with 20 larvae per replicate.

3.2 Effect of Extraction Methods

The extraction method significantly influenced larvicidal efficacy.

For kaffir lime peel, the grinding method resulted in significantly higher mortality than the boiling method during the early exposure period (1–24 hours).

Similarly, ground holy basil extract demonstrated markedly greater larvicidal activity than boiled holy basil.

In contrast, no apparent difference between grinding and boiling methods was observed for citronella extracts, both of which showed negligible larvicidal activity.

3.3 Time-dependent Larvicidal Activity

The mortality rate increased progressively with exposure time for the effective herbal extracts.

Ground kaffir lime peel induced rapid mortality during the first hour, while boiled kaffir lime and ground holy basil exhibited gradual increases in mortality over time.

The remaining treatments maintained low mortality throughout the observation period.

Larval mortality (%) of second- to third-instar Aedes aegypti following exposure to herbal extracts prepared by grinding and boiling methods. Values represent the mean mortality percentage of three independent replicates. Ground kaffir lime peel extract produced the fastest larvicidal response, reaching 100% mortality within 24 hours.

3.4 Statistical Analysis

Control mortality reached 11.7% at 48 h; therefore, mortality values at this observation time were corrected using Abbott's formula before comparisons among treatment groups. Ground kaffir lime peel produced 100.0% corrected mortality, whereas boiled kaffir lime peel and ground holy basil each produced approximately 98.1% corrected mortality. Boiled holy basil and both citronella preparations produced corrected mortality values of 0%.

Statistical comparisons were performed using replicate-level mortality data. Significant differences were observed among the treatment groups at 48 h ($p < 0.05$). However, no statistically significant difference was observed among ground kaffir lime peel, boiled kaffir lime peel, and ground holy basil after 48 h.

Table 5
Multiple Comparison (Tukey's HSD)

Comparison	Mean Difference	p
Kaffir Ground vs Control	17.67	< 0.001
Kaffir Ground vs Holy Basil Ground	0.33	0.041
Kaffir Ground vs Kaffir Boiled	0.33	0.038
Holy Basil Ground vs Holy Basil Boiled	19.00	< 0.001
Kaffir Boiled vs Citronella Ground	19.33	< 0.001

4. Discussion

The present study demonstrated that both the type of herbal material and the extraction method significantly influenced larvicidal activity against *Aedes aegypti* larvae. Among all treatments, ground kaffir lime (*Citrus hystrix*) peel extract exhibited the highest larvicidal efficacy, achieving complete larval mortality within 24 hours. These findings suggest that extraction method plays a crucial role in preserving biologically active compounds responsible for larvicidal activity.

The superior performance of ground kaffir lime peel extract may be attributed to its high concentration of volatile essential oils, particularly citronellal, limonene, β-pinene, and sabinene, which have been reported to possess insecticidal and larvicidal properties. Mechanical grinding is likely to preserve these volatile compounds more effectively than boiling, which may result in evaporation or thermal degradation of active constituents. Consequently, ground extracts produced faster and higher larval mortality than boiled extracts.

These findings are consistent with previous studies reporting the larvicidal activity of *Citrus hystrix* peel against *Aedes aegypti*. Several investigators have demonstrated that essential oils extracted from kaffir lime peel effectively disrupt larval respiration, damage the cuticle, and interfere with neurological function, ultimately leading to larval death. Similar observations have also been reported for other citrus-derived essential oils.

Ground holy basil (*Ocimum tenuiflorum*) extract also demonstrated considerable larvicidal activity, although its effect was slower than that of kaffir lime peel. Holy basil contains several bioactive compounds, including eugenol, methyl chavicol, and linalool, which have previously been reported to exhibit insecticidal activity against mosquitoes and agricultural pests. The delayed mortality observed in the present study suggests that these compounds may require longer exposure periods to exert their toxic effects.

In contrast, citronella (*Cymbopogon citratus*) extracts showed minimal larvicidal activity under the present experimental conditions. Although citronella essential oil is widely recognized as an effective mosquito repellent, its larvicidal efficacy appears to depend strongly on extraction procedures, formulation, and concentration. The simple aqueous extraction methods employed in this study may not have efficiently extracted the hydrophobic essential oil components responsible for insecticidal activity.

The comparison between grinding and boiling methods further highlighted the importance of extraction procedures. Grinding consistently produced higher larvicidal activity than boiling for both kaffir lime and holy basil. This finding indicates that extraction methods should be carefully considered when developing botanical larvicides for practical applications. Simple grinding techniques may provide an inexpensive and accessible approach for household mosquito control, particularly in rural communities where sophisticated extraction equipment is unavailable.

From a public health perspective, botanical larvicides offer several advantages over conventional synthetic insecticides. Plant-derived products are generally biodegradable, environmentally friendly, locally available,

and less likely to induce insecticide resistance. Therefore, herbal extracts may represent a sustainable alternative for integrated mosquito vector management, particularly in dengue-endemic countries.

Nevertheless, several limitations should be acknowledged. First, only one extract concentration (10%) was evaluated. Second, the study was conducted under laboratory conditions and may not fully represent field environments. Third, the chemical composition of each extract was not analyzed using analytical techniques such as gas chromatography–mass spectrometry (GC–MS), preventing identification of the specific compounds responsible for larvicidal activity. Finally, lethal concentration values (LC_{50} and LC_{90}) were not determined, limiting comparison with other published studies.

Future investigations should evaluate multiple extract concentrations, determine LC_{50} and LC_{90} values, characterize active compounds using chromatographic techniques, and assess field efficacy under natural breeding conditions. Such investigations would provide stronger evidence supporting the development of standardized botanical larvicides for sustainable mosquito control.

5. Conclusion

This study demonstrated that both herbal species and extraction methods significantly affected the larvicidal efficacy against *Aedes aegypti* larvae. Among the tested treatments, ground kaffir lime (*Citrus hystrix*) peel extract exhibited the highest larvicidal activity, producing complete larval mortality within 24 hours. Boiled kaffir lime peel and ground holy basil (*Ocimum tenuiflorum*) extracts also showed considerable effectiveness, whereas citronella (*Cymbopogon citratus*) extracts exhibited minimal larvicidal activity under the experimental conditions. The grinding method consistently produced higher larvicidal efficacy than boiling, indicating that extraction techniques play an important role in preserving bioactive compounds.

These findings suggest that ground kaffir lime peel extract has strong potential as an inexpensive, environmentally friendly, and locally available botanical larvicide for household mosquito control. The use of herbal larvicides may contribute to reducing dependence on synthetic insecticides and support sustainable vector management programs. Future studies should optimize extraction procedures, identify active compounds, determine LC_{50} and LC_{90} values, and evaluate long-term efficacy under field conditions before large-scale implementation.

6. Practical Implications

The findings of this study have several practical implications.

1. 6.1 Ground kaffir lime peel extract can be developed as a low-cost botanical larvicide for household mosquito control.
2. 6.2 The extraction method should be considered carefully because grinding preserved larvicidal activity better than boiling.

3. 6.3 Herbal larvicides may reduce the use of synthetic insecticides and minimize environmental contamination.
4. 6.4 Communities in dengue-endemic regions may utilize locally available medicinal plants for sustainable mosquito control.
5. 6.5 The results provide baseline information for developing standardized herbal larvicide formulations suitable for public health applications.

7. Limitations

Several limitations should be acknowledged.

Only one extraction concentration (10%) was investigated.

The study was conducted under laboratory conditions.

Only second- to third-instar larvae were examined.

Chemical composition of the extracts was not analyzed.

LC_{50} and LC_{90} values were not determined.

Only aqueous extraction methods were evaluated.

Declarations

Ethics approval and consent to participate

This study did not involve human participants or vertebrate animals. The experimental work was conducted using laboratory-reared *Aedes aegypti* larvae obtained from the National Institute of Health, Department of Medical Sciences, Thailand. Therefore, ethical approval and informed consent were not required for this study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

N.B. conceived the study, performed the experiments, collected the data, analyzed the results, and drafted the manuscript.

C.W. contributed to the study design, experimental procedures, data analysis, interpretation of the results, and critically revised the manuscript.

P.E. supervised the study, contributed to the conception and methodology, interpreted the findings, critically revised the manuscript for important intellectual content, and approved the final version of the manuscript.

All authors read and approved the final manuscript.

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Figures

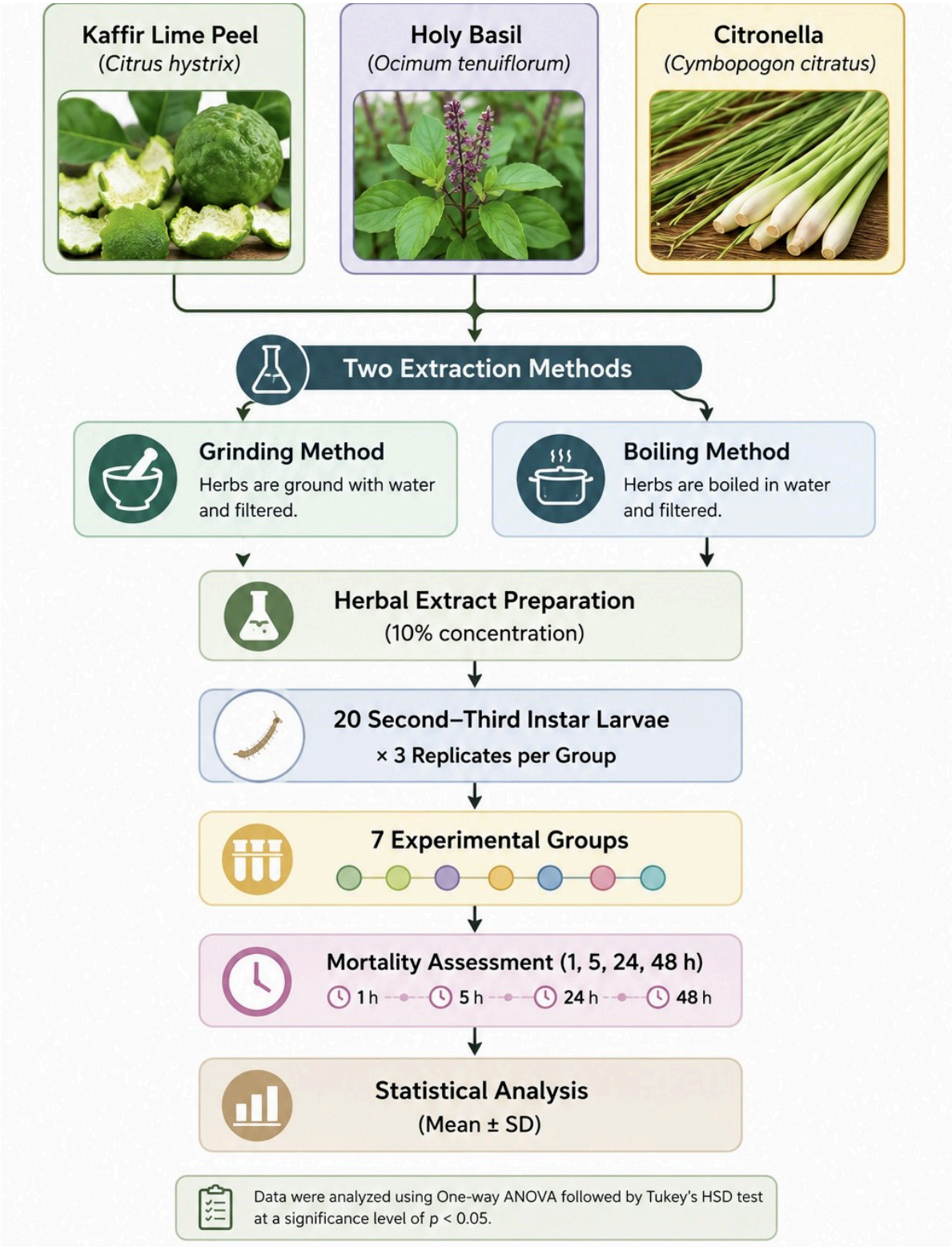

Figure 1

Overall experimental workflow for evaluating the larvicidal activity of herbal extracts against Aedes aegypti larvae.

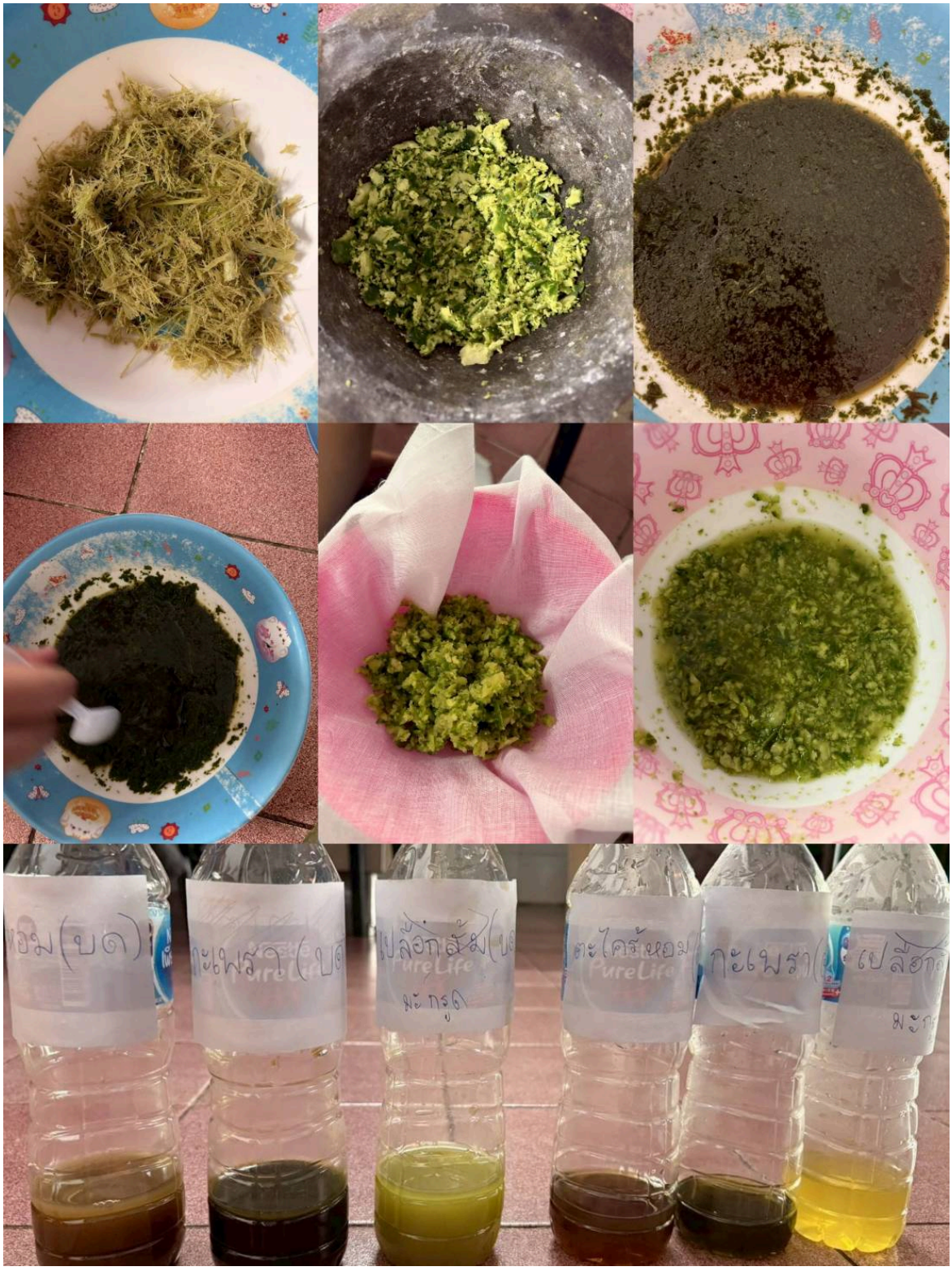

Figure 2

Preparation of herbal extracts using grinding and boiling methods.

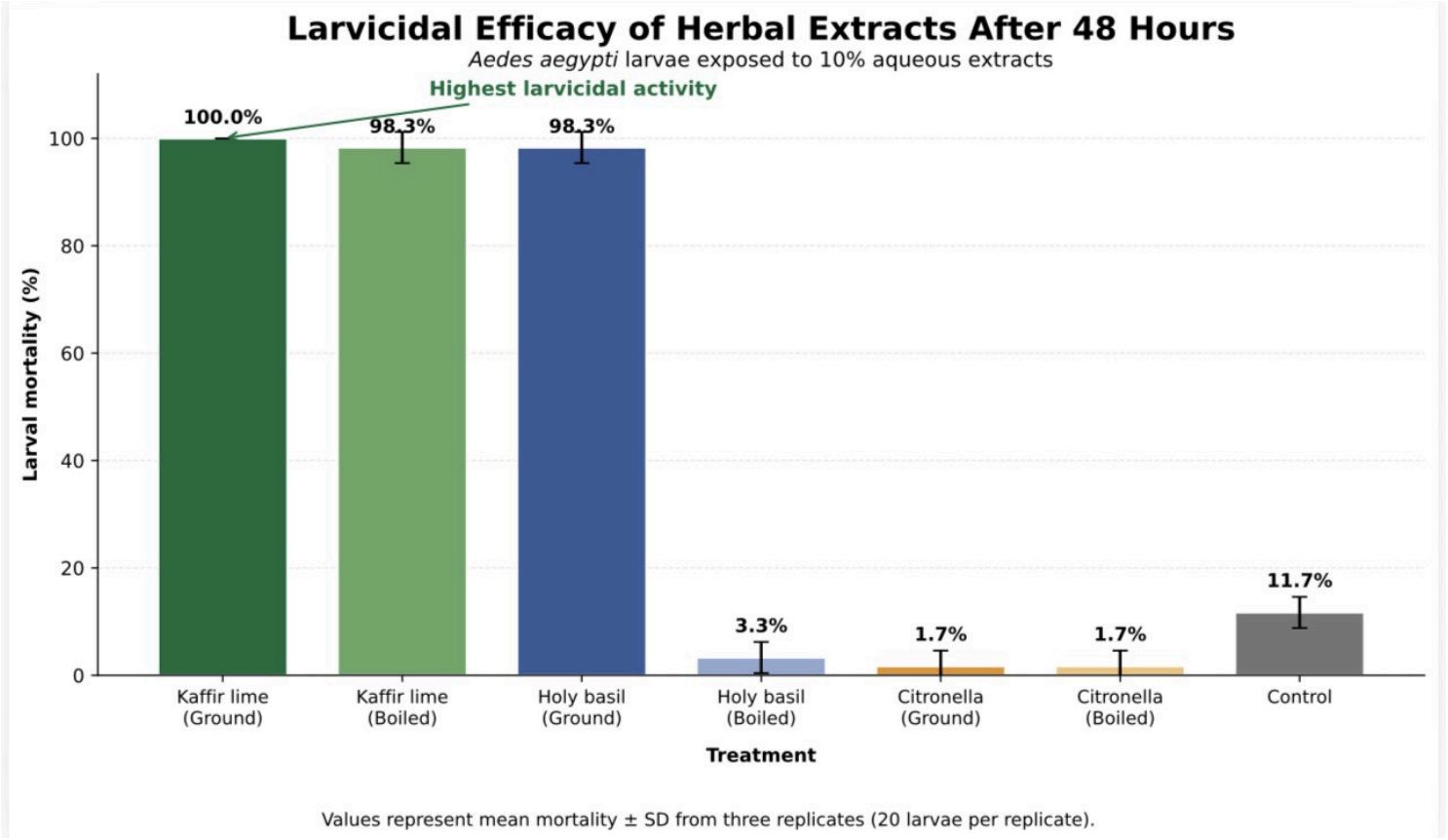

Figure 3

Larvicidal Efficacy of Herbal Extracts After 48 Hours

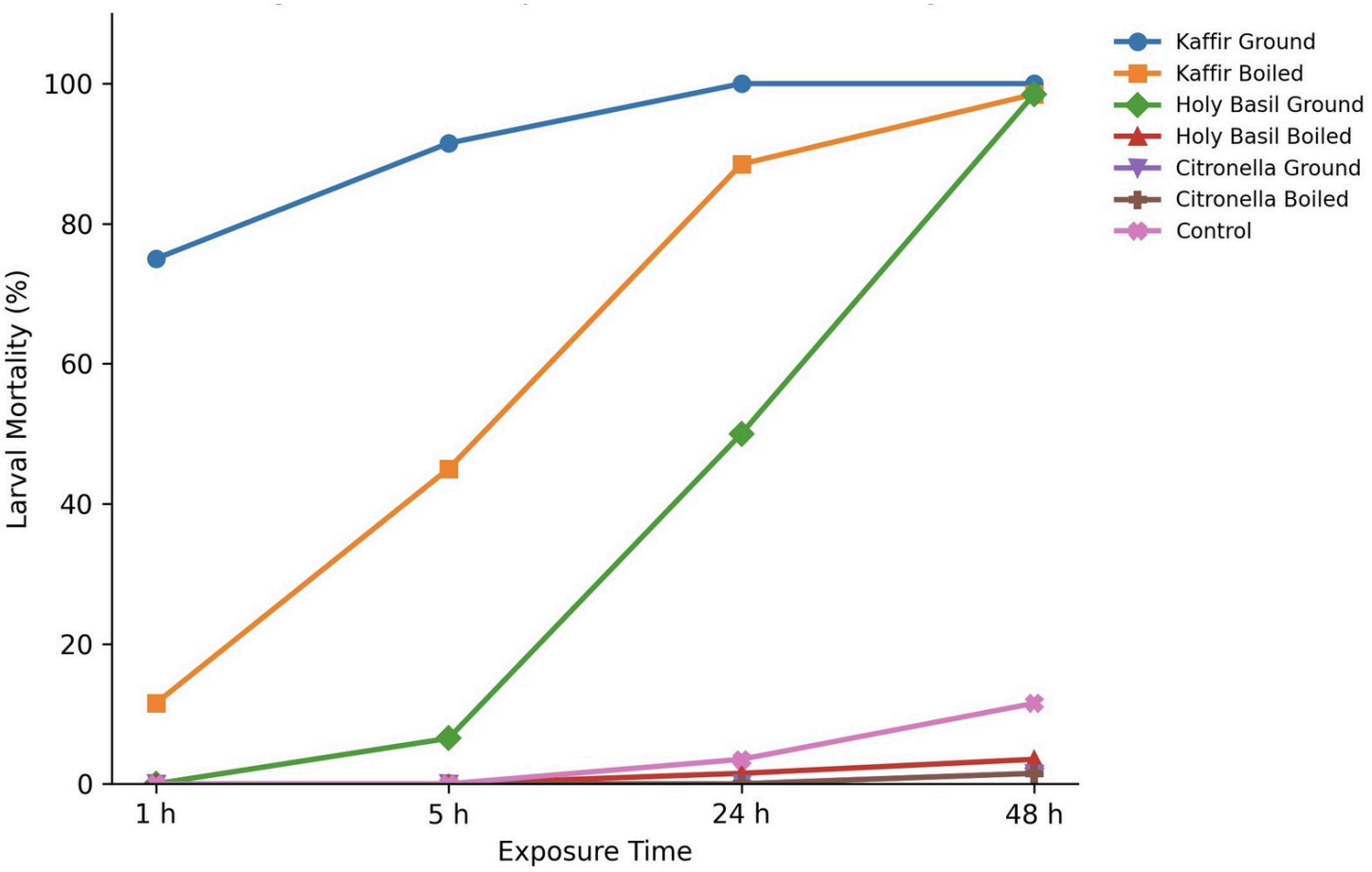

Figure 4

Time-course of larval mortality in Aedes aegypti exposed to herbal extracts prepared by different extraction methods.

Figure 5

Proposed application pathway of herbal extracts for sustainable mosquito vector control and dengue prevention.