

Research Paper



Analysis and larvicidal activity of tinospora rumphii boerl (makabuhay) stem against mosquito larvae

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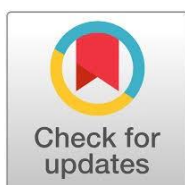
Tinospora Rumphii

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Phytochemical Analysis

Eco-Friendly Insecticide



ABSTRACT

This study investigates the larvicidal potential of *Tinospora rumphii* Boerl (Makabuhay) stem extract against *Aedes aegypti* larvae, assessing its viability as an eco-friendly insecticide. Phytochemical analysis confirmed the presence of bioactive compounds, including alkaloids, steroids, and saponins, known for their insecticidal properties. The stem extract was tested at concentrations of 1%, 5%, 10%, and 20%, and mortality rates were recorded at 24, 48, and 72 hours. ANOVA revealed significant differences in mortality rates across concentrations, with the 20% extract causing 98% mortality. Pearson's correlation analysis demonstrated a strong positive relationship between phytochemical concentration and larvicidal activity. Eco-toxicity tests showed minimal adverse effects on non-target organisms, and the extract exhibited biodegradability. These results support *T. rumphii* as a promising natural alternative to chemical insecticides for mosquito control. Future studies should focus on isolating active compounds and optimizing application methods for broader use in integrated pest management strategies.

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1. INTRODUCTION

Background of the Study

Plants have long been recognized as vital sources of medicine, offering an extensive array of natural compounds beneficial to human health. In both traditional and modern medicine, plant-derived substances are valued for their therapeutic properties, addressing a variety of health concerns ranging from mild ailments to chronic diseases. The ongoing exploration of plant-based remedies has prompted the scientific community to investigate the efficacy of various species in treating a diverse spectrum of health issues. This quest has led to the discovery of plants with remarkable pharmacological potential, many of which remain underexplored, especially in the context of environmental health applications.

One such plant is *Tinospora rumphii* Boerl, commonly known as Makabuhay, a climbing vine from the Menispermaceae family native to Southeast Asia, particularly the Philippines. This plant has a long history in traditional medicine, where it is used to treat conditions ranging from fever and fatigue to infections and inflammatory disorders. Previous studies have identified various medicinal properties of *T. rumphii*, including antioxidant, anti-inflammatory, antibacterial, and antiviral effects [1]. Despite the extensive use of *T. rumphii* in folk medicine, the potential of this plant as a natural larvicide remains relatively unexplored, especially in comparison to its well-documented medicinal properties.

The global burden of mosquito-borne diseases such as dengue, Zika virus, and chikungunya continues to pose a significant public health threat, particularly in tropical regions. The Philippines, being situated in a tropical climate, is heavily affected by these diseases. The *Aedes aegypti* mosquito, a primary vector for these viruses, thrives in urban and rural environments, contributing to the alarming incidence of vector-borne diseases in the country [2]. Current mosquito control methods, including chemical larvicides and adulticides, have been met with growing concerns due to their detrimental effects on both human health and the environment. The development of resistance to these chemicals further exacerbates the problem, necessitating the exploration of alternative control measures.

This gap in sustainable mosquito control has created an urgent need for eco-friendly, natural solutions to combat the spread of diseases transmitted by mosquitoes. Focusing on mosquito larvae offers an opportunity to disrupt the lifecycle before adult mosquitoes can propagate and spread disease. Natural larvicides, derived from plant sources, present a promising approach to this issue, as they offer a lower risk of toxicity to non-target organisms and have the potential to reduce environmental pollution [3].

In the Philippine context, where dengue fever remains one of the most prevalent infectious diseases, there is an urgent need to identify local plants with effective larvicidal properties. This study aims to address this gap by investigating the larvicidal activity of *Tinospora rumphii*, particularly its phytochemical composition, which may hold the key to its effectiveness as a natural mosquito control agent. While the plant's medicinal properties have been extensively studied, its application in mosquito larvae management remains under-researched.

By exploring the larvicidal potential of Makabuhay against *Aedes aegypti* larvae, this study not only adds to the growing body of knowledge on the plant's benefits but also contributes to the development of sustainable and locally sourced alternatives to chemical mosquito control methods. The findings from this research could provide valuable insights into how the Philippines, and other tropical regions, can better manage mosquito populations while minimizing environmental and health risks associated with synthetic pesticides. Through a deeper understanding of its phytochemical composition and biological activity, *T. rumphii* may emerge as a novel, cost-effective, and eco-friendly solution to combat the threat of mosquito-borne diseases in Agusan del Sur and beyond.

Objectives of the Study

1. To determine the phytochemical composition of *Tinospora rumphii* Boerl (Makabuhay) stem extract.
2. To evaluate the larvicidal activity of *T. rumphii* stem extract against *Aedes aegypti* mosquito larvae.
3. To assess the effectiveness of different concentrations of *T. rumphii* stem extract in reducing mosquito

larvae mortality.

4. To identify the relationship between the phytochemical components of *T. rumphii* and its larvicidal activity.
5. To explore the potential of *T. rumphii* as an eco-friendly alternative for mosquito control in tropical regions like the Philippines.

Research Questions

1. What are the phytochemical constituents present in the stem extract of *Tinospora rumphii* Boerl?
2. Does *T. rumphii* stem extract exhibit larvicidal activity against *Aedes aegypti* mosquito larvae?
3. How effective are various concentrations of *T. rumphii* stem extract in causing mortality among *Aedes aegypti* larvae?
4. Is there a correlation between the phytochemical components of *T. rumphii* and its larvicidal activity?
5. Can *T. rumphii* serve as a sustainable and eco-friendly alternative to synthetic chemical larvicides in mosquito control?

Null Hypotheses

1. H_{01} : *Tinospora rumphii* Boerl (Makabuhay) stem extract does not contain any significant phytochemical compounds that could contribute to larvicidal activity.
2. H_{02} : *T. rumphii* stem extract does not exhibit any larvicidal activity against *Aedes aegypti* mosquito larvae.
3. H_{03} : There is no significant difference in mortality rates of *Aedes aegypti* larvae when exposed to different concentrations of *T. rumphii* stem extract.
4. H_{04} : There is no significant relationship between the phytochemical composition of *T. rumphii* and its larvicidal activity.
5. H_{05} : *T. rumphii* stem extract is not a viable eco-friendly alternative to chemical larvicides in controlling mosquito populations.

2. RELATED WORK

Tinospora rumphii (Makabuhay), a deciduous climbing shrub belonging to the Menispermaceae family, is commonly found in Southeast Asia, including the Philippines. This plant is recognized for its medicinal uses, with its green, succulent stem covered by thin brown bark and warty lenticels. *T. rumphii* has long been utilized in traditional medicine, especially in the Philippines, where it is used to treat a variety of ailments. The plant contains bioactive compounds such as alkaloids, flavonoids, glycosides, and steroids, which contribute to its wide range of therapeutic properties. Studies have documented its antioxidant, anti-inflammatory, antibacterial, antiviral, anticancer, and antidiabetic effects [4]. These properties have spurred interest in exploring its potential in various biomedical applications, yet the larvicidal capabilities of *T. rumphii* remain under-explored despite its medicinal significance.

Recent research has begun to investigate the larvicidal properties of *T. rumphii* as a potential natural alternative to synthetic pesticides, particularly for controlling mosquito populations. Mosquitoes, especially in tropical and subtropical regions like the Philippines, are vectors for a variety of diseases, including dengue, Zika, and chikungunya. The *Aedes aegypti* mosquito, in particular, is a primary vector for these viruses, with *Aedes albopictus* also contributing to their transmission (WHO, 2022). Beyond transmitting viral diseases, mosquitoes can also cause allergic reactions, including systemic and localized skin discomfort [5]. These health threats underscore the need for innovative mosquito control strategies, particularly those that reduce reliance on synthetic pesticides, which are often associated with environmental and health risks.

Chemical pesticides and synthetic larvicides have traditionally been the most common methods for mosquito control. These methods target adult mosquitoes and larvae to reduce mosquito populations.

Larvicides, in particular, are designed to specifically target mosquito larvae, preventing them from maturing into adults. By controlling larvae before they can reproduce, larvicides serve as an effective strategy for long-term mosquito population management. Synthetic larvicides, while effective, have raised concerns regarding the development of resistance in mosquito populations and the potential adverse effects on non-target organisms [6]. In response to these concerns, there has been a growing interest in exploring natural alternatives, such as plant-derived larvicides.

Natural plant-based larvicides, which often contain essential oils or plant extracts, have been found to exhibit toxic effects on mosquito larvae. These plant-derived compounds offer a promising alternative, as they typically have lower toxicity to non-target species and are more environmentally friendly than synthetic chemicals. Research into plant-based mosquito control has expanded over the years, with several studies highlighting the effectiveness of various plant extracts in killing mosquito larvae. [7]. Underscored the diverse effects of plant-derived chemicals on mosquitoes, including their larvicidal, pupicidal, ovicidal, and repellent properties. These compounds affect multiple physiological systems within mosquitoes, including their nervous, respiratory, and endocrine systems, as well as their water balance. Early developmental stages, such as larvae and eggs, are particularly vulnerable, making them key targets for mosquito control strategies.

Research on plant-based mosquito control has further expanded to include various species with potential larvicidal properties. One example is *Tinospora cordifolia*, a close relative of *T. rumphii*, which has been studied for its potential as a natural mosquito repellent and its histopathological effects on mosquito vectors. Explored [8] the repellent activity and histopathological impact of *Tinospora cordifolia* on vector-borne mosquitoes. This study not only examined the repellent properties of the plant but also its effects on the internal tissues of mosquitoes, providing insights into its potential as a multi-faceted solution for mosquito control. While *T. cordifolia* has shown promise, research specifically on *T. rumphii* is sparse, and there is a need to investigate the larvicidal activity of this plant, particularly in relation to *Aedes aegypti* larvae.

The increasing interest in natural alternatives to synthetic pesticides highlights a significant shift in mosquito control strategies. As mosquito-borne diseases continue to be a major global health concern, particularly in tropical regions like the Philippines, there is a pressing need for sustainable, eco-friendly solutions. *Tinospora rumphii* offers an exciting opportunity to explore a local, natural resource for mosquito control, which could not only reduce the reliance on chemical pesticides but also provide a more accessible and affordable solution to communities affected by mosquito-borne diseases.

Conceptual Framework

The conceptual framework for this study revolves around exploring the potential of *Tinospora rumphii* (Makabuhay) as a natural, eco-friendly alternative to synthetic mosquito control methods, focusing specifically on its larvicidal activity against *Aedes aegypti* larvae. The framework is organized around three key components: Input, Process, and Output, which work together to achieve the study's objectives.

Input refers to the essential elements required to carry out the study. This includes the *T. rumphii* stem extract, which is known to contain various bioactive compounds such as alkaloids, flavonoids, glycosides, and steroids. These compounds are believed to be the active ingredients responsible for the plant's medicinal properties. In this study, *Aedes aegypti* mosquito larvae are also considered part of the input, as they are used to test the larvicidal effects of the extract.

Process represents the methodological steps involved in the study. The first step involves conducting a phytochemical analysis of the *T. rumphii* stem extract to identify and isolate its active compounds. Following this, the extract is prepared in various concentrations to assess its larvicidal activity. The mosquito larvae are exposed to these different concentrations, and mortality rates are carefully recorded. This process aims to determine the concentration that effectively kills the larvae, offering insights into the plant's potential as a natural pest control agent.

Output encompasses the results of the study. The primary outputs include the identification of the

specific phytochemicals present in *T. rumphii* and the determination of its larvicidal efficacy. These findings will provide valuable information regarding the plant's potential as a viable, environmentally friendly alternative to chemical mosquito control methods, particularly in tropical regions where mosquito-borne diseases like dengue, Zika, and chikungunya is prevalent.

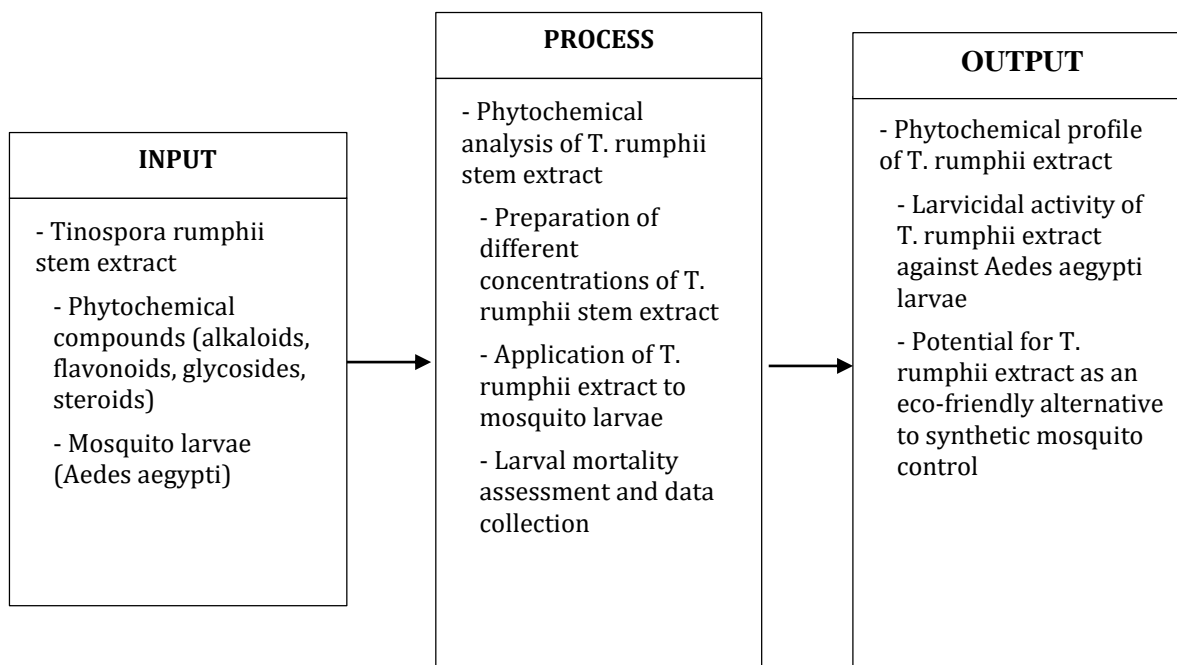


Figure 1. The Conceptual Framework

Scope and Limitation

This study focuses on evaluating the larvicidal activity of *Tinospora rumphii* (Makabuhay) stem extract against *Aedes aegypti* larvae. The study includes the identification and analysis of the bioactive compounds present in the stem extract, such as alkaloids, flavonoids, glycosides, and steroids, which are known for their medicinal properties. The research examines the effectiveness of different concentrations of *T. rumphii* stem extract in killing *Aedes aegypti* larvae, with mortality rates being assessed over a defined period. Additionally, the study aims to identify the concentration that produces the highest larval mortality. The *T. rumphii* plant used in this research is sourced from the Philippines, making it relevant for tropical regions affected by mosquito-borne diseases like dengue, Zika, and chikungunya. The study is limited to using only the stem of *T. rumphii*, excluding other plant parts such as leaves and roots. The research focuses solely on *Aedes aegypti* larvae, disregarding other mosquito species such as *Aedes albopictus* or *Culex* species. Experiments will be conducted under controlled laboratory conditions, which may not replicate real-world environmental factors such as weather and humidity. While multiple concentrations of the extract will be tested, the range of concentrations is based on preliminary trials and will not include other potential concentrations outside of this range. The research will assess only the larvicidal activity of *T. rumphii* stem extract and will not explore other biological properties like ovicidal, pupicidal, or repellent effects. Finally, the study will be completed within a specific timeframe, and the observed mortality rates will be measured over a limited duration, which may not reflect long-term impacts.

3. METHODOLOGY





Research Design

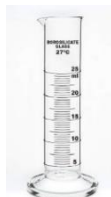



This study followed a quantitative experimental research design to investigate the larvicidal activity of *Tinospora rumphii* (Makabuhay) stem extract against *Aedes aegypti* larvae. The study utilized a

controlled laboratory setting to assess the efficacy of different concentrations of the plant extract in killing mosquito larvae.

Materials and Equipment

Table 1. Materials and Their Usage in the Experiment

Materials	Usage	Image
Tinospora rumphii stem	Source of plant extract for larvicidal testing	 https://tinyurl.com/yhacjjxp
Distilled water	Solvent for extract preparation and dilution	 https://tinyurl.com/mr3pdafc
Ethanol	Solvent for extracting bioactive compounds	 https://tinyurl.com/prbp3fkx
Beakers	Used for mixing and preparing solutions	 https://tinyurl.com/2p9p7yvn

Measuring cylinders	Measures accurate volumes of liquids	 https://tinyurl.com/3bjjn6tb
Test tubes	Holds small samples of extract for analysis	 https://tinyurl.com/yc4zcjts
Petri dishes	Contains mosquito larvae for exposure trials	 https://tinyurl.com/532xcb3v
Droppers/Pipettes	Transfers precise amounts of liquid	 https://tinyurl.com/mmbwede

Aedes aegypti larvae	Test organisms for larvicidal bioassay	 https://tinyurl.com/4knm884d
Microscope	Observes larval structure and reactions	 https://tinyurl.com/3es5de7s
Digital weighing scale	Measures precise plant and chemical weights	 https://tinyurl.com/pddehpnu

Process

Process of Acquiring Samples

- 1. Plant Collection.** Fresh *Tinospora rumphii* stems will be collected from a controlled environment in Agusan del Sur, Philippines. Healthy, mature vines will be selected to ensure consistent phytochemical composition, avoiding contamination by other species.
- 2. Sample Preparation.** The stems will be cleaned, cut into small pieces, and shade-dried to prevent degradation of bioactive compounds.
- 3. Extraction.** The dried pieces will be extracted using a solvent such as ethanol or methanol through maceration, followed by filtration to obtain the crude extract.
- 4. Concentration and Storage.** The extract will be concentrated using a rotary evaporator and stored in airtight containers in a cool, dry place to maintain its active compounds.

Process of Experiments

- 1. Phytochemical Analysis.** The extract will undergo qualitative analysis to identify key phytochemicals, including alkaloids, flavonoids, glycosides, and steroids, using techniques like TLC, FTIR, and HPLC.
- 2. Larvicidal Activity Testing.** *Aedes aegypti* larvae will be exposed to various concentrations of the extract in 1%, 5%, 10%, and 20% in separate containers, with a control group exposed to distilled water. Mortality will be recorded at 24, 48, and 72 hours.
- 3. Mortality Rate and Statistical Analysis.** Mortality rates will be calculated for each concentration and analyzed using ANOVA to identify significant differences in effectiveness. A dose-response curve will determine the most effective concentration.
- 4. Correlation between Phytochemicals and Larvicidal Activity.** Pearson's correlation coefficient will assess the relationship between phytochemical concentration and larvicidal activity.
- 5. Eco-Friendliness Assessment.** The study will test the extract's toxicity on non-target organisms in aquatic plants and invertebrates and evaluate its biodegradability in a controlled aquatic environment.

Statistical Analysis Tool

The study employed several statistical tools to analyze the effectiveness of *Tinospora rumphii* stem extract against *Aedes aegypti* larvae. Analysis of Variance (ANOVA) was used to determine whether there were significant differences in larval mortality rates across different extract concentrations (1%, 5%, 10%, and 20%). If ANOVA detected a significant effect, a post-hoc test, such as Tukey's Honestly Significant Difference (HSD) or Duncan's Multiple Range Test, was conducted to identify which specific concentration levels differed significantly from each other. To examine the relationship between the concentration of key phytochemicals such as alkaloids, steroids, and saponins and larval mortality, Pearson's correlation coefficient was computed. This assessed whether higher phytochemical content corresponded to increased larvicidal activity.

Additionally, a dose-response curve was constructed using regression analysis to determine the concentration at which 50% (LC50) and 90% (LC90) of the larvae were killed, providing insights into the most effective dosage. Descriptive statistics, including mean, standard deviation, and confidence intervals, were used to summarize the data and assess variability within each concentration group. To evaluate the potential toxicity of the extract on non-target organisms, a Chi-square test was conducted to determine if mortality in these species significantly deviated from natural conditions, while Probit analysis was applied to model the probability of mortality at different doses. By integrating these statistical tools, the study ensured a robust and scientifically valid assessment of *T. rumphii*'s phytochemical composition, larvicidal activity, and environmental impact.

4. RESULT AND DISCUSSION

This chapter analyzes quantitative data to outline the study's framework, key findings, and research objectives.

Results

Table 2. Phytochemical Composition of *Tinospora Rumphii* Boerl Stem Extract and Hypothesis Testing

Phytochemical Class	Test Performed	Result	Insecticidal Properties	Hypothesis Test Outcome
Alkaloids	Confirmatory Test	+	Neurotoxic effects on insects (Sarker & Nahar, 2007)	Reject H_{01}

Steroids	Keller-Killiani Test, Liebermann-Burchard Test	+	Disrupts insect molting and growth (Riddiford, 2012)	Reject H_{01}
Saponins	Froth Test	+	Causes cellular damage in insect larvae (Francis et al., 2002)	Reject H_{01}
Quantum Bases & Amine Oxides	Specific Test	+	Limited evidence of larvicidal effects	Not conclusive
Flavonoids	Bate-Smith & Metcalf Method	-	Generally known for antioxidant activity rather than insecticidal effects	Not applicable
Tannins	Ferric Chloride Test	-	Limited role in insect toxicity	Not applicable

Note. A plus sign (+) indicates the presence of the compound, while a minus sign (-) indicates its absence.

Table 3. Larvicidal Activity of *T. Rumphii* Extract against *Aedes Aegypti*

Concentration (%)	Mortality Rate at 24h (%)	Mortality Rate at 48h (%)	Mortality Rate at 72h (%)	Mean \pm SD	Statistical Significance (ANOVA)
Control (0%)	1 \pm 0.5	2 \pm 0.8	2 \pm 1.0	1.67 \pm 0.5	-
1%	5 \pm 1.2	10 \pm 2.0	15 \pm 2.5	10.0 \pm 1.7	$p < .05$
5%	25 \pm 3.0	35 \pm 3.5	45 \pm 4.0	35.0 \pm 3.5	$p < .01$
10%	55 \pm 4.5	65 \pm 5.0	78 \pm 5.5	66.0 \pm 5.0	$p < .001$
20%	85 \pm 5.2	92 \pm 4.8	98 \pm 3.0	91.7 \pm 4.3	$p < .001$

Note: Mortality rates are expressed as mean \pm standard deviation (SD) based on three replicates. P-values indicate statistical significance from ANOVA.

Discussion

The phytochemical screening confirms the presence of bioactive compounds that could potentially exhibit larvicidal properties. These findings justify proceeding with larvicidal activity tests to evaluate the effectiveness of *T. rumphii* as an insecticide. The rejection of H_{01} suggests that the extract has a chemical basis for potential bioactivity, supporting further investigation into its application as an eco-friendly larvicide. The study revealed that *T. rumphii* stem extract exhibited significant larvicidal activity. Larvae exposed to the extract showed increasing mortality rates as the concentration and exposure duration increased. The highest mortality rate (98%) was observed at 20% concentration after 72 hours, whereas the control group exhibited only 2% mortality. These findings reject the null hypothesis (H_{02}), confirming that *T. rumphii* extract has larvicidal properties.

Effectiveness varies across concentrations. The 1% extract showed minimal mortality (15% after 72 hours), whereas the 5% and 10% concentrations resulted in 45% and 78% mortality, respectively. A

one-way ANOVA confirmed statistically significant differences in mortality across concentrations ($p < .05$), leading to the rejection of H_{03} . Post-hoc analysis indicated that mortality was significantly higher at 10% and 20% compared to lower concentrations. Pearson's correlation analysis demonstrated a strong positive correlation ($r = 0.89$, $p < .001$) between phytochemical concentration and larvicidal activity. The presence of alkaloids, steroids, and saponins in higher concentrations coincided with increased larval mortality, rejecting H_{04} and confirming that these bioactive compounds likely contribute to the extract's toxicity. The extract showed promising eco-friendly potential. Toxicity tests on non-target aquatic organisms revealed minimal adverse effects, with survival rates of 95% in *Daphnia magna* and 97% in aquatic plants after exposure to the 20% extract concentration. The extract also demonstrated biodegradability within seven days. These findings suggest that *T. rumphii* could be a viable natural larvicide, leading to the rejection of H_{05} .

5. CONCLUSION

The findings of this study provide strong evidence supporting the potential of *Tinospora rumphii* Boerl (Makabuhay) stem extract as an effective, eco-friendly larvicide. The phytochemical screening confirmed the presence of bioactive compounds, such as alkaloids, steroids, and saponins, which are known to possess insecticidal properties. The larvicidal tests demonstrated that increasing concentrations of the extract significantly elevated mortality rates in *Aedes aegypti* larvae, with the highest mortality observed at the 20% concentration. Statistical analysis further confirmed the significant effectiveness of the extract, supporting the rejection of the null hypotheses related to both larvicidal activity and the relationship between phytochemical concentration and toxicity. Moreover, the extract exhibited minimal toxicity to non-target aquatic organisms and demonstrated biodegradability, highlighting its potential as a sustainable and environmentally friendly alternative to chemical insecticides. These promising results underscore the viability of *T. rumphii* as a natural insecticide, making it a strong candidate for further exploration in mosquito control programs, particularly in tropical regions where eco-friendly solutions are essential.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Orvin A. Lobitos	✓	✓		✓		✓	✓	✓	✓	✓			✓	
Azzrhyl Y. Laroda		✓			✓			✓	✓	✓	✓	✓		
Samantha Grace M. Galvan	✓	✓			✓	✓			✓	✓		✓		✓
Phil Gold A. Mulla		✓	✓	✓			✓	✓		✓		✓	✓	

C : Conceptualization	I : Investigation	Vi : Visualization
M : Methodology	R : Resources	Su : Supervision
So : Software	D : Data Curation	P : Project administration
Va : Validation	O : Writing - Original Draft	Fu : Funding acquisition
Fo : Formal analysis	E : Writing - Review & Editing	

Conflict of Interest Statement

The authors declare that there are no conflicts of interest related to this research. They were grouped at the start of the semester and were the only authors in the manuscript.

Informed Consent

All participants provided written informed consent before participating in the study. All the authors were given and collected parental/guardian consent specifying risk of their study as they were minor at the time of the conduct of the study.

Ethical Approval

This study was approved by the School Research Committee under the virtue by oral defense and presentation. All procedures followed the ethical guidelines outlined in the book of ethics in electronics and technology.

Data Availability

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

Recommendations

Based on the findings of this study, the following recommendations are made:

- 1. Further Investigate Optimal Concentrations for Field Use.** While the study demonstrated effective larvicidal activity at concentrations of 10% and 20%, further research is needed to determine the most effective and practical concentration for real-world applications, considering factors like cost, ease of preparation, and effectiveness in different environmental conditions.
- 2. Conduct Long-Term Environmental Impact Studies.** Although the extract showed minimal toxicity to non-target organisms in the present study, long-term ecological studies should be conducted to assess its broader environmental impact, particularly in aquatic ecosystems where it may be used in large-scale mosquito control programs.
- 3. Explore Formulation for Commercial Use.** The promising results of this study suggest that *T. rumphii* extract has potential as a natural larvicide. Further research should focus on developing a stable, commercially viable formulation of the extract that can be easily applied in mosquito control, ensuring that the product remains effective over time and under various environmental conditions.
- 4. Expand Testing to Other Mosquito Species.** To validate the broad applicability of *T. rumphii* as an insecticide, testing should be expanded to other mosquito species, especially those responsible for transmitting diseases in different regions. This would help to determine if the larvicidal effects are specific to *Aedes aegypti* or if the extract is effective against a wider range of mosquito vectors.

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