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# Activity Assay and Determination of Active Larvicidal Compounds from Cat's Whiskers (*Orthosiphon aristatus* Blume miq.)

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## Abstract

The tropical climate of Indonesia presents a significant risk for the prevalence of various mosquito-borne diseases, particularly Dengue Hemorrhagic Fever (DHF), which has become a critical public health concern in recent years. The control of mosquito populations, specifically targeting larvae, is a strategic approach in managing the transmission of DHF. Traditional methods involving synthetic larvicidal agents, however, raise concerns regarding potential resistance among mosquito populations and adverse effects on human health. Consequently, there exists an urgent need for alternative solutions that are both effective and safe for human exposure. One promising avenue is the exploration of natural larvicidal agents sourced from local flora, such as the white-purple variety of cat's whiskers (*Orthosiphon aristatus* Blume miq.). This study investigates the larvicidal potential of this plant, focusing on its main secondary metabolites, namely flavonoids and phenolics, which are hypothesized to possess larvicidal activities. Conducted extensive larvicidal activity assaying on various extracts and fractions derived from the white-purple cat's whiskers. Notably, the ethyl acetate fraction demonstrated superior efficacy, evidenced by a calculated LC50 value of 737.71 ppm, underscoring its potential as a sustainable larvicidal agent. Subsequent analyzes of this active fraction revealed the presence of the compound sinensetin, which further validates the insecticidal application of this plant in controlling mosquito populations effectively. This research contributes to the paradigm shift toward natural alternatives in pest management, highlighting the importance of harnessing local biodiversity in the fight against vector-borne diseases.

**Keywords :** Cat's Whiskers**,** Larvicidal, Dengue Hemorrhagic Fever, Active Compound, Traditional Plant

## 1. Introduction

The tropical climate of Indonesia, characterized by high humidity and temperatures conducive to mosquito proliferation, presents a notable risk for the prevalence of several mosquito-borne diseases. Dengue Hemorrhagic Fever (DHF) is one such disease that has been identified as a critical public health concern, contributing to significant morbidity and mortality within affected populations.[1] The transmission dynamics of DHF are largely influenced by the *Aedes aegypti* mosquito, a primary vector responsible for the spread of the dengue virus. Efforts to mitigate the transmission of DHF necessitate effective strategies for mosquito population control, particularly through larvicidal interventions targeting mosquito larvae, which are typically found in stagnant water sources prevalent throughout urban and rural environments of Indonesia.[2]

Traditional methodologies for mosquito control primarily involve the use of synthetic larvicidal agents. While these chemical agents have been effective in controlling mosquito populations, there are growing concerns related to their safety and environmental impact.[3] Issues such as the development of resistance among mosquito populations to these chemical agents have led researchers to explore alternative, more eco-friendly solutions.[4] Moreover, the potential adverse effects of synthetic larvicidal agents on non-target organisms, including beneficial insects and human health, necessitate an urgent need for environmentally safe and effective larvicidal agent alternatives. [5]

In this context, the exploration of natural larvicidal agents derived from local flora represents a promising avenue. *Orthosiphon aristatus* , commonly known as cat's whiskers, has gained significant attention due to its potential larvicidal properties.[6,7] Studies emphasize the role of secondary metabolites, particularly flavonoids and phenolics—bioactive compounds that have been reported to exhibit insecticidal activities against various mosquito species.[8] Research on the larvicidal effects of extracts from plants similar to *Orthosiphon aristatus* indicates that such natural products can yield substantive results in larvicidal efficacy.[9,10]

Study focuses on the larvicidal potential of extracts from the white-purple variety of *Orthosiphon aristatus*, where extensive assaying has demonstrated the effectiveness of its extracts, particularly the ethyl acetate fraction. Remarkably, this fraction exhibited a calculated LC50 value of 737.71 ppm, surpassing the efficacy of many conventional synthetic larvicidal agents.[11] This elevated activity is hypothesized to stem from the presence of bioactive compounds such as sinensetin, a flavonoid that has shown significant insecticidal properties against vectors like Aedes.[12,13]

The implications of employing plant-derived larvicidal agents extend beyond effective mosquito control; they also pave the way for sustainable pest management approaches, thereby reducing reliance on synthetic chemicals. This shift toward using natural resources for pest management highlights the importance of integrated pest management strategies, which can be crucial in addressing public health challenges posed by vector-borne diseases.

The findings from this study reinforce the potential of using *Orthosiphon aristatus* as a sustainable, botanically sourced larvicidal agent. Through harnessing the biochemical capabilities of local flora, it is possible to establish a more balanced ecosystem capable of sustaining mosquito population control efficiently and effectively, thus addressing the public health priorities of dengue prevention in Indonesia.[14,15]

## 2. Method

### Collection and Processing of Assay Materials

Preparation of white-purple variety cat's whiskers leaves was obtained from the medicinal plant garden of the Faculty of Pharmacy, UNJANI. The material was determined at the Central Laboratory of Padjadjaran University. Preparation of Aedes aegypti mosquito larvae for assaying was obtained from the Toxicity Assaying Laboratory of the School of Life Sciences and Technology, Bandung Institute of Technology.

### Extract Preparation

100 g of purple variety cat's whiskers powder was extracted by maceration using 1000 ml of 70% ethanol for 3 x 24 hours. It was then filtered and evaporated using a rotary evaporator to obtain a thick extract.

### Fractionation

The next process was fractionation using liquid-liquid extraction. The thick extract obtained from the extraction was dissolved in 100 mL of water. The aqueous extract was then transferred to a separating funnel and added with n-hexane (1:1). Afterwards, the solvents were shaken and allowed to stand until the two solvents were completely separated. This process was repeated three times. The water layer was separated and returned to a separating funnel. Ethyl acetate (1:1) was added, then shaken and allowed to stand until separated. This process was repeated three times. The water, ethyl acetate, and n-hexane layers were evaporated using a rotary evaporator to obtain a thick fraction. The extracts and fractions were assayed for larvicidal and

repellent activity. Thin-layer chromatography profiles of the extracts and fractions were also monitored.

**Vacuum Liquid Chromatography Packaging**

A total of 70.6 grams of G 60 H silica was weighed and loaded into a vacuum liquid chromatography KCV column and compressed until there was no air between the silica particles. Next, the silica was moistened with 200 mL of N-Hexane until a column was formed. Afterward, 10 grams of the sample impregnated with silica gel was loaded onto the top of the packed column.

**Separation of Compounds Using Vacuum Liquid Chromatography**

The separation was performed using a solvent gradient. The solvents used were N-Hexane and Ethyl Acetate. The solvent gradient ratios used were N-Hexane:Ethyl Acetate (10:0), (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9), and (0:10). The solvents were introduced into the column sequentially, based on the highest to lowest N-Hexane ratio. The separation results were collected and labeled in order of their ratio. After the solvent gradient was completed, the column was washed with methanol. 300 mL of methanol was added to the column. The wash results were collected in glass bottles and labeled.

**Sub-Fraction TLC Assay**

The mobile phase was saturated in the chamber. The mobile phase used was N-Hexane:Ethyl Acetate (3:7).

**Larvicide Assay Preparation**

Prepare a plastic tray filled with distilled water, then put Aedes aegypti eggs into it for 4-6 days until the eggs develop into instar III or IV and are ready to be used in assaying.

**Larvicidal Activity Assaying**

A preliminary assay was first conducted to determine the optimal concentration range. Each concentration solution was pipetted into a plastic cup and added with a certain amount of distilled water. A total of 25 larvae of Aedes aegypti, Anopheles gambiae, Culex sp that had reached instar III or IV were placed into the plastic cup and distilled water was added to the volume up to 100 mL. A negative control was made by adding only 1 mL of ethanol made in 100 mL of distilled water, while a positive control was used Temephos 1% (Abate). The assay was repeated three times and carried out at room temperature of 25-28°C. After 24 hours of assaying, the number of dead and live mosquito larvae was counted in each plastic cup. The results obtained were recorded in the table. Mortality of the treatment group was corrected using the Abbott formula:

X = larvae that are still alive in the control Y = larvae that are still alive in the assay substance

$$\%\ Mortality = \frac{X-Y}{X} \text{ x } 100\%$$

The Lethal Concentration 50 (LC50) value was calculated using the probit analysis method. LC50 was calculated by converting the percentage (%) of mortality into a probit value using the Finney table and conducting a linear regression against the logarithm of the concentration.

## 3. Result

**Assay Introduction Larvicidal extracts and fractions**

The number of larvae deaths in the preliminary assay of the N-Hexane and Ethyl Acetate fractions is presented in Table 1, Table 2, Table 3 and Table 4.

**Table 1 Results Assay Introduction Larvicide Ethanol Extract**

| Ethanol extract (ppm) | Number of Larval Death | | | Average | % Death |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 1000 | 7 | 7 | 8 | 7.33 | 48.89 |
| 1500 | 8 | 9 | 8 | 8.33 | 55.56 |
| 2000 | 9 | 10 | 8 | 9.00 | 60.00 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

**Table 2 Results Assay Introduction N- Hexane Fraction Larvicide**

| n-Hexane Fraction (ppm) | Number of Larval Death | | | Average | % Death |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 50 | 1 | 1 | 0 | 0.67 | 4,445 |
| 500 | 1 | 2 | 3 | 2 | 13.33 |
| 1000 | 3 | 3 | 3 | 3 | 20 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

**Table 3 Results Assay Introduction Ethyl Acetate Fraction Larvicide Acetate**

| Ethyl Acetate Fraction (ppm) | Number of Larval Death | | | Average | % Death |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 50 | 8 | 7 | 7 | 7.33 | 48.89 |
| 500 | 8 | 7 | 9 | 8 | 53.33 |
| 1000 | 13 | 8 | 11 | 10.67 | 71.11 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

**Table 4 Results Assay Introduction Water Fraction Larvicide Acetate**

| Water fraction (ppm) | Number of Larval Death | | | Average | % Death |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| 50 | 0 | 0 | 0 | 0 | 0 |
| 500 | 0 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 | 0 |
| Negative control | 0 | 0 | 0 | 0 | 0 |

Preliminary assaying showed that the ethyl acetate fraction of *O. aristatus* had the greaassay potential as a larvicidal agent, as a concentration of 500 ppm killed 50% of mosquito larvae. Further larvicidal agent assaying was conducted on the ethyl acetate fraction.

The potential of the ethyl acetate fraction of *Orthosiphon aristatus* as a larvicidal agent has garnered attention due to its demonstrated efficacy against mosquito larvae. Preliminary assays indicate that this fraction exhibits significant lethality, achieving a 50% mortality rate at a concentration of 500 ppm.[16,17]

The linear equation obtained is Y = 0.0244x + 32 with an $r^2$ value of 0.968. From this equation, the LC50 value was found to be 737.71 ppm. This figure indicates that at a concentration of 737.71 ppm, the ethyl acetate fraction of *O. aristatus* can kill 50% of the assay larval population (Figure 1).The findings regarding the toxicity of the ethyl acetate fraction of *O. aristatus* can be discussed through the lens of methodology and results, specifically referencing the derived linear equation, (Y = 0.0244x + 32), which yielded an ($r^2$) value of 0.968, signifying a robust correlation between the concentration of the isolate and its lethal

effect on assay larvae. This strong ($r^2$) value denotes that a significant proportion of the variation in larvicidal efficacy can be attributed to the concentration of the ethyl acetate fraction, thus validating its use as a reliable predictor for estimating the lethal concentration needed to kill 50% of the population (LC50) of the assayed larvae, which is calculated to be 737.71 ppm.

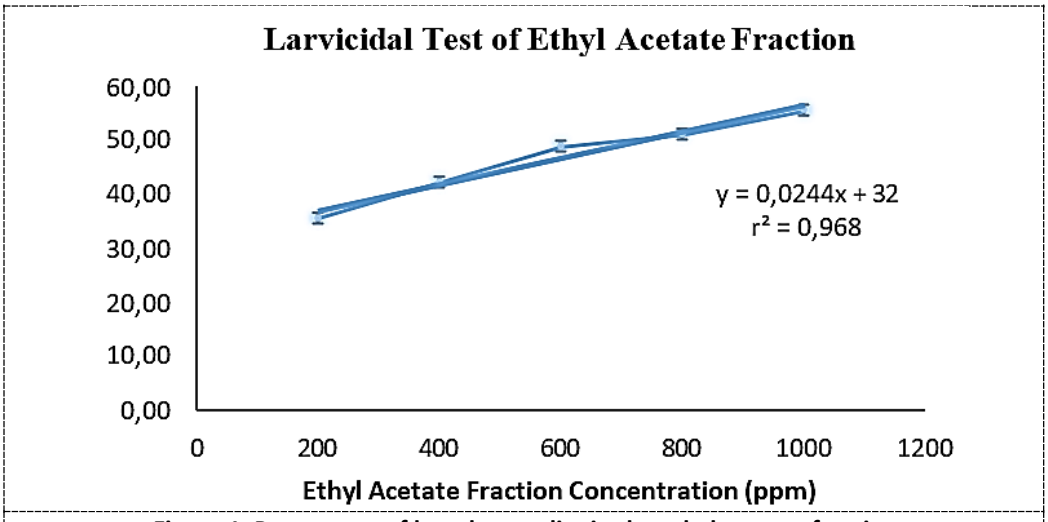


**Figure 1. Percentage of larval mortality in the ethyl acetate fraction**

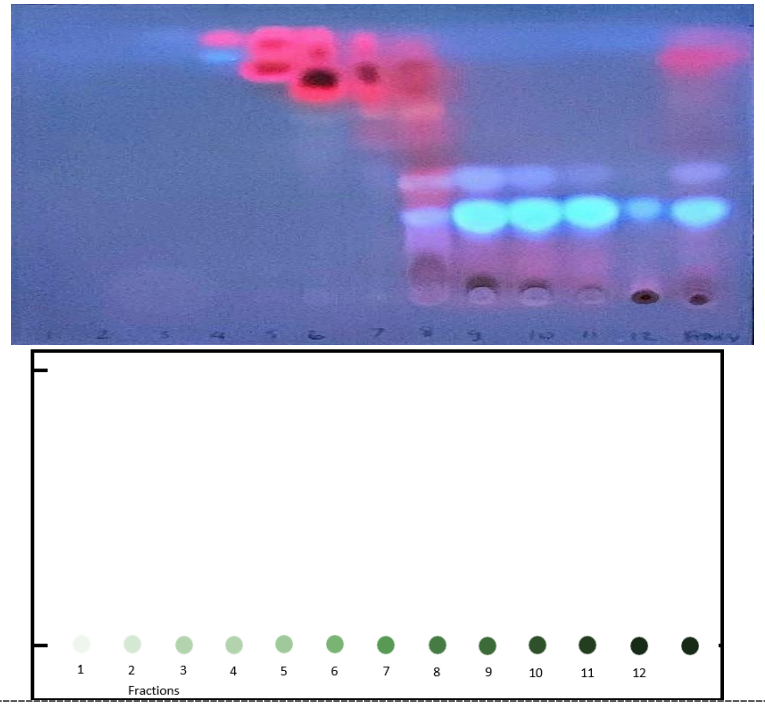


**Figure 2. Sub-fractions of the ethyl acetate fraction**

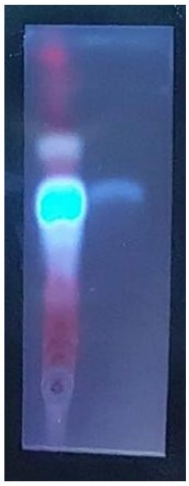


**Figure 3. TLC results comparing combined sub-fractions with sinensetin standards. (a) Combined Sub-Fractions, (b) Sinensetin Standards**

**Compound Identification**

Further separation of the ethyl acetate fraction using vacuum liquid chromatography yielded 12 sub-fractions (Figure 2). Sub-fractions 9-11 were combined into an evaporator dish and left in *a water bath* until the remaining solvent evaporated. The dried sub-fractions were then weighed. The resulting sub-fractions were 1.02 grams. The combined sub-fractions were monitored using TLC, with sinensetin, a marker of O. *aristatus*, as the standard compound (Figure 3).

## 4. Discussion

The extract's capacity as an efficient larvicidal agent may be attributed to the presence of several key phytochemicals, such as rosmarinic acid and sinensetin, which enhance the extract's biological activity.[18] These compounds have shown not only antioxidant and anti-inflammatory effects but also larvicidal activities that can disrupt the life cycle of mosquito populations effectively. Phytochemical analyzes reinforce that such active compounds are responsible for the observed biological functions.[19]

Compellingly, it has been documented that the effectiveness of plant extracts, including those from O. aristatus, lies in their complex chemical composition.[20] Research suggests that the efficacy of larvicidal activity can be significantly influenced by the method of extraction and the solvents employed. Ethyl acetate appears to optimally extract bioactive compounds due to its balanced polarity, which aids in solubilizing both polar and non-polar phytochemicals from plant sources.[21] Thus, the ethyl acetate fraction stands out not merely as a larvicidal agent but also as a potential source of bioactive principles for broader applications in pest control strategies.

Additionally, support for the larvicidal potential of *O. aristatus* is illustrated through comparative studies which analyze larvicidal efficiency relative to other traditional and herbal alternatives. Research indicates that numerous plant extracts, including O. aristatus, can outperform synthetic larvicidal agents, showcasing a greener approach to pest management in agricultural and residential contexts.[22,23] This underscores the urgent need for sustainable alternatives in managing mosquito populations, particularly as resistance to conventional insecticides becomes increasingly prevalent.[24,25]

In vitro studies emphasize the significance of concentration in the efficacy of *O. aristatus* extracts, revealing that higher concentrations lead to accelerated larval mortality, establishing a clear dose-response relationship.[26] This response can be linked back to the concentration of active compounds, including flavonoids, which hold promise for the development of future larvicidal agents. Investigations into the potential mechanisms of action reveal that these compounds may disrupt cellular processes essential for larvae survival, indicating a multi-faceted biochemical approach to pest management.[27]

Several studies corroborate the implications of LC50 values as critical indicators of toxicity in various biological systems. Notably, Eriadi et al. emphasize that semi-polar solvents like ethyl acetate are preferred for extraction purposes due to their favorable properties, including low toxicity levels compared to more toxic solvents such as n-hexane and methanol.[28] This positions ethyl acetate as a suitable candidate for extracting bioactive compounds from plant materials while minimizing potential harm to non-target organisms.

Moreover, Gad et al. explored the varying toxicity levels associated with different solvent fractions from plant extracts, although their primary focus was on bioactive compounds in ethyl acetate-MeOH fractions, which presents a more nuanced context of toxicity assessments.[29] The concept of LC50 aligns closely with the findings from O. aristatus, where the 737.71 ppm concentration signifies an effective threshold for mortality in the larval stage. In a related vein, Ruttanaphan et al. also underlined the necessity to explore less toxic alternatives for pest management, suggesting that compounds derived from ethyl acetate extracts can be leveraged in developing sustainable agricultural practices.[30]

It is essential to contextualize the observed LC50 value within existing literature on ethyl acetate's effects on marine and aquatic organisms. For example, Ramadhan et al. reported notable toxicity of ethyl acetate extracts from marine sponges, where extracts displayed varying LC50 values indicating biological activity.[31] Such studies support the notion that concentrations of ethyl acetate extracts can exert potent lethal effects on larvae, akin to the effects observed in this research, thereby reinforcing the potential of *O. aristatus* extracts for broader applications in biological pest control.

The synthesis of literature across extending various taxa indicates a rich tapestry of interactions influenced by ethyl acetate's physicochemical properties and biological activity. Chen et al. explicate the mechanisms behind the production of ethyl acetate during stress responses in living cells, suggesting that its generation could signal potential toxicity and metabolic roles in various organisms.[32] This indicates that the targets of ethyl acetate could be extrapolated beyond mere pathogen suppression to include broader ecological implications, particularly concerning non-target marine fauna impacted by its application.

Addressing concerns of application risks, the literature also enunciates that while toxicity is a primary consideration, the advantages of using less harmful solvents like ethyl acetate could outweigh the risks, especially in agricultural settings. Windyaswari et al. found that secondary metabolites extracted using ethyl acetate exhibited promising bioactivity, suggesting a potential for dual use: acting ethically in pest control while minimizing ecological disturbances.[33] It also aligns well with the findings of Syam et al., highlighting how various solvent extracts yielded significant toxicity values via established bioassays,

crucially emphasizing the safety margins associated with natural extracts.[34]

In the context of the LC50 value derived for O. aristatus, continuous research into dosage-dependent toxicity is vital. The concept of balancing efficacy against potential ecological consequences remains critical to establishing sustainability in employing natural extracts for biocontrol. Thus, the 737.71 ppm designation derived from the study can guide future research and application practices, limiting dosages to maintain non-lethal thresholds for non-target organisms.

Notably, toxicity evaluations conducted across other studies emphasize the necessity of integrating the resultant findings with broader agricultural practices. For example, Zakaria et al. explored antibacterial activities related to the composition of different extracts, which intertwine with the biological assessments of extracts involving various applications, including those akin to the evaluated larvicidal potential of $O. aristatus$.[35] This points to an interdisciplinary approach, melting toxicology, agricultural science, and ecological conservation.

In the ethyl acetate sub-fraction of *O. aristatus* which has potential as a larvicidal agent, the presence of the compound sinensetin was detected. The presence of the compound sinensetin in the ethyl acetate sub-fraction of *Orthosiphon aristatus* (*O. aristatus*) has garnered attention due to its potential as a larvicidal agent against mosquito vectors. This plant has been documented to possess various biological activities, particularly through the extraction of its bioactive compounds using ethyl acetate, which effectively produces phytochemicals that can exhibit activity against larval stages of mosquitoes.[36,37] However, the specific presence of sinensetin has been reported in the ethyl acetate sub-fraction rather than in the ethyl acetate fraction of O. aristatus, thus warranting further investigation into its larvicidal properties within the appropriate solvent extraction context.[38]

The effectiveness of *O. aristatus* extracts can be informed by studies demonstrating the efficacy of different plant extracts in achieving significant larvicidal activity against *Aedes aegypti*. Ethyl acetate fractions of plants, including those comparable to O. aristatus, displayed high potency against mosquito larvae, showing significant dose-dependent effects.[39] Similarly, Jayaraman et al. emphasized that the effectiveness of botanical extracts in larvicidal applications often correlates with the concentration of specific compounds, such as flavonoids, which can interfere with physiological functions in larvae [11].

Sinensetin, as part of the flavonoid class, can be aligned with these findings. Its role in larvicidal efficacy may be related to its potential to disrupt critical enzymatic pathways linked to neurotransmission and metabolism in mosquito larvae, such as interference with acetylcholinesterase, which is essential for muscle contraction.[40] Studies have noted that plant-derived compounds frequently affect acetylcholinesterase activity, which can lead to impaired movement and subsequent mortality of the larvae.[41]

Moreover, the efficiency of ethyl acetate extraction has been highlighted for its selective ability to enrich extracts with compounds contributing to biological effects in pest management systems.[42] This selective enrichment supports the notion of using *O. aristatus* as a potential source of bioactive compounds that could have applications in insecticide formulations, especially regarding minimizing resistance in mosquito populations.[43]

Research into other botanical extracts has also shown larvicidal effects, reinforcing

the applicability of plant-derived compounds in managing vector-borne diseases. For example, investigations into *Senna occidentalis* have revealed phytochemical constituents that contribute to its larvicidal activity, suggesting that *O. aristatus* could serve as an alternative or complementary bio-insecticide in efforts to control mosquito vectors.[44] Additionally, studies on *Terminalia chebula* have highlighted multifaceted mechanisms of action involving both direct larvicidal toxicity and ecological disruption within mosquito habitats.[45]

## 5. Conclusion

The findings presented in this study advocate for the integration of bio-based larvicidal agents derived from local plant species as a significant advancement in the field of mosquito population control and the mitigation of DHF risks. By pursuing sustainable and effective alternatives to synthetic pesticides, health authorities can fortify their combat strategies against dengue fever while promoting ecological balance and community health in Indonesia and similar ecologies around the world. The research reinforces our responsibility to innovate within the context of local biology, ultimately ensuring that health interventions are aligned with the principles of environmental stewardship and public empowerment. Future policy implementations should focus on the standardized incorporation of such natural solutions into existing public health frameworks, thereby enhancing overall community resilience against epidemic diseases.

## 6. Acknowledgment

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