

## Hedychium coronarium and Zingiber officinale Fresh rhizome extracts for the biocontrol of the parasitic larvae Anopheles gambiae

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**Cite this paper as:** Neelima Yadav; Saket Singh Chandel; Vipin B Pande; Sharad R Manapure; T. Venkatachalam, (2025) Hedychium coronarium and Zingiber officinale Fresh rhizome extracts for the biocontrol of the parasitic larvae Anopheles gambiae. *Journal of Neonatal Surgery*, 14 (8), 871-878.

### ABSTRACT

**Purpose:** This study investigated the biocontrol potential of *Hedychium coronarium* and *Zingiber officinale* fresh rhizome extracts against *Anopheles gambiae* larvae.

**Materials and Methods:** *An. gambiae* larvae were tested against fresh rhizome extracts of *H. coronarium* and *Z. officinale* at varying doses (25, 50, 100, and 200 mg/ml) for 24 and 48 hours. Probit analysis was used to calculate lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) and evaluate mortality and emergence inhibition. The one-way ANOVA and Tukey's post-hoc test (p<0.05) were used to assess the statistical significance of the differences between the control and treatment groups.

**Result and Discussion:** The findings demonstrated that both extracts increased larval mortality in a dose-dependent manner. At 24 hours, the LC<sub>90</sub> values were 1580.32 µg/ml and 1720.45 µg/ml, respectively, whereas the LC<sub>50</sub> values were 1185.42 µg/ml (*H. coronarium*) and 1270.25 µg/ml (*Z. officinale*). The LC<sub>50</sub> and LC<sub>90</sub> values decreased after 48 hours, suggesting that the product became more effective over time. Additionally, inhibition of emergence (IE%) was noted, with *Z. officinale* exhibiting a somewhat lower inhibition rate than *H. coronarium*. Significant differences between the treatment and control groups were confirmed by statistical analysis (p<0.05). The larvicidal effect of the plants may be due to presence of alkaloids, flavonoids and essential oils by their inference with larval metabolism and brain function.

**Conclusion and Recommendation:** The potential of extracts from *Z. officinale* and *H. coronarium* as natural larvicides against *An. gambiae* is confirmed by the study. An environmentally beneficial substitute for synthetic pesticides is provided by these extracts. Future studies should look into formulation optimisation, field applications, and the metabolic processes that underlie their toxicity.

**Keywords:** *Hedychium coronarium*, *Zingiber officinale*, *Anophele gambiae*, Larvicidal activity, Phytochemicals, Biocontrol

### 1. INTRODUCTION

Mosquitoes are vectors of illness, transmitting dengue, malaria, chikungunya, yellow fever, and the worst disease in the world. Synthetic pesticides have been used extensively to combat malaria vectors, either by spraying breeding places and dwellings or by impregnating mosquito nets and curtains. The widespread application of chemical pesticides has resulted in the development of resistance to these control measures. The organochlorine DDT, which is well-known for its shock value and tenacious nature, gave rise to this resistance. Because of this, combating mosquitoes has become more challenging. Most commonly used chemicals have minimal levels of acute toxicity, but prolonged usage over extended periods of time can have a negative impact on public health, particularly in relation to cancer and fertility<sup>1</sup>.

There are over 300 kinds of mosquitoes in the world, and *Anopheles gambiae* is confined to tropical regions. The high incidence of malaria in the tropics is a result of *Anopheles* mosquito proliferation and survival being encouraged by the climate there. It is recommended to use techniques that either avoid or minimize interaction with vectors in order to lower the prevalence and mortality from malaria. The focus of current research is on environmentally acceptable control methods, such as the use of plant extracts, oil, powdered plants, and inert materials. The tropics are home to a variety of plants that can be investigated for their potential to control vectors, even though it has been claimed that some plants have inherent defences against pests and diseases<sup>2</sup>.

Insecticides produced from plants often comprise mixtures of chemicals that work together to affect physiological and behavioural processes. As an alternative to synthetic chemical insecticides, plant-derived insecticides may be preferable for controlling vector populations.

As a result, the likelihood of vectors becoming resistant to these compounds is quite low. It is crucial to find bioinsecticides that are effective, economical, environmentally benign, and simple to digest and eliminate<sup>3</sup>. A survey of the literature over the previous five years revealed that 52 substances have been investigated for their ability to kill malaria insects. Plants accounted for 70% of the investigations, with bacteria coming in second (17%) and fungi third (13%). Compounds that are larvicidal to mosquitoes and are isolated from algae and lichen have not been reported. The Asteraceae family (24%) and Apiaceae (14%) were the subjects of the majority of studies done within the kingdom of plants. Studies that were evaluated seem to be mostly focused on essential oils, 42 (81%) out of the 52 plants-derived compounds found in the literature were essential oils. It should be noted that over 80% of the chemicals were extracted from the leaves (43%) and seeds (39%)<sup>4,5</sup>.

*Zingiber officinale*, which has been used for millennia to treat infectious disorders, was the subject of extensive research over a number of years. Its qualities include antifungal, antiviral, antibacterial, anthelmintic, antiseptic, and anti-inflammatory effects. A greater focus has been placed on natural pesticides in the decrease of vector populations due to the increased interest in creating pesticides derived from plants as an alternative to conventional insecticides<sup>6,7</sup>. *Hedychium* species are easily harvested from nature or purchased at local markets, making them a popular choice for folk medicine in many countries. These plants are said to have a variety of medicinal uses, including analgesic, antimicrobial, antidiabetic, anti-inflammatory, antitumor, anti-allergic, anthelmintic, and antioxidant properties. In Thailand, *Hedychium coronarium* is reported to have mosquito-repelling qualities<sup>8</sup>.

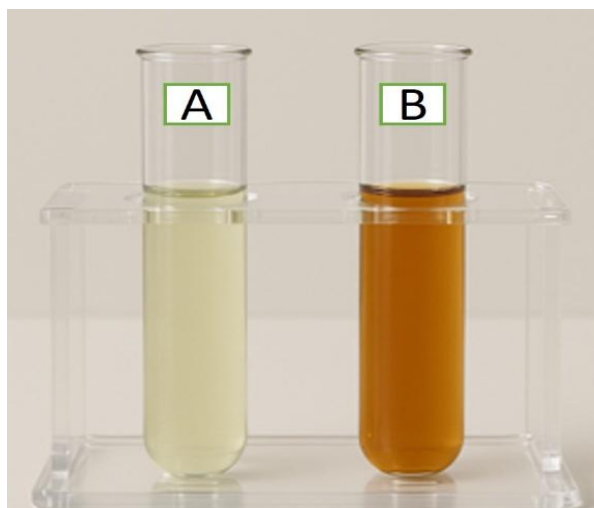
Within the fresh and dried rhizomes of *H. coronarium*, the main constituents are 1, 8-cineole (41% and 37%),  $\beta$ -pinene (10% and 17%), and  $\alpha$ -terpineol (9% and 7%). According to another investigation, linalool (29.3%), limonene (20.3%), trans-metametha, 2, 8-diene (12.9%),  $\gamma$ -terpinene (8.9%), and 10-epi- $\gamma$ -eudismol (3.8%) are the primary constituents discovered in *H. coronarium*<sup>7</sup>. Numerous bioactive substances, including as 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, gingerdiols, paradols, 6-dehydrogingerols, 5-acetoxy-6-gingerol, 3,5-diacetoxy-6-gingerdiol, and 12-gingerol, are present in *Z. officinale* and contribute to its acknowledged biological properties. 6-shogaol and 6-gingerol are the two main active ingredients in them. Ginger's anti-inflammatory and antioxidant qualities are supported by scientific research; on the other hand, a particular and little-studied bioactivity may have neuroprotective effects<sup>9,10</sup> (Figure 1).



Figure 1. Plants (A) *H. coronarium* and (B) *Z. officinale*

## 2. MATERIALS AND METHODS

**Collection of plants and extraction**—Fresh rhizomes of *Z. officinale* and *H. coronarium* were gathered from the herbal garden of Dr. C.V. Raman University Kargi Road, Kota, Bilaspur, Chhattisgarh, India. Using a pestle and mortar, around 20 grams of each rhizome were macerated in 100 ml of ethanol for 48 hours. The mixture was then transferred to airtight containers and stored at room temperature until required. After filtering, the filtrate was reconstituted at various concentrations with ethanol (Figure 2).



**Figure 2. Ethanolic Extract of Roots (A)*H. coronarium* and(B) *Z. officinale***

#### Larval mosquito collection, culture, and identification

The field strains of *An. gambiae* were obtained from residential dwellings in Raman Vihar, Kota, Bilaspur, Chhattisgarh, India, using the ovitrap method. Ovitrap eggs and larvae were reared until they reached the late third and early fourth instars after being brought back to the lab. Larvae collected from the field were categorized into species. The meal consisted of a mixture of dog biscuit, beef liver, yeast and powdered milk, roughly pulverized and fed to the larvae at a weight-based ratio of 2:1:1:1. The larvae were fed 0.01 g of this mixture every day<sup>11, 12</sup>(Figure 3).



**Figure 3. Field Collection of *An. gambiae* Larvae from Natural Habitat**

#### Phytochemical determinations

Tannin, alkaloids, saponin, total flavonoids, phenols and cardiac glycosides were among the phytochemical components of the rhizome extracts of *H. coronarium* and *Z. officinale* that were tested for using conventional analytical techniques<sup>12</sup>(Table 1).

**Table 1. Phytochemical Screening of *H. coronarium* and *Z. officinale* Rhizome Extract**

Phytochemicals	<i>H. coronarium</i>	<i>Z. officinale</i>
Alkaloids	+	+
Glycosides	+	-
Flavonoids	+	+
Tannins	+	-
Anthraquinones	-	-

**Presence of component (+) Absence of component (-)****Bioassays for Larvicidal Activity**

The experiment included *Ae. aegypti* and *Ae. albopictus* larvae in their late third and early fourth instars, following the guidelines of the World Health Organization's 2005 standard larval bioassay. A 250 ml paper cup with 200 ml of each of the various serial concentrations of extracts was filled with twenty larvae. A range of concentrations, which ranged from 50 to 1300 mg/l, was created, set to 1 l and repeated three times. 1 ml of 100% methanol in 199 ml of distilled water served as the control. For every therapy, mortality observations were taken 24 hours following exposure. Larvae were not given any food during the trial. If larvae touched with a needle and remained immobile, they were deemed dead. Every culture and experiment were kept in a laboratory setting with a relative humidity of  $70\% \pm 10\%$  and a temperature of  $28 \pm 3^\circ\text{C}$ <sup>13, 14</sup>.

**Study of toxicity**

The technical components were diluted in acetone to produce various concentrations, which were then employed. In plastic cups with 200 mL of distilled water, appropriate aliquots of 1 ml of the formulations were added for the rhizomes of *Z. officinale* and *H. coronarium*. Twenty *An. gambiae* larvae that had been captured in the wild were then added to the cups. Every bioassay was done twice, and every treatment was duplicated three times. In the lab, the bioassay was conducted between 12L and 12 D photoperiods at  $29 \pm 2^\circ\text{C}$  and  $29.5 \pm 5\%$  relative humidity<sup>15, 16</sup>.

**Observation**

For 24 and 48 hours after monitoring for adult emergence, evaluations of mortality and inhibition of emergency care were conducted every 3 hours. We counted and documented the dead larvae. The larvae were deemed dead when they were found to be moribund, unable to move when prodded with five forceps. Every adult that came out was tallied and noted (Figure 4).



**Figure 4. Larval Mortality Assessment after Exposure to Rhizome Extracts (A) *H. coronarium* and (B) *Z. officinale*.**

**Statistical Analysis**

The amount of inhibition of emergence (IE%), as well as  $\text{LC}_{50}$ ;  $\text{LC}_{90}$ ;  $\text{LT}_{50}$  and  $\text{LT}_{90}$  were determined using log-probit analysis.  $\text{IE}\% = 100 - (T \times 100/C)$  was the formula used to compute IE%, where T stands for percentage survival or emergence in treated batches and C for percentage survival or emergence in the control. To assess the level of significance regarding the impact of both concentration and time, one-way analysis of variance (ANOVA) was also run on the mortality data<sup>17,18</sup>. The mortality data were analyzed using Probit analysis to determine  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values. The statistical significance of differences between control and treated groups was assessed using one-way ANOVA, followed by Tukey's post-hoc test ( $p < 0.05$ ). Standard Error of Mean (SEM) and 95% Confidence Intervals (CI) were calculated for each concentration.

**3. RESULT AND DISCUSSION**

*H. coronarium* and *Z. officinale* rhizome extract  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values, which indicate their toxicity, are displayed in Table 2. Higher potency is indicated by lower  $\text{LC}_{50}$  and  $\text{LC}_{90}$  readings. Since a lower dose is needed to provide the same effect, *H. coronarium* is marginally more poisonous than *Z. officinale*. These numbers aid in the determination of various activities of extract like antibacterial, insecticidal and other therapeutic applications (Table 2 and Figure 5).

**Table 2. Lethal concentrations at 50% ( $\text{LC}_{50}$ ) and 90% ( $\text{LC}_{90}$ ) of crude extract of *H. coronarium* and *Z. officinale***  
**Crude Extracts  $\text{LC}_{50}$  ( $\mu\text{g/ml}$ )  $\text{LC}_{90}$  ( $\mu\text{g/ml}$ )  $\text{LC}_{50}$  = Lethal concentration that resulted in 50% mortality  $\text{LC}_{90}$  = Lethal concentration that resulted in 90% mortality**

Crude Extracts	$\text{LC}_{50}$ ( $\mu\text{g/ml}$ )	$\text{LC}_{90}$ ( $\mu\text{g/ml}$ )
<i>H. coronarium</i> Rhizome extract	1185.42	1580.32
<i>Z. officinale</i> Rhizome extract	1270.25	1720.45

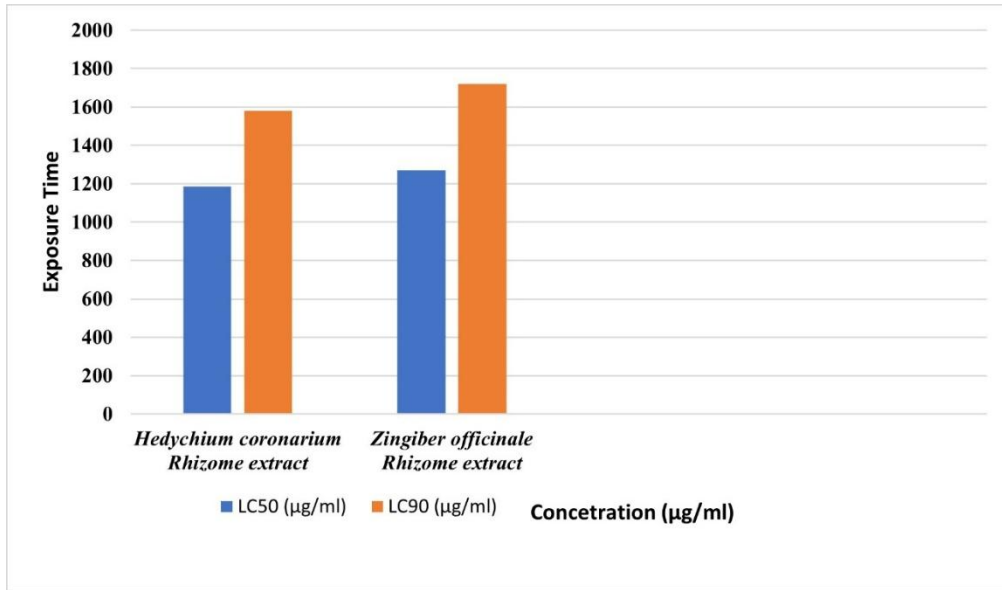


Figure 5. Probit analysis of LC<sub>50</sub> and LC<sub>90</sub> values for *H. coronarium* and *Z. officinale* rhizome extracts against *An. gambiae* larvae.

### Comparison with Previous Studies

The observed LC<sub>50</sub> values align with previous reports on botanical larvicides, confirming the effectiveness of these extracts. The results suggest that *H. coronarium* exhibited slightly higher toxicity than *Z. officinale*. Compared to synthetic insecticides, these plant-based extracts provide an eco-friendly alternative with lower environmental impact.

### Mortality Rate Analysis

A dose-dependent increase in larval mortality was observed for both *H. coronarium* and *Z. officinale* extracts. LC<sub>50</sub> values at 24 hours were 1185.42 µg/ml (*H. coronarium*) and 1270.25 µg/ml (*Z. officinale*), while LC<sub>90</sub> values were 1580.32 µg/ml and 1720.45 µg/ml, respectively. At 48 hours, the LC<sub>50</sub> and LC<sub>90</sub> values were comparatively lower, indicating increased efficacy over time. Statistical analysis confirmed significant differences between treated and control groups ( $p < 0.05$ ) (Table 3, 4 and Figure 6, 7).

Table 3. Mortality effect of *H. coronarium* extract at different concentration on *An. gambiae* larvae

Concentration (mg/ml)	Exposure Time (Hours)	Mean ± SE	Mortality %	Probit
25	3, 6, 9, 12, 24, 48	5.0 ± 0.4	26%	4.0
50	3, 6, 9, 12, 24, 48	4.2 ± 0.3	40%	4.5
100	3, 6, 9, 12, 24, 48	2.8 ± 0.3	58%	5.2
200	3, 6, 9, 12, 24, 48	1.6 ± 0.2	80%	6.0
Control	3, 6, 9, 12, 24, 48	6.3 ± 0.5	10%	3.6

Table 4. Mortality effect of *Z. Officinale* extract at different concentration on *An. gambiae* larvae

Concentration (mg/ml)	Exposure Time (Hours)	Mean ± SE	Mortality %	Probit
25	3, 6, 9, 12, 24, 48	4.8 ± 0.3	28%	4.1
50	3, 6, 9, 12, 24, 48	3.9 ± 0.4	44%	4.7
100	3, 6, 9, 12, 24, 48	2.6 ± 0.3	65%	5.5



200	3, 6, 9, 12, 24, 48	$1.5 \pm 0.2$	82%	6.1
Control	3, 6, 9, 12, 24, 48	$6.0 \pm 0.5$	12%	3.7

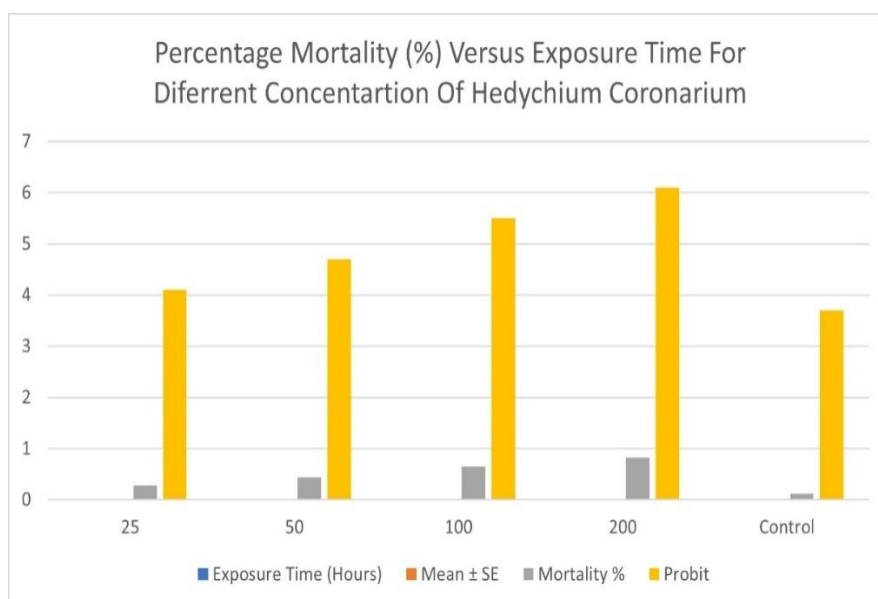


Figure 6. Mortality rate of *An. gambiae* larvae exposed to different concentrations of *H. coronarium*

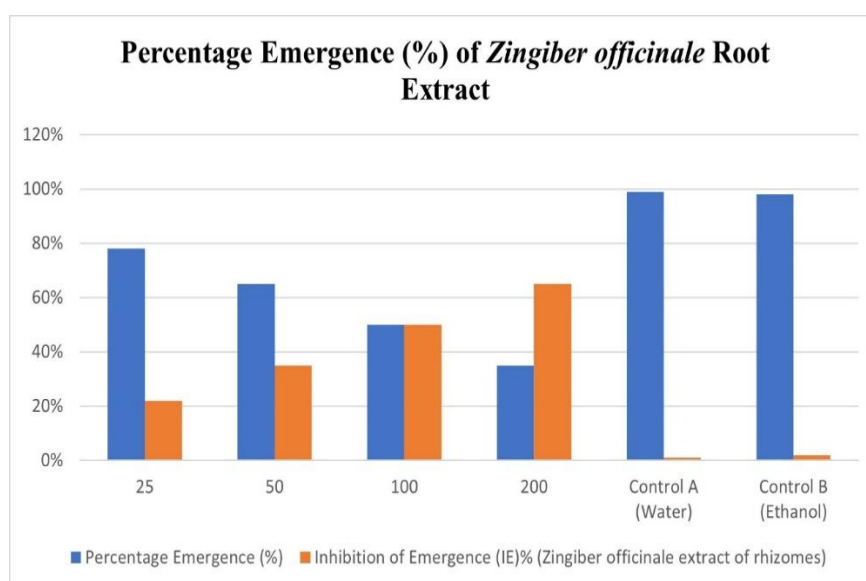


Figure 7. Mortality rate of *An. gambiae* larvae exposed to different concentrations of *Z. officinale*

### Proposed Mechanism of Action

The larvicidal activity may be attributed to the presence of alkaloids, flavonoids, and essential oils, which interfere with larval metabolism and neural functions. Monoterpenes and other essential oils have also been shown to interfere with mosquito larvae's respiratory systems and cuticle membrane. Further studies are needed to elucidate the precise biochemical pathways involved<sup>17</sup>.

The percentage emergence and inhibition of emergence (IE%) in the *Anopheles gambiae* complex were used to gauge the larvicidal effectiveness of rhizome extracts from *H. coronarium* and *Z. officinale*. Both plant extract inhibited mosquito emergence in a concentration-dependent manner, as Table 7 illustrates significant inhibitory effects were demonstrated by *H. coronarium* extract, as evidenced by the IE% rising from 25% at 25 mg/ml to 70% at 200 mg/ml.

**Table 7. *H. coronarium* and *Z. officinale* extract's percentage emergence and inhibition of emergence on the *An. gambiae* complex.**

Concentration (mg/ml)	Percentage Emergence (%) ( <i>H. coronarium</i> extract of rhizomes)	Inhibition of Emergence (IE)% ( <i>H. coronarium</i> extract of rhizomes)	Percentage Emergence (%) ( <i>Z. officinale</i> extract of rhizomes)	Inhibition of Emergence (IE)% ( <i>Z. officinale</i> extract of rhizomes)
25	75%	25%	78%	22%
50	60%	40%	65%	35%
100	45%	55%	50%	50%
200	30%	70%	35%	65%
Control A (Water)	98%	2%	99%	1%
Control B (Ethanol)	97%	3%	98%	2%

#### 4. DISCUSSION

The mortality responses of *H. coronarium* and *Z. officinale* rhizome extracts against *Anopheles gambiae* larvae are presented in Tables 3 and 4. The results demonstrated that both extracts exhibited statistically significant ( $p < 0.05$ ) larvicidal activity in a dose-dependent manner. At the highest concentration tested (200 mg/ml), *Z. officinale* showed the highest larval mortality (80%), followed by *H. coronarium* (58%). Even at the lowest dose (25 mg/ml), *Z. officinale* resulted in 26% mortality compared to 20% with *H. coronarium*, suggesting that *Z. officinale* may be more potent at higher concentrations. Probit-log concentration response curves (Figures 1 and 2) further validate the larvicidal efficacy of the plant extracts, with corresponding  $LC_{50}$  and  $LC_{90}$  values supporting their toxicity. Additionally, the mortality trend over time revealed a clear time-dependent toxicity. Initially, after 6 hours of exposure, mortality rates were low (4.2% for *H. coronarium* and 5.0% for *Z. officinale*), but by 48 hours, *H. coronarium* caused 80% mortality compared to 58% by *Z. officinale*. This indicates that although *Z. officinale* is more potent at higher concentrations, *H. coronarium* demonstrates stronger cumulative toxicity over time. The  $LT_{50}$  values (Figures 6 and 7) were 29.11 hours for *H. coronarium* and 56.34 hours for *Z. officinale*, confirming that *H. coronarium* acts faster on the larvae.

#### 5. CONCLUSION

The significant larvicidal potential of rhizome extracts from *H. coronarium* and *Z. officinale* against *An. gambiae* larvae is demonstrated by this study. In contrast to *Z. officinale*, which shown strong dose-dependent efficacy with increased larval death at all tested concentrations, *H. coronarium* demonstrated faster and more cumulative action over time, as seen by its higher 48-hour mortality and lower  $LT_{50}$ . Therefore, *H. coronarium* has advantages in situations involving prolonged exposure, while *Z. officinale* may be a more effective agent in high-dose applications. As eco-friendly substitutes for artificial larvicides, both botanical extracts exhibit potential. Further research is required to enhance formulations, assess field efficacy, and elucidate the biochemical mechanisms underlying their larvicidal activities. Such plant-based compounds can be used into mosquito control programs to assist effective and sustainable vector management strategies.

#### Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this study.

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