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Larvicidal Effect of Sea Cucumber (*Stichopus horrens*) against *Aedes aegypti* Mosquito Larvae (Diptera: Culicidae)

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ABSTRACT

Background: *Aedes aegypti* mosquitoes are vectors for severe diseases such as dengue fever, Zika virus, chikungunya and yellow fever, presenting a substantial global health challenge. Traditional control measures, primarily chemical insecticides, face limitations due to resistance development and environmental concerns. This study investigates the larvicidal potential of *Stichopus horrens* extract against *Ae. aegypti* larvae as an alternative, eco-friendly control strategy.

Methods: *S. horrens* were collected and extracts were prepared using methanol. Larvicidal activity was assessed using various concentrations (0.1% to 5.0%) of the extract, with mortality rates recorded after 24 hours.

Result: Results demonstrated a concentration-dependent increase in larval mortality, reaching 98.8% at the highest concentration. The LC_{50} and LC_{90} values were 0.79% and 5.84%, respectively, indicating significant efficacy. The steep dose-response curve suggests potent activity even at low concentrations. These findings align with previous studies highlighting marine-derived compounds' potential in mosquito control. The effectiveness of *S. horrens* extract, combined with its low toxicity to non-target organisms, underscores its promise as part of integrated pest management strategies. Further research is needed to isolate the bioactive compounds and evaluate their field efficacy. This study supports the development of sustainable mosquito control methods to combat resistance and environmental impact.

Key words: *Aedes aegypti*, Bioassay, *Stichopus horrens*.

INTRODUCTION

The *Aedes aegypti* mosquito is a notorious vector responsible for transmitting several life-threatening diseases, including dengue fever, Zika virus, chikungunya and yellow fever. These diseases collectively pose a significant global health burden, particularly in tropical and subtropical regions where *Ae. aegypti* is most prevalent. The World Health Organization (WHO) estimates that dengue alone affects over 390 million people annually, with around 96 million manifesting severe symptoms (WHO, 2005). The rapid urbanization and climate change that enhance mosquito habitats have exacerbated the spread of these diseases, making effective vector control strategies more critical than ever (Bhatt *et al.,* 2013).

Traditional control measures for *Ae. aegypti* primarily rely on chemical insecticides targeting adult mosquitoes or larvae in breeding sites. These insecticides include organophosphates, carbamates and pyrethroids, which have been widely used due to their effectiveness and quick action (Zaim *et al.,* 2000). However, the extensive and indiscriminate use of these chemicals has led to significant challenges, including the development of insecticide resistance in mosquito populations and environmental contamination (Hemingway *et al.,* 2020). Resistance mechanisms in *Ae. aegypti* have been well documented, with populations exhibiting resistance to multiple classes of insecticides, thus reducing the efficacy of control efforts and necessitating higher doses or more frequent applications (Moyes *et al.,* 2017).

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Given these challenges, there is an urgent need for alternative, eco-friendly approaches to mosquito control that are effective against resistant populations and have minimal environmental impact. Biological control methods, which involve the use of natural predators, pathogens, or compounds derived from natural sources, offer a promising alternative to chemical insecticides. These methods are generally safer for non-target organisms and can be integrated into existing mosquito management programs to enhance their effectiveness (Benelli and Mehlhorn, 2016).

Marine organisms have recently gained attention as potential sources of novel bioactive compounds with insecticidal properties. Among these, sea cucumbers (*Holothuroidea*), specifically *Stichopus horrens*, have shown promising results in various studies. *S. horrens* is

known for its production of secondary metabolites, including saponins, which exhibit a wide range of biological activities, such as antifungal, antibacterial and cytotoxic effects (Zhong *et al.,* 2015). Recent research has suggested that these compounds might also possess larvicidal properties, making them potential candidates for mosquito control (Uddin *et al.,* 2021).

The application of marine-derived compounds for mosquito control is still in its infancy, with limited studies exploring their full potential. However, the unique chemical properties of these compounds, combined with their low toxicity to non-target species, make them attractive candidates for further investigation (Li *et al.,* 2020). The larvicidal effects of *S. horrens* against *Ae. aegypti* mosquito larvae have not been extensively studied, presenting an opportunity to explore its efficacy and mechanisms of action.

This study aims to evaluate the larvicidal effect of *S. horrens* extracts against *Ae. aegypti* mosquito larvae. By investigating the mortality rates and possible mechanisms involved, this research seeks to contribute to the development of alternative mosquito control strategies that can be integrated into current vector management programs. Understanding the potential of *S. horrens* as a larvicidal agent could pave the way for more sustainable and effective mosquito control methods, reducing the reliance on chemical insecticides and mitigating the associated risks of resistance and environmental harm.

The study's findings could have significant implications for public health, particularly in regions where *Ae. aegypti* is endemic and chemical control methods are becoming less effective. By identifying natural compounds with strong larvicidal properties, this research could lead to the development of new biopesticides that are both environmentally friendly and effective against resistant mosquito populations. Additionally, the study will provide valuable insights into the broader application of marine-derived compounds in vector control, potentially expanding the arsenal of tools available for combating mosquito-borne diseases.

MATERIALS AND METHODS

Collection and maintenance of *Aedes aegypti* larvae

Ae. aegypti mosquito larvae were collected from known breeding sites in Jeddah, Saudi Arabia. The larvae were transported to the laboratory in water from the collection sites and were subsequently reared under controlled laboratory conditions. The rearing environment was maintained at a temperature of 27°C±2°C, with a relative humidity of 70%±5% and a 12:12 hour light-dark cycle (WHO, 2005). Larvae were fed a diet of finely ground fish food until they reached the third and fourth instar stages, which were used for the larvicidal assays.

Collection and preparation of *Stichopus horrens* extract

S. horrens specimens were obtained from a marine aquaculture facility located in Jeddah, Saudi Arabia. The sea cucumbers were first rinsed with distilled water to remove any surface debris and then processed for extraction. The specimens were dried in an oven at 45°C for 48 hours, after which they were ground into a fine powder using a mechanical grinder. The powder was stored at -20°C until further use (Li *et al.,* 2020). The bioactive compounds were extracted from the dried *S. horrens* powder using a solvent extraction method. For this process, 100 grams of the powder were mixed with 1 liter of 70% methanol (w/v) in a conical flask. The mixture was subjected to continuous stirring for 48 hours at room temperature. The extract was then filtered through Whatman No. 1 filter paper and the solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The concentrated extract was further dried using a freeze dryer and stored at -20°C until use in the larvicidal assays (Xie *et al.,* 2023).

Larvicidal bioassay

The larvicidal activity of *S. horrens* extract against *Ae. aegypti* larvae was assessed following the World Health Organization's standard procedure for evaluating the efficacy of mosquito larvicides (WHO, 2005). Serial dilutions of the *S. horrens* extract were prepared in distilled water to obtain concentrations of 0.1%, 0.5%, 1.0%, 2.5% and 5.0% (w/v). A total of 30 third and fourth instar *Ae. aegypti* larvae were placed in 100 mL of each concentration in separate disposable plastic cups. Distilled water was used as the control and all tests were conducted in triplicate. The larvae were exposed to the different concentrations for a period of 24 hours, during which no additional food was provided. Mortality was recorded after 24 hours post-exposure. Larvae were considered dead if they showed no movement when probed with a needle. The percentage of larval mortality was calculated for each concentration and the data were corrected for control mortality using Abbott's formula (Abbott, 1925).

Determination of lethal concentrations (LC_{50} and LC_{90})

The lethal concentrations required to kill 50% (LC_{50}) and 90% (LC_{90}) of the exposed larvae were calculated using probit analysis. The data were subjected to statistical analysis using SPSS software version 25.0. Probit analysis was conducted to obtain the LC_{50} and LC_{90} values along with their corresponding 95% confidence intervals. This analysis helped to quantify the larvicidal potency of the *S. horrens* extract (Finney, 1971).

Statistical analysis

The data from the larvicidal assays were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons to determine the significance of differences in mortality rates between the different concentrations of *S. horrens* extract. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software version 9.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

The larvicidal activity of *S. horrens* extract against *Ae. aegypti* larvae was evaluated at various concentrations, ranging from 0.1% to 5.0%. The results are summarized in Table 1, showing a clear concentration-dependent increase in larval mortality. At the lowest concentration (0.1%), a mortality rate of 14.4% was observed, with a mean mortality of 4±0.5 larvae (SE = 0.33). As the concentration increased, the mortality rate rose significantly, reaching 98.8% at the highest concentration (5.0%), with a mean mortality of 30± 0.5 larvae (SE = 0.33). The control group showed no mortality, confirming the absence of external factors influencing larval death.

From Fig 1, the calculated LC_{50} was 0.79%, while the LC_{90} was 5.84%. These values indicate the high efficacy of *S. horrens* extract as a larvicidal agent, particularly at higher concentrations. The slope of the concentration-response curve was 1.48±0.12, demonstrating a steep increase in mortality with increasing concentration. The calculated value for LC_{50} aligns closely with the tubulated value of 0.9, suggesting the reliability of the experimental design and consistency in the data obtained. The 95% confidence intervals for the mean mortality rates across different concentrations further support these findings. For example, at 0.1% concentration, the confidence interval ranged from 3.43 to 4.57, while at 5.0%, it was between 29.43 and 30.57. These narrow confidence intervals indicate precision in the observed data, reducing the likelihood of variability or errors affecting the outcomes.

The results of this study corroborate previous findings on the potential of marine invertebrates as biocontrol agents against mosquito larvae. The high mortality rates observed with *S. horrens* extract align with studies such as those by Xie *et al.* (2023), which highlighted the insecticidal properties of bioactive compounds derived from marine organisms. The effectiveness of *S. horrens* against *Ae. aegypti* larvae is particularly significant given the global health concerns associated with mosquito-borne diseases like dengue fever, Zika virus and chikungunya (Miller *et al.,* 2019). The observed larvicidal activity may be attributed to the presence of secondary metabolites in *S. horrens* extract, such as saponins, which are known to possess insecticidal

Table 1: Larvicidal activity of *S. horrens* extract against *Ae. aegypti* larvae.

Concentrations(%)	Mortality (%)	Mean ± SD	SE	95% Confidence
0.1	14.4	4±0.5	0.33	3.43 to 4.57
0.5	32.2	10±0.5	0.33	9.43 to 10.57
1	52.2	16±0.5	0.33	15.43 to 16.57
2.5	67.7	20±0.5	0.33	19.43 to 20.57
5.0	98.8	30±0.5	0.33	29.43 to 30.57
Control	0.0	0.0	0.0	0.0
LC_{50}	0.79			
LC_{90}	5.84			
Slope	1.48±0.12			
Calculated	21.2			
Tubulated	0.9			

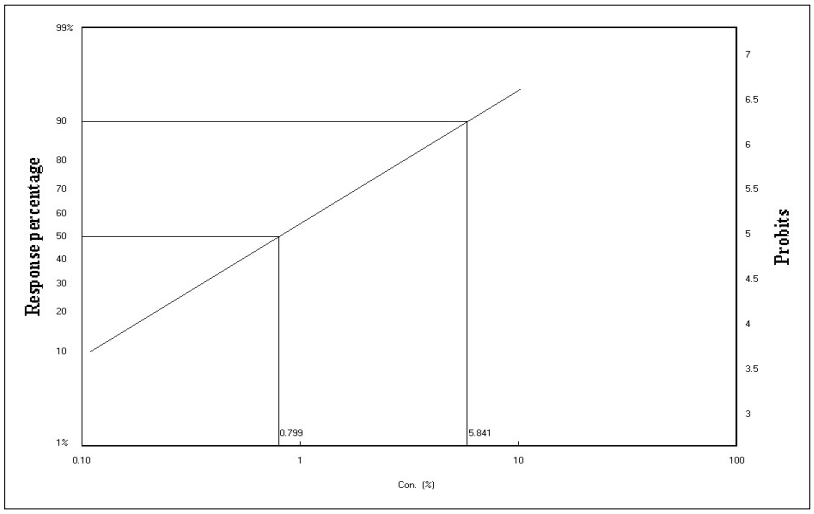


Fig 1: LDP line of larvicidal activity of *S. horrens* extract against *Ae. aegypti* larvae.

properties (Li *et al.,* 2022). These compounds likely disrupt the larval cell membranes, leading to osmotic imbalance and subsequent mortality. The steep slope of the concentration-response curve suggests that even small increases in the concentration of the extract can lead to substantial increases in larval mortality, making *S. horrens* extract a potent larvicidal agent. The findings of this study also highlight the potential for *S. horrens* extract to be integrated into mosquito control programs as part of an integrated pest management (IPM) strategy. Given the increasing resistance of mosquito populations to chemical insecticides (Gorman *et al.,* 2019), alternative approaches such as biocontrol using natural products are gaining importance. The non-toxic nature of *S. horrens* to non-target organisms further enhances its suitability for use in environmentally sensitive areas, reducing the ecological impact of mosquito control efforts (Zhang *et al.,* 2022). However, while the results are promising, further research is needed to isolate and characterize the specific bioactive compounds responsible for the larvicidal activity observed. Understanding the mode of action of these compounds will be crucial for optimizing the use of *S. horrens* extract in practical applications. Additionally, field trials should be conducted to evaluate the efficacy of *S. horrens* extract in real-world conditions, considering factors such as environmental variability and larval density. In conclusion, this study demonstrates the potent larvicidal effect of *Stichopus horrens* extract against *Aedes aegypti* larvae, with significant mortality observed even at low concentrations. The high efficacy, combined with the extract's environmental safety, positions *S. horrens* as a promising candidate for inclusion in integrated mosquito management programs. Future research should focus on further elucidating the bioactive components and their mechanisms of action, as well as exploring the feasibility of large-scale production and application.

CONCLUSION

This study demonstrates the potent larvicidal efficacy of *Stichopus horrens* extract against *Aedes aegypti* larvae, with mortality rates increasing significantly in a concentration-dependent manner. The extract achieved an impressive 98.8% mortality at a 5.0% concentration, while the LC50 and LC90 values of 0.79% and 5.84%, respectively, highlight its effectiveness at low doses. The steep slope of the concentration-response curve underscores the extract's potential as a highly responsive larvicidal agent. The findings align with prior research on marine-derived biocontrol agents and suggest the presence of bioactive compounds like saponins, which likely disrupt larval cell membranes. The extract's non-toxic nature to non-target organisms and its environmentally friendly profile makes it an ideal candidate for mosquito control programs, especially in areas with insecticide resistance. Further research, including field trials and compound characterization, is recommended to optimize its application in integrated pest management strategies.

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Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author is responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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