ORIGINAL ARTICLE

Preliminary Evaluation of the Larvicidal Effectiveness of *Ananas comosus* Flesh Extracts on *Aedes albopictus*

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ABSTRACT

Introduction: The control of mosquito populations to prevent disease transmission relies profoundly on the application of larvicides. However, the surge of resistance poses a significant threat. Botanical insecticides serve as an alternative approach for vector control, and thus, this study aimed to evaluate the larvicidal capabilities of *Ananas comosus* flesh against *Aedes albopictus*. **Methods:** An aqueous extract of *A. comosus* was concentrated using the double-boiling method. The crude extract was diluted through the serial dilution technique. Mosquito eggs were collected using ovitraps and reared until adulthood under standard insectarium conditions. Late third and early fourth instar of *Ae. albopictus* larvae (30 larvae/replicate, n = 3) were exposed to the extract, and the mortality rate was recorded following 24 h and 48 h exposure. **Results:** Probit analysis revealed that the median lethal concentration, LC50 and 90% lethal concentration after 24 h were 17.24 (9.62 – 21.03) and 24.53 (19.74 – 31.13) %, respectively. The LC50 and LC90 values for 48 h were 8.45 (6.49 – 10.06) and 11.59 (9.72 – 14.81) %, respectively. **Conclusion:** The findings suggest that *A. comosus* flesh revealed activity against *Ae. albopictus*, making it a potential botanical-derived larvicide. Future studies should focus on investigating the phytochemicals responsible for larval mortality and their mode of action.

Keywords: *Aedes albopictus*; Larvicidal activity; *Ananas comosus*; Natural insecticides

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INTRODUCTION

Concerns regarding the growth of the mosquito population and the arboviral diseases they transmit are escalating in the public health sphere. Chemical insecticides have been commonly employed over the years to tackle this, despite the rising incidences of insecticide resistance and the associated risks both to human health and the ecosystem (1-2). This scenario adds to the complexity of vector control efforts, emphasizing the need to explore alternative initiatives that are targeted, sustainable and environmentally sound.

To address this, botanical insecticides have been introduced as potential solutions. Numerous studies have scrutinised the larvicidal activity of various fruits and plants. These natural sources possess bioactive compounds that can effectively target specific insects while being environmentally friendly. An earlier investigation, for instance has recorded the larvicidal and pupicidal impacts of essential oil extracted from Illicium verum and Zanthoxylum limonella (3). Larvicidal properties were also discovered in *Averrhoa bilimbi* extract (4). In addition, a combination of essential oils from *Alpinia galanga*, *Curcuma zedoaria*, *Zingiber cassumunar* and *Eucalyptus globulus* essential oils exhibited ovicidal and adulticidal actions against *Ae. albopictus* and Anopheles minimus (5). These findings highlight the probable usage of plants and their extracts as effective alternatives for mosquito population control efforts.

Ananas comosus is a tropical fruit widely found in Malaysia, with significant commercial value as a fruit crop in the country. This fruit is a rich source of vitamins, minerals, proteins and dietary fibres that offer a wide range of health benefits (6). *Ananas comosus* contains phytochemicals such as flavonoids, polyphenols, vitamin C and β-carotene (7). It also contains bromelain, an enzyme that aids digestion

and possesses various therapeutic properties, including anti-inflammatory, antioxidant and anti-cancer effects (8). In a recent study, the larvicidal efficacy of pineapple extract was investigated (9), the findings of which serves as a foundation for the current research, utilizing *Ae. albopictus*.

MATERIALS AND METHODS

Plant material and preparation of extract

This study utilized MD2 pineapples, sourced from a local distributor in Selangor, Malaysia. The pineapples were rinsed thoroughly to ensure cleanliness. The peel was removed, and the flesh was then diced into small pieces. Subsequently, the flesh was blended at room temperature. The mixture was then filtered using a cloth strainer and concentrated using the double boiling method. Finally, the concentrated mixture was subjected to centrifugation at 3000 rpm for 15 minutes.

Larvae collection and rearing

A total of twenty ovitraps were distributed in Puncak Alam Selangor, Malaysia. These ovitraps were filled with tap water and contained paddles to facilitate mosquito oviposition. The paddles were carefully examined for eggs; while the water was inspected for the larvae. The larvae collected during sampling were reared until adulthood within one week of sample collection at the Vector Control and Disease Research Laboratory, UiTM Selangor Campus Controlled laboratory conditions were meticulously maintained to establish cyclical colonies of mosquitoes. The cage was kept at 28°C ± 2°C, with a relative humidity of 70±10%, and 12:12 h (light: dark) photoperiod, as previously described (10). Female mosquitoes were identified and carefully selected based on morphological characteristics and standard taxonomic keys (11).

Larvicidal assay

The larvicidal bioassay was conducted according to the standardized protocol described by the World Health Organization (12). A total of 30 larvae in the late third instar stages were exposed to varying concentrations of the test medium containing water in petri dishes (100%, 50%, 25%, 12.5%, 6.25%). Three replicates were prepared for each concentration. Temephos (1 ppm) was used as a positive control, while distilled water served as the negative control. The larvicidal assay was executed at a constant temperature of 27±2 C and 12:12 h (light: dark) photoperiod. The mortality rate was recorded following 24 hours and 48 hours of exposure.

Statistical analysis

The larvae mortality was expressed as the mean ± standard deviation of three replicates. Probit analysis was employed to determine the median lethal concentration (LC50) and 90% lethal concentration (LC90) of the population exposed, analysed with a 95% confidence interval (CI) level using the SPSS 20.0 software. One Way Analysis of Variance (ANOVA) was conducted, and the significance level was set at $p < 0.05$.

RESULTS

Mortality rates following exposure to *A. comosus*

The absence of movement in response to stimuli and the inability to reach the eater surface were indicators used in this study to determine larval death. After 24 hours of exposure, the larval mortality rates exhibited an increase from 0% at the 6.25% concentration to 10% at 12.50% and steadily increased. At 48 hours of treatment, the mortality rates revealed similar patterns of increased larval mortality rates with increased plant concentration (Figure I). The results obtained indicate that there is no difference in mortality rates after the larva was exposed to 50% and 100% concentration of extracts for 24 and 48 hours. The mortality ± Standard Error Mean (SEM) of Temephos and distilled water were 100 ± 0.00 and 0 ± 0.00 respectively, across all three replicates. Statistical comparisons using one-way ANOVA revealed significant differences in larval mortality between all groups for both 24 hours ($F(4, 10) = [519.167]$, $p = 0.01$) and 48 hours of exposures ($F(4, 10) = [6889.000]$, $p = 0.01$) (Table I).

Lethal concentration (LC50 and LC90)

Probit analysis performed following 24-hour exposure revealed that the LC50 and LC90 of the extract were determined to be 17.24 and 24.53%. Comparatively, after 48-hour exposure, the values were determined to be 8.45 (LC50) and 11.59% (LC90), respectively (Table II). These findings demonstrated that the concentration and duration of the exposure improved the larvicidal activity of the Ananas extract.

Table I : ANOVA of *Ae. albopictus* larvae mortality rates following exposure to *A. comosus* flesh extract for 24 and 48 hours

Exposureperiod (h)	df	MS	F	*P*-value
24	4	7541.348	519.167	0.01
48	4	459.267	68889.00	0.01

Table II : Probit analysis of *Ae. albopictus* larvicidal activity following exposure to *A.comosus* flesh extract for 24 and 48 hours

Extract	24 h	48 h
Median lethal concentration (LC_{50},%) (LCL-UCL)	**17.24** (9.62 – 21.03)	**8.45** (6.49 – 10.06)
90% lethal concentration (LC_{90}, %) (LCL-UCL)	**24.53** (19.74 – 31.13)	**11.59** (9.72 – 14.81)
df	4	4
p-value	0.01	0.01

DISCUSSION

Plant-based products are recognized for their rich reserves of biologically active compounds, rendering them valuable in managing vectors. These compounds, including secondary metabolites can act through multiple modes, often with synergistic effects to combat mosquito vectors. Its utilization can trigger diverse physiological and biochemical processes, hindering further development of resistance. Integrating botanical agents into current pest management strategies provides a sustainable, eco-friendly approach, and can minimize ecological disruption. In tandem, previous studies have explored the potential use of natural products as repellents, insecticides, oviposition deterrents, and insect growth regulators (13-15).

This study aimed to evaluate the larvicidal activity of the aqueous extract *A. comosus* against *Ae. albopictus* using standard bioassays. The results revealed that as the exposure time increased, the observed results become more pronounced. Our findings indicate a direct correlation between the mortality rate, the duration of the exposure and the concentration of extracts to which they were subjected. Comparable results have been reported in other studies involving different species, such as studies on Artemisia vulgaris (16), Piper betle L. (17) and Allium macrostemon Bunge (18). This could be attributed to the impact of phytochemicals on the midgut epithelium, subsequently influencing the structure of gastric cecal and malformed tubules in the larvae (17). In addition, our findings demonstrated the use of distilled water is effective as extraction solvent based on the mortality rates. Similarly, the aqueous extract of Cedrus deodara (Roxb.) Loud. and Nicotiana tabacum Linn. exhibited complete larval mortality following a 24-hour exposure to Culex quinquefasciatus. (19).

The larvicidal activity of the aqueous extract of *A. comosus* flesh against *Ae. albopictus* was evaluated and the LC50 and LC90 values for 24- 46 hours were determined. The aqueous extract of *A. comosus* flesh demonstrated larvicidal capabilities. The findings align with a study previously conducted, which documented the larvicidal efficacy of *A. comosus* flesh extract against Ae. aegypti larvae. (9) However, slight differences in the larval mortality rates were observed.

It is important to note that the quality of the extract is influenced by the extraction method, solvent polarity, concentration and the organ of the plant utilised (20). Our study revealed that. *Ae. albopictus* displayed susceptibility to the aqueous extract of *A. comosus* flesh. This finding is in agreement with previous reports. The aqueous leaves extract of Annona glabra exhibited lower LC values to Ae aegypti and *Ae. albopictus* respectively, which indicate Ae albopictus was more vulnerable than Ae. aegypti (21) The same pattern was observed using Eucalyptus nitens extract (22). However, Ae. aegypti was more susceptible to S. aromaticum compared to other mosquito (13). These findings suggest mosquito species exhibit different susceptibilities to plant extract, which in turn is dependent on its phytochemicals constituents.

Based on our findings, it is evident that *A. comosus* flesh extract exhibits larvicidal activity, and thus shows promise as a natural alternative for controlling *Ae. albopictus* mosquito populations. Future study including phytochemical profiling and the identification of constituents with larvacidal activity is imperative. This can in turn reveal the mechanism of action such as by inhibiting insensitive acetylcholinesterase (23) or inhibiting conformational changes on the voltage-gated sodium channel that prevent insecticide binding (24).

CONCLUSION

In conclusion, the aqueous extract of *A. comosus* flesh in this study has demonstrated strong larvicidal activity against *Ae. albopictus*. The observed mortality rates and lethal concentration values suggest that

A. comosus flesh holds promise as a valuable source of natural larvicidal agent that could be adopted in vector control programmes.. Further research is pivotal to reinforce these findings.

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