Ethanolic extract of red dragon fruit inhibits growth of mosquito larvae

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Abstract

Controlling the vector by eradicating mosquito larvae is frequently done by spreading Abate powder containing 1% Temephos as a larvicide agent. However, the use of Abate powder will possibly increase pesticide residue contamination in the water. This study aimed to explore the potential use of ethanolic extract from red dragon fruit skin to kill mosquito larvae. An experimental laboratory study was performed to examine the larvicidal activity of ethanolic extract from red dragon fruit skin. Maceration method was used to extract active compounds from the fruit skin. Subsequently, the viscous extract was formulated using the wet granulation method to obtain powder form. The larvicide test was done by comparing the lethal concentration (LC_{50}) of mosquito larvae after treatment using the extract and the reference larvicide agent, 1% Temephos Abate. The treatment groups consisted of four different extract concentrations of 1%, 2%, 3%, and 4%. The LC_{50} of each treatment groups was then calculated against the negative control group. Phytochemical screening showed that the ethanol extract of red dragon fruit skin contains alkaloids, flavonoids, saponins, triterpenoids, and tannins. The larvicide test on *Aedes aegypti* mosquito larvae showed that the extract treatment hardly killed the *Aedes aegypti* mosquito larvae. However, on *Culex* mosquito larvae, the ethanolic extract of red dragon fruit skin was able to kill mosquito larvae, with LC_{50} value of of 19.06%. The ethanolic extract of red dragon fruit skin was effective on killing the *Culex* mosquito larvae but not the *Aedes aegypti* mosquito larvae.

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Introduction

Vector-based infectious diseases account for over 17% of all infectious diseases, resulting in more than 400,000 deaths annually and afflicting 219 million people worldwide. In Indonesia, these diseases are prevalent health issues in various regions and cities, often leading to outbreaks with a high risk of mortality. Being a tropical country, Indonesia experiences relatively high humidity and rainfall, creating favorable conditions for the proliferation of vector populations.[1,2] Mosquitoes can transmit several vector-based infectious diseases, with some of the most prevalent ones in Indonesia being Dengue Hemorrhagic Fever (DHF),[3] *Japanese encephalitis*, and filariasis. These diseases require particular attention due to their high incidence in the country.

The vector responsible for transmitting Dengue Hemorrhagic Fever (DHF) is the Aedes aegypti mosquito, while *Japanese encephalitis* (JE) is transmitted by the *Culex* mosquito. In the case of filariasis, it can be transmitted by various mosquito species, including *Mansonia, Anopheles, Culex, Aedes,* and *Armigeres* mosquitoes. Several studies have emphasized that global climate change has had an impact on the risk of disease vector transmission, particularly in the case of mosquitoes.[4–7] As of the end of 2022, Indonesia had reported approximately 143,000 cases of Dengue Hemorrhagic Fever (DHF), while there were an average of 35,000 Japanese encephalitis (JE) cases in Asia each year. In 2021, Indonesia reported 9,354 cases of filariasis. These vector-based infectious diseases have the potential to cause severe phys-

ical and mental disabilities and can increase mortality.[8–10] Given these facts, disease prevention measures are crucial to prevent their spread and reduce mortality.

One of the most efficient preventive actions is the control of disease vectors by eradicating mosquito larvae of *Aedes aegypti* and *Culex* using larvicidal agents, such as 1% Temephos in the form of Abate powder. It has been in use since 1976 and has been proven effective in killing *Aedes aegypti* and *Culex* mosquito larvae. However, the accumulation of Abate residue from its long-term use may lead to water contamination, especially in drinking water, and may create selection pressure that induces larvae to become resistant to the larvicidal agent.[11–13] Therefore, there is an urgent need for an alternative larvicide that is safer, more selective, less toxic for the environment, and yet has a similar effect to Abate.

Red dragon fruit skin is a natural ingredient that has the same function as abate. Commonly, red dragon fruit skin is often discarded despite containing various active compounds, including flavonoids, tannins, alkaloids, and saponins.[14–16] Flavonoids are known to be toxic to insects' digestive and respiratory tracts.[17,18] Tannins can inhibit insects in the process of food metabolism.[17] Alkaloids have the ability to degrade insect cells,[19] and saponins can reduce metabolic work and protein work in insects.[17] There is a lack of research exploring red dragon fruit skin as a mosquito larvicide agent. This study aimed to explore the larvicidal activity of red dragon fruit skin (*Hylocereus polyrhizus*) ethanolic extract against *Aedes aegypti* and *Culex* mosquito larvae.

Materials and Methods

This study was an experimental laboratory research that using *Aedes aegypti* and *Culex* mosquito larvae as subjects. The material used in this study was red dragon fruit peel, ethanol 96%, HCl 2N, dragendorf reagen, mayer reagen. Magnesium powder, chloroform, acetic acid, sulfuric acid, $FeCl_3$, PGA, aquadest, and amylum. The independent variables were the compound content in the ethanol extract of red dragon fruit peel and variations in herbal Abate concentrations. The dependent variable was the mortality of Aedes aegypti and Culex mosquito larvae, determined by counting the number of mosquito larvae that survived after treatment.

Red dragon fruits (*Hylocereus polyrhizus*) were obtained from Jember area, East Java. The fruits were then cleaned and separated from their fruit flesh. Subsequently, the fruit skin was dried and grounded to form powder. The maceration process was carried out using 96% ethanol to obtain thick extract followed by wet granule formulation to get powder form. Besides, the viscous extract was subjected for phytochemical screening to asses its active ingredients. The larvicidal activity of red dragon skin ethanolic extract against mosquito larva was determined using lethal concentration (LC_{50}). Briefly, the *Aedes aegypti* and *Culex* mosquito larvae were treated using different concentrations of powdered extract at 1%, 2%, 3%, and 4%. The 1% Temephos Abate was used as a positive control. The dead larva populations after incubation were counted[20–23]. Subsequently, the LC_{50} was determined using the probit linear regression test[24].

Results

Plant determination was carried out to determine the tribe and type of the red dragon fruit (*Hylocereus polyrhizus*) plant. The determination results showed that the sample used was a red dragon fruit plant (*Hylocereus polyrhizus*).

Phytochemical screening test of red dragon fruit

The results showed that the ethanolic extract of red dragon fruit peel contains alkaloids, flavonoids, saponins, triterpenoids, and tannins (Table 1). Differences in the content of plant secondary metabolites can occur due to differences in light, temperature, pH, altitude, and soil conditions in the sample planting areas. Unfortunately, we hardly detected steroid compounds using non-specific color changes testing as well as the quantitative testing using. This may occur due to low steroid content in the extract. Based on the phytochemical screening, red dragon fruit skin extract contains potential compounds that can be used as a biolarvicidal agent for *Aedes aegypti* and *Culex* mosquitoes.

Larvicidal activity test

The larvicidal activity test of red dragon fruit skin ethanolic extract of was carried out *Aedes aegypti* and *Culex* mosquito lar-

Table 1. Phytochemical screening results.

Secondary metabolites	Test result
Alkaloid	(+)
Flavonoid	(+)
Saponin	(+)
Triterpenoid and steroid	Triterpenoid (+) Steroid (-)
Tanin	(+)

Table 2. Data on observation results of effectiveness tests on *Culex* mosquito larvae.

No.	Rep.	Number of dead larvae					
		Negative control	Red dragon fruit skin extract concentration				Positive control (1% Themefos Abate)
			1%	2%	3%	4%	
1	I	5	7	16	21	24	25
2	II	4	13	15	20	25	22
3	III	6	9	18	15	24	26
Amount		15	29	49	56	73	73
Average		5	9,7	16,3	18,7	24,3	24,3
Percentage (%)		19	36	60	69	90	90
Profit value		4.12	4.64	5.25	5.50	6.28	6.28

vae. Selection of third instar larvae because these larvae have a larger size. In addition, third-instar mosquito larvae have relatively good resistance to the external environment and mechanically stronger resistance when transferring the larvae. Each test group, 25 mosquito larvae were used in 100 mL of water. The test was carried out for 24 hours with three replications. Our results revealed hat the ethanolic extract of red dragon fruit skin could not kill the *Aedes aegypti* mosquito larvae. However, the larvicidal activity of the extract on *Culex* mosquito larvae are shown in the Table 2.

Based on Table 2, it can be seen that 1% ethanolic extract of red dragon fruit peel can kill 36 % of the population *Culex* mosquito larvae. The 2% concentration of ethanolic extract killed about 60% of the *Culex* mosquito larvae and at 3% extract, about 69% of the population *Culex* mosquito larvae died. At 4% extract concentration 90% of the population *Culex* mosquito larvae were died, the same death percentage as seen in the positive control. It can be concluded that the higher concentration of the ethanolic extract of red dragon fruit skin, the higher the death precentage of *Culex* mosquito larvae. The probit linear regression test showed that the LC_{50} value of the ethanolic extract of red dragon fruit peel against *Culex* mosquito larvae was 19.06% and proven to be toxic for *Culex* mosquito larvae.

Discussion

This research was conducted to determine the effectiveness of red dragon fruit peel extract (*Hylocereus polyrhizus*) against *Aedes aegypti* and *Culex* mosquito larvae. Previous studies using soursop and *Angsana* leaf extract had LC_{50} values of 0.736% and 0.83%, respectively, on *Culex* mosquito larvae.[25,26] From the results of this study, the LC_{50} value of the ethanol extract of red dragon fruit peel against *Culex* mosquito larvae was 19.06%. This shows that the herbal abate 96% ethanol extract of red dragon fruit peel is more toxic to *Culex* mosquito larvae than soursop and *angsana* leaf extract.

Compounds that may be toxic from the ethanol extract of red dragon fruit peel (*Hylocereus polyrhizus*) are alkaloids, flavonoids, saponins, triterpenoids, and tannins. Alkaloids are salts that can degrade cell membranes so that they damage cells and can also interfere with the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme.[24] Flavonoids can interfere with energy metabolism in mitochondria by inhibiting the electron transport system so that ATP production is inhibited and causes a decrease in oxygen usage by mitochondria. This can inhibit the respiratory chain, oxidative phosphorylation, and break the chain between the respiratory chain and oxidative phosphorylation so that flavonoids can work as respiratory inhibitors in mosquitoes.[17] Saponins can reduce the surface tension of the mucous membrane of the larvae's digestive tract so that the larvae's walls become corrosive and eventually damaged.[27] Triterpenoids as stomach poisons.[28] Tannin compounds can bind to protease enzymes by binding to enzymes by tannins, so the work of these enzymes will be hampered so that cell metabolic processes can be disrupted, and the larvae will lack nutrition.[27]

This finding can be used in developing an abate product from red dragon fruit peel extract, which is environmentally friendly and has almost the same effectiveness as temefos abate. The herbal abate of red dragon fruit peel is expected to replace the role of temefos abate as a larvicide so that it can reduce environmental pollution, especially water pollution. The limitation of this study is that it only examined one herbal abate formula and only at concentrations of 1%, 2%, 3%, and 4%. Further research can be carried out so that the herbal abate of red dragon fruit peel can be even better in terms of formulation, concentration, and appearance and can be used as a larvicide on all mosquito larvae that act as disease vectors. This study has a limitation namely only examining the chemical compound content ethanol extract of red dragon fruit peel and proving the effectiveness ethanol extract of red dragon fruit peel against *Aedes aegypti* and *Culex* mosquito larvae by calculating the LC_{50} value.

Conclusions

The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, triterpenoids, and tannins in the red dragon fruit peel. However, the ethanol extract of red dragon fruit peel (*Hylocereus polyrhizus*) did not demonstrate effectiveness as a larvicide against *Aedes aegypti* larvae. Conversely, it was effective against *Culex* mosquito larvae, with an LC50 value of 19.06%. Future research should investigate the larvicidal efficacy of the ethanol extract of red dragon fruit peel on different mosquito species.

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