Larvicidal Activity of *Citrus aurantifolia* Decoction against *Aedes aegypti* Larvae

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Abstract

Background: Infected female *Aedes mosquito* is the primary vector of virus transmission for dengue hemorrhagic fever (DHF). Natural phytochemical larvicide is becoming a complementary way for vector control management. The citrus plant extract has natural chemical reactions against mosquito larvae. This study aimed to identify the larvicidal activity of *Citrus aurantifolia* leaves decoction against *larvae* of *Aedes aegypti* as an effort to discover natural phytochemical repellant.

Methods: This was an analytic experimental study using twenty-five *Aedes aegypti* larvae. The larvae were placed in translucent cups containing different concentrations of *C. aurantifolia* leaves decoction. The cups were filled with Abate as positive controls and water as negative controls. The experiment was repeated for three consecutive days, and the mortality of larvae was monitored for 48 hours as described in the World Health Organization (WHO) guidelines for laboratory and field testing for mosquito larvicides (WHOPES).

Results: *C.aurantifolia* decoction significantly decreased the number of larvae. The highest mortality was shown in 30% concentration with a total of 224 dead larvae. Probit analysis showed LC50 was 38.5% and 6.6% at 24 and 48 hours, respectively. The highest rate of killing the larvae was taken at LC60 with 91.6% for 24 hours and LC65 64.4% for the 48 hours; thus LC90 could not be determined. The significance of the decoction concentration was analyzed by one way ANOVA preceded with Post-hoc test (p-values 0.000).

Conclusions: Decoction of *C.aurantifolia* leaves has proved to have larvicidal activity against *larvae* of *Aedes aegypti* and could be used as phytochemical larvicides in controlling vector of DHF.

Keywords: *Aedes aegypti,* larvae*, Citrus aurantifolia,* dengue, fever

Introduction

Dengue fever is transmitted through a bite of infected female Aedes mosquito, the *Aedes aegypti*. This mosquito is the primary transmission vector for dengue virus infection.[1] The *Aedes mosquito* is also the main vector for several other diseases such as Yellow Fever and Chikungunya.[2] In year 2006, Indonesia reported quite a number of dengue cases in South East Asian region, with 57% contributing to the southern hemisphere countries.[3] Due to the increasing incidence rate of dengue, the Indonesian government has focused on vector controlling which can be directed by biological, chemical or environmental management with the usage of larvicides as a complementary way in eradicating dengue vector development according to the World Health Organization (WHO) guideline. One percent has been used for resistance management tool in mosquito abatement programs. However, it also causes toxicity in higher dosage due to overstimulation of the nervous system in humans.[1,4] Thus, alternative natural phytochemicals are preferred to encounter toxicity problems.

The previous study on citrus-derived essential oils has proved that citrus has natural elements effective against *Aedes mosquito* as larvicides.[5] *Citrus aurantifolia* is a citrus genus consisting of chemical coumarins such as phenolics, scopoletin, flavonoids and limonin, which are favorable as phytochemical

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larvicides.[6,7] Research in Thailand[8] has shown that *Citrus hystrix's* fruit peel has higher mortality compared to the plant against *Aedes aegypti* larvae. By using the decoction process, the extracted herbs which are boiled at 90^{0}C with a specific volume of water in 30 minutes is convenient for extracting heat-stable and water-soluble ingredients.[9] This procedure is the inexpensive and can be easily performed. However, citrus-derived leaves have not been proved to have higher mortality on *Aedes* larvae. This study aimed to identify the effectiveness of *Citrus aurantifolia* leaves in decoction form against larvae of *Aedes aegypti* and to determine the lethal concentration at 50% (LC50) and 90% (LC90) mortality as an effort to promote natural phytochemicals to eradicate dengue vector transmission.

Methods

This research was an analytic experimental study in accordance as described by WHO guidelines for laboratory and field testing of mosquito larvicides.[10] This study was conducted in the Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran in October to November 2012.

Leaves of key lime (*Citrus aurantifolia*) were collected from the Tanjung Sari market, Sumedang and identified at the Biology Herbarium, Universitas Padjadjaran. Leaves were weighed for 400g for each time repetition. *Aedes aegypti* larvae were obtained from the Department of Biology, Institute of Technology Bandung and were further identified at the Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran. The larvae eggs were bred until becoming Instar III or IV, and the larvae were fed with fish powder as its food source.

For the decoction process, fresh *Citrus aurantifolia* leaves (400g) was mixed with 400 mL water into the decoction equipment, designated as 100% concentration. The mixture was heated at 90^{o}C for 30 minutes, on an electric stove and stirred occasionally. Then, the solution was filtered and a certain amount of hot water was added until the final volume of 400 mL. This mixture was further diluted using distilled water. Concentrations were made for 15%, 30%, 45%, 60%, 75%, and 90%. For example, 15 mL of the prepared 100% concentration of decocting *Citrus aurantifolia* was mixed with 85 mL of distilled water to obtain 15% of concentration with 100 mL in total for each concentration.

Abate (Temephos 1%) was used as the control positive in this experiment and a solution of 100 mL Abate was prepared according to the Abate sachet procedure. As a control negative, 100 mL distilled water was used. Four replicates were prepared in containers for each concentration. Twenty-five actively motile larvae were inserted into each container and the mortality rate of larvae was recorded at an interval of 24 hours and 48 hours. The experiment was repeated in three different days with a minimal interval of one day. Room temperature was recorded as a control variable that might influence the experiment from the beginning until the end.

The significant difference of the *Citrus aurantifolia* leaves decocts concentrations were statistically analyzed by One-Way Analysis of Variance (ANOVA) which was a parametric method for numerical unpaired observation. The test was followed with a Post-hoc test. The statistical analysis was conducted using Statistical Product and Service Solutions (SPSS) for Windows (version 15.0). The LC50 and LC90 values were determined using Probit analysis.

Table 1 Cumulative Frequency and Percentage of the Mortality Rate of *Aedes Aegypti* Larvae in Different Concentrations of *Citrus aurantifolia* Decoct at 48 Hour Observation

	Replicate	Control negative	Concentration of *C.auratifolia*						Control Positive
			15%	30%	45%	60%	75%	90%	
Cumulative frequency	1	1	45	61	44	44	51	50	75
	2	-	35	59	41	48	47	51	75
	3	1	38	52	42	45	47	59	75
	4	-	33	52	45	37	48	54	75
% mortality rate		0.6	50.3	74.6	57.3	58.0	64.3	71.3	100

Note. The number of dead larvae

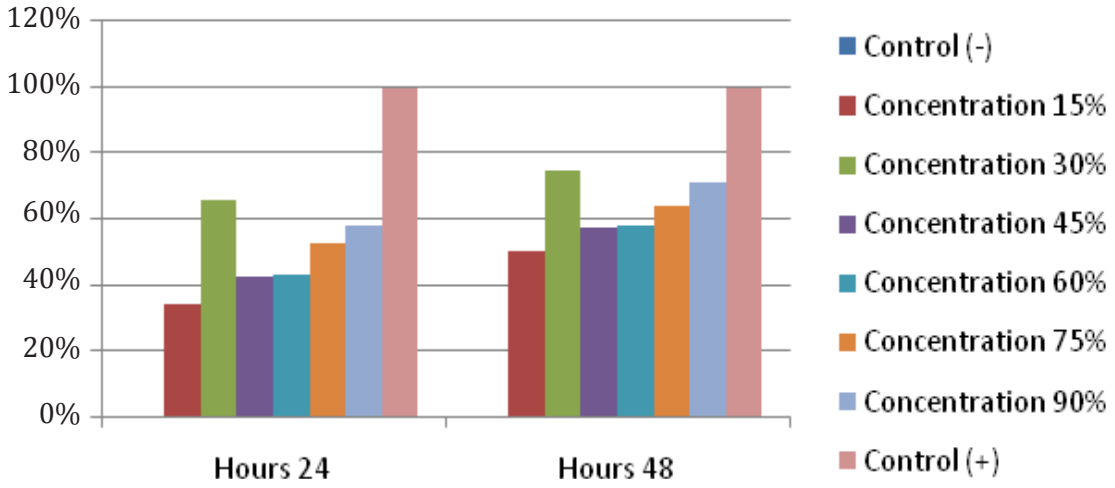

Figure 1 Percentage of mortality rates of Aedes aegypti at 24 hours and 48 hours of Observation

Results

The highest mortality rate was shown at the concentration of 30% with a total of 224 dead larvae; while control negative had shown 2 dead larvae and control positive with complete 100% mortality rate with a total of 300 dead larvae (Table 1). The highest mortality rate of *Aedes aegypti* larvae of the *Citrus aurantifolia* leaves decoction concentrations was shown the highest rate at 30% with 65.6% on the 24 hours of observation and 74.6% on the 48 hours of observation; whereas the lowest mortality rate was shown by 15% concentration of *Citrus aurantifolia* with 34.3% at 24 hours of observation and 50.3% on the 48 hours of observation. Distilled water has 0.6% and Abate with 100% mortality. The higher killing power was shown by decocts of *Citrus aurantifolia* at 30% concentration, and there was no difference mortality rate that was observed in 24 hours or 48 hours (Figure 1).

ANOVA and Post-hoc tests were exerted to identify the effect of *Citrus aurantifolia* leaves decoction at various concentrations with the mortality of *Aedes aegypti* larvae. When comparing the number of dead larvae's in various concentrations of *Citrus aurantifolia* leaves and distilled water, it showed that even concentration of 15% had a significantly high number of dead larvae (p-value 0.000), suggesting that decoction of *Citrus aurantifolia* leaves has good larvicidal activity on *Aedes aegypti* larvae.

Table 2 The Comparison between the Various Concentration of *Citrus aurantifolia* and Distilled Water

Concentration	No. dead larvae (n=300)	P-value†
Distilled water*	0.5±0.57	
15%	37.75±6.95	0.000
30%	56±2.94	0.000
45%	43±2.00	0.000
60%	43.5±6.76	0.000
75%	48.25±2.50	0.000
90%	53.5±4.20	0.000
Abate **	75	

Note: * negative control ** positive control †One way ANOVA, Post-hoc test compared to distilled water as well as abate

Table 3 Probit Analysis Result at 24 Hour and 48 Hour for Each Concentration

Lethal Concentration (LC)	Percentage (%)	
	24 h	48 h
0.01	.014	.000
0.05	.140	.000
0.1	.483	.003
0.2	2.173	.046
0.3	6.423	.299
0.4	16.219	1.483
0.5	38.547	6.613
0.6	91.617	29.494
0.65	143.824	64.269
0.7	231.330	146.043
0.8	683.921	949.644
0.9	3075.295	12739.750
0.95	10642.584	1.087E5
0.99	1.093E5	6.069E6

The same result was shown when the various concentrations of *Citrus aurantifolia* leaves were compared to positive controls; there were statistically significant differences in the effectiveness of decoction of *Citrus aurantifolia* leaves and Abate. The median differences between *Citrus aurantifolia* decoction and Abate showed that Abate had a higher median. It indicated that Abate was the most effective in killing *Aedes aegypti* larvae. This study also revealed larvicidal activity of decoction form of *Citrus aurantifolia* leaves against larvae of *Aedes aegypti* within 48 hours observation.

Based on Probit analysis the chances of killing 50% of *Aedes aegypti* larvae were achieved at a concentration of 38.5% for 24 hours and 6.6% for the 48 hours of observation. However, the LC90 could not be defined. This was probably due to the highest lethal concentration taken by decoct form of *Citrus aurantifolia* leaves in killing *Aedes aegypti* larvae was at LC60 with 91.6% and at LC65 was 64.4% for 24 hours and 48 hours of observation, respectively (Table 3).

Discussion

This study has identified the larvicidal activity of *Citrus aurantifolia* leaves decoction against larvae of *Aedes aegypti* as an effort to discover natural phytochemicals. The results showed that *C.aurantifolia* decoction significantly decreased the number of larvae. The highest mortality has proved that 30% concentration of *Citrus aurantifolia* decoction was effective in killing almost 75% of the larvae as also shown with the 90% concentration. According to a study done in India[11], the higher the concentration, the higher the mortality rate achieved by *Citrus sinensis*, a species of Genus Citrus. However, our result did not show the linear similarities by *Citrus aurantifolia*, indicating that there might be other factors that could have influenced the result of the experiment. Minor trauma would have occurred while handling the transfer of actively motile *Aedes aegypti* larvae into containers, or there might be changes of the chemical mixture while preparing the needed concentrations. Other possible contributing factors could be due to the lack of photo period of 12 hours light and 12 hours dark as recommended by WHO guidelines.[10]

Table 1 has shown 0.6% mortality in the negative control, which is still acceptable according to WHOPES guidelines of less than 5% mortality, LC50 have shown 38.5% which is 385,470 ppm at 24-hour observation and 6.6% which is 66,130 ppm. Furthermore, LC90 could not be defined due to the highest killing effect of *Citrus aurantifolia* decoction, therefore, LC60 has been taken with 91.6% which is 916,170 ppm for 24 hours and LC65

64.4% which is 644,940 ppm at 48 hours. Study in Nigeria[12] has proved that Citrus species, Citrus sinensis has lower ppm with LC50 0.4 ppm and LC90 with 0.9 ppm using ethanolic extraction method against *Aedes aegypti* larvae. Another study showed that *Citrus limonia* and *Citrus Sinensis* fruit peels essential oils have high mortality against *Aedes aegypti* larvae with lower ppm, suggested that alcohol extract of used herbs contained more chemical components compared with decoction method.[13,14] In line to those studies, the chemical content of Citrus-derived plant is better in killing the *Aedes* larvae by the ethanolic extraction method compared to the decoction method as the decoction method has weakness in extracting chemical constituent of ingredients or herbs being used.

In conclusion, *Citrus aurantifolia* leaves have larvicidal activity against *Aedes aegypti* larvae. Further study should be conducted in the narrower range to determine larvae mortality at LC90. Government and health authorities should encourage the usage of *Citrus aurantifolia* leaves as a bio-insecticide for vector controlling against dengue hemorrhagic fever. Further research is suggested on ethanolic extractions or essential derived oil from *Citrus aurantifolia* leaves against *Aedes aegypti* as larvicides or mosquito repellent.

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