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Original papers

In-vitro study on the larvicidal activity of *Manihot glaziovii* peel extract against *Aedes aegypti* larvae

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ABSTRACT. Use of chemical method to control *Aedes aegypti* population in the Dengue endemic areas caused the emergence of a resistant strain of this species to insecticide compounds, including temephos. Exploration of the alternative compounds that have larvicidal effects is needed, such as natural products derivate from plants. The current study was designed to evaluate the effective dosage of methanolic extract of *Manihot glaziovii* peel against the third instar larvae of *Aedes aegypti*. Bioassay test was performed based on the WHO procedure to occupy the 24 hours exposure of five levels dosages of *Manihot glaziovii* peel extract, namely 1,800, 2,400, 3,000, 3,600 and 4,200 ppm respectively. As many as 25 of healthy *Aedes aegypti* larvae were subjected into each group, five-time replicated and compared with tap water control group. The mortality rate of *Aedes aegypti* larvae has increased due to the increase in treatment groups dosages, ranged from 44–97.6%. It is significantly different in larvae mortality rate between different treatment groups. There was no observation of killed larvae in the control group. LD_{50} and LD_{90} of methanolic extract of *Manihot glaziovii* peel were measured 2,027 ppm and 3,772 ppm, respectively. This result suggests the possible use as an ecofriendly larvicide against *Aedes aegypti* larvae. Further investigation is needed to obtain secondary metabolites of this plant.

Keywords: *Aedes aegypti*, bitter cassava, larvicidal activity, *Manihot glaziovii*, peel extract

Introduction

Cassava (Euphorbiaceae) is one of common horticulture plant found in Indonesia, mainly in Java island [1]. The most common species of this plant are *Manihot utlilisima* Pohl, *Manihot esculenta* Crantz, and *Manihot carthaginensis* subspecies *glaziovii* (called *Manihot glaziovii*) Müll. Arg. The two of *Manihot* genus were grouped into the close-related species [2] that called the bitter cassava, namely *Manihot esculenta* and *Manihot glaziovii*. *Manihot glaziovii* has a toxic effect on animal and human because it contains a chemical compound that has various enzymatic and inhibitory activities [3]. The leaves and leathers of *Manihot glaziovii* have a toxic effect when fed to cattle [4]. The previous study reported that another species from Manihot genus, *Manihot utilissima* Pohl has a toxic effect against *Aedes aegypti* larvae [5]. The toxic effect of *Manihot glaziovii* must be compared with the other cassava species.

Reducing the *Aedes aegypti* population is the principal effort for preventing Dengue virus transmission in the Dengue endemic areas by implementing the biological, physical and chemical methods [6]. Unfortunately, the community in these areas prefer to use chemical methods, mainly fogging [7]. This control measure is often done inappropriately by the community without skilled personnel. The frequent of inappropriate insecticide pressure for a long time caused the emergence of resistant strain in *Aedes aegypti* populations [8,9].

Studies in many countries report that *Aedes aegypti* has been resistant to several insecticidal compounds such as deltamethrin, cypermethrin, permethrin, and malathion [9–17]. *Aedes aegypti* larvae were resistant to temephos in several countries, namely Cabo Verde [10], India [18], Brazil [11,19–21], Pakistan [22], and Colombia [23]. In Indonesia, the resistance of *Aedes aegypti* larvae to temephos was reported from Jakarta [24], West Java [25,26], Surabaya [27], Semarang [28],

and Southeast Sulawesi [29]. This condition shows that the chemical methods are not effective to control of Dengue vectors including a high density of *Aedes aegypti* population in the Dengue endemic areas [30].

Development of alternative methods is an important point to control of Dengue vectors, especially based on the natural or herbal chemical compounds. Previous studies show that several local herbal extracts have different values of larvacidal activity [31–33], such as *Manihot utilissima* Pohl [5]. The ethanolic extract of *Manihot utilissima* leaf has a lethal effect on *Aedes aegypti* larvae with LC90 of 2.613%. This cassava species contains saponin and flavonoid [5] and cyanide acid compounds [4]. *Manihot glaziovii* is believed to contain higher concentrations of the chemical compounds [34] that can cause respiratory disorders in mammals including humans [35]. Other plant species containing saponin have a toxic effect against *Aedes aegypti* larvae too. The chemical compounds enter through the digestive system of larvae and make their death via inhibiting acetylcholinesterase, increasing AMP cyclic, disorganization of epithelial midgut cells, and disturbing co-transport of Na^+, Cl^-, and K^+ ions [36]. Peel of *Manihot glaziovii* contains several chemical compounds, including cyanide acid [34], so that the part is suggested to be studied. This laboratory study was designed to evaluate the larvicidal activity and effective dosage of methanolic extract of *Manihot glaziovii* peel against the third instar larvae of *Aedes aegypti*.

Materials and Methods

Species determination and extraction of *Manihot glaziovii*. As many as fifteen kilograms of *Manihot glaziovii* peel were obtained from cassava farmers in Kandangmas village of Kudus district, Central Java Province, Indonesia on March 2018. Species determination of the bitter cassava was done in Plant Taxonomy Laboratory, Department of Biology, Mathematical and Sciences Faculty of Universitas Padjadjaran, Bandung. The bitter cassava peel extraction by using the polar solvent, methanol was done in Natural Material Laboratory of Mathematical and Sciences Faculty, University of Jenderal Ahmad Yani, Bandung, Indonesia based on the previous study [5,34].

***Aedes aegypti* larvae and bioassay test.** Larvae were reared in Epidemiology Laboratory of Public Health Faculty, Universitas Muhammadiyah Semarang. Larvae were hatched from F7 of insecticide-susceptible *Aedes aegypti* eggs that obtained from Rowosari village of Semarang municipality, Central Java Province, Indonesia. Larvae were maintained at the air temperature and humidity of 28°C and 75%. Preliminary bioassay test was performed based on the previous study [5] with slight modification in a concentration range, namely 0.1 to 0.5 percent (1,000 – 5,000 ppm), and resulted in mortality rate 12–100 percent. Based on the results, we arranged the new range concentration namely 1,800, 2,400, 3,000, 3,600, and 4,200 ppm respectively in 100 ml aqua dest solution. Bioassay test was performed based on the WHO procedure [37]. Briefly, five to six days old and healthy larvae were subjected to those five different concentration groups of *Manihot glaziovii* peel extract. Twenty-five *Aedes aegypti* larvae were entered to each treatment group for twenty-four hours. Two (positive and negative) controls groups of the experiment were 0.02 ppm of temephos and aqua dest, respectively. Each control group consists of twenty-five *Aedes aegypti* larvae. Each experiment group was performed into five times replication. The knockdown larvae were observed after 30, 60, 120, 480 and 1,440, and the mortality rate was counted after 1,440 minutes contacted with the solution treatment. The effective concentration of *Manihot glaziovii* peel extract was determined with the lethal doses (LD) 50 and 90 that represented 50% and 90% of mortality rate by using the probit analysis [38].

Data analysis. Data analysis was used SPSS (version 11.5) software and expressed as the mean and percentage. One-way ANOVA was used to compare mean differences between groups of concentration with significant value was $p \leq 0.05$.

Ethical consideration. Ethical review and approval were obtained from the Health Research Ethics Commission of Faculty of Public Health, Universitas Muhammadiyah Semarang.

Results

The number of knockdown of *Aedes aegypti* larvae in each treatment level showed an increase over the observation period. Increasing the concentration level of treatment followed by an increase in the percentage of knockdown larvae ranged of 44–97.6%. A similar phenomenon was observed in the positive control group (Temephos

Table 1. The trend of knockdown larvae during the exposure time of methanolic extract of *Manihot glaziovii* peel

Concentration (ppm)	Replication	Total larvae	Knockdown larvae based on observation time (minutes)				
			30	60	120	480	1440
1,800	1	25	0	0	0	0	11
	2	25	0	1	1	3	10
	3	25	1	2	2	4	11
	4	25	0	0	1	2	9
	5	25	0	1	1	3	14
Mean			0,2	0,8	1,0	2,4	11,0
Percentage			0.8	3.2	4.0	9.6	44.0
2,400	1	25	0	0	1	2	13
	2	25	1	2	1	3	19
	3	25	0	0	1	3	13
	4	25	1	1	1	4	16
	5	25	0	1	1	6	17
Mean			0,4	0,8	1,0	3,6	15,6
Percentage			1.6	3.2	4.0	14.4	62.4
3,000	1	25	0	1	1	3	20
	2	25	0	1	1	5	19
	3	25	0	1	1	4	20
	4	25	1	1	1	5	19
	5	25	1	1	2	3	21
Mean			0,4	1,0	1,2	4,0	19,8
Percentage			1.6	4.0	4.8	16.0	79.2
3,600	1	25	1	2	5	10	24
	2	25	2	3	3	8	23
	3	25	1	1	2	7	22
	4	25	1	1	4	10	24
	5	25	1	1	4	11	23
Mean			1,2	1,6	3,6	9,2	23,2
Percentage			4.8	6.4	14.4	36.8	92.8
4,200	1	25	1	1	4	12	24
	2	25	1	1	5	12	25
	3	25	1	2	4	14	24
	4	25	1	1	3	13	24
	5	25	1	3	5	10	25
Mean			1,0	1,6	4,2	12,2	24,4
Percentage			4.0	6.4	16.8	48.8	97.6
Positive control *	25		6	22	25	25	25
Negative control#	25		0	0	0	0	0

*) Temephos 0.02 ppm #) Aquadest

The number of knockdown larvae increases progressively according to the observation period; shows the effect of exposure period on knockdown larvae. This condition occurs gradually where the higher exposure dose is followed by a higher number of knockdown larvae in each observation period.

0.02 ppm) and reached the peak of the knockdown number of larvae at the 4th hour of observation. There were no knockdown larvae observed in the negative control group (Table 1). Larvae mortality rates after 24 hours of exposure period showed an increasing trend with increasing levels of treatment concentration. Larvae mortality rates in the positive control group reached 100%, and no dead larvae were found in the negative control group (Table 2). Overall, there was a significant difference in larval mortality rate based on a treatment level, except for couples of concentrations of 2,400 with 3,000 (b),

Table 2. The mortality rate of *Aedes aegypti* larvae after twenty-four hours exposure of a methanolic extract of *Manihot glaziovii* peel

Concentration (ppm)	Total tested larvae	Killed larvae after 24 hours exposure			
		Minimum	Maximum	Average	Percentage
1,800	25	9	14	11.0	44.0
2,400	25	13	19	15.6	62.4
3,000	25	19	21	19.8	79.2
3,600	25	22	24	23.2	92.8
4,200	25	24	25	24.4	97.6
Positive control*	25	25	25	25.0	25.0
Negative control#	25	0	0	0.0	0.0

*) Temephos 0.02 ppm #) Aquadest

The results of observations after 24 hours of exposure showed the mortality rate of larvae increased with the level of exposure to the dose. There were no dead larvae in the negative control, and 100 percent of the larvae died in positive control.

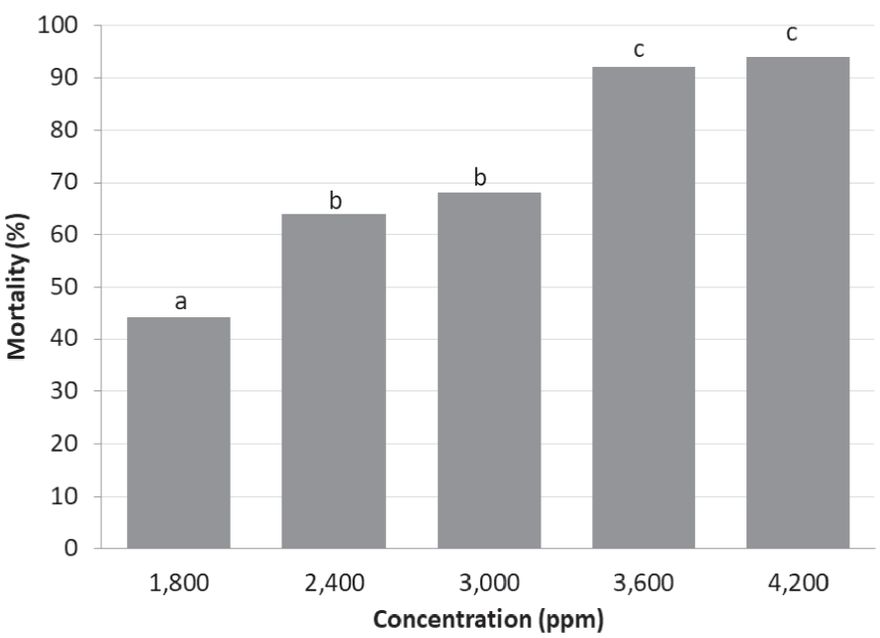

Fig. 1. The difference of mortality rate of *Aedes aegypti* larvae after 24 hours exposure of a methanolic extract of *Manihot glaziovii* peel. The mortality rate of the larvae showed by alphabetic symbol above the shape.

and 3,600 with 4,200 (c) ppm (Fig. 1). Effective concentrations (LC50 and LC90) of methanolic extract of *Manihot glaziovii* peel against *Aedes aegypti* larvae were achieved at 2,104 and 3,446 ppm (Table 3).

Table 3. Result of probit analysis of LD50 and LD90

Lethal Dosage	Concentration (ppm)		
	Mean	Lower bound	Upper bound
LD50	2,104	1,749.459	2,378.990
LD90	3,446	3,119.361	3,953.634

Discussion

Result of this study shows that the peel extract of *Manihot glaziovii* has lethal activity against *Aedes aegypti* larvae indicating by the high mortality rate. The lethal doses of the extract are equal to the lethal concentration (LD_{50} and LD_{90}) 0.2104% and 0.3446%, respectively. These lethal doses of *Manihot glaziovii* peel extract are lower than the flower extract of this bitter cassava when applied to *Apis mellifera*, namely 0.5 to one percent. The results indicate that the bitter cassava peel extract

has higher larvicidal potency, and to be more effective for *Aedes aegypti* larvae rather than the bee species [3]. Allegedly, bees have a higher expression of esterase enzyme that supports their survival rather than mosquitoes [39].

Manihot glaziovii is classified in one family with *Croton nepetaefolius* and *C. zehntneri.* Results of the current study show that *Manihot glaziovii* peel extract is more effective compared to *Croton nepetaefolius* and *C. zehntneri* extract with 100% mortality of *Aedes aegypti* larvae in the concentration 8.3% and 7.8%, respectively. It means that the lethal effect of *Manihot glaziovii* peel extract against *Aedes aegypti* larvae are 22 and 21 times higher than the steam distillation extract of both *Croton* species [40]. Although this toxicity is lower than Temephos 0.02 ppm, this finding shows that the *Manihot glaziovii* peel extract has a good larvicidal potency.

The mortality rate of *Aedes aegypti* larvae after exposure by *Manihot glaziovii* peel extract is caused due to the presence of chemical compounds, such as saponin, linamarin and hydrogen cyanides [34]. Toxicity of hydrogen cyanide in mosquito larvae may have similar mechanisms with another animal describing in the previous study. Briefly, the compound will inhibit the activity of cytochrome oxidase enzyme causing the decrease of oxygen utility in the tissues, and resulting general cell hypoxia including the nervous system, and death [35].

This study used the methanolic extract of *Manihot glaziovii* peel. The use of high polar solvent, methanol in *Manihot glaziovii* peel extraction results the polar chemical properties such as phenolic acids, phenylpropanoids, polar-flavonoids, glycosides, and alkaloids. On the other hand, some phytochemical groups may be isolated using semi-polar and non-polar solvents such as tannins, terpenoids, and semi-polar and non-polar flavonoids [41]. However, this study has not been able to explore the other kind of phytochemicals by using the other solvents, and determine the kind of phytochemical which has high toxicity to the *Aedes aegypti* larvae, so that the further research is needed.

Based on the chemical compounds contained, *Manihot glaziovii* peel is classified as hazardous organic waste to the environment and animals. This waste is produced in large amount annually in cassava farm in rural regions of the tropics [42]. Cassava production in Indonesia was more than twenty-two million ton in 2015 [43]. This data is related to the quantity of waste production post-harvest processes, especially cassava peel. The percentage weight and phytochemical content of cassava peel are eleven percent of the tuber and 15 to 400 ppm cyanogenic glycoside, respectively [42,44]. When converted from cassava production data per year [43], the production of cassava peel waste in Indonesia is estimated to reach around 2.42 million tons a year. This data shows a large amount of the toxic of organic waste disposal that can adversely affect human, environment, and animals.

Some other studies found that the ethanolic extract of raw tuber cassava contains several phytochemical compounds such as alkaloid, flavonoid, tannin, [45], and beside of those compounds, cassava leaves also contain saponin and cyanogenic glycoside [46]. Cyanogenic glycoside produces cyanide acid, one of the phytochemical compounds that have high toxicity effect to animals, mainly livestock species [47]. The use of *Manihot glaziovii* peel has public health importance in reducing one of the environmental pollution sources and producing the bioactive compounds that are useful for larvicidal ingredients. Indonesia has large scale cassava farming land on thousands of islands. This condition indicates that the production of waste cassava peels also occurs widely and continues, and ensures the availability of raw materials for the isolation of various phytochemicals contained [41].

In conclusion, the *Manihot glaziovii* peel extract has larvacidal potency to *Aedes aegypti* larvae with low effective dosage. Further investigation is needed to understand its larvicidal effect to the field caught and the insecticide-resistant *Aedes aegypti* larvae, especially to cypermethrin, malathion dan temephos compounds. The other extraction solvents are also important to be studied in determining the best solvent for extraction the bitter cassava peel. The specific chemical compounds are important to be isolated and quantified from bitter cassava peel in obtaining the specific herbal bioactive compound for larvicide formula.

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