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The potentials of pangi leaf extract for *Aedes* spp. mosquito control

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ABSTRACT

Aedes spp. are the primary vectors of arboviruses such as dengue, Zika, and chikungunya. To date, neither an approved vaccine nor a drug that can definitively prevent or treat these diseases. Vector control continues to be an essential method of disease prevention. Plant-based insecticides are an alternative to chemical insecticides because they are less toxic to non-target insects and degrade more quickly. The Papuan people have used Pangi leaves for generations as a potent natural chemical against head lice and mosquito larvae. This study aimed to evaluate the potential of pangi (*Pangium edule*) leaf extract as a biolarvicide and mosquito repellent. Fresh pangi leaves were extracted with water by using a blender to pulverize the leaves. One-way ANOVA was used to examine the variance in mortality rates, and the LC_{50} value was calculated for probit analysis. Dead larvae were identified and counted. In addition to testing the effectiveness of the extracts as biolarvicide, the extract was also tested as potential insect repellents. The findings demonstrated that pangi leaf extract can kill mosquito larvae. The concentration of the extract affected larval mortality. Furthermore, the extract demonstrated its effectiveness as an insect repellent. Pangi leaf extract contains alkaloids, flavonoids, tannins, saponins, phenolic compounds, and cyanide compounds, with alkaloids being the most abundant. The study found that there was a direct correlation between the concentration of the insecticide and the number of dead *Aedes* spp. larvae. The LC_{50} probit test revealed that pangi leaf extract belongs to the category of toxic substances. Several compounds, including alkaloids, flavonoids, tannins, saponins, and cyanide, may be responsible for the toxicity of pangi leaf extract. Thus, pangi leaf extract might be established as a means of overcoming numerous health issues caused by mosquito vectors.

INTRODUCTION

Dengue fever is currently the most prevalent virus transmitted by mosquitoes in Indonesia. It is an acute febrile illness caused by the dengue virus (DENV) and transmitted by the *Aedes* spp. mosquito vector [1]. *Aedes aegypti* and *Ae. albopictus* are two members of the genus *Aedes* that serve as important disease vectors in Indonesia [2]. One of the pathogens transmitted by *Aedes spp*. mosquitoes is a flavivirus, whose members include the yellow fever virus, dengue virus, and Zika virus [3]. The dengue virus is a member of the flavivirus family that infects people and contributes to the world's most serious health issues in terms of the quantity of patients and quality of facilities it demands [4]. Dengue virus can cause both primary and secondary infections. Primary infection occurs when acute fever or dengue fever subsides approximately 7 days after the formation of a complex immune system, whereas secondary infection is more severe and causes dengue shock syndrome [5].

Controlling mosquito vectors is crucial for preventing flavivirus transmission. Widely used vector control today is derived from synthetic materials that, when used for

extended periods of time, can cause mosquito resistance, human health problems, and environmental pollution [6]. Previous research has demonstrated that the use of synthetic insecticides, such as coil smoke, can pose serious health risks [7].

Eliminating mosquito larvae with the help of natural substances derived from plants is a risk-free alternative method for controlling disease vectors. Plant extracts with their enhanced phytochemical constituents have the potential to replace conventional mosquito larvicides and repellents [8]. Numerous previous studies have demonstrated the efficacy of plants as larvicides. For instance, crude leaf extracts of *Acanthospermum hispidum* were effective against *Anopheles stephensi*, *Ae. aegypti*, and *Culex quinquefasciatus* [9].

Pangi (*Pangium edule*) is one example of a plant species that possesses the potential to act as a natural larvicide. The development of this natural larvicide has attracted the interest of numerous researchers. Aside from being consumed as vegetable [10], the Papuan people have used pangi leaves for eradicating head lice and mosquito larvae. This practice has been carried out for many generations. Therefore, researchers are strongly encouraged to conduct in-depth testing of the larvicide-potential components of the Pangi plant, and the doses required to kill mosquito larvae. So that it can later be recognized by the community as a means of overcoming numerous health issues caused by mosquito vectors.

MATERIALS AND METHODS

This research was conducted in Maybrat Regency, West Papua Province, Indonesia, in collaboration with the Regional Health Laboratory of Sorong, Indonesia, to identify mosquito larvae using specific criteria.

Pangi leave extract preparation

Pangi leaves are sliced and crushed in water using a blender mixture until smooth, after which they are filtered and placed in a container. The filtrate was then used as a test material after being subjected to several different dilutions. The concentrations used were 5%, 10%, 25%, 50%, and 75%.

Preparation of mosquito larvae and adults

Oviposition traps were installed inside the house, next to the door, and next to the window. After a few days, the mosquito larvae were found inside the traps, which were then transferred into collection containers. These larvae were collected for the larvicide test's mortality evaluation. In addition, the remaining larvae were allowed to develop into adults and were used as specimens for repellent tests. Each container was equipped with a mosquito net to prevent mosquitoes from escaping.

Evaluation of pangi leaf extract as a larvicide

Containers containing pangi leaf extracts of varying concentrations (5%, 10%, 25%, 50%, and 75%) were placed in the home and yard. To serve as a control, distilled water was used instead of the extract. Observations were made by calculating the number and percentage of larval mortality within each container after 24th hours with four repetitions, referring to the Federer formula as follows:

$$p(n-1) \geq 16$$

where p is the number of experiment and n is the number of repetitions. The results were analysis statistically using analysis of variance (ANOVA) test and Probit LC_{50}. At a *p*-value less than 0.05, the results were considered to be significant [11]. The level of toxicity of an extract is as follows: $LC5_0$ 30 mg/l = very toxic; LC_{50} 1,000 mg/l = toxic; LC_{50} > 1000 mg/l = non-toxic [12].

Evaluation of pangi leaf extract as a mosquito repellent

The effectiveness of the repellent was evaluated using adult mosquitoes. The mosquitoes were placed in cages covered with mosquito nets. Pangi leaf extracts of varying concentrations (5%, 10%, 25%, 50%, and 75%) were used in this evaluation. Each concentration was applied to the surface of the subject tests. The tests were conducted by inserting the subject's arm alternately into the treated and control (untreated) mosquito cages and counting the number of mosquitoes that landed every 10 minutes, 20 minutes, 30 minutes, and 60 minutes. Before the repellent test, the research subjects provided informed consent indicating their willingness to participate in the study.

Phytochemical analysis of the pangi leaf extract

The pangi leaf extract was subjected to phytochemical analysis, which included testing for the presence of cyanide, alkaloids, flavonoids, tannins, saponins, and phenolics. The pangi leaves are chopped into small pieces and then dried for two to three days at 50^0C. Approximately 10 grams of dried leaves are pulverized at maximum speed in a blender. After adding 100 ml of distillated water, the mixture was blended again. The filtered results were then analyzed phytochemically. The analysis was carried out by following the previous methods [11,13,14].

RESULTS

Effect of concentration of pangi leaf extract on mosquito larvae survival

Table 1 displays the findings of an experiment that was conducted to determine the mortality rate of mosquito larvae exposed to various concentrations of solutions over the course of four separate trials. At a concentration of 5%, only 0.5 larvae died on average, with a mortality percentage of 2.5%. The highest mortality percentage (42.5%) was observed at 75% extract concentration.

The relationship between mosquito larval mortality and the concentration of pangi leaf extract is displayed in Table 2. The results demonstrated that the average mortality of mosquito larvae was 14, with a standard deviation of 15.04, the lowest mortality being 2, and the highest mortality being 75. Meanwhile, the average concentration of pangi leaf extract was 30% with a standard deviation of 31.02, with 5% being the lowest and 75% being the highest.

The investigation continued with probit analysis and ANOVA tests. To demonstrate the toxicity of pangi leaf extract, a probit analysis was administered. At each concentration of pangi leaf extract, an ANOVA test was performed to determine if there were differences in the mortality of larvae. Table 3 displays the results of the probit test, while Table 4 displays the correlation test results. The results of the probit test indicated that the LC_{50} of pangi leaf extract was 95.39, with an interval of 70-179. According to the

results of statistical tests using the ANOVA, the value of p in Table 4 is 0.027 (p<0.05). Thus, it can be concluded that the mortality of larvae differs depending on the concentration of pangi leaf extract. The greater the concentration of pangi leaf extract, the greater the larval mortality.

Table 1. Mortality of mosquito larvae at various concentrations of extract.

Extract concentration	Replication	Number of larvae observed	Number of dead larvae	Average	% Mortality
0%	0	20	0	0	0
5%	1	20	0	0.02	2
	2	20	1		
	3	20	1		
	4	20	0		
10%	1	20	0	0.02	2
	2	20	1		
	3	20	0		
	4	20	1		
25%	1	20	1	0.07	7
	2	20	1		
	3	20	2		
	4	20	3		
50%	1	20	2	0.27	27
	2	20	3		
	3	20	8		
	4	20	14		
75%	1	20	4	0.34	34
	2	20	5		
	3	20	10		
	4	20	15		

Table 2. Mortality of mosquito larvae in relation to the concentration of pangi leaf extract.

Variable	Mean	Standard deviation	Minimum - maximum	95% CI*
Mortality of mosquito larvae	14	15.04	2.34	-4.28-33.08
Concentration of pangi leaf	30	31.02	5-75	-8.52-68.52

*CI = confidence interval

Table 3. Results of probit LC_{50} analysis.

Concentration (%)	Number of larvae observed	Number of dead larvae	*LC_{50}
0	20	0	
5	20	2	
10	20	2	95.39(70-179)
25	20	7	
50	20	27	
75	20	34	

*LC_{50} = the concentration of the extract required to kill 50% of the mosquito larvae.

Table 4. Results of ANOVA test.

Concentration (%)	Mean	Standard deviation	95%CI	*p*-value*
5	24.7	2.49	2.29-2.64	0.027
10	24.7	2.49	2.29-2.64	
25	27.2	2.41	3.56-28.89	
50	343	27	32-35	
75	376	38	35-40	

*The obtained values were considered significant at $p \leq 0.05$

Effect of pangi leaf extract as mosquito repelent

Table 5 shows the results of the evaluation of various concentrations of pangi leaf extract as a mosquito repellent. According to the findings, the higher the concentration of pangi leaf extract, the fewer mosquitos perched.

Table 5. The number of mosquitoes that landed on the arms of volunteers after applying pangi leaf extract at varying concentrations.

Time (minute)	0%	5%	10%	25%	50%	75%
10	3	2	1	0	0	0
20	25	8	5	2	0	0
30	38	15	10	6	3	1
60	45	22	18	10	5	2

Results of phytochemical analysis of pangi leaf extract

Table 6 displays the phytochemical analysis results of the pangi leaf extract. Alkaloids, flavonoids, tannins, saponins, phenolic compounds, and cyanide compounds are all found in the extract of pangi leaf. Pangi leaf contains a greater concentration of alkaloids than other compounds.

Table 6. Qualitative phytochemical test results from pangi leaf extract.

Group of compounds	Results	Color change
Alkaloids	+++	Dragendorf: orange
		Wagner: brown
		Meyer: white precipitate
Flavonoids	+	Red
Tannins	+	Green
Saponins	+	Bubble/foam
Phenolics	+	Brown, orange
Cyanide	+	Brown, orange

DISCUSSION

Female mosquitoes are anthropophilic because they prefer the blood of humans over that of animals [15]. Blood proteins provide the amino acids necessary for the synthesis of vitellogenin, which is essential for egg production [16]. *Aedes* species are known to repeatedly bite their hosts until their stomachs are completely full of blood. Therefore, these mosquitoes have the high potential to act as disease-transmitting vectors.

Some plants have been shown to have the potential to act as natural larvicides, as was demonstrated by the findings of previous studies. There are approximately 1,200 plant

species that have potential insecticidal value, and there are 344 plant species that have only shown activity against mosquitoes [17].

The ANOVA results of this study's research using pangi leaf extract showed a significance value of 0.027, indicating that there are differences in the mortality of *Aedes* mosquito larvae at each concentration. The group of toxic compounds found in Pangi leaf extract is evidence that the extract has larvicide potential. The higher the concentration of pangi extract solution, the higher the mortality of larvae.

The phytochemical analysis of pangi leaf extract revealed the presence of alkaloids, flavonoids, tannins, saponics, phenolics, and cyanides, with the alkaloid content being the most abundant. It has been previously reported that the alkaloid group has larvicidal activity [18,19]. In addition, saponin compounds can irritate the mucosa lining of the digestive tract, rendering them toxic to mosquito larvae. Additionally, saponin compounds can damage the cell membranes of mosquito larvae [20].

The relationship that exists between the levels of chemical substances and the effects that they cause is the aspect of a compound that is most responsible for determining whether or not it poses a risk to human health or not. The results of cyanide testing performed on pangi leaf extract with water serving as the solvent in the first minute showed 6 ppm, while after 1x24 hours there was only 2.22 ppm remaining. This occurs as a result of cyanide's physical properties of being water-soluble and volatile. Young leaves of the pangi plant were reported to have a higher cyanide acid content than the old leaves [21]. The amount of cyanide in the pangi leaf extract is also affected by the testing duration. The longer the required time, the quicker cyanide will evaporate. It has been reported that cyanide has larvicidal properties [22]. Cyanide can bind to cytochrome oxidase, an enzyme that functions in the electron transport chain [23], thereby causing mosquito larvae to die. The Food and Agriculture Organization of the United Nations recommends a safe limit of 10 ppm for cyanide consumption [24]. If consumed or inhaled in low concentrations, the compound can cause headaches, dizziness, nausea, and vomiting. At higher concentrations, approximately 50 to 60 mg, cyanide acid can induce paralysis or death.

CONCLUSIONS

Pangi leaf extract has the potential to be a natural larvicide, the higher the concentration, the greater the mortality of the larvae. Pangi leaf extract may also be useful as a natural repellent. Nonetheless, it is suggested that further research be conducted with larger *Aedes* spp. samples. Furthermore, multiple solvents should be considered in the extraction of pangi leaves.

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AUTHOR CONTRIBUTIONS

YK and JMEM were involved in conception and design of the experiments. YK and NK contributed to perform the experiments. YK and NK analyzed data. YK, MT, JSBT, TET contributed to drafting the article. JMEM and TET contributed to revising it critically for important intellectual content. TET made the final approval of the version to be published.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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