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Bio-insecticide's Extract of Scented Root (*Polygala paniculata*) in Controlling the Mosquito *Aedes aegypti* (L.)

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Abstract

Controlling *Aedes aegypti* mosquitoes with chemical insecticides causes resistance on humans, environmental residues, and contaminates food and water. *Polygala paniculata* is a potential alternative to insecticides in controlling *Ae. aegypti.* This study aimed to determine the effect of the Insecticide Score of *P. paniculata* extracts on the mortality of *Ae. aegypti* mosquitoes based on KT_{50}. A total of 20 mosquitoes for each concentration were used to examine the effectiveness of 10%, 15%, 20%, and 25% concentration of *P. paniculata* extracts compared to a positive and a negative control with four replications. The results showed that *P. paniculata* extracts had an effect on *Aeaegypti* mosquito mortality. There were significant differences in mortality rate between concentrations (Kruskal-Wallis test, p = 0.001). Spearman Correlation test gave a p-value of 0.008 < 0.05. Similarly, there was a weak but significant correlation between plant extract concentration and mortality (Spearman correlation: r = +0.312, p = 0.008). The 10% and 15% concentrations had KT50 with Insecticide Scores of 1 and 2, respectively, implying that both had no knockdown effect. A 20% concentration had a KT50 with an Insecticide Score of 3, indicating a weak knockdown effect, whereas a 25% concentration had KT50 with an Insecticide Score of 5, signifying a quick knockdown effect. These results show that a 25% concentration has a quick knockdown time on *Ae. aegypti* mosquitoes. Therefore, extract *P. paniculata* extract at a concentration of 25% has a potential for use as a bio-insecticide in controlling *Ae. aegypti* mosquitoes.

Keywords: *Aedes aegypti mosquito, bio-insecticides, extract P. paniculata*

Introduction

The *Aedes aegypti* mosquito (L), which belongs to the Order Diptera and family Culicidae, is the main vector, often neglected, transmitting human diseases includingYellow Fever, Zica, Chikungunya, Dengue HaemorhaegicFever and other arbo viruses [1–4]. *Ae. aegypti* transmits the human arboviral disease, a global public health threat[5] that causes significant morbidity and mortality in developing countries [1]. The transmission of dengue fever is increasing in urban and semi-urban areas in tropical countries worldwide [6]. It is estimated that 40% or 50 to 528 million people worldwide are at risk of becoming infected with dengue fever and around 10,000–20,000 people die yearly [7]. According to WHO, about 390 million cases of dengue virus infection occur every year, of which 96 million manifest clinically with high severity. Furthermore, WHO reports that about 3.9 billion people are at risk of being infected with the dengue virus [8].

Indonesia is one of the countries where dengue hemorrhagic fever (DHF) occurs and is an unresolved health problem. In 2020, there were 15,132 DHF cases and 145 deaths in Indonesia, and a DHF incident rate of 31.23% [9]; indicating problems in controlling the disease.

Long-term application and extensive use of synthetic insecticides cause accumulation of residues in food, water, and soil, and affect human and ecosystem health [10–11]. Use of insecticides leaves residues that pollute the environment [3–12] and cause resistance in *Ae. aegypti* populations[4, 5, 13–17].

Controlling *Ae. aegypti* mosquitoes using synthetic insecticides involves fumigation with pyrethroids and larvacides containing temephos [18]. *Ae. aegypti* develops resistance to pyrethroid and multiply rapidly increasing the incidence of dengue fever worldwide. This puts almost half of the world's population at the risk of being infected with the disease [5]. Using temephos to kill larvae has a similar effect. This has been reported in several countries, such as Brazil [4], Mexico [19] and countries in south-east Asia, including Indonesia, Malaysia, Philippines, Thailand, Singapore, Laos, and Myanmar [5].

There is a need to obtain alternative insecticides effective in controlling the *Ae. aegypti* population [20]. This could be achieved by using natural plant chemical compounds that are environmentally friendly and avoid the side effects of synthetic insecticides [10–22]. Bioactive plant compounds are biodegradable, environmentally friendly, and non-toxic to non-target insects [2]. They only affect the target insect without destroying beneficial natural enemies. Generally, they are a safe, economical, target-specific, biodegradable, and residue-free [23].

Polygala L. is one of the largest genera belonging to the *Polygalaceae* tribe. This genus consists of 500 species and can be found in tropical, sub-tropical, temperate, and mountainous areas throughout the world except New Zealand. Most of these species grow in Central and South Tropical America. There are several species of *Polygala L*. that can be used as medicine including *Polygala chinensis L., Polygala paniculata L., Polygala polifolia Presl., and Polygala sibirica L.*[24]. *Polygala paniculata*, also known as vetiver in Riau, Indonesia, is a good-smelling annual herbaceous plant belonging to the family Polygalaceae. The plant is often used in traditional medicine as a tonic, and for controlling inflammatory cases of asthma, bronchitis, arthritis, and kidney disorders[25]. Moreover, *P. paniculata* is used for *in vivo* protection against the neurotoxic effects of methylmercury (Hg) [26], bronchitis, neurahenia, inflammation, amnesia, topical anesthetic, and expectorant drugs [27]. *Polygala* extracts contain secondary metabolites, including alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [27]. These bioactive compounds make *P. paniculata* potentially useful as a bio-insecticide. The bioactive compounds extracted from *P. paniculata* have not yet been explored as bio-insecticides in controlling the *Ae. aegypti* mosquito. Therefore, the main objective of this study was to evaluate the Insecticide Score of the toxin contained in the bioactive compounds of *P. paniculata* in killing the *Ae. aegypti* mosquito.

Materials and Methods

Materials and equipment. This study used 2500 g *P. paniculata*, 5 liter of 96% ethanol, 5 liter of distilled water, one bottle of synthetic insecticide Baygon (cypermethrin), and 480 *Ae. aegypti* mosquitoes. The equipment used in this study included a blender, analytical scales, Rotary Vacuum Evaporator, stopwatch, shaker water bath, thermometer, hygrometer, Buchner funnel, stick, basin, test box, syringe, and a spray bottle.

Test animal preparation. Test animals were bred in media containing clean water in a cool place and protected from direct sunlight for the *Ae. aegypti* mosquitoes to lay their eggs. The larvae were reared in an aquarium at 24.2 - 24.4 °C, with a relative humidity of 67–70%. The larvae were fed with coconut water [28] until they reached instar III and IV stages before becoming mosquitoes. The adult *Ae. aegypti* mosquitoes were then used as test animals.

Extraction process. A mass of 2500 g of *P. paniculata* plants were washed and air-dried at room temperature, and blended to give a final mass of 400 g of powder. The powder was macerated with 96% ethanol until completely submerged for three days. The resulting solution was filtered using a Buchner funnel and placed in a dark bottle. The dregs from the first filtering were soaked again for one day, filtered, and the process repeated for the third time. The filtrates from the three maceration processes were combined and concentrated using a Vacuum Rotary Evaporator to evaporate 96% ethanol and obtain an extract. The extract obtained was stored in the refrigerator to be used later [29].

Tests of extracts of *P. paniculata* against *Ae. aegypti* mosquitoes. Tests of *P. paniculata* extracts against *Ae. aegypti* were performed by transferring 20 *Ae. aegypti* in each test box before spraying with different concentrations of *P. paniculata* extracts with four repetitions per concentration, as well as for C (+) and C (-). The effect of the extracts on the *A. aegypti* mosquito was observed by looking at the changes in behavior, movement, and physical condition until death. Deaths of *Ae. Aegypti* mosquitoes were counted every 5 min for one hour. Moreover, the Insecticide Score of *P. paniculata*was determined from the number of *Ae. aegypti* mosquitoes considered dead at 5-minute intervals. *Ae. aegypti* mosquitoes that remained alive were left to die or killed with Baygon.

Data analysis. Data were analyzed using the One-Way Analysis of Variance test. However, when the data did not conform to the assumptions of parametric tests the non-parametric Kruskal–Wallistest and the Spearman test were conducted to examine the relationship between the independent and dependent variables.

Results and Discussion

Observations on deaths of *Ae.aegypti* Mosquitoes after spraying the *P. paniculata* plant extracts. The *Ae. aegypti* mosquitoes died after exposure to concentration of 10% and 15 % *P. paniculata*plant extracts. They died slowly by flying irregularly and actively before falling in a tilted body position. After falling, the mosquitoes appeared weak, with limited leg movements, before becoming paralyzed, and eventually dying. The death was faster at 20% and 25% concentrations. In the positive control (K+), death occurred in less than five minutes, while in the negative control (K-), there was no deaththe *Ae.*

Aegypti mosquitoes tried to avoid the spray during the four experiment repetitions.

Total mortality of *Ae. aegypti* mosquitoes at each concentration. After exposure to *P. paniculata* plant extract at 10%, 15%, 20%, and 25% concentration, total mortalities of 11, 18, 27 and 54 individuals, respectively, were recorded in the first 5 min of observation (Figure 1). After 60 mins, the total mortalities of *Ae. aegypti* mosquitoes were 52 at 10%, 80 each at 15%, 20%, and 25%. In the positive control (K+), which was a synthetic insecticide called cypermethrin, 76 *Ae. aegypti* mosquitoes fell and died in less than 5 min (Figure 1). In the negative control (K-), which was distilled water, mosquitoes did not die. There were 80 *Ae. aegypti* mosquitoes deaths after 51–55 min at a concentration of 15%, after 41–45 min at 20% concentration and after 21–25 minutes at 25% concentration.

Mean mortalities of *Ae. aegypti* at each concentration at five-minute intervals. After exposure to *P. paniculata* plant extract at concentrations of 10%, 15%, 20%, and 25%, the mean mortality of *Ae. aegypti* mosquitoes were 2.75 individuals (13.75%), 4.5 individuals (22.5%), 6.75 individuals (33.75%), and 13 individuals (67.5%), respectively, in the first 5 min of observation (Figure 2). Mean mortality rate of *Ae.aegypti* mosquitoes after 60 min of exposure were 13 individuals (65%) at 10%, 20 individuals (100%). at each of concentrations of 15%, 20%, and 25% (Figure 2). All (100%) *Ae. aegypti* individuals fell and died in positive control (K+) in less than 5 min. In the negative control (K-) no deaths (0% mortality) were recorded. All (100%) of *Ae. aegypti* individualsdied after 51–55 min at 15% concentration, after 41–45 min at 20%concentration, and after 21–25 min at 25% concentration (Figure 2).

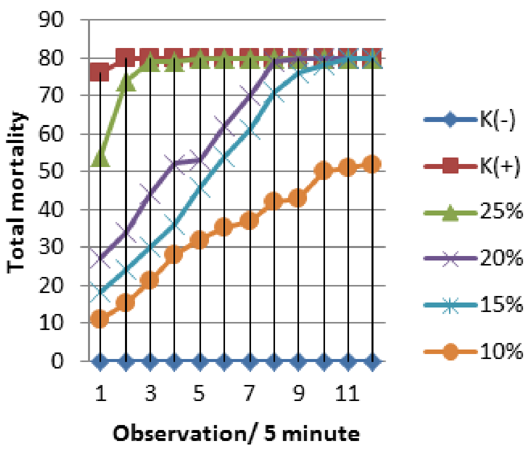

Figure 1. Total Mortality *of Ae. aegypti* Mosquitoes at Each Concentration at Five-minute Intervals

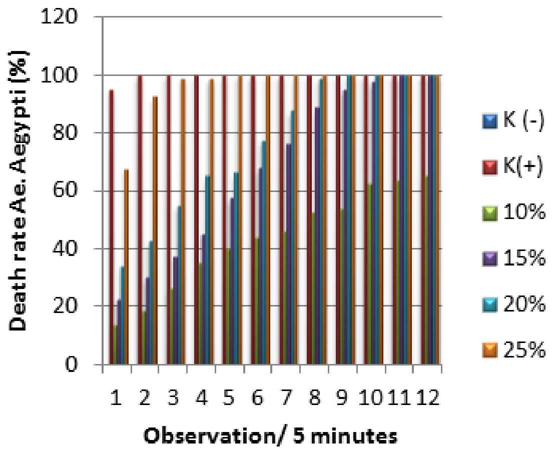

Figure 2. Mean Mortality of *Ae. aegypti* at each Concentration at five-minute intervals

These results indicate that the different concentrations of *P. paniculata* plant extracts had different effects on mortlaity of *Ae. aegypti* individuals. Mortality tended to increase with the concentration of *P. paniculata* plant extracts, indicating that the higher the concentration of extract used, the higher of potency of the *P. paniculata* plant extract as a bio-insecticide against the *Ae. aegypti*. This was supported by the results of the Kruskal–Wallis test (p= 0.001), which means that there was a significant difference mortality rate of the *Ae. aegypti* mosquito between the concentrations of *P. paniculata* plant extracts (10%, 15%, 20%, 25%). The results of the Kruskal–Wallis test concurred with those of the Spearman correlation test which showed a significant correlation (p = 0.008) between concentration of the extracts and the knockdown time. These results indicate that the higher the concentration, the shorter the time to death of *Ae. aegypti* mosquito, the strength of the correlation is denoted by a correlation coefficient 0.312 (31.2%). The higher concentrations of *P. paniculata* plants extract then the shorter of knockdown time. It was also noted that mortality increased with increased duration of exposure to the plant extracts

Insecticide Score of *P. paniculata plant extracts*. Knockdown Time$_{50}$ (KT$_{50}$) is the time to knockdown of *Ae. aegypti* mosquitoes. The highest KT$_{50}$ of between 36 and 40 minutes was recorded for the10% concentration of *P. paniculata* plant extract followed by that of the15% concentration of between 21 and 25 min, and that of the 20% concentration between 11 and 15 min, and that of the 25% concentration of less than 5 min (Table 1). There were no *Ae. aegypti* mosquitoes that died in the negative control and the KT$_{50}$ of the positive control was less than 5 min (Table 1). These results indicate that the different concentrations of *P. paniculata* plant extracts had different KT$_{50}$ on *Ae. aegypti* mosquito.

Table 1. Mean Falling Down Times of *Ae. aegypti* at Various Treatment Concentrations with Knockdown Time$_{50}$ (KT$_{50}$)

Time	10%	15%	20%	25%	K (+)	K(-)
5	2,75	4,5	6,75	13,5	19	0
10	3,75	6	8,5	18,5	20	0
15	5,25	7,5	11	19,75	20	0
20	7	9	13	19,75	20	0
25	8	11,5	13,25	20	20	0
30	8,75	13,5	15,5	20	20	0
35	9,25	15,25	17,5	20	20	0
40	10,5	17,75	19,75	20	20	0
45	10,75	19	20	20	20	0
50	12,5	19,5	20	20	20	0
55	12,75	20	20	20	20	0
60	13	20	20	20	20	0

: *Ae. Aegypti* Knockdown Time (KT_{50});
Source: (Primary Data, 2020)

Table 2. Bio-Insectiside score of *P. paniculata* plant extracts based on Knockdown Time 50

Group Control	KT50 (Time)	*Knockdown Effect*	Insecticide Score	Interpretation
Concentration 10%	36–40	-	1	-
Concentration 15%	21–25	-	2	-
Concentration 20%	11–15	+	3	Weak Knockdown
Concentration 25%	< 5	+++	5	Quick Knockdown
Positive Control	< 5	+++	5	Quick Knockdown

Interpretation of data (+ *weak Knockdown* +++ *Quick Knockdown*) [30]
(Primary Data, 2020)

Table 2 shows the effectiveness level of the four *P. paniculata* plant extract concentrations and positive control based on KT_{50}. A 10% concentration of the *P. paniculata* plant extract had a $KT_{50,}$ between 36 and 40 min giving an Insecticide Score of 1 or no knockdown effect. Similarly, a 15% concentration of the plant extracts had a KT_{50} of, between 21and 25 min, indicating an Insecticide Score of 2 or no knockdown. A 20% concentration of the plant extract had a KT_{50} of between 11–15 min, giving an Insecticide Score of 3 or a weak knockdown effect. Furthermore, a 25% concentration of *P. paniculata* plant extract had a KT_{50} of less than 5 min, indicating an Insecticide Score of 5 or a quick knockdown effect. The 25% concentration had a KT_{50} of less than 5 min, similar to that of the positive control, with an Insecticide Score of 5 or a quick knockdown effect. Therefore, the 25% concentration of *P. paniculata* plant extract was the most effective in knocking out *Ae. Aegypti* mosquitoes, with an Insecticide Score of 5 or a quick knockdown effect.

The results showed that the number and percentage of *Ae. aegypti* mosquito deaths increased with the concentration of *P. paniculata* plant extract. This was expected as higher concentrations of the *P. paniculata* plant extract a higher exposure of *Ae. aegypti* mosquitoesto higher levels of the bioactive compounds.

Kosini reported similar results for *Callosobruchus maculatus* larvaeexposed to *Gnidia kaussiana* plant extractsand attributed them to anincreased absorption of toxic compounds in by the larvae at higher concentration of the plant extract, which accelerates the mortality process of larvae. Absorption of toxic plant bioactive compounds disrupts the endocrine system due to the presence of secondary metabolites such as terpenoids, alkaloids, and flavonoids [31]. da Botas et al attributed the larcide effect of BaccharisreticulariaDC and *limonene* on *Ae. aegypti* to its ability to inhibit the formation of acetylcholinesterase , which can cause death and paralysis in *Ae. Aegypti* larvae. The more the larvae of *Ae. aegypti* absorb the toxic compounds in *B. reticularia* essential oil, the higher the *Ae. aegypti* mortality rate. Also, the longer the exposure to *B. reticularia* essential oil compounds the higher the level of toxicity [32].

Our results corroborate those of many previous studies stating that bioactive plant compounds have insecticidal, larvicidal, effects insects. According to Suluvoy, essential oils, flavonoids, alkaloids, glycosides, esters, and fatty acids can be used as an alternative to chemical compounds because they have anti-insect effects [21]. Previous studies reported that *Polygala* plants contain bioactive compounds with various biological activities such as alkaloids, saponins, flavonoids, phenols, tannins, steroids, and terpenoids [27]. The bioactive compounds in *P. paniculata* extracts able to cause mortality of *Ae. aegypti* mosquitoes are flavonoids, saponins, tannins, alkaloids, steroids, and terpenoids. In this study, the mortality of *Ae. aegypti* mosquitoes was most probably caused by compounds in the *P. paniculata* plant extract they entered the mosquito's body through contact or respiratory poisoning and the mouth and digestive tract, causing stomach poisoning.

The results showed that the mortality rate of *Ae. aegypti* mosquitoes when exposed to the *P. paniculata* plant extract varied with the concentration of the extract. Therefore, the higher the concentration of the plant extract the more effective it was as an insecticide against mosquitoes.

This study compared the effectiveness of the four *P. paniculata* plant extract concentrations with that of the positive control based on Insecticide Knockdown Time$_{50}$ (KT_{50}). A 25% concentration of the plant extract had a KT_{50} of less than five minutes, which was similar to that of the positive control. This KT_{50} implies an Insecticide Score of 5 or a quick knockdown effect. This is in line with the 2006 WHO standard, which stated that an insecticide has a knockdown time required to drop a vector when the median knockdown ranges between 3–5 min [30]. Furthermore, an insecticide has a quick knockdown effect when it has a KT_{50} of less than 5 min. According to Norris, a good insecticide requirement for controlling disease vector insect species is that it must cause a rapid knockdown of the target species[17].

Therefore, the 25% concentration of the *P. paniculata* plant extract was the most effective concentration in knocking down *Ae. aegypti* mosquitoes as it had an Insecticide Score of 5 or a quick knockdown effect. The positive control treatment was a benchmark for comparing the quality of *P. paniculata* plant extracts. In contrast, the negative control treatment was used to compare its effectiveness with that of the plant extract. Chang highlighted the need to use bio-insecticides as an alternative insect controller. It is necessary to develop safe alternative insecticides, larvicides, and repellents effective for humans, animals, the environment, and the ecosystem. Natural insecticides are needed to suppress vector resistance and slow down their genetic adaptation [34]. According to Sulovoy and Grace, plant insecticides only affect target insects, do not destroy beneficial natural enemies, and are a safe and residue-free food source. [21]. It is more environmentally friendly, effective, cheap, and naturally available [12].

Conclusion

The bioactive compounds contained in the *P. paniculata*plant extracts have the ability to drop and kill *Ae. aegypti* mosquitoes. Therefore, the plant extracts *P. paniculata* are a potential alternative insecticide for controlling *Ae. aegypti* mosquitoes. Like other plant insecticides, it is expected that *P. paniculata* extracts will not leave residues in the environment and will be environmentally friendly. Also, the bioactive compounds in the extracts will not cause resistance against *Ae. aegypti* mosquitoes.

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