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Research Article

Larvicidal Effect of Ether and Chloroform Extract of *Kaempferia galanga* Against the Larvae of *Aedes aegypti* (Diptera : Culicidae)

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ABSTRACT

Dengue is a mosquito-borne viral disease. Many insecticidal programs are in practice to eradicate this notorious vector of dengue virus, *Aedes aegypti*. Due to increased risk of pollution because of chemical insecticide, researches have been carried out to identify suitable biopesticide to eradicate *Ae.aegypti*. A study has demonstrated that *Kaempferia galanga* rhizome to possess larvicidal properties against mosquito larvae. Aim of the study is to determine the larvicidal activity of ether and chloroform extract of *K.galanga* rhizome against larvae of *Ae.aegypti*. Furthermore, to determine which of the two extract used is more potent. The larvicidal test was performed by exposing the 3-4th instar larvae of *Ae.aegypti* to various concentration of ether and chloroform extract of *K.galanga* according to standards recommended. The LC_{50} for the ether extract of *K.galanga* rhizome was 64.08 ppm, while the LC_{50} of chloroform extract was 105.02 ppm. The low LC_{50} level of both ether and chloroform extract proves that the *K.galanga* is an effective larvicide against the larvae of *Ae.aegypti*. However, ether extract is more effective as a larvicide than chloroform extract.

Keywords: *Aedes aegypti, Kaempferia galanga*, larvicidal activity.

INTRODUCTION

Dengue is the most rapidly spreading arthropod-borne or mosquito-borne viral disease in the world causing it to be the main concern of public health departments of many countries[1]. This disease is most prevalent in tropical and subtropical regions of the world, predominantly in urban-suburban areas[2]. Dengue is transmitted through the bites of mosquitoes. *Aedes aegypti* and *Aedes albopictus* is two of the mosquitoes which belongs to the genus *Aedes*, acts as the principal vectors of the dengue virus[3,4]. As there is no effective vaccine currently available against the dengue virus, disease prevention largely depends on the mosquito vector control or interrupting the vector-human contact[3,4]. Various strategies have been employed in the past and continued to be used now, including environmental control, biological control, chemical control, and active case surveillance. Chemical control involves the use of space spraying and larvicidal insecticide and it is one of the most important methods of controlling vectors of medical significance[5,6]. More specifically, larviciding acts as the first step in chemical control of mosquitoes, as larvicides kill the mosquitoes at their breeding site, before they could disperse and infest a community[6].

Mosquito's resistance towards these chemical agents has become a major threat for vector control[7]. Furthermore, use of conventional larvicides in water sources poses many risks to the environment and people. These pesticides have also been proven to be harmful to other beneficial organism present in the same area[7,8]. Natural pesticides, particularly phytochemicals however eliminate such risks[8]. In a study conducted previously, a rhizome called *Kaempferia galanga* demonstrated effective larvicidal activity towards mosquitoes[9].

Kaempferia galanga (Zingiberaceae) is an acaulescent perennial which is well known in Indonesia as "kencur". It has a broad range of medicinal use and well known as expectorant and carminative[10]. The major constituents of *k.galanga* essential oil are namely, ethyl p-methocycinnamate, ethyl-cinnamate, 3-carene, 2-propionic acid and pentadecane are thought to cause the larvicidal effect of this rhizome[11]. In this research, ether and chloroform extract of *K.galanga* will be tested against *Ae.aegypti* larvae. Furthermore, the results obtained will be used to determine which one of it possesses greater larvicidal effect. Probit analysis was done on the results obtained from the experiment and dose-response graph was plotted to determine the LC_{50} value of ether and chloroform extract.

MATERIALS AND PROCEDURE

The research is an experimental study. It aimed on determining the LC_{50} of the *K.galanga* extract. The subjects, *Ae.aegypti* larvae was colonized and reared in Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada. The method used in the experiment is maceration extraction method[9]. The extract was prepared using 1.5 kg of fresh rhizome which is then dried (dried powder, 300 grams) and macerated with ether (1.5 liter) as solvent several times to obtain the crude extract of *K.galanga*. The filtrate then was air dried till the final volume of concentrated extract (9 grams) obtained.

The same method was carried out to obtain the chloroform extract of *K.galanga*. The final volume of concentrated chloroform extract obtained is 10 grams. Ten different ether and chloroform extract solutions each with

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Table 1a: Results of bioassay test using the ether extract of *K.galanga* against 3rd- 4th instar larvae

Concentration (ppm)	Total larvae tested	Total mortality of larvae			Percentage of average mortality (%)	Standard deviation
		N1	N2	N3		
10	10	0	0	0	0.00	0.00
20	10	0	0	0	0.00	0.00
30	10	0	0	0	0.00	0.00
40	10	3	2	3	26.67	0.58
50	10	4	2	3	30.00	1.00
60	10	2	4	4	33.33	1.15
70	10	6	3	4	43.33	1.53
80	10	7	9	7	76.67	1.15
90	10	7	7	9	76.67	1.15
100	10	9	10	6	83.33	2.08
125	10	10	10	8	93.33	1.15
150	10	10	10	10	100	0.00

Table 1b: Final test results of the bioassay test using the chloroform extract of *K.galanga* against the 3rd-4th instar larvae of *Ae.aegypti* after 24 hours.

Concentration (ppm)	Total larvae tested	Total mortality of larvae			Percentage of average mortality (%)	Standard deviation
		N1	N2	N3		
50	10	0	0	0	0.00	0.00
60	10	1	1	0	6.67	0.58
70	10	1	0	0	3.33	0.58
80	10	3	2	3	26.67	0.58
90	10	4	3	3	33.33	0.58
100	10	6	4	4	46.67	1.15
110	10	5	6	3	46.67	1.53
120	10	4	7	7	60.00	1.73
130	10	9	8	8	83.33	0.58
140	10	10	10	10	100.00	0.00
150	10	10	10	10	100.00	0.00

concentration ranging from 10000 to 0.1 ppm were prepared. Ten larvae were placed in each plastic cups containing a 100ml of ether or chloroform extract of the prepared solution with known concentration. To improve the reliability of the test, a second preliminary was performed using the data obtained from the first test using concentration ranging from 10 ppm to 500 ppm. The second test was replicated twice.

The result of both the ether and chloroform extract was then analyzed to determine the percentage mortality. The concentration that has percentage mortality ranging from 10% to 95% was selected to be used in final test. The test involves the same procedure performed in the preliminary test and the final test was replicated twice more. Final results obtained were analyzed using probit analysis and log-probit graph was plotted to determine the LC_{50} and LC_{90} value of both the extracts.

RESULT AND DISCUSSION

In ether extract, the larval mortality ranging from 10% to 95% fell in between 50 ppm to 500ppm. The 50 ppm caused a mortality of 40%, in 100 ppm it was 50 % and 100% in 500 ppm. While in chloroform extract, it was between 10 ppm to 500 ppm. 10 and 50 ppm presented a larval mortality of 10%, 100 ppm was 30% and 500 ppm was 100%. An accurate range of 10% to 95% percent mortality could not be obtained from the first test. The range between the concentrations was too wide giving rise to inconsistency in result obtained.

While in second preliminary study, in ether extract, 10 ppm dosage concentration presented average percentage mortality of 3%, 50 ppm was 13%, 100 ppm was 70%, while in 150 ppm it was 100% after an exposure of 24 hours. The chloroform extract demonstrated 6.67% mortality in 50 ppm, 36.67% in 100 ppm and 100% mortality at 150 ppm. In both the extract, the range above 150 ppm produced absolute larval mortality. Thus in the final test concentration ranging from 10 to 150 ppm was used. The result obtained is presented in Table 1a and 1b. The controls of the experiment consisting 10 larvae in 100ml of water produced no larval mortality.

The probit unit corresponding to the result obtained it can be noted that increasing concentration of the extracts increases the percentage of larval percentage of average mortality was determined using Finney's table[12]. Data from Table 2a and 2b was analyzed using statistical packaged software. The input data in the form of mean mortality percentages of the larvae after 24 hours exposure

Table 2a: Analysis of the data of ether extract. Probit unit was determined using finney's[12] table.

Percentage of Average Mortality	Probit unit	Dosage Concentration (ppm)	Log of Concentration
0.00	-	10	1.0000
0.00	-	20	1.3010
0.00	-	30	1.4771
26.67	4.38	40	1.6021
30.00	4.48	50	1.6990
33.33	4.57	60	1.7782
43.33	4.83	70	1.8451
76.67	5.73	80	1.9031
76.67	5.73	90	1.9542
83.33	5.97	100	2.0000
93.33	6.50	125	2.0970
100	-	150	2.1761

Table 2b: Analysis of the data of chloroform extract.probit unit was determined using Finney's [12] table.

Percentage of Average Mortality	Probit Unit	Dosage Concentration (ppm)	Log of concentration
0.00	0.00	50	1.6990
6.67	3.50	60	1.7782
3.33	3.16	70	1.8451
26.67	4.38	80	1.9031
33.33	4.57	90	1.9542
46.67	4.92	100	2.0000
46.67	4.92	110	2.0414
60.00	5.25	120	2.0792
83.33	5.97	130	2.1140
100.00	0.00	140	2.1461
100.00	0.00	150	2.1761

was analyzed using probit analysis to determine the LC_x (lethal concentration X) of the extract. The data obtained from analysis is presented in Table 3a and 3b.

If graph is to be plotted to determine the dose response relationship between the percentage of larval mortality and its corresponding concentration of extract, a sigmoid curve will be obtained. The sigmoid relationship can be linearized by transformations such as probit. The analysis involves transforming the percentage mortality using probit transformation into probit value and the concentrations to $\log_{10}$[13].

By using the linear equation determined through the analysis, a linear regression line can be plotted on a log-probit graph paper representing dose response relationship between larval mortality and extract concentration. The graph will be represented by dosage concentration in X-axis and percentage mortality on Y-axis. The regression line was fitted by eye.

From the analysisit is determined that the LC_{50} of the ether extract is 64.1 ppm while the LC_{50} of the chloroform extract is 105.02 ppm. This provides evidence that the *K.galanga* ether and chloroform extract have considerable amount of larvicidal effect on larvae of *Ae.aegypti* at low doses of the extract. However the LC_{50} of the ether extract is much lower than the chloroform extract. Hence, ether fraction is more potent than the chloroform. This probably due to greater active compound extraction ability of ether compared to chloroform. The polarity index of ether is 2.8, while of chloroform are 4.1. The ether is less polar compare to chloroform[9].

Consequently, ether can extract more non-polar compounds compared to chloroform. From the statement above, it can be concluded that the active compounds in *K.galanga* is non-polar in nature and better extracted in non-polar organic solvents such as ether. This correlated with the result of a study conducted, the hexane fraction of *K.galanga* (highly non-polar in nature) provided the maximum yield and highest potency. Highly polar solvents such as methanol had the lowest potency with a LC_{50} of 1052 ppm[9].

In the past various researches have been tested to determine the larvicidal activity of *K.galanga* against various types of mosquito species. A related research produced results of hexane fraction (LC50=42.33 ppm), non-alkaloid dichloromethane fraction (LC50=75.91 ppm), crude alkaloids of dichloromethane (LC50=141.51 ppm), methanolic (LC50=1052 ppm) when tested against larvae of *Cx.quinquefasciatus*[9]. Microscopic examination of the anal gill of *Cx. quinquefasciatus* exposed to ethanolic fraction revealed damage of irregular ridge-like epithelium on their surface which functions as ionic regulator. Thus the ionic disregulation due to damage in anal gill was found to be the mode of action of *K.galanga* extract[14].

The essential oil of the *K.galanga* forms a viscous liquid with golden yellow colour and cineolic-like odor[15]. Out of the 98.89% of indentified compounds of the essential oil,

Table 3a: Probit analysis of bioassay test using ether extract of *K.galanga* against 3rd-4th instar larvae of *Ae.aegypti*.

Mortality percentage	Dosage/LC_x (PPM)	95% Confidence limits		Variance
		Lower	Upper	
10	34.53	28.04633	42.50482	2.12169
20	42.70	36.46600	49.99638	1.222329
30	49.77	43.90678	56.40740	7.704089
40	56.72	51.19887	62.83606	5.148782
50	64.08	58.65309	70.01342	3.847443
60	72.40	66.46485	78.86467	3.591659
70	82.52	74.99996	90.78525	4.47821
80	96.17	85.31837	108.4114	7.043113
90	118.94	100.8706	140.2379	1.332635

Y = -3.622267 + 4.77229X
Regression coefficient, b: 4.77229
Intercept, a: -3.622267
Chi square (X^2): 7.423062
Degree of freedom: 6

Table 3b: Probit analysis of bioassay test using chloroform extract of *K.galanga* against 3rd-4th instar larvae of *Ae.aegypti*.

Mortality Percentage	Dosage/LC_x (PPM)	95% Confidence Limits		Variance
		Lower	Upper	
10	72.04	64.30172	80.70873	6.339337
20	82.00	75.52154	89.03000	3.323632
30	90.02	84.40387	96.04148	2.038846
40	97.49	92.19584	103.0927	1.531834
50	105.02	99.28444	111.0884	1.548946
60	113.13	106.0695	120.6617	2.039307
70	122.52	113.174	132.6342	3.090174
80	134.51	121.5815	148.8077	5.011874
90	153.10	133.8042	175.1807	8.910896

Y = -10.82584 + 7.829632X
Regression coefficient, b: 7.829632
Intercept, a: - 10.82584
Chi square (X^2): 5.04047
Degree of freedom: 5

the most abundant compound in *K.galanga* is 2-propeonic acid, pentadecane and ethyl p-methoxycinnamate; while the other compounds found in smaller percentage[11,15]. However, compounds such as methyl cinnamate, carvone, eucalyptol was also found to be present in large percentages. This shows that the variation and climatic and geographic conditions play a role in the rhizome's essential oil content[16]. In addition, the major constituent of the petroleum ether extract is sterols, triterpenoids and resins[17].

Consequently, it is possible that the *K.galanga* found abundantly in Indonesia to have different essential oil content. Yet, in both extract and essential oil, ethyl p-methoxycinnamate found to be present in highest percentage; (25.96 to 87.4% in essential oil)[11]. The compound that mainly contributes to the larvicidal activity of *K.galanga* is primarily Ethyl p-methoxycinnamate, whereas, ethyl-cinnamate, 3-carene, 2-propionic acid and pentadecane was also found to possess high larvicidal properties[11]. In conclusion, it can be clearly defined that the above mentioned constituents of the *K.galanga* rhizome is what causes the toxicity symptoms and mortality observed among the *Ae.aegypti* larvae.

CONCLUSION AND SUGGESTIONS

Both ether and chloroform extract of the *K.galanga* have larvicidal activity against *Ae.aegypti* larvae. However the ether extract is a more potent larvicide than the chloroform extract. Further researches have to be conducted to determine the specific phytochemicals that serve as active compound in the *K.galanga* that demonstrates insecticide properties.

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