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Research article

Toxicity test of clove leaf extract compared to clove flower extract (*syzygium aromaticum*) as aedes aegypti linnaeus larvacide

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) is an epidemic disease. In early 2019 the World Health Organization (WHO) determined it as a potential threat. Prevention efforts have been made in biology, chemistry, and behavior. However, the use of chemicals that become instant measures has a negative impact on the occurrence of mosquito resistance and environmental pollution. Natural materials are alternative solutions as natural potentials that are easy, cheap, and safe for human health and environmental health. Cloves are Indonesia's natural potential which contains eugenol, saponins, flavonoids, and tannins which have the potential as active ingredients for *Aedes aegypti* larvicidal. This study aims to compare the results of the toxicity test of leaf extract with clove flower extract which has the potential as *Aedes aegypti* larvicidal. The research method uses a laboratory experimental design with a posttest-only control group design. The research sample in the form of third-instar larvae was obtained from the laboratory of the School of Life Sciences and Technology (SITH) Bandung Institute of Technology (ITB) using a purposive sampling method. Comparative data analysis with a numerical variable scale. The results showed that the death effect within 24 hours was between 19% - 92% mortality with clove leaf extract and 64% - 100% the death effect of *Aedes aegypti* larvae with clove flower extract. Toxicity test with Clove leaf extract obtained LC50 of 7.761% and LC90 of 37.014%. While the clove flower extract obtained LC50 of 3.374% and LC90 of 4.441%.

Keywords: Toxicity test, Larvicide, Cloves, control of *Aedes aegypti* larvae, Natural ingredients

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is well developed an epidemic in Indonesia, which has a tropical climate. The cause of DHF is a viral infection of the Flaviviridae species, namely the Flavivirus genus with DEN-1, DEN-2, DEN-3, and DEN-4 serotypes[1]. Urban development, climate change, increased mobility of population density, and low awareness of keeping the environment clean can be risk factors for DHF as an environment-based disease[2]. Prevention efforts have been carried out by the Indonesian Ministry of Health through the Eradication of Mosquito Nests (PSN) 3M Plus Consisting of 3M. Chemicals as a prevention effort have entered the stage of resistance in adult mosquitoes. Where it is resistant to 0.8% malathion, 0.05% cypermethrin, and lambda-cyhalothrin and is still tolerant to alpha-cypermethrin. From the research results, it was found that almost all strains of *Aedes aegypti* from North Sumatra and Jambi Provinces were resistant to 0.025% deltamethrin, only strains from Deli Serdang Regency were still tolerant to 0.025% deltamethrin[3]. Heni (2016) states that the use of malathion and temephos in the long-term cause's resistance to *Aedes aegypti*.

Replacing insecticides with active ingredients that have been tested for their effectiveness and increasing efforts to eradicate mosquito nests in the community are urgently needed[4].

Biolarvicide is the right alternative solution considering that natural ingredients are potentially available in nature as local potential. So eradication efforts can be maximized with local wisdom that has leverage because it is easy to obtain, cheap, and of course safe for public health and environmental health.

The increasing number of diseases and the demands for treatment make researchers research medicinal plants as alternative therapies. Natural ingredients are becoming popular in developing and developed countries due to their natural nature and low side effects that may occur. The World Health Organization states that there are 21,000 plants that have potential for treatment and the like throughout the world[5]. Evaluation through research on medicinal plants is carried out to determine the effectiveness and safety of drugs so that the profile of the effectiveness of therapy and the toxicity of plant products can be known for various benefits [6].

Various attempts have been made to prevent mosquito bites, including mosquito coils, use of mosquito sprays, and generally use repellents in the form of repellants [7]. The efforts mentioned above are included in chemical control. However, chemical control has been proven to have a negative impact on health and the environment. For example, the use of repellants made from chemicals and containing the active ingredient N,N-diethyl-m-toluamide (DEET). Then another effort is needed to control vector-borne diseases by avoiding the use of DEET chemicals on health. Natural ingredients are an alternative to natural mosquito repellent preparations to replace DEET [8]. Natural materials besides being safe are also very promising because of course people will like them, considering that natural materials are safer for health and the environment. The development of citronella oil micro emulsion natural ingredients as mosquito repellents can be an alternative repellant to reduce the prevalence of DHF [9].

Some natural ingredients have even been researched before. Lime peel extract (*Citrus aurantifolia)* has the ability to kill third instar *Aedes aegypti* mosquito larvae. The effective concentration of lime peel extract *(Citrus aurantifolia)* which can kill 50% (LC50) is 3.419% within 24 hours[10]. The ethanol extract of citronella *(Cymbopogon nardus L)* has the potential as a natural larvicide of *Aedes aegypti* caused larvae death in the treatment group with concentrations of 0.05%, 0.1%, 0.2%, 0.5%, 1%, and 2% with a p-value <0.05[11].

Previous special research related to cloves, namely by Sakriani (2016) stated that the number of larvae that died in clove leaf juice was higher than the number of larvae that died in papaya leaf juice at each concentration. with concentrations of 10%, 15%, and 20% much more than a solution containing papaya leaf juice[12]. There is a larvicidal effect of brewing clove leaves (*Syzygium aromaticum* L.) on the mortality of *Anopheles* sp mosquito larvae, where the effective concentration is 6% and has an LC50 value in killing *Anopheles sp* larvae of 0.89%. Clove leaf phytochemicals have a fast response to the mortality of *Anopheles sp*. in the form of saponins, flavonoids, and tannins as larvicides through the mechanism of damaging cell membranes or interfering with larval metabolic processes[13]. Where the content of essential oil of clove leaves of the Zanzibar variety has a larvicidal effect on *Aedes aegypti* larvae. The concentration of 100 ppm of clove leaf essential oil of the Zanzibar variety has the same effect as temephos as the gold standard larvicide (WHO recommendation)[14].

Clove oil has many benefits, including as a natural remedy for dealing with dry skin, treating stomach aches, increasing blood flow and hair growth, preventing the risk of developing cancer, being able to kill herbs or being antimicrobial, reducing toothache and joint pain, helping to control blood sugar, improving liver function, maintain bone mass, prevent stomach ulcers, can boost the immune system and has a natural antioxidant content in euganol to ward off free radicals. Cloves are one of the natural ingredients that have the potential to be used as larvicides. The potential of saponins, flavonoids, and tannins in natural products is an alternative source of larvicides from nature. Contained in cloves. Extracts from cloves, especially leaf extracts and clove flower extracts, need to be tested for toxicity against Aedes aegypti larvae.

Based on the description above, the researcher was interested in comparing the results of the toxicity test with clove leaf extract and clove flower extract. The purpose of this study was to compare the results of toxicity tests with clove leaf extract and clove flower extract which have the potential to become *Aedes aegypti Linnaeus* larvae.

MATERIALS AND METHODS

The design of this research is laboratory experimental with Posttest Only Control Group Design. The research sample was obtained through a purposive sampling method so that it immediately matched the criteria needed by the researcher, where the inclusion criteria were live or moving third instar larvae. The characteristics of the third instar larvae are 4-5 mm in size, the chest spines are starting to become clear, the respiratory funnel is blackish brown, the larvae are easier to identify than instars I and II and they do not quickly turn into pupae. The third instar larvae of *Aedes aegypti* were obtained from the laboratory of the School of Life Sciences and Technology (SITH) of the Bandung Institute of Technology (ITB). The sample size in this study referred to WHO provisions in larvicidal testing, namely 25 *Aedes aegypti* larvae in each group divided into 7 groups,

which were divided into 1 control group and 6 treatment groups using leaf extract compared to *Syzygium aromaticum* clove flowers.

Based on Federer's formula to determine the number of repetitions in the study, 3 repetitions of the toxicity test were obtained on instar III *Aedes aegypti* larvae. The total sample of Aedes aegypti larvae needed in the study was: 25 larvae x 7 groups x 3 repetitions x 2 ratios or 1050 plus 10%, namely 1155 or rounded up 1200 larvae from SITH ITB.

The extracts used in this study were clove leaf and flower extracts obtained from the soxhlet extraction process with 96% ethanol solvent. The extracts used varied from concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56%. Considering that these compounds have the potential as larvicides with active ingredients from natural ingredients, they can be obtained using suitable solvents

RESULTS AND DISCUSSION

The research was carried out at the Biological Pharmacy Laboratory and the UPT Basic Laboratory at Bhakti Kencana University. Biological Pharmacy Laboratory to make extracts from clove flowers and leaves and UPT Basic laboratory of Bhakti Kencana University for toxicity tests. Clove leaves and flowers as research samples were obtained from clove plantations in the Bandung Regency area.

Based on the results of the research that has been done, the following data are obtained:

Table 1: Toxicity test results for *Aedes aegypti* larvae with clove leaf extract

SESSION I							
Concentration	**Time of Death (minutes)**						**24-hour mortality**
	15	**30**	**45**	**60**	**120**	**480**	
50%	2	3	5	6	10	14	22
25%	0	4	6	6	8	8	21
12.50%	2	3	4	4	4	4	20
6.25%	2	3	3	4	6	6	11
3.12%	1	2	2	2	2	2	7
1.56%	0	2	2	2	2	2	5
Control	0	0	0	0	0	0	0
SESSION II							
50%	3	3	5	5	7	7	24
25%	2	3	3	3	5	6	21
12.50%	2	3	4	4	4	4	19
6.25%	2	4	4	5	6	6	10
3.12%	1	2	2	2	2	2	8
1.56%	0	2	2	2	2	2	4
Control	0	0	0	0	0	0	0
SESSION III							
50%	3	3	5	5	8	10	23
25%	4	4	4	5	6	9	22
12.50%	2	3	4	4	4	4	18
6.25%	2	2	2	3	3	6	11
3.12%	1	2	2	2	2	2	6
1.56%	0	2	2	2	2	2	5
Control	0	0	0	0	0	0	0

The cloves used are of the Zanzibar variety. Toxicity tests on larvae were carried out on clove leaf extract and clove flower extract. The test was carried out 3 (three) times with repetition of both treatment and control.

Table 2: Toxicity Test Results for Aedes aegypti larvae with Clove Flower extract

SESSION I							
Concentration	**Time of Death (minutes)**						**24-hour mortality**
	15	**30**	**45**	**60**	**120**	**480**	
50%	23	24	25	25	25	25	25
25%	20	23	23	24	24	24	25
12.50%	19	23	24	25	25	25	25
6.25%	17	22	23	23	25	25	25
3.12%	9	15	18	19	21	21	19
1.56%	5	8	10	10	16	18	17
Control	0	0	0	0	0	0	0
SESSION II							
50%	20	22	24	25	25	25	25
25%	17	23	23	24	24	25	25
12.50%	16	21	21	25	25	25	25
6.25%	13	20	23	23	25	24	25
3.12%	10	17	19	21	22	24	18
1.56%	3	7	11	11	15	20	16
Control	0	0	0	0	0	0	0
SESSION III							
50%	23	24	25	25	25	25	25
25%	22	24	23	24	24	24	25
12.50%	21	24	24	25	25	25	25
6.25%	18	22	23	20	25	25	25
3.12%	11	15	18	19	22	21	18
1.56%	6	8	11	11	16	19	15
Control	0	0	0	0	0	0	0

Each test was repeated 3 times using a sample containing 25 *Aedes aegypti* larvae. Concentration in the test according to the geometric progression limited to concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.56%. The test started with each container filled with 100 ml of extract solution according to the concentration of the treatment and 25 *Aedes aegypti* larvae. In the control, each replicate was filled with water and 25 larvae. The next process is to observe the condition of the larvae both fainted for one hour and for 24 hours to find out whether the larvae have died[15].

The results of observations that have been made show that there are different effects in each treatment process at different doses from 1.56% - 50% of clove leaf extract. The condition of dead *Aedes aegypti* larvae varied at different concentrations and time intervals. The higher the concentration and over time the more *Aedes aegypti* larvae that die.

Observations in the research process described in table 2 show that at different doses of 1.56% - 50% clove flower extract there are different effects in each treatment process. The mortality of *Aedes aegypti* larvae were already high in the first minute, namely the 15th minute, it increased along with the increase in the concentration of the extract in each treatment.

Table 3: The percentage of death of *Aedes aegypti* larvae with leaf extract compared to clove flower

Concentration	SESSION I	SESSION II	SESSION III	Dead	% Dead
Clove leaves					
50%	22	24	23	69	92
25%	21	21	22	64	85
12.50%	20	19	18	57	76
6.25%	11	10	11	32	43
3.12%	7	8	6	21	28
1.56%	5	4	5	14	19
Control	0	0	0	0	0
Clove Flower					
50%	25	25	25	75	100
25%	25	25	25	75	100
12.50%	25	25	25	75	100
6.25%	25	25	25	75	100
3.12%	19	18	18	55	73
1.56%	17	16	15	48	64
Control	0	0	0	0	0

The effect of different mortality in the 24-hour observation obtained the mortality interval between 19% - 92% in *Aedes aegypti* larvae treated with clove leaf extract. In clove flower extract, the death effect of *Aedes aegypti* larvae in 24 hours of observation was 64% - 100%.

Table 4: LC50 and LC90 in *Aedes aegypti* Larvae Toxicity Test

Ekstrak Tanaman	LC_{50}	LC_{90}
Clove leaves	7,761 % (3.655 – 11.663 %)	37,014 % (27.678 – 54.557 %)
Clove Flower	3,374 % (2.634 – 5.242 %)	4,441 % (3.679 – 38.581%)

Table 4. Probit analysis shows that the concentration of 50% of the dead test animals (LC50) in clove leaves ranges from 7.761% (3.655 – 11.663%) with LC90 ranging from 37.014% (27.678 – 54.557%). Meanwhile, the LC90 for clove flowers was 4.441% (3.679 – 38.581%) with a concentration of 50% of dead test animals (LC50) for clove flowers ranging from 3.374% (2.634 – 5.242%).

Figure 1: Percentage of Fainting Effect of *Aedes aegypti* larvae on Treatment of Clove Leaf Extract Compared to Clove Flower Extract at 15 Minutes of Observation

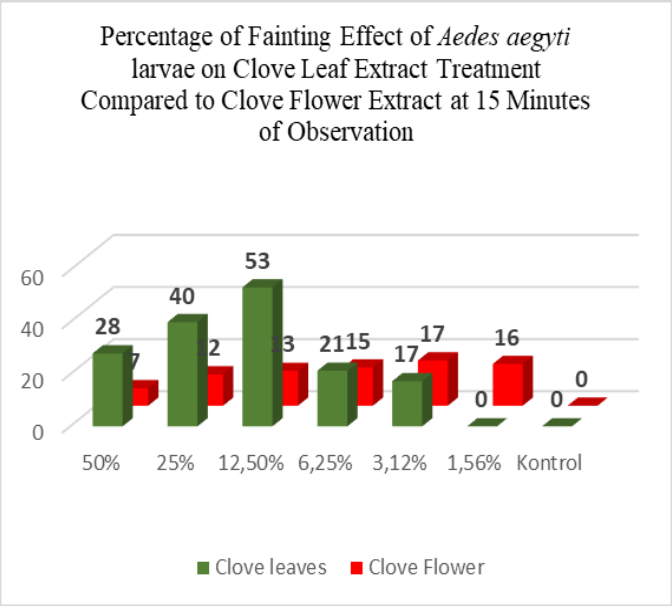
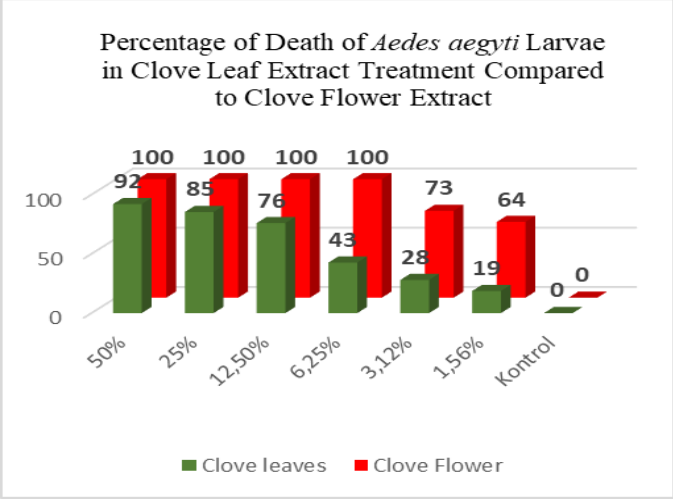


Figure 2: Percentage of *Aedes aegypti* Larvae Mortality in Clove Leaf Extract Treatment Compared to Clove Flower Extract at 24 hours of observation



There are many cloves as natural ingredients in Indonesia. Based on the results of research conducted by Kurniawati previously stated that both clove leaves and flowers contain flavonoids, saponins, tannins, quinones, and steroids/triterpenoids[16]. This compound is a larvicidal compound [12,14,17].

In Tables 1 and 2, the death effect is involved with varying concentrations and periods. Different effects in each treatment process at different doses from 1.56% - 50% of clove leaf extract. The condition of dead *Aedes aegypti* larvae varied at different concentrations and time intervals. The higher the concentration and over time the more *Aedes aegypti* larvae that die. Likewise, clove flower extract at a concentration dose of 1.56% - 50% had a different death effect where the mortality of the test larvae of *Aedes aegypti* was already high in the early minutes. The study showed that a reaction occurred since the minimum concentration in each extract, both leaf extract, and clove flower extract, resulted in a difference in the number of deaths in the test larvae of Aedes aegypti. In the 24-hour observation, the mortality interval was between 19% - 92% of *Aedes aegypti* larvae treated with clove leaf extract. Meanwhile, the death effect of *Aedes aegypti* larvae on clove flower extract was 64% - 100% (Table 3).

In addition to the death effect, observations were also made of the fainting effect on *Aedes aegypti* in clove leaf extract was different in each process with a dose of 1.56% - 50% (Chart 1). The number of fainted *Aedes aegypti* larvae varied at different concentrations and time intervals. Toxicity effects on species are strongly influenced by the concentration of chemical compounds in the body of the target species. The higher the concentration of the extract, the higher the content of the active ingredient in the substance, especially if the active ingredient functions as a pesticide that can kill in large quantities[18]. Chart 2 shows the different percentages of death at each treatment concentration and the time

added to clove leaf and flower extracts.

The higher the concentration and over time, the fainter *Aedes aegypti* larvae faint. Flavonoids act as strong inhibitors of respiration or as respiratory poisons[19]. This is what can cause test larvae to faint due to the content of flavonoids with varying concentrations of each treatment. Based on the results of preliminary research the highest levels of flavonoids in Clove leaves were obtained through a reflux process with 96% Ethanol solvent where the flavonoid content was 29.53% and saponin levels in previous studies were found in Clove leaf extract through the soxhlet process with 96% Ethanol solvent at 9.92 %[16].

In line with Handito's research that clove leaf extract has the potential as an insecticide against *Aedes aegypti* where the most effective extract concentration is a concentration of 50%. This concentration is effectively used as a basic ingredient for liquid electric mosquito repellents for *Aedes aegypti* mosquitoes[20]. Infusion of clove leaves (*Syzygium aromaticum* L) has an effective larvicidal effect on the mortality of Anopheles sp mosquito larvae which is 6% effective. The brew also has an LC50 value of 0.89% on the death of *Anopheles sp*. larvae. The phytochemical content of clove leaves has a quick response to the death of Anopheles sp larvae, namely the presence of saponins, flavonoids, and tannins which act as larvicides through the mechanism of damaging cell membranes or interfere with the metabolic processes of larvae or known as stomach poisoning. The content of saponins, flavonoids, and tannins can increase the mortality of *Aedes aegypti L.* larvae[13].

A literature study conducted by Tiara states that clove leaf extract containing eugenol, saponins, tannins, and flavonoids is larvicidal against Aedes aegypti mosquitoes[21]. Where is the effectiveness of the Afo variation of clove leaf extract against *Anopheles subpictus* in the 10th minute after treatment with a concentration of 80%, the mortality rate reached 33.3% and increased with the length of application time. A concentration of clove leaf infusion of 20% is effective against the death of *Aedes aegypti* in the first 3 hours of application and achieved 85% larval mortality after 15 hours of application. Cloves of the Afo variety have the potential as a natural larvicide for controlling malaria and dengue mosquito vectors[22]. Hasil penelitian menunjukkan LC_{50} pada daun Cengkih berkisar 7,761 % dan LC_{90} berkisar 37.014 %. Sedangkan LC_{90} pada bunga Cengkih 4.441 % dan LC_{50} pada bunga Cengkih berkisar 3.374 % (Table 4). This means that the content of potentially larvicidal compounds is greater in the content of clove flower extract where as much as 3.374% can cause the test animals to die.

The mortality of the test larvae increased with the increase in the concentration of the extract, this was due to the number of active ingredients present in the extract, both leaf extract, and clove flower extract. The presence of bioactive compounds such as saponins and tannins acting as stomach poisons is suspected to be the trigger for the death of *Aedes aegypti* larvae. Tannins with digestive target organs can irritate the stomach. Tannins have a bitter and sharp taste and work by inhibiting the eating process[23].

In this study, clove leaf extract and clove flower extract were obtained from the results of the soxhlet extraction process with the consideration that the soxhlet process produced perfect extraction results. The process in Soxhlet extraction is carried out continuously where the extraction is carried out repeatedly with a relatively constant amount of solvent. This process causes the chemical compounds in the sample to be isolated properly. Soxhlet extraction is often used because the extraction process occurs perfectly so that the extract yields are also greater and there is a heating process that can help speed up the extraction process. Setyowati stated that the movement of molecules is faster if the extraction temperature is higher, including the circulation (movement) of the solvent. Temperature and solvent circulation increase the rate of mass transfer of compounds from leaf or flower cells. So the contact of the solute with the solvent is more frequent so that more extracts are obtained[24].

The results of this study can be used as basic research for further research to utilize natural materials with potential larvicides that must be developed. It is hoped that the use of natural materials with the potential for larvicide can encourage an increase in PSN 3M Plus efforts which have not been able to show maximum results. Where people's behavior is a challenge in consistent efforts to implement PSN 3M Plus. This follows Kurniawati's research that behaviors such as draining water reservoirs, closing water reservoirs, recycling used items that can hold water, and the habit of hanging clothes, are related to the incidence of DHF [25]. So it is necessary to intensify and optimize community empowerment in PSN 3M activities, one of which is by developing larvicides from natural materials.

CONCLUSION

Since Parkinson's disease is a disease that causes various disorders through complex mechanisms, effective treatment intervention methods must be included in the process of constructing a rehabilitation program for the treatment of PD. This study investigated the effects of training using PNF, which is applied in clinical neuro rehabilitation, on balance and gait required for functional activities in Parkinson's patients. In the results of the study, the experimental group to which PNF was applied showed improvements in balance and walking ability compared to the control group. Based on these results, the rehabilitation program for Parkinson's patients should include training using PNF.

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