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Testing the larvacides activities of clove leaves extract (*Syzygium aromaticum*) on the mortality of *Aedes Aegypti* L

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A B S T R A C T

Dengue Hemorrhagic Fever (DHF) is an infection caused by the dengue virus which is transmitted by the Aedes Aegypti mosquito. Clove leaves (Syzigum aromaticum) is a part of a plant that contains eugenol, saponins, flavonoids and tannins which are useful as natural larvicides against Aedes Aegypti mosquito larvae.This study aims to determine the effect of larvicidesClove leaf extract (Syzigium aromaticum) on Aedes aegypti L larvae and to determine the LC50 value that can kill Aedes aegypti L larvae. This study was an experimental study with a post test only group control design. Clove leaves were extracted by maceration using 96% w/v ethanol.This study was divided into 7 groups using Aedes aegypty Instar III larvae, namely:2 control groups namely K1 NaCMC 1% as negative control and K6 Abate 0.1% b/v as positive controland 5 treatment groups each with a concentration of clove leaf extract K20.010 % w/v, K3 0.025 % w/v, K4 0.05 % b/v, K5 0.075 % b/v and K6 0.100 % b/v.The number of larvae deaths was counted after 24 hours.HThe results of the study showed that the number of deaths of Aedes aegypti mosquito larvae for each group was: K1 0%, K2 3.33%, K3 8.33%, K4 23.33%, K5 53.33%, K6 80% and K7 100 %Analysis resultsstatistics using Anova .and the LSD follow-up test isthere was a significant difference in larval mortality at each concentration (p 0.00 <0.05) And K2 showed a non-significant effect with K3 (sig p 0.089 > 0.05). From the research results it can be concluded that clove leaf extract(Syzygium aromaticum L)has a larvicidal effect on Aedes aegypti larvae.Clove leaf extract(Syzygium aromaticum) can kill Aedes aegypti mosquito larvae with an LC50 value of 600 ppm and is included in the toxic category.

INTRODUCTION

Dengue Fever (DHF) is an infection caused by the dengue virus which is transmitted through a vector, namely the Aedes Aegypti mosquito. (Adrianto.H et al. 2022). Based on Indonesia's health profile from the Ministry of Health, in 2021 there were 73,518 cases of DHF with a total of 705 deaths. In 2022 the cumulative number of Dengue cases up to week 22 will reach 45,387 cases with the number of deaths reaching 432 cases.Riau Archipelago Province has the highest DHF Incidence Rate (IR) of 80.9 per 100,000 population, South Sulawesi ranks 10th with a DHF IR of 40.0 per 100,000 population. Until now there is no drug to eradicate the virus or vaccine to prevent dengue. An effective way to completely tackle this disease is to control vectors/transmitting mosquitoes (Ministry of Health, 2014). Controlling the immature or larval stages is relatively easier than the adult stages, besides that the density of the pre-adult stages or mosquito larvae is directly proportional to the extraordinary incidence of DHF (Suwasono H. 1997). Treatment of this disease is not easy and one way that can be done to prevent dengue outbreaks is to break the chain of transmission. Mosquitoes as vectors that can transmit the virus that causes DHF population must be controlled. Some of the ways that are often done are the 3 M plus program, namely draining, closing, burying and avoiding mosquito bites (RI Ministry of Health, 2022).

Chemical control is still the most popular using insecticides. Spraying with LUV malathion is still a common way to kill adult mosquitoes. Unfortunately, besides being

able to have a negative impact on the environment, it also cannot kill larvae in water. One way to control this mosquito vector is by using lervasida.Larvicidation is the eradication of larvae by sprinkling larvicidal powder. Larvicidation aims to eradicate mosquito larvae, especially in water reservoirs that cannot be drained or cleaned..The larvicide that is often used is Temophos (Abate®). abate (temephos) in Indonesia has been used since 1976. Four years later, namely in 1980, abate (temephos) was established as part of the Aedes aegypti mass eradication program in Indonesia. Abate (temephos) has been used for more than 30 years (Natadisastra, D and Agoes, R.2009). However, prolonged use creates resistance to Aedes aegypti population, so higher doses are needed which have toxic effects on humans, animals and the environment.Fuadzy et al., 2015., Mulyatto, KC.2012). Themepos organophosphate is a larvicide that is widely used as a vector control for dengue in Martinique (French West Indies) which has shown resistance in Southeast Asia, South America and the Caribbean (Torres, SM et al., 2014.,Marcombe, Sebastien et all 2011).

Based on this, it is necessary to make an effort to obtain alternative larvicides, namely by using natural larvicides. Natural larvicides are larvicides derived from plants that have toxic content against insects. The use of natural larvicides has no side effects on the environment, a low level of toxicity for humans (Pratiwi, 2014).

Indonesia is famous for its biodiversity. There are many plants that can be used as vegetable larvicides that have been used by the community, one of which is clove (*Syzigum aromaticum*). Clove (Syzygium aromaticum L.) is a type of herbaceous plant that has large, hard woody tree trunks. Clove (Syzygium aromaticum L.) has a single leaf, stemmed, thick, stiff, ovate to elongated lanceolate, pointed tip, tapered base, flat edge, pinnate leaf veins, glossy upper surface, 6-13.5 cm long, wide 2.5-5 cm, light green or light brown when young and dark green when old Kardinan A. (2003). Clove leaves (Syzigum aromaticum) have phytochemical compounds such as eugenol, saponins, tannins, flavonoids which have a larvicidal effect on Aedes aegypti larvae (Chintithia, 2015)

In a previous study by Haditomo.I (2010) the results showed an increase in the number of deaths of Aedes aegypti larvae by increasing the concentration of clove leaf extract. The minimum concentration of 0.025% kills 35% of the larvae while the concentration of 0.100%, 0.125% and the maximum concentration of 0.150% kills 100% of the larvae of aedes aegypti with a Lethal Concentration (LC50) value of 400 ppm.

Formulation of the problem

1. Is clove leaf extract(Syzygium aromaticum) has a larvicidal effect on Aedes aegypti larvae ?
2. What is the LC50 value of clove leaf extract(Syzygium aromaticum) which can kill Aedes aegyti larvae for 24 hours ?

Research purposes

1. To determine the larvicidal effect of clove leaf extract(Syzygium aromaticum) derived from the larvae of Aedes aegypti
2. To determine the LC50 value of clove leaf extract(Syzygium aromaticum) which can kill Aedes aegyti larvae for 24 hours.

METHODS

Design

The research design isexperimentallaboratory with post test only control design group design. Research is divided into2 control groups namely K1 NaCMC 1% as a negative control, K6 Abate and as a positive controland 5 treatment test groups each with a concentration of clove leaf extract K20.010 % w/v, K3 0.025 % w/v, K4 0.05 % b/v, K5 0.075 % b/v and K6 0.100 % b/v.The data measured was the death of Aedes aegypti L Instar III larvae after 24 hours after being treated.The test was carried out with 3 repetitions.

Time and Place of Research

The research was conducted in October - December 2022 at the Pharmacology Laboratory of Pancasakti University, Makassar

Population and Sample

The population of this study were the larvae of insects that cause disease in humans. The sample for this research was Instar III larvae of Aedes aegyptiobtained from entemology laboratory, Faculty of Medicine, Hasanuddin University.

Tools and materials

The tools used were maceration vessels, porcelain cups, measuring cups, measuring pipettes, simplicia drying cabinets, ovens, rotary evaporators, analytical balances, UV-vis spectrophotometers. The materials used were clove leaves (Syzygium aromaticum L) originating from Lemo Village, Polewali City, West Sulawesi, distilled water, 96% ethanol, NaCMC, abate.

Manufacture of Clove Leaf Extract (Syzygium aromaticum)

Clove leaves(Syzygium aromaticum) was collected and washed using running water to separate the adhering dirt and then cut into small pieces to speed up the drying process and then air dried. 250 mg of clove leaf simplicia was weighed and then extracted by maceration using 96% w/v ethanol. Left for 3x24 hours. Then filtered, the filtrate obtained was evaporated in a rotary evaporator until a thick extract was obtained.

Phytochemical Test

Flavonoid Test

Weighed 50 mg of the extract and added 100 ml of hot water, then boiled for 5 minutes and filtered. 0.05 mg of magnesium powder was added to the filtrate as much as 5 ml and 1 ml of concentrated HCl, then shaken vigorously. The magenta red color formed indicates the presence of flavonoid compounds (Harborne, 1996).

Tannin Test

Weighed 50 mg of the extract dissolved in 2 mL of water then added 2 drops of 1% FeCl3 solution. The presence of tannins was indicated by the formation of black-blue and black-green colors (Harborne, 1996)

Saponin Test

The extract was weighed as much as 50 mg then added 10 mL of water and shaken for 1 minute. After that, 2 drops of 1 N HCl were added. The presence of saponins was indicated by the formation foam that remains stable for 7 minutes (Harborne, 1996) .

Distribution of Treatment Groups

This study was divided into 7 treatment groups randomly, namely:

a. The negative control group (K1) consisted of 20 aedes aegypti larvae which were given 1% w/v NaCMC suspension in media
b. Group K2 is20 Aedes aegypti larvae fed clove leaves with concentration0.010 % w/vv in media
c. Group K3 is20 Aedes aegypti larvae fed clove leaves with concentration0.025 % w/vv in media
d. Group K4 is20 Aedes aegypti larvae fed clove leaves with concentration0.05 % w/vv in media
e. Group K5 is20 Aedes aegypti larvae fed clove leaves with concentration0.075 % w/vv in media
f. K6 group is20 Aedes aegypti larvae fed clove leaves with concentration0.10 % w/vv in media
g. Group K7 as the positive control is20 Aedes aegypti larvae given Abate with concentration0.10 % w/vv in media

LC50 determination

20 larvae a*edes aegypti*put into the test solution with a concentration of 0.010% w/v, 0.025% w/v, 0.05% w/v, 0.075% w/v and 0.100% w/v, K6Na. CMC 0.5% w/v and Abate 0.1% w/v

The number of dead larvae was counted after 24 hours after being treated. The criteria for dead larvae at the time of treatment were larvae that did not move and did not respond when touched and larvae that were half dead.The test was carried out with 3 repetitions using the formula:

$P(n - 1) \geq 16$
Where P : Number of treatments
n : Number of repetitions
16 : Constants

The percentage of death of Aedes aegypti larvae was calculated using the following equation:

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100\ \%$$

Abbot's formula is used when any control is off

$$\% \text{ Mortality} = \frac{\% \text{ Mortality in test} - \% \text{ Mortality in control}}{100\ \% \text{ Mortality in control}} \times 100\ \%$$

Then the value of Lethal Concentration 50 (LC50) was calculated using the probit analysis method to determine the toxicity of clove leaf extract to 50% of Aedes aegypti larvae.

Data analysis

Data were analyzed using SPSS (Statistical Product and Service Solution). Furthermore, the data was tested for normality using the Kolmogorov Smirnov Test while the homogeneity of the data was tested using the Lavene Test. If the data is normal and homogeneous, then the analysis is continued using parametric statistics, namely one way ANOVA (ANOVA). Difference) to see the average difference between treatment groups

RESULTS

Phytochemical Test

Phytochemical test results of Clove Leaf Extract (Syzygium aromaticum)can be seen in table 1

Table 1. Phytochemical test results
Clove Leaf Extract (Syzygium aromaticum) can be seen in table 1

Types of Compound Results Conclusion
Greenish yellow tannins +
Flavonoids Red, yellow +
Saponin Foam +

Tannin Test

Based on the results of the tannin test from clove leaf extract with FeCl reagent$_3$0.1% shows a positive test, namely the color of the solution becomes greenish yellow, this occurs because of the reduction reaction.

Flavonoid Test

The flavonoid test is carried out by adding Mg powder and concentrated HCl to produce red, yellow or orange due to the reduction of Mg and concentrated HCl. The test results showed that clove leaf extract positively contained flavonoids

Saponin Test

Saponin test results from clove leaf extract(Syzygium aromaticum)showed positive results which were marked with aquadest reagent showing a positive test, namely the formation of consistent foam for 7 minutes. According to Prihatna (2001), saponins have a characteristic in the form of foam so that when it is reacted with water and shaken with water, foam will form which lasts a long time.

Observation Results of Aedes aegypti Larvae Mortality

Table 2. Results of observations on the mortality of Aedes Aegypti mosquito larvae after administration of clove leaf extract (Syzygium aromaticum) for 24 hours.

Replication	Number of test larvae	Concentration (% w/v)						
		K1 0.01	K2 0.025	K3 0.05	K4 0.075	K5 0.10	K6 NaCMC	K7 Abate
1	20	1	1	4	11	13	0	20
2	20	0	1	5	11	14	0	20

3	20	1	3	5	10	11	0	20
Total dead		2	5	14	32	38	0	60
Average		0.67	1.67	4.67	10.67	12.66	0	20
Percentage (%)		3,33	8.33	23,33	53,33	80	0	100

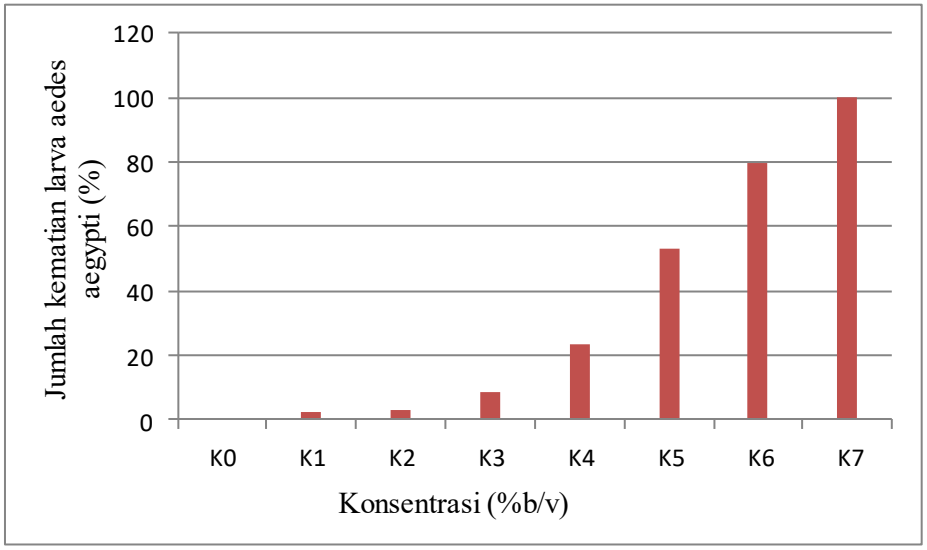

Figure 1. Histogram of the percentage of death of Aedes aegypti larvae in each concentration group

DISCUSSION

Clove leaf extract is made by maceration extraction method. Extraction is a process for separating a mixture of several substances into separate components which include powder preparation, wetting, filtering and concentration (Ansel, 1989; William, 1981). Meanwhile, maceration is a liquid preparation made by soaking vegetable materials using non-water solvents or half-water solvents such as dilute ethanol for a certain time (Marjoni, 2016). until it seeps and softens the cell arrangement so that what is easily soluble will dissolve and this method is good for components that are not heat resistant. The maceration method was chosen because the work and equipment used are simple. The solvent used is ethanol because it has polar properties. Solvents will tend to dissolve active compounds from the same group while semipolar solvents will dissolve some or all of the active compounds which are polar and nonpolar.

The results of the phytochemical test on clove leaf extract (Syzygium aromaticum L.) extracted using 96% ethanol obtained bioactive compounds such as tannins, flavonoids and saponins, while the results of the phytochemical screening test for the ethanol extract of clove leaves (Syzygium aromaticum L.) were obtained by Dewi, CIDY et al (2021) are bioactive compounds of saponins, phenols, terpenoids, glycosides, flavonoids and tannins. According to Septiani et al. (2017) 96% ethanol is widely used as a solvent in the extraction of bioactive compounds because ethanol is easier to penetrate cell membranes to extract intracellular materials from plants so it is well used to extract compounds such as tannins, saponins, terpenoids, phenols and flavonoids. Differences in the results of the phytochemical screening test in several studies could also be influenced by the geographical conditions of the location where clove leaves (Syzygium aromaticum L.) were planted. Clove leaves (Syzygium aromaticum L.) used in this study were taken from Gentuh, Madenan Village, Singaraja. In this study, clove leaves came from Lemo Village, Polewali City, West Sulawesi. Altitude is related to climate, plant temperature, water availability, and adequacy of light in the photosynthesis process which can disrupt physiological functions and plant life cycles. This factor may affect the compounds found in clove leaves. (Lambiju, EM et al. 2017). In this study, clove leaves came from Lemo Village, Polewali City, West Sulawesi. Altitude is related to climate, plant temperature, water availability, and adequacy of light in the photosynthesis process which can disrupt physiological functions and plant life cycles. This factor may affect the compounds found in clove leaves. (Lambiju, EM et al. 2017). In this study, clove leaves came from Lemo Village, Polewali City, West Sulawesi. Altitude is related to climate, plant temperature, water availability, and adequacy of light in the photosynthesis process which can disrupt physiological functions and plant life cycles. This factor may affect the compounds found in clove leaves. (Lambiju, EM et al. 2017).

In the larvicidal effect test, third instar Aedes aegypti larvae were used.The larval stage greatly affects the reaction to toxic substances.The reason for choosing third instar larvae is because these larvae are large enough in size so they are easy to identify besides that third instar larvae are research samples which are WHO standards (2015).Based on the results of research on the larvicidal effect of clove leaf extract on the death of Aedes aegypti larvae, it was found that the higher the concentration of clove leaf extract

So the percentage of death of Aedes aegypti larvae is getting bigger. The results of the research in table 1 and figure 1 show that the death of aeges aegypti larvae after 24 hours began to increase, namely at a concentration of 0.01% b/v; 0.025%w/v; 0.075%w/v; 0.5% w/v each 3.33 %; 8.33%; 23.33; 53.33 and showed the highest mortality at a concentration of 0.10% with a percentage of 80% larval mortality. The mortality rate of the larvae is not

onlyinfluenced by the chemical components contained therein but closely related to the concentration of the extract against Aedes aegypti larvae.These results indicate that clove leaves from Lemo Village, Polewali City, West Sulawesi have a larvicidal effect. According to WHO (2005) The concentration of larvicides is considered effective if it can cause the death of 10 - 95% of the test larvae. To find out whether there is a significant difference in each treatment group's data on larval mortality, the data was analyzed using Analysis of Variance (ANOVA). The results of the data normality test using the Kolmogorov-Smirnov and the data homogeneity test using the Lavene test obtained a p value > 0.05, which means that the data is in the normal group and has a homogeneous distribution. each treatment group on the death of Aedes aegypti larvae. The Post Hoc LSD (Least Square Difference) follow-up test proved that there was a significant difference in each treatment group except K1 0.010% non-significant with K2 0.025% sig p value 0.087 > 0.00. This shows that K1 and K2 have no different effect on the death of Aedes aegypti larvae.

L value*et al*Concentration (LC50) using the probit method is at a clove leaf extract concentration of 0.06% or 600 ppm which can kill 50% of Aedes aegypti larvae and is included in the toxic category.

According to the results of probit analysis, the 24-hour LC50 value of clove leaf extract on the death of Aedes aegypti mosquito larvae was obtained at a concentration of 0.06%, which means that at a concentration of 0.06%, clove leaf extract was able to kill 50% of Aedes aegypti larvae for 24 hours, so it can be said that clove leaf extract is effective against Aedes aegypti larvae.

The LC50 value obtained in this study was different from the LC50 obtained by Haditomo I, who stated that the LC50 of clove leaf extract was 400 ppm (Haditomo, I., 2010.) This difference in LC50 value was influenced by several factors including the place of origin of the plant, methodology, solvent used, extraction method and population used and (Fayemiwo et al., 2014). In this study, clove leaves (Syzygium aromaticum) came from Lemo Village, Polewali City, West Sulawesi, while Haditomo I, came from Tawangmangu Region, Central Java. Differences in where plants grow in each area greatly affect plant growth and plant content. The difference is influenced by several factors such as biological factors which include the identification of plant species, the location of the plant of origin, harvesting period of plant products, storage of plant material and plant age. Chemical factors that can affect the extraction results include internal factors such as the type of active compound in the material, the qualitative composition of the active compound, the quantitative composition of the active compound and the average total content of the active compound. External factors in the form of extraction methods, characteristics of materials and solvents used in extraction (Depkes RI, 2006). Extraction is a process of separating solid and liquid materials with the help of a solvent (Ansel, 1989). The solvent used must be able to extract the desired substance without dissolving other materials. The type of solvent in extraction can affect the level of active substances obtained from plants. Therefore, using the best solvent will further enhance optimization in extraction. Differences in extraction solvents will affect the total content of bioactive compounds (Santoso, et al. 2012). This is due to differences in the polarity of the solvents (Meqha et al. 2014). Based on the results of this study it was found that differences in solvents would cause differences in the percentage of larval mortality even though the concentration of clove leaf extract used was the same. In the use of 96% ethanol, the mortality of Aedes aegypti larvae was obtained respectively: 3.333% for a concentration of 0.025%w/v, 23.33% for 0.050%b/v, 53.33% for a concentration of 0.075%w/v and 80% for concentration of 0.100% w/v with an LC50 value of 600 ppm while in the study of Haditomo.I (2010) which extracted clove leaves with 70% ethanol, the number of larvae deaths was found to be: 35% for 0.025% concentration, 63% for 0.050%, 94% for concentration 0, 075% and 100% for a concentration of 0.100% with an LC50 value of 400 ppm. This is probably due to differences in the concentration of ethanol used in the extraction process. Based on the principle of extraction that the withdrawal of a compound is based on its polarity. 70% ethanol can extract both polar and nonpolar compounds such as alkaloids, flavonoids, saponins, steroids and tannins, while 96% ethanol can extract compounds from the alkaloids, flavonoids, steroids and tannins. Differences in the concentration of ethanol solvent affect its polarity and concentration. active compounds obtained (Riwanti.P et al. 2020). Another factor that can cause differences in LC50 values is differences in extraction methods. Extraction is a process for separating a mixture of several substances into separate components which include the manufacture of powders, wetting, filtering and concentration (Ansel, 1989; William, 1981). Differences in extraction methods will affect the amount of active substance obtained. Percolation is better than the maceration method due to the presence of the solvent which causes a change of solution to occur with a solution of lower concentration, causing differences in the degree of concentration and the presence of spaces between the grains of the simplicia powder to form capillary channels where the liquid of the extractor flows causes an increase in the difference in concentration (Fatmawati, S. , 2019). This is in line with Haditomo's research (2010) which used the percolation method and obtained a higher larvicidal effect compared to the appropriate maceration method used in this study. Differences in extraction methods will affect the amount of active substance obtained. 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The toxic effect on clove leaf extract is probably due to the compounds it contains, namely flavonoids, tannins and alkaloids. flavonoid alkaloids. Symptoms of toxicity in the larvae are reduced movement and then the larvae are under the vessel with tremors and convulsions which then end in death. Tannins act as stomach poisons. Alkaloids are in the form of salts so that they can degrade cell membranes to enter and damage cells and can also interfere with the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme (Cania and Setyaningrum, 2012). Tannin compounds will reduce the activity of protease enzymes in converting amino acids. Cell metabolism processes in the larvae will be disrupted so that the larvae will lack nutrition. In addition, tannins will also bind to proteins in the digestive system that larvae need for their growth. The response of the larvae to this compound is a decrease in growth rate and nutritional disturbances if it continues continuously it will result in the death of Aedes aegypti larvae (Tandi, E., J. 2010; Gautar et al. 2013; NCBI , 2015). Saponins can reduce the activity of digestive enzymes and food absorption, saponins can cause corrosion of the walls of the digestive tract of larvae due to the ability of saponins to damage membranes. Saponins can interfere with the lipoid layer on the epicuticle and the protein layer on the endocuticle, making it easier for toxic substances to enter the body of the larvae (Kusumawati, WD et al. 2018).

To find out whether there is a significant difference in each treatment group's data on larval mortality, the data was analyzed using Analysis of Variance (ANOVA). The results of the data normality test using the Kolmogorov-Smirnov and the data homogeneity test using the Lavene test obtained a p value > 0.05, which means that the data is in the normal group and has a homogeneous distribution. each treatment group on the death of Aedes aegypti larvae. The Post Hoc LSD (Least Square Difference) follow-up test proved that there was a significant difference in each treatment group except K1 0.010% non-significant with K2 0.025% sig p value 0.087 > 0.00. This shows that K1 and K2 have no different effect on the death of Aedes aegypti larvae.

LC50 is the concentration needed to kill 50% of the population of test larvae. L value*et al*Concentration (LC50) using the probit method is at a concentration of clove leaf extract of 0.06% or 600 ppm which can kill 50% of Aedes aegypti larvae and belongs to the toxic category. obtained at a concentration of 0.06% which means that at a concentration of 0.06% clove leaf extract was able to kill 50% of Aedes aegypti larvae for 24 hours so that it can be said that clove leaf extract was effective against Aedes aegypti larvae. The LC50 value obtained in this study was different from the LC50 obtained by Haditomo I, who stated that the LC50 of clove leaf extract was 400 ppm (Haditomo, I., 2010.) This difference in LC50 value was influenced by several factors including the place of origin of the plant, methodology, the solvent used in the extraction process and the population used and (Fayemiwo et al., 2014). In this study, clove leaves (Syzygium aromaticum) came from Lemo Village, Polewali City, West Sulawesi, while Haditomo I, came from Tawangmangu Region, Central Java. Differences in where plants grow in each area greatly affect plant growth and plant content. The difference is influenced by several factors such as biological factors which include identification of plant species, location of plant origin, harvesting period of plant products, storage of plant material and plant age. Chemical factors that can affect the extraction results include internal factors such as the type of active compound in the material, the qualitative composition of the active compound, the quantitative composition of the active compound and the average total content of the active compound. External factors in the form of extraction methods, characteristics of materials and solvents used in extraction (Depkes RI, 2006). Differences in solvents will cause differences in the percentage of larval death. This is in line with the research of Haditomo.I (2010) who extracted clove leaves with 70% ethanol. Increasing the concentration of clove leaf extract caused an increase in the number of larvae deaths, namely: 35% for a concentration of 0.025%, 63% for 0.050%, 94% for a concentration of 0.075% and 100% for a concentration of 0.100%. At the same concentration it turned out to show a different percentage of larval mortality from this study using 96% ethanol, namely 8.33% for a concentration of 0.025%, 23.33% for 0.050%; 80% for a concentration of 0.075% and 80% for a concentration of 0.100%. This is probably due to differences in the concentration of ethanol used in the extraction process. Based on the principle of extraction that the withdrawal of a compound is based on its polarity. 70% ethanol can extract both polar and nonpolar compounds such as alkaloids, flavonoids, saponins, steroids and tannins, while 96% ethanol can extract compounds from the alkaloids, flavonoids, steroids and tannins. Differences in the concentration of ethanol solvent affect its polarity and concentration. active compounds obtained (Riwanti.P et al. 2020).

The toxic effect on clove leaf extract is probably due to the compounds it contains, namely flavonoids, tannins and alkaloids. flavonoid alkaloids. Symptoms of toxicity in the larvae are reduced movement and then the larvae are under the vessel with tremors and convulsions which then end in death (SutthanontN, et al. 2010). Tannins act as stomach poisons, alkaloids in the form of salts so that they can degrade cell membranes to enter and damage cells and can also interfere with the larval nervous system by inhibiting the action of the acetylcholinesterase enzyme (Cania and Setyaningrum, 2012). Tannin compounds will reduce the activity of protease enzymes in converting amino acids. Cell metabolism processes in the larvae will be disrupted so that the larvae will lack nutrition. In addition, tannins will also bind to proteins in the digestive system that larvae need for their growth. The response of the larvae to this compound is a decrease in growth rate and nutritional disturbances if it continues continuously it will result in the death of Aedes aegypti larvae (Tandi, E., J. 2010; Gautar et al. 2013; NCBI , 2015). Saponins can reduce the activity of digestive enzymes and food absorption, saponins can cause corrosion of the walls of the digestive tract of larvae due to the ability of saponins to damage membranes. Saponins can interfere with the lipoid layer on the epicuticle and the protein layer on the endocuticle, making it easier for toxic substances to enter the body of the larvae (Kusumawati, WD et al. 2018).

CONCLUSIONS

Based on the results of research and data analysis, it can be concluded that:

1. Clove leaf extract (Syzygium aromaticum) has a larvicidal effect on Aedes aegypti larvae
2. Clove leaf extract (Syzygium aromaticum) can kill the Aedes aegypti mosquito larvae by

LC50 value of 600 ppm and is included in the toxic category.

SUGGESTION

It is necessary to isolate the active compounds that act as larvicides in clove leaves and to test the toxicity of the ethanol extract of clove leaves on animals and on humans before being used in society.

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