

TOXICITY STUDY OF PARE LEAF EXTRACTS (*MOMORDICA CHARANTIA*) TO *CULEX PIPIENS* MOSQUITO LARVAE

¹*Rahmi Annisa, ² Muhammad Amir, ² Hadi Kuncoro

¹Department of Pharmacy, Faculty of Medicine and Health Sciences
Universitas Islam Negeri Maulana Malik Ibrahim, Malang, Indonesia
² Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia

* Email : apt.rahmiannisa@gmail.com

ABSTRACT

This study aims to determine the yield, toxicity, and toxic concentration of crude extracts of ethanol, n-hexane fraction extract, ethyl acetate fraction extract and n-butanol fractions extract pare leaf (*Momordica charantia*L.) larvae *Culex pipiens*. The method used to determine the toxicity of extract larvasida test larvae *Culex pipiens* to obtain the LC₉₉ values obtained using the analysis of Reed and Muench. Extraction and fractionation processis known to yield a ethanol crude extract obtained 8.86%, n-hexane fraction extract of 1%, ethyl acetate fraction extract 1.80% and n-butanol fractions extract of 0.80% and the toxicity obtained test results that pare leaf (*Momordica charantia* L.) have the potential toxicity larvae larvae of *Culex pipiens* as indicated by the value by Lethality Concentration 99 % (LC₉₉) in the crude extract ethanol 7416.52 ppm, n-hexane fraction extract of 2564.48 ppm, ethyl acetate fraction extract 2702.09 ppm and n-butanol fractions extract of 2665.63 ppm.

Keywords: Pare leaf (*Momordica charantia* L.), toxicity, larvae *Culex pipiens*

INTRODUCTION

Since ancient times the people of Indonesia know and use medicinal plants as one of the efforts to overcome health problems before formal health care with synthetic chemicals touched the community. Utilization of pare leaves as one of the plants that can be used as insecticide mosquito larvae larvae (larvasida) that can be obtained from natural materials.

Larvacide is an ingredient insecticides capable of inhibiting life cycle or killing the larvae stage in their natural habitat or at potential habitat. A mosquito larvaside that effective must have fast work persistent at various breeding places mosquitoes, either on clean water or on polluted water (Haeni, 2008).

Mosquitoes are among the species of insects that receive great attention in human health, because it has the potential as a vector in the transmission of a disease. Insect borne diseases are a common problem in Indonesia. Efforts to control a lot of vector is done

spraying several types of insecticides. The use of chemicals that are less cautious can pose a danger to human health that is shortness of breath when the smell is stinging and allergic to the skin, causing environmental pollution problem, besides synthetic chemicals can cause resistance in mosquito (Araujo, *et al.*, 2006). In order to achieve optimal effort and minimize the impact on the environment, an appropriate method should be developed for different areas of the environment. Therefore it is necessary to find another alternative that is more simple and not detrimental to environmental health (Samsuhidayat and Hutapea, 1991).

MATERIAL AND METHOD

Materials

Pare leaf Extract (*Momordica charantia* L.), aquadest, ethanol, *n*-heksana, etil asetat, *n*-butanol, tween 80, larva nyamuk *Culex pipiens* instar III

Procedure

Sample Preparation leaf of *Momordica charantia* L were collected at Kutai Kartanegara town, Indonesia. The leaves were cleaned and dried. The dried sample was pulverized until 60 mesh.

Extraction of Maseration The air-dried sample (250 gr) was extracted using Ethanol 96% (1000 mL) until 48 hours and filtrated. The residue obtained after extraction was reextracted with Ethanol 96% (1000 mL) or until the filtrat was transparent. The extract were drained and concentrated under reduce pressure using Rotary Evaporator (40-50°C). The ethanol extract was allowed to dry and its constant weight was recorded.

Fractionated extracts performed with liquid-solid fractionation method. In this fractionation will be made *n*-heksana extract fraction, ethyl acetate and *n*-butanol.

Preparation of bio-indicators (*Culex pipiens* mosquito larvae)

Culex pipiens mosquito larvae selected by criteria such as size, shape, and color of the uniform. In the third instar spines and funnel chest began to clear respiratory blackish brown. The test larva used was instar larvae of instar mosquitoes III.

Design of Larvicides Bioactivity Test of Pare Leaf Extract

Table 1. Treatment of variation concentration of pare leaf extract

Replication	Concentration variation Pare Leaf Extract (<i>Momordica charantia</i> L.)				
	1	2	3	4	5
1	KEK ₁ U ₁	KEK ₂ U ₁	KEK ₃ U ₁	KEK ₄ U ₁	KEK ₅ U ₁
2	KEK ₁ U ₂	KEK ₂ U ₂	KEK ₃ U ₂	KEK ₄ U ₂	KEK ₅ U ₂
3	KEK ₁ U ₃	KEK ₂ U ₃	KEK ₃ U ₃	KEK ₄ U ₃	KEK ₅ U ₃

Information

KEK₁U₁ : Treatment of the first crude extract concentration on the first replay

KH₁U₁ : Treatment concentration n-hexane fraction first in first repeat

KEA₁U₁ : Treatment concentration of ethyl acetate fraction first in first repeat

KB₁U₁ : Treatment concentrations of n-butanol fraction first in first repeat

Larvicides bioactivity test of Pare Leaf Extract to *Culex pipiens* mosquito larvae

Entered 10 *Culex pipiens* mosquito larvae was added to the vial containing water up to 10 mL. In each vial was treated differently according to the prepared test group then covered with aluminum foil and given a small hole above it. Observed, noting the number of *Culex pipiens* mosquito larvae mortality after 24 hours. LC₉₉ values are calculated crude extract ethanol, n -heksana fraction, of ethyl acetate and n -butanol fraction pare leaf (*Momordica charantia* L.) to *Culex* mosquito larvae mortality *pipiens*.

Analysis of Data The data was described with table and graph. The lethal dose of 99% (LD₉₉) was determined by Reed and Muench method.

RESULTS AND DISCUSSION

Extract and Fractionation of Pare Leaf Extract

Pare leaf extract (*Momordica charantia* L.) is a leaf pare that has beeb extracted by maceration by maceration method using ethanol andwith bulbs of 250 g. The maceration process causes the solvent to penetrate the cell wall and into the cell cavity containing the active substance. The active substance will dissolve together with organic solvent in the presence of different concentrations between the active substance solution inside the cell and outside the cell, then the concentrated solution will be pushed out. The event will be repeated

resulting in a concentration equilibrium between the solution outside and inside the cell. After conducting the subsequent sampling, the concentration of the sample solution which is aimed to obtain the concentrated extract. Concentration is done by using *a rotary evaporator*. This method aims to compounds Metab secondary olit not damaged by high temperatures. To avoid the use of temperature on the concentration used process vacuum pumps, resulting in reduced pressure and the solvent will evaporate at a temperature below its boiling point (Dervish, 2000).

Extracts were obtained from 250 g dry sample is 22:16 g, so that can know the amount of the yield obtained is at 8.86. To identify the components of a mixture based on the level of the polarity of a compound fractionation process is carried out. The purpose of this fractionation is to see bandage dengan pare leafextract bioactivity larvicides against the mosquito larvae *Culex pipens*. Rendemen analysis faction aims to know the percentage of fraction produced from the extract by fractionation method. Results yield extracts and fractions can be seen in Table 2 the following:

Table 2. The results yield leaf extract of pare leaf extract (*Momordica charantia* L.)

No	Extract	Extract Weight(g)	Rendemen I (%)	Rendemen II (%)	Rendemen III (%)
1	Ekstrak etanol	22.16	2.67	8.86	-
2	Fraksi <i>n</i> -heksana	2.50	0.30	1.00	13.16
3	Fraksi Etil asetat	4.50	0.54	1.80	23.68
4	Fraksi <i>n</i> -butanol	2.00	0.24	0.80	10.53

Larvicides bioactivity test of Pare Leaf Extract to *Culex pipiens* mosquito larvae

To determine the bioactivity of larvicides leaf extract of pare leaf extract (*Momordica charantia* L.) then tested the mosquito larvae mortality with the use of acute toxicity tests. This test is designed to determine the toxic effect of a compound that will occur in a short time or administration with a certain dose (Department of Therapeutic Pharmacology, 2000). This test is a toxicology bioactivity test that aims to determine the biological activity of the extract or fraction of plants by observing the death response in experimental animals. The death of the experimental animal is considered as a response to the influence of certain compounds. This test is important to obtain information regarding the concentration relationship with death response of experimental animals (Colagate and Russel, 1993).

Test the efficacy of the ethanol extract of pare leaf extract (*Momordica charantia* L.) against larvae of *Aedes aegypti* has been investigated by Lianawati (2008). The results of these studies conclude that the leaf extract of pare leaf extract (*Momordica charantia* L.) has the ability to kill mosquito larvae of *Aedes aegypti* with LC₅₀ value of 654.24 ppm and the LC₉₀ for 1588.40 ppm. This study aimed at testing the rough ethanol extract, extract fraction of *n-hexane*, ethyl acetate fraction extract and extract of *n-butanol* fraction of the *Culex pipiens* mosquito larvae.

This study uses the mosquito *Culex pipiens* larva the third instar. First instar larvae are stable and resistant to changes in temperature and humidity (Hanti, 1996). The mosquito larvae in the third instar is more perfect than the physiological and morphological third instar larvae thus more resistant to the materials of larvicides. Therefore it can be concluded that the concentration that kills 100% of larvae of mosquitoes instar III also can kill mosquito larvae instar III and not vice versa.

Results of testing the bioactivity of larvicides ethanol crude extract obtained from the leaves of bitter melon five concentration variations with 3 repetitions. The test results are shown in Table 3 :

Table 3. The results of bioactivity test larvicides ka sar ethanol extract of the 1 pare leaf extract (*Momordica charantia* L.) against *Culex pipiens* mosquito larvae

Concentration	Log Concentration	Amount		Accumulate		Ratio to	Mortality
		Die	Life	Die (x)	Life (y)	tally dead	(%)
						Accumulate	Ratio x
						x : (x+y)	100 %
3750 ppm	3.57	9	21	9	45	0.1667	16.67
5000 ppm	3.69	15	15	24	24	0.5000	50.00
6250 ppm	3.79	21	9	45	9	0.8384	83.84
7500 ppm	3.87	30	0	75	0	1	100
8750 ppm	3.94	30	0	105	0	1	100

In Table 3 above shows the test results crude extract ethanol, in which the lowest test concentration of 3750 ppm has been giving activity in killing mosquito larvae *Culex pipiens* and the high concentration of 8750 ppm level *Culex pipiens* mosquito larvae mortality give effect to kill all the test animals. This is in accordance

with Harborne (1987) which states that the higher the concentration is given, the higher the death rate bioindikator.

The influence of the concentration of ethanol crude of pare leaf extract (*Momordica charantia* L.) the death of *Culex pipiens* mosquito larvae in shows that their effect on mortality of *Culex pipiens* concentration of crude extract ethanol. The results of test analysis using the method of Reed and Muench LC₉₉ values obtained ethanol crude extract of 7416.52 ppm. The increase of extract concentration is directly proportional to the number of test animals that died and vice versa inversely to the number of live test animals. Increasing concentrations of ethanol crude extract of leaves of bitter melon (*Momordica charantia* L.) provides an increased ability to kill mosquito larvae *Culex pipiens*, this is because the more concentrated solution concentration, the more substances contained in the leaf extract of pare leaf extract (*Momordica charantia* L.), which means the more toxins consumed *Culex pipiens* mosquito larvae, so the number of mosquito larvae mortality is also higher. This is in accordance with Nurhayati (2005) states the more concentrated solution concentration means more active ingredient which can interfere with the metabolism of the test animals.

Larvicides bioactivity test of Pare Leaf fraction *n*-hexane to *Culex pipiens* mosquito larvae

Results bioactivity test of Pare Leaf fraction *n*-hexane to *Culex pipiens* mosquito larvae can be seen in Table 4 :

Table 4. Bioactivity test of Pare Leaf fraction *n*-hexane to *Culex pipiens* mosquito larvae

Concentration	Log Concentration	Amount		Accumulate		Ratio totally dead	Mortality %
		Die	Life	Die (x)	Life (y)	Accumulate x: (x + y)	Ratio x 100%
750 ppm	2.87	18	12	18	29	0.2429	38.29
1250 ppm	2.99	21	9	39	17	0.6964	69.64
1750 ppm	3.24	25	5	64	8	0.8887	88.87
2250 ppm	3.35	27	3	91	3	0.9681	96.81
2750 ppm	3.44	30	0	121	0	1	100

Effect of Pare Leaf fraction *n*-hexane the death of *Culex pipiens* in shows that increasing the concentration of *n*-hexane fraction is directly proportional to the number of test animals that die and instead is inversely proportional to the number of test animals were alive. Increasing concentrations of *n*-hexane fraction leaves of Pare Leaf fraction *n*-hexane improve the ability to kill mosquito larvae of *Culex pipiens*. Analysis using the method of Reed and Muench LC₉₉ values obtained *n*-heksana extract fraction of 2564.48 ppm.

Larvicides Bioactivity test of Pare Leaf Fraction Ethyl Acetate to *Culex pipiens* mosquito Larvae

Results bioactivity test of Pare Leaf fraction *ethyl acetate* to *Culex pipiens* mosquito larvae can be seen in Table 5 :

Table 5. Bioactivity test of Pare Leaf fraction *Ethyl Acetat* to *Culex pipiens* mosquito larvae

Concentration	Log Concentration	Amount		Accumulate		Ratio totally dead	Mortality %
		Die	Life	Die (x)	Life (y)	Accumulate x: (x + y)	Ratio x 100%
750 ppm	2.87	6	24	6	72	0.0769	7.69
1250 ppm	2.99	9	21	15	48	0.2380	23.80
1750 ppm	3.24	15	15	30	27	0.5263	52.63
2250 ppm	3.35	18	12	48	12	0800	80.00
2750 ppm	3.44	30	0	78	0	1	100

Effect of Pare Leaf fraction *ethyl acetate* the death of *Culex pipiens* in shows that increasing the concentration of *ethyl acetate* fraction is directly proportional to the number of test animals that die and instead is inversely proportional to the number of test animals were alive. Increasing concentrations of *ethyl acetate* fraction leaves of Pare Leaf fraction *ethyl acetate* improve the ability to kill mosquito larvae of *Culex pipiens*. Analysis using the method of Reed and Muench LC₉₉ values obtained *ethyl acetate* extract fraction of 2702.09 ppm.

Larvicides bioactivity test of Pare Leaf fraction *n*-butanol to *Culex pipiens* mosquito larvae

Results bioactivity test of Pare Leaf fraction *n*-butanol to *Culex pipiens* mosquito larvae can be seen in Table 6 :

Table 6. Bioactivity test of Pare Leaf fraction *n*-butanol to *Culex pipiens* mosquito larvae

Concentration	Log Concentration	Amount		Accumulate		Ratio totally dead	Mortality %
		Die	Life	Die (x)	Life (y)	Accumulate x: (x + y)	Ratio x 100%
750 ppm	2.87	9	21	9	60	0.0869	8.69
1250 ppm	2.99	12	18	21	39	0.3500	35.00
1750 ppm	3.24	15	15	36	21	0.6316	63.16
2250 ppm	3.35	24	6	60	6	0.9091	90.91
2750 ppm	3.44	30	0	90	0	1	100

Effect of Pare Leaf fraction *n*-butanol the death of *Culex pipiens* in shows that increasing the concentration of *n*-butanol fraction is directly proportional to the number of test animals that die and instead is inversely proportional to the number of test animals were alive. Increasing concentrations of *n*-butanol fraction leaves of Pare Leaf fraction *ethyl acetate* improve the ability to kill mosquito larvae of *Culex pipiens*. Analysis using the method of Reed and Muench LC₉₉ values obtained *ethyl acetate* extract fraction of 2665.63 ppm.

Based on these descriptions, it is known that the crude extract of ethanol and the three extract fractions tested had as larvicidal activity, it is in accordance with Dalimartha (2008), which explains that bitter melon plants can be used to kill insects. Pare leaf extract and the fractions have the activity level is different, the difference in larvicidal activity of extracts and fractions caused some fractionation process wherein the compound contained in the extract chemical separation based on the degree of polarity so as to provide a different larvicidal activity.

The content of compounds in bitter melon leaves are flavonoids, alkaloids, saponins and terpenoids (Syamsuhidayat and Hutapea, 1991). Based on the study h acyl phytochemical test extracts or extract fractions pare leaf by Hernawati (2006) in his research on the potential p acre fruit (*Momordica charantia* L.) as a herbal ntifertilitas show that ethanol crude extract contains alkaloids, saponins, flavonoids, and triterpenes oid. Fraction *n*-hexane containing triterpenoids, flavonoids containing ethyl acetate and *n*-butanol containing flavonoids.

Fortunately (1993) and Kristanti (2008) explains that the insecticide can get into the insect's body through various ways, among others: as a stomach poison (*stomach poison*) that goes into the insect's body through the digestive tract of insects, gastrointestinal tract mosquitoes have a structure such as a wall body. Thus the absorption of insecticides in the digestion equals absorption of the body wall. Contact poison (*contact Poisoning*) that enter through the skin or body wall, finding body part that can absorb large amounts of insecticide. The body wall has a base layer that are semipermeable membrane so that it can choose the types of compounds that can pass through and the last one *fumigant* or breathing into the insect's body through the respiratory system. The respiratory tract is called the trachea. Air and oxygen enter the trachea by diffusion assisted by abdominal movement. Oxygen will be directly related to the network. Insecticides enter the respiratory system in the form of gas or fine grains that are brought to the living tissue.

Function alkaloids, triterpenoids, saponins and flavonoids in the leaves of bitter melon can hinder eating larvae (*antifedant*). These compounds can enter the digestive through immersion extract concentration ingested by the larvae of the mosquito *Culex pipiens*, then enter the digestive tract and are absorbed by the intestinal wall and then circulate along the blood that would interfere with the metabolism of the body of the mosquito larvae *Culex pipiens* that lack of energy for activities that lead to Mosquitoes finally die. The workings of these compounds is to act as *stomach poisoning* or stomach poison. Therefore, if these compounds are included in the larval body, it can be disturbed digestion (Cahyadi, 2009).

CONCLUSION

The crude extract of bitter melon leaves ethanol and fractions have an activity as larvicides against the mosquito larvae *Culex pipiens* the LC₉₉ value of each is at 7416.52 ppm ethanol extract, extract *n*-heksana fraction 2564.48 ppm, extract ethyl acetate fraction 2702.09 ppm and extract *n*-butanol fraction 2665.63 ppm.

ACKNOWLEDGEMENTS

The development of this plant needs to be done because this plant is easy to find and the number is many. Extract and pure leaf extract fraction having as larvicidal activity. Extract fraction *n*-heksana known to have the most excellent larvicidal activity, these results can be used as a reference in use are the most excellent than extract as larvicides for further research so that the development of further research on bitter melon leaves that leads

to the use of these crops is necessary, the isolation of pure compound From leaf pare is a way of development further to do in the future so that it can be used as a natural larvicidal derived from leaf extracts of bitter melon (*Momordica charantia* L.).

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