The effectiveness of dichloromethane extract of various plants on eggs hatchability, and life cycle of *Aedes aegypti* L. mosquitoes

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Abstract. The research in effectiveness of dichloromethane extract of Eggs Hatchability, the life cycle of larvae-pupae, pupae-adult, phytochemical screening of bitter gourd leaf (*Momordica charantia* L.), basil leaf (*Ocimum basilicum* L.), and lemon grass (*Cymbopogon winterianus* Jowitt.,) has been conducted using Completely Randomized Factorial Designs, 3 times iterations, concentration (0, 200, 400, 600, 800, 1000 ppm) on 25 eggs of *Aedes aegypti* and were analyzed using SPSS release 23 computer application. The result showed that the Eggs hatchability rate of eggs-larvae and the survival rate of larvae-pupae experienced a decrease of 4%-40% and 16%-64% respectively. However this does not effect the life cycle of pupae-adult. The decrease of eggs hatchability, survival rate of larva-pupae, pupae-adult is greater in the extract of bitter gourd leaf (*M. charantia*), and basil (*O. basilicum*) than in the extract of lemon grass (*C. winterianus*). The result of phytochemical screening showed that there are 1 secondary metabolite compounds contained in bitter gourd leaf (saponin), 1 in basil leaf(tanin), 3 in lemon grass (flavonoid, tanin, saponin).

1. Introduction

Dengue fever cases in Indonesia tend to increase every year, including North Sumatra province. Started from 5.688 cases with 44 of them were pronounced dead in 2015 to 7.777 cases in 2016 with 48 victims died. In North Sumatra, Medan has the most numbers of dengue fever cases at 1470 and 7 died in 2015, 1540 and 10 died in 2016 [7]. The higher occurrence rate of dengue fever in Medan is due to the rapid development of industries, all the small, medium and large industries, and educational institutions in Medan, forging Medan as the favorable destination for job seekers and as the center of education for the countryside citizens of North Sumatra. This certainly causes the high rate of population growth in Medan. Unfortunately this is not followed by the adequate residence development for the citizens which resulted the decrease in quality of the citizen health.

To overcome the epidemic of dengue fever in Medan, the government has tried numerous precaution activities, one of which is conducted mosquitoes larvae control program using synthetic insecticides Temephos (Abate) which is larvicides. The frequent use of temephos (abate) is thought to have caused
resistance to the mosquito *A. Aegypti*. The reports of this resistance were came from, Tamil Nahdu district, India [12], Mansehra district, Pakistan [11] and Jakarta, Indonesia [14].

Alternative insecticide, such as botanical insecticide in the form of chemical compounds contained in the plants, is required to overcome the issue [2, 3, 12, 16]. Botanical insecticides can be toxic, inhibit food, and inhibit the plants growth with different performances, and it will reduce the possibility of resistance occurrence and the environmental damage [13]. Bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*) and lemon grass (*C. winterianus*) are the potential plants whose chemical compounds can be used as insecticides [5].

2. Materials and Methods

2.1 Experimental Animals

This research used the experimental eggs of *A. Aegypti* obtained from Center for Environmental Health and Infectious Disease Control Medan.

2.2 Research Design

The effectiveness test of dichloromethane extracts of, bitter gourd (*M. charantia*), basil (*O. basilicum*) and lemon grass (*C. winterianus*) on eggs hatchability and life cycle of *A. aegypti* mosquitoes conducted using Completely Randomized Factorial Designs with 3 times of iteration. It is consisted of 2 factors, which are Factor (Plants type: bitter gourd (*M. charantia*), basil (*O. basilicum*), lemon grass (*C. winterianus*)), and (Extracts Concentration: 0, 200, 400, 600, 800, 1000 ppm).

2.3 Procurement of dichloromethane extract of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), and lemon grass (*C. winterianus*)

The ethanol extract of bitter gourd (*M. charantia*), basil leaf (*O. basilicum*), and lemon grass (*C. winterianus*) were macerated using hot water with a temperature of 40°C. The solvent was then filtered to form a filtrate. Then filtrate was inserted into separating funnel and participated by adding dichloromethane solvent multiple times until it showed the original color. The dichloromethane liquid extract obtained was evaporated with rotavapor (40°C) to obtain a dichloromethane bitter gourd leaf (*M. charantia*) extract, basil leaf (*O. basilicum*), and lemon grass (*C. winterianus*) [6].

2.4 Test Parameter Observation Chemical compound test of dichloromethane extract of bitter gourd (*M. charantia*), basil (*O. basilicum*), and lemon grass (*C. winterianus*)

Qualitative tests using the compounds of saponin, tannins, alkaloids, terpenoids, and flavonoids were conducted to identify the chemical compound of dichloromethane extract of bitter gourd (*M. charantia* L.), basil (*O. basilicum* L.), and lemon grass (*C. winterianus* Jowitt.) [6].

2.5 Phytochemical screening

2.5.1 Saponin

Dichloromethane extract of bitter gourd (*M. charantia*), basil (*O. basilicum*), lemon grass (*C. winterianus*) were diluted up to 10 times with hot water, filtered, shaken for five minutes, left then seen whether the foam is formed or not. If the foam formed, it may contain Saponin. The test is continued by adding a few drops of concentrated sulfuric acid into the formed foam. The existence of saponin
compound can be identified qualitatively if the formed foam is stable after a few drops of concentrated sulfuric acid.

2.5.2 Tannin

Dichloromethane extract of bitter gourd (M. charantia), basil (O. basilicum), lemon grass (C. winterianus) were diluted up to 10 times with hot water, and filtered into the filtrate with addition of gelatin. The existence of tannin compounds can be identified if white sediment is formed after few drops of gelatin.

2.5.3 Alkaloid

Dichloromethane extract of bitter gourd (M. charantia), basil (O. basilicum), lemon grass (C. winterianus) (4 drops) put each 2 drops into 2 drop plate. The first plate with 2 dichloromethane extract drops of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus) + Dragendorff reagents formed orange to brownish red sediment, while the second plate with 2 dichloromethane extract drops of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus) + Mayer reagents formed white sediment.

2.5.4 Terpenoid

Dichloromethane extract of bitter gourd (M. charantia), basil (O. basilicum), lemon grass (C. winterianus) + 2 drops in addition of 2-3 drops of anhydride acetic acid (AC2O) in one, and 1-2 drops of H2SO4 in the other hole as the comparison. The part which added with AC2O was stirred gently until it dried then added 1-2 drops of concentrated H2SO4. Green or bluish green coloring gives an indication of terpenoids.

2.5.5 Flavonoid

Dichloromethane extract of bitter gourd (M. charantia), basil (O. basilicum), lemon grass (C. winterianus) + 2 drops were put into the drop plate + 2 drops of concentrated HCl + magnesium powder. If it gives the orange color, it indicates the existence of flavonoid.

2.5.6 Dichloromethane extract tests of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus) in A. Aegypti eggs hatchability and life cycle

The test on eggs hatchability was conducted by setting 6 ethanol extract concentrates of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus) under LC50 concentration and one control, each with 3 iterations. In the glass, added 100 ml of solvent of each dichloromethane extract concentrate of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus) then the experimental 25 eggs were added into the solvent and observed 24 hours in 15 days. Later, the number of eggs-larvae, larvae-pupae, and pupae-adult were observed.

2.6 Data Analysis

Data collected and analyzed by Anova level of 5% is in bootstrap with SPSS 23.
3. Results and Discussion

From the research of dichloromethane extract effectiveness of various plants on eggs hatchability and development of *Aedes aegypti* mosquitoes, obtained results as shown in the following table and figure: From Figure 1, 2, and 3, it is seen that the eggs hatchability and the life cycle of *A. aegypti* mosquitoes (the development of larvae-pupae, pupae-adult) are inhibited. The inhabitation occurs on all treatment concentration and is directly proportional to the increase in dichloromethane extract concentration of all three types of plants, i.e. bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), lemon grass (*C. winterianus*).

The value of eggs-larva hatchability for all treatment concentration in the dichloromethane extract of bitter gourd is at the range of 15-23 (60%-92%), basil at 16-23 (64%-92%), lemon grass at 17-24 (68%-96%). Generally, when compared to control, there was a decrease in the number of eggs developed into larvae (4.0%-40.0%) i.e. bitter gourd (8.0%-40.0%), basil (8.0%-34.0%) and lemon grass (4.0%-32.0%). However, the treatment between concentrations in one plant was not significantly different. The impact caused by extract of bitter gourd was similar to basil and lemon grass, however the downturn caused by the extract of bitter gourd and basil was greater than the one caused by the extracts of lemon grass (Figure 1).

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Eggs hatchability (Egg-larvae) of *A. aegypti* in the dichloromethane extract of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), lemon grass (*C. winterianus*)

Note: BG = Bitter Gourd, B= Basil, LG= Lemon Grass. Graphic with the same letter notation towards the same and different group does not differ significantly with Tukey test at level 5%.

The maturation value of larvae-pupae of all treatment concentration in the extract of bitter gourd is at the range of 8-21 pupae (32.0% - 80.0%), basil at 9-21 (36.0%-84.0%), lemon grass at 9-22 (36.0%-88.0%). In general, when compared to control, there was a decrease in number of larvae-pupae development (16.0%-64.0%) i.e. bitter gourd (16.0%-64.0%), basil (16.0%-68.0%) and lemon grass (12.0%-64.0%). However, the treatment between concentrations in one plant was not significantly different. The impact caused by extract of bitter gourd was similar to basil and lemon grass, however the downturn caused by
the extract of bitter gourd and basil to the development of larvae-pupae was greater than the one caused by
the extracts of lemon grass (Figure 2).

Figure 2. Development (larvae-Pupae) of A. aegypti in the dichloromethane extract of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus)

Note: BG = Bitter Gourd, B= Basil, LG= Lemon Grass. Graphic with the same letter notation towards the same and different group does not differ significantly with Tukey test at level 5%.

The development value of pupae-adult of all treatment concentration in the extract of bitter gourd is at the range of 8-21 (100%-100%), basil at 9-21 (100%-100%), lemon grass at 9-22 (100%-100%) (Figure 3). In accordance with the data, it shows that generally, there was no decrease in the number of pupae developed into adults in the extract of bitter gourd leaf (M. charantia), basil leaf (O. basilicum), lemon grass (C. winterianus), indicating that all pupae were successfully transformed into adulthood in all treatment concentration.
Figure 3. Development (Pupae-Adult) of *A. aegypti* in the dichloromethane extract of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), lemon grass (*C. winterianus*).

Note: BG= Bitter Gourd, B= Basil, LG= Lemon Grass. Graphic with the same letter notation towards the same and different group does not differ significantly with Tukey test at level 5%.

Based on Figure 1 2 and 3, it can be seen that there is a difference in mortality and inhibition of *A. aegypti* development ie: eggs hatchability, development of larvae-pupae, and pupae-adult of *A. aegypti* in the extracts of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), and lemon grass (*C. winterianus*). This may be caused because there is a difference of secondary metabolite content in the plants. The number of secondary metabolite content found in the plants are: 1 in bitter Gourd (saponin), 1 in basil (Tanin), 3 in lemon grass (flavonoid, tanin, saponin) (Table 1). As explained by [11], Secondary metabolites of plants as self-defense can give different effects towards insect among others are toxic and inhibit insect reproduction. Mortality and inhibition of *Aedes aegypti* development also occurs in *Callitris glaucophylla* extract [4], *Moringa oleifera* seed extract [10], *Ipomoea cairica* extract [17].

In this research (Figure 2), it can also be seen in all treatments that inhibition was occurred in the development of larvae into pupae, but if the larvae was successfully developed into pupa, then the pupa will transform to adulthood by 100%. Meaning larval mortality occurs when it develop into pupae, but larva mortality is not occurs in pupae when it develop into adult, it happens because the given treatment concentration has caused cell death (apoplosis) in larvae, but has not caused cell death (apoplosis) at the pupa level. As described by [8], *Momordica charantia* seed extract and DMPA at high concentrations can cause cell death (apoplosis).

The capability of the dichloromethane extracts of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*), lemon grass (*C. winterianus*) to inhibit fecundity (egg-larvae) and development of larvae-pupae, pupae-adult may have an impact as growth hormone on insect growth regulating hormone. Halophenozide molting hormone (RH-0345) [1].
Table 1. Phytochemical Test Result of The dichloromethane Extract of bitter gourd leaf (*M. charantia*),
basil leaf (*O. basilicum*), lemon grass (*C. winterianus*).

| Phytochemical Test | *M. charantia* | *O. basilicum* | *C. winterianus* |
|--------------------|----------------|----------------|------------------|
| Flavonoid          | -              | -              | +                |
| Alkaloid           | -              | -              | -                |
| Terpenoid          | -              | -              | -                |
| Tanin              | -              | +              | +                |
| Saponin            | +              | -              | +                |

Note: + : Detected, - : Not Detected

From Table 1, it can be seen the number of secondary metabolite contents contained in various
plants, which are: 1 in bitter gourd (saponin), 1 in basil (Tanin), 3 in lemon grass (flavonoid, tannin,
saponin) the differences will certainly affect the eggs hatchability and the life cycle of *A. Aegypti*.

4. Conclusions

From the research of dichloromethane extract effectiveness of various plants that are that potential to be
botanical insecticides against *A. aegypti* mosquitoes, can be deduced as follows:

- All dichloromethane extract treatments of bitter gourd leaf (*M. charantia*), basil leaf (*O. basilicum*),
  lemon grass (*C. winterianus*) caused a decrease on eggs hatchability (eggs-larvae) (4-40%), and larvae-
pupae development of (16-64%), this did not affect on pupae-adult development.
- The decrease on the eggs hatchability and the development of larvae-pupae, pupae-adult caused by the
dichloromethane extract of bitter gourd (*M. charantia*) and basil (*O. basilicum*) is greater than by the
dichloromethane extracts of Lemon Grass (*C. winterianus*).
- There is differences in the plants secondary metabolite contents, i.e. 1 in bitter gourd leaf (saponin), 1
  in basil leaf (Tanin), and 3 in lemon grass (flavonoid, tannin, saponin).

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