CHAPTER 6

DISCUSSION

6.1 Active ingredients in *Acalypha indica linn* root

The *Acalypha indica linn* root contains active ingredients flavonoids and saponins (Rajaselvam et al., 2008). During the study, a qualitative test was conducted to prove the presence of the saponin and flavonoid in the *Acalypha indica linn* root extract. The saponins presence was tested by treating the extract with hydrochloric acid(HCL) and 3 drops of olive oil. The presence of saponin is proven when there was small bubbles and colour changes(greenish yellow) in the dark green extract. The flavonoid was tested by adding magnesium powder(0.5 g),1ml hydrochloric acid(HCL) and amyl alcohol. The positive result is seen when the extract colour turns orange in the amyl alcohol layer. Hence, the presence of saponin and flavonoid is confirmed in the *Acalypha indica linn* root extract. The active ingredient flavonoids, inhibits development of the egg by entering into the egg at polygonal points on the egg surface through process of diffusion. Flavonoid is also capable of affecting the insulin-like peptide hormone known as ILP and an ovary ecdysteroid-ogenic hormone known as OEH,which helps and protects eggs to develop into larvae (Gonzales, 2015). Saponin will also inhibit the the growth of the *Aedes aegypti* eggs by lysing the cytoplasma membrane until the serous layer is damaged (Xu, 2006).

6.2 Ovicidal activity of *Acalypha indica linn* root

The research was conducted to prove that the root extract of *Acalypha indica linn* has ovicidal effect against *Aedes aegypti* eggs. The roots of *Acalypha indica linn* is used in this research because it is often used for herbal treatment for diarrhea and its root extract can be rapidly degraded so it is less harmful to human health as accumulation of the substance does not occur. The extract in this study is used with ethanol. The use of natural materials as ovicide is selected because it is less dangerous to use in the community to kill the eggs of *Aedes aegypti* (Dalimartha, 2002). In this study, positive control(abate) showed the highest percentage of ovicidal activity but the 2.0% of root extract is still found to be very close to the value of the positive control. This shows the *Acalypha indica linn root* extract still can be used as a bioovicide.

6.3 The effect of *Acalypha indica linn* root on *Aedes aegypti* egg morphology

In the 48 hours trials conducted, *Aedes aegypti* eggs are damaged in both the 0.5% extract and 2.0% extract but their differences can be clearly seen in the depth of damage. The egg seen in 0.5% and 1.0% are just damaged at their exochorion layer and their outer layer looks smoother. However when left in 1.5% *Acalypha indica linn* root extract, the *Aedes aegypti* egg tend to have a sharp ridge where the outer layer undergoes lysis and have disappeared. Meanwhile in the 2.0% extract, the egg is damaged further into the endochorion layer and the exochorion layer tend to disappear or becomes very light brown colour.

In this experiment, well water is selected as a negative control based on research conducted by Santoso and Sayono (2007) which proved that *Aedes aegypti* lays more eggs on ovitrap containing well water than on rainwater. In this study, well water is used to dilute the *Acalypha indica linn* root extract. The results obtained in the negative control is, almost 80% of *Aedes aegypti* eggs hatch after 48 hours of observation. The positive control test(by giving abate 10%) is done to compare the results with the *Acalypha indica linn* root extract.

The variation in number of unhatched eggs in each trials with same concentration occurs due to the difference in sensitiveness and resistancy of each *Aedes aegypti* egg to toxic substance. Resistancy occurs because *Aedes aegypti* and other dengue vectors are able to develop immunity against insecticides which are often used. Some studies also indicate the existence of cross-resistance, the emergence of resistance to an insecticide through exposure of other insecticides (Soegijanto, 2004).

6.4 Elaboration of the data analysis

Based on the Kolmogorov-Smirnov test, data on the number of unhatched *Aedes aegypti* eggs is not normal. Meanwhile Levene test revealed the data on number unhatched *Aedes aegypti* eggs has a range that is not homogeneous, so the one way ANOVA test cannot be used, instead the statistical analysis is done using Kruskal Wallis test. The Kruskal Walis test is used to know if there is difference in effect of *Acalypha indica linn* root extract against the number of *Aedes aegypti* eggs that did not hatch. The criteria used for Kruskal Wallis test is when the probability value is lower than level of significance($p < 0.05$) or value of

Chi Square test ≥ Chi Square$_{table}$ (7.815), there is a minimal of one group of *Acalypha indica linn* root concentration which is significantly different. Based on the Kruskal Wallis test, we know that the *Chi Square* value 51.392 is bigger than 7.815 and probability is 0.000 which is lower than 0.05, thus proving there is a minimal of one group of *Acalypha indica linn* root concentration which is significantly different. The analysis result is continued with *Bonferroni test* to determine the group of *Acalypha indica linn* root concentration against number of unhatched *Aedes aegypti* eggs which is significantly different. Bonferroni Test is used when the root extract of *Acalypha indica linn* produces a lower probability than the level of significance (p = 0.05). Based on the probability and notation of Bonferroni test table, we can see that 2% concentration of *Acalypha indica linn* root shows the difference significantly. Thus analysis shows, that the *Acalypha indica linn* root extract of 2% resulted in the highest number of unhatched *Aedes aegypti* eggs and significantly different from the eggs in *Acalypha indica linn* root extract of 0.5%, 1%, 1.5%, control positive and control negative. Meanwhile, provision of well water(negative control) resulted in the lowest number of unhatched(damaged) *Aedes aegypti* eggs which differs significantly from the 2% *Acalypha indica linn* root extract, but didn't differ significantly from 0.5%, 1%, 1.5%, *Acalypha indica linn* root extract and control positive. The number of unhatched eggs from abate is higher when compared with number of unhatched eggs from 2% Acalypha indica linn root extract because the concentration of extract used is still not sufficient to cause a higher effect than the abate.

In another study conducted, using the extract from trifoliate orange against dengue vectors, the ovicidal activity was observed in 100 mg/l for 3hours and at 200 mg/l for 15-18 hours.The flavonoid compound from the trifoliate orange was

tested and found to be in very high level. In this investigation, the ovicidal activity of trifoliate orange against Culex. *quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* depended on the factors like concentrations of the extract, age of the eggs and mosquito species involved in the manifestation of ovicidal activity (Rajkumar et.al, 2008).

Based on the results obtained in the study and analysis from above data, it can be concluded that the *Acalypha indica linn* root extract has a moderate bioovicidal effect. Further test should also be done on the eggs of Culex and Anopheles due to the lack of information on the ovicidal activity of *Acalypha indica linn* root extract against the eggs of this mosquitoes. This suggests that further studies need to be done on the working mechanism of *Acalypha indica linn* root extract so that the results can be applied in current society.

6.5 The limitation of this study

When conducting this study, we had few limitations where we could not conduct the quantitave test to see the level of saponin and flavonoid present in the *Acalypha indica linn* root extract due to the small sample of extract available.