

## THE BIOOVICIDAL ACTIVITY OF *ACALYPHA INDICA LINN* AGAINST *AEDES AEGYPTI* EGGS

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### ABSTRACT

*Aedes aegypti* was chosen as the specimen for this study because it serves as an important mechanical vector for Dengue Hemorrhagic Fever. Man made chemical Ovicide have proven to be highly effective in eradicating this pest but its negative effects upon human health and environmental safety are major drawbacks to its continued use. Hence, the search for alternative, natural substances such as *Acalypha indica linn*, that are both environmentally safe as well as being effective Ovicide. The extract of *Acalypha indica linn* is known to contain high concentrations of *Flavonoid and Saponin*. These substances are thought to have ovicidal properties. This study aims to identify the effect of *Acalypha indica linn* extract against *Aedes aegypti* eggs using a contact poison method. A true experimental study was conducted, using specimens of *Aedes aegypti*. at four concentrations of *Acalypha indica linn* extract; 0,5%, 1,0%, 1,5%, and 2,0% with 1 negative control using well water, and also 1 positive control using abate 10%. The experiment was repeated four times and each repetition was observed at four time intervals (12<sup>th</sup> hour, 24<sup>th</sup> hour, 36<sup>th</sup> hour, and 48<sup>th</sup> hour). There were no significant difference between a concentration of 0.5%, 1.0% and 1.5%. The result of the effect of ethanol extract of root(*Acalypha indica linn*) as ovicidal to the eggs of mosquitoes (*Aedes aegypti*) was performed using Bonferroni test informs that the negative control (water wells) producing eggs of mosquitoes (*Aedes aegypti*), which become larvae most numerous and differ significantly from the positive control (abate 10%). Analysis results of the effect of treatment time of 48 hours, produced number of unhatched eggs of mosquitoes (*Aedes aegypti*), which differed significantly with treatment time of 12 hours. It is concluded that *Acalypha indica linn* root extract has ovicidal activity against *Aedes aegypti* eggs and the higher the concentration, the greater the ovicidal activity.

Keywords : *Acalypha indica linn* extract, Ovicide, *Aedes aegypti*.

## ABSTRAK

Telur *Aedes aegypti* dipilih karena merupakan vektor mekanis untuk penyakit seperti *Demam Berdarah*. Ovisidal kimiawi memiliki efektifitas tinggi dalam membunuh telur tetapi juga memiliki efek samping yang merugikan terhadap manusia dan lingkungan. Oleh karena itu sebaiknya digunakan ovisidal alami seperti akar *Acalypha indica linn* karena bersifat mudah terurai sehingga penggunaannya relatif aman. *Acalypha indica linn* diduga mengandung zat aktif flavonoid dan saponin. Zat-zat ini diduga mempunyai efek ovisidal. Penelitian ini bertujuan untuk menguji efek ekstrak etanol kulit jeruk nipis sebagai ovisidal terhadap telur *Aedes aegypti*. Penelitian ini merupakan 'penelitian *true experimental-post test only control group design*' dengan sampel yang digunakan adalah telur *Aedes aegypti*. Pengulangan dilakukan sebanyak empat kali dengan jumlah perlakuan sebanyak enam jenis yaitu kontrol negatif (air sumur), konsentrasi larutan ekstrak kulit jeruk nipis sebesar 0,5%, 1,0%, 1,5% dan kontrol positif (abate). Setiap perlakuan diamati pada empat interval waktu yaitu pada 12 jam, 24 jam, 36 jam, dan 48 jam. Hasil penelitian menunjukkan bahwa pada konsentrasi 1,5%, sudah tidak ada telur yang menetas menjadi larva pada pengamatan 48 jam. Tidak didapatkan perbedaan yang signifikan dan bermakna antara konsentrasi 0,5%, 1,0%, 1,5%, dan 2,0%. Hasil uji pengaruh pemberian ekstrak etanol kulit jeruk nipis (*Citrus aurantiifolia*) sebagai ovisidal terhadap telur nyamuk (*Aedes aegypti*) yang dilakukan menggunakan Bonferroni Test menunjukkan bahwa kontrol negatif (air sumur) menghasilkan telur nyamuk (*Aedes aegypti*) yang menjadi larva paling banyak dan berbeda signifikan dengan kontrol positif (abate 10%). Hasil analisis pengaruh waktu perlakuan dengan telur yang tidak menetas menunjukkan bahwa konsentrasi perlakuan 2,0% menghasilkan telur nyamuk (*Aedes aegypti*) yang tidak menetas paling banyak dan berbeda signifikan dengan konsentrasi perlakuan 0,5%. Kesimpulan dari penelitian ini adalah ekstrak akar *Acalypha indica linn* mempunyai efek sebagai ovisidal terhadap Telur *Aedes aegypti* dan semakin tinggi konsentrasi maka efek ovisidal semakin besar.

Kata kunci : ekstrak akar *Acalypha indica linn*, ovisidal, *Aedes aegypti*.

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## INTRODUCTION

### Background

Dengue fever is a disease caused by dengue virus which is spread by the *Aedes aegypti* mosquito. The virus is distributed through each of the bite. Most common type of dengue which occurs in the community is the Dengue Hemorrhagic Fever (DHF). Dengue fever is common in most cities in Malaysia and also in many tropical countries. Based on the Ministry of Health (MOH), from the cases of dengue which occurred in January, the state of Selangor has the highest prevalence of total 3,789 cases.

The *Aedes aegypti* mosquitoes can spread the dengue fever within 8-12 days after sucking blood from a human suffering from dengue fever. Female mosquitoes infected with dengue virus can transmit the virus to the next generation through transovarial transfer (through the ovary). Dengue virus can only be passed on to others in the next 18 hours and at least 3 days after the onset of symptoms<sup>17</sup>.

There are several solutions to address diseases that are transmitted by mosquitoes. Ovicide is one of the most useful ways to prevent the spread of disease by inhibiting or development of mosquito eggs or kill the mosquito eggs. The mostly used ovicide today is Abate. Abate is an organophosphate which acts by contact, inhibiting an enzyme that is

important in the normal functioning of the egg<sup>4</sup>.

The use of abate (temephos) in Malaysia has been since year 1973. It could be said Abate (temephos) has been used for more than 40 years<sup>4</sup>. In addition, one important thing to note is the emergence of resistancy in this mosquitoes towards abate. It is possible, because the use of Abate (temephos) is said to be more than 40 years in Malaysia thus causing resistancy in the mosquitoes. Reports of *Aedes aegypti* eggs resistancy towards abate (temephos) also have been found in several countries such as Brazil, Bolivia, Argentina, Cuba, the Caribbean, and Thailand<sup>2</sup>.

Along with public awareness about the use of abate that are likely to lead to resistancy, it is necessary to choose bioovicidal selectively to inhibit the development of *Aedes aegypti* eggs. The active substance like saponin and flavonoid obtained from plants are the alternatives that should be considered. In a study conducted, the wild asparagus extract with saponin and flavonoid tend to show remarkable ovicidal activity against the *Aedes aegypti* eggs<sup>8</sup>. A research study also shows the effect of *Acalypha indica linn* leaves containing flavonoids and tannins as active ingredients were proven to be effective anthelmintic whereas root extracts of *Acalypha indica linn* with active ingredients like flavonoids and saponins are still unknown as an bioovicide.

Thus in this study, we will investigate the effect of *Acalypha indica* linn root extract as an bioovicide towards *Aedes aegypti* eggs.

## STUDY METHOD

### Study Design

This research is an experimental laboratory research with true experimental - post test only control group design that aims to determine the potential of the *Acalypha indica* linn root extract to kill *Aedes aegypti* eggs.

Sample of research used are eggs that has the complete potential to grow into larvae after observing under microscope.

### Materials

Materials used in this research are roots of *Acalypha indica* linn, blender, jar to soak the root which is made powder (with a volume of 1.5 liters), cooling spiral, plastic hoses, water bath and vacuum, distilled water, container extraction tube, freezer, clear glass, tweezers (pinset), pipete, timer.

### Methods

The provision of extracts are from :

Plant material ( roots ) were collected and dried for several days under dry air. Secondly, roots pulverized with the aid of an electric blender. After that, root powder is

extracted with alcohol in Polinema, Malang. Test samples that have

been provided were concentration of 0,5%, 1,0%, 1,5% and 2.0%. Later petri dishes , pipete and bottles are sterilized at the opening. Next abate 10% was prepared as positive control. Nextly, abate and well water (air sumur) are prepared. Then, *Aedes aegypti* eggs collected is prepared. The extract solutions are pipeted and put in the petri dish. Petri dish is labelled as below.

- A – Well water (air sumur)
- B- Abate 10%
- C- root extract of 0.5 %
- D- root extract of 1.0%
- E – root extract of 1.5%

After that, 20 eggs were left in each petridish labelled A,B,C,D and. Then observations were made every 12 hours for 2 days. Later, for each extract with different concentrations , they were all repeated 4 times each. Next, the number of *Aedes aegypti* eggs that hatch during the procedure were calculated. The outcome data of the number of eggs were analyzed Kruskal Wallis hypothesis to see whether there are significant differences between various concentrations of *Acalypha indica* linn root extract and the Ovicidal Activity is tested to see the percentage of extract that inhibits growth and kills the mosquito eggs.

### Methods for Qualitative Test

Saponin test method:

1. 0.5 g of extract is diluted with 10 ml of hot water.



2. Wait 10 seconds to let the solution to cool down.
3. Hydrochloric acid 2 M is added.
4. The solution is shaken and 3 drops of olive oil is added .
5. The solution is shaken and observed for bubbles which indicates positive for presence of saponin.

#### Flavonoid test:

1. 5 ml of root extract is added with 0.5 g magnesium powder.
2. 1 ml of hydrochloric acid is added to the solution.
3. Amyl alcohol is added then the solution is shaken slightly.
4. Colour changes in the solution is observed, a red, yellow or orange colour solution indicates the presence of saponin in the extract.

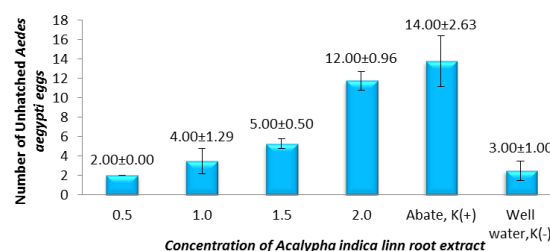
#### Data analysis

The potential data is obtained using the computer program SPSS 21.0 using the *kruskal walis* test with criteria probability value is lower than level of significance ( $p < 0.05$ ) or value of Chi Square test  $\geq$  Chi Square table (7.815). The normality of data distribution is tested using *kolmogrov smirgrov* test, while the data variance is tested its homogeneity using *Levene Test*. When there is minimal of one group which has significant difference, *Benferonni Test* is used to see which

concentration of *Acalypha indica linn* has the significant differences.

#### RESULTS

The graph below was done to know the mean number of unhatched *Aedes aegypti* eggs against the concentration of *Acalypha indica linn* root extract for 48 hours.



Based on the above graph, it can be seen that the highest average number of *Aedes aegypti* eggs that did not hatch for 48 hours is  $14.00 \pm 2.63$  after the administration of abate (positive control). Then the second highest average number of *Aedes aegypti* eggs that did not hatch for 48 hours is about  $12.00 \pm 0.96$  after giving 2% of *Acalypha indica linn* root extract. The third highest average number of eggs that did not hatch is  $5.00 \pm 0.50$  after giving 1.5% of *Acalypha indica linn* root extract. Furthermore, the average number of *Aedes aegypti* eggs that did not hatch is  $4.00 \pm 1.29$  after giving 1% of *Acalypha indica linn* root extract. Then the average number of eggs that did not hatch for 48 hours is  $3.00 \pm 1.00$  when well water (negative control) is used and the lowest average number of *Aedes aegypti* eggs that did not hatch for 48 hours is  $2.00 \pm 00.00$  after giving 0.5% of *Acalypha indica linn* root extract.

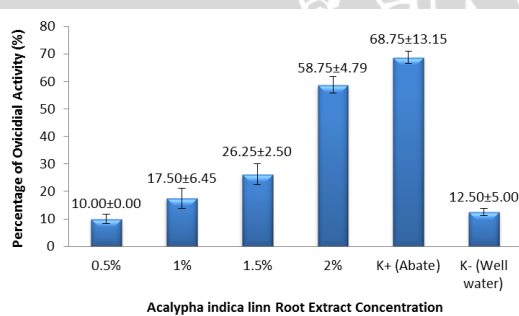
### Percentage of Ovicidal Activity

Ovicidal activity percentage is used to determine ability of *Acalypha indica linn* root extract to inhibit the hatching of *Aedes aegypti* egg. Percentage of ovicidal activity can be calculated through the formula as shown below.

Formula: Ovicidal activity % =

$$\frac{\text{No. of unhatched eggs}}{\text{No. of total eggs}} \times 100$$

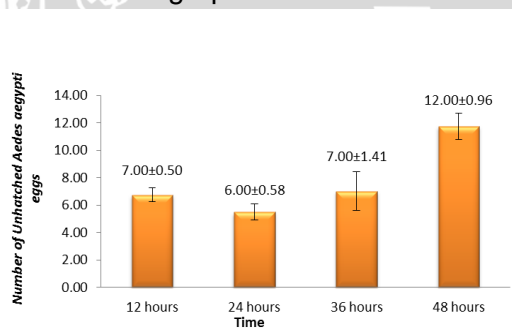
Based on the ovicidal activity formula, we can find the mean for each concentration used for this experiment as seen in the graph below



Based on the above graph, it can be seen that the highest average percentage of ovicidal activity in 48 hours is 68.75 ± 13.15 after administration of abate (positive control). Then the second highest average percentage of ovicidal activity obtained in 48 hours is 58.75 ± 4.79 after giving 2% of *Acalypha indica linn* root extract. The third highest average percentage of ovicidal activity in 48 hours is 26.25 ± 2.50 after giving 1.5% of *Acalypha*

*indica linn* root extract. Furthermore, the average percentage of ovicidal activity at the 48th hour is 17.50 ± 6.45 when given 1.0% of *Acalypha indica linn* root extract. Then the average percentage of ovicidal activity is 12.50 ± 5.00 after administration of well water (negative control) and 10.00 ± 00.00 after giving 0.5% of *Acalypha indica linn* root extract in 48 hours.

The mean number of unhatched *Aedes aegypti* eggs against the time taken in concentration of 2.0% *Acalypha indica linn* root extract is shown in the graph below.



From the graph, we know that mean number of *Aedes aegypti* eggs that remained unhatched after leaving in 2.0% concentration of *Acalypha indica linn* root extract for 48 hours gives the highest value of 12.00 ± 0.96. The second highest value obtained is 7.00 ± 1.41, after leaving the eggs in 2.0% concentration of *Acalypha indica linn* root extract for 36 hours. The value of 7.00 ± 0.50 is obtained when the *Aedes aegypti* eggs are left in 2.0% *Acalypha indica linn* concentration for 12 hours. Meanwhile, when the eggs are left in 2.0% *Acalypha indica linn*



extract concentration for 24 hours, a value of  $6.00 \pm 0.58$  is produced.

## DISCUSSION

The *Acalypha indica linn* root contains active ingredients flavonoids and saponins. 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<sup>7</sup>. Saponin will also inhibit the the growth of the *Aedes aegypti* eggs by lysing the cytoplasmic membrane until the serous layer is damaged<sup>25</sup>.

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*<sup>3</sup>. 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.

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<sup>18</sup>.

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  $\geq$  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 trifoliolate 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 trifoliolate orange was tested and found to be in very high level. In this investigation, the ovicidal activity of trifoliolate 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<sup>16</sup>.

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. Last but not least, when conducting this study, we had few limitations where we could not conduct the quantitative 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.

## CONCLUSION

1. The *Acalypha indica linn* root extract is proven to have saponin and flavonoid which causes the ovicidal activity by preventing the hatching of *Aedes aegypti* egg.
2. The *Acalypha indica linn* root extract is proven to have ovicidal effect by preventing the hatching of *Aedes aegypti* eggs since the extract concentration of 2.0%.
3. The highest Ovicidal Activity of *Acalypha indica linn* root extract can be seen at concentration of 2.0% based on the graph of percentage of ovicidal activity.

## RECOMMENDATIONS

1. Further study should be done to know the possible side effects of *Acalypha indica linn* root extract to humans and the surroundings when used at high concentration.
2. Further study about the quantitative test should be conducted to determine exact concentration of the

active ingredient saponin and flavonoid contained in the *Acalypha indica linn* root extract.

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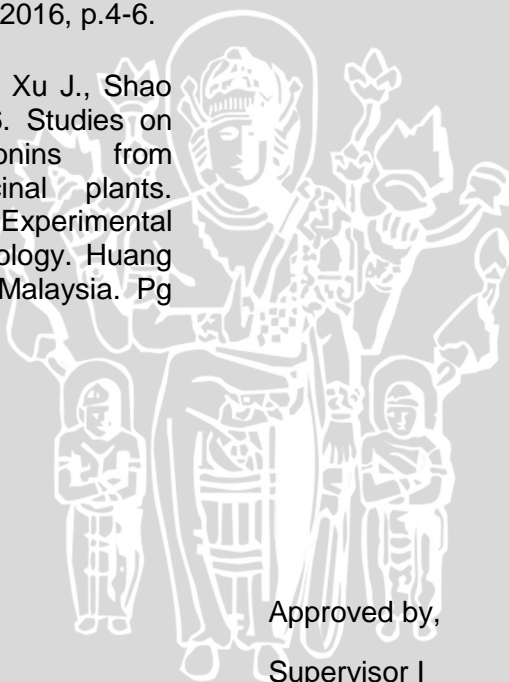
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