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# Effectivity Test of 96% from Soe (Citrus sinensis L.) Sweet Orange Rind Ethanol Extract as Biolarvaside

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## Abstract

One of the plants that can be used as larvicides is sweet orange (Citrus sinensis). Sweet orange rind containing saponins, tannins, flavonoids, and triterpenoids have a characteristic of aromatic odor and bitter taste which contain 96% essential oils containing limonene, glucoside, hesperidium, and resin that can function as biolarvasides. The Regency of the Middle East South is one of the areas in Indonesia which is known as the center of sweet orange. Soe sweet orange fruit has a distinctive color, aroma, and taste compared to other oranges in Indonesia. The objectives of this study are to discover the effectiveness of the orange rind extract in killing the Aedes aegypti larvae and to identify the minimum concentration of the extract of the sweet, dry rind in killing the larvae. This type of research is experimental post-test only control group design. This research was conducted at the Medical Laboratory Technology of Kupang Poltekkes (Health Polytechnic) in January 2019. The third (III) instar larvae were placed in 5 vials, each containing 15 larvae. The total number of samples needed was 375 larvae. It was mixed with 15 ml of Soe sweet orange rind with a concentration of 0.075%, 0.1%, 0.25%, 0.50%, 0.75%. One-Way Anova test results obtained sig p-value=0,000 ($p<0.05$), meaning that there is an influence of sweet orange rind ethanol extract on the death of larvae or orange rind extract is effective as a natural insecticide. Significant values were obtained for all concentrations p=0.008 ($p> 0.05$) meaning that there was an average difference of each concentration. The conclusion of the study shows that the ethanol extract of sweet orange rind (Citrus sinensis L.) is effective in killing Aedes aegypti mosquito larvae with a minimum concentration of ethanol extract sweet orange rind (Citrus sinensis L.) which can kill 0.075% of larvae.

**Keywords:** Biolarvaside, orange rind, Aedes aegypti

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## 1. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is an important public health problem in Indonesia and often causes an Extraordinary Cases with a large death (Astuti et al., 2016). In 2013, DHF cases were found as many as 2986 cases or as many as 0.6/1000 population (Rahmawati, E., & Tarigan, L. (2013). In 2014, the number of DHF patients was 487 cases, whereas in 2015, it was 665 cases, and the highest case occurred in Kupang City (Dinas Kesehatan NTT, 2015).

Dengue Hemorrhagic Fever is an infectious disease by the Dengue virus which is transmitted through the bite of the Aedes aegypti mosquito, with symptoms of being infected by a mosquito bite such as sudden high fever accompanied by manifestations of bleeding and potentially causing shock and death Aedes aegypti develops in a place that has a lot of puddle (Nofyan et al., 2013) (Faridah et al, 2017). Efforts to eradicate mosquitoes can be conducted by breaking the chain or life cycle of the Aedes aegypti mosquito and avoiding direct contact with mosquitoes (Astuti et al., 2016). The easiest and most effective way to break the life cycle of Aedes aegypti is to kill mosquito larvae. It is caused by mosquito larvae living in puddles. Eradication of larvae is a key strategy for vector control programs throughout the world (Alfiantya et al., 2018). The use of insecticides as larvasides is the most common way by people to control the growth of these vectors. The most commonly used insecticide in Indonesia is Abate (Alfiantya et al., 2018).

An alternative safe way is to use natural ingredients from plants. It is because materials made from nature are biodegradable so they do not pollute the environment and are relatively safe for humans (Ekawati, 2017). There are several plants that can be used as larvasides (Aritonang et al, 2017). Some plants that have been reported to have biolarvicidal activity are peel of lime leaves (Yuniarty, 2016)(Rachmawati, 2019), torch ginger leaves (Virgianti, 2015), dogfruit epidermis (Ayuchecaria et al., 2019), mahogany seed extract (Koneri & Pontororing, 2017), Langsat fruit peel extract (Mirnawaty et al., 2012), a combination of purut lime and lemongrass leaves (Filansari & Erna Susanti, 2017), extracts of purut lime, limau lime and grapefruit (Adrianto et al., 2014).

The results of research on the biocontrol of Aedes aegypti mosquito larvae from endemic plants in Dieng, Central Java have been reported by Supono et al.,(2015), where carica seed waste extract has the potential to be used for biocontrol of Aedes aegypti larvae. In addition, ethanol extracts from fragrant root oil refining can also be used as biolarvasides against *Aedes aegypti* and *Anopheles sundaicus larvae* (Lela et al., 2010).

One of the plants that can be used as larvasides is sweet orange (Citrus sinensis). Sweet orange (Citrus sinesis) contains lemonen which is suspected to be larvaside, besides sweet orange (Citrus sinensis) is easily found in our environment but its use is still lacking by the community. Sweet orange that can be used for its skin as biolarvaside is widely distributed in Soe City, which is one of the areas located in Timor Tengah Selatan Regency, East Nusa Tenggara Province. However, in general, people only use fruit for consumption and seeds for planting while other parts of the fruit such as the peels are not utilized properly. Based on the literature on sweet orange peel, there are chemical contents such as saponins, tannins, flavonoids and triterpenoids (Sari, 2012). Sweet orange peel has a characteristic of aromatic odor and bitter taste, which

contains 96% essential oil containing lemonen, glucoside, heperidina, and resin that can function as biolarvasides (Rini et al., 2009).

According to Aritonang et al., (2017) limonide compounds extracted from orange leaves cause a bitter taste and have a larvicidal effect. Extract of purut lime (Citrus hytrix D, C) and kalamondin lime (Citrus mitis Blanco) leaf produce saponins, tannins, flavonoids and triterpenoids, and produce essential oils and produce inactive compounds. However, these compounds can help increasing the overall activity of the extract (Aritonang et al., 2017). It allows insects not easily become resistant. Based on the results of Andriana's research (2006), the toxicity of purut lime extract (Citrus hystrik D.C) against Aedes aegypti mosquitoes was 3500 ppm and kalamondin orange peel extract (Citrus mitis Balanco) against Aedes aegypti L. mosquitoes was 4200 ppm. The lethal period (90%) of kalamondin purut lime extract (Citrus mitis Balanco) which caused larvainstar III death of Aedes aegypti L. mosquito was 13 hours.

Timur Tengah Selatan Regency is one of the regions in Indonesia known as sweet orange centers. Oranges in Timur Tengah Selatan Regency were originally planted in Tobu Village, North Mollo District, Timur Tengah Selatan Regency, known by the local people as "*Chinese Lemon*". Soe sweet orange has a distinctive color, aroma and taste when compared to other tangerines in Indonesia. In 2003 in the national superior fruit competition, Soe sweet orange was determined as the best superior fruit (Martosupono et al., 2007). The objective of this study is to determine the effectiveness of the Sweet Orange (Citrus sinesis) peel extract from Soe Regency, TTS Province, NTT to kill Aedes aegypti mosquito larvae. It is also supported by materials that are easily obtained so using sweet orange (Citrus sinesis) to conduct larvaside test research.

## 2. RESEARCH METHOD

This study was an experimental study with a post test only control group design. The population of this study was Aedes aegypti III instar larvae obtained from the Laboratory of the Faculty of Veterinary Medicine, Nusa Cendana Kupang. Instar III larvae were placed in 5 vials, each containing 15 larvae. Replicated 3 times at 5 concentrations.

The sample in the form of orange peel was washed thoroughly (physically visible), then dried with aerated until the water was drained. Samples of clean orange peel were chopped into small pieces to facilitate the drying process. The drying process was conducted in an oven at 60oC for 5 days. The sample was then pollinated with a size of 45 mesh. The prepared sample was dried orange peel powder.

The extraction method used was adapted from Saraswati (2015) with several modifications. 400g orange peel powder was soaked in 70% ethanol solvent with a ratio of 1:4 (w/v) for 3x24 hours, where every 24th hour, the extract was filtered and the residue was macerated again using a new solvent. Maceration was conducted at room temperature and occasionally assisted with stirring. The maceration filtrate was then combined and concentrated with a rotary evaporator vacuum with a temperature of 60oC. The concentrated extract produced was then evaporated in a water bath at 60°C to obtain a thicker filtrate.

Ethanol extract of sweet orange peel (Citrus sinensis) weighed 0.075 gr, 0.1 gr, 0.25 gr, 0.50 gr and 0.75 gr for concentrations of 0.075%, 0.1%, 0.25% respectively, 0.50% and 0.75%. After weighing, the extract was then dissolved with 100 ml of distilled water in the Erlenmeyer, and then homogenized. The homogeneous extract was put into a 15 ml vial, with repetition 3 times in each concentration. A total of 15 Aedes aegypti larvae were inserted into a vial that contained a solution of Soe sweet orange extract (Citrus sinensis). Then, the larvae were observed every 12 hours and 24 hours,

then the number of dead larvae was counted. Data analysis was performed using the One-Way ANOVA parametric statistical test. To determine which concentration has meaningfulness, a Post Hoc analysis was performed using the LSD Test.

## 3. RESULTS AND DISCUSSION

The sample used was a sweet orange fruit from Soe (*Citrus sinensis L.*) which was ripe, yellowish red skin. Slippery and easy to peel. The collection of citrus fruits was taken from Soe City, Timor Tengah Selatan Regency. Fruit was bought at fruit sellers around Haumeni Terminal, Soe City. Furthermore, the fruit was sorted wet to clean the dirt still attached to the sweet orange peel. Furthermore, the fruit is peeled and aerated at room temperature then dried in an oven at 60°C. The dried orange peel was then blended by blending and sifted using a 45 mesh sieve and then followed by maceration with 70% ethanol.

The Aedes aegypti mosquito larvae that were used were obtained from the Faculty of Veterinary Medicine of Nusa Cendana University which has been bred. Aedes aegypti mosquito larvae in this study used instar III larvae because they have better resistance to mechanical factors when moving, shaking and instar III also have a long time to turn into adult mosquitoes (Saragih et al., 2015).

Table 1. Overview of Death of the 12 Hour and 24 Hour Larvae

| Observation Time | N | Minimum | Maximum | Mean |
|---|---|---|---|---|
| 12 Jam | 15 | 4,00 | 15,00 | 10,8 |
| 24 Jam | 15 | 10,00 | 15,00 | 14,3 |

Based on table 1, it can be seen at the time of observation of 12 hours, the maximum number of Aedes aegypti larval death was 15, and the minimum number of death was 4 with an average death of 11 individuals. Meanwhile, at the 24-hour observation period, the maximum number of larval deaths was 15 and the minimum number of deaths was 10 with an average of 14 deaths.

Table 2. Normality Test for Larval Death

| Observation Time | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | Df | Sig. | Statistic | Df | Sig. |
| Death in 12 hours | 0,174 | 15 | 0,200 | 0,903 | 15 | 0,106 |
| Death in 24 hours | 0,474 | 15 | 0,000 | 0,523 | 15 | 0,000 |

Based on the normality test conducted, the results obtained at death 12 hours p value=0.106 and at 24 hours death p value=0.000. Significant value of the observation in 12-hour death was (p-value>0.05) which means that 12-hour mortality was normally distributed, then it was continued with the data analysis stage using One-Way Anova. Meanwhile, 24-hour deaths were not normally distributed so it was continued with the Kruskall-wallis test.

Table 3. Homogeneity Test for Larvae Mortality

| Observation Time | Sig. |
|---|---|
| Death in 12 hours | 0,016 |
| Death in 24 hours | 0,004 |

Based on Table 3, it can be indicated that the significant value of 12-hour mortality was p=0.016 (p> 0.05) which means that the data was homogeneously distributed while at 24 hours of death the value of p=0.004 (p <0.05) which means the data was not homogeneously distributed.

Table 4. One-Way Anova Test (12 Hours Death)

| Observation Time | Sig. | Note |
|---|---|---|
| Death in 12 hours | 0,000 | There is an effect |
| Death in 24 hours | 0,000 | There is an effect |

Based on Aedes aegypti mosquito larvae mortality, a One-Way Anova test was obtained sig (significant) from the results of Aedes aegypti mosquito mortality after being provided ethanol extract of Soe sweet orange peel (Citrus sinenis L.) which is p-value=0.000 (p<0.05), it can be stated that there is an effect of ethanol extract of sweet orange peel on the death of Aedes aegypti larvae or it can be stated that the extract of Soe sweet orange peel (Citrus sinensis L.) is effective as a natural insecticide against Aedes aegypti larvae

Table 5. Kruskal-wallis test

| Concentration | | Sig. | Note |
|---|---|---|---|
| 0,075% | 0,1% | 1,000 | No difference |
| | 0,25% | 0,000 | There is difference |
| | 0,50% | 1,000 | No difference |
| | 0,75% | 1,000 | No difference |
| 0,1% | 0,075% | 1,000 | No difference |
| | 0,25% | 0,000 | There is difference |
| | 0,50% | 1,000 | No difference |
| | 0,75% | 1,000 | No difference |
| 0,25% | 0,075% | 0,000 | There is difference |
| | 0,1% | 0,000 | There is difference |
| | 0,50% | 0,000 | There is difference |
| | 0,75% | 0,000 | There is difference |
| 0,50% | 0,075% | 1,000 | No difference |
| | 0,1% | 1,000 | No difference |
| | 0,25% | 0,000 | There is difference |

| | | | |
|---|---|---|---|
| | 0,75% | 1,000 | No difference |
| 0,75% | 0,075% | 1,000 | No difference |
| | 0,1% | 1,000 | No difference |
| | 0,25% | 0,000 | There is difference |
| | 0,50% | 1,000 | No difference |
| Total | 0,075, 0,1, 0,25, 0,50, 0,75% | 0,008 | No difference |

Based on the test results in tables 1 and 2 obtained, a significant value at 24 hours of death was the normality test p=0.000 (p<0.005) which means that the data was not normally distributed and the homogeneity test p=0.004 (p<0.005) which means the data was not homogeneously distributed. Thus, the Kruskal-wallis test was performed to determine the difference between the treatment and the average cumulative number of larvae deaths. Based on the test results in table 5, it obtained significant values for all concentrations p=0.008 (p>0.005) which means there was no difference in the average of each concentration. Therefore, it can be said that all concentrations are capable of killing Aedes aegypti larvae.

Selection of 70% ethanol was because 70% ethanol can be a preservative to prevent the growth of fungi and bacteria during maceration. This ethanol solvent will also penetrate the cell wall and enter the cell cavity of the active substance, so that the active substance can be dissolved. The 70% ethanol solvent used was universal, selective, and semi-polar in nature so that it is able to dissolve polar and non-polar chemical compounds (Ekawati, 2017). In this case, compounds contained in sweet orange peel including flavonoids, saponins, and tannins can be dissolved. Maceration method used in making this extract through 2 processes, which were maceration for 5 days and remaseration for 2 days with a ratio of 4:1 where 400 gram simplicia and 1600 ml of 70% ethanol. After the maceration and remaceration filtrate were combined, it was continued in the evaporation and waterbath processes at 60°C and a thick extract was obtained.

The high mortality of test larvae can be caused by the presence of chemical compounds in sweet orange peel that play a role in biological activity in the growth and development of larvae (Adrianto et al., 2014). Various types of plants have been known to contain bioactive compounds such as phenylpropane, terpenoids, alkaloids, acetogenin, steroids and tannins which are as insecticides (Aritonang et al., 2017). The compounds contained in orange peel include limonoids, saponins and tannins. Limonoids and saponins act as food inhibitors in insects (antifeedant), working to wither nerves in the respiratory system of insects and tannins can influence the failure of moulting in larvae so that they die before developing into pupae (Rachmawati et al., 2019).

The condition of Aedes aegypti instar III larvae after being treated with ethanol extract of sweet orange peel showed a change that was moving slower and looked like death. If the Aedes aegypti larva is dead, the larva will sink and not move. However, in this study, there was no difference between the lowest concentration of 0.075% and the highest concentration of 0.75% while in the intermediate concentration of 0.1%, 0.25% and 0.50% obtained confusing results in which larval deaths were smaller than lowest concentration of 0.075%. This is due to an error in the dilution process of concentration

variation where the thick extract did not dissolve completely in the solvent and at medium concentrations most of the larvae have reached instar IV which was close to the pupa so that it affected the yield.

## 4. CONCLUSION

Based on research that has been conducted, it can be concluded that the ethanol extract of sweet orange peel (Citrus sinensis L.) is effective in killing Aedes aegypti mosquito larvae. The minimum concentration of ethanol extract of sweet orange peel (Citrus sinensis L.) which can kill is 0.075%.

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