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LARVICIDE ACTIVITIES OF EXTRACT AND ESSENTIAL OIL FROM SUNKIST ORANGE PEEL (*Citrus sinensis* (L.) Osbeck)

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ABSTRACT

Orange peel (*Citrus sinensis* (L.) Osbeck) contains flavonoids and saponins potentially to be larvicides. The research aimed to compare between the activities of extract and essential oil in orange peel against *Aedes aegypti* mosquito larvae determined by LC_{50} value. First, the orange peel was extracted by maceration using ethanol while the essential oils were obtained by water distillation with aquadest at 70°C . The larvicide was assayed at the concentration of 1000 ppm, 500 ppm and 250 ppm also with a negative control (DMSO 1%) and a positive control (Abate®). Five samples of *Aedes aegypti* larvae were added into the vial containing solution and store for 24 hours. Next, the dead larvae were observed and replicated 3 times. The result showed that the ethanol extract of orange peel had effective larvicidal activity with LC_{50} value of $562.34 \mu\text{g/mL}$ compared to essential oils with LC_{50} value $1047.12 \mu\text{g/mL}$.

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Keywords: Orange peel (*Citrus sinensis* (L.) Osbeck), extract, essential oil, larvicide, *Aedes aegypti*

ABSTRAK

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Kulit jeruk Sunkist (*Citrus sinensis* (L.) Osbeck) mengandung flavonoid dan saponin yang berpotensi sebagai larvasida. Tujuan penelitian ini untuk mengetahui perbandingan antara ekstrak etanol dan minyak atsiri kulit jeruk Sunkist (*Citrus sinensis* (L.) Osbeck) yang memiliki aktivitas terhadap larva nyamuk *Aedes aegypti* berdasarkan nilai LC_{50} . Pada penelitian ini kulit jeruk Sunkist diekstraksi secara maserasi dengan menggunakan pelarut etanol sedangkan minyak atsiri diperoleh dengan metode destilasi air. Pengujian larvasida pada kedua sampel yaitu ekstrak etanol dan minyak atsiri dilakukan dengan konsentrasi 1000 ppm, 500 ppm, 250 ppm, Kontrol negatif (DMSO 1%) dan kontrol positif (Abate®). Larva nyamuk *Aedes aegypti* ditambahkan sebanyak 5 ekor kedalam vial yang berisi larutan uji, disimpan selama 24 jam lalu diamati jumlah larva yang mati dan dilakukan 3 kali replikasi. Hasil penelitian menunjukkan bahwa ekstrak etanol kulit jeruk Sunkist (*Citrus sinensis* (L.) Osbeck) memiliki aktivitas larvasida yang efektif dengan nilai LC_{50} $562,34 \mu\text{g/mL}$ dibandingkan dengan minyak atsiri kulit jeruk Sunkist (*Citrus sinensis* (L.) Osbeck) yang tidak aktif dengan nilai LC_{50} $1047,12 \mu\text{g/mL}$.

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Kata kunci: Kulit jeruk Sunkist (*Citrus sinensis* (L.)), Ekstrak etanol, Minyak atsiri, Larvasida, *Aedes aegypti*

INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is caused by the *Aedes aegypti* mosquito. The *Aedes aegypti* mosquito usually lives in a tropical atmosphere, one of the countries with a tropical climate is Indonesia. DHF disease is increasingly widespread when the *Aedes aegypti* mosquito continues to breed (Ratnaningsih 2010, p. 11).

Until now, prevention of DHF is still prioritized on vector control because there are still no effective vaccines and antiviral drugs available. The intended vector control is to prevent the growth of *Aedes aegypti* mosquitoes by controlling the *Aedes aegypti* mosquito larvae. The pesticide used to control *Aedes aegypti* larvae is Abate® (Mulyatno et al, 2012).

According to Susanna (2003) and Panghiyangani (2012) the use of pesticides as larva control can harm the environment, other predators if used for a long time can harm humans and even cause cancer.

The many negative impacts of using synthetic pesticides have encouraged research on natural larvicides, one of them is orange peel from various species.

According to Robinson (1991), sunkist orange peels contain chemical

compounds including limonoids, flavonoids and saponins.

Based on research from Megawati and Rosa 2015, field tests conducted on insects, limonene acts as a food inhibitor in insects (anifeedant). According to Novizan (2002), limonene is a relatively new botanical pesticide product. In addition, limonene is an essential oil derived from orange peel extract. Orange oil contains about 90% limonene.

Apart from the essential oil of orange peel, Hamidah 2012 stated that the methanol extract of kaffir lime peel has high biolarvicide potential against *Aedes Aegypti* larvae. Meanwhile, in Ery Maryanti's research, the ethanol extract of kaffir lime leaves was declared effective as a larvicide because it could kill *Ae. aegypti* larvae.

In research by Adrianto et al, 2014 extracts of kaffir lime leaves (*Citrus hystrix*), lime (*Citrus amblycarpa*), and grapefruit (*Citrus maxima*) contain secondary metabolites, namely essential oils, flavonoids, saponins and terpenes, which have the potential as a larvicide.

From the description above, this research has conducted a comparative test or comparison of the ethanol extract and essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) against *Aedes aegypti*

mosquito larvae with the aim of providing information of using Sunkist orange peels against *Aedes aegypti* mosquito larvae preferably in the form of ethanol extract or essential oil

RESEARCH METHOD

Equipments

The equipments used are a set of steam distillation tools (stahl distillation), Rotary Vacum Evaporator, a set of glass tools, analytical scales (carat series), vials.

Materials

The materials used are Abate®, *Aedes aegypti*, Distilled water, Aluminum Foil, DMSO 1%, Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) and ethanol.

Work Procedures

Making of Sunkist Orange peel (*Citrus sinensis* (L.) Osbeck) Extract

The peel of the Sunkist orange (*Citrus sinensis* (L.) Osbeck) was cleaned and cut into small pieces in order to make drying and crushing process easier. The extract of Sunkist orange peel was carried out by maceration, by weighing 250 g of Sunkist orange peel powder and putting it in an Erlenmeyer flask, then add ethanol solvent. After that, it was filtered and the filtrate is evaporated to remove the solvent using a rotary vacuum evaporator.

Extraction to obtain Essential Oil

Sunkist orange peel samples (*Citrus sinensis* (L.) Osbeck) were separated from the fruit then cut into small pieces and weighed 250 grams then put into a round bottom flask filled with distilled water to about half of the flask and distillation was carried out at 70°C. The extraction process temperature is maintained at less than 100°C because the boiling point of limonene or limonoid is 112°C, if the extraction temperature is higher than 100°C, it may cause the essential oil to evaporate. After the distillation is complete, the tool is allowed to cool (about 15 minutes) and the distillate results are obtained (Hanani 2014, p. 216).

Chemical Compound Test of Extract

a. Flavonoid Test

Tests were carried out with a number of extracts dissolved in ethanol, then add a little Mg and concentrated HCl powder was added to produce yellow, orange, or red colors indicating the presence of flavonoids (Nafisah et al., 2014).

b. Alkaloid Test

The test solution was added with 1 mL of HCl 2N and shaken. 2 different tubes were prepared then the filtrate was inserted. Add 1 drop of Mayer reagent in the first tube and add 1 drop of Dragendorff reagent in the second tube.

The formation of a yellow sediment shows a positive result for Mayer reagent and Dragendorff reagent red sediment (Mariana, Suryanti & Suyono 2005).

c. **Saponnin Test**

Saponin identification was carried out by dissolving the extract in 10 mL hot water and add HCl, then shaking it vigorously for 10 seconds. If it's positive, foam will form (Setyowati et al. 2014, p. 3).

d. **Tannin Test**

1 mL of hot water is added to the test solution with 3-4 drops of FeCl₃, if a blackish blue or blackish green color appears, it indicates that the sample contains tannins (Hakim A R and Saputri R 2017, p. 36).

e. **Parchment Paper Test**

Drop the extract components onto parchment paper. If there is no residue, the essential oil is still in the extract (Ahmad, 2014).

Identification essential oil of the Extract using TLC

In the mobile phase, a mixture of n-hexane-ethyl acetate (5: 5) was put into the chamber and allowed to saturate. On the TLC F254 plate, approximately 5 µL of ethanol extract is placed and put into the chamber, eluted to the mark, take out and let dry. Then sprayed using Vanillin-sulfuric acid extract

positively containing essential oils if it is blue, or red purple (Stahl Egol, 1969).

Preparation of Test Solution

The essential oil pipetted as much as 0.02 mL is then added with 10 mL of 1% DMSO to form a 2000 ppm stock solution. After that, a concentration of 1000 ppm, 500 ppm and 250 ppm was made. Concentration of 1000 ppm is made by pipetting 5 mL from the stock solution, add up to 10 mL, concentration of 500 ppm, pipetted 2.5 mL from stock solution, add to 10 mL and 250 ppm pipetted 1.25 mL from stock solution, then add to 10 mL.

Larvacide Test

The prepared solution containing essential oil from the peel of Sunkist orange (*Citrus sinensis* L. Osbeck) was transferred into the prepared vial and divided into 8 treatment groups with 2 control groups. Group 1 Negative control (DMSO 1% + 5 larvae), Group 2 Positive control (Temephos 1% + 5 larvae), Group 3 Essential oil 0.1% v / v + 5 larvae, Group 4 Essential oil 0, 05% v / v + 5 larvae, Group 5 essential oil 0.025% v / v + 5 larvae, Group 6 0.1% v / v ethanol extract + 5 larvae, Group 7 Ethanol extract 0.05% v / v + 5 larvae and Group 8 ethanol extract 0.025% v / v + 5 larvae.

Toxicity Test

The vials containing the larvae and essential oil of each concentration were stored. After 24 hours, the number of dead larvae was observed.

RESULTS AND DISCUSSIONS

Dengue hemorrhagic fever is a disease caused by the *Aedes aegypti* mosquito that carries the dengue virus. Until now, the handling of dengue hemorrhagic fever has been carried out by eradicating vectors aimed at *Aedes aegypti* larvae.

The use of chemical insecticides as larvicides is the most common way to kill larvae. However, it has a negative impact on the environment and organisms. For this reason, a *hit and run* natural larvicide is used, that is, if it is used it will kill pests at that time and after the pest is killed it will quickly

One of the plants that has the potential as a larvicide is sunkist orange (*Citrus sinensis* (L.) Osbeck), the part used is the skin of sunkist orange (*Citrus sinensis* (L.) Osbeck) which contains several secondary metabolites including flavonoids, saponins and essential oils which are in the form of limonene. Limonene and saponins act as food inhibitors in insects (antifeedant) (Asikin S, 2013).

In making the ethanol extract of sunkist orange peel (*Citrus sinensis* (L.)

Osbeck), the extraction was carried out by the maceration method because the sample used was the skin of the fruit which had a soft consistency so that without heating the chemical components could be extracted (Fachruddin, 2001).

This maceration method was chosen because it does not go through a heating process, it is feared that there is a class of flavonoid compounds that are not heat-resistant because flavonoid compounds are easily oxidized at high temperatures (Rahayu, 2009). Maceration is the process of immersing a sample using organic solvents at room temperature. In the immersion process, the plant samples will experience the breakdown of the cell wall and membrane due to the difference in pressure between inside and outside the cell, so that the secondary metabolites in the cytoplasm will dissolve in organic solvents. (Lenny, 2006).

The solvent used is ethanol because ethanol has a greater polarity (dielectric constant of 24.6) and the compounds extracted by ethanol are polar and semi-polar compounds such as secondary metabolites of the flavonoid, alkaloid and natural-bound groups on glycosides (Rochmat et al, 2016). And according to research conducted by Astuti (2008) ethanol can affect larvae

mortality 24 hours after treatment is at concentrations above 10%.

The essential oil was extracted using the water vapor distillation method. According to Nainggolan (2002) the essential oil yield value with the water vapor distillation method was higher than the water distillation method and the steam distillation method. Where the yield value of water distillation method obtained 0.35-0.37%, the yield value of steam distillation method obtained was 0.6% while the yield value of water vapor distillation method obtained was 2.38%.

A screening test is carried out to prove the chemical content present in the extract. Where according to Robinson (1991) the orange peel contains compounds including limonoids, flavonoids and saponins.

Based on the results of research conducted through phytochemical screening tests, it is known that the chemical content of the ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) are as follows:

Table 1. Chemical Content Test of the Ethanol Extract of Sunkist Orange Peel (*Citrus sinensis* (L.) Osbeck)

No	Content	Reagent	Result	Note
1	Flavanoid	Mg powder, Thick HCl	+ +	Yellow Red or Orange
2	Alkaloid	Bauchardat Lp	-	(+) brown to black sediment was formed

3	Saponin	HCl 2 N	+	foam was formed for 10 minutes
4	Tanin	FeCl ₃	-	(+) blackish-blue or blackish-green color was formed

Table 2. Chemical Compound Test of Sunkist Orange Peel (*Citrus sinensis* (L.) Osbeck) Essential Oil

No	Content	Reagent	Result	Note
1	Essential Oil	vanillin sulfuric acid	+	Purple or Reddish-purple
2	Essential Oil	Parchment Paper test	+	No residue on parchment paper

Positive results obtained in the flavonoid test were shown by showing a yellow color when the extract was added with Mg powder and concentrated HCl (Nafisah et al., 2014). And in the saponin test, positive results were obtained, indicated by the formation of foam for 10 minutes when the extract was added with 10 mL of hot water and HCl and then shaken vigorously (Setyowati et al, 2014, p. 3). The specific test of essential oils was carried out by using the TLC profiling where the mobile phase used was a mixture of n-hexane: ethyl acetate (5: 5) then sprayed with Vanillin-sulfuric acid, heated and if positive the essential oil was shown in blue or red purple (Stahl Ego, 1969). And also by testing on parchment paper, by dripping ethanol extract and essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) on parchment paper, if

there is no residue then it is said that the essential oil is still in the sample (Ahmad, 2014).

In a study conducted to determine the effect of ethanol extract and essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) on *Aedes aegypti* larvae, each was divided into 5 test groups with concentrations of 250 ppm, 500 ppm and 1000 ppm and positive control (Abate®) and negative control (DMSO 1%), which to test and observe the mortality of larvae after 24 hours.

The larvae used were larvae *Aedes aegypti* instar III / IV because the larvae of instar III already had complete body parts and the structure of their body walls had not yet hardened so they were suitable for treatment. Larvae Instar III have better resistance to mechanical factors when there is displacement, shaking of place, and instar III has a long time to develop into imago (adult mosquitoes). In addition, instar III / IV larvae are a research sample that is the WHO standard (WHO, 2005).

The following table is the observation data on the mortality of *Aedes aegypti* larvae using ethanol extract and essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck):

Table 3. Percent of larval mortality in the ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck)

Sample	Replication	The number of mosquito larvae that died per concentration $\mu\text{g} / \text{mL}$ (5 tails)		
		250	500	1000
Ethanol extract of Sunkist orange peel (<i>Citrus sinensis</i> (L.) Osbeck)	1	1	3	5
	2	0	1	2
	3	0	2	4
Death total		1	6	11
Death Percentage		6,66 %	40 %	73,33 %

Table 4. Percent of larval mortality in the Essential Oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck)

Sample	Replication	The number of mosquito larvae that died per concentration $\mu\text{g} / \text{mL}$ (5 tails)		
		250	500	1000
Essential Oil of Sunkist (<i>Citrus sinensis</i> (L.) Osbeck) peel	1	0	0	2
	2	0	0	2
	3	0	1	0
Death Total		0	1	4
Death Percentage (%)		0	6,66 %	26,66 %

Based on the table above, it shows that the greater the concentration of ethanol extract and Sunkist orange peel oil (*Citrus sinensis* (L.) Osbeck), the higher the number of dead larvae. Judging from the concentration of 1000 ppm of ethanol extract and oil of Sunkist orange peel (*Citrus*

sinensis (L.) Osbeck) percentage of death larvae, respectively 73.33% and 26.66%. Whereas in the control group, for negative controls (DMSO 1%) there were 0% deaths and positive controls (Abate®) there were 100% deaths. The active ingredient contained in Abate® is temephos which has an effect that can disrupt the nervous system of mosquito larvae so that it can cause death in larvae and other animals (Fahmi 2006). This shows that there is an effect of giving ethanol extract and essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) on the mortality of *Aedes aegypti* larvae.

From the data obtained, proceed to the calculation to determine the LC50 value. LC50 (Lethal Concentration-50) is the level or concentration of a substance, which can cause 50% of deaths in experimental animals from a group of species after the experimental animal is exposed for a certain time expressed in parts per million or ppm (Inayah, Ningsih, & Adi 2013, p. 93). LC50 (Lethal Concentration) obtained from ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) is $552.34 \mu\text{g} / \text{mL} \pm 0.37$ and for essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) is $1047,12 \mu\text{g} / \text{mL} \pm 0.28$. Komalamisra et al. states that if $\text{LC50} < 750 \mu\text{g} / \text{mL}$ is effective against *A. aegypti* larvae, while $\text{LC50} > 750 \mu\text{g} / \text{mL}$ is

declared inactive. This shows that only the ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) is toxic to the *Aedes aegypti* mosquito larvae.

The ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) has an effect on larval mortality. This is due to the compound content of the ethanol extract of the Sunkist orange peel (*Citrus sinensis* (L.) Osbeck). Based on the screening test, the ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) contains secondary metabolites in the form of flavonoids and saponins.

Based on research from Wahyulianto (2005), flavonoids can enter through the cuticle lining the larva's body so that it can damage the larval cell membrane. Saponins, which are bioactive compounds as toxic substances or toxins. Saponins are included in the contact poison group because they can enter through the body walls of the larvae and poison the stomach through its mouth. In addition, saponins also have a bitter taste which can reduce larvae appetite, then the larvae will die of starvation (Novizan, 2002).

In larvicide testing using essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck), the number of larvae deaths was diverse, the mortality of larvae was more at higher concentrations. This difference is

influenced by the amount of chemical content of the essential oil. The chemical compounds contained in essential oils are the result of plant secondary metabolites.

Based on the results of research by Megawati and Rosa (2015), the largest component of essential oil from Sunkist oranges (*Citrus sinensis* (L.) Osbeck) is limonene (96.69%). The more limonene contained in the essential oil of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck), the better the quality of the essential oil. According to Novizan (2002) The way limonene works causes loss of organ coordination. Limonene is a contact poison that can be used to control insects..

CONCLUSION

Based on the results of the research conducted, it can be concluded that the ethanol extract of Sunkist orange peel (*Citrus sinensis* (L.) Osbeck) has an effect on *Aedes aegypti* mosquito larvae with the LC50 value in the ethanol extract of 562.34 $\mu\text{g} / \text{mL}$ while the essential oil is inactive due to LC50 1047.12 $\mu\text{g} / \text{mL}$.

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Wrong Form You may have used the wrong form of this word.



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Wrong Article You may have used the wrong article or pronoun. Proofread the sentence to make sure that the article or pronoun agrees with the word it describes.



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Article Error You may need to use an article before this word. Consider using the article **the**.



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S/V This subject and verb may not agree. Proofread the sentence to make sure the subject agrees with the verb.



Article Error You may need to remove this article.



Run-on This sentence may be a run-on sentence. Proofread it to see if it contains too many independent clauses or contains independent clauses that have been combined without conjunctions or punctuation. Look at the "Writer's Handbook" for advice about correcting run-on sentences.



Article Error You may need to use an article before this word.



Article Error You may need to remove this article.



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Missing ", " You may need to place a comma after this word.



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Proofread This part of the sentence contains a grammatical error or misspelled word that makes your meaning unclear.



Missing ", " You may need to place a comma after this word.



Article Error You may need to use an article before this word. Consider using the article **the**.



Article Error You may need to use an article before this word.



Sp. This word is misspelled. Use a dictionary or spellchecker when you proofread your work.



Missing ", " You have a spelling or typing mistake that makes the sentence appear to have a comma error.



Article Error You may need to use an article before this word. Consider using the article **the**.



Article Error You may need to use an article before this word. Consider using the article **the**.



P/V You have used the passive voice in this sentence. Depending upon what you wish to emphasize in the sentence, you may want to revise it using the active voice.



Confused You have used **Where** in this sentence. You may need to use **we're** instead.



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