Research Article

***In Vitro* Test on the Effectiveness of Citrus limon and Allium sativum as Pediculicides**

Uji Efektivitas *Citrus limon* dan *Allium sativum* sebagai Pedikulisida secara *In Vitro*

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ABSTRACT

Excessive usage of synthetic pediculicides, such as permethrin, lindane, and malathion, can induce resistance, environmental pollution, health problems, and even death. Efforts to prevent the side-effects of synthetic pediculicides are needed, one of which is by shifting to powerful yet safe natural pediculicides, such as *Citrus limon* (lemon) and *Allium sativum* (garlic). This study aimed to observe the effective concentration of *Citrus limon* and *Allium sativum* in vitro on the mortality of *Pediculus humanus capitis* (*P. h. capitis*). This research was an experimental laboratory study conducted in July to September 2019 in the Laboratory of Parasitology at Faculty of Medicine, University of Riau, while the making of *Allium sativum* extract was done at the Faculty of Mathematics and Science, University of Riau. A total of 288 samples of adult *P. h. capitis* was taken from children in two orphanages in Pekanbaru. *Citrus limon* juice and *Allium sativum* extract of 25%, 50%, 75%, and 100% concentrations, permethrin 1% as the positive control, and distilled water as the negative control were used in this study, and repetition was done three times. *P. h. capitis* mortality was observed every 10 - 120 minutes, characterized by the absence of movement of the legs and antenna. Data analysis was performed after 120 minutes with the one-way ANOVA test, LSD ($=0.05$), and Lethal Concentration 50 test (LC50). The one-way ANOVA test results of this study showed that statistically *Citrus limon* juice and *Allium sativum* extracts showed differences in each treatment formulation of juice on *P. h. capitis* mortality (Sig 0.017<0.05 and Sig 0.000<0.05) and LC50 juice of *Citrus limon* and *Allium sativum* extract by 51.999% and 72.426%. In this study, *Citrus limon* juice and *Allium sativum* extract had effect as pediculicides.

Keywords: *Allium sativum*, *Citrus limon*, Pediculicide

ABSTRAK

Pemakaian pedikulisida sintetik secara berlebihan seperti *permethrin*, *lindane*, *malathion* dan lain-lain, dapat menimbulkan resistensi, pencemaran lingkungan, gangguan kesehatan bahkan kematian. Upaya pencegahan akibat efek samping pedikulisida sintetik sangat dibutuhkan, salah satunya dengan pedikulisida alami yang ampuh dan aman seperti *Citrus limon* (lemon) dan *Allium sativum* (bawang putih). Penelitian ini bertujuan untuk menguji efektivitas *Citrus limon* dan *Allium sativum* secara *in vitro* terhadap mortalitas *Pediculus humanus capitis* (*P.h.capitis*). Penelitian ini merupakan penelitian eksperimental laboratorium yang dilaksanakan pada bulan Juli-September tahun 2019 di Laboratorium Parasitologi Fakultas Kedokteran Universitas Riau dan pembuatan ekstrak *Allium sativum* di FMIPA Universitas Riau. Sebanyak 288 Sampel *P.h. capitis* dewasa diambil dari anak-anak di dua panti asuhan di Kota Pekanbaru. Perasan *Citrus limon* dan ekstrak *A.sativum* dengan konsentrasi 25%, 50%, 75%, 100%, permethrin 1% sebagai kontrol positif, dan aquadest sebagai kontrol negatif digunakan dalam penelitian ini, serta dilakukan pengulangan sebanyak tiga kali. Mortalitas *P.h.capitis* diamati setiap 10 menit sampai 120 menit, ditandai dengan tidak adanya perpindahan/pergerakan kaki dan antenna. Analisa data dilakukan setelah 120 menit dengan uji *one way* ANOVA, uji beda dengan LSD ($=0,05$) dan uji *Lethal Concentration* 50 (LC50). Hasil uji *one way* ANOVA penelitian ini menunjukkan bahwa secara statistik perasan *Citrus limon* dan ekstrak *Allium sativum* terdapat perbedaan tiap perlakuan formulasi perasan terhadap mortalitas *P.h. capitis* (Sig 0,017<0,05 dan Sig 0,000<0,05) dan LC50 perasan *Citrus limon* dan ekstrak *Allium sativum* sebesar 51,999% dan 72,426%. Pada penelitian ini Perasan *Citrus limon* dan ekstrak *Allium sativum* memiliki efek sebagai pedikulisida.

Kata Kunci: *Allium sativum*, *Citrus limon*, Pedikulisida

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INTRODUCTION

Pediculus humanus capitis (*P. h. capitis*) is head louse that causes infection in the scalp or head hair called pediculosis capitis (1). This disease is one of the health problems in the world, both developed countries such as the United States and developing countries such as Indonesia (2). Millions of capitis pediculosis cases per year occur throughout the world. Cases of capitis pediculosis in the United States are recorded to 6-12 million per year (3), whereas there is no exact number of capitis pediculosis patients in Indonesia (1).

Diseases caused by insects such as *Pediculosis capitis* have several treatment methods, one of which is by using insecticide (4). Insecticides made from synthetic chemicals to overcome capitis pediculosis are called chemical pediculicides such as permethrin, lindane, and malathion (5). Insecticide resistance, environmental pollution, poisoning other insects, health problems, and even death are the side effects of using chemical insecticides if used excessively (4). Side-effects caused by lindane that have been reported are skin irritation, numbness, dizziness, headache, diarrhea, nausea and vomiting, respiratory problems, seizures, and death, while, permethrin which is widely used in Indonesia can cause eye irritation, ataxia, and seizures (5). The compound of *Citrus limon* and *Allium sativum*, such as flavonoids, saponins, and essential oils, are used as insecticides or pediculicides (4,6). Allicin contained in *A. sativum* also has an insecticidal effect (7).

Based on the side effects caused by synthetic chemical pediculicides and the resistance that can arise, the researchers were interested in researching natural ingredients that were safer and more effective as pediculicides such as *Citrus limon* (lemon) and *Allium sativum* (garlic). This study aimed to determine the effectiveness of *Citrus limon* and *Allium sativum* on *P. h. capitis* mortality.

METHOD

Time and Place

This research was an experimental laboratory study conducted in July to November 2019 in the Parasitology Laboratory of the Faculty of Medicine, University of Riau, while the *Allium sativum* extract was made in the laboratory of the Faculty of Mathematics and Science, University of Riau. The ethical clearance of this research has been obtained from the Medical and Health Research Ethics Unit of the Faculty of Medicine, University of Riau through decree number 142/UN 19.5.1.1.8/UEPKK/2019.

Collection of *P.h. capitis*

The samples used in this study were 288 adult *P.h. capitis* measuring 1-3 mm in length and having white gray to black color, triangular-shaped heads that had eyes, a pair of antennas, a suction-stick-like mouth, and three pairs of legs. *Pediculus humanus capitis* were taken from children in orphanages in Pekanbaru using wet lice combs and placed in a glass jar that was not tightly closed and then brought to the Parasitology Laboratory, Faculty of Medicine, University of Riau. In this study, *P.h. capitis* sampling at two different orphanages and the effectiveness test of *Citrus limon* juice and *A. sativum* extract were carried out at different times since at the first sampling, the number of adult *P.h. capitis* was not sufficient for the tests.

Preparation of *Citrus limon* Juice and Extract *A. sativum*

Citrus limon used were imported *Citrus limon* purchased at a supermarket in Pekanbaru. *Citrus limon* imports are more oval in shape compared to local *Citrus limon*. *Citrus limon* were squeezed using a lemon squeezer, collected in an Erlenmeyer flask with glass stopper so that the essential oil content did not evaporate quickly, and followed by filtering using cheesecloth (cloth filter). *Citrus limon* juice produced was considered as a 100% concentration. *Citrus limon* 25% concentration was made by mixing 2.5ml *Citrus limon* with 7.5ml distilled water, 50% concentration was made by mixing 5ml *Citrus limon* with 5ml distilled water, and 75% concentration was made by mixing 7.5ml *Citrus limon* with 2.5ml distilled water.

Allium sativum used in this research was *Allium sativum* locally produced in Bukit Tinggi. *A. sativum* extract was made using maceration. As much as 2kg of cleaned garlic was sliced obtaining a thickness of 1-2mm and roasted to dry for 1 hour until the color turned brown and the water content reduced. The dried garlic was then crushed using a food processor and stored in airtight plastic. *A. sativum* simplicia was taken as much as 500grams, placed in a jar, and added with 70% ethanol in a ratio of 1: 5 (500gr of *A. sativum* simplicia mixed to 2500ml of ethanol), and then stirred until homogeneous. The solution was allowed to stand for 24 hours and filtered using filter paper, and the pulp obtained was macerated with the same solvent, 70% ethanol. This stage was carried out twice until obtaining clear filtrate. The filtrate was concentrated using a rotary evaporator at 400°C until a concentrated extract (solution) was obtained. In this study, *A. sativum* extract obtained was as much as 150ml and was considered as a 100% concentration. *A. sativum* extract of 25% concentration was made by mixing 2.5ml *A. sativum* extract with 7.5ml distilled water, 50% concentration was made by mixing 5ml *A. sativum* extract with 5ml distilled water, and 75% concentration was made by mixing 7.5ml *A. sativum* extract with 2.5ml distilled water.

Treatment and Statistical Analysis

Six petri dishes were used for the formula of 100% concentration, 75% concentration, 50% concentration, and 25% concentration, permethrin 1% was used as the positive control, and distilled water was used as the negative control. Whatman No. 1 filter paper was cut following the size of the petri dish used and put in the base of a petri dish. The effectiveness test of *Citrus limon* formula and *A. sativum* extract was performed three times.

The test for the *Citrus limon* was a modification from Suprobawati and Suliati (8) and Shrivastava, *et al.* (9). A number of ten *P.h. capitis* were put in each petri dish of *Citrus limon* juice extract formula, followed by dripping 2.5 ml of *Citrus limon* juice extract concentration of 100%, 75%, 50%, 25%, permethrin 1%, and distilled water, respectively. *P.h. capitis* mortality was observed macroscopically every 10-120 minutes, characterized by the absence of movement in the foot/antenna of *P.h. capitis*.

The test of *Allium sativum* extract was based from Tigauw, *et al.* (10). A number of six *P.h. capitis* were put in each petri dish of *A. sativum* extract formula, followed by dripping 2.5ml of *A. sativum* extract concentration of 100%, 75%, 50%, 25%, permethrin 1%, and distilled water, respectively. *P.h. capitis* mortality was observed macroscopically every 10-120 minutes, characterized by the absence of movement in the foot/antenna *P.h. capitis*.

Table 1. The behavior of lemon citrus juice on *P. h. Capitis* mortality

Concentration	Mean of <i>P. h. capitis</i> Mortality (%) Time Series in Minutes											
	10 Minutes	20 Minutes	30 Minutes	40 Minutes	50 Minutes	60 Minutes	70 Minutes	80 Minutes	90 Minutes	100 Minutes	110 Minutes	120 Minutes
25%	10	10	10	10	10	10	10	10	10	10	20	20
50%	0	10	10	10	20	20	30	30	40	50	50	50
75%	10	10	10	10	10	20	30	30	40	40	50	50
100%	20	20	20	30	30	40	50	60	60	70	80	90
Positive control	90	90	100	100	100	100	100	100	100	100	100	100
Negative control	0	0	0	0	0	0	0	0	0	0	0	0

The technique used to analyze the data was by conducting a Kolmogorov-Smirnov normality test to find out if the sample was from a normally distributed population. Data analysis was continued by using the Homogeneity of Variance test to find out whether the sample was homogeneous, followed by the one-way ANOVA test to determine the effect of Citrus lemon juice on *P. h. capitis* mortality, Post Hoc with LSD ($\alpha=0.05$) and Probit test for Lethal Concentration 50 (LC50) to determine the concentration needed to kill 50% of *P. h. capitis*. The tests of normality, variance, differentiation, and LC 50 were performed at the 120th minutes.

RESULTS

Effectiveness Test on *Citrus limon*

Data on the behavior results of lemon citrus juice with various concentrations on *P. h. capitis* mortality can be seen in Table 1.

Table 1 showed that the average mortality of *P. h. capitis* after 120 minutes with a treatment of the 25% *Citrus limon* concentration was 20%, the 50% concentration was 50%, the 75% concentration was 50%, and the 100% concentration was 90%. Positive control could kill all *P. h. capitis* in the 30th minute, while negative control could not kill *P. h. capitis* until the 120th minute. Results showed that the data were normally distributed ($p=0.200$) and homogeneous ($p=0.841$). The one-way ANOVA test results in this study indicated that statistically there were differences in each treatment of *Citrus limon* juice formulation on *P. h. capitis* mortality ($p=0.017$). The results of LSD test in this study showed that in the effect of *Citrus limon* juice test material, on the mortality of *P. h. capitis* was found only between 25% concentration and the 100% concentration.

Table 2. Probit analysis for lethal concentration (LC)

Lethal Concentration	Concentration (%)		
	Estimation	Lower Limit	Upper Limit
LC50	51.999	30.836	76.118

Based on statistics analysis, the effective concentration of *Citrus limon* to kill 50% *P. h. capitis* (LC50) was 51.999 (%CI: 30.836%-76.118%).

Allium sativum Effectiveness Test

Data from the behavior of *Allium sativum* extract with various concentrations of *P. h. capitis* mortality can be seen in table 3.

Result show that the average mortality of *P. h. capitis* after 120 minutes by the administration of *Allium sativum* extract 25% concentration could not kill *P. h. capitis*, the 50% concentration by 15%, the 75% concentration by 33%, the 100% concentration by 83%. Positive control could kill all *P. h. capitis* samples in the 40th minute, while negative control could not kill *P. h. capitis* until the 120th minute.

Data were normally distributed ($p=0.200$) and homogeneous ($p=0.154$). The one-way ANOVA test results in this study indicated that statistically, there were differences in each treatment of the *Allium sativum* juice formulation against *P. h. capitis* mortality. ($p=0.001$). The results of LSD test in this study showed that in the test material of *Allium sativum* extract, there were differences in the mortality of *P. h. capitis* for the 25% concentration with the 50%, 75%, and 100% concentrations, the 50% concentration with the 25%, 75%, and 100% concentrations, the 75% concentration with 25%, 50%, and 100% concentration, while 100% concentration with 25%, 50%, and 75% concentrations.

Table 4. Probit Analysis for Lethal Concentration (LC)

Lethal Concentration	Concentration (%)		
	Estimation	Lower Limit	Upper Limit
LC50	72,426	61,826	85,792

Based on statistics, the effective concentration of *Allium sativum* to kill 50% *P. h. capitis* (LC50) was 72.426% (CI: 76.118% - 85,792%)

Table 3. The behavior of *Allium sativum* extract on *P. h. capitis* mortality

Concentration	Mean of <i>P. h. capitis</i> Mortality (%) Time Series in Minutes											
	10 Minutes	20 Minutes	30 Minutes	40 Minutes	50 Minutes	60 Minutes	70 Minutes	80 Minutes	90 Minutes	100 Minutes	110 Minutes	120 Minutes
25%	0	0	0	0	0	0	0	0	0	0	0	0
50%	0	0	17	17	17	17	17	17	17	15	15	15
75%	0	0	0	0	0	0	17	17	33	33	33	33
100%	0	0	0	0	17	17	17	50	50	50	50	83
Positive control	83	83	83	100	100	100	100	100	100	100	100	100
Negative control	0	0	0	0	0	0	0	0	0	0	0	0

DISCUSSION

The results of this study showed that in the 120th minute, the 100% concentration of *Citrus limon* killed *P. h. capitis* the most (90%). This was probably due to the concentration of 100% pure fresh *Citrus limon* juice without the addition of distilled water, so there were many active ingredients contained. This result was supported by Shrivastava V, *et al.* (7) who stated that a higher concentration of an ingredient would result more active ingredients contained and higher killing power. This study was the same as the study conducted by Suprobawati and Suliati (8) that showed at the 120th minute, the 100% concentration of lime killed more *P. h. capitis* than any other concentrations. According to Nurhaifah and Sukesu (11), the high mortality rate in the larvae test carried out was due to the presence of chemical compounds in sweet oranges that played a role in the biological activities in the growth and development of larva.

The results of this study indicated that the negative control containing distilled water could not kill *P. h. capitis*, while the lowest concentration of *Citrus limon* (25%) could kill *P. h. capitis* by 20%. This proved that there was an effect of the administration of *Citrus limon* juice on *P. h. capitis* mortality. This is in line with research conducted by Nurhaifah and Sukesu (11) that stated negative control (distilled water) could not kill *Aedes aegypti* (*A. aegypti*) larvae, whereas low concentration at 0.2% of lime juice could kill 57.32% of *A. aegypti* larvae. The results of research conducted by Shrivastava, *et al.* (9) showed that *Citrus limon* could be used as a pediculicide, and *Citrus limon* juice without dilution or addition of distilled water could kill 95.5% *P. h. capitis* within 3 hours. The research results conducted by El Akkad, *et al.* (12) showed that *Citrus limon* juice had a higher pediculicidal effect (100%) compared to olive oil (96.7%) and tea tree oil (23.3%).

The one-way ANOVA test results in this study indicated that statistically there were differences in the behavior of *Citrus limon* juice formulation on *P. h. capitis* mortality (Sig 0.017<0.05). The results of LSD test in this study showed that in the *Citrus limon* juice test material, there was a difference in the mortality of *P. h. capitis* for the 25% concentration with the 100% concentration, and the 100% concentration with the concentrations of 25%, 50%, and 75%. The difference in *Citrus limon* juice extraction might be due to differences in the amount of active ingredients contained. The results of this study also showed that based on statistics effective concentration of *Citrus limon* to kill 50% *P. h. capitis* (LC50) was 51.999%.

Various types of plants have been known to contain bioactive compounds such as phenylpropane, terpenoids, alkaloids, acerogenins, steroids, and tannins that are insecticides (11). Some of the contents of *Citrus limon* are vitamin C, citric acid, essential oils, bioflavonoids, polyphenols, coumarin, flavonoids, and volatile oils on the skin such as limonents ($\pm 70\%$), α -terpinenes, α -pinen, β -pinen, coumarin, and polyphenols (6). *Citrus limon* can be used as a pediculicide because it contains flavonoids and essential oils (5). Flavonoids are phenol compounds that are water-soluble and can be used as active ingredients for insecticides (4). Flavonoid metabolites such as isoflavones, biochanin, and pinocembrin have toxic effects on insects (4,13). Isoflavone has a toxic effect on insects by influencing the behavior, growth, and development of insects (14). Rotenone is a type of isoflavone as a mitochondrial poison by blocking the

electron transport chain leading to inhibition of energy production (15). Insect fertility can be reduced by the presence of biochanin metabolite compounds (13), pinocembrin and limonoid metabolite compounds contain antifeedant that reduces the appetite of insects and prevents insects from digesting food so that their stomachs are disturbed (11,13).

The results of this study indicated that the formula of pediculicide *Allium sativum* with 100% concentration is significantly the most effective in killing *P. h. capitis* in vitro (83%). The one-way ANOVA test results in this study indicated that statistically there were differences in each treatment of the *Allium sativum* juice formulation against *P. h. capitis* mortality (Sig 0.000<0.05). The results of LSD test in this study showed that in the *Allium sativum* juice test material, there were differences in the mortality of *P. h. capitis* in the 25% concentration of with the concentrations of 50%, 75%, and 100%, the 50% concentration with the concentrations of 25%, 75%, and 100%, the 75% concentration with the concentrations of 25%, 50%, 100%, and the 100% concentration with the concentrations of 25%, 50%, and 75%.

The results of this study indicate that *Allium sativum* can be used as a pediculicide. This is supported by the research results by Meriga, *et al.* (16) that stated *Allium sativum* had a pediculicidal effect. The results of this study were the same as the study conducted by Pritacindy, *et al.* (17) that stated there were differences in the effectiveness of *Allium sativum* extracts based on *Allium sativum* concentration, and the higher the concentration level, the faster the *P. h. capitis* killing time. The number of active compounds that have direct contact with *P. h. capitis* causes *P. h. capitis* mortality at various concentrations (4). The results of the study conducted by Rusdy (7) with *Allium sativum* extract concentrations of 5%, 10%, 15%, and 20% in the golden snail pest showed that the golden snail's dead speed was dominated by the high concentration level (20%). It means that concentrations of active ingredients that were highly concentrated, such as diallyl sulfide contained in *Allium sativum*, were more effective for controlling snails. The results of this study were also the same as the study by Sasmilati (4) that reported based on the results of the one-way ANOVA test there were significant differences in the effects of larvicides in the different concentrations of *Allium sativum* solution groups with p-value <0.05(sig) and found significant differences between groups that was p-value <0.05(sig). The results of a study conducted by Pritacindy, *et al.* (17) showed that *Allium sativum* proved to be effective as a pediculicide, and a higher concentration of *A. sativum* extract would be a more effective insecticide for hair lice. The results of research conducted by Al-Zanbagi and Al-Hasdi (18) showed that *A. sativum* was a strong natural pediculicide, medicinally substance with no toxicity; further, the results also proved that higher concentrations of *Allium sativum* would kill *P.h.capitis* more effectively.

Allium sativum contains metabolite compounds such as allicin, adenosine, ajoene, flavonoids, saponins, tuberholosides, scordinin (10,17), alliin, scordinin, methylene trisulfide, saltivine, essential oils (4). Sulfide derivatives, namely allicin, are stomach poisons that kill the target organism if allicin enters the digestive organs and is absorbed by the intestinal wall (7). Allicin is produced from alliin and alliinase enzymes formed after the processing of *Allium sativum*, and it is very reactive and unstable (19). This instability causes allicin to turn into a compound of allyl disulfide, which is classified as an

organic synthetic pesticide (7,19) and inhibits or blocks the action of the cholinesterase and ganglion enzymes in the central nervous system terminal (7).

The production of RNA and lipids is also associated with allacin, that is when the production of RNA in small amounts or not produced, the protein synthesis is reduced and results in the cessation of each phase because there is no or a little RNA messenger, ribosomal (20). Proteins and amino acids are not produced that will interfere with the growth and development of insects (16). Research by Nejad, *et al.* (20) on the growth of *Staphylococcus aureus* on hamburgers added with 3ml garlic seasoning and stored for three months showed less amount of *Staphylococcus aureus* compared with those added with 1 ml and 2 ml of garlic. This was because allacin contained in *Allium sativum* entered the bacteria through the lipid membrane and inhibits RNA synthesis.

Flavonoids contained in *Citrus limon* and *Allium sativum* work as respiratory inhibitors (13). Flavonoids enter the insect's body through the respiratory system, namely spiracles that can be found on the surface of the body so that paralyze the nerve and damage the spiracles, and it causes the insects not be able to breathe and eventually die (21). Disruption of energy metabolism in the mitochondria caused by flavonoids by inhibiting the electron transport system blocks the ATP production and causes a decrease in oxygen consumption by the mitochondria (4). Other metabolite compounds contained in *Citrus limon* and *Allium sativum*, saponins, enter through the walls of the insect's body and are toxic, which results in the death of the insects (21).

Essential oils contained in *Citrus limon* and *Allium sativum* plants are 1,3-Dithiane, di-2-propenyl, 1-Propene, 3,3'-thiobis, methyl 2-propenyl, 3-vinyl-1,3- dithiin, 2-vinyl-1,3-dithiin, di-2- propenyl, 3-vinyl-1,2 dithiin-1-chloro-4-(1-ethoxy)-2-methylbut-2-ene, methyl 2-propenyl, diallyl disulfide, 3-vinyl-1,2 dithiin, methyl-1-methyl-2-butenyl sulphide, octane 4-bromine (7), which have effectiveness as insecticides, atypic microbes, and anti parasites (22). Essential oils have biological activity against insects that are repellent, attractive, contact poisons, respiratory toxins, reducing appetite, inhibiting egg-laying, inhibiting growth, and reducing fertility (5). Direct or indirect effects of essential oils through absorption in the cuticles make essential oils effective pediculicides (23).

Phytochemical test results showed that essential oils contain secondary metabolites in the form of alkaloids, terpenoids, and tannins (4). Toxic alkaloids and nerve poisons are alkalic substances and contain one or more nitrogen atoms (24). The research results conducted by

Samilati, *et al.* (4) showed that the death of mosquito larvae given *Allium sativum* solution was through four phases, namely stimulation (changes in larval behavior from normal circumstances), convulsions, paralysis, and ended with death. Another secondary metabolite contained in essential oils is terpenoids, which have a very low molecular weight so that they can act mechanically by penetrating the cuticle of *P. h. capitis* up to the trachea so that they are suffocated due to the inability to breathe (23). Terpenoids do not eradicate but are antifeedant or reduce the digestive enzymes (21) and the appetite in larvae because these terpenoids have a bitter and sharp taste, which causes larvae not to eat, so they starve and die (25). Tannins found in *Citrus limon* and *Allium sativum* are toxic, have a bitter taste, and have an antifeedant effect (23). Tannins play a role in disrupting the process of absorption of proteins needed for growth by binding to proteins in the digestive system of insects (24).

Essential oils are effective pediculicides due to their direct effects through absorption in the cuticles and indirect absorption through oil vapor (23). This causes the essential oil to have better contact against *P. h. capitis* than permethrin because direct absorption of permethrin is only through the cuticle (5). Differences in the number of *P. h. capitis* deaths in this study might have been caused by differences in the sensitivity of *P. h. capitis*. Some conditions that affected the differences of number of died insects from each concentration were differences in the insect sensitivity to the solution concentrations, the condition of each insect when test materials were given and when samples were taken, the environmental conditions such as the temperature at which experiment was conducted, extract storage (4,25), the extract quality (test material) which was affected by plant species, plant age, parts used, and etc. (19) The results of research conducted by Alzowahi, *et al.* (25) showed that there were differences in the ability of *Allium sativum* as an antibacterial that were stored at 4°C and room temperature of 30°C. The essential oil will evaporate and will be followed by an oxidation process that affects the quality if it is placed uncovered during the storage process (19).

Based on the results of this study, it can be concluded that *Citrus limon* and *Allium sativum* have pediculicides effect and kill 50% *P. h. capitis* (LC50) at a concentration 51.999% for *Citrus limon* and 72.426% for *Allium sativum*.

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