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TOXICITY TEST OF DAYAK ONION BULBS EXTRACT *(Eleuthherine palmifolia (L) Merr)* ON *Artemia salina* LEACH USING BSLT METHOD

(Preface Study As Root Canal Irrigation Materials)

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ABSTRACT

Background*: Dayak onion (Eleutherine palmifolia (L) Merr) is a native plant from Central Kalimantan, which contains active compounds as anti-bacterials that can be used as an alternative material for root canal irrigation. High toxicity is one of the causes of root canal failure, so it takes a toxicity test to determine the toxic effects of the dayak onion bulbs extract.* ***Purpose****: To analyze the difference of toxic effects with various concentrations of dayak onion bulbs extract on Artemia salina Leach using BSLT method.* ***Material and Methods****: This study used true experimental research design, namely post-test only with control group design treated with 11 different treatments, which were dayak onion bulbs extract with concentrations 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml and sea water as its negative control, and done with 3 times repetitions.* ***Result****: The result of the probit analysis in the amount of LC_{50} was 70,371 mg/ml. The data analysis used Shapiro-Wilk and Levene's Test results p > 0,05 means that all the data was normally distributed and homogenous. Further test with One Way Anova obtained result p < 0,05 which means there was a significant difference in the number of dead larvae between concentrations. Further test with Pos-hoc LSD obtained result that there was a significant difference at concentration 100 mg/ml with 10 mg/ml with value p=0,001.* ***Conclusion:*** *The highest toxic effect on dayak onion bulbs extract on Artemia salina Leach is at concentration of 100 mg/ml.*
Keywords: *Toxicity Test, Dayak Onion, Artemia salina* Leach, *BSLT*
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INTRODUCTION

The most common pulp disease cases found in dentistry is pulp necrosis.[1] Pulp necrosis is treated with root canals treatment to clear the root canals of the necrotic tissue. Root canal treatment has three stages: biomechanical preparation, sterilization and obturation.[2] 60% of root canal treatment failure is caused by unclean biomechanical preparation actions and poor filling.[3] At the biomechanical preparation stage requires irrigation fluid to remove debris and debris necrotic tissue.[4] Unclean root canal walls will result in bacteria colonizing and causing persistent inflammation.[5]

A good irrigation solution can kill bacteria, dissolve debris and necrotic tissue, a durable endodontic tool lubricant, not toxic, and economical.[4] Sodium hypochlorite irrigation materials (NaOCl) have the best antibacterial power.[6] The disadvatages of sodium hypochlorite (NaOCl) are toxic, can not dissolve inorganic debris, corrosive to endodontic devices, and causes irritation to healthy tissue.[7]

Selection of appropriate irrigation materials is critical for successful root canal treatment. Incorrect administration of irrigation solutions is one of the reasons for the high irrigation materials toxicity that can irritate healthy tissue.[8] Natural materials can be used to reduce the toxicity of root canal irrigation materials.[4] One of the natural ingredients that can be used as root canal irrigation materials is the dayak onion (*Eleuthherine palmifolia (L) Merr*) from Central Kalimantan.[9] Dayak onion has been found to contain compounds such as alkaloids, glycosides, flavonoids, phenolics, streroids, tannins, naphthoquionones and polyphenols which are antimicrobial compounds.[9,10] Dayak onions are also known to have inhibitory

effects to the fungi and bacteria growth in root canals such as *Enterococcus faecalis* and *Staphylococcus aureus.* Based on this, a test is needed to determine the safety of the dayak onion bulbs extract by performing toxicity test. [9,11,12]

The effects of toxicity of dayak onion bulbs extract can be identified by the method of Brine Shrimp Lethality Test (BSLT) with the animal test used is *Artemia salina* Leach larvae.[13] The use of BSLT method is more advantageous because the results obtained faster (24 hours), inexpensive and easy to process. It is the initial screening method of toxicity testing to know the presence of toxic compounds and not requiring special equipment.[14]

Based on Sari research (2017) showed that the concentration of bacterial inhibitory power of dayak onion bulbs extract (*Eleutherine palmifolia (L) Merr*) on *Enterococcus faecalis* bacteria using concentration variation 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml and 80 mg/ml using 96% ethanol solvent, and the most effective concentration in inhibiting *Enterococcus faecalis* bacteria is at concentration 80 mg/ml.[12] The purpose of this study is to analyze the effect of toxic effects of dayak onion (*Eleuthherine palmifolia (L) Merr*) bulbs extract at concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml , 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml and 100 mg/ml against *Artemia salina* Leach larvae.

MATERIALS AND METHODS

This research began with the making of research permit and ethical clearance issued by Dentistry Faculty of Lambung Mangkurat University No. 007/KEPKGFKG-ULM/EC/VIII/ 2017. This research was a pure experimental study with post-test design only with control group design. The samples used in this study were dayak onion bulbs extract grouped in 11 treatment groups, were concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml and seawater as a negative control. Repetition on each treatment in this study obtained using *Federer*'s formula that was 3 times.

Extraction of Dayak Onion Bulbs

Extraction of dayak onion bulbs was using the maceration method. The criterion of the dayak onion bulbs used was 3-4 months old. Dayak onion bulbs dried in the open air and protected from direct sunlight which covered by a black cloth. The weight of the simplicia is weighed using the analytical balance and the dayak onion bulbs was dried for 3 days, then blended to a fine powder. Dayak onion bulbs powder weighed again with an analytical balance, then the powder was put into a maceration vessel and soaked using 96% ethanol solvent with several stir. Filtering done after 3 days to receive its filtrate. The filtrate was concentrated using a rotary evaporator at a temperature of 40^{o}C until a dayak onion bulbs extract was obtained.

Ethanol Free Test

The ethanol-free test was performed to liberate the extract from ethanol, thus proving no ethanol content contained in dayak onion bulbs extract. The ethanol-free test was performed by adding potassium dichromate which did not produce discoloration after reagents were added.

Sea Water Making

Sea water could be obtained by two ways, obtained from the sea and artificial. Artificial seawater treatment was done by adding 35 grams of non iodized salt to 1 liter of PDAM water. The artificial sea water was measured using salinometer, pH meter and thermometer to achieve the criteria of salinity 35%, pH 7 and water temperature 27^{o}C.

Hatching of *Artemia salina* Leach Larva

Artemia salina Leach larvae hatching was done by several stages, the selection of eggs by aquadest, *Artemia salina* Leach eggs hatching done in plastic containers which divided into 2 types of room, the bright room given lighting with incandescent lamps and dark room covered with black cloth and both spaces was limited by styrofoam, the seawater poured into a plastic container and inserted with 1 gram of *Artemia salina* Leach egg. After 48 hours the eggs would hatch. Healthy *Artemia salina* Leach would move towards light.

Toxicity Test with BSLT Method

Testing of toxicity of dayak onion bulbs extract by BSLT method was done by preparing 33 vials filled with 10 larvae of *Artemia salina* Leach on each vial. Each vial was filled with dayak onion bulb extract and a vial without giving the extract as a negative control. The sea water was put into the vial with a total volume of 10 ml. The BSLT method toxicity test was carried out for 24 hours. After 24 hours, the larvae stimulated with a stir bar for 10 seconds observed with the help of the loop, the non-moving larva was declared to be dead. Dead larvae calculated by the total number of each concentration minus the number of live larvae

RESULTS

The concentrations used in this study were 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml and 100 mg/ml with 3 repetitions and 0 mg/ml concentration as negative control. The mean diagram of the description of data results in this study is presented in Figure 1.

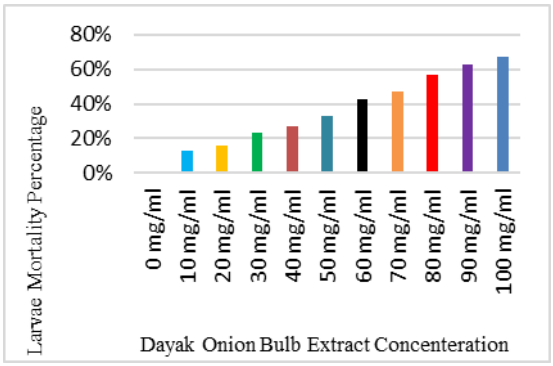

Figure 1. Chart diagram of the mean number of *Artemia salina* Leach larvae deaths at various concentrations of dayak onion bulbs extract after 24 hours figure

Based on the above diagram shows that the higher the concentration of dayak onion bulbs extract, the higher the number of larvae deaths indicating the higher the toxic properties of the extract. The negative control group found no larvae mortality.

The results of Shapiro-Wilk normality test showed all results obtained in the form p value> 0.05 which means all data was normally distributed. Levene's Test results showed that the value of p = 0.981 (p> 0.05) which means homogeneous data.

One Way Anova test results showed that the value of Sig. = 0.006, so p <0.05 means there was a significant difference in the number of dead larvae from various concentrations.

Result of Post-hoc LSD test in this research, there was a significant difference at concentration 100 mg/ml with concentration 10 mg/ml got value (Sig.) = 0,001, so p <0,05. The LSD Post-hoc test results also showed no significant concentrations of concentrations at 10 mg/ml with 20 mg/ml with value (Sig.) = 0.841. At concentrations of 100 mg/ml has a greater toxic effect than other concentrations. It can be showed that the higher concentration of dayak onion bulbs extract potentially has a higher toxicity as well. The next process was a probit analysis to determine the LC_{50} value which was the concentration required to kill 50% of the population of the test larvae. Lethal Concentration 50 (LC_{50}) is used to assess the toxicity of the larvaside. The value of LC_{50} was determined based on the number of test larva deaths obtained at each concentration. The graph below shows the concentration of probit values obtained from the percentage of larvae deaths. The graph of the probit value is shown in Figure 2.

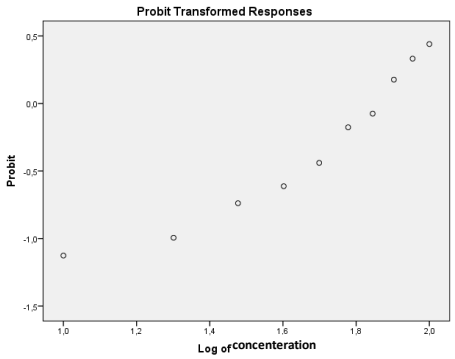

Figure 2. Regression analysis of larvae mortality of *Artemia salina* Leach on various concentrations of dayak onion bulbs extract graphic

The graphic of regression analysis above showed that the greater the concentration of dayak onion bulbs extract used, the greater the percentage value of larvae mortality. Based on probit analysis result got LC_{50} value on larva was 70,371, which mean that 50% of larvae were death at concentration of 70,371 mg/ml.

DISCUSSION

The results of this study suggest that there are significant differences in toxic effects. The greater the concentration of dayak onion bulbs extract, the greater the toxicity. The results of this study showed the greatest toxicity found in dayak onion bulbs extract with concentration of 100 mg/ml.

The value of Lethal Concentration 50 (LC_{50}) from dayak onion bulbs extract to *Artemia salina* Leach mortality in this study was obtained by using probit analysis method. Toxic effects on dayak onion bulbs extract are considered on the basis of magnitude of LC_{50} with Confidence Limit (CL) of 95%. The result of probit analysis showed that the LC_{50} value of dayak onion bulbs extract on mortality of *Artemia salina* Leach can cause toxic. This result is consistent with Mayer's 1982 theory which states that if LC_{50} of an extract that less than or equal to 1000 mg/ml were toxic. The use of *Artemia salina* Leach larvae as a test animal due to the structure of the Ribonucleid Acid (RNA) polymerase II subunit in *Artemia salina* Leach which similar to RNA polymerase II HeLa cells, so it can be used as an indicator of the presence of toxic compounds.[15] The use of BSLT method is more advantageous, inexpensive, faster (24 hours), does not require special equipment and used as a preliminary screening to determine toxic compounds.[14]

The toxic effect of the dayak onion bulbs extract on BSLT method can be seen by observing the number of *Artemia salina* Leach larvae deaths. The submerged or immobile larvae 10 s after the given stimulus declared to be dead.[16] Toxic effects are caused by several active compounds in the dayak onion bulbs extract such as alkaloids,

glycosides, flavonoids, phenolics, steroids, tannins and naphthoquinones.[9,10] Such compounds may inhibit the feeding of the larvae (antifedant). The working mechanisms of these compounds are acting as stomach poisoning which resulted in disturbed larvae digestion. These compounds also inhibited the taste receptor at the mouth area of the larvae. This results in the larvae inability to get flavor stimulus and recognize its food so it will left the larvae to starve. This caused the *Artemia salina* Leach larvae death.[10,17,18]

The largest bioactives on dayak onion bulbs are phenolic compounds by 34.20%.[19] This indicates that the phenolic compounds contained in dayak onion bulbs extract are more dominant in *Artemia salina* Leach larvae mortality. In general, phenolic compounds have potential as antibacterial, antioxidant and antiseptic. Phenolics have a sharp odor and mostly soluble in water or organic solvents. Toxic mechanisms of phenolic compounds can inhibit larvae growth by interfering the work of the brain hormone, edikson hormone and growth hormone, so that the larvae cannot grow and cause death.[20]

Several factors that influence the hatches of *Artemia salina* Leach larvae are salinity, pH, temperature and oxygen level. Salinity levels of seawater should be up to 35%, if the salinity were less than 35%, then *Artemia salina* Leach eggs will sink due to unsuitable levels of salinity. Sea pH conditions range from 7-8.5, because the pH can cause hatching enzymes to work optimally. At pH <7, adult Artemia can not grow optimally and the larvae growth decreases. Warm temperatures can accelerate hatching of eggs, especially at sea water temperature with a range of 25- 28^{o}C which is the optimal temperature for hatching. Sea temperature below 25^{o}C causes eggs to hatch more slowly. *Artemia salina* Leach eggs begins to hatch after exposure to light, so a light stimulus also affects in egg hatching. The use of air pumps with medium pressure and good lighting is very important in hatching eggs, because sea water requires at least 90% oxygen and warm water temperatures for successful egg hatching.[21,22]

Based on the research that has been done, the room used as hatchery and maintenance of the larvae of the treatment group and the negative group is the same as sea water condition. *Artemia salina* Leach in the BSLT method at the 48-hour-old stage of nauplii, since it was the most sensitive age to perform a test and based on its morphology. The 48-hour-old *Artemia salina* Leach larva has begun to have the mouth and digestive tract to consume certain particles, because the yolk (egg yolk) contained food reserves are running out. This is in contrast to the 24-hour *Artemia salina* Leach larvae, although it has a gastrointestinal tract, but is still unable to contact the environment that the extract or external compound can not be absorbed by the larvae.[13,23,24] In this research it can be concluded that the highest toxic effect on dayak bulbs extract on *Artemia salina* Leach is at concentration of 100 mg/ml.

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