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THE EFFECTIVENESS OF DAYAK ONION BULBS EXTRACT (*ELEUTHERINE PALMIFOLIA (L) MERR.*) AGAINST ROOT CANAL MIXED BACTERIAL

(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 and has been trusted as a multifunctional plant, one of them is as an antibacterial. **Purpose:** To find out the difference in the effectiveness of Dayak onion bulbs extract with various concentrations on the growth of mixed bacterial of root canal to be used as an alternative root canal irrigation. **Methods:** This study used true experimental research design namely post-test only with control group design treated with 5 different treatments, Dayak onion bulbs extract at concentrations of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 5,25% sodium hypochlorite, and done with 5 times repetitions. The antibacterial effect test was performed using diffusion method. **Result:** The result of average diameter of inhibition zone formed on Dayak onion bulbs extract at concentrations of 20 mg / ml, 40 mg / ml, 60 mg / ml, 80 mg / ml and 5.25% sodium hypochlorite to the root canal mixed bacterial in sequence i.e. 12.74 mm , 15.82 mm, 20.38 mm, 25.90 mm and 23.52 mm. One Way Anova Test result and Post Hoc LSD test obtained the value $p=0,000$ ($p<0,05$). It proved that there were difference of antibacterial activity of Dayak onion bulbs extract concentration 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and sodium hypochlorite 5,25% against mixed bacteria of the root canal. **Conclusion:** Dayak onion bulbs extract with concentration of 80 mg/ml has greatest antibacterial activity to inhibit the growth of root canal mixed bacterial.

Keywords: Dayak Onion, Effectiveness, Root canal mixed bacterial, Sodium Hypochlorite 5,25%

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INTRODUCTION

The necrosis of pulp is the death of cells in the root canal which resulted in the loss of blood flow and nerve death in pulp tissue which is accompanied by infection.¹ There are some bacteria in the tooth root canal that can cause necrosis, such as *Bacteroides*, *Prevotella*, *Porphyromonas gingivalis*, *Enterococcus faecalis*, *Fusebacterium*, *Veillonella*, *Propionibacterium*, *Eubacterium*, *Actinomyces*, *Peptostreptococcus*.^{1,2,3} Those bacterias that cause infection in the root canal are called mixed bacteria. On the Borzini (2016) research, it was explained that root canal mixed

bacteria consists of over 700 different species of anaerobic bacteria in root canal so in that research the bacteria was directly taken from patients which have necrosis teeth due to caries. Treatments that can be performed on necrosis teeth is root canal treatment.^{4,5}

The succeed of root canal treatment is directly measured by the reduction of microorganisms number in the root canal. Root canal treatment consists of 3 important stages, namely the biomechanics preparation, sterilization and root canal obturation.⁵ Root canal irrigation is one of the procedures that affect the success of root canal treatment. Irrigation aims to remove the smear

layer, as the antibacterial lubrication agent that will eliminate bacteria in the root canal.⁶

Sodium hypochlorite (NaOCl) is one of the widely used irrigation solutions for root canal treatment. According to Chunzhu et al (2013), sodium hypochlorite is an antibacterial and has bacteriocidal characteristic which is used as a lubricant for the root canal preparation. It can dissolve pulp tissue necrosis and organic components in the root canal.⁷ The concentration of sodium hypochlorite usually used is from 0.5%-5.25%. The use of sodium hypochlorite with concentrations of 5.25% cause very toxic effects against the vital tissue which is primarily the dental periapical tissue. The effect of the toxicity of sodium hypochlorite has resulted in the rise of pain in periapical tissue and swelling or oedema in soft tissue.^{8,9} Based on that, it takes irrigation materials derived from nature that does not cause toxic effects and more compatible with the tissue, but have the same antibacterial ability with non-biological materials. One of the natural ingredients that can be used as an alternative root canal irrigation, that is Dayak onion.^{10,11}

Dayak onion is a traditional Central Kalimantan plant and also known as (*Eleutherine palmifolia* (L) Merr) for its scientific name.¹² The part of dayak onion that can be used is the bulb.¹³ The compounds of Dayak onion consists of alkaloids, glycosides, flavonoids, phenolic, triterpenoid saponin, and tannin. The compound can provide antibacterial effect.¹⁴ Based on Sari research (2017), the consents of bacteria power on Dayak onion (*Eleutherine palmifolia* (L) Merr) in inhibiting the *Enterococcus faecalis*'s growth was at concentration of 40 mg/ml, 50 mg/ml, 60 mg/ml, and 80 mg/ml use solvent ethanol 96%, and the concentration of the most effective in inhibiting *Enterococcus faecalis* is found at concentration of 80 mg/ml. It is known as the inhibitory of zone 21, 28 mm.¹⁴

Based on that description, the researcher is interested in examining the effectiveness of Dayak onion (*Eleutherine palmifolia* (L) Merr) at concentration of 20mg/ml, 40mg/ml, 60 mg/ml, and 80mg/ml against the growth of root canal mixed bacterial. The purpose of this research was to know the difference in effectiveness of Dayak onion extract (*Eleutherine palmifolia* (L.) Merr) at concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, and 80 mg/ml and 5.25% sodium hypochlorite

against the growth of the root canal mixed bacterial.

MATERIALS AND METHODS

This research was begun by taking research permit from an ethical clearance issued by Fakultas Kedokteran Gigi Universitas Lambung Mangkurat No.057/KEPKG-FKGULM/EC/IX/2017. This research was a pure research experimental (true experimental) with the design of the post-test only control group design. Sample of these studies was the extracts of Dayak onion which divided into 4 groups at concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml and 80 mg/ml and 5.25% sodium hypochlorite as positive controls. Repetitions of each treatment in this research were obtained using the Federer formula that was 5 times.

Extraction Of Dayak Onion Bulbs

The extraction of Dayak onion was using maceration method. Bulbs was dried in the open air which was protected from the sun exposure with a black fabric-cover. The weight of simplistic was measured by using analytic scale and Dayak onion were dried for 3 days, then crushed using a mixer to a get the powder. Dayak onion powder have been weighed again with analytic scale then inserted into the maceration vessel and solvent soaked in 96% ethanol used as the solvent by stirring a few times. The filtering was done after 3 days to get the filtration. That filtration was concentrated using a rotary evaporator at a temperature of 40°C until it was obtained the thick Dayak onion extract, and then evaporated in the waterbath so it obtained fixed weights. The various concentration of Dayak onion extract was obtained from 100% extract where 4 ml Dayak onion extract without the addition of any materials incorporated in to reaction tube I. The dilution was then performed until it was obtained a solution at concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and then mixed for 60 seconds. The next step was ethanol-free trial addition of Potassium dichromate (K₂Cr₂O₇). If there was no color change, then the Dayak onion extract stated as free-ethanol.

Uptake of Root Canal Mixed bacterial

The researchers introduced and explained to the patient about the purpose and the course of this research. After giving an explanation to the patient, researchers was seeking permission to patients willing to follow procedures of research, then researchers used a universal precaution like masks and handsocon.

Root canal mix bacteri were taken on patient who had necrosis due to caries in Gusti Hasan Aman Dental Hospital. Those teeth were isolate by using a rubber dam. Caries lesions were eliminated and we made access to the pulpa cavity using sterile round bur. The bacteria were taken by using sterile paper points that were entered into the root canal for 1 minute and the pulp necrosis was diagnosed to get mixed bacterial. Paperpoint that contain the bacteria was incorporated into Bouillon media and then closed. Then, the media was put into the incubator for 24 hours.

Antibacterial Power Testing

Mixed bacteria that had been standardized with the Mc Farland 1 for 3×10^8 CFU/ml applied topically with sterile cotton rib on the Mueller Hinton media. Then the paper disk soaked in Dayak onion bulbs extract (*Eleutherine palmifolia* (L.) Merr) at concentration of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, and 5.25% sodium hypochlorite for 3 hours. Next, that media were incubated for 24 hours at a temperature of 37°C. After that we read the measurement drag zone bacterial growth results using a caliper.

RESULT

Based on the research results obtained, average value of root canal mixed bacterial inhibitory zone is as follows:

Table 1. Table of the mean value and standard deviation of inhibitory zone from each treatment of root canal mixed bacterial

Group	Mean \pm SD
EUBD 20 mg/ml	12,74 \pm 0,41
EUBD 40 mg/ml	15,82 \pm 0,20
EUBD 60 mg/ml	20,38 \pm 0,81
EUBD 80 mg/ml	25,90 \pm 0,79
NaOCl 5,25%	23,52 \pm 0,25

Note :

EUBD : Ekstrak Umbi Bawang Dayak (Dayak Onion Bulbs Extract)

NaOCl : 5,25% Sodium Hypochlorite

Based on Shapiro-Wilk normality test, all data of the inhibitory zone were distributed normally with p -value > 0.05 . That distributed normal data was further tested with homogeneity test using the Levene's test. The test result of antibacterial inhibitory zone against root canal mixed bacteria has p -value= 0,637 ($p > 0.05$) which means the distribution of data was homogenized.

The results of One Way Anova statistical tests showed a value of 0.001 ($p < 0.05$) indicating that there were significant differences in each Dayak onion bulbs extract concentration and 5.25% sodium hypochlorite.

Tabel 2. Result Test of Post Hoc LSD Dayak Onion Bulbs Extract Inhibitory Zone and 5.25% NaOCl Against the Growth of Root Canal Mixed bacteria

	NaOCl 5,25%	EBD 20 mg/ ml	EBD 40 mg/ ml	EBD 60 mg/ ml	EBD 80 mg/ ml
NaOCl 5,25%	-	0,00*	0,00*	0,00*	0,00*
EBD 20 mg/ml		-	0,00*	0,00*	0,00*
EBD 40 mg/ml			-	0,00*	0,00*
EBD 60 mg/ml				-	0,00*
EBD 80 mg/ml					-

* = There is a significant difference ($p < 0.05$)

Table 2 shows that the Dayak onion bulbs extract at concentration of 20 mg/ml group has significant difference compared to the group of Dayak onion bulbs extract at concentration of 40 mg/ml, 60 mg/ml, 80 mg/ml and 5.25% sodium hypochlorite. The Dayak onion bulbs extract at concentration of 40 mg/ml group showed a significant difference with the Dayak onion bulbs extract at concentration of 60 mg/ml group, 80 mg/ml and sodium hypochlorite 5.25%. The group of Dayak onion bulbs extract at concentration of 60 mg/ml showed a significant difference with the group of Dayak onion at concentration of 80 mg/ml and 5.25% sodium hypochlorite. The group of Dayak onion bulbs extract at concentration of 80 mg/ml showed a significant difference with the group with 5.25% sodium hypochlorite. These results indicate that the most effective Dayak onion bulbs extract was at concentration of 80 mg/ml to inhibit the growth of root canal mixed bacteria with a mean inhibitory zones of 25.90 mm and has a higher resistance than the positive control that is 5.25% NaOCl with an average of 23.52 mm.

DISCUSSION

The results of this study demonstrated that the best effectiveness of Dayak onion bulbs extract is at concentration of 80 mg/ml against the root canal mixed bacterial mix growth compare to Dayak onion bulbs extract at concentration of 20 mg/ml, 40 mg/60 mg/ml, and 5.25% sodium hypochlorite. According to Mufti et al (2017), the ability of antibacterial ingredient depends on the antibacterial concentration. The magnitude of the power inhibition was increased along with the enhancement of extract concentration.¹⁵ This is because Dayak onion content which consist of secondary metabolites.¹⁴

Secondary metabolites found in Dayak onion are flavonoids, phenolics, alkaloids, saponins, tannins, glycosides, Quinones, steroids and essential oils.¹² Phenol is one of the largest secondary metabolites that contained in onion Dayak amount 34.32%.¹⁶ Phenol interaction with microorganisms cell walls will result in the denaturation of the protein and a decrease permeability of microorganisms.^{11,17}

The bacteria cell wall can be damaged because of permeability reduction which caused by phenol. The permeability reduction will resist the onset of ions displacement – organic ions into the cells of bacteria, so it can inhibit the growth and may even cause cell death in bacteria. Phenol are able to denaturate protein so that all the activity of the cell metabolism is stalled by hydrogen bonds against the protein. The bacteria cell membrane can be damaged because phenol was able to decipher the Phospholipid molecules in the bacteria by ion H^+ attacks on the phosphate groups and causes the cell membrane to leak.^{11,17} Other influential metabolite compounds which act as antibacterial on Dayak onion are essential oil, flavonoids, tannins, saponins and alkaloids.¹⁰

The mechanisms of essential oils as antibacterial start from the process of bacterial cell wall degradation that continued by damaging the cytoplasmic membranes and protein membrane so that the contents of cytoplasm blow out from bacteria cell walls and lead to cell death in bacteria.¹¹ The mechanisms of flavonoids according to Hendra et al (2011), is by the presence of flavonoids to kill bacteria. This metabolite is

classified as nucleic acid synthesis inhibitor and cytoplasmic membrane synthesis inhibitor. The mechanism of alkaloid is by using the content of nitrogen in alkaloid compounds which react with the amino acids that build bacteria cell walls and bacterial DNA. This reaction changes the structure of the amino acids arrangement so that it will change the genetic balance on DNA chain and cause cell death in bacteria.^{10, 19, 20}

Mechanisms of saponin as antibacterial on Dayak onion is by reacting with transmembrane protein on the outer cell walls membranes of bacteria, then formed a strong bond to the polymer resulting in transmembrane protein destruction. The destruction of transmembrane protein will reduce the permeability of the bacteria cell membranes that will result in the lack of nutrients for bacteria so that bacterial growth will be stunted.^{18, 19, 20}

Sodium hypochlorite (NaOCl) is an irrigation solution which often used in root canal treatment. The ideal concentration of sodium hypochlorite for root canal treatment is between 0.5% to 5.25%.²² According to Chunzhu et al (2013), sodium hypochlorite is antibacterial and has bactericidal activity which is used as a lubricant for the preparation of the root canal. It can dissolve necrosis pulp tissue and the organic components in the root canal. Chlorine is the active substances of sodium hypochlorite. Mechanism of chlorine is firstly by damaging the bacteria cell walls. The structure of the bacterial cell wall will be broken by inhibiting cell wall formation. Second, by changing the molecules of protein with denaturation and by damaging nucleic acids without any improvements to the structure as like before. Last, by inhibiting the reaction of biochemical enzyme and disrupting metabolism or causing bacterial cells to die.^{7, 23, 24} In this research, we can conclude that the Dayak onion bulbs extract in concentration of 80 mg/ml has the greatest antibacterial activity against the growth of the root canal mixed bacteria.

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