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ANTIBACTERIAL EFFECT OF RADISH TUBERS (*Raphanus sativus L.*) ON *F.nucleatum* AND *P.gingivalis* AS AN ALTERNATIVE MATERIAL FOR ROOT CANAL MEDICAMENT (*IN-VITRO STUDY*)

CUT NURLIZA & DENNIS

Department of Conservative Dentistry Faculty of Dentistry, University of Sumatera Utara Jl. Alumni Kampus USU, Medan, Indonesia

ABSTRACT

The success of root canal treatment is affected by the elimination of pathogenic bacteria in the root canal aided with the application of intra canal medicament such as calcium hydroxide, but it has a resistance to some bacteria such as Fusobacterium nucleatum and Porphyromonas gingivalis. Development of alternative medicament from natural agent that meets the requirements of medicament materials is needed, such as radish tuber that is biocompatible and has antibacterial properties. Objective: This study was aimed to determine the antibacterial effect of the ethanol extract of the radish tuber (R.sativus L.) against F.nucleatum and P.gingivalis by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Methods

This study began by drying 5 kg radish tuber and 300 grams of simplicia was obtained by extraction with 5 L of 70% ethanol then evaporated with rotary evaporator until 250 gr extracts was obtained. Antibacterial testing using the method of dilution by diluting the extract in Trypticase Soy Broth (TSB) with multiple dilutions to obtain a concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0, 78%, 0.39%. In determining the MIC, 4 ml of each concentration was taken and added to 1 ml of bacterial suspension, vortexed and incubated at 37°C for 24 hours in the CO_2 incubator. Turbidity of each tube was observed visually and compared to control McFarland. Each group was vortexed and taken 100mL, dropped into the petri then poured Trypticase media Soy Agar (TSA) were replicated four samples, then incubated at 37°C for 24 hours in the CO_2 incubator. Counting the number of bacteria formed by using a pour plate method to obtain the value of MBC.

Results

The results indicate that begin to appear clear tube is at concentration of 12.5% and 6.25% for F.nucleatum and P.gingivalis consecutively. There was absence of F.nucleatum at concentration of 100% to 25%. Meanwhile the diffusion test of P.gingivalis showed petri of a concentration of 100%, 50%, 25% and 12.5% in the fourth replication showed sterile petri. Conclusion: The conclusion of the study was the ethanol extract of radish tuber has an antibacterial effect against F.nucleatum and P.gingivalis with the MIC values at a concentration of 12.5% and 6.25% and the MBC at a concentration of 25% and 12.5% consecutively.

KEYWORDS: Root Canal Medicament, Radish Tuber, F.nucleatum & P.gingivalis

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Original Article

INTRODUCTION

Primary root canal infections involve various microorganisms from approximately 4-7 species in root canal which are anaerobic gram-negative bacteria. Some studies have shown there is an obligate anaerobic bacteria which total 90% of all species in the infected root canal.[1]According to Laevi et al (2004) microorganisms involved in chronic periapical lesions are Bacteroides, Treponema, Porphyromonas, Fusobacterium, Peptostreptococcus, Streptococcus, Eubacterium, Actinomyces and Camphylobacter. While the microorganisms involved in acute periapical lesions are Porphyromonas, Treponema, Fusobacterium, Bacteroides, Prevotella, Streptococcus and Peptostretococcus. [2]

Fusobacterium nucleatum is an obligate anaerobic gram-negative bacteria found in the oral cavity and mucous membranes of humans.[3] Presence of these microorganisms in the oral cavity play a role in initiating the course of infection in patients with bad oral hygiene conditions.[3] According to research from Kipalev et al. (2014),Fusobacterium nucleatum is more often found in the primary root canal infection using the Polymerase Chain Reaction (PCR) in the amount of 52.8% compared to secondary root canal infections.[4] Molecular biology research has demonstrated that Porphyromonas gingivalis is one bacterium which has a sizeable percentage in either primary endodontic infections associated with chronic apical periodontitis and acute apical abscess. [5]

Root canal medicaments are the materials used to minimize or eliminate the population of microorganisms in the root canal system after preparation and irrigation process of the canal.[6]Characteristics needed for root canal medicament materials are that it is able to eliminate bacteria that live in the entire root canal system which is not covered with the chemomechanical preparation, reducing inflammation and periradicular pain, helps reduce any periapical exudates, and prevents reoccurring inflammation.[7]

Some ingredients of root canal medication that is often used are calcium hydroxide, antibiotics, biocides non-phenol, biocides phenol and iodine compound. Of all the above medicament material, calcium hydroxide is most widely used in the world since 1920 until now. According to research from Siqueira et al. (2007) it was discovered that bacterium Fusobacterium nucleatum isolated from the root canal after administration of calcium hydroxide for 1 week.[8]

Due to the weakness of some of the ingredients of a medicament in eliminating bacteria, alternative herbal medicament materials are used from plant extracts which have eligible characteristics for a root canal medicament.[9] One plant species has been reported to have antibacterial activity in the study of Raphanus sativus L.[10,11]Beevi et al. (2009) shows the results of the MIC acetone extract with a concentration of 1 mg / ml horseradish root against Bacillus bacteria, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Salmonella typhimurium, Enterobacter aerogenes, Enterobacter cloacae.[10]

Currently there is no research on the antibacterial activity of the ethanol extract of the tuber radish (Raphanus sativus L) against the bacterium Porphyromonas gingivalis and Fusobacterium nucleatus which are one of the bacteria found in the root canal. It is necessary for testing the antibacterial activity of the ethanol extract of the tuber radish (Raphanus sativus L) as an alternative material for root canal medicaments find the value of MIC and MBC.

MATERIAL AND METHOD

Suspension Manufacture Tested Material

Radish tuber extract in ethanol were weighed using an electronic balance and mass adjusted to the desired concentration by way of the media reconstituted with Trypticase Soy Broth (TSB). TSB made with 3 grams of powder in

100 ml TSB aqueduct.TSB is then sterilized in an autoclave for 15 minutes at a pressure of 2 atm and a temperature of 121 ° C. The starting concentration of Radish tuber extract in ethanol is 100% because the concentration capable of inhibiting the growth of Fusobacterium nucleatum dan Porphyromonas gingivalis is unknown, therefore testing began with the greatest concentration.

6 tubes were provided, one tube contained 8ml 100% concentration TSB, while each of the other tubes contained 4 ml of TSB. In the first tube 8 grams of lumpy tuber radish extract was inserted and mixed using a vortex to obtain the tuber radish extract ethanol with a concentration of 100%. Then double dilution was done by taking half of the 100% concentration of the ethanol extract of the tuber turnip using a micropipette and placed in a second tube to get the 50% ethanol extract of the tuber turnip (dilution multiple). The same process was done to obtain a concentration of 25% and 12.5%. Each tube is labeled according to its concentration.

Specimen Culture

The Fusobacterium nucleatum was used from a specimen stem cells Fusobacterium nucleatum ATCC 25 586 and Porphyromonas gingivalis yang was used from a specimen of stem cell Porphyromonas gingivalis ATCC 33 277 where both bred purely on media TSA in an atmosphere of anaerobes in CO2 incubators to obtain healthy growth, which means for the bacteria to thrive. Take as much as 1-2 ose of pure cultured bacteria and suspend it in a 0.9% NaCl solution to obtain turbidity according 0,5 McFarland Standard or equivalent to the amount of 1 x 108CFU / ml (CFU: Colony Forming Unit).

Antibacterial Effectiveness Test

In testing the antibacterial effect of horseradish root extract against Fusobacterium nucleatum and Porphyromonas gingivalis observations were done of for each concentration of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0, 78%, and 0.39%, Determination of the concentration is based on the standards of the laboratory of Tropical Disease at Airlangga University using the dilution method (double dilution).

RESULTS

Observations of Fusobacterium nucleatum obtained MIC at concentration of 12.5%, since at concentrations below 12.5% began to look turbid when compared to positive control (Mc. Farland) after 24 hours of incubation. Furthermore, the calculation of the number of bacterial colonies using Pour Plate method that aims to prove that the turbidity level at each concentration showed the ability of the material trying to kill the bacteria by 99.9% -100%, which is called KBM. In this study, it was found that extracts of tuber radish (Raphanus sativus L.) has an antibacterial effect against Fusobacterium nucleatum with KBM value of25%. (Table 1)

Table 1: Test Results of Antibacterial Effects of ethanol Radish Extract (Raphanus sativus L.) against Fusobacterium Nucleatum at a Concentration of 100%, 50%, 25%, 12.5%.

Tested Material	Replication (CFU/ml)*	Concentration				Positive Control (with Bacteria) (CFU/ml)	Negative Control (without Bacteria) (CFU/ml)
		100%	50%	25%	12,5%		
Ethanol tuber radish extract (*Raphanus sativus L.*)	1	0	0	0	7×10^{6}	$2,1\times10^{8}$	0
	2	0	0	0	5×10^{6}	$2,1\times10^{8}$	0
	3	0	0	0	5×10^{6}	$2,8\times10^{8}$	0
	4	0	0	0	3×10^{6}	$3,2\times10^{8}$	0
	X	0	0	0	5×10^{6}	$2,55\times10^{8}$	0

Description: 0 CFU / ml = sterile, found no bacterial growth

Each CFU / ml has been multiplied by 10 (the multiplier)

Table 1 shows the test results of antibacterial effects (counting the number of colonies formed) against Fusobacterium nucleatum on materials tested with a concentration of 100%, 50%, 25%, 12.5%. In the media given the ethanol extract of the tuber radish (Raphanus sativus L.) with a concentration of 100%, 50%, 25%, negative control showed the results of 0 CFU / ml or sterile and showed the same results in each replication, which means that after planting the TSA media and incubated for 24 hours in a temperature of 37 ° C is growth of bacteria Fusobacterium nucleatum was not found or all of the bacteria had died. While the positive control media encountered by the bacteria growth - average number of all replication is 2.55 × 108 CFU / ml and media were given ethanol extract of the tuber radish (Raphanus sativus L.) with a concentration of 12.5% was found for the growth of bacteria - average number of all replication is 5 × 106 CFU / ml.

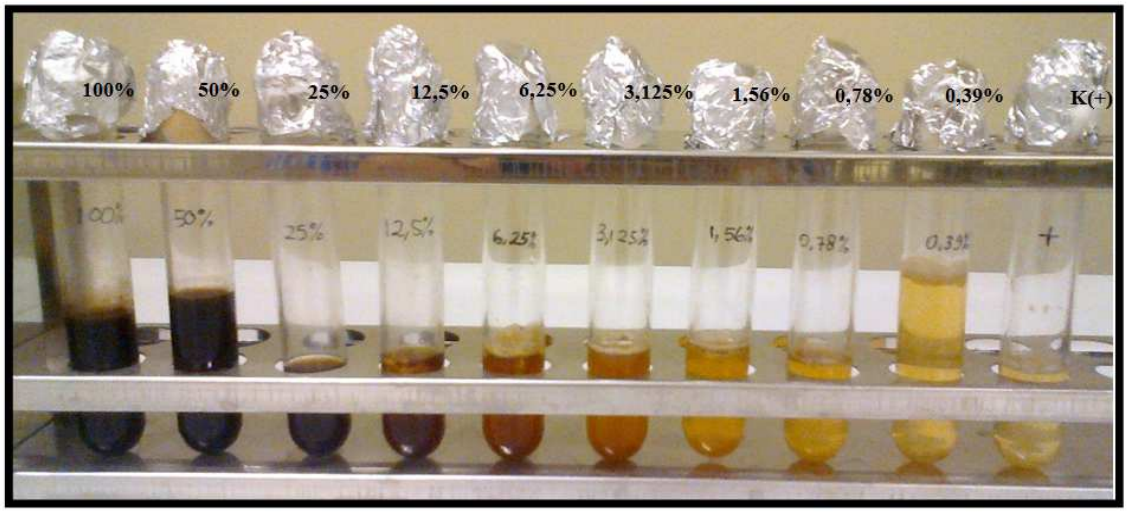

Figure 1: Test Result of KHM at Concentration 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, 1,56%, 0, 78%, 0, 39%, and positive control after 24 Hour Incubation at a Temperature of 37°C against *Fusobacterium nucleatum*

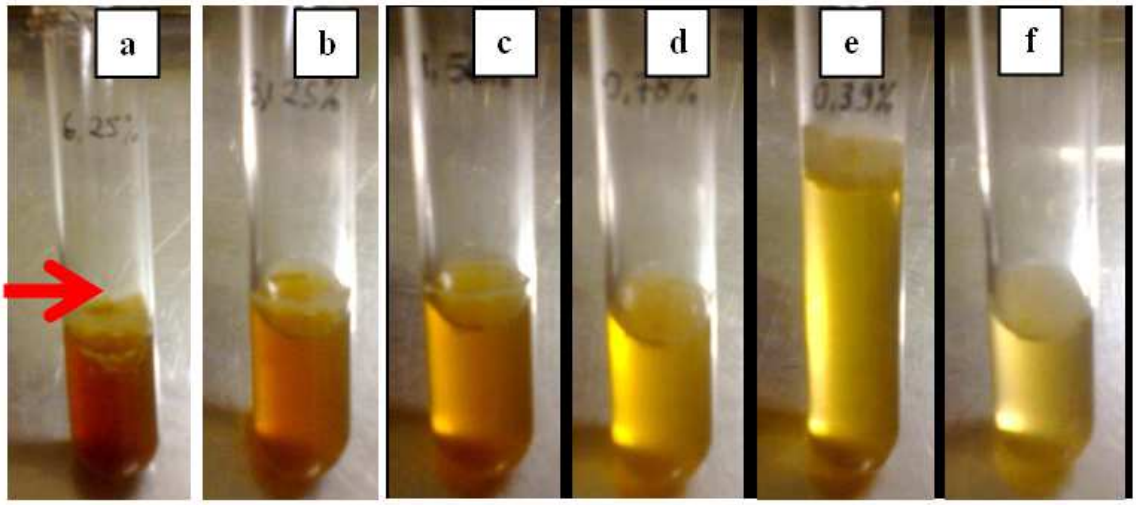

Figure 2: Test Results of KHM Showed Turbidity at (a) 6, 25%, (b) 3,125%, (c) 1, 56%, (d) 0, 78%, (e) 0, 39% Compared with Positive

Control against *Fusobacterium nucleatum Bacteria*

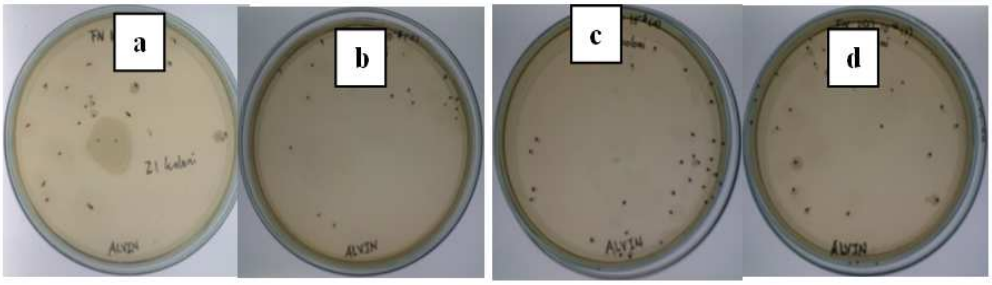

Figure 3: Test Result of Positive Test Material (a) Replication I 2, 1×10^8 CFU/ml, (b) Replication II 2, 1×10^8 CFU/ml, (c) Replication III 2, 8×10^8 CFU/ml, (d) Replication IV 3, 2×10^8 CFU/ml

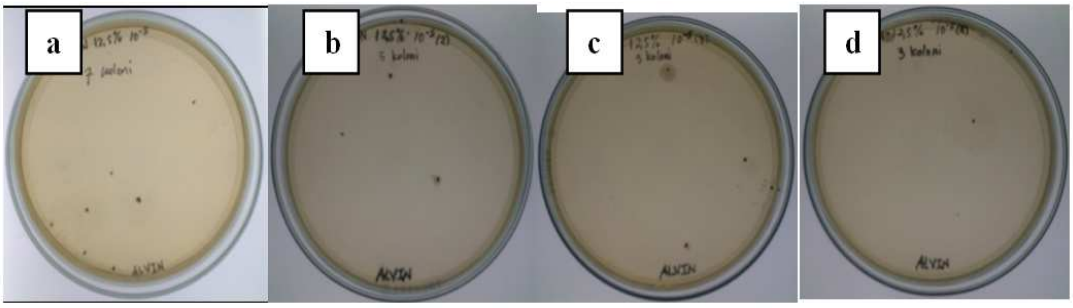

Figure 4: Test Results of Concentration 12, 5% Which Shows Bacterial Growth at (a) Replication I 7×10^6 CFU/ml, (b) Replication II 5×10^6 CFU/ml, (c) Replication III 5×10^6 CFU/ml, (d) Replication IV 3×10^6 CFU/ml

Table 2: Test Results of Non-Parametric Kruskal-Wallis

No.	Concentration	N	Median ± *Interquartile Range*	P *Value*
1.	100%	4	0	0,000
2.	50%	4	0	0,000
3.	25%	4	0	0,000
4.	12,5%	4	5 ± 3	0,000
5.	Control +	4	245 ± 100	0,000
6.	Control -	4	0	0,000

Table 3: Test Results of Non-Parametric Mann-Whitney

No.	Concentration	Concentration	P *Value*
1.	100%	50%	1,000
		25%	1,000
		12,5%	0,013
		Control +	0,013
		Control -	1,000
2.	50%	25%	1,000
		12,5%	0,013
		Control +	0,013
		Control -	1,000
3.	25%	12,5%	0,013
		Control +	0,013
		Control -	1,000
4.	12,5%	Control +	0,019
		Control -	0,013
5.	Control +	Control -	0,013

From the results of the Kruskal-Wallis statistic test obtained p-value = 0.000 (p <0.05) which states that the ethanol extract of the tuber radish (Raphanus sativus L.) has significant antibacterial effect against Fusobacterium nucleatum. From the test results that have medians and interquartile range is a concentration of 12.5% with a median value of 5 and interquartile range of 3, and the median value of the positive control at 245 and interquartile range of 100 (Table 2). The test results comparing concentrations ranging from 100% to 50%, 25%, and a negative control, indicates the value of p> 0.05, which means there is no significant difference. Concentration ratio of between 50% to 25% and a negative control indicates the value of p> 0.05, which means there is no significant difference between the 25% concentration. Comparison with the negative control, showing the value of p> 0.05, which means there is no significant difference. (Table 3)

MIC determination is seen by comparing treatment tube which began to appear clearer when compared with controls Mac. Farland. From the test results tuber extract radish (Raphanus sativus L) against the bacterium Porphyromonas gingivalis after mixed using a vortex and incubated for 24 hours, it was found that in all four tubes replication at a concentration of 3.125%, 1.56%, 0.78%, 0.39%, 0.195% and the positive control there is a visual of a white coating on the surface and if vortex then the solution became turbid while the four other tube replication at a concentration of 100%, 50%, 25%, 12.5% and 6.25% there was no white coating on the surface of the extract so it can be concluded that the extract of the tuber radish (Raphanus sativus L) has a MIC at a concentration of 6.25%.

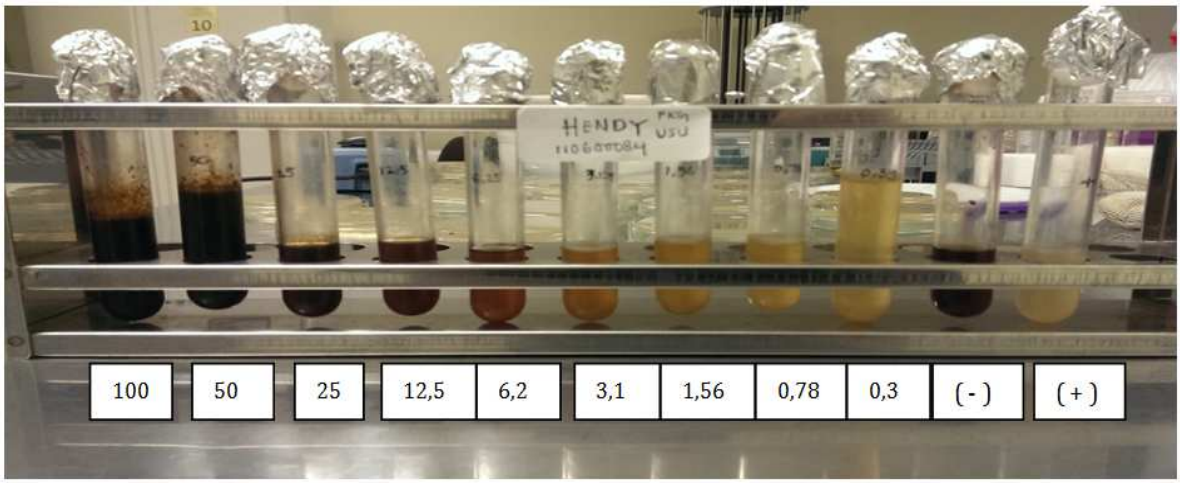

Figure 5: Test Tubes of Concentration 100%, 50%, 25%, 12, 5%, 6, 25%, 3,125%, 1, 56%, 0, 78%, 0, 39%, Positive Control and Negative Control

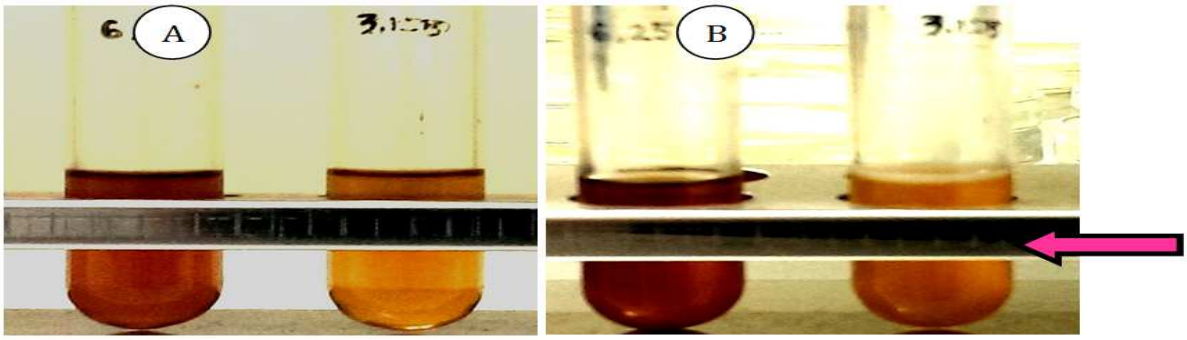

Figure 6: Difference in Turbidity (Arrow) when Compared Concentration 6, 25% with 3,125% (A) Before and (B) After Incubation for 24 Hours

Table 2: Test Results of Diluted Radish Extract against *Porphyromonas gingivalis* at a Concentration of 100%, 50%, 25%, 12, 5%, 6, 25% and 3,125%, Sign (+) Shows Turbidity and (–) Indicated Clear

Test Material	Concentration	Replication			
		1	2	3	4
Radish extract (*Raphanus sativus L*)	100%	-	-	-	-
	50%	-	-	-	-
	25%	-	-	-	-
	12,5%	-	-	-	-
	6,25%	-	-	-	-
	3,125%	+	+	+	+
Control + (*P.gingivalis*)		+			
Control – (radish)		-			

Antibacterial test results on a petri obtained that at a concentration of 100% up to 12.5% there was no bacterial colonies growing at the concentration of 100% up to 12.5 in the fourth replication. At a concentration of 6.25% it appears that there are two colonies of bacteria on second replication, while at the other three there was no visible replication of bacteria. At a concentration of 3.125% at the first replication encountered 8 colonies of bacteria, second replication found 35 colonies of bacteria, third replication found 30 colonies of bacteria, fourth replication found 29 colonies of bacteria. This indicates that the radish root extract has MIC at a concentration of 12.5%.

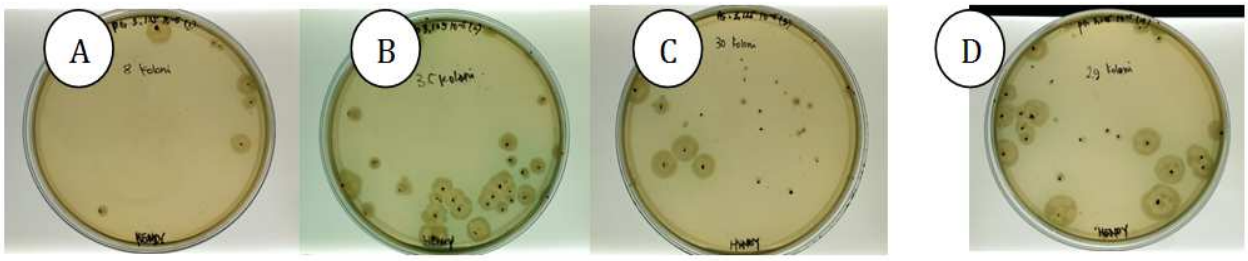

Figure 7: Test Results at Concentration 3,125% Replication (A) 1 (B) 2, (C) 3, (D) 4

Table 3: Test Results of diffused radish Extract against *Porphyromonas gingivalis* at a Concentration of 100%, 50%, 25%, 12, 5%, 6, 25% and 3,125%

Test Material	Concentration	Replication (CFU/ml)				Mean (CFU/ml)
		1	2	3	4	
Tuber radish extract (*Raphanus sativus L*)	100%	0	0	0	0	0
	50%	0	0	0	0	0
	25%	0	0	0	0	0
	12,5%	0	0	0	0	0
	6,25%	0	2.10^6	0	0	$0,5.10^6$
	3,125%	8.10^6	35.10^6	30.10^6	29.10^6	$25,5.10^6$
Control + (*P.gingivalis*)		10.10^6	20.10^6	90.10^6	30.10^6	$37,5.10^6$
Control – (*Raphanus sativus L*)		0				

Table 3 shows the test results of antibacterial activity of extracts of tuber radish (Raphanus sativus L) to the growth of Porphyromonas gingivalis with a concentration of 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. Calculation of the number of colonies at all concentrations from 100% to 3,125% was done using dilution as much as 5 times. In the media given the ethanol extract of the tuber radish (Raphanus sativus L) at a concentration of 100%, 50%, 25% and 12.5% showed the same results in each replication which means that planting on TSA media and incubation for 24 hours in a temperature of 37 ˚ there was no bacteria Porphyromonas gingivalis growth found or it died. At a concentration of 6.25%

there was two bacterial colonies found on second replication, while at the third there was no growth replication of the bacteria Porphyromonas gingivalis. While at a concentration of 3.125% with replication to-1 encountered 8 colonies, second replication encountered 35 colonies, third replication was found 30 colonies, fourth replication showed bacterial colonies found 29 Porphyromnas gingivalis. All colonies of bacteria is then converted to units of CFU / ml using the formula:

Bacterial Σ = Number of colonies x multiplier / dilution factor

Table 4: Kruskal-Wallis Test Concentration of Tuber Radish Extract (*Raphanus sativus L*).

Concentration	Median±Inerquartil Range	Statistic Test Results
100%	0,00 ± 0,000	0,000
50%	0,00 ± 0,000	
25%	0,00 ± 0,000	
12,50%	0,00 ± 0,000	
6,25%	0,00 ± 1,000	
3,125%	29,50 ± 11,958	
Positive control	25,00 ± 35,940	

Table 4 shows the Kruskal-Wallis test showed the average of each tuber extract concentration radish (Raphanus sativus L) and standard deviation and statistical significance using the Kruskal-Wallis test. Median shows the middle value of each concentration. Interquartile range shows the value of the upper limit and lower limit while the Test Results Statistics show the value of p which states that there are differences in the data tested. In Table 4 it was obtained p-value of 0.000 where p <0.05 so it can be concluded that there are significant differences from the data being tested.

DISCUSSIONS

Based on the research method of determining the value of MIC and MBC to begin the search shows that the MIC value of all concentrations of the ingredients tested, it was obtained that at concentrations below 12.5% there was turbidity equivalent when compared to the positive control after 24 hours of incubation. Ethanol extract of root radish (Raphanus sativus L.) at a concentration of 100% up to 25% did not encounter bacterial growth (sterile media) the number of colonies 0 CFU / ml (sterile), at a concentration of 12.5% whereas the colonies formed by the average - average 5 × 106CFU / ml. Based on the results of the study, the research hypothesis is accepted that no antibacterial effect of the ethanol extract of the tuber radish (Raphanus sativus L.) against Fusobacterium nucleatum ATCC 25 586 with a value of 12.5% MIC and MBC values of 25%, with statistical testing that showed significant results.

Based on this research, MIC values obtained at a concentration of 6.25%. Acquisition of MIC can be observed visually by using dilution method. On the tube with a concentration of 3.125%, 1:56%, 0.78% and 0.39% there was a white coating seen on the surface of the extracts and appeared cloudy. It shows that the extract of the tuber radish (Raphanus sativus L) is not effective against the bacteria Porphyromonas gingivalis at these concentrations. While at a concentration of 100%, 50%, 25%, 12.5% and 6.25% seen that there is no formation of white coating on the surface of the liquid extract and the extract is clear after the vortex. This shows that the extract is effective at concentrations so that the MIC values obtained at a concentration of 6.25%. In this study it was found that the average number of colonies in the positive control is 3, 75.107 CFU / ml while the average number of colonies at a concentration of 6.25% and 3.125% is 0, 05.107 CFU / ml and 0 CFU / ml. Results of calculating the number of colonies at a concentration of 6.25% with positive control guidelines kill 98.66% and at a concentration of 3.125% the bacteria die 100%. This indicates that the radish root

extract has KBM at a concentration of 3.125%.

In Indonesia there are many researches on the development of the natural ingredients in herbal remedies and alternative materials as well as root canal medicaments. Research conducted by Amalia (2013) showed that the ethanol extract siwak (Salvadora persica L.) against the bacterium Fusobacterium nucleatum a value of KBM 100% was obtained and the study of Vika (2014) regarding the ethanol extract of leaves Africa (Vernonia amygdalina) which can kill bacteria Fusobacterium nucleatum with KBM value of 12.5%.[12,13] Differences in the antibacterial effects of various natural ingredients can be caused by differences in the levels and content types of antibacterial active substance of each extract material.

CONCLUSIONS

Based on the results of the study the antibacterial effect of the ethanol extract of the tuber radish (Raphanus sativus L) against Porphyromonas gingivalis in vitro can be concluded that the ethanol extract of the tuber radish (Raphanus sativus L) has an antibacterial effect against Porphyromonas gingivalis, with results 6.25% MIC and MBC 12, 5%.Based on the results of experimental studies to find antibacterial effect by finding the value of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Kill Concentration), t shows the antibacterial effect of the ethanol extract of the tuber radish (Raphanus sativus L.) against Fusobacterium nucleatum ATCC 25 586 in vitro obtained MIC values in concentration of 12.5% and the value of KBM at a concentration of 25%.

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