ORIGINAL RESEARCH

Journal of Dentomaxillofacial Science (*J Dentomaxillofac Sci*) December 2018, Volume 3, Number 3: 156-161
P-ISSN.2503-0817, E-ISSN.2503-0825

Differences in effectiveness of guava leaf extract (psidium guajava linn) and lime water (citrus aurantifolia) as irrigation material of root canal as inhibitors of bacteria enterococcus faecalis (Laboratory of Microbiology Faculty of Pharmacy University of Muslim Indonesia (UMI) Makassar 2018)

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Abstract

Objective: Analyze the differences in inhibition zone diameter between 60% guava leaf extract (psidium guajava linn.) and 100% lime juice (citrus aurantifolia) when inhibiting the growth of enterococcus faecalis bacteria.

Material and Methods: This study was a laboratory experimental method with post-test only control group design. Sampling with purposive sampling using 4 treatments and 5 repetitions. Statistical test used One-way ANOVA.

Results: The results of this study showed 60% guava leaf extract (Psidium guajava linn.) inhibited the growth of enterococcus faecalis bacteria with a diameter of inhibition zone of 11.75 ± 0.680 while 100% lime juice (citrus aurantifolia) had a a diameter of inhibition zone of 20.67 ± 2.655 mm, and based on statistical test determined $p = 0.0046 < p = 0.05$.

Conclusion: The alternative hypothesis of this study was accepted and the results of this study indicate that there was a difference of effectiveness between 60% guava leaf extract (psidium guajava linn.) and 100% lime juice (citrus aurantifolia) extract as root canal irrigation material that can inhibit bacterial growth of enterococcus faecalis.

Keywords: Root canal irrigation, Guava leaf extraxt, Lime juice, Enterococcus faecalis

Cite this Article: Syam S, Arifin NF, Irkan NY. 2018. Differences in effectiveness of guava leaf extract (psidium guajava linn) and lime water (citrus aurantifolia) as irrigation material of root canal as inhibitors of bacteria enterococcus faecalis (Laboratory of Microbiology Faculty of Pharmacy University of Muslim Indonesia (UMI) Makassar 2018). *Journal of Dentomaxillofacial Science* 3(3): 156-161. DOI: 10.15562/jdmfs.v3i3.780

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Introduction

Microorganisms could enter the root canal through multiple routes. Generally, the main entrance of microorganisms into the pulp was in the presence of dental caries. Microorganisms might also enter the pulp cavity due to mechanical or traumatic injury through the gingival sulcus and bloodstream.[1]

Several studies had proven that nearly 90% of infections in root canals were bacteria. One of which is a species of gram-positive bacteria known as enterococcus faecalis. Studies of bacterial and molecular cultures confirm that enterococcus faecalis is one of the most prevalent bacteria found in post-root canal root canals that fail. The high resistance of enterococcus faecalis is caused by, among others reasons, the ability of enterococcus faecalis to survive in unfavourable environments.[2-4]

Infected root ducts due to microorganisms play an important role in the occurrence of necrosis of the dental pulp and the development of periapical disorders such as abscesses, granulomas and cysts. Therefore, root canal treatment should be aimed at eliminating or reducing the microbial population and biomechanically removing the necrotic tissue in the root canal system that can act as a microbial growth medium and preventing re-infection by closing the chamber.[4,5]

Root canal treatment can be divided into 3 main stages of root canal biomechanical preparation or cleaned and shaping, disinfection and obturation of root canals. Irrigation is one of the important stages in supporting the success of root canal treatment, since irrigation facilitates the removal of necrotic tissue, microorganisms, and dentine debris from infected root canals. An ideal origin should be non-toxic, inexpensive, and easy to use.[6-8]

Currently there were several commonly used irrigation materials, namely sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA) solution, mixture of tetracycline, an acid and a detergent (MTAD), chlorhexidine, and iodine potassium iodide (IPI). However, the most frequently used is 0.5% - 5.25% NaOCl. This is because NaOCl is considered to be quite effective as an irrigation solution and is considered to represent the ideal

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Received: 29 July 2018
Revised: 2 October 2018
Accepted: 7 November 2018
Available Online 1 December 2018

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conditions of the irrigation solution compared to other irrigation solutions. In addition, several in vitro studies showed that a 5.25% NaOCl solution was able to kill Enterococcus faecalis within 30 seconds. However, NaOCl can create a disruption of the periradicular tissue causing pain, swelling and ulceration. One disadvantage of NaOCl is that it is unable to make good contact with all tissues and not all bacteria in the root canal can be removed.[7,2]

The use of materials derived from nature can be an option as an alternative to root canal irrigation materials because some of these materials can be inhibit the growth (bacteriostatic) or kill bacteria (bactericide). The use of traditional medicines was considered to have fewer side effects compared to drugs derived from chemicals and ultimately, the price is more affordable.[8]

Guava plants (Psidium guajava linn.) are well-known by the people of North Sulawesi and often used to treat dengue fever by means of the juice, but the guava leaf was rarely used as a medicinal plant when the leaves have been proven to treat various diseases such as diarrhoea, dysentery, dengue fever, swollen gums, canker sores, heart and diabetes. Guava leaves can be used as anti-inflammatory and hemostatic remedies. Guava leaf contains active ingredients, including antibacterial tannins (precipitating proteins from bacteria), quercetin, polyphe nolate, quinone, saponins, alkaloids, and flavonoids that can inhibit bacterial growth, leukocyanidin, essential oils, malic acid, resin and oxalic acid.[9]

Another natural plant that has developed in the field of Dentistry as an alternative substance of root canal irrigation is lime (Citrus aurantifolia). Lime has antimicrobial activity that is effective against gram-positive and gram-negative bacteria. The advantages of using lime juice as an antibacterial is that it is easy to obtain. The acid content in lime juice serve as a source of coagulant. In addition, citric acid compounds contained in lemon juice can prevent microbial growth.[2]

Based on research at Muhammadiyah University of Yogyakarta, that 60% guava leaf extract is more effective than other concentration in inhibiting growth of Enterococcus faecalis bacteria. Another study at Lambung Mangkurat University stated that 100% lime juice is more effective than other concentrations in inhibiting the growth of enterococcus faecalis bacteria. Therefore, the researchers are interested in determining the effectiveness of 60% guava leaf extract and 100% lime juiceas irrigation agents of Enterococcus faecalis inhibitor root canal.

Material and Methods

This research used laboratory experimental method with post-test only control design research form. The type of research conducted was true experimental laboratory. In this research method, the sampling technique used was purposive sampling, using 4 treatments with 5 times repetition obtained from the calculation using the formula Lukito.

The research tools used in this research were stationery, autoclave, incubator, split 10 mL, 5 mL and 1 mL, paper disk, analytical scales, Petri dish, sterilizer (oven), stirring bar, reaction cylinder, spoon horn (flatware), round oceans, beaker, spiral light, tweezers, slipper, plastic warp, gloves, aluminum foil, erlenmeyer tube, buchner mouthpiece coated filter paper, label paper, mask and vial bottle.

The ingredients used in this study were bacterium enterococcus faecalis, guava leaf, lime, aquades, 2% chlorhexidine, Mueller Hinton Agar (MHA), 96% ethanol and spirits.

The tools used were washed thoroughly and then dried, for sterilized glassware using an oven at 180°C for 2 hours. Heavy-scale and non-heat-resistant glassware and plastics was sterilized in an autoclave at 121°C for 15 minutes.

Gauva leaves were collected while they were still fresh green and then the guava leaf was washed, dried, cut into small pieces and put into oven with temperature 500°C. Guava leaf was said to be dry if it appeared brownish. A total of 200 g of guava leaf was weighed and dissolved with 2000 mL of 96% ethanol for 24 hours. The result obtained was filtered using a Buchner funnel, after which it was evaporated from the rest of the solvent with a three-hour evaporator with a temperature of 70°C.

The pure extract obtained was placed into a 40°C oven at for two hours then poured into a sterile glass bottle, covered and stored in a refrigerator.

The weighted guava leaf extract was then dissolved with 1 mL of aquades to obtain a 60% concentration. The resulting dilution of guava leaf extract was stored in a vial bottle and labelled.

Limes were washed with clean water and rinsed with 96% ethanol then cut into 2 parts. The limes were then juiced into a 100 mL into the Erlenmeyer flask and filtered using filter paper and then re-filtered using a 0.2 µm membrane filter. The 100% lime juice was covered with sterile aluminum foil and stored at 4°C until use.

MHA (3.4 grams) was dissolved with 100 mL of aquades in an Erlenmeyer flask and covered with gauze and wrapped in paper. The medium was sterilized in an autoclave at 121°C. for 25 minutes. Next, use split to insert 10 mL of medium into a sterile vial bottle. Take 1 ose of bacteria then insert into vial containing medium then homogenize. Pour into a Petri dish and leave it to solidify. Where the bottom of the petri dish was divided according

to the number of paper disks to be assigned to determine the area boundary of each treatment on the MHA.

After the medium was solidified, the paper disk was inserted into 60% guava leaf extract using tweezers and then placed on the surface of the media which is cultured enterococcus faecalis. The other surfaces of the media are given different treatments, ie paper disks that have been inserted into 100% lime juice, aquades and also 2% Chlorhexidine (CHX) using tweezers. They were incubated at 37°C for 24 hours.

Table 1 **Inhibitory zone diameter of 60% guava leaf extract, positive control (K+) and negative control (K-) inhibiting the growth of enterococcus faecalis bacteria**

	Cashew Leaf Extract Concentration 60% (mm)			Control (mm)		
Replication		Mean ± SD	K-	Mean ± SD	K+	Mean ± SD
1.	12.00		0.00		25.70	
2.	11.09		0.00		32.19	
3.	12.60	11.75 ± 0.680	0.00	0.00 ± 0.000	41.08	31.91 ± 6.414
4.	12.04		0.00		26.02	
5.	11.01		0.00		34.58	

Table 2 **Inhibition zone diameter of 100% lime juice, the positive control (K+) and the negative control (K-) on the growth of enterococcus faecalis bacteria**

	Lime juice (Citrus aurantifolia) concentration 100% (mm)			Control (mm)		
Replication		Mean ± SD	K-	Mean ± SD	K+	Mean ± SD
1.	19.05		0.00		25.70	
2.	17.82		0.00		32.19	
3.	24.84	20.67 ± 2.655	0.00	0.00 ± 0.000	41.08	31.91 ± 6.414
4.	20.87		0.00		26.02	
5.	20.79		0.00		34.58	

Table 3 **Differences in inhibition zone diameter of 60% guava leaf extract, 100% lime juice, the positive control (K+) and the negative control (K-) inhibiting the growth of enterococcus faecalis bacteria**

Group	Comparison	Mean Difference	Std. Error	p
60% Guava leaf extract	100% Lime juice	-8.92600*	2.20564	0.0046*
	K+ (CHX)	-20.16600*	2.20564	0.0000*
	K- (Aquades)	11.74800*	2.20564	0.0000*
100% Lime juice	K+ (CHX)	-11.24000*	2.20564	0.0010*
	K- (Aquades)	20.67400*	2.20564	0.0000*
K+ (CHX)	K- (Aquades)	31.91400*	2.20564	0.0000*

The inhibitory power was based on the measurement of inhibition zone diameter (clear zone or clear area without microorganism growth) formed around the paper disk. The measurement used a digital threshold expressed in millimetres (mm).

Results

Inhibition zones were measured with the inhibitory test using 60% guava leaf extract, sterile aquades as a negative control and 2% CHX as a positive control against the growth of enterococcus faecalis bacteria using five replicates on each group. The results obtained are listed in table 1.

Table 1 shows an inhibition zone has been formed in the medium around the paper disk given 60% guava leaf extract and 2% chlorhexidine. The results of the measurements in the table above show that the largest inhibition zone was observed in 60% guava leaf extracton replication 3 of 12.60 mm and the smallest inhibition zone is formed on replication 5 of 11.01 mm. The mean value of five replications of 60% guava leaf extract was 11.75 ± 0.680 mm. The positive control showed the largest inhibition zone in replication 3 of 41.08 mm and the smallest inhibition zone in replication 1 of 25.70 mm. The mean value of the five replicates of 2% chlorhexidine was 31.91 ± 6.414 mm. In the negative control, there was no zone of inhibition around the paper disk.

The inhibition zone was measured with the inhibitory test using 100% lime juice, 2% CHX as a positive control and aquades as a negative control on the growth of enterococcus faecalis bacteria by using five replications on each for each group. The results obtained are listed in table 2.

Table 2 shows that inhibition zones have been formed in the medium around the paper disk given 100% lime juice and 2% chlorhexidine against the growth of enterococcus faecalis bacteria. The results showed that the largest inhibition zone in 100% lime juice was replication 3 of 24.84 mm and the smallest inhibition zone was replication 2 of 17.82. The average value of the five replicates of 100% lime juice was 20.67 ± 2.655 mm.

The inhibition zone was measured with the inhibitory test using 60% guava leaf extract, 100% lime juice, aquades as negative control and 2% chlorhexidine as a positive control on the growth of Enterococcus faecalis bacteria by doing each of five replications, the result of difference of diameter of inhibition zone according to table 3.

Table 3 shows the difference in inhibition zone diameter between 60% guava leaf extract, 100% lime juice, as well as a positive control and

negative control on the growth of enterococcus faecalis bacteria. Based on the results of post hoc multiple comparisons statistical test or further test, it was found that the difference in inhibition zone diameter between 60% guava leaf extract and 100% lime juice was -8.926 mm with a statistically significant p-value of 0.0046. The positive control and 60% guava leaf extract had a difference 20.166 mm and also had a statistically significant p-value of 0.0000. The 60% guava leaf extractand negative control differed by 11.748 mm with had a statistically significant p-value of 0.0000. The 100% lime juice and positive control differed by 11.240 mm and had a statistically significant p-value of 0.0010, The 100% lime juice and negative control differed by 20.674 mm and had a statistically significant p-value of 0.0000. Finally, the positive control and the negative control differed by 31.914 mm and had a statistically significant p-value of 0.0000.

Discussion

Based on the results of the research, the presence of inhibition zones, or clear zones, formed on the medium around the paper disk containing 60% guava leaf extractand 2% CHX. The diameter of the inhibition zone formed showed that the presence of antibacterial activity in 60% guava leaf extract and 2% CHX against the growth of enterococcus faecalis bacteria. In the agar medium given a paper disk containing only sterile aquades as a negative control did not form a clear inhibition zone around the paper disk. This was in line with a study at Muhammadiyah University of Yogyakarta that stated 60% guava leaf extract inhibits the growth of enterococcus faecalis bacteria.[10]

Based on this research, zones of inhibition or clear zones formed on the medium around the paper disk containing 100% lime juice. The diameter of the inhibition zone formed shows that the antibacterial activity of 100% lime juice against the growth of enterococcus faecalis bacteria. This was inline with research at Lambung Mangkurat University stating that 100% lime juiceis effective in inhibiting the growth of enterococcus faecalis bacteria[2]

Looking at the average diameter of inhibition zones, the positive control group produced the largest inhibition zone diameters of 31.91 ± 6.414 mm compared to the inhibition zone diameters of 60% guava leaf extract(11.75 ± 0.680 mm) and 100% lime juice (20.67 ± 2.655 mm). This shows that 2% CHX has strong antibacterial activity when compared to 60% guava leaf extract and 100% lime juice.

The category of inhibition zone inhibition assessment can be drawn from the measurements of diameters classified as no inhibition zone (0 mm), weak (<5 mm), moderate (5-10 mm), strong (11-20 mm) and very strong (>20 mm). Based on the above criteria, the zone of inhibition formed around the paper disk containing 2% chlorhexidine as positive control can be categorized as very strong, the 60% guava leaf extract can be categorized as strong, and the 100% lime juicecan be categorized as very strong when inhibiting the growth of enterococcus faecalis bacteria. The negative control (sterile aquades) showed no inhibition zone diameter is formed, so it can be said that the negative controls used do not have antibacterial power in inhibiting the growth of enterococcus faecalis bacteria.[11]

Based on the research results obtained, the data has homogeneous research results. Homogeneity was tested using the Levene's Test. Research result are said to be homogeneous if homogeneity test results have a significance value of p>0.05. In this study, the normality test was done using the Shapiro-Wilk test because the number of samples was less than 50 samples. Normality test results showed that the test bacteria group had a significance value of >0.05, therefore the data of the research results were said to be of normal distribution.

Based on Levene's test homogeneity test and Shapiro-Wilk normality and then tested using One Way ANOVA analysis. The results showed significance value p=0.000 (p <0.05). This means there were significant differences. Furthermore, the post-hoc multiple comparisons test was used to see whether there was a difference or not between each treatment group.

The post-hoc multiple comparisons test found that there are significant differences seen in all groups. The difference in mean diameter of the largest inhibition zone exists between 2% chlorhexidine as a positive control with sterile aquades as a negative control and the difference in mean diameter of the smallest inhibition zone is between 100% lime juice and 60% leaf extract. This difference is due to the different content of active substances present in each group.

The greatest inhibition zone was found in 2% chlorhexidine because the active ingredients are bactericidal (kill bacteria). CHX is often used in endodontic treatment and it is recommended as a root canal irrigation solution but not a major irrigation material because it is unable to dissolve the remnants of necrotic tissue, it is less effective against gram-negative bacteria, and it has side effects such as toxicity. The use of natural derived ingredients, such as guava leaf extract and lime juice, can be an option

as an alternative to root canal irrigation materials. In addition to the fact that some of these ingredients can inhibit growth (bacteriostatic) and kill bacteria (bactericidal), these ingredients are also considered to have less side effects compared to chemicals, and are more affordable.[7,8]

The second largest inhibition diameter after 2% CHX was 100% lime juice. Lime juice had a difference in mean diameter of the greater inhibition zone compared to guava leaf extract. Both treatments contain flavonoids and other active substances that can act as antibacterials. However, lime juice contains citric acid causing a low pH (2). At that pH, the bacteria enterococcus faecalis is unable to grow because the bacteria prefers a pH environment around 4-11. Changes in pH in the bacterial cell will inhibit the process of sending amino acids from RNA that inhibits bacterial growth.[12]

The antibacterial ability of lime juice and guava leaf extract has been proven in various previous studies. A study conducted by Lauma.[13] said that lime juice has an antibacterial effect on the growth of colonies of staphylococcus aureus characterized by an inhibitory zone formed. This is due to the presence of antibacterial active compounds in the lime juice obtained from the chemical content contained therein, such as essential oils, capable of inhibiting the growth of staphylococcus aureus bacteria. The study conducted by Berlian.[14] has similarities with this study which states that lime juice has an antibacterial effect, indicated by the zone of inhibition that occurs on the growth of escherichia coli bacteria. Inhibition zones occur due to the presence of antibacterial active compounds such as citric acid, flavonoids, and saponins contained in lime juice that are able to inhibit the growth of microbes, such as bacteria escherichia coli.[13,14]

Guava leaf extract has an antibacterial effect on the growth of lactobacillus acidopilus colonies characterized by formation of inhibition zones. Guava leaf extract can inhibit the growth of bacteria because contains active compounds, such as flavonoids, tannins and saponins. This research has similarities with previous studies that state that the guava leaf has an antibacterial effect, indicated by the zone of inhibition formed against the growth of staphylococcus aureus bacteria. The factors that affect the ability of guava leaf extract that is bacteriostatic and bactericidal that contain active compounds saponin, tannin, triterpenoid and flavonoids.[15,16]

Conclusion

Based on the results of research conducted by researchers, it can be concluded: 60% guava leaf extract (psidium guajava Linn.) has an inhibition zone diameter of 11.75 ± 0.68 mm inhibiting the growth of enterococcus faecalis bacteria, 100% lime juice (citrus aurantifolia) has an inhibition zone diameter of 20.67 ± 2.65 mm inhibiting the growth of enterococcus faecalis bacteria, the difference of inhibition zone diameter between 60% guava leaf extract (psidium guajava linn.) with 100% lime juice (citrus aurantifolia) of -8.926 mm in inhibiting growth of Enterococcus faecalis bacteria with p-value = $0.046 < \alpha = 0.05$.

Acknowledgment

Laboratory of Microbiology Faculty of Pharmacy, Indonesian Muslim University for providing facilities required for the successful completion of the research work.

Conflict of Interest

The authors report no conflict of interest.

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