DOI: 10.21276/sjds

Scholars Journal of Dental Sciences (SJDS) **ISSN 2394-496X (Online)**
Sch. J. Dent. Sci., 2017; 4(6):262-266 **ISSN 2394-4951 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjds.2017.v04i06.003

Original Research Article

The Inhibitory Effect of *Carica papaya cv.* Thailand Leaf Extract to the Growth of *Enterococcus faecalis* In Vitro

Yetti Wilda[1], F. M. Satryani[1], S. Maemonah[1], Badriyah[1], J. Christyaningsih[1]*
[1]Health Polytechnic Surabaya, Indonesia

***Corresponding author**
Yetti Wilda
Email: yettiregina@gmail.com

Abstract: *Enterecoccus faecalis* is one of resistant bacteria in the medication of root canal treatment. ChKM is mostly used as sterilization agent. *Carica papaya* leaf extract has been reported to have antibacterial effect from gram-negative bacteria, so could be potentially developed as a root canal sterilization agent. The aim of this study was to determine the inhibitory effect of Thailand varietas of papaya leaf extract to the growth of *Enterococcus faecalis*. This study was an experimental study with post test only control group design and were tested by diffusion methods with 3 groups concentration of 25%, 50%, 75% and 2 controls groups: Aquadest as negative control, and ChKM as positive control, each group consisted of 6 samples. The inhibition effects were examined by measure the diameter of the clear zone around the disc. Data were analyzed by One Way ANOVA test and followed by LSD test. Result showed that there were clear zone around the disc, the greater concentration of the extract the greater diameter of the clear zone. Mean of inhibitation zone at concentration of 25% (6,30mm), 50% (7,54mm), 75%(8,36mm), Aquadest (6mm), and ChKM (11,32mm). It had been proved that papaya leaf extract could inhibit the growth of *Enterococcus faecalis* ($p<0,05$). The largest diameter of the clear zone was in the concentration of 75%. Thailand varietas of papaya leaf extract could inhibit the growth of *Enterococcus faecalis* and the most effective inhibitory concentration is 75% but is smaller than positive control (ChKM).
Keywords: root canal treatment, antibacterial, *Carica papaya* cv. Thailand, *Enterococcus faecalis*

INTRODUCTION

The main purpose of root canal treatment is to remove the bacteria as much as possible of the root canal and create an environment that is not conducive for any remaining organisms to survive [1]. Determinants of successful root canal treatment namely access and working length, sterilization, filling or root canal obturation. Root canal sterilization is required for the action of the preparation with irrigation cannot be freed from the root canal bacteria, given the pulp chamber anatomy is quite complicated and away bacterial penetration into dentinal tubules and aims to obtain the antimicrobial activity in the root canal, neutralize remnants debris in the root canal, control and painful prevent [2].

Root canal treatment requires the use of drugs in order to be able to eliminate the sterilization of bacterial endotoxin that has been attached to the tooth structure that is not perfectly eliminated during the process of root canal instrumentation. Use of drugs for the treatment of root canal sterilization should be able to sterilize and reduce the amount of pathogenic microorganisms in the root channel [3]. Terms of root canal sterilization drug is not irritate periapical tissues and has an antimicrobial effect. Root canal sterilization drug most often used today are ChKM, Camphorated monoparachlorophenol (CMCP), Ca $(OH)_2$ and formocresol [1,9]. Drug sterilization phenols such as ChKM most widely used because it has the advantage of being able to spread because it has a wide spectrum and effective against microorganisms that can destroy a variety of microorganisms, but ChKM also has some drawbacks, namely a pungent odor, bad taste, it can be absorbed by temporary fillings and can spread to the mouth so that the patient will complain of bad taste and are allergens that can cause an immune reaction that can harm dental pulp [4].

Considering the weakness of the material of root canal sterilization, the current root canal sterilization materials with natural materials began to be developed because it is cheap, durable, readily available, low toxicity, and resistance to mikroba [5]. Natural ingredients can be developed as an alternative to root canal sterilization material is papaya leaf.

Papaya is a tree which is so useful, in addition to the fruit which is rich in vitamins, the leaves have so many benefits behind the bitterness they contains, that's why it is the old habits of the people to frequently use papaya leaves as vegetables to eat or boiled for medication. Young papaya leaves can also be used to soften the meat, because the sap in the young leaves of papaya contains papain which is one of the proteolytic enzymes contained in papaya latex. In addition to karpain, papaya leaves contain alkaloids which are compounds produced by plants typical papaya. Alkaloids are nitrogen-heterocyclic compounds, which have toxic properties against microbes so as to effectively kill bacteria and viruses, as antiprotozoal and antidiarrheal [6].

Although the types of papaya are so many, the farmers are often cultivated varieties of Thailand. Cibinong and Hawaiian varieties cultivated only on a limited basis. Thailand papaya cultivation can be found in East Java, for example in the area of Blitar. One MSME (Micro, Small and Medium Enterprises) in the agribusiness sector in plantation cultivation of papaya that have bright prospects are cultivating papaya Thailand because of advantages such as sweet and juicy flesh, as well as large-sized fruit.

Papaya leaf extract can inhibit the growth of bacteria Streptococcus mutans [6], but it is not known activity against the bacteria *Enterococcus faecalis* [9]. This study wanted to determine whether there are inhibition Thailand varieties of papaya leaf extract against bacteria *Enterococcus faecalis*. The general objective of this study was to determine the inhibitory Thailand varieties of papaya leaf extract against bacteria *Enterococcus faecalis*.

Therefore, researchers are interested in papaya leaves of papaya as the herbal ingredients are many and easy to grow in Indonesia as an antibacterial against bacterial growth *Enterococcus faecalis*.

MATERIALS AND METHODS

This research was true experimental design with the post test only control group design. Materials used include the suspension of bacteria *Enterococcus faecalis*, BHI liquid, BHI agar, Thailand varieties of papaya leaf extract with various concentrations (25%, 50% and 75%), Mc solution. Farland 0.5, and sterile distilled ChKM.

Papaya leaf samples taken from the Thai variety plantation Blitar District Agriculture Office. An extraction process was using maceration method. Papaya leaves washed, weighed, dried (drying process was carried out at room temperature without exposure to direct sunlight to avoid damage to the active ingredient contained in the sample) [14], Drying is done until completely dry samples are characterized by a brownish color in all parts of the leaves then the weight is recorded and then finely pulverized by means of blended and sifted through a fine sieve, and blended until it becomes a powder [11]. Put in a flask and added ethanol 96% and shaken by using a water bath at 120 rpm (rotation per minutes) to achieve homogeneous conditions for 1 hour. Furthermore, the solution was macerated for 24 hours at room temperature, then filtered or separated by filters Bunchner. Then filtration residue was aerated and remacerated for 24 hours, maceration repeated up to 3 times. Distillate 1-3 mixed and concentrated by Rotary vacuum evaporator with a temperature of 50°C so that produced creamy papaya leaf extract. Condensed extract of papaya leaves made three series of concentration (25%, 50% and 75%) by using distilled water diluent solution steril [13].

Enterococcus faecalis bacteria breed a pure culture in BHI liquid form that has been incubated for 24 hours in an anaerobic atmosphere, further turbidity is comparable to the standard 0.5 Mc Farland. The study was conducted by the method of diffusion in the media BHI agar. The filter paper was placed in each zone BHI media in order to use sterile tweezers and a bit pressed-press. On the negative control group of filter paper drops of distilled using a micropipette with thoroughness 10 µL, Whereas the positive group spilled ChKM filter paper using a micropipette with a precision 10 µL. in the group treated filter paper spilled Thailand varieties of papaya leaf extract at various concentrations using a micropipette with thoroughness 10 µL. then patridish included in the anaerobic jar and incubated in inkibator during 2x24 hours with a temperature of 37^{o}C. Then measure the diameter of inhibition zone in the form of a clear area around the filter paper using digital calipers (in mm). The measurement is made of a clear boundary past which is adjacent to the colony on the left to the right side of the area measured at the longest distance. Large diameter inhibition zone arising showed antibacterial inhibition at each concentration of varieties of papaya leaf extract Thailand.

RESULT

Table-1: Descriptive statistical tests

Groups	N	Average diameter of inhibition zone (mm)
X_0	6	6,00 ± 0,00
X_1	6	11,32 ± 0,93
X_2	6	6,30 ± 0,68
X_3	6	7,54 ± 0,17
X_4	6	8,36 ± 0,10

The data were analyzed by descriptive analysis to obtain a picture of the distribution and summarizing data to clarify the presentation of the results. Research data that showed bacterial growth inhibition zone *Enterococcus faecalis* with varieties of papaya leaf extract of Thailand at various concentrations are analyzed and tested statistical

significance with a significance level of error of 5 % ($p < 0.05$).

Each treatment group and the positive control test tested the normality using the Shapiro – Wilk [4]. The results Shapiro - Wilk shows that the normal distribution of data and Levene test results obtained significance value of 0.053, so it can be concluded that the research data homogeneous ($p > 0.05$).

The research data were normally distributed and the variance are homogenized and analyzed using parametric tests are one way ANOVA to determine the difference between the positive control group to the treatment group concentration of 25 %, 50 % and 75 % of papaya leaf extract varieties of Thailand on sample.

One way ANOVA test results showed a significance value of 0.000 ($p < 0.05$). This means that there was a significance difference between the positive controls with each treatment group who had a different concentration. These conditions were followed by LSD. From the test results of LSD, it is known that papaya leaf extract Thai variety of all treatments showed significant differences ($p < 0.05$). The higher concentration of papaya extract given to the Thai variety of bacteria, the greater the diameter of inhibition zone formed around the paper disc.

DISCUSSION

The root canal treatment is a dental procedure that aims to eliminate the bacteria that infect the root canal and then preventing the tooth ongoing bacterial infection after treatment. The success of a root canal treatment depends on the reduction or elimination of the microorganisms. The existence of microorganisms after root canal treatment can lead to failure of root canal treatment. It is often found that a polymicrobial growth in root canals is dominated by obligate anaerobic bacteria and facultative anaerobes [3, 15, 16].

Species of anaerobic bacteria such as *Enterococcus faecalis*, Streptococcus anginosus, Bacteroides gracilis and Fusobacterium nucleatum contained in root canal therapy which faced failure . Where Enterococcus usually found in small amounts in root canals that have not been treated but it is often found in root canal treatment failed and can cause persistent infection root canal [3]. Therefore, it needs a root canal sterilization drug which is able to eliminate the bacteria *Enterococcus faecalis* from the root cana [14,15].

The root canal sterilization drug administration considered vital for successful root canal treatment because it can help remove microorganisms reduce pain eliminate the apical exudates, accelerate healing and the formation of hard tissue. Root canal sterilization drug use with the aim of eliminating bacteria that cannot be destroyed by chemo-mechanical processes such as instrumental and irrigation [7].

At this time the root canal sterilization drug commonly used ChKM (Chlorophenol camphor-menthol) besides Cresophene and formocresol. Where the use of drugs sterilization ChKM as a channel to inhibit the growth of microorganisms because it is volatile and has high flowability because of its more liquid / non-viscous so that it can contact directly with microorganism [1]. Addition ChKM able to spread because it has a wide spectrum and effective against microorganisms, can destroy a variety of microorganisms, root canal sterilization is the material most widely used. ChKM consists of two parts para-chlorophenol and three parts of camphor. Disinfectant power and irritating properties smaller than formocresol camphor as a means of thinning and reducing irritation properties of the pure-chlorophenol. Additionally ChKM may prolong the effects of the antimicrobial properties and has a pH that is acidic so easy irritation [3]. However ChKM also has some drawbacks, namely a pungent odor, bad taste, it can be absorbed by a temporary fillings and can spread to the mouth so that the patient will complain of taste unwell and are allergens that can cause an immune reaction that can harm pulpa [8].

Considering the weakness of the material that root canal sterilization, the current root canal sterilization materials with natural materials began to be developed because it is cheap, durable, readily available, low toxicity, and resistance to microbe natural ingredients that can be developed as an alternative to root canal sterilization material is papaya leaf [6].

Research on the antibacterial activity of papaya extract in vitro in bacteria Streptococcus mutans and the results of papaya leaf extract can inhibit the growth of Streptococcus mutant [9]. Where is the gram-positive bacterium that has the same characteristics as the bacteria that will be examined in this study.

The results mentioned with a concentration of 50% papaya extract are able to inhibit the growth of bacteria, followed by administration of the concentration of 75%, and 100%. The higher concentration of papaya leaf extract is used, the greater the inhibition antibacterial. So the researchers chose to decrease the concentration of papaya leaf extract to 25%, 50%, and 75% for the bacteria to be tested for inhibitory namely *Enterococcus faecalis* is gram-positive bacteria. Selected positive control is ChKM because it has a broad antibacterial spectrum and is effective against bacteria and can destroy a variety of microorganisms in the root canal. ChKM also a root canal sterilization materials most widely used consists of two parts para-chlorophenol and three parts of camphor. Disinfecting power and irritating properties

smaller than the formocresol. Camphor as a means of thinning and reducing irritation properties of the pure-chlorophenol. Besides extending the effects of nature antimicrobe [12]. Negative control used is sterile because Aquadest not have antibacterial properties that will affect the inhibition of bacteria and is used as a diluent papaya extract is based on the results of preliminary research that has been done.

The research to see their inhibitory papaya extract varieties of Thailand (*Carica papaya* cv thailand) on the growth of the bacteria *Enterococcus faecalis* performed by diffusion method that uses the media to BHI as reasonably practicable to do with high validity and effectively used to determine the growth of bacteria *Enterococcus faecalis* that is a bacteria Gram positive anaerob [8]. Papaya extract solvent selected Thai variety is distilled sterile because it has antibacterial properties that will affect the inhibition of bacteria and is used as a diluent papaya extract is based on the results of preliminary research that has been done. In this study, it appears that Thailand varieties of papaya leaf extract can inhibit the growth of bacteria *Enterococcus faecalis* [13] in all treatment groups with a concentration of 25%, 50% and 75%. It is known in the research of papaya leaf extract showed significant inhibition on the growth of Gram-positive bacteria [12]. This fact caused by broad-spectrum antibacterial component found in papaya leaves include alkaloids, tochopenol, and flavonoids. Alkaloid has a working mechanism that is associated with the ability to interact with DNA. The working mechanism of inhibition by interfering with peptidoglycan constituent component of the bacterial cell, so the cell wall layers are not fully formed and caused the death of these cells. In alkaloid compounds also contained basic groups containing nitrogen reaction that will react with the amino acid compound preparing cell walls of bacteria and bacterial DNA. This reaction resulted in changes in the structure and composition of amino acids that will cause genetic changes in the balance on the DNA chain that would damage that will promote cell lysis bacteria that will cause the death of cells in bacteria [3].

Tochopenol is typical of phenolic compounds in the papaya plant. Where these phenol compounds can disrupt the cell wall constituent that causes increased permeability of cell membranes and cause a loss of the components of the cell resulting in lysis (dissolved) cell [11]. Flavonoids are the largest group of phenolic compounds having a carbonyl group with an extract of cells and soluble proteins, with such ties can inhibit protein synthesis of the bacterial cell. This is what gives the antibacterial activity. Phenol compounds from plants also have the ability to form a complex with the protein through hydrogen bonding, which can damage cell membranes bacteri[3]. In this study, researchers used ChKM as a positive control. ChKM included in the derivatives of phenol, which is where the mechanism of action of phenolic compounds in inhibiting bacterial cell, by way denature protein bacterial cells, inhibits the function of cell membranes (transport of substances from the cell one cell to another) and inhibit the synthesis of nucleic acid that bacteria growth can obstructed labor [11,12]. Mechanism for inhibiting bacterial ChKM same as the mechanism of action of flavonoids which are within the content of papaya varieties Thailand.

Diameter of inhibition zone was measured and statistically tested using one-way ANOVA test with an error rate of 5%. Then to compare the relationship between the zones of inhibition at concentrations from one another are used post hoch test in the form of LSD or a real smaller difference test. From the statistical analysis shown significant difference between the positive control group (ChKM) the negative control group (distilled sterile), with the treatment group (Thai variety of papaya extract with a concentration of 25%, 50% and 75%).

At the same statistical tests, it was found that the diameter of inhibition zone on providing varieties of papaya leaf extract Thailand with 75% concentration showed a significant difference when compared with the other treatment groups. From the findings, it seemed that the greater the concentration of papaya leaf extract Thai variety, the greater the diameter of inhibitory zone. The concentration of 75% has the highest zone of inhibition when compared with a concentration of 25% and 50%. This is because flavonoids function as an antimicrobial compound soluble in water [13].

This research was qualitative in nature, namely to show the differences in inhibition of papaya leaf extract varieties of Thailand to the growth of the bacteria *Enterococcus faecalis* by using a concentration of 25%, 50% and 75% compared with ChKM which showed that the inhibition of the highest concentration of 75% is still smaller than inhibition ChKM as a positive control so we need to do further research to compare the toxicity of Thailand varieties of papaya leaf extract compared with root canal sterilization materials ChKM.

CONCLUSION

Based on the results was revealed papaya leaf extract can inhibit the growth of Thailand varieties of bacteria *Enterococcus faecalis* at concentrations of 25 %, 50 % and 75 %. The extract of papaya leaf at a concentration of the largest varieties of Thailand (75 %) is the most effective concentration to inhibit the growth of *Enterococcus faecalis*, the largest concentration in this study (75 %) have inhibitory smaller than ChKM as a positive control.

REFERENCES

1. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as

antimicrobial medicaments in endodontics. Australian Dental Journal. 2007 Mar 1;52(s1).
2. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics. 2006 Feb 28;32(2):93-8.
3. Newman MG, Van Winkelhoff AJ. Antibiotic and antimicrobial use in dental practice. 2001.
4. Suchitra U, Kundabala M, Shenoy MM. In search of endodontic pathogens.
5. Deppe C. The problem of endodontitis and managing it through conservative dentistry.
6. Amri E, Mamboya F. Papain, a plant enzyme of biological importance: a review. Am J Biochem Biotechnol. 2012;8(2):99-104.
7. Johnson WT, Noblett WC. Cleaning and shaping. Endodontics: Principles and practice. 4th ed. St. Louis: Elsevier Health Sciences. 2009:258-87.
8. Becton D. Brain Heart Infusion Agar. USA. 2011, 1-2.
9. Souza-Filho FJ, Soares AD, Vianna ME, Zaia AA, Ferraz CC, Gomes BP. Antimicrobial effect and pH of chlorhexidine gel and calcium hydroxide alone and associated with other materials. Brazilian dental journal. 2008;19(1):28-33.
10. Juntavee A, Peerapattana J, Ratanathongkam A, Nualkaew N, Chatchiwiwattana S, Treesuwan P. The antibacterial effects of apacaries gel on Streptococcus mutans: An in vitro study. International journal of clinical pediatric dentistry. 2014 May;7(2):77.
11. Basting RT, Gonçalves FR, França FM, do Amaral FL, Flório FM. Antimicrobial potential of papain chemomechanical agent on Streptococcus mutans and Lactobacillus casei followed by the use of self-etching adhesive systems. Journal of Clinical Pediatric Dentistry. 2016 Jan;40(1):62-8.
12. Bhardwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*: An in vitro study. Journal of conservative dentistry: JCD. 2012 Jul;15(3):293.
13. Manjunath HM , Chandrashekhar GJ, Vijay R, Mahesh M. Comparative Analysis of Papain from Different varieties of Papaya Plant Latex, International Journal of Agricultural and Food Science, 2014; 4(4): 123-127.
14. Suchitra U, Kundabala M. *Enterococcus faecalis*-An Endodontic Pathogen. Endodontology. 2006;18(2):11-3.
15. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics. 2006 Feb 28;32(2):93-8.
16. Portenier I, Waltimo TM, Haapasalo M. *Enterococcus faecalis*–the root canal survivor and 'star'in post-treatment disease. Endodontic topics. 2003 Nov 1;6(1):135-59.