ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2017; 3(1): 89-93

www.oraljournal.com
Received: 17-11-2016
Accepted: 18-12-2016

Dennis
Department of Conservative Dentistry, Faculty of Dentistry, University of Sumatera Utara, Jl. Alumni No. 2 Kampus USU Medan, Sumatera Utara, Indonesia

Cut Nurliza
Department of Conservative Dentistry, Faculty of Dentistry, University of Sumatera Utara, Jl. Alumni No. 2 Kampus USU Medan, Sumatera Utara, Indonesia

Wulandari Savitri
Department of Conservative Dentistry, Faculty of Dentistry, University of Sumatera Utara, Jl. Alumni No. 2 Kampus USU Medan, Sumatera Utara, Indonesia

Correspondence
Dennis
Department of Conservative Dentistry, Faculty of Dentistry, University of Sumatera Utara, Jl. Alumni No. 2 Kampus USU Medan, Sumatera Utara, Indonesia

Antibacterial effect of ethanol extract of the avocado seed (*Persea Americana* Mill.) as an alternative root canal Irrigants against *Porphyromonas Gingivalis* (*In Vitro*)

Dennis, Cut Nurliza and Wulandari Savitri

Abstract
Porphyromonas gingivalis is one of the bacterial that often found in primary endodontic infection and able to form biofilm. Root canal irrigation is very important step to eliminate bacterial and biofilm, but currently there is no ideal irrigant solution. Avocado seed (*Persea americana* Mill.) is one of the herbal product which has antibacterial effect and able to developed as an alternative irrigants. The purpose of this study is to know the antibacterial effect of ethanol extract of the avocado seed (*Persea americana* Mill.) by finding the minimum inhibitory concentration (MIC) and minimum bactericidal concentration. This study design is posstest only control group and that collected by doing the experiments. 300 gram of avocado seed (*Persea americana* Mill.) simplicia is being macerated with 70% alcohol in 15 minutes, then percolated until percolator fluid become clear, after that evaporated with rotary evaporator till get 60 gram of viscous extract. MIC value determination is done using dilution method by dilute the extract in *Trypticase Soy Broth* (TSB) with concentration 100%, 50%, 25%, 12,5%, 6,25%, and 3,125%. 4 ml of each concentrations is taken and added 100 µl of bacterial suspension, vortexed and incubated in temperature 37°C for 24 hours. The turbidity of each tube is observed visually and compared with *Mc Farland* control. The tube that start to look clear is MIC. Next, each concentration vortexed and took 100 100 µl, dropped in petri then poured it in *Trypticase Soy Agar* (TSA) media and replicated into 4 samples, incubated in temperature 37°C for 24 hours, then counting the number of bacterial colony with Pour Plate method to determine MBC value. In dillution test the tube start to look clear in concentration 50%, in bacterial colony counting using Pour Plate method showed the bacterial growth in concentration 50% with average $1{,}75.10^7$ *CFU*/ml.Further study was done to determine MBC value and found concentration 60%. Kruskal-Wallis test showed that avocado seed (*Persea americana* Mill.) ethanol extract had antibacterial effect against *Porphyromonas gingivalis* (p=0,000) and Mann-Whitney test showed there are significant differentiation between concentration 50% with 100%, 80%, and 60%. The conclusion of this study is ethanol extract of the avocado seed (*Persea americana* Mill.) had antibacterial effect against *Porphyromonas gingivalis* with MIC value in concentration 50% and MBC value in concentration 60%.

Keywords: root canal irrigation, avocado seed ethanol extract, *Porphyromonas gingivalis*

1. Introduction
Pulp and periapical disease occurs because of an opportunistic infection by pathogenic bacteria that invade the pulp and periapical tissues [1]. Therefore, the succesful of endodontic treatment depends on a decrease in the number of microorganisms in the root canal. An estimated 700 oral bacterial species have been identified by nucleotide sequence analysis of the 16S rRNA subunit and less than 50% of these species cannot, as yet, be cultured in the laboratory [2]. Some studies indicate that in infected root canal is more than 90% dominated by obligate anaerob bacteria [3]. In the root canal infections, bacteria not only as planktonic form or another aggregate, but also can form a biofilm. Biofilms are composed of microcolonies of bacterial cells that are distributed in a matrix which consists of exopolysaccharides, proteins, salts and cell material in an aqueous solution [4]. Study shows that microorganisms in biofilms 1000-1500 times more resistant to antimicrobials [5]. Black pigmented bacteria (BPB) is one of the most often found bacteria in root canal infections as Prevotella and Porphyromonas [4]. Porphyromonas gingivalis is a group of Porphyromonas, anareob obligate, gram-negative and black pigmented bacteria normally found in a cavity mouth and plays an important role in the occurrence of periodontal disease, but also often found in root canal infections [6].

In studies using the PCR method Porphyromonas gingivalis was found with a prevalence of 28%-43.3% [4, 7, 8]. The prevalence of Porphyromonas gingivalis with various forms of periapical lesions showed a fairly high number, this is indicated by several studies that found Porphyromonas gingivalis on the primary root root canal infection accompanied apical periodontitis at 39.5% -70% [9, 10].

The existence of Porphyromonas gingivalis and Porphyromonas endodontalis in the necrotic root canal respectively 43% and 28% often associated with the occurrence of periapical disease and acute abscess accompanied by pain and swollen [4, 11]. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia are a group of red complex bacteria that dominate the periodontal pocket and have a role in the development of periodontitis [6]. In addition, the study shows red complex bacteria is also found in the root canal infection with endo-perio lesions. Rocas *et al.* (2001) found 33 out of 50 pulp necrosis with periapical lesion teeth contained at least one group of bacteria in red complex [12]. Porphyromonas gingivalis has virulence factors include lipopolysaccharide, fimbria, capsules, gingipain, outer membrane vesicles, proteinase, fibrinolysin, phospholipase, acid phosphatase, DNase, hialironidase, chondroitin sulfatase, hemolysin, metabolites, and heat-shock proteins [13]. This virulence factors can trigger the body defense mechanism that leads to tissue damage [6].

The successful of root canal treatment are directly affected by the removal of microorganisms in the root canal. So that, to eliminate the microorganism completely from root canal we need chemomechanical technique preparation. Chemomechanical preparation is a combination of mechanical instrumentation, root canal irrigation, and medicaments containing mantibacterial [14]. Root canal irrigation aims to prevent the accumulation of hard and soft tissue in the apical portion, eliminate microorganisms on the root canal, lubricate dentin walls, remove debris, and capable of dissolving the smear layer [15].

To achieve the goal of endodontic treatment, an ideal irrigation solution is needed. The ideal irrigation solution are have characteristics such as not toxic, capable of dissolving pulp tissue is necrotic or still vital, eliminate microorganisms, can act as a lubricant, dissolving smear layer, removing debris, and have a low surface tension, however there is no irrigation material which possesses all of the ideal characteristics [15, 16]. NaOCl has some weakness such as bad smell, toxic to periodontium tissue, incapable of removing smear layer, interfere bonding and polymerization sealer resin [15, 17]. EDTA barely have antimicrobial properties so that EDTA would be more effective if combined NaOCl [17]. Chlorhexidine is not a major irrigation material because is not able to dissolve necrotic tissue and less effective against gram negatif [16, 17]. Study showed antibiotic in MTAD can cause stains on teeth. In addition, some bacteria found in root canals it was likely to be resistant to tetracycline antibiotic used in high concentrations [18].

Avocado seed (*Persea americana* Mill.) is one of the natural products that are currently widely studied. *Persea americana* Mill. Is a plant that originated in Central America (Mexico, Guatemala, Antilles) which is also found in many tropical regions like Indonesia because it can adapt well. Avocado seed (*Persea americana* Mill.) is known to have a hypoglycemic effect and can be used as a traditional medicine to treat toothache, chronic gastritic, hypertension, and diabetes mellitus [19, 20].

Phytochemical analysis results show that the avocado seed has secondary metabolites such as flavonoids, saponins, tannins, alkaloids, steroids and terpenoids. The active component act as antibacterial of the seed extract of avocado (*Persea americana* Mill.) are flavonoids, tannins, saponins and steroids. The experiment by Idris S *et al.* (2009) of ethylacetate extracts of avocado seed (*Persea americana* Mill.) 3.25 gram against *S. aureus* and *S. pyogenes* showed antibacterial activity with inhibition zone 37 mm and 25 mm [21]. Asri D (2014) conducted a study of antibacterial effect of ethanol extract of the avocado seed (*Persea americana* Mill.) as an alternative irrigation against *E. faecalis* show that at a concentration of 10% avocado seed extract showed antibacterial activity with inhibition zone 2.32 ± 0, 12 mm [22].

The purpose of this study was to determine the antibacterial effect of the ethanol extract of the avocado seed (*Persea americana* Mill.) as an alternative root canal irrigants against Porphyromonas gingivalis to find the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) ethanol extract of seeds of avocado (Persea americana Mill.)

2. Materials and methods

This study design is posstest only control group and that collected by doing the experiments. The population of this study is *Porphyromonas gingivalis* bacteria. The sample in this study are *Porphyromonas gingivalis* ATCC 33277 colonies which isolated and cultured with *Trypticase Soy Agar* (TSA) media. Sampel yang digunakan dalam penelitian ini adalah koloni adalah bakteri *Porphyromonas gingivalis* ATCC 33277 yang telah diisolasi dan dibiakkan dengan media *Trypticase Soy Agar* (TSA).

Total sample is determined by the experimental formula and obtained 28 samples respectively for MIC and MBC.

2.1 Avocado Seed Extraction

Avocado seed (*Persea americana* Mill.) 2 kg washed and dried in the drying cabinet. After that, blended and sifted in order to obtain 300 grams of simplicia powder. Then simplicia powder soaked in 70% ethanol for 15 minutes, then transfer to a perkulator and added 70% ethanol and closed with aluminum foil for 24 hours. Liquid dripped and added 70% ethanol to obtain a clear liquid. The liquid extract is evaporated at vaccum rotapavor until viscous consistency, then stored in a sealed plastic bottle, stored in a cool place.

Fig 1: Dried avocado seed

Fig 2: Blended avoado seed

Fig 3: Macerated avocado seed

Fig 4: Percolated avocado seed

Fig 5: Viscous avocado seed extract

2.2 Antibacterial Activities Test with Dilution Method

Antibacterial test of avocado seed extract (Persea americana Mill.) performed by dilution method. The ethanol extract of avocado seed (Persea americana Mill.) replicated four times with concentration of 100%, 80%, 60%, 50%, 25%, 12.5%, 6.25%, 3.125% (each concentration = 1 ml) added to the suspension of the bacteria Porphyromonas gingivalis (100 mL), and then incubated in a CO2 incubator at a temperature of 37○C for 24 hours. Then, all of avocado seed extract concentration of ethanol compared with Mc. Farland standard to determine the value of the MIC. After that each group concentration was mixed using a vortex, taken 100 mL and dropped on the solid medium (Trypticase Soy Agar), and then put in a CO2 incubator at a temperature of 37○C for 24 hours. Then, calculate the number of bacterial colonies on each petri to determine the value of MBC. Antibacterial test data were analyzed computerized using a statistical Kruskal Wallis and Mann-Whitney test.

3. Results

The extraction of avocado seed (Persea americana Mill.) obtained 60 grams of viscous extract. This viscous extract stored in sealed plastic bottles and placed in the refrigerator before the effectiveness of antibacterial activity test. Testing for antibacterial activity begins with determining the value of the MIC. On the results of observations MIC showed at concentrations 50% because at the concentrations below 50% visually visible white layer on the surface of extracts and then in the vortex the solution becomes cloudy when compared to the positive control (Mc Farland) after incubated 24 hours in a temperature of 37○C, while at concentrations above 50% is clear and there are no white coating on the surface of the extract. After that, the calculation of the number of bacterial colonies using Pour plate method which aims to get the value of MBC. MBC is the concentration that able to kill bacteria by 99.9% -100% after incubated for 24 hours in a temperature of 37○C. MBC value of avocado seed extract (Persea americana Mill.) in this study was obtained at a concentration of 60% because in the concentration of 50% is still a bacterial growth with an average 1,75.107 CFU / ml.

Statistical test results of Kruskal-Wallis obtained p value = 0.000 ($p < 0.05$). This means that the ethanol extract of the seeds of avocado (Persea americana Mill.) had a significant antibacterial effect against Porphyromonas gingivalis. The results of Mann-Whitney test showed significant differences between each concentration of 100% compared with a concentration of 50% and a positive control, a concentration of 80% compared with a concentration of 50% and a positive control, a concentration of 60% compared to 50% and a positive control, concentration of 50% compared to the positive control and negative control, as well as the negative control compared to positive controls which show the p values <0.05. Comparison between the concentration of 100% to 80%, 60%, and a negative control showed the p value> 0.05 means that there is a significant difference.

4. Discussion

In this study, the avocado seed extraction is done with ethanol. Ethanol is a solvent that can dissolve the entire active substances contained in a natural product, both active substances are polar, semipolar or nonpolar. Ethanol used in this study was 70%, which is able to attract the active substances in the seeds of avocado (Persea americana Mill.). In addition, the use of 70% ethanol is also safer than the methanol.

The test of antibacterial activity against Porphyromonas gingivalis done by dilution method to search MIC value followed by Pour Plate method to find the MBC value. Dilution method selected because the substances can contact directly with microorganisms so that can be known MIC value by observe the changes in turbidity in the test tube. Pour Plate method was conducted to determine the value by counting the number of colonies of bacteria that has been given the extract in accordance with the concentration so that the results will be more representative.

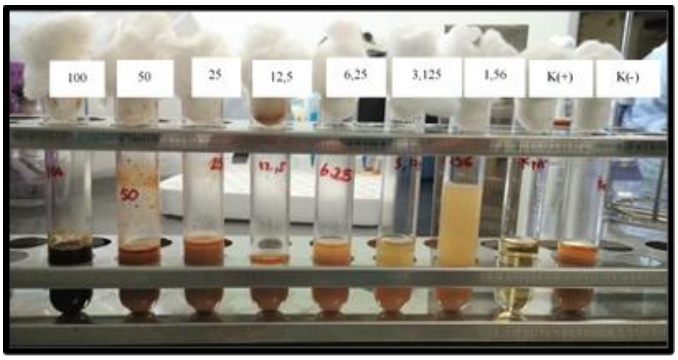

Fig 6: Dilution tubes for MBC test at concentration 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, 1,56%, Positive control, and Negative control before incubated 24 hours at temperature 37°C.

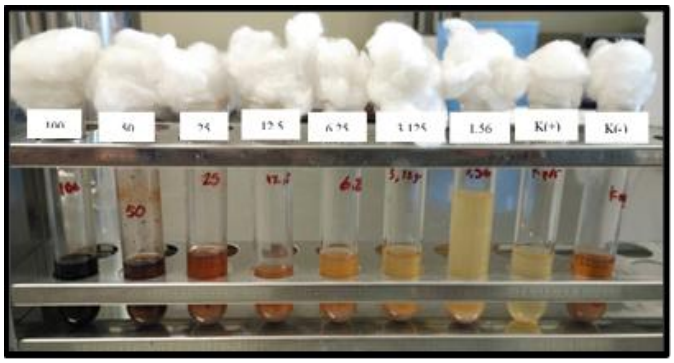

Fig 7: Dilution tubes for MBC test at concentration 100%, 50%, 25%, 12, 5%, 6,25%, 3,125%, 1,56%, Positive control, and Negative control after incubated 24 hours at temperature 37°C.

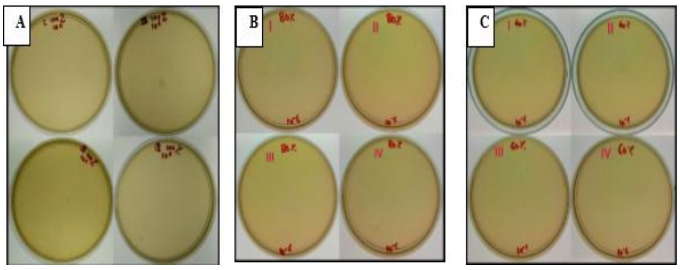

Fig 8: Pour plate test results of concentration (a) 100%, (b) 80%, (c) 60% at all replications showed no bacterial

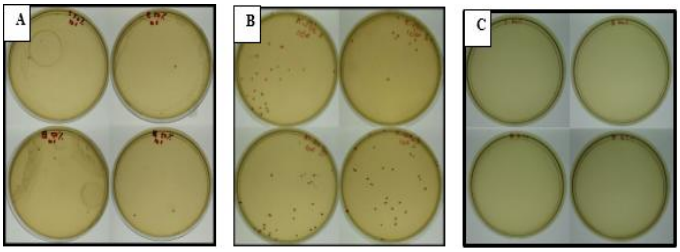

Fig 9: Pour plate test results of concentration (a) 50%, (b) Positive control showed bacterial growth, and (c) Negative control showed no bacterial growth.

The test of antibacterial effect of the ethanol extract of avocado seed begins with the search for MIC. MIC shows minimal concentrations that could inhibit bacterial growth after 24 hours incubation is marked by a change of color in the tube dilution becomes clear. Initially concentrations used are double dilution of 100%, 50%, 25%, 12.5%, 6.25% and 3.125%. The results showed concentrations below 50% begin to look turbid when compared to the positive control after incubated for 24 hours. Therefore it can be concluded MIC of ethanol extract avocado seed (Persea americana Mill.) is in the concentration of 50%.

Furthermore, the ethanol extract of avocado seed (Persea americana Mill.) with a concentration of 100%, 50%, and positive control on the dilution tube, planting on TSA with the pour plate method and replicated 4 times repetition. The results showed the extract at a concentration of 100% is not encountered bacterial growth (sterile) while the concentration of 50% form a bacterial colonies with an average 1,75x107 CFU / ml and a positive control to form a bacterial colonies with an average 35,5X107 CFU / ml.

There are a wide range between 100% - 50%, further research is done by the same dilution and pour plate methods with concentration 80% and 60% to get a more specific MBC. The results of both concentrations showed no turbidity and the result of counting the number of colonies indicate 0 CFU / ml (sterile). Therefore, we can conclude KBM value avocado seed extract (Persea americana Mill.) In this study is at concentration 60%.

The study by Asri D of ethanol extract of avocado seed (Persea americana Mill.), shows that at concentrations 10% avocado seed extract can inhibit the growth of E. faecalis with inhibition zone diameter of 0.12 2.32 mm [22]. Research by Idris S *et al.* the extract of avocado seed (Persea americana Mill.) also showed antibacterial effect against C. ulcerans and S. aureus with inhibition zone on each of the ethyl acetate extract of 32 mm and 12 mm, while the methanol extract of the seeds of avocado (Persea americana Mill.) have inhibitory zone of 37 mm and 15 mm [21].

Many differences in the results of some research likely due to the quality of the extract and different bacteria tested. Some factors that affect the quality of the extract are the type of solvent used and the quality of the ingredients. Extract with methanol better at binding to the active substances which are polar than ethanol, chloroform, and water, but it has the disadvantage that methanol is more toxic than others. In this study ethanol was selected because it is relatively safe, not toxic and can be used to dissolve various compounds that are not water soluble. The quality of materials can also try to influence the quality of the extract like biological factors such as different geographic areas and state lands are likely to affect the levels of active compound contained in the avocado seed (Persea americana Mill.). The duration of the drying process avocado seed before extraction also may affect active compounds avocado seed, because the longer the drying process more active substance is lost due to evaporation.

Different bacteria also became one of the causes of differences in the results. This is due to the different morphologies of gram positive and negative bacteria. In gram-negative bacteria outer membrane is composed of protein, phospholipids and lipopolysaccharide (LPS). LPS in gram-negative is also known as endotoxin causes more pathogenic gram-negative bacteria than gram-positive bacteria. In addition, the structure of the outer membrane layer is also capable of blocking the entry of large molecules to the outside such as antibacterial agents. Vesicle is a virulence factor that belongs only other gram-negative bacteria. Vesicle is able to produce enzymes that inactivate antibacterial and is also capable of transferring an antibacterial material into the other bacterial cell [23].

Some studies have also been developed regarding the use of natural materials as antibacterial against Porphyromonas gingivalis. Research conducted by Vivi L (2014) showed that ethanol extracts of lerak has antibacterial activity against Porphyromonas gingivalis by obtaining the MBC value at concentration 25% [24]. Hendy (2015) conducted research horseradish root extract against Porphyromonas gingivalis showed MIC and MBC values obtained respectively 6.25% and 12.5% [25]. The difference in the antibacterial activity of some of these results can be caused by differences in the types and levels of active compounds that exist in each of the natural product.

Antibacterial effect of ethanol extract of avocado seed (Persea americana Mill.) against Porphyromonas gingivalis likely caused by the active compounds. The ethanol extract of avocado seed (Persea americana Mill.) content such as flavonoids, saponins, tannins, and a steroid which acts as an antibacterial. The mechanism of flavonoids as antimicrobial can be divided into three parts, it can inhibit the nucleic acid synthesis, inhibits the function of cell membranes by forming a complex compound with extracellular proteins and dissolved thus destroying the bacterial cell membrane and followed by the release of intracellular compounds, as well as inhibit the metabolism of energy required for the biosynthesis of macromolecules. Saponins can be antibacterial due to the active substance surface act like detergent, consequently saponin will lower the surface tension of the bacterial cell wall and damage the membrane permeability, so that saponin can diffuse into the cells then bind to the cytoplasmic membrane that disrupt and reduce the stability of the cell membrane causes the cytoplasm to leak out of the cell lead to cell lysis [23].

Tannin is phenolic compounds that have antibacterial and astringent properties (which are shrinking). The antibacterial effect of tannins associated with its ability to precipitate protein, react with the cell membrane, inactivation of the enzyme and function of the genetic material, inactivate cell adhesion and disrupt protein transport in the inner layer of cells. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds causing decreased membrane integrity and changes the cell membrane morphology causing fragile and cell lysis [22, 23].

This study proves that the ethanol extract of avocado seed (Persea americana Mill.) has an antibacterial effect *in vitro* against Porphyromonas gingivalis with minimal inhibition concentration at concentrations of 50% and a minimum bactericidal concentration at concentrations of 60%. It may show different results if applied in a root canal because of the bacteria present in the root canal is polymicrobial and the bacterium Porphyromonas gingivalis as well as one of the bacteria in the root canal can have the ability to establish and

support a biofilm layer. Based on the above discussion, the hypothesis of this study there is antibacterial effect of ethanol extracts of avocado seed (Persea americana Mill.) against Porphyromonas gingivalis accepted.

5. References

1. Baumgartner JC, Siquiera JF, Sedgley CM, Kishen A. Microbiology of Endodontic Disease. In: Ingle's Endodontic 6. India: BC Decker Inc. 2008, 221-286.
2. Svensäter G, Chávez de Paz L, Theilade E. The microbiology of the necrotic pulp. In: Textbook of endodontolgy. 2nd ed. Singapore: Wiley-Blackwell. 2010, 95-110.
3. Ercan E, Dalli M, Yavuz İ, Özekinci T. Investigation of Microorganisms in Infected Dental Root Canals. Biotechnology & Biotechnological Equipment. 2006; 20(2):166-172.
4. Peciuliene V, Maneliene R, Balcikonyte E, Drukteinis S, Rutkunas V. Microorganism in root canal infections: a review. Stomatologija, Baltic Dental and Maxillofacial Journal. 2008; 10(1):4-9.
5. Mohammadi Z, Palazzi F, Giardino L, Shadavi S. Microbial biofilm in endodontic infections: an update review. Biomed J. 2013; 36(2):59.
6. Yoshino T. Genotypic and penotyphic characterization of Porphyromonas gingivalis in realtion to virulence. Thesis. Sweden: Göteborg (Univ). 2007, 11-12.
7. Tomazinho LF, Avila-Campos MJ. Detection of Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, and Prevotella nigrescens in chronic endodontic infection. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 103(2):285-8.
8. Saito D, Countinho LL, Saito CPB, *et al*. Real-time polymerase chain reaction quantification of Porphyromonas gingivalis and Tannarella forsythia in primary endodontic infections. JOE. 2009; 35(11):1518-23.
9. Lačevic S, Foschi F, Pojskić L, Pojskić N, Bajrović K, Izard J. Correlation of periodontal pathogens in concurrent endodontic-periodontal diseases. AODR. 2015; 2(4):1-5.
10. Loo TYW, Jin LJ, Cheung MNB, Dou Y. Detection of Bacteroides forshythus and Porphyoromonas gingivalis in infected root canal during periapical periodontitis by 16S rDNA. Afr. J. Biotechnol. 2009; 8(10):2021-26.
11. Latoo S, Shah AA *et al*. Endodontic Microbiology: Review Literature. IJCCI, 2011; 2(6):24-36.
12. Tokunaga C *et al*. Literature Review Article Microbiology aspect of endoperiodontal lesion. RSBO, 2013; 10(2):176-81.
13. Siquiera JF, Rôças. Bacterial pathogenesis and mediators in apical periodontitis. Braz Dent J. 2007; 18(4):267-280.
14. Young GR, Parashos P, Messer HH. The principles of techniques for cleaning root canals. Aust Dent J. 2007; 52(1):S52-S63.
15. Venghat S, Hegde M, Shetty C. Irrigants used in endodontics. Int. J. Curr. Microbiol. App. Sci. 2014; 3(3):126-132.
16. Basrani B, Rani A, Chopra A. Endodontic irigation. 1 Mei, 2011. http://www.oralhealthgroup.com/news/endodonticirrigation/1425964/?&&er=NA. (24 Agustus 2015).
17. Ema M. Peran bahan disinfeksi pada perawatan saluran akar. Maj Ked Gi. 2011; 18(2):205-9.
18. Schäfer E. Irrigation of the root canal. ENDO. 2007; 1(1):11-27.
19. Dewi, Sulistyowati. Penggunaan ekstrak biji buah alpukat (Persea americana) sebagai antibakteri Proteus mirabilis dan Aerobacter aeorgenes. STIGMA. 2013; 6(2):31-34.
20. Jiménez-Arellanes *et al*. Antiprotozoal snd antimycobacterial activities of Persea americana seeds. BMC Complement Altern Med. 2013; 13:109.
21. Idris S, Ndukwe GI, Gimba CE. Preliminary phytochemical screening and antimicrobial activity of seed extract of Persea americana (Avocado pear). Bajopas. 2009; 2(1):173-6.
22. Asri D. Efektivitas antibakteri ekstrak etanol biji alpukat (Persea americana) sebagai bahan irigasi saluran akar terhadap pertumbuhan bakteri Enterococcus faecalis. Skripsi. Surakarta: FKG UMS, 2014, 9.
23. Rika PR. Uji aktivitas antibakteri ekstrak etanol daun mangga bacang (Mangifera foetid L) terhadap Staphylococcus aureus secara *in vitro*. Skripsi. Pontianak: FK Universitas Tanjungpura. 2014, 11-14.
24. Vivi L. Efek antibakteri ekstrak etanol lerak (Sapindus rara DC) sebagai alternatif bahan irigasi saluran akar terhadap Porphyromonas gingivalis (Penelitian *In Vitro*). Skripsi. Medan: FKG USU. 2014, 57.
25. Hendy. Efek antibakteri ekstrak etanol umbi lobak (*Raphanus sativus* L) sebagai alternatif bahan medikamen saluran akar terhadap Porphyromonas gingivalis (Penelitian *In Vitro*). Skripsi. Medan: FKG USU. 2015, 37-40.