Denta, *Jurnal Kedokteran Gigi*
Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

RESEARCH ARTICLE

Comparison Between Antibacterial Extract of Gambier (UncariagambirRoxb) and Chlorhexidine 2% to Enterococcus Faecalis

Sari Dewiyani*, Selfina Muthia Sari**
*Department of Conservative Dentistry, Faculty of Dentistry, Universitas Prof. Dr. Moestopo (B)
**Dental student, Faculty of Dentistry, Universitas Prof. Dr. Moestopo (B)

Online submission : 17 May 2021
Accept Submission : 25 May 2021

ABSTRACT

Background: *In the case of reinfection after endodontic treatment, Enterococcus faecalis is a bacterium that is commonly found in root canals. Irrigation was carried out to eliminate Enterococcus faecalis from the root canal. Chlorhexidine 2% irrigating agent was effective in reducing the growth of Enterococcus faecalis bacteria and fungi, but it could not dissolve tissue. Gambir extract (UncariagambirRoxb) has antioxidant and antibacterial with phenolic content in the form of catechins.* ***Purpose:*** *Describe the antibacterial comparison of gambier extract (Uncaria gambir Roxb) and 2% chlorhexidine against Enterococcus faecalis bacteria.* ***Material and Methods:*** *This research is a laboratory experimental research. Enterococcus faecalis ATCC 29212 is a microbiological sample. Extract of gambier (UncariagambirRoxb) with concentrations of 1%, 1.5%, 2%, 2.5%, and 3%. 2% chlorhexidine solution and a paper disc to measure the inhibition zone were placed on a petri dish containing Mueller-Hinton agar medium, and incubated 1x24 hours. Gambier extract was made by maceration and rotavapor methods.* ***Results:*** *Gambier extract (UncariagambirRoxb) and 2% chlorhexidine affected the growth inhibition of Enterococcus faecalis bacteria by forming a clear zone around the paper disc in each petri dish. Concentration of 3% is the largest value of the inhibition zone formed in the extract of gambeir (UncariagambirRoxb), while chlorhexidine 2% shows the largest inhibition zone, this can be seen from the average inhibition zone formed of 17.20 mm.****Conclussion:*** *There are differences in the antibacterial of gambier extract (UncariagambirRoxb) and 2% chlorhexidine against Enterococcus faecalis bacteria.*

Keywords: *Enterococcus faecalis, 2% chlorhexidine, gambier extract (UncariagambirRoxb).*

Correspondence: *Sari Dewiyani, Departement of Conservative Dentistry, Faculty of Dentistry Prof.Dr. Moestopo (B), Bintaro Permai Raya No. 3, Jakarta, Indonesia, Phone 08158050182, Email: sari.drg@gmail.com*

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2

Accredited No. 28/E/KPT/2019
p-ISSN: 1907-5987 e-ISSN: 2615-1790

INTRODUCTION

Endodontic treatment is the treatment of pulp disease by removing bacteria and their metabolic products from the root canal system. The purpose of endodontic treatment are to clean and disinfect the root canal system so it can reduce bacteria, remove necrotic tissue, and assist the periapical healing process.[1] Endodontic treatment can be divided into three main stages: access, preparation, and obturation.[2]

One of the steps of biomechanical preparation in endodontic treatment is root canal irrigation which aims to eliminate bacteria in the root canal. Root canal irrigation has two purposes, mechanical and biological. The mechanical purpose is to remove debris, lubricate the root canal and remove organic and inorganic tissue while the biological purpose is as an antimicrobial.[3]

To choice of irrigation solution need a good knowledge and understanding of the properties of various irrigation solutions, appropriate irrigation methods and knowledge of various microorganisms that use in the process of root canal infection which contributes to the effectiveness of irrigation solutions. In addition, the effectiveness of the irrigating agent depends on the amount of irrigation solution, the diameter of the root canal, and the condition of the pulp.[4]

Infections that occur in the root canal are caused by microorganisms that gain access to the pulp tissue and periapical. In the case of failure of endodontic treatment, Enterococcus faecalis(E. faecalis) is one of the most bacteria found in root canals. E. faecalisis found up to 77% of endodontic treatment failure cases. These bacteria are difficult to remove even though chemo mechanical instrumentation and root canal medication have been performed. Clinical features due to the virulence of this bacterium are acute apical periodontitis, chronic periodontitis, exacerbation of apical periodontitis, marginal periodontitis and periradicular abscess.[5]

Materials that can be used as root canal irrigation solutions are sodium hypochlorite solution, chelator solution or athylene diamine tetraacetic acid (EDTA), mixture of tetracycline, anacid and a detergent (MTAD), chlorhexidine, and potassium iodide iodine (IPI). Irrigation materials that are commonly used are those that have antiseptic properties and materials that can inhibit the growth of microorganisms in vitro and in vivo in living tissue.[6,7]

Chlorhexidine is one of the solutions used as root canal irrigation. Chlorhexidine has been used in various concentrations of 0.1-2%. Chlorhexidine 2% is recommended as a root canal irrigant because non-toxic, reduces and eliminates microorganisms from the root canal. Chlorhexidine is used as a disinfectant because it has good antimicrobial properties against gram-positive, gram-negative bacteria, bacterial spores, lipophilic viruses, fungi and dermatophytes. Chlorhexidine 0.1%-0.2% is an antiseptic that is generally used to control oral plaque. Chlorhexidine cannot be used as a sole irrigation solution in root canal treatment because it does not have the ability to dissolve necrotic tissue.[7]

In Indonesia, there are various types of plants that can be used as medicinal plants, one of which is the gambier plant (UncariagambirRoxb). Gambier (UncariagambirRoxb) comes from Sumatra and Kalimantan. Gambier is a plant with a height of 1-3 cm.[8] Gambier has been used as a complement to chewed betel and is believed to strengthen teeth. The main content of Gambier extract is about 7-33% catechins, a polyphenolic compound that has the potential as an antioxidant and antibacterial.[9]

Based on several studies on gambir, Hafsah (2016) researched the benefit of antibacterial gambier and obtained the results that Gambier extract had antibacterial activity against Entrococcusfaecalisbacteria with a minimal inhibitory concentration of 1%.[10] A similar study conducted by Merta (2013) resulted in the concentration of the inhibitory capacity of gambir extract being 80-90%

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2

effective against the growth of Staphlococcus aureus.[11] Krisnawati's researched (2009) stated that the combination of concentration and contact time affects the antibacterial effect of gambier extract.[12]

METHODS

The type of research is experimental laboratory research. This research was conducted at the Bandung Institute of Technology Laboratory. Bacteria Enterococcus facalis ATCC 29212 microbiological samples in all experimental stages have been cultured. Extract of gambier (Uncaria gambir Roxb) with concentrations of 1%, 1.5%, 2%, 2.5%, and 3% and 2% chlorhexidine solution. Each study group used 6 samples, therefore for the 3 groups required 18 samples.

Gambier extract was made using maceration and rotavapor methods.[13] Gambier weighed as much as 500 g, then the gambir put into a round bottom flask and 1 L of ethanol was added, after that it stored for 48 hours. During storage, stir 2 times in 1 day.[14]

The macerated gambier was filtered and then evaporated at a temperature of 60 C for ± 1 hour until a thick extract was obtained. After that, let it free for 2 hours so that the extract dries. The gambier extract was weighed using an analytical balance, each weighing 0.1 g, 0.15 g, 0.2 g, 0.25 g, 0.3 g, which was obtained from previous studies.[15,16,17]

The gambier extract dissolved with 2 ml of 10% DMSO solution to obtain a concentration of 1%, 1.5% ,2% ,2.5%, 3%. After that, the concentration of gambier extract was put into a vial bottle and labeled according to its concentration.

BHIB media (37 gr/1 liter or 3.7gr/100ml) in a closed tube was sterilized by autoclaving at 121 C for 15 minutes. Then pure bacteria Enterococcus faecalis which in the reaction tube was inserted into the BHIB media using a round loop. Then incubated at 37°C for 24 hours. After that, the bacteria were put back into sodium agar in a petri dish using a round loop with three quadrants etched and then incubated at 37°C for 24 hours to see if any bacterial colonies were formed.

Eighteen petri dishes containing Mueller Hinton Agar (MHA) media were prepared. A cotton swab was dipped into a reaction tube which containing Enterococcus faecalis bacteria. The cotton swab was scratched on the surface so that the MHA medium was in a petri dish and spread evenly. The next step, the paper disc was placed on a filled petri dish with MHA agar and incubated for each concentration of gambier extract and 2% chlorhexidine using a micropipette. Replication was made three times.[15]

Petri dishes were incubated at 37°C for 1x24 hours and after 24 hours the zone of inhibition was measured using a caliper.

RESULT

The results of the research on the measurement of the inhibition zone can be seen in the figure and table as follows:

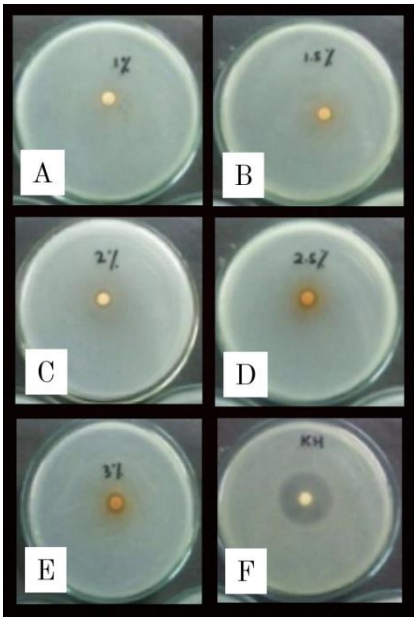
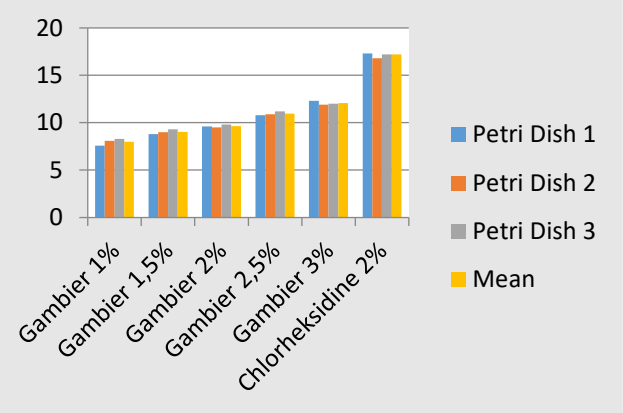


Fig 1. Comparison test results of gambier extract (UncariagambirRoxb) and 2% chlorhexidine against Enterococcus faecalis bacteria after incubation for 24 hours at 37 C. (A) 1% gambier extract (B) 1.5% gambier extract (C) gambier extract 2 % (D) 2.5% gambier extract (E) 3% gambier extract (F) 2% chlorhexidine.

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2

In Figure 1 gambier extract (UncariagambirRoxb) and 2% chlorhexidine affect the growth inhibition of Enterococcus faecalis bacteria which can be seen in the clear zone around the paper disc in each petri dish.

Chart 1. Measurement Results Inhibition Mean (milimeter)



Based on chart 1, it can be seen that the average diameter of the inhibition zone formed around the paper disc extract of gambier (UncariagambirRoxb) concentration of 3% is the largest value compared to the inhibition zone formed at concentrations of 1%, 1.5%, 2%, 2,5%, while chlorhexidine 2% showed the largest inhibition zone, this can be seen from the average inhibition zone formed of 17.20 mm.

Data normality test uses Shapiro-Wilk because the research sample is less than 50 data. Data normality test determines whether the data collected from 18 samples is normally distributed or not, the data distributionis said normal if p>0.05. The results of normality test antibacterial comparison of gambier extract (UncariagambirRoxb) and 2% chlorhexidine against Enterococcus faecalis bacteria showed that all samples studied had Sig. values>0.05 (p>0.05) then the data distribution is normal, continued to test variance.

The results of Anova test shows there is a significant difference in antibacterial power from the results of the inhibition zone measurements of various concentrations of gambier extract (UncariagambirRoxb) with 2% chlorhexidine against Enterococcus faecalis bacteria. This is showed by the value of Sig. 0.000 (p<0.05). One Way Anova test can only show whether or not there is a difference in antibacterial power between gambier extract (UncariagambirRoxb) and 2% chlorhexidine against Enterococcus faecalis bacteria. For this reason, it is necessary to test using the Multiple Comparison LSD (Least Significant Difference) test in order to know how big the difference in the antibacterial power of each group.

The results of Multiple Comparison (LSD) test show there is a significant difference in the average comparison of the inhibition zones between one group and another. This is showed by the value of p<0.05 in all comparisons between various concentrations of gambier extract (UncariagambirRoxb) and 2% chlorhexidine.

DISCUSSION

Enterococcus faecalishas been known as a bacterium that can survive in endodontic treatment. The frequency of the presence of E. faecalis in cases of post-endodontic infection was found to be nine times higher than that of primary endodontic infections.[3,4,5]

The ability of a bacterium to survive against medicine can be known by looking at the decreasing colonies of a bacterium and the concentration of medicine liquid is one of the factors that affect the effectiveness of the medicine in killing E faecalis.[18,19]

The results are each concentration of gambir extract there was an inhibition zone against E faecalis bacteria. In the 2% chlorhexidine test,[20] the inhibition zone against the bacteria E. faecalis formed had a larger size than the inhibition zone in the gambier extract. The size of the inhibition zone in 2% chlorhexidine on average is 17.20 mm, while the gambier extract with a concentration of 1% has an inhibition zone of 8.00 mm, a concentration of 1.5% with an inhibition zone of 9.03 mm, a concentration of 2% with an inhibition zone of 9.63 mm, a concentration of 2.5% with an inhibition zone of 10.97 mm and a concentration of 3% having an inhibition zone of 12.07 mm.

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2

The inhibition zone formed after an incubation period of 24 hours indicates that the bacteria in the area cannot grow due to the influence of the test material, which is gambier extract.[21] In this study, it shown that the concentration of gambier extract which increased from 1%, 1.5%, 2%, 2.5% and 3% to E faecalis bacteria for 24 hours showed a significant increase in the inhibition zone ($p<0.05$) much bigger the concentration of the gambier extract solution, much bigger the inhibition zone formed. This research is in line with the research by Hafsah et al. showed that the concentration of 1% gambier extract with a contact time of 24 hours against Enterococcus faecalis bacteria effectively inhibited the growth of Enterococcus faecalis bacteria.[10]

Research by Istiyaningsih (2012), also used gambier extract (UncariagambirRoxb) against root canal microbes in necrotic teeth with various concentrations, found that gambier extract was also effective in inhibiting the growth of Streptococcus mutans bacteria.[22]

Research by Kresnawaty and Zainuddin (2009) stated that methyl derivatives of ethanol extract of gambier leaves has antioxidant and antibacterial activity.[12,18]

Chlorhexidine is an irrigation solution that has been proven effective against Enterococcus faecalis bacteria. Darmetto et al (2005) assessed that the antimicrobial power of 2% chlorhexidine against E. faecalis bacteria was more effective than NaOCl, because 2% chlorhexidine is effective as a root canal disinfection agentecause its antibacterial capacity can destroy the integrity of bacterial cell membranes, which is changes in cytoplasmic membrane permeability can increase cytoplasmic protein deposition, change cellular osmotic balance, interfere with metabolism, growth and division of bacterial cells, so that Enterococcusfaecalis cell walls can be damaged, lysed and eventually die.[19,23]

Based on the results of the research, it was found that gambier extract (UncariagambirRoxb) and 2% chlorhexidine had antibacterial ability in inhibiting the growth of Enterococcus faecalis ATCC 29212 bacteria. Gambir extract with the smallest concentration of 1% still could inhibit the growth of Enterococcus faecalis ATCC 29212 bacteria. This is indicated by the presence of an inhibition zone formed. Thus gambier extract can inhibit the growth of Enterococcus faecalis ATCC 29212 bacteria, but 2% chlorhexidine has greater antibacterial power.[20]

CONCLUSION

There is a difference in the antibacterial of gambier extract (UncariagambirRoxb) and 2% chlorhexidine against Enterococcus faecalis bacteria. Chlorhexidine 2% has a greater zone of inhibition against Enterococcus faecalis bacteria than gambier extract (UncariagambirRoxb).

REFERENCES

1. Gutmann JL, Paul EL. Problem Solving in Endodontics: Prevention Identification, and Management. 5th Edition. Elsevier/Mosby. 2011. p: 209-16.
2. Jiang LM, Bram L, Leonardus M, Lucas WMV, Wesselink P. Comparison of the Ceaning of Different Final Irrigation Techniques. J.Endod 2012;38 (6):838-41
3. Zaparolli D, Saquy PC, Cuz-filho AM. Effect of Sodium Hypochlorite and EDTA Irrigation, Individually and in Alternation, on Dentin Microhardness at the Furcation Area of Mandibular Molars. Brazilian Dental Journal: 2012; 23(6): 654–8.
4. Cohen, S. & Burns, R.C. Pathway of the pulp (12th ed.). St Louis: Mosby, 2020.
5. Grossman's. Endodontic Practice 13th Edition. Wolters Kluwer Health, 2014
6. Alagl AS, Bedi S, Almas K Phytosolutions for Enterococcus faecalisin Endodontics. Oral Health and Dental Management. 2016;15(5):332-6.
7. Kumar A, Kumar A, Thakur P,PatilS,Payal C,. Antibacterial activity of green tea(Camellia sinensis) extracts against various bacteria isolated from environmental sources. Recent Research in Science and Technology. 2012;4(1):19-23.

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2

8. Rahmawati N.,Bakhtiar A., Prima PutraD. Isolasi Katekin dari Gambir (UncariaGambirRoxb) untuk Sediaan Farmasi dan Kosmetik. Jurnal Penelitian Farmasi Indonesia; 2013;1(2).
9. Azmi Dhalimi, Permasalahan Gambir (UncariagambirRoxb) di Sumatera Barat dan Alternatif Pemecahannya. Perspektif Jurnal Pertanian. 2015. 3; 5(4):46-59.
10. Hafsah K, Suminarti, Indya KM, Rasmidar S, Moch H, Suryani A. Inhibitory Concentration and Minimun Contact Time Gambir Extract (UncariagambirRoxb) Against Bacterial Growth Enterococcus faecalis. International Journal of Sciences: Basic and Applied Research. 2016;27(3): 239-146.
11. Merta IW., NuidjaIN,.Mawati NM. Ekstrak gambir memiliki daya hambat terhadap pertumbuhan Staphylococcus aureus secara in vitro. Jurnal Husada. 2013;10: 39-43.
12. Kresnawati I, Zainuddin A. Aktivitas antioksidan dan antibakteri dari derivate metal ekstrak daun gambir. Jurnal Littri. 2009;15(4):145-151.
13. Isnawati A, Raini N, Dwi Sampurno O, Mutiatikum D, Widowati L,Gitawati R. Karakteristik Tiga Jenis Ekstrak Gambir (UncariaGambirRoxb). Sumatera Barat. Bul. Peneliti Kesehatan. 2012; 40(4)
14. Hendri M, Gustri Y, Wilsn H, Yulia H. Pembuatan Konsentrat Polifenol Gambir (UncariaGambirRoxb) Sebagaai Bahan Antioksidan Pangan. Jurnal Riset Industri, 2010:IV(2):71-82
15. Wijayanti AI. Pengaruh ekstrak gambir (uncariagambirroxb) sebagai alternative bahan larutan irigasi saluran akar terhadap mikroba saluran akar pada gigi nekrosis [Skripsi]. Makassar, Universitas Hasanuddin, 2011.
16. Suraini, Chairani, Enlita. Uji Aktivitas Anti jamur Ekstrak Gambir (UncariaGambir) terhadap Candida albicans. Scientia jurnal farmasi kesehatan. 2015;5(2)
17. Chabib L, Triasuti A, irianti RD. Fomulasi Tablet Hisab ekstrak Gambir (UncariagambirRoxb) dengan Variasi Bahan Pengikat Gom Arab. Majalah Obat Tradisional. 2010;15(2):75-79
18. Ayu Hapsari, M. Uji Aktivitas Antibakteri (+)(–) Katekin dan Gambir (Uncariagambir) terhadap Beberapa Jenis Bakteri Gram Negatif dan Mekanismenya [Skripsi]. Jakarta, Universitas Islam Negeri Syarif Hidayatullah, 2010.
19. Sukadana, I.M. Senyawa antibakteri golongan flavonoid dari buah belimbing manis(Avverhoa carambola Linn.L). Jurnal Kimia. 2009;(2):109-66.
20. Mulyawati,Elma.Peran bahan disinfeksi pada perawatan saluran akar. Majalah Kedokteran Gigi. 2011; 18(2):205-9.
21. Husain N. Efektifitas ekstrak gambir (Uncariagambir) sebagai alternative bahan larutan irigasi saluran akar terhadap pertumbuhan bakteri Entrococcusfaecalis pada konsentrasi dan waktu kontak yang berbeda. [Skripsi] Makassar, Universitas Hasanuddin. 2011
22. Istiyaningsih Alvia. PengaruhEkstrakGambir (UncariagambirRoxb) sebagai Alternatif Bahan Irigasi Saluran Akar Terhadap Mikroba Saluran Akar pada Gigi Nekrosis. [Thesis] Universitas Hasanuddin. 2012.
23. Gupta R, Chandavarkar V, Galgali SR, Mishra M. Chlorhexidine, a medicine for all the oral disease. Global Journal of Medicine and Public Health (GJMPH). 2012; 201;1(2):43.

Denta Jurnal Kedokteran Gigi, Agustus 2021; Vol.15 No.2; Hal 64-69
Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/14
DOI: 10.30649/denta.v15i2.2