JOURNAL OF DENTISTRY INDONESIA

UNIVERSITAS INDONESIA

P-ISSN : 16939697 E-ISSN : 23554800 Subject Area : Health

0
Impact Factor

2176
Google Citations

Sinta 2
Current Acreditation

Google Scholar Garuda Website Editor URL

History Accreditation

2018 2019 2020 2021 2022 2023 2024

Garuda Google Scholar

Treatment of Oral Vascular Malformations with Ethamolin: A Report of Two Cases
UI Scholars Hub Journal of Dentistry Indonesia
2023 DOI: - Accred : Sinta 2

Ramsay Hunt Syndrome with Oral Findings: A Rare Case
UI Scholars Hub Journal of Dentistry Indonesia
2023 DOI: - Accred : Sinta 2

Treatment of Gingival Fenestration using Mucoperiosteal Flap with Connective Tissue Graft: A Report of Two Cases
UI Scholars Hub Journal of Dentistry Indonesia
2023 DOI: - Accred : Sinta 2

Do Nasal Anatomical Variations Affect the Maxillary Sinus? A CBCT Volumetric Analysis
UI Scholars Hub Journal of Dentistry Indonesia
2023 DOI: - Accred : Sinta 2

Assessment of DNA Damage of Oral Mucosa due to Ceramic Bracket Using Comet Assay and Mutagenicity of Orthodontic Bonding System Using Ames Test
UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

A Prospective-Randomized Study: The Impact of Four Different Caries Removal Method on Pain and Clinical Evaluations

UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

Studentsâ?? Experience, Self-Confidence, and Perception Toward Endodontic Learning: National Survey Among Malaysian Dental Schools

UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

Management of Tooth Hypersensitivity Under a Cantilever Fixed Partial Denture Using a Novel Low-Level Laser Therapy Protocol: A Case Report

UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

Class III Malocclusion Camouflage Treatment Using a Conventional Orthodontic Appliance in a Non-Growing Patient

UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

A Two-Year Retrospective Evaluation of Cone Beam Computed Tomography Indications in Pediatric, Adolescent and Adult Patients

UI Scholars Hub Journal of Dentistry Indonesia

2023 DOI: - Accred : Sinta 2

View more ...

P-ISSN 1693-9697
E-ISSN 2355-4800
Volume 27
Number 2
August 2020

Journal of Dentistry Indonesia

The Official Publication of
The Faculty of Dentistry, Universitas Indonesia
in Collaboration with
Indonesian Dental Association

Journal of Dentistry Indonesia

EDITORIAL TEAM

EDITOR IN CHIEF

Yuniardini Septorini Wimardhani (https://www.scopus.com/authid/detail.uri?authorId=56784363100) , (SCOPUS ID=56784363100) Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Indonesia, Indonesia

SECTION EDITORS

Lisa Rinanda Amir (https://www.scopus.com/authid/detail.uri?authorId=14048047100) , (SCOPUS ID = 14048047100) Department of Oral Biology, Faculty of Dentistry Universitas Indonesia, Indonesia

Bramma Kiswanjaya (https://www2.scopus.com/authid/detail.uri?authorId=26664624100) , (SCOPUS ID = 26664624100) Department of Dentomaxillofacial Radiology, Faculty of Dentistry, Universitas Indonesia

Erik Idrus (ttp://www.scopus.com/authid/detail.url?origin=resultslist&authorId=51763635000&zone=) , (SCOPUS ID = 51763635000)Department of Oral Biology, Faculty of Dentistry Universitas Indonesia, Indonesia

Sunarso (https://www.scopus.com/authid/detail.uri?authorId=55191249900) , (SCOPUS ID = 55191249900) Department of Dental Materials Science, Faculty of Dentistry, Universitas Indonesia

EDITORIAL TEAM

Anne-Marie Kuijpers-Jagtman (https://www.scopus.com/authid/detail.uri?authorId=35237602100) , (SCOPUS ID = 35237602100) Department of Orthodontics, University Medical Center Groningen, The Netherlands

Adrian Yap (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=7103215953&zone=) , (SCOPUS ID = 7103215953) National University of Singapore, Singapore

Anak Iamaroon (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=6701786341&zone=) , (SCOPUS ID = 6701786341) Faculty of Dentisry, Chiang Mai University, Thailand, Thailand

Benny Mulyono Soegiharto, (https://www.scopus.com/authid/detail.uri?authorId=24478917200) (SCOPUS ID = 24478917200) Department of Orthodontics, Faculty of Dentistry, Universitas Indonesia, Indonesia

Budi Kusnoto (http://www.experts.scival.com/uic/expertPubs.asp?n=Budi+Kusnoto&u_id=1175&oe_id=1&o_id=154) , (SCOPUS ID = 6603330742) Department of Orthodontics, College of Dentistry, University of Illinois at Chicago, United States

BSM Samadarani Siriwardena (https://www.scopus.com/authid/detail.uri?authorId=16246416800) , (SCOPUS ID = 16246416800) Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka

Chun Hung Chu (http://hub.hku.hk/cris/rp/rp00022) , (SCOPUS ID= 7404345729) The University of Hong Kong, Hong Kong

Clive Wright (https://www.researchgate.net/researcher/39161650_Clive_Wright) , (SCOPUS ID = 55453620500) University of Sydney, Australia

Cortino Sukotjo (https://www.scopus.com/authid/detail.uri?authorId=6508194317) , (SCOPUS ID = 6508194317) Department of Restorative Dentistry, College of Dentistry, University of Illinois at Chicago, United States

Diah Ayu Maharani (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=32867911000&zone=) , (SCOPUS ID = 32867911000) Department of Preventive and Public Health Dentistry Faculty of Dentistry Universitas Indonesia, Indonesia

Diana Cassi (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=56062239400&zone=) , (SCOPUS ID = 56062239400) Department of Orthodontics University of Modena and Reggio Emilia, Italy

Hamid Nurrohman (http://www.researchgate.net/profile/Hamid_Nurrohman3) , Hamid Nurrohman, (SCOPUS ID = 52564067000) Department of Restorative and Prosthodontics, The University of Texas School of Dentistry, Houston, Texas, USA.

Izzet Yavuz (https://www.scopus.com/authid/detail.uri?authorId=35967243400) , (SCOPUS ID = 35967243400) Faculty of Dentistry, University of Dicle, Turkey, Turkey

Kobkan Thongprasom (https://www.scopus.com/authid/detail.uri?authorId=6601959811) , (SCOPUS ID = 6601959811) Department of Oral Medicine, Faculty of Dentistry, Chulalongkorn University, Thailand, Thailand

Koichi Kawabuchi (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=7003523337&zone=) , (SCOPUS ID = 7003523337) Tokyo Medical and Dental University, Japan

Marco Meleti (https://www.scopus.com/authid/detail.uri?authorId=55926088200) , (SCOPUS ID = 55926088200) Dental School at the University of Parma, Italy

Masita Mandasari (https://www.scopus.com/authid/detail.uri?authorId=57008957900) , (SCOPUS ID= 57008957900) Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia

Nieka Adhara Wahono (https://www.scopus.com/authid/detail.uri?authorId=57204112724) , (SCOPUS ID= 57204112724) Department of Pediatric Dentistry, Faculty of Dentistry, Universitas Indonesia

Nina Ariani (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=55301181500&zone=) , (SCOPUS ID = 55301181500) Department of Prosthodontics, Faculty of Dentistry, Universitas Indonesia

Pham Anh Vu Thuy (http://www.scopus.com/authid/detail.url?origin=resultslist&authorId=51562199400&zone=) , (SCOPUS ID = 51562199400) University of Medicine and Pharmacy, Viet Nam

Contents of Vol. 27, No.2, August 2020

Journal of Dentistry Indonesia

Journal of Dentistry Indonesia

Volume 27
Number 2 *August*

Article 2

8-30-2020

Effectiveness of Lentinus edodes Mushroom Extract on Eradication of Enterococcus faecalis Biofilm

Putri Lestari Anggiarini
Postgraduate Program, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Meiny F Amin
Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Juanita A Gunawan
Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Armelia Sari Widyarman
Department Head of Microbiology, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia, armeliasari@trisakti.ac.id

Effectiveness of Lentinus edodes Mushroom Extract on Eradication of Enterococcus faecalis Biofilm

Cover Page Footnote

ACKNOWLEDGEMENT The authors would like thank Faculty of Dentistry, Trisakti University, for invaluable support in this study. Authors also would like to thank Mario, SSi and Aradhea Monica Drestia, SSi from the Microbiology Center of Research and Education (MiCORE) laboratory for their assistances for this study.

Journal of Dentistry Indonesia 2020, Vol. 27, No. 2, 56-60
doi: 10.14693/jdi.v27i1.1126

ORIGINAL ARTICLE

Effectiveness of *Lentinus edodes* Mushroom Extract on Eradication *of Enterococcus faecalis* Biofilm

Putri L. Anggiarini[1], Meiny F. Amin[2], Juanita A. Gunawan[2], Armelia S. Widyarman[3]

[1]Postgraduate Program, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
[2]Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
[3]Department Head of Microbiology, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
Correspondence e-mail to: armeliasari@trisakti.ac.id

ABSTRACT

Sodium hypochlorite is a commonly used irrigation solution in endodontic procedures, but it irritates tissues and has toxic effects. *Lentinus edodes* is a mushroom that has antibacterial properties. *Enterococcus faecalis* is an anaerobic bacterium that can cause root canal treatment failure. **Objective:** This study aimed to determine the effect of *L. edodes* extract on the eradication of *E. faecalis* biofilms. **Methods**: Phytochemical tests of *L. edodes* were performed to analyze alkaloids, steroids, triterpenoids, phenolics, tannins, flavonoids, and glycosides from this extract qualitatively. *E. faecalis* ATCC 29212 was cultured in brain heart infusion broth for 24 h at 37°C in an anaerobic atmosphere. Biofilm assay was performed to analyze the eradication of *E. faecalis* biofilm after treatment with *L. edodes* extract. The application times were 5, 15, and 30, and 10%, 20%, 40%, and 80% concentrations were used. Distilled water was used as a negative control, and NaOCl was used as a positive control. Data were statistically analyzed via one-way analysis of variance, where $p < 0.05$ was set as the level of significance. **Results:** *L. edodes* mushroom extract was effective in eradicating *E. faecalis* biofilms in all concentrations and incubation times compared with the control ($p < 0.05$). Significant differences were found between the application times of 5 and 15 min compared with 30 min ($p < 0.05$). The most effective concentration in eradicating *E faecalis* biofilms was 40% with an application time of 30 min. **Conclusion:** *L. edodes* mushroom extract proves its antibiofilm activity against *E. faecalis* biofilm. Further study is necessary to determine which substances are have the most influence on the effectiveness of *L. edodes* extract in eradicating *E. faecalis* biofilm in vivo.

Key words: biofilm eradication, *Enteroccus faecalis*, *Lentinus edodes*, mushroom extract

How to cite this article: Anggiarini PL, Amin MF, Gunawan JA, Widyarman AS. Effectiveness of *Lentinus edodes* mushroom extract on eradication of *Enterococcus faecalis* biofilm. J Dent Indones. 2020; 27(2):56-60

INTRODUCTION

Caries can develop over a long period of time on the entire surface of the tooth covered by biofilms.[1] Bacteria present in carious lesions can invade the pulp, causing reversible pulpitis. Pulps in irreversible pulpitis and pulp necrosis should be treated with root canals.[2] The most important factors that indicate the success of root canal treatment are good preparation, sterilization, and apical closure of obturation. The removal of necrotic pulp tissue and bacteria is an important factor. Imperfect obturation is a result of inadequate preparation and irrigation, leaving bacteria to grow in the root canal. Bacteria left in the root canal form biofilms and cause further inflammatory reactions in the periapical region. If not treated properly, inflammation may worsen.[3]

Tooth root canal preparation supports the sterilization process and produces good filling; thus, optimal results are obtained. Preparation is carried out alternately with root canal irrigation solutions. Root canal irrigation is an important step in root canal treatment. It removes organic and inorganic necrotic tissue, kill microorganisms, and dissolve the smear layer during root canal preparation.[2] The ideal irrigation solution should be non-toxic, have antibacterial properties, function as a lubricant, have low surface tension, and be

able to dissolve the remaining necrotic pulp tissue and the smear layer formed during root canal preparation. Until now, there has not been an ideal irrigation solution that has all these properties. Existing irrigation solutions must be combined with other irrigation solutions, such as a combination of EDTA, sodium hypochlorite (NaOCl), and chlorhexidine.[3] NaOCl is a very commonly used solution for root canal irrigation. However, besides its being an irrigation solution, it also has a toxic effect on tissues: i.e., the higher its concentration, the worse its toxic effect. Some studies mention that NaOCL toxicity can cause pain, soft tissue edema, and paresthesias.[4]

Natural ingredients from traditional plants have been widely investigated as potentially better sources of antimicrobials. Mushrooms are alternative natural materials that have antibacterial effects. Fungi have bioactive metabolite ingredients that function as immunomodulators, protect the liver and cardiovascular system, and have antifibrotic, anti-inflammatory, antidiabetic, antiviral, antioxidant, and antitumor effects. Mushrooms can be a source of natural antibiotics.[5] Mushrooms contain peptides and proteins. Components found in fungi include sesquiterpenes, steroids, anthraquinones, benzoic acid derivatives, quinolones, oxalic acids, and phenols that function as antibacterial agents.[6]

Lentinus edodes is the most studied fungus. It appears to have broad antimicrobial effects on gram-negative and gram-positive bacteria. Extracts using chloroform, ethyl acetate, and water inhibit various gram-positive and gram-negative bacteria at concentrations below 50 mg/ml.[7] Ciric et al. examined the effectiveness of *L. edodes* mushroom extract to determine its antigingivitis effect.[8] Some literature has shown the effectiveness of *L. edodes* mushroom extract against *Streptococcus mutans* and *E. faecalis*.[6,7] *E. faecalis* is an anaerobic bacteria found in the root canal that can cause infection and inflammation. It is often associated with various forms of periradicular disease, as both primary (4%–44%) and persistent endodontic infections (24%–74%).[9] Some studies on the antibacterial effect of *L. edodes* mushroom extract on *E. faecalis* found that *L. edodes* has the ability to inhibit *E. faecalis*.[6,10] However, Carvalho stated that further investigation is required.[11] The present study was carried out to examine the effectiveness of the *L. edodes* mushroom extract in various concentrations and time applications in eradicating biofilm of *E. faecalis*. Previous studies focused on the effects of the extract on *E. faecalis*, not on its biofilm. Biofilm is more difficult to eliminate than planktonic bacterium. The results of this study can be used as baseline information on the use and selection of an irrigation solution in root canal treatment, which is more effective in reducing the prevalence of secondary infections after root canals caused by *E. faecalis*.

METHODS

This experimental laboratory study was designed to determine the antibacterial effectiveness of fungal extracts of *L. edodes* on the eradication of *E. faecalis*. Concentrations of 10%, 20%, 40%, and 80% were applied for 5, 15, and 30 min. The study employed a post-treatment design with a control. This study was conducted at the Microbiology Center of Research and Education Laboratory of the Faculty of Dentistry, Trisakti University, Jakarta.

L. edodes identification and phytochemical testing

The sample of *L. edodes* was taken from the Cibodas Bionic Farm, Puncak, West Java, Indonesia. The identification of the *L. edodes* mushroom plant was carried out at the Bogor Institute of Sciences, Bogor, Indonesia, and the phytochemical assay analysis was performed at the Indonesian Medicinal and Aromatic Crops Research Institute (BALITTRO) in Bogor, Indonesia.

L. edodes extract preparation

A total of 200 g of dried *L. edodes* mushroom was immersed in 300 mL of water at 4°C overnight. The wet *L. edodes* material was crushed with a mixer, added to 1600 mL of chloroform, and stirred at 4°C overnight. Chloroform and insoluble material were separated by filtering or centrifugation. The chloroform extract was evaporated under low pressure to produce 876 mg of a light-yellow oily liquid. This insoluble material was then mixed with 1000 mL of water and stirred overnight at 4°C. The resulting suspension was then filtered or centrifuged to remove insoluble materials. After the pressure was reduced, the aqueous supernatant concentration was reduced to 200 mL. This concentration was then extracted by mixing with 200 mL of ethyl acetate three times. The organic layer was separated and evaporated at low pressure to produce a light-yellow ethyl acetate extract (114 mL). The aqueous extract was then freeze-dried and returned to a full water suspension. The solution obtained was diluted with sterile Distilled water to obtain concentrations of 10%, 20%, 40%, and 80%.

Bacterial Culture and Biofilm Assay

E. faecalis ATCC 29212 was cultured in brain heart infusion broth for 24 h at 37°C in an anaerobic atmosphere. Subsequently, 200 µl of bacterial suspension (1×10^7 CFU/mL) was inserted into a 96-well plate and incubated for 24 h. The well plate was then rinsed with 200 µl phosphate buffered saline (PBS) and discarded. Each irrigation solution was put into the well plate up to 200 µl. The irrigation solution was incubated for 5, 15, and 30 min.[12] Moreover, the irrigation solution was discarded, and the well plate was rinsed again with 200 µl of PBS and discarded. Staining was then performed by adding a 200-µL solution of

Table 1. Phytochemical screening of the ethanol extracts of temu kunci

Plant constituents	Qualitative assay
Alkaloids	+
Saponins	-
Tannins	+
Phenolics	+
Flavonoids	+
Triterpenoids	+
Steroids	-
Glycosides	+

crystal violet into each well of the microtiter plate for 15 min and then rinsed with PBS. Ethanol acetone (200 µL) was put into each microtiter well plate to check for biofilm formation. A microtiter plate reader was used to measure the optical density of violet crystals at 450 nm.[13] Sterile distilled water was used as a negative control and NaOCl as a positive control. The concentration of NaOCl used as the positive control was 5.25% as it is used as the golden choice for irrigation solution in root canal treatment. All treatments were done in three independent experiments in triplicates.

Statistical Analysis

Data were analyzed using two-way analysis of variance and post-hoc tests to reveal significant differences between treatment groups. Data normality test was performed using the Kolmogorov–Smirnov test. Statistical significance was set at $p < 0.05$. Statistical calculations were performed using SPSS Statistics for Windows software version 20 (IBM Corp., Armonk, NY, USA).

RESULTS

The results of the phytochemical tests showed that the aqueous extract of *L. edodes* contained alkaloids, tannins, phenolics, flavonoids, triterpenoids, and glycosides (Table 1).

The results showed that *L. edodes* mushroom extract was effective in eradicating *E. faecalis* biofilms. This extract reduced the biofilm mass significantly in all concentrations and application times compared with the negative control ($p < 0.05$). The average optical density and standard deviation of *E. faecalis* biofilms is shown in Figure 1-3. The most effective concentration is 40% applied for 30 mins, which eradicated 99.2% of biofilms compared with the negative control (without *L. edodes*).

DISCUSSION

A comparative multiple test was performed using the post-hoc test to prove the significance of the

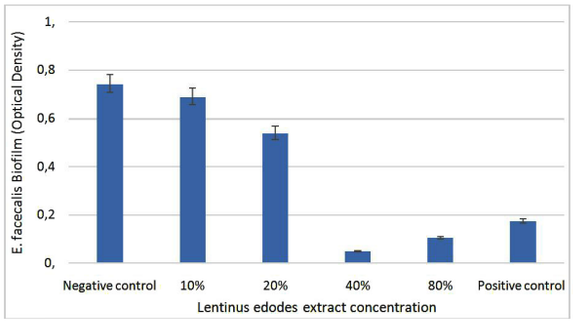

Figure 1. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 5 min of incubation time. The concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20%, and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

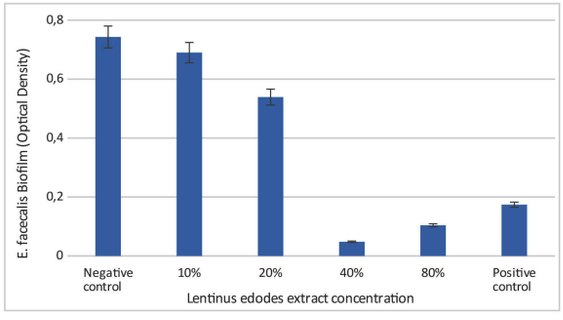

Figure 2. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 15 mins of incubation time. The concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20% and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

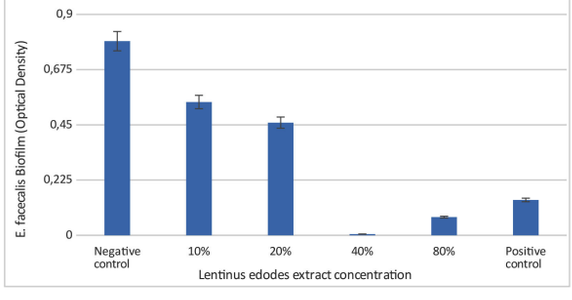

Figure 3. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 30 mins of incubation time. Concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20% and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

differences between each ingredient. *L. edodes* extract with a concentration of 10%, 40%, and 80% showed significant differences compared with the negative control ($p < 0.05$). When compared with 80% concentration, 40% did not give significant results, meaning that the antibacterial effect was not

significantly different between the two concentrations. Based on the application time, when the results of multiple applications, e.g., 5 and 15 mins, were compared with 30 mins, the results were significant ($p < 0.05$).

E. faecalis reinfection and bacterial resistance are the most common causes of root canal treatment failure. In a study of 30 cases of endodontic failure, *E. faecalis* was found in 20 cases.[14] In primary endodontic cases, the prevalence *of E. faecalis* was 18%. Secondary endodontic cases had a higher prevalence of 67%.[15] *E. faecalis* can form biofilms in the root canal that are resistant to destruction. They are 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than other organisms that do not form biofilms. In addition, biofilms can reduce the quality of the covering of the root canal filling material.[4]

This study aimed to develop a natural ingredient as a safer irrigation solution for use in endodontic procedures. *L. edodes* was chosen because it is easy to find; it is a food ingredient that is used in various types of cooking. Hearst investigated the antimicrobial activity of the aqueous extract of the fungus *L. edodes* against *E. faecalis*. However, Carvalho showed the opposite results.[10, 11]

In this study, the content of *L. edodes* is consistent with those in the study by Alves and Macoris, who examined the phenolic content of the aqueous extract of the fungus *L. edodes*. They examined various phenolic components contained in the fungus *L. edodes*, namely, p-Hydroxybenzoic acid, protocatechuic acid, and cinnamic acid, which have antimicrobial power against *E. faecalis* by changing the permeability of cell membranes and damaging DNA.[7, 16]

Kuznetsov showed that a 5% concentration solution of *L. edodes* mushroom extract had antimicrobial power against *E. faecalis*.[17] Many studies have shown that some herbs, such as *Boesenbergia rotunda*, *Clitoria ternatea*, and *Myrmecodia pendans*, have been proven effective in inhibiting *E. faecalis*, *F. nucleatum*, *T. denticola,* and *P. gingivalis* as endodontic pathogen biofilms. These herbals are also known to have antibacterial substances, such as flavonoids, alkaloids, and tannins.[18, 18, 20]

Alkaloids can penetrate into the bacterial membrane and cause cytoplasmic leakage. Tannin is a type of compound that belongs to the polyphenol group. Tannin is able to deactivate bacterial adhesin, enzymes, and cell wall transport proteins and form complex compounds against extracellular proteins, which interfere with the integrity of the bacterial cell membrane. Its mechanism of action is by denaturing bacterial cell proteins and causing irreversible damage to cell membranes. Flavonoids form complexes with cell walls and bind to adhesin. Triterpenoids can cause damage to bacterial cell membranes.[21,22,23]

The longer application time and higher concentration of *L. edodes* mushroom extract showed a significant difference when compared with the negative control, and the optical density value was lower. This demonstrates its effectiveness in eradicating *E. faecalis* biofilms. The results of this study are consistent with those of Hearst. However, the results are inconsistent with those of Carvalho et al., who concluded that *L. edodes* was not effective against *E. faecalis*. This difference may be attributed to differences in the part of the fungus studied, how it was extracted, and the incubation period.[10,11]

CONCLUSION

L. edodes mushroom extract was effective in eradicating *E. faecalis* biofilms. The most effective *L. edodes* extract was at a concentration of 40% applied for 30 min. This extract may be useful as antibiofilm agent. However, further research is needed on the effectiveness of *L. edodes* extract in vivo. Furthermore, it is necessary to study which substances have the most influence on the effectiveness of *L. edodes* extract in eradicating biofilms.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest related to this study.

REFERENCES

1. Marsh PD, Takahashi N, Nyvad B. Dental Caries The Disease & Its Clinical Management. 3rd ed. Fejerskov, O. Nyvad, B. Kidd E, ed. John Wiley & Sons;2015:107-29.
2. Fouad A, Levin LG. Pulpal Reactions to Caries and Dental Procedures. In: Hargreaves KM, Cohen S, editors. Pathways of The Pulp. 11Th ed. ST. Louis: Mosby;2011:573-95.
3. Torabinejad M, Walton ERE, Fouad AF. Endodontics Principles and Practice. 5th ed. Elsevier. 2015:37-45.
4. Tanumihardja M. Solution for root canal irrigation. (Indonesian). Dentofasial. 2010;9(2):108-15.
5. Zaura E, Buijs MJ, Hoogenkamp MA, Ciric M, Papetti A, et al. The effects of fractions from shiitake mushroom on composition and cariogenicity of dental plaque microcosms in an in vitro caries model. J. Biomed Biotech. 2011:135034.
6. Alves MJ, Fereira ICFR, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity

of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med. 2012:78;1707-18.
7. Macoris JDM, Brugnari T, Boer CG, Contato AG, Peralta RM, Souza CGM. Antioxidants properties and antimcrobial potential of aqueous extract of Bbsidioma from Lentinus edodes (Berk.) Sing. (Shitake). Int J Curr Microb Appl Sci. 2017;6(9):3757-67.
8. Ciric L, Tymon A, Zaura E, Lingstrom, P. Stauder M, et al. In vitro assessment of shiitake mushroom (Lentinula edodes) extract for its antigingivitis activity. J Biomed Biotechnol. 2011:507908.
9. Kurian B, Swapna DV, Nadig RR, Ranjini MA, Rashmi K, Bolar SR. Efficacy of calcium hydroxide, mushroom, and Aloe vera as an intracanal medicament against Enterococcus faecalis: An in vitro study. Endodontol. 2016;28(2):137-42.
10. Hearst R, Nelson D, McCollum G, Millar C, Maeda Y, et al. An examination of antibacterial and antifungal properties of constituents of shiitake (Lentinula edodes) and oyster (Pleurotus ostreatus) mushrooms. Complement Ther Clin Pract. 2009;15(1):5-7.
11. Carvalho MP, Sand ST, Rosa EAR, Germani JC, Ishikawa NKI. Investigation of the antibacterial activity of basidiomycetes Lentinula boryana and Lentinula edodes. Biociencias Porto Alegre. 2009;15(2):173-9.
12. Carpio-PerochenavA, Duarte MAH, Graeff MS, Andrade FB. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. JOE. 2011;37(8):1134-6.
13. Widyarman AS, Widjaja SB, Idrus E. Strawberry extract's effects on Enterococcus faecalis and Porphyromonas gingivalis biofilms in vitro. Sci Dent J. 2017; 1(1):1–5.
14. Rocas IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J. Endod. 2004;30(5):315-20.
15. Bergenholtz G. Pathogenic mechanisms in pulpal disease. J Endod. 1990;16(2):98-101.
16. Flemming HC, Wingender J. The Biofilm Matrix. Nat Rev Microbiol. 2010 Sep;8(9):623-33.
17. Kuznetsov O, Milkova EV, Sosnina AE. Antimicrobial action of L Kentinus edodes juice on Human Microflora. J Microb Epid immunobiol. 2005;1:80-2.
18. Widyarman AS, Cynthia E, Theodorea CF, Amtha RT. Antibiofilm activity of temu kunci (Boesenbergia rotunda), an Indonesian medicinal plant extract, against root canal pathogens. Drug Invention Today. 2019;12(11):2486-92.
19. Widyarman AS, Sumadi S, Agustin TP. Antibiofilm effect of Clitoria ternatea flower juice on Porphyromonas gingivalis in vitro. J Indones Dent Assoc. 2018;1(1):7-12.
20. Soviati N, Widyarman AS, Binartha CTO. The effect ant-nest plant (Myrmecodia pendans) extract on Streptococcus sanguinis and Treponema denticola biofilms. J Indones Dent Assoc. 2020;3(1):11-15.
21. Ishikawa ON, Kasuya MCM, Vanetti MCD. Antimicrobial activity of Lentinula edodes grown in liquid medium. Brazilian J Microb. 2001;32(3):206-10.
22. Signoretto C, Burlacchini G, Marchi A, Grillenzoni M, Cavalleri G, et al. Testing a low molecular mass fraction of a mushroom (Lentinus edodes) extract formulated as an oral rinse in a cohort of volunteers. J Biomed Biotech.2011;2011:1-7.
23. Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RM, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microb. 2013;115:346-57.

(Received March 5, 2020; Accepted June 19, 2020)

Effectiveness of Lentinus edodes Mushr eness of Lentinus edodes Mushroom Extr oom Extract on Eradication of Enterococcus faecalis Biofilm

by Meiny Fuadah Amin

Submission date: 27-Jun-2022 10:22AM (UTC+0700)
Submission ID: 1863428434
File name: ctiveness_of_Lentinus_edodes_Mushroom_Extract_on_Eradication.pdf (1,018.99K)
Word count: 3553
Character count: 19806

P-ISSN 1693-9697
E-ISSN 2355-4800
Volume 27
Number 2
August 2020

Journal of Dentistry Indonesia

The Official Publication of
The Faculty of Dentistry, Universitas Indonesia
in Collaboration with
Indonesian Dental Association

Contents of Vol. 27, No.2, August 2020

Journal of Dentistry Indonesia

Journal of Dentistry Indonesia

Volume 27
Number 2 *August*

Article 2

8-30-2020

Effectiveness of Lentinus edodes Mushroom Extract on Eradication of Enterococcus faecalis Biofilm

Putri Lestari Anggiarini
Postgraduate Program, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Meiny F Amin
Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Juanita A Gunawan
Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia

Armelia Sari Widyarman
Department Head of Microbiology, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia,
armeliasari@trisakti.ac.id

Effectiveness of Lentinus edodes Mushroom Extract on Eradication of Enterococcus faecalis Biofilm

Cover Page Footnote

ACKNOWLEDGEMENT The authors would like thank Faculty of Dentistry, Trisakti University, for invaluable support in this study. Authors also would like to thank Mario, SSi and Aradhea Monica Drestia, SSi from the Microbiology Center of Research and Education (MiCORE) laboratory for their assistances for this study.

Journal of Dentistry Indonesia 2020, Vol. 27, No. 2, 56-60
doi: 10.14693/jdi.v27i1.1126

ORIGINAL ARTICLE

Effectiveness of *Lentinus edodes* Mushroom Extract on Eradication *of Enterococcus faecalis* Biofilm

Putri L. Anggiarini[1], Meiny F. Amin[2], Juanita A. Gunawan[2], Armelia S. Widyarman[3]

[1]Postgraduate Program, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
[2]Department of Conservative Dentistry, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
[3]Department Head of Microbiology, Faculty of Dentistry, Trisakti University, Jakarta 11440, Indonesia
Correspondence e-mail to: armeliasari@trisakti.ac.id

ABSTRACT

Sodium hypochlorite is a commonly used irrigation solution in endodontic procedures, but it irritates tissues and has toxic effects. *Lentinus edodes* is a mushroom that has antibacterial properties. *Enterococcus faecalis* is an anaerobic bacterium that can cause root canal treatment failure. **Objective:** This study aimed to determine the effect of *L. edodes* extract on the eradication of *E. faecalis* biofilms. **Methods**: Phytochemical tests of *L. edodes* were performed to analyze alkaloids, steroids, triterpenoids, phenolics, tannins, flavonoids, and glycosides from this extract qualitatively. *E. faecalis* ATCC 29212 was cultured in brain heart infusion broth for 24 h at 37°C in an anaerobic atmosphere. Biofilm assay was performed to analyze the eradication of *E. faecalis* biofilm after treatment with *L. edodes* extract. The application times were 5, 15, and 30, and 10%, 20%, 40%, and 80% concentrations were used. Distilled water was used as a negative control, and NaOCl was used as a positive control. Data were statistically analyzed via one-way analysis of variance, where $p < 0.05$ was set as the level of significance. **Results:** *L. edodes* mushroom extract was effective in eradicating *E. faecalis* biofilms in all concentrations and incubation times compared with the control ($p < 0.05$). Significant differences were found between the application times of 5 and 15 min compared with 30 min ($p < 0.05$). The most effective concentration in eradicating *E faecalis* biofilms was 40% with an application time of 30 min. **Conclusion:** *L. edodes* mushroom extract proves its antibiofilm activity against *E. faecalis* biofilm. Further study is necessary to determine which substances are have the most influence on the effectiveness of *L. edodes* extract in eradicating *E. faecalis* biofilm in vivo.

Key words: biofilm eradication, *Enteroccus faecalis*, *Lentinus edodes*, mushroom extract

How to cite this article: Anggiarini PL, Amin MF, Gunawan JA, Widyarman AS. Effectiveness of *Lentinus edodes* mushroom extract on eradication of *Enterococcus faecalis* biofilm. J Dent Indones. 2020; 27(2):56-60

INTRODUCTION

Caries can develop over a long period of time on the entire surface of the tooth covered by biofilms.[1] Bacteria present in carious lesions can invade the pulp, causing reversible pulpitis. Pulps in irreversible pulpitis and pulp necrosis should be treated with root canals.[2] The most important factors that indicate the success of root canal treatment are good preparation, sterilization, and apical closure of obturation. The removal of necrotic pulp tissue and bacteria is an important factor. Imperfect obturation is a result of inadequate preparation and irrigation, leaving bacteria to grow in the root canal. Bacteria left in the root canal form biofilms and cause further inflammatory reactions in the periapical region. If not treated properly, inflammation may worsen.[3]

Tooth root canal preparation supports the sterilization process and produces good filling; thus, optimal results are obtained. Preparation is carried out alternately with root canal irrigation solutions. Root canal irrigation is an important step in root canal treatment. It removes organic and inorganic necrotic tissue, kill microorganisms, and dissolve the smear layer during root canal preparation.[2] The ideal irrigation solution should be non-toxic, have antibacterial properties, function as a lubricant, have low surface tension, and be

able to dissolve the remaining necrotic pulp tissue and the smear layer formed during root canal preparation. Until now, there has not been an ideal irrigation solution that has all these properties. Existing irrigation solutions must be combined with other irrigation solutions, such as a combination of EDTA, sodium hypochlorite (NaOCl), and chlorhexidine.[3] NaOCl is a very commonly used solution for root canal irrigation. However, besides its being an irrigation solution, it also has a toxic effect on tissues: i.e., the higher its concentration, the worse its toxic effect. Some studies mention that NaOCL toxicity can cause pain, soft tissue edema, and paresthesias.[4]

Natural ingredients from traditional plants have been widely investigated as potentially better sources of antimicrobials. Mushrooms are alternative natural materials that have antibacterial effects. Fungi have bioactive metabolite ingredients that function as immunomodulators, protect the liver and cardiovascular system, and have antifibrotic, anti-inflammatory, antidiabetic, antiviral, antioxidant, and antitumor effects. Mushrooms can be a source of natural antibiotics.[5] Mushrooms contain peptides and proteins. Components found in fungi include sesquiterpenes, steroids, anthraquinones, benzoic acid derivatives, quinolones, oxalic acids, and phenols that function as antibacterial agents.[6]

Lentinus edodes is the most studied fungus. It appears to have broad antimicrobial effects on gram-negative and gram-positive bacteria. Extracts using chloroform, ethyl acetate, and water inhibit various gram-positive and gram-negative bacteria at concentrations below 50 mg/ml.[7] Ciric et al. examined the effectiveness of *L. edodes* mushroom extract to determine its antigingivitis effect.[8] Some literature has shown the effectiveness of *L. edodes* mushroom extract against *Streptococcus mutans* and *E. faecalis*.[6,7] *E. faecalis* is an anaerobic bacteria found in the root canal that can cause infection and inflammation. It is often associated with various forms of periradicular disease, as both primary (4%–44%) and persistent endodontic infections (24%–74%).[9] Some studies on the antibacterial effect of *L. edodes* mushroom extract on *E. faecalis* found that *L. edodes* has the ability to inhibit *E. faecalis*.[6,10] However, Carvalho stated that further investigation is required.[11] The present study was carried out to examine the effectiveness of the *L. edodes* mushroom extract in various concentrations and time applications in eradicating biofilm of *E. faecalis*. Previous studies focused on the effects of the extract on *E. faecalis*, not on its biofilm. Biofilm is more difficult to eliminate than planktonic bacterium. The results of this study can be used as baseline information on the use and selection of an irrigation solution in root canal treatment, which is more effective in reducing the prevalence of secondary infections after root canals caused by *E. faecalis*.

METHODS

This experimental laboratory study was designed to determine the antibacterial effectiveness of fungal extracts of *L. edodes* on the eradication of *E. faecalis*. Concentrations of 10%, 20%, 40%, and 80% were applied for 5, 15, and 30 min. The study employed a post-treatment design with a control. This study was conducted at the Microbiology Center of Research and Education Laboratory of the Faculty of Dentistry, Trisakti University, Jakarta.

L. edodes identification and phytochemical testing

The sample of *L. edodes* was taken from the Cibodas Bionic Farm, Puncak, West Java, Indonesia. The identification of the *L. edodes* mushroom plant was carried out at the Bogor Institute of Sciences, Bogor, Indonesia, and the phytochemical assay analysis was performed at the Indonesian Medicinal and Aromatic Crops Research Institute (BALITTRO) in Bogor, Indonesia.

L. edodes extract preparation

A total of 200 g of dried *L. edodes* mushroom was immersed in 300 mL of water at 4°C overnight. The wet *L. edodes* material was crushed with a mixer, added to 1600 mL of chloroform, and stirred at 4°C overnight. Chloroform and insoluble material were separated by filtering or centrifugation. The chloroform extract was evaporated under low pressure to produce 876 mg of a light-yellow oily liquid. This insoluble material was then mixed with 1000 mL of water and stirred overnight at 4°C. The resulting suspension was then filtered or centrifuged to remove insoluble materials. After the pressure was reduced, the aqueous supernatant concentration was reduced to 200 mL. This concentration was then extracted by mixing with 200 mL of ethyl acetate three times. The organic layer was separated and evaporated at low pressure to produce a light-yellow ethyl acetate extract (114 mL). The aqueous extract was then freeze-dried and returned to a full water suspension. The solution obtained was diluted with sterile Distilled water to obtain concentrations of 10%, 20%, 40%, and 80%.

Bacterial Culture and Biofilm Assay

E. faecalis ATCC 29212 was cultured in brain heart infusion broth for 24 h at 37°C in an anaerobic atmosphere. Subsequently, 200 µl of bacterial suspension (1×10^7 CFU/mL) was inserted into a 96-well plate and incubated for 24 h. The well plate was then rinsed with 200 µl phosphate buffered saline (PBS) and discarded. Each irrigation solution was put into the well plate up to 200 µl. The irrigation solution was incubated for 5, 15, and 30 min.[12] Moreover, the irrigation solution was discarded, and the well plate was rinsed again with 200 µl of PBS and discarded. Staining was then performed by adding a 200-µL solution of

Table 1. Phytochemical screening of the ethanol extracts of temu kunci

Plant constituents	Qualitative assay
Alkaloids	+
Saponins	-
Tannins	+
Phenolics	+
Flavonoids	+
Triterpenoids	+
Steroids	-
Glycosides	+

crystal violet into each well of the microtiter plate for 15 min and then rinsed with PBS. Ethanol acetone (200 µL) was put into each microtiter well plate to check for biofilm formation. A microtiter plate reader was used to measure the optical density of violet crystals at 450 nm.[13] Sterile distilled water was used as a negative control and NaOCl as a positive control. The concentration of NaOCl used as the positive control was 5.25% as it is used as the golden choice for irrigation solution in root canal treatment. All treatments were done in three independent experiments in triplicates.

Statistical Analysis

Data were analyzed using two-way analysis of variance and post-hoc tests to reveal significant differences between treatment groups. Data normality test was performed using the Kolmogorov–Smirnov test. Statistical significance was set at $p < 0.05$. Statistical calculations were performed using SPSS Statistics for Windows software version 20 (IBM Corp., Armonk, NY, USA).

RESULTS

The results of the phytochemical tests showed that the aqueous extract of *L. edodes* contained alkaloids, tannins, phenolics, flavonoids, triterpenoids, and glycosides (Table 1).

The results showed that *L. edodes* mushroom extract was effective in eradicating *E. faecalis* biofilms. This extract reduced the biofilm mass significantly in all concentrations and application times compared with the negative control ($p < 0.05$). The average optical density and standard deviation of *E. faecalis* biofilms is shown in Figure 1-3. The most effective concentration is 40% applied for 30 mins, which eradicated 99.2% of biofilms compared with the negative control (without *L. edodes*).

DISCUSSION

A comparative multiple test was performed using the post-hoc test to prove the significance of the differences between each ingredient. *L. edodes* extract with a concentration of 10%, 40%, and 80% showed significant differences compared with the negative control ($p < 0.05$). When compared with 80% concentration, 40% did not give significant results, meaning that the antibacterial effect was not

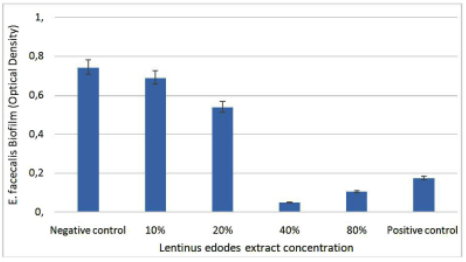

Figure 1. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 5 min of incubation time. The concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20%, and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

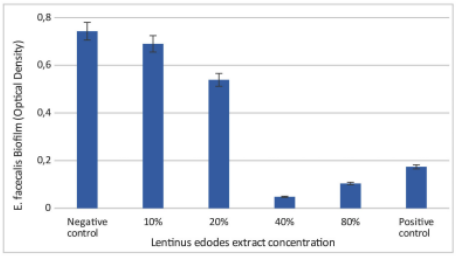

Figure 2. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 15 mins of incubation time. The concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20% and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

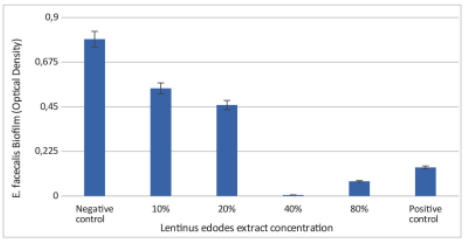

Figure 3. The biofilm assay of the *Lentinus edodes* extract against *E. faecalis* with 30 mins of incubation time. Concentrations of *Lentinus edodes* extract applied were 80%, 60%, 40%, 20% and 10%. Untreated biofilm wells were used as a negative control, while 5.25% sodium hypochlorite was used as a positive control.

significantly different between the two concentrations. Based on the application time, when the results of multiple applications, e.g., 5 and 15 mins, were compared with 30 mins, the results were significant ($p < 0.05$).

E. faecalis reinfection and bacterial resistance are the most common causes of root canal treatment failure. In a study of 30 cases of endodontic failure, *E. faecalis* was found in 20 cases.[14] In primary endodontic cases, the prevalence *of E. faecalis* was 18%. Secondary endodontic cases had a higher prevalence of 67%.[15] *E. faecalis* can form biofilms in the root canal that are resistant to destruction. They are 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than other organisms that do not form biofilms. In addition, biofilms can reduce the quality of the covering of the root canal filling material.[4]

This study aimed to develop a natural ingredient as a safer irrigation solution for use in endodontic procedures. *L. edodes* was chosen because it is easy to find; it is a food ingredient that is used in various types of cooking. Hearst investigated the antimicrobial activity of the aqueous extract of the fungus *L. edodes* against *E. faecalis*. However, Carvalho showed the opposite results.[10, 11]

In this study, the content of *L. edodes* is consistent with those in the study by Alves and Macoris, who examined the phenolic content of the aqueous extract of the fungus *L. edodes.* They examined various phenolic components contained in the fungus *L. edodes*, namely, p-Hydroxybenzoic acid, protocatechuic acid, and cinnamic acid, which have antimicrobial power against *E. faecalis* by changing the permeability of cell membranes and damaging DNA.[7, 16]

Kuznetsov showed that a 5% concentration solution of *L. edodes* mushroom extract had antimicrobial power against *E. faecalis*.[17] Many studies have shown that some herbs, such as *Boesenbergia rotunda*, *Clitoria ternatea*, and *Myrmecodia pendans*, have been proven effective in inhibiting *E. faecalis*, *F. nucleatum*, *T. denticola,* and *P. gingivalis* as endodontic pathogen biofilms. These herbals are also known to have antibacterial substances, such as flavonoids, alkaloids, and tannins.[18, 18, 20]

Alkaloids can penetrate into the bacterial membrane and cause cytoplasmic leakage. Tannin is a type of compound that belongs to the polyphenol group. Tannin is able to deactivate bacterial adhesin, enzymes, and cell wall transport proteins and form complex compounds against extracellular proteins, which interfere with the integrity of the bacterial cell membrane. Its mechanism of action is by denaturing bacterial cell proteins and causing irreversible damage to cell membranes. Flavonoids form complexes with cell walls and bind to adhesin. Triterpenoids can cause damage to bacterial cell membranes.[21,22,23]

The longer application time and higher concentration of *L. edodes* mushroom extract showed a significant difference when compared with the negative control, and the optical density value was lower. This demonstrates its effectiveness in eradicating *E. faecalis* biofilms. The results of this study are consistent with those of Hearst. However, the results are inconsistent with those of Carvalho et al., who concluded that *L. edodes* was not effective against *E. faecalis*. This difference may be attributed to differences in the part of the fungus studied, how it was extracted, and the incubation period.[10,11]

CONCLUSION

L. edodes mushroom extract was effective in eradicating *E. faecalis* biofilms. The most effective *L. edodes* extract was at a concentration of 40% applied for 30 min. This extract may be useful as antibiofilm agent. However, further research is needed on the effectiveness of *L. edodes* extract in vivo. Furthermore, it is necessary to study which substances have the most influence on the effectiveness of *L. edodes* extract in eradicating biofilms.

CONFLICT OF INTEREST

The authors declare there are no conflicts of interest related to this study.

REFERENCES

1. Marsh PD, Takahashi N, Nyvad B. Dental Caries The Disease & Its Clinical Management. 3rd ed. Fejerskov, O. Nyvad, B. Kidd E, ed. John Wiley & Sons;2015:107-29.
2. Fouad A, Levin LG. Pulpal Reactions to Caries and Dental Procedures. In: Hargreaves KM, Cohen S, editors. Pathways of The Pulp. 11Th ed. ST. Louis: Mosby;2011:573-95.
3. Torabinejad M, Walton ERE, Fouad AF. Endodontics Principles and Practice. 5th ed. Elsevier. 2015:37-45.
4. Tanumihardja M. Solution for root canal irrigation. (Indonesian). Dentofasial. 2010;9(2):108-15.
5. Zaura E, Buijs MJ, Hoogenkamp MA, Ciric M, Papetti A, et al. The effects of fractions from shiitake mushroom on composition and cariogenicity of dental plaque microcosms in an in vitro caries model. J. Biomed Biotech. 2011:135034.
6. Alves MJ, Fereira ICFR, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity

of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med. 2012:78;1707-18.
7. Macoris JDM, Brugnari T, Boer CG, Contato AG, Peralta RM, Souza CGM. Antioxidants properties and antimcrobial potential of aqueous extract of Bbsidioma from Lentinus edodes (Berk.) Sing. (Shitake). Int J Curr Microb Appl Sci. 2017;6(9):3757-67.
8. Ciric L, Tymon A, Zaura E, Lingstrom, P. Stauder M, et al. In vitro assessment of shiitake mushroom (Lentinula edodes) extract for its antigingivitis activity. J Biomed Biotechnol. 2011:507908.
9. Kurian B, Swapna DV, Nadig RR, Ranjini MA, Rashmi K, Bolar SR. Efficacy of calcium hydroxide, mushroom, and Aloe vera as an intracanal medicament against Enterococcus faecalis: An in vitro study. Endodontol. 2016;28(2):137-42.
10. Hearst R, Nelson D, McCollum G, Millar C, Maeda Y, et al. An examination of antibacterial and antifungal properties of constituents of shiitake (Lentinula edodes) and oyster (Pleurotus ostreatus) mushrooms. Complement Ther Clin Pract. 2009;15(1):5-7.
11. Carvalho MP, Sand ST, Rosa EAR, Germani JC, Ishikawa NKI. Investigation of the antibacterial activity of basidiomycetes Lentinula boryana and Lentinula edodes. Biociencias Porto Alegre. 2009;15(2):173-9.
12. Carpio-PerochenavA, Duarte MAH, Graeff MS, Andrade FB. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. JOE. 2011;37(8):1134-6.
13. Widyarman AS, Widjaja SB, Idrus E. Strawberry extract's effects on Enterococcus faecalis and Porphyromonas gingivalis biofilms in vitro. Sci Dent J. 2017; 1(1):1–5.
14. Rocas IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J. Endod. 2004;30(5):315-20.
15. Bergenholtz G. Pathogenic mechanisms in pulpal disease. J Endod. 1990;16(2):98-101.
16. Flemming HC, Wingender J. The Biofilm Matrix. Nat Rev Microbiol. 2010 Sep;8(9):623-33.
17. Kuznetsov O, Milkova EV, Sosnina AE. Antimicrobial action of L Kentinus edodes juice on Human Microflora. J Microb Epid immunobiol. 2005;1:80-2.
18. Widyarman AS, Cynthia E, Theodorea CF, Amtha RT. Antibiofilm activity of temu kunci (Boesenbergia rotunda), an Indonesian medicinal plant extract, against root canal pathogens. Drug Invention Today. 2019;12(11):2486-92.
19. Widyarman AS, Sumadi S, Agustin TP. Antibiofilm effect of Clitoria ternatea flower juice on Porphyromonas gingivalis in vitro. J Indones Dent Assoc. 2018;1(1):7-12.
20. Soviati N, Widyarman AS, Binartha CTO. The effect ant-nest plant (Myrmecodia pendans) extract on Streptococcus sanguinis and Treponema denticola biofilms. J Indones Dent Assoc. 2020;3(1):11-15.
21. Ishikawa ON, Kasuya MCM, Vanetti MCD. Antimicrobial activity of Lentinula edodes grown in liquid medium. Brazilian J Microb. 2001;32(3):206-10.
22. Signoretto C, Burlacchini G, Marchi A, Grillenzoni M, Cavalleri G, et al. Testing a low molecular mass fraction of a mushroom (Lentinus edodes) extract formulated as an oral rinse in a cohort of volunteers. J Biomed Biotech.2011;2011:1-7.
23. Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RM, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microb. 2013;115:346-57.

(Received March 5, 2020; Accepted June 19, 2020)

Effectiveness of Lentinus edodes Mushr eness of Lentinus edodes Mushroom Extr oom Extract on Eradication of Enterococcus faecalis Biofilm

ORIGINALITY REPORT

21%	16%	11%	6%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	acikerisim.antalya.edu.tr Internet Source	3%
2	www.contempclindent.org Internet Source	2%
3	en.fkg.trisakti.ac.id Internet Source	2%
4	Submitted to Our Lady of Fatima University Student Paper	1%
5	digitalcommons.aaru.edu.jo Internet Source	1%
6	www.karger.com Internet Source	1%
7	www.scielo.br Internet Source	1%
8	Submitted to Fakultas Kedokteran Gigi Universitas Trisakti Student Paper	1%

9	Masatomo Hirasawa, Naoto Shouji, Tomotake Neta, Kazuo Fukushima, Kazuko Takada. "Three kinds of antibacterial substances from Lentinus edodes (Berk.) Sing. (Shiitake, an edible mushroom)", International Journal of Antimicrobial Agents, 1999 Publication	1 %
10	pharmacy.mahidol.ac.th Internet Source	1 %
11	blog.balimedicaljournal.org Internet Source	1 %
12	cwww.intechopen.com Internet Source	1 %
13	docplayer.com.br Internet Source	1 %
14	bioone.org Internet Source	1 %
15	Ranty Dwi Farasayu, Mayu Winnie Rachmawati, Ika Dewi Ana, Ahmad Syaify, Dyah Listyarifah. " The Effect of Hibiscus Flower Extract ( ) on the Growth of Bacteria ", BIO Web of Conferences, 2021 Publication	1 %
16	Maria Alves, Isabel Ferreira, Joana Dias, Vânia Teixeira, Anabela Martins, Manuela Pintado. "A Review on Antimicrobial Activity of	1 %

Mushroom (Basidiomycetes) Extracts and Isolated Compounds", Planta Medica, 2012
Publication

17	Samsu Adi Rahman, Yanti Mutalib, Frederik Dony Sangkia, Admi Athirah, Marlan, Muhlis Kadir, Chrisoetanto P Pattirane. "Evaluation of inhibitory potential of mangrove leaves extract Avicennia marina for bacteria causing ice-ice diseases in seaweed Kappaphycus alvarezii", IOP Conference Series: Earth and Environmental Science, 2020 Publication	1 %
18	docshare.tips Internet Source	1 %
19	www.parasitol.kr Internet Source	1 %

Exclude quotes	On	Exclude matches	< 15 words
Exclude bibliography	On		

Effectiveness of Lentinus edodes Mushr eness of Lentinus edodes Mushroom Extr oom Extract on Eradication of Enterococcus faecalis Biofilm

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10