Research Article

Antibiofilm activity of *temu kunci (Boesenbergia rotunda)*, an Indonesian medicinal plant extract, against root canal pathogens

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ABSTRACT

Introduction: A medicinal extract from an Indonesian plant, *temu kunci* (*Boesenbergia rotunda*) has an antibacterial effect. The objectives of this study is to determine the effects of *temu kunci* extract against *Enterococcus faecalis*, *Fusobacterium nucleatum*, and *Treponema denticola* biofilms. **Materials and Methods:** This experimental laboratory study used the post-test control group design method. The *E. faecalis*, *F. nucleatum*, and *T. denticola* were each cultured in brain heart infusion broth, for 24 h, at 37°C, under anaerobic conditions. Each bacterium was distributed into 96-well microplate and treated with an ethanol extract of *temu kunci* in different concentrations and incubated for 5 min, 1 h, 6 h, and 24 h. The biofilm without treatment was used as the negative control, and sodium hypochlorite was used as the positive control. The biofilm mass was read using a microplate reader (490 nm). One-way analysis of variance and a *post hoc* test was used to analyze the results. **Results:** The *temu kunci* extract significantly reduced *E. faecalis*, *F. nucleatum,* and *T. denticola* in the monospecies and multispecies biofilms for all incubation periods ($P < 0.05$). Inhibitory efficiency of each concentration of the *temu kunci* extracts in preventing biofilm formation against *E. faecalis*, *T. denticola*, *F. nucleatum* monospecies, and multispecies. Moreover, the type of bacteria, the concentration, and the incubation time period were statistically significant compared to the control ($P < 0.05$). **Conclusion:** *Temu kunci* has antibiofilm activity against root canal pathogen biofilms, and it may be used as an alternative for root canal irrigation. However, further works are needed to determine the detailed mechanism by which *temu kunci* affects biofilm formation.

KEY WORDS: Antibacterial effect, *Enterococcus faecalis*, Ethanol extract of *temu kunci*, *Fusobacterium nucleatum*, Root canal bacteria, *Treponema denticola*

INTRODUCTION

Post-endodontic infection is a persistent infection inside the dental pulp and periapical tissue that cannot be cured after endodontic treatment.[1] Endodontic treatment failures are primarily due to the complexity of the root canal anatomy and restricted instrument. Periodontal-periradicular lesions are another reason for treatment failure; these are caused by bacteria and the endodontic procedure, such as sterilization or imperfect obturation.[2] Primary or secondary infection of a root canal is caused by microorganism colonization, which is dominated by anaerobic bacteria.[3] Anaerobic facultative bacteria and anaerobic obligate bacteria also play a role. The most common type of bacteria found in pulp and periapical disease is *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus gordonii*, *Prevotella intermedia*, *Prevotella denticola*, *Enterococcus faecalis*, *Actinomyces* spp., *Fusobacterium nucleatum*, *Eubacterium* spp., and *Treponema denticola*.[4] The presence of bacteria in the root canal or periapical tissue will determine the success of endodontic treatment.[5] Among those bacteria, *E. faecalis, F. nucleatum*, and *T. denticola* have been found to be the predominant species in endodontic failure cases.[6]

E. faecalis is facultative anaerobic Gram-positive cocci that normally inhabit the intestine and can be present in the oral cavity and gingival sulcus. *E. faecalis* can form biofilm and survive in poor nutrient environment, including the root canals.[4] *E. faecalis* is found in

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Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 10-06-2019; Revised on: 16-07-2019; Accepted on: 17-08-2019

approximately 77% of all endodontic failure cases.[7] In some cases, *E. faecalis* can be found in an obturated root canal with a periapical lesion.[8,9] *F. nucleatum* is primarily found in root canal culture bacteria with an apical lesion (48%).[10] According to Siquiera's research on acute apical abscess, *F. nucleatum* was found in 27 cases from 42 individuals. Thus, the prevalence of *F. nucleatum* was 64% from 27 cases.[11]

F. nucleatum is a Gram-negative anaerobic oral commensal and a periodontal pathogen.[12] *F. nucleatum* can inhibit the proliferation of fibroblast cells in the gingiva. This inhibition enables *F. nucleatum* to penetrate into the gingiva epithelial. The butyric acid that is produced by *F. nucleatum* can irritate the tissue. Butyric acid has been proven to be the greatest inhibitor of blastogenesis in T-cells; it stimulates the production of interleukin-1, which is related to bone resorption.[11] In general, lipopolysaccharides are related to the endotoxin substrate; its biological effects include complement activation, cytotoxicity, and bone resorption. Lipopolysaccharides play a primary role in attachment, and they can be diluted in saliva. The lipopolysaccharides produced by *F. nucleatum* lead other bacteria to attach to the hydroxyapatite, serum, and cementum. This demonstrates that lipopolysaccharides from *F. nucleatum* play a primary role in attachment to the epithelial tissue and the tooth surface.[13]

T. denticola is a spiral spirochaeta species; it is Gram-negative, motile, and has flagella.[14] *T. denticola* is one of the most common types of bacteria in the oral cavity of humans; it can ferment amino acids, such as cysteine and serine, to form acetate acid, carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide.[15] Research has shown that *T. denticola* from an infected root canal can stimulate bone resorption and spread to the organ host; it was found to be associated with the highest prevalence (56.5%) of endodontic failure in the sample cases.[16]

E. faecalis, *F. nucleatum,* and *T. denticola* can form a biofilm in a root canal. Intracanal biofilms are microbial biofilms formed on the root canal dentin of the infected tooth. Biofilm was first identified by Nair in 1987 using transmission electron microscopy.[17,18] The attachment of bacterial cells to the surface results from signals to express biofilm-forming genes. These genes code proteins resulting in the synthesis of communication signals and the formation of polysaccharides. During this process, bacterial cells in the matrix produce chemical signals. These signal molecules play a role in creating the characteristics of the biofilm so that it can mature and coordinate its activities.[19] The action of these signals is a quorum-sensing process, which is intercellular communication; the molecule's ability to do an action depends on the signal concentration in the surrounding area. Intercellular communication is important for the development and maintenance of biofilm.[20] The structure and basic physiology of biofilm enable it to naturally resist antimicrobial agents, such as antibiotics, disinfectants, and germicides. This can be seen in the significant difference in the resistance of antibiotics to biofilm cells and their planktonic cells.[21,13]

Therefore, a new type of root canal irrigation that is safer and easier to obtain is needed. The use of natural ingredients, such as plants and fruits, has become one of the alternative choices to replace chemical drugs.[22] Some *in vitro* research studies have shown that herbal extracts and juice from fruit can inhibit the formation of oral pathogen biofilms.[23-25] *Temu kunci* (*Boesenbergia rotunda*) is one of the plants that have many medicinal benefits. The rhizomes of *temu kunci* contain essential oils, such as 1,8-sineol, camphor, borneol, kalkon, pinnen, zingiberon, curcumin, panduratin, boesenbergin, and zeodarin.[26]

In general, *temu kunci* is effective in curing rheumatoid arthritis, gastritis, inflammation of the mucous membrane, urinary problems, malaria, stomatitis, dry cough, diarrhea, ascariasis, gassiness, colon problems, and skin diseases. In addition to being an analgesic, *temu kunci* also has an antibacterial effect.[27] Essential oils from the rhizome of *temu kunci* inhibit pathogen bacterial growth in the oral cavity.[28] Based on this information, we investigated the antibacterial effect of an ethanol extract obtained from the rhizomes of *temu kunci* against root canal bacteria, such as *E. faecalis, F. nucleatum*, and *T. denticola*.

MATERIALS AND METHODS

Research Design

Temu kunci plants and extract preparation

The *temu kunci* plants were obtained from Sweetberry Agrowisata, Green Apple Cipanas, Puncak, West Java, Indonesia. The plants were manually collected in April 2018. After being collected, around 4 kg of fresh *temu kunci* was dried at 40°C for 6 days and then stored under controlled conditions (dry air, dark, at 20°C). The *temu kunci* extract was prepared with the maceration method using ethanol (70%) under stirring at room temperature for 24 h. After filtering, the extract was concentrated on the rotary evaporator attached to a vacuum pump and then used for phytochemical screening.

Phytochemical screening

The ethanol extracts of the plants were tested for the presence of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides.

The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.

Alkaloid detection assay

Extracts were treated with hydrochloric acid and filtered. A few drops of potassium mercuric iodide solution (Mayer's reagent) were added into the filtrate. The presence of alkaloid compounds was indicated by the formation of the white creamy-colored precipitate.[29,30]

Saponins detection assay

Extracts were diluted in a tube containing distilled water. The tube was shaken continuously in a shaker for 15 min. The formation of persistent foam layer at the top of the tube indicates the presence of saponins.[31]

Tannins detection assay

About 1% of the gelatin-sodium chloride solution was added into the tube-containing extract. The presence of tannins was indicated by formation of white precipitate at the bottom of the tube.[32]

Phenolic compounds detection assay

The ethanolic extract was spotted on a piece of filter paper, added with a drop of phosphomolybdic acid reagent, and exposed to ammonia vapors. The presence of phenolic compounds was indicated by the formation of blue coloration of the spot.[31]

Flavonoids detection assay

A piece of magnesium ribbon was added into 2–3 mL of mangosteen pericarp ethanolic extract, followed by the addition of 1 mL of concentrated hydrochloric acid. The presence of flavonoids was indicated by reddish-pink or red coloration in the solution.[31]

Triterpenoids detection assay

Triterpenoids detection was carried out using the Liebermann-Burchard test. Mangosteen pericarp extract (100 mg) was shaken with chloroform in a tube. A few drops of acetic anhydride were added into the solution. The tube was boiled in a water bath and cooled in iced-water, followed by the addition of concentrated sulfuric acid (2 mL) into the tube. The presence of triterpenoids was indicated by formation of deep red color in the solution.[29]

Plant steroids detection assay

1 mL of chloroform was added into 1 mL of extract, followed by the addition of 2–3 mL of acetic anhydride and 1–2 drops of concentrated sulfuric acid. The presence of plant steroids was indicated by dark green coloration in the solution.[31]

Glycosides detection assay

HCl was added into the extract as a pre-treatment to induce hydrolysis. Then, the extract was treated with ferric chloride solution and immersed in boiling water for 5 min. After cooling down, an equal volume of benzene was added into the extract. The benzene layer was separated and added with NH_3 solution. The presence of plant glycosides was indicated by the formation of rose-pink color in the solution (Tiwari *et al*. 2011).[32]

Bacterial strains and growth conditions

The following bacterial strains were obtained from American Type Culture Collection (ATCC): *E. faecalis* ATCC 29212®™, *F. nucleatum* ATCC25586®™, and *T. denticola* ATCC35405®™. The strains were cultured in brain heart infusion broth medium and incubated under anaerobic conditions at 37°C (10% CO_2, 10% H, 80% N_2) or using GasPak™ EZ Gas Generating Container System for 24 h.

Effect of temu kunci on biofilm formation

To generate a biofilm on the microtiter plate wells, 200 µL of the bacterial suspension that was cultured overnight was placed in each well of polystyrene 96-well flat-bottom microtiter plates (Greiner Bio-One; Frickenhausen, Germany), Subsequently, the *temu kunci* extract in different concentration were added into the biofilm containing well then incubated at 37°C under anaerobic conditions for 5 min, 1 h, 3 h, and 24 h. Following these incubation periods, the spent medium was aspirated, and the wells were washed with phosphate-buffered saline (PBS) solution to remove the unattached cells; the plates were then air-dried. Each well was then stained for 15 min with 200 µL of 1% crystal violet solution (CV Gram-stain, Merck, Germany) at room temperature. The dye was washed with PBS solution and air-dried at room temperature. After drying the stained plates, 200 µL of 96% ethanol was distributed into the microplate and incubated for 15 min at room temperature. Then, the microplate was measured with a microplate reader (490 nm wavelength; optical density [OD]: OD_{490}). Each assay was performed in triplicate at different concentrations (50%, 25%, 12.5%, 6.25%, 3.1%, 1.5%, 0.75%, and 0.35%), and the mean absorbance values were used to measure the extract's ability to inhibit biofilm formation (OD). About 5.25% sodium hypochlorite was used as the positive control; Biofilm wells without treatment were used as the negative control.

Statistical Analysis

The obtained results were statistically analyzed using one-way analysis of variance to determine a significant differences in the biofilm reduction between the experimental groups. $P < 0.05$ was considered to be statistically significant. The Shapiro–Wilk test was used to test for normality; Statistical calculations were performed using SPSS Statistics for Windows version 20 software (IBM SPSS Statistics of Windows, Version 20.0. Armonk, NY: IBM Corp).

RESULTS

Preliminary Qualitative Phytochemical Screening Analysis

The present study's findings demonstrate that the ethanol extract of *temu kunci* contained alkaloids, saponins, phenolics, flavonoids, triterpenoids, and glycosides, but not tannins or steroids [Table 1].

The Inhibitory Effects of the *Temu Kunci* Extract Against Biofilm Formation

The inhibitory efficiency of each concentration of the *temu kunci* extracts in preventing biofilm formation against *E. faecalis*, *T. denticola*, and *F. nucleatum* mono-species [Figures 1-3]. After examining the biofilm formation, it was concluded that the type of bacteria, the concentration, and the incubation time period were statistically significant compared to the negative control ($P < 0.05$).

Overall, based on the mean of the inhibitory effects of the *temu kunci* extracts at different incubation times, these extracts were better able to inhibit biofilm formation in the *E. faecalis* monospecies (mean range OD = 0.315–0.919) in comparison to the *T. denticola* monospecies (mean range OD = 1.202–1.838) and the *F. nucleatum* monospecies (mean range OD = 2.542–2.649). Moreover, based on the mean of the inhibitory effects of the *temu kunci* extracts at different concentrations, the *T. denticola* monospecies (OD = 0.035 ± 0.021) and *F. nucleatum* monospecies (OD = 0.142 ± 0.009) were better able to inhibit biofilm formation than the negative control, and the difference was significant ($P < 0.05$). In the case of the *E. faecalis* monospecies, the greatest inhibition of biofilm formation was observed for the 1-h incubation time period with the *temu kunci* extract concentration of 3.10% (OD = 0.092 ± 0.127) in comparison to the negative control. We also found that the *E. faecalis* monospecies were less susceptible to 25% and 50% concentrations of the *temu kunci* extract after 6 h of incubation.

Our results demonstrate that the *temu kunci* extract with a 50% concentration and a 6-h incubation period (OD = 0.015 ± 0.018) was the most effective for inhibiting the formation of the multispecies biofilm [Figure 4].

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

Plant constituents	Interference
	Ethanol 70%
Alkaloids	+
Saponins	+
Tannins	−
Phenolics	+
Flavonoids	+
Triterpenoids	+
Steroids	−
Glycosides	+

+: Presence, −: Absence

DISCUSSION

The aim of endodontic therapy is to eliminate the infection from the inner root canal system and prevent re-infection by obturation.[33] However, some

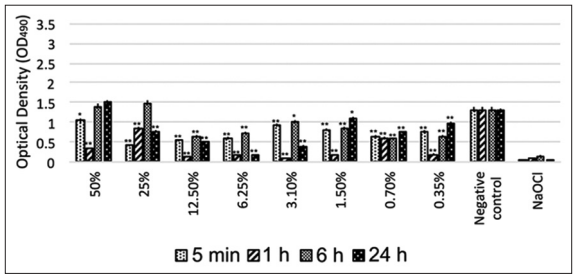

Figure 1: The *in vitro* biofilm assay of the *temu kunci* extract against *Enterococcus faecalis* monospecies. The incubation time ranged from 5 min to 24 h. All given concentrations of *temu kunci* extract were 50%, 25%, 12.5%, 6.25%, 3.10%, 1.05%, 0.70%, 0.70%, and 0.35%. Biofilm wells without treatment was used as a negative control, while 5.25% sodium hypochlorite was used as a positive control in this study

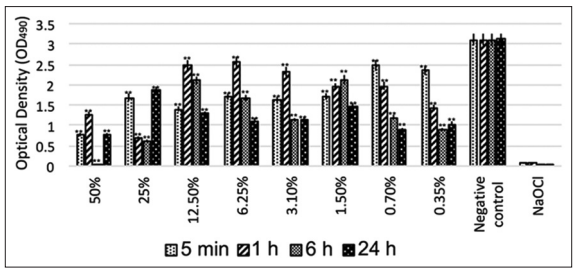

Figure 2: The *in vitro* biofilm assay of the *temu kunci* extract against *Treponema denticola* monospecies. The incubation time ranged from 5 min to 24 h. All given concentrations of *temu kunci* extract were 50%, 25%, 12.5%, 6.25%, 3.10%, 1.05%, 0.70%, 0.70%, and 0.35%. Biofilm wells without treatment was used as a negative control, while 5.25% sodium hypochlorite was used as a positive control in this study

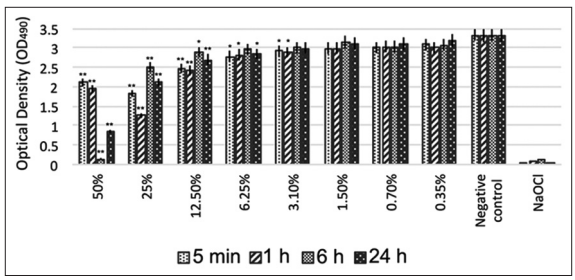

Figure 3: The *in vitro* biofilm assay of the *temu kunci* extract against *Fusobacterium nucleatum* monospecies. The incubation time ranged from 5 min to 24 h. All given concentrations of *temu kunci* extract were 50%, 25%, 12.5%, 6.25%, 3.10%, 1.05%, 0.70%, 0.70%, and 0.35%. Biofilm wells without treatment was used as a negative control, while 5.25% sodium hypochlorite was used as a positive control in this study

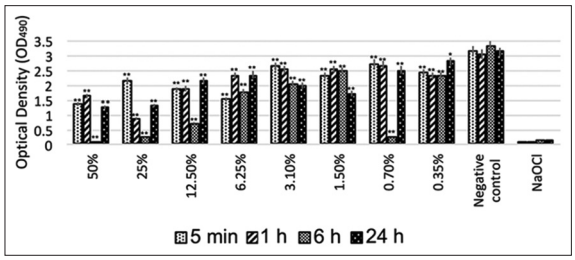

Figure 4: The *in vitro* biofilm assay of the *temu kunci* extract against multispecies. The incubation time ranged from 5 min to 24 h. All given concentrations of *temu kunci* extract were 50%, 25%, 12.5%, 6.25%, 3.10%, 1.05%, 0.70%, 0.70%, and 0.35%. Biofilm wells without treatment was used as a negative control, while 5.25% sodium hypochlorite was used as a positive control in this study

reports have recognized that the presence of residual microorganisms after endodontic therapy is one of the main causes of root canal treatment failure.[34] This study investigated the bacterial communities commonly found in the root canal system. *E. faecalis*, *F. nucleatum*, and *T. denticola* can form a biofilm in the root canal. Intracanal biofilms are microbial biofilms formed on the root canal dentin of the infected tooth.[17,18]

In this study, we used an extract of *temu kunci*, a plant that is known to have many medicinal benefits. The phytochemical analysis showed the presence of alkaloids, saponins, phenolics, triterpenoids, glycosides, and flavonoids, which give this plant its anti-inflammatory and antibacterial effects.[35-38]

In many Asian countries, *temu kunci* is a common edible ingredient. The fresh rhizomes of *temu kunci* are used to treat inflammatory diseases, such as dental caries, dermatitis, dry cough and cold, tooth and gum diseases, swelling, wounds, diarrhea, and dysentery; it is also used as a diuretic.[28,39] According to a previous study, *temu kunci* contains flavonoids as its major secondary metabolites that categorized in three main classes: Chalcones, flavanones, and flavones.[40] Since long, flavonoids have been proven to possess direct antibacterial activity through various mechanisms, including cytoplasmic membrane damage, inhibition of nucleic acid synthesis, and inhibition of energy metabolism.[41]

The rhizomes of *temu kunci* also contain essential oils, such as 1,8-sineol, camphor, borneol, pinnen, sesqueterpene, zingiberon, curcumin, and zeodarine. Other ingredients are kardamonin, pinosembrin (5,7-dihydroxyflavone), pinostrobin (5-hydroxy-7-methoxyflavone), panduratin A, and 4-hydroxypanduratine. The rhizomes of *temu kunci* also contain starch, resin, saponins, boesenbergin A, boesenbergin B, kavinat acid, flavone compounds (5,7-dimethoxyflavone, 3,5,7,4-tetramethoxyflavone, and 3,5,7,4-pentamethoxyflavone), flavanone (5-hydroxy-7,4-dimethoxyflavanone), chalcone compounds (2-hydroxy-4,6-dimethoxychalcone, 2,6-dihydroxy-4-methoxychalcone, 2-hydroxy-4,4,6-trimethoxychalcone, and 2,4-dihydroxy-6-methoxychalcone), panduratinB1, and panduratinB2.[28] The essential oils of *temu kunci* extracts have been reported to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria due to its ability to interfere with the membrane cell permeability and alter salt tolerance of bacterial cells.[40]

In the present study, the *temu kunci* extract was found to inhibit the biofilm formation of the *E. faecalis*, *F. nucleatum,* and *T. denticola* monospecies, as well as the biofilm formation of multi-species. *Temu kunci* extract, in the form of crude extracts, essential oils, and purified compounds have been proven to strongly combat various strains of bacteria, including cariogenic bacteria, acne-causing bacteria, and other pathogens even in low concentrations.[40] The antibacterial effect in an ethanol extract from the rhizomes of *temu kunci* is due to the synergism between the hydrophilic and hydrophobic chemical components of the plant. Hydrophilic compounds contain hydroxyl group that interacts with bacterial lipopolysaccharides and might be able to pass through bacterial outer membrane through porin proteins,[42] while hydrophobic compounds can easily bind to protein and lipid in bacterial membrane, thus providing membrane disruption activity.[43] The hydrophobic compound will interact with hydrophobic peptidoglycan; peptidoglycans can be easily penetrated by hydrophobic molecules that can affect both membrane stability and bacterial cytoplasm. These processes will disrupt and damage membrane permeability of the bacterial cell, leading to the leakage of intracellular material of the membrane.[42] In addition, bioactive compound found in the rhizome of *temu kunci* possesses antibiofilm activity by inhibiting oral bacteria from forming biofilm and disrupting existing biofilm *in vitro*.[44] These observations demonstrate that *temu kunci* can act as an antibacterial agent, especially against bacteria in the root canal, such as *E. faecalis*, *F. nucleatum*, and *T. denticola*.

The efficacy of an antibacterial agent can be influenced by many factors, such as: (1) Bacterial status (susceptibility and resistance, tolerance, and persistence biofilm) and inoculum size, (2) the concentration level, (3) time-dependent incubation, and (4) host responses.[45,46] This study focused on the concentration level of the *temu kunci* extract and the time-dependent incubation period to determine the ability of this plant to inhibit the biofilm formation of monospecies and multispecies, *in vitro*. Based on the mean of the inhibitory effects of the *temu kunci* extracts at different concentrations, the greatest

inhibition of biofilm formation was observed against the *T. denticola* monospecies (OD = 0.035 ± 0.021) and the *F. nucleatum* monospecies (OD = 0.142 ± 0.009) in comparison to the negative control when the *temu kunci* extraction concentration was 50% [Figures 1-3].

This result showed that it is better to use a higher concentration of the tested material because the active substances in that material might create a stronger inhibition effect. However, as the concentration of the extract increased, the inhibition effect might decrease, even with a higher concentration. The inhibition effect against the *E. faecalis* monospecies decreased for the 50% concentration of the *temu kunci* extract at incubation times of 6 h and 24 h [Figure 1]. Therefore, it was suspected that, in the high concentration solution, the active substance and the inhibition effect might be decreased. Crude plant extracts contain many bioactive constituents that may affect and interact with another compounds, thus providing either synergistic or antagonistic effects toward another compound at certain concentrations.[47]

The bioactive components in plant extracts may vary depending on the solvent used in the extraction methods.[48] Polar solvents are often used to generate antimicrobial compounds from plants. For example, ethanol has been known to provide higher phenolic compounds from plant materials.[49] At low concentrations, phenolic compounds can diffuse into phospholipid bilayer membrane and interfere with bacterial enzymes involved in the adenosine triphosphate production, while at higher concentrations, they can bind to proteins and may cause protein denaturation in bacterial cells.[42] The rhizome of *temu kunci* plants contains many phenolic compounds[28] These mechanisms can damage the physiological functions of bacterial cell, leading to the death of the cell.

The incubation time also had an impact on the inhibition mechanism of the antibacterial substance. We used a range of incubation times to determine which had the greatest effect on enabling the rhizome *temu kunci* extract to inhibit the biofilm formation. The time was chosen to obtain the optimum antibacterial activity. During the lag phase, bacterial growth was very low, because the bacteria must adjust their metabolism to the new environmental condition after the addition of the extract before continuing the stationary phase.[50] The duration of the lag phase was influenced by the presence of extract because the extract acted as inhibitors for bacterial growth.[46] The more complex the compound, the longer the time it took to hydrolyzed. Some complex molecules with high molecular weight might require longer contact time to dissociate and interfere with bacterial membrane, affecting the death of the cells.[51] Further, these findings can be used as a basis to evaluate the cytotoxic activity of *temu kunci*.

CONCLUSION

Based on the research about the antibacterial effect from ethanol extract of rhizome of *temu kunci* (*B. rotunda*) against bacterium in a root canal, among four incubation periods and eight different concentrations, we can conclude that the most effective antibiofilm activity is provided by 3.10% extract with 1 h of incubation for *E. faecalis*, 50% extract with 6 h of incubation for both *T. denticola* and *F. nucleatum* and 50% extract with 3 h of incubation for the multispecies bacterial growth. However, further works are needed to determine the detailed mechanism by which *temu kunci* affects biofilm formation.

ACKNOWLEDGMENT

The authors thank the Faculty of Dentistry, Trisakti University, and MiCORE Laboratory, Trisakti University, for invaluable support in this study. The authors also thank Aradhea Monica Drestia, S.Si, for her laboratory assistance.

REFERENCES

1. Ingle JI, Bakland LK, Baumgartner JC. Ingle's Endodontics. Hamilton, Ont.: B C Decker; 2008. Available from: http://www.online.statref.com/document.aspx?FxId=93&DocID=1&grpalias=. [Last accessed on 2018 May 19].
2. Iqbal A. The factors responsible for endodontic treatment failure in the permanent dentitions of the patients reported to the college of dentistry, the university of Aljouf, Kingdom of Saudi Arabia. J Clin Diagn Res 2016;10:ZC146-8.
3. Dubey S, Saha SG, Rajkumar B, Dhole TK. Comparative antimicrobial efficacy of selected root canal irrigants on commonly isolated microorganisms in endodontic infection. Eur J Dent 2017;11:12-6.
4. Narayanan LL, Vaishnavi C. Endodontic microbiology. J Conserv Dent 2010;13:233-9.
5. Estrela C, Holland R, Estrela CR, Alencar AH, Sousa-Neto MD, Pécora JD, *et al.* Characterization of successful root canal treatment. Braz Dent J 2014;25:3-11.
6. Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, *et al.* PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol 2002;40:3223-31.
7. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
8. Dumani A, Yoldas O, Yilmaz S, Koksal F, Kayar B, Akcimen B, *et al.* Polymerase chain reaction of *Enterococcus faecalis* and *Candida albicans* in apical periodontitis from Turkish patients. J Clin Exp Dent 2012;4:e34-9.
9. Karale R, Thakore A, Shetty V. An evaluation of antibacterial efficacy of 3% sodium hypochlorite, high-frequency alternating current and 2% chlorhexidine on *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent 2011;14:2-5.
10. Bolstad AI, Jensen HB, Bakken V. Taxonomy, biology, and periodontal aspects of *Fusobacterium nucleatum*. Clin Microbiol Rev 1996;9:55-71.
11. Siqueira JF Jr. Rôças IN. The microbiota of acute apical abscesses. J Dent Res 2009;88:61-5.
12. Han YW. Fusobacterium nucleatum: A commensal-turned

pathogen. Curr Opin Microbiol 2015;23:141-7.
13. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001;9:34-9.
14. Carranza FA, Newman MG, Takei HH, Klokkevold PR. Carranza's Clinical Periodontology. St. Louis, Mo: Saunders Elsevier; 2006.
15. Dumitrescu AL. Etiology and PATHOGENESIS of Periodontal Disease. Heidelberg: Springer; 2010. Available from: https://www.trove.nla.gov.au/version/49809203. [Last accessed on 2018 May 19].
16. Nóbrega LM, Delboni MG, Martinho FC, Zaia AA, Ferraz CC, Gomes BP, *et al.* Treponema diversity in root canals with endodontic failure. Eur J Dent 2013;7:61-8.
17. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29-39.
18. Jhajharia K, Parolia A, Shetty KV, Mehta LK. Biofilm in endodontics: A review. J Int Soc Prev Community Dent 2015;5:1-2.
19. Jefferson KK, Cerca N. Bacterial-bacterial cell interactions in biofilms: Detection of polysaccharide intercellular adhesins by blotting and confocal microscopy. Methods Mol Biol 2006;341:119-26.
20. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15:167-93.
21. Lewis K. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001;45:999-1007.
22. Street RA, Prinsloo G. Commercially important medicinal plants of South Africa: A review. J Chem 2013;2013:1-6.
23. Widyarman AS, Widjaja SB, Idrus E. Strawberry extract's effects on *Enterococcus faecalis* and *Porphyromonas gingivalis* Biofilms *in vitro*. Sci Dent J 2017;1:1-5.
24. Widyarman AS, Suhalim OP, Nandary D, Theodorea CF. Pomegranate juice inhibits periodontal pathogens biofilm *in vitro*. Sci Dent J 2018;2:101-8.
25. Widyarman AS, Sumadi S, Agustin TP. Antibiofilm effect of *Clitoria ternatea* flower juice on *Porphyromonas gingivalis in vitro*. J Indones Dent Assoc 2018;1:7-12.
26. Ongwisespaiboon O, Jiraungkoorskul W. Fingerroot, *Boesenbergia rotunda* and its aphrodisiac activity. Pharmacogn Rev 2017;11:27-30.
27. Isa NM, Abdelwahab SI, Mohan S, Abdul AB, Sukari MA, Taha MM, *et al. In vitro* anti-inflammatory, cytotoxic and antioxidant activities of boesenbergin A, a chalcone isolated from *Boesenbergia rotunda* (L.) (fingerroot). Braz J Med Biol Res 2012;45:524-30.
28. Eng-Chong T, Yean-Kee L, Chin-Fei C, Choon-Han H, Sher-Ming W, Li-Ping CT, *et al. Boesenbergia rotunda*: From ethnomedicine to drug discovery. Evid Based Complement Alternat Med 2012;2012:473637.
29. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei darussalam. J King Saud Univ Sci 2015;27:224-32.
30. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. Int J Adv Res Chem Sci 2015;2:25-32.
31. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 2011;8:1-10.
32. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Int Pharm Sciencia 2011;1:98-106.
33. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004;15:348-81.
34. Siqueira JF Jr., Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:85-94.
35. Xie Y, Chen J, Xiao A, Liu L. Antibacterial activity of polyphenols: Structure-activity relationship and influence of hyperglycemic condition. Molecules 2017;22:E1913.
36. Souto AL, Tavares JF, da Silva MS, Diniz Mde F, de Athayde-Filho PF, Barbosa Filho JM, *et al.* Anti-inflammatory activity of alkaloids: An update from 2000 to 2010. Molecules 2011;16:8515-34.
37. Lucchini JJ, Corre J, Cremieux A. Antibacterial activity of phenolic compounds and aromatic alcohols. Res Microbiol 1990;141:499-510.
38. Ríos JL, Recio MC, Maáñez S, Giner RM. Natural triterpenoids as anti-inflammatory agents. In: Atta-ur-Rahman, editor. Studies in Natural Products Chemistry. Vol. 22. Bioactive Natural Products (Part C). New York: Elsevier; 2000. p. 93-143.
39. Salguero CP. A Thai Herbal: Traditional Recipes for Health and Harmony. Finland: Findhorn Press; 2013.
40. Chahyadi A, Hartati R, Wirasutisna KR, Elfahmi E. *Boesenbergia pandurata* Roxb., an Indonesian medicinal plant: Phytochemistry, biological activity, plant biotechnology. Procedia Chem 2014;13:13-37.
41. Cushnie TP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents 2011;38:99-107.
42. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel) 2013;6:1451-74.
43. Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control 2014;46:412-29.
44. Yanti, Rukayadi Y, Lee KH, Hwang JK. Activity of panduratin A isolated from *Kaempferia pandurata* Roxb. Against multi-species oral biofilms *in vitro*. J Oral Sci 2009;51:87-95.
45. Stewart PS. Antimicrobial tolerance in biofilms. Microbiol Spectr 2015;3:1-13.
46. Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, *et al.* Antimicrobial activity and resistance: Influencing factors. Front Pharmacol 2017;8:364.
47. Chouhan S, Sharma K, Guleria S. Antimicrobial activity of some essential oils-present status and future perspectives. Medicines (Basel) 2017;4:E58.
48. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel) 2017;6:E42.
49. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al.* Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J Food Drug Anal 2014;22:296-302.
50. Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron AD, *et al.* Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. J Bacteriol 2012;194:686-701.
51. Kenawy el-R, Worley SD, Broughton R. The chemistry and applications of antimicrobial polymers: A state-of-the-art review. Biomacromolecules 2007;8:1359-84.

Source of support: Nil; Conflict of interest: None Declared