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Research Article

Aloe Vera Extract Is More Effective in Inhibiting the Growth of Enterococcus faecalis Compared to Betel Leaf *(Piper betle L.)* Extract

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KEYWORDS

Root canal irrigation, Enterococcus faecalis, betel leaf, aloe vera

ABSTRACT

Introduction: Oral health is increasingly recognized as critical, as the oral cavity serves as a primary entry point for pathogens, potentially leading to oral diseases and systemic health complications.
Materials and Methods: This experimental study employed a post-test only control group design to evaluate the antibacterial efficacy of *Piper betle* L. (betel leaf) extract compared to *Aloe vera* extract against *Enterococcus faecalis*, a key contributor to root canal treatment failure due to its high resistance and persistence in harsh environments. The study aimed to confirm the potential of these extracts as alternatives for managing *E. faecalis* infections in root canal therapy. Extracts were prepared via maceration using 96% ethanol as solvent, and inhibition zones were measured at 100% concentration against *E. faecalis* cultured on Mueller-Hinton agar.
Results and Discussion: *E. faecalis* cultures were grown on Mueller-Hinton agar. Both extracts demonstrated inhibitory effects on bacterial growth, with betel leaf extract exhibiting greater efficacy than *Aloe vera* extract.
Conclusion: Betel leaf extract showed superior antibacterial activity against *E. faecalis* compared to *Aloe vera* extract, supporting its potential as an adjunct in root canal disinfection.

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INTRODUCTION

Oral health is essential for maintaining overall well-being, as the oral cavity serves as the primary entry point for nutrients from food and drink, providing the body with vital energy. A healthy oral environment supports optimal physiological function. Furthermore, the oral cavity acts as a gateway for pathogens, where microbial entry can compromise oral health and influence systemic health in other organs.[1]

Indonesian Basic Health Data in 2018 shows that most dental and oral problems are caries or toothache, which is around 45.3%, and most oral problems are gingival inflammation or abscess, around 14%. This data shows that oral diseases that occur must be resolved in a pandemic situation to prevent an Increase in the prevalence of dental and oral problems. According to the WHO (2012), maintaining dental and oral hygiene is one of the efforts to improve health because it can prevent various oral cavity diseases.[1] The main problem in the oral cavity is dental caries.[2] Various factors cause caries, one of the main factors is frequent consumption of *cariogenic foods and drinks* with very high sucrose content. Treatment actions that can be carried out on patients with cavities or caries, the aim for the first visit is to relieve pain, which can be done with a temporary medication which given on the *cavity.* Administration of medication can be done in a local way, or it can be done directly with *zinc oxide eugenol*. This drug is given especially for pain relief, and the benefit is to prevent caries. If the pain has gone, then treatment can be continued. [3] If the level of caries becomes wider and reaches the pulp tissue, it can potentially cause problems with the pulp. Teeth with pulp disease are one of the indications for *endodontic treatment*/root canal treatment (RCT).

RCT is a treatment for pulp disease by removing the vital pulp or *necrotic pulp.* The most common dental and oral problems are caries or toothache, which is around 45.3%, and most oral problems are gingival inflammation or *abscess,* which is around 14%.[1] *Enterococcus faecalis* is bacteria which can cause root canal treatment failure and can lead to persistent root canal infections. *Enterococcus faecalis* is a gram-positive bacterium with a coccus shape and is a facultative anaerobic bacterium.[4] *Enterococcus faecalis* is a bacterium known as the most resistant species found in root canals. This occurs due to the virulence factor of *Enterococcus faecalis.*[5] *Enterococcus faecalis* can adapt to the conditions of the root canal that has been treated. maintenance, as well as its own defense, which is strong against infection of root canals, when nutrients are very limited. *Enterococcus faecalis* can survive in environments with a pH of 11.5 and 6.7. It can be killed using sodium hypochlorite with high concentration, that is, 5.25 % until with 6 %. If *sodium hypochlorite is* used with very high concentrations, such as 5.25% to 6 % can increase the risk of *toxic effects.*[5]

Root canal treatment is performed to preserve the tooth so it can survive in the oral cavity, using the principle called the triad endodontic. There are three stages called triad endodontic. Stage one is with access opening, which is directly to the root canal. The second stage is root canal preparation, which aims to remove all irritant which include sterilization process. The next stage is obturating to prevent infection by microorganisms and treat inflammation on the periapex. Preparation that does not extend beyond the root canal provides a good prognosis. Generally, RCT is performed in several visits to ensure sterility of the root canal, but with advances in technology, RCT can be performed in a single visit.[7.] Root canal preparation is

one of the stages that must be carried out, where each needle change and irrigation are performed. Root canal irrigation is an important stage in supporting the success of root canal treatment because it facilitates the removal of *necrotic tissue,* microorganisms, and dentin fragments from the infected root canal. The action of rinsing this irrigation solution is one of the principles of triad endodontics. In addition, the irrigation solution removes and dissolves infected hard/soft tissue deposits in the periapical and periapical tissues. In addition to having an antibacterial effect, irrigation materials are toxic in nature and can cause pain if they enter the periapical tissue.[8] The ideal irrigation solution should have a broad antibacterial spectrum, be non-toxic, be able to dissolve necrotic pulp tissue residues, prevent the formation of a smear layer during root canal preparation, or be able to dissolve it immediately after cleaning. However, according to various studies conducted, no irrigation solution compound has ideal criteria.

On the other hand, research shows that using a combination solution rinse can increase the effectiveness of the rinse and support the success of the treatment. The popular irrigation solution for root canal is a sodium hypochlorite, a chelator/ethylene diamine tetra-acetic acid (EDTA) solution, a mixture of tetracycline, an acid, and a detergent (MTAD), chlorhexidine, and iodine potassium iodide (IPI). The advantages of sodium hypochlorite are the ability to dissolve vital and necrotic pulp tissue, flush debris out of the root canal, its broad-spectrum antimicrobial, sporicidal, virucidal, lubricating, economical, and readily available properties. However, sodium hypochlorite solution can irritate if pushed into the periapical tissue, it is unable to dissolve inorganic components, it causes white spots if it gets on the patient's clothing, and it has an unpleasant odor.[8]

In Indonesia, many plants are very well-known and frequently used by the majority of people as herbal medicines. Herbal medicines are plant-derived preparations processed or extracted into powders, pills, or liquids without the use of chemicals. As is known, herbal medicines can cure diseases with minimal side effects because they are made from natural ingredients. Unlike synthetic drugs, which can cause side effects either directly or after long-term use, herbal medicines are generally considered safer.[10] One plant that has potential as an alternative herbal medicine for root canal treatment is from the Piperaceae family, namely betel leaf (*Piper betle* L.). Betel leaf is a natural antiseptic, used as a gargle, a feminine cleanser, and a drug for eye inflammation. The benefits of betel that originate from a number of the active compounds it contains include alkaloids, flavonoids, polyphenols, tannins, and essential oils. Alkaloids are detoxifying and can neutralize toxins. Flavonoids and polyphenols are antioxidants, antidiabetics, anticancer, antiseptics, and anti-inflammatories. Tannins could bind and precipitate proteins and are antibacterial. The part of the betel leaf that contains antibacterial properties is phenol, and its derivative compounds can denature bacterial cell proteins. In addition to betel leaves, plants that have potential as alternative herbal ingredients for root canal medication include *Aloe vera* (*Aloe barbadensis Miller*), which belongs to the Liliaceae family. *Aloe vera* contains beneficial compounds, including tannins, amino acids, anthraquinones, enzymes, hormones, minerals, salicylic acid, sterols, sugars, and vitamins. The active substance contained in *Aloe vera* is the polysaccharide acemannan. Acemannan can be used as a therapy for tumors, diabetes, leukemia, metastases, sarcomas, melanomas, and other malignancies. *Aloe vera* also contains antibacterial substances, namely

anthraquinones, saponins, and tannins.[13] Based on the explanation above, researchers are interested in examining whether there is a difference in the effectiveness of betel leaf extract *(Piper betle* L.*)* compared to aloe vera extract (*Aloe* vera) against bacterial growth of *Enterococcus faecalis.*

MATERIALS AND METHODS

This study was a true experimental laboratory study using a post-test only control group design. The bacterial sample used was Enterococcus faecalis ATCC 29212 obtained from the stock culture of the Microbiology Laboratory, Faculty of Medicine, Airlangga University.

The study population consisted of Enterococcus faecalis bacteria, which were randomly allocated into four groups. Group P1 received 100% betel leaf extract, Group P2 received 100% aloe vera extract, Group K1 served as the positive control and was treated with chlorhexidine, and Group K2 served as negative control and was treated with distilled water. Observations were conducted to determine the antibacterial inhibitory effect in each group.

The aloe vera used in this study was obtained from Bali, and the extraction process was carried out in Karangasem Regency, Bali. Five kilograms of aloe vera were washed thoroughly and air-dried for seven days. The dried material was ground using a food processor until smooth and then macerated with 96% ethanol for 72 hours. The macerated solution was filtered three times using a Buchner funnel lined with filter paper and collected in an Erlenmeyer flask. The filtrate was evaporated using a vacuum rotary evaporator and further heated in a water bath at 40°C to obtain a thick extract. To prepare a 100% extract, 25 grams of the extract were dissolved in 25 mL of distilled water.

The betel leaves used in this study were also obtained from Bali. Two kilograms of betel leaves were washed thoroughly, dried at room temperature, and ground into simplicial. The powdered material was macerated with 96% ethanol for 72 hours. The filtrate was obtained through triple filtration using a Buchner funnel and evaporated using a vacuum rotary evaporator at 40°C to obtain a thick extract. A 100% extract was prepared by dissolving 25 grams of extract in 25 mL of distilled water.

The Enterococcus faecalis suspension was prepared by culturing bacteria obtained from the Microbiology Laboratory, Faculty of Medicine, Airlangga University. The antibacterial activity test was conducted using Mueller-Hinton Blood Agar media. The prepared media were sterilized in an autoclave at 121°C for 15 minutes. A loopful of pure bacterial culture was inoculated and incubated at 37°C for 24 hours. The bacterial suspension turbidity was adjusted to the McFarland 0.5 standard (1×10^8 CFU/mL).

The antibacterial inhibitory activity of aloe vera and betel leaf extracts was tested using the Kirby-Bauer diffusion method. The standardized bacterial suspension was spread evenly on the surface of Mueller-Hinton Blood Agar using a sterile cotton swab and allowed to stand for 5-15 minutes. The samples were divided into four groups: 100% aloe vera extract, 100% betel leaf extract, 0.2% chlorhexidine (positive control), and sterile distilled water (negative control). The plates were incubated at 37°C for 24 hours. The inhibition zone, defined as the clear area around the paper disc, was measured using a caliper.

Data analysis was performed using SPSS software. Normality was tested using the Shapiro-Wilk test because the sample size was less than 30. Homogeneity of variance was assessed using Levene's test. Since the data were not normally distributed, the Kruskal-Wallis test was used to determine significant differences among groups. If the Kruskal-

Wallis test showed p < 0.05, further analysis was conducted using the Mann-Whitney test to identify differences between treatment groups.

RESULTS AND DISCUSSIONS

The results of testing betel leaf extract with 100% concentration and aloe vera extract with 100% concentration in inhibiting the growth of *Enterococcus faecalis bacteria* on *Mueller-Hinton* Agar media show the results as in Table 1 below.

Table 1. Test results of betel leaf and aloe vera extracts

Repetition	K+	K-	100% Betel Leaf	100% Aloe Vera
I	20.60	-	15.80	13.55
II	20.20	-	15.60	13.75
III	20.40	-	15.80	13.60
IV	20.55	-	15.75	13.40
V	20.60	-	15.55	13.05
VI	20.55	-	15.60	13.20

Table 1 shows that the antibacterial inhibitory power of betel leaf extract with a concentration of 100% and aloe vera extract with a concentration of 100% is included in the strong category because it is in the range of 10-20mm, where in aloe vera extract with a concentration of 100% the average is 13.42 mm, betel leaf extract with a concentration of 100% the average is 15.68 mm, while in positive control B it is included in the strong category with an average value of 20.4 mm.

Based on the results of Tukey's further test, comparisons between groups showed significant differences in the bacterial growth inhibition zones. K (+) group compared with Betel Leaf has an average difference of 4,800 with p -value = 0.000, which indicates significant results. Group K (+) compared to aloe vera has a different average of 7.05833 with p -value = 0.000, which also shows significant results. The betel leaf group compared with K (+) had an average difference of -4,800 with a p-value of 0.000, indicating a significant difference between the two groups. The betel leaf group compared with the aloe vera average difference of 2.258 with p -value = 0.000, which also shows a significant difference. Group aloe vera compared to K(+) had a different average of -7.058 with p -value = 0.000, indicating a significant difference. Group aloe vera compared to betel leaf as an average difference of -2.258 with p value = 0.000, indicating a significant difference.

Table 2. Descriptive test

Group	N	Average	Standard Deviation	Minimum	Maximum
K (+)	6	20.48	0.157	20.20	20.60
Betel leaf	6	15.68	0.112	15.55	15.80
Aloe vera	6	13.42	0.262	13.05	13.75

The results of this study indicate that there are differences in the effectiveness of the growth inhibition zone of *Enterococcus faecalis bacteria* among the three treatment groups. The positive control group (K+), which used as the main comparison, had an average inhibition zone of 20.48 mm with a standard deviation of 0.157 mm, indicating the highest effectiveness in inhibiting growth bacteria. The minimum and maximum inhibition zones in this group ranged from 20.20 mm to 20.60 mm.

The group that used betel leaf as the test material showed a lower inhibition zone compared to the positive control, with a mean of 15.68 mm and a standard deviation of 0.112 mm. Although lower, betel leaf still demonstrated fairly good antibacterial properties. The inhibition zone produced in this group ranged from 15.55 mm to 15.80 mm. Meanwhile, the aloe vera group showed the lowest average inhibition zone among all groups, that is 13.42 mm with a standard deviation of 0.262 mm. The range of inhibition zones in this group ranged from 13.05 mm to 13.75 mm, indicating that the effectiveness of aloe vera which lowest in inhibiting the

growth of *Enterococcus faecalis* compared to the other groups. These results confirm that the positive control was the most effective treatment, followed by betel leaf and aloe vera.

Table 3. Normality test

Group	N	*p-value*
K+	6	0.054
Betel leaf	6	0.121
Aloe vera	6	0.849

Based on the results of the normality test, all data groups are normally distributed, because the p-value in each group is greater than 0.05. Control positive (K+) own p-value as big as 0.054, leaf betel of 0.121, and aloe vera as big as 0.849. It can be concluded that the distribution data on the third group fulfils the assumptions of normality, which is required for further statistical analysis.

Table 4. Levene's Test

F	df1	df2	*p-value*
2.448	2	15	120

Based on the results of the data homogeneity test in the table above, the *p-value* is 0.120, which is greater than the 0.05 significance level. This indicates that the three data groups have homogeneous *variance.*

Table 5. ANOVA Test

	Sum of Squares	df	Mean Square	F	p- value
Between Groups	155.920	2	77.960	2.205	0.000
Within Groups	0.530	15	0.035		
Total	156.451	17			

Based on Table 5, it is known that the *p-value* is 0.000, which is smaller than 0.05, so that H0 is rejected. This means there is a significant difference between zones, the growth of bacteria which is produced by group K (+), betel leaf, and aloe vera. Next, a Tukey follow-up test was conducted to determine the average difference between the two groups. The basis for decision-making in the Tukey follow-up test is as follows: If *p-value* < 0.05, then there is a significant difference. If *p-value* > 0.05, then there is a significant difference.

Table 6. Tukey's Test

Group	Comparison	Different Average	p-value
K (+)	Betel leaf	4.800	0.000
	Aloe Vera	7.058	0.000
Betel leaf	K (+)	-4.800	0.000
	Aloe Vera	2.258	0.000
Aloe Vera	K (+)	-7.058	0.000
	Betel leaf	-2.258	0.000

Based on the results of Tukey's further test, comparisons between groups showed significant differences in the bacterial growth inhibition zones. The explanation is as follows: The K (+) group compared with betel leaves has an average difference of 4.800 with a p-value = 0.000, which indicates a significant difference. Eight groups K (+) compared to aloe vera had a different average of 7.05833 with a p-value = 0.000, which also shows a significant difference. The betel leaf group compared with K (+) had an average difference of -4.800 with a p-value of 0.000, indicating a significant difference between the two groups. The betel leaf group compared with the aloe vera own average difference of 2.258 with a p-value of 0.000, which also shows a significant difference. Group aloe vera compared to K (+) showed a different average of -7.058 with p-value = 0.000, indicating a significant difference. Group aloe vera compared to betel leaf has an average difference of -2.258 with a p-value of 0.000, indicating a significant difference. All comparisons between groups showed significant differences in the bacterial growth inhibition zone.

The results of this study showed that there was an inhibition zone for bacterial growth. *Enterococcus faecalis* on extract betel leaf with concentration

100% and aloe vera extract with concentration 100% is quite significant due to the treatment of betel leaf extract and aloe vera extract, this is proven by the formation of an inhibition zone or clear zone on the agar medium where in the positive control group the inhibition zone produced was an average of 20.4 mm then in the treatment group with betel leaf extract with concentration 100% obtained average as big as 15.6 mm, and on the treatment group with aloe vera extract at a concentration of 100% obtained an average of 13.4 mm. Clear zone indicates existence growth of microorganism by antimicrobial agent on media surface, the diameter of the inhibition zone is categorized according to its antibacterial power based on the *Davis* and *Stout classifications* (21 mm or more means very strong inhibition), a clear zone with diameter 11-20 mm means strong inhibition, a clear zone with diameter 6-10 mm means moderate inhibition, a clear zone diameter 2-5 mm means weak inhibition.[14.] Based on the results of the inhibition zone measurements, extract betel leaf and extract aloe vera can hinder the growth of *Enterococcus faecalis* bacteria, but betel leaf extract can inhibit bacterial growth better than aloe vera extract because diameter zone resistor on extract leaf betel bigger but still both in the strong category.

This study shows the improvement of the diameter zone resistor in a strong category. Betel leaf has antibacterial potential due to its active compounds such as flavonoids, alkaloids, saponins, tannins, and triterpenoids. It can be proven that the existence of the compound is active with the phytochemical test identification. The results of the phytochemical identification test on betel leaf extract showed that tannin compounds were positive, while alkaloids, flavonoids, saponins, and triterpenoids were negative. The results of the phytochemical identification test on aloe vera extract showed that alkaloids, flavonoids, and triterpenoids were positive. saponins, triterpenoids, and tannins. Compound tannin and saponins were found positive, while for triterpenoid, alkaloid, and flavonoid compounds, it was found negative, compounds the own antibacterial properties. Compound Active *flavonoids* have an antibacterial mechanism by forming complex compounds with extracellular and dissolved proteins, so that they can damage bacterial cell membranes and are followed by the release of intracellular compounds.[15] *Saponin* works as an antimicrobial because the *saponin compound* can carry out an inhibitory mechanism by forming a complex compound with the cell membrane through *hydrogen bonds*, so that it can destroy the permeability of the bacterial cell wall and cause bacterial cell death[16]. The active compound *tannin* has antibacterial activity, which is related to with his abilities for activate *adhesive* microbial cells. It also inactivates enzymes and disrupts protein transport in the inner layer of cells17. The antibacterial mechanism *of triterpenoids* is that they react with *porins* (trans membrane proteins) on the outer membrane of the bacterial cell wall and form bonds. A *polymer* that is strong enough to cause damage to the *porin.* The damage to *porin* is the entry and exit point for nutrients, so inhibitory compounds will reduce the permeability of the bacterial cell wall. This permeability of the bacterial cell wall will interfere with the entry and exit of nutrients and other compounds, so that bacterial growth is inhibited or the bacterial cell will die. *Alkaloids* work as antibacterials by disrupting the peptidoglycan components in bacterial cells, so that the cell wall layer does not form completely, causing bacterial cell death.[18.] Secondary metabolite compounds, namely tannins, which are tested against Gram-positive and Gram-negative bacteria, show that tannin more effective in inhibiting the growth of Gram-positive bacteria compared to

Gram-negative. Bacteria *Enteroccocus faecalis* is bacteria grams positive that. This means it can be inhibited by the tannins contained in betel leaf extract and aloe vera extract.

The data obtained from the test results are normally distributed and homogeneous data so that parametric tests are then carried out using the One-Way ANOVA test. The results of the SPSS test using the One-Way ANOVA test show that the *p-value* = 0.000 ($p<0.05$). This indicates that there is a significant difference between the growth inhibition zones of *Enterococcus faecalis* bacteria in the groups using positive controls, betel leaf extract, and aloe vera extract. Furthermore, a Tukey further test was carried out to determine the average difference in the two groups, with the results of all groups showing significant differences in the growth inhibition zones of *Enterococcus faecalis bacteria.*

CONCLUSION

Based on the literature review, research results, and discussion, this research can be concluded that betel leaf extract (*Piper betle* L.*)* with 100% concentration and aloe vera extract with 100% concentration can hinder the growth of bacteria *Enterococcus faecalis.* Betel leaf extract (*Piper betle* L.*) is* more effectives hinder growth of bacteria *Enterococcus faecalis* compared with aloe vera extract.

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