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Antibacterial Potential Test of Ethanol Extract of Basic Leaf (Ocimum Basilicum) against Enterococcus Faecalis ATCC 29212

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ABSTRACT

Introduction: *Enterococcus faecalis* is a bacteria found in failed root canal treatment cases. *Enterococcus faecalis* can tolerate significant environmental changes and invade the dentinal tubules, so under certain conditions, irrigants cannot altogether remove them in the chemomechanical process. Basil (*Ocimum basilicum*) is a natural ingredient with many benefits and is known to have high antibacterial activity against Gram-positive and Gram-negative bacteria. This study aimed to determine the antibacterial effect of ethanol extract from basil leaves (*Ocimum basilicum*) against *Enterococcus faecalis* ATCC 29212.

Methods: This research was conducted by testing the bacteria *Enterococcus faecalis* ATCC 29212 with ethanol extract of basil (*Ocimum basilicum*) leaves made from 256,000 ppm – 1,000 ppm, using the *disc diffusion method*, carried out with three repetitions. Precise zone measurements were carried out using a caliper.

Results: There were no clear zones produced by the ethanol extract of basil leaves (*Ocimum basilicum*) against *Enterococcus faecalis* ATCC 29212 at various test concentrations.

Discussion: the test results in this study can be influenced by (i) the solubility factor of the extract, (ii) the active compounds contained in the plant, and (iii) the method used in this study.

Conclusion: The ethanol extract of basil leaves (*Ocimum basilicum*) has no antibacterial potential against *Enterococcus faecalis* ATCC 29212. Further research can be carried out using the agar diffusion method or by conducting a minimum inhibitory concentration (MIC) test to confirm the antibacterial effect.

KEYWORDS: *Enterococcus faecalis* ATCC 29212, *Ocimum basilicum,* antibacterial activity, ethanol extract of basil leaves.

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I. INTRODUCTION

Root canal infection is a polymicrobial disease consisting of anaerobic bacteria and facultative anaerobic bacteria, which are usually found together in cases after endodontic treatment.[1] *Enterococcus faecalis* is a bacteria found in 38% of teeth with failed root canal treatment cases. *Enterococcus faecalis* was found in 71% of teeth with apical periodontitis cases.[2,3] *Enterococcus faecalis* can tolerate significant environmental changes, survive in root canals without a nutrient supply, and reproduce through contact with human serum.[2,3]

The fundamental goal of root canal treatment is to eliminate bacteria and prevent recurrent infections. In the root canal cleaning process, there is a reduction in the number of bacteria. Still, this stage can only partially remove the bacteria because the root anatomy is very complex. Thus, medicaments or irrigants enhance bacterial elimination before root canal filling.[1] several intracanal medicaments are often used in dentistry, including Calcium Hydroxide [Ca(OH) 2], Chlorhexidine (CHX), and Sodium Hypochlorite (NaOCl).

Chlorhexidine is the bisbiguanide class of intracanal medicament with a broad spectrum of antibacterial effects.[1,4] Chlorhexidine is known to be more effective in inhibiting gram-positive bacteria compared to gram-negative bacteria.[5,6] Chlorhexidine 2% is often used as an irrigant in endodontic treatment, but chlorhexidine is known to be unable to remove organic matter and necrotic tissue in root canals.[7] Chlorhexidine 2% has limitations in eliminating *Enterococcus faecalis* due to weak penetration into the dentinal tubules. Chlorhexidine 2% is also known to be ineffective in removing biofilms.[8] Several studies suggest that chlorhexidine at a concentration of 2% is known to have a toxic effect on tissues.[8,9]

Sodium hypochlorite is a root canal irrigation material often used in dentistry. Sodium hypochlorite can remove organic substances.[10] Sodium hypochlorite is usually used concomitantly with chlorhexidine in root canal treatment. Still, previous studies have shown that the interaction of the two materials can cause oxidation reactions which can cause tooth discoloration.[1,8] Sodium hypochlorite is also known to have an unpleasant chlorine odor.[1,8] Case reports indicate that sodium hypochlorite in contact with mucosal tissue can cause acute inflammation, ulceration, tissue necrosis, and swelling. [11,12]

Based on the explanation above, it is known that the intracanal medicaments used today are quite effective in eliminating *Enterococcus faecalis* in root canals but still have some drawbacks, such as uncomfortable taste and smell and potentially corrosive. A cytotoxic effect on periradicular tissue,[2] so researchers wanted to see the potential of other materials that could be effectively used as irrigants in endodontic treatment. Other materials that can be selected one of which is natural materials. A natural ingredient that is relatively easy to find in Indonesia, and the author wants to know about its efficacy against *Enterococcus faecalis,* is basil (*Ocimum basilicum*).

Basil (*Ocimum basilicum*) is a natural ingredient with many benefits, but it is most commonly used for culinary needs. Basil essential oil is also widely used in the cosmetic industry.[13] Traditionally, *Ocimum basilicum* is used to treat various diseases such as hypertension, migraines, stomach cramps, acne, gum ulceration, and mouth sores.[13,14] Previous studies have shown that *Ocimum basilicum* has an antibacterial effect on gram-positive and gram-negative bacteria.[15] *Ocimum basilicum* in the form of essential oil contains *chavicol methyl ether (estragole), linalool,* and *eugenol,* which are known to have high antibacterial activity.[16,17]

Based on the results of the author's search through *databases for the* past 10 years, it is known that information or research regarding the effects of basil leaf extract (*Ocimum basilicum) on Enterococcus faecalis* bacteria is still minimal, so the authors are interested in conducting this research so that it can be used as an alternative to root canal medicaments.

II. METHOD

A. Types of Research

This study was conducted to test the antibacterial potential of ethanol extract from basil leaves (*Ocimum basilicum*) against *Enterococcus faecalis* ATCC 29212. This research was conducted by providing treatment or intervention to *Enterococcus faecalis* ATCC 29212 bacteria with various test concentrations of ethanol extract of basil leaves (*Ocimum basilicum*) to observe the effect of its antibacterial. The test was carried out in triplicate and using 0.1% chlorhexidine and DMSO solvent as the control group. This study met the criteria for experimental research based on Kumar, 2011, in the book *Research Methodology 3rd Edition*. In the book of *Research Methodology 3rd Edition*.[46]

B. Research Sample

The samples used in this study were basil (*Ocimum basilicum*) leaves obtained from Lembang, which were determined at the Herbarium of the School of Life Sciences and Technology, Bandung Institute of Technology and *Enterococcus faecalis ATCC 29212* bacteria obtained from the Eyckman Microbiology Laboratory, Faculty of Medicine, Padjadjaran University.

C. Research Variable

As for the identification of variables:

1. Independent variable: concentration of basil (*Ocimum basilicum*) leaf extract;
2. Dependent variable: diameter of the inhibition zone of *Enterococcus faecalis ATCC 29212.*

D. Operational Definition

The antibacterial potency test was carried out using the *disc diffusion method* or the *Kirby Bauer test,* which is a method for testing the antibacterial power of a material against certain bacteria by calculating the diameter of the inhibition produced on the agar media, which is indicated by the presence of a clear zone. The drag diameter was measured using a vernier caliper in millimeters (mm).

According to David and Stout, if the diameter of the inhibition zone produced is 20 mm or more, then the antibacterial compound is said to be very strong. The diameter of the inhibition zone of 10-20 mm means the antibacterial blend is potent, and the diameter of 5-10 mm means the antibacterial compound is moderate. The diameter of the inhibition zone is less than 5 mm antibacterial compound is said to be weak. [47]

The ethanol extract of basil leaves is an extract of a viscous dark green liquid with an intense aroma, produced through a maceration technique using ethanol as a solvent to extract the active components of basil leaves. The resulting extract was prepared at 256,000 ppm, 128,000 ppm, 64,000 ppm, 32,000 ppm, 16,000 ppm, 8,000 ppm, 4,000 ppm, 2,000 ppm, 1,000 ppm in units of ppm or µg/ml.[48]

Enterococcus faecalis bacteria ATCC 29212 is a Gram-positive, oval-shaped bacterium with a diameter of 0.5 – 1 µm, nonmotile and facultative anaerobic and is a bacterium commonly found in failed root canal treatments. *Enterococcus faecalis* ATCC 29212 came with a turbidity standard of 0.5 McFarland (1.5 x CFU/ml). [25]

E. Research Tools and Materials

This study used tools, including ovens, *stainless mixing spoons*, sterile glass bottles, analytical scales, *vacuum rotary evaporator*, ultrasonic, spatulas, Petri dishes, *cotton swabs*, oese, *paper* discs, *candle jars*, Bunsen, lighter, *beaker glass*, *parafilm*, tweezers, *microtube*, test tube, test tube rack, 0.1% chlorhexidine, autoclave, incubator, micropipette and tip, caliper.

The materials used are basil (*Ocimum basilicum*) leaves, *Enterococcus faecalis* ATCC 29212, 70% ethanol, methanol, DMSO, Mueller Hilton Broth powder, and Bacteriology Agar. Basil leaves (Ocimum *basilicum*) were extracted from the Lembang area using 96% ethanol by maceration technique. The test microorganism in this study was *Enterococcus faecalis* ATCC 29212, obtained from the Eyckman Microbiology Laboratory, Faculty of Medicine, Padjadjaran University.

Figure I – 1 Research Tools and Materials

Figure II – 2 Laminar Air Flow and Maserator

Figure I – 3 Vacum Rotaru Evaporator and Ultrasonic

F. Research Procedure

This research was conducted by referring to methods used in previous studies and the *Clinical and Laboratory Standards Institute*.

1. Identification and determination of plants

   Identification and determination of a plant are made to find out the truth of the identity of a plant. This aims to avoid errors in the collection of samples to be studied.

2. Preparation of Ethanol Extract from Basil Leaves (*Ocimum basilicum*)

   Basil leaves (*Ocimum basilicum*) from Lembang were washed, cut into small pieces, and dried at room temperature for several days. 9 500 grams of dried leaves were then extracted by maceration method by soaking the dried leaves in 10 L of 96% ethanol as a solvent for 5 x 24 hours and stirring periodically. After 5 days, the maserate was filtered using filter paper. The solvent in the extract was evaporated using *a rotary evaporator* with a temperature of 50. The

extract was stored in sterile glass vials at a temperature of −20°C.[48]

3. Preparation of agar media and bacterial culture media

Mueller Hinton Broth (MHB) powder was weighed as much as 2.1 grams and added with 2% Agar Bacteriology powder as much as 2 grams dissolved in 100 ml of distilled water. The media was sterilized by autoclaving for 2 hours at 121 °C, and the agar was printed on sterile glass petri dishes.

Mueller Hinton Broth (MHB) powder weighed as much as 2.1 grams and dissolved in 100 ml of distilled water. The media was sterilized in an autoclave at 121 °C for 2 hours. Media can be stored in the refrigerator if not used immediately.

4. Test bacterial culture and preparation of inoculation suspension

Rejuvenation of the test bacteria was carried out by inoculating Mueller Hilton agar sterile by streaking with *oese*, then incubated for 48 hours at 37°C in a *candle jar* and stored in an incubator.

Furthermore, the test bacteria that had grown on the surface of the MHA agar were taken using *an oese*, then cultured in a test tube containing 50 ml of Mueller Hilton Broth to make a liquid bacterial culture. The liquid bacterial culture was then incubated at 37°C for 48 hours anaerobically, up to standard turbidity of 0.5 McFarland (1.5 x 10^8 CFU/ml).[6,47,49]

5. Antibacterial potency test with *agar disc-diffusion test*

First, prepare the extract by weighing 51.2 mg of the extract and dissolving it with 20 µl DMSO 100% and 180 µl distilled water to form a concentration of 256,000 ppm as the initial stock. After that, do serial dilutions until 1000 ppm is obtained.

Then, the inoculation suspension that has been made will then be spread on 20 mL of Muller Hilton Agar (MHA) surface using sterile *cotton swabs.* Place 7 sheets of sterile paper discs with a diameter of 6 mm on the surface of the agar. Leave sufficient space between paper discs. Ensure that the disc paper surface is in perfect contact with the agar media. After that, 20 µl of the extract was dripped with a micropipette on 5 paper discs, 1 other paper disc was dripped with 10% DMSO as a negative control, and 1 other paper was dripped with Minosep chlorhexidine 0.1% as a positive control, then incubated at 37° C for 48 hours. [6,47,49]

The bacterial inhibition zone around the disc paper was measured using a caliper and recorded after incubation in millimeters. The shortest distance from the outer edge of the paper disc to the initiation point of bacterial growth or the clear zone formed was considered the inhibition zone. All experimental procedures were carried out under aseptic conditions and repeated 3 times using three separate culture plates with the same inoculum. [49]

G. Data Analysis

In the inhibition measurement, data will be obtained from basil leaf extract inhibition diameter (*Ocimum basilicum) against Enterococcus faecalis* bacteria. The diameter of inhibition was obtained by measuring the clear zone formed on the agar—3 measurement data obtained from each extract concentration. The data will then be processed using Microsoft Excel 2011, and the *mean will be obtained*.

III. RESULTS

A. Results of Ethanol Extract of Basil Leaves

The ethanol extract of basil leaves obtained has a dark green color, thick consistency, and intense aroma. The extract got weights 89.0635 grams.

Figure 0-1Ethanol extract of basil leaves

B. Bacterial Culture Results

Enterococcus faecalis ATCC 29212 is the raw research material grown on Mueller Hilton Agar (MHA). Colonies formed were round, smooth, and white with a creamy consistency.

Figure 0-2Colonies of E. faecalis on MHA

C. Results of Making Bacterial Suspension

Bacterial suspensions are prepared by mixing liquid bacterial cultures with sterilized Mueller Hilton Broth. The liquid bacterial culture incubated for 48 hours was observed for its absorbance using a *spectrophotometer*. After seeing the absorbance, dilution was carried out by adding Mueller Hilton Broth until the absorbance was obtained according to the standard 0.5 McFarland turbidity.

Figure 0-3 Results of bacterial suspension

D. Results of Antibacterial Activity Test of Ethanol Extract of Basil Leaves Against E. faecalis ATCC 29212

In this study, the antibacterial test activity of the ethanol extract of basil leaves was carried out using the *disk diffusion test method*. On agar that has been swabbed with E. faecalis suspension with standard turbidity of 0.5 McFarland, 7 paper discs are placed, with 5 paper discs dripped with ethanol extract of basil leaves and 2 other paper discs dripped with the positive control (CHX 0.1 %) and negative control (DMSO 10%), each of 20 µl. Then, incubation was carried out at 37°C for 48 hours anaerobically. This study was carried out with three repetitions using the same bacterial suspension.

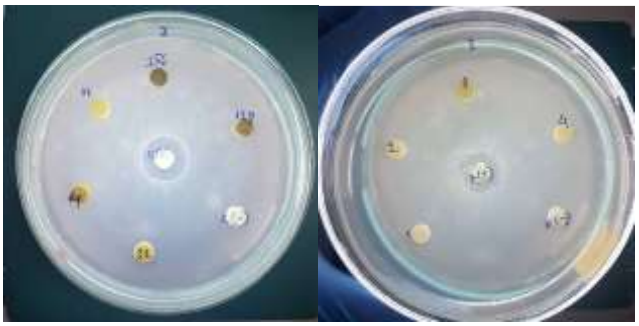

Figure 0-4 Test results of antibacterial activity of the ethanol extract of basil leaves against Enterococcus faecalis ATCC 29212 first repetition; second repetition; third repetition

Table 0-1 Test results of antibacterial activity of ethanol extract of basil leaves against E.faecalis ATCC 29212

Concentrati	Repetition 1	Repetition 2	Repetition 3	Average
256,000	-	-	-	-
ppm	-	-	-	-
128,000	-	-	-	-
ppm	-	-	-	-
64,000	-	-	-	-
ppm	-	-	-	-
32,000	-	-	-	-
ppm	-	-	-	-
16,000	-	-	-	-
ppm	14.45	13,9	14.5	14,28
8,000 ppm	13,9	14,1	14,2	14.06
4,000 ppm	-	-	-	-
2,000 ppm	-	-	-	-
1,000 ppm				
CHX 0.1%				
DMSO 10%				

- : no inhibition zone was measured

The antibacterial activity of the ethanol extract of basil leaves was seen by measuring the clear zone formed on the agar plate. In the three repetitions, no clear zones were seen resulting from the ethanol extract of basil leaves against *Enterococcus faecalis* ATCC 29212.

IV. DISCUSSION

This research on antibacterial potential was carried out using the *disc diffusion method* to determine whether the ethanol extract of basil leaves has antibacterial potential against *Enterococcus faecalis* ATCC 29212 bacteria. Based on the antibacterial activity test results, the ethanol extract of

basil leaves did not appear to have any effect on *Enterococcus faecalis* ATCC bacteria. 29212. The results of this study can be seen in Figure (1V-4) and Table (IV-1). The test concentration of 256,000 ppm – 1,000 ppm did not produce an inhibition zone against *Enterococcus faecalis* ATCC 29212. The antibacterial activity test was initiated by diluting the extract by dissolving 51.2 mg of the extract with 10% DMSO in a *microtube.* A vortex mixer and ultrasonic waves assisted this dissolved extract for 5 minutes. The extracts to be tested were found to be completely insoluble, as evidenced by the presence of extracts attached to the *microtube*. This led the authors to assume that the active compound content of the extract tested on the *E. faecalis* ATCC 29212 bacteria was not maximally dynamic because the solubility level of the extract was not good.

Several journals suggest that the content of active compounds in plants will vary depending on climate, geographical conditions, temperature, soil conditions, age of leaves taken, humidity, and treatment of plants. [50,51] Hussain et al. in 2008 tested basil *essential oil* grown in various seasons. [14] The results of his research *are that essential oils* derived from leaves planted in summer have lower antibacterial power than plants grown in winter or rainy seasons. This difference in results is likely due to the high enough temperature to cause evaporation of some of the expected contents. [14] In this study, the samples of basil leaves used were taken in March. This supports the researchers' assumption that the absence of the effect of ethanol extract from basil leaves on *Enterococcus faecalis* ATCC 29212 could be due to the active compound content contained in the basil leaf samples, which were taken poorly due to the ongoing dry season.

In addition, Kaya et al., in 2008, conducted research on the antibacterial effect of basil leaf extract on 10 types of bacteria, one of which was *Enterococcus faecalis* ATCC 29212, and 4 types of fungi with various solvents. [52] The antibacterial testing method was carried out using the *disc diffusion method*. The solvents used in this research were chloroform, acetone, and methanol, prepared in two concentrations. The study results showed that the extract with chloroform, acetone, and methanol had no effect on *Enterococcus faecalis* ATCC 29212 but is known to be effective on several other bacteria. Kaya et al. concluded that the differences in active compounds in plants are caused by several factors such as soil structure, daily and seasonal changes that occur during the collection of plant material, the physiological growth period of the plant, the part of the plant studied, the extraction process, the solvent and the type of bacteria used.

Judging from the method used, the *disc diffusion* method is the most frequently used method to see the antibacterial potential of a material. This method is often used because the costs incurred in testing are cheaper, and the way to work is easier than the *agar method diffusion.* [53,54] However, what is often criticized from this method is the ability of the tested extract to diffuse onto the agar. The extract that diffuses into the agar and away from the *disc* can create a concentration gradient that follows the color of the extract, so this gradient that appears can be considered a potential antibacterial. All antibacterial test methods, which use poorly soluble extracts in water, cannot reflect antibacterial activity. [53] Thus, it is recommended to test the minimum inhibitory concentration in more detail to determine the antibacterial activity.

V. CONCLUSION

Based on the study's results, it can be concluded that the ethanol extract of basil leaves has no antibacterial effect against *Enterococcus faecalis* ATCC 29212

VI. SUGGESTION

Based on the results of this study, suggestions that the author can give are:

1. Conduct a phytochemical test on the extract to determine the active compounds contained in the extract in detail.
2. Carrying out further research using different methods, namely the agar diffusion method and conducting a minimum inhibitory concentration test.
3. Conducting research using fractionated extracts on Enterococcus faecalis ATCC 29212 bacteria.

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