Antibacterial Potential Test of Basil Leaf (*Ocimum Basilicum*) Against *Enterococcus Faecalis* ATCC 29212

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ABSTRACT

Introduction: Bacteria and their virulence factors are the main causes of apical periodontitis after endodontic treatment. The bacteria that is often found and considered as pathogens in root canals after endodontic treatment is *Enterococcus faecalis*. The main goal of endodontic treatment is the elimination of microorganisms and preventing re-infection after root canal treatment which can be achieved through mechanical-chemical debridement using an irrigation solution. Basil leaves (*Ocimum basilicum*) which is an herbal plant are thought to have antibacterial compounds that can inhibit *Enterococcus faecalis*. This research was conducted to determine the antibacterial potential of the n-hexane extract of basil leaves against *Enterococcus faecalis* ATCC 29212.

Methods: This experimental laboratory study tested the effectiveness of n-hexane extract of basil leaves (*Ocimum basilicum*) against *Enterococcus faecalis* ATCC 29212 using *disk diffusion* method with three times of repetition.

Results: The results of this study indicated that the n-hexane extract of basil leaves did not have an inhibition zone against *Enterococcus faecalis* ATCC 29212.

Discussion: The concentration of n-hexane extract of basil leaves was not directly proportional to the diameter of the resulting inhibition zone. This is due to several factors that can affect the test results.

Conclusion: The n-hexane extract of basil leaves does not have antibacterial potential effect against *Enterococcus faecalis* ATCC 29212.

KEYWORDS: Antibacterial potential test, basil leaf n-hexane extract, *Enterococcus faecalis* ATCC 29212.

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I. INTRODUCTION

Bacteria and its virulence factors are a major cause of apical periodontitis following endodontic treatment. The bacteria that is often found in root canals after endodontic treatment is *Enterococcus faecalis* (*E. faecalis*).¹ *E. faecalis* is the normal commensalism of the oral cavity, digestive tract and vagina.² These bacteria were found in 38% of cases of failed root canal treatments and 71% of cases of apical periodontitis.^{3,4} Several studies suggest that *E. faecalis* has a pathogenic role in the failure of endodontic treatments.²⁻⁵ *E. faecalis* can survive unfavorable conditions such as an environment with a pH of 9.6 (alkaline), without nutrients and can reproduce when it

comes into contact with human serum.^{3,6} *E. faecalis* is resistant to ultraviolet light, heat, sodium hypochlorite, calcium hydroxide, hydrogen peroxide, and ethanol.⁷

The main goals of endodontic treatment are to eliminate microorganisms and prevent re-infection after root canal treatment. These goal can be achieved by mechanical-chemical debridement with irrigation solutions.⁸ Chlorhexidine (CHX) is an irrigation solution which has a broad spectrum antibacterial effect, effective against gram-positive and gram-negative bacteria, one of which is *Enterococcus faecalis*.⁹ Chlorhexidine 1% showed better effectiveness against *E. faecalis* compared to 1% calcium

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hydroxide with a contact time of less than 30 seconds and 20 minutes.¹⁰ This material cannot dissolve tissue, so it should be preceded by sodium hypochlorite, however sodium hypochlorite has an unpleasant aroma and irritant to periapical tissue.¹¹ The use of these two materials has a negative effect due to their interaction.⁹

Products with natural ingredients are considered useful and attractive as substitutes for pharmaceuticals, because of their low side effects and low cost.¹² Interest in using natural ingredients in treating diseases has begun to develop and it is hoped that they can be biocompatible and reduce the side effects of synthetic antimicrobials.¹³ Research on the antibacterial activity of basil essential oil (*Ocimum sanctum*) against *E. faecalis*, obtained results in the form of a minimum inhibitory concentration of 31.25 ppm of basil essential oil.¹⁴ Other species of *Ocimum*, the type *basilicum*, also been used extensively for fragrances, tooth paste and mouthwashes.¹⁵

Basil leaves (*Ocimum basilicum*) in the form of essential oils and extracts are thought to have antibacterial compounds that are effective against gram-positive and negative bacteria.¹⁶ One of the gram-positive bacteria that can be inhibited by basil is *E. faecalis*.¹⁷ *Ocimum basilicum* has a strong inhibitory effect against *Staphylococcus*, *Enterococcus*, and *Pseudomonas*. The chemical elements of basil leaves include *linolene*, *linalool*, *eugenol*, *methylchavicol*, and *methylcinnamate*. *Linalool*, which is a *monoterpenoid*, is thought to have a role in antibacterial activity. *Ocimum basilicum* still not well investigated regarding its antibacterial activity so there is little information about the antibacterial effectiveness of basil leaves against microorganisms.¹⁷

Based on the description above, the authors are interested in conducting research on the antibacterial potential test of n-hexane extract of basil leaves against *E. faecalis*, especially in the field of dentistry in Indonesia. Basil leaves (*Ocimum basilicum*) are expected to be developed as a component of root canal irrigation.

II. METHOD

A. Description of this research

Type of this research was an in vitro experimental laboratory study. The population in this study were all basil leaves grown in the province of West Java. The sample used *basilicum* that was grown by farmers in Lembang, West Bandung Regency. Determination is carried out to ensure the taxonomy of basil leaves carried out at the Herbarium of the School of Life Sciences and Technology, Institute of Technology Bandung.

B. Materials and Tools

Tools used include a *macerator*, *beaker glass*, *rotary evaporator*, autoclave, analytical balance, *vortex*, *erlenmeyer tube*, *laminar air flow*, *oese* round incubator, *candle jar*, spirit lamp, refrigerator, petri dish, test tube, *micropipette*, *spectrophotometer*. The materials used were basil (*Ocimum*

basilicum), *E. Faecalis* ATCC 29212, n-Hexan, filter cloth, aquades, *Mueller Hinton Broth* 21 gr / L(MHB) powder, powder *bacteriological agar* 2%, *dimethyl sulfoxide* (DMSO), *minosep®* / *chlorhexidine* 0.1%, *cotton swab* sterile, *paper disk*, *mmicropipette tip*, *parafilm wax*, *cling wrap*.

C. Preparation of n-hexane extract of Basil Leaves (*Ocimum basilicum*)

Basil leaves (*Ocimum basilicum*) are washed and dried by drying in a greenhouse for 24 hours. Basil leaves that have been dried weigh 109 grams. The dried leaves are put into a *macerator* that has been given a cotton pad and filter cloth, then soaked in 3-4 L of n-hexane and let stand for 24 hours. The macerate was removed from the *macerator outlet* to obtain a dilute extract and pulp. The new N-hexane is added to the dregs in the macerator, then it is left to stand for 24 hours, then the second mass is removed again from the *outlet*. This procedure is repeated until the solvent that comes out is clear. Repetitions are carried out for 5-6 times of immersion. The dilute or macerate extract obtained from the *macerator* is concentrated using a *rotary evaporator* at a temperature of 50 until no more solvent drips from the *rotary evaporator outlet*. The concentrated extract is stored in a sterile glass bottle.

D. Media

Mueller Hinton Broth (MHB) media was made by dissolving 2.1 grams of media powder in 100 ml of distilled water in tube *Erlenmeyer*. The media is sterilized in an autoclave at a temperature of 121 for 2 hours, then stored in a refrigerator until needed.

Mueller Hinton agar (MHA) procedure is almost the same as making MHB liquid media, only 2% powder is added for *bacteriological agar* to get a hard and clear enough agar. The procedure for making the above media is adjusted to the instructions from the manufacturer regarding the product information.

E. Rejuvenation and Bacterial Culture

Rejuvenation of the tested bacteria was carried out by inoculating the bacteria on the MHA using *streak plate* method. Bacterial colonies were taken from agar preparations with *oese*, then scratched on the agar surface. The petri dishes that already contain bacteria are covered using *parafilm wax* and *cling wrap*.

Bacterial culture was carried out by inoculating the bacteria on the MHB using *oese*. Bacterial colonies were taken from agar preparations, then *oese* was immersed in MHB. Petri dishes and test tubes that have been inoculated are then incubated for 18-24 hours with a temperature of 37 ° C and atmosphere *anaerobic* in the *candle jar*. The rejuvenation procedure and bacterial culture were carried out in a sterile state in *laminar air flow*.

F. Preparation of Suspended Early Bacterial Inoculation

Procedure refers to the standards issued by the *Clinical and Laboratory Standards Institute* (CLSI). The initial inoculum of the bacteria used was adjusted to the

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number of colonies in the high-risk colony count conditions. The initial inoculum colony count for *E. faecalis* was 0.5 McFarland (1.5x CFU/mL).^{18,19} Incubated bacterial suspensions and sterile MHB were inserted into a separate cuvette of 500 to measure turbidity. The turbidity of the suspension and sterile MHB were measured using a *spectrophotometer* with a wavelength of 600nm. Bacterial turbidity is adjusted through dilution so that an absorbance value of 0.08-0.1 is obtained or equivalent to 0.5 McFarland (1.5x CFU/ mL). Adjustment for bacterial turbidity was carried out using the following formula:

$$m1.v1 = m2.v2$$

The description for those formula are: m1 is the difference in absorbance of bacteria from liquid culture and sterile MHB, v1 is volume of bacteria from liquid culture needed to adjust the absorbance of 0.1, m2 is absorbance value equivalent to 0.5 McFarland (absorbance value = 0, 1) and v2 is volume of the bacterial suspension preparation.

G. Preparation of n-hexane extract *Basilicum* (*Ocimum basilicum* Concentrated)

Extract and 10% DMSO are dissolved in *microtube* then homogenized with *vortex* until dissolved. DMSO 10% solvent is prepared beforehand by mixing 20 DMSO 100% into 180 distilled water. A concentration of 100 extracts of 512,000 ppm was obtained by mixing 51.2 mg of concentrated basil extract with 100% DMSO. Extract preparations of 256,000 ppm, 128,000 ppm, 64,000 ppm to 1000 ppm were obtained by performing serial dilution dilutions on 100 *microtubes* that had been filled with 10% DMSO. 100 500,000 ppm concentration of extract preparations were taken, then put into the *microtube* second and homogenized to obtain the extract. with a concentration of 256,000 ppm. 100 extracts of 256,000 ppm concentration were taken, then put into the *microtube* third and homogenized to obtain an extract with a concentration of 128,000 ppm. This procedure was carried out until the final result was 1000 ppm concentration in the *microtube* tenth, then 100 of the *microtube* was tenth discarded.

H. Antibacterial Potential Test of n-hexane

Extract of basil leaves test for the antibacterial potential of n-hexane extract of basil leaves was carried out using *agar disk diffusion* method. Dip the dry sterile bacterial suspension until wet, then wipe the *cotton swab* to the surface of the MHA plate and also wipe the edges of the agar with three repetitions while rotating the plate 60° each time, so that the distribution of bacteria is evenly distributed.

The n-hexane extract of basil leaves, 0.1% chlorhexidine (K +) and 10% DMSO (K-) is piped onto the surface of each *paper disk* as much as 20, then the *paper disk* is placed onto the surface of the MHA plate using sterile tweezers while pressed to fix the *paper disk* to keep it on place during the incubation period. One MHA plate consists of 7 *paper disks*, namely 5 *paper* basil leaf extract disks, 1 *paper disk* 1% chlorhexidine and 1 *paper disk* 10% DMSO. The test MHA plate was closed and then glued using *parafilm wax* and

cling wrap, then put into a *candle jar* and stored in an incubator at 37 for 16-18 hours.

III. RESULTS

A. Results of Basil Leaf Extract (*Ocimum basilicum*)

n-Hexane Basil leaf maceration was carried out by soaking the dried leaves using n-hexane organic solvent. The maceration process produces an extract in the form of a very thick liquid that is dark green in color and has a strong basil aroma.

Figure 1. Extract of n-hexane basil (*Ocimum basilicum*)

B. Bacteria culture

Enterococcus faecalis ATCC 29212 grown on the MHA plate has round, smooth, white colonies with consistency *creamy*. The results of the water culture look cloudy, this indicates the presence of bacterial growth on sterile MHB that has been implanted with bacterial colonies.

Figure 2 Culture on MHA (A), liquid culture of *Enterococcus faecalis* ATCC 29212 (B), sterile MHB (C)

C. Potential Test Results Antibacterial extract of n-hexane leaves of basil (*Ocimum basilicum*) against *Enterococcus faecalis* ATCC 29212

Inhibition zone of n-hexane extract of basil leaves (*Ocimum basilicum*) was tested using *agar disk diffusion* method. 5 pieces of *paper disk* were dropped with 20 series of diluted extracts, one dropped by solvent control (DMSO 10%), the other was dropped by positive control (Chlorhexidine 0.1 %). These procedure is performed by triple repetition.

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Table I. ANTIBACTERIAL POTENCY TEST OF n-HEXANE EXTRACT OF BASIL LEAVES AGAINST *ENTEROCOCCUS faecalis* ATCC 29212

Basil leaf extract concentration (ppm)	Diameter of Inhibition Zone (mm)			Average
	Repetition			
	I	II	III	
512,000	-	-	-	-
256,000	-	-	-	-
128,000	-	-	-	-
64,000	-	-	-	-
32,000	-	-	-	-
16,000	-	-	-	-
8,000	-	-	-	-
4,000	-	-	-	-
2,000	-	-	-	-
1,000	-	-	-	-
K +	6.7	4.5	5.7	5.63
	4 ,3	5,2	5,4	4,97
K-	-	-	-	-
	-	-	-	-

IV. DISCUSSION

Research with the *disk diffusion* method was carried out with the aim of knowing the antibacterial potential of the n-hexane extract of basil (*Ocimum basilicum*) against *Enterococcus Faecalis* ATCC 29212. The results showed that there was no inhibition zone in the form of a clear area around the *paper disk* dripped with basil leaf extract. This shows that the extract does not have antibacterial potential against *E. faecalis* ATCC 29212. The concentration of n-hexane extract of basil leaves is not directly proportional to the diameter of the resulting inhibition zone. This can be caused by several factors such as the composition of the leaf extract, the solubility of the extract and the method used.

Factors that can affect the composition of the extract include the harvest season and the phenological stage of the plant which includes vegetation, early blooming, full bloom and seeding, and drying techniques can provide an evaporation effect on temperature-labile components such as *linalool*, *methyl chavicol*, and *methyl cinnamate*.^{17,20,21} The basil leaves used by researchers to extract were obtained from

farmers in the Lembang area in March 2019, in that month the dry season is taking place, besides the drying process is carried out by drying basil leaves in a greenhouse and under the light sun. This drying process can cause the evaporation of some temperature-labile compounds from basil leaves such as *linalool* and *eugenol* which are antibacterial agents.^{17,21} This could explain that the researcher did not have complete quality control regarding these factors as long as the basil leaves were planted, grown, harvested and reached the researchers' hands in dry form.

The difficulty in dissolving the extract is one of the factors that can reduce the possibility of inhibition of the extract. Concentrated extract that was dissolved using DMSO 10% produced a suspension of the extract that was not completely dissolved. DMSO 10% solvent did not produce clear zones on the *paper disk* as a negative control. This indicates that DMSO 10% does not have an antibacterial effect against *E. faecalis* ATCC 29212, so that the inhibition zone results are more accurate because there is no antibacterial effect from DMSO solvent. This insoluble extract makes it possible for the active compound to be difficult to carry into the extract preparation which will be dropped on a *paper disk* during the inhibition test of the basil leaf extract.

This study uses the method *disk diffusion*. The method of *disk diffusion* has the advantages of a simple technique, relatively cheap price, minimal use of test substances, and can be done by researchers who are beginners. The use of the method *disk diffusion* also has its drawbacks, depends on the ability of the extract to diffuse through the agar and each extract component that diffuses away from the disc will create a concentration gradient, as well as the antibacterial compounds present in the extract. All antibacterial test methods that use water-based solvents for the dilution of the test substance, either by diffusion of agar or with *broth*, have the possibility of not producing true antibacterial activity when used in extracts that have limited solubility in water-based media such as extracts containing essential oils.²²

Basil leaves are known for their large amount of essential oil content. This indicates that the absence of inhibition zone of the n-hexane extract of basil leaves can be caused by the lack of the method used. In addition to the concentration in preparations diluted extract has a concentration inaccurate because of incomplete solubility, concentration on gradient *paper disk* can also affect the test results that appear because of the narrower concentration formed on the outer periphery of *paper disk*.

One of the same studies as this study was the study of the antibacterial activity of basil (*Ocimum basilicum*) extract with various extract solvents against 10 bacteria, one of which was *E. faecalis* ATCC 29212, and the method was used *disc diffusion*. The results of this study did not result in the inhibition zone of Basil leaf extract against *E. faecalis* ATCC 29212. The results of *in vitro studies* showed that basil

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leaf extract was not effective against *E. faecalis* ATCC 29212.¹⁵ This is in line with the results of research conducted by researchers, so there is a possibility that basil leaves were used by researchers to produce an extract that does not contain active compounds that can provide antibacterial effects against *E. faecalis* ATCC 29212.

V. CONCLUSION

Based on the results obtained, it can be concluded that n-hexane extract of basil leaves (*Ocimum basilicum*) does not have antibacterial potential against *E. faecalis* ATCC 29212.

VI. SUGGESTION

Further research can be carried out by:

- 1) Doing antibacterial potency test with other methods such as *well diffusion* to compare which method is the most optimal for similar research in future studies.
- 2) Performing phytochemical screening to determine the active compounds contained in the n-hexane extract of basil leaves (*Ocimum basilicum*).
- 3) Fractionation of the extract to separate compounds based on polarity in order to obtain an extract with a more concentrated compound than the n-hexane extract of basil (*Ocimum basilicum*).

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