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Antibacterial activity of moringa leaf extract (*Moringa oleifera*, Lamk) against the growth of *Enterococcus faecalis* bacteria

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Abstract
Dental and oral health problems in Indonesia are very diverse, one of which is a health problem due to damage to hard dental tissue caused by bacteria. *Enterococcus faecalis* bacteria is a bacteria that often causes problems, especially in teeth that have had root canal treatment. Root canal treatment consists of several stages, one of which is irrigation. Root canal irrigation is an important step to eliminate bacteria. Chlorhexidine 2% is recommended as a root canal irrigation solution, because it has broad and long-lasting antimicrobial effects due to its ability to adhere to the root canal walls., but cannot be used as a root canal irrigant and has several drawbacks. The use of materials derived from plants can be an alternative for irrigation materials which are part of root canal treatment, one of which is Moringa leaves (*Moringa oleifera*). This study aims to determine the effectiveness of Moringa leaf extract as an antibacterial against *E. faecalis* bacteria. The method used is a laboratory experimental designposttest only control group. Extraction of Moringa leaves using 70% ethanol solvent. Bacterial growth can be inhibited with 2.5%, 5%, and 10% Moringa leaf extract. Moringa leaf extract concentration of 10% has antibacterial power close to that of 2% chlorhexidine against *Enterococcus faecalis* bacteria.

Keywords: *Enterococcus faecalis*, antibacteri, moringa leaf extract

Introduction

Damage to the hard tooth tissue by bacteria which results in infection of the pulp tissue and gain access to the root canal causing pulpal necrosis (Kartinawanti & Khoiruza Asy'ari, 2021) [1]. Pulp necrosis is an indication for root canal treatment to prevent disease extension to the periapical tissues [2]. There is a biomechanical preparation and sterilization step in root canal treatment that requires irrigating agents to remove microorganisms, necrotic tissue, and dentine debris. (Kartinawanti & Khoiruza Asy'ari, 2021) [1].

Chlorhexidine 2% irrigant is recommended because of its broad and long-lasting antimicrobial effect and its ability to adhere to the root canal walls. Chlorhexidine 2% has the disadvantage that it cannot be used as a single irrigation solution in root canal treatment because it does not have the ability to dissolve necrotic tissue and is less effective against gram-negative bacteria [2].

Bacteria as microorganisms that predominate in cases of root canal infection consist of various types, one of which is *Enterococcus faecalis* [3]. *Enterococcus faecalis* includes gram- positive bacteria which are facultative anaerobes which have the ability to live and reproduce with or without oxygen and are the most frequently isolated bacteria in the root canal system with an amount of 45.8%. Persistent root canal infection can be caused by *Enterococcus faecalis* [4]. These bacteria are able to penetrate the dentinal tubules, and form biofilms in anaerobic and nutrient deficient conditions, and are able to survive in acids and bases [5].

Alternative root canal irrigation materials can be developed through natural materials, one of which is the Moringa plant. Moringa plants are plants that are often found because of their ability to grow easily. Moringa leaves are part of the Moringa plant which has many benefits, because it contains flavonoids, saponins, tannins, alkaloids [6]. Therefore, Moringa leaves are believed to have antibacterial properties that can reduce or inhibit the growth of *Enterococcus faecalis* bacteria [7].

Materials and Methods

This research method is Experimental laboratory posttest only control group [4]. Sample groups were used, namely the positive control group (K+) with 2% Chlorhexidine irrigation, and the treatment group, namely Moringa leaf extract concentration of 2.5%, 5% concentration, and 10% concentration. Samples of Moringa leaf plants were taken by random sampling with the inclusion criteria for leaves that were green, not wilted, not dry, and without stems. The independent variables in this study were Moringa leaf extract concentrations of 2.5%, 5% and 10%. The dependent variable is the inhibition zone of Moringa leaf extract on the growth of *E. faecalis* bacteria Preparation of Moringa Leaf Extract (*Moringa oleifera*).

Moringa leaves without stalks as much as 1 kg, washed with running water, drained and then dried for 2-4 days at room temperature. Then grind it in a blender and then sift it and produce moringa leaf powder. As much as 100 grams of dried moringa leaf powder was taken and macerated with 500 ml of 70% ethanol. Stirring is done every 6 hours to speed up the extraction process of the compounds. Every 24 hours the solution was replaced using a new 70% ethanol. The solution resulting from soaking Moringa leaves is put together in a container that has been filtered with a Buchner filter. The maserate was then concentrated to evaporate 70% ethanol using a Rotary Vacuum Evaporator at a temperature of 30-40oC with a pressure of 75 mmHg to obtain a thick extract of Moringa leaves with a concentration of 100% and then diluted using 70% ethanol to a concentration of 2.5%, 5%.

Resistance Test

Culture and Preparation of *Enterococcus faecalis* Bacterial Suspension One ose of *E. faecalis* isolate was added to a test tube containing 5 mL of MHB liquid medium to make a suspension of *E. faecalis*. The test tube was closed using cotton and put into a desiccator and incubated at 37oC for 24 hours. After 24 hours, *E. faecalis* in a test tube was vibrated using a vortex and standardized with Mc Farland 0.5 (1.5 x 108 CFU/ml).

Testing of moringa leaf extract

Testing of Moringa leaf extract against bacteria *Enterococcus faecalis* [6] times with disc diffusion method (Kirby-Baurer). The test bacteria were inoculated onto the MHA agar surface by streaking motion. The petridish was closed and left at room temperature for 3-5 minutes until the surface of the media dried. Each disc was dripped with 10 µl using a micropipette. The MHA medium containing *E. faecalis* bacteria was divided into 4 parts and given instructions for placing disks containing Moringa leaf extract concentrations of 2.5%, 5% and 10% and 2% chlorhexidine as positive controls. the next step the media was incubated at 37oC for 18-24 hours and observed the growth of bacteria from the clear zone that formed on each disc. The clear zone is measured using a caliper to measure the strength of the drag force.

Data analysis

Data analysis was carried out in the SPSS program with a degree of meaningα= 0.05. normality test with the Shapiro Wilk test and homogeneity test using the Levene test. nonparametric statistical tests using the Kruskal Walis test to determine whether there is a significant difference in the Mann-Whitney test to determine specifically which groups have significant differences using the test.

Results

Moringa leaves that have been dried and mashed to produce a fine powder weighing 217 grams. Then 100 grams were taken and extracted using 70% ethanol. The extraction process produces a thick dark brown extract of Moringa leaves as much as 10 ml with a concentration of 100%. Then it was diluted using distilled water to produce Moringa leaf extract with a concentration of 2.5%, 5% and 10%.

Fig 1: Diluted Extraction of Moringa Leaves

This research was carried out 6 repetitions to obtain valid data on the antibacterial activity of Moringa leaf extract. The difference in the average diameter of the antibacterial inhibition zone of Moringa leaf extract against *Enterococcus faecalis* can be seen clearly using a bar chart. The following are the results of the inhibition zones produced after 24 hours of incubation.

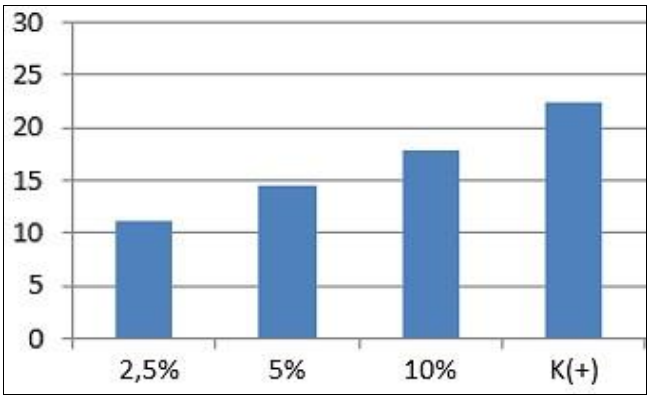

Fig 2: Bar Diagram of Inhibition Zone of Moringa Leaf Extract Against *E. faecalis* Bacteria

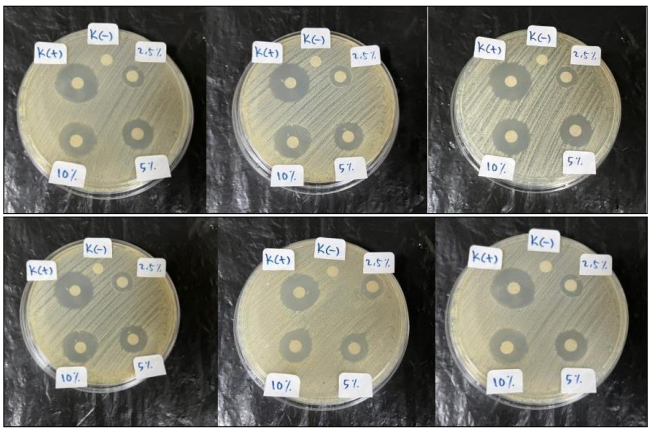

Fig 3: Antibacterial Activity Test of Moringa Leaf Extract Against *E. faecalis* Bacteria

Based on the measurement of the diameter of the clear zone which describes the inhibition of Moringa leaf extract, the following results were obtained.

Table 1: The results of measuring the diameter of the clear zone were carried out

No	K (+)	10%	5%	2.5%
1	21.60	17.80	14.40	11.20
2	23,40	18.60	15,20	11.40
3	21,20	17.40	14,20	11.15
4	23,20	18.35	14.75	11.05
5	21.95	17.75	14,15	11.15
6	22.95	17.60	14.05	11.00

Based on the results of the normality test by Shapiro Wilk on 24 samples, the data (α<0.05) were normally distributed. Then, a homogeneity test was carried out using the Levene test (α<0.05) and the result was that the data was not homogeneous. Subsequent nonparametric data were tested with the Kruskal-Wallis test resulting in a significant difference in the results of measuring the diameter of the inhibition zone for *Enterococcus faecalis* bacteria between the positive control group (chlorhexidine 2%) and the

treatment group of Moringa leaf extract concentrations of 2.5% 5% and 10%. The follow-up test, namely the Mann-Whitney test, showed that all study groups produced results ($\alpha<0.05$) that were significantly different to both the 2% chlorhexidine control group and the Moringa leaf extract treatment group.

Table 2: Mann-Whitney Test Results

Group	2.5%	5%	10%	KP
2.5%		0.004*	0.004*	0.004*
5%			0.004*	0.004*
10% KP				0.004*

Discussion

Plants that can be used both as food and medicine are moringa plants (*Moringa oleifera* L.). Moringa leaf extract has active compounds of alkaloids, flavonoids, saponins, tannins (Pratama Putra *et al*., 2017) [6]. Moringa leaf extract used in this study has a concentration of 2.5%, 5% and 10%. Moringa leaf extract concentration of 2.5% has the widest zone yield with a value of 11.40 mm. Moringa leaf extract concentration of 10% showed the widest zone with a value of 18.60 mm. based on the data obtained, the higher the concentration of the extract the wider the diameter of the antibacterial inhibition zone against *E. faecalis* is produced.

Based on the classification of antibacterial power in Widiani (2020) [10] the antibacterial zone >10 mm is categorized as strong antibacterial, Moringa leaf extract concentration of 2.5% with the smallest value of 11.00 mm, while the highest value is 11.40 mm. The diameter of the antibacterial inhibition produced by Moringa leaf extract with a concentration of 5% had the lowest yield of 14.05 mm and the highest yield was 15.20 mm. Moringa leaf extract concentration of 10% resulted in the diameter of the antibacterial inhibition zone with the lowest value being 17.40 mm while the highest value was 18.60 mm. This shows different results from research (Rante *et al*, 2017) [7]. Moringa leaf extract 20% showed an antibacterial inhibition zone with a value of 9.15 mm. Differences in the habitat of the moringa plants used can affect the active compound content of the resulting extract. (Widowati *et al*., 2018; Rante *et al*., 2017) [7, 8]. The maturity level of Moringa leaves to be used also has an influence on the amount of active ingredients it has. Mature Moringa leaves contain optimal active compounds and leaf collection can be done in the morning or evening with the characteristics of the petiole angle of 45-90o, dark green color and no yellow parts. (Tri Akbar *et al*., 2019; Widiani & Pinatih, 2020) [9, 10].

Positive control with 2% chlorhexidine is an irrigant that has an effective antibacterial ability against *Enterococcus faecalis*. Chlorhexidine has a substantive property, namely the ability to release antibacterial effects continuously and gradually, so that it can increase the antibacterial effect so as to produce an antibacterial effect in the long term. Moringa leaf extract concentration of 2.5% consists of several active compounds such as alkaloids, flavonoids, saponins, tannins which have not been purified. Moringa leaf extract concentration of 10% has higher results in producing antibacterial inhibition zones compared to Moringa leaf extract concentrations of 5% and 2.5%. The ability of Moringa leaf extract at a concentration of 10% has antibacterial activity that approaches 2% chlorhexidine (Kuntari *et al*., 2014; Primary Putra *et al*., 2017) [11].

The number of compounds in the ethanol extract of Moringa leaves in Kusmiati., *et al* (2022) showed that total flavonoids had the highest amount, followed by taniin, saponin, and alkaloid compounds. The antibacterial mechanism of flavonoids occurs through the formation of complex flavonoid compounds with bacterial extracellular proteins which then dissolve and damage the bacterial cell membrane followed by the release of intracellular compounds. Antibacterial activity carried out by Moringa leaf extract includes penetrating and disrupting metabolic activity in bacteria resulting in protein precipitation in bacterial cells, inhibition of bacterial nucleic acid and protein synthesis, modification of bacterial cell membrane permeability, damage to cell membranes and cell walls, inhibition of bacterial metabolism. (Kusmiyati *et al*.,2022; Yan *et al*., 2021; Lusi *et al*., 2016) [12, 13, 14].

Moringa leaves contain better antioxidant activity in dark green leaves compared to light green Moringa leaves. The process of photosynthesis in Moringa leaves as an active plant converts CO2 and H2O compounds into C6H12O6 and O2 and energy (ATP) with the help of sunlight. This energy is used by plants to carry out the process of forming plant nutrient elements. Sufficient nitrogen content contained in leaves can make leaves more green and become part of the chlorophyll molecule which controls the ability of plants to carry out photosynthesis. Leaves that have low nitrogen levels tend to have a pale yellow to dark green leaf color (Rahmawati and Parfati, 2020; Rai Saputri and Indah Permatasari, 2019) [15, 16].

Conclusion

Moringa leaf extract concentrations of 2.5%, 5% and 10% have antibacterial properties against *Enterococcus faecalis* bacteria. Moringa leaf extract concentration of 10% has antibacterial activity ability close to 2% chlorhexidine.

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