



Comparison of the Antibacterial Effectiveness of Moringa Leaf Extract (*Moringa oleifera*) on the Growth of *Enterococcus faecalis* and *Streptococcus mutans* Bacteria in Vitro

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Abstract: *Streptococcus mutans* and *Enterococcus faecalis* are bacterial pathogens of dental caries and root canal treatment failure. The growth of bacteria that cause dental and oral disease can be inhibited by using antibacterials derived from plants that have antibacterial properties. The aim of the research was to compare the antibacterial effectiveness of *Moringa oleifera* leaf extract against the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria in vitro. This type of research is a laboratory experiment with a post test only control group design. The research samples were pure cultures of *Streptococcus mutans* and *Enterococcus faecalis*. There were seven treatment groups, namely *Moringa* leaf extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, positive control chlorhexidine and negative control DMSO with four repetitions each. Testing the antibacterial effectiveness of *Moringa* leaf extract used the diffusion and dilution method, then the data was analyzed using one way ANOVA and post hoc LSD statistical tests. Based on the research results, it was stated that there was effective inhibitory and killing power of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria. From the research results, it can be concluded that *Moringa oleifera* leaf extract is more effective as an antibacterial against *Streptococcus mutans* compared to *Enterococcus faecalis*.

Keywords: Antibacterial; Effectiveness; *Enterococcus faecalis*; *Streptococcus mutans*.

Introduction

Dental and oral health is an integral part of overall body health. This dental and oral disease will limit an individual's capacity for activities and psychosocial activities (Bakhitah et al., 2021; Rumate et al. (2023). The Global Burden of Disease Study 2017 states that dental caries is a disease experienced by almost half of the world's population. Globally, it is estimated that 2.3 billion adults and more than 530 million children suffer from caries (Rumate et al., 2023). In Indonesia, the results of Basic Health Research in 2018, the largest proportion of dental and oral problems with a fairly high degree of severity is dental caries at 57.6% (Adam dan Ratucia, 2022).

Caries is a hard tissue disease of the teeth (Listrianah, 2018). Some experts believe that bacteria are the cause of dental caries. The large number of *S. mutans* bacterial colonies indicates a very close causal relationship with caries (Kusiak et al., 2021; Wulandari, et al., 2022). Its acidogenic properties can ferment carbohydrates and produce acids. As a result, salivary pH decreases, and the growth of cariogenic bacteria increases. Biofilms and white spots appear as the beginning of the caries process (Al-Shami et al., 2018; Babaeekhou et al., 2020; Wulandari et al., 2022).

Caries is chronic and takes a long time to develop (Boy dan Khairullah, 2019; Giacaman 2022). If caries is left untreated, there is a risk of pulp and periapical disease which must be treated with root canal treatment

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(Bakhitah, et al. 2021; Rumate et al., 2023). The main reason for failure of this treatment is often associated with the presence of *Enterococcus faecalis* (Hafizha et al., 2018; Blancas et al., 2021).

Root canal treatment requires irrigation materials, including chlorhexidine gluconate 0.2% which is the gold standard because it is bactericidal and bacteriostatic against various types of bacteria (Alibasyah et al., 2018). Its use over a long period of time can have side effects such as changes in taste sensation and xerostomia, so other alternative ingredients are needed to overcome these problems (Putranto, 2019; Puteri et al., 2022).

The use of antibacterial plant extracts has been widely studied to overcome the shortage of chemical ingredients, one of which is *Moringa oleifera* leaves (Tarigan, et al., 2022). *Moringa oleifera* is the most commonly used moringa leaf species. This plant contains various phytochemical substances such as alkaloids, tannins, flavonoids, saponins and triterpenoids (Natsir et al., 2023).

Research by Ervianingsih et al., (2019) showed that *Moringa oleifera* leaf extract 8%, 4% and 2% could inhibit *S.epidermidis* bacteria. Tarigan et al. (2019) found that 20% *Moringa* leaf extract gel was the most effective concentration against *P. acnes*. Riswana, et al. (2022) stated that *Moringa oleifera* leaf extract 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56% has antibacterial activity against *S.aureus*, *S.epidermidis*, and *P.acnes*. Savitri et al. (2018) shows that the ethanol extract of *Moringa* leaves has inhibitory power against *S.aureus*.

Based on this background, the author is interested in researching the comparative antibacterial effectiveness of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria in vitro.

Method

The type of research is laboratory experiment with post-test only control group design. The samples are pure cultures of *E. faecalis* and *S. mutans* bacteria. This research consisted of seven groups. The concentrations of *Moringa oleifera* leaf extract were 50%, 25%, 12.5%, 6.25%, 3.125%, 2.5% chlorhexidine (positive control), and DMSO (negative control). The results of the sample size calculation were using the Federer formula ($t =$ number of groups $r =$ repetition) seven groups used in the final research and 4 replications were obtained for each group.

Antibacterial testing using two methods, namely diffusion using disk disks and the average diameter of the inhibitory zone of *moringa oleifera* leaf extract against *Enterococcus faecalis* bacteria and dilution of the average number of colonies of *Moringa oleifera* leaf extract against

Enterococcus faecalis to obtain KHM and KBM (is the lowest concentration that can inhibit bacteria, identified with *E.Faecalis* and *S.Mutans* which can still grow on the results of scratches on the cup then KBM is the lowest concentration that can kill bacteria) Next, all data were analyzed using the one-way ANOVA statistical test.

Result and Discussion

Result

Effectiveness of Inhibitory Power of *Moringa oleifera* Leaf Extract on the Growth of *Enterococcus faecalis* Bacteria. The results of testing the effectiveness of the inhibitory power of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* bacteria using the oneway ANOVA statistical test are as follow.

Table 1. Effectiveness of Inhibitory Power of *Moringa oleifera* Leaf Extract on the Growth of *Enterococcus faecalis* Bacteria

Concentration	Mean±SD	p
Mo leaf leaf extract 3.125%	8.13±1.024	
Mo leaf leaf extract 6.25%	8.60±0.161	
Mo leaf leaf extract 12.5%	8.88±1.001	
Mo leaf leaf extract 25%	9.68±0.826	0.0001*
Mo leaf leaf extract 50%	9.95±0.592	
K (+)	12.18±0.403	
K (-)	0	

One way ANOVA test results * Significant

The results of the research in Table 1 above state that there is a significant difference in the mean inhibitory diameter with a value of $p=0.0001$ ($p \leq 0.05$), which means that there is effective inhibition of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* bacteria.

Effectiveness of Killing Power of *Moringa* Leaf Extract on the Growth of *Enterococcus faecalis* Bacteria

Testing the effectiveness of the killing power of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* bacteria using the oneway ANOVA statistical test as follows.

Table 2. Effectiveness of Killing Power of *Moringa oleifera* Leaf Extract on the Growth of *Enterococcus faecalis* Bacteria

Concentration	Mean±SD	p
Mo leaf leaf extract 3.125%	950.50±13.964	
Mo leaf leaf extract 6.25%	865.00±10.863	
Mo leaf leaf extract 12.5%	735.00±19.916	
Mo leaf leaf extract 25%	630.25±12.685	0.0001*
Mo leaf leaf extract 50%	518.25±2.986	
K (+)	372.00±3.162	
K (-)	1746.00±10.863	

One way ANOVA test results * Significant

The research results in Table 2 above state that there is a significant difference in the mean number of colonies with a value of $p=0.0001$ ($p\leq 0.05$), which means that there is effectiveness in killing *Moringa oleifera* leaf extract against the growth of *Enterococcus faecalis* bacteria.

Effectiveness of Inhibitory Power of Moringa oleifera Leaf Extract on the Growth of Streptococcus mutans Bacteria

Testing the effectiveness of the inhibitory power of *Moringa oleifera* leaf extract on the growth of *Streptococcus mutans* bacteria using the oneway ANOVA statistical test as Table 3.

Table 3. Effectiveness of Inhibitory Power of *Moringa oleifera* Leaf Extract on the Growth of *Streptococcus mutans* Bacteria

Concentration	Mean±SD	p
Mo leaf leaf extract 3.125%	7.05±0.129	0.0001*
Mo leaf leaf extract 6.25%	7.55±0.370	
Mo leaf leaf extract 12.5%	8.34±0.340	
Mo leaf leaf extract 25%	8.85±0.436	
Mo leaf leaf extract 50%	9.48±0.330	
K (+)	12.18±0.403	
K (-)	0	

One way ANOVA test results * Significant

The results of the research in Table 3 above state that there is a significant difference in the mean inhibitory diameter with a value of $p=0.0001$ ($p\leq 0.05$) which means that there is effective inhibition of *Moringa oleifera* leaf extract on the growth of *Enterococcus faecalis* bacteria.

Effectiveness of Killing Power of Moringa oleifera Leaf Extract on the Growth of Streptococcus mutans Bacteria

Testing the effectiveness of the killing power of *Moringa oleifera* leaf extract on the growth of *Streptococcus mutans* bacteria using the oneway ANOVA statistical test as Table 4.

Table 4. Effectiveness of Killing Power of *Moringa oleifera* Leaf Extract on the Growth of *Streptococcus mutans* Bacteria

Concentration	Mean±SD	p
Mo leaf extract 3.125%	533.25±19.568	0.0001*
Mo leaf extract 6.25%	480.25±5.377	
Mo leaf extract 12.5%	311.50±8.266	
Mo leaf extract 25%	283.75±3.50	
Mo leaf extract 50%	165.50±9.609	
K (+)	258.75±6.994	
K (-)	1721.75±30.203	

Hasil uji one way ANOVA * Signifikan

The research results in Table 4 above state that there is a significant difference in the mean number of colonies

with a value of $p=0.0001$ ($p\leq 0.05$) which means that there is effectiveness in killing *Moringa oleifera* leaf extract against the growth of *Streptococcus mutans* bacteria.

Discussion

This research has a general objective, namely comparing the antibacterial effectiveness of *Moringa oleifera* leaf extract against the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria. These two bacteria cause diseases in the oral cavity. *E.faecalis* has been associated with root canal infections, while *S.mutans* plays a role in the etiology of dental caries (Blancas et al., 2021).

According to Sari and Febriawan (2021), the antibacterial testing methods commonly used are dilution and diffusion. This study used these two antibacterial methods to determine the diameter of the inhibition zone, number of colonies, minimum inhibitory content (MIC) and minimum kill content (KBM) of *Moringa* leaf extract on the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria.

Based on the research results, it can be stated that *Moringa* leaf extract has significant antibacterial effectiveness against the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria with $p\leq 0.05$. The results of this study support research by Tunas et al (2019) that there is antibacterial activity from the ethanol extract of *Moringa oleifera* leaf against *Staphylococcus aureus* bacteria.

The research results of Munira et al (2021) state that *Moringa oleifera* leaf extract has the effect of inhibiting the growth of *S.aureus* and *E.coli*. Research by Savitri et al (2019) states that *Moringa oleifera* leaf extract has antibacterial properties against the growth of *S. aureus* bacteria. Likewise, research by Ginarina et al (2020) shows that there is an antibacterial ability of *Moringa oleifera* leaf extract against the growth of *S.aureus* bacteria.

Moringa oleifera leaves are the plant most widely used by the public because of their antioxidant, antihyperuricemia, analgesic, anticancer, antihyperglycemia, antiinflammatory, antihyperlipidemia, and antibacterial activities (Israqi et al., 2020; Tarigan, et al., 2022). The antibacterial effectiveness of *Moringa* leaf extract in this study can be caused by the active compounds contained in it, namely alkaloids, flavonoids, saponins, triterpenoids, tannins and steroids (Savitri et al., 2019; Israqi et al., 2020). *Moringa* leaves also contain several micronutrient elements such as calcium, beta carotene, thiamin, riboflavin, niacin, iron, phosphorus, magnesium, zinc, vitamin C (Israqi et al., 2020).

Alkaloids have an antibacterial mechanism by disrupting the peptidoglycan components in bacterial

cells, so that the cell wall layer does not form completely and cell death occurs (Munira et al., 2021). Furthermore, flavonoids consist of three types of antibacterial mechanisms of action, namely inhibiting energy metabolism, nucleic acid synthesis, and cell membrane function (Ginarana et al., 2020).

According to Isyraqi et al (2020), the mechanism of saponin as an antibacterial is by reducing the surface tension of bacterial cell walls which causes cell permeability to increase, so that intracellular compounds will come out. Tannins have antibacterial action by causing damage to the permeability of bacterial cell walls, microsomes and lysosomes as a result of the interaction of flavonoids with bacterial DNA so that they can dissolve extracellular protein complexes (Villanueva et al., 2023).

The results of this research show that Moringa leaf extract is most effective in inhibiting and killing the growth of *Streptococcus mutans* bacteria rather than *Enterococcus faecalis*. According to Savitri et al (2019), apart from the active compounds contained in a material, antibacterial activity can also be influenced by the type of bacteria. *Enterococcus faecalis* and *Streptococcus mutans* are gram-positive bacteria whose cell walls are composed of peptidoglycan and teichoic acid, so these bacteria are more susceptible to mechanical damage (Amin et al., 2023; Robertson, and Willet, 2023).

Even though Moringa leaf extract appears to have antibacterial effectiveness against the growth of *Enterococcus faecalis* and *Streptococcus mutans* bacteria, this research did not find MIC or KBM values. This result is different from research by Dima et al (2016) in Isyraqi et al (2020) that Moringa leaf extract in vitro has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Minimum Inhibitory Level for *Escherichia coli* bacteria is 12 mm and *Staphylococcus aureus* is 11 mm.

There are several factors that influence the results of antibacterial tests, one of which is concentration (Gajic et al., 2022; Wenzlet et al., 2023). According to Tunas et al (2019), the small concentration of the extract in the preparation affects the diameter of the inhibition zone it causes. The highest concentration of Moringa leaf extract, namely a concentration of 50% in this study, was not able to kill >99% of bacteria and the smallest concentration of 3.125% also still found obstacles, so the minimum inhibitory level (MIC) and minimum kill level (KBM) were not found.

Conclusion

Based on research results, Moringa oleifera leaf extract is more effective as an antibacterial against *Streptococcus mutans* compared to *Enterococcus faecalis*

then the KBM value < 3.125% concentration and KBM > 50% concentration.

Author Contribution

Concepts and methods in research by Firdha muharraran; validation Suci Erawati; remainder of research by Dini Arta Lestari Dalimunthe. The published version of the work has been read approved by all of the writers.

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Conflicts of Interest

The authors declare no conflict of interest.

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