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RESEARCH ARTICLE

The inhibition of leaf extract Moringaoleifera on the formation biofilm bacteria Enterococcus faecalis

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ABSTRACT

Background: *Enterococcus faecalisis the most common bacteria that cause failure to root canal treatment, which can become very resistant under biofilms. Moringaoleifera has antibacterial properties and may affect the multidrug-resistant bacteria.* ***Purpose:*** *This study aimed to observe the inhibition of Moringaoleifera leaf extract on the biofilm formation of bacteria Enterococcus faecalis.* ***Method:*** *This study was true experimental laboratory research with post-test only control group design and tested using biofilm method, divided into six groups, each group consisted of eight samples. The control groups were: K- (CMC 0,1%), K+ (ChKM), and four treatment groups were: P1 (Moringaoleifera 20%), P2 (Moringaoleifera 40%), P3 (Moringaoleifera 60%), P4 (Moringaoleifera 80%). Antibacterial inhibition was determined by the value of Optical Density in the ELISA Reader. Data analysis using Kruskal-wallis followed by Mann-Whitney test.* ***Results:*** *There were significant differences (p <0.05) seen from the percentage value of biofilm inhibition, on the K - (0 %) group compared with K+ (47,69%), P1(7,68%), P2 (21,13%), P3 (42,33%) and P4 (55,78%), as well on K + group (ChKM) compared with P4 group (Moringaoleifera 80%).* ***Conclusion:*** *Moringaoleiferaleaf extract has inhibition effect for the formation of bacteria Enterococcus faecalis biofilm and the effect is 80% greater than ChKM.*

Keywords: *Biofilm, Enterococcus faecalis, Moringaoleifera*

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INTRODUCTION

Root canal treatment is a treatment in dentistry that aims to maintain teeth in oral cavity so that they can be accepted biologically by the surrounding tissues[1]. Root canal treatment also aims to eliminate the bacteria that cause infection in the pulp tissue and periapex, prevent any damage to hard or soft tissue in teeth and prevent reinfection due to bacterial contamination[2,3,4].

The success of root canal treatment can be evaluated based on clinical, radiographic, and histological examination. Research states that the success rate of root canal treatment reaches 90-95%, and the failure rate of root canal treatment which causes the need for re-treatment reaches 5-10%[5]. Microorganisms remaining after root canal treatment are the main factors causing reinfection[6].*Enterococcus faecalis* is a Gram-positive facultative anaerobic bacteria, known as the most resistant species, are often found in cases with abnormalities after endodontic treatment in a high percentage of up to 77% [7,8,9].*Enterococcus faecalis* bacteria are facultative gram-positive anaerobic bacteria that can live with or without oxygen supply[10].*Enterococcus faecalis* is able to survive in an environment that does not support, survive at low temperatures, in a nutrient-poor environment, invasion of the dentinal tubules, suppress the work of lymphocytes, form biofilms[6,11].

Bacteria *Enterococcus faecalis* has virulent factors that are high resistance to root canal medicamentbecause this bacterium has the ability to maintain a pH balance[12].*Enterococcus faecalis* is also able to penetrate the dentinal tubules to allow the bacteria to avoid instrumentation of the preparations and irrigation materials used during biomechanical treatment[6,13]. Biofilms are collection of bacterial cells either of a kind or several types attached to the substrate, tissue, or cells covered by a polysaccharide binding layer resulting from the excretion of bacterial cells, causing damage to the surface of the cells, mucosa and the tissue that it attaches[14]. Biofilms are one of the causes *Enterococcus faecalis* resistant to antimicrobial materials that can allow this bacterium to be 1000 times more resistant to antibodies, antimicrobials, and phagocytosis[10].

Root canal preparation measures are accompanied by insufficient irrigation to be able to free the root canals from bacteria properly so that it is necessary to administer root canal drugs[15] .ChKM also has better antibacterial, antiseptic, and disinfectant properties than other ingredients such as povidone iodine, chlorhexidine digluconate, and polyhexanide and other phenol groups (para-monochlorophenol, thymol, and cresol). ChKM also has shortcomings such as having toxic effects on periapex tissue, can cause irritation and soft tissue necrosis, it feels bad, has a pungent odor, and can cause allergic reactions[15,16]. The lack of root canal drugs encourages the development of research on natural ingredients that contain antibacterial as an alternative ingredient as a drug that can be used to reduce bacterial activity, because the use of natural ingredients has lower levels of danger, risk, and side effects when compared to the use chemical drugs[17].

Plants that can be used as food and medicine are *Moringaoleifera* plants[18]. *Moringaoleifera* is known as The Miracle Tree because it is proven to be a natural source of food nutrition and is efficacious as a drug whose contents are larger than in other plants[19]. All parts of *Moringaoleifera* plants include: leaf, roots, seeds, bark, fruit, flowers, and adult pods, can be used as a heart and blood circulation stimulant, anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer , anti-spasmodic, diuretic, anti-hypertensive, cholesterol-lowering, antioxidant, anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal[20]. The content of secondary metabolite compounds from *Moringaoleifera* leaf is flavonoids, saponins, tannins, and alkaloids[21].

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The results of the antibacterial activity test of *Moringaoleifera* leaf extract against *Escherichia coli* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria) showed strong antibacterial power in all treatment groups, and the treatment of Escherichia coli with *Moringaoleifera* leaf extract , the concentration of 80% has the strongest antibacterial power[22]. The purpose of this study was to determine the ability of *Moringaoleifera* leaf extracts to inhibit the formation of *Enterococcus faecalis* biofilms.

MATERIAL AND METHOD

This study is a true experimental laboratory study, with the Randomized Post Test Only Control Group Design research design, there are 6 groups, namely negative control (CMC solvent 0.1%); positive control (ChKM); P1 (*Moringaoleifera* extract 20%); P2 (Moringaoleifera extract 40%); P3 (*Moringaoleifera* extract 60%); P4 (80% *Moringaoleifera* extract). and each group consisted of 8 samples. In this research, a method has been conducted to qualitatively test the formation of *Enterococcus faecalis*biofilms by using Congo red to incubate for 48 hours and form a black colony that shows a strain of bacteria capable of producing biofilms.

The making of *Moringaoliefera* leaf extract solution was done by maceration technique. A total of 100 g of *Moringaoliefera* leaf simplex powder was put into erlernmeyer, then soaked with 96 mL of 96% ethanol solution, covered with aluminum foil and left for 5 days while occasionally stirring. After 5 days, the samples soaked were filtered using filter paper to produce filtrate 1 and residue 1. The residue was then added with a 96% ethanol 96% solution, covered with aluminum foil and left for 2 days while occasionally stirring. After 2 days, the samples were filtered using filter paper to produce filtrate 2 and residue 2. Filtrate 1 and 2 were mixed into one, then evaporated using a rotary evaporator, to obtain a thick extract of *Moringaoliefera* leaves. The resulting viscous extract was put into a water bath and evaporated until all the ethanol solvents evaporated. The extract was weighed and stored in a closed glass container before being used for testing. 20% test solution is made; 40%; 60% and 80% by weighing 0.2 gram; 0.4 gram; 0.6 gram and 0.8 gram of Moringa leaf thick extract were then dissolved in 1 mL of 0.1% CMC Na solution, respectively. 22% CMC Na solution was used as a negative control and (ChKM) used as a positive control.

The biofilm inhibition test was carried out by mixed bacteria on BHIB and equalized to 0.5 McFarland I standard. Suspension of *Enterococcus faecalis*biofilm bacteria that had been equalized was then diluted to 1: 100. Bacterial suspension was inserted into a 96-well round- bottomed plastic tissue culture plate (microtiter plate) with a total volume of 0.1 mL (100 µl) in each well using a micropipette. The study was conducted on 1 test plate and 1 blank plate. The test plate was filled with bacterial suspension, then the microtiter plate was put in a special container and then incubated at 37°C for 24 hours. After 24 hours the microtiter plate was removed from the incubator then the test extract solution was put into the microtiter plate filled with bacterial suspension, and on the blank plate the test extract solution was filled without bacteria. The microtiter plate is put back into the incubator for 24 hours. After that the microtiter plate is removed from the incubator, then the test solution is removed and washed 3 times with 0.2 mL phosphate buffer saline. The microtiter plate was dried, then each well was added with 1% crystal violet of 0.2 mL (200 µl) and allowed to stand for 15 minutes. Then rinsed using distilled water and dried for 15 minutes in an incubator of 37 ° C. Add Tween 80 2% 0.2 mL to each microtiterplate.The test results in the form of optical density (OD) values are read using an ELISA reader at a wavelength of 570 nm. The inhibitory power of biofilm formation from the test solution is calculated by the following formula[23].

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RESULT

The research data were analyzed descriptively to obtain an overview of the distribution and summary of the data in order to clarify the presentation of results.

Table 1. The mean value of *Enterococcusfaecalis* biofilms

Group	Replication	Mean	SD
K(-)	8	0	0
K(+)	8	47,69	6,70
P1	8	7,68	0,92
P2	8	21,13	4,45
P3	8	42,33	3,31
P4	8	55,78	3,68

Note: K- (CMC 0.1%); K (+) (ChKM); P1 (*Moringaoleifera* extract 20%); P2 (*Moringaoleifera* extract 40%); P3 (*Moringaoleifera* extract 60%); P4 (80% *Moringaoleifera* extract).

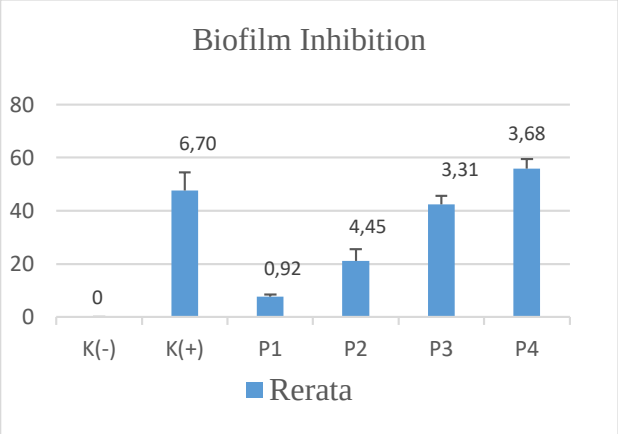

Figure 1. Average bar chart of Enterococcus faecalis biofilm inhibition

The data above shows the largest inhibitory biofilm of *Enterococcus faecalis* is the largest group P4 (*Moringaoleifera* extract 80%), the lowest in the P1 group. Increasing the inhibitory value in the treatment group was seen by the increasing concentration of *Moringaoleifera* leaf extract combination. In K (-) (CMC 0.1%) there was no inhibition, while the K (+) group (ChKM) had inhibitory effect that inhibited the formation of *Enterococcus faecalis* biofilm.

Table 2. Results of normality test with Shapiro-Wilk

Group	Sig
K (+)	0,829*
P1	0,826*
P2	0,238*
P3	0,883*
P4	0,101*

Note : * (p>0,05)

The results of the normality test with Shapiro-Wilk can be seen that each treatment group has a significance value of p> 0.05. This shows that the inhibitory value of biofilms is normally distributed.

Table 3. Test results for the variance homogeneity with Levene

Levene Test	Sig.
5,394	0,001

Note : (p>0,05)

Based on the results of the variance homogeneity test with Levene it is known that the significance value is 0.001 or can be interpreted as p <0.05, so it can be concluded that the biofilm inhibition value is not homogeneous (p <0.05).

Tabel 4. The result of *Kruskal-Wallis* Test

Kruskal-Wallis	**Sig.**
AntarKelompok	0,000*

Note : * (p<0,05)

Kruskal-Wallis test results showed that the value of siginification was 0,000 (p <0.05), it can be concluded that there was a significant biofilm inhibition between the treatment group with the negative control group and the positive control group. To see To see the significant difference in antibacterial power of each group, the Mann-Whitney test was performed

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Table 5. Mann-Whitney Test

Group	K(-)	K(+)	P1	P2	P3	P4
K(-)		0,000*	0,000*	0,000*	0,000*	0,000*
K(+)			0,001*	0,001*	0,059	0,012*
P1				0,001*	0,001*	0,001*
P2					0,001*	0,001*
P3						0,001*
P4						

Note: Group (average inhibition of biofilms); * (p <0.05)

The Mann-Whitney test results showed that there were significant differences in the value of biofilm inhibition (p <0.05), between K (-) with K (+), P1, P2, P3 and P4; between K (+) with P1, P2 and P4; between P1 and P2, P3 and P4; between P2 and P3 and P4; and between P3 and P4.

RESULT

This study aims to determine the inhibitory power of *Moringaoleifera* leaf extracts against the formation of the bacterial biofilm *Enterococcus faecalis.* Biofilms are a community of bacteria that are often well organized, attached to the surface and embedded in the extracellular slime layer which is a sticky material and as a protection produced by bacteria[24]. The formation of biofilms is one of the common mechanisms of a collection of bacteria as a form of defense for survival. Biofilms can protect bacteria from the host's body defenses and can protect from adverse environmental influences such as extreme pH (pH <4.4 and> 9.1), low oxygen content and extreme pressure and temperature (> 100 ° C). Bacteria can become very resistant under biofilm[25].

The method used to identify biofilm formation qualitatively is congo red agar. The results of identification on the media in order to show that the bacteria *Enterococcus faecalis* has the ability to form biofilms shown by the formation of black colonies. While the method used to test the formation of bacterial biofilms quantitatively is a microtiter plate assay. This method is used to observe microbial biofilm attachment[26]. This method was chosen because it is fast, easy, simple, accurate and has a sensitivity of 97.1%, specificity of 97.5% and accuracy of 97.2% in detecting biofilm attachment[27,28].

Statistical test found a significant difference between groups K (-) to K (+), this shows that ChKM is a broad-spectrum antibacterial active against gram-positive and gram-negative bacteria. ChKM began to be known in 1905, ChKM is known to have good antiseptic and disinfectant properties for root canals. This drug is often used in the case of root canal treatment even though ChKM has a toxic content in its use.16 In group K (-) there was no biofilm inhibition, this shows that the 0.1% CMC solution is only a neutral solvent, and is completely has no inhibition and does not have any effect on *Moringaoleifera* leaf extract in inhibiting the formation of *Enterococcus faecalis* biofilms. In all treatment groups P1, P2, P3 and P4 all have significant biofilm inhibitory values this means *Moringaoleifera* leaf extracts at concentrations of 20%, 40%, 60% and 80% contain antibacterial compounds so that they can inhibit the formation of bacterial biofilms *Enterococcus faecalis* .

Moringaoleifera leaves have potential as an antibacterial because the extract contains secondary metabolite compounds including flavonoids, saponins, tannins, and alkaloids. Flavonoids work by interrupting signaling between bacterial cells, so that it will result in the formation of colonies and permanent attachment of bacteria inhibited[21].Saponins work by releasing bonds between bacteria on biofilms into free planktonic bacteria, so that the content of other active substances that are anti microbial can work better[29,30]. Tannins work by coagulating microbial protoplasms, so that

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stable bonds with bacterial proteins are formed which cause inactivation of bacterial proteins[29]. Alkaloids work by reacting with bacterial DNA and amino acid compounds so that the cell nucleus becomes lysis[25]. The magnitude of the active ingredient *Moringaoleifera* leaf extract is directly proportional to the large concentration of the extract, this can be seen from the results of the average inhibition of bacterial biofilms, the greater the concentration of *Moringaoleifera* leaf extracts, the inhibition of *Enterococcus faecalis*biofilms is also greater. There was a significant difference in inhibition of the formation of *Enterococcus faecalis* biofilms (p <0.05) between all treatment groups and in group P4 (*Moringaoleifera* 80% leaf extract) had the greatest inhibitory power. The P4 group and the positive control group (ChKM) also had significant differences (p <0.05) where P4 had a greater inhibition of the formation of *Enterococcus faecalis* biofilms than K (+).

CONCLUSION

Moringaoleifera leaf extract can inhibit the formation of biofilm bacteria *Enterterococcusfaecalis*. *Moringaoleifera* leaf extract concentration of 80% had the greatest inhibitory effect on the formation of *Enterococcus faecalis*bacterial biofilms compared with ChKM as K (+) and *Moringaoleifera* leaf extract concentrations of 20%, 40%, 60%.

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