

Biomass index and viscosity values of *Moringa oleifera* that influenced by *Enterococcus faecalis*

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ABSTRACT *Enterococcus faecalis* (*E. faecalis*) has been reported as the primary infectious agent in root canals. *Moringa oleifera* (*M. oleifera*) is said to have the ability to prevent the development of *E. faecalis*. The purpose of this study was to measure the biomass index and viscosity of the ethanol extract of *Moringa* (*Moringa oleifera*) leaves, which were affected by *E. faecalis*. This study used *Moringa oleifera* and *E. faecalis*. The biomass index of *Moringa oleifera* extracts using the Biomass Assay method and the viscosity with the Ostwald Viscometer. The biomass index of *M. oleifera* affected by *E. faecalis* at a concentration of 12.5% for 48 hours was better than other concentrations. CHX has a perfect biomass index at 24 hours, while at 48 hours, the biomass index increases to close to 10%. Meanwhile, *M. oleifera* has a high viscosity at a concentration of 12.5% (0.81 Cp). The results of the viscosity examination were in line with the biomass index with a positive correlation (0.92) and $p < 0.05$ between the *M. oleifera* concentrations between the two treatments. *M. oleifera* has very good biomass and viscosity index at a concentration of 12.5%. Both are determinants of the development of *E. faecalis* under the influence of *M. oleifera*.

KEYWORDS bomassa indek, viskositas, *E. faecalis*

INTRODUCTION

The root canal of the tooth contains nerves and blood vessels. Root canal infection is an indicator of damage to the tissue, especially the nerves.¹ In general, root canal infections are influenced by pathogenic bacteria found in root canals.² *Enterococcus faecalis* was reported as the leading agent as the most dominant biofilm-forming bacteria in root canal infections. These bacteria are complicated to eliminate, especially in cases of chronic infection.² In addition, *E. faecalis* attacks the root canal system of teeth, thereby inducing an immunoinflammatory response that can cause apical tissue damage. The infection also causes abscesses to form and causes swelling of the face, neck, and head. In addition, discharge from the site

of infection and in the long term can cause loss of alveolar bone and tooth roots.³

Elimination of *E. faecalis* has been the focus of research in the field of endodontics for decades.⁴ The use of irrigation solutions such as 5.25% NaOCl and 17% EDTA aims to prevent infection and stimulate new blood vessels. In addition, the use of these chemicals can cause surface roughness of the root canal and leave a smear layer.⁵ Both of these problems can provide opportunities for *E. faecalis* to increase its viability by forming large amounts of biofilm mass. So that it helps and provides opportunities for bacteria to grow and improve the pathogenesis of infection.⁶

Preventive measures in the pathogenesis of root canal infection caused by *E. faecalis* need

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attention. One of them is research using natural ingredients such as Moringa leaf plants. Moringa leaves (*M. oleifera*), in addition to containing antioxidant compounds, anti-inflammatory is also antibacterial.⁷ Yan (2020) reported that Moringa oleifera could prevent the growth and formation of bacterial biofilms of *E. faecalis*.⁸ This potential provides an opportunity for Moringa oleifera to be used as a test material, both as an irritant and as an antibacterial. One of the abilities of natural materials

to inhibit or disrupt the development of bacteria is characterized by the biological properties of these materials. The physical properties of raw materials of viscosity and biomass formation can indicate the response to pathogen interaction. This study aims to determine the biomass index and viscosity of Moringa oleifera solution after interaction with *E. faecalis*. Biomass and viscosity index as a reference for bacterial development

MATERIALS AND METHODS

This research passed the ethical clearance No. 126/KE/FKG/2019 from the Faculty of Dentistry ethics committee, Syiah Kuala University, Darussalam, Banda Aceh, Indonesia. This study used Moringa oleifera 5 concentrations (75%, 50%, 25%, 12.5%, 6.25%). While the *E. faecalis* ATCC 43062 as a research subject was obtained from the Unsyiah FKG Research Laboratory. Furthermore, interactions were carried out to measure the biomass index of Moringa oleifera and the viscosity influenced by *E. faecalis*.

Enterococcus faecalis Culture

Cultures of *E. faecalis* were planted on Mueller Hinton Agar (MHA). It was performed using the T streak method (streak T). The petri dish was divided into three parts using a marker pen. Culturing is by heating the needle loop and waiting for it to cool, then taking one circle of pure culture inoculated in area 1, half a cup with zigzag strokes. Then reheat the oase needle and wait for it to cool, then proceed with zigzag strokes in area 2, which is perpendicular to the first stroke, then continue with zigzag strokes in area 3 perpendicular to the second stroke. Petri dishes that bacteria have scratched are then tightly closed and incubated for 24 hours at 37°C in an anaerobic atmosphere.⁹

Extraction of *Moringa oleifera*

Moringa leaves (*Moringa oleifera*) separated from the stalks are collected in quantities up to 1 kg and then washed with water. Drying time was two days until wilted, then 48 hours in an oven at 50 °C. *M. oleifera* is ground into Moringa leaf powder using a blender. The received powder is then sealed in an airtight container. The powder is soaked in 100 mL of 96 percent ethanol in a clean flat-bottomed glass container. The separation of residue and filtrate was carried out for three days using the same solvent. The filtrate is collected and concentrated using a

rotary vacuum evaporator at a temperature of 50 °C and a pressure of 75 mmHg to produce the extract.¹⁰

Viscosity assay

The viscosity was measured with an Ostwald viscometer after the density was measured with a pycnometer. The empty pycnometer is weighed and covered with an analytical balance in the first step. The extracted liquid in the tube was then taken with a 5 mL dropper pipette and placed in the pycnometer and pycnometer, which were then closed. In addition, the pycnometer containing 5 mL of extract was weighed with an analytical balance (Ohaus, max cap 210 gr).¹¹

Biomassa Assay

The ethanol extract of Moringa leaves that had been prepared was then taken 3 mL in various concentrations in the dosage bottle and weighed using an analytical balance. Then it was incubated at 37 °C for 24 h, 48 h, then weighed again. This treatment was repeated on the ethanol extract of Moringa leaves added with 100 L of *E. faecalis*. The scale value (g/mL) became an indicator of biomass before and after interaction with *E. faecalis*. with the formula $A=X1-Y$; $B=X2-Y$; $C=A-B$, where A= biomass value before incubation; B= biomass value per incubation mass; C= total biomass value; X1= The value of the bottle after administration of the extract; X2= Value after incubation; and Y = bottle value before extract administration.¹²

Statistical analysis

Kruskal Wallis Test analyzed viscosity and biomass index data. The probability value of 0.05% is the limit of significance. The relationship between biomass and viscosity variables was analyzed by Spearman Ro, with $r = 1$ indicating a strong correlation.

RESULTS

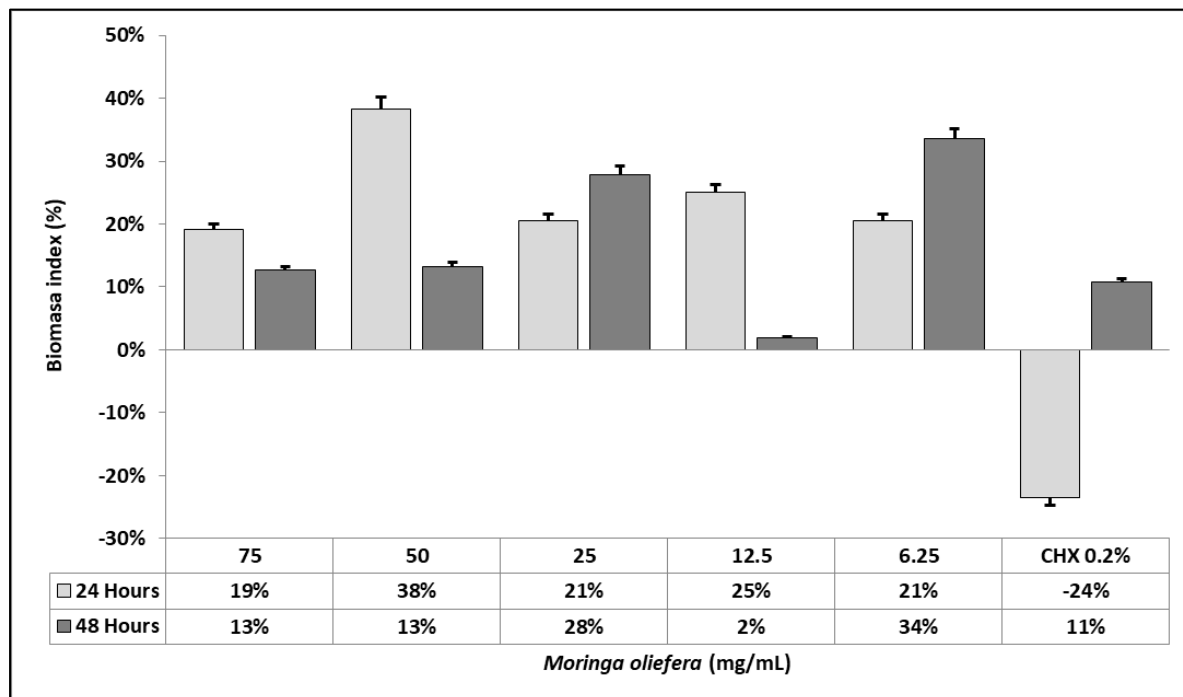


Figure 1. Biomasa index of *M. oleifera* extracts after its interaction with *E. faecalis*. Moringa leaf biomass index at a concentration of 12.5% at 48 hours was better than other concentrations. CHX has a very good biomass index at 24 hours, while at 48 hours, the biomass index increases to close to 10%. Bar (biomasa index) and Bar Error (percentage error).

Figure 1 shows the biomass index of Moringa leaf extract after interaction with *E. faecalis* ($p > 0.05; 0.346$). The lowest percentage value indicates the best biomass index, meaning that there is no decomposition of the active compound of the test material so that there is no change in biomass

and can even reduce it as in 24-hour CHX. For example, at a concentration of 12.5%, Moringa leaf prevented bacterial adaptation to the influence of the active ingredients of the test material so that no decomposition occurred. It can be explained that this test material can be bacteriostatic.

Table 1. The viscosity of *M. oleifera* ethanol extract after interaction with *E. faecalis*

Concentration	pH	Density (gr/mL)	Viscosity (Cp)
6,25 %	5.60	0.9865	0.8042
12,5 %	5.70	0.990	0.8154
25%	5.70	0.995	0.8655
50%	5.77	1.007	0.9881
75%	5.80	1.018	1.1191

Table 1. shows that at a concentration of 12.5%, the lowest Cp value is 0.81, no significant among concentrations ($p > 0.05; 0.284$) so that at this concentration it has a high viscosity, meaning that at this concentration, Moringa leaves can provide a better response to *E. faecalis*, so that bacteria are

unable to decompose several active ingredients of the test material and Moringa leaves can maintain its viscosity. The viscosity test results are in line with the biomass index, which at a concentration of 12.5% has the same ability to prevent bacterial activity.

DISCUSSION

This study evaluated Moringa oil (*M. oleifera*) ethanol extract to suppress the activity of *E. faecalis* based on the biomass index and viscosity of *M. oleifera* formed after interaction with *E. faecalis*. Both of these results can provide an overview of the biological activity of *E. faecalis* survived under the influence of *M. oleifera*. Furthermore, *M. oleifera* suppresses the formation of biomass during interaction with *E. faecalis* shows that *M. oleifera* can prevent bacterial activity because the biological activity of bacteria strongly influences the frequency of the biomass index. Furthermore, *M. oleifera*, as the test material used in this study, was able to increase the viscosity after interacting with *E. faecalis*, meaning that *M. oleifera* maintained its adaptability to the development of *E. faecalis*.

The decrease in the viscosity value indicates the higher the workforce of the bacteria. On the other hand, if the viscosity is high, then *M. oleifera* can control the growth of bacteria because there is no metabolic activity or decomposition of the active compounds contained in *M. oleifera* during the interaction phase. This concept has been reported by Lin (2020), who emphasized that bacteria tend to carry out metabolic activities in all solutions, including antibacterial materials, because bacterial metabolic activity can increase the specific gravity of the solution, which causes a decrease in viscosity.¹³

The results at 1 show that the concentration of 12.5% has an excellent effect on reducing the biomass index, meaning that it can provide tolerance for bacteria to adapt to the test material. Fuentes (2016) reported that biomass is a product formed due to the metabolism of pathogens against bacteria.¹⁴ In this perspective, Llado (2016) adds that the decomposition of the active components of plant extracts by bacteria is indicated by changes in the

pH of the solution. Because the products of synthesis or bacterial metabolism tend to be acidic.¹⁵ In addition, the decrease in biomass from bacterial activity with antibacterials indicates that antibacterial agents have an excellent ability to disrupt metabolism and reduce the biostability of the bacterial environment.¹⁶

Table 1 reports that a concentration of 12.5% has a high viscosity, meaning that Moringa leaves can provide a better response to the development of *E. faecalis* because there is no biosynthesis or decomposition of antibacterial substances. Low viscosity can indicate bacterial metabolism, meaning that bacterial activity is more dominant than the antibacterial role. The bacteria can release all the virulent enzymes that can solidify the products of metabolism. High coagulation of bacterial metabolic waste material can reduce the viscosity of the solution, so this can be an indicator of the work of antibacterial agents.¹⁷ Reported that the low viscosity of the solution (saliva) tends to be caused by protein metabolism factors. The increase in protein that bacterial hydrolysis enzymes have changed can cause a decrease in viscosity, thus causing an increase in bacterial metabolism and growth.¹⁸

This study provides information about the biological properties of *M. oleifera* in reducing the bioactivity of *E. faecalis*. Biomass and viscosity are two references to measure bacterial bioactivity during interactions with antibacterial agents. Further studies are highly expected so that the role of *M. oleifera* as an antibacterial (*E. faecalis*) can be used as a reference as an irrigation material for root canal infections.

CONCLUSIONS

M. oleifera has a very good biomass index and viscosity values at a concentration of 12.5%. Both

can be a reference in disrupting or maintaining the biological activity and development of *E. faecalis*.

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