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

Cut SORAYA*, Zulfan M. ALIBASYAH1, Basri A. GANI3

1 Department of Conservative Dentistry, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.
2 Department of Periodontia, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia
3 Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia
*Corresponding author address: E-mail: sorayaaldine@unsyiah.ac.id

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 biomass index, viscositas, 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

How to cite this article: SORAYA C, Alibasyah ZM, Gani, BA. Biomass index and viscosity values of moringa oleifera that influenced by Enterococcus Faecalis. JDS. 2021; 6(1): 1-5
© 2021 Syiah Kuala University Press
e-ISSN 2502-0412
www.jurnal.unsyiah.ac.id/JDS
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. Yan (2020) reported that Moringa oleifera could prevent the growth and formation of bacterial biofilms of E. faecalis. 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 Ace, 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.

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.

**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).

**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.

**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. Biomass 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 (biomass index) and Bar Error (percentage error).

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

| Concentration | pH  | Density (g/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.

ACKNOWLEDGMENT

This research was supported by Universitas Syiah Kuala of Lektor Kepala Research: No.247/UN11.2.1/PT.01.03/PNBP/2020. Thank Laboratorium Microbiology and Laboratorium Riset of Veterinary Faculty, Laboratory Chemistry, Education Faculty, Universitas Syiah Kuala, Darussalam Banda Aceh, Indonesia
REFERENCES

[1]. Walsh LJ. Novel Approaches to Detect and Treat Biofilms within the Root Canals of Teeth: A Review. Antibiotics 2020;9(3):129.

[2]. Narayanan LL, Vaishnavi C. Endodontic microbiology. Journal of conservative dentistry: JCD 2010;13(4):233-39.

[3]. Rôças IN, Siqueira Jr JF. Frequency and levels of candidate endodontic pathogens in acute apical abscesses as compared to asymptomatic apical periodontitis. PLoS One 2018;13(1):e0190469.

[4]. Diogo P, Fernandes C, Caramelo F, et al. Antimicrobial photodynamic therapy against endodontic Enterococcus faecalis and Candida albicans mono and mixed biofilms in the presence of photosensitizers: A comparative study with classical endodontic irrigants. Frontiers in microbiology 2017;8:498.

[5]. Vinothini K. Comparative Evaluation of Antimicrobial Efficacy of Triphala, Azadiracta Indica, Metronidazole and 3% Sodium Hypochlorite as Root Canal Irrigants: An In Vivo study [Tamil Nadu Government Dental College and Hospital, Chennai; 2020].

[6]. Wojnicz D, Tichaczek-Goska D, Korzekwa K, Kicia M, Hendrich AB. Study of the impact of cranberry extract on the virulence factors and biofilm formation by Enterococcus faecalis strains isolated from urinary tract infections. International journal of food sciences and nutrition 2016;67(8):1005-16.

[7]. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: A review on nutritive importance and its medicinal application. Food science and human wellness 2016;5(2):49-56.

[8]. Yan G, Liping S, Yongliang Z. UPLC-Q-Orbitrap-MS2 analysis of Moringa oleifera leaf extract and its antioxidant, antibacterial and anti-inflammatory activities. Natural product research 2020;34(14):2090-94.

[9]. Soraya C, Mubarak Z, Gani BA. The growth and biofilm formation of Enterococcus faecalis in ethanol extract of Citrus aurantiifolia Indonesian species. Journal of Pharmacy & Pharmacognosy Research 2020;8(6):558-68.

[10]. Zhang T, Jeong CH, Cheng WN, et al. Moringa extract enhances the fermentative, textural, and bioactive properties of yogurt. LWT 2019;101:276-84.

[11]. Mwanzia B. Characterization Of Telfairia Pedata (Smith Ex Sims) Hook., Seed Kernel Oil For Nutritional Value And Antioxidant Activity [University of Nairobi; 2020].

[12]. Łukajtis R, Holowacz I, Kucharska K, et al. Hydrogen production from biomass using dark fermentation. Renewable and Sustainable Energy Reviews 2018;91:665-94.

[13]. Lin M-C, Chen C-C, Wu I-T, Ding S-J. Enhanced antibacterial activity of calcium silicate-based hybrid cements for bone repair. Materials Science and Engineering: C 2020;110:110727.

[14]. Fuentes JL, Garbayo I, Cuaresma M, et al. Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. Marine drugs 2016;14(5):100.

[15]. Lladó S, Žifčáková L, Větrovský T, Eichlerová I, Baldrian P. Functional screening of abundant bacteria from acidic forest soil indicates the metabolic potential of Acidobacteria subdivision 1 for polysaccharide decomposition. Biology and fertility of soils 2016;52(2):251-60.

[16]. Schmidt H, Thom M, Wieprecht S, Manz W, Gerbersdorf SU. The effect of light intensity and shear stress on microbial biostabilization and the community composition of natural biofilms. Research and Reports in Biology 2018;9:1-16.

[17]. Najjar A, Sabri S, Al-Gaashani R, Kochkodan V, Atieh MA. Enhanced fouling resistance and antibacterial properties of novel graphene oxide-Arabic gum polyethersulfone membranes. Applied Sciences 2019;9(3):513.

[18]. Gurung N, Ray S, Bose S, Rai V. A broader view: microbial enzymes and their relevance in industries, medicine, and beyond. BioMed research international 2013;2013:329121-21