ORIGINAL ARTICLE

The Effect of *Moringa oleifera's* antibacterial and antibiofilm properties against *Fusobacterium nucleatum* and *Staphylococcus aureus*: a laboratory experiment

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ABSTRACT

Introduction: *Fusobacterium nucleatum* and *Staphylococcus aureus,* both associated with pulp and periapical diseases, must be effectively eliminated during irrigation. Natural agents are generally more biocompatible and less harmful to bodily tissues, making them a promising alternative to chemical irrigants such as sodium hypochlorite. Fractions of *Moringa oleifera* have demonstrated potential as an alternative irrigant due to their antibacterial and antibiofilm properties. This study aimed to evaluate the antibacterial and antibiofilm efficacy of *Moringa oleifera* fractions against *Fusobacterium nucleatum* and *Staphylococcus aureus*. **Methods:** The tube dilution method was used for antibacterial tests, while the biofilm assay method measured the optical density (OD) in a 96-well plate to evaluate antibiofilm effects. *Moringa oleifera* fractions were tested at 20%, 40%, 60%, and 80% concentrations. A one-way ANOVA test was applied for normally distributed data, and the Kruskal Wallis test was used for non-normally distributed data. Post-hoc analyses were conducted to determine significant differences between groups, with the significance level set at 0.05. **Results:** The antibacterial test revealed statistically significant differences between *Moringa oleifera* groups in eliminating *Fusobacterium nucleatum* ($p = 0.003$, $p < 0.05$) and *Staphylococcus aureus* with p value ($p = 0.001$, $p < 0.05$). The antibiofilm test also showed statistically significant differences among the concentration groups for both bacteria. **Conclusion**: This study demonstrates the antibacterial and antibiofilm effectiveness of *Moringa oleifera's* fractions against *Fusobacterium nucleatum* and *Staphylococcus aureus,* supporting its potential as an alternative irrigant.

KEYWORDS

Moringa oleifera, antibacterial, antibiofilm, *Fusobacterium nucleatum*, *Staphylococcus aureus.*

INTRODUCTION

According to the Indonesian Health Survey, dental caries, gingivitis, and abscesses remain the most common oral health problems in Indonesia, accounting for 45.3% and 14% of cases, respectively. Microorganisms or bacteria are the primary cause of caries, pulp disease, and periapical illness. One of the key bacteria implicated in root canal infections is *Fusobacterium nucleatum,* an anaerobic gram-negative bacterium considered an endodontic pathogen. This species can survive in the root canal even after biomechanical preparation.

Staphylococcus aureus, a facultative anaerobic gram-positive bacterium, is also frequently found in acute abscesses, with a reported prevalence of 0.7-15%. Numerous microorganisms capable of forming oral biofilms are responsible for infections in oral tissues. *Fusobacterium nucleatum* can coaggregate with other species, including *Staphylococcus aureus*, facilitating their coexistence in biofilms. According to Lukic et al., *Staphylococcus aureus* is one of six endodontic bacteria contributing to biofilm production. Coaggregation of these bacteria in biofilms is commonly associated with failed endodontic treatment and may increase the risk of endodontic infection.[1–6]

Due to the intricate structure of the root canal system, necrotizing tissue, germs, and microbes frequently accumulate, and their removal often requires effective irrigation materials. The most widely used and recommended irrigating agent is sodium hypochlorite (NaOCl), which has antimicrobial properties and is effective in dissolving pulp tissue, necrotizing tissue, and biofilms. However, if NaOCl escapes the apex, it can become hazardous. Based on the research and data currently available, the optimal irrigant for endodontic therapy success remains a topic of debate. The bactericidal properties of a well-established irrigation material is a crucial component. Natural materials can be considered as alternatives to conventional agents, as they are less harmful to bodily tissues and more suitable for clinical use.[7–10]

Moringa oleifera has long been recognized for its nutritional value and pharmacological properties, including anti-inflammatory, antitumor, anticonvulsant, antibacterial, and antibiofilm properties. Because *Moringa oleifera* contains active compounds such as flavonoids, tannins, saponins, alkaloids, phenolics, and triterpenoids, it exhibits antibacterial and antibiofilm properties. The broad-spectrum antibacterial activity of *Moringa oleifera* leaf extract with ethanol has been demonstrated in research conducted by Amabye TK et al. According to research by Su-Kyung Jwa, *Moringa oleifera* leaves exhibit antibacterial properties capable of eradicating biofilm. Numerous studies have shown that *Moringa oleifera* possesses antibacterial properties effective against various bacteria and biofilms. However, neither of these studies directly explained how *Moringa oleifera's* antibacterial and antibiofilm properties affect *Staphylococcus aureus* and *Fusobacterium nucleatum* in comparison with sodium hypochlorite (NaOCl) as an irrigation material used in endodontic treatment.[11–15]

The aim of this study is to evaluate the antibacterial and antibiofilm properties of *Moringa oleifera* methanolic fractions against *Staphylococcus aureus* and *Fusobacterium nucleatum.*

METHODS

In order to ascertain the antibacterial and antibiofilm effects of *Moringa oleifera* methanolic fractions on *Fusobacterium nucleatum* and *Staphylococcus aureus*, this laboratory study was conducted at MiCore Laboratory, Faculty of Dentistry, Universitas Trisakti, Jakarta. The antibacterial and antibiofilm characteristics of the Moringa oleifera methanolic fraction were tested at concentrations of 20%, 40%, 60%, and 80%, following identifications by Herbarium Jatinangoriensis with letter number 191/LBM/IT.V.2023. Distilled water was used as a negative control and NaOCl as a positive control. Antibacterial testing was carried out using the tube dilution method, while antibiofilm testing was performed through the biofilm assay method on 96-well plates. Each 96-well plate was divided into six groups, 80%, 60%, 40%, and 20% of the *Moringa oleifera* methanolic fraction, NaOCl as a positive control and distilled water as a negative control. Each treatment was repeated four times.[16,17]

One milliliter of bacterial suspension was added to each well, followed by 24-hour incubation to perform the antibacterial test via the dilution method. The Minimum Inhibitory Concentration (MIC) was calculated using the first minimal

concentration of the Moringa oleifera methanolic fraction that caused the bacterial suspension on *Brain-heart Infusion Broth* (BHI-B) to become transparent after incubation. Following that, the incubation results were transferred to *Brain-heart Infusion Agar* (BHI-A) and incubated once again for 24 hours at 37°C. Using the Standard Plate Count method, the absence of bacterial growth on BHI-A medium indicated the Minimum Bactericidal Concentration (MBC).[16,17]

Fusobacterium nucleatum mono-species biofilm, *Staphylococcus aureus* mono-species biofilm, and *Fusobacterium nucleatum-Staphylococcus aureus* dual-species biofilm were all subjected to the antibiofilm test, which was separated into two parts, the biofilm attachment inhibition test and the biofilm attachment degradation test. The biofilm attachment inhibition test was carried out by mixing 200 µL of bacterial suspension into 200 µL of *Moringa oleifera* fractions in different concentrations, 5.25% NaOCl, and distilled water, then adding the mixture to each well of the 96-well plates. The well plates were then incubated for 24 hours at 37°C.[18–20]

The entire mixture was discarded after incubation and the wells were rinsed three times with phosphate-buffered saline (PBS) before being dried. After adding 150 µL of 1% crystal violet dye to each well, the wells were allowed to stand at room temperature for 15 minutes. After the incubation was finished, three further PBS rinses were performed, followed by drying and a 15-minute incubation with 200 µL of 96% ethanol. Optical Density (OD) measurements in the biofilm attachment inhibition test were obtained using spectrophotometry at a wavelength of 570 nm. Biofilms formed by mono-species *Fusobacterium nucleatum*, mono-species *Staphylococcus aureus*, and dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* were subjected to the biofilm attachment inhibition test.[18–20]

The bacterial suspension (200 µL) was initially added to a 96-well plate and incubated for 24 hours to begin the biofilm attachment degradation test. After discarding the bacterial culture results and rinsing the wells with 300 µL PBS to remove any detached planktonic cells, they were dried. Then, 200 µL of *Moringa oleifera* fractions, distilled water, and different doses of NaOCl (5.25%) were placed in a 96-well plate and incubated for 24 hours at 37°C. The entire mixture was discarded after incubation, rinsed three times with PBS, and then dried. After staining for 15 minutes with 150 µL of crystal violet at room temperature, the wells were rinsed three times with PBS, dried, then treated with 200 µL of 96% ethanol for 15 minutes. OD measurements in the biofilm attachment degradation test were obtained using spectrophotometry at a wavelength of 570 nm.

This biofilm attachment degradation test was carried out on mono-species *Fusobacterium nucleatum*, mono-species *Staphylococcus aureus* and dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* biofilms. A one-way ANOVA test was used to analyze the results if the data were normally distributed. If the data did not meet normality assumptions, the Kruskal-Wallis test was applied. Post-hoc tests were subsequently conducted to determine significant differences between the groups. The significance level that was applied in this study is 0.05.[17-19]

RESULTS

The Kruskal-Wallis test on antibacterial activity against *Fusobacterium nucleatum* (Table 1) showed a significant difference between the groups, with p value of 0.003 ($p < 0.05$). The post-hoc test results (Table 2) indicated significant differences between the *Moringa oleifera* fractions at 80% and 60% compared to 20%. *The* 20% fraction also differed significantly from the positive control. Significant differences were further observed between the negative control (distilled water) and all *Moringa oleifera* fraction groups.

The 60% and 40% concentrations of *Moringa oleifera* fractions also differed significantly from the positive control and the 20% concentration. Statistically significant differences were observed between the positive and negative control groups across all Moringa oleifera fraction concentrations, as evident on BHI-A media (Figure 1).

The Kruskal-Wallis test on antibacterial activity against *Staphylococcus aureus* (Table 1) also showed a significant difference between each group, with p value of 0.001 ($p<0.05$). According to the post-hoc test (Table 3), the 80% *Moringa oleifera* fraction differed significantly from the 60%, 40%, and 20% fractions, as well as the positive control.

Table 1. Kruskal Wallis Test Results for the Antibacterial Activity of *Moringa oleifera* fractions against *Fusobacterium nucleatum* and *Staphylococcus aureus*.

	Fusobacterium nucleatum		***Staphylococcus aureus***	
	Bacterial colonies (10^4 CFU/ml) Median (Min - Max)	p-value	Bacterial colonies (10^4 CFU/ml) Median (Min - Max)	p-value
MO 80%	0 (0 - 0)	0,003*	0 (0 - 0)	0,001*
MO 60%	0 (0 - 1)		2 (0 - 4)	
MO 40%	1,5 (0 - 4)		3 (2 - 5)	
MO 20%	1,5 (1 - 5)		14 (7 - 19)	
Positive Control	0 (0 - 0)		0 (0 - 0)	
Negative Control	637,5 (633 - 645)		775,5 (771 - 783)	

*Significant difference with a p-value < 0.05
*MO: *Moringa oleifera* fraction
*Positive Control: NaOCl
*Negative Control: Aquades

Table 2. Mann-Whitney Post-hoc Test for *Moringa oleifera's* Antibacterial Efficacy against *Fusobacterium nucleatum*.

	Moringa oleifera fraction 20%	Positive control	Negative control
Moringa oleifera fraction 80%	0,013*	0,000*	0,014*
Moringa oleifera fraction 60%	0,034*		0,013*
Moringa oleifera fraction 40%			0,020*
Moringa oleifera fraction 20%		0,013*	0,020*
Positive control (NaOCl)			0,014*

*Significant difference with a p-value < 0.05

Table 3. Mann-Whitney Post-hoc Test for *Moringa oleifera's* Antibacterial Activity against *Staphylococcus aureus*.

	Moringa oleifera fraction 60%	*Moringa oleifera* fraction 40%	*Moringa oleifera* fraction 20%	Positive control	Negative control
Moringa oleifera fraction 80%	0,047*	0,013*	0,014*	0,000*	0,014*
Moringa oleifera fraction 60%			0,021*	0,047*	0,021*
Moringa oleifera fraction 40%			0,020*	0,013*	0,020*
Moringa oleifera fraction 20%				0,014*	0,021*
Positive control (NaOCl)					0,014*

*Significant difference with a p-value < 0.05

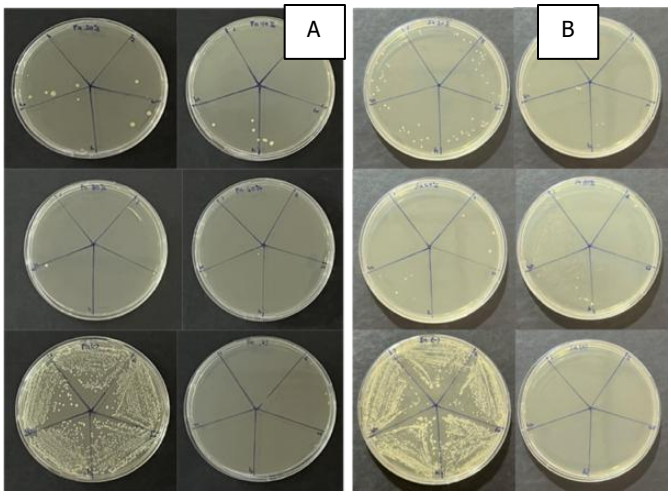

Figure 1. Results of the antibacterial efficacy test of Moringa oleifera methanolic fraction at 80% concentration against (A) *Fusobacterium nucleatum* (B) *Staphylococcus aureus*.

The Kruskal-Wallis test on the biofilm attachment inhibition of *Fusobacterium nucleatum* mono-species (Table 4) revealed a statistically significant difference between groups ($p = 0.001$; $p < 0.05$). The Mann-Whitney post-hoc test showed that the 80% *Moringa oleifera* fraction differed significantly from the 60%, 40%, and 20% concentrations. All *Moringa oleifera* fractions, except 20% concentration, also differed significantly from negative control (Table 5).

The one-way ANOVA test on the biofilm attachment inhibition of *Staphylococcus aureus* mono-species (Table 6) demonstrated a significant difference between groups ($p = 0.000$; $p < 0.05$). According to Tukey's post-hoc test, significant differences were observed between the 80%, 60%, and 40% *Moringa oleifera* fractions compared to both the 20% fractions and the negative control (Table 7).

Similarly, the one-way ANOVA test on the biofilm attachment inhibition of the dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* (Table 8) revealed a statistically significant difference, with a p-value of 0.000 ($p < 0.05$). According to Tamhane's post-hoc analysis (Table 9), the *Moringa oleifera* fractions at 80% and 60% concentrations differed significantly from the negative control.

Table 4. Kruskal-Wallis test on biofilm attachment inhibition of mono-species *Fusobacterium nucleatum* by *Moringa oleifera* fraction.

	Optical Density Median (Min - Max)	P value
Moringa oleifera fraction 80%	0,8555 (0,704 - 0,942)	0,001*
Moringa oleifera fraction 60%	1,297 (1,043 - 1,392)	
Moringa oleifera fraction 40%	1,3455 (1,119 - 1,409)	
Moringa oleifera fraction 20%	1,651 (1,239 - 1,745)	
Positive control (NaOCl)	0,1045 (0,042 - 0,202)	
Negative control (aquades)	1,7755 (1,647 - 1,782)	

*Significant difference with a p-value < 0.05

Table 5. Mann-Whitney post-hoc test on biofilm attachment inhibition of mono-species *Fusobacterium nucleatum* by *Moringa oleifera* fraction.

	Moringa oleifera fraction 60%	*Moringa oleifera* fraction 40%	*Moringa oleifera* fraction 20%	Positive control
Moringa oleifera fraction 80%	0,021*	0,021*	0,021*	0,021*
Moringa oleifera fraction 60%				0,021*
Moringa oleifera fraction 40%				0,021*
Positive control (NaOCl)			0,021*	

*Significant difference with a p-value < 0.05

Table 6. One-way ANOVA test on biofilm attachment inhibition of mono-species *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Sum of Square	*Mean Square*	P value
Between groups	6,857	1,371	0,000*
Within groups	0,765	0,043	
Total	7,623		

*Significant difference with a p-value < 0.05

Table 7. Tukey post-hoc test on biofilm attachment inhibition of mono-species *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Moringa oleifera fraction 20%	Negative control
Moringa oleifera fraction 80%	0,000*	0,000*
Moringa oleifera fraction 60%	0,001*	0,000*
Moringa oleifera fraction 40%	0,003*	0,000*
Positive control (NaOCl)	0,000*	0,000*

*Significant difference with a p-value < 0.05

Table 8. One-way ANOVA test on biofilm attachment inhibition of dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Sum of Square	*Mean Square*	P value
Between groups	1,616	0,323	0,000*
Within groups	0,456	0,025	
Total	2,073		

*Significant difference with a p-value < 0.05

Table 9. Tamhane post-hoc test on biofilm attachment inhibition of dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Negative control
Moringa oleifera fraction 80%	0,001*
Moringa oleifera fraction 60%	0,002*
Positive control (NaOCl)	0,002*

*Significant difference with a p-value < 0.05

The one-way ANOVA test on the biofilm attachment degradation test of mono-species bacteria *Fusobacterium nucleatum* (Table 10) revealed a significant difference, with p-value of 0.000 (p< 0.05). The Tukey post-hoc test (Table 11) indicated that all groups differed significantly, except for the 20% concentration group and the negative control. The Kruskal Wallis test on the biofilm attachment degradation test toward mono-species bacteria *Staphylococcus aureus* (Table 12) also revealed a significant difference, with a p-value of 0.000 (p< 0.05). According to the Mann Whitney post-hoc test results (Table 13), there were statistically significant differences between each group.

Similarly, the one-way ANOVA test on the biofilm attachment degradation of dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* (Table 14)

revealed a statistically significant difference, with a p-value of 0.000 (p< 0.05). The Tukey post-hoc test (Table 15) demonstrated that all groups differed significantly, except between the 20% *Moringa oleifera* fraction and the negative control (Figure 2).

Table 10. One-way ANOVA test on biofilm attachment degradation of mono-species *Fusobacterium nucleatum* by *Moringa oleifera* fraction.

	Sum of Square	*Mean Square*	P value
Between groups	10,478	2,096	0,000*
Within groups	0,053	0,003	
Total	10,531		

*Significant difference with a p-value < 0.05

Table 11. Tukey post-hoc test on biofilm attachment degradation of mono-species *Fusobacterium nucleatum* by *Moringa oleifera* fraction.

	Moringa oleifera fraction 60%	*Moringa oleifera* fraction 40%	*Moringa oleifera* fraction 20%	Positive control	Negative control
Moringa oleifera fraction 80%	0,000*	0,000*	0,000*	0,000*	0,000*
Moringa oleifera fraction 60%		0,035*	0,000*	0,000*	0,000*
Moringa oleifera fraction 40%			0,000*	0,000*	0,000*
Positive control (NaOCl)			0,000*		0,000*

*Significant difference with a p-value < 0.05

Table 12. Kruskal–Wallis test on biofilm attachment degradation of mono-species *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Optical Density Median (Min - Max)	P value
Moringa oleifera fraction 80%	1,1275 (1,121 - 1,141)	0,000*
Moringa oleifera fraction 60%	1,595 (1,593 - 1,628)	
Moringa oleifera fraction 40%	1,8085 (1,721- 1,821)	
Moringa oleifera fraction 20%	2,142 (2,096- 2,157)	
Positive control (NaOCl)	0,3925 (0,356 - 0,452)	
Negative control (aquades)	2,2875 (2,243 - 2,295)	

*Significant difference with a p-value < 0.05

Table 13. Mann–Whitney post-hoc test on biofilm attachment degradation of mono species *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Moringa oleifera fraction 60%	*Moringa oleifera* fraction 40%	*Moringa oleifera* fraction 20%	Positive control	Negative control
Moringa oleifera fraction 80%	0,021*	0,021*	0,020*	0,021*	0,021*
Moringa oleifera fraction 60%		0,021*	0,020*	0,021*	0,021*
Moringa oleifera fraction 40%			0,020*	0,021*	0,021*
Moringa oleifera fraction 20%				0,020*	0,020*
Positive control (NaOCl)					0,021*

*Significant difference with a p-value < 0.05

Table 14. One-way ANOVA test on biofilm attachment degradation of dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Sum of Square	*Mean Square*	P value
Between groups	7,281	1,456	0,000*
Within groups	0,039	0,002	
Total	7,320		

*Significant difference with a p-value < 0.05

Table 15. Tukey post-hoc test on biofilm attachment degradation of dual-species of *Fusobacterium nucleatum* and *Staphylococcus aureus* by *Moringa oleifera* fraction.

	Moringa oleifera fraction 60%	*Moringa oleifera* fraction 40%	*Moringa oleifera* fraction 20%	Positive control	Negative control
Moringa oleifera fraction 80%	0,000*	0,000*	0,000*	0,000*	0,000*
Moringa oleifera fraction 60%		0,000*	0,000*	0,000*	0,000*
Moringa oleifera fraction 40%			0,000*	0,000*	0,000*
Positive control (NaOCl)			0,000*		0,000*

*Significant difference with a p-value < 0.05

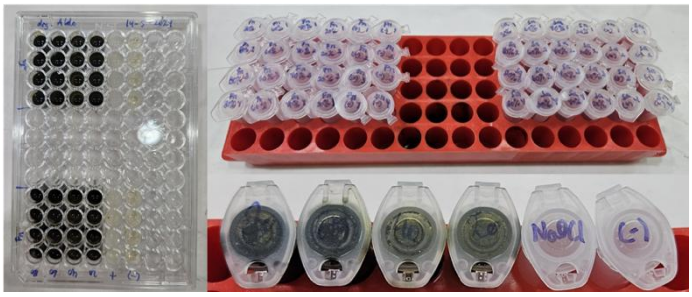

Figure 2. Biofilm attachment inhibition and degradation tests.

DISCUSSION

According to the study's findings on Tables 1-3, *Moringa oleifera* exhibits antibacterial properties against *Staphylococcus aureus* and *Fusobacterium nucleatum*. The data suggest that *Moringa oleifera* inhibits the growth of both bacterial species at a 20% concentration, which corresponds to its Minimum Inhibitory Concentration (MIC). The absence of bacterial colonies on BHI-A media, as observed in Figure 1, indicates that the *Moringa oleifera* fraction is capable of bactericidal activity against *Fusobacterium nucleatum* and *Staphylococcus aureus.* Therefore, the 80% concentration can be considered the Minimum Bactericidal Concentration (MBC) for both bacterial strains. These results are consistent with those reported by Amin et al. (2024), who also demonstrated the antibacterial efficacy of *Moringa oleifera* against *Fusobacterium nucleatum*, although their reported MIC and MBC values were 25% and 12.5%, respectively.[13]

Angestia et al. (2020) conducted a similar study to evaluate the antibacterial properties of *Moringa oleifera* against Staphylococcus aureus. Their research employed two distinct methods: particularly agar diffusion and microdilution. The microdilution showed a MIC value of > 1 µg/ml. The agar diffusion method supported these findings, showing inhibitory zones of 17.1 + 0.3 mm at 3.9 mg/ml, 18.85 + 0.05 mm at 7.8 mg/ml, and 20.55 + 0.25 mm at 16.6 grams/ml. The results demonstrate promising inhibitory activity of *Moringa oleifera* against Staphylococcus aureus.[21]

This study also demonstrated the antibiofilm properties of *Moringa oleifera*. The biofilm attachment of mono-species *Fusobacterium nucleatum*, mono-species *Staphylococcus aureus*, and dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* was effectively inhibited and degraded by the *Moringa oleifera* fraction. *Moringa oleifera* was more efficient against mono-species *Staphylococcus aureus* (40%) than mono-species *Fusobacterium nucleatum* (80%) and dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* (60%) when it came to inhibiting biofilm attachment, in accordance with Tables 4-9. However, based on Tables 10-15, *Moringa oleifera's* capacity to degrade biofilm attachment was similarly effective at an 80% concentration. Therefore, rather than disrupting biofilm attachment, the *Moringa oleifera* is more effective at preventing it (Figure 2).

Another study by Su-Kyung Jwa (2019) also investigated *Moringa oleifera's* antibiofilm properties. In this study, *Streptococcus mutans* was used to investigate the efficacy of *Moringa oleifera* extracts against cariogenic biofilm. The extracts were diluted with phosphate-buffered saline at a concentration of 50µg/mL and were added to a 96-well plate. The prepared bacterial suspension (20µL) was inoculated into the wells. The plate was incubated at 37°C underaerobic conditions for 24h. Bacterial growth was calculated by measuring OD at a wavelength of 660 nm using a spectrophotometer. *Moringa oleifera* significantly reduced bacterial growth at and above concentrations of 6.25 µg/mL and 25 µg/mL, respectively. This study showed that *Moringa oleifera* extracts have antimicrobial activities against cariogenic bacteria and biofilm.[15]

In addition, Amabye TK et al. (2016) conducted a study on the phytochemical and antibacterial activity of *Moringa oleifera.* Their study revealed that *Moringa oleifera* contains phytochemical constituents such as flavonoids, alkaloids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthrax quinones. The antibacterial activity of *Moringa oleifera* was also demonstrated against several bacteria namely *Staphylococcus aureus,* etc.[14]

The bioactive substances present in *Moringa oleifera* are essential to its antibacterial and antibiofilm properties. Among these are flavonoids, which comprise 5.42% and represent the most bioactive substances. Flavonoid bioactive compounds exert antibacterial effects by damaging bacterial membrane function, preventing biofilm formation and inhibiting virulence factors. Flavonoids bind to the phospholipids of the bacterial membrane, thereby disrupting the processes such as osmoregulation, respiration, transportation, biosynthesis, peptidoglycan cross-linking, and lipid biosynthesis, ultimately impairing bacterial metabolism and leading to cell death.[23-25]

Additionally, these flavonoid bioactive compounds help regulate immune cells, reduce inflammation, and act as antioxidants. Flavonoids prevent oxidative damage by reducing lipid peroxidation and free radicals. They mitigate inflammation by inhibiting inflammatory proteins (IL-1, IL-4, IL-10, and IL-13), protein kinases (COX, cyclooxygenase, LOX, lipoxygenase and PLA2, phospholipase A2), and associated transcription factors (NF-κB, GATA-3, and STAT-6). Flavonoid also modulate immune responses by stimulating immune cells (T cells, macrophages, PMN, and Th2 cells) and suppressing dendritic cells, histamine, prostaglandins, proinflammatory, and mast cell cytokines, in order to reduce excessive inflammation. Therefore, it appears that the presence of flavonoid bioactive compounds in *Moringa oleifera* contributes to bacterial elimination and biofilm inhibition, as demonstrated by the finding in Figure 1 and Figure 2.[24,25]

The limitation of this study is that the *Moringa oleifera* methanolic fraction was too dense and exhibited high viscosity, which made it difficult to determine the MIC. Therefore, it is suggested that future research focus on reducing viscosity. Furthermore, since this study aims to develop *Moringa oleifera* as an alternative irrigation material in endodontic treatment, achieving a lower viscosity is essential to access complex roots canal anatomy.

CONCLUSION

This study demonstrated that *Moringa oleifera* possesses effective antibacterial and antibiofilm properties against *Fusobacterium nucleatum* and *Staphylococcus aureus,* with its antibacterial activity observed at a concentration of 80%. The attachment of biofilms formed by mono-species *Fusobacterium nucleatum*, mono-species *Staphylococcus aureus*, and dual-species *Fusobacterium nucleatum* and *Staphylococcus aureus* was both inhibited and disrupted by the *Moringa oleifera* fraction. However, *Moringa oleifera* was more effective at inhibiting biofilm formation than disrupting established biofilm attachment. The implication of this study is that *Moringa oleifera* has potential for development as an alternative irrigant for endodontic treatment.

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