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ASSESSING THE ANTIBACTERIAL EFFICACY OF MORINGA OLEIFERA LEAF EXTRACT VERSUS SODIUM HYPOCHLORITE IN ROOT CANAL THERAPY: A COMPARATIVE STUDY

Yousra Samir Helmy[1]* *BDS,* Mai Hamdy Ragab[2]*PhD,* Yousra Samir Helmy[3]*PhD*

ABSTRACT

BACKGROUND: Root canal treatment aims to eliminate bacterial presence and debris from within the root canal system. **MATERIAL AND METHODS:** This study utilized sixty mandibular premolars with single roots, which underwent decoronation and root canal shaping. Post-sterilization, the specimens were inoculated with Enterococcus faecalis and incubated at 37°C for 48 hours. Subsequently, the specimens were segregated into six groups, differentiated by their irrigation solutions: 2.5% sodium hypochlorite, 5.25% sodium hypochlorite, a solution derived from Moringa oleifera leaves, a mixture of 2.5% sodium hypochlorite with M. oleifera leaf solution, a mixture of 5.25% sodium hypochlorite with M. oleifera leaf solution, and a control group treated with saline. Microbial sampling occurred pre-irrigation, post-irrigation, and seven days post-irrigation to assess bacterial reduction via colony-forming unit (CFU) counts.
RESULTS: ANOVA, or two-way repeated measures and Tukey's HSD revealed a notable decline in CFU/ml across all experimental groups after irrigation. Noteworthy is the significant microbial reduction observed in groups irrigated with sodium hypochlorite solutions combined with M. oleifera leaf extract compared to those irrigated with sodium hypochlorite alone. **CONCLUSION:** The findings suggest the potential of Moringa oleifera leaf extract as a viable endodontic irrigant, offering a significant antibacterial effect, particularly when used in conjunction with traditional sodium hypochlorite solutions.
KEYWORDS: Irrigant, Enterococcus faecalis, Moringa, Sodium hypochlorite

1. Assistant Professor, Department of Pediatric Dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University
2. Associate professor, Department of Endodontics, Faculty of Dentistry, Suez Canal University
3. Department of Pediatric Dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University

Corresponding authors:
Email: yousra_samir@dent.suez.edu.eg

INTRODUCTION

Root canal treatment aims to eliminate bacteria and debris from within the root canal system. The effectiveness of this therapy depends on various elements such as the appropriate instruments, cleaning procedures, and obturation techniques.[2] Even though removing infected tissue with instruments is an important step, it's not possible to get rid of all bacteria this way. Bacteria left behind in the root canal has the potential to spread infection in the pulp and surrounding tissues. [3] Thus, the primary objectives of root canal therapy are to eradicate bacteria from the root canal and to create conditions conducive to tissue regeneration and healing.[4] The complex shape of root canals, with their many branches, makes it hard to clean them thoroughly. This is why root canal treatment can sometimes fail, especially in deciduous teeth. If bacteria stay in the infected canals, the treatment won't be successful. [5, 6]

One strategy that has been suggested for cleaning the root canal system is the use of antibacterial irrigants in combination with mechanical instruments and dissolving infected dentin. This approach has the potential to remove a significant portion of the bacterial species that contaminate the canal and facilitate the drainage of purulent exudate. [7] Studies have identified a variety of bacterial pathogens associated with infected root canals, including Treponema denticola, Porphyromonas gingivalis, Enterococcus faecalis (E. faecalis), and fungal species. [8, 9] E. faecalis is a facultative anaerobic Gram-positive bacterium that is recognized as one of the most resistant endodontic pathogens, with a prevalence ranging from 29% to 77% in infected root canals .[10] The inherent antibiotic resistance of E. faecalis, along with its capacity to infiltrate deeply into dentinal tubules [11,12], and form biofilms, make it a persistent challenge and a common cause of root canal treatment failures.[13] Consequently, removing E. faecalis from the root canal system is difficult due to these characteristics.[14]

It is well accepted that sodium hypochlorite (NaOCl) is the best irrigation agent for root canals. Its potent antibacterial activity and efficient tissue-

dissolving ability have been demonstrated. [15–16] Its detrimental effects on the tissues surrounding the root apex, inability to eliminate the smear layer, and unpleasant taste and odor, especially problematic for young patients, represent notable drawbacks. [17, 18] Researchers are focusing on using herbal materials in endodontic therapy due to negative effects of NaOCl and the ongoing rise of antibiotic-resistant strains. [19] Herbal products are becoming more and more common in dental and medical practices these days because of their long shelf life, low toxicity, increased antibacterial activity, antioxidant, and anti-inflammatory qualities. [1] Numerous natural extracts, including Morinda citrifolia, green tea polyphenols, triphala, and neem, have been discussed in the literature for endodontic purposes. [20–21] The Moringa oleifera (M. oleifera), often referred to as the "drumstick" or "horseradish" tree, originates from India and belongs to the Moringaceae family. [22] It is highly nutritious and has a variety of medical applications. [23] It has been utilized in the management of conditions such as malaria, myeloma, malnutrition, and colon cancer. [24] The presence of many bactericidal mechanisms in the form of flavonoids, saponins, phenolics, alkaloids, tannins, and triterpenoids accounts for the antibacterial activity of M. oleifera leaf extract. [25] Moreover, a number of investigations have revealed that M. oleifera leaf extract significantly reduced the growth of E. faecalis. [26–27] A new endodontic irrigant that performs as well as NaOCl but is less dangerous and more favored by patients is still being researched. Consequently, this study aimed to furnish critical insights evaluating and comparing the antibacterial efficacy of M. oleifera leaf extract, NaOCl, and their combinations as intracanal irrigants against E. faecalis. The null hypothesis of this study that there was no significant reduction in E. faecalis CFU/ml after intra-canal irrigation with M. oleifera leaf extract, NaOCl, and their combinations.

MATERIALS AND METHODS

Ethical approval

Faculty of Dentistry's Research Ethics Committee (REC) No. 665 / 2023 gave its clearance for the in-vitro study to be conducted. That was entirely compliant with the 2008 version of the Helsinki Declaration of the World Medical Association.

Sample Size

G-power version 3.1, a statistical power analysis tool, was employed to determine the sample size. [28] There was a 95% power and a 0.05 significance level. In order to identify the 0.25 effect size at a 90% power (1-β=0.90) at a significant probability level of $p < 0.05$ and a partial eta squared of 0.06, a total sample size of 60 samples was sufficient. [28]

Specimen Collection and Preparation

For the investigation, sixty single-root, unidentified, mandibular premolars were selected, which were extracted for orthodontic or periodontal reasons and exhibited fully developed root apices. Teeth with prior endodontic procedures, visible cracks, fractures, curved roots, root decay, and root resorption were excluded. To ensure a single open canal in each tooth, radiographic evaluations were performed. The teeth were meticulously cleaned to remove any calculus and soft tissue remnants and preserved in 0.9% saline solution to avert desiccation until they were prepared for instrumentation. Each tooth underwent decoronation to a standard length of about 14.1 ± 1 mm employing a diamond disc with constant water irrigation, ensuring a uniform baseline for instrumentation. [29] The working length for each root canal was ascertained by deducting 1 mm from the point where a #15 k-file tip was discernible at the apical foramen. A glide path for each canal was established using stainless steel k-files up to size #25. Canal shaping was then performed using the ProTaper Next system by Dentsply Maillefer up to an X4 file size, with irrigation carried out using 5 ml of 2.5% NaOCl delivered through a 23-gauge needle in a disposable syringe. The apical foramen was sealed with a light-cured composite resin, and the prepared root specimens were subjected to sterilization via autoclaving at 121°C for 20 minutes in glass tubes filled with 3 ml of Brain Heart Infusion broth, ensuring complete sterility. Following this, the tubes were incubated at 37°C for 24 hours. [30]

Collection and Processing of Plant Material

Fresh M. oleifera leaves were harvested from a local garden center, meticulously washed under running water to eliminate debris and foreign particles, followed by immersion in purified, sterile water. Subsequently, the foliage was left to air-dry at ambient conditions throughout the night. To ensure complete dehydration, the leaves were then placed in a heated air oven at 45°C until all moisture was eradicated. The dehydrated leaves were ground, combined, and kept at 4°C in airtight receptacles until needed. One M. oleifera plant source was used for the entire experiment. [31]

Preparation of *M. oleifera* Leaves Extract

Upon adding 200 grams of M. oleifera leaf powder into 1,000 milliliters of 60% ethanol, the mixture was allowed to sit at ambient temperature for a duration of 48 hours. Following this period, the mixture underwent a homogenization process, facilitated by magnetic stirring at a velocity of 800 revolutions per minute for 4 hours. This step was crucial for ensuring the optimal extraction of active ingredients, after which the solution was refrigerated at 4°C for 24 hours to further facilitate the separation of active compounds. [32, 33] Subsequent to initial filtration through muslin cloth, further purification was achieved using Whatman

No. 1 filter paper. The filtrate was then subjected to a controlled environment at 37°C for several days, permitting the gradual evaporation of the solvent, thereby concentrating the extract to a final volume of 100 ml. This concentrated, 100% pure extract was meticulously stored under sterile conditions in sealed containers until required for use. [34]

Preparation of *E. faecalis* Suspension

A strain of E. faecalis (ATCC 29212) was cultured for 24 hours at 37°C on a bile esculin agar plate. After cultivation on solid media, individual colonies were transferred to BHI broth. The optical density of these suspensions was standardized to approximately 1.5 x 10^8 colony-forming units (CFU)/ml by spectrophotometrically measuring their turbidity against a 0.5 McFarland standard.[35]

Inoculation of Root Canals

Two milliliters of sterile BHI broth were removed from the previously autoclaved tubes containing root specimens, and replaced by 2 ml of E. faecalis suspension. Following their secure sealing, these tubes were incubated at a temperature of 37°C for a duration of 48 hours.[36]

Sample randomization and grouping:

Random Assignment: Each specimen, labeled 1 through 60 based on the type of irrigation solution utilized, was allocated into one of six categories (each consisting of 10 samples) through the use of the randomization tool available at www.randomizer.org.

- Group 1 was treated with 2.5% sodium hypochlorite (NaOCl).
- Group 2 received 5.25% sodium hypochlorite.
- Group 3 was administered a 100% extract from M. oleifera leaves.
- Group 4 involved a combination of 2.5% NaOCl and 100% M. oleifera leaf extract.
- Group 5 employed a mixture of 5.25% NaOCl and 100% M. oleifera leaf extract.
- Group 6, serving as the control, utilized 0.9% isotonic saline.

Microbial Sampling and Bacterial Counting

After the incubation phase, each root was meticulously extracted from its tube in a sterile environment. To remove any loosely attached bacteria and residual culture media, 5 milliliters of sterile physiological saline were employed for irrigation. Immediately following the incubation yet before applying the test irrigants, a sterile, dry paper point size 40 was utilized for the initial bacterial sampling (S1). This paper point was left within the root canal for a duration of five minutes, thereafter, transferred to a sterile vial containing one milliliter of sterile saline, and then subjected to vortexing for thirty seconds. For bacterial quantification, each specimen underwent serial dilution four times (102, 103, 104, and 105). The number of proliferating E. faecalis colonies was tallied and adjusted according to the dilution rate to determine the CFU-1/ml concentration. Subsequently, bile esculin agar plates were cultivated with 10µl aliquots of every dilution and incubated for an entire day at 37°C.[37, 38]

Antibacterial Activity of the Experimental Irrigants

For each specimen, 5 mL of the designated experimental irrigation solution, corresponding to its assigned group, was introduced into the canal and allowed to remain for ten minutes. The irrigation needle was positioned 4 mm short of the determined working length. Following this, a concluding flush was administered using 4 mL of sterile physiological saline. Subsequent to these steps, a second set of bacterial cultures (S2) was collected to determine the colony-forming units per milliliter (CFU-2/ml), employing the previously described procedure. A third bacterial sample (S3) was collected seven days later to assess the CFU-1/ml value.[39]

Statistical Analysis

The software utilized for data gathering and management was Microsoft Excel® (Version 365). To check if the data were normal and to identify if they were parametric or nonparametric, Shapiro-Wilk was utilized. Data were analyzed for both graphical and numerical descriptive statistical descriptions. ANOVA, or two-way repeated measures, was used to evaluate differences at significance levels of 0.05. Tukey's HSD (THSD) was used to compare treatment subgroups after an ANOVA. SPSS® (Statistical Package for the Social Sciences, version 22) was used to analyze the data. Data were collected and handled using Microsoft Excel® (Version 365). Shapiro-Wilk was used to test the normality of the data and determine whether the data were parametric or nonparametric. Both graphical and numerical descriptions of the data were obtained by descriptive statistical analysis. Differences were assessed using two-way repeated measures (ANOVA) at significance levels of 0.05. ANOVA were followed by Tukey's HSD (THSD) to compare between treatment subgroups. Data analyses were performed using SPSS® (Statistical Package for the Social Sciences- ver. 22).

RESULTS

Concerning the treatment groups through 3 stages, differences in bacterial count were investigated in 5 treatment groups as well as, saline group (control). Overall, there were highly significant differences separately between groups (p<0.001), time, and the combination between them (Table 1, Figure 1).

During the pre-intervention stage, the first bacterial sampling (S1) results show slightly significant differences in bacterial count between the 6 different sample groups (57.4±4.79, 57.5±3.92, 57.9±3.87, 58.9 ±5.22, 62.1±4.20 and 63.1±4.31) respectively (Table 1, Figure 1).

After the treatment phase, a subsequent bacterial culture (S2) was obtained following the application of the specified irrigants, which included Sodium hypochlorite at both 2.5% and 5.25% concentrations, pure M oleifera leaf extract, and their blends with 2.5% and 5.25% NaOCl, as well as, saline solution, respectively. Analysis revealed a marked decrease in bacterial levels in contrast to those recorded before the intervention, though without notable variances among the experimental groups. Conversely, the group treated with saline solution (control group) did not exhibit a discernible change in bacterial levels before and after the intervention (Table 1, Figure 1).

After 7 days of intervention, the third bacterial sampling (S3) was tested and the results showed that irrigant groups (1, 2, 3, 4, 5) continued showing an enhancement in the antibacterial efficiency when compared with the control group which revealed high bacterial count (Table 1, Figure 1).

Inside each group, significant differences through 3 stages of investigation were detected (p<0.001). For irrigant groups (1, 2, 3, 4, 5), an enhancement in antibacterial efficiency was obtained when comparing both pre- and post- intervention samples. However, post-7-days of intervention, antibacterial efficiency decreased slightly but with maintaining of high efficiency against bacterial activity. As for group 6 the saline (control) group, there was an increase in the bacterial count which signifies a clear decrease in the antibacterial efficiency through time (Table 1, Figure 1).

Table (1). Effect of different treatment and control group through three stages (pre-, post and post-7-days) on bacterial count.

Fig. (1). Bacterial count in different treatment and control group through three stages (pre-, post and post-7-days)

Correlation coefficient and regression analysis:

Finding the association between the number of bacteria and the timing interval or stages of treatment showed that the timing length of treatment and the number of bacteria had negative moderate values of Pearson's correlation coefficient. On the other hand, the timing duration and control group showed a very positive Pearson's correlation coefficient (Table 2). By applying regression analysis, negative relationship between bacterial count and timing were obtained in treatment groups. Although, positive relationship was detected in control group (Figure 2). Accordingly, time of investigation showed an inverse correlation with bacterial count of NaOCl 2.5%, NaOCl 5.25%, Moringa Leaf extract, Moringa Leaf extract + NaOCl 2.5% and Moringa Leaf extract +NaOCl 5.25%, and positively with bacterial count in saline solution. The heatmap is based on Pearson's correlation (figure 3).

Table (2). *Pearson's correlation coefficient* (r) between bacterial count and different timing (pre-, post and post-7-days)

Fig. (3). Heatmap presenting the interrelationships between variables, red for negative correlation, blue for positive correlation, shaded boxes for significant correlation.

Table (1). Effect of different treatment and control group through three stages (pre-, post and post-7-days) on bacterial count.

Group	Bacterial count CFU, x 10 4									Sign.
	Pre			Post			Post-7-days			
	Mean	SD	THSD	Mean	SD	THSD	Mean	SD	THSD	
2.5% NaOCl	**57.4**	4.79	C	**0.43**	0.46	d	**3.14**	0.50	D	<0.001***
5.25% NaOCl	**57.5**	3.92	C	**0.91**	0.42	d	**2.98**	0.25	D	<0.001***
Moringa oleifera leaf	**57.9**	3.87	C	**1.91**	0.14	d	**4.44**	0.31	D	<0.001***
M+2.5% NaOCl	**58.9**	5.22	bc	**0.05**	0.03	d	**1.53**	0.28	D	<0.001***
M+5.25% NaOCl	**62.1**	4.20	bc	**0.11**	0.07	d	**1.26**	0.17	D	<0.001***
Saline	**63.1**	4.31	B	**63.10**	4.31	b	**87.00**	6.96	A	<0.001***
Sign.	0.013*			<0.001***			<0.001***			
Two-way repeated measures ANOVA										
Corrected model	<0.001***									
Group	<0.001***									
Time	<0.001***									
Group x Time	<0.001***									

* Significant at p<0.05
*** significant at p<0.001
Multiple comparison obtained using Tukey's. HSD post hoc test (THSD)
SD= Standard deviation.

Table (2). Pearson correlation coefficient (r) between bacterial count and different timing (pre-, post and post-7-days)

Group	Correlation with time	
	R	P
2.5% NaOCl.	-0.472	0.008**
5.25% NaOCl.	-0.472	0.008**
Moringa oleifera leaf	-0.472	0.008**
M+2.5% NaOCl	-0.472	0.008**
M+5.25% NaOCl	-0.472	0.008**
Saline	0.711	<.001***

** significant at p<0.01, *** significant at p<0.001

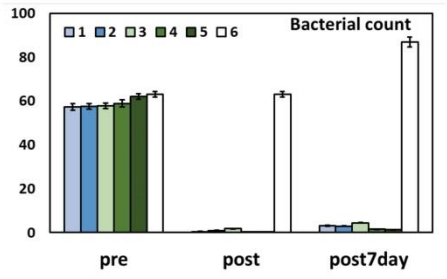

Fig. (1). Bacterial count in different treatment and control group through three stages (pre-, post and post-7-days)

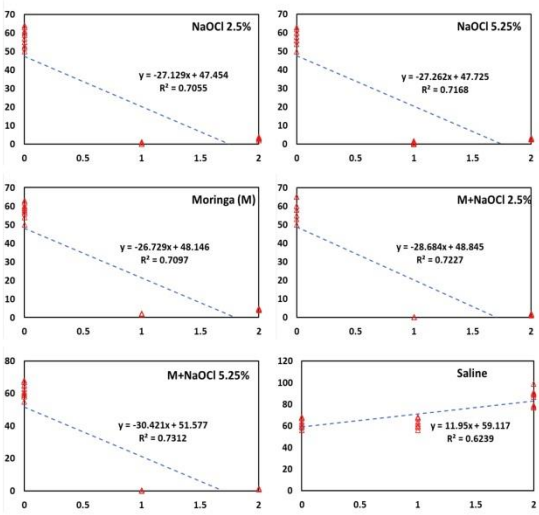

Fig. (2). Regression analysis of different treatment on timing (pre-, post and post-7-days)

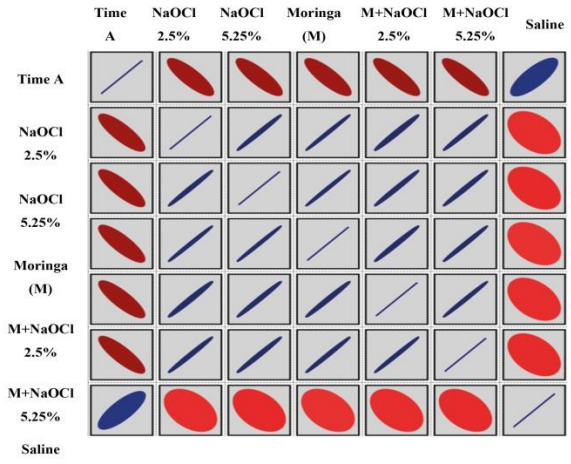

Fig. (3). Heatmap presenting the interrelationships between variables, red for negative correlation, blue for positive correlation, shaded boxes for significant correlation.

DISCUSSION

This study aimed to assess the antibacterial effectiveness of different irrigation solutions against Enterococcus faecalis (E. faecalis). Findings indicated notable variations in colony-forming units (CFU/ml) among the groups under investigation, leading to the rejection of the null hypothesis. E. faecalis was selected as the focus of this research due to its clinical significance and documented resistance to chemomechanical treatment protocols. This is because its thick cell wall layer, peptidoglycan, can block many antibacterial agents. [16, 30, 31] Therefore, finding new antibacterial irrigants that are effective against E. faecalis is important for improving the prognosis of endodontic treatments. [28] The strain of E. faecalis used in this study was ATCC 29212, which has been used in previous studies. [15, 20, 32, 33] This research examined the antibacterial properties of several substances, including 2.5% sodium hypochlorite (NaOCl), 5.25% NaOCl, pure M. oleifera leaf extract, a mixture of the leaf extract with 2.5% NaOCl, a combination of the leaf extract with 5.25% NaOCl, and saline solution serving as the control group.

The current gold standard for irrigating and disinfecting root canals is sodium hypochlorite (NaOCl). However, it can have harmful side effects, such as damaging tissues around the tooth and having an unpleasant taste and smell, especially at higher concentrations.[40] In this investigation, the efficacy of M. oleifera leaf extract as a viable substitute for sodium hypochlorite (NaOCl) in root canal irrigation was explored. Prior research studied the antibacterial and antifungal activities of aqueous extracts of plant M. oleifera in comparison to chlorohexidene gluconate and deionized water, has demonstrated the antibacterial capabilities of M. oleifera leaves [27]. The study focused on comparing the antibacterial effectiveness of three distinct solutions: 2.5% NaOCl, 5.25% NaOCl, and 100% M. oleifera leaf extract. They also tested two combinations of NaOCl and Moringa extract, and a saline solution as a control.

The findings indicated that M. oleifera leaf extract effectively eradicated bacteria, outperforming sodium hypochlorite (NaOCl) in some cases. The pure Moringa extract proved to be the most potent, outperforming both the 5.25% and 2.5% NaOCl solutions. While the mixtures of NaOCl and Moringa extract showed antibacterial activity, they did not match the effectiveness of the pure Moringa extract. These results indicate that M. oleifera leaf extract holds potential as a viable substitute for NaOCl in root canal irrigation due to its bacterial elimination capabilities without NaOCl's adverse side effects. Nonetheless, further investigation is necessary to verify these preliminary findings and to ascertain the most effective concentration of Moringa extract for root canal irrigation purposes. The objective of this research was to assess the

antimicrobial effectiveness of M. oleifera leaf extract in relation to traditional root canal irrigation substances. They chose M. oleifera because it's been shown to have antibacterial properties. The standard solution they used was sodium hypochlorite (NaOCl), which is commonly used in root canals but can have unpleasant side effects.

The researchers tested five different solutions on bacteria. The solutions were: A 60% ethanol extract of M. oleifera leaves, two solutions with both NaOCl and the Moringa extract, in different concentrations, two concentrations of NaOCl on its own and a saline solution, which was the control.

The investigation demonstrated the bactericidal capacity of M. oleifera leaf extract, showcasing superior effectiveness in certain scenarios compared to sodium hypochlorite (NaOCl). Among the solutions tested, the pure Moringa extract emerged as a potent antibacterial agent, surpassing both the 5.25% and 2.5% concentrations of NaOCl. The synergistic solutions of NaOCl and Moringa extract also displayed antibacterial properties, as they were more potent than the pure Moringa extract solution alone.

This evidence positions M. oleifera leaf extract as a potential alternative to NaOCl in the context of root canal irrigation, given its efficacy in bacterial eradication without the adverse effects associated with NaOCl. Nevertheless, additional research is imperative to corroborate these preliminary outcomes and identify the optimal Moringa extract concentration for root canal irrigation application.

Regarding the treatment groups through three stages, variations in the number of bacteria were examined in five treatment groups in addition to the saline group (control). Significant variations were observed across different metrics, including time, the interaction between groups, and the groups independently, with a statistical significance of $p<0.001$. This aligns with the results presented by Alharby et al.[37], which encompassed a range of treatments: 2.5% sodium hypochlorite (NaOCl), 0.1% octenidine dihydrochloride (OCT), pure Moringa oleifera leaf extract, a blend of pure Moringa oleifera leaf extract with 1.25% NaOCl, pure Moringa oleifera leaf extract with 0.1% OCT, and 0.9% saline solution as the control group.

Following the findings of Berber et al., [41] who observed that a 10-minute exposure to NaOCl effectively eradicated E. faecalis strains, a similar contact time was maintained for all irrigants within the canals. Prior to collecting the second bacterial sample (S2), a final flush was performed using sterile saline. [8, 19, 32, 41]

During the post-treatment phase, the second set of bacterial cultures (S2) was obtained following the irrigation process. The results, which showed a significant decrease in the bacterial count when compared with the pre-intervention bacterial count across all groups, but with no significant changes across the treated groups (1, 2, 3, 4, 5), demonstrated that the assigned irrigant groups (1, 2, 3, 4, and 5) increased the antibacterial efficiency.

The results align with research by Alharby et al.[37] in 2023, demonstrating that E. faecalis present in root canals can be effectively eradicated using M. oleifera extract solutions at concentrations of 75% and 100%. Additionally, these findings concur with those of Nugroho et al. [42] who evaluated the antimicrobial properties of Moringa leaf nanoparticle paste against E. faecalis, comparing its effectiveness to different concentrations of calcium hydroxide paste. They came to the conclusion that M. oleifera was highly successful in preventing E. faecalis from growing.

Alkaloids, saponins, tannins, and flavonoids are included in the extract, which has demonstrated antibacterial action against E. faecalis, according to Wang et al. [4], who examined the phytochemical content of moringa leaves. This outcome could be the consequence of saponins' interference with the permeability of bacterial cell walls, which is thought to be the source of their antibacterial action. On the other hand, denaturation of proteins and nucleic acids results from the disruption of the tertiary structure of proteins by flavonoid chemicals. This denaturation disrupts the metabolism and physiological processes of bacteria and induces protein coagulation. Flavonoid Inhibits the use of oxygen by bacteria so that energy metabolism is inhibited. It also inhibits cell membrane synthesis and aggregate effects on all bacterial cells. [35]

Tannin chemicals have the ability to reduce cell walls and prevent the synthesis of proteins needed for cell wall formation, which can cause permeability issues and ultimately lead to cell death. [16] The antibacterial activity of alkaloids stems from their ability to disrupt the peptidoglycan elements of bacterial cells. This interference hampers the synthesis of complete cell wall structures, leading to bacterial cell demise. [4] Additionally, when terpenoid compounds compromise the bacteria's outer cell membrane, agents that reduce the cell wall's permeability can infiltrate. This invasion deprives the bacterial cell of essential nutrients, inhibiting its proliferation. [22]

While the bacterial cell count did not significantly differ between the treated groups before and after the intervention, the superior outcome of NaOCL against herbal Moringa extract was consistent with several research that employed various herbal extracts. In contrast to NaOCl, Goud et al. [19] investigated the effects of three herbal irrigants on E. faecalis bacteria: green tea, miswak, and chamomile. They came to the conclusion that compared to other herbal irrigants, NaOCl had a stronger antibacterial action against E. Faecalis.

However, there was no discernible change in the bacterial count between the pre- and post-intervention samples in the saline irrigant (control group) samples. This outcome is consistent with that of Murray et al.'s study [21] and, El Mansy et al. [43] who investigated, the efficacy of two standard irrigation solutions, 0.5% sodium hypochlorite (NaOCl) serving as a positive control and 0.9% saline solution as a negative control, was evaluated against the extracts of moringa, costus, and star anise and they found that All the Herbal irrigants tested in this study demonstrated an agreeable antibacterial effect against both E. faecalis when used in root canals of primary molars.

When the third bacterial sampling (S3) was evaluated after the intervention lasted for seven days, the results showed that the irrigant groups (1, 2, 3, 4, and 5) kept demonstrating an improvement in the antibacterial efficiency when compared to the control group, which had a high bacterial count.

Inside each group, significant differences through 3 stages of investigation were detected ($p<0.001$). For irrigant groups (1, 2, 3, 4, 5), an enhancement in antibacterial efficiency was obtained when comparing both pre- and post- intervention samples. However, post-7-days of intervention, antibacterial efficiency decreased slightly but with maintaining of high efficiency against bacterial activity. This result is in accordance with Alharby et al, [37] who observed a significant decrease in the colony-forming units per milliliter (CFU/ml) of E. faecalis following the use of irrigants, highlighting a noteworthy reduction. In contrast, for Group 6—the saline (control) group—an escalation in bacterial counts was noted, indicating a discernible reduction in antimicrobial efficacy over time.

Limitations and recommendations:

One limitation of this research is its focus on using uniform, single-rooted teeth with only one canal, which does not accurately represent the diverse anatomical complexities seen in both permanent and deciduous teeth, including the presence of accessory canals, root bifurcations, or teeth with multiple canals and roots. Moreover, extracting bacterial samples from areas beyond the primary canal, such as dentinal tubules, poses an additional hurdle. The study's in vitro setup also fails to account for the physiological reactions and the compatibility of the tested extract with living tissues. To address the limitations of NaOCl, such as its cytotoxic effects, unpleasant odor, burning sensation, potential for allergic reactions, and ability to damage permanent tooth follicles, after prolonged incubation times, the study recommends more research be done on the materials that were examined and their combinations, as well as, longer-term follow-up to assess the persistence of the antibacterial effect and evaluate the long-term success of the irrigating material. This study advocates for the investigation of root canal irrigants beyond sodium hypochlorite (NaOCl) and highlights the promise of herbal remedies, such as the extract from M. oleifera leaves, in the root canal treatment of primary teeth in children.

CONCLUSION

The investigation revealed a marked decrease in bacterial levels post-irrigation with both concentrations of Sodium hypochlorite (2.5% and 5.25%), pure M. oleifera leaf extract, and their blends with 2.5% NaOCl and 5.25% NaOCl, compared to the initial bacterial counts. Although there was a decrease, the differences in results across the different treatment groups were not statistically significant. Additionally, even against the backdrop of elevated bacterial counts in the control group, a discernible enhancement in antimicrobial effectiveness was noted after a period of 7 days. The findings suggest the potential of M. oleifera leaf extract as a viable endodontic irrigant, offering a significant antibacterial effect, particularly when used in conjunction with traditional sodium hypochlorite solutions.

Declaration:

Competing interests: The authors declare no conflict of interest.

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