Evaluation of the Antibacterial Effect of Miswak and Neem as Root Canal Irrigant on Enterococcus Faecalis of Primary Anterior Teeth

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Abstract

Background

Root canal management is a necessary procedure because caries spread quickly in deciduous teeth, causing pulpal damage by contamination of the pulpal tissue by bacteria and their toxins. The search for a superior root canal irrigant is ongoing due to the drawbacks of conventional root canal irrigants. Chewing sticks made from Salvadora persica (Miswak), a popular oral hygiene product, have a variety of biological qualities.

Aim: to evaluate the antibacterial effect of 12.5% miswak, and 7.5% Neem extracts as novel irrigant solutions in comparison with 0.2% chlorhexidine irrigant solution against Enterococcus Faecalis microorganisms in primary anterior single-rooted teeth.

Methods: A total of forty-five extracted single-rooted primary anterior teeth were used in this study, the teeth were taken from children patients (between 2 to 8 years) who lost those teeth due to trauma that resulted in avulsion or intrusion or extraction of primary anterior teeth or due to delayed exfoliation of primary anterior teeth. This study was categorized into three groups; the first group; 12.5% miswak extract, the second group; 7.5% Neem extract as a novel irrigant solution as the "study group", while, the third group; 0.2% chlorhexidine irrigant solution as a "control group".

Results: all tested irrigant solutions (Miswak, Neem, chlorhexidine) showed a statistically significant antibacterial effect against Enterococcus Faecalis microorganisms. Also, the results of this study exposed a statistically significant difference against Enterococcus Faecalis microorganisms among all tested irrigant solutions. The results showed that the use of 0.2% chlorhexidine irrigant solution showed a statistically significant effect in reducing the Enterococcus Faecalis microorganisms count when compared to 12.5% miswak and 7.5% Neem extracts. Moreover, the results of this study exposed that 7.5% Neem extract had a statistically higher significant antibacterial effect against Enterococcus Faecalis microorganisms when compared to 12.5% Miswak

Conclusion: 12.5% Miswak and 7.5% Neem extracts presented significant antibacterial effect against Enterococcus Faecalis microorganisms and can be used as effective alternative irrigant solutions in primary teeth. Chlorhexidine irrigant solution is more effective against Enterococcus Faecalis microorganisms when compared to 12.5% Miswak and 7.5% Neem extracts as irrigant solutions in primary anterior teeth. Chlorhexidine irrigant solution is still the gold standard irrigant solution in primary anterior teeth.

Keywords: Antibacterial Effect, Miswak, Neem, Root Canal Irrigant, Enterococcus Faecalis, Primary Anterior Teeth

INTRODUCTION

The maintenance of arch length, the optimum function of speech and mastication, the optimal development of occlusion, and the preservation of deciduous teeth are all necessary for the oral tissues to remain in excellent health **(1).** Root canal management is a necessary procedure because caries spread quickly in deciduous teeth, causing pulpal damage by contamination of the pulpal tissue by bacteria and their toxins **(2).**

Due to the vast gaps in the bone marrow, the weak bone trabeculae, and the close proximity of the developing buds of permanent teeth to the roots of primary teeth, the infection spreads quickly in primary teeth **(3).** Because of their understanding of the complexity of root canal systems and the microbial flora of baby teeth, dentists are able to use efficient antibacterial agents during pulpectomy treatments **(4).**

It is more crucial to use irrigation solutions along with mechanical instruments in order to clean the root canal system because proper irrigation of the root canal system with chemical agents at the time of mechanical cleaning will remove soft and hard tissue remnants from various parts of the root canal system that are inaccessible by instruments in comparison to permanent teeth **(3, 5).** These characteristics include the fact that primary teeth have root canals that are shaped like ribbons and have more accessory canals, foramina, and porosities on the pulpal floor **(3, 4).**

The most frequent bacteria cultivated from unsuccessful root canals that require retreatment as well as from initial endodontic infection cases is *Enterococcus faecalis* (*E. faecalis*) (4). It is a facultative, gram-positive anaerobic organism that develops by the formation of a biofilm, is resistant to chemical and mechanical cleaning, and is capable of withstanding root canal medicine and obturation. If left untreated, it may cause the root canal procedure to fail **(6).**

Sodium hypochlorite (NaOCl), hydrogen peroxide (H2O2), chlorhexidine (CHX), and normal saline solution (NSS) are the most often utilized intracanal irrigation solutions (**7**). By oxidizing the sulfide group in the bacterial enzymatic system, NaOCl prevents the metabolism of bacteria **(6).** Sodium hypochlorite has equivalent antibacterial activity at doses of 0.5% and 5% **(8).** In terms of reducing microbial flora, CHX is more effective than NaOCl and is accessible in concentrations of 0.2%, 1%, and 2% **(9).** By adding 0.2% CHX, a negative bacterial culture can be produced in 30 to 1 minute. In the root canal, extensive irrigation greatly reduces bacterial colonies **(8, 9).**

In addition to being renewable in nature, using herbal extracts in endodontics is more cost-effective than using chemical ones **(3, 10).** *Neem*, or *Zadirachta indica*, is utilized as a potent root canal irrigation agent because of its potent antiviral, antifungal, antibacterial, and anticancer properties. Neem extract is also used to treat gingivitis and tooth plaque **(8, 11, 12).**

Chewing sticks made from *Salvadora persica* (*Miswak*), a popular oral hygiene product, have a variety of biological qualities **(5, 11).** Numerous chemicals, including cyanogenic glycoside and benzyl-isothiocyanate, as well as high concentrations of sodium chloride (NaCl) and potassium chloride (KCl), salvadourea, salvadorine, saponins, tannins, vitamin C, silica, and resin, are thought to be responsible for its antimicrobial and cleaning properties **(13).**

The search for a superior root canal irrigant was ongoing due to the drawbacks of conventional root canal irrigants. Therefore, the purpose of this study is to compare the effects of chlorhexidine solution with Neem and Miswak extracts as root canal irrigants on *Enterococcus faecalis* in primary anterior teeth.

MATERIALS AND METHODS

This study was conducted in pediatric dentistry clinic at Faculty of Dental Medicine of Al-Azhar University for Girls. Ethical approval was obtained from the research and ethical committee of the Faculty of Dental Medicine of Al-Azhar University for Girls Cairo-Egypt.

Sample size calculation

According to a previous study by Al Qarni et al. (2020) (11), the mean bacterial count (CFU) using Miswak was 4500±4100, in comparison to 500±400 using Neem and 1000±3000 using chemical root canal irrigant. A medium effect size of approximately 0.48 was expected. A total sample size of 45 (15 in each group) was sufficient to detect an effect size of 0.48, with a power (1-β error) of 0.8, using a two-sided hypothesis test and a significance level (α error) 0.05 for data. G power version 3.1.9.2 was used for sample size calculation.

A total of 45 tooth samples were used in this study, and were divided into three equal groups (n=15) according to the type of irrigant used as the following:

Group I:	Group II:	Group III:
• 15 samples were irrigated with 12.5% Miswak solution	• 15 samples were irrigated with 7.5% Neem solution	• 15 samples were irrigated with 0.2% CHX.

Inclusion criteria [13]**:**

All teeth were deciduous anterior teeth single rooted free of caries and have complete root formation. Teeth were taken from children patients aged between (2 to 8 years) and were extracted due to:

1- Trauma that results in avulsion (contradiction for replantation).
2- Trauma that results in an intrusion that requires extraction (to avoid ankylosis).
3- Delayed exfoliation of primary teeth that require an extraction (to allow eruptions of successors).
4- In serial extraction cases (e.g. upper C).

Exclusion criteria:

1- Permanent teeth.
2- Posterior teeth.
3- Multi-rooted teeth (mostly resorbed at the time of extraction).
4- Carious teeth were discarded.

Materials:

Material	Description	Manufacturer
Miswak	Miswak chewing sticks	Nawah Company, Almokattam Cairo, Egypt
Neem	Fresh Neem leaves	Nawah Company, Almokattam Cairo, Egypt
CHX	0.2% CHX solution	JK Dental Co., Cairo, Egypt.
Ringer's lactate	Ringer's lactate solution	Egypt Otsuka Pharmaceutical Co
NaOCl	1% NaOCl	Clorox Co, Egypt.
Thymol	Thymol solution	Sigma Chemical Co., Cairo, Egypt.

Two appropriate media were used to culture *E. Faecalis* microorganisms and to identify these microorganisms as follows;

Three suitable agar plate (Brain- Heart Infusion (BHI) broth) was used to identify *E. Faecalis* microorganisms' colonies **Figure (1)**

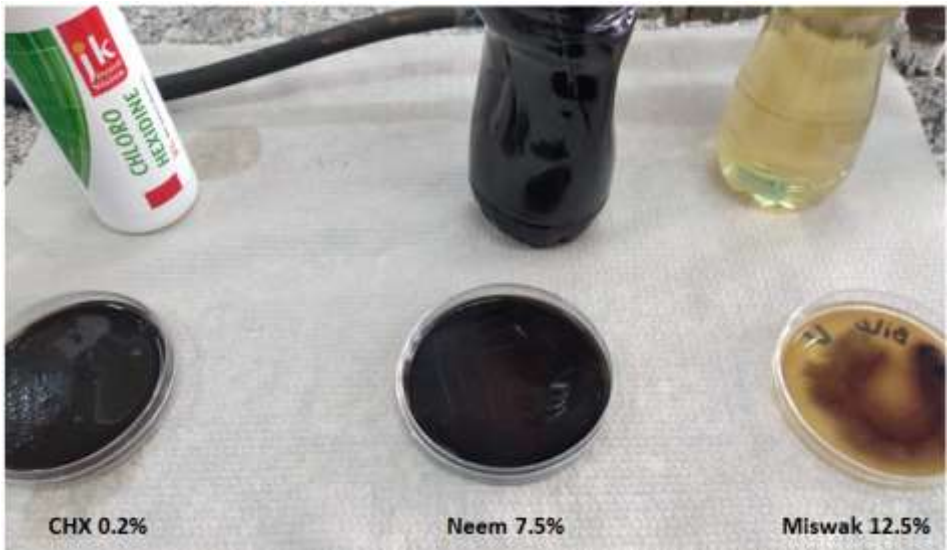

Figure (1): Photograph showing three suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies prepared with three irrigations (CHX, Neem and Miswak irrigations).

Methods:

Preparation of irrigating solutions: -

Preparation of miswak irrigating solution: -

Using a food processor, 800 grams of Miswak chewing sticks were reduced to powder. **Figure (2)**

In a clean, tightly closed bottle, 120 mL of 60% ethanol and 40 grams of powder were combined

After 3 days at room temperature, the mixture was filtered through quick filter paper. The extract was chilled in sterile screw-capped vials until needed after being dried in an incubator at 37°C.

Using 1 g of dried extract and 2.5 mL of Ringer's lactate, which had 100% concentration, a Miswak solution with a 12.5% concentration was created.

Figure (2): Photograph showing Miswak (*Salvadora Persica*).

Preparation of Neem irrigating solution:

After being gathered, weighed, and cleaned with distilled water, fresh Neem leaves were added to 50 mL of 100% ethanol and macerated for one to two minutes. **Figure (3)**

A muslin cloth was used to filter out any gritty debris from the mixture. With 25 mL of absolute ethanol in a glass flask at room temperature and left overnight, this procedure was repeated once more for the coarse residue. The fast filter paper was used to combine and filter these two extracts. The extract was reduced to a 25 mL solution and then placed in a water bath to eliminate the alcohol component.

107.5 grams of yield extract were produced after letting the resulting extract dry. One component of it was diluted by adding one part of sterile, distilled water. The resulting solution, which included 7.5% turmeric extract, was utilized for irrigation. The prepared solution was maintained in an airtight amber-colored container.

Figure (3): Photograph showing Neem (*Azardiractaindica*) used in this study.

Teeth preparation:

The teeth were scrubbed with hard brush on the outside to remove any soft or hard tissue debris, washed with water, sterilized with 1% NaOCl, and then preserved in a thymol solution until they were used. **Figure (4)**

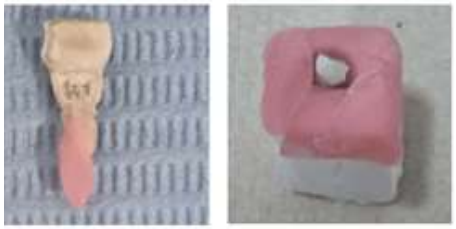

Figure (4): Photograph showing single-rooted primary anterior tooth used in this study.

Preparation of root canal samples:

A number 3.30 pear-shaped carbide bur in a high- speed hand-piece contra angle with water coolant was used for the removal of superficial caries and endodontic access opening

Coronal pulp amputation was done with a sterile large round carbide bur (size 2 or size 4).

Working length determination was determine by the visual eye with #8 K- file was introduced into the root canal, all canals were flushed with 5 ml normal saline and dried with absorbent paper points. Different three types of irrigations (CHX, Neem and Miswak irrigations) were

injected with a 30-guage insulin syringe into the root canals.

To ensure that the canals were open, an access cavity was created, and a K-file size 10, 15, 20 was inserted into each tooth's root canal until its tip could be seen at the apical foramen. After measuring the file's length (root length), the working length was estimated and documented by deducting 1 mm from each tooth's anatomical root length. The preparation was completed by using step-back technique with files of size 10 to 20 with recapitulation.

Rotary canal preparation by M Pro Rotary Pedo files was used by 16 mm length NiTi files driven by a hand piece at speed 300 rpm as recommended by the manufacturer. A total of two instruments (D1, El as recommended by the manufacturer) was used to prepare canals up to the determined working length or not less than 12mm (working length of the files) (Figure 11), each instrument was not used more than 4 times, after root canal was located using appropriate size K-file the D1 rotary file was first used followed by E1 rotary file to prepare the root canal.

Figure (5): Photograph showing the K-files, (a), (b) for initial working length, (c) hand file kit for manual instrumentations.

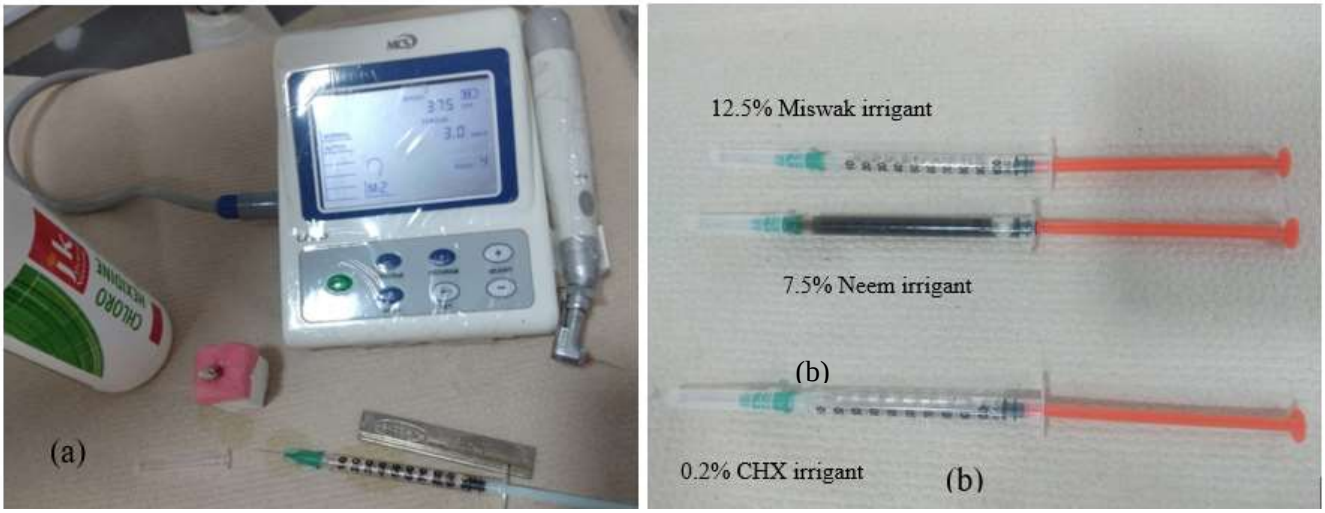

Figure (6): (a) Photograph showing root canal tooth preparation with 0.2% CHX irrigant

solution by pedo rotary files. (b) Photograph showing three different types of irrigant solutions (Miswak, Neem and CHX irrigants).

Sterilization of root canal samples:

After mechanical preparation and apical sealing of the root samples, the root samples were sterilized in an autoclave at 121-celsius degrees for 15 minutes before the inoculation of *E. faecalis*.

After that, the sterilization was verified and the culture media was checked for bacterial growth to make sure there are no microbes inside the canal.

Figure (7): Photograph showing sterilized single-rooted primary anterior tooth used in this study.

Bacterial sample contamination:

E. faecalis, after that were cultured in BHI broth at 37°C. In order to inject the broth suspension inside the root canal to the full working length, a 30-gauge needle was used. All specimens were immersed in broth at 37°C to allow bacterial growth. Medium (BHI broth) in this process was changed once a week. Two weeks period was chosen for the inoculation of bacteria, as a recognizable number of colonies of bacteria were produced after it.

After this process, root samples were removed from the bacterial culture. After that, the end of each tooth specimen was wiped with 3% NaOCl in order to disinfect the outside of the tooth before irrigation treatment.

Irrigation procedure:

After removal of root samples from the bacterial culture, all root samples were divided randomly into three groups, for the experimental procedure of irrigation materials. After that, the root canals of all samples were irrigated with normal saline (normal saline here acts as good media for inoculation of the content of canal) and then preoperative swap (first culture) was taken with a sterile paper point. Each group sample was irrigated with its irrigant solution (Miswak, Neem or CHX) and final irrigation with normal saline.

Each tooth in each group received 10 mL of the irrigating solution. For 5 minutes, the irrigation solution was left in the canal. Use of absorbent paper points allowed for the removal of extra irrigant from the canal.

Bacterial investigation:

The sterile paper points were used for taking post-irrigation samples (the second culture) from each root canal in each tested group. Both pre and post-sample (1^{st} and 2^{nd} culture), were kept in

Eppendorf tubes, which contain 1ml of normal saline.

Then the collected cultures were subjected to serial dilution (1:10), 1:100, and 1:1000) performed in saline. A culture of points on BHI Agar was used to identify bacterial infections. After anaerobically incubation for 2-3 days at 37 °C, a colony counter with a magnifying glass was used to count the number of colonies and they were expressed as the number of colony-forming units per ml (CFU/ml) of the sample. Gram staining was used using a light microscope and an oil immersion lens to analyze the morphology of bacterial growth.

Quantification of the number of colonies was carried out by multiplying the actual colony count by 10^2 or 10^3 (depending on the calibrated loop utilized).

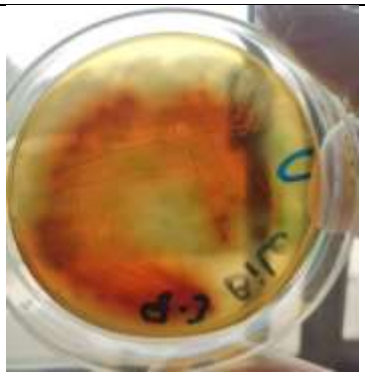

Figure (8): Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies before 12.5% Miswak irrigant solution.

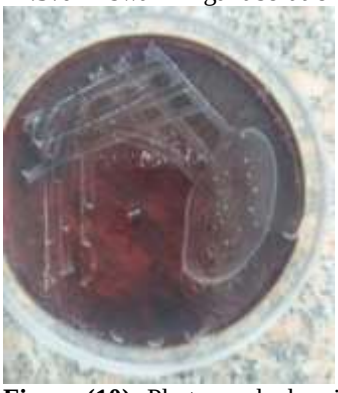

Figure (10): Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies before 7.5% Neem irrigant solution.

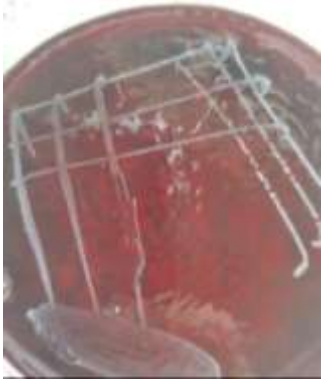

Figure (12): Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies before

Figure (9): Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies after 12.5% Miswak irrigant solution in bacterial incubator.

Figure (11): Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies after 7.5% Neem irrigant solution in bacterial incubator.

0.2% CHX irrigant solution.	 **Figure (13):** Photograph showing one suitable agar plate (BHI Agar) was used to count E. Faecalis microorganisms' colonies after 0.2% CHX irrigant solution in bacterial incubator.

Statistical Analysis:

Data were collected, tabulated, and statistically analyzed using SPSS ® statistics Version 20. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. Numerical data were described as mean and standard deviation. F-test (ANOVA) for normally distributed quantitative variables, to compare between more than two groups. A dependent *t*-test was used to compare sample means for quantitative data with normal distribution. The level of significance was set at $P<0.05$. All tests were two-tailed.

RESULTS:

The involved root canals among the studied groups were compared regarding the ***E. faecalis*** baseline count and represented as CFU/mL, and then were statistically analyzed using **One-way ANOVA (Figure 26)**.

The statistical analysis results of the ***E. faecalis*** baseline count (CFU/mL), which showed mean ± SD of the involved root canals among the studied groups were summarized in (**Table 1**) and graphically represented in (**Figure 15**).

There was no statistically significant difference in ***E. faecalis*** baseline count (CFU/ml) between the involved root canals among the studied groups with a P-value of (P= 0.9280) as indicated by the **One-way ANOVA test**.

The involved root canals that received Miswak irrigant solution showed a higher (mean ± SD) ***E. faecalis*** count (3.59±0.26) followed by the root canals that received Neem irrigant solution (3.58±0.22). However, the involved root canals that received CHX irrigant solution showed a lower (mean ± SD) ***E. faecalis*** count (3.56±0.22).

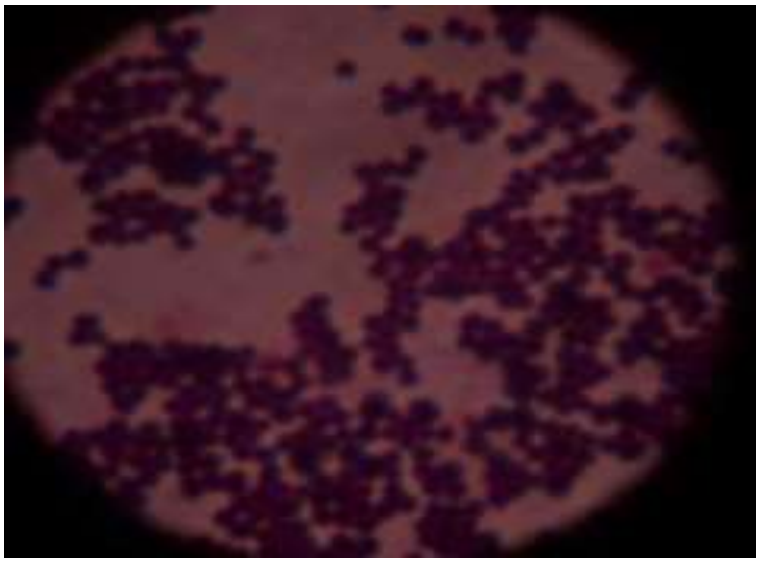

Figure (14): Photograph showing identification stained *E. faecalis* by gram positive stain under a light microscope.

Table (1): Comparison of *E. faecalis* baseline count (CFU/ml) throughout the study

Variable	Mean	SD	f-ratio	P-value
Neem	3.58	0.22	0.074	0.9280ns
Miswak	3.59	0.26		
CHX	3.56	0.22		

Comparison of *E. faecalis* count (CFU/ml) before and after the use of Neem irrigation:

The group of root canals involved to received Neem irrigant solution before and after irrigation were compared regarding the *E. faecalis* count and represented as CFU/mL and then were statistically analyzed using paired *t*-test.

The statistical analysis results of the *E. faecalis* count (CFU/mL), which showed mean ± standard deviation (SD) of the involved group of root canals those received Neem irrigant solution before and after irrigation was summarized in (Table 2)

There was a statistically significant difference in *E. faecalis* count (CFU/ml) before and after the use of Neem irrigant solution with a P-value of (<0.0001*) as indicated by Paired t-test. By conventional criteria, this difference is considered to be extremely statistically significant.

The involved root canals showed a statically significant reduction in *E. faecalis* (CFU/ml) count after Neem irrigant solution from (3.58±0.22) at the baseline to (1.61±0.21) after irrigation.

Table (2): Comparison of E. faecalis count (CFU/ml) before and after the use of Neem irrigation.

Variable	**Before irrigation**		**After irrigation**		**df**	**t-value**	***p*-value**
Neem irrigant	**Mean**	**SD**	**Mean**	**SD**	**14**	**21.64**	**<0.0001***
	3.58	**0.22**	**1.61**	**0.21**			

Comparison of *E. faecalis* count (CFU/ml) before and after the use of Miswak irrigation:

The group of root canals involved to received Miswak irrigant solution before and after irrigation were compared regarding the *E. faecalis* count and represented as CFU/mL and then were statistically

analyzed using paired *t*-test.

The statistical analysis results of the *E. faecalis* count (CFU/mL), which showed mean ± standard deviation (SD) of the involved group of root canals those received Miswak irrigant solution before and after irrigation was summarized in (Table 3).

There was a statistically significant difference in *E. faecalis* count (CFU/ml) before and after the use of Miswak irrigant solution with a P-value of (<0.0001*) as indicated by Paired t-test. By conventional criteria, this difference is considered to be extremely statistically significant.

The involved root canals showed a statically significant reduction in *E. faecalis* (CFU/ml) count after Miswak irrigant solution from (3.59±0.26) at the baseline to (2.15±0.24) after irrigation.

Table (3): Comparison of E. faecalis count (CFU/ml) before and after the use of Miswak irrigation.

Variable	Before irrigation		After irrigation		df	t-value	*p*-value
Miswak irrigant	Mean	SD	Mean	SD	14	18.05	<0.0001*
	3.59	0.26	2.15	0.24			

Comparison of *E. faecalis* count (CFU/ml) before and after the use of CHX irrigation:

The group of root canals involved to received CHX irrigant solution before and after irrigation were compared regarding the *E. faecalis* count and represented as CFU/mL and then were statistically analyzed using paired *t*-test.

The statistical analysis results of the *E. faecalis* count (CFU/mL), which showed mean ± standard deviation (SD) of the involved group of root canals those received CHX irrigant solution before and after irrigation was summarized in (Table 4).

There was a statistically significant difference in *E. faecalis* count (CFU/ml) before and after the use of CHX irrigant solution with a P-value of (<0.0001*) as indicated by Paired t-test. By conventional criteria, this difference was considered to be extremely statistically significant.

The involved root canals showed a statically significant reduction in *E. faecalis* (CFU/ml) count after CHX irrigant solution from (3.56±0.22) at the baseline to (1.31±0.14) after irrigation.

Table (4): Comparison of *E. faecalis* count (CFU/ml) before and after the use of CHX irrigation.

Variable	Before irrigation		After irrigation		df	t-value	*p*-value
CHX irrigant	Mean	SD	Mean	SD	14	31.46	<0.0001*
	3.56	0.22	1.31	0.14			

Results of *E. faecalis* count (CFU/ml) after irrigation along the study:

The involved root canals among the studied groups were compared regarding the *E. faecalis* count after the use of irrigation and represented as CFU/mL, and then were statistically analyzed using One-way ANOVA.

The statistical analysis results of the *E. faecalis* count (CFU/mL) after the use of irrigation, which showed mean ± SD of the involved root canals among the studied groups were summarized in (Table 5)

There was a statistically significant difference in *E. faecalis* count (CFU/ml) between the involved root canals among the studied groups after the use of irrigation with a P-value of (P<0.00001) as indicated by the One-way ANOVA test.

The involved root canals that received Miswak irrigant solution showed a higher (mean ± SD) *E. faecalis* count (2.15±0.24) followed by the root canals that received Neem irrigant solution (1.61±0.21). However, the involved root canals that received CHX irrigant solution showed a lower (mean ± SD) *E. faecalis* count (1.31±0.14).

Table (5): Comparison of *E. faecalis* count (CFU/ml) after irrigation throughout the study.

Variable	Mean	SD	f-ratio	P-value
Neem	**1.61^{B}**	**0.21**	**65.62**	**<0.00001***
Miswak	**2.15^{A}**	**0.24**		
CHX	**1.31^{C}**	**0.14**		

*; Significance level at P≤0.05.
; ns= Non-significant level at P>0.05.
; Different uppercase litters mean statistically significant.

- Between Neem and Miswak (P =0.00000*)
- Between Neem and CHX (P =0.0005*)
- Between Miswak and CHX (P =0.00000*)

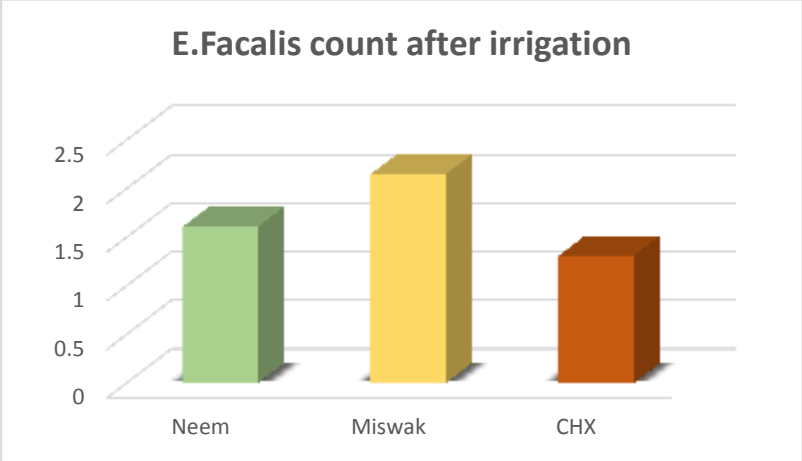

Figure (15): Comparison of *E. faecalis* count (CFU/ml) after irrigation throughout the study.

DISCUSSION

In order to create the ideal environment for tissue repair and healing, root canal therapy tries to eradicate microorganisms from the root canal system. Bacteria in the root canal are frequently connected to failure during and after endodontic therapy **(14).** The removal of pathogens from infected root canals is still a difficult task. Although the population of microorganisms is greatly reduced after mechanical preparation of the root canal system, not all germs can be eradicated **(14, 15).**

Due to complicated internal anatomies especially in primary anterior teeth, such as fins, apical deltas, isthmuses, or other anomalies that are typically missed by instrumentation alone, chemical debridement is particularly necessary **(16, 17).** Consequently, the root canal system is cleaned using a variety of chemicals and therapeutic agents. Instrumentation and irrigation work together to help remove pulp tissue and/or bacteria **(18)** Therefore, the aim of this study was to evaluate and compare the in vitro antibacterial effect of one commercial synthetic irrigant (CHX) with that of two herbal medicines (Neem and Miswak) used as root canal irrigants against *E. faecalis* microorganisms.

An antibacterial agent with a broad spectrum is chlorhexidine. In low concentrations, it is bacteriostatic, whereas, in high concentrations, it is bactericidal **(19)** Additionally, CHX is incapable of disintegrating tissue **(20).** Therefore, it was selected in this present study as a control irrigant

solution.

Chlorhexidine has been used in endodontics in concentrations ranging from 0.12 to 2% as an irrigant and intracanal medication. However, if CHX is expressed outside of the boundaries of the root canal, it may have toxic effects on host tissue and slow healing at 2% concentration **(18)** Hence in this current in-vitro study, 0.2% of CHX was selected to simulate the common and safe concentration that is used in pediatric dentistry. However, it was found that when chlorhexidine and sodium hypochlorite are combined, parachloroanaline is a carcinogenic **(18)**

Because of the negative side effects of synthetic medications, researchers are looking for herbal substitutes. The importance of medicinal plants as possible sources of bioactive chemicals has been recognized by pharmacological investigations (181). According to a WHO assessment, conventional medicines are the major treatment option for 80% of the world's population. Herbs as antimicrobial medicines provide a number of benefits, including few side effects, cost-effectiveness, improved patient tolerance, renewable nature, and non-toxicity **(18)**

Numerous herbal extracts, including Neem, Miswak, tulsi, Aloe vera, Circuma longa, and Turmeric, have been studied for use as endodontic irrigants and medications because of their antibacterial, anti-inflammatory, and therapeutic properties **(18, 21, 22).** The active ingredients in Neem that give it its antibacterial properties include Nimbidin, Nimbin, Nimbolide gedunin, Azadirachtin, Mahmoodin, Margolone, and cyclic trisulfide **(18, 22).** In an in vitro investigation, it was demonstrated to be a powerful antibacterial agent against *E. faecalis* **(21, 22).** Therefore, in this present study Neem was selected as a tested irrigant solution against *E. faecalis.*

Miswak chewing sticks offer many beneficial biological characteristics and are a well-liked oral hygiene tool. Numerous compounds, including cyanogenic glycoside and benzyl-isothiocyanate, as well as high concentrations of NaCl and KCl, salvadourea, salvadorine, saponins, tannins, vitamin C, silica, and resin, are thought to be responsible for its antibacterial and cleansing properties **(22, 23).** It was proven to be a potent antibacterial agent against *E. faecalis* in vitro research. As a result, Miswak was chosen for this study's testing as an irrigant solution against *E. faecalis* **(22).**

Microorganisms found in the canal system are mostly to blame for root canal infection and/or reinfection **(22).** *Enterococcus faecalis* is Gram-positive cocci and can appear alone, in pairs, or in short chains. They are facultative anaerobes, meaning they can grow either with or without oxygen. Since of their complexity, *E. faecalis* presents a significant problem to dentists because it allows them to endure difficult conditions including extremely alkaline pH, high salt concentrations, and temperatures of 60 °C **(23).** Due to its resilience in challenging conditions, capacity to build biofilms, and capacity to penetrate dentinal tubules, *E. faecalis* is the primary source of secondary infection (**22**). Consequently, it is necessary to consider treatment regimens aimed at eliminating or avoiding the infection of *E. faecalis* and other germs. In order to evaluate the antibacterial impact of irrigants, *E. faecalis* was studied in this in-vitro investigation.

Primary molar root canal systems usually have multiple ramifications and deltas between canals, making thorough debridement challenging **(24).** However, primary anterior teeth were used in this present study because the primary anterior root canal systems are generally more straightforward, have fewer abnormalities, and are reasonably easy to treat endodontically **(24).** It is also worth mentioning that, the present study used roots of primary anterior teeth. Operating on roots of primary molars may show different results due to the presence of lateral canals, anastomoses, and apical ramifications, in which other factors may contribute to the effectiveness of the antibacterial irrigation such as accessibility and substantiveness **(25).**

Natural teeth were employed in this study instead of artificial ones because artificial dentine cannot imitate real dentine in a clinical setting. The elimination of intracanal bacteria will be potentiated by reaching inaccessible places and expanding the dentinal tubules to allow antimicrobials to enter more

effectively. This is accomplished by instrumenting the apical portion of the root canal to a bigger file-size **(3, 22).**

Subsequently, the teeth were sterilized using an autoclave before the inoculation of *E. faecalis*, which, according to earlier research, effectively eliminated all types of germs that might be present in the root canal system **(26).** To guarantee there were no microorganisms inside the canal, we then validate sterilization and check the culture material for bacterial growth **(3).**

There are numerous ways to induce *E. faecalis* bacterial inoculation and calculate an irrigant's antibacterial action. However, the results of previous studies demonstrate that the methodology utilized "BHI broth suspension" for the current investigation was appropriate since, after 48 hours of incubation, cultures of viable, pure microorganisms were found to be present in all samples **(3, 27).**

To standardize the microbial suspensions employed through the testing processes described by Sassone et al. **(28)**, the microbial suspensions used in the current investigation were adjusted to match the turbidity of the No. 1 MacFarland scale. According to a study by Shabahang and Torabinejad (2003) **(29)**, the optimal period for the irrigation material to assess the antibacterial impact was found to be five minutes of exposure to irrigating solutions. For the purpose of estimating the antimicrobial impact of the various irrigants under research in the current study, samples were taken from the root canals using sterile paper points before, and after the irrigation **(3).**

Moreover, in the agar diffusion test, microbial growth was seen around all of the wells with various volumes and concentrations, indicating that the organisms were resistant to the aqueous and alcoholic neem extract. This might have happened as a result of the Neem's inability to efficiently diffuse through the agar media **(30).** In light of this finding, we chose to avoid bias in the outcomes of the current investigation by determining the antibacterial effect using the serial broth dilution method.

According to Nayak et al. **(30).** findings' 7.5% Neem leaf extract as the minimal inhibitory concentration, were solvent-dependent and Neem leaf extracts in ethanolic and dichloromethane were shown to be more effective than the other extracts in the study against the bacterial species tested. Therefore, in this present study ethanolic extract for Neem with a concentration of 7.5% as minimum inhibitory concentration was selected to ensure the antibacterial activity of Neem extract in this present study.

Moreover, Daga et al. **(22)** stated that the use of 12.5% Miswak ethanolic extract was solvent-dependent and was shown to be more effective against *E. faecalis* microorganisms. Therefore, in this present study ethanolic extract for Miswak with a concentration of 12.5% concentration was selected to ensure the antibacterial activity of Miswak extract in this present study.

This present study showed that the post-irrigation *E. faecalis* bacterial count in all the groups was significantly lower than the pre-irrigation values of *E. faecalis* bacterial count. These results agree with the results of the previous study which show that CHX, Neem, and Miswak irrigants haw significant antibacterial effects against *E. faecalis* microorganisms **(18, 21, 22).**

The results of this present study revealed that 0.2% CHX showed a significant reduction in the bacterial count when used as an irrigant in the infected root canals. This is because CHX is a cationic bis-biguanide with a broad antibacterial spectrum. Using an active or passive transport mechanism, CHX, a positively charged hydrophobic and lipophilic molecule, interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria. It works by interacting with negatively charged phosphate groups on the cell walls of microorganisms and changing the osmotic balance within the cell **(18)**

This result was following the results of Rathee et al. **(18)** which showed the use of 0.2% CHX in infected root canals for irrigation, a highly significant decrease in the number of *E. faecalis* microorganisms was seen in the CHX-treated specimens. Also, Esmail et al. **(3)** stated that the use of

CHX can significantly reduce the *E. faecalis* bacterial count.

Moreover, the results of this study showed that 7.5% of Neem had a significant reduction effect on the *E. faecalis* microorganisms' bacterial count when used as an irrigant in the infected root canals. This was because Alkaloids, glycosides, terpenoids, steroids, and tannins are only a few of the active phytoconstituents that give Neem its antibacterial characteristics **(31).** These results were in accordance with Dutta and Kundabala **(32)** who exported that following irrigation with test irrigants, the preoperative and postoperative samples were examined for colony-forming units. Neem was found to have good anti-microbial efficacy and might be used in endodontic procedures.

Additionally, the results of this study showed that 12.5% of Miswak had a significant reduction effect on the *E. faecalis* bacterial count when used as an irrigant in the infected root canals. This was because the main component of Miswak is benzyl thiocyanate, which inhibits the growth of *E. faecalis **(22)*.** Additionally, the scientists discovered that the limonoid acts as a broad-spectrum antibiotic against a variety of Gram-positive and Gram-negative bacteria when they isolated the active component. Moreover, the previous studies discovered that the active ingredient of *Salvadora persica's* 10% aqueous extract interfered with additional polysaccharides and glycosidase enzymes produced by bacteria when they tested the extract's capacity to limit bacterial growth **(33, 21).**

Furthermore, the results of this study revealed that CHX had a significantly higher antimicrobial effect against *E. faecalis* microorganisms when compared to the tested herbal solutions (Neem and Miswak). This could be attributed to the fact that chlorhexidine gluconate can adhere to hard tissues and maintain its antibacterial effect is one of the factors contributing to its widespread use. This is caused by the constant interaction of many CHX molecules with the dentin **(34).** This could explain the statistically significant difference between the reduction in the *E. faecalis* bacterial count after using the CHX irrigant solution compared with the other herbal irrigant solutions (Neem and Miswak).

These results are in accordance with the results of previous studies which show that the CHX irrigant had a higher antimicrobial effect than the herbal solutions **(3, 120).** Chandrappa et al. **(19)** found that a significant antibacterial effect against *E. faecalis* was observed with CHX followed by neem extract.

Moreover, the results of this current investigation showed that Neem had a higher and statistically significant antibacterial effect against *E. faecalis* microorganisms. Chandrappa et al. **(19)** reported that the active ingredients in Neem that give it its antibacterial properties include Nimbidin, Nimbin, Nimbolide, Gedunin, Azadirachtin, Mahmoodin, Margolone, and cyclic disulfide significantly reduces *E. facials* adhesion to dentin. This could explain the significant antibacterial effect of Neem against *E. facials* microorganisms in this present study when compared with Miswak. These results agreed also with the results of Daga et al. **(22)** who reported that Neem demonstrated better antimicrobial efficacy against *E. facials* followed by Miswak.

CONCLUSION

According to the study's findings, it was established that:

1- 12.5% Miswak and 7.5% Neem extracts presented significant antibacterial effect against *Enterococcus Faecalis* microorganisms and can be used as effective alternative irrigant solutions in primary teeth.

2- Chlorhexidine irrigant solution exhibited significant potential against *Enterococcus Faecalis* microorganisms.

3- Chlorhexidine irrigant solution is more effective against *Enterococcus Faecalis* microorganisms when compared to 12.5% Miswak and 7.5% Neem extracts as irrigant solutions in primary anterior teeth.

4- Chlorhexidine irrigant solution is still the gold standard irrigant solution in primary anterior

teeth.

Recommendations

Considering the findings of the current study, we may recommend that:

1. Herbal products should be researched and taken into consideration as an alternative to conventional effective irrigants.
2. Neem and Miswak extracts should be researched as substitute irrigant solutions at various concentrations.
3. Future research should examine various Neem and Miswak extract techniques.

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