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Antibacterial effect and smear layer removal ability of herbal intra-canal irrigants in primary teeth: an *ex-vivo* study

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Natural irrigants, specifically when used with children are important as they have fewer adverse effects. **Objectives:** To evaluate the antibacterial effect and smear layer removal ability of sage versus turmeric irrigants. **Methods:** 60 roots of primary teeth were inoculated with *E. faecalis* and randomly allocated to the following subgroups (12 roots each): Subgroup (I): 0.2% sage solution, Subgroup (II): 12.5% turmeric solution, Subgroup (III): 2% chlorhexidine solution, Subgroup (IV): contaminated only, Subgroup (V): negative controls. Microbiological culturing was performed. Assessment of the smear layer removal ability, 40 roots (10 roots in each group) were randomly divided; Subgroup 1: 0.2% Sage solution. Subgroup 2: 12.5% turmeric solution. Subgroup 3: 2% chlorohexidine. Subgroup 4: saline. The evaluation of smear layer removal was performed by SEM. **Results:** Chlorohexidine showed the highest percentage reduction in bacterial count followed by turmeric, then the sage group. A comparison of the smear layer removal, scores showed significant differences in the middle section of the different groups, with the turmeric group having a significantly higher median score than the sage group. **Conclusion:** chlorohexidine showed the highest antibacterial properties and smear layer removal ability followed by turmeric then sage.

Keywords: Anti-bacterial agents. Smear layer. Plant extracts. Root canal irrigants. Tooth, deciduous.

Introduction

The main goal for successful endodontic treatment is achieving a suitable environment for the recovery of periapical lesions, which mainly rely on the chemo-mechanical debridement of pulpal tissue, dentin debris, and elimination of infective microorganisms[1]. Pulpectomy in primary teeth is more challenging than that in permanent teeth due to the complex anatomy of the root canal systems and their proximity to the successors teeth, in addition to the difficulty in behavioural management of children[2]. Disinfection of severely infected primary teeth can be difficult because both obligate anaerobic and aerobic microorganisms as streptococci and gram-negative aerobic rods are usually found[3], with evidence that the most prevalent species of bacteria in primary teeth root canals are *Enterococcus faecalis, Porphyromonas gingivalis and Treponema denticola*[4].

Irrigation plays a pivotal role during and after instrumentation in root canal treatment, as it accommodates the removal of microorganisms, dentin chips and smear layer that were formed during instrumentation through the flushing mechanism of tissue remnants, as it was proofed that mechanical debridement only is not sufficient for convenient disinfection[5].

There are various synthetic intracanal irrigants that are used in primary teeth. These include chlorhexidine gluconate, sodium hypochlorite, Ethylene diamine tetra acetic acid (EDTA), hydrogen peroxide, and others[6]. The purpose of these irrigants is to make the instrumentation process easier, prevent the packing of apical debris, and help in the extrusion of infected material. Additionally, some of these irrigants have antibacterial properties[7].

Chlorhexidine (CHX) is considered a potent irrigant as it is bacteriostatic and bactericidal agent due to its substantivity and affect both Gram-positive and Gram-negative strains as well as fungi[8]. As some irrigants are ineffective as antibacterial agents plus posing possible adverse consequences and security issues[9], herbal and natural products have been proven effective as alternatives to synthetic agents in both medical and dental practice. They are becoming increasingly popular in various fields of dentistry, especially in pediatric dentistry due to their high biocompatibility, antimicrobial activity, anti-inflammatory, and antioxident properties[10,11]. Therefore, they have the potential to be used as root canal irrigants[12].

It is widely known that the Lamiaceae family possesses enormous pharmacological, industrial, and therapeutic capabilities. The largest member of the Lamiacea, or mint family, with over 900 species worldwide is *Salvia Officinalis*, also known as sage[13]. Sage essential oils possess anticancer, antimicrobial, antioxidant, and antiseptic properties[14]. This encouraged dentists to use herbal extracts containing *Salvia officinalis* as intra-canal irrigant[15], cariostatic agent[16], antifungal agent[17], and antiplaque agent[18].

Turmeric, also known as curcuma longa, is a member of the ginger family Zingiberaceae. Its active component is curcumin, and it has been used for thousands of years as a medicinal herb owing to its antimicrobial, antioxidant, anti-inflammatory, and antimutagenic properties[19]. Turmeric has been used in several forms to relieve dental problems; some authors suggested using it as a staining agent in dental-plaque detection systems, and others used it as mouth rinse and pastes to relieve the pain of gingivitis and periodontitis[20].

The amorphous and irregular particles that coat all instrumented surfaces of the prepared root canals as a result of root canal instrumentation are referred to as the "smear layer." A number of variables, such as the size and form of the root canal, the form and sharpness of the instruments, and the kind and volume of irrigating solution, can affect how thick the smear layer is in a given tooth[21]. There are several methods of smear layer removal, including Ultrasonic system[22], laser[23], and several chelating agents such as ethylenediaminetetraacetic acid, citric acid, and maleic acid[21]. However, using these chemical formulations has resulted in a number of drawbacks, including dentinal erosions, allergies, decreased dentin microhardness, and issues with biocompatibility[24].

That is why, the current study aimed to assess the antibacterial effect and smear layer removal ability of sage and turmeric herbal extracts as root canal irrigants in deciduous teeth and compare them with the chlorohexidine irrigation solution as one of the most used irrigants in primary teeth as recently, herbal medicine has gained popularity due to its beneficial properties, availability, and decreased side effects[24].

The hypotheses of the current study were: 1- The antibacterial effect of CHX, sage, and turmeric are equal. 2- The smear removal ability of the three interventions is the same.

Materials and Methods

A power analysis was designed to have adequate power to apply a statistical test of the null hypothesis that the capacity to remove the smear layer and the number of bacteria present in each examined group are the same. By assuming an alpha level of (0.05) a beta of (0.2) i.e. power=80% and an effect size (f) of (0.466) calculated based on previous studies results[25,26]; the predicted sample size for testing the antibacterial effect (n) was obtained to be about (60) samples and for smear layer removal (n) was obtained to be about (39) samples. Sample size calculation was performed using G*Power version 3.1.9.7[27].

One hundred deciduous anterior teeth were selected from the outpatient clinic of the Pediatric Dentistry Department at the Faculty of Dentistry, Ain Shams University. Inclusion criteria for selection of specimens: teeth with minimal apical resorption (at least 2/3 of root length), no internal or external root resorption as determined by x-ray, exclusion criteria: teeth with root caries, cracks or fractures[47]. The teeth were preserved in distilled water at room temperature for no more than three months[29].

Preparation of aqueous solution of 0.2% Sage extract: The mechanical grinder was used to turn the dried parts of the Ariel plant into a fine powder. This powder plant material (200 g) was then soaked in 3 liters of 80% ethanol and kept for 3 days. Afterward, the solvent was removed using a rotary evaporator and kept at a temperature of 4°C until it was ready to be used. To prepare the extract, it was diluted using Dimethyl sulfoxide (DMSO), using the following equation advocated by Summerlin (1981) (law of dilution): volume (1) x concentration (1) = volume (2) x concentration (2). A 0.2% dilution was prepared[30].

Preparation of aqueous solution of 12.5% turmeric extract: The rhizomes of the plant were cleaned with distilled water, dried, and then ground into a fine powder using a mechanical grinder. A flask containing 250 grams of powdered curcuma longa rhizomes was filled with 1000 milliliters of absolute ethanol[31]. After covering the flask with aluminium foil, it was kept at ambient temperature for the whole night.

Whitman's filter paper, available from Whitman Ltd. in England, was used to filter the final extract. The extract was separated by submerging the flask in the water bath of Heidolph Instruments GmbH & Co. KG's LABORATA 4000 eco rotary evaporator. The rotary evaporator was operated under reduced pressure at 400C. The temperature of the water bath was continuously monitored to ensure it did not exceed 45-50°C. The liquid was then stored in a sealed container[32].

Preparation of the teeth

Decoronating (100 teeth) below the cementoenamel junction was performed using double-sided diamond disc (NTI diamond disc, Axis Dental, USA), mounted on a high-speed contra-angle with coolant. To confirm the canal's patency, File #15 (MANI INC, Japan) was placed into the root canal of the teeth until its tip showed at the apical foramen[33,34]. Using hand files (K-type) and enlarging them to size #40 35, mechanical preparation was carried out to standardize the internal diameter of the root canals. To avoid clogging the canal, irrigation with 20 ml of saline was carried out for five minutes following the use of each file[35]. After that, α-cyanoacrylate glue was used to seal each specimen's apical foramina from the outside in order to stop bacterial microleakage[36]. All specimens were placed in sterilization pouches and autoclaved for 15 minutes at 121 °C and 15 PSI pressure to guarantee sterilization[37]. The specimens were then split into two main groups at random in the following ways:

Group A: Evaluation of the antibacterial effect (n = 60): 60 roots were inoculated with *E. faecalis* and randomly allocated to one of the following subgroups (n=12) according to the irrigant used: Subgroup (I): (0.2% sage solution), Subgroup (II): 12.5% turmeric solution, Subgroup (III): 2% chlorhexidine digluconate solution (Gluco-CHeX; Cerkamed, Poland).Each Sample was irrigated with 5 ml of the solution for 5 min. 24 roots were randomly allocated to one of the following subgroups: Subgroup (IV): in which 12 roots that were contaminated only (positive control), Subgroup (V): in which 12 roots that were neither contaminated nor irrigated (negative control).

Evaluation of the antibacterial effect of the herbal extracts

Microbiological culturing was conducted in the Department of Microbiology, Faculty of Medicine, Ain Shams University. A clinical isolate of *E. faecalis* was obtained from the Microbiology laboratory at the Central Laboratories of the Ministry of Health, Egypt, in order to facilitate the production of biofilms. The bacterial strain was then inoculated in Brain Heart Infusion (BHI) broth and incubated for 24 hours at 37 °C. It was finally calibrated to the MacFarland turbidity standard, Number 1.

Using the same incubation conditions, a biological marker was grown on the surface of Brain Heart Infusion agar (BHIA) to form the suspensions utilized in the experiment. The final concentration of the bacterial cells utilized to infect the samples was about 3 x 108 cells/mL, which was achieved by suspending the cells in saline solution. The roots of the experimental groups were inoculated with the *E. faecalis* suspension and then placed in sealed vials at 37 °C for 10 days. The fluid was replaced every 72 hours with freshly prepared 0.9% physiologic saline solution from El Nasr Pharmaceutical Chemicals in Egypt. Every 72 hours, sterile saline was added to the samples in the negative control group after they had been inoculated with sterile BHI broth.

After the samples were contaminated, a 27-gauge irrigation needle was used to irrigate them for five minutes using a 5 ml irrigant that was allotted to each group[38]. After that, the irrigation fluid from the canals was absorbed by sterile paper tips and moved to a test tube with 1.0 milliliter of saline. Twenty seconds were spent vortexing the contents. Aliquots of 0.1 ml were placed onto BHI agar plates following a 10-fold serial dilution, and the plates were then incubated for 48 hours at 37 °C[39].

Following an examination of the plates, the total colony forming units (CFU) per milliliter (ml) of sample was calculated by counting the number of bacterial colonies and multiplying that number by the appropriate dilution factor[40].

Group B: for evaluation of smear layer removal ability (n = 40): The roots were randomly divided into 4 subgroups (10 roots each) as follows, Subgroup 1: irrigated with 0.2% Sage solution. Subgroup 2: irrigated with 12.5% turmeric solution. Subgroup 3: irrigated with 2% CHX gluconate. Subgroup 4: irrigated with normal saline.

Evaluation of the smear layer removal ability of the herbal extracts: By passively inserting a sterile (27 gauge) plastic syringe 2 mm from the working length within the canal, each group's roots received the prescribed irrigation[26]. Following another irrigation with 5 milliliters of distilled water, the specimens were dried using paper points.

Scanning Electron Microscopy (SEM): assessment of smear layer removal was carried out using scanning electron microscopy (SEM, Figure 1). The roots were split into two halves with a diamond disc along two longitudinal grooves that were prepared on both the lingual and buccal surfaces. The canal was not drilled through. Each root had only one-half selected[41]. The sections were placed on metallic stubs, gold-sputtered, and analyzed under SEM (JSM-5400LV, JEOL, TOKYO, Japan). The tested group was kept anonymous during the numbering of the specimens and the blind photo shoot. Next, each root's clearest third was chosen, and it was enlarged at 1500- and 2000-times magnifications. The scores were blindly recorded by two impartial observers.

Scores were recorded according to the scoring system of modified Hülsmann et al.[42] as follows:

Score 1 = Score 1: There is no smear layer and the dentinal tubule orifices are patent.

Score 2 = Smear layer is little, and 75% of the dentinal tubules are patent.

Score 3 = All of the canal wall's homogenous smear layer is present, and 50% of the dentinal tubules are patent.

Score 4 = There are extremely few patent dentinal tubules (less than 25%) and a uniform smear layer covering the majority of the root canal wall.

Score 5 = a substantial layer of non-homogenous smears that covers the whole root canal wall.

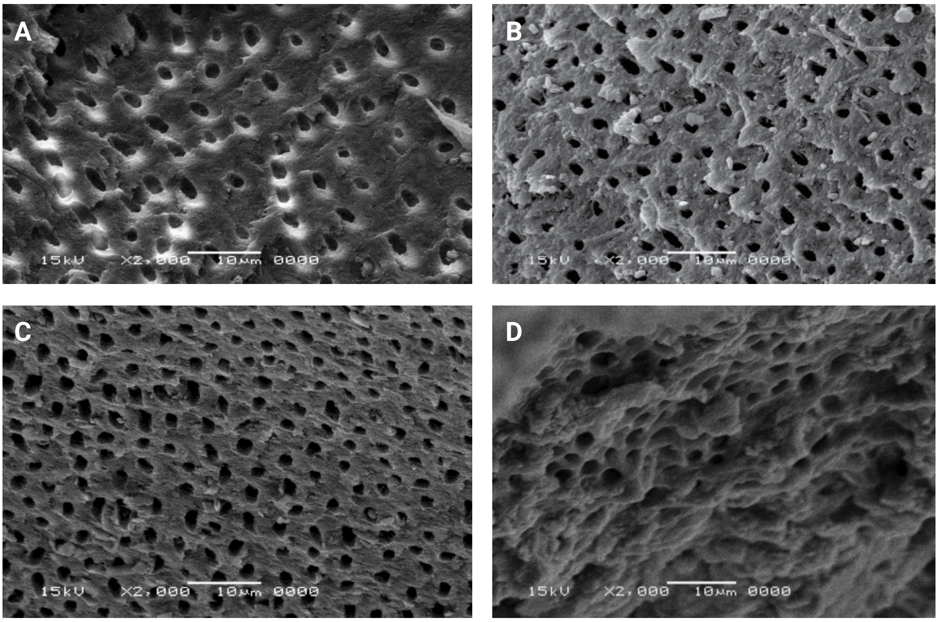

Figure 1. a. SEM image of root canal after using 12.5% turmeric showing open dentinal tubules under magnification (x2000). Score 2. c. SEM image of root canal after using 2% CHX gluconate showing most of dentinal tubules are open under magnification (x 2000). Score 2. b. SEM image of root canal after using 0.2 % sage showing homogenous layer of smear layer under magnification (x2000). Score 3. d. SEM image of root canal after using saline reveals that the dentinal tubules are scarce, and the root canal wall is nearly entirely covered with a uniform smear layer under magnification (x2000). Score 4

Results

Statistical descriptions for log bacterial counts and smear layer removal scores are presented in tables 1 and 2 respectively.

Table 1. Statistical descriptions for log bacterial count

Group	Mean	95% CI		SD	Median	IQR
		Lower	Upper			
Turmeric	7.68	7.61	7.75	0.12	7.68	0.16
Sage	9.72	9.55	9.90	0.28	9.74	0.29

Continue

Continuation

Chlorhexidine	4.81	4.45	5.17	0.57	4.62	0.69
Saline	12.24	12.14	12.34	0.17	12.25	0.24
No contamination/ No irrigation	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Statistical descriptions for smear layer removal scores

Root section	Group	Mean	95% CI		SD	Median	IQR
			Lower	Upper			
Coronal	Turmeric	2.60	2.12	3.08	0.55	3.00	1.00
	Sage	1.60	1.12	2.08	0.55	2.00	1.00
	Chlorhexidine	2.00	1.38	2.62	0.71	2.00	0.00
Middle	Turmeric	2.60	2.12	3.08	0.55	3.00	1.00
	Sage	1.20	0.81	1.59	0.45	1.00	0.00
	Chlorhexidine	1.80	1.41	2.19	0.45	2.00	0.00

95% CI= 95% confidence interval for the mean; SD=standard deviation; IQR=interquartile range

Results of intergroup comparison of log bacterial counts and bacterial counts percentage reduction that are presented in tables 3 and 4 respectively showed that The groups differed significantly from one another (p<0.001). The highest count was in the saline group followed by the sage group, turmeric group, and chlorhexidine group respectively. In contrast, the uncontaminated samples showed no signs of bacterial growth. The highest percentage reduction of bacterial count was in the chlorohexidine group followed by the turmeric group . The lowest reduction was found in the sage group. All pairwise comparisons were statistically significant (p<0.001).

Table 3. Intergroup comparison for bacterial count

Log bacterial count (Mean±SD)					p-value
Turmeric	Sage	Chlorhexidine	Saline	No contamination/ No irrigation	
7.68±0.12[C]	9.72±0.28[B]	4.81±0.57[D]	12.24±0.17[A]	0.00±0.00[E]	<0.001*

Means with different superscript letters are significantly different (p<0.05) *significant (p<0.05)

Table 4. Intergroup comparison of bacterial count reduction (%)

Bacterial count reduction (%) (Mean±SD)			p-value
Turmeric	Sage	Chlorhexidine	
98.79±0.47[B]	91.60±2.20[C]	99.94±0.05[A]	<0.001*

Means with different superscript letters are significantly different (p<0.05) *significant (p<0.05)

Results of inter and intragroup comparison of smear layer removal score presented in Table 5 showed that in the coronal section samples, there was no significant difference in the scores of different groups (p=0.075), while in the middle section, the difference was statistically significant (p=0.012), with turmeric group having a significantly higher median score than sage group (p<0.001). In all groups, the difference in the score between the coronal and middle sections were not statistically significant (p>0.05). Median and interquartile range values for smear layer scores for different groups are presented in Figure 2.

Table 5. Inter and intragroup comparison of smear layer removal scores

Root section	Smear layer score [Median (IQR)]			p-value
	Turmeric	Sage	Chlorhexidine	
Coronal	3.00 (1.00)^{Aa}	2.00 (1.00)^{Aa}	2.00 (0.00)^{Aa}	0.075
Middle	3.00 (1.00)^{Aa}	1.00 (0.00)^{Ba}	2.00 (0.00)^{ABa}	0.012*
p-value	1	0.424	0.773	

Within the same horizontal row and vertical column, respectively, medians with differing upper and lowercase superscript letters show a significant difference (p<0.05). * Significant (p<0.05)

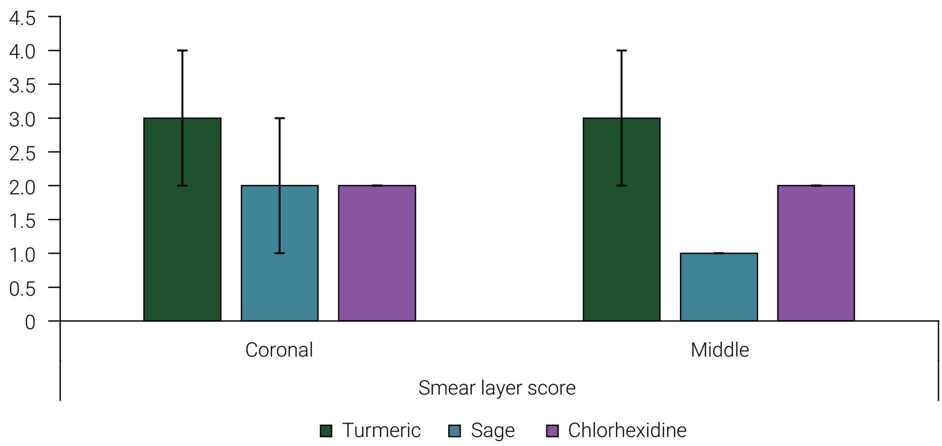

Figure 2. Bar chart displaying median and interquartile range values of smear layer removal scores

Statistical analysis

Using the Shapiro-Wilk test and examining the data distribution, numerical data were examined for normality. The data of bacterial count revealed a non-parametric distribution with positive skewness. After the data were log-transformed to account for their skewness, their parametricity was confirmed by re-examining them for normalcy.

The data were displayed as the mean and standard deviation, and one-way ANOVA was used to compare them between groups before Tukey's post hoc analysis was performed.

The data on the ordinal smear layer removal score were displayed as the median and interquartile range. They were analysed using the Kruskal-Wallis test for intergroup comparisons and Dunn's post hoc test with Bonferroni correction for pairwise comparisons. The Wilcoxon signed rank test was used to evaluate the intragroup comparisons. For every test, the significance threshold was set at $p<0.05$. R statistical analysis software, version 4.1.2 for Windows[1], was used to conduct the statistical analysis.

Discussion

For children, the primary objective of root canal therapy is to remove any contaminated tissues before sealing the canal(s) with a material that is safe and biocompatible.When dealing with infected baby teeth, where the root canal structure is complex and there is an active microbial circumference, it is critical to choose a highly effective antibacterial irrigation agent[38].

Considering that children are more sensitive to drug adverse effects than adults, therefore, there is a great tendency towards using natural products in pediatric dentistry[43].

Accordingly, this study aimed to shed light on the antibacterial effect and the smear layer removal ability of potent natural irrigants such as sage and turmeric, that might compete with the intracanal efficacy of the available chemical irrigants, besides the advantage of having fewer adverse effects.

For this study, irrigation was carried out using four different solutions: 2% CHX gluconate, 12.5% turmeric solution, 0.2% sage, and sterile saline. To ensure consistency among all the specimens, the volume of the irrigants and the time of irrigation were standardized[44]. The specimens were exposed to the irrigating solutions for five minutes, which has been identified as the optimal duration for the solutions to have an antibacterial effect[45].

According to Gomes et al.[45] (2001), 2% CHX gluconate is considered as a standard antibacterial irrigant. At this concentration, CHX has a bactericidal effect, which means it kills bacteria. On the other hand, if used at a lower concentration (0.12%), it only has a bacteriostatic effect, which means it only inhibits bacterial growth. Previous research has demonstrated that *E. faecalis* could not be eradicated by low concentrations of CHX in whatever period of time[46]. Similarly, previous studies have recommended using 0.2% sage and 12.5% turmeric concentrations as they possess the most effective antibacterial properties[30,31].

Inoculation with *E. faecalis* facultative anaerobic bacteria in the root canals of extracted primary teeth was chosen in this study as it was documented to be the most common and widespread bacterium in infected canals of primary teeth[47].

Since the culture method is thought to be a primary investigation method to identify, quantify predominant species, and also make a correlation between certain bacteria and the presence of clinical findings, as reported by Cogulu et al.[47] (2007), it was used in this study instead of PCR (polymerase chain reaction) for the detection of *E. faecalis* in root canals[28].

[1] R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

In the current study, the saline group was designated to guarantee sample contamination and establish the baseline bacterial count prior to irrigation. The purpose of the negative control group was to make sure the samples were sterilized[28].

A study by Saber and El-Hady[39] (2012) found that it is clinically feasible to reduce the irrigation time for smear layer removal to two minutes for each irrigant. Scanning Electron Microscopy (SEM), the most efficient technique for acquiring precise data regarding dentin surfaces, was used to assess smear layer removal. SEM makes it possible to evaluate the prepared canal walls' morphological characteristics along their whole length[41].

The present study found that CHX, turmeric, and sage extracts have antibacterial properties when compared to saline. CHX demonstrated a significantly higher antibacterial effect than turmeric and sage irrigation, leading to the rejection of the null hypothesis. This is in agreement with Kalaiselvam et al.[48] (2019), where 2% CHX exhibited the highest antibacterial activity against *E. faecalis* compared to herbal medicaments.

Although 2% CHX showed a significantly higher antibacterial effect than the other tested extracts in the present study, the herbal extracts may be considered a safer alternative to synthetic antimicrobials with reported side effects such as allergic contact dermatitis, desquamative gingivitis, teeth, or tongue discoloration as well as dysgeusia[9,49].

The findings of this investigation, however, were at odds with an in vivo study conducted by Al-Bazzaz[49] (2011) which demonstrated that normal saline was incapable of eliciting any antimicrobial effects and that there was no discernible difference between the antimicrobial effects of 0.2% ethanolic extract of Sage, 0.2% ethanolic extract of Rue, and 0.2% CHX on aerobic and anaerobic microorganisms. The disparity in the CHX concentrations utilized could be the cause of this dispute.

Although several studies investigated the smear layer removal ability of chemical irrigants, only a few studies investigated the effect of medical herbs on smear layer removal ability. To the best of the knowledge of the authors of this study, our study is the first to investigate the effect of *Salvia officinalis* (sage) on smear layer removal.

According to this study on the efficacy of CHX, turmeric, sage extract, and saline in removing the smear layer, the highest score mean value for the remaining smear layer was found in saline solution. This finding aligns with the results of Serafino et al.[50] (2006). On the other hand, the CHX solution showed the lowest score value for the remaining smear layer, followed by turmeric and sage solutions. Hence, the null hypothesis is rejected.

It is possible to draw these conclusions within the limitations of the current study, CHX remains the gold standard for irrigation in endodontic infections. Turmeric followed by sage had effective antibacterial properties and smear layer removal ability, which suggests their potential use as root canal irrigants for infected primary teeth. However, in vivo studies will also be required to recommend ideal clinical protocols using these materials.

In conclusion, chlorohexidine showed the highest antibacterial properties and smear layer removal ability followed by turmeric then sage.

Why this paper is important to pediatric dentists:

- The findings from this study highlight the importance of using natural irrigants that have fewer adverse effects than chemical irrigants, and this is specifically important in children who are more sensitive to drug formulations than adults.
- The novelty and uniqueness of the current study are represented in its being one of the very few studies that explored the smear layer removal ability of natural irrigants in primary teeth.

Further studies are recommended to evaluate different concentrations of the sage and turmeric extracts as irrigants in primary teeth. Besides, we recommend comparing the antibacterial effect and smear layer removal ability of sage, turmeric and other herbal extract irrigations with EDTA solution in future studies.

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Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

Datasets related to this article will be available upon request to the corresponding author.

Author Contribution

Gehan Allam: Conceptualization, Writing the manuscript, and final revision and approval of the version to be published. **Amira Badran:** Assistance in writing the manuscript and final revision. **Reem Mansour:** Contributed to the practical part. **Soha El Hady:** Contributed to the analysis of the antibacterial properties. **Reham Elghazawy:** Revised the manuscript and contributed to the practical part. All authors have revised and approved the final version of the manuscript.

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