EGYPTIAN DENTAL JOURNAL
VOL. 66, 2739:2750, OCTOBER, 2020
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
www.eda-egypt.org • Codex : 62/2010 • DOI : 10.21608/edj.2020.38341.1200

EVALUATION OF THE ANTIBACTERIAL EFFICACY OF TWO HERBALS AND THEIR EFFECT ON DENTIN MICROHARDNESS (A COMPARATIVE IN VITRO STUDY)

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ABSTRACT

Objectives: The aim of the study was to compare the antibacterial efficacy and the effect on dentin microhardness of turmeric extract, thymus vulgaris oil, sodium hypochlorite and chlorhexidine when used as root canal irrigants.

Material and methods: Sixty four permanent mandibular single rooted premolars were classified into 4 groups of 16 teeth each according to the final irrigant used. For the antibacterial test, 11 teeth per group (n=11), and the microhardness test, 5 specimens per group (n=5): Group I: 2% Turmeric extract. Group II: 2% Thymus vulgaris oil. Group III: 5.25% NaOCl. Group IV: 2% CHX. Forty four Samples were inoculated with *E. faecalis*, incubated then chemo-mechanically prepared according to the assigned group, sampled and incubated for 24 hours then Colony Forming Units were counted. Twenty samples were used to create dentin blocks, then were kept in airtight containers saturated with 5 ml of each irrigant for 5 minutes. Microhardness test was performed at different time intervals; After 24 hours, 3 days and 7 days using Vickers hardness indentation machine.

Results: The highest antibacterial efficacy against *E. faecalis* was in 2% CHX followed by 5.25% NaOCl, 2% Thymus vulgaris oil and finally 2% Turmeric extract. In microhardness test 2% CHX showed the highest statistically significant value of dentin microhardness, followed by 2% Turmeric extract, 2% Thymus and finally 5.25% NaOCl.

Conclusion: Herbals may serve as alternatives to conventional root canal irrigants as they possess antibacterial properties and cause minimal alteration in dentin microhardness when compared to chemical irrigants.

KEY WORDS: Antibacterial efficacy, dentin microhardness, herbal, Irrigation, sodium hypochlorite.

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INTRODUCTION

The primary objective of root canal therapy is the elimination of microorganisms and their by-products from the root canal system. To do so, a combination of mechanical instrumentation and irrigating solutions has to be used to disinfect the canal. [1]

Antimicrobial solutions must possess certain qualities such as the ability to penetrate the infected site, suppress or destroy microbial growth, and to avoid the possible development of resistant strains.

However, chemical irrigants show several drawbacks as sensitivity, cytotoxic reactions and inability to completely eliminate bacteria from root canals.

It has been reported that some irrigants used during endodontic procedures are capable of causing surface changes to dentin which in turn cause reduction in microhardness causing dentin to be structurally weak and nonsupportive which may affect the final restored tooth. [2]

Sodium hypochlorite (NaOCl) is one of the most widely used endodontic irrigants for its antibacterial action and its organic tissue dissolving capacity. However, its dissolving property is non-selective, and it can be toxic to periapical tissues, especially at high concentrations. [3]

Chlorhexidine gluconate (CHX) has been used in endodontics as both an irrigant and an intracanal medicament. It is a relatively non-toxic, broad spectrum antimicrobial agent with less potential for adverse effects, thus offering a clinical advantage over sodium hypochlorite.

To avoid the side effects of the chemical irrigants, wide varieties of herbal and natural products have been recently used. Their low toxicity, microbial resistance, side effects, cost effectiveness and their easy availability have made these products very popular. [4]

Turmeric (Curcumin longa) and thymus oil exhibit an antibacterial activity and had been useful in dental practice. [4]

However, the use of Turmeric and thymus oil as root canal irrigants wasn't previously studied. This is why the following study was undertaken to evaluate their antibacterial efficacy and their effect on dentin microhardness.

The aim of the present study was to compare the antibacterial efficacy and the effect on dentin microhardness of turmeric extract, thymus vulgaris oil, sodium hypochlorite and chlorhexidine when used as root canal irrigants.

The null hypothesis was that there would be no statistically significant difference between turmeric extract, thymus vulgaris oil, sodium hypochlorite and chlorhexidine in the antibacterial efficacy and their effect on dentin microhardness.

MATERIALS AND METHODS

Sample size Calculation

For antibacterial efficacy: Based on a previous study by Saha et al, 2015 [5] sample size was calculated as 11 teeth in each group, using power 80% and 5% significance level.

For microhardness: Based on a previous study by Prabhakar et al, 2013 [6] sample size was calculated to be 5 specimens in each group, using power 80% and 5% significance level. Sample size calculation was achieved using PS: Power and Sample Size Calculation software Version 3.1.2 (Vanderbilt University, Nashville, Tennessee, USA).

Selection of Samples

Sixty four extracted human permanent mandibular single rooted premolars were utilized in this study. Teeth were collected from the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Cairo University; due to periodontal disease from patients aged between 20-50 years old. Initial radiographs were taken to ensure that all teeth had mature apices, with no internal or

external resorption, calcifications or root caries. The collected teeth were immersed in 5.25% Sodium hypochlorite solution for half an hour for disinfection and removal of any debris. Followed by mechanical ultrasonic scaling to remove any remaining bone, calculus or soft tissue. Then, teeth were restored in saline solution until use.

Samples Classification:

Samples were classified into 4 groups of 16 teeth each according to the final irrigant used. Each group was further divided into two subgroups: Subgroup (A): 11 teeth for the evaluation of the antibacterial efficacy and Subgroup (B): 5 specimens for the evaluation of effect on dentin microhardness: Group I: 2% Turmeric extract. Group II: 2% Thymus vulgaris oil. Group III: 5.25% NaOCl. Group IV: 2% CHX.

Preparation of Samples:

Antibacterial Efficacy: forty four teeth were used; crowns were removed at the cementoenamel junction by using a double sided disc rotating in a low speed handpiece to obtain a standard root length of 16 mm. After working length determination; root canals were mechanically prepared from #15 to #25 stainless steel K-files, irrigation with 3ml of 5.25% Sodium hypochlorite between each subsequent file was done by means of 27-gauge needle. Then, the canals were flushed with 3ml of distilled water. Auto polymerizing acrylic resin was placed at the apex of each root canal, then; all teeth were sterilized in a class B autoclave at 121°C for 15 minutes.

Inoculation of *E. faecalis* in The Root Canal

Each root canal of the sterile teeth was filled with 30μ *E. faecalis* suspension strain ATCC 29212. The roots were incubated in a closed container for one week at 37°C. Refreshing broth with 20μ was added by using 1 ml insulin syringe every 48 hours throughout the one week incubation period. After incubation, 3 sterile paper points (Size #25) were separately placed in each root canal as close to the full working length as possible for one min. Once saturated, the paper points were transferred to a sterile test tube containing 1 ml of sterile brain heart infusion broth and kept for 3 minutes with shaking before it was smeared on plate agars. Then, the incubation of plate agars and examination of colonies took place as will be discussed.

Preparation of the Root Canal System:

Cleaning and shaping was performed on the contaminated root canals by using the Protaper Universal rotary files system both shapers and finishers until size F4. A gear reduction hand piece of 20:1 ratio was used with a torque controlled ENDO-MATE electric motor DT set at 3 Ncm and a speed of 300 rpm. After the use of each file, the root canal was irrigated with 3ml of the tested irrigant solution by means of Endo-Eze 27-gauge needle placed 2 mm short of the working length as follow: Group (I); 2% Turmeric extract. Group (II); 2% Thymus Vulgaris oil. Group (III); 5.25% NaOCl. Group (IV); 2% CHX. Then, a final flush for one minute was done with 3ml of the tested irrigant. Followed by flushing for another minute with 3ml of distilled water.

Antibacterial Efficacy Evaluation using Brain Heart Infusion Agar Method:

After the chemo-mechanical preparation of the root canals, 3 sterile Protaper paper points (Size #40) were separately placed in the canal as close to the full working length as possible for one min then transferred to a sterile test tube containing 1 ml of sterile brain heart infusion broth and kept for 3 minutes with shaking. Before placing the paper points into the tubes, the mouth of each tube was heated on the flame to prevent contamination. Sterile micropipettes with yellow tips were used to take 30μ from tubes and smear it on the agar plates by using sterile L-shaped glass rod. Then, the plates were incubated at 37°C for 48 hours. Examination of bacterial growth and colonies then took place by visualization of individual white pinpoint colonies

on the agar plates, additional confirmation was determined by microscopic observation of gram +ve cocci arranged in a cross-chain pattern. Visible colonies of *E. faecalis* were counted on each plate and expressed as confirmed by colony forming unit (CFU) plate, and then the number of CFU/samples was calculated.

Dentin Microhardness Test:

Twenty single rooted human mandibular premolars were selected for the test. A rotary diamond disk was used to decoronate the teeth 5 mm below the cementoenamel junction and cut the apical part of the root to obtain 6 mm of the middle third of the root. Gates Glidden drills no. 3 was used with a low speed handpiece to standardize the internal diameter of the root canals. Then, the blocks were put in sterilization pouches and autoclaved at 121°C for 15 minutes. Dentin blocks were randomly and equally divided into 4 groups (n= 5) according to the irrigant used as follows: Group (I): 2% Turmeric extract. Group (II): 2% Thymus Vulgaris oil. Group (III): 5.25% NaOCl. Group (IV): 2% CHX. Blocks were polished with abrasive papers and embedded in acrylic resin leaving their dentin surface exposed. Then, blocks were kept in airtight containers saturated with 5 ml of each irrigant for 5 minutes, then, flushed with distilled water and left to dry in open air for 24 hours.

The microhardness test was performed at different time intervals; After 24 hours, 3 days and 7 days using Vickers hardness indentation machine at magnification 20x and a depth of 400 μm from canal lumen and blocks were kept in airtight containers between measuring periods. Three separate indentations were made by using 200 gm load and dwell time of 15 seconds at three different points and mean was calculated. The representative hardness values were recorded as Vickers hardness number (VHN) then tabulated and statistically analyzed.

Preparation of herbal irrigants

1) ***Turmeric Extract:*** Turmeric rhizomes were washed with distilled water and dried, then cut into irregular large pieces and dried in an oven by tray drying process at a temperature of 45±5 °C for about 9 -10 days till they were moisture-free. Then, they were ground to form a coarse powder. 500 gm of the powder were placed in two large glass chambers each. 2500 ml of distilled water was added to one glass chamber to prepare the aqueous extract and to the other one; 1550 ml of water and 850 ml of ethanol (95%) were added to get a hydro-alcoholic extract. The liquid was then strained and the solid residue was pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained were mixed and clarified by filtration. 2000 ml of liquid was obtained and stored in a refrigerator at 4 °C in two beakers. China dishes were used for evaporation of the liquid over a water bath. Then a thick dark brown colored sticky mass was obtained as the aqueous extract and a yellowish brown colored sticky mass as the hydro -alcoholic extract. These extracts were mixed with distilled water and stored in a refrigerator at 4 C in a dark colored pre-sterilized airtight container ready to be used. [7]

2) ***Thymus Vulgaris Oil:*** Five hundred grams of fresh Thymus vulgaris were crushed and extracted by conventional steam distillation using a Clevenger apparatus for 3 hours. Then, they were condensed continuously at 4°C in cold water. The essential oil was then dried over sodium sulfate (Sigma-Aldrich, St Quentin-Fallaveier, France) and stored at 4°C in dark vials until use. 1 mg/ml solution of Thymus vulgaris oil was prepared in 10% aqueous dimethyl sulfoxide containing 0.5% Tween 80 (for easy diffusion). [8]

Statistical Analysis

Data was analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Numerical data were described as mean and standard deviation or median and range. Data was explored for normality

TABLE (1): Materials and devices used in this study:

	Materials	Source
1	Abrasive paper	3M™ , Egypt
2	Auto polymerizing acrylic resin	Acrostone, Dental and Medical Supplies, Cairo, Egypt
3	Brain heart infusion agar	Biolife, Milano-Italy
4	Brain heart infusion agar plate incubator	Precision, dual program illuminated incubator
5	Brain heart infusion broth	Biolife, Milano-Italy
6	Chlorhexidine	Cadila Pharmaceuticals Ltd.
7	Class B Autoclave	Biobase, China
8	Endo-Eze size 27 gauge needle	Ultradent products inc., South Jordan, UT, USA
9	Endo-Mate electric motor	NSK, Japan
10	*Enterococcus faecalis* strain (ATCC 29212)	Egyptian Ministry of Health
11	Gates Glidden drills no. 3	Mani Inc, Tachigi-ken, Japan
12	Gear reduction handpiece	MPA F-type contra angle head, NSK, Japan
13	K- files	Mani Inc, Tachigi-ken, Japan
14	Low-speed handpiece	NSK, Japan
15	Protaper Universal paper points	Dentsply, Millefer, USA
16	Protaper Universal rotary files	Dentsply, Millefer, USA
17	Sodium hypochlorite (NaOCl)	Egyptian Company for household products under license of Clorox Co. USA
18	Thymus vulgaris oil	Faculty of Pharmacology. Cairo University
19	Turmeric extract (Curcuma Longa)	Faculty of Pharmacology. Cairo University
20	Woodpecker Ultrasonic scaler	Foshan Vimel Dental Equipment Co. Ltd, China

using Kolmogrov-Smirnov test ad Shapiro-Wilk test. Comparison between 4 groups for normally distributed numeric variables was done using the ANOVA while for non-normally distributed numeric variables was done by Kruskal Wallis test. A p-value less than or equal to 0.05 was considered statistically significant. All tests were two tailed.

RESULTS

Antibacterial Efficacy:

A) Intergroup Comparison of The Antibacterial Effect

The highest antibacterial effect was recorded with Group (IV); 2% CHX with a mean of (33.64 ± 45.23 CFU), followed by Group (III); 5.25% NaOCl with a mean of (68.18 ± 68.18 CFU), then Group (II); 2% Thymus with a mean of (163.64 ± 71.03 CFU), and Group (I); 2% Turmeric showed the lowest antibacterial effect with a mean of (172 ± 56.41 CFU). There was a statistically significant difference among the four groups ($p < 0.001$) (Table 2)

B) Pairwise Comparison between groups:

Both Group (I); 2% Turmeric and Group (II); 2% Thymus showed significantly lower antibacterial effect than Group (III); 5.25% NaOCl and Group (IV); 2% CHX ($p < 0.0001$). However, there was neither a statistically significant difference between Group (I); 2% Turmeric and Group (II); 2% Thymus nor between Group (III); 5.25% NaOCl and Group (IV); 2% CHX. (Table 2, Figure 1)

Effect on Dentin Microhardness:

Dentin microhardness values were measured for all specimens pre-exposure to irrigants with a mean of (59.49 ± 14.72) VHN.

TABLE (2): Mean, standard deviation (SD) and results of ANOVA test for comparison of antibacterial effect in CFU between the four groups.

	Group(I) 2%Turmeric	Group(II) 2%Thymus	Group(III) 5.25%NaOCl	Group(IV) 2%CHX	P-Value
Mean	172.73	163.64	68.18	33.64	<0.001
SD	56.41	71.03	40.45	1.23	

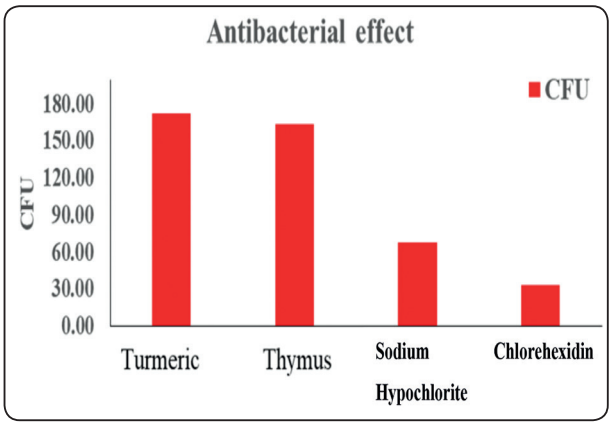

Fig. (1): Bar chart representing difference in antibacterial effect between the four groups

A) Intergroup Comparison of The Effect of Different Irrigants on Dentin Microhardness:

At day 1, Group (II); 2% Thymus showed the highest microhardness with a mean of (44.24 ± 19.90) VHN, followed by Group (III); 5.25% NaOCl with a mean of (42.13 ± 9.37) VHN, then Group (I); 2% Turmeric with a mean of (39.56 ± 5.88) VHN and the lowest microhardness was recorded with Group (IV); 2% CHX with a mean of (34.49 ± 5.55) VHN. However, there was no statistically significant difference in microhardness at day 1 between the four groups (p=0.599).

At day 3, Group (IV); 2% CHX had the highest microhardness with a mean of (61.81 ± 5.53) VHN, followed by Group (I); 2% Turmeric with a mean of (60.58 ± 2.12) VHN, then Group (III); 5.25% NaOCl with a mean of (57.89 ± 7.73) VHN and the lowest microhardness was reported to Group (II); 2% Thymus with a mean of (44.65 ± 7.77) VHN. There was a statistically significant difference in microhardness among the four groups (p=0.002).

At day 7, Group (IV); 2% CHX had the highest microhardness with a mean of (61.87 ± 16.72) VHN, followed by Group (II); 2% Thymus with a mean of (54.39 ± 6.22) VHN, then Group (I); 2% Turmeric with a mean of (48.63 ± 9.61) VHN and the least microhardness was reported to Group (III); 5.25% NaOCl with a mean of (46.83 ± 11.54) VHN. There was no statistically significant difference in microhardness among the four groups (p=0.209). (Table 3, Figure 2)

TABLE (3): Results of Tukey post hoc test for pairwise comparison of antibacterial effect in CFU between the four groups.

		P - Value
Turmeric	Thymus	0.979
	NaOCl	<0.0001*
	CHX	<0.0001*
Thymus	NaOCl	0.001*
	CHX	<0.0001*
NaOCl	CHX	0.456

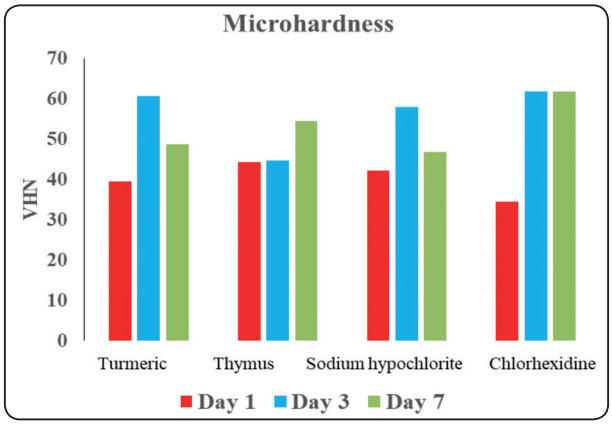

Fig. (2): Bar chart representing difference in microhardness between the four groups

TABLE (4): Mean, standard deviation (SD) and results of ANOVA test for comparison of microhardness in VHN between the four groups.

	Group (I) 2% Turmeric		Group (II) 2% Thymus		Group (III) 5.25% NaOCl		Group (IV) 2% CHX		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P - Value**
Day 1	39.56	5.88	44.24	19.90	42.13	9.37	34.49	5.55	0.599
Day 3	60.58	2.12	44.65	7.77	57.89	7.73	61.81	5.53	0.002*
Day 7	48.63	9.61	54.39	6.22	46.83	11.54	61.87	16.72	0.209

(B) Pairwise Comparison between groups:

At day 3, Group (II); 2% Thymus showed a significant lower microhardness values than Group (I); 2% Turmeric, Group (III); 5.25% NaOCl and Group (IV); 2% CHX (p=0.005, 0.019 and 0.002 respectively). (Table 4)

(C) Intragroup Comparison:

Group (I); 2% Turmeric showed a significant increase in the microhardness from Day 1 to Day 3 and from Day 3 to Day 7 (p= 0.001 and 0.036 respectively). Group (II); 2% Thymus showed a non-statistically significant increase in the microhardness (p= 0.396), while Group (III); 5.25% NaOCl showed a non-statistically significant decrease in the microhardness (p= 0.063), and Group (IV); 2% CHX showed a significant increase in the microhardness from Day 1 to Day 3 and from Day 3 to Day 7 (p= 0.004 and 1.0 respectively). (Tables 5 and 6, Figure 3)

TABLE (5): Results of Tukey post hoc test for pairwise comparison of microhardness in VHN between the four groups at Day 3.

		P - Value
Turmeric	**Thymus**	0.005*
	NaOCl	0.903
	CHX	0.988
Thymus	**NaOCl**	0.019*
	CHX	0.002*
NaOCl	**CHX**	0.751

TABLE (6): Mean, SD and results of ANOVA test for comparison of microhardness (VHN) at different time intervals within the same group: The values are mean ± SD

	Group (I) 2% Turmeric		Group (II) 2% Thymus		Group (III) 5.25% NaOCl		Group (IV) 2% CHX	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Day 1	39.56	5.88	44.24	19.90	42.13	9.37	34.49	5.55
Day 3	60.58	2.12	44.65	7.77	57.89	7.73	61.81	5.53
Day 7	48.63	9.61	54.39	6.22	46.83	11.54	61.87	16.72
P-Value	0.001		0.396		0.063		0.002	

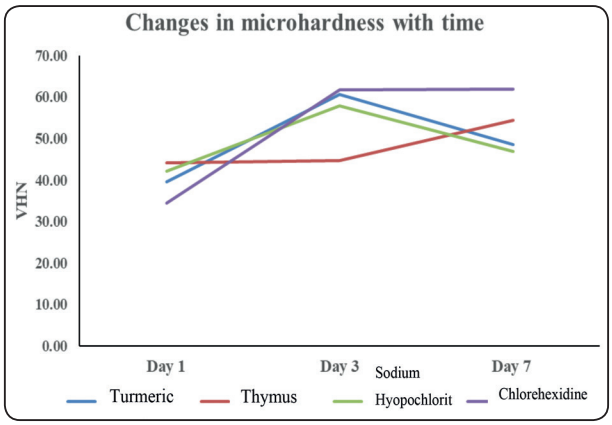

Fig. (3): Line chart representing changes in microhardness with time in the four groups

DISCUSSION

The main goal of endodontic treatment is to establish a bacteria-free environment, without compromising the structural integrity and microhardness of root canal dentin which would consequently weaken the tooth structure. [9]

Sodium hypochlorite remains the most recommended irrigant due to its antibacterial activity, dissolving capacity on pulp tissue, ease of availability and cheap price. Where, 5.25% NaOCl can effectively eradicate *E. faecalis* as confirmed by Alazzawi (2015) and Garg et al. (2014) [10,11]. On the other hand, CHX inhibits *E. faecalis* in 80% of the cases due to its substantivity and broad-spectrum antimicrobial effects against Gram-positive and Gram-negative organisms. [12]

However, the multiple side effects associated with the use of chemical irrigants as unpleasant taste and odour, cytotoxic effect upon extrusion into the periapical tissues, as well as the harmful effects on dentin structural integrity upon prolonged exposure had led the researchers to explore natural and herbal irrigants as an alternative option. [13] Thereby, the selection of Turmeric (Curcumin longa) as an intervention was attributed to its anti-inflammatory and antimicrobial potential.[14] Also, Thymus vulgaris was selected for its antibacterial, antifungal and anti-inflammatory properties as well. [15]

Since *E. faecalis* is considered the most resistant intracanal bacteria and one of the main causes for endodontic failures and flare ups.[16] The bacteria have the ability to tolerate high pH levels, high salt concentration, and long periods of starvation.[17] In the present study, *E. faecalis* strain (ATCC 29212) was incubated aerobically for 1 week at 37°C [18] to allow its growth. The brain heart infusion agar method was used to test the antibacterial efficacy of each irrigant, because it is the most widespread technique in the antimicrobial activity assessment; as it provides both qualitative and quantitative information.

For the microhardness test, teeth were decoronated and apices were removed to create 6 mm dentin blocks as to expose the dentin surface and facilitate the exposure to the irrigant solution. [6]

The experimental period of the present study was set between 1 and 7 days, at day 1, day 3 and day 7; to evaluate the demineralizing capacity of the irrigants used; this was on account of the reports conducted by Metzler et al. (1989) [19] who found that cleaning of the canal and isthmuses was successful when using intracanal Calcium hydroxide dressing for 7 days with subsequent instrumentation, and Sjogren et al. (1991) [20] who also suggested that a 7-days dressing with Calcium hydroxide efficiently eliminated the bacteria that survived biomechanical preparation.

While, Vicker's hardness tester is the most commonly used device in measuring dentin hardness and it is considered a suitable and practical method to evaluate the changes in the surface in deeper hard tissue structures. This test is widely accepted because of its extremely accurate readings and the fact that in this method just one type of indentation was used for all types of surface treatment. [21]

Results of this study showed that 2% chlorhexidine group had the highest antibacterial efficacy against *E. faecalis*, followed by 5.25% NaOCl, then 2% Thymus Vulgaris oil and finally the least antibacterial effect was reported to 2% Turmeric extract group ($p < 0.001$).

The high antibacterial activity of 2% CHX (Mean = 33.64 CFU) could be attributed to its positively-charged molecule; where CHX binds to the negatively charged sites on the cell wall of *E. faecalis*, destabilizing the cell wall and interfering with osmosis. Once the cell wall is damaged, chlorhexidine crosses inside the cell and attacks the cytoplasmic membrane causing intracellular components leakage; leading to cell death. [22]

This result came in accordance with the work of Prabhakar et al. (2013)[6], Sinha et al. (2015) [23], Mathew et al. (2015) [24], and Yadav et al. (2018) [25].

While the significant antibacterial effect exerted by 5.25% sodium hypochlorite against *E. faecalis* (Mean = 68.18 CFU) is based on its high pH which interferes with the cytoplasmic membrane integrity causing an irreversible enzymatic inhibition and biosynthetic alterations in the cellular metabolism by formation of chloramines and phospholipid destruction [26].This comes in accordance with Al-Azzawi (2015)[10], Divia et al. (2018) [27] and Siddique et al. (2019) [28].

Regarding the 2% Thymus in our present study; it demonstrated an antibacterial efficacy against *E. faecalis*. (Mean = 163.64 CFU), Much of the antibacterial activity of Thymus vulgaris oil is related to its phenolic compounds thymol and carvacrol. It is mostly believed that the hydroxyl group on these two compounds interacts with the cytoplasmic membrane; it changes its permeability and affects the lipid ordering and stability of its bilayer, resulting in an increase of proton passive flux across the membrane, which leads to disruption of the cytoplasmic membrane and leakage of cellular contents [29, 30]. This was in agreement with Jafari et al. (2015) [31].

In our study, 2% Turmeric extract showed the lowest antibacterial effect against *E. faecalis* among all groups (Mean = 172 CFU) as confirmed by Prabhakar et al. (2013) [6], Sinha et al. (2015) [23] and Saha et al. (2015) [5].

Turmeric's antibacterial effect is thought to be via the antibacterial action of its ingredients that are responsible for its biologic activity which is Curcumin. Curcumin suppresses the bacterial cytokinesis through induction of filamentation. It also markedly suppresses the cytokinetic Z-ring formation in bacteria without significantly affecting the segregation and organization of the nucleoids. It suppresses the bacterial cell proliferation by the inhibition of assembly dynamics of FtsZ which is an essential cell division protein that forms a contractile ring structure (Z ring) at the future cell division site [32].

In this study, CHX produced a significant increase of microhardness from day 1 to day 3 and from day 3 to day 7 ($p= 0.004$ and 1.0 respectively). Chlorhexidine's positive effect on dentin microhardness is understandable as it has been proven by several studies to act on maintaining root dentin integrity, owing to phosphate (PO4) and calcium (Ca) ionic fixation and to the proteolytic inhibition capacity of Matrix MetalloProteinase (MMPs) enzymes 2, 8 and 9, which are responsible of the dentin organic matrix degradation [33]. Those findings came in contrast to the results presented by Prabhakar et al. (2013) [6]. This may be attributed to the difference in the experimental design.

In the present study, 2% Turmeric showed a significant increase of microhardness values from day 1 to day 3 and from day 3 to day 7 ($p= 0.001$ and 0.036 respectively), those results were in agreement with Prabhakar et al. (2013)[6]. This could

be attributed to Curcumin's ability to inhibit Matrix MetalloProteinase (MMP-9) action; which causes dentin organic matrix degradation. This interaction of Curcumin with MMP can be explained by their ability to chelate the catalytic Zinc ions essential for MMP activity [34].

Thymus also showed a statistically non-significant increase of the microhardness at different days. Wissam et al. (2018) concluded that Thymus extract had low chelating capacities in comparison with Ethylenediamine tetraacetic acid (EDTA); due to its phenolic compounds thymol and carvacrol, though the exact mode of action of these compounds is not clearly understood yet [35].

The results from the current study revealed a statistically non-significant decrease of dentin microhardness values exerted by 5.25% sodium hypochlorite. In agreement with the work of Saha et al. (2017) [5]. Those findings could be attributed to the organic dissolving capacities of NaOCl on the collagen component of dentin, magnesium and phosphate ions. In addition, it was reported that NaOCl treatment significantly alters the Calcium/Phosphorus ratio of the root dentin surface, ultimately decreasing the dentin microhardness [36, 37]. In 2004, Goldberg et al. [36] concluded that the higher the concentration of NaOCl, the higher the detrimental effect on dentin hardness and strength properties.

Up to this date, literature lacks evidence in reference to plant extract having any effect on microhardness of root dentin, thus it is required to conduct more studies to obtain a clearer evidence based data.

CONCLUSIONS

Within the limitations of this study we concluded that:

- Herbal irrigants like Turmeric and Thymus may serve as alternatives to conventional root canal irrigants as they cause minimal alteration to dentin microhardness when compared to chemical irrigants.
- Though herbal alternatives may not be as potent as chemical irrigants used in root canal disinfection due to their delayed antibacterial action, they have the potential to be used as intracanal medicaments.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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