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The Effect of Herbal Medicine against *Enterococcus faecalis* on Infected Root Canal Substrate: An *Ex-vivo* Study

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Authors' contributions

This work was carried out in collaboration between both authors. Authors EMA and SAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SAA managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate the antimicrobial effects of ginger, cinnamon and their combination against *Enterococcus faecalis* located in infected root canals.
Methodology: A quasi experimental pre-test post-test design was applied through microbiological testing upon prepared root canals of 50 extracted single-rooted teeth. The roots were divided into a total of 5 groups, each group consisted of 10 root canals; three groups contained extracts of ginger, cinnamon, and their combination in a gel form. In addition; a positive control group of calcium hydroxide with Iodoform paste and a negative control group of solely infected root canals. All root canals in each group were infected by *Enterococcus faecalis*. The colony forming units (CFU) in each root canal were recorded before and after the procedure, and the minimum inhibitory concentration for each test solution was also obtained. Comparisons between the different variables were conducted through the Kruskal Wallis, Wilcoxon and Mann Whitney U tests with the level of significance set at $P \leq 0.05$.

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Results: Extracts of ginger, cinnamon and their combination showed a reduction in (CFU) from an initial count of 83 colonies to a mean (CFU) of 26.5, 77.8 and 49.7, respectively. Ginger showed the greatest antibacterial efficacy in comparison with the others. Upon comparison of the negative control group and the test solutions of ginger, cinnamon and their combination, significant differences were observed ($P < 0.05$), while no statistical differences ($P > 0.05$) were observed when comparing with the Calcium Hydroxide with Iodoform group to these test solutions.
Conclusions: An inhibitory effect against *Enterococcus faecalis* occurred upon testing ginger, cinnamon and their combination. Ginger was found to have the highest effect in comparison to other test groups.

Keywords: Cinnamon; Enterococcus faecalis; ginger; infected root canals.

1. INTRODUCTION

Among factors investigating the etiology of failures in endodontic therapy, it is generally believed that the major cause of failure is the persistence of microorganisms in the apical part of root filled teeth [1].

Enterococci, common inhabitants of the human body, have been associated with colonization and infection in a variety of sites including the oral cavity [2]. *E. faecalis* appears to possess the requisites to establish an endodontic infection and maintain an inflammatory response potentially detrimental to the host. *E. faecalis* has been frequently found in obturated root canals exhibiting signs of chronic apical periodontitis [3]. Once established in the root canal, *E. faecalis* faces several challenges for survival, including the ability to withstand antimicrobial agents used during treatment and endure potential starvation in the cleaned and obturated canal [1]. Its ability to survive these extreme challenges by tolerating harsh environmental conditions may act as an advantage over other species [3]. *E. faecalis* that invades the dentinal tubules may survive chemo mechanical instrumentation and intra canal medication; it can colonize the tubules and re-infect the obturated root canal [4]. This may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments [3]. Recent studies have also shown that *E. faecalis* is highly resistant to commonly used intra canal medicaments such as calcium hydroxide [4,5].

The use of calcium hydroxide has also been suggested as a factor contributing to the continual presence of *E. faecalis* after endodontic treatment because of its relative inefficiency as an antimicrobial agent against this organism [3,6,7].

Herbal medicine is becoming the new turning point in medicine through its growing popularity and its implication in dental procedures, which may eliminate the challenge of resistant bacteria to conventional forms of treatment [8-11]. There are phytotherapeutic substances in dentistry that have been used as anti-inflammatory, antibiotic, analgesic and sedative agents as well as for miscellaneous purposes including endodontic irrigants, medicaments and in endodontic retreatment [12]. Of the herbs having antimicrobial properties against oral pathogens, ginger from the plant *Zingiber officinale* and cinnamon from *Cinnamomum zeylanicum* have shown significant activity against isolates of enterococci. This has been proven in an *in vitro* study that investigated the antibacterial activity of spices against clinical isolates of enterococci with a result of all different species of enterococcus maximally inhibited by ginger and cinnamon [13]. With no previous *ex vivo* studies tackling this issue.

The null hypothesis (H_0) is that ginger, cinnamon and the combination of ginger and cinnamon will have no antibacterial efficacy against *Enterococcus faecalis* on infected root canal substrate *(Ex-vivo)* or no similar or greater efficacy to calcium hydroxide paste. Another null hypothesis includes that the combination of ginger and cinnamon will not have a synergistic effect against *E. faecalis* on infected root canal substrate. Otherwise, the alternative hypothesis (H_1) that ginger, cinnamon and their combination will have antibacterial efficacy against *E. faecalis* on infected root canal substrate with a similar or greater efficacy to calcium hydroxide paste. Alternatively, the combination of ginger and cinnamon will have a synergistic effect against *E. faecalis* on infected root canal substrate.

The main objective of this study was to evaluate the antimicrobial potential of ginger (*Zingiber officinale*) and cinnamon (*Cinnamomum zeylanicum*), alone and in combination, against *Enterococcus faecalis* species on infected root

canal substrate, *ex vivo*. While the specific objectives were to assess the efficacy of ginger and cinnamon in comparison to calcium hydroxide with Iodoform paste against *Enterococcus faecalis* on infected root canal substrate. Also another specific objective was to evaluate the synergistic effect of combining herbs, cinnamon and ginger, in their antimicrobial potential towards *Enterococcus faecalis*.

2. MATERIALS AND METHODS

2.1 Study Design, Duration and Power of the Study

Quasi Experimental, Pre Test Post Test Design. The course of the study was made between October 2013 and February 2014. Statistical Power of the Study with a power level of 80% determined the size of 50 extracted single rooted teeth [14].

2.2 Tested Population

Extracted Human Teeth comprised a total of 50 roots: Permanent sound single maxillary or mandibular premolar teeth. Severely curved canals, root fractures; dental caries, root resorption and any presence of anomalies were excluded.

Materials used were Ginger [15], Cinnamon [16] and Calcium Hydroxide with Iodoform Paste [I-CAL Plus, MedicinosLinija UAB, Lithuania]. Microorganism used was *Enterococcus faecalis (family Enterococcaceae, species Faecalis, Gram Positive bacterium; non- motile and Facultative Anaerobe)* [17].

2.3 Methods

2.3.1 Herb/Plant collection and authentication

The herbs *Zingiber officinale* and *Cinnamomum zeylanicum* were collected from markets in Khartoum State and were authenticated at the Medicinal and Aromatic Plants Research Institute (MAPRI) at the National Center for Research Herbarium- Khartoum State – Sudan.

2.3.2 Herb preparation and extraction

500 grams of each ginger and cinnamon were cleaned using distilled water air-dried and the prepared ginger was grinded into fine pieces. Both herbs were placed into separate sterile glass flasks containing 1000 ml of 70% ethanol [15].

The suspended ginger and cinnamon were then subjected to the process of cold maceration for 4 hours per day throughout 2 days of 48 hours and were filtered. The obtained residues of their filtrates were subjected to Rotaevaporation to facilitate complete evaporation of ethanol from the filtrate. They were then stored at room temperature for further use. Prior to usage of the herbs, the extracts were pulverized into fine powder using an electric food processor.

2.3.3 Preparation of extracted teeth

Freshly human extracted (for orthodontic treatment), sound, single rooted teeth were cleaned and sterilized in an autoclave for 15 minutes at 121℃. The teeth were stored in deionized water [14] for one week before the experiment.

In order to standardize the length of the roots, the teeth were decoronated using a diamond disc on a low speed hand piece reaching 12 mm (as this was the greatest length achievable from the various root lengths present) for all roots.

The root canals were all chemo mechanically prepared through the step-back technique with simultaneous irrigation between each instrument using 3% sodium hypochlorite up to a master file size of 40 mm (ISO) and stepping back up to a size 55 (ISO). Flowable composite (Nexcomp Flow, META Biomed) was placed over the root apices [17] and to ensure no leakage of microbial media, the roots surfaces were sealed using clear acid resistant nail varnish. Then these 50 roots were mounted on five blocks of self-cured acrylic resin; 10 per block and stored in sterile deionized water for the day of the experiment.

2.4 Microorganism

Standard cultures of the following organism were obtained: Gram positive bacterium: *Enterococcus faecalis (family Enterococcaceae, species Faecalis, Gram Positive bacterium; non- motile and Facultative Anaerobe* [ATCC 29212] according to study by Neelakantan et al. [17], and were obtained from microbiology laboratory of the University of Medical Sciences and Technology, Sudan.

2.5 *In vitro* Pilot Trial

2.5.1 Cup plate method

Twenty ml of sterile nutrient agar medium was poured into sterile Petri dishes and allowed to solidify. The Petri dishes were incubated at 37℃ for 24 hours to check for sterility. The medium was seeded with the organisms by the pour plate method using sterile top agar (20 ml) contained 0.2 ml of culture. Bores/wells were made on the medium using a sterile borer (10 mm in diameter) [18]. 0.1 g of the dried, powdered ginger, cinnamon and their combination was measured using a balance in Bijou bottles and each dissolved in 1 ml of methanol. 0.1 ml of each test solution was placed into their wells using a micropipette. The plates were incubated overnight at 37℃ for 24 hours [18] and the zones of inhibition were observed, measured and recorded in millimeters (mm).

2.5.2 Disk diffusion method

Using an aseptic technique, a sterile swab was placed into the broth culture of *E. faecalis* and any excess liquid was removed by gently pressing the swab against the inside of the tube.

Using the same swab, the nutrient agar plate was streaked in one direction in order to maintain uniform growth [19]. Using flame sterilized forceps; each disc was gently pressed into the agar to ensure that it is attached to the agar. 0.02 ml of each test solution (ginger, cinnamon and combination extracts), at a concentration of 10% each, was placed onto each disk using a micropipette. The plates were then incubated overnight at 37℃ for 24 hours and the zones of inhibition were observed, measured and recorded in millimeters (mm) [19].

2.6 Minimum Inhibitory Concentrations (MIC)

Minimum inhibitory concentration (MIC)is defined as the lowest concentration of extract that exhibited no growth by visual reading. It is expressed in microgram/millimeter (µg/ml). The MICs were determined for each test solution: Ginger alone, Cinnamon alone and the combination of the ginger and cinnamon (1:1 ratio). Serial dilutions of the three test solutions were made in 5 test tubes that contained 10 ml of sterile distilled water (SDW) giving final concentrations of 10, 5, 2.5, 1.25 and 0.625. One gram of the test solution was dispensed into the first tube and mixed well. After wards, 10 ml from test tube #1 (10% concentration) was removed and dispensed into test tube #2 (5% concentration), followed by removal of 10 ml from test tube #2 which was dispensed into test tube #3 (2.5%), another 10 ml removed from test tube #3 and dispensed into test tube #4 (1.25%), and a final dilution of 10 ml from test tube #4 into test tube #5 leaving a final concentration of 0.625%.

Four other test tubes contained 0.02 nutrient broths each. 10 ml from each previously diluted test solutions were dispensed into their corresponding nutrient broth-containing test tubes.

The bacterial suspension (0.02 ml) was prepared in nutrient agar on 4 Petri dishes. A swab was placed into each of the four test tubes and streaked onto their corresponding Petri dish containing nutrient agar. The plates were sealed and incubated at 37℃ for 24 hours [20] and then observed for the presence or absence of growth for each concentration of the herbal extract (ginger, cinnamon and combination).

2.6.1 Gel preparation and formulation

Polyethylene Glycol (PEG) 200:6000 was used to formulate a gel [21] in order to mimic the clinical procedure of medicament placement. A powder to liquid ratio of 1:4; 1 g of powder and 4 ml of liquid were stirred into a glass beaker using a sterile glass rod. The mixture was placed into a water bath at a starting temperature of ~ 66℃ and was observed until ensured that a homogenous mixture was created. 0.1 g of ginger, 0.1 g of cinnamon, and 0.1 g of their combination (0.05 g ginger + 0.05 g cinnamon) were each mixed separately with 0.9 g of the gel – hence a concentration of 10% for each herb.

2.6.2 Inoculums preparation

Cultures of *E. faecalis* were kept on nutrient agar overnight under aerobic conditions. The turbidity of the suspension was standardized by comparison with a 0.5 McFarland standard to give an approximate count of 10^8 colony – forming units (CFU/ml) [14].

The root canals were dried using sterile paper points (ISO size 40). Inoculum of this culture (0.02 ml) was suspended into each canal of the fifty roots using sterile micropipettes. This 0.02 ml retrieved from the McFarland standard that produced a total of 10^8 colonies was dispensed into nutrient agar in order to measure the initial number of CFU/ml which gave a colony count of

83 colonies. 0.02 ml of nutrient broth was also dispensed into each root canal to ensure bacterial survival.

The inoculated roots were re-mounted onto self-cured acrylic resin blocks and randomly divided into 5 study groups of 10 roots each as per test solution used (each block was labeled accordingly):

- Ginger Group (10%),
- Cinnamon Group (10%),
- Ginger + Cinnamon Group (10%),
- Positive Control group (Calcium Hydroxide Paste with Iodoform) (I-CAL Plus, MedicinosLinija UAB, Lithuania)
- Negative Control Group (bacterial inoculated canal; no test solution used).

After 24 hours, the bacteria were recovered from the root canals using sterile paper points (Size 40 mm – ISO), which were rubbed against the walls of the root canals and allowed to draw up their full capacity with liquid for 30 seconds for each. The paper points were then transferred into bijou bottles containing 10 ml of sterile saline. After 30 minutes, 0.1 ml of the paper point containing saline was taken up using sterile micropipettes and dispensed into a Petri dish containing 20 ml nutrient agar.

These plates were incubated overnight at 37℃ for 24 hours to allow bacteria growth. *E. faecalis* colony forming units (CFU/ml) were then enumerated using a colony counter and recorded.

2.7 Fractional Inhibitory Concentration (FIC)

2.7.1 Synergy testing

The *checkerboard method*, which is commonly used for measurement of interactive inhibition, was used for the determination of synergy between the ginger and cinnamon. The checkerboard method is often combined with calculation of a *fractional inhibitory concentration (FIC) index* to test the antimicrobial potencies of drugs in medical laboratories. The FIC value for each agent was calculated using the formula:

FIC *(Ginger)* = (MIC of Ginger + Cinnamon)/MIC of Ginger

FIC *(*Cinnamon*)* = (MIC of Ginger + Cinnamon)/ MIC of Cinnamon

FIC index= FIC of Ginger + FIC of Cinnamon

Combinations are classified as:

Synergistic, if the FIC indices were ≤1,
Additive, if the FIC indices were = 1,
Indifferent, if the FIC indices were between 1 and 2 and
Antagonistic, if the FIC indices were ≥ 2 [22].

2.8 Statistical Analysis

Data were analyzed by SPSS statistical software package (SPSS for Windows, Rel. 20.0. 2011. Chicago: SPSS Inc.). A Kruskal Wallis test was used to compare mean colony forming units (CFU) between groups; Wilcoxon test, in order to test the efficacy of each group, as well as the Mann Whitney U test, to measure the association between groups. The significance level was set at $P \leq 0.05$.

2.9 Ethical Considerations

This study was approved by the ethical committee of the University Medical Sciences and Technology. Permission was obtained from the National Centre for Research laboratories to conduct this study. Patients whom their teeth were used in this study signed an informed written consent to leave their teeth voluntarily for the purpose of this study.

3. RESULTS

Descriptive statistic of the results was displayed in form of Tables (1-7). The mean colony forming units for all groups were displayed in Tables (1 and 2).

According to the Wilcoxon Test, *P* values for all groups were less than 0.05 indicating a statistical significance; each group has a certain efficacy against the bacterium *E. faecalis*. Having the least *P* value, ginger had the greatest efficacy in comparison to the other tested groups (Table 3).

According to the Mann Whitney U test, there were significant differences between the negative control group and the test solutions of ginger, cinnamon and their combination as shown by the *P* values of less than 0.05 (Table 4). Upon comparing the positive control group Ca (OH_2) to the tested groups, no significant differences were found (Table 5). Comparison within the test solutions showed no association between ginger or cinnamon with their combination and no significant difference upon comparing ginger and cinnamon alone as their *P* values were greater than 0.05 (Table 6).

Table 1. Colony Forming Units (CFU/ml) before and after study

Root canal#	Initial CFU/ml	Ginger	Cinnamon	Ginger + Cinnamon	Calcium hydroxide with Iodoform	Negative control
1	83	65	0	78	0	349
2	83	109	29	31	3	23
3	83	4	134	64	3	307
4	83	45	7	0	1	760
5	83	19	90	1	14	67
6	83	6	205	89	0	800
7	83	7	4	35	180	171
8	83	0	79	195	3	79
9	83	4	9	1	41	420
10	83	6	221	3	26	69
Mean	83	26.5 (3×10^1)	78.9 (8×10^1)	49.7 (5×10^1)	27.1 (3×10^1)	304.5 (3×10^2)

Table 2. Distribution of the study sample according to the mean colony forming units (CFU/ml) per group

Test groups	N	Min	Max	Mean	Standard deviation
Ginger	10	0	109	26.5	35.94
Cinnamon	10	0	221	77.8	55.41
Ginger + Cinnamon	10	0	195	49.7	61.12
Calcium Hydroxide with Iodoform	10	0	180	27.1	55.41
Negative Control*	10	23	800	304.5*	284.43

**Negative Control (bacteria only) had the highest mean CFU/ml compared to other*

Table 3. Efficacy of each test group based on the mean CFU/ml

Test group	P value
Ginger	0.004
Cinnamon	0.005
Ginger + Cinnamon	0.005
Calcium Hydroxide with Iodoform	0.005
Negative Control	0.005

Table 4. Association between the negative control group and ginger, cinnamon and their combination based on their mean CFU/ml

Associations	P – value
Negative Control & Ginger	0.003
Negative Control & Cinnamon	0.015
Negative Control & Ginger & Cinnamon	0.03

According to the Kruskal Wallis test the comparison of Mean Colony Forming Units (CFU/ml) between each group, with a *P*= 0.02, Thus there is a difference between the mean colony forming units in each test group.

The synergy between the ginger and cinnamon was determined by checkerboard method which is often combined with calculation of a *fractional inhibitory concentration (FIC) index* to test the antimicrobial potencies of the two herbs. The result revealed that:

FIC Ginger= 1.25/ 2.5 = 0.5
FIC Cinnamon=1.25/ 1.25 = 1

FIC Index = 0.5 + 1 = 1.5
FIC Index = 1.5

With an FIC index of 1.5 (valued between 1 and 2), this indicates that cinnamon and ginger have an ***indifferent*** effect towards one another.

Table 5. Association between the calcium hydroxide with iodoform group and ginger, cinnamon and their combination based on their mean CFU/ml

Associations	P – value
Calcium Hydroxide with Iodoform & Ginger	0.252
Calcium Hydroxide with Iodoform & Cinnamon	0.269
Calcium Hydroxide with Iodoform & Ginger + Cinnamon	0.235

Table 6. Association between combination and ginger and cinnamon based on their mean CFU/ml

Associations	P – value
Ginger+cinnamon & Ginger	0.252
Ginger+cinnamon & Cinnamon	0.269
Cinnamon & Ginger	0.235

Table 7. Minimum inhibitory concentration (MIC) – *In vitro test* results

Herb	5%	2.5%	1.25%	0.625%
Ginger	S	**S**	R	R
Cinnamon	S	S	**S**	R
(ginger + cinnamon)	S	S	**S**	R

S = Susceptible, R = Resistant

4. DISCUSSION

This study demonstrated the presence or absence of antimicrobial activity of the herbs ginger, cinnamon and their combination as well as a comparison of their efficacy and tests of synergism on root canal substrate infected with the bacterium *E. faecalis*. In accordance with the results obtained, all three test groups (ginger, cinnamon and their combination) have shown antibacterial activity against this organism.

Revati S, et al. [13] found the maximum zones of inhibition were 20 mm for ginger and 34 mm for cinnamon; However, Anjan G, et al. [15] used the same concentration of 10% ginger extract, gave a maximum zone of inhibition of 14 mm for *E. faecalis* at a volume of 75 µL, which is near to the value achieved in this study; 17 mm at a volume of 1000 µL. As the volumes were different, thus the results would be.

Components of ginger and cinnamon, named Gingerol and cinnameldehyde, are the primary compounds responsible for the antimicrobial action of these herbs [22,23]. Cinnameldehydes disrupt the cell membrane of the bacteria and its structures leading to ion leakage [24] and in terms of ginger, the active constituent that inhibits the growth of oral bacteria associated with Periodontitis has not yet been revealed [26].

It was hypothesized that both ginger and cinnamon would exhibit an antimicrobial effect against this organism which has been proven in the previously stated results. Both herbs presented an exponential reduction in their colony counts when compared to the original number of colonies present when the canals were initially infected. When these two herbs were tested *in vitro* by Revati S, et al. [13], they showed 100% antibacterial activity against *E. faecalis*, which was not demonstrated according to *mean* values of CFU/ ml (Table 1) but rather when deducing the trials separately. Each tested group, ginger, Cinnamon and Ginger + Cinnamon showed one trial result out of the ten root canals per group having zero colonies present post experimentation i.e. 100% antibacterial action.

Upon comparison of the efficacy of the three tested groups, the CFU results were ginger> pilot study cont> combination > cinnamon >Negative control group which was shown in Table 1. This may be attributed to the active component of ginger, as previously mentioned, to be more powerful than that of cinnamon. The minimum inhibitory concentrations that were revealed for each test group (ginger, cinnamon and combination) represented the minimum concentration of the herb extract that could demonstrate an antibacterial effect against *E. faecalis*. In the study conducted by Anjan G, et al. [15]; the minimum inhibitory concentration was found for ethanolic ginger extract in which *E. faecalis* was found to be susceptible at a minimum concentration of 5% ginger extract while the following study found the MIC of ginger to be 2.5%, showing *E. faecalis* to be susceptible to even less concentration of ginger by half the amount that resulted in this previous study. Through these concentrations attained, the FIC for ginger and cinnamon were calculated to test synergy between them as they were used as a combination in this study. It was revealed that ginger and cinnamon did not portray a synergistic but rather indifferent interaction, and as such their effects in combination will not yield a result greater than the concentration and effectiveness of the most active substance in the combination which goes against the hypothesis in that synergism would be produced between the two.

According to the results of this study, the negative control group, that was only infected with *E. faecalis* and not subjected to any test solutions showed an increased number of colony counts in comparison to the initial 83 CFU/ml (mean of 304.5 CFU/ml), yet there were also certain trial results showing inhibition for this group which is reason for additional investigation.

It is shown in Table 2 that the maximum values of the tested groups (ginger, Cinnamon and

Ginger + Cinnamon) and positive control (Calcium Hydroxide with Iodoform) to be greater than the initial count (109, 221, 195, 180 CFU/ml for ginger, cinnamon, combination and positive control respectively). This demonstrates inconsistent results as the *in vitro* trial for diffusion (pilot study) confirmed antimicrobial action for the tested groups, which warrants additional investigation.

Further inconsistency in results may have occurred, as it is possible that upon removal of the bacteria from the canals using paper points these paper points may not fully absorb the contents within the canal. This may be due to differences in root shape cross sectional configurations (round, oval, long oval, bowling pin, kidney bean, ribbon and hour glass) and these different shapes can occur at any level within the root [25] and the round shape of the paper point may have not coincided with the root canal shape; all occurring even whilst the canal preparations were standardized according to diameter and length.

In this study, after ensuring that the paper points have absorbed the canal contents they were each placed into a bijou bottle containing saline, and a sample of the saline was taken and deposited onto nutrient agar – this may have left room for error in which some of the canal contents could have dispersed throughout the saline and may have not been taken up for colony growth on agar. Another possibility leaving room for error may concern the age range of the individual from which the extracted tooth was taken from. Based on a study entitled "the effect of age on bacterial penetration of radicular dentin" the effect of the patient's age on the prevalence and depth of bacterial penetration inside dentinal tubules was determined through histological analysis of extracted single rooted teeth. It was found that the number of tubules that were invaded by bacteria in the young group (18 - 25 years) of individuals as well as the depth of invasion, was significantly higher than in the old group (≥ 60 years) [26]. It should also be taken into account the presence of microbial biofilms [6] formed by the organism that may prevent the proper absorption of the bacteria present fully by the paper point and can act as a barrier for the test solutions to diffuse into the dentinal tubules. All of these possibilities may have affected the outcomes of the test and control groups and account for any discrepancies in their results.

A pilot study was carried out *in vitro* prior to experimentation on the extracted teeth through diffusion testing on agar plates by two methods, both of which demonstrated the combination of ginger and cinnamon with the greatest zone of inhibition compared to cinnamon and ginger alone. The cup plate diffusion technique also, at the same level of effectiveness, has shown cinnamon to have a large zone of inhibition as well. While in both the cup plate and Kirby Bauer methods, ginger had the smallest zone of inhibition, even showing an intermediate rather than susceptible effect towards *E. faecalis*.

An inconsistency arose when comparing the results from the *in vitro* versus that of the *ex vivo* experiments may be attested to the basis of biofilms being formed within root canals and since the testing of antimicrobial agents against bacterial biofilms; is yet to be standardized and no *in vitro* method accurately reflects the conditions under which microorganisms grow *in vivo* [17]. It may be also because this is a preliminary study; further research is warranted on these two herbs prior to their clinical application as intra canal medicaments. Certain variables should be considered such as; different time intervals as Ca $(OH)_2$ requires a maximum of 10 days to complete its action, different concentrations of ginger and cinnamon, different vehicle preparations (solutions, oil based, etc.), age of person from which extracted tooth was obtained (as bacterial invasion differs according to the age of radicular dentine) and *Enterococcus faecalis* biofilm testing within root canals.

5. CONCLUSION

The alternative hypothesis was correct; all three tested groups of extracts (ginger, cinnamon and ginger + cinnamon) have shown antibacterial effects against the organism *Enterococcus faecalis* within infected root canal substrate.

In terms of efficacy, ginger was found to produce a more desirable result when compared to the other tested groups (cinnamon and ginger + cinnamon). Ginger was found to have a similar efficacy to calcium hydroxide with Iodoform, however when testing for synergism, an indifferent interaction was observed between ginger and cinnamon.

DISCLAIMER

Some part of this manuscript was previously presented and published in the following conference.
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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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