Original Article

Evaluation of antimicrobial efficacy of four medicinal plants extracts used as root canal irrigant on Enterococcus faecalis: An in-vitro study

I. N. Radwan1, B. Randa2, A. N. Hend2, G. Camilia3

1Department of Endodontics, Cairo University, Giza, Egypt, 2Department of Endodontics, Faculty of Oral and Dental Medicine, Cairo University, Giza, Egypt, 3Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Giza, Egypt

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Abstract

Aim: Evaluation and comparison of the antimicrobial efficiency of the medicinal plant extracts, when used as root canal irrigants, on single species of Enterococcus faecalis.

Materials and Methods: A comparative bioactivity-guided fractionation of four medicinal plants was carried out using solvents with different polarities (ethanol, hexane, ethyl acetate, butanol, and water). The organism was freshly inoculated in brain-heart infusion broth for 24 h prior to the test, and the inoculum was standardized. Cultures were spread over the surface of the plate, and wells were punched out in every plate. Each well was inoculated by each extract and incubated for 24 h and the zone of inhibition around each well was recorded. A total of 50 extracted human single rooted teeth were disinfected decapitated and prepared, then divided into six groups: Group I: Neem leaf extract, Group II: Ginger extract, Group III: Miswak extract, Group IV: Lemon solution, Group V: NaOCl 5.25% (+ve control), and Group IV: Saline (−ve control). Each root canal was completely filled with bacterial suspension and incubated at 37°C for 24 h, then completely filled with one of the medicinal plants extract according to each group. Root specimens were then incubated for 24 h at 37°C. The root canals were sampled, and the samples were contained and incubated for 24 h at 37°C. The colony forming units (CFU) were calculated. Data presented as mean and standard deviation values. One-way analysis of variance (ANOVA) was used for comparisons between more than two groups. Tukey’s post-hoc test was used for pair-wise comparison between the groups when ANOVA test was significant.

Results: NaOCl 5.25% showed significantly less CFU/ml count, when compared with all tested plant extracts (P < 0.001). This was followed by fresh lemon solution which showed significantly less CFU/ml count when compared with all of the other tested plant extracts. Butanol fraction of ginger showed significantly less CFU/ml count than ethyl acetate fraction of miswak and neem.

Conclusions: Medicinal herbs may offer a new source of antibacterial agents for use; medicinal plants are natural antimicrobial agents and might be used in the development of a promising irrigants, which might be safer than other chemical compounds used in the endodontic treatment process.

Introduction

The success of endodontic therapy depends on many factors, starting from access cavity preparation, biomechanical preparation, and three-dimensional obturation of the root canal system. Eradication of the root canal infection is very important during endodontic treatment, since residual infection is one of the chief factors leading to post-treatment failure.1
Enterococcus faecalis is one of the most frequent isolated microorganisms from endodontic infections. This is explained by its various survival and virulence factors; including its ability to compete with other microorganisms, invasion of dental tubules and resistance to nutritional deprivation.\(^1\)

Because of the cytotoxic reactions of most of the commercial irrigants used and their inability to totally eliminate bacteria from root canals, trend of recent medicine attempts to use biologic irrigants extracted from natural plants. Herbal products have been used since ancient times, involving both eastern and western medicinal traditions.\(^2\)

Many plants with biological and antimicrobial properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse and misuse. In dentistry, phytomedicines have been used as anti-inflammatory, antibiotic, analgesic, and sedative agents. Hence, it seems beneficial to study and evaluate the antibacterial efficiency of some medicinal plants extracts when used as root canal irrigants on E. faecalis.

Materials and Methods

Selection and preparation of medicinal plants

In this study, the medicinal plants used for the experimental purpose were:

- **Neem (Azadirachta indica A. Juss)** leaves were purchased from the Experimental Station of Faculty of Pharmacy, Cairo University, Giza, Egypt
- **Ginger (Zingiber officinale Roscoe)** rhizomes and Miswak (Salvadora persica L.) dry sticks were purchased from the local market (herbalist Haraz)
- **Lemon (Citrus limonum Risso)** fruits were purchased from the Agricultural Research Centers, Faculty of Agriculture, Cairo University, Giza, Egypt.

The taxonomical feature of Neem, Ginger and Miswak were kindly confirmed by Faculty of Pharmacy, Cairo University, Egypt and that of C. limonum by Faculty of Agriculture, Cairo University, Egypt.

Preparation of extracts

About 2 kg of fresh neem leaves were thoroughly washed with distilled water and shade dried at room temperature with continuous rotation. The air-dried leaves were coarsely powdered by an electric mill and extracted by maceration until exhaustion with ethanol 70\% (El-Gomhoria Co., Cairo, Egypt). The total ethanolic extract together with its fractions (petroleum ether, ethyl acetate, butanol and remaining aqueous fractions) of neem, ginger, miswak and fresh lemon solution were tested for their antimicrobial activity against E. faecalis. Sodium hypochlorite 5.25\% (CLOROX™, Egyptian Household Cleaners Co., 10th of Ramadan city) was used as a positive control, while dimethyl sulfoxide (DMSO) (El-Gomhoria Pharmaceutical Co., Cairo, Egypt) and saline served as the negative control. The collected ethanolic extract was filtered using what Mann filter paper No.6 (Whatman® qualitative filter paper) and concentrated to dryness using a rotary evaporator (SINYCO Technology Co., China) at 60°C under reduced pressure. The obtained residue was transferred to a porcelain dish and kept in a desiccator until constant weight and then weighed using a three digits sensitive electric balance to calculate the percentage yield of the total ethanol 70\% extract.

A specified weight of the neem ethanolic residue (111 g) was suspended with distilled water in a separating funnel and extracted with petroleum ether (50 ml x 4), ethyl acetate (50 ml x 4) and butanol saturated with water (50 ml x 4). Each fraction was separated and concentrated to dryness under reduced temperature and pressure using a rotary evaporator. Finally, different fractions were kept in desiccator and weighed similarly like the ethanol 70\% extract to calculate the percentage yields.

Similarly, 2 kg of ginger rhizome and miswak sticks were grinded, extracted with ethanol 70\%, the percentage yield was calculated, and finally the ethanolic extracts were fractionated separately with petroleum ether, ethyl acetate and butanol saturated with water. The remaining aqueous suspensions of the three tested medicinal plants (neem, ginger and miswak) were similarly concentrated to dryness and all fractions were refrigerated in dark containers at 3°C for 3 weeks for further antimicrobial and endodontic irrigation evaluation. Finally, 2 kg of lemon fruits were freshly squeezed just before use using an electric squeezer.

Selection and preparation of bacterial micro-organism

A single species of E. faecalis strain no. ATCC 29212 was kindly provided by the Regional Center for Mycology and Biotechnology, Al-Azhar University. The microorganism was maintained in a frozen (−80°C) cultural broth containing 50\% glycerol. A culture of E. faecalis was prepared in sterile brain heart infusion (BHI) broth (Oxoid, England) and adjusted spectrophotometrically to an optical density of 625 nm corresponding to match the turbidity of a McFarland 0.5 scale.

Antimicrobial evaluation

In the present study, the antimicrobial evaluation was performed using two methods:

Antimicrobial sensitivity test

1. Agar well-diffusion test
   
   The total ethanolic extract together with its fractions (petroleum ether, ethyl acetate, and remaining aqueous fractions) of neem, ginger, miswak and fresh lemon solution were tested for their antimicrobial activity against E. faecalis.

   Sodium hypochlorite 5.25\% (CLOROX™, Egyptian Household Cleaners Co., 10th of Ramadan city) was used as a positive control, while dimethyl sulfoxide (DMSO) (El-Gomhoria Pharmaceutical Co., Cairo, Egypt) and saline served as the negative control. The tested plant extract fractions were dissolved in DMSO at concentration of 20 mg/ml.

   Six petri dishes of 16 cm diameter were used. In each petri dish, Muller-Hinton agar was poured and swirled to distribute the medium homogenously. After setting of the agar, the freshly prepared inoculum containing the bacterial suspension in BHI broth was spread over the surface of the Muller-Hinton agar plates using sterile cotton swabs. Wells of 6 mm diameter and 4 mm in depth were bored using a cack borer into the medium. Using pipettes, the wells were filled with 50 \( \mu \)l of each plant extract and the same volume of the controls. The plates were left for 30 min at room temperature to allow diffusion of the plant.
extracts through the agar, and then they were incubated at 37°C for 24 h.

After incubation, the plates were observed for zones of inhibition of the microbial growth around the wells containing the extracts. The diameters of these zones were measured in millimeters [Figure 1] with a transparent ruler and recorded. Diameter <6 mm indicated no effect and was neglected.

2. Minimum inhibitory concentration (MIC)

The plant extracts showing the most potent antimicrobial effect against *E. faecalis* were selected for the MIC test. The microorganism was cultured using Muller-Hinton agar 24 h before the test. A suspension of the freshly cultured organism in normal saline equivalent to 0.5 McFarland was also prepared. 100 μl of sterile BHI broth was dispensed into every test tube. 100 μl of each extract was added to the first test tube of each row. Serial dilution from column 1 to column 10 was done, and the last 100 μl was discarded (the final volume in each test tube was 100 μl). 5 μl of bacterial suspension adjusted to 0.5 McFarland was added starting from test tubes with the highest dilution to the test tubes with lowest dilution. Trays were covered and then incubated for 24 h in a laminar air flow cabinet at 37°C. The MIC results of each extract were then recorded in μg/ml.

**Direct contact test**

After the antibacterial screening by the agar well-diffusion method and the MIC, the extract fractions having the highest antimicrobial effect against *E. faecalis* were tested on prepared root canals of extracted human teeth.

**Selection of samples**

About 50 extracted human permanent teeth with single completely-formed roots with no visible cracks or caries were selected. The teeth were soaked in 5.25% NaOCl solution, for surface disinfection before the removal of soft tissues remnants on the root surface, and then washed with tap water to remove any soft tissue tags followed by proper scaling to remove any calculus deposits or bone. Teeth were then stored in normal saline at room temperature to avoid dehydration until used.

**Preparation of samples**

Teeth were decapitated using a rotating diamond disc under water coolant to get a standard root length of 16 mm. The coronal and middle 2/3 of the root canal were prepared using Gates Glidden drills (#2-4) (DENTSPLY Maillefer), and the apical 1/3 was prepared using (#15-50) stainless steel K-file (MANI, Japan) at the working length which was 1 mm shorter than the apex. Root canal patency was maintained using #15 stainless steel file. NaOCl 5.25% was used as an irrigant through the preparation procedure. To remove the smear layer, the root canals were treated with 3 ml of NaOCl 5.25% followed by 3 ml ethylenediaminetetraacetic acid (EDTA) 17% for 3 min each. Teeth apices were etched using 37% phosphoric acid for 30 s then they were rinsed and dried followed by the placement of a bonding agent (VOCO GmbH) which was cured for 20 s and finally sealed with composite resin (VOCO GmbH) and covered by double layer of nail polish to prevent leakage.

The root specimens were then placed in sterilization pouches and sterilized twice in an autoclave at 120°C for 30 min. To insure the sterility and to avoid contamination a swab was taken from a tooth specimen and then it was cultured before being used in the direct contact test.

**Classification of samples**

The 50 root specimens were assigned to six groups according to the plant extracts to be tested and the control groups (positive and negative). To facilitate both handling and identification of the root specimens, they were mounted vertically in sterile plastic eppendorf vials, and arranged inside an eppendorf vial holder and autoclaved at 120°C for 30 min.

**Specimen inoculation**

An overnight culture of *E. faecalis* was grown on agar plates. Test tubes containing BHI were inoculated with freshly grown cultures and adjusted to 0.5 McFarland scale. Each root canal was completely filled with the corresponding bacterial suspension using a sterile 3 ml plastic syringe with a 27 gauge needle. Sterile #15 K-files were used to carry the bacterial suspension to the entire root canal length. All procedures were done aseptically in a laminar air flow cabinet.

Root specimens were incubated at 37°C for 24 h. After incubation, the root canals of each experimental group were completely filled with one of the tested plant extracts. The plant extracts were inserted to the canal lumen with sterile 3 ml plastic syringes and 27 gauge needles until the canal was completely filled. After plant extracts insertion into the canals, the eppendorf caps were tightened, and incubated for 24 h at 37°C. After incubation, the root canals were sampled, using three sterile paper points. The paper points were immediately transferred to tubes containing 2 ml of saline buffer and vortexed for 1 min.

**Figure 1:** Sample of Muller-Hinton ager plate, showing the zone of inhibition measured in (mm) from the outer margin of the well to the point of initial microbial growth.
Serial dilution was performed by taking 1 ml of the vortexed saline buffer and placing it into 9 ml of saline, this produces 10 ml of the dilute solution, producing a 10-fold dilution. Then, 100 μl of the samples were pipetted and cultured onto Muller-Hinton agar plates, and incubated for 24 h at 37°C. The colony forming units (CFU) that were grown were counted and calculated to give CFU/ml, and transferred to log_{10} number to normalize the data before statistical evaluation.

Statistical analysis
Data obtained in this study were presented as mean and standard deviation values. One-way analysis of variance (ANOVA) was used for comparisons between more than two groups. Tukey’s post-hoc test was used for pair-wise comparison between the groups when ANOVA test was significant. The significance level was set at $P \leq 0.05$.

Results

Antimicrobial sensitivity tests

Agar well diffusion test
The results revealed that some of the tested plants extracts showed antimicrobial activity, with significantly varying magnitudes ($P < 0.01$). The antimicrobial effect of the tested plant extracts is presented in Chart 1. The positive control (5.25% NaOCl) showed a mean growth inhibition zone diameter of 22.40 ± 1.5 mm with *E. faecalis*. While in the negative control (saline) and DMSO a confluent growth was observed (no growth inhibition zone).

The antimicrobial effect of the tested plant extracts was as follows:
- Ethyl acetate fraction of neem exhibited a mean growth inhibition zone diameter of 13.3 ± 0.58 mm, while total ethanolic extract, petroleum ether, butanol and the remaining aqueous fractions exhibited no growth inhibition zone
- Butanol fraction of ginger exhibited a mean growth inhibition zone diameter of 19.20 ± 0.58 mm, followed by total ethanolic extract with a mean growth inhibition zone diameter of 17.10 ± 1.2 mm and ethyl acetate fraction with a mean growth inhibition zone diameter of 16.30 ± 0.63 mm. Petroleum ether and the remaining aqueous fractions exhibited no growth inhibition zone
- Ethyl acetate fraction of Miswak exhibited a mean growth inhibition zone diameter of 14.5 ± 0.63 mm, while total ethanolic extract, petroleum ether, butanol and the remaining aqueous fractions exhibited no growth inhibition zone
- Fresh lemon solution exhibited a mean growth inhibition zone diameter of 20.60 ± 1.2 mm.

ANOVA depicted a significant difference among the zones of inhibition of the tested plant extracts ($P < 0.01$).

Post-hoc tukey’s test showed that:
- NaOCl 5.25% was associated with significantly wider mean inhibition zone diameter when compared with all of the tested plant extracts
- Fresh lemon solution showed significantly larger mean inhibition zone diameter in comparison with all of the tested plant extracts, but significantly narrower than NaOCl [Table 1]
- Butanol fraction of ginger showed significantly wider mean inhibition zone diameter compared to ginger total ethanolic extract, ginger ethyl acetate, Miswak ethyl acetate and neem ethyl acetate fractions
- There was no statistically significant difference in inhibition zone diameter between ginger total ethanolic extract and ginger ethyl acetate fraction. Both showed significantly wider inhibition zone diameter compared to Miswak ethyl acetate and neem ethyl acetate fractions
- Miswak ethyl acetate showed statistically significant wider mean inhibition zone diameter compared to neem ethyl acetate fraction.

MIC test
The MIC test was carried out for the most potent plant extracts against *E. faecalis*. It was found that the positive control (5.25% NaOCl) exhibited MIC of 62.5 μl/ml, followed by the following four plants extracts:

<table>
<thead>
<tr>
<th>Tested agents</th>
<th>MIC (μl/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem (ethyl acetate) fraction</td>
<td>13.30b</td>
</tr>
<tr>
<td>Ginger (ethyl acetate) fraction</td>
<td>16.30d</td>
</tr>
<tr>
<td>Ginger (butanol) fraction</td>
<td>19.20c</td>
</tr>
<tr>
<td>Ginger (total ethanolic extract)</td>
<td>17.10d</td>
</tr>
<tr>
<td>Miswak (ethyl acetate) fraction</td>
<td>14.05e</td>
</tr>
<tr>
<td>Lemon fresh solution</td>
<td>20.60b</td>
</tr>
<tr>
<td>NaOCl (5.25%)</td>
<td>22.40a</td>
</tr>
</tbody>
</table>

$P$ value <0.0001*

*Significant at $P \leq 0.05$. Different letters in the same column indicate significant differences between the tested agents. SD: Standard deviation
• Ethyl acetate fraction of neem with MIC of 1000 μg/ml
• Butanol fraction of ginger with MIC of 250 μg/ml
• Ethyl acetate fraction of miswak with MIC of 2000 μg/ml
• Fresh lemon (C. limonum) solution with MIC of 125 μl/ml.

Direct contact test

To normalize the data, a Log10 transformation of each CFU count was performed before statistical evaluation. Values of P < 0.01 were considered significant. ANOVA showed a significant difference among the CFU/ml count of the tested plants extracts (P < 0.01).

The results of pairwise comparison using post-hoc Tukey’s test are shown in Table 2 and Chart 2.

- NaOCl 5.25% showed significantly less CFU/ml count, when compared with all tested plant extracts (P < 0.001)
- This was followed by a fresh lemon solution which showed significantly less CFU/ml count when compared with all of the other tested plant extracts
- Butanol fraction of ginger showed significantly less CFU/ml count than ethyl acetate fraction of miswak and neem
- Ethyl acetate fraction of miswak showed the significantly highest CFU/ml count when compared to all the tested plant extracts.

Discussion

A broad antimicrobial spectrum against anaerobic and facultative microorganisms, biofilms and ability to remove smear layer during instrumentation or dissolve it once it has formed are among the main requirements of endodontic irrigants. They should be non-toxic and non-caustic to periodontal tissues.11

The most effective and commercially used is sodium hypochlorite. However, it has several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential, disagreeable smell and taste.2 To overcome the problems associated with currently used irrigants, the use of natural plant extracts as endodontic irrigants might be of interest to professionals as part of a growing trend to seek natural remedies in dental treatment.12 E. faecalis is a normal inhabitant of the oral cavity; it is associated with different forms primary endodontic infections and recurrent infections.4 The selection of the tested plants was based on their antimicrobial efficacy.8,9 The tested plant extracts fractions were concentrated to dryness under reduced pressure using rotary device to give the dry fractions of each solvent. They were dissolved in DMSO to facilitate testing and usage as an endodontic irrigant, since DMSO doesn’t possess any antimicrobial effect.8,9 The microbial suspensions used in this study were adjusted to match the turbidity of 0.5 McFarland scales, to standardize the microbial suspensions in the susceptibility procedure.10,11 The antimicrobial potential in this study was tested using the agar well-diffusion method and the direct contact test.10 The well-diffusion method has been used in this study instead of the disc-diffusion method because it showed larger activity, when used in screening of medicinal plants due to the larger amount of tested material which can be placed in the wells, in relation to that used to saturate the paper disc, as reported by Essawi and Srour (2002).12

Muller-Hinton agar culture medium was used in this study, since it is widely used in sensitivity tests and commonly used to culture E. faecalis.17 Sodium hypochlorite (5.25% NaOCl) was used as a positive control because it is a well-known antimicrobial endodontic irrigant,10,13,14 while saline was used as a negative control. The Muller-Hinton agar plates were left for 30 min at room temperature, before incubation to ensure the diffusion of the tested material through the agar.15 The agar well-diffusion method was used to screen the antimicrobial action of the plant extracts and the four most potent plant extracts were additionally tested by MIC to determine the effective concentration which will be used in the direct contact test. It is believed that the contact test is a trustworthy method that requires a simple execution with good reproducibility that simulates contact between the bacteria and the irrigation solution inside the root canal.10

The MIC of these extracts was determined by micro broth dilution technique according to the method described in the National Committee for Clinical Laboratory Standards NCCLS (2003).16 Extracted human teeth were used for the direct contact test to simulate the clinical condition and to test the efficiency of the plants extracts within the root canal system.14,16 The selected extracted teeth were single canal teeth to exclude the anatomical variations and complexity factors.17 The extracted teeth in this study were prepared using Gates Glidden drills (#2-4), and reaching a final apical preparation of size 50 K-files.18 During the mechanical preparation, NaOCl 5.25%

Table 2: Statistical comparison of the direct contact test results between the tested plants extracts along with the positive control (5.25 NaOCl)

<table>
<thead>
<tr>
<th>Tested agents</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem (ethyl acetate) fraction</td>
<td>4.7253</td>
<td>0.06743</td>
</tr>
<tr>
<td>Ginger (butanol) fraction</td>
<td>3.8217</td>
<td>0.04166</td>
</tr>
<tr>
<td>Miswak (ethyl acetate) fraction</td>
<td>4.8340</td>
<td>0.05328</td>
</tr>
<tr>
<td>Lemon fresh solution</td>
<td>2.6617</td>
<td>0.06682</td>
</tr>
<tr>
<td>5.25% NaOCl (+ve control)</td>
<td>2.4614</td>
<td>0.07281</td>
</tr>
<tr>
<td>Saline (–ve control)</td>
<td>7.6877</td>
<td>0.11544</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at P<0.05. Different letters in the same column indicate significant differences between the test agents. SD: Standard deviation

Chart 2: Effect of the tested plants extracts and the control groups on the colony forming units/ml count of Enterococcus faecalis
was used for lubrication and debridement, EDTA 17% was used to remove the smear layer before inoculation, to allow the tested microorganisms to penetrate the dentinal tubules. Teeth apices were sealed with composite resin and the root surface covered with a double layer of nail polish to prevent leakage of the microbial suspension also to maintain the extracts within the root canal.  

The sensitivity test of all neem fractions proved that the ethyl acetate fraction inhibited the growth of *E. faecalis*. Such antimicrobial activity may be attributed to the presence of triterpinoids nimbidin, nimbolide, and nimbin reported to be present in *Azadirachta indica*. These compounds have been reported to have antimicrobial activity.  

The present study has proven that ethyl acetate fraction of neem had an antimicrobial effect against *E. faecalis*, which comes in disagreement with Bohora et al. (2010) in which they have shown that the active extract was the total ethanolic extract. This difference might be attributed to the fact that the ethyl acetate fraction is a part of the total ethanol 70% extract, and that the bioactive constituents were specifically extracted by the ethyl acetate. It also comes in disagreement with Almas (1999) who found that the active extract was aqueous extracts of neem; this might be attributed to the difference in susceptibility of the tested extract against different types of microorganism since he tested the antimicrobial efficacy against *Streptococcus faecalis* and *Streptococcus mutans*. Moreover, it comes in agreement with Dutta and Kundabala (2013) who found that the neem leaf extract (total extract) exhibited no antimicrobial effect on *E. faecalis*, but when mixed with seed-bark powder mixture dissolved in DMSO it showed antimicrobial activity against *E. faecalis*, this might be due to environmental factors affecting the quality and quantity of active constituents in the different plant organs.  

The sensitivity test of all ginger fractions proved that the butanol, total ethanolic and ethyl acetate fractions inhibited the growth of *E. faecalis*. Such antimicrobial activity may be due to the presence of gingerol and shagaeol present in *Z. officinale* and reported to have antimicrobial activity. Likewise, the total ethanolic extract of ginger exhibited antimicrobial effect which is in agreement with previous studies. However, it comes in disagreement with Gull et al. (2012) who found that the total ethanolic ginger extract possessed poor antimicrobial effect. This might be attributed to the difference in susceptibility of the tested extract against different types of microorganisms, since the extract was tested against eight types of clinical bacterial isolates, other than *E. faecalis*. Unfortunately, none of the reviewed studies tested the antimicrobial efficacy of the butanol fraction of ginger, but this study has proven that the butanol fraction of ginger showed higher antimicrobial efficacy against *E. faecalis* when compared with the total extract.  

The sensitivity test of all miswak fractions proved that the ethyl acetate fraction inhibited the growth of *E. faecalis*. Such antimicrobial activity may be due to the presence of flavonoids and alkaloids reported to be present in *S. persica* and reported to have antimicrobial activity. This study has proven that the ethyl acetate fraction of miswak exhibited antimicrobial effect against *E. faecalis*. This is in disagreement with Almas (1999) who found that the active extract was aqueous extracts of miswak. This might be attributed to the difference in susceptibility of the tested extract against different types of microorganism since he tested the antimicrobial efficacy against *S. faecalis* and *S. mutans*. Most of the reviewed studies revealed that miswak extract possessed high antimicrobial efficacy against different types of microorganisms rather than *E. faecalis*, when used as mouthwash or toothpaste in the treatment of periodontal diseases.

The sensitivity test of lemon (*C. limonum*) proved that the fresh lemon solution inhibited the growth of *E. faecalis*. Such antimicrobial activity may be due to the presence of flavonoids, alkaloids, volatile oil and citric acid reported to be present in *C. limonum*. These compounds have been reported to have antimicrobial activity.  

This study has proven that the fresh lemon (*C. limonum*) solution exhibited antimicrobial effect against *E. faecalis* which is in agreement with the findings of Abuzied and Eissa. The direct contact test has proven that the ethyl acetate fraction of neem exhibited antimicrobial effect against *E. faecalis* when used as root canal irrigant in disagreement with Vinothkumar et al. (2013) since they have proven that the active fraction was the total ethanolic extract, and it was superior to NaOCl 5.25%. This difference in findings might be attributed to the use of 99% ethanol during fractionation and the use of real-time quantitative polymerase chain reaction test in their study.

Unfortunately, none of the reviewed studies in relation to ginger, miswak and lemon performed the direct contact test, as done in this study. All plants extracts used in the present study have been found to be less effective against *E. faecalis* than (NaOCl 5.25%), this might be attributed to the high concentration of NaOCl in comparison to other studies that used (NaOCl 2%) as a positive control.

## Conclusion

Under the limitations and conditions of this study, it could be concluded that:

- Sodium hypochlorite (5.25%) showed the highest antimicrobial effect among all of the tested groups
- Lemon (*C. limonum* Risso) fresh solution, butanol fraction of ginger (*Z. officinale* Roscoe), ethyl acetate fraction of miswak (*S. persica* L.) and of neem (*A. indica* A. Juss), showed a considerable antibacterial effect against *E. faecalis*.

## Recommendation

Further studies should be carried out using these herbal extracts; lemon (*C. limonum* Risso) fresh solution and butanol fraction of ginger (*Z. officinale* Roscoe) to determine, their effect on smear
layer removal during endodontic treatment and to determine the effect of cleaning on the sealing ability, adaptability and push out bond strength of different sealers and core materials used in root canal obturation.

References


