ORIGINAL RESEARCH

Evaluation of the Antibacterial Effect of Virgin Olive oil on *Enterococcus Faecalis*: An *In Vitro* Study

Shashit Shetty Bavabeedu[1], Vinod Babu Mathew[2], Abdalla N Fahad[3], Mohammed Saleh H Al Hutaylah[4], Saad Abdullah O Al Ahmari[5], Mohammed Saeed A Al Yami[6]

ABSTRACT

Background: Repeated endodontic failures are due to pathogens like *Enterococcus faecalis* which are resistant to the common intracanal medicaments available. Plant extracts have shown promising results against pathogens in the recent literature. One such extract is virgin olive oil which has shown its efficacy against some pathogens.

Aim: This study aims to determine the role of commonly used virgin olive oil against root canal pathogen *E. faecalis*, and to compare its efficacy with chlorhexidine (CHX), a popularly used intracanal medicament.

Materials and Methods: Bacterial strains of *E. faecalis* were tested against virgin olive oil and CHX. 10 agar plates were prepared using brain heart infusion (BHI) agar. The BHI agar dishes were then stored at room temperature for 2 days before use to verify that they had remained sterile. BHI broth was also prepared and stored in 5 ml vials for 2 days. *E. faecalis* was maintained on BHI broth and cultures of *E. faecalis* were grown overnight at 37°C in BHI broth for 24 h, and bacterial growth was checked by the presence of turbidity. The BHI broth was inoculated with *E. faecalis* from a freshly grown culture on an agar plate. The broth culture was incubated at 37°C for 24 h. 100% olive oil and CHX gluconate gel were used as test specimens. Well diffusion method was used to derive the results. The inoculated plates were kept in an incubator at 37°C for 48 h and inhibition zones were measured.

Results: The CHX group showed a consistent zone of inhibition in all the agar plates. The average diameter of the zone of clearance shown by Group 1 (CHX 2%) was 24.2 ± 2.39 mm. The test group of extra virgin olive oil showed no zone of clearance in all the plates tested. This shows a lack of direct antibacterial activity by this group.

Conclusion: In the present study, virgin olive oil did not show any antibacterial activity against *E. faecalis* which is common root canal pathogens which are responsible for repeated endodontic failures when compared with medicaments like CHX.

Keywords: Chlorhexidine, *Enterococcus faecalis,* Virgin olive oil.

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[1,2]Assistant Professor, [3]Lecturer, [4-6]Intern

[1,2]Departmernt of Restorative Dental Science, King Khalid University, College of Dentistry, Abha, Kingdom of Saudi Arabia

[3]Department of Clinical Laboratory Science, King Khalid University, College of Applied Medical Science, Abha, Kingdom of Saudi Arabia

[4-6]King Khalid University College of Dentistry, Abha, Kingdom of Saudi Arabia

Corresponding Author: Dr. Shashit Shetty Bavabeedu, Assistant Professor, Department of Restorative Dental Science, King Khalid University, College of Dentistry, Abha, Kingdom of Saudi Arabia. Phone: +966 599332854. e-mail: sbavabeedu@kku.edu.sa

INTRODUCTION

Microbial invasion of the root canal system has an important role in initiating and sustaining periapical disease.[1] The aim of root canal therapy is to eliminate bacteria and their by-products from the root canal system.[2] Although chemomechanical cleaning and shaping of the canal are effective in reducing bacterial counts, microorganisms may persist in the anatomical complexities of root canal system and increase the risk of treatment failure.[3,4] Therefore, intracanal medication is advocated to further reduce bacteria in the root canal system and increases the success of root canal treatment.[5] *Enterococcus faecalis* is the most frequently isolated strain in failed endodontic therapy. *E. faecalis* is a Gram-positive facultative anaerobic bacteria species. Chlorhexidine gluconate (CHX) can be used in endodontics as an irrigant and intracanal medicament due to its biocompatibility, substantivity, and wide antimicrobial activity. In recent times, many naturally occurring medicaments have shown promising results against *E. faecalis*.[6] Antimicrobial activity in olive products against certain strains of bacteria has been demonstrated and documented.[7] The aim of the present study was to evaluate *in vitro* the antibacterial efficacy of virgin olive oil against *E. faecalis*.

MATERIALS AND METHODS

The present study was conducted at Clinical Laboratory Science Department, College of Applied Medical Science, King Khalid University, Abha, KSA. 10 agar plates were prepared using brain heart infusion (BHI) agar (Figure 1). Agar was mixed according to manufacturer's

directions, and enough agar was poured to cover the surface of a 125 mm Petri dish. The BHI agar dishes were then stored at room temperature for 2 days before use to verify that they had remained sterile. BHI broth was also prepared and stored in 5 ml vials for 2 days.

E. faecalis (MTCC 51299) (obtained from Armed Forces Hospital, Southern Region, Khamis Mushait) (Figure 2) was maintained on BHI broth and cultures of *E. faecalis* were grown overnight at 37°C in BHI broth for 24 h, and bacterial growth was checked by the presence of turbidity (Figure 3). The BHI broth was inoculated with *E. faecalis* from a freshly grown culture on an agar plate. The broth culture was incubated at 37°C for 24 h (Figure 4).

100% olive oil (Figure 5) and CHX gluconate gel (Figure 6) were used as test specimens. Well diffusion method was used to derive the results. Wells of 7 mm diameter and 4 mm depth were punched in agar plates and filled with 10 µl of medicaments to be tested. Plates were inoculated for 72 h at 37°C. All manipulations of the specimens were performed under a laminar flow to avoid contamination. Microbial zones of inhibition were measured in millimeters (Figures 7 and 8).

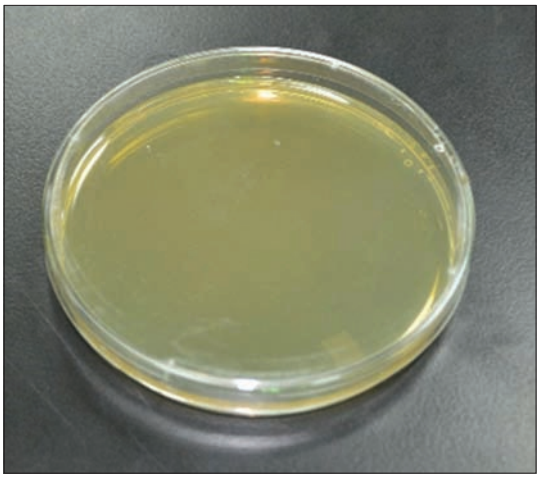


Figure 1: Brain heart infusion agar plates

Figure 2: *Enterococcus faecalis*

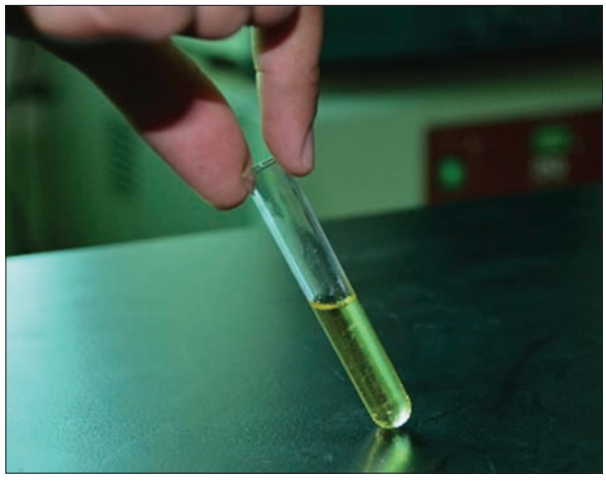


Figure 3: Turbidity

RESULTS

The data were measured from the agar plates and tabulated for statistical analysis.

Figure 4: Incubator

Figure 5: Virgin olive oil

Figure 6: Chlorhexidine gluconate gel

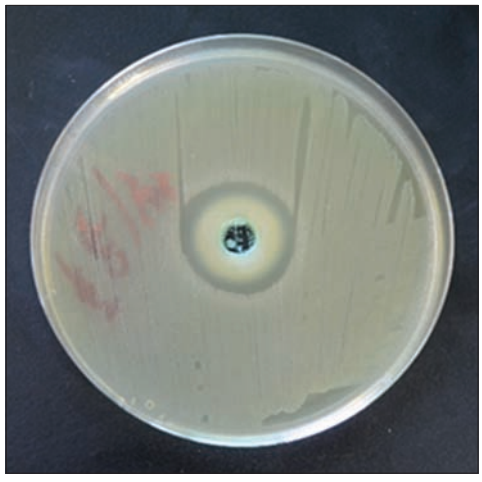


Figure 7: Specimen sample showing zone of inhibition in chlorhexidine sample

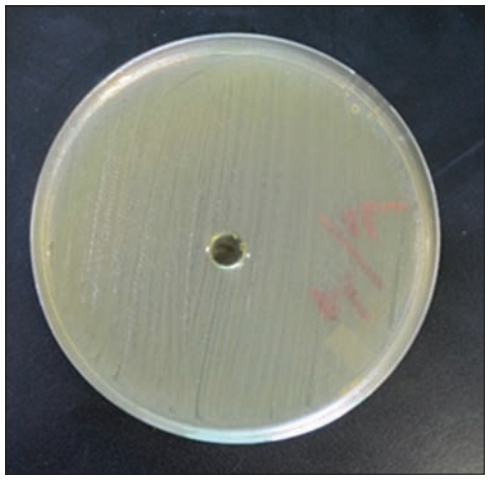
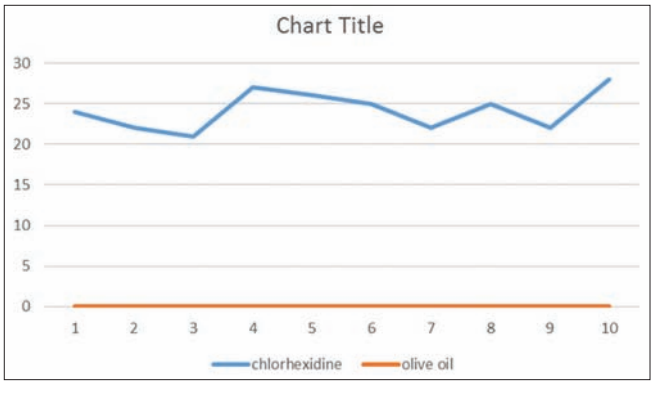


Figure 8: Specimen sample showing no zone of inhibition in virgin olive oil sample



Graph 1: The diameter of the zone of inhibition of *Enterococcus faecalis* by chlorhexidine and extra virgin olive oil

The CHX group showed a consistent zone of inhibition in all the 10 agar plates. The average diameter of the zone of clearance shown by Group 1 (CHX 2%) was 24.2 ± 2.39 mm.

The test group of extra virgin olive oil showed no zone of clearance in all the plates tested. This shows a lack of direct antibacterial activity by this group. Since they were consistent in their inactivity, no statistical test was required to analyze the variation with the other groups (Graph 1).

DISCUSSION

E. faecalis is a Gram-positive facultative anaerobic bacteria species. It is one of the most CH-resistant microorganisms of the root canal system. Although it comprises a small portion of the root canal flora in initial endodontic infections, environmental changes can be advantageous to *E. faecalis*, resulting in persistent infections. Some resistance factors of this bacterial species are deep dentinal penetration ability, high pH tolerance, surviving in food deprivation condition, and surviving without any support from other microbial species.

CHX gluconate has been used in endodontics as an irrigant and intracanal medicament due to its biocompatibility, substantivity, and wide antimicrobial activity. The antimicrobial property of CHX is attributed to its cationic molecule, which is adsorbed to the negatively charged inner cell membrane, resulting in the leakage of intracellular components. It is an effective agent against Gram-positive and Gram-negative bacteria.

In vitro research has shown that olive oil phenolic compounds have antimicrobial properties.[8] Particularly, the phenolic compounds oleuropein, hydroxytyrosol, and tyrosol have demonstrated potent antimicrobial activity against several strains of bacteria responsible for intestinal and respiratory infections. Secoiridoides (oleuropein and derivatives), one of the major classes of polyphenol contained in olives and olive oil, have recently been shown to inhibit or delay the rate of growth of a range of bacteria and microfungi. However, in our study, they were found to be ineffective against *E. faecalis.* This may be attributed to the non-availability or non-leachability of the chemical compounds in the present form of the olive oil.

CONCLUSION

With time, plant extracts have been understood to encompass the attributes accounted not only for their fragrance and flavor but also for their antimicrobial nature. In the present study, CHX -2% has again proved its efficacy against *E. faecalis* - one of the common organisms responsible for root canal failure. However, virgin olive oil showed negative results in elimination of *E. faecalis*. However, further studies could be carried out to determine the use of virgin olive oil in combination with other medicaments as an intracanal medicament in endodontics.

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