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In vitro evaluation of propolis as an intracanal medicament against *Enterococcus faecalis*

Rendah. O. I. Elsayed[1]*,Sanaa. O. Yagoub[2], and Neamat. H. Abubakr[3]

[1]Department of Dental Materials, Faculty of Dentistry, Al Neelain University, Khartoum, Sudan.
[2]Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan.
[3]School of Dental Medicine, University of Nevada, Las Vegas, USA
Corresponding author E-mail: Randaosman909@hotmail.com

Abstract

Root canal failure is considered to be a common occurrence, and the most common organism that causes failure is *Enterococcus Faecalis*. *E. faecalis* is resistance to the commonly used intracanal medicament. This study aimed to evaluate efficiency of propolis as root canal medicament against *E. faecalis*, as well as determine the minimal inhibitory concentration of propolis that inhibit the growth of *E. faecalis*. To conduct this study, Ethanolic extract of propolis was prepared according to the method described by Sukhdev et. al. (2008), then dissolved in dimethyl sulfoxide to make 100% stock, preparation of different concentration of propolis were done as 80%, 60%, 40%, 20% and 10%. Wells were made in a sterile Muller Hinton agar containing 1 ml of standardized bacterial stock suspension and filled with 0.1 ml of the different concentrations of the propolis extract, chlorhexidine 2% gel was used as control. Different concentrations of propolis were added using automatic microlitre pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 24 hours. After the incubation period the diameters of the resultant growth inhibition zones were measured in mm, averaged and then the mean values were tabulated. The minimum inhibitory concentration (MIC) of propolis was found to be 60% concentration. Propolis was found to be effective against *E. faecalis* bacteria, It is comparable to chlorhexidine gel 2%.

Keywords: Propolis, MIC of propolis, Agar diffusion method, E .faecalis.

Introduction

The pulp of the tooth can prone to microbial infections, such affected tooth can be restored through the root canal treatment (Niemiec, 2005). There are more than 150 bacterial species present in root canal (Siqueira Jr et al., 1996) . The microbial flora in canals after the failure of root-canal treatment are mainly *E. faecalis* (Pinheiro et al., 2003). *Enterococci* species are not associated with the disease in endodontic infections (Kaufman et al., 2005)(Ozbek et al., 2009), however, they are the main species cause endodontic failure (Gomes et al., 2008) (Zoletti et al., 2011) (Sakamoto et al., 2008). This bacteria is commonly detected in asymptomatic persistent endodontic infection, its prevalence in such infections ranges from 24% - 77% (Stuart et al., 2006)(Siqueira & Rôças, 2004). They can grow in extremely alkaline pH, salt concentrated environment, in a temperature range of 10–45°C, and survive a temperature of 60°C for 30 min. (Sedgley et al., 2005).

Chemo-mechanical preparation of the root canal is not enough to eliminate infection because many microorganisms are hidden in lateral canals which cannot be reached during the cleaning of the main canal. Use of an intracanal medicament between visits is essential to eliminate the microorganisms (Lima et al.,2012)(Siqueira, Magalhães & Rôças 2007). The correct choice of the antimicrobial agent as inter-appointments medicament is important to remove pathogens from infected root canals (Tang et al., 2004).

Although currently the used of intracanal medicaments have considered to fulfill many of the properties of an ideal root canal dressing, their effect on E Faecalis are controversial (Estrela et al., 1995)(Beltes et al., 1997)(Mohammadi & Dummer, 2011). Some intracanal medicaments are effective

against this bacteria but they reduced the success of root canal treatment (Ng. Mann & Gulabivala, 2011), while the other types containing antibiotics develop resistance by E. Faecalis and cause tooth discoloration (Kirchhoff et al., 2015). Moreover, allergic contact dermatitis, occupational asthma and anaphylactic shock have been reported after use of some synthetic intracanal medicaments (Krautheim et al., 2004) (Snellman & Rantanen, 1999).

Treatment with bee products (e.g honey, pollen, propolis, fortified honey, herbs honey…etc) is an old tradition that have been revitalized recently *(Banskota et al., 2001)*. Research on chemical composition of propolis started at the twentieth century *(Kuropatnicki et al., 2013).* More than 300 compounds have been identified in propolis such as, phenolic compounds, aromatic acids, essential oils, waxes and amino acids (Anjum et al., 2019).

Propolis has gained popularity as traditional medicine to increase the immunity and prevent diseases (Teixeira et al., 2010). It has been used in dentistry as mouth wash and toothpastes to prevent caries and treat gingival inflammations (Gómez-Caravaca et al, 2006). Several studies revealed that propolis had good antibacterial activity against *E. faecalis* in the root canals and suggested that it could be used as an alternative intracanal medicament (Oncag et al., 2006)(Ramos et al., 2012)(Ahangari et al., 2012) (Al-Shaher et al., 2004).

Justification:

- To draw the attention of health care workers towards propolis as natural remedy.
- To avoid the drawback of many synthetic intracanal medicaments
- To find effective material that can reduce cases of root canal failures.

Objectives:

- To determine the antibacterial efficacy of propolis against *Enterococcus Faecalis* microorganism.
- Try to find natural products material (Propolis) to work as intracanal medicament
- Evaluate propolis as intracanal medicament against *E.faecalis*
- Determine the MIC of propolis (use Agar diffusion).

Materials and Methods

This study was conducted at the Chemistry laboratory, National Center for Researches, Khartoum, Sudan, and the Laboratory of the Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan. Ethical consent was obtained from Al Neelain University Ethical and Research Committee.

Preparation of the propolis extracts

Hundred grams of propolis sample were extracted, extraction was carried out according to method described by Sukhdev *et. al.* (2008) (Gennaro, 2008). 1000 ml of 80 % ethanol (S D Fine Chemicals India) was used to dissolve the sample. The mixture was shaken using shaker apparatus (Suart UK) for about seventy two hours with daily filtration with filter paper Whatman no. 1 (Macherey – Nagel Germany), the evaporation of the solvent was done under reduced pressure using rotary evaporator apparatus (Buchi Switzerland). Finally, extract was allowed to exposure to air till complete dryness, and the yield percentage was calculated as followed:

Weight of extract obtained / weight of propolis sample ×100

Preparation of the tested organism

E. faecalis obtained from Department of Microbiology, Sudan University of Science and Technology, Khartoum, Sudan, was used. The isolate was reconfirmed by the morphological appearance, cultural characteristic and biochemical reactions.

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the *E. faecalis* were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms per ml of the stock suspension was determined by using the surface viable counting

technique (Miles et al., 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Minimal inhibitory concentration (MIC) for propolis

To determine MIC dose of propolis, 200mg of propolis was weighted and dissolved in Dimethyl Sulfoxide (DMSO) solution to make stock of propolis. Dilution of the stock in DMSO was done to get concentration of 80%, 60%, 40%, 20% and 10% (figure 1).

Figure 1: Different concentration of Propolis

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts by using sterile Muller Hinton agar (Kavanagh, 1972). One ml of the standardized bacterial stock suspension 10^8 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile Muller Hinton agar and were distributed into sterile Petri-dishes. The agars plate were left to set and in each of them cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar disc was removed. Cups were filled with 0.1 ml of the different concentrations of the propolis and Chlorhexidine gel 2% using automatic microtittre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 24 hours. After the incubation period the diameters of the resultant growth inhibition zones were measured in mm., averaged and the mean values were tabulated.

Results and Discussion

The 100 gm of the propolis was yield as 38.26 gm ethanol extract (figure2).

The inhibitory zones around the wells containing medicaments were seen clearly in the chlorhexidine gel 2%, it was 24 mm. The zone of bacterial growth inhibition around propolis was 15 mm at concentrations of 100%, 80% and 60% (figure 2). There is no zone of bacterial inhibition around the wells containing propolis extract of 40%, 20% and 10%. (Figure 3).

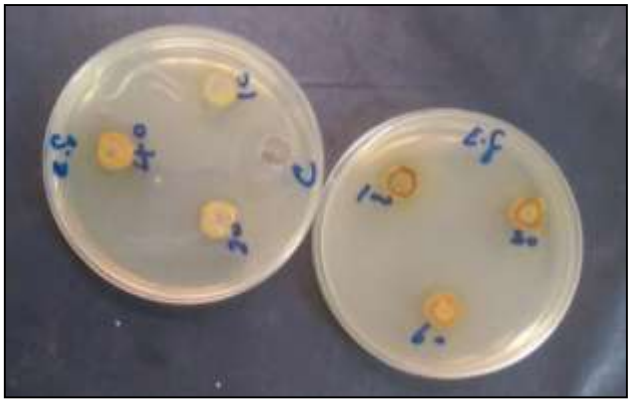

Figure 2: Zone of bacterial inhibition around different concentrations of propolis and chlorhexidine 2%

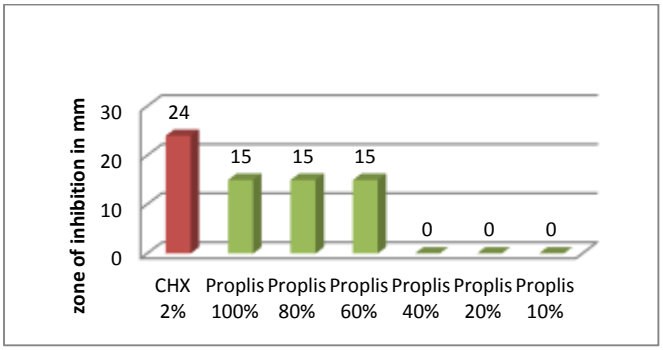

Figure 3: The effect of the different concentration of the ethanol extracted propolis and chlorhexidine 2% gel on *E. faecalis* (zone of inhibition in mm)

The result showed that concentration of 100%, 80% and 60% of ethanolic extracted propolis (EEP) was active against *E. faecalis*, these confirm the finding of the many researches who revealed that propolis is effective against *E. faecalis* bacteria (Oncag et al., 2006)(Ramos et al., 2012)(Ahangari et al., 2012)(Silva et al., 2004)(Al-Shaher et al., 2004). The activity of the propolis might be due to its ability to reduce adhesion of *E. faecalis* to smooth surface, and it had bactericidal effects against *E.faecalis* as reported by (Wahjuningrum & Subijanto, 2015). *E. Faecalis* is the main species cause endodontic failure *(Gomes et al., 2008) (Zoletti et al., 2011)* (Sakamoto et al., 2008). Intracanal medicaments are defined as the temporary placement of medicaments with good biocompatibility into root canals to inhibit coronal invasion of bacteria. Propolis is a biocompatible material, since it give antibacterial activity against *E faecalis*, so it can be used as intracanal medicament. It is well known that the use of intracanal medicament is important between endodontic visits to eliminate bacteria hidden in lateral canals and dentinal tubules so prevent treatment failure. The major advantages of herbal substances are that it safe, easy availability, increased shelf life, cost effectiveness and lack of microbial resistance (Jain & Ranjan, 2014), propolis also has these advantages. It is well known that propolis cannot be used as raw material, and it must be purified by extraction with solvents (Pietta et al., 2002). In this study extraction of propolis was done with ethanol, this solvent did not influence the antimicrobial effect of propolis extract (de Andrade Ferreira et al., 2007). Several methods have been proposed to evaluate the potential antimicrobial activity of intracanal medications for treating endodontic infections, such as the agar diffusion test, the broth microdilution test, and the dentin infection and disinfection tests (Pimenta et al., 2015). Agar diffusion method was used to test the antimicrobial activity of propolis in this study.

Although results of this study showed that 40% propolis has no antibacterial activity against *E. faecalis*, others were found that the bacterial growth of *E. faecalis* was inhibited by 40% propolis extract (Pimenta et al., 2015). On the other hand, Fatemeh *et al.,* (2017) found that the strongest effect against different type of bacteria including *E. faecalis* was demonstrated by 60% propolis diluents in dimethyl sulfoxide (DMSO) (Ghasemi et al., 2017). Herrera Sandova suggested that use of propolis as an alternative of irrigation and medication in endodontics because of its powerful effect against *E. faecalis (Sandoval, 2019*).

Chlorhexidine 2% was used as control substance because it is known to be effective against *E. faecalis*, it was given the higher antimicrobial action in compare to propolis. In this study it was seem that propolis has a comparable effect to chlorhexidine 2%. It was reported that two percent chlorhexidine is one of the most versatile irrigant and an intracanal medicament in vital and non-vital teeth (Sinha et al., 2015). Carbajal Mejía, (2014) reported that chlorhexidine is the most patent medicament against both *E.faecalis* and *C. albicans* (Carbajal Mejía, 2014), Bhardwaj *et.al* (2012) compare chlorhexidine gel, calcium hydroxide and natural products, they reported that chlorhexidine gel showed the highest antimicrobial activity against *E. faecalis* (Bhardwaj et al., 2012). All these findings are in accordance with the finding of this study. Moreover, the results of this study were similar to study conducted by Maryam Ehsani *et al.* (2013), who found that ethanolic extracted propolis showed high antibacterial activity against *E. faecalis* comparable to that of Chlorhexidine (Ehsani et al., 2013*)*. Use of propolis as alternative for synthetic intracanal medicament can decrease cases of root canal failure because it is active against *E. faecalis* which play the main rule in the failure of endodontic treatment.

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