

## ANTIMICROBIAL EFFICACY OF NANOPROPOLIS COATED VS SILVER-CURCUMIN NANOPARTICLES COATED GUTTA-PERCHA POINTS ON VARIOUS MICROBIAL SPECIES

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### ABSTRACT

**Introduction:** Nanopropolis, nanosilver and curcumin have a wide antimicrobial action range. They are to be tested as a gutta-percha coating. **Objectives:** Comparing the antimicrobial efficacy of silver-curcumin nanoparticles versus nanopropolis coated gutta-percha against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* and assessing the continuity of both coatings.

**Materials and Methods:** Suspensions of both materials were loaded on filter discs, placed on the surface of agar plates inoculated with tested microbes and incubated for 24 hours. Gutta-percha cones were inserted in Eppendorf tubes containing the tested materials for 24 hours, placed on the surface of agar plates inoculated with the tested microbes and incubated for 24 hours. The antimicrobial activity of the discs was determined by measuring the diameter of the inhibition zone whereas it was measured by positive response and its degree around gutta-percha. Coated gutta-percha cones were scanned under scanning electron microscope to assess the continuity of the coating.

**Results:** Mean and Standard Deviation of the inhibition zone diameter for Silver-curcumin nanoparticles around discs were  $12.35 \pm 0.71$ mm for *E.faecalis*,  $20.6 \pm 0.39$ mm for *S.aureus*,  $14.6 \pm 0.61$ mm for *E.coli* and  $18.2 \pm 0.67$ mm for *C.albicans*. For nanopropolis they were  $8.95 \pm 0.69$ mm for *E.faecalis*,  $15.4 \pm 0.7$ mm for *S.aureus*,  $9.75 \pm 0.86$ mm for *E.coli* and  $14.8 \pm 0.63$ mm for *Candida*. The inhibition zones around gutta-percha were more pronounced for Silver-curcumin nanoparticles. Statistically significant difference was found between the inhibitory effect of silver-curcumin nanoparticles and nanopropolis against all tested microorganisms  $P < 0.001$ . Nanopropolis coating was more uniform and closely adherent to the gutta-percha.

**Conclusion:** Silver-curcumin nanoparticles have a greater antimicrobial effect compared to nanopropolis.

**Keywords:** *E. faecalis*; *Staphylococcus aureus*; *Candida albicans*; Nanoparticles; Gutta-percha.

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## INTRODUCTION

Several microbial species, predominantly facultative anaerobe bacteria are key factors in failing endodontic cases<sup>(1-3)</sup>. These microorganisms either survive all endodontic disinfection protocols and persist inside the canal and dentinal tubules or enter the canal after obturation through coronal or apical leakage<sup>(4-6)</sup>. The most common microbial species that contribute to endodontic failures and flare ups include gram positive bacteria such as *Enterococcus faecalis* and *staphylococcus aureus*, gram negative bacteria such as *Escherichia coli* and yeasts such as *Candida albicans*<sup>(6-9)</sup>.

Few studies were conducted to investigate the antimicrobial effect of either sealers or gutta-percha, combined with antimicrobial agents in combating residual infection. Among these antibacterial materials are calcium hydroxide, zinc oxide, chlorohexidine, antibiotics and iodoform<sup>(7,10-13)</sup>.

Curcumin is a natural pigment extracted from the dried rhizome of turmeric *Curcuma longa* that was shown to have anti-inflammatory, antimicrobial and antioxidant activities by inhibiting biofilm formation<sup>(14-18)</sup>. The antimicrobial effects of curcumin were significantly enhanced upon nanoparticle formation<sup>(19)</sup>. On the other hand, the ability of curcumin, as a polyphenol, to release H<sup>+</sup> atom permits it to reduce metal ions as a precursor to nanoparticles formation. Therefore, silver nanoparticles (AgNPs) could be prepared based on curcumin<sup>(20,21)</sup>.

Silver nanoparticles possess antibacterial and antifungal properties through the release of Ag<sup>+</sup> ions which increases the permeability of bacterial cell wall causing damage to the bacterial cytoplasm and DNA<sup>(22,23)</sup>.

Propolis is a sticky resinous material produced by honey bees from different plant sources such as leaves, flowers, and bud exudates, modified by bee secretions and wax<sup>(24)</sup>. It has various pharmacological effects as an antioxidant, antimicrobial, antifungal, antiviral, anti-inflammatory, anticancer and wound healing<sup>(25-31)</sup>.

The aim of the present study is to assess and compare the antimicrobial efficacy of silver-curcumin nanoparticles coated gutta-percha versus nanoproplis coated gutta-percha against gram positive (G+ve) *Enterococcus faecalis*, G+ve *Staphylococcus aureus*, gram negative (G-ve) *Escherichia coli* and *Candida albicans*, which could be effective in reducing flareups and failures and to assess the continuity of both coatings on the gutta-percha cones.

**Null hypothesis:** There is no difference between the effect of silver-curcumin nanoparticles or nanoproplis on all tested microorganisms.

## MATERIALS AND METHODS

### External coating materials preparation

#### *Preparation of Silver-curcumin nanoparticles*

In situ reduction of Ag<sup>+</sup> ions using nano curcumin was started by adding 100 µl of 0.1 M silver nitrate solution to a 20 ml of nano curcumin dissolved in Poly Ethylene Glycol (PEG) with 0.05 gm Poly Vinyl Pirlidone (PVP) as an adhesive polymer. The mixture was stirred for 10 min at room temperature.

#### *Preparation of Nanoproplis*

One hundred grams of raw propolis was placed in a flask with 500 mL of 80 % ethanol, which was then placed on hot plate and stirred with a stirrer (MSH-20A, Wetige®, Wertheim, Germany) for 7 days. After that the product was filtered using filter papers. The filtered solution was added at 1:10 ratio to distilled water to isolate pure propolis particles. The suspension was placed in an ultrasonic bath for 20-30 minutes to obtain propolis nanoparticles. Poly vinyl alcohol (PVA) was added as an adhesive.

### Filter paper discs preparation

After preparation of both materials, a volume of 200 micrograms of each material was used to load the filter paper discs.

### Coated gutta-percha preparation

An International Organisation for Standardisation (ISO) size 40 taper 6% gutta-percha cones (META-BIOMED, Korea) were autoclaved and then inserted in the Eppendorf tubes containing the tested coating materials for 24 hours (Figure 1). After that the cones were removed from the tubes and allowed to dry in air for 24 hours.

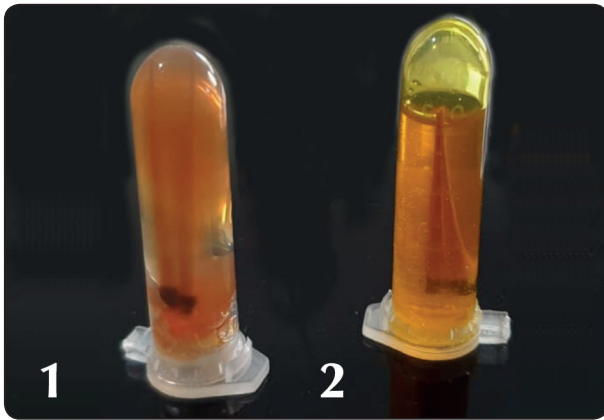


Fig. (1) The gutta-percha cones immersed in the coating agents. Nanopropolis (1) and Silver-curcumin nanoparticles (2).

### Scanning electron microscope

The external surface of the coated guttapercha cones were scanned under scanning electron microscope (QUANTA FEG 250) with 200 X magnification. The guttapercha cones were cut horizontally and the cross sections of the cones were scanned to obtain cross sectional scanning images.

### Microorganisms preparation and agar diffusion test

The tested microorganisms used in this study were *E. faecalis* ATCC29212, *E. coli* ATCC 25922, *Staph aureus* ATCC 6538 and *Candida albicans* ATCC 10231.

Eighty nutrient agar plates were heavily seeded uniformly with 0.1ml of  $10^5$ - $10^6$  cells/ml of the tested microorganism (20 plate for each microorganism). The plates of each microorganism were then divided into 2 groups (filter paper group n=10 and coated gutta-percha group n=10).

Filter paper discs saturated with silver-curcumin nanoparticles or nanopropolis were placed on the surface of inoculated agar plates. The plates were kept at low temperature (4°C) for 2-4 hours to allow maximum diffusion. The plates were then incubated at 37°C for 24 hours to allow maximum growth of the microorganisms. The longest diameter of the inhibition zone around each disc was measured in millimeters.

The same procedures were repeated using the coated gutta-percha cones where they were placed on the surface of agar plates inoculated with the tested microorganisms and were incubated for the same period. The antibacterial activity was measured by positive response and its degree.

### Statistical analysis of data

Numerical results were statistically analyzed with IBM® SPSS® Statistics Version 20 for Windows using analysis of variance (ANOVA) followed by student t-test for group comparisons. The p-value <0.001 was considered statistically significant.

## RESULTS

Both coatings produced positive inhibitory action on all tested microbial species. The mean and standard deviation of the diameter of the inhibition zone for Silver-curcumin nanoparticles around filter paper was the largest for *Staphylococcus aureus*  $20.6 \pm 0.39$  mm followed by  $18.2 \pm 0.67$  mm for *Candida albicans* followed by  $14.6 \pm 0.61$  mm for *Escherichia coli* and the least was  $12.35 \pm 0.71$  mm for *Enterococcus faecalis*. As for Nanopropolis the largest action was on *Staphylococcus aureus*  $15.4 \pm 0.70$  mm followed by  $14.8 \pm 0.63$  mm for *Candida albicans* followed by  $9.75 \pm 0.86$  mm for *Escherichia coli* and the least action was  $8.95 \pm 0.69$  mm for *Enterococcus faecalis*. There is an extremely significant difference between the inhibitory effect of silver-curcumin nanoparticles and nanopropolis in each of the groups of the tested microorganisms ( $P < 0.001$ ) (Table1) (Figures 2,3).

TABLE (1) Mean and standard deviation of the inhibition zones of Nanopropolis (1) and Silver-curcumin nanoparticles (2) loaded on filter paper discs against different tested microbes representing G+ve bacteria (*S. aureus* & *E. faecalis*), G-ve bacterium (*E. coli*) and Yeast (*C. albicans*).

	Inhibition zone (fmm)			
	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Nanopropolis	8.95±0.69	15.4±0.70	9.75±0.86	14.8±0.63
Silver-curcumin nanoparticles	12.35±0.71	20.6±0.39	14.6±0.61	18.2±0.67
P value	*** P<0.001	*** P<0.001	*** P<0.001	*** P<0.001

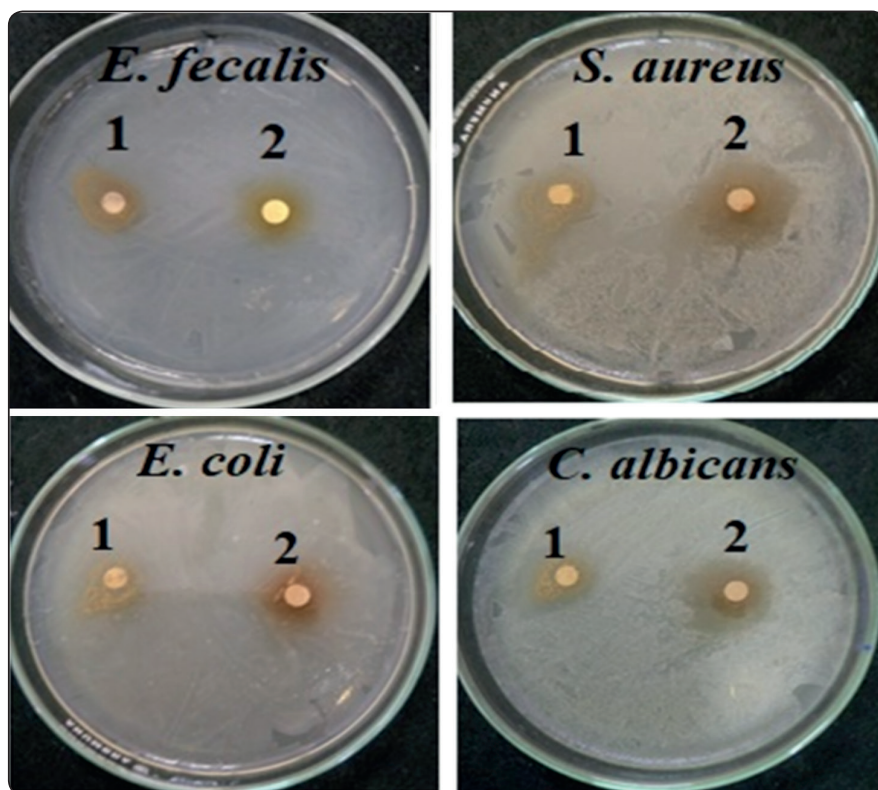


Fig. 2 The antimicrobial activity of Nanopropolis (1) and Silver-curcumin nanoparticles (2) loaded on filter paper discs against different tested microbes representing G+ve bacteria (*S. aureus* & *E. faecalis*), G-ve bacterium (*E. coli*) and Yeast (*C. albicans*).

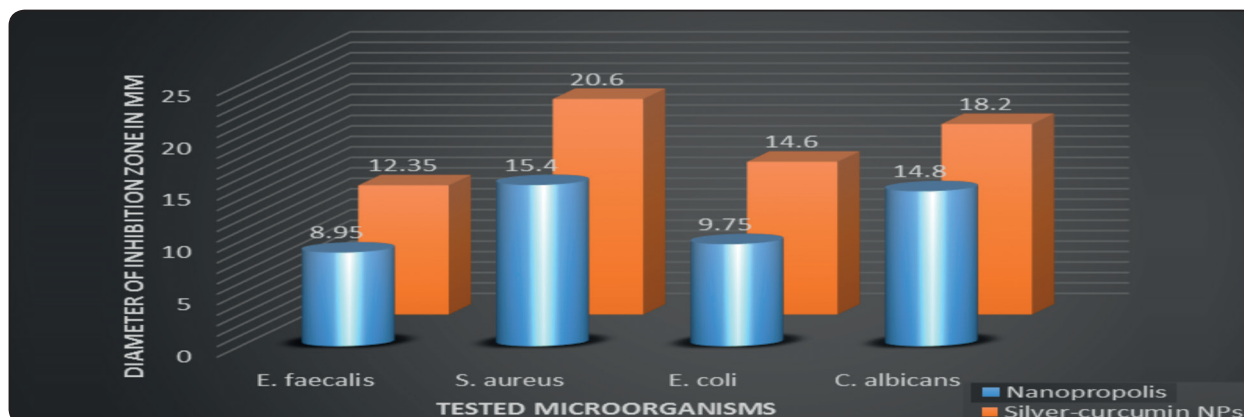


Fig. (3) Chart comparing the antimicrobial activity of Silver-curcumin nanoparticles versus Nanopropolis on all tested microorganisms.

Both coatings produced positive inhibitory action on all tested microbial species but with different potencies. The inhibition zones around coated gutta-percha cones were more pronounced for silver-curcumin nanoparticles than nanopropolis in all groups. Silver-curcumin nanoparticles produced the largest inhibitory effect on *Staphylococcus aureus* and *Candida albicans* (++++) followed

by *Escherichia coli* and *Enterococcus faecalis* (+++). Nanopropolis also produced the largest inhibitory effect on *Staphylococcus aureus* and *Candida albicans* (+++), but with less effect than that of silver-curcumin nanoparticles, followed by *Escherichia coli* and *Enterococcus faecalis* (++) (Table 2) (Figure 4).

Table (2) The antimicrobial activity of Nanopropolis (1) and Silver-curcumin nanoparticles (2) coated gutta-percha cones against different tested microbes representing G+ve bacteria (*S. aureus* & *E. faecalis*), G-ve bacterium (*E. coli*) and Yeast (*C. albicans*).

	Inhibition zone			
	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>
Nan opropolis	++	+++	++	+++
Silver-curcumin nanoparticles	+++	++++	+++	++++

+ Inhibition zone of < 7mm

++ Inhibition zone of 7mm - 12mm

+++ Inhibition zone of 12mm - 17mm

++++ Inhibition zone of > 17mm

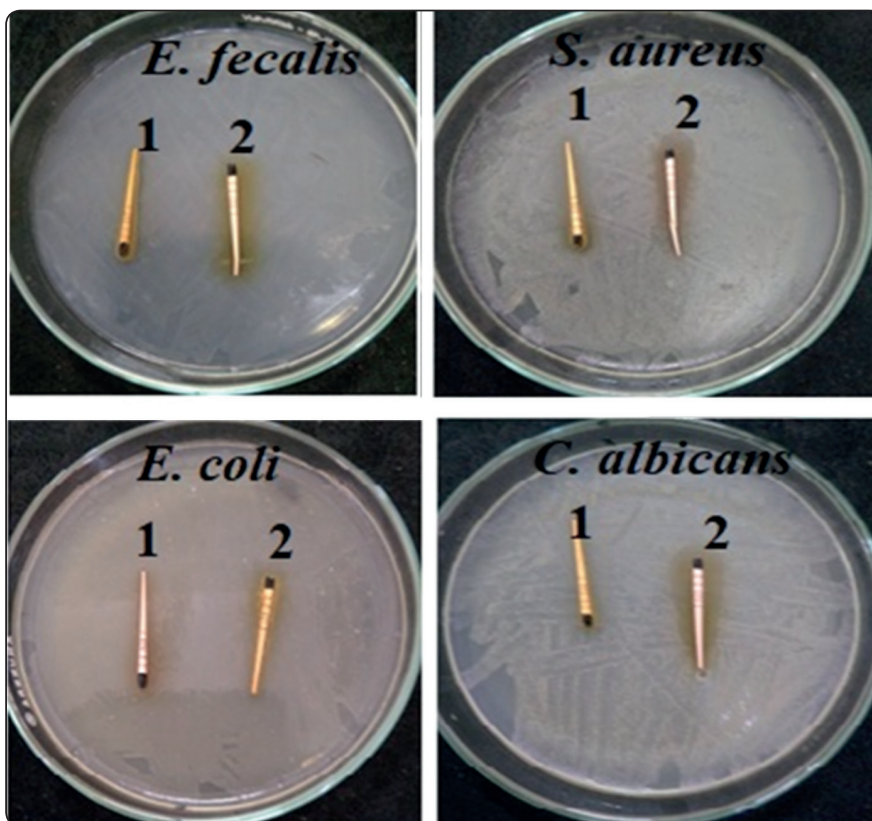


Fig. 4 The antimicrobial activity of Nanopropolis (1) and Silver-curcumin nanoparticles (2) coated gutta-percha against different tested microbes representing G+ve bacteria (*S. aureus* & *E. faecalis*), G-ve bacterium (*E. coli*) and Yeast (*C. albicans*).

**Fig. 4** The antimicrobial activity of Nanopropolis (1) and Silver-curcumin nanoparticles (2) coated gutta-percha against different tested microbes representing G+ve bacteria (*S. aureus* & *E. faecalis*), G-ve bacterium (*E. coli*) and Yeast (*C. albicans*).

The nanopropolis coating was more uniform and closely adherent (Figure 5) to the gutta-percha cone while the silver-curcumin nanoparticles coating appeared to be crazed and not well adapted (Figure 6) in some areas under scanning electron microscope.

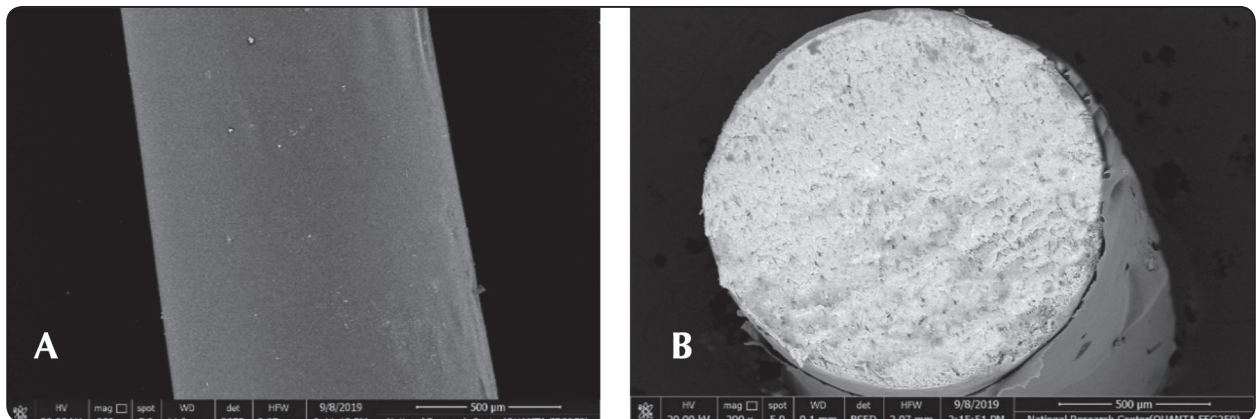


Fig. (5) Scanning electron microscopic image showing the external surface (A) and cross section (B) of Nanopropolis coated gutta-percha at 200x.

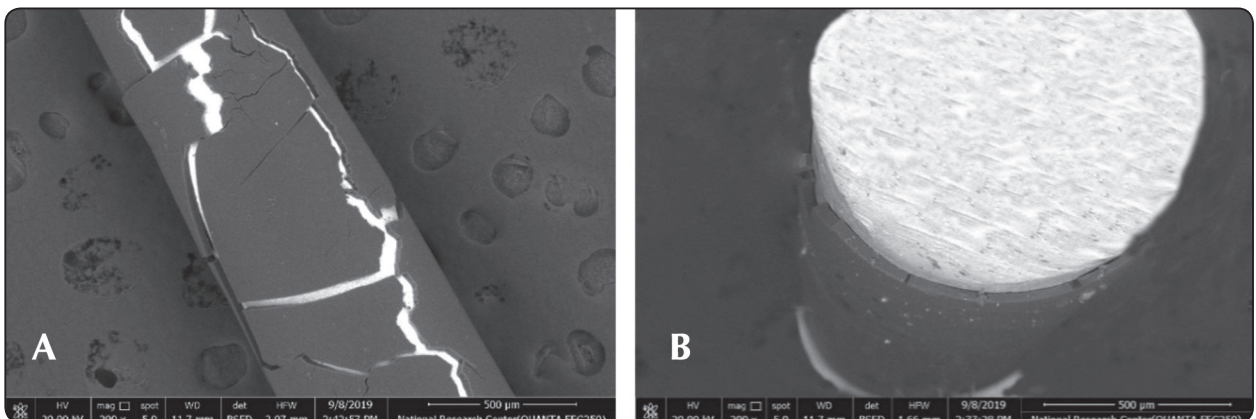


Fig. (6) Scanning electron microscopic image showing the external surface (A) and cross section (B) of Silver-curcumin nanoparticles coated gutta-percha at 200x.

## DISCUSSION

Some bacteria may survive all the cleaning and shaping procedures and intracanal medications due to its high virulence which causes their persistence after obturation. Other species may enter the canal due to improper final restoration that leads to coronal leakage. So, there is a need for new strategies to combat residual or new coming infections after obturation is completed (1, 32-34).

It was proven that each of the silver nanoparticles and curcumin has an antimicrobial action when used alone (14-23), Therefore we combined both materials to increase the antimicrobial action of curcumin and decrease the toxicity of silver nanoparticles that was demonstrated in previous studies (35-39).

In the present study silver-curcumin nanoparticles were bounded to gutta-percha using poly vinyl piloridone (PVP) while propolis was

bounded by Poly vinyl alcohol because it was found that poly vinyl alcohol (PVA) binds Poly ethylene glycol (PEG) in which curcumin is dissolved via cross linking causing the precipitation of curcumin and that would affect the inhibitory action of the material<sup>(40)</sup>.

Agar diffusion test was used in this study to evaluate the antimicrobial effect of discs loaded with silver-curcumin nanoparticles and nanopropolis and gutta-percha cones coated with the same materials. This method was used in previous studies to evaluate the antimicrobial activity of various materials used in dentistry such as irrigants, sealers, gutta-percha, medicated sealers or gutta-percha and other products. The used of this method in contact with the coated gutta-percha cones simulates the clinical condition inside the canal where the gutta-percha is in close contact to canal walls<sup>(1,41-43)</sup>.

A size 40 guttapercha cone was chosen in this study as it is an intermediate size that can be used widely in most of the canals and most of the obturation techniques<sup>(44)</sup>.

The results of the present study showed great antibacterial action of silver curcumin nanoparticles and nanopropolis over all the tested microorganisms, although the effect of silver-curcumin nanoparticles was far more pronounced. The results of this study were in agreement with Shantiaee et al.<sup>(45)</sup> who evaluated the effect of nanosilver gutta-percha on *E. faecalis* via bacterial leakage test and concluded that nanosilver gutta-percha could be very effective in endodontic treatment. This study was also in agreement with ALfhham and AL-Haidar<sup>(46)</sup> who evaluated the effect of silver nanoparticles as an irrigant on *Enterococcus faecalis* in vitro and Wu et al.<sup>(47)</sup> who demonstrated that the use of silver nanoparticles medicating gel was more effective than using it as an irrigant and that is very close to the nanosilver- curcumin nanoparticles coating. Also the results were in agreement with Moghadas et al.<sup>(48)</sup> who found that nanosilver has a great antibacterial

action against *E. faecalis* and *S. aureus* and with Tyagi et al.<sup>(49)</sup> who showed that curcumin has strong antibacterial effect against *E. faecalis*, *S. aureus* and *E. coli* and Mandroli and Bhat<sup>(50)</sup> who concluded that curcumin has antibacterial potential against standard strains of common endodontic bacteria. Regarding the antimicrobial effect of propolis, the results of this study was in agreement with Abdel Raheem IA et al.<sup>(51)</sup> who tested nanopropolis containing sealers, Carbajal Mejia JB<sup>(52)</sup> who tested propolis as intracanal medicament, Al Waili N et al.<sup>(53)</sup>, Seibert JB et al.<sup>(54)</sup>, Kharsany K et al.<sup>(55)</sup> who all concluded that propolis has an antibacterial action on wide range of organisms including *E. faecalis*, *S. aureus*, *E. coli* and *Candida albicans*.

The results of this study were in disagreement with Rodrigues et al.<sup>(56)</sup> who stated that nanosilver was not effective in elimination of *E. faecalis* biofilm.

## CONCLUSION

Silver-curcumin nanoparticles has a greater antimicrobial effect compared to nanopropolis either alone or as a gutta-percha coating. Guttapercha cones coated with either materials have an inhibitory action on wide range of microbes and may be of great help in combating residual microbes in the root canals after obturation, thus limiting flare ups and decreasing failure rates in endodontic treatments. Nanopropolis coating is more uniform and closely adherent to the gutta-percha cones than silver-curcumin nanoparticles coating.

## RECOMMENDATIONS

A more stable adhesive needs to be found for silver-curcumin nanoparticles to obtain better adaptation on the gutta-percha cones without reacting with the coating materials. Future clinical trials are needed to assess the clinical benefit for these antibacterial coatings.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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