

Antimicrobial efficacy of silver nanoparticles of *Aloe vera*

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ABSTRACT

The aim of this study is to compare the antimicrobial activity of *Aloe vera* nanoparticles to that of calcium hydroxide in persistent endodontic infections. Microorganisms that cause persistent apical periodontitis usually exhibit a high level of resistant to disinfection and are able to survive against harsh conditions of root canal system and organize a mature biofilm. Therefore, these microorganisms are not easily eliminated from the root canal by common irrigation solutions or intracanal medicaments. It may be stated that efforts to eliminate these bacteria may equate with achieving successful disinfection. The study consists of four groups of intracanal medicament, Group A - calcium hydroxide (positive control), Group B - sterile water (negative control), Group C - *A. vera*, and Group D - silver nanoparticles (Ag-NPs) of *A. vera* which was tested against *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans* that were common in persistent endodontic infections. Strains of these microorganisms were isolated and were subcultured. Disc diffusion was done for the primary evaluation of antimicrobial susceptibility, in which microorganisms were incubated in agar plates, following which 50 µL of each medicament was added in filter paper and placed on the plates. The plates were incubated, and zone of inhibition around each disc was measured. Ag-NPs of *A. vera* demonstrated antimicrobial activity; henceforth, research should be directed toward the use as an intracanal medicament in root canal treatment.

Keywords: *Aloe vera*, silver nanoparticles, antimicrobial efficacy

Introduction

Pulpal and periapical pathologies are associated with microorganisms that are polymicrobial in nature. Ultimate aim of root canal treatment is to completely eliminate microorganisms from root canal system through mechanical and chemical debridement. Although mechanical debridement can reduce the bacteria from the root canal, chemical debridement is associated with high success rates in bacterial elimination from the root canal system.^[1]

Most persistent root canal infection in root canal system is *Enterococcus faecalis* that has the ability to survive harsh environment and it possess a potential difficulty in removing from the root canal system.^[2] Fungi have occasionally been found in primary root canal infections, but they appear to occur more often in filled root canals of teeth in which treatment has failed. *Candida albicans* is by far the fungal species most commonly isolated from infected root canals. *Candida* species is one of the commonly isolated microorganisms from root canals in 55% of the cases and been associated with necrotic pulp in 15.3% of the

cases.^[3] *Streptococcus mutans* though commonly associated with dental caries, it has also been reported to be associated with root canal infections such as apical periodontitis.^[4]

Calcium hydroxide is the most commonly used intracanal medicament due to its high alkaline pH that has seem to have the inhibitory effect on bacterial growth. However, it is not proven to be effective against all endodontic pathogens.^[5] Although synthetic chemical medicaments such as calcium hydroxide and chlorhexidine are commonly used, development of antibiotic-resistant strains and the side effects associated with it have directed the researchers to look for the use of herbal alternatives for root canal disinfection.^[6]

Aloe barbadensis Mill is a short succulent herb resembling a cactus, with green dagger-shaped fleshy, spiny, and marginated leaves, filled with a clear viscous gel belong to family Liliaceae. It has been used in root canal disinfection due to its antimicrobial property both as an intracanal medicament and as an irrigant. The antimicrobial effects of *Aloe vera* have been attributed to the plant's natural anthraquinones that have demonstrated in numerous vitro studies.^[7-10]

Nanoparticles offer distinct properties such as particle size, increased chemical reactivity, and increased surface area/mass ratio compared to their bulk counterparts. These distinct properties of nanomaterials have made them an immensible tool in dentistry. Advantages of these nanoparticles are that they interact with at the subcellular and molecular level of the human body to achieve maximal therapeutic efficacy with minimal side effects.^[11]

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Among all the nanomaterials, clusters of silver atoms, which are defined as silver nanoparticles (Ag-NPs), have the highest degree of commercialization, and it been used exponentially.^[12] In endodontics, Ag-NPs have been used to inhibit microbial development and prevent infections; as a disinfectant in root canal treatments to eliminate bacteria, bacterial products, and debris from the root canal system, this rapid spread of Ag-NP use in endodontic materials was mainly because of its proven antimicrobial activity against nearly 650 different disease-causing microorganisms, including multidrug-resistant strains. Another reason that has encouraged the use of Ag-NPs for therapeutic and clinical purposes was information showing that silver, in the form of nanoparticles, would be less toxic to cells and tissues.^[13-16]

Therefore, this study was conducted to assess the synergistic action of nanoparticles and *A. vera* in root canal disinfection.

Aim

The aim of this study was to evaluate the antimicrobial efficacy of Ag-NPs of *A. vera* against *E. faecalis*, *S. mutans*, and *C. albicans*.

Materials and Methods

Test materials used are as follows:

1. Calcium hydroxide (RC Cal; Prime Dental Products, India) - positive control
2. Normal saline - negative control
3. *A. vera* extract
4. Ag-NPs of *A. vera*.

Microorganisms:

- *Candida albicans* (ATCC 10556)
- *Enterococcus faecalis* (ATCC 29212)
- *Streptococcus mutans*
- Brain heart infusion (BHI) broth
- Vernier callipers.

Preparation of *A. vera* extract

A. vera gel was extracted from the plant's leaves. The leaves of the plants were washed with distilled water, and the surfaces of the leaves were disinfected with 70% ethyl alcohol. After cutting and opening the leaves, the fresh pulp was collected and homogenized.

Synthesis of Ag-NPs

30 g portion of *A. vera* leaves were thoroughly washed and finely cut. Leaves were boiled in 100 ml sterile distilled water. The resulting extract was used for the synthesis of nanoparticles.

25 ml of ammonia was added to the 50 ml of 0.01M silver nitrate solution followed by the addition of 50 ml leaf extract. The concentration of silver nitrate was adjusted to 0.001M by making up the final volume to 500 ml using distilled water. The observation of color change after 24 h incubation in dark place indicates the formation of Ag-NPs. These mixtures were centrifuged at 10,000 rpm

for 10 min at room temperature. The pellet was collected and dried in a hot plate. The dried powder was collected in Eppendorf tubes and stored in refrigerator and which was further characterized by spectrophotometer [Figure 1].

Preparation of experimental solutions

A. vera gel and Ag-NPs of *A. vera* were diluted with distilled water to obtain a concentration of 500 µg, 1000 µg, and 2000 µg.

Agar diffusion test

Microorganisms were subcultured, and they were streaked in Petri dish containing BHI agar. After drying the inoculums, sterile filter paper discs were applied. 0.1 ml of each solution was pipetted onto the sterile paper disks. Five replicates were prepared for each organism. The plates were incubated at 37°C for 48 h.

Microbial inhibition was measured around the papers disks, and the test was repeated under aseptic conditions to ensure consistency. The antimicrobial activity of each medicament was expressed in terms of the mean of the diameter of zone of inhibition (in mm).

Statistics

The normality tests Kolmogorov–Smirnov and Shapiro–Wilks tests results reveal that the variable follows a normal distribution. Therefore, to analyze the data, parametric methods are applied. To compare the mean values between groups, one-way ANOVA is applied. SPSS version 22.0 is used to analyze the data. Significance level is fixed as 5% ($\alpha = 0.05$).

Results

The mean values of growth inhibition produced by different test groups against the test microorganisms are given. Table 1 shows the mean zone of inhibition of calcium hydroxide, *A. vera* gel (500 µg), and Ag-NPs of *A. vera* (500 µg), and there was a statistical significant difference between the groups. Table 2 shows the mean zone of inhibition of calcium hydroxide, *A. vera* gel (1000 µg), and Ag-NPs of *A. vera* (1000 µg), and there was a statistical significant difference

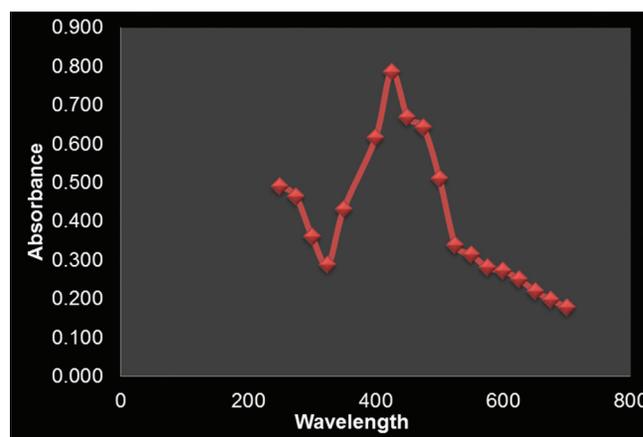


Figure 1: Ultraviolet absorption spectrum

between the groups. Table 3 shows the mean zone of inhibition of calcium hydroxide, *A. vera* gel (2000 µg), and Ag-NPs of *A. vera* (2000 µg), and there was a statistical significant difference between the groups.

Calcium hydroxide performed better than all the test groups, followed by Ag-NPs of *A. vera* (2000 µg > 1000 µg > 500 µg) and *A. vera* extract (2000 µg > 1000 µg > 500 µg) against all the tested microorganisms after 48 h incubation [Tables 1-3].

Mean zone of inhibition for *C. albicans* (Group I - calcium hydroxide, Group II - *A. vera* at a concentration of 500 µg, 1000 µg, and 1500 µg and Ag-NPs of *A. vera* at a concentration of 500 µg, 1000 µg, and 1500 µg) [Figure 2].

Table 1: Mean zone of inhibition calcium hydroxide versus *Aloe vera* (500 µg) versus silver nanoparticles of *Aloe vera* (500 µg)

Variables	Group	Mean±SD	F value	P value
<i>C. albicans</i>	Group I	24.00±1.225	177.143	<0.001
	Group II	12.00±0.707		
	Group III	14.00±1.225		
<i>E. faecalis</i>	Group I	35.00±2.000	393.781	<0.001
	Group II	11.20±1.304		
	Group III	14.20±0.837		
<i>S. mutans</i>	Group I	38.00±1.581	760.000	<0.001
	Group II	14.00±0.707		
	Group III	16.00±0.707		

E. faecalis: *Enterococcus faecalis*, *S. mutans*: *Streptococcus mutans*, *C. albicans*: *Candida albicans*, SD: Standard deviation

Table 2: Mean zone of inhibition calcium hydroxide versus *Aloe vera* (1000 µg) versus silver nanoparticles of *Aloe vera* (1000 µg)

Variables	Group	Mean±SD	F value	P value
<i>C. albicans</i>	Group I	24.00±1.225	40.000	<0.001
	Group II	14.00±2.121		
	Group III	16.00±2.121		
<i>E. faecalis</i>	Group I	35.00±2.000	297.404	<0.001
	Group II	16.20±0.837		
	Group III	17.00±1.000		
<i>S. mutans</i>	Group I	38.00±1.581	429.684	<0.001
	Group II	18.40±0.548		
	Group III	22.00±1.000		

E. faecalis: *Enterococcus faecalis*, *S. mutans*: *Streptococcus mutans*, *C. albicans*: *Candida albicans*, SD: Standard deviation

Table 3: Mean zone of inhibition calcium hydroxide versus *Aloe vera* (2000 µg) versus silver nanoparticles of *Aloe vera* (2000 µg)

Variables	Group	Mean±SD	F value	P value
<i>C. albicans</i>	Group I	24.00±1.225	23.407	<0.001
	Group II	20.00±0.707		
	Group III	21.20±0.837		
<i>E. faecalis</i>	Group I	35.00±2.000	140.211	<0.001
	Group II	21.80±0.837		
	Group III	23.00±1.000		
<i>S. mutans</i>	Group I	38.00±1.581	156.364	<0.001
	Group II	24.00±1.581		
	Group III	26.00±0.707		

E. faecalis: *Enterococcus faecalis*, *S. mutans*: *Streptococcus mutans*, *C. albicans*: *Candida albicans*, SD: Standard deviation

Mean zone of inhibition for *E. faecalis* (Group I - calcium hydroxide, Group II - *A. vera* at a concentration of 500 µg, 1000 µg, and 1500 µg and Ag-NPs of *A. vera* at a concentration of 500 µg, 1000 µg, and 1500 µg) [Figure 3].

Mean zone of inhibition for *S. mutans* (Group I - calcium hydroxide, Group II - *A. vera* at concentration of 500 µg, 1000 µg, and 1500 µg and Ag-NPs of *A. vera* at concentration of 500 µg, 1000 µg, and 1500 µg) [Figure 4].

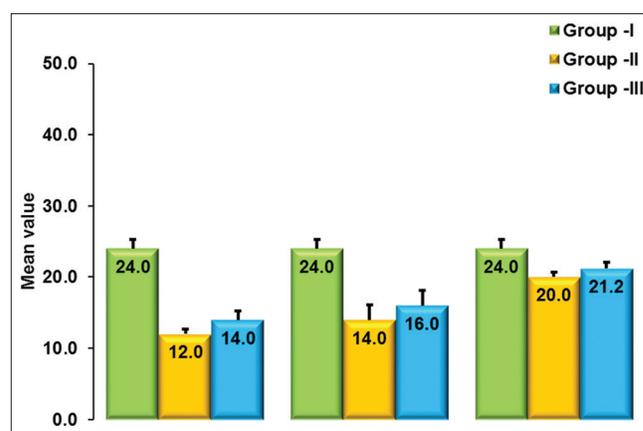


Figure 2: *Candida albicans*

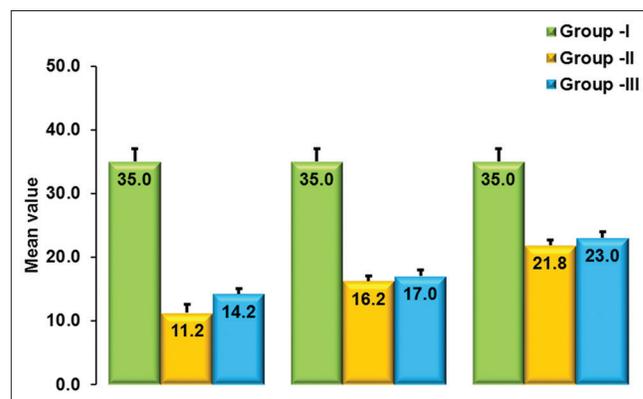


Figure 3: *Enterococcus faecalis*

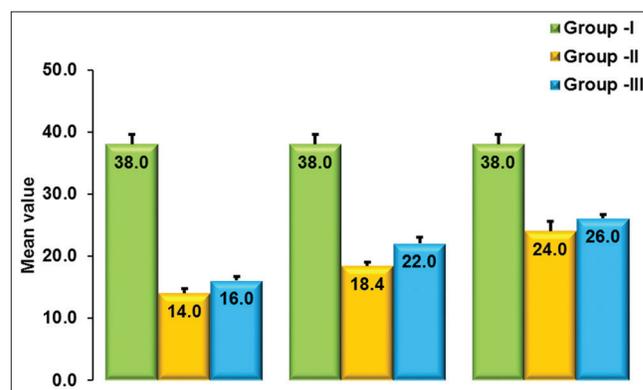


Figure 4: *Streptococcus mutans*

Discussion

A variety of laboratory methods are available to evaluate the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disc diffusion method. It is commonly used because of advantage of providing direct estimation of its antimicrobial activity against a specific microorganism and added advantages of simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease of results interpretation. Although there are new technologies in the field of microbiology, disc diffusion is still one of the preliminary tests to assess the antimicrobial activity of a material.^[17-19] To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent). The incubation time of 48 h was chosen owing to the reason majority of these intracanal medicaments were placed for a period of 7–10 days in root canal system.

As expected, calcium hydroxide had better antimicrobial property when compared to other medicaments. Antimicrobial activity of Ca (OH)₂ is related to the release of highly reactive hydroxyl ions in an aqueous environment, which mainly affects cytoplasmic membranes, proteins, and DNA.^[20,21] Among the microbes, *C. albicans* was found to have a minimal zone of inhibition signifying that they require more amount of hydroxyl ions release to inhibit its growth.

Plants and other natural sources provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites, and new synthesized molecules as potential antimicrobial agents. The fact that a plant extract exhibits antimicrobial activity is of interest, *A. vera* had 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids which were possible reasons for its antimicrobial action. It also contains curcumin, nimbodin, myristic acid, and anthraquinones, all of which can account for its antimicrobial activity.^[22]

Biologically synthesized Ag-NPs are commonly used in the field of medicine. Extracellular biosynthesis of Ag-NPs was carried out using medicinal plant extracts. It has been reported that Ag-NPs are non-toxic to humans and most effective against bacteria, virus, and other eukaryotic microorganism at low concentrations and without any side effect.^[23] Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents. In small concentrations, silver is safe for human cells but lethal for microorganisms.^[24] Silver is known to produce an antibacterial effect by acting on multiple targets starting from interaction with the sulfhydryl groups of proteins and DNA, alter the hydrogen bonding/respiratory chain, unwind DNA, and interfere with cell-wall synthesis/cell division. Ag-NPs are known to further destabilize the bacterial membrane and increase permeability, leading to leakage of cell constituents. This could be the possible reason for Ag-NPs of *A. vera* to demonstrate antimicrobial activity.

Conclusion

Within the limitations of the study, it can be concluded that Ag-NPs of *A. vera* demonstrated antimicrobial activity against *E. faecalis*, *S. mutans*, and *C. albicans*.

Henceforth, future research should be carried out for using Ag-NPs of *A. vera* in root canal disinfection by increasing its concentration and future research oriented toward testing its biocompatibility.

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