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Original Article

Feasibility of the crude extracts of *Amorphophallus paeoniifolius* and *Colocasia esculenta* as intracanal medicaments in endodontic therapy in comparison to the 940 nm diode laser: An *in vitro* antimicrobial study

Didar Sadiq Hama Gharib [a*], Raid Fahim Salman [b]

[a] *Department of Conservative Dentistry, College of Dentistry, University of Sulaimani, As Sulaymaniyah, 46001, Iraq*
[b] *Department of Conservative Dentistry, College of Dentistry, Hawler Medical University, Erbil, Iraq*

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Intracanal medicaments

Abstract *Background/purpose:* The elimination and debridement of intracanal bacteria are credited with long-term effectiveness in endodontic therapy. This study aimed to compare the antimicrobial efficacies of *Amorphophallus paeoniifolius* (Suran), *Colocasia esculenta* (Aravi) crude extracts as intracanal medicaments with calcium hydroxide (CH), 2% chlorhexidine (CHX) gel, and 940 nm diode laser.
Materials and methods: Fifty-eight intact, single-root, extracted human mandibular premolar teeth were prepared. The samples were sterilized, transferred into microcentrifuge tubes, and inoculated with *E. faecalis*. The samples were placed in an incubator for three weeks to allow the biofilm to grow. Then the samples were randomly divided into five experimental groups (n = 10), disinfected with Suran, Aravi crude extracts, CH, 2% CHX gel, and a 940 nm diode laser. The negative control group (n = 4) and the positive control group (n = 4). Then the samples were observed under light and scanning electron microscopy to monitor the *E. faecalis* biofilm. The sampling method was carried out in paper point (intracanal) and Peeso bur (intradentinal). Later the number of colony-forming units was counted and analyzed.
Results: Colony-forming units were significantly reduced in the 2% CHX gel in both sampling methods, while Suran showed lower colony-forming units compared to Aravi and CH. The differences between experimental groups were not statistically significant ($P > 0.05$) in both sampling methods.

* Corresponding author. Department of Conservative Dentistry, College of Dentistry, University of Sulaimani, As Sulaymaniyah, 46001, Iraq.
E-mail address: didar.hamagharib@univsul.edu.iq (D.S. Hama Gharib).

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Conclusion: This study showed that the application of Suran and Aravi crude extracts as intracanal medicaments leads to a significant reduction in the number of bacterial colonies compared to CH, 2% CHX gel, and 940 nm diode laser.

Introduction

Microorganisms have long been suspected of being involved in the initiation and progression of endodontic infections. As a result, the primary goal of endodontic therapy is to prevent or cure periapical pathosis produced by these bacteria.[1] The tooth is "chemo-mechanically prepared" by mechanical instrumentation of the root canal area combined with the administration of chemical agents to achieve thorough infection elimination and promote healing.[2]

The structure of the root canals is complex, and effectively cleaning this intricate system is one of the most challenging parts of endodontic therapy. Endodontists accomplish this goal by utilizing irrigants and intracanal medicaments.[3] Microorganisms observed in the infected root canal space colonize in two ways: as free-floating planktonic cells or as biofilms linked to one another or the root canal walls.[4] While planktonic microorganisms can be removed in various ways, eradicating biofilm bacteria from the root canal remains a primary objective.[5]

A biofilm is a population of bacteria encased in an extracellular polymeric matrix and adhering to a solid surface. It has been hypothesized that biofilm bacteria exhibit a range of phenotypes within this community. As a result, attempts to remove germs may be linked to successful disinfection. Intracanal administration of medicaments such as calcium hydroxide (CH) is usually recommended due to its high pH, which changes the lipopolysaccharides in the bacterial cell wall.[6] However, it has been demonstrated that this elevated pH level is not sustained.[7] Additionally, a rise in pH can facilitate bacterial adhesion to dentine collagen fibers, preserving them from disinfection operations.[8] It weakens dentine, even when used in short spans,[9] and has a limited capacity to penetrate dentinal tubules.[10]

The most prevalent bacteria discovered in endodontically infected root canals is *Enterococcus faecalis* (*E. Faecalis*).[11,12] *E. faecalis* is a Gram-positive, facultative anaerobe usually found in chronic periodontitis and root canal retreatment cases.[13,14] Numerous investigations have revealed that *E. faecalis* is one of the most frequently isolated species from root canals following the endodontic treatment.[15,16] Enterococcus species, particularly *E. faecalis*, can fail root canal treatments and persistent apical periodontitis in endodontics.[17] One explanation for treatment failure may be virulence factors such as aggregation substance, lipoteichoic acid, and pheromones, which enable *E. faecalis* to persist in dentinal tubules up to 400 μm depth.[18,19] *E. faecalis* was isolated from 38% of unsuccessful root canal treatments.[20] Because of its ability to withstand extreme environmental changes, *E. Faecalis* has become a treatment-resistant microbe,[21] which is considered due to its high alkaline tolerance[22] and tubular invasion ability, protecting it from intracanal endodontic medicaments.

Calcium hydroxide (CH) is a popular intracanal medicament used in endodontic treatments. The high pH of CH destroys the bacterial cell membrane and protein components.[23] Some studies show that CH is ineffective against *E. faecalis* because it persists in dentinal tubules.[21,24]

Chlorhexidine digluconate (CHX) gel is another alternate root canal treatment with well-known broad-spectrum antibacterial properties. Also, it has been demonstrated that CHX induces intracellular component leakage by adhering to the cell walls of microorganisms.[23] In the dentinal tubules, it has been demonstrated that CHX is more successful than CH at eliminating germs such as *E. faecalis* that are resistant to CH.[25,26]

Researchers examined a wide range of natural compounds in their quest for novel irrigant and intracanal medicaments with high biocompatibility and antibacterial activity.[1] They believed that the proper herb in the right combination kept the biological system balanced in ancient times. Herbs include various components with varying degrees of therapeutic activity, including essential oils, flavonoids, tannins, and alcohols.[27]

Amorphophallus paeoniifolius (Suran), often known as elephant foot yam or Araceae, is a tuberous, robust indigenous herbaceous medicinal plant commonly cultivated as a vegetable and readily available tuber plant.[28] This plant is widely grown in tropical and subtropical areas, notably in Southeast Asia. It is cultivated Commercially in India, China, Malaysia, Sri Lanka, Indonesia, Thailand, the Philippines, and in tropical regions of Africa. It is a tuberous, robust, indigenous, herbaceous medicinal plant commonly grown as a vegetable and is readily available as a tuber plant.[29] Fresh yam has expectorant, stimulant, and caustic properties.[30] The plant's roots are tonic, stomachache-relieving, and tasty.[31] Anti-protease activity[32] and antibacterial, antifungal, cytotoxic, and analgesic effects have been shown in the tuber.[33]

Colocasia esculenta belongs to the Araceae family and is known in northern India as Aravi or Arbi. The corm root of a plant was employed in this experiment. The plant has long heart-shaped or arrowhead-shaped leaves that point downward and are hardy succulent. It has an upright stem that is green, red, black, or variegated. Vitamin C is abundant in the young leaves, whereas carbohydrates are abundant in the roots. The nutrients discovered are thiamine, riboflavin, niacin, oxalic acid, calcium oxalate, and sapotoxin.[34] It has traditionally been used to soothe the stomach, reduce swelling and pain, and lessen fever. Its corm treats tuberculous ulcers, lung congestion, paralyzed

limbs, fungal abscesses in animals, and anthelmintics. Its leaves are used as a styptic and a poultice.[35] The *C. esculenta* extract demonstrated substantial antibacterial activity against several tested bacteria and fungi.[36]

Recently, root canal disinfection with a Diode Laser has gained attention as a potential alternative antibacterial disinfection regimen for drug-resistant microbes.[37] Diode lasers emitting at 940–980 nm deliver energy through thin, flexible fibers to the root canals. These lasers have a power output of 0.5–7 W and operate in two modes: continuous wave or pulsed. The utilization of contemporary laser technology enables access to previously inaccessible locations.[38]

As a result, the current study was carried out to investigate the antibacterial potential of crude extracts *A. paeoniifolius* (Suran) and *C. esculenta* (Aravi) as intracanal medicaments compared to CH, 2% CHX gel and 940 nm diode laser against intraradicular *E. Faecalis* biofilm.

Materials and methods

Plant preparation and extraction

Plant materials collected freshly in Carrefour Hypermarket in Dubai, United Arabs of Emirates, were obtained using their vernacular and botanical names. Both plants are washed with sterile distilled water and then sliced into thin slices to naturally dry them under the shade of the sunlight at room temperature. Next, the dried plants are ground to a coarse powder by an electrical grinder. After that, the ground powder was kept in a closed container tightly.

In a sterilized amber glass bottle, 100 g of ground powder from each plant was soaked in (150 ml Methanol + 150 ml Acetone + 50 ml distilled water) and then mixed for 48 h with a magnetic stirrer. Afterward, the mixture was poured into a 10 ml centrifuge tube and centrifuged for 20 min at 10000 rpm (revolution per minute); the supernatant separated from the coarse residue of the mixture. Finally, the supernatants through number one Whatman filter paper (Merck, Darmstadt, Germany) are filtered, and the filtrates are stored in an amber glass bottle and kept in a refrigerator at 4 °C. Filtrates from both plants were poured into glass Petri dishes, then placed in a vacuum drying oven (Daihan LabTech, Jakarta, Indonesia) under 40 °C to evaporate solvents. Eventually, a semi-solid residue (dark brown for Suran and dark orange for aravi) was left on the bottom of the Petri dishes. These residues are collected and stored in a 3-mL sterile glass bottle.

Consequently, 1000 mg of each extract is dissolved in 1 ml of 10% Dimethyl Sulfoxide (DMSO) (Merck, Darmstadt, Germany) and stored in a dark container not to be affected by light (because some plant extract components are sensitive to light).

Sample (teeth) selection

Fifty-eight single-rooted mandibular premolars with fully formed apices and roots with an angle of curvature less than ten degrees (mild curvature) were assessed by Schneider's approach: "teeth with straight canals on preparation reveal rounder prepared canals than the curved canal."[39,40] The selected teeth were planned for extraction due to periodontal disease, caries, or orthodontic concerns. Mandibular premolar teeth were used in this investigation because of their oval-shaped root canals; however, to verify this, a series of conventional buccolingual and mesiodistal periapical radiographs were taken and visually inspected. Multiple root canals, fractured roots or calcified root canals, internal or external root resorption, severe curvature or open apices, and internal or external root resorption were all ruled out before endodontic therapy. All teeth were kept in 10% formalin following extraction. The selected teeth were methodically cleaned from soft and hard tissues on the roots using a hand curette and scalpel blades during the preparation phase. This procedure was completed by immersing the teeth for 2 h in a 5.25% NaOCl solution (Chloraxis, Cerkamed, Poland) and then thoroughly washing them in flowing tap water. They were then submerged in the saline solution until they were ready for use again.[40,41]

Sample preparation and standardization of working length

The samples were decoronated at or below the cementoenamel junction (CEJ) using a low-speed water-cooled diamond disc bur (Edenta AG, St. Gallen, Switzerland). A standardized root length of around (15 mm ± 1) was created, and a digital caliper was used to measure the lengths of the samples from the apex to the CEJ. Next, a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to make sure the canal was open at the apex and was then pushed down until the file tip was barely visible beyond the apex; the working length was then measured after subtracting one millimeter from this length.[42] Cleaning and shaping were performed using a crown-down instrumentation technique with Wave One Gold primary (25/0.07) rotary files (Dentsply Maillefer, Ballaigues, Switzerland) and a 16:1 gear reduction handpiece driven at a constant rotation speed of 250 revolutions per minute by a torque-controlled electric motor X-smart (Dentsply Maillefer).[40] All samples were prepared using Wave One Gold (Dentsply Maillefer) Primary size (25/0.07) and pecked in and out until the working length was attained. Light apical pressure was applied to the file during the preparation phase, and the file was utilized in a brushing motion to ensure that the volumes of all canals were standardized to the same taper. Following the usage of each instrument, all debris, including the smear layer, was removed using a final irrigant of 5.25% sodium hypochlorite (Cerkamed) and 17% ethylenediaminetetraacetic acid (EDTA) ENDO-Solution (Cerkamed) for 1 min and then the canals were rinsed with sterile distilled water to eliminate solution residues. Sterile Wave One Gold paper points (Dentsply Maillefer) were then used to dry the root canals.

Sterilization of root specimens

Autoclave pouches were used to pack the prepared teeth, then autoclaved at 121 °C for 30 min at a pressure of 15 psi.[43] Furthermore, all specimens were placed in microcentrifuge tubes containing sterile modeling wax to insert

the root specimens upright. Then, four specimens were randomly selected and checked to ensure sterilization of samples (negative control), i.e., there was no cross-contamination to prove that the root samples were completely sterile prior to root canal inoculation with *E. faecalis* bacteria.

Contamination of root specimens

The test organism used for this study was *E. faecalis* (ATCC 29212), a gram-positive facultative anaerobic bacterium common in root-filled teeth with post-treatment infection. *E. faecalis* was grown in thioglycolate broth (NEOGEN, Lansing, MI, USA) for 24 h. Each root canal was inoculated with 24 h old cultured broths of bacterial solution of *E. faecalis* using a sterile Insulin needle in a microbiological safety cabinet. Following the inoculation, the samples were kept in closed microcentrifuge tubes and incubated at 37 °C for 21 days under aseptic conditions. The canals were re-inoculated with fresh bacterial samples at every three days intervals to ensure the viability of bacteria. After 21 days, another four teeth were randomly selected to ensure that the bacterial biofilm was present and the bacterial cells (*E. Faecalis*) penetrated deep into the dentinal tubules (positive control).

Antimicrobial assessment (root canal disinfection)

The canal contents were aspirated after 21 days of incubation, rinsed with 5 mL saline, and dried with sterile paper points. The remaining 50 root specimens were then randomly divided into five groups (n = 10 each):

Group one (G1): root canal disinfected with crude extract of Suran as intracanal medicament.

Group two (G2): root canal disinfected with crude extract of Aravi as intracanal medicament.

Group three (G3): root canal disinfected with CH paste (ApexCal, Ivoclar Vivadent, Schaan, Liechtenstein) as intracanal medicament.

Group four (G4): root canal disinfected with 2% CHX gel - GLUCO-CHEX (Cerkamed) as intracanal medicament.

Group five (G5): root canal disinfected with 940 nm diode laser (Epic X) (Biolase, San Clemente, CA, USA).

The root samples in groups one and two were filled with both crude extracts in gel form (by mixing them with carboxymethyl cellulose powder as a thickening agent) and, with the help of Lentulo spiral filler (Dentsply Maillefer) transferred to the root canals.

In both groups, three and four CH paste (Ivoclar Vivadent) and 2% CHX gel (Cerkamed) respectively were inserted into the root canal samples through special syringe tips following manufacturer instructions.

In group five, sterilized normal saline was used to rinse the canals. The canals were then irradiated with a laser at a wavelength of 940 nm and output power of 0.5 W in continuous mode using an Epic X BIOLASE (Biolase) and an endodontic tip (ezTip Endo, 14mm/200 μm).[44] The laser irradiation was performed with circular motions from apical to coronal (step-back technique); the root canals were irradiated four times in each canal for 15 s, with a linger time of 20 s, for a total of 1 min,[45] because of concerns about possible temperature changes in dentin and in accordance with 940 nm diode laser-assisted root canal disinfection and transmission investigations, the power was set at 0.5 W.[44–46] The laser was inserted directly into the root canal without any root canal treatment.

Specimens of all groups are sealed apically with flowable composite resin (Beautifil-Bulk Flowable, SHOFU INC., Japan) and coronally with temporary filling material (CAVITON, GC, Japan) and placed in sterile microcentrifuge tubes containing modeling wax under aseptic conditions. The specimens remain wet and stand in an upright position. They were incubated in an aerobic environment for 37 °C at the end of seven days.

Determination of the colony forming unit (CFU)

The root specimens are rinsed with sterile normal saline as a transfer fluid after removing the coronal seal. Then, a sterile paper point was inserted into each root specimen and left for 1 min,[47] and they were dipped in one milliliter of sterile saline solution and vortexed for 30 s. Following sampling with paper points, Peeso burs #3 (Mani Inc., Tochigi, Japan) with micro-electromotor (STRONG, Fujian, China) at 2000 rpm were used to attain samples from dentin.[48] Each bur was used once up to the working length of the canal, and dentine chops (debris) were transferred into one milliliter of sterile saline solution and vortexed for 30 s. The resulted bacterial suspensions were serially diluted, and 10 μL of the diluted solution was plated on Mueller-Hinton blood agar (Condalab, Madrid, Spain) plates then incubated at 37 °C for 24 h in an aerobic environment to count bacterial colonies in CFU/mL.[49] The total CFU/mL was calculated from the number of colonies along with the dilution factor using this formula:[50]

$$\mathrm{CFU}\Big/\mathrm{mL}=\frac{\text{No.of bacterial colonies}\times\text{dilution factor}}{\text{volume of culture transferred to plate}}$$

Eventually, colonies were counted by using a magnifying lens and were performed by two observers. The presence of *E. faecalis* biofilm and dentinal tubules penetration was confirmed by scanning electron microscopy (SEM) and light microscopy.

Gram staining technique

Gram staining was performed on root sample from each group. The staining procedure was as follows: 4-5 h of demineralization in 40% hydrochloric acid. Following that, the samples were hydrated with increasing concentrations of ethanol. Following that, xylene was used to clean the area to enable the penetration of the paraffin wax. The samples were positioned such that the root segment's cross-section was parallel to the floor and then immersed in paraffin wax. Three μm histological slices were produced using a microtome. To stain the slides, a Gram stain kit (Atom Scientific, Cheshire, United Kingdom) was used, and the manufacturer's slide preparation instructions were followed. The slides were inspected at a magnification of 100× with a binocular microscope (Olympus CX-22, Japan) to trace the region of blue dots that filled and adhered to

the lumen of the dentinal tubules, indicating the presence of Gram-positive bacteria (*E. Faecalis*).[51] (Figs. 1 and 2).

Scanning electron microscopy (SEM) technique

A root sample from each group was assigned for scanning electron microscopy (SEM). A longitudinal groove was cut along the entire length of the root from the buccal and lingual side, and then they were split into two halves with a sterile bone cutter. The pressure was applied manually on the grooved tooth until the sample split. This procedure was better than using a disc where debris accumulates in the cut dentin surface that plugs the opening of dentinal tubules, preventing penetration by the microorganisms.[52] The samples were soaked in 100% ethanol for 10 min. The segments were sputter-coated with gold/platinum for 180 s in a coating machine (Emithech K550X, Kent, United Kingdom). Then the sample segments were scanned with SEM (CamScan 3200 LV, Waterbeach, United Kingdom).[51] (Figs. 3–6).

Statistical analysis

The SPSS computer software version 25.0 (SPSS Inc., Chicago, IL, USA) was used to analyze data. All the data were submitted to the analysis of distribution using the Shapiro-Wilk test. The reported data are presented as means and standard deviations. The CFU/mL mean values were log-transformed (base 10) for the statistical analysis. The values of CFU/mL from all test groups were analyzed with the non-parametric Kruskal and Wallis test to demonstrate statistical differences between the groups. Probability values of $P \leq 0.05$ were set as the reference for statistically significant results.

Result

For the control groups

The negative control samples were to ensure that no contamination occurred throughout the study period. No colonies were observed on agar plates in the negative control group for the paper point and Peeso bur sampling methods (Figs. 1 and 3). Meanwhile, bacterial growth was observed in both sampling methods from the positive control group for paper point and the Peeso bur sampling method. Also, both gram staining technique and scanning electron microscopy showed the presence of bacterial cells. This result was strong proof of *E. Faecalis* biofilm presence (Figs. 1 and 4) (Tables 1 and 2).

For paper point (intracanal) sampling method

Group four, disinfected with 2% CHX, showed the least means of CFU/mL, while group three was disinfected with Aravi extract and showed the highest mean of CFU/mL among all the five experimental groups (Table 1). Statistically, there was a non-significant difference between all experimental groups ($P = 0.088$). However, 2% CHX gel and diode laser was more effective than both plant extracts. But Suran demonstrated superior antibacterial effectiveness than CH and Aravi (Table 1) (Figs. 2 and 5).

For peeso bur (intradentinal) sampling method

In this method, the colony-forming units counted for those dentinal shavings (debris) obtained by Peeso bur. The sequence of the mean of CFU/mL of all experimental groups was 2% CHX gel < Diode laser < Suran < CH < Aravi. Statistically, there was a non-significant difference between all experimental groups ($P = 0.062$). These data presented in Table 2. However, the 2% CHX gel and diode laser was more effective than Suran and Aravi in reducing bacterial count (CFU), but Suran was more effective than CH and Aravi (Figs. 2 and 6).

Bacterial survival rate

The bacterial survival rate in the intracanal sampling method, group four (2% CHX) showed the lowest survival rate. Meanwhile, group two (Aravi) and group three (CH) exhibited a highest survival rate (Fig. 7). While in the intradentinal sampling method the sequence of the survival rate lowest to highest was as following: 2% CHX < diode laser < Suran < Aravi < CH (Fig. 8). These findings illustrate that the bacterial survival rate was higher in the intradentinal sampling method compared to the intracanal sampling method in all five experimental groups.

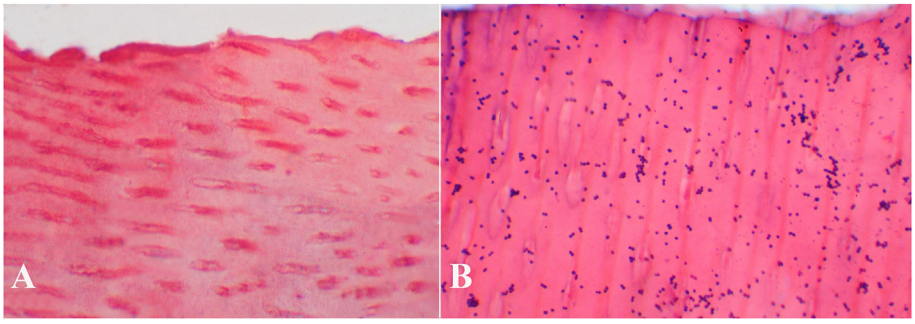

Figure 1 Light microscopic images of Gram-stained sections for control groups. (A) Negative control: No *E. faecalis* cells; (B) Positive control: *E. faecalis* cells are present as a dark blue dot.

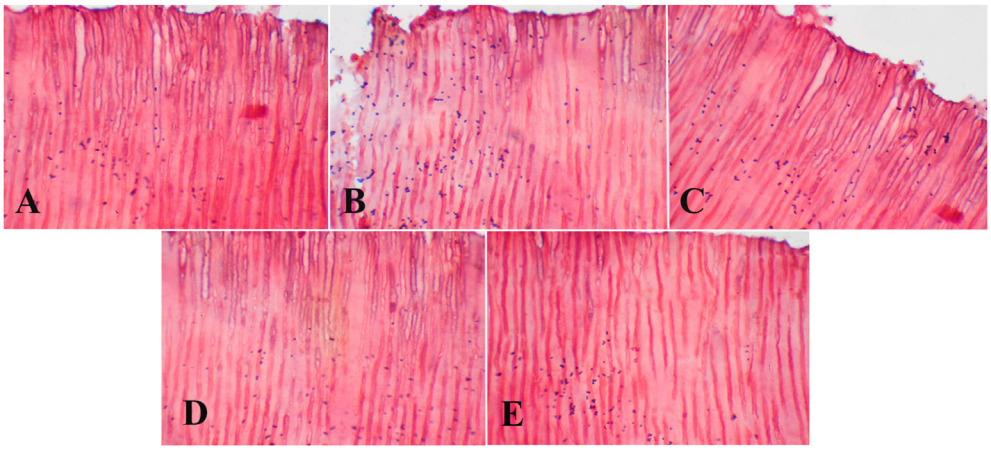

Figure 2 Light microscopic images of Gram-stained sections for experimental groups and the presence of *E. faecalis* as a dark blue dot. (A) Suran; (B) Aravi; (C) Calcium hydroxide; (D) 2% Chlorhexidine; (E) Diode laser.

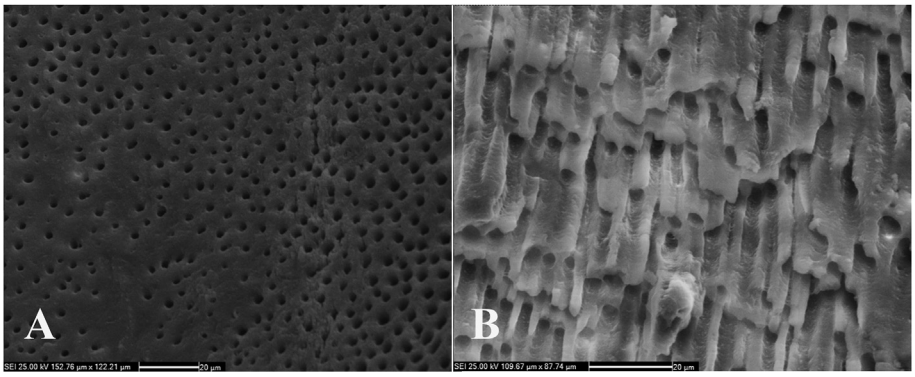

Figure 3 Scanning electron microscope images of the negative control. (A) Root canal wall dentine surface in the paper point (intracanal) sampling method; (B) Root canal dentinal tubules in the peeso bur (intradentinal) sampling method.

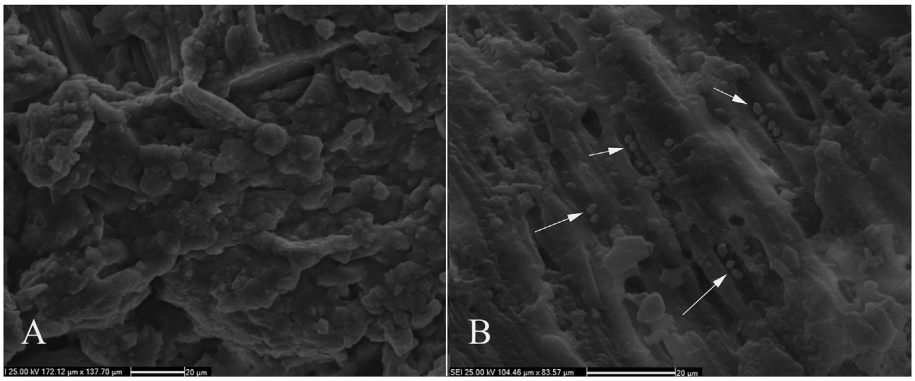

Figure 4 Scanning electron microscope images of the positive control. (A) Mature *E. faecalis* biofilm at 21 days; (B) Deep penetration of *E. faecalis* (white arrows) into dentinal tubules.

Discussion

This study aimed to compare the efficacy of crude extracts of Suran and Aravi as intracanal medicaments and calcium hydroxide paste, 2% CHX gel and 940 nm diode laser in root canal disinfection. Nonetheless, only a few actions are necessary to treat endodontic infections in clinical situations. Additionally, the antimicrobial control step incorporates standardized irrigants and mechanical instrumentation.

The test species, *E. faecalis*, was chosen due to its shown microbial role in chronic root canal infection. Also, permanent human teeth were utilized as samples to replicate the clinical setting. A methodology was assessed for its

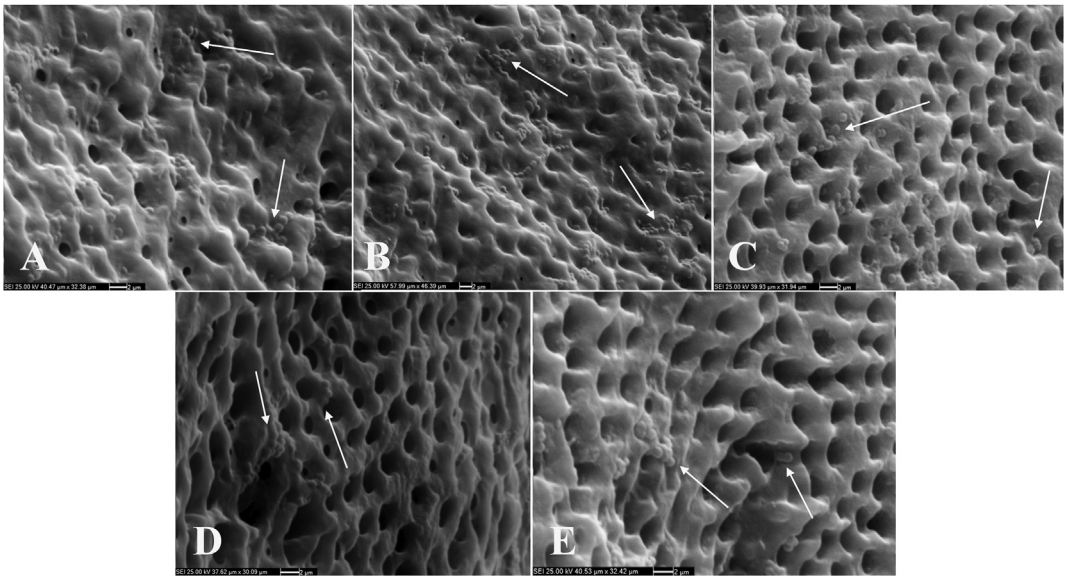

Figure 5 Scanning electron microscope images of the experimental groups in the paper point (Intracanal) sampling method, (white arrow) shows *E. faecalis* cells. (A) Suran; (B) Aravi; (C) Calcium hydroxide; (D) 2% Chlorhexidine; (E) Diode laser.

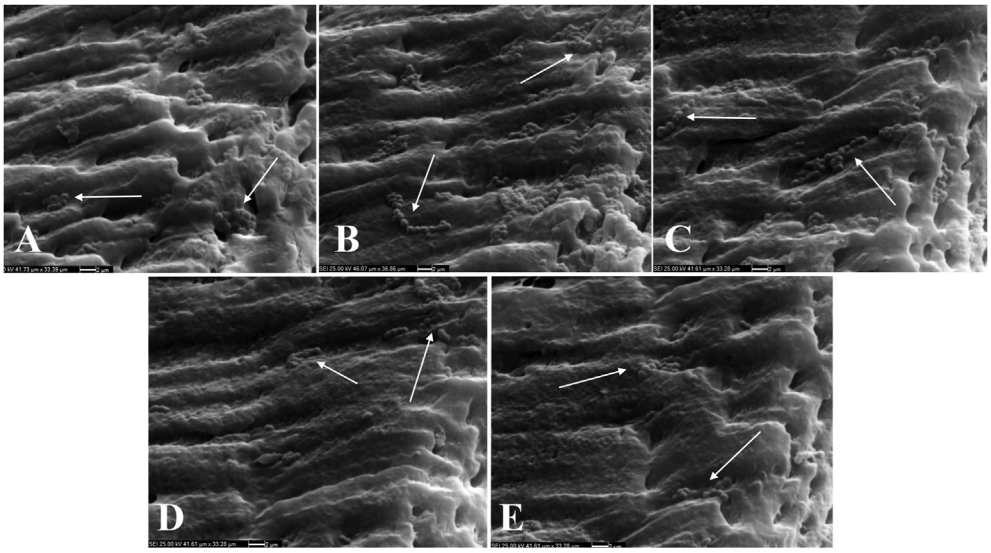

Figure 6 Scanning electron microscope images of the experimental groups in the peeso bur (Intradentinal) sampling method, (white arrow) shows *E. faecalis* cells. (A) Suran; (B) Aravi; (C) Calcium hydroxide; (D) 2% Chlorhexidine; (E) Diode laser.

efficiency in eliminating biofilms developed dentin samples.[53] However, the bacterial colonization structure on dentine, which includes dentinal tubules and a significant quantity of unmineralized type I collagen that acts as an adhesion substrate for oral streptococci, is a more reasonable explanation.[54,55] Antimicrobial susceptibility testing requires strict quality control due to many factors influencing the results. The primary control is provided by a challenging reference strain of *E. faecalis* (ATCC 29212). The optimal control strains have susceptibility endpoints in the middle of the antimicrobial concentration ranges investigated and have little susceptibility pattern changes over time.[56] This explains the choice of this specific microbial strain in this study.

Single straight root canals were used in this study to compare the bactericidal impact of various agitation procedures under normal conditions. However, many teeth in clinical practice have curved root canals. In this case, the bactericidal effect of laser systems may be compromised if the optical fiber cannot be moved readily via the canals.

The contamination period of samples was 21 days, and the microbial broth was renewed every three days. Haapasalo and Ørstavik reported that after three weeks of incubating the root sample with *E. faecalis*, a dense infection from the canal side reached 300—400 μm.[57] In earlier investigations, bacteria were collected using sterile paper points from the root canal system.[58,59] This sampling method has limitations because paper points may identify

Table 1 Mean (±SD) of the of log^{10} (CFU/mL) values of all experimental groups with significant levels between groups in paper point (Intracanal) sampling method.

	Groups	*N*	Mean log^{10} (±SD)	*P*-value
Control groups	Negative control	4	0	
	Positive control	4	5.20 (4.47)	
Experimental groups	G1-Suran	10	2.97 (3.17)	**0.088**
	G2-Aravi	10	3.08 (3.17)	
	G3-Calcium hydroxide	10	3.04 (3.13)	
	G4-2% CHX gel	10	0	
	G5-Diode laser	10	2.54 (2.79)	

n; number of samples, SD; standard deviation, CFU; colony forming unit, mL; milliliter, CHX; Chlorhexidine, log^{10}; logarithm with the base = 10, *P*-value; significant level ($P < 0.05$).

Table 2 Mean (±SD) of the of log^{10} (CFU/mL) values of all experimental groups with significant levels between groups in peeso bur (Intradentinal) sampling method.

	Groups	*n*	Mean log^{10} (±SD)	*P*-value
Control groups	Negative control	4	0	
	Positive control	4	5.32 (4.76)	
Experimental groups	G1-Suran	10	3.49 (3.63)	**0.062**
	G2-Aravi	10	3.85 (3.77)	
	G3-Calcium hydroxide	10	3.60 (3.54)	
	G4-2% CHX gel	10	2.72 (3.05)	
	G5-Diode laser	10	3.20 (3.44)	

n; number of samples, SD; standard deviation, CFU; colony forming unit, mL; milliliter, CHX; Chlorhexidine, log^{10}; logarithm with the base = 10, *P*-value; significant level ($P < 0.05$).

only planktonic microorganisms. Paper points cannot access irregularities and other root canal system locations. As a result, this method may be ineffective at harvesting live bacteria from the biofilm and some parts of the root canal system.[60] Thus, in the current study, the canal wall was instrumented using a sterile #3 Peeso bur to remove dentine, allowing for a more predictable sample. Additionally, specific *E. Faecalis* cells in biofilms might reach a stationary phase, rendering them undetectable using standard culture techniques.[61] As a result, statistics derived from CFU counts should be evaluated cautiously.[62] For standardization purposes, all samples were assessed after one week of incubation.

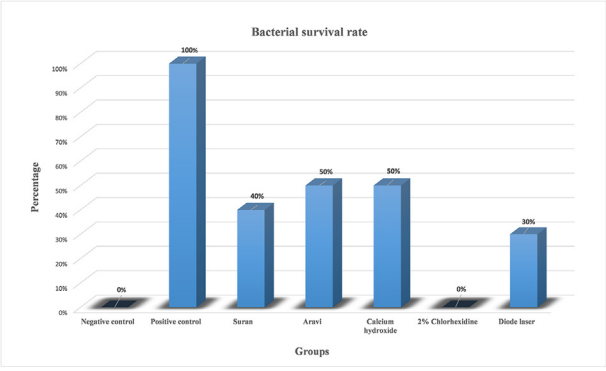

Figure 7 Shows the bacterial survival rate in the paper point (Intracanal) sampling method for both the control and experimental groups.

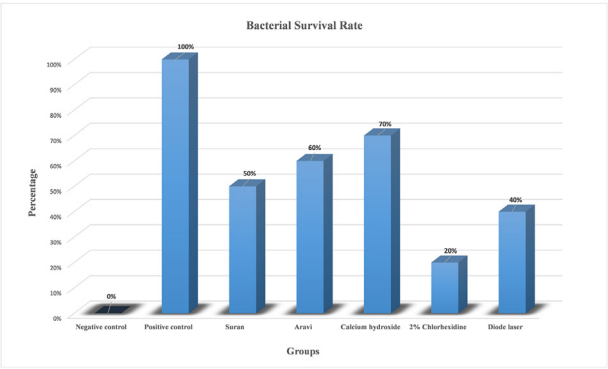

Figure 8 Shows the bacterial survival rate in the peeso bur (Intradentinal) sampling method for both the control and experimental groups.

The current study used two herbal extracts (Suran and Aravi) as intracanal medicaments. There were no previous data and studies on the application of these two plants in dentistry in general and in the endodontic field, mainly which has been no previous report regarding its antibacterial efficacy against *E. faecalis* in the root canals, so for comparison of our results, most common intracanal medicaments used as (gold Standard) which are CH. it has been demonstrated to be an efficient intracanal medicament[10] and is the dressing of choice for treating infected root canals. Regrettably, a new issue has emerged over CH's low antibacterial efficacy against some microbes often seen in infected root canals, particularly *E. faecalis*, which can tolerate its high pH.[63,64] CH did not efficiently remove *E.*

faecalis from infected root canals.[49] This has been linked to the insufficient number of hydroxyl ions achieved after one week[65,66] or dentin's buffering activity.[7] These findings support our study since in our research, CH disinfection of infected root canal and dentinal tubules was likewise inferior to that of other medications except was higher than Aravi only, also showed highest bacterial survival rate among other tested medicaments in both sampling method. As a result, another antimicrobial agent with equivalent capabilities to the tested calcium hydroxide paste but without the associated adverse side effects is required. So, in the present study, two herbal (Suran and Aravi) extracts were used as intracanal medicaments and compared their antibacterial efficacies with CH, 2% CHX gel and, 940 nm diode laser.

Chlorhexidine gluconate gel is an alternative root canal medicament due to its broad-spectrum antimicrobial activity. The antibacterial impact of 2% CHX gel in the root canal has been observed after seven days of chlorhexidine treatment of root dentine.[67] In our study, 2% CHX gel showed the greatest microbial reduction after seven days for both sample techniques, also depicts lowest bacterial survival rate. Since it is a positively charged hydrophobic and lipophilic molecule, it interacts with negatively charged phospholipids and lipopolysaccharides on the microorganism's cell membrane and enters the cell via an active or passive transport mechanism, altering the osmotic balance of the cells. This increases the permeability of the cell wall, allowing the CHX molecule to enter the microorganism, followed by the leaking of internal contents, especially phosphate entities such as adenosine triphosphate and nucleic acids. It binds to hydroxyapatite and soft tissues, altering their electrical field to compete with microbial adhesion and so reducing microbial adhesion.[68] The result of the present study is supported by previous research that has demonstrated that 2% chlorhexidine gel has a greater antibacterial effect than Calcium hydroxide.[68,69] These findings explain the significantly lowest bacterial colony count (CFU/mL) of *E. faecalis* in both sampling methods for 2% CHX gel and lowest survival rate of *E. faecalis* cells.

Previous studies have shown the antibacterial effect of Suran and Aravi extract, attributed to its saponins, flavonoids, tannins, alkaloids, phenolics, and triterpenoids.[70] Saponins' antibacterial activity is ascribed to their interfering with the permeability of the bacterial cell wall.[71,72] Meanwhile, flavonoid compounds form complexes with proteins, disrupting the protein's tertiary structure and resulting in the denaturation of proteins and nucleic acids. This denaturation results in protein coagulation and impairs bacteria's metabolic and physiological processes.[73] Additionally, flavonoids prevent cell membrane production and have a cumulative effect on all bacterial cells.[74]

Tannin compounds prevent protein synthesis required for cell wall construction and cause cell walls to contract, impairing cell permeability and ultimately resulting in cell death.[75,76] The antibacterial activity of alkaloids is owing to their interference with peptidoglycan components in bacterial cells, resulting in the breakdown of the development of complete cell wall layers and, ultimately, cell death.[77] Terpenoid chemicals disrupt the outer cell membrane, allowing substances to enter that reduce the permeability of the bacterial cell wall, depriving the bacterial cell of nutrition and inhibiting growth.[78] These findings explained the low CFU means of the Suran group in both sampling methods in comparison to the Aravi group, this might be due to the low level of phytochemicals mentioned previously in this statement that are responsible for antibacterial properties of the plant extracts, even though, there was a non-significant difference between them (Tables 1 and 2). Earlier investigations conducted by Awawadeh et al. support the results of Suran; meanwhile, it is against the results obtained by Aravi.[79] These results obtained from both Suran and Aravi groups might be due to the properties of dentine that buffer the antibacterial effectiveness of the medicaments,[79] i.e., Suran is less affected by the dentine, while Aravi more affected. *E. faecalis* is also reported to create organic compounds and release them intercellularly during starvation; this may play a part in deactivating the antibacterial action of the medication.[80] Moreover, removing the smear layer enables the *E. faecalis* to penetrate the dentinal tubules deeply.[81]

Many diode lasers are effective against germs, but research on the antimicrobial activity of lasers in root canal systems remains debatable. Our findings indicated a significant decrease in CFU in the laser group due to the direct supply of laser energy via the thin diameter optic cable (200–320 μm) utilized, which allowed for efficient laser light delivery deep into the root canal. The bacteria absorbed the laser energy, which resulted in a photothermal interaction, which had a bactericidal impact.[82] Because the diode laser has high permeability and minimal interaction with dentin, it is efficient against germs that have reached the dentinal tubules.[83] Coluzzi observed that diode lasers had a 10,000-fold higher depth of penetration per pulse than Er: YAG lasers and hence may act more deeply into the dentinal tubules.[84] According to Gutknecht et al., Irradiation with a 940-nm diode laser may eliminate germs that have migrated deep into the dentine (up to 500 μm), whereas chemical treatments can only reach 100 μm.[85] These findings explain the significantly low bacterial colony count (CFU/mL) of *E. faecalis* in both sampling methods for the diode laser group. Statistically, there was no significant difference between Suran, Aravi crude extracts as intracanal medicaments and diode laser in Intraluminal and intradentinal sampling methods in reducing bacterial counts (CFU/ml); this was supported by previous research performed by Zohra et al. in their findings showed non-significant difference between diode laser and calcium hydroxide in a mean of CFU/mL count.[86]

The antimicrobial evaluation was only done once, which was one of the study's limitations. Various intracanal medications may take varying lengths of time to show their best possible results. Therefore, more research is needed to determine how time affects the efficacy of Suran and Aravi in disinfecting the dentinal tubules. Root canals were exclusively infected with *E. Faecalis* for this investigation; however, clinical endodontic infections typically contain many species of bacteria. As a result, more research into mixed infections is required.

Within the constraints of this experimental study, it can be stated that the crude extracts of *A. paeoniifolius* and *C. esculenta* as intracanal medicaments exhibit remarkable

antibacterial efficiency against the *E. faecalis* biofilm in comparison to CH, 2% CHX gel and 940 nm diode laser. It is advised that these medicaments be evaluated for their toxicity, as well as the effects on the dentine's mechanical and chemical characteristics, before they may be approved for clinical use.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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