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RESEARCH ARTICLE

IN VIVO STUDY EVALUATES EFFECTIVENESS OF ADDING CIPROFLOXACIN TO CALCIUM HYDROXIDE VERSUS *SAUSSUREA COSTUS* TO CALCIUM HYDROXIDE MEDICATION AGAINST BACTERIAL BIOFILM IN ROOT CANAL TREATMENT

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Abstract

Background and objectives: Elimination of root canal bacteria is a perquisite for a successful root canal treatment. This research aims to determine the effectiveness of adding ciprofloxacin versus *Saussurea costus* extract to calcium hydroxide medicament against bacterial biofilm in root canal treatment *in vivo* study.
Materials and methods: *In vivo* study on 70 single-rooted human teeth at Sana'a University dental clinics used *Saussurea costus* extract preparation, including washing, drying, crushing, mixing, centrifugation, drying, and homogenization.The intracanal drug concentration was standardized by mixing 1 g of calcium hydroxide powder with 1 ml of ciprofloxacin or 1 ml of *Saussurea costus* extract in both groups. Three bacteriological samples were taken before root canal treatment, before Medicaments were placed, and 7 days after, and CFU and bacterial isolation were performed using standard methods.
Results: An intragroup analysis showed that all drug groups had a significant decrease in the number of intracanal bacterial cells from S1 to S2 and from S2 to S3 (p<0.0001). When comparing quantitative S1 or S2 data, there was no significant difference between the groups; however, at Sample 3, the *Saussurea costus* extract calcium hydroxide (34.05±21.8) had considerably higher bacterial counts than the calcium hydroxide + Ciprofloxacin group (11.9±29 CFU). The most isolated bacteria in S1 and S2 were *Staphylococcus aureus* (58.6%, 51.4%), followed by *Klebsiella* species (27.1%, 18.6%), *Escherichia coli* (25.7%, 17.1%), *Enterococcus faecalis* (22.9%, 15.7%) and *Pseudomonas aeruginosa* (21.4%, 12.9%).
Conclusions: The addition of Ciprofloxacin to calcium hydroxide and *Saussurea costus*- calcium hydroxide provided further antibacterial effectiveness when used as an intracanal medicament *in vivo* during root canal treatment.
Keywords: Bacteria, biofilm, calcium hydroxide, ciprofloxacin, root canal treatment, *Saussurea costus*.

INTRODUCTION

Since apical periodontitis is mostly caused by bacterial infection, minimizing or getting rid of bacteria is essential to maximizing treatment results[1,2]. The bacterial burden in the root canal system has been demonstrated to be significantly decreased by mechanical instrumentation combined with chemical disinfection[3]. Nonetheless, chemomechanical instrumentation is not sufficient to eradicate all root canal bacteria, according to multiple studies[4-7]. Intracanal medicine has been suggested as a means of achieving this goal, as it facilitates a notable increase in bacterial clearance during chemo-mechanical preparation[8,9]. Due to its antibacterial and biologic properties, calcium hydroxide is the most often utilized intracanal medication[10,11]. Nevertheless, calcium hydroxide has a limited antibacterial impact, especially in situations of persistent infections[12].

In their evaluation of the effectiveness of calcium hydroxide nanoparticles in eliminating *Enterococcus faecalis* in human root dentine, Louwakul *et al.*[13], found that calcium hydroxide was not able to destroy the bacteria. Comparably, Javidi *et al.*[14], assessed calcium hydroxide's antibacterial efficiency in terms of killing *E. faecalis* in root canals, both with and without

a silver nanoparticle suspension. They found that calcium hydroxide was less effective than calcium hydroxide mixed with other antibacterial agents. Nonetheless, prior research assessing the antibacterial efficacy of calcium hydroxide as an intracanal medication has yielded inconsistent results. While some research[1,4] observed further bacterial clearance following intracanal treatment with calcium hydroxide, other studies[15,16] reported an increase in the percentage of positive cultures and bacterial counts. It has been previously established that the presence of bacteria at the time of root filling has a substantial impact on the root canal treatment's success[16-18].

Medicinal plant extracts have been utilized extensively around the world for therapeutic purposes, including the treatment of numerous microbial illnesses[19]. For a variety of reasons, including their ease of availability, low cost, and lack of side effects, plants are used as a natural source of medications and are seen as an alternative to modern treatment, particularly in developing nations[20,21]. These traditional medicinal herbs can be investigated for their antimicrobial therapeutic potential in order to find and develop new antimicrobial medicines. *Saussurea costus*, sometimes known as *S. costus*, is one of the more significant medicinal herbs that have been utilized from ancient times. In addition to being used in traditional Chinese and Indian medicine, S. costus is well-known in Islamic medicine[22]. Some active biochemical components of *S. costus*, which have various physiological effects on the body, are what give it its medicinal usefulness. Flavonoids, alkaloids, sesquiterpenes, phenolic compounds, tannins, sugars, and glycosides are the primary biochemically active substances[23]. Many studies have documented the therapeutic benefits of *S. costus* root, including its ability to heal wounds, stimulate the immune system, be anti-inflammatory, choleretic, hepatoprotective, antiulcerogenic, larvicidal, gastro-protective, cytotoxic, cardiotonic, and anticancer properties[24].

Ciprofloxacin, a second generation fluoroquinolone, has been used successfully as an intracanal drug for root canal treatment. It is effective against enteric bacteria, including *E. coli* and *Enterococcus* species with strong tubular penetration[25-29].The purpose of this study is to compare the effectiveness of *S. costus* extract versus ciprofloxacin in addition to calcium hydroxide medication against bacterial biofilm in root canal treatment in an *in vivo* setting.

MATERIALS AND METHODS

S. costus extract preparation

Botanist details of the plant's roots: The kingdom is *Plantae*, the *Clade* is *Tracheophytes*, the order is *Asteraceae*, the genus is *Dolomiaea*, and the species is *D. costus*. The plant's roots was brought from China by a documented *S. costus* dealer, as commercial records for this plant date back to the year 2024 AD, Junuary first. The roots of the plant were thoroughly dried after being thoroughly cleaned three times with tap water. The root was first reduced to tiny bits and then to a fine powder using an electronic grinder. After combining 25 g of powdered dried roots with 250 ml of solvent (water) and letting it sit for 24 hours, the solutions were filtered through gauze, centrifuged for 15 minutes at 3000 rpm, and then allowed to dry for 48 hours at 45 °C in a hot air oven. A sterile container is used to store the dried extract. The crude was then ready for use after the dried plant combination was steeped in 70% ethanol and homogenized using magnetic stirrers.

The preparation of the calcium hydroxide mixture:

In order to create a uniform combination (ratio of 1:1) with 1 ml of either Ciprofloxacin in group I or *S. costus* extract in group II, the concentration of intracanal medications was standardized across all groups using 1 g of calcium hydroxide powder.

Ethical Consideration: The Medical Ethics and Research Committee of Sana'a University's Faculty of Dentistry granted consent for this study, which took place between January 2024 and May 2024 (approval number: 77; dated January 1, 2024). The review committee's set of ethical guidelines was regularly adhered to.

Patient selection

Patients in need of endodontic treatment who attended Sana'a University clinics were chosen. Every patient gave their informed consent to take part. Following that, a thorough medical and dental history was taken, and each patient underwent a clinical and radiographic examination.

Inclusion criteria:

- The age of the patients was within a range of 19 to 65 years.
- All selected teeth are single root teeth.
- The patient is clinical and X-ray diagnostic with necrotic pulp and asymptomatic apical periodontitis around 2-4 mm.
- All selected teeth for making the root canal have enough crown structure for adequate isolation with a rubber dam.

Exclusion criteria:

- Patients who received antibiotic treatment during the last 3 months.
- Patients had or have a systemic disease.
- Pregnant females.
- The relevant tooth shows periodontal pockets deeper than 4 mm during clinical examination.
- Apical radiolucency is smaller than 2 mm or bigger than 4 mm in preapical digital x-ray.
- Non-restorable teeth.
- No previous endodontic treatment.
- Incomplete root formation or root resorption, perforation, or calcification.

Root canal treatment and microbiological sampling procedures:

The study's patients' teeth were sized and polished prior to receiving treatment[30]. The patients were instructed to rinse their mouths with chlorhexidine digluconate (a chemical manufactured by the CHX DEXA firm). Before beginning the endodontic process, each tooth was topically anesthetized with 20% benzocaine gel (Prime-Dent). This was followed by a local anesthetic injection with 0.9 ml of a solution comprising 2%

Lidocaine HCL and 1:100000 epinephrine (Lignospan standard). Aseptic approach and a rubber dam were used to clean the operative field, which included the tooth, during the endodontic process. The area was then disinfected with 30% hydrogen peroxide (H_2O_2) and 2.5% sodium hypochlorite. Sterile spherical burs were used to remove the decayed coronal structures (Mani, Inc.). The crown surface and the surrounding structures (the clamp and dam) were cleaned for thirty seconds, as previously mentioned. After that, the impact of the NaOCl was neutralized using 5% sodium thiosulfate. Sterile high-speed round and fissure bursts were used to create the access cavity in an aseptic environment. A tapered low-speed stone (Mani, Inc.) was used to smooth the access cavity in an aseptic setting. As previously mentioned, the disinfection treatment was carried out once more following the completion of the access cavity preparation. As stated by Karataş *et al*.[31], sterile Teflon tape and cotton pellets were employed to seal the pulp chamber's floor and roof in order to stop disinfectants from penetrating the coronal and radicular pulp spaces. Following the inactivation of the NaOCl effect using sodium thiosulfate, sterility control samples (SR) were collected from the coronal surface of the tooth, rubber dam, and clamp using a sterile swab with an ejectable tip (Sample 1). The swab was inserted into 1 milliliter of Brain Heart Infusion Broth (BHI Broth) and immediately inoculated into Brain Heart Infusion agar for the purpose of isolating and counting bacteria. It was then cultured for 48 hours at 37°C, after which the colonies were counted and the bacteria were identified. Next, using Fanta (AF) nickel titanium rotary files (Fanta) and an E-connect endo motor (Eighteeth Medical) in rotation mode with a setting of 325 rpm and 1.8 N cm torque as advised by the manufacturer, the root canal system was prepared till AF4 35/04. Hand files #40–60 (Micromega) were used for additional shaping in accordance with the architecture of each canal until clean, white dentine chips were clearly visible at the apical 3 mm of the master file. After every file use, 2 ml of sterile saline was irrigated using side-vented needles of size #30 G, positioned 1 mm below the WL. following the instrumentation of the canal up to the master apical file (MAF). Using an Irrisafe ultrasonic tip, irrigation was carried out using sterile saline solution with the assistance of passive ultrasonic irrigation (PUI). To activate the irrigant in each canal, the Varios 370 (woodpecker device) ultrasonic device's tip was inserted 1 mm from the working length at medium power. A 2–3 mm apical–coronal movement was used to produce the activation (3 cycles×20 s= 1 min). For a total volume of 3 ml, 1 ml of the irrigating solution was added to the root canal device in each cycle. In order to get the second microbiological sample (Sample 2), the canal content was collected, dried using three sterile absorbent paper points that matched the MAF size, and aspirated. The tested intracanal medications were then inserted into the appropriate root canals, stopping about 1 mm short of the WL. To administer the intracanal medications, Dentsply Maillefer's lentulo- spiral was utilized. inside groups I, II, and III of the canals. Using a Dentsply Maillefer plugger, the medications were packed up to the level of the opening. Following that, sterile Teflon tape was used to cover the canal orifices. The second allocator sealed the access cavity using sterile cotton and glass ionomer fillin (Medifil, Promedica Dental Material GmbH).

After seven days, patients were summoned back for another appointment. Leak detection was performed on the interim restoration during the second visit. No single case of teeth with leaky or damaged restorations was included in the study. Every tooth was isolated using a rubber dam, and the surgical field was cleaned as previously indicated. After removing the temporary restoration, the pulp chamber was thoroughly cleansed and saline flushed once more. With careful strokes, the intracanal medication was extracted from the canals using Headstorm hand files. Using syringes with 30-gauge needles, a circumferential filing was performed with sterile saline (10 ml) until the irrigant exited the canal devoid of any leftover medication[32]. The third postmedication sample (Sample 3) was obtained following the removal of the intracanal medication using three sterile paper points, and it was inculcated into one milliliter of peptone water as previously mentioned. Using the cold lateral condensation technique, gutta percha cones (Meta Biomed) and AH Plus sealer (Dentsply/Maillefer) were used to obturate the canals after they had been dried with sterile paper points. A composite restoration was placed on the tooth right away, and a follow-up radiograph was obtained to assess the level of obscuration.

RESULTS

Table 1 shows the sex and age distribution of patients included in the study at the Sana'a University dental clinics in Sana'a city. The male patients counted 40%, and the female patients were 60%. The mean age of our patients was 31.9 years, with SD equal to 11.3 years, and ages ranged from 14 to 58 years. Most of our patients were in the age group of 25-34 years (47.1%), followed by the group of 15-24 years (20%), while other age groups were less frequent.

Table 1: Sex and age distribution of patients included in root canal treatment.

Characters	N (%)
Sex	
Male	28 (40)
Female	42 (60)
Age groups (years)	
15 - 24	14 (20)
25 – 34	33 (47.1)
35- 44	11 (15.7)
≥ 45	12 (17.1)
Total	70 (100)
Mean age	31.9 years
SD	11.3 years
Median	30 years
Mode	25 years
Min – Max	14 - 58 years

Table 2: Type of materials used for reduction of endodontic microbiota for patients included in root canal treatment study.

Materials	N (%)
Saussurea costus	26 (37.2)
Calcium Hydroxide + Ciprofloxacin	22 (31.4)
Calcium Hydroxide + *Saussurea costus*	22 (31.4)
Total	70 (100)

Table 2 shows the type of mixed materials used to reduce endodontic microorganisms for patients included in the root canal treatment study. The study evaluated *S. costus* alone, calcium hydroxide-ciprofloxacin, and calcium hydroxide- *S. costus* in reducing endodontic microorganisms in 70 Yemeni patients. In 26 patients, *S. costus* was applied, calcium hydroxide-ciprofloxacin was applied to 22 patients, and calcium hydroxide was applied to 22 patients. Table 3 shows the types of bacterial isolates before performing root canal drilling from the infected tooth and surrounding tissues (sample 1).

Table 3: Types of bacterial isolates before performing root canal drilling of the infected tooth (sample 1).

Bacteria	N (%)
E. coli	18 (25.7)
Klebsiella species	19 (27.1)
P. aeruginosa	15 (21.4)
S. aureus	41 (58.6)
E. faecalis	16 (22.9)
Mixed growth	23 (32.9)
Total isolates	104 (100)

The most isolated bacteria was *Staphylococcus aureus* (*S. aureus*) (58.6%), followed by *Klebsiella* species (27.1%), *Escherichia coli* (*E. coli*) (25.7%), *Enterococcus faecalis* (*E. faecalis*) (22.9%), and *Pseudomonas aeruginosa* (*P. aeruginosa*) (21.4%). Mixed growth was observed in 23 samples (32.9%). Table 4 shows the types of bacterial isolates after performing root canal drilling before irrigation with antibacterial agents (sample 2). The most isolated bacteria was *S. aureus* (51.4%), followed by *Klebsiella* species (18.6%), *E. coli* (17.1%), *E. faecalis* (15.7%), and *P. aeruginosa* (12.9%). Mixed growth was observed in 13 samples (18.6%).

Table 5 shows the types of bacterial isolates after performing root canal and after irrigation with antibacterial agents (sample 3). The most isolated bacteria was *S. aureus* (11.4%), followed by *Klebsiella* species (7.1%), *E. coli* (11.4%), *E. faecalis* (4.3%), and *P. aeruginosa* (8.6%). Mixed growth was not observed, and 40% showed complete sterilization for aerobic bacteria.

Table 4: Types of bacterial isolates after performing root canal drilling before irrigation with antibacterial agents (sample 2).

Bacteria	N (%)
E. coli	12 (17.1)
Klebsiella species	13 (18.6)
P. aeruginosa	9 (12.9)
S. aureus	36 (51.4)
E. faecalis	11 (15.7)
Mixed growth	13 (18.6)
Total isolates	94 (100)

Table 5: Types of bacterial isolates after performing root canal and after intracanal medication irrigation with antibacterial agents (sample 3).

Bacteria growth	N (%)
E. coli	8 (11.4)
Klebsiella species	5 (7.1)
P. aeruginosa	6 (8.6)
S. aureus	8 (11.4)
E. faecalis	3 (4.3)
Mixed growth	0 (0.0)
No growth	40 (57.1)
Total	70 (100)

Table 6 shows the comparison between the frequency of bacterial isolates from the root canal after applying antibacterial mixtures to the canal. For *E. coli*, there was a non-significant difference equal to 5.7% reduction after applying the antibacterial mixture ($p=0.33$). Considering *Klebsiella* species, there was a significant difference equal to 11.5% reduction after applying the antibacterial mixture (95% CI = 0.23-22.8 and p=0.04). Considering *P. aeruginosa*, there was a non-significant difference equal to 4.3% reduction after applying the antibacterial mixture (95% CI = 6.4- 15.2 and p=0.41). Considering *S. aureus*, there was a significant difference equal to a 40% reduction after applying the antibacterial mixture with a 95% CI of 25-552 and $p<0.0001$.

Table 6: Comparing between frequency of bacterial isolates from root canal after applying antibacterial mixtures.

Bacteria growth	Sample 2 N (%)	Sample 3 N (%)	Difference	95% CI	*p* value
E. coli	12 (17.1)	8 (11.4)	5.7	6.1-17.5	0.33
Klebsiella species	13 (18.6)	5 (7.1)	11.5	0.23-22.8	0.04
P. aeruginosa	9 (12.9)	6 (8.6)	4.3	6.4-15.2	0.41
S. aureus	36 (51.4)	8 (11.4)	40	25-52	<0.0001
E. faecalis	11 (15.7)	3 (4.3)	11.4	1.2-22	0.025
Mixed growth	13 (18.6)	0 (0.0)	18.6	9.5-29.3	0.0002
No growth	0 (0.0)	40 (57.1)	57.1	44.3-68	<0.0001
Total bacteria growth, n=220	81 (36.8)	30 (13.6)	32.2	8.8-36.4	0.0016

Table 7: Comparison of the effect of antibacterial agents in inhibiting bacterial growth and biofilm formation after root canal procedure and after irrigation with antibacterial agents (sample 3).

Bacterial growth	*Saussurea costus* N (%) A	Calcium Hydroxide Ciprofloxacine N (%) B	Calcium Hydroxide + *S. Costus* N (%) C
E. coli	4 (28.4)	1 (4.5)	3 (13.6)
Klebsiella species	3 (11.5)	1 (4.5)	1 (4.5)
P. aeruginosa	0 (0.0)	0 (0.0)	6 (27.3)
S. aureus	0 (0.0)	1 (4.5)	7(31.8)
E. faecalis	2 (7.7)	0 (0.0)	1 (4.5)
No growth	17 (65.4)	19 (86.4)	4 (18.2)
Mixed growth	0 (0.0)	0 (0.0)	0 (0.0)
Total	26 (37.1)	22 (31.4)	22 (31.4)

Table 8: Comparison of the effect of antibacterial agents in inhibiting bacterial growth and biofilm formation after root canal procedure and after irrigation with antibacterial agents (sample 3).

Bacterial growth	Calcium Hydroxide Ciprofloxacine N (%)	Calcium Hydroxide + *S. costus* N (%)	Difference	95% CI	*p* value
E. coli	1 (4.5)	3 (13.6)	9.1	-10.3-29	0.29
Klebsiella species	1 (4.5)	1 (4.5)	0.0	-17-17	1.0
P. aeruginosa	0 (0.0)	6 (27.3)	27.3	6.7-48.1	0.009
S. aureus	1 (4.5)	7(31.8)	27.3	4.1-48.4	0.02
E. faecalis	0 (0.0)	1 (4.5)	4.5	-10-21.7	0.31
No growth	19 (86.4)	4 (18.2)	68	39.7-82.1	<0.0001
Mixed growth	0 (0.0)	0 (0.0)	0	-14.8-14.8	
Total growth	3 (13.6)	18 (81.8)	68.2	39.9-82.2	<0.0001

Considering *E. faecalis*, there was significant difference equal to 11.4% reduction after applying the antibacterial mixture with 95% CI equal to 1.2–22 and p=0.025. Considering mixed growth there was significant difference equal to 18.6% reduction after applying the antibacterial mixture with 95% CI equal to 9.5–29.3 and p=0.0002. Considering no bacteria growth there was significant difference equal to 57.1% reduction after applying the antibacterial mixture with 95% CI equal to 44.3–68 and p<0.0001. Considering Total bacteria growth there was significant difference equal to 32.2% reduction after applying the antibacterial mixture with 95% CI equal to 8.8–36.4 and p=0.0016.

Table 7 shows the comparison of the effect of antibacterial agents in inhibiting bacterial growth and biofilm formation after root canal procedure and after irrigation with antibacterial agents (sample 3). For *E. coli*, the lowest number occurred in mixtures B (Calcium Hydroxide-Ciprofloxacine) and C (1, 4.5%), and with mixture B, a higher negative growth occurred (86%) than other mixtures (A, D). Table 8 shows the comparison of the effect of the 3 tested antibacterial agents in inhibiting bacterial growth and biofilm formation after root canal procedure and after irrigation with antibacterial agents (sample 3). Considering the total absent growth, the highest rate (86.4%) occurred for mixture A (Calcium Hydroxide-Ciprofloxacine), while for mixture B (Calcium Hydroxide- *S. costus)*, the inhibition growth was only 18.2%, with a positive difference growth inhibition equal to 68% and a 95% CI equal to 39.7-82.1 with p=0.0001).

Table 9: The mean, the median, the mode and SD of colony counts and the reductions after mixers used to sterilize the root canals.

Variables		*S. costus* CFU/ml N=26	Ca $(OH)_2$+ Ciprofloxacine N=22	Calcium Hydroxide + *S. costus* N=22
Sample 1	Mean	613.8	294.7	228.9
	Median	571	183	167
	Mode	555	104	
	Range	520 - 789	104 –727	121 – 799
	SD	187.6	186.5	183.9
Sample 2	Mean	598.3	72.5	290.3
	Median	599	65.5	255
	Mode	601	65	235
	Range	530 – 699	35–121	226 – 452
	SD	50.04	26.8	121.4
Sample 3	Mean	11.7	11.9	34.05
	Mode	0	0	0
	Median	0	0	36
	Range	0 -141	0 -84	0 – 84
	SD	27.7	29	21.8

Calcium Hydroxide-Ciprofloxacine showed good effect by killing completely *P. aeruginosa* and *E. faecalis*, while 4.5% growth rates occurred for *E. coli*, *Klebsiella* species, and *S. aureus*. Calcium Hydroxide- *S. Costus* mixture showed lower effect in killing *P. aeruginosa* with a survival rate equal to 27.3%, and for *E. coli* it was 13.6%, while for *E. faecalis* and *Klebsiella* species the survival rate was 4.5% for the mixture and B. Table 9 shows the mean, median, mode, and SD of the number of bacterial colonies and reductions after using mixers to sterilize root canals. The mean±SD for *S. costus* was 598.3±50.04 CFU/ml before application and decreased to 11.7±27.7 CFU/ml after application. The mean±SD of calcium hydroxide-Ciprofloxacine was 72.5±26.8 CFU/ml before application and then decreased to 11.9±29 CFU/ml after application. Mean±SD for calcium hydroxide- *S. costus* was 290.3±121.4 CFU/ml before application and then decreased to 34.05±21.8 CFU/ml after application.

DISCUSSION

The current study assessed the antibacterial activity of calcium hydroxide in conjunction with ciprofloxacin or *S. costus* extract on the bacterial biofilm found in patient teeth root canals. The colony count in the groups receiving *S. costus* extract and calcium hydroxide + ciprofloxacin was inhibited, according to the results. The major study question's alternative hypothesis was verified by colony counting, which demonstrated that the *in-vivo* antibacterial effects of CH paste on bacterial biofilm were enhanced when calcium hydroxide was combined with either ciprofloxacin or *S. costus*. Bacteria can enter the pulp tissue when a carious lesion spreads to the pulp chamber and the hard tissue barrier is broken. Bacteria spread and colonize the entire root canal system in apical periodontitis. Consequently, bacteria within the root canal system need to be removed in order to facilitate the healing of apical periodontitis and cleaning of the root canal system.

Within-group analysis in the present investigation showed a statistically significant decrease in the quantity of intra-canal bacterial cells from S1 to S2 and from S2 to S3 across all tested treatment groups ($p< 0.0001$). Intracanal medicine and chemo-mechanical methods both markedly decreased the amount of comparable bacterial cells from infected root canals. This result is consistent with other research that found that intra-canal medicine and chemo-mechanical treatments significantly decreased the number of germs in the treated area[33,34]. On the other hand, a number of earlier investigations[5,35] noted a rise in bacteria following intracanal treatment. The increase in bacterial counts following intra-canal treatment in earlier investigations may have been caused by bacterial leakage through transient repair or contamination. Another possibility is that bacteria were present in areas of the chemo-mechanical preparation that were not treated, such as the isthmus, ramifications, or dentinal tubules, and that this led to the regrowth of leftover bacteria after intra-canal therapy. The current study's findings indicate that there was no discernible variation in the groups' S1 or S2 bacterial counts. The calcium hydroxide+Ciprofloxacin group (mean=11.7, range from 0-81CFU/ml) had considerably lower bacterial counts in S3 compared to the pure *S. costus* (11.7, 0-141 CFU) and calcium hydroxide + *S. costus* (mean=34.05, range from 0-84) groups. Furthermore, compared to the pure *S. costus* and calcium hydroxide+ *S. costus* groups, the percentage of the decrease in bacterial counts from S1 to S3 and from S2 to S3 was larger in the calcium hydroxide+Ciprofloxacin group.

Notable antibacterial action is shown by the second-generation fluoroquinolone ciprofloxacin, which has good pharmacokinetic qualities and little adverse effects[36]. Quinolones have been found to have antibacterial action and to be well tolerated (low toxicity, low allergenicity, and high intrinsic solubility) making them appropriate for the prophylaxis of infectious problems[37]. With a frequency of 0.4% to 2%[38], immediate hypersensitivity reactions to quinolones are uncommon and can cause urticaria, itching, erythema, skin rash, and shock. Furthermore, ciprofloxacin, which has a sensitivity rate ranging from 90% to 100%, is among the most effective antibiotics in Yemen[39-41].

This study also revealed that the use of pure *S. costus* in intracanal medication, calcium hydroxide + Ciprofloxacin, and calcium hydroxide + *S. costus* in calcium hydroxide + *S. costus* resulted in 17 of 26, 19 of 22, and 4 of 22 root canals being negative for bacteria, respectively (Table 7). On the other hand, no cases of chemo-mechanical preparation were found to be negative for root canal bacteria, indicating that while chemo-mechanical preparation is effective in significantly lowering the amount of intracanal bacteria, it cannot completely eradicate all bacteria from root canals. Therefore, intracanal medication provides additional antibacterial effect in the form of bacteria-free root canals, which is consistent with the findings of earlier studies[5,33].

The intracanal application period in this trial was seven days. Regarding the intracanal administration period of calcium hydroxide or other combinations, there is inconsistent evidence available. In the past, Sj€ogren *et al.*[42], revealed that all it took to achieve bacteria-free root canals was a seven days administration of a calcium hydroxide medication. Similar to this, Shuping *et al.*[8], study showed that 92.5% of root canals with negative cultures were treated after applying calcium hydroxide for one week. On the other hand, data addressing the timing of calcium hydroxide intracanal application indicates that the pH in the outer root dentine rises 1-6 days before peaking, at least 1-3 weeks later[43]. In a similar vein, Estrela *et al.*[44], observed that microbial inactivation required a 60-day application of calcium hydroxide. Nonetheless, it has been noted that applying calcium hydroxide intracanally for at least 30 days reduces the resistance to root fracture[45]. For this reason, calcium hydroxide was applied for seven days in the current investigation.

Following that, sterile Teflon tape was used to cover the canal orifices in the current investigation. To stop

bacteria from entering the root canal, the second allocator sealed the access cavity using sterile cotton and glass ionomer fillin. Cavit is a substance made of zinc oxide and ethylene that could leak tiny amounts. According to Weston *et al*.[46], Cavit only stops bacterial leaks when it is applied with a minimum thickness of 4 mm. In the current investigation, access cavities were sealed with Cavit-G and canal orifices were sealed with a sterile Teflon pellet. Nevertheless, not every teeth's Cavit thickness was standardized or measured. The significant number of negative instances found in S3 for bacteria could indicate that the Cavit shielded root canals against bacterial leaks. It has been shown in the past that Cavit, when used to treat root canals with calcium hydroxide, offers sufficient protection against bacterial leakage for a month[47]. Finally, despite a significant decrease in colony counts across all experimental groups, no intra-canal medication was able to completely remove all germs from each patient's root canal. This fact highlights the value of mechanical instruments that can be used in conjunction with various chemical antibacterial treatments.

According to Table 3, Table 4, and Table 5, the most frequently identified aerobic bacteria from root canals in the current investigation were *S. aureus*, *E. coli*, *Klebsiella* species, *E. faecalis*, and *P. aeruginosa*. It is well recognized that the etiology of endodontic infections cannot be determined just by culturing and isolating aerobic bacteria. Therefore, molecular techniques have been widely employed to characterize bacterial communities in various contexts[48], and their capacity to incorporate as-yet-uncultured species in analysis[49] is one of their major advantages in this respect. Analyses of the endodontic microbiota using community profiling produced some intriguing results: (a). Different endodontic infections have been shown to consist of mixed communities[50,51], including persistent/secondary infections linked to treated teeth[52]. (b). Underrepresented uncultivated bacteria may frequently be found in infected root canals[53,54]. (c). Depending on the clinical situation (chronic apical periodontitis, acute apical abscesses, treated teeth), bacterial communities may follow a particular pattern[51,52]. (d). Endodontic communities linked to the same clinical disease exhibit significant interindividual variation[51], meaning that each individual has a distinct microbiota in terms of species richness and abundance. (e). This interindividual variation is further pronounced when analyzing individuals from different geographic locations. Nonetheless, endodontic infections are still largely caused by cultivated aerobic bacteria.

Since endodontic infections arise in previously sterile areas, their microbiota is abnormal. As a result, any species found in the canal has the potential to cause endodontic infections or at the very least contribute to the ecology of the microbial community. The aerobic bacteria that we recovered for current study also play a part in this process. For obvious ethical considerations, practically all studies to date that have attempted to identify the genotype or phenotype of endodontic bacteria have used a cross-sectional approach. It is only from these investigations that species prevalence and, consequently, species-disease correlation can be deduced. Causality can be reinforced by possible pathogenicity (in animal models or inferred from relationship with other human diseases), in addition to repeat detection. Numerous species have surfaced as candidates or suspected endodontic pathogens based on cross-sectional research; candidate or putative categories should be kept in place until a particular involvement in disease pathogenesis is demonstrated[56].

Limitation of the study

Similar to earlier research, bacteriological samples were gathered for this investigation utilizing a similar methodology[5,6]. In this study, various tooth kinds (incisors and canines) with various root canal morphology were treated. The structure of the root canal may have an impact on bacteria that are sampled with paper points. Since adherent biofilms and inaccessible regions such dentinal tubules, accessory canals, and fins are not included in the microbiological root canal sample method utilizing paper points, it only provides information on the bacteriological conditions of the main root canal space[12]. Nonetheless, the present study's findings indicate that there was no statistically significant variation in the distribution of teeth among the groups. Furthermore, anaerobic culturing was not used to separate only anaerobic bacteria.

CONCLUSIONS

The adding of Ciprofloxacin to calcium hydroxide and *S. costus*- calcium hydroxide given extra antibacterial effectiveness as used as an intracanal medicament *in vivo* during root canal treatment. Additional research is required to assess the antibacterial efficacy of calcium hydroxide + Ciprofloxacin and *S. costus* - calcium hydroxide.

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AUTHOR'S CONTRIBUTIONS

Al Kareem AL Muhalel AA: Formal analysis, conceptualization, data organization, and clinical and laboratory examinations. **Al-Shamahy HA:** Methodology, formal analysis, visualization. **Al-Maswary AA:** supervision, clinical part. All authors reviewed the article and approved the final version.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICT OF INTEREST

There are no conflicts of interest in regard to this project.

REFERENCES

1. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985 Oct;1(5):170-5. *https://doi.org/10.1111/j.1600-9657.1985.tb00652.x*
2. Orstavik D. Root canal disinfection: A review of concepts and recent developments. Aust Endod J 2003 Aug;29(2):70-4. *https://doi.org/10.1111/j.1747-4477.2003.tb00515.x*
3. Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002 Nov; 28(11):779-83. *https://doi.org/10.1097/00004770-200211000-00008*
4. McGurkin-Smith R, Trope M, Caplan D, Sigurdsson A. Reduction of intracanal bacteria using GT rotary instrumentation, 5.25% NaOCl, EDTA, and $Ca(OH)_2$. J Endod 2005 May;31(5):359-63. *https://doi.org/10.1097/01.don.0000145035.85272.7c*
5. Zandi H, Rodrigues RC, Kristoffersen AK, *et al*. Antibacterial effectiveness of 2 root canal irrigants in root-filled teeth with infection: A randomized clinical trial. J Endod 2016 Sep;42(9):1307-13. *https://doi.org/10.1016/j.joen.2016.06.006*
6. Rodrigues RCV, Zandi H, Kristoffersen AK, *et al*. Influence of the apical preparation size and the irrigant type on bacterial reduction in root canal-treated teeth with apical periodontitis. J Endod 2017 Jul;43(7):1058-1063. *https://doi.org/10.1016/j.joen.2017.02.004*
7. Gazzaneo I, Vieira GCS, Pérez AR, *et al*. Root canal disinfection by single- and multiple-instrument systems: effects of sodium hypochlorite volume, concentration, and retention time. J Endod 2019 Jun; 45(6):736-741. *https://doi.org/10.1016/j.joen.2019.02.017*
8. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000 Dec;26(12):751-5. *https://doi.org/10.1097/00004770-200012000-00022*
9. Paiva SS, Siqueira JF Jr, Rôças IN, *et al*. Clinical antimicrobial efficacy of NiTi rotary instrumentation with NaOCl irrigation, final rinse with chlorhexidine and interappointment medication: A molecular study. Int Endod J. 2013 Mar;46(3):225-33. *https://doi.org/10.1111/j.1365-2591.2012.02111.x*
10. Desai S, Chandler N. Calcium hydroxide-based root canal sealers: A review. J Endod. 2009 Apr;35(4):475-80. *https://doi.org/10.1016/j.joen.2008.11.026*
11. Özdemir MB, Karataş E, Albayrak M, Bayır Y. Effect of intracanal medicaments on matrix metalloproteinase-9 and vasoactive intestinal peptide secretion in periapical lesions of re-treated canals: A randomized controlled clinical study. Clin Oral Investig 2019 Feb;23(2):921-928. *https://doi.org/10.1007/s00784-018-2517-8*
12. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: A systematic review and meta-analysis. Int Endo J 2007, 40, 2–10. *https://doi.org/10.1111/j.1365-2591.2006.01197.x*
13. Louwakul P, Saelo A, Khemaleelakul S. Efficacy of calcium oxide and calcium hydroxide nanoparticles on the elimination of *Enterococcus faecalis* in human root dentin. Clin Oral Investig. 2017 Apr;21(3):865-871. *https://doi.org/10.1007/s00784-016-1836-x*
14. Javidi M, Afkhami F, Zarei M, Ghazvini K, Rajabi O. Efficacy of a combined nanoparticulate/calcium hydroxide root canal medication on elimination of Enterococcus faecalis. Aust Endod J. 2014 Aug;40(2):61-5. *https://doi.org/10.1111/aej.12028*
15. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002 Jan;35(1):13-21. *https://doi.org/10.1046/j.0143-2885.2001.00447.x*
16. Waltimo T, Trope M, Haapasalo M, Ørstavik D. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. J Endod 2005 Dec;31(12):863-6. *https://doi.org/10.1097/01.don.0000164856.27920.85*
17. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997 Sep;30(5):297-306. Erratum in: Int Endod J 1998 Mar;31(2):148. PMID: 9477818. *https://doi.org/10.1046/j.1365-2591.1997.00092.x*
18. Zandi H, Petronijevic N, Mdala I, *et al*. Outcome of endodontic retreatment using 2 root canal irrigants and influence of infection on healing as determined by a molecular method: A randomized clinical trial. J Endod 2019 Sep;45(9):1089-1098.e5. *https://doi.org/10.1016/j.joen.2019.05.021*
19. Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of antimycobacterial agents. J Ethnopharmacol 2007 Mar 21;110(2):200-34. *https://doi.org/10.1016/j.jep.2006.12.031*
20. Abdallah EM, Qureshi KA, Ali AM, Elhassan GO. Evaluation of some biological properties of *Saussurea costus* crude root extract. Biosc Biotech Res Comm 2017; 10: 601-611. *https://doi.org/10.21786/bbrc/10.4/2*
21. Nadda RK, Ali A, Goyal RC, Khosla PK, Goyal R. *Aucklandia costus* (Syn. *Saussurea costus*): Ethnopharmacology of an endangered medicinal plant of the himalayan region. J Ethnopharmacol 2020 Dec 5;263:113199. *https://doi.org/10.1016/j.jep.2020.113199*
22. Deabes MM, Fatah AE, Sally I, Salem SHE, Naguib KM. Antimicrobial activity of bioactive compounds extract from *Saussurea costus* against food spoilage microorganisms. Egypt J Chem 2021; 64: 9-10.
23. Abdelwahab SI, Taha MME, Alhazmi HA, *et al*. Phytochemical profiling of costus (*Saussurea lappa* Clarke) root essential oil and its antimicrobial and toxicological effects. Trop J Pharm Res 2019; 18: 2155-2160.
24. Al Otibi F, Rizwana H, Alharbi RI, Alshaikh N, Albasher G. Antifungal effect of *Saussurea lappa* roots against phytopathogenic fungi and resulting morphological and ultrastructural changes. Gesunde Pflanzen 2020; 72: 57-67. *https://doi.org/10.1007/s10343-019-00483-5*
25. Sharafuddin A H, Alshameri B, AL-Haddad KH, Al-Najhi MM, Al-Shamahy HA. The effect of dental implants on increasing the colonization rate of aerobic bacteria in the oral cavity. Universal J Pharm Res 2023, 8(3):1-6. *https://doi.org/10.22270/ujpr.v8i3.944*
26. Al-Hamzi MA, Sharafuddin AH, Al-Shamahy HA, *et al*. The effect of dental implants on aerobic bacteria colonization in the oral cavity and the antibiotic profile of common isolated aerobic bacteria. Universal J Pharm Res 2023; 8(4):1-6. *https://doi.org/10.22270/ujpr.v8i4.969*
27. Ali Nagi IA, Howilah AA, Al-Shamahy HA, *et al*. The prevalence and associated factors of *Staphylococcus aureus* colonization in the oral cavity of adults. Universal J Pharm Res 2024; 9(2):1-6. *https://doi.org/10.22270/ujpr.v9i2.1084*
28. Al-Shameri B HH, Alsubari RAS, Al-Shamahy HA, *et al*. Detection of biofilm production for oral bacterial isolates and it's impact on dental caries occurance. Universal J Pharm Res 2024; 9(1):1-6. *https://doi.org/10.22270/ujpr.v9i1.1055*
29. Abbas AK M, Al-Kibsi TAM, Al-Shamahy HA, *et al*. Characterization and antibiotic sensitivity of bacteria in orofacial abscesses of odontogenic origin. Universal J Pharm Res 2021;5(6):1-6. *https://doi.org/10.22270/ujpr.v5i6.510*
30. Teles R, Teles F, Frias-Lopez J, Paster B, Haffajee A. Lessons learned and unlearned in periodontal microbiology. Periodontol 2000 2013 Jun; 62(1):95-162.

https://doi.org/10.1111/prd.12010

31. Karataş E, Baltacı MÖ, Uluköylü E, Adıgüzel A. Antibacterial effectiveness of calcium hydroxide alone or in combination with Ibuprofen and Ciprofloxacin in teeth with asymptomatic apical periodontitis: A randomized controlled clinical study. Int Endod J 2020 Jun;53(6):742-753. *https://doi.org/10.1111/iej.13277*
32. Zhang GF, Liu X, Zhang S, Pan B, Liu ML. Ciprofloxacin derivatives and their antibacterial activities. Eur J Med Chem. 2018 Feb 25;146:599-612. *https://doi.org/10.1016/j.ejmech.2018.01.078*
33. Manzur A, Gonzalez AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: A randomized clinical trial. J Endodontics 2007; 33, 114–8.
34. Arruda MEF, Neves MAS, Diogenes A, *et al*. Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: A randomized clinical trial. J Endod 2018 Oct;44(10):1474-1479. *https://doi.org/10.1016/j.joen.2018.07.001*
35. Peters LB, van Winkelhoff A-J, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endodontic J 2002; 35 (1), 13–21.
36. Sabrah AH, Yassen GH, Gregory RL. Effectiveness of antibiotic medicaments against biofilm formation of *Enterococcus faecalis* and *Porphyromonas gingivalis*. J Endod 2013;39(11):1385-9.
37. Beck R, van Keyserlingk J, Fischer U, Guthoff R, Drewelow B. Penetration of Ciprofloxacin, norfloxacin and ofloxacin into the aqueous humor using different topical application modes. Graefe's archive for clinical and experimental ophthalmology 1999; 237: 89–92.
38. Andriole VT. The future of the quinolones. Drugs 1999; 58 (Suppl. 2), 1–5.
39. Al-Yosaffi E A, Al-Shamahy HA, Othman AM, Al-Haddad AM, Al-Moyed KA. Antibiotic sensitivity of bacterial bloodstream infections in the intensive care unit patients of university hospitals in Sana'a city, Yemen. Universal J Pharm Res 2023; 8(5):1-6. *https://doi.org/10.22270/ujpr.v8i5.1004*
40. Al-Khamesy KSA, Al-Shamahy HA. Assessment of the present bacteriological profile and antibiotic sensitivity pattern in chronic suppurative otitis media in Sana'a, Yemen. Universal J Pharm Res 2023; 8(5):7-12. *https://doi.org/10.22270/ujpr.v8i5.1010*
41. Al-Shami HZ, Al-Haimi MA, Al-Shamahy HA, *et al*. Patterns of antimicrobial resistance among major bacterial pathogens isolated from clinical samples in two tertiary's hospitals, in Sana'a, Yemen. Universal J Pharm Res 2021; 6(5):12-18. *https://doi.org/10.22270/ujpr.v6i5.674*
42. Sjögren U, Figdor D, Spångberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991 May;24(3):119-25. *https://doi.org/10.1111/j.1365-2591.1991.tb00117.x*
43. Nerwich A, Figdor D, Messer HH. pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. J Endod 1993 Jun;19(6):302-6. *https://doi.org/10.1016/s0099-2399(06)80461-9*
44. Estrela C, Estrela CRdA, Pecora JD. A study of the time necessary for calcium hydroxide to eliminate microorganisms in infected canals. J Apllied Oral Sci 2003; 11: 133–7.
45. Zarei M, Afkhami F, Malek Poor Z. Fracture resistance of human root dentin exposed to calcium hydroxide intervisit medication at various time periods: an *in vitro* study. Dent Traumatol 2013 Apr;29(2):156-60. *https://doi.org/10.1111/j.1600-9657.2012.01158.x*
46. Weston CH, Barfield RD, Ruby JD, Litaker MS, McNeal SF, Eleazer PD. Comparison of preparation design and material thickness on microbial leakage through Cavit using a tooth model system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008 Apr;105(4):530-5. *https://doi.org/10.1016/j.tripleo.2007.10.014*
47. Barthel CR, Zaritzki FF, Raab WH, Zimmer S. Bacterial leakage in roots filled with different medicaments and sealed with Cavit. J Endod 2006 Feb;32(2):127-9. *https://doi.org/10.1016/j.joen.2005.10.023*
48. Nocker A, Burr M, Camper AK. Genotypic microbial community profiling: a critical technical review. Microb Ecol. 2007 Aug; 54(2):276-89. *https://doi.org/10.1007/s00248-006-9199-5*
49. Siqueira JF, Jr, Roˆc¸as IN, Rosado AS. Application of denaturing gradient gel electrophoresis (DGGE) to the analysis of endodontic infections. J Endod 2005; 31: 775_82.
50. Machado de Oliveira JC, Siqueira JF, Jr, Roˆc¸as IN, *et al*. Bacterial community profiles of endodontic abscesses from Brazilian and USA subjects as compared by denaturing gradient gel electrophoresis analysis. Oral Microbiol Immunol 2007; 22: 14_8.
51. Siqueira JF, Jr, Roˆc¸as IN, Rosado AS. Investigation of bacterial communities associated with asymptomatic and symptomatic endodontic infections by denaturing gradient gel electrophoresis fingerprinting approach. Oral Microbiol Immunol 2004; 19: 363_70.
52. Roˆc¸as IN, Siqueira JF, Jr, Aboim MC, Rosado AS. Denaturing gradient gel electrophoresis analysis of bacterial communities associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 741_9.
53. Machado de Oliveira JC, Gama TG, Siqueira JF, *et al*. On the use of denaturing gradient gel electrophoresis approach for bacterial identification in endodontic infections. Clin Oral Investig 2007; 11: 127_32.
54. Siqueira JF, Jr, Roˆc¸as IN, Cunha CD, Rosado AS. Novel bacterial phylotypes in endodontic infections. J Dent Res 2005; 84: 565_9.
55. Siqueira JF, Roˆc¸as IN, Debelian G, *et al*. Profiling of root canal bacterial communities associated with chronic apical periodontitis from Brazilian and Norwegian subjects. J Endod 2008; 34: 1457_61.
56. Siqueira JF Jr, Rôças IN. Distinctive features of the microbiota associated with different forms of apical periodontitis. J Oral Microbiol 2009 Aug 10;1. *https://doi.org/10.3402/jom.v1i0.2009*