ORIGINAL RESEARCH

Comparative Evaluation of Antifungal Efficacy of 3% Sodium Hypochlorite, 2% Chlorhexidine Gluconate, Ozonated Water, Alum Water, and Normal Saline Solutions against Endodontopathogenic Microorganism, *Candida Albicans*: A Microbiological *In Vitro* Study

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Abstract

Aims and objective: To compare and evaluate the antifungal efficacy of 3% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX) gluconate, 4 mg/mL ozonated water, and 2M alum water against *Candida albicans* (*C. albicans*).

Materials and methods: A total of 35 patients were selected from those attending the outpatient department of Pedodontics and Preventive Dentistry at Santosh Dental College and Hospitals, Ghaziabad. Their salivary samples were taken and cultured on a Sabouraud's dextrose agar (SDA) plate. The antifungal efficacy of 3% NaOCl, 2% CHX gluconate, 4 mg/mL concentration of ozonated water, and 2M alum water was assessed against clinical strains of *C. albicans* with the help of agar well diffusion method. The microbial isolates were inoculated into 10 mL of sterile peptone water and incubated at 37°C for 8 hours. The cultures were swabbed on the surface of sterile Mueller–Hinton agar plates using a sterile cotton swab. Five wells of 6 mm diameter were punched in each Petri dish. Around 100 µL of each test solution was poured into the designated wells. Further, the plates were incubated in an upright position at 37°C for 24 hours. The antifungal activity of the test solutions was determined by measuring the diameter of the inhibition zone in mm produced against the Candida isolates, and means were calculated.

Results: It was observed that all test solutions used in this study were inhibitory against *C. albicans* but with a variation in the size of inhibitory zones. According to the means of the diameter of inhibitory zones for all test solutions, the 3% NaOCl represented the statistically significant largest average zones of inhibition against *C. albicans*, followed by 2% CHX when compared with the other two test solutions alum water and ozonated water. Ozonated water produced the smallest mean inhibitory zone.

Keywords: Alum water, Chlorhexidine gluconate, Ozonated water, Sodium hypochlorite.

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Introduction

Endodontic infections are polymicrobial in nature and are directed toward anerobic species.[1] In a classical study, Kakehashi et al.[2] showed that bacteria were the origin of pulpal disorders; yet, a number of subsequent investigations have suggested that fungi, and more recently, viruses, may also play a role in endodontic infection occurrence. *Actinomyces*, *Candida albicans* (*C. albicans)*, and *Enterococcus faecalis* are the most common bacteria linked to endodontic therapy failure, according to the literature.[1]

Various instrumentation techniques, irrigation schedules, and intracanal medications have all been reported as ways to lower the amount of microorganisms in the root canal system. A crucial component of a successful endodontic treatment is cleaning every part of the root canal system with chemical agents during biomechanical preparation.[3]

Numerous substances have been employed as irrigants for root canals, such as phosphate, citric, and lactic acids; proteolytic enzymes; chelating agents such as ethylenediaminetetraacetic acid (EDTA); alkaline solutions such as potassium, sodium hydroxide, urea, and hypochlorite; oxidative agents such as hydrogen peroxide and glyoxide; local anesthetic solutions; chlorhexidine (CHX); and mixtures of tetracycline and normal saline.[3]

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Since its introduction in endodontics in 1920, sodium hypochlorite (NaOCl) has been the most widely utilized irrigating solution. One of the irrigants that is currently most frequently utilized in endodontic therapy is NaOCl, a clear, straw-colored reducing agent that can be employed in concentrations ranging from 0.5 to 5.25%. Moreover, it has a low cost, high durability, and wide accessibility.[4]

Strong antiseptic CHX is frequently used to chemically control plaque in the oral cavity. CHX has a broad spectrum of antimicrobial action, which is why it has been used in endodontics as root canal irrigants and medicaments. For this reason, aqueous solutions with a concentration of 0.1–0.2% are advised, whereas the endodontic literature often uses solutions with a 2% concentration for root canal irrigation.[3]

Ozone has been the subject of recent discussion as a potential substitute antiseptic agent in dentistry due to its great antibacterial potency without causing medication resistance.[5] Aqueous ozone has no cytotoxicity and is highly biocompatible compared with other antiseptics. When irrigating the specimen using sonication, it was discovered that the antibacterial properties of ozonated water and 2.5% NaOCl were nearly equal. Before ozonated water is utilized as a root canal irrigant, more research and adjustments are required.[5,6]

Being an astringent, alum has been utilized extensively. An efficacious daily alum rinse (0.02M) in lowering salivary levels of *Streptococcus mutans* was also documented in a recent investigation.[7]

Alum also has antibacterial and antifungal effects. After the establishment of the potential antimicrobial effects of alum as a mouthrinse and cariostatic agent, its potential use as a root canal irrigant can be explored.

Thus, keeping the above in mind, the antifungal efficacy of 3% NaOCl, 2% CHX gluconate, ozonated water, and alum water was studied as potential root canal irrigants against *C. albicans*.

Materials and Methods

The study was performed on samples collected from 35 children in the age-group of 3–15 years. Patients were selected from those attending the outpatient department of Pedodontics and Preventive Dentistry at Santosh Dental College and Hospitals, Ghaziabad. Selected patients of parents were informed of the experimental design, and before conducting the study, written informed consent was obtained from all the participants. The study sample consisted of children who had healthy periodontal status and met at least one of the inclusion criteria specified below. Children who have more than six carious teeth, widespread caries, and caries in breastfeeding bottles.

The present study employed swab samples from 35 patients to obtain clinical isolates of *C. albicans*.

The antifungal activity of the following materials was to be studied:

- Around 3% NaOCl.
- Around 2% CHX gluconate.
- Around 4 mg/mL concentration of ozonated water.
- Around 2M alum water.
- Normal saline as control.

The purpose of the study is to examine the oral cavity's mycological characteristics. A sterile cotton swab was gently rubbed across the tongue's dorsal surface, the vestibular sulci, and the apex of the palatal vault in order to gather samples for fungal analysis (Fig. 1).

The samples were grown on Sabouraud's dextrose agar (SDA) culture plates (Figs 2 and 3). Aerobic incubation of the culture plates was done at a temperature of 37°C for

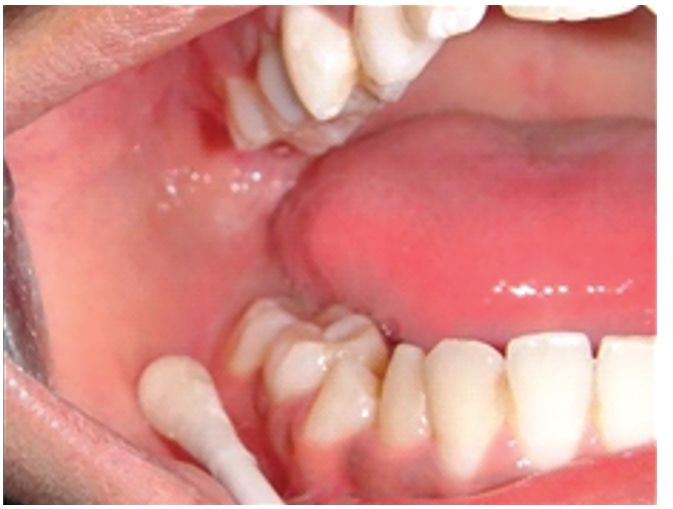

Fig. 1: Swab sample collection

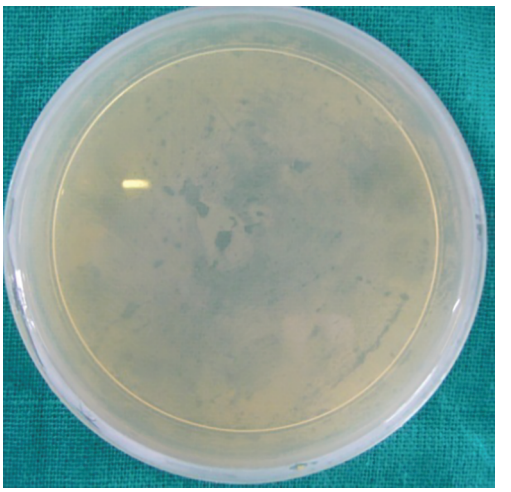

Fig. 2: Sabouraud's dextrose agar (SDA)

Fig. 3: Swab sample smeared on the SDA plate

24–48 hours. After 48 hours of incubation, colonies on SDA were small to medium-sized, became larger, round margin, cream colored, pasty, and easily emulsifiable on further incubation (Fig. 4). Gram staining was done to determine the morphology of *Candida*. Gram-positive, budding oval yeast cells were seen.

For species identification of *Candida*, germ tube test was performed.

Germ tube formation is a process of hyphal development that occurs directly from a yeast cell. This process is characterized by the emergence of a tubular structure with parallel walls from the yeast cell surface. Of all the species of *Candida*, *C. albicans* forms a germ tube. On the basis of germ tube formation, it can be differentiated from non-*C. albicans* (Fig. 5).

The strains of *C. albicans* were added to a broth made of peptone water and cultured for an additional three to 6 hours, or until the culture reached a turbidity of 0.5 McFarland units (Fig. 6). Inoculation of the Mueller–Hinton plate was done (Fig. 7). In order to assess the susceptibility of *C. albicans*, this study followed the agar well diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) (Figs 8 and 9).[8]

The result was observed at 24 and 48 hours intervals and determined antifungal activity by observing zones of inhibition around the solutions in the cavities, which was measured in mm for all the individual isolated (Fig 10).

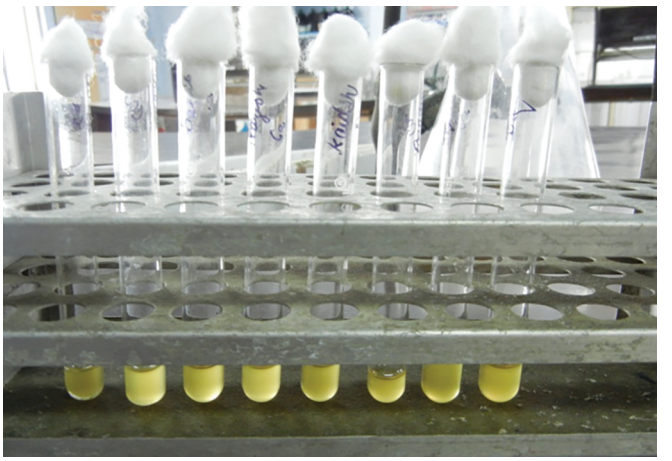

Fig. 6: *C. albicans* showing turbidity in peptone water

Fig. 4: Fungus growth on SDA

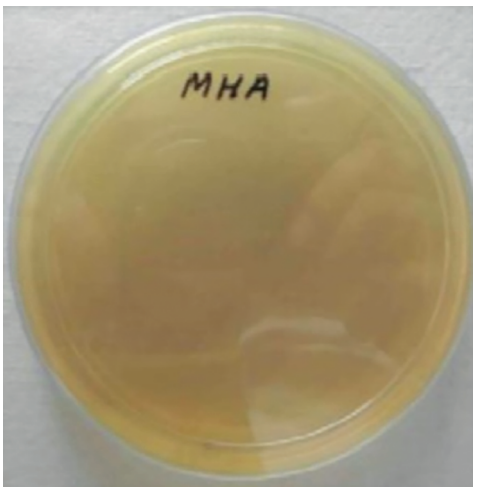

Fig. 7: Mueller–Hinton agar

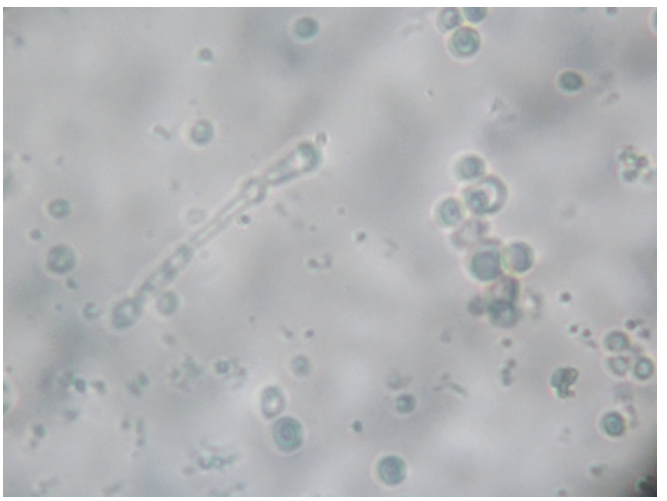

Fig. 5: Germ tube test showing germ tube formation under a microscope

Fig. 8: Test materials were dropped in punched wells

Observation and Results

In the study, the antifungal efficacy of four antimicrobial agents was compared *in vitro* by microbiological testing, which showed that they exhibited antifungal efficacy to varying degrees. Around 3% NaOCl showed the maximum antifungal efficacy, followed by 2% CHX gluconate. Next in the order was 2M alum water, and the least efficacy was observed in ozonated water. Normal saline, which was used as a control, showed no antifungal efficacy.

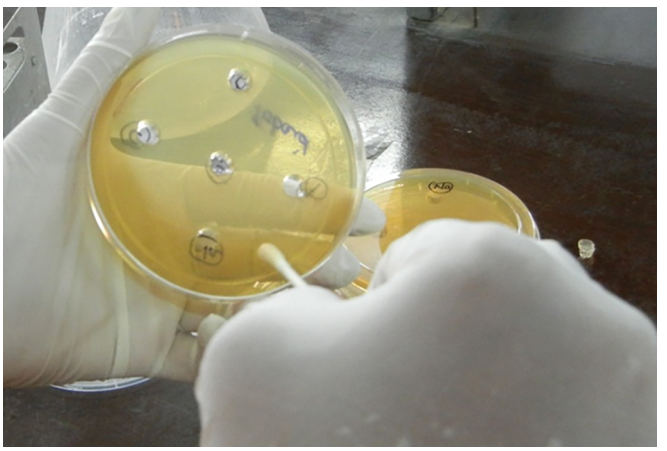

Fig. 9: Streaking of *C. albicans* on Mueller–Hinton agar plate for antisusceptibility testing

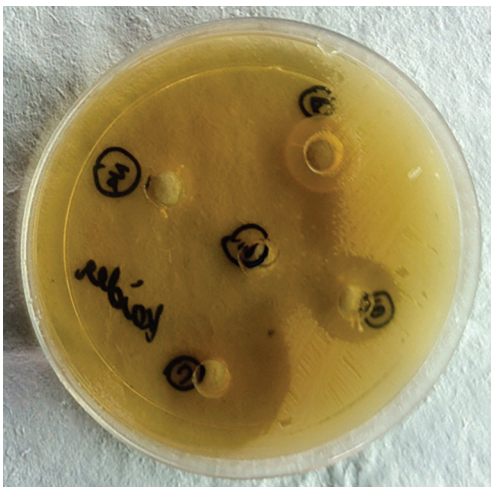

Fig. 10: Zone of inhibition for test irrigants after susceptibility test

The observations are summarized in Tables 1 and 2.

Table 3 illustrates the frequency, mean, and standard deviation of the diameter of zones of inhibition of test irrigants in comparison to negative control (saline) using a *t*-test. The mean diameter of zones of inhibition of NaOCl was 30.23 ± 1.40, CHX gluconate 21.957 ± 0.988, alum water 16.786 ± 1.0592, ozonated water 8.806 ± 1.1156, and normal saline 0.00 ± 0.000.

Table 4 illustrates a comparison among the various test agents of mean and standard deviation, and the significance of the

Table 1: Mean diameter of zones of inhibition of the various antifungal test agents used

Antifungal agents	*N*	*Mean diameter of zones of inhibition (in mm) with SD (mean ± SD; range)*
3% NaOCl	35	30.23 ± 1.40; 26.0–32.0
2% CHX gluconate	35	21.957 ± 0.988; 20.0–24.0
Alum water (2M)	35	16.786 ± 1.0592; 14.0–18.5
Ozonated water (4 mg/mL)	31	8.806 ± 1.1156; 6.0–10.0
Normal saline	35	0.00 ± 0.00; 0.0–0.0

Mean, arithmetic mean of the diameter of the zones of activity; N, number of sample size; range, minimum diameter of the zones in millimeter-maximums diameter of the zones in millimeters observed; SD, standard deviation

Table 2: Comparative evaluation of mean diameter of zones of inhibition of various test agents with respect to negative control (saline) using *t*-test

	Mean	*N*	*SD*
Saline vs 3% NaOCl	0.00 30.229	35 35	0.000 1.3898
Saline vs 2% CHX gluconate	0.00 21.957	35 35	0.000 0.9880
Saline vs Alum water (2M)	0.00 16.786	35 35	0.000 1.0592
Saline vs Ozonated water (4 mg/mL)	.00 8.806	31 31	0.000 1.1156

Mean, arithmetic mean of the diameter of the zones of activity; N, number of sample size; SD, standard deviation

Table 3: Comparison of mean, standard deviation, and test of significance of paired differences of diameter of zones of inhibition of test irrigants with respect to negative control (saline) using *t*-test

	Paired differences			
		95% confidence interval of the difference		
	Mean ± SD	*lower*	*upper*	*p-value*
Saline—3% NaOCl	−30.2286 ± 1.3898	−30.7060	−29.7512	0.000
Saline—2% CHX gluconate	−21.9571 ± 9880	−22.2965	−21.6178	0.000
Saline—alum water (2M)	−16.7857 ± 1.0592	−17.1496	−16.4219	0.000
Saline—ozonated water (4 mg/mL)	−8.8065 ± 1.1156	−9.2157	−8.3972	0.000

Mean, arithmetic mean of the diameter of the zones of activity; N, number of sample size; *p*-value < 0.05, statistically significant; SD, standard deviation

Table 4: Comparative evaluation, among the various test agents for mean diameter, of zones of inhibition of test irrigants using *t*-test

		Paired differences		
		Mean	*SD*	*p-value*
Pair 1	3% NaOCl —2% CHX gluconate			
Pair 2	3% NaOCl —alum water (2M)	13.4429	1.7937	0.000
Pair 3	3% NaOCl —ozonated water (4 mg/mL)	21.4677	1.8571	0.000
Pair 4	2% CHX gluconate—alum water (2M)	5.1714	1.5289	0.000
Pair 5	2% CHX gluconate—ozonated water (4 mg/mL)	13.1935	1.3582	0.000
Pair 6	Alum water (2M)—ozonated water (4 mg/mL)	8.0484	1.7529	0.000

Mean, arithmetic mean of the diameter of the zones of activity; *p*-value < 0.05, statistically significant; SD, standard deviation

diameter of zones of inhibition of test irrigants is tested using a *t*-test. The mean value of paired difference of diameter of zones of inhibition of pair 1 was 8.2714 ± 1.7377, pair 2 was 13.4429 ± 1.7937, pair 3 was 21.4677 ± 1.8571, pair 4 was 5.1714 ± 1.5289, pair 5 was 13.1935 ± 1.3582, and pair 6 was 8.0484 ± 1.7529.

A statistically significant difference was found between all six pairs (*p*-value < 0.05).

As per the results given above, it was observed that all test solutions used in this study were inhibitory against *C. albicans* but with a variation in the size of inhibitory zones. The means of diameter of inhibitory zones for all test solutions are given in Table 1.

Around 3% NaOCl represented the statistically significant largest average zones of inhibition against *C. albicans*, followed by 2% CHX when compared with the other two test solutions, alum, and ozonated water. Ozonated water produced the smallest mean inhibitory zone.

The paired difference of means of the diameter of inhibitory zones of test solutions is given in Table 4. There was a statistically significant difference between all the test pairs. On comparison of NaOCl with different test solutions, the largest difference of means of the diameter of inhibitory zones was seen between NaOCl and ozonated water, that is, 21.4677 mm and the least difference was between NaOCl and CHX gluconate, that is, 8.2714. On comparison of CHX gluconate with other test solutions, the maximum difference was with ozonated water, that is, 13.1934, followed by NaOCl, that is, 8.2714, and the least difference was with alum water, that is 5.1714.

Discussion

Endodontic infections have a polymicrobial etiology. Reducing the bacteria count is one of the main goals of endodontic therapy, as this helps the periodontal tissues repair normally.[9]

Oftentimes, root canal therapy is not effective in treating apical periodontitis, and the inflammation around the treated area may continue for months or even years. This is due to the root canal infection. The treatment of persistent cases may fail for a number of reasons. These characteristics are typically associated with challenges in the chemomechanical preparation of the root canals.[10] In recent years, researchers have shown a growing curiosity about the presence and role of yeasts in endodontic infections that are resistant to traditional root canal therapy.

As part of the regular flora, yeasts can be found in many different places throughout the human body. The most significant oral yeasts in terms of clinical use are found in the genera *Candida* and Saccharomycetaceae families.[11]

Candida albicans (*C. albicans)* is a ubiquitous fungal species that colonizes the oral cavity of both healthy and medically compromised individuals. It appears that *C. albicans* primarily inhabits the dorsum of the tongue, with secondary colonization occurring elsewhere in the mouth. These locations consist of the periodontal pockets, the root, the mucosa and supragingival, the dentin, and the subgingival.[12]

Over the past 50 years, microbiological studies on apical periodontitis have shown that yeasts can also be isolated from diseased root canals. Around 7–18% of root canals had a yeast infection.[12] *C. albicans* was discovered in a patient's periapical granulomas and root canals in a different case report. The patient had chronic urticaria.[13] In obturated teeth, when therapy has failed, fungi appear to be more prevalent in the root canals.

Sundqvist et al.[14] isolated *C. albicans* from two of 24 canals of the teeth in which endodontic treatment had failed.

Many intracanal medications, including calcium hydroxide, have also been proven to be resistant to *C. albicans*. Cases of persistent root canal infections may be linked to *C. albicans* due to their capacity to infiltrate dentinal tubules and resistance to routinely used intracanal medications.[12]

No matter where or how yeasts enter the root canal, their presence in cultivable numbers may be clinically significant, particularly in cases that are chronic. In this investigation, *C. albicans* was chosen in an effort to find a more recent and potent antibacterial irrigant. For endodontic therapy to be successful, the root canal system needs to be totally removed and cleaned. Root canal irrigants are crucial to the optimization of the root canal preparation process, which is primarily a chemomechanical one, despite the morphological constraints posed by the internal root anatomy.[15]

Sodium hypochlorite (NaOCl) and CHX gluconate are the two most frequently employed endodontic irrigants.

The choice of 3% NaOCl and 2% CHX gluconate for the present study was due to significant use in endodontic practice. NaOCl is the most widely used irrigant. In addition to their broad-spectrum, nonspecific killing effects on all microorganisms, preparations containing hypochlorite also exhibit sporicidal and virucidal properties, and they dissolve tissues much more readily in necrotic tissues than in live tissues.[16] NaOCl solutions also have a long shelf life, are easily accessible, and are reasonably priced.

However, NaOCl has been shown to have some side effects; the most common is that at high concentrations, it can cause tissue irritation upon contact, and it is highly virulent. The majority of NaOCl-related problems seem to arise from inadvertent injections of the medication past the root apex, which can result in severe tissue reactions that include pain, swelling, bleeding, and, in rare situations, the emergence of secondary infections and paresthesia.[17]

Root canal therapy for deciduous teeth carries a risk of damaging permanent tooth follicles. Additionally, cases of open

apices, such as immature permanent teeth, carry a higher risk of accidental NaOCl injection.

The NaOCl has an unpleasant smell, damages clothing when it comes into touch with it and is harmful to vital tissues. There have also been reports of hypersensitivity reactions to NaOCl.[18]

In the late 1940s, Imperial Chemical Industries Ltd's research labs in Macclesfield, England, created CHX. Strong antiseptic CHX is frequently used to chemically reduce plaque in the oral cavity. For that reason, aqueous solutions containing 0.1–0.2% are advised, although the endodontic literature often uses solutions with a 2% concentration for root canal irrigating solutions.[19]

At physiological pH, CHX gluconate, the most widely used oral formulation, easily dissociates and releases the positively charged CHX component.[20]

It has been suggested that CHX be used instead of NaOCl. Its low-grade toxicity and strong, broad-range antibacterial activity are its two main advantages. Most researchers believe that NaOCl would be more caustic than CHX.

Chlorhexidine (CHX) is helpful as a last irrigant, but in typical endodontic cases, it cannot be recommended as the primary irrigant due to two reasons—(1) it cannot dissolve necrotic tissue remnants, and (2) it works less well on gram-negative bacteria than on gram-positive bacteria. The quest for new alternatives is required because of its inability to dissolve tissue and the drawbacks of the current antimicrobial irrigants.

Two novel irrigants that are being researched are ozonated water and alum water. Due to its great antibacterial power without causing medication resistance, ozone is currently being explored as a possible replacement antiseptic agent in dentistry.[21]

Ozone is a powerful oxidizing agent and has been used in water and food industry for many years to kill microorganisms.[22] It has modest levels of cytotoxicity and antibacterial properties. Its effectiveness as an antimicrobial in endodontics has only been assessed in a few papers.[9]

It has been demonstrated that ozone has unique properties and can be used in clinical dentistry and medicine. Ozone is known to have a number of functions, including antibacterial (bactericidal, viricidal, and fungicidal), immunostimulatory, immunological modulatory, anti-inflammatory, biosynthetic (activating the metabolism of proteins, carbohydrates, and lipids), bioenergetic, antihypoxic, analgesic, hemostatic, etc.[23]

The benefits of ozone in the aqueous phase—its potency, simplicity of handling, lack of mutagenicity, and quick microbicidal effects were utilized in the current study to create ozonated water.[24] According to Huth et al., the highest level of biocompatibility was observed with aqueous ozone in comparison to gaseous ozone, NaOCl, and hydrogen peroxide.[25]

Alum is used both internally and externally; it is styptic and astringent. When consumed orally, it enters the body and is absorbed; it has been found in the urine, spleen, and liver. A teaspoonful dose of powdered alum works well as an emetic. It coagulates albumen and produces a copious flow of saliva, forming white, membrane-like flakes from the salivary albumen and buccal mucus.

It mostly has an astringent impact on mucosal membranes. When applied topically to areas that are relaxed or bleeding, it acts as an astringent by corrugating the surrounding tissues and causing the capillaries to constrict. In a more recent study, aluminum has demonstrated antimicrobial efficacy against periodontal pathogens, cariogenic streptococci, and normal oral flora by drastically reducing oral bacteria's colloidal stability and limiting streptococci's capacity to colonize enamel surfaces. Due to its astringent qualities, alum is frequently used. The Food and Drug Administration's over-the-counter advisory council has recommended it as a category-I active component for mouthwashes.[26]

An efficacious daily alum rinse (0.02M) in lowering salivary levels of *Streptococcus mutans* was also documented in a recent investigation.[27] As alum solutions may have antimicrobial properties as well, the purpose of the current study was to evaluate and compare the antifungal efficacy of 2M alum solution as a practical root canal irrigant.

To the best of my knowledge, this is the first study to look into the antifungal effectiveness of alum water against *C. albicans*.

In the present study, a higher concentration was used; that is, a 2M concentration of alum water was compared with other solutions as root canal irrigant. The concentration of 0.2M or 0.02M used for mouthwash is too low for root canal irrigation, so a 2M concentration of alum water was used. Since there are no known reports of the antifungal efficacy of alum water and its comparison with the known irrigants, this *in vitro* study was an attempt to find out the same. The aim of the present study was thus to comparatively evaluate the antifungal efficacy of 3% NaOCl, 2% CHX gluconate, 4 mg/mL ozonated water, and 2M alum water against *C. albicans*. The zone of inhibitions that each test irrigant produced against *C. albicans* was measured in the current study using the agar well diffusion method to compare the antifungal efficacy of the test irrigants. According to the study's findings, all of the test solutions employed in this study were inhibitory against *C. albicans*; however, the sizes of the inhibitory zones varied.

As per the means of the diameter of inhibitory zones for all test solutions given in Table 1, the 3% NaOCl represented the statistically significant largest average zones of inhibition against *C. albicans*, followed by 2% CHX when compared with the other two test solutions alum water and ozonated water. Ozonated water produced the smallest mean inhibitory zone.

The current study's findings showed that the most effective antifungal agent was 3% NaOCl, followed by 2% CHX gluconate. This result was consistent with the findings of Ignatius and Pradeep,[28] whose results showed that 2.5% NaOCl was effective in 90% of the samples of *C. albicans* harvested from root canals compared to zinc oxide + 2% CHX and calcium hydroxide + 2% CHX combinations, which was 70 and 60% effective, respectively. Sena et al.[29] reported that single-species biofilms of either *C. albicans* or *Escherichia faecalis* could be eradicated in 30 seconds using 5.25% NaOCl, both with and without mechanical agitation. They also showed that agitating 2% CHX eliminated both organisms in 30 seconds, whereas applying the same dose of CHX without agitation required a longer contact period (5 minutes) to prevent *C. albicans* development.

Conversely, the current study's findings disagreed with those of Radeva et al.,[30] who demonstrated that 2% rather than 3% of CHX gluconate was more efficacious. NaOCl in eliminating *C. albicans*. Similar results were obtained by Lau et al.[31] According to their study, 0.2% CHX showed better antifungal efficacy against *C. albicans* than 2.5% NaOCl.

The least effective antifungal agent in the current study's settings was 4 mg/mL ozonated water. The findings were corroborated by a study by Wali et al.,[9] which showed that 2% CHX and 5.25% NaOCl were significantly more effective against *C. albicans* than even 24 mg/L ozonated water. On the contrary, in the present study results, Nagayashi et al.[26] examined the effects of 4 mg/mL ozonated water on *C. albicans* for 10 seconds

and discovered that *C. albicans* in pure culture may be effectively killed by the ozonated water. This was further supported by Arita et al.,[32] who investigated how *C. albicans* responded to ozonated water on an acrylic denture plate. Within one minute of being exposed to ozonated water (2 or 4 mg/L), viable *C. albicans* cells were almost nonexistent. Controversial *in vitro* studies have shown that ozonated water has antibacterial properties. Numerous *in vitro* studies have demonstrated that the antimicrobial activity of ozone is dependent on several factors, including the type of microorganism used, whether it is planktonic or in a biofilm; the concentration of ozone, its flow rate, contact time; the delivery system (gaseous ozone, ozonated water, or ozone bubbled in the experimental model at constant flow rate); the depth of action; the use of sonication; and the time-dependent degradation of ozone in water.[9]

Table 4 presents the paired difference of means of the inhibitory zone diameters of the test solutions. Every test pair showed a statistically significant difference. On comparison of ozonated water with different test solutions, the largest difference of means of the diameter of inhibitory zones was seen between NaOCl and Ozonated water, that is, 21.4677 mm, followed by 13.1935 mm with CHX gluconate and least with alum water, that is, 8.0484 mm.

This study clearly indicates that ozonated water has an antifungal activity but is in no comparison to NaOCl and CHX gluconate. Ozone undergoes autodecomposition with no residual power effects in aqueous solution, which can be explained by its relative instability. Ozone, thus, has a time-varying concentration, unlike NaOCl and CHX, whose concentrations stay high and steady during the experiment. As per the result from Table 4, the efficacy of 2M alum water was comparable to 2% CHX as the difference in the means of the diameter of inhibitory zones was the lowest, that is, 5.1714 mm. The maximum difference was seen with NaOCl, that is, 13.4429 mm.

The study's findings reveal that alum water may be utilized as a root canal irrigant since it exhibits antifungal efficacy similar to that of CHX gluconate. However, there is a lack of research on the antifungal properties and possible applications as a root canal irrigant.

In conclusion, ozonated water and alum water both confirm the antifungal activity, but ozonated water was in no comparison to NaOCl and CHX, whereas the antifungal efficacy of alum water was comparable to CHX.

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