

Evaluation of Antimicrobial Efficacy of Commercially Available Herbal Products as Irrigants and Medicaments in Primary Endodontic Infections: *In Vivo* Study

Goldy Rathee¹, Monika Tandan², Rakesh Mittal³

ABSTRACT

Aim: The purpose of this study was to comparatively evaluate the antimicrobial efficacy of two commercially available herbal products as root canal irrigants and medicaments—an *in vivo* study.

Materials and methods: Thirty patients of age group 15–50 years with single-rooted teeth were selected. After access opening and working length (WL) determination, the pretreatment sample (S1) was obtained using a sterile paper point dipped in transport media. Biomechanical preparation (BMP) was done with master apical file size #40 and step-back up to size #60. The patient was randomly divided into three groups and irrigation was done. Group I—2% chlorhexidine gluconate (CHX), group II—*neem* juice extract, and group III—*tulsi* juice extract. Post-instrumentation sample (S2) was taken in the same manner. Canals were dried and three solutions were dispensed as intracanal medicaments. A double seal of Cavit was placed. After 7 days, post-medicament sample (S3) was collected in the same manner as S1. Microbiological samples (S1, S2, S3) were incubated and then plated on brain heart infusion agar and the colonies were counted. Collected data were statistically analyzed using repeated measures of analysis of variance (ANOVA).

Results: The results of this study demonstrated that a statistically significant reduction was seen among all the groups during the intragroup comparison. The reduction in S3 from S1 was found to be significantly higher than that found in S2 from S1, which was further significantly higher than that found in S3 from S2. In intergroup comparison, the difference in colony counts reached the level of statistical significance in S2 from S1 and in S3 from S1 in all the three experimental solutions. Among these stages, a significant reduction was seen in group I and group II, group I and group III.

Conclusion: Therefore, within the limitations of this study, it can be concluded that herbal products have shown significant antimicrobial activity in primary endodontic infections when compared to 2% CHX. Hence, they can be recommended as endodontic irrigants and medicaments.

Clinical significance: Herbal products can be recommended as endodontic irrigants and medicaments.

Keywords: Antimicrobial, Chlorhexidine gluconate, Herbal medicine, *Neem* juice extract, Primary endodontic infection, *Tulsi* juice extract.

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INTRODUCTION

It is well-known that microorganisms in the root canal are responsible for pulp and periradicular infection. Root canal therapy aims to eliminate microorganisms from the root canal to provide an appropriate environment for tissue healing.¹ It has been observed from several studies that mechanical instrumentation alone does not remove the microorganisms completely, but the use of intracanal irrigants and medicaments helps in the removal of the bacteria during and after cleaning and shaping.² Various irrigants/medicaments are advised for disinfecting the root canal as well as for the removal of microorganisms from inaccessible sites.³

Sodium hypochlorite has been universally used as a root canal irrigant.⁴ However, it has various side effects, such as, unpleasant taste and odor, tissue toxicity, inability to remove the smear layer, inability to fully eradicate microbes from the infected canals, allergic potential, risk of emphysema on overfilling, and discoloration of clothes.⁵

Chlorhexidine is a broad-spectrum antimicrobial agent. It is bacteriostatic in low concentration and bactericidal in high concentration.² At 2% concentration, it may have toxic effects on host tissue if expressed beyond the confines of the root canal and may impair healing. It also lacks tissue dissolving capacity.⁶ Chlorhexidine when mixed with sodium hypochlorite produces a carcinogenic product, i.e., parachloroaniline.⁷

¹Department of Conservative Dentistry and Endodontics, SGT Dental College, Gurugram, Haryana, India

^{2,3}Department of Conservative Dentistry and Endodontics, Sudha Rustagi Dental College, Faridabad, Haryana, India

Corresponding Author: Monika Tandan, Department of Conservative Dentistry and Endodontics, Sudha Rustagi Dental College, Faridabad, Haryana, India, Phone: +91 9999063631, e-mail: drmonikatanadan@gmail.com

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Calcium hydroxide is the most commonly used and effective intracanal medicament. Some microorganisms, such as, *Enterococcus faecalis* and *Candida albicans* are resistant to its antimicrobial activity.⁸ Side effects of synthetic drugs have prompted researchers to search for herbal alternatives. Pharmacological studies have acknowledged the value of medicinal plants as potential sources of bioactive compounds.⁹ As per the World Health Organization (WHO) report, 80% of the world population relies mainly on traditional therapies. There are many advantages of using herbs



Fig. 1: Experimental solution



Fig. 2: Root canal sample taken with a paper point

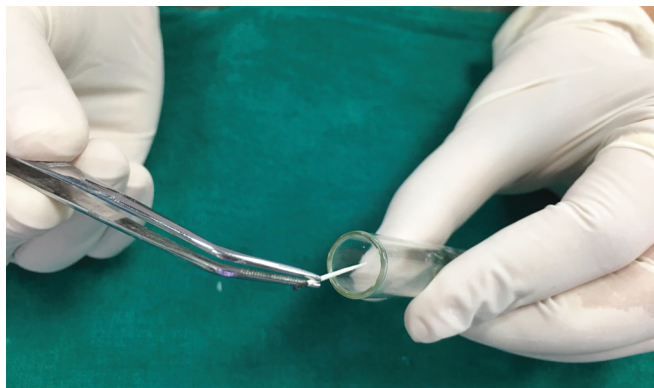


Fig. 3: Paper point sample insertion into transport media

as antimicrobial agent's minimal side effects, cost-effective, better patient tolerance, renewable in nature, and non-toxic.³ Various herbal extracts, such as, *neem*, *tulsi*, *aloe vera*, *Circuma longa*, and turmeric having antimicrobial, anti-inflammatory, and therapeutic effects have been evaluated to be used as endodontic irrigants and medicaments.⁷ *Neem* has several active constituents like nimbidin, nimbin, nimbolide gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulphide which are responsible for its antibacterial action. It is proved to be an effective antimicrobial agent against *E. faecalis* in an *in vitro* study.¹⁰ *Tulsi* (*Ocimum sanctum*) has been tested for its antimicrobial properties against *Escherichia coli*, *Klebsiella*, *C. albicans*, *Staphylococcus aureus*, *E. faecalis*, and *Proteus* and has shown positive results.¹¹ Antimicrobial activity of *tulsi* is due to its constituents, ursolic acid and carvacrol.¹² Both *neem* and *tulsi* extracts are commercially available.

Hence, the purpose of this study was to comparatively evaluate the antimicrobial efficacy of two commercially available herbal products as root canal irrigants and medicaments—an *in vivo* study.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Ethical Committee of the Post Graduate Institute of Dental Sciences, Rohtak, Haryana, India (PGIDS/IEC/2016/62). This *in vivo* study was carried out in the Department of Conservative Dentistry and Endodontics, Sudha Rustagi College of Dental Sciences and Research, Faridabad, Haryana.

Selection of Teeth

Thirty patients with 30 single-rooted teeth in the age group of 15–50 years were selected for the study. Prior clearance of the protocol from the Ethical Committee and informed consent from each patient was taken. Patients with a non-contributory medical history, intact permanent teeth without any previous restoration, teeth with necrotic or infected pulp as diagnosed clinically and radiographically, teeth with an adequate coronal structure for proper isolation, temporization, and restoration were included. Patients with systemic conditions, acute periapical abscess, retreatment cases, patients on antibiotic therapy within the last 3 months, teeth with calcified canals, sinus opening, immature apex, internal or external resorption, and teeth with periodontal pockets >5 mm were excluded.

INSTRUMENTATION

First Treatment Session

Each tooth was anesthetized (Biocaine-ADR Biochem Pharmaceutical Industries Ltd., India) and isolated with a rubber dam (Hygienic® Dental Dam Kit, Coltene Whaledent, Switzerland) followed by caries removal. The access cavity was prepared using a high-speed endo access bur #2 (Dentsply Maillefer, Switzerland) under water spray with an air rotor handpiece (NSK Pana Air, Japan). The working length (WL) was determined radiographically using RVG (Kodak 5100, Eastman Kodak Company, France) and a size #10 K-file (Mani, Inc., MDCI Ltd., Japan) to the apex. Pretreatment sample (S1) was obtained by injecting normal saline (5 mL) (0.9% v/w, Lifusion™, India) into the root canal and circumferentially pumping a #10 K-file (1 mm short of WL). A sterile paper point (Meta Biomed, India) was inserted after immersing it into the transport media and placed into the canal for 60 seconds (Fig. 1). It was then immediately transported to the test tube containing transport media (Peptone water, HiMedia Laboratories Pvt. Ltd., India) (Fig. 2). Three samples were taken for each tooth. Biomechanical preparation (BMP) was done using step-back technique up to master apical size #40 K-file. The patients were randomly divided into three groups (Fig. 3):
Group I: 2% chlorhexidine gluconate (CHX).
Group II: *Neem* juice extract (Sahyog herbal, Pvt. Ltd., India).
Group III: *Tulsi* juice extract (Sahyog herbal, Pvt. Ltd., India).

Irrigation was done with 6 mL of the irrigants. Post-instrumentation sample (S2) was similarly collected after irrigation as S1. It was sent to the laboratory for processing within 2 hours. After second sampling, the canals were dried and the solutions were used as an intracanal medicament and placed inside the root canals with the help of a paper point:

Group I: 2% Chlorhexidine gluconate.

Group II: *Neem* juice extract (Sahyog herbal, Pvt. Ltd., India).

Group III: *Tulsi* juice extract (Sahyog herbal, Pvt. Ltd., India).

A cotton pellet was placed inside the pulp chamber. A double seal of Cavit G (Orafil-G™, PREVEST DenPro, India) was placed.

Second Treatment Session

Patients were recalled after 7 days. The tooth was isolated using a rubber dam and disinfection was done by Betadine solution. The temporary restoration was removed using a round diamond bur and an air rotor handpiece. The cotton pellet was removed, irrigation was done with saline, and an H file was used to remove the medicaments. Post-medication sample (S3) was collected similarly as S1. Teeth were obturated by lateral condensation technique using #40 gutta-percha (Meta Biomed, India) as a master cone (after radiographic verification) and AH Plus sealer (Dentsply, Germany). The access cavities were then restored with composite (Spectrum, Dentsply India Pvt. Ltd., India). Microbiological samples (S1, S2, S3) were preincubated for 30 minutes and shaken vigorously in a vortex mixer for 60 seconds and then were plated on brain heart infusion agar (HiMedia Laboratories Pvt. Ltd., India) and colonies were counted after 24 hours using classic bacterial counting method. Both the session of treatment was performed by the same examiner for each patient. The examiner was aware of the material being used in every patient. No blinding was not done.

STATISTICAL ANALYSIS

The normality of data was checked using the Shapiro–Wilk test. Data reached normality. Thus, inferential statistics were performed using parametric tests of significance. Inter- and intragroup comparison was done using repeated measures of analysis of variance (ANOVA).

Post hoc Tukey analysis was done for pairwise comparison. The level of significance was set at <0.05.

RESULTS

The antibacterial efficacy was measured by counting colony-forming units from the samples taken at different stages; i.e., after access opening (S1), after BMP and irrigation with respective irrigants (S2), and 7 days after placing the same solutions as intracanal medicaments (S3). Their mean reduction was calculated shown in Table 1.

The results showed a statistically significant reduction among all the groups during the intragroup comparison. The reduction in S3 from S1 was found to be significantly higher than that found in S2 from S1, which was further significantly higher than that found in S3 from S2. On intergroup comparison, the difference in colony counts reached the level of statistical significance in S2 from S1 and in S3 from S1 in all the three experimental solutions. Among these stages, a significant reduction was seen in group I and group II, group I and group III. The percentage reduction observed was in the following decreasing order: CHX > *neem* > *tulsi*.

DISCUSSION

Root canal therapy aims to eliminate bacteria from the root canal system to provide an appropriate environment for tissue repair and healing. Failure during and after endodontic treatment is often linked to the presence of bacteria in the root canal.⁴ Elimination of microorganisms from infected root canals always remains a challenging task.³ Although after mechanical preparation of the root canal system, the population of microorganisms is significantly decreased but all the microorganisms cannot be eliminated.¹³ Chemical debridement is especially needed due to complex internal anatomies, such as, fins, apical deltas, isthmuses, cul de sacs, or other irregularities that are usually missed by instrumentation alone.¹⁴

Therefore, several chemicals and therapeutic agents are used to disinfect the root canal system. Irrigation is complementary to instrumentation in facilitating the removal of pulp tissue and/or microorganisms. Ideally, an irrigant should provide a mechanical flushing action, be microbicidal, and dissolve remnants of organic

Table 1: Mean and standard deviation of bacterial colony count (CFU/mL × 10⁵) of each group at S1, S2, and S3 stages

		Colony count × 10 ⁵			p ^a value	Post hoc
		S1	S2	S3		
Group I	Group I: chlorhexidine gluconate				<0.0001, S	S1 > S2 > S3
	Mean	0.91	0.30	0.20		
	N	10	10	10		
	Standard deviation	0.37	0.16	0.13		
Group II	Group II: <i>Neem</i>				<0.0001, S	S1 > S2 > S3
	Mean	0.80	0.47	0.30		
	N	10	10	10		
	Standard deviation	0.31	0.25	0.16		
Group III	Group III: <i>Tulsi</i>				<0.0001, S	S1 > S2 > S3
	Mean	0.89	0.64	0.48		
	N	10	10	10		
	Standard deviation	0.38	0.39	0.31		
p ^a value	0.456, NS	0.451, NS	0.54, NS			
Post hoc	NA	NA	NA			

^aRepeated measures of ANOVA. Level of significance

tissues without damaging the periradicular tissues if extruded into the periodontium.⁸

The gold standard irrigants are sodium hypochlorite (NaOCl), and 2% chlorhexidine, which have different antibacterial spectrum. Sodium hypochlorite is the most commonly used irrigating solution since its introduction by Walker in 1936. Its superior properties of tissue dissolution and antibacterial efficacy makes it the irrigating solution of choice.¹⁵ The antimicrobial effectiveness of sodium hypochlorite is based on its high pH which interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degradation observed in lipid peroxidation.¹⁶ Siqueira et al.¹⁷ showed that the antibacterial effectiveness of 4% NaOCl and 2.5% NaOCl were significantly >0.5% NaOCl; 0.2% chlorhexidine digluconate; 2.0% chlorhexidine digluconate; 10% citric acid; and 17% ethylenediamine tetraacetic acid (EDTA). In endodontic therapy, NaOCl solution can be used in a concentration varying from 0.5 to 5.25%.¹⁸ The major disadvantages of sodium hypochlorite as an irrigant are its cytotoxicity when injected into periradicular tissues, foul smell and taste, ability to bleach clothes, and ability to cause corrosion of metal objects. In addition, it does not kill all bacteria, nor does it remove the smear layer completely. It also alters the flexural strength and elastic modulus of dentin.¹⁹

Another popular irrigant for root canal therapy is CHX. It is a cationic bis-biguanide and is a broad-spectrum antimicrobial agent. It has been used as an irrigant and intracanal medicament in endodontics in concentrations ranging from 0.12 to 2%.²⁰ CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through an active or passive transport mechanism. Its efficacy is due to the interaction with negatively charged phosphate groups on the microbial cell walls, thereby altering the cell's osmotic equilibrium.²¹ Delany et al.²² evaluated 0.2% CHX-gluconate in infected root canals. A highly significant reduction in the number of microorganisms in the CHX-treated specimens after instrumentation and irrigation was observed. The disadvantages of chlorhexidine included no tissue solvent capacity, the cytotoxic effect on host tissue if expressed beyond the confines of the root canal, contact dermatitis, desquamative gingivitis, discoloration of the teeth and tongue or dysgeusia.²³

Intracanal medicaments may be utilized in endodontic therapy to eliminate bacteria, reduce inflammation (and thereby reduce pain), induce healing of calcified tissues, help eliminate apical exudate, control inflammatory root resorption, and prevent contamination between appointments. Since its introduction in 1920, calcium hydroxide has been widely used in endodontics as a root canal medicament. It is a strong alkaline substance, which has a pH of approximately 12.5. Various biological properties have been attributed to this substance, such as, antimicrobial activity, tissue-dissolving ability, inhibition of tooth resorption, and induction of repair by hard tissue formation.²⁴ The high concentration of hydroxyl ions from calcium hydroxide alters the pH gradient of the cytoplasmic membrane, thereby damaging the bacterial protein.²⁵ Cvek et al.²⁶ revealed that 90% of the samples taken from root canals 3 months after medication with calcium hydroxide mixed with Ringer's solution showed no microbial growth. However, several studies have attested the inefficacy of calcium hydroxide in eliminating bacterial cells inside dentinal tubules. Haapasalo

and Orstavik²⁷ reported that a calcium hydroxide paste failed to eliminate, *E. faecalis* in the tubules even superficially.

As the incidence of potential side effects, increased resistance by pathogenic bacteria, safety concerns, and cytotoxic reactions to currently used chemotherapeutics agents is more, the researchers are developing interest in alternative treatment options and products for oral diseases. Hence, the natural phytochemicals isolated from plants used in traditional medicine are being researched as alternatives to synthetic chemicals.²⁵ According to the WHO, as many as 80% of the world's population depends on traditional medicines (herbal) for their primary healthcare needs. Herbal remedies have a long history of use for gum and tooth-related problems. In many cultures, the use of herbal "chewing sticks" taken from plants, shrubs, or trees with high anti-microbial activity are common.²⁸

Azadirachta indica (neem) is a commonly seen tree in India. Popularly known as "Indian neem/Margosa tree or Indian lilac", it is one of the most versatile medicinal plants having a wide spectrum of biological activity. The importance of the *neem* tree has been recognized by the US National Academy of Science, where *neem* is entitled as "a tree for solving global problems". Several biologically active agents have been isolated from different parts of the *neem* plant, such as, azadirachtin, meliacin, gedunin, salanin, nimbin, and valassin. *Neem* has an anti-adherence activity by altering bacterial adhesion and the ability of the organism to colonize. Oil from leaves, seeds, and bark of *neem* tree has shown a wide spectrum of antibacterial action against gram-positive and gram-negative microorganisms and is proved to be effective against *E. faecalis*, *C. albicans*, etc. *Neem's* antiviral, antifungal, antibacterial, and anticarcinogenic activity makes it a potential agent for its use as a root canal irrigant.²⁹ Vinothkumar et al.¹⁰ and Ghonmode et al.³⁰ found that *neem* was highly effective against *E. faecalis* and *C. albicans* when compared to 5.25% sodium hypochlorite. *C. longa*, *Aloe barbadensis*, *Myristica fragrans*, *Terminalia chebula*, and grape seed extracts. Hedge and Kesaria³¹ compared the antimicrobial activity of 2% sodium hypochlorite, propolis, *neem* leaf extract, turmeric, and licorice and concluded that the highest zone of inhibition against *E. faecalis* and *C. albicans* was observed with the *neem* leaf extract.³³

Ocimum sanctum (Holy basil, *Tulsi*) is a plant native to India with known medicinal properties since the Vedic period. It is classified as a "rasayana"—a herb that nourishes a person's growth to perfect health and promotes long life. It has known antibacterial, antifungal, and antiviral properties. The antibacterial effect of *O. sanctum* is due to the presence of linoleic acid, eugenol (1-hydroxy-2-methoxy-4-allylbenzene), carvacrol, and linolenic acid. The mechanism of antibacterial action is due to the formation of malondialdehyde, an aldehyde formed as a breakdown product of linoleic acid and linolenic acid. Extracts of *O. sanctum* inhibit acute and chronic inflammation and are used in the treatment of arthritis. In addition, its oil has a strong analgesic effect. The dried leaves of the plant can be powdered, mixed with mustard oil to make a dentifrice which prevents dental caries and aphthous ulcers in the mouth.³² Prabhakar et al.³³ proved that *Tulsi*—"Ocimum sanctum" had a better antimicrobial efficacy against periapical pathogens isolated from the root canals of primary molars. Mishra et al.³² documented the antibacterial and anti-inflammatory properties of the essential oil extract of *O. sanctum*, for its proposed use as an intracanal medicament.

Many *in vitro* studies have been done on the evaluation and comparison of antimicrobial efficacy of herbal products as root canal irrigants and medicaments but none had reported an *in vivo* study. Therefore, this study was undertaken to comparatively evaluate the antimicrobial efficacy of two commercially available herbal products used as intracanal irrigants and medicaments by counting the colonies formed after culturing the samples taken from the patients at different stages i.e., pre-instrumentation sample (S1), post-instrumentation sample (S2), and post-medicament placement (S3).

The results of the present study demonstrated that during intragroup comparison statistically significant reduction was seen among all the groups at the three stages. The reduction in S3 from S1 was found to be significantly higher than that found in S2 from S1, which was further significantly higher than that found in S3 from S2. In intergroup comparison, the difference in colony counts reached the level of statistical significance in S2 from S1 and in S3 from S1 in all the three experimental solutions. Among these stages, a significant reduction was seen in group I > group II > group III.

These results are in accordance with the results obtained from several *in vitro* studies.^{2,10,30} In the present study, 2% CHX showed the highest reduction of mean bacterial count as root canal irrigant and medicament followed by *neem* and the least reduction was seen with *tulsi*. Kusuma et al.³⁴ evaluated *neem*, aloe vera, chlorhexidine, and calcium hydroxide as an intracanal medicament against *E. faecalis* and showed that chlorhexidine produced better results followed by *neem*, calcium hydroxide, and least with aloe vera. Bhardwaj et al.³⁵ compared the antimicrobial efficacy of *neem*, *tulsi*, Guduchi extract, and chlorhexidine against *E. faecalis*, when used as an intracanal medicament and showed that the reduction in the bacterial count is maximum for chlorhexidine followed by *neem*, *tulsi*, and least by Guduchi. The plausible reason for better antimicrobial activity of CHX in comparison to *neem* could be increased diffusion of medicament into the dentinal tubules and its substantivity. In the present study also, *neem* has shown better results than *tulsi*. *Neem* contains different active phytoconstituents, such as, alkaloids, glycosides, terpenoids, steroids, and tannins.³⁶ Its antimicrobial activity is due to nimbidin and nimbolide, which causes lysis of the bacterial and fungal cell wall. Vinothkumar et al.¹⁰ showed that *neem* leaf extract had a significant antimicrobial efficacy against *E. faecalis* and *C. albicans* when compared to 5.25% sodium hypochlorite. Ghonmode et al.³⁰ showed that *neem* leaf extract showed significantly greater zones of inhibition when compared to 3% sodium hypochlorite and microbial inhibition potential of *neem* extract opens perspectives for its use as an intracanal medicament. *Tulsi* has shown the least antimicrobial activity in the present study.³⁷ Chandrappa et al.² assessed the antimicrobial activity of herbal medicines (*tulsi* extract, *neem* extract) and CHX against *E. faecalis* and showed that herbal extracts (*neem*, *tulsi*) can be used alternatively as endodontic irrigants/medicaments.⁴

CONCLUSION

Therefore, within the limitation of this study, it can be concluded that herbal products have shown significant antimicrobial activity in primary endodontic infections when compared to 2% CHX. Hence, they can be safely used as endodontic irrigants and medicaments.

MANUFACTURER NAME

Sahyog Herbal Pvt. Limited, India

REFERENCES

- Shruti S, Rashmi N, Hemant A. Comparative evaluation of propolis metronidazole with chlorhexidine, calcium hydroxide and *Curcuma longa* extract as intracanal medicament against *Enterococcus faecalis*. An *in vitro* study. J Clin Diagn Res 2015;9(11):ZC19–ZC21.
- Chandrappa PM, Dupper A, Tripathi P, et al. Antimicrobial activity of herbal medicine (tulsi extract, neem extract) and chlorhexidine against *Enterococcus faecalis* in endodontics: an *in vitro* study. J Int Soc Prevent Communit Dent 2015;5(2):89–92.
- Saxena D, Saha SG, Saha MK, et al. An *in vitro* evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against *Enterococcus faecalis*. Indian J Dent Res 2015;26(5):524. DOI: 10.4103/0970-9290.172080.
- Arora T, Kang RS, Mann JS, et al. Antimicrobial activity of herbal extracts against recalcitrant endodontic pathogens. An original *in vitro* study. Saint Int Dent J 2015;1(1):28–32. DOI: 10.4103/2454-3160.161798.
- Murray PE, Farber RM, Namerow KN, et al. Evaluation of morinda citrifolia as an endodontic irrigant. J Endod 2008;34(1):66–70. DOI: 10.1016/j.joen.2007.09.016.
- Mistry KS, Sanghvi Z, Parmar G, et al. Antibacterial efficacy of *Azadirachta indica*, *Mimusops elengi* and 2% chlorhexidine gluconate on multispecies dentinal biofilm. J Conserv Dent 2015;18(6):461–466. DOI: 10.4103/0972-0707.168810.
- Babaji P, Jagtap K, Lau H, et al. Comparative evaluation of antimicrobial effect of herbal root canal irrigants (*Morinda citrifolia*, *Azadirachta indica*, *Aleo vera*) with sodium hypochlorite: an *in vitro* study. J Int Soc Prevent Communit Dent 2016;6(3):196–199.
- Rahimi S, Janani M, Lotfi M, et al. A review of antibacterial agents in endodontic treatment. Iran Endod J 2014;9(3):161–168.
- Sedigh-Shams M, Badiie P, Adl A, et al. *In vitro* comparison of antimicrobial effect of sodium hypochlorite solution and Zataria multiflora essential oil as irrigants in root canals contaminated with *Candida albicans*. J Conserv Dent 2016;19(1):101–105. DOI: 10.4103/0972-0707.173212.
- Vinothkumar TS, Rubin MI, Balaji L, et al. *In vitro* evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent 2013;16(2):167–170. DOI: 10.4103/0972-0707.108208.
- Samak G, Vasudevan DM, Kedlaya R, et al. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against enteric pathogens. Indian J Med Sci 2001;55:434–438.
- Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: an *in vitro* study. Indian J Dent Res 2010;21(3):357–359. DOI: 10.4103/0970-9290.70800.
- Bazvand L, Aminozarbian MG, Farhad A, et al. Antibacterial effect of triantibiotic mixture, chlorhexidine gel, and two natural materials propolis and aloe vera against *Enterococcus faecalis*: An *ex vivo* study. Dent Res J 2014;11(4):469–474.
- Kandaswamy D, Venkateshbabu N. Root canal irrigants. J Conserv Dent 2010;13(4):256–264. DOI: 10.4103/0972-0707.73378.
- Bhargava K, Kumar T, Aggarwal S, et al. Comparative evaluation of the antimicrobial efficacy of neem, green tea, triphala and sodium hypochlorite: an *in vitro* study. J Dent Res Rev 2015;2(1):13–16. DOI: 10.4103/2348-2915.154638.
- Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58(6):329–341. DOI: 10.1111/j.1875-595X.2008.tb00354.x.
- Siqueira JF Jr, Batista MM, Fraga RC, et al. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod 1998;24(6):414–416. DOI: 10.1016/S0099-2399(98)80023-X.

18. Schäfer E. Irrigation of the root canal. ENDO (Lond Engl) 2007;1(1):11–27.
19. Abraham S, Raj JD, Venugopal M. Endodontic irrigants: a comprehensive review. J Pharm Sci Res 2015;7(1):5–9.
20. Rani A, Thakur S, Gupta S, et al. Comparative evaluation of antimicrobial activity of different herbal extracts and 2% chlorhexidine gluconate against *E. faecalis* & *C. albicans*: an in vitro study. Ind J Dent Sci 2015;7(1):20–23.
21. Kanisavaran ZM. Chlorhexidine gluconate in endodontics: an update review. Int Dent J 2008;58(5):247–257. DOI: 10.1111/j.1875-595X.2008.tb00196.x.
22. Delany GM, Patterson SS, Miller CH, et al. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol 1982;53(5):518–523. DOI: 10.1016/0030-4220(82)90469-8.
23. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J 2009;42(4):288–302. DOI: 10.1111/j.1365-2591.2008.01540.x.
24. Siqueira JF, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32(5):361–369. DOI: 10.1046/j.1365-2591.1999.00275.x.
25. Ba-Hattab R, Al-Jamie M, Aldreib H, et al. Calcium hydroxide in endodontics: an overview. Open J Stomatol 2016;6(12):274–289. DOI: 10.4236/ojst.2016.612033.
26. Cvek MI, Hollender LA, Nord CE. Treatment of non-vital permanent incisors with calcium hydroxide. VI. A clinical, microbiological and radiological evaluation of treatment in one sitting of teeth with mature or immature root. Odontol Revy 1976;27(2):93–108.
27. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66(8):1375–1379. DOI: 10.1177/00220345870660081801.
28. Badole GP, Bahadure RN, Kubde R. Herbal medicines in endodontics: an overview. J Dent Oral Disord 2016;2(9):1–7.
29. Agrawal A, Srikumar GPV. Herbal root canal irrigants – a review. Ann Essen Dent 2016;8(3):7c–10c.
30. Ghonmode WN, Balsaraf OD, Tambe VH, et al. Comparison of the antimicrobial efficacy of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *Enterococcus faecalis* – an in vitro study. J Int Oral Health 2013;5(6):61–66.
31. Hedge V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, prpolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* - an in vitro study. Endodontology 2013;25(2):38–45.
32. Mishra N, Logani A, Shah N, et al. Preliminary ex-vivo and an animal model evaluation of *Ocimum sanctum*'s essential oil extract for its antibacterial and anti-inflammatory properties. Oral Health Dent Manag 2013;12(3):174–179.
33. Prabhakar AR, Vallu VVRKM, Yavagal C. *Ocimum sanctum* as an intracanal irrigant in contemporary paediatric endodontics – an in vivo study. Int J Oral Health Med Res 2015;2(4):6–9.
34. Kusuma CS, Manjunath V, Gehlot PM. Comparative evaluation of neem, aloe vera, chlorhexidine and calcium hydroxide as an intracanal medicament against *E. faecalis* - an in vitro study. J Clin Diagn Res 2018;12(3):ZC21–ZC25. DOI: 10.7860/JCDR/2018/29382.11293.
35. Bhardwaj A, Srivastava N, Rana V, et al. How efficacious are neem, tulsi, Guduchi extracts and chlorhexidine as intracanal disinfectants? A comparative *ex vivo* study. Ayu 2017;38(1-2):70–75. DOI: 10.4103/ayu.AYU_72_16.
36. Mistry KS, Sanghvi Z, Parmar G, et al. The antimicrobial activity of *Azadirachta indica*, *Mimusops elengi*, *Tinospora cardifolia*, *Ocimum sanctum* and 2% chlorhexidine gluconate on common endodontic pathogens: an *in vitro* study. Eur J Dent 2014;8(02):172–177. DOI: 10.4103/1305-7456.130591.
37. Singhal A, Gurtu A, Guha C. Comparison of antimicrobial efficacy of conventional irrigants and herbal products alone and with calcium hydroxide against *Enterococcus faecalis*. Guident 2012;5(2):77–79.