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Comparative evaluation of the antimicrobial efficacy of five different herbal extracts as root canal irrigants - an ex vivo study

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Abstract

Introduction: The undesirable side effects caused by synthetic drugs used as canal irrigants have prompted researchers to look for alternatives. The herbal alternatives for endodontic usage might prove to be advantageous.

Aim: To check the antimicrobial efficacy of Neem, Green Tea, Triphala, Babool, Tulsi and sodium hypochlorite against the endodontic microflora.

Materials and Methods: Mixed cultures of bacteria were isolated from carious teeth associated with periapical radiolucencies by inserting paper point into the canal. These paper points were cultured and agar diffusion test was done for the different irrigants. The irrigants are divided into Group I: Neem, Group II: Green Tea, Group III: Triphala, Group IV: Tulsi, Group V: Babool

Herbal extracts were prepared by soxhlet method.

These plates were incubated for 24 h at 37°C in an incubator. After incubation, plates were assessed for zones of inhibition.

Statistical analysis was done by one-way analysis of variable and compared by the Mann Whitney U test.

Result: Sodium hypochlorite showed the maximum antimicrobial activity, followed by neem. This was followed by Triphala and babool, whereas tulsi and green tea showed the lowest values.

Conclusion: Within the limitations of this study, 3% NaOCl and Neem showed maximum antibacterial activity against the endodontic microflora.

Keywords: Herbal extracts, root canal irrigants, antimicrobial efficacy

Keymessage: Herbal alternatives used for root canal irrigation will prove to be preferable considering the various unenviable properties of NaOCl.

Introduction

Microorganisms play an essential role in dental caries the most common dental pathology. If left untreated, it eventually leads to pulp and periapical inflammation. Hence it is crucial to disinfect the root canal during pulp therapy for a successful outcome. In contemporary endodontics, chemomechanical preparation associated with antiseptic medication has been recommended for infection control. Despite this, residual microorganisms

may persist in root canals.(1) As the microflora present in root canal space is of the vast spectrum, various therapeutic and chemical solutions are used in disinfection of the root canal. The gold standard is sodium hypochlorite (NaOCl), and other commonly used are 2% solution of chlorhexidine and calcium hydroxide, which vary in their antibacterial activity. 2

An irrigant serves to flush out debris from within the instrumented root canals, dissolve organic tissue remnants, disinfect the root canal space, and provide lubrication during instrumentation without causing irritation to biological tissues.(3) Sodium hypochlorite has gained popularity since its introduction in 1936 by Walker.(4) It's superior properties of tissue dissolution and antibacterial efficacy make it the irrigating solution of choice for treatment of teeth with pulp necrosis. However, it has a few shortcomings like high toxicity(5), reduction in elastic modulus as well as flexural strength of dentin, corrosive to instruments, [6] unpleasant taste and inability to remove the smear layer.[7] There is a constant increase in antibiotic-resistant strains and in the side effects caused by synthetic drugs.

Herbal medicines have a long history of their use. Due to their safe and promising results, they are studied widely to replace contemporary synthetic drugs. Herbal medicine is defined as a plant- derived substance which contains raw ingredients from one or multiple plants with therapeutic uses (WHO). [8] The advantageous reasons for using herbal irrigants are low toxicity and lack of microbial resistance and easy availability. [9]

Neem or Azadirachta indica has shown to be effective against various microorganisms found in the oral microflora such as Enterococcus faecalis and Candida albicans. Its excellent antibacterial properties make it a material of choice for root canal irrigation and also an alternative to harmful chemical irrigants such as NaOCl. [10, 11]

Green tea polyphenols are the traditional and most widely consumed beverage of China and Japan, obtained from Camellia sinensis. [12] Cathechins and the flavins are present in GTPs, and they are considered as microbiologically active against Gram-positive and Gram-negative bacteria, and E. fecalis [13]

Triphala consists of Terminalia chebula Terminalia bellerica and Emblica officinalis, the three medicinal plants which are dried and powdered to get an Indian Ayurvedic herbal formulation. [14] It has a potential of antibacterial activity against enteric pathogens, [15] and also has anti- inflammatory activity. [16]

Ocimum sanctum, popularly known as Tulsi is a time-tested premier medicinal herb that is used in ayurvedic medicine since ancient times. It has made an important contribution to modern research due to its large number of medicinal properties.

Different parts of the plant have shown antimicrobial, anti-inflammatory, analgesic, antipyretic, antiulcer, antidiabetic, antioxidant and anticancer activity. [17]

Acacia nilotica (Babool) A. nilotica as reported in literature possesses good antimicrobial, antioxidant, antifungal, antiviral and antibiotic activity. Khan et al. [18] in their study have proved the antibacterial action of extract of babool against S. mutans and E. faecalis. In another study, the authors reported that A. nilotica at a 50% concentration, had the highest activity against E. faecalis [19].

This study was carried out to evaluate and compare the efficacy of five different herbal extracts as root canal irrigants.

Materials and Methods

The disease-free, fresh, young, and green leaves were collected from the neem, tulsi and babool plants. The fresh

leaves were harvested, properly washed in tap water, and rinsed in sterile distilled water. (Fig 1). They were shade dried for 2 weeks in a cool and dry place. Green tea (Lipton™) and Triphala powder (Patanjali™) were used for the study. It was coarsely powdered with the help of a blender. (Fig 2) The coarse powder of leaves was then exhaustively extracted in a Soxhlet apparatus. In this extraction process, accurately weighed 60 g of powdered leaf sample was extracted with 500 ml methanol. This process was repeated until the residual marc got exhaustively extracted and finally extracts were pooled and evaporated in rota-evaporator. The extracts were concentrated under partial vacuum at 80°C to dryness, leaving behind thick semi-solid residue. This extract was dissolved in 2ml of 10 % dimethyl sulfoxide (DMSO) to obtain an equal concentration and was stored in a refrigerator until used. (Fig 3)

Cultures of bacteria were isolated from patients reporting to Department of Pedodontics and Preventive Dentistry, Rural Dental College Loni with carious teeth associated with dentoalveolar radiolucencies. This was thus a mixed culture.

Coronal access was gained to the root canals of the involved teeth under rubber dam isolation. A paper point was inserted into the canal and held there for 3–5 min to obtain a sample of the mixed culture of microorganisms. This paper point was then immediately transferred to the sterile Muller Hilton agar plates. These agar plates were taken to the Biotechnology Department for further processing. Bacteria were grown overnight at 37°C on agar plates for 24 h.

To check the antimicrobial efficacy of herbs and 3% NaOCl:

Agar disk diffusion method was performed. The irrigants were divided into

- Group I: Neem (60mg/ml in 10% DMSO),
- Group II: Green Tea Polyphenols (60mg/ml in 10% DMSO),
- Group III: Triphala (60 mg/ml in 10% DMSO),
- Group IV: Tulsi (60mg/ml in 10% DMSO),
- Group V: Babool (60mg/ml in 10% DMSO),

Agar plates were prepared, and cultures (200 µl) were spread on the agar plates. (Fig 4) Depending upon the group, irrigants were pipetted into the filter paper disks placed on agar media. (Fig 5) Two subgroups (50µl and 100 µl) were created within each group depending upon the quantity of the irrigant that had been pipetted. (Fig 6) These plates were incubated for 24 h at 37°C in an incubator. After incubation, plates were assessed for zones of inhibition. (Fig 7)

Statistical analysis

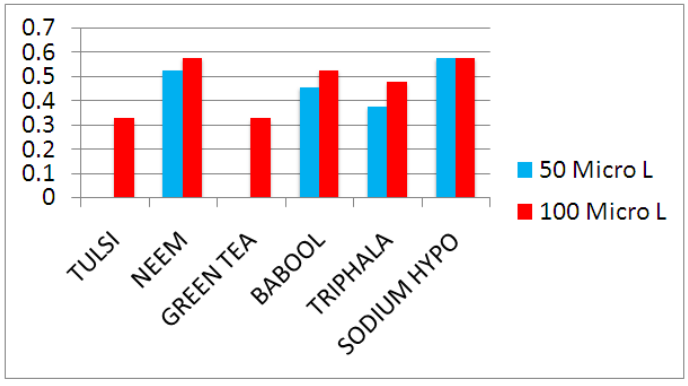
Statistical analysis was done by one-way analysis of variable and compared by the Mann Whitney U test.

Results

Sodium hypochlorite showed the maximum antimicrobial activity, followed by neem. There was no statistical difference between NaOCl and neem. This was followed by Triphala and babool, whereas tulsi and green tea showed the lowest values. There was a statistical difference between the efficacy of NaOCl and Triphala, babool, green tea, and tulsi. The efficacy of neem was also significantly more than that of Triphala, babool, green tea, and tulsi. At lower concentration, there was no efficacy of green tea and babool.

NO OF SAMPLE - 5			
50 MICRO ML		100 MICRO ML	
ZONE OF INHIBITION PLATE NO 1	VALUE	ZONE OF INHIBITION PLATE NO 1	VALUE
TULSI EXTRACT	0	TULSI EXTRACT	0.325
NEEM EXTRACT	0.525	NEEM EXTRACT	0.575
GREEN TEA EXTRACT	0	GREEN TEA EXTRACT	0.325
BABOOL EXTRACT	0.455	BABOOL EXTRACT	0.525
TRIPHALA EXTRACT	0.375	TRIPHALA EXTRACT	0.475
SODIUM HYPOCHLORITE	0.575	SODIUM HYPOCHLORITE	0.575

Case Record Format							
NO OF SAMPLE – 5							
Zone of inhibition plate	**TULSI**	**NEEM**	**GREEN TEA**	**BABOL**	**TRIPHALA**	**SODIUM HYPO**	**MEAN**
50 micro ml	0	0.525	0	0.455	0.375	0.575	0.321
Standard deviation	0	0.14425	0	0.094752	0.0381838	0.1796051	
100 micro ml	0.325	0.575	0.325	0.525	0.475	0.575	0.466
Standard deviation	0.0997021	0.077075	0.099702	0.041719	0.006364	0.0770746	
Mann Whitney U Test value	not comparable	1.985	not comparable	3.16	8.42	1.66	
P Value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	



Discussion

The major goal of root canal treatment is to clean canal by considering biological, chemical, and mechanical objectives. [20] After mechanical debridement, a biofilm may remain undisturbed in the anatomically challenging areas such as fins, lateral or furcal canals, apical deltas, webs, and isthmus. Effective disinfection in endodontics is only achieved by augmenting mechanical preparation with antimicrobial irrigants. [3]

NaOCl has been considered as an irrigant of choice for root canal irrigation because of its antimicrobial activity and tissue-dissolving capacity. High pH of NaOCl interferes with the cytoplasmic membrane integrity and causes biosynthetic alterations in cellular metabolism,

attributing to its antimicrobial nature. Tissue-dissolving action and dissolution rate of NaOCl are directly proportional to its concentration.[21] However, not only its actions such as antimicrobial activity, tissue-dissolving capacity, and smear layer remove ability but also the caustic potential and toxicity of NaOCl also increase with the increase in concentration.[22]

Herbal products have been used in dental practice and have become more common now due to their high antibacterial activity, biocompatibility, and antioxidant properties.[23] Herbal and alternative medicine is gaining popularity among the public but as dental practitioners, we have to understand these products and imply them in our clinical practice.[24] The present study evaluated the antibacterial efficacy of herbal irrigants in comparison with 3% NaOCl.

Pulpitis is a polymicrobial infection, so there is a need to use irrigant with high efficacy. In this study, NaOCl showed the best antimicrobial followed by Neem, Triphala, Babool, Tulsi and Green Tea. Saline showed no antibacterial activity. About 3% NaOCl was best among all the groups. Neem was also as efficient as NaOCl against the endodontic microflora. There was no significant difference between the two.

Neem extract has been shown to have a wide spectrum of antibacterial efficacy against Gram-positive, as well as Gram-negative microorganisms. Major crude bitter principle extract of neem has been shown to be the key reason for its antibacterial activity are nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulphide. These active constituents uncouple mitochondrial oxidative phosphorylation; thus, inhibiting the respiratory chain. This results in its anti- adherence activity by altering the bacterial adhesion and the ability of the microorganism to colonize thereby causing a maximum reduction in adherence of *E. fecalis* to dentin.[25,26,27,28]

Triphala is an ayurvedic rasayana consisting of Amulaki (emblica officinalis), Bibhitaki (terminalia bellirica) and Halituki (terminalia chebula) in equal proportions.[24,26] In such formulations, different compounds may be of help in enhancing the potency of the active compounds resulting in an additive or synergistic positive effect. The strong antioxidant activity of triphala may be partially responsible for many of the biological properties.[29] *T. belerica* was the most active antioxidant followed by *E. officinalis* and *T. chebula*. The major ingredients of *T. bellerica* are ellagic and gallic- acid; *E. officinalis* has several gallic acid derivatives including epigallocatechingallate and in *T. Chebula*gallic acid is the major ingredient.[29] The presence of these active ingredients of phenolic nature may be responsible to scavenge the free radicals generated by the bacteria. Tannic acid represents the major constituent of the ripe fruit of *T. chebula, T. belerica* and *E. officinalis*. Earlier studies have reported that tannic acid is bacteriostatic or bactericidal to some Gram-positive and Gram negative pathogens. Prabhakar *et al.*,[9] conducted a study on the antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols (GTP)), MTAD, and 5% NaOCl against *E. fecalis* biofilm formed on tooth substrate in which, Triphala achieved 100% killing of *E. fecalis* at 6 min.[9]

Acacia nilotica (Babool) *A. nilotica* as reported in literature possesses good antimicrobial, antioxidant, antifungal, antiviral and antibiotic activity. Khan et al. [18] in their study have proved the antibacterial action of extract of babool against *S. mutans* and *E. faecalis*. The antimicrobial function is believed to be due to tannins, phenolics compounds, essential oil, and flavinoids and is effective against E-faecalis. [18, 24, 26]

Green tea is very well known for its antioxidant and healing property. Most of the biological activities of green tea, particularly its antibacterial properties, have been associated with the polyphenol catechin fractions which constitute up to 30% of solid green tea leaves.[30] There are four main types of catechins: Epigallocatechin- 3- gallate (EGCG), epigallocatechin, epicatechin- 3- gallate and epicatechin.[31] EGCG is the most abundant of these catechins, comprising about 50% of the catechin pool.[30] EGCG has been shown to cause irreversible membrane disruption in both Gram- positive and Gram- negative bacteria [32] and also to inhibit bacterial DNA gyrase preventing DNA supercoiling and leading to bacterial cell death. EGCG neutralizes toxic end metabolites such as collagenase, protein tyrosine phosphatase; alkaline phosphatase of pathogenic bacteria.[30] Prabhakar *et al*.,[9] showed that GTP can eradicate the *E. fecalis* biofilm formed on tooth substrate in 6 minutes.

Tulsi, herb with high medicinal value is a holy plant which is commonly found everywhere. The biological properties of the plant have been attributed to the presence of active compounds like Ursolic acid, flavonoids (epigenin, orientin, and vicenin),[33] and phenolic compounds (cirsilineol, circimaritin, isothymusin, eugenol).[34] The leaves of Tulsi contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol.[35] Eugenol is the most prominent phytoconstituents present in this plant which may be responsible for antimicrobial activity and also has strong COX-2inhibitor effect which has an analgesic effect.[36]

Dimethyl sulfoxide DMSO was used as a solvent for extracts. DMSO is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of the herb being dissolved. Antibacterial inertness of 10% DMSO was confirmed with the disc diffusion test.[9]

Agar disk-diffusion method Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6mm in diameter), containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. The drawbacks of this method are that it cannot distinguish bactericidal and bacteriostatic effects. Moreover, the agar disk-diffusion method is not appropriate to determine the minimum inhibitory concentration (MIC), as it is impossible to quantify the amount of the antimicrobial agent diffused into the agar. But medium disk-diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. [37]

Due to the undesirable side effects of a chemical used for treatment, there has been a huge interest to revaluate herbal traditional medicine. The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, and no known microbial resistance reported so far. The findings of this present study agreed with those previous studies and supported the findings that herbal extracts can inhibit the growth of microbies. The zones of inhibition of bacterial growth attained by NaOCl were greater than that obtained for other extracts. This indicates that it has the highest efficacy compared to other herbal agents. Neem showed promising results compared to NaOCl, which can be further studied. While triphala and babool had

comparable results. However, tulsi and green tea had significantly fewer values compared to standard. Although these results had some sort of significance as our study emphasized whether herbal products would provide acceptable antimicrobicity in routine endodontic practice as it is well known that herbal products are more biofriendly to human tissues. The effectiveness of these herbal extracts was likely due to their active ingredients, as listed in Table

Herbs	Botanical name	Active ingredient
Neem	Azardirachta indica	Azadirachtin, nimbin, gallic acid, catechin
Babool	Accacia nilotica	Tannins,Alkaloids
Triphala	Amalak (Emblica officinalis), Bibhitaki (Terminalia bellirica), and Haritaki (Terminalia chebula)	Gallic acid, chebulagic acid, and chebulmic acid.
Green tea	*Camellia sinensis*	epicatechins and flavanols, epicatechin gallate
Tulsi	Ocimum sanctum	Eugenol & Essential Oils, Carvacrol, Vitamin A, C, Zinc & Iron

Hence, from the present study, it can be evaluated that, NaOCl was the best antimicrobial irrigant which is followed in descending order by the neem, Triphala, babool, green tea, and tulsi.

Conclusion

- Within the limitations of this study, 3% NaOCl and Neem showed maximum antibacterial activity against the endodontic microflora.
- Triphala and Babool also showed antimicrobial efficacy. Green tea and Tulsi also had antimicrobial properties but it was not much significant.
- Herbal alternatives used for root canal irrigation will prove to be preferable considering the various unenviable properties of NaOCl. Further research is required to recommend the use of herbal alternatives as a root canal irrigant

Limitation of the study

- The present study was an in vitro study with limited sample size.

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Legends Images

Figure 1: Sample collection

Figure 2: Powder Form Of sample

Figure 3: Sample Prepared after Soxhlet

Figure 4: Culturing the Plate

Figure 5: Adding Filter paper to the Culture

Figure 6: Adding sample to filter Paper

Figure 7: Incubated Plates