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ANTIMICROBIAL EFFICACY OF CURCUMA LONGA (TURMERIC), AZADIRACTA INDICA (NEEM) AND SODIUM HYPOCHLORITE AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

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ABSTRACT:

Aim and objectives: This in-vitro study was designed to comparatively evaluate the antimicrobial efficacy of Curcuma longa (Tumeric), Azadiracta indica (Neem) and 3% sodium hypochlorite against *E. faecalis* when used as an endodontic irrigant.

Material and Methods: Agar plates were prepared using brain-heart infusion (BHI) agar. Cultures of E. faecalis were grown in BHI broth at 37°C and agar diffusion test was done for the different irrigants. The irrigants were divided into Group 1: Turmeric in Sterile Distilled Water (T+D), Group 2: Neem in Sterile Distilled Water (N+D),Group 3: Neem in Absolute Ethanol (N+E) Group 4: 3% Naocl (control) Group 5: Absolute Ethanol (control) (E). Plates were inoculated for 24h at 37°C and microbial zones of inhibition were recorded. The experiment was carried out simultaneously on 5 agar plates and the mean zones of inhibition was calculated.

Statistical analysis: Statistical analysis was performed using Kruskal Walis anlysis of variance (ANOVA) test.

Results: Neem extract in Ethanol showed highest zones of inhibition followed by aqueous extract of turmeric and aqueous extract of neem. The zones of inhibition of neem with ethanol were highly significant to 3% Sodium Hypochlorite ($p<0.05$)

Conclusion: Sodium hypochlorite and Ethanolic extract of neem showed maximum antibacterial activity against *E. faecalis* compared to aqueous turmeric and neem extract.

Keywords: *Curcuma longa* (Turmeric), *Azadiracta indica* (Neem), sodium hypochlorite, Endodontic Irrigant, agar diffusion test.

INTRODUCTION:

The eradication of bacteria from the root canals determines the success of endodontic treatment. Root canal treatment comprises of chemo-mechanical preparation of the canals followed by obturation to seal the root canals and prevent microleakage. The process of chemico-mechanical

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preparation makes use of irrigants along with mechanical means for the debridement of the root canals.[1] Endodontic infections are often polymicrobial in nature. Many authors have demonstrated the role of microorganisms in the growth and progress of pulpal and periapical diseases.[2] The commonly encountered endodontic microbes are Gram positive anaerobic cocci and facultative rods, Gram negative anaerobic rods, lactobacillus species.[3] In a study conducted by Lana and Ribeiro-Sobrinho et al. showed that necrotic teeth contained obligate and facultative anaerobes, microaerophilic bacteria and yeast.[4] Amongst these pathogens Enterococcus *faecalis* has gained popularity in endodontic literature because of its association with persistent periapical infections resulting in endodontic treatment failure.[3]

Endodontic irrigating solutions must have qualities such as the ability to penetrate the infected site, to suppress or eliminate microbial growth, and avoid the development of resistance. Sodium hypochlorite (NaOCl) is a commonly used irrigant during root canal therapy and has the ability to destroy a broad range of microbes.[5] However it poses some undesirable properties such as tissue toxicity, risk of emphysema, allergic potential, disagreeable taste and odour. Along with these drawbacks, the rise in antibiotic resistant strains has encouraged researchers to find other herbal alternatives.[3,6]

Herbal remedies have been used since ancient times in folk medicine. Numerous plant extracts have demonstrated the presence of bioactive compounds which have antimicrobial, anti-inflammatory, analgesic and other therapeutic properties.[6-8] Phytochemical extracts of *Cucurma longa* (Turmeric) and *Azadiracta indica* (Neem) have bioactive ingredients such as cucurmin and nimbidin respectively.[9,10] Cucurmin, a phytochemical present in the range of 2-5% is responsible for its therapeutic effects.[11] Whereas Nimbidin, Nimbin, Nimbolide, Azadirachtin, Gallic acid etc. are the phytochemicals present in *Azadiracta indica* (Neem). Amongst these chemicals Azadirachtin, Nimbidin and Nimbinin show antimicrobial property.[12,13]

The aim of this invitro study was to evaluate the antimicrobial efficacy of Curcuma longa, Azadiracta indica and 3% sodium hypochlorite against Enterococcus faecalis when used as an endodontic irrigant.

MATERIALS AND METHODS:

Pure neem powder (Himalaya, India), Turmeric rhizomes, 3% Sodium hypochlorite, Absolute alcohol, Sterile distilled water, E. *faecalis* (ATCC 29212), Brain Heart Infusion(BHI) Broth and Agar plates.

Preparation of turmeric extract: Dried turmeric rhizomes were grounded into a coarse powder. 50mg of coarsely ground turmeric powder was dissolved in 100ml

of sterile distilled water. The mixture was allowed to stand for 7 days with occasional stirring. The mixture was then filtered using whatman filter paper to remove the coarse residues. The aqueous extract obtained was then stored in an airtight coloured container until use.

Preparation of neem extract in sterile distilled water: 50gm of neem powder was dissolved in 100ml of sterile distilled water. The extracts were then filtered through whatman filter paper to remove the residues. The extracts were stored in an airtight coloured container until use.

Preparation of neem extract in Ethanol: 50gms of neem powder was added to 100 ml of absolute ethanol. Mixture was macerated for 1-2 min, extract was filtered. Extraction process was repeated again using residue and 100ml ethanol.

The samples were divided into 5 groups

Group 1: Turmeric in Sterile Distilled Water (T+D)

Group 2: Neem in Sterile Distilled Water (N+D)

Group 3: Neem in Absolute Ethanol (N+E)

Group 4: 3% NaOCl (control)

Group 5: Absolute Ethanol (control) (E)

Agar diffusion test: Cultures of E. *faecalis* were grown and maintained on BHI broth and agar. The cultures were grown overnight at 37°C for 24 hours.

To evaluate the antimicrobial efficacy of Turmeric in sterile distilled water, Neem in sterile distilled water, Neem in absolute ethanol, 3% NaOCl and Absolute Ethanol, Agar diffusion method was used. BHI agar plates were prepared. Wells of 6mm diameter were punched on the agar surface, to which 20 µl of each sample was added. The plates were incubated at 37°C for 24 hours and the zones of inhibition was recorded for each group. The experiment was carried out simultaneously on 5 agar plates and the mean zones of inhibition was calculated.

RESULTS:

The zones of inhibition was measured as shown (figure 1). The results were analysed statistically using Kruskal Walis ANOVA. The values for the means zones of inhibition are summarized (Table 1, graph 1). Neem extract in absolute ethanol and 3% Sodium Hypochlorite showed similar values against E. *faecalis*. When aqueous turmeric extract was compared with aqueous neem extract the latter seemed to exhibit less antimicrobial efficacy. There was significant differences observed between the 3 study groups and 3% NaOCl. But there was no significant difference observed between absolute ethanol and the other groups.

DISCUSSION:

The complete eradication of microorganisms from the root canals is not possible regardless of the antimicrobial properties of

chemomechanical preparation. This could be attributed to the anatomic complexities within the canals or due to the unpredictable vulnerability of different species.[14,15] Phytochemical extracts of plants such as *Cucurma longa* (Turmeric) and *Azadiracta indica* (Neem) have been extensively studied and used in other forms of medicine such as Ayurveda, Unani and Homeopathy. [16]

Many researchers have demonstrated the antimicrobial action of *Cucurma longa* (Turmeric) against different pathogens. [17] It has a wide range of therapeutic actions such as anti-inflammatory, antibacterial and antifungal. [18] The anti-inflammatory action of curcumin (diferuloylmethane) is suggested due to its ability to downregulate the expression of COX-2 enzyme.[19,20] In a study conducted by Kumar H. to comparatively evaluate the antimicrobial efficacy of Curcuma longa, Tachyspermum ammi (ajwain), chlorhexidine (CHX) gluconate gel and calcium hydroxide as intracanal medicaments against Enterococcus faecalis using well diffusion method, it was concluded that C. longa had the potential to be used as an intracanal medicament in endodontic failure cases.[21] In our study the aqueous extract of turmeric exhibited zones of inhibition against *E. faecalis* within the range of 7.5mm to 9.0 mm which was better than mean inhibition zone of neem in distilled water (1.41mm) but was less effective then neem in ethanol and NaOCl. For an irrigant to be effective against biofilms, the action should involve the eradication of the EPS (extracellular polymeric substance) matrix as well as the bacteria, for instance this matrix could act as an additional source of nutrients and/or an appropriate surface promoting cell growth. It has been hypothesized that curcumin causes the perturbation of the GTPase activity of FtsZ assembly which is lethal to bacteria.[22,23]

Authors T. Haukvik and E. Bruzell conducted a study to evaluate the phototoxic effects of curcumin and concluded that aqueous preparations of curcumin were effect in photokilling of both Gram positive and Gram negative bacteria. The antibacterial effect appeared to be dependent on the concentration, radiant exposure, post-irradiation incubation time, bacteria species and pharmaceutical preparation of the turmeric extract. [24] The mechanism by which curcumin causes light induced cell death is unclear, but it may be suggested that the binding of the photosensitizer to the outer membrane of the microbial cell causes the photosensitisation of the cell. Thus opening up avenues for research on the use of C. longa (turmeric) in photodynamic therapy for the effective disinfection of root canals. [25]

Several pharmacological activities and medicinal properties of A. indica (neem) are well known. Neem possess antimicrobial, antioxidant, anti-inflammatory, analgesic properties making it a suitable substance to be used as an irrigant. [10,26,27] In the present

study the ethanolic extract of neem (11.20mm) showed significant antibacterial action against E. faecalis when compared to its aqueous counterpart as demonstrated by different researchers. Authors have demonstrated the antibacterial activity of neem especially against S. mutans and E. faecalis. Ethanloic extracts of neem have been shown to exhibit significant antibacterial properties when compared to their aqueous extracts.[28-30] Some authors have suggested that the *Neem* leaf extract has exhibited significant antimicrobial effect against *E. faecalis* obtained from infected root canal samples and the extract was found to be as effective as compared with 2% sodium hypochlorite.[31] Authors Polaquini and colleagues evaluated the hydrophobicity, biofilm formation and adhesion of aqueous neem extract on C. albicans and demonstrated that the substance exhibited anti-adherence property and prevented colonisation of C. albicans suggesting it as a good alternative to sodium hypochlorite.[32]

Several authors have conducted studies on the antimicrobial activity of irrigation solutions such as 0.5%,1%, 2.5%, 5% sodium hypochlorite.[33],[34] Sodium hypochlorite in full concentration is well known for its antimicrobial action and cytotoxicity.[35],[36] Authors Bothelo and colleagues have demonstrated that neem was highly effective in treatment of periodontal disease thus demonstrating its biocomaptibilty to human PDL fibres. [37] Therefore, the use of neem as an endodontic irrigant can be advantageous due to its biocompatibility and thus not likely to cause injury that might occur due to sodium hypochlorite accidents. To improve patient compliance and acceptance sweetening agents and flavours can be added to alter the taste. [31]

The results obtained in this in vitro study showed that neem leaf extract in ethanol is a viable irrigant against E. faecalis when compared to aqueous turmeric and neem extract with the mean zones of inhibition being 11.20mm, 8.66mm and 1.41 mm respectively. There was no significant difference observed between 3% sodium hypochlorite and ethanolic extract of neem suggesting that neem extract can be used as an herbal alternative to sodium hypochlorite as an irrigant.

CONCLUSION:

Under the limitations of this study, it was concluded that 3% sodium hypochlorite and Ethanolic extract of neem showed maximum antibacterial activity against *E. faecalis* compared to aqueous turmeric and neem extract. Herbal alternatives used for root canal irrigation serve as a good cost effective alternative with fewer side effects and least resistance developed by species.

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TABLES:

Table 1: statistical analysis of the zones of inhibition (mean values)

Groups	Mean
T+D	8.66
N+D	1.41
N+E	11.20
NaOCL	11.02
E	24.40
H-value	17.6668
P-value	0.0005*

GRAPHS:

Graph 1: mean zones of microbial inhibition