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Comparative study of the antimicrobial efficiency of Neem leaf extract , Sodium hypochlorite and Biopure MTAD - An in vitro study

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ABSTRACT:

AIM- The present study was aimed to evaluate alternative inexpensive, simple and effective method for the sanitization of the root canal system. The antimicrobial efficacy of neem leaf extract as irrigant was evaluated and compared with the standard irrigant sodium hypochlorite and Biopure MTAD.

METHODOLGY-Neem leaf extract was prepared from neem leaves and pure ethanol. To check the antimicrobial efficacy of neem leaf extract, Biopure MTAD and 2.5% NaOCl, agar well diffusion method was performed. BHI agar plates were prepared and cultures were spread onto agar plates. The plates were incubated for 24 hours at 37°C aerobically. Following incubation the diameters of zone of bacterial inhibition (clear zone) were measured in millimetre.

RESULTS- Biopure MTAD had the maximum antibacterial efficacy, exhibiting broader zones of inhibition and sodium hypochlorite the least.

CONCLUSIONS - Under the conditions of this study Biopure MTAD offers maximum antibacterial advantage over neem leaf extract and 2.5% NaOCl.

Key words: Brain heart infusion broth, Neem leaf extract, Biopure MTAD, *Enterococcus faecalis*, *Candida albicans*.

INTRODUCTION

Microorganisms and their by products are considered to be the primary etiologic agents in endodontic diseases.¹ Failure, during and after endodontic treatment, are linked to the presence of bacteria in the root canal.²

The *Enterococcus faecalis* (*E. Faecalis*) and *Candida albicans* (*C. albicans*) are known to be important resistant species in infected root canals, and they may cause treatment failures.³ This result hence emphasizes the importance of completely eliminating bacteria from the root canal system.⁴ The most effective way to achieve this aim is by means of instrumentation and irrigation.

Numerous irrigants have been recommended for use in the treatment of root canal infections. Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics by Walker in 1936.⁵

However, unpleasant taste and Odour⁶, toxicity⁷, resorption⁸, inability to remove smear layer and fully eradicate microbes from the infected canals⁹ are the main disadvantages of this popular irrigant.

Recently a new irrigant called MTAD (a mixture of tetracycline isomer, an acid and a detergent) has been introduced into the market¹⁰ as a final irrigant to be used after NaOCl for disinfecting the root canal.

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The results of some investigations have shown that MTAD can effectively remove the smear layer and abolish *E. faecalis*,^{11,12} though some researchers are still adamant that NaOCl is more effective.^{13,14}

The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs have prompted researchers to look for herbal alternatives.

The literature has shown that neem (*Azadirachta indica*) has antimicrobial and therapeutic effects, suggesting its potential to be used as an endodontic irrigant,¹⁵ but there is lack of documentation or data regarding neem research in endodontics

AIMS & OBJECTIVES

The purpose of this in-vitro study was to compare the antimicrobial activity of Biopure MTAD, 2.5% sodium hypochlorite and neem leaf extract against *Enterococcus faecalis*, *Candida albicans* and to assess the antimicrobial property of neem leaf extract against endodontic pathogens, using the agar diffusion method.

MATERIALS & METHODS

Neem leaf extract, 2.5% sodium hypochlorite, Biopure MTAD, absolute ethanol, *Enterococcus faecalis* (ATCC 29212), *Candida albicans* (ATCC 10231) culture were the materials used in this study.

PREPARATION OF HERBAL EXTRACTS

25gms of fresh neem leaves were added to 50ml of absolute ethanol. Mixture was macerated for 1-2 minutes, then extract was filtered through muslin cloth for coarse residue. Extraction process was repeated again using coarse residue and 25ml ethanol.

Both the extracts were pooled together and filtered through fast filter paper. Alcohol part was removed from the extract on water bath till the volume was about 25ml. Extract was prepared and stored in airtight amber coloured container.

Agar - diffusion test

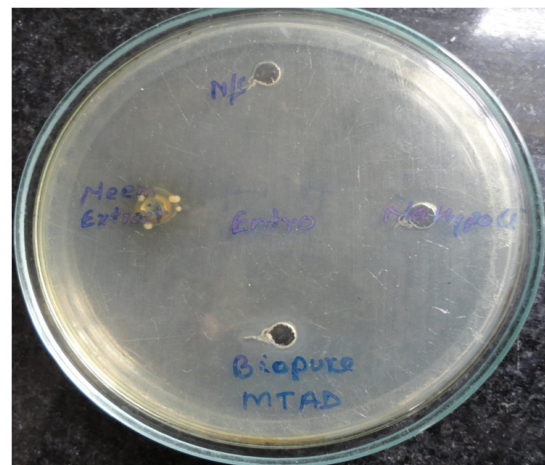
Cultures of *E. faecalis*, *C. albicans* were maintained on brain heart infusion (BHI) broth and agar.

Cultures grown overnight at 37°C in brain heart infusion (BHI) broth on a rotary shaker (150 rpm) and bacterial growth was checked by changes in turbidity after 24hrs.

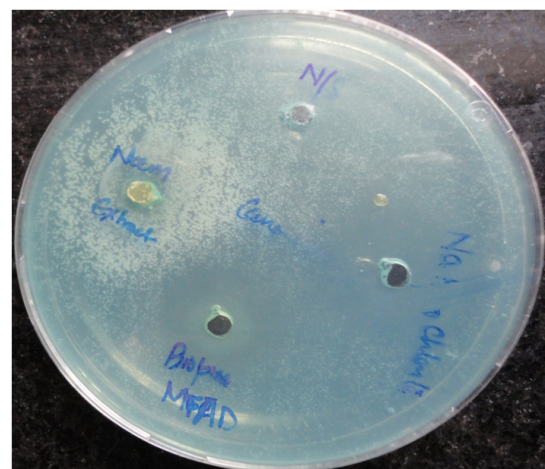
To check the antimicrobial efficacy of herb, 2.5% NaOCl, Biopure MTAD, agar well diffusion method was performed.

BHI agar plates were prepared and cultures (200µl) were spread on agar plates. Wells of 6mm diameter were made in the agar surfaces.

Biopure MTAD, Neem leaf extract, sodium hypochlorite and control, each 50µl, were added to the respective wells and the plates were incubated for 24hrs at 37°C in an incubator. Control used in the study was ethanol. After incubation period, plates were removed and zones of inhibition were recorded.



Zone of inhibition against enterococcus faecalis



Zone of inhibition against candida albicans

RESULTS

The results were tabulated and statistically analyzed using analysis of variance (ANOVA). In table, ANOVA shows that there is significant difference between the zone diameters of *Biopure MTAD* and others against *E.faecalis* and *C.albicans* ($p<0.05$). No significant difference was observed between zone of inhibition for Neem leaf extract and 2.5%NaOCl.

Table : Zone of Inhibition

Zone diameter (mm)	<i>E.faecalis</i> (Mean±SD)	<i>C.albicans</i> (Mean±SD)
Biopure MTAD	24.67±1.15	12.50±0.50
2.5%NaOCl	17.67±0.58	9.50±0.50
Neem leaf extract	18.67±0.58	10.97±0.45
Ethanol	0.00±0.00	0.00±0.00
Statistical Analysis (P value)	P<0.05 Significant	P<0.05 Significant

DISCUSSION

In this study, all the tested irrigants were shown to inhibit and eliminate the tested strains.

Torabinejad et al (2003) showed that MTAD was significantly more effective than NaOCl in killing *E. faecalis*.¹⁶

Soley Arslan et al (2011) showed that MTAD was more effective in lower concentrations in both *C. albicans* and *E. faecalis* than NaOCl.¹⁷ This superior antimicrobial effect of MTAD was also shown in this study.

Biopure MTAD also showed a high antimicrobial activity than Neem extract. The superior bactericidal effect of Biopure MTAD may have been caused by a carry over effect of doxycycline. Furthermore, the antimicrobial effect of doxycycline against several oral pathogens was shown in previous studies.¹⁸⁻²⁰

Neem is a biocompatible antioxidant and not likely to cause severe injuries to patients. Thus it might be advantageous to use neem as root canal

irrigant.²¹ Neem is bitter in taste and can be altered by different formulations due to addition of sweeteners and flavours to increase the patient compliance and acceptability.²²

The results obtained in this in-vitro study showed that Neem leaf extract is a viable medicament against *C. albicans*, *E. Faecalis* and supports Bokhara and co-workers who concluded that Neem leaf extract has a significant antimicrobial effect against *Enterococcus faecalis* and *Candida albicans*.

CONCLUSION

Within the limitations of this study, Biopure MTAD showed the best results amongst the three solutions used. Neem leaf extract showed statistically significant activity against *Enterococcus faecalis* and *Candida albicans*. Further research is needed to conclusively recommend herbal solutions as a root canal irrigant.

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