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Comparative Analysis of Ozonated Oil and Neem Extract as a Root Canal Irrigant – An In-Vitro Study

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Abstract: Ozone, an allotropic form of oxygen occurring naturally in the Earth's atmosphere has a wide range of applications in Dentistry. Ozone oils can be used to sterilize the root canal systems by virtue of ozone's bactericidal and effervescent properties. Neem (azadirachta indica) twig has antimicrobial and therapeutic effects suggesting its potential to be used as an endodontic irrigant, but there is scanty documentation regarding the comparison of efficacy of neem extract and ozonated oil against a common root canal pathogen, Enterococcus fecalis (E. fecalis) . Thus this research focuses on testing and comparing the efficacies of neem extract and ozonated oil against E. fecalis.

Aim: Evaluation of the efficacy of neem extract and ozonated oil as a root canal irrigant against root canal pathogen Enterococcus fecalis (E. fecalis).

Materials and Methods: A total of 45 samples of the bacterial strain of E. faecalis were incubated on Mueller Hinton agar plates. The antimicrobial activity of Ozonated oil and Neem extract was evaluated against E. faecalis by the agar well diffusion technique. 3% Sodium hypochlorite was used as a positive control. Zone of inhibition was observed and compared with that of Ozonated oil and Neem extract.

Results: Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). A highly significant difference between the diameters of the zone of inhibition of neem extract and ozonated oil against E. faecalis was observed ($p < 0.001$). The diameter of zone of inhibition for neem against E. faecalis appeared to be greater as compared to ozonated oil.

Conclusion: Neem extract and ozonated oil have a significant antimicrobial effect against *E. faecalis* and can be used as root canal irrigants. Neem extract has a greater microbial inhibition potential as compared to ozonated oil for its use as a root canal irrigant.

Keywords: Neem extract, Ozonated oil, Sodium hypochlorite, E. fecalis, Root canal irrigant

Introduction

A detailed literature search from the past has revealed a possible role of E. faecalis for post treatment root canal failures. This has been a proven fact from multiple previous studies.[1-5]

Thus a proper sterilization of root canals during and post-treatment is essential to maintain the integrity of the tooth. Alongwith the use of proper sterilized instrumentation, there is a necessity of adequate use of root canal irrigants

to obtain clean environment of the root canals before obturation.

Sodium hypochlorite has been widely recommended as an irrigant for chemico-mechanical debridement of root canals because of its tissue dissolution and antimicrobial activity, thus making it an irrigating solution of choice. However, it's several undesirable characteristics ranging from severe pain, edema, and necrosis of subcutaneous tissues and mucosa [6] to urticaria, oedema, shortness of breath and bronchospasm [7-9] tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste motivate us to look for novel alternatives.

One such alternative that has engrossed the researchers is Ozone. Ozone occurs in the environment either in gaseous form or as ozonated water. [10] It is a strong antimicrobial agent against bacteria, fungi, viruses and protozoa. [11] Failure of root canal therapy is mainly caused by microorganisms, thus demanding an increased research into clinical application of mild or non-toxic root canal irrigants such as aqueous ozone as well as ozonated oil. [12] Ozonated oils have been used in the past as antiseptics obtained from the chemical reaction between ozone and unsaturated fatty acids of vegetable oils. [13] Recent studies have concluded from their findings a positive and a promising role of ozonated oil against various microorganisms observed in the oral cavity. [13-15]

Apart from ozone, there has been an increasing demand in various herbal root canal irrigants due to their non-toxic nature as well as their economic advantage. Amongst various herbal products, neem extracts have been proven effective as well as non-toxic root canal irrigants which have been time tested and extensively studied. The phytotherapeutic applications of various parts of neem attribute properties like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, analgesic, immunostimulant to it.. [16-19]

Moreover, its anti-adherence activity by altering bacterial adhesion and ability of organism to colonize also stimulated the study of this substance as an anti bacterial agent against E. fecalis. [20-21]

Despite its excellent efficacy against common root canal bacteria like enterococcus fecalis, few undesirable characteristics of sodium hypochlorite warrant extensive research into safer and natural alternatives like ozonated oil and neem extracts. This study attempts to evaluate and compare the efficacy of neem extract and ozonated oil as a root canal irrigant.

Material and methods

The armamentarium used for the present study was as follows:

1. Strains of enterococcus fecalis (ATCC 29212)
2. Ozonated oil
3. Neem extract
4. Sodium hypochlorite
5. Mac Farland's standard
6. Culture media
7. Culture broth
8. Petri dishes
9. Cotton swabs
10. Cork borer
11. Incubator
12. Vernier Calliper

Methodology

The bacterial strain used for the study was E. feacalis (ATCC 29212). The culture was grown overnight in brain heart infusion (BHI) broth at 37°C (FIG- 1**)**.

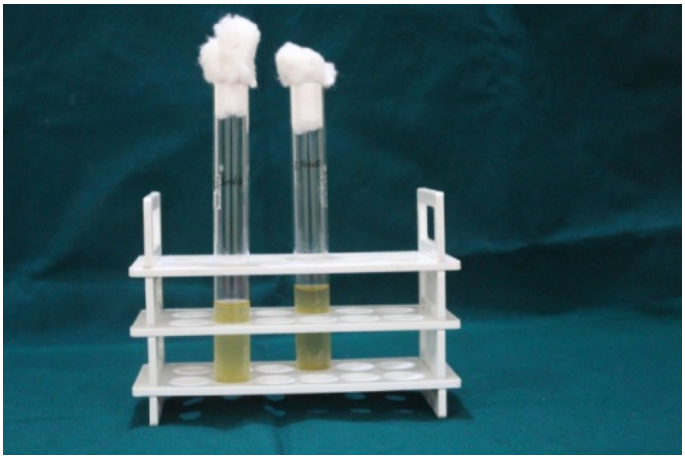

Figure 1: BHI broth with E. faecalis

Broth culture of the test organism was compared to Mac Farland's standard (1.5 × 108 bacteria/ml). Lawn culture of the test organism was made on the Mueller Hinton agar plates using sterile cotton swab and the plates were dried for 15 minutes (FIG- 2,3**)**.

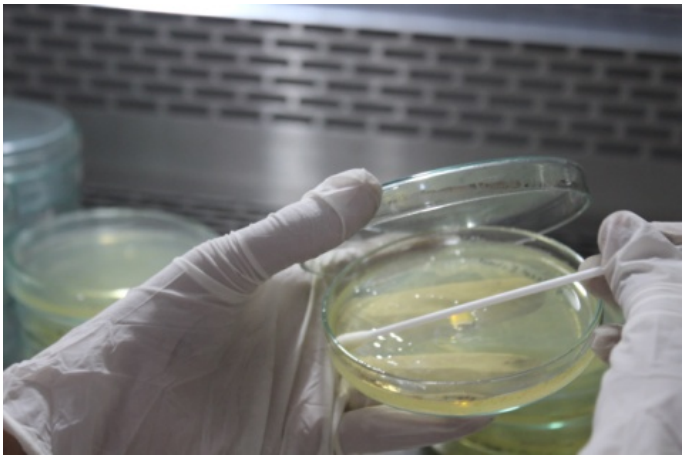

Figure 2: Preparation of lawn culture of test organism

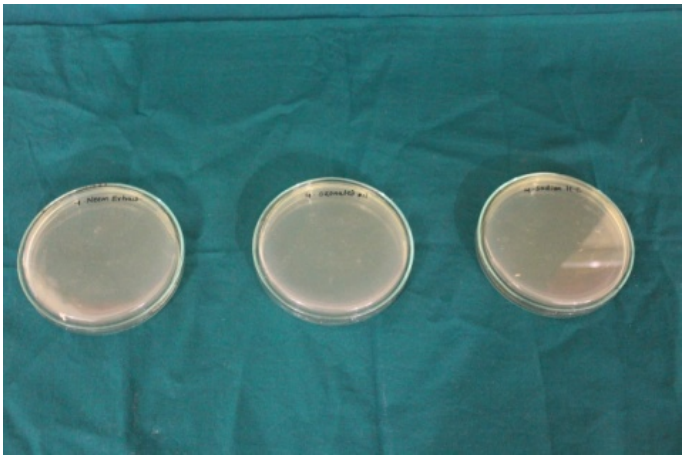

Figure 3: Culture plates with test organism

Wells measuring 4mm in depth were made on the agar using sterile cork borer. Ozonated oil and neem extract were added to the wells on separate plates (15 plates each for ozonated oil and neem extract) (FIG- 4**).**

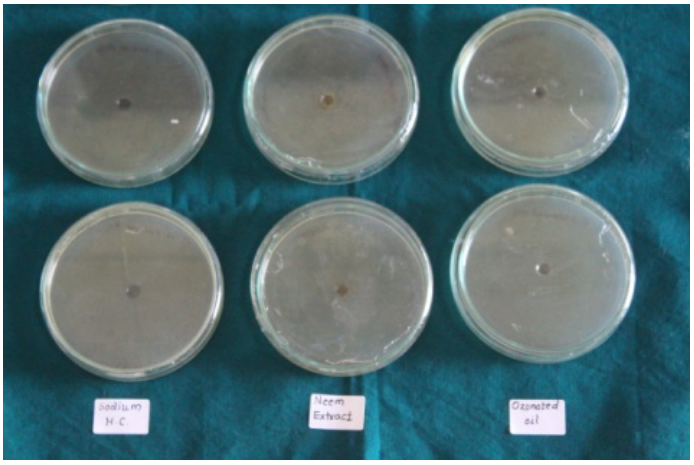

Figure 4: Culture plates with central well containing ozonated olive oil, aqueous neem extract and 3% sodium hypochlorite solution

Neem extract was obtained by a process of reflux where 25g of neem leaves were allowed to boil with 250ml of distilled water and a concentrate of 50 ml was obtained after 24 hours (FIG- 5, 6**).**

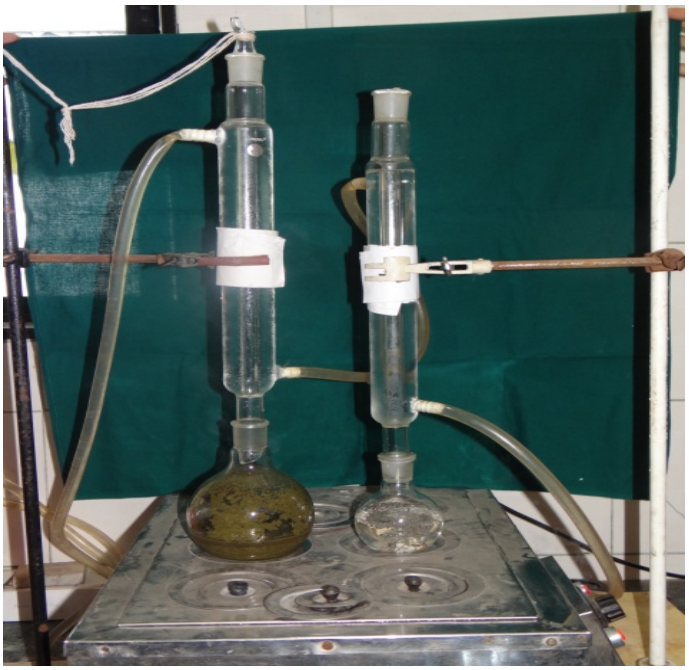

Figure 5: Obtaining of aqueous neem extract by reflux

Figure 6: A concentrate of 50 ml of aqueous neem extract

A concentration of 3% sodium hypochlorite was used as the positive control that was added separately on 15 agar plates (FIG- 3**).** The plates were incubated at 37 degree Celsius for 48 hours. Various zones of inhibition of bacterial growth were observed (clear zone for ozonated oil, yellow zone for neem extract and brownish zone for 3% sodium hypochlorite) which were measured in mm diameter (FIG- 7,8,9**)**

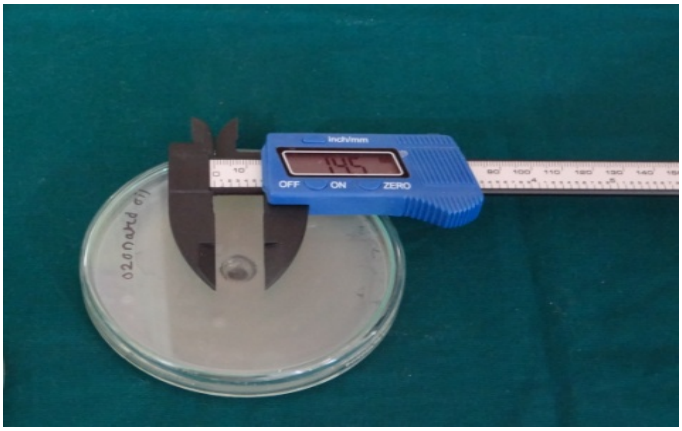

Figure: 7 Clear zone of ozonated oil

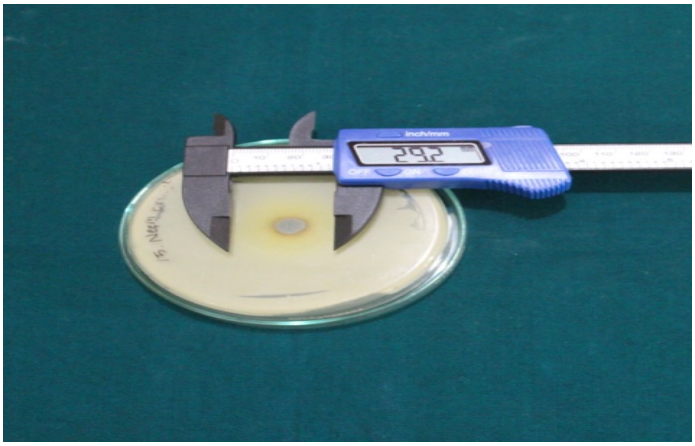

Figure 8: Yellow zone of aqueous neem extract

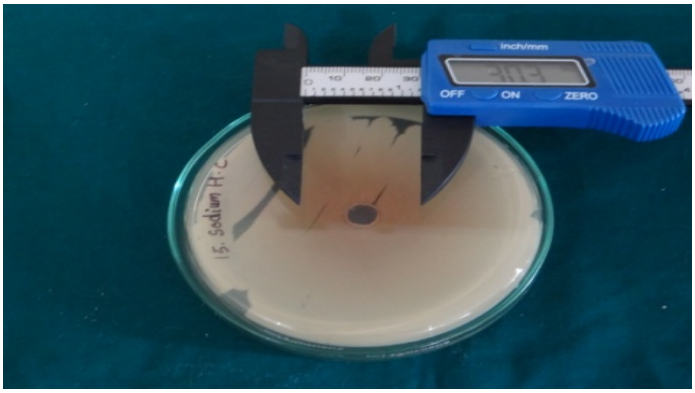

Figure 9: Brownish zone of 3% sodium hypochlorite

All the tests were done in triplicate to minimize the test error. Thus the Antimicrobial activity of Ozonated oil and Neem extract were evaluated against E. feacalis by the agar well diffusion technique.

Results:

The study sample was divided into three groups as follows:

Group 1 – Sodium Hypochlorite (positive control)

Group 2 – Aqueous Neem extract

Group 3 – Ozonated oil

Sodium hypochlorite (Group 1) was used as a positive control and a comparison was made between the efficacy of aqueous neem extract (Group 2) as well as ozonated oil (Group 3) by observing the zone of bacterial growth inhibition. Each sample was incubated on 15 agar plates separately at 37 degree Celsius for 48 hours. The zone of inhibition was measured and statistical analysis was performed.

Statistical analysis

The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data. Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). A highly significant difference between the diameters of the zone of bacterial growth inhibition of neem extract and ozonated oil against E. faecalis was observed ($p < 0.001$) by ANOVA test (Table 1**,** FIG- 10**)**.

As sodium hypochlorite is being used as the gold standard root canal irrigant, the highest zone on bacterial growth inhibition was observed on the agar plates containing 3% sodium hypochlorite (Mean of 29.467mm), followed by neem extract (Mean of 28.733mm) and ozonated oil (Mean of 14.267).

Group	N	Mean	Std. Deviation	F value	P value
Group 1	15	29.467	0.7584	**1363.090**	**<0.001****
Group 2	15	28.733	0.9507		
Group 3	15	14.267	0.9730		
Total	45	24.156	7.1323		

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

Table 1: Comparison of the zone of inhibition when treated with different agents in terms of {Mean (SD)} against E. Fecalis using ANOVA test

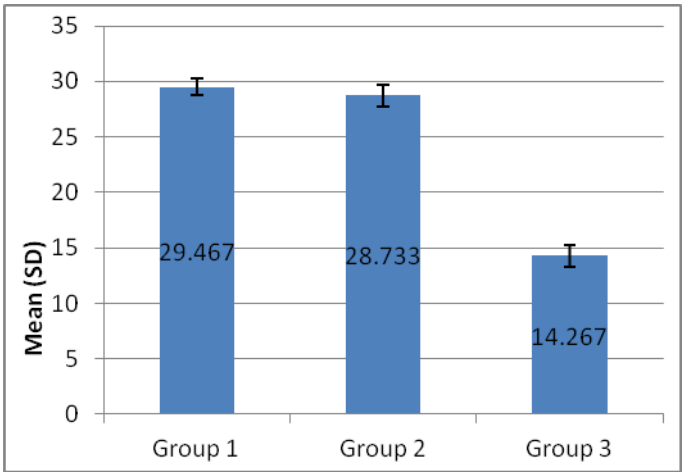

Figure 10: Comparison of the zone of inhibition when treated with different agents in terms of {Mean (SD)} against E. Fecalis using ANOVA test

Discussion

The present study was conducted to check the efficacy of neem extract and ozonated oil on E. faecalis. As E. faecalis is responsible for and is isolated from most of the post treated failed root canal infections [2] , it was chosen for this study to test the efficacies.

The use of Sodium Hypochlorite has been extensive in dentistry since its inception during World War I by the chemist Henry Drysdale Dakin and the surgeon Alexis Carrel for irrigation of infected wounds. [22] The concentration of sodium hypochlorite most effective against microorganisms especially *E faecalis* appeared to be 5.25% in one of the studies from the past [23] , however, lower concentrations of the same, viz. 3% sodium hypochlorite also appeared to be very effective in elimination of *E faecalis.* [24] In the present study, a concentration of 3% was used on bacterial agar plates and an effective zone of inhibition of bacterial growth was observed (Mean of 29.467mm) as compared to ozone and neem extract. Our study is not in conjunction with the study done by Vinothkumar TS et al where they observed the efficacy of neem leaf to be more as compared to sodium hypochlorite against E. faecalis . [25]

A German chemist, Christian Friedrich Schonbein, first discovered ozone in 1840 but it was not

until 1936, that Dr. Fish, a Swiss dentist used ozone either as gas or ozonated water in his dental practice. [26] Further researches were carried out with different treatment modalities using ozonated gas, aqueous ozone and ozonated oil to check the efficacy of ozone in Dentistry till date. [27-32]

Hems RS et al evaluated the potential of ozone as an antibacterial agent using Enterococcus faecalis as the test species and concluded that Ozone had an antibacterial effect on planktonic E. faecalis cells and those suspended in fluid, but little effect when embedded in biofilms. [33]

Adriana M et al, in their study found 77% success rate in cases where ozonized oil was used as the intracanal medication. The results of their study demonstrated that ozonized oil may potentially be used as an intracanal medication. [14]

Montevecchi M et al, investigated the antimicrobial effectiveness of a commercially available ozonated oil (O3-Oil), in comparison with 0.2% chlorhexidine digluconate (CHX) and 10% povidone-iodine (PVP-I) through a disk diffusion test. They concluded the potential applicability of ozonated oil against *Staphylococcus aureus* (Sa) and *Porphyromonas gingivalis in periodontology*. [13]

The present study however compared the efficacy of ozonated oil and neem extract on E. fecalis and observed that ozonated oil has a potential antibacterial effect against E.fecalis but to a lesser extent than neem extract.

Çiğdem Eda Balkan et al, 2016, compared the antimicrobial activities of commercially obtained thyme, rose, centaury and ozone oils against the clinically important bacteria and yeasts. They found no zone of bacterial growth invasion when ozonated oil was used against micro-organisms and concluded that thyme oil had a stronger antimicrobial activity than the rose, ozone and centaury oils. [15]

In the present study, a clear zone of bacterial growth inhibition was found around the well containing ozonated oil using disk diffusion method.

Various studies have been conducted by numerous researchers to evaluate the antimicrobial efficiency of neem leaf extract when used in endodontics.

Hegde *et al*. [34] in 2013 concluded in their study that the neem leaf extract showed the highest zone of inhibition against *E. faecalis* and *C. albicans* while comparing the antibacterial efficacy of 2% sodium hypochlorite, propolis, neem leaf extract, turmeric and liquorice against *E. faecalis* and *C. albicans*, using the agar diffusion method. The results of the present study is not in conjunction with this study. Ghonmode *et al.* [35] in 2013 found that neem leaf extracts showed significantly greater zones of inhibition as compared to grape seed extracts, 3% sodium hypochlorite, and absolute ethanol, against *E. faecalis* culture by performing an agar diffusion test. The present study however compared the efficacy of aqueous neem extract and ozonated oil using 3% sodium hypochlorite as a positive control and found the zone of inhibition of E.fecalis on agar plates to be highest with 3% sodium hypochlorite followed by neem and ozonated oil.

The present study goes in accordance with the studies by Dutta *et al*., Chandrappa *et al*, Babaji *et al* , Sundaram *et al* and Prasad et al [36-40] which suggests an antibacterial efficacy and potential role of ozonated oil and aqueous neem extract in the future of endodontics.

Conclusion

Literature reports from the past support the use of ozonated olive oil and aqueous neem leaf extract as antimicrobial root canal irrigant but the efficacies of both differ according to this study. The present study found that aqueous neem leaf extract possesses greater antimicrobial activity against E. faecalis. Since the present study was performed in vitro, more clinical trials are required for the use of both the agents as root canal irrigants. This research may predict the use of aqueous neem leaf extract with certain additives for palatability as a herbal alternative to the root canal irrigants. Further studies on the role of ozone as an irrigant are also warranted.

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