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Comparative Evaluation of Antibacterial Efficacy of *Aloe Barbadensis Miller*, *Camellia Sinensis, Azadirachta Indica* and Doxycycline with Sodium Hypochlorite as Root Canal Irrigant Against E. *Faecalis*: An *In-Vitro* Study

By Dr. Tanzeem Qureshi, Dr. Mayuri Singh, Dr. Vartika Vashistha, Dr. Amit Singh, Dr. Garvita Sahu & Dr. Rajiv Prajapati

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Keywords: *Enterococcus faecalis, root canal irrigants, doxycycline, sodium hypochlorite, camellia sinensis and azadirachta indica, and aloe barbadensis miller.*

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Comparative Evaluation of Antibacterial Efficacy of *Aloe Barbadensis Miller*, *Camellia Sinensis*,*Azadirachta Indica* and Doxycycline with Sodium Hypochlorite as Root Canal Irrigant Against E. *Faecalis*: An *In-Vitro* Study

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Abstract- ***Aims and objectives:*** Successful root canal treatment involves the complete elimination of pathogenic microorganisms from the root canal system. Enterococcus faecalis is one of them which is most commonly detected in asymptomatic persistent endodontic infections. Chemical irrigation of the root canals along with biomechanical preparation helps in the elimination of such microorganisms. Thus, the present study was done to evaluate and compare the antibacterial efficacy of Doxycycline, *Camellia Sinensis*, *Aloe barbadensis Miller*, *Azadirachta indica* and saline with Sodium hypochlorite as an intracanal irrigant against E.*faecalis*.

Material and Method: Five different intracanal irrigants were selected for this study (*ie.* Doxycycline, *Camellia Sinensis*, *Aloe barbadensis Miller*, *Azadirachta indica,* and normal saline)and their comparison was done with Sodium hypochlorite against E.*faecalis*to check their antimicrobial efficacy. The extracts were prepared, and MIC was determined.A total of 6 wells per plate were made on the agar plate with the help of cork borer. Once wells on plates are made, the testing irrigatingsolutions was pipetted into each well,and then the zone of inhibition was determined after 72hrs by Agar well diffusion method.

Results: The average size of zones of inhibition after 72 hours were: Doxycycline has the highest zone of inhibition (34.00 ± 0.71) followed by *Azadirachta indica*(16.00 ± 0.63), *Camellia Sinensis*(14.00± 0.71), Sodium hypochlorite (10.17 ± 0.98) (positive control), and least zone with *Aloe barbadensis Miller*(8.17 ± 0.75) and no zone of inhibition was observed with normal saline (negative control). Results were subjected to statistical analysis by using *Mann-Whitney U test* and Kruskal-Wallis test.

Conclusion: Tested herbal irrigants like *Aloe barbadensis Miller*, *Camellia Sinensis* and *Azadirachta indica* showed inhibitory zone against E. *faecalis*. Hence, these can be used as root canal irrigating solutions.

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Keywords: Enterococcus faecalis, root canal irrigants, doxycycline, sodium hypochlorite, camellia sinensis and azadirachta indica, and aloe barbadensis miller.

Running Title: Qureshi *et al.* Antibacterial Efficacy of root canal Irrigants against E. *faecalis*.

I. Introduction

The long term success of endodontic treatment completely depends upon the debridement of non-commensal microorganisms from the root canal system.[1] However, due to complex root canal configuration, complete debridement through mechanical instrumentation alone cannot remove the entire bacterial load.Since it is a difficult task to completely eradicate microorganisms from the infected root canal system, intracanal irrigant seems necessary for eradication of infected tissues and microorganisms in addition to mechanical debridement.Moreover, low oxygen tension, less nutrient availability and enormous bacterial interactions lead to predominant colonization of facultative anaerobic species prevailing in the root canals.[2]

Persistence of microorganisms in apical third of the root canals leads to failure of endodontic treatment. *E. faecalis* microorganism is one of them which is the most prevalent (24-77%) gram positive and facultative anaerobe persistently found in root canal failures. Thus intracanal irrigant play's an important role in eliminating residual bacteria after biomechanical preparation and providing a favourable environment for obturation and periapical healing.[3]Nowadays, various irrigants are used for root canal disinfection, which includes sodium hypochlorite, gly-oxide, EDTA (ethyl diamine tetra acetic acid), and citric acid but none of them fulfil the requirement of ideal root canal irrigant.[4]

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An ideal irrigation should be:-

- Nonirritating to periapical tissues.
- Remain stable in solution
- Be active in the presence of blood, serum and protein derivatives of tissue
- Low surface tension
- Prolonged antimicrobial effect
- Not stain tooth structure
- Do not interface with repair of perapical tissues
- Completely remove smear layer
- Disinfect the underlying dentin & its tubules
- Inexpensive
- Dissolve pulp tissue and organic debris
- Should be a good lubricant
- Be systemically non toxic, non caustic to periodontal tissue.

Therefore in the present study various plants extracts were used such as Green tea Aloe-vera, and Neem which has anti-inflammatory, anti-bacterial, anti-fungal, antimicrobialproperty.[5]

Green tea is harvested from leaves of the young *Camellia sinensis* tree. Its antibacterial activity is due to inhibition of the DNA gyrase bacterial enzymes, which actby binding to the ATP binding sites of the ATP subunit. Ramezanali *et al.* found that green tea has antibacterial activity against E. *faecalis*.[6]

Aloe Vera (Aloe barbadensis Miller) is a kind of plant that is well known for its numerous biologic as well as therapeutic functions such as wound healing, hypoglycemic effects, anti inflammatory, immune-modulation features and also antimicrobial properties. It has been proven in several studies that Aloe Vera shows considerable antimicrobial activity against various species such as Enterococcus faecalis, Candida albicans and staphylococcus aureus.[5]

It is well known that neem leaves (*A. indica* or AI) have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, and analgesic effects without any side effect. AI has several active constituents like nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulfide which are responsible for its antibacterial action. AI causes maximum reduction in adherence of *E. faecalis* to dentin.[7]

Although many irrigating solutions are used routinely,the search for biocompatible, non-cytotoxic and therapeutic solution is still on. Thus, the present study was done to evaluate and compare the antibacterial efficacy of Doxycycline, *Camellia Sinensis*, Aloe barbadensis Miller*, Azadirachta indica* and saline with Sodium hypochlorite as an intracanal irrigant against E.*faecalis*

II. Materials and Method

The present *in-vitro* study was done in the Department of Paedodontics and Preventive Dentistry, Hitkarini Dental College and Hospital, Jabalpur, Madhya Pradesh, in collaboration with Daksh Laboratories and Research Center, Jabalpur which was aimed to evaluate the antibacterial efficacy of Green tea (Tetley Green Tea Regular, Tata Global Beverages Ltd., Bengaluru, India), Aloe vera (Fresh Leaves), Neem (Fresh Leaves), Doxcycyline HCL (100mg) (Doxycept Cap, Concept Biosciences (P) Ltd. Baddi Distt.,Solan (H.P), India.) and their comparison was done with Sodium hypochlorite(3%) (Vishal Dentocare Pvt. Ltd., Ahmedabad, Gujarat, India) against E.*faecalis* (ATCC-11700) (Himedia Laboratories Pvt. Ltd.,Mumbai, India).

a) Preparation of test organism and Agar plates

The organism *E. faecalis* strain (ATCC 29212) is obtained from Himedia Laboratories. The vial containing the lyophilized bacteria was opened under complete aseptic conditions, then the test tube containing 5 - 6 ml of Brain Heart Infusion broth, 0.5 ml to 1 ml was pipette out using a sterile pipette and incorporated into vial to rehydrate the pellet. After mixing it well the suspension was transferred to the test tube containing the broth, again it is mixed properly and then several drops of this suspension was used to inoculate the Brain Heart Infusion agar plate and kept for incubation at a temperature of 37° C for 24 hours. Pure cultures were initially identified according to their staining characteristics and ability to produce catalase. By using sterile cotton buds bacteria from broth is spread over the Muller-Hinton agar plate.

b) Preparation of intracanal irrigant's and determination of MIC for each irrigating solution

i. *Preparation of Green Tea Extract*

Green tea extract was prepared by a soxhlet extractor by mixing 10gm of the green tea bag in 250ml ethanol at a temperature of 40°C for 6-7 hrs under reduced pressure in a rotary evaporator. Once concentrated to 10gm volume, the extract was transferred to a pre-weighed beaker and allowed to dry completely in a bench-top oven (30°C) overnight. A final concentration of dried powder of 10gm was added to 500ml of distilled water to obtain a concentration of 0.05mg/ml.[8]

ii. *Preparation of Doxycycline solution*

The enteric-coated hard gelatin capsule shell seal is broken into two halves, and the powdered ingredients were mixed with 100ml of distilled water to make a homogenous aqueous solution of concentrated 1mg/ml.

iii. *Preparation of Neem leaf extract*

100 g of neem leaves were tied in a muslin cloth and socked in 800 ml of distilled water in a beaker. This beaker was boiled under low flame until the extract reduced to 400 ml to obtain a 25% concentration of aqueous neem extract. After the extract cooled, it was filtered using a filter paper and stored for usage.[9]

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iv. *Preparation of Aloe vera extract*

Aloe Vera gel is extracted from the plant's leaves. The leaves of the plants are washed with distilled water, and the surfaces of the leaves are disinfected with 70% ethyl alcohol. After cutting, the fresh pulp was collected and homogenized. 80 gram of the gel was dissolved in 20 milliliters distilled water to prepare 80% Aloe Vera solution.[10]

c) Determination of Minimum Inhibitory Concentration of the Prepared intracanal irrigants

100 µl of each dilution is added to 100 µl of Mueller Hinton broth. 5 µl of bacterial suspension, which is adjusted to 0.5 McFarland, was added, and this mixture is then incubated at 37^{0}C for 24 hrs. After 24 hours, the tubes were visually checked for turbidity. The lowest dilution inhibiting the growth is taken as MIC.

After determination of MIC, the following irrigants were randomly divided into six groups:

Group A= NaOCl (positive group)

Group B=Saline(negative group)

Group C=Green Tea

Group D= Doxycycline HCL

Group E=Aloe vera

Group F=Neem.

d) Placement of intracanal irrigants in Agar plates

A total of 6 wells per plate with a diameter of 6mm and depth 4mm were made equidistant from each other on a prepared agar plate with the help of cork borer. Once wells on plates were made, spreading of bacteria from the nutrient broth was done by using sterile cotton buds, and then 50 µl of the test irrigant solution was pipetted into each well, and then zone of inhibition is determined after 72hrsfor the experimental groups The size of the resulting zones of inhibition were measured by an independent observer with sliding calipers and calculated as follows:

Size of growth inhibition zone = (diameter halo – diameter specimen) / 2.

The results were recorded in terms of average diameter of growth inhibition zone. Each specimen was tested 15 times. (Fig. 1)

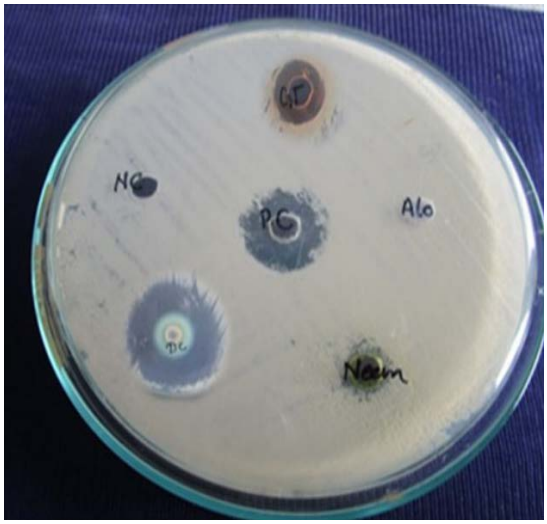


Fig. 1: Zone of inhibition of different irrigating solutions after 72hrs

III. Results

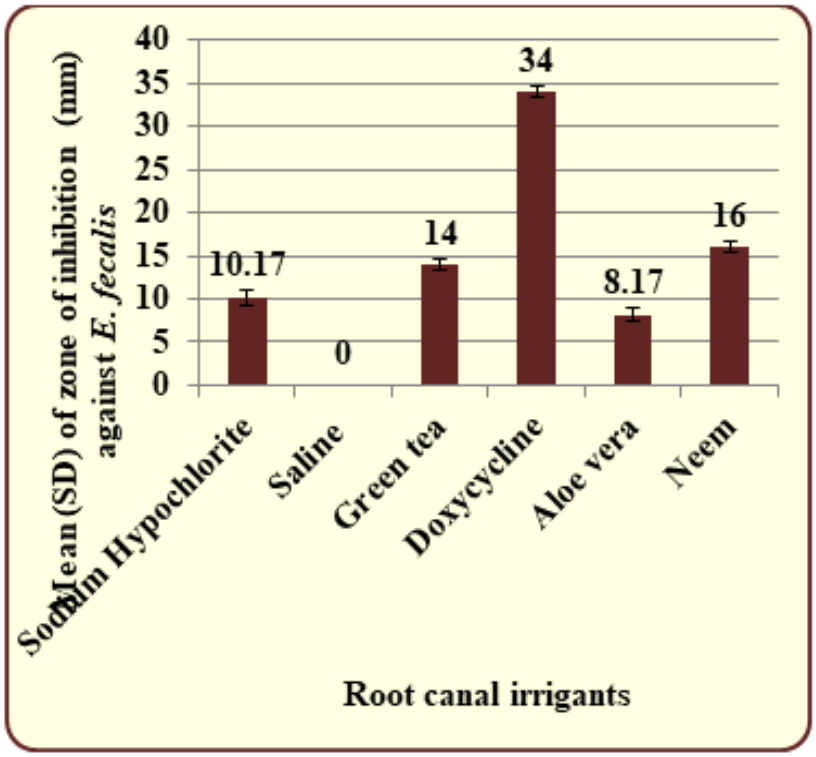
Mean, and standard deviation are calculated for all the root canal irrigants, *i.e.*, Sodium hypochlorite (positive control), saline (negative control), Aloe vera, Neem, Green tea and Doxycycline (Experimental group). Table-1 shows Means of the Zones of inhibition showed by all the root canal irrigants against E. *faecalis.*

In Table-1 and Graph-1, there was a significant difference among the groups as the p-value was <0.001 (Kruskal-Wallis test). Doxycycline had the highest zone of inhibition (34.00 ± 0.71). In herbal groups, Neem had the higher zone of inhibition (16.00 ± 0.63) followed by Green tea (14.00± 0.71) and least in Aloe vera (8.17 ± 0.75). Whereas, there was no significant difference between Aloe vera and Sodium hypochlorite with p-value = 1.000 against E. *faecalis.*

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Table 1: Comparision of antibacterial efficiency of root canal irrigants against E. faecalis

Root canal irrigants	*n*	Zone of inhibition (mm) against E. faecalis		
		Mean ± SD	Median	Min-Max
Sodium Hypochlorite (Positive Control)	06	10.17 ± 0.98	10.50	9.00-11.00
Saline (Negative Control)	06	0.00 ± 0.00	0.00	0.00-0.00
Green tea	06	14.00 ± 0.71	14.00	13.00-15.00
Doxycycline	06	34.00 ± 0.71	34.00	33.00-35.00
Aloe vera	06	8.17 ± 0.75	8.00	7.00-9.00
Neem	06	16.00 ± 0.63	16.00	15.00-17.00
Kruskal wallis test	χ^2 = 34.086, df = 5, P = 0.000 (<0.001), Sig. diff.			
Mann Whitney U test	Doxycycline > Neem > Green tea > Sodium Hypochlorite > Aloe vera > Saline			



Graph 1: Mean (SD) Zones of inhibition in mm between different irrigating agents against *E. faecalis.*

IV. Discussion

A successful endodontic treatment completely depends on proper diagnosis, thorough biomechanical preparation and elimination of the infective microorganism from the root canal system, which favours the periapical healing. Regardless of these treatment protocols, pathogenic microorganism still persists in the root canal system because of the complex root canal configurations such as ramifications,

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webs and fins.[5] The most common species is *Enterococcus faecalis* which is agram positive, facultative anaerobic and coccoid bacterium which is persistently found in root canal failure. Thus, in order to reduce such bacteria from root canal, intracanal irrigant seems necessary.[6]

Various studies have been done to evaluate intracanal irrigant such as sodium hypochlorite, glyoxide, EDTA (ethyl diamine tetra acetic acid), and citric acidagainst *Enterococcus faecalis*. Despite of number of studies, none of the intracanal irrigant shows promising result in eradication of *E. faecalis*. Henceforth, the search for a potent substance with high antimicrobial potential and low cytotoxicity continues to be a relevant issue for endodontics.[7]

Doxycycline is broad-spectrum antibiotics effective against a wide range of microorganisms. It inhibits collagenase and matrix metalloproteinases of bacteria and consequently releases antigenic by products such as endotoxins. It also increases the level of interleukin-10, an anti-inflammatory cytokine.Jaju *et al.* stated that Doxycycline is the most potent anticollagenase antibiotic among commercially available tetracyclines. This is in confirmity with the present study as a significant difference in zone of inhibition was observed (34.00±0.71), which suggests that doxycycline has substantial, the therapeutic effect on E. *faecalis* but it has demerits, the calcium chelates are formed within the dentinal tubules which may later cause discoloration of tooth.[11]

Sodium hypochlorite is another most ideal irrigant, as it covers most of the requirements for endodontic irrigant than any other known compound, which has a unique capacity such as antimicrobial activity, tissue dissolving ability, lack of tooth discoloration and availability. The present study revealed that the zone of inhibition (10.17 ± 0.98) against E. *faecalis* obtained with 3% of NaOCl can also be used as an endodontic irrigating solution. However, toxicity and potential for the severe inflammatory response in the periradiculararea, making its concern for the search of other alternating solutions.[12]The results of the present study were similar to the research conducted by Kini *et al.* where sodium hypochlorite and doxycycline shows effectivity in eliminating E. *faecalis*.[13]

Recently, plant extracts are introduced in dentistry. Green tea, Aloe vera and Neem has not only antimicrobial, antifungal, anti- immunomodulatory but also stimulates dental pulp proliferation, differentiation, and extracellular matrix mineralization.[5]

Rosaline *et al.* used NaOCl, EDTA, saline, *Moriandacitrifolia*, *Azadiractaindica,* and *Camellia sinensis*as a final irrigant. Significantly, less bacterial adhesion is noticed in samples treated with Neem, NaOCl, Green tea and *Moriandacitrifolia*, respectively.[14]

Neem (*Azadiracta indica*) consists of isoprenoid (diterpenoids, triterpenoids, and steroids) and isoprenoid compounds containing proteins, amino acids, and flavonoids, etc. The antibacterial effect of neem marks it as a good root canal irrigant with the highest inhibition zone of (16.00±0.63) which concur with current studies of Ghonmode *et al*., Hegde *et al*., Damre *et al*. between neem and NaOCl.[9]

Green tea extracts have shown to completely eradicate E. *faecalis* in 6 min.In the present study, green tea showed good antibacterial activity against E. *faecalis* compared to the control group with the minimum inhibitory zone of 14.00 ± 0.71. It's healing properties such as antioxidant activity, anti-inflammatory, and radical scavenging properties because of catechins present in it make it appropriate for intra-canal irrigation.[6] No report of microbial resistance of green tea is documented yet.

The antibacterial activity of Aloe Vera is related to anthraquinones and also contains a derivative of 2-ethylanthraquinone, used in the production of H_2O_2. According to Sureshchandra *et al*. observed that chloroform extract of Aloe vera had a significant anti-microbial effect against E.*faecalis* (9mm).The results of the current study shows that Aloe vera does have an inhibitory effect on E. *faecalis,* but it is less than the antimicrobial effect of doxycycline and NaOCl and also with both herbal irrigants *i.e*., green tea and neem with the zone of inhibition (8.17±0.75). Karkare *et al*. concluded that aloe vera showed the highest zone of inhibition against E. *faecalis* similar to NaOCl.[10] The low inhibitory zone of aloe vera could be because of pH of substrate and incubation period of drug which may have neutralize the effect of aloe vera.

Although Doxycycline has high inhibition zone and is considered as one of the best root canal irrigant, but research is continuing for the option of biocompatible, non-cytotoxic, therapeutic solutions.

V. Conclusion

Based on the findings of the present study *Aloe barbadensis Miller*, *Camellia Sinensis*, and *Azadirachta indica* showed inhibitory zone against E. *faecalis*. Hence, these can be used as an alternative root canal irrigating solutions.

Limitation of the study

The present study was an *in-vitro* study a large sample size with a wider spectrum of bacteria needs to be tested to validate its use as an intracanal irrigant.

Further research

Herbal irrigants are proven to be effective antimicrobial agents against *E. faecalis.* Results in this study proved herbs as antimicrobial, anti-inflammatory, cost-effective but further research is needed to evaluate the role of herbs against root canal microbes also.

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Conflicts of interest
There are no conflicts of interest.

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