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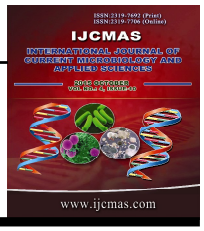

Original Research Article

Comparative Evaluation of Antimicrobial Activity of Green Tea Extract, Garlic Extract, Neem Leaf Extract and Sodium Hypochlorite as Root Canal Irrigants against *E. faecalis* and *C. albicans* – An *In Vitro* Study

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Keywords

Garlic extract, Green tea extract, Neem leaf extract, *Candida albicans*, *Enterococcus faecalis*, Sodium hypochlorite

A B S T R A C T

The main aim of this study to analyse the anti-microbial efficacy of different herbal extracts like green tea, garlic and neem leaf in comparison with 2.5 % sodium hypochlorite against *Enterococcus faecalis* and *Candida albicans*. The antimicrobial activity was determined using agar diffusion test. Extracts were divided into 4 groups: Group I- Green tea, Group II- Neem leaf, Group III- Garlic extract and Group IV- 2.5 % Sodium hypochlorite. The zone of inhibition of growth was recorded. Statistical analysis was performed using one way ANOVA with post-hoc Turkey's HSD. Values obtained were statistically analysed ($P < 0.05$). 2.5 % sodium hypochlorite showed maximum inhibitory effect against *Enterococcus faecalis* followed by green tea, garlic and neem. Against *Candida albicans* also 2.5 % sodium hypochlorite exhibited the maximum inhibitory effect followed by garlic extract, green tea extract and neem leaf extract. Results obtained were statistically significant. Herbal extracts showed significant inhibitory effects against *Enterococcus faecalis* and *Candida albicans* when compared with 2.5% sodium hypochlorite.

Introduction

Primary root canal infections are polymicrobial, typically dominated by obligatory anaerobic bacteria. *E. faecalis* in particular has gained special attention, as it is one of the primary organisms present in patients with post treatment endodontic infection and *C. albicans* is the other main microorganism forming the biofilm.

The prime objective of endodontic treatment is to clean the root canal system thoroughly and making it free of microbiota and debris, as they have definite role in the initiation and perpetuation of pulpal and periapical diseases (Kandaswamy and Venkateshbabu, 2010). This process mainly revolves around the chemomechanical preparation wherein chemically active solutions are used along with mechanical instrumentation of the root

canal space. Several studies have demonstrated that a large proportion of areas the main root canal wall remains untouched during instrumentation (Peters *et al.,* 2002). Therefore the use of irrigant solutions is an essential complement to mechanical preparation aiding in the removal of pulp remnants and in the elimination of residual bacteria from the unreachable areas of the complex root canal system (Kandaswamy and Venkateshbabu, 2010).

Numerous irrigants have been recommended for use in the treatment of root canal infections for many years. Sodium hypochlorite is the primary irrigant of choice in root canal instrumentation used in a concentration ranging from 0.5% to 6%. It is a potent antimicrobial agent killing most of the bacteria on direct contact (Siqueira *et al.,* 1998). It also effectively dissolves the pulpal remnants and removes the smear layer (Spano *et al.,* 2001). Accidental injection of sodium hypochlorite beyond the root apex can cause tissue reactions characterized by pain, swelling, tissue necrosis and haemorrhage. Not only the actions but also the toxicity and caustic potential of Sodium hypochlorite are dose-dependent (Pashley *et al.,* 1985).

Herbal products with less toxic effects have been used since ancient times in both eastern and western medicinal traditions. Various natural plant extract shave known antimicrobial effects suggesting their ability to be used as endodontic irrigant. Antibacterial, antioxidant, anti-inflammatory and immune modulatory actions of garlic extract, neem extract and green tea extract have been extensively investigated.

The purpose of the present study is to explore and compare the antimicrobial efficacy of newer herbal irrigating agents like green tea extract, neem leaf extract and garlic extract against *E. faecalis* and *C. albicans* which would probably be as effective or more and at the same time less irritating to the tissues than sodium hypochlorite.

Materials and Methods

In the present study, the extracts of green tea, neem leaf and garlic were selected as the experimental groups and 2.5% sodium hypochlorite was the positive control group. Experimental groups were named: Group I – green tea extract, Group II - neem leaf extract, Group III- garlic extract and Group IV was 2.5% sodium hypochlorite. *Enterococcus faecalis* (ATCC 29212), and *Candida albicans* (ATCC 10231) strains were used in this study to check antimicrobial activity.

Preparation of the green tea extracts (Group I): Green tea extract is supplied in the form of powder which is readily soluble in water. Green tea extract dilutions are prepared in the concentrations ranging from 0.5 to 6% with difference of 0.5 serial dilutions by weighing the required amount of powder necessary over an electronic weighing balance and mixing it with 100 ml of sterile boiling distilled water for 5 minutes and filter sterilized. The dilutions are kept for sterility check overnight at 37°C. For the agar diffusion method 1–3% concentrations are used.

Preparation of neem leaf extract (Group II): Mature fresh neem leaves are collected from the medicinal garden of Tropical Institute Ecological Sciences, Velloor, Kottayam, Kerala after taxonomic identification of the plant. Leaves are washed in sterilized distilled water and weighed in a sterile disposable cup. 25gms of fresh neem leaves are added to 50ml of absolute ethanol. Mixture is macerated for 1–2 minutes and 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 combined together and filtered through fast filter paper. Alcohol part was removed and extract was prepared and stored in airtight amber coloured container.

Preparation of garlic extract (Group III): Fresh peeled cloves of garlic were shade dried and powdered. 5 grams of garlic powder was macerated with 100 ml of distilled water. The homogenate of garlic powder was filtered using Whatman's filter paper no. 1. This extract was heated over water bath for 5–6 hours till it become viscous.

Agar - diffusion test: Hundred microliters each of *E. faecalis* and *C. albicans* suspensions were taken from prepared cultures and inoculated in 130 mm culture plates with previously set layers of Mueller Hinton and Sabouraud dextrose agar, respectively.

Inoculations were performed using sterile spreader across media. After that four uniform wells of 6mm diameter were made in the agar plates containing cultures of *E. faecalis* and *C. albicans*. 50µl of neem leaf extract, garlic extract and green tea extract were added to the respective wells on each plate and were incubated for 24hours at 37^0C in an incubator. Positive control used in the study was 2.5% sodium hypochlorite. After incubation period, plates were removed and the diameters of zones of inhibition of bacterial growth attained by all four groups against the two tested organisms were recorded in centimetre (Fig. 1 and Fig. 2). Agar diffusion tests had performed 6 times for obtaining significant results.

Results and Discussion

The results were tabulated and statistically analysed using computer software, Statistical Package for Social Sciences (SPSS) version 16. Table 1 shows that significant difference exist between the diameters of zones of inhibition of bacterial growth obtained for green tea extract, neem leaf extract, garlic extract and sodium hypochlorite against *E. faecalis* and *C.albicans* ($p<0.05$). This finding was found out using one way ANOVA.

Table 2 and 3 shows post hoc tests- Turkey's HSD forthe inter comparison of the antimicrobial efficacy of different groups against *C. albicans* and *E. faecalis* respectively. Group IV (2.5% sodium hypochlorite) had statistically significant superior antibacterial activity against both *C. albicans* and *E. faecalis* than all other groups. Group III (Garlic extract) and Group I (Green tea extract) showed greater antimicrobial activity against both microbes in comparison with neem extract and was statistically significant. Group III (Garlic extract) had greater antibacterial activity against *C. albicans* and lesser activity against *E. faecalis* when compared to Group I (green tea extract) which were statistically insignificant.

The main objective of root canal therapy is to eradicate the microbes from the root canal system and to prevent the microbial recontamination after treatment. The anatomical complexities like lateral or furcal canals, fins, webs, apical deltas and isthmus may limit mechanical debridement. Therefore, endodontic irrigant solutions are necessary to complement the mechanical action and they facilitate maximum removal of microorganisms (Gomes *et al.*, 2006).

Bacteriologic examinations of infected root canals showed that 85% to 98% of the isolated bacteria were anaerobic. The most frequently isolated microorganisms before

root canal treatment include Gram-negative anaerobic rods, Gram-positive anaerobic cocci, Gram-positive anaerobic and facultative rods, Lactobacillus species, and Gram positive Streptococcus species. The obligate anaerobes are rather easily eradicated during root canal treatment (Chavez De Paz *et al.,* 2003). *E. faecalis* is frequently isolated from root canals in cases of failed root canal treatments (Stuart *et al.,* 2006). In addition, *C. albicans* may also be found in root canals associated with therapy resistant apical periodontitis as they are more resistant to antimicrobial agents usually used in endodontics (Gomes *et al.,* 2006). In the present study, sodium hypochlorite, garlic extract and green tea extract were shown to inhibit the *E. faecalis C. albicans* effectively. But neem extract showed very minimal activity against *E. faecalis C. albicans* in present study.

For irrigation of root canals, sodium hypochlorite concentrations ranging from 0.5- 6% have been advocated. It is cheap, easily available, having good shelf life and demonstrate optimum tissue dissolving ability and antibacterial property (Kandaswamy and Venkateshbabu, 2010). Chlorine in sodium hypochlorite presents an antimicrobial action by inhibiting bacterial enzymes leading to the irreversible oxidation of SH groups (sulfhydryl group) of essential bacterial enzymes. Antimicrobial activity of sodium hypochlorite is also based on its high pH. The high pH of sodium hypochlorite interferes with the cytoplasmic membrane integrity and also biosynthetic alterations in cellular metabolism, and phospholipid degradation (Estrela *et al.*, 2002). Green tea polyphenols have significant antioxidant, anti-cariogenic, anti-inflammatory, probiotic and antimicrobial properties (Mukherjee *et al.,* 2006). Its action against dental plaque might be due to its antioxidant properties. Green tea polyphenols can effectively inhibit the biofilm formation (Prabhakar *et al.,* 2010). Tea also contains natural fluoride, which may be helpful in preventing dental caries. Many of these beneficial effects of green tea are related to its catechin, particularly epigallocatechin-3-gallate, content.

Neem is an antioxidant and not likely to cause severe injuries to patients. Thus it might be advantageous to use neem as root canal irrigant. Neem is bitter in taste and can be altered by different formulations due to addition of sweeteners and flavours to increase patient compliance and acceptability.

Garlic contains many sulphur compounds and minerals such as selenium. The sulphur compounds are responsible both for garlic's pungent odour and many of its medicinal effects. Allicin, an unstable compound formed from the allinin is proposed to be responsible for the inhibition of fungal growth (Amagase *et al.,* 2001). Allicin inhibits both germination of spores and growth of hyphae. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes (Ankri *et al.,* 1999).

In the present study, sodium hypochlorite showed a mean diameter of 4.2cm for *C. albicans* and 4.3cm for *E. faecalis*. The zones of inhibition of bacterial growth attained by 2.5% sodium hypochlorite were greater than that obtained for other extracts for the tested organisms. This indicates that it has the highest efficacy against the tested organisms than other herbal agents. Siqueira *et al.* (2000) reported good antibacterial action of 2.5% sodium hypochlorite against *E. faecalis* with inhibition zones measuring 21.5 mm that are much lesser than those obtained by us, probably because of different methodology.

Table.1 Analysis of variance (One Way ANOVA) of mean diameter of zone of inhibition of bacterial growth comparing antimicrobial activity four irrigant

Organism	material	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	Pvalue
						Lower Bound	Upper Bound		
C. albicans	Green tea	6	2.7667	.30768	.12561	2.4438	3.0896		
	Neem	6	.7667	.12111	.04944	.6396	.8938		
	Garlic	6	2.8000	.17889	.07303	2.6123	2.9877	322.620	<0.01**
	NaOCl	6	4.2833	.11690	.04773	4.1606	4.4060		
	Total	24	2.6542	1.29009	.26334	2.1094	3.1989		
E. faecalis	Green tea	6	2.8333	.16330	.06667	2.6620	3.0047		
	Neem	6	.2500	.15166	.06191	.0908	.4092		
	garlic	6	2.4667	.23381	.09545	2.2213	2.7120	524.203	<0.01**
	NaOCl	6	4.3000	.15492	.06325	4.1374	4.4626		
	Total	24	2.4625	1.49050	.30425	1.8331	3.0919		

**P value <0.01 - Test is highly significant

Table.2 Post Hoc test -Turkey's HSD for inter comparison of antimicrobial activity different irrigants against*Candida albicans*

Organism	GROUP	GROUP	Mean difference in diameter	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
C. albicans	Green tea	Neem	2.00000*	.11365	.000	1.6819	2.3181
		Garlic	-.03333	.11365	.991	-.3514	.2848
		NaOCl	-1.51667*	.11365	.000	-1.8348	-1.1986
	Neem	Green tea	-2.00000*	.11365	.000	-2.3181	-1.6819
		Garlic	-2.03333*	.11365	.000	-2.3514	-1.7152
		NaOCl	-3.51667*	.11365	.000	-3.8348	-3.1986
	Garlic	Green tea	.03333	.11365	.991	-.2848	.3514
		Garlic	2.03333*	.11365	.000	1.7152	2.3514
		NaOCl	-1.48333*	.11365	.000	-1.8014	-1.1652
	NaOCl	Green tea	1.51667*	.11365	.000	1.1986	1.8348
		Neem	3.51667*	.11365	.000	3.1986	3.8348
		Garlic	1.48333*	.11365	.000	1.1652	1.8014

*. The mean difference is significant at the 0.05 level.

Table.3 Post Hoc tests- Turkey's HSD for inter comparison of antimicrobial activity different irrigants against *Enterococcus faecalis*

Organism	GROUP	GROUP	Mean difference in diameter	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
E. faecalis	Green tea	Neem	2.58333*	.10341	.000	2.2939	2.8728
		Garlic	.36667*	.10341	.010	.0772	.6561
		NaOCl	-1.46667*	.10341	.000	-1.7561	-1.1772
	Neem	Green tea	-2.58333*	.10341	.000	-2.8728	-2.2939
		Garlic	-2.21667*	.10341	.000	-2.5061	-1.9272
		NaOCl	-4.05000*	.10341	.000	-4.3394	-3.7606
	Garlic	Green tea	-.36667*	.10341	.010	-.6561	-.0772
		Neem	2.21667*	.10341	.000	1.9272	2.5061
		NaOCl	-1.83333*	.10341	.000	-2.1228	-1.5439
	NaOCl	Green tea	1.46667*	.10341	.000	1.1772	1.7561
		Neem	4.05000*	.10341	.000	3.7606	4.3394
		Garlic	1.83333*	.10341	.000	1.5439	2.1228

*. The mean difference is significant at the 0.05 level.

Fig.1 Culture plates showing zones of inhibition against *Candida albicans*

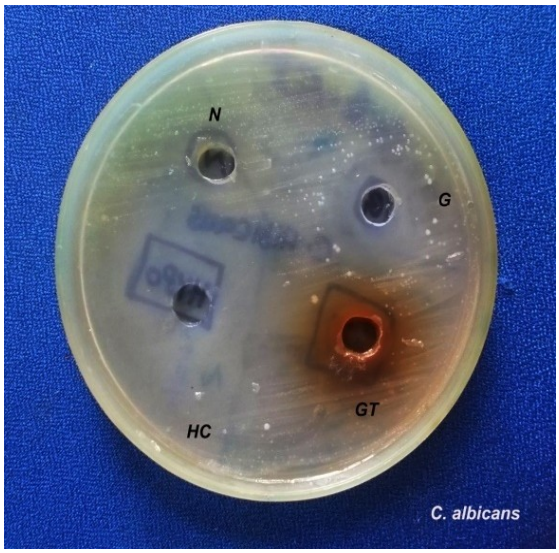
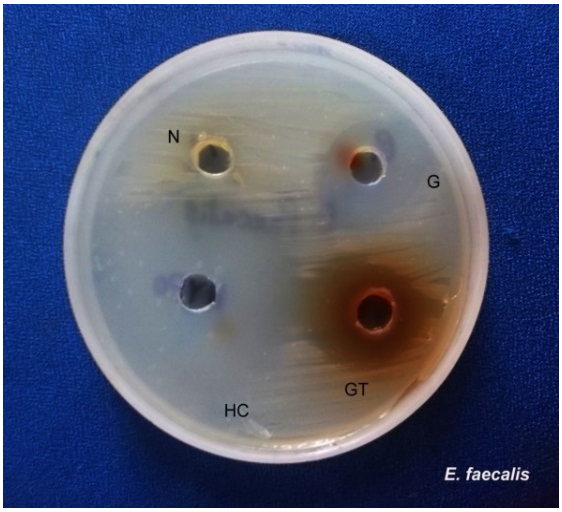


Fig.2 Culture plates showing zones of inhibition against *Enterococcus faecalis*



Green tea extracts showed superior antibacterial activity against the *E. faecalis* than neem extract and garlic extract. The mean diameter of inhibition zone obtained by green tea extracts for *E. faecalis* was 2.8cm whereas neem and garlic extract demonstrated mean value of 0.2cm and 2.4cm respectively. When considering the antibacterial activity against *C. albicans*, green tea extract demonstrated a mean diameter of inhibition zone of 2.7cm. This was significantly less when compared to sodium hypochlorite and marginally inferior in comparison with garlic extract which has got mean diameter of 2.8cm. This finding is in agreement with a study carried out by Leea *et al.* (2011) according to which garlic extract has good antibacterial activity against *C. albicans*.

Hence from the present study it can be evaluated that against *Candida albicans*, sodium hypochlorite was the best antimicrobial irrigant which is followed in descending order by the garlic extract, green tea extract and neem leaf extract. Again sodium hypochlorite showed the best anti-microbial property against *E. faecalis*, followed in descending order by green tea extract, garlic extract and neem leaf extract.

In conclusion, the present *in vitro* study aimed to evaluate the antimicrobial potential of certain herbal extracts viz. green tea extract, neem leaf extract and garlic extract when used as root canal irrigants against *E. faecalis* and *C. albicans* in comparison with 2.5% sodium hypochlorite. Within the limitations of this study, 2.5% sodium hypochlorite showed the best results amongst the four tested solutions. Green tea extract and garlic extract showed statistically significant activity against *Enterococcus faecalis* and *Candida albicans* which was less than that of 2.5% sodium hypochlorite. Though less effective, these experimental solutions are less tissue toxic when compared with sodium hypochlorite. Further research is required to conclusively establish the efficacy of these herbal extracts as root canal irrigants.

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