Antimicrobial Efficacy of Herbal Extracts

Zeenath Ambareen[1,*], Sapna Konde[2], Sunil Raj N[3], Kumar N C[4]

[1]Senior Lecturer, Department of Pedodontics and Preventive Dentistry,
Sri Siddhartha Dental College and Hospital, Tumkur - 572107
[2]Professor and Head, [3,4]Professor, Department of Pedodontics and Preventive Dentistry,
Aecs Maaruti College of Dental Sciences and Research Centre, Bangalore

***Corresponding Author:**
Email: drzeenie@gmail.com

ABSTRACT:

Background and objectives: *The aim of the study was to evaluate and compare extracts of ginger, garlic, aloe vera, neem, turmeric and sodium hypochlorite as root canal irrigants.*

Methods: *Freshly prepared extracts of ginger, garlic, aloevera, turmeric, neem and 2% sodium hypochlorite were used and compared against Enterococcus faecalis using the agar diffusion test. Agar plates were incubated at 37^0 C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm.*

Results and interpretation: *Higher mean zone of inhibition was recorded in Sodium Hypochlorite followed by Garlic Extract, Neem Extract, Ginger Extract and Turmeric Extract respectively. The lowest mean zone of inhibition was found in Aloe Vera Extract.*

Conclusion: *There was no significant difference between the antimicrobial property of garlic extract and 2% sodium hypochlorite.*

Keywords: *Antimicrobial, Herbal extracts, Irrigants*

INTRODUCTION

Enterococcus faecalis is the most commonly isolated species from the canals of teeth presenting post-treatment diseases. Enterococcus faecalis accounts for up to 77% of therapeutic failures[1].

Among the procedures involved in the control of endodontic infection, irrigation is an important step in eliminating microorganisms from the root canal system. Sodium hypochlorite is the most widely used root canal irrigating solution. The advantages of sodium hypochlorite are its ability to dissolve necrotic tissues and its antibacterial properties against most microorganisms. However, it has several undesirable characteristics such as tissue toxicity, risk of emphysema on overfilling, allergic potential, and disagreeable smell and taste[2].

As the irrigants currently used in the field of endodontics have their share of limitations, the search for an ideal root canal irrigant continues[3]. The use of herbal extracts as endodontic irrigants is today gaining popularity. In ginger rhizome there are several components which have antibacterial and antifungal effect. The gingerol and shagelol were identified as more active agents[4]. The main active component of garlic is allicin. It is antibacterial and has immune regulatory functions. Allicin destroys cell wall and cell membrane of root canal bacteria[5]. Aloe vera contains alloins and barbadoins as main chemical constituents. Aloe Vera has gel has inhibitory effects on S-pyogens and E.faecalis because of the presence of anthra quinine as antimicrobial[6]. Neem contains Nimbidin which demonstrated antiinflammatory, antiarthritic, antipyretic, hypoglycaemic, antigastric ulcer, spermicidal, antifungal, antibacterial and diuretic properties[7]. Curcumin [diferuloylmethane], the main yellow bioactive component of turmeric has shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities[8].

The aim of the study was to evaluate and compare extracts of ginger, garlic, aloe vera, neem and turmeric with sodium hypochlorite as a root canal irrigant.

MATERIALS AND METHODS

Freshly prepared extracts of ginger, garlic, aloevera, turmeric, neem and 2% sodium hypochlorite were used and compared against Enterococcus faecalis.

Preparation of ginger extract: Outer covering of ginger was peeled off. 20 g of ginger was kept in closed containers after being chopped into small pieces. 50 ml of diethyl ether was added to it and the mixture was left for 6 hours. The mixture was periodically agitated during this period every 15 minutes. It was then filtered and the ether was vaporized in an evaporator (60° C)[9]. The extract obtained at the end of this process was used in a non-diluted form for analysis on the same day.

Preparation of garlic extract: Fresh peeled garlic (80gm) was chopped and homogenized in 100ml sterile distilled water, centrifuged, filtered through

Whattman No 1 filter paper and kept at -70^{0}C until use[10].

Preparation of aloevera extract: The pulp was removed from 100 gm of aloevera leaves and converted into liquid form in a mixer. The liquid was mixed well with distilled water in 1:5 ratio and the mixed solution was placed in a crucible on a water bath for dehydration. Precipitate of extract was dissolved in methanol and used[11].

Preparation of neem extract: 100 gms of neem leaves were obtained and tied in muslin cloth which was soaked in 800 ml of distilled water in a beaker. The beaker was boiled over low flame till the extract reduced to 400 ml to obtain a 25% concentration of aqueous neem extract. After the extract cooled down, it was filtered in Whatman filter paper and the extract was stored in amber bottle for further usage[12].

Preparation of turmeric extract: The extract was prepared by elemental hydro-distillation method. 100g dried turmeric and 500 ml distilled water were mixed in a 1,000 mL round bottomed flask. Distillation was conducted continuously for 60 h. After turning on the valve to remove the distilled water, the essential oil was collected as stock solutions and kept into a dark bottle and stored at 4°C until use[13].

Agar-diffusion test: The bacterial stock culture Enterococcus faecalis was obtained and culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller- Hinton agar plates. Inoculation was performed by using sterile swab brushed across the media. One round well, 4 mm deep and 8 mm diameter was punched in each agar plate using sterile cork-borer and the prepared irrigants were added to the wells. Agar plates were incubated at 37^0 C for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm. The results were tabulated and statistically analyzed using analysis of variance (ANOVA) (Table 1).

RESULTS

Higher mean zone of inhibition was recorded in Sodium Hypochlorite followed by Garlic extract, Neem extract, Ginger extract and Turmeric extract respectively. The lowest mean zone of inhibition was found in Aloe Vera extract.

There was no statistically significant difference between sodium hypochlorite and garlic extracts. In order to find out among which pair of groups there existed a significant difference, multiple comparisons were carried out using Tukey's test (Table 2).

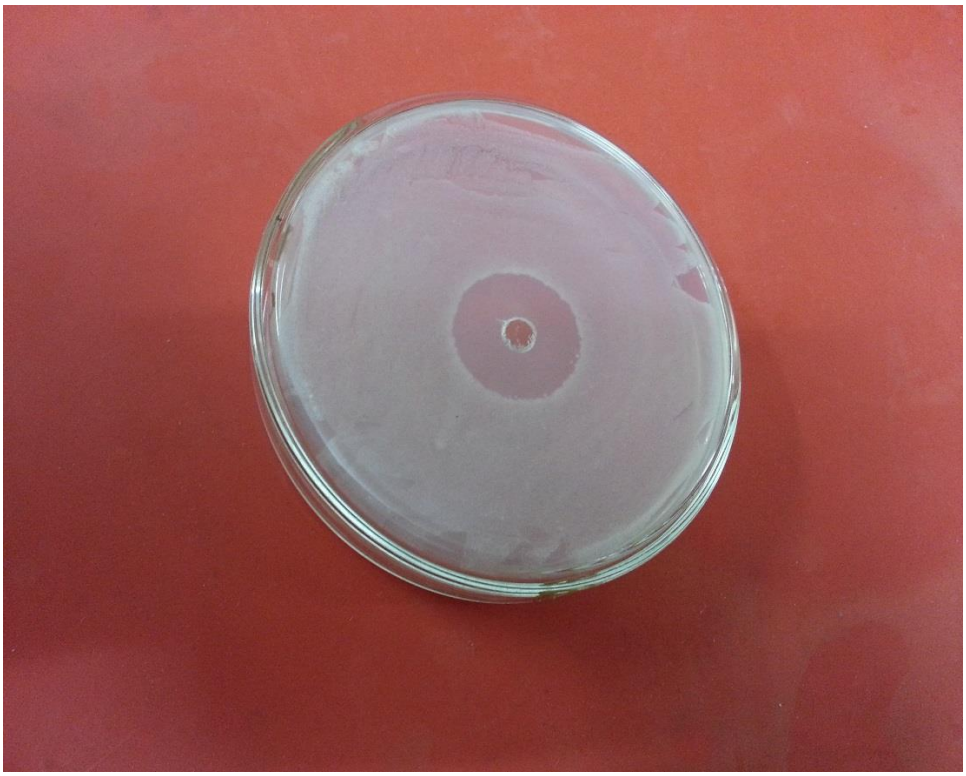

Fig 1: Aloevera extract- least zone of inhibition

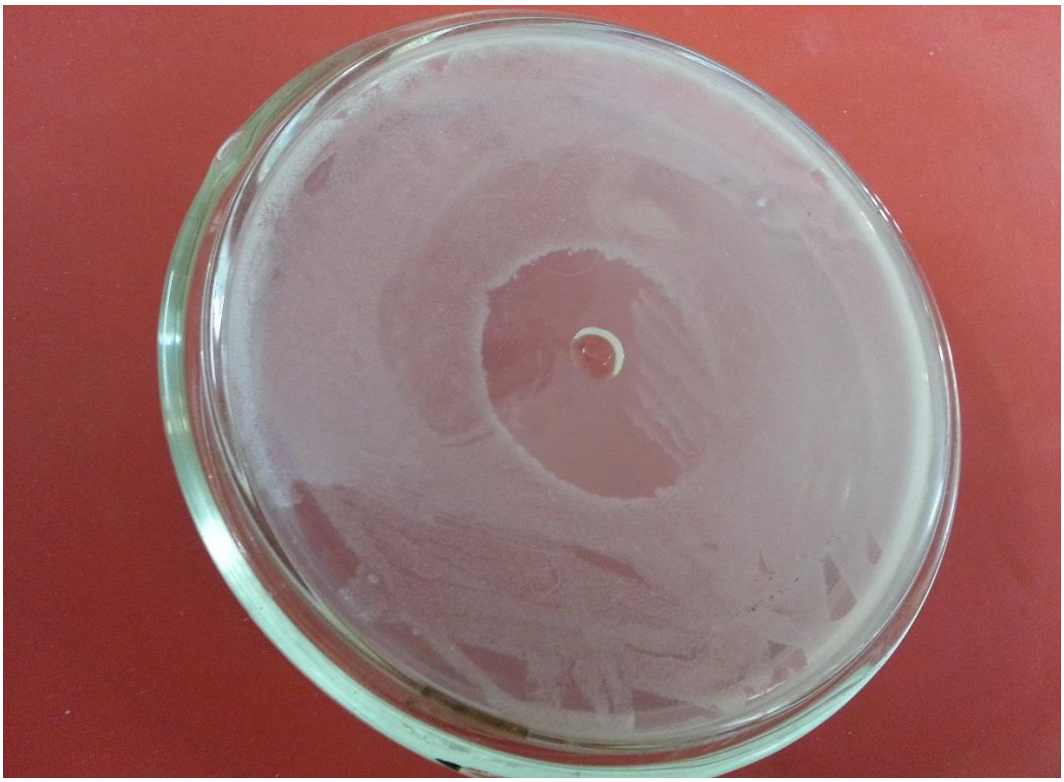

Fig 2: Garlic extract- highest zone of inhibition

Table 1: Mean zone of inhibition of different herbal extracts

Irrigant	Mean zone of inhibition
Sodium Hypochlorite	23.00
Turmeric Extract	8.00
Ginger Extract	11.00
Garlic Extract	22.00
Neem Extract	16.00
Aloe Vera Extract	6.40

Table 2

(I) Irrigant	(J) Irrigant	Mean Difference (I-J)	Std. Error	95% CI for mean difference		P-Value
				Lower Bound	Upper Bound	
Sodium Hypochlorite	Turmeric Extract	15.000	0.787	12.565	17.435	<0.001*
	Ginger Extract	12.000	0.787	9.565	14.435	<0.001*
	Garlic Extract	1.000	0.787	-1.435	3.435	0.798
	Neem Extract	7.000	0.787	4.565	9.435	<0.001*
	Aloe Vera Extract	16.600	0.787	14.165	19.035	<0.001*
Turmeric Extract	Ginger Extract	-3.000	0.787	-5.435	-0.565	0.010*
	Garlic Extract	-14.000	0.787	-16.435	-11.565	<0.001*
	Neem Extract	-8.000	0.787	-10.435	-5.565	<0.001*
	Aloe Vera Extract	1.600	0.787	-0.835	4.035	0.355
Ginger Extract	Garlic Extract	-11.000	0.787	-13.435	-8.565	<0.001*
	Neem Extract	-5.000	0.787	-7.435	-2.565	<0.001*
	Aloe Vera Extract	4.600	0.787	2.165	7.035	<0.001*
Garlic Extract	Neem Extract	6.000	0.787	3.565	8.435	<0.001*
	Aloe Vera Extract	15.600	0.787	13.165	18.035	<0.001*
Neem Extract	Aloe Vera Extract	9.600	0.787	7.165	12.035	<0.001*

*denotes significant difference

The difference in mean zone of inhibition was found to be statistically significant between Sodium Hypochlorite & Turmeric Extract (P<0.001), Sodium Hypochlorite & Ginger Extract (P<0.001), Sodium Hypochlorite & Neem Extract (P<0.001), Sodium Hypochlorite & Aloe Vera Extract (P<0.001), Turmeric Extract & Ginger Extract (P<0.05), Turmeric Extract & Garlic Extract (P<0.001), Turmeric Extract & Neem Extract (P<0.001), Ginger Extract &Garlic Extract (P<0.001), Ginger Extract & Neem Extract (P<0.001), Ginger Extract & Aloe Vera Extract (P<0.001), Garlic Extract & Neem Extract (P<0.001), Garlic Extract & Aloe Vera Extract (P<0.001) as well as between Neem Extract & Aloe Vera Extract (P<0.001).

DISCUSSION

Healing potential of plants is an ancient idea, but in recent times it has gained renewed interest and importance. The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of reported microbial resistance.[14]

The present study showed that garlic extract had good inhibitory efficacy against Enterococcus faecalis. The antimicrobial action of garlic is primarily attributed to allicin whose mechanism of action partially inhibits DNA and protein synthesis, and entirely inhibits RNA synthesis. DNA transcription and other DNA activities are also influenced by allicin[15].

In a study antibacterial activities of two natural plants (freshly minced garlic extract and fresh lemon solution) were compared with that of three traditional intracanal medications (10% citric acid, 5.25%naocl, and CPCP) on mixed root canal flora on blood agar plates as well as inside prepared sterile root canals. The results showed that both freshly minced garlic and CPCP represented the statistically significant largest inhibitory zones when compared with other three medications used[16].

Various parts of the neem tree have been used as traditional ayurvedic medicine in India from time immemorial. Its use for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. Nimbidin, a major crude

bitter principle extracted from the oil of seed kernels of A. indica demonstrated antiinflammatory, antiarthritic, antipyretic, hypoglycaemic, antigastric ulcer, spermicidal, antifungal, antibacterial and diuretic properties[7]. Neem also has an anti-adherence activity by altering bacterial adhesion and ability of organism to colonize[17].

Use of neem as an endodontic irrigant might be advantageous because it is a biocompatible antioxidant and thus not likely to cause any adverse reactions. Bitter taste associated with this plant can be altered by modifying the formulations with the addition of sweeteners and flavors thereby increasing the patient compliance and acceptability. A study using neem leaf extract has shown that it is a viable medicament against C. albicans, E. faecalis and even mixed culture when compared to sodium hypochlorite.[18].

In the present study neem extract showed significant zone of inhibition compared to the other extracts, but the zone of inhibition was less compared to garlic extract.

Aloevera has various therapeutic uses in arthritis, asthma, digestive and bowel disorders, skin problems (eg: eczema, psoriasis, acne, burns), ulcers etc in general. In dentistry aloevera is used in cases of apthous ulcers, lichen planus, alveolar osteitis[6]. Aloevera leaves contain alloins and barbadoins as main chemical constituents.

In a study aloevera extract has shown significant antibacterial activity against E. fecalis and C.albicans[11]. But in the present study aloevera extract showed the least zone of inhibition against E.fecalis.

A study has shown that ginger extract had good inhibitory efficacy against Enterococcus faecalis[19]. Another study has reported significant antifungal activity of ginger extract on oral species of Candida albicans[20]. Studies have shown that the extract of ginger can inhibit the growth of oral bacteria[21]. However in the present study ginger extract showed lesser zone of inhibition when compared to the previous studies.

Traditional Indian medicine uses turmeric powder for the treatment of biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis[8].

Curcumin [diferuloylmethane], the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities[8].

The lower antimicrobial efficacy of turmeric, aloevera, ginger, neem in the present study as compared to the earlier studies could be attributed to the constituents of the herbs depending on the area where these were grown, the method of extract preparation or the use of these preparations. However, several disadvantages of herbal irrigants like fresh solutions have to be prepared each time, the unacceptable odour and taste, short shelf life have to be overcome The smell and taste of the herbal irrigants has to be modified by adding flavouring agents to make it palatable and acceptable by the patient. More research for prolonging the shelf life of these irrigants has to be done so that these irrigants are more widely accepted. There is not much evidence about the overdose reactions of these herbal irrigants. More research about the use of particular concentrations of these irrigants to achieve maximum therapeutic effects have to be done.

CONCLUSION

Garlic extract showed to be a potent antimicrobial against E.fecalis when compared to the other herbal extracts used in this study. There existed no statistical difference between antimicrobial activity of sodium hypochlorite and garlic extract. However, further research has to be done in the field of herbal extracts as endodontic irrigants to identify the concentration to be used, the method of extract preparation and differences between the efficacy of the herbal extracts and traditional root canal irrigants.

ACKNOWLEDGEMENTS: Wingene Biotech Research Labs, Bangalore.

REFERENCES

1. Siqueira JF, Rocas IN. Exploiting molecular methods to explore endodontic infections: Part 2 – Redefining the endodontic microbiota. J Endod 2005; 31: 488-98.
2. Segura JJ, Jimenez-Rubio A, Guerrero JM, Calvo JR. Comparative effects of two endodontic irrigants, chlorhexidine digluconate and sodium hypochlorite on macrophage adhesion to plastic surface. J Endod 1999; 25: 243-46.
3. G.Seelan, JE Sam, A.Kumar. Comparative evaluation of antimicrobial efficacy of four different herbal extracts and 5.25% sodium hypochlorite against E.fecalis and C.albicans. An agar disk diffusion method. JIADS RES Vol 1 issue 1 April-June 2012.
4. Ficker C, Smith M L, Akpagana K, Bioassay-guided isolation and identification of antifungal compounds from ginger. J Phytother Res 2003; 17: 897-903.
5. Traditional Chinese medicine used in root canal disinfection research. Pharmacy papers (Online article). http://eng.hi138.com/?b106.
6. Wynn RL. Aloe Vera gel: update for dentistry. General dentistry 2005: 53(1): 6-9.
7. P. Sudhir Kumar, Debasis Mishra, Goutam Ghos and Chandra S. Panda. Biological action and medicinal properties of various constituent of *Azadirachta indica* (Meliaceae)" an Overview. Annals of Biological Research, 2010, 1 (3):24-34.
8. Neelakantan P, Subbarao C, Subbarao CV. Analysis of Antibacterial Activity of Curcumin against Enterococcus Fecalis. International Journal of Current Research and Review, 2011; 3: 37-42.
9. Rahman MSA, Thangaraj S, Salique SM, Khan KF, Natheer SE. Antimicrobial and biochemical analysis of some spices extract against food spoilage pathogens. Int J food Safety 2010; 12: 71-75.

10. Bakri IM and Douglas CW. Inhibitory activity of garlic extract on oral bacteria. Arch Oral Biol 2005;50:645-51
11. B. Sureshchandra, Arun J. Kumar. Antibacterial efficacy of aloe vera extract on resistant antimicrobial strains in endodontics.
12. Sheila R.B. Polaquini, Terezinha I.E. Svidzinski, Carlos Kemmelmeier, Andre´ Gasparetto. Effect of aqueous extract from Neem (Azadirachta indica A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by Candida albicans. Archives of Oral Biology (2006) 51, 482—490
13. Pattanan Dansai Warawut Krusong. Effect of turmeric extract, fermented vinegar and their mixture on Salmonella Typhimurium Reduction in vitro 2011 2nd International Conference on Biotechnology and Food Science IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore
14. Abascal K, Yarnell E, Herbs and drug resistance. Part 2 – clinical implications of research on microbial resistance to antibiotics. Altern Complementary Therapies 2002; 8: 284-90.
15. Eja ME, Asikong BE, Abriba C, Arikpo GE, Anwan EE, Enyi-Idoh KH. A comparative assessment of the antimicrobial effects of garlic (Allium Sativum) and antibiotics on diarrheagenic organisms. Southeast Asian J Trop Med Public Health. 2007; **38:** 343 – 348.
16. Abuzied ST, Eissa SAL. Comparative study on antibacterial activities of two natural plants versus three different intracanal medicaments. (Online article) http://www.kau.edu.sa/Files/165/Researches/19240_Comparative%20Study%20On.pdf
17. Da Silva F B, De Almeida J M, De Sousa S M G. Natural medicaments in endodontics – a comparative study of the anti-inflammatory action. Braz Oral Res 2004;18(2):174-9.
18. Bohora A, Hegde V, Kokate S. Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against E. faecalis, C. albicans and mixed culture - An in vitro study. Endodontology 2010; 22: 8-12.
19. Meenal N Gulve, Nitin D Gulve. Comparison of Antimicrobial Efficacy of Ginger Extract and 2% Sodium Hypochlorite against *Enterococcus faecalis* using Agar Diffusion Method. *JIDA, Vol. 4, No. 10, October 2010*
20. Atai Z, Atapour M, Mohseni M. Inhibitory effect of ginger extract on candida albicans. Am J Applied Sci 2009; 6 : 1067-69.
21. Park M, Bae J, Lee DS. Antibacterial activity of (10) – Gingerol and (12) – Gingerol isolated from ginger rhizome against periodontal bacteria. J Phytother Res 2008; 22: 1446-49.