ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Comparative Evaluation of Antimicrobial Activity Of herbal vs chemical Root Canal Irrigants against E. Faecalis - An in Vitro Study

Dr. Prajkta G. Damre

(PG Student Dept. Of Conservative Dentistry And Endodontics; Saraswati-Dhanwantari Dental College And Hospital And Post Graduate Research Institute Parbhani)

Manuscript Info

Manuscript History:

Received: 14 July 2015
Final Accepted: 25 August 2015
Published Online: September 2015

Key words:

Corresponding Author

Dr. Prajkta G. Damre

Abstract

Enterococcus faecalis is the most predominant microorganism found in the root canals of teeth where, previous treatment has failed. Effective endodontic treatment is attained through debridement and elimination of pathogens from the infected root canal.

In recent times, interest in drugs derived from medicinal plants has peaked. Most of the commercial intracanal medicaments have cytotoxic reactions and are unable to eliminate bacteria completely from dentinal tubules. Instead, Phytomedicines are used which are biologic medication extracted from plants. These have multiple uses in dentistry as anti-inflammatory, antibiotic, analgesic, sedative and endodontic irrigant.

The aim of the study was to explore the antimicrobial efficacy of herbal irrigating agents which would be more efficacious and less irritating to the periapical tissues compared to sodium hypochlorite.

The objective of this in vitro study was to compare the antimicrobial activity of 5% sodium hypochlorite Vs neem leaf extract, turmeric, honey and aloe vera against E. Faecalis, using the commonly used agar diffusion method.

Results: Honey showed the highest zone of inhibition against E.faecalis followed by neem, sodium hypochlorite, haldi and aleo-vera.

INTRODUCTION

Primary endodontic infections are caused by oral microorganisms which are usually opportunistic pathogens that can invade necrotic tissue and establish an infectious process. The number of facultative anaerobes increase when the root canal remains infected for a longer period. The success of the endodontic treatment depends on the elimination of the bacteria from the infected root canal[1].

Enterococcus faecalis is the most common organism cultured from failed root canals that undergo retreatment and also from non - healing endodontic cases[1,2]. It is a facultative, gram positive anaerobic organism, which grows through the formation of a biofilm and can survive chemomechanical preparation, intracanal medication and can reinfect the obturated root canal[1].

Intracanal irrigants should possess a good antimicrobial property to enhance the outcome of the instrumentation procedures. Chemomechanical preparation is the most important step in endodontic disinfection[2].

Sodium hypochlorite is the most commonly used irrigant because it f+ulfills most of the requirements as an ideal endodontic irrigant. But it has disadvantages like high tissue toxicity, unpleasant smell & taste, allergic

potential and inability to remove the smear layer. To overcome the problems of currently used irrigants, the use of herbal root canal irrigants is gaining interest[4].

Various natural plant extracts have antimicrobial properties & therapeutic effects suggesting their potential to be used as an endodontic irrigant[5]. The advantages of using herbal extracts in endodontics are that they have few side effects, less expensive, better tolerated by patients and renewable in nature. Herbal extracts such as neem, turmeric, aloe vera and honey have an antimicrobial, anti-inflammatory & antioxidant properties[5,6].

Aim And Objective

- The aim of this study was to explore the antimicrobial efficacy of herbal irrigating agents which would probably be as effective or more and at the same time less irritating to the tissues than sodium hypochlorite.
- The objective of this in vitro study was to compare the antimicrobial activity of 5% sodium hypochlorite Vs neem leaf extract, turmeric, honey and aloe vera against E. Faecalis, using the commonly used agar diffusion method.

Materials and Methods

Neem leaf extracts, Turmeric, Honey, Aloe vera and 5% Sodium hypochlorite, absolute ethanol, normal saline, nutrient broth, sterile cotton swab, Enterococcus faecalis (ATCC 29212) cultures, chocolate agar were the materials used in the study.

Preparation of Neem Leaf Extract

Mature fresh Azadirachta indica leaves were collected from the medicinal garden of Krushi Vidyapeeth parbhani & taxonomic identification of the plant was performed. Leaves were washed in sterilized distilled water and weighed in sterile disposable cup. 25 gms of fresh neem leaves were added to 50 ml of absolute ethanol. Mixture as macerated for 1-2 min, extract was filtered with muslin cloth for coarse residue. Extraction process was repeated again using coarse residue and 25 ml 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 kept ready and stored in airtight container[7].

Preparation of Ethanolic Extract of Turmeric

Curcuma longa commonly known as turmeric, is an herbaceous perennial plant which belongs to the family zingiberaceae. The plant is distributed throughout the tropical and subtropical regions of the world and widely cultivated in South East Asian countries[8]

The rhizomes were washed with distilled water and dried. They were then cut into irregular large pieces and dried in an oven by tray drying process at a temperature of 45±5 C for a period of about 9˚-10 days till they were completely moisture-free.

The irregular large sized pieces were ground to form a coarse powder. Maceration process of extraction was then performed on this coarse powder of the rhizomes. 500 gms of coarsely ground powder of the Curcuma longa rhizomes was placed in two large glass chambers each. To one glass chamber 2500 ml of sterile distilled water was added to prepare the aqueous extract and to another one, approximately 1550 ml of water and 850 ml of ethanol (95%) was added in a 70:30 ratio of water and alcohol to get a hydro-alcoholic extract. Both the glass chambers were closed with a glass lid to prevent evaporation of the menstruum and this system was allowed to stand for 7 days with occasional stirring.

The liquid i.e. the menstruum was then strained and the solid residue, called marc, was pressed to recover as much occluded solution as possible. The strained and expressed liquid thus obtained were mixed and clarified by filtration. The filtration was carried out in a beaker using a Whatman's filter paper no 1. 2000 ml of menstruum was obtained which was stored in a refrigerator at 4C in two beakers .˚China dishes were used for evaporation of the menstruum. These china dishes containing the menstruum were placed on a water bath.

After evaporation of the menstruum a thick dark brown colored sticky mass was obtained as the aqueous extract and a yellowish brown colored sticky mass as the hydro -alcoholic extract . These extracts were stored in a dark colored pre -sterilized airtight container . The same procedure was performed for the remaining menstruum . It was then stored in a refrigerator at 4°C in a dark colored pre-sterilized airtight container until its further use[9].

Preparation of aloe vera juice

Leaves of Aloe vera were collected from the medicinal garden of Krushi Vidyapeeth parbhani & taxonomic identification of the plant was performed. Mature, healthy and fresh leaves of A. vera were washed in the running tap water for 5 min and rinsed with sterile distilled water, then dissected longitudinally and the colourless parenchymatous tissue (aloe gel) was scraped out using a sterile knife without the fibres[10] . The gel was ground with DMSO using the mortar and pestle. The extracts were filtered using Whatman No. 1 filter paper and the filtrate was centrifuged at 5000 rpm for 5 min[11]. The supernatant was collected and stored in refrigerator at 4°C[11].

Preparation of Honey

The honey used in this study was collected from honey comb.It was collected in sterile container and checked for purity on chocolate agar by streaked on chocolate agar plate and incubated overnight. The honey sample was diluted by saline to 50%

Preparation of filter discs: Whatman No 1 filter paper was obtained. Round discs of 6 mm diameter were cut from the filter paper. They were dried and sterilized in hot air oven at 60 degree C. Each of these discs were then soaked with 0.1 ml of the honey suspensions Pure culture of microorganisms was grown on nutrient broth which was cultured on chocolate agar plate using inoculation loops. The plates were incubated at 37 degree C for 24 hours under aerobic condition and were there after examined for zones of inhibition[12,13]

Test for Antimicrobial Assay

E. faecalis was cultured on chocolate agar

Fig 1

Agar well-diffusion method was followed to determine the antimicrobial activity chocolate agar plates were swabbed (sterile cotton swabs) with 8 hour using nutrient broth culture of E. Faecalis Wells (6mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Neem leaf extract, ale vera solution ,sodium hypochlorite solution, honey extract, turmeric extract and control 50ìl each was added to the respective wells and the plates were incubated for 24hrs at 37degC in an incubator[14,15].

Control used in the study is the solvent used for extraction of neem and turmeric – ethanol. The activity of the solvent was checked earlier and didn't give any zone of inhibition against cultures. After incubation period, plates were removed and zones of inhibition were recorded. Experiment was performed 3 times and mean of the zone of inhibition was recorded in mm

Culture plate showing zones of inhibition of the sodium hypochlorite against E. Faecalis

Fig 2

Culture plate showing zones of inhibition of the neem against Enterococcus Faecalis

Fig 3

Culture plate showing zones of inhibition of the turmeric against Enterococcus Faecalis

Fig 4

Culture plate showing zones of inhibition of the Honey against Enterococcus Faecalis

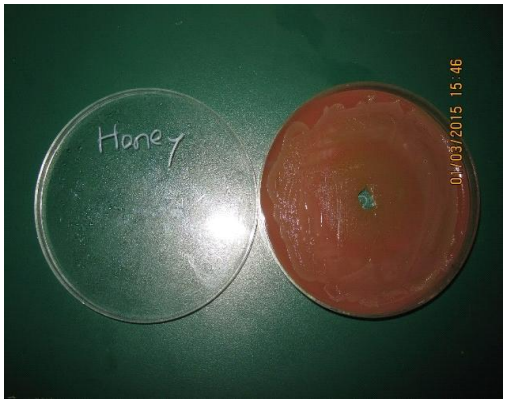

Fig 5

Culture plate showing zones of inhibition of the Aloe Vera against Enterococcus Faecalis

Fig 6

Comparative antimicrobial assay of herbal vs sodium hypochlorite showing different zone of inhibition

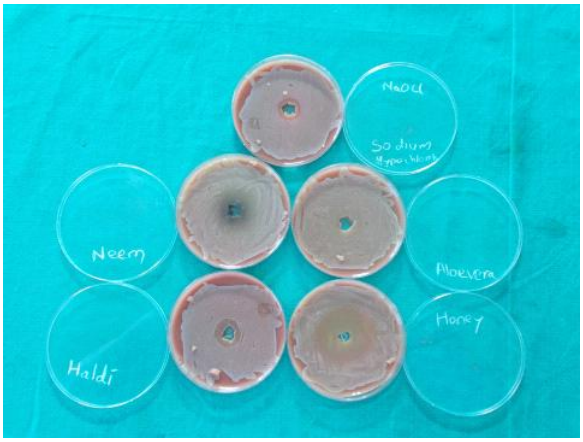

Fig 7

Results

The results were tabulated and statistically analyzed using analysis of variance (ANOVA).

Sr no	Zone of inhibition (Diameter in mm)	E. Faecalis
1	5% NaOCl (Control Group)	3mm
2	Honey	7mm
3	Neem	4mm
4	Haldi	2mm
5	Aloe vera	0mm

Table 1

- The antimicrobial activity of honey is more than the other agents against E. Faecalis, the difference being statistically significant.
- The antimicrobial activity of sodium hypochlorite solution is only less than neem extract and honey.
- The turmeric shows the weak antimicrobial activity.
- The aloe vera extract shows no antimicrobial activity.

STASTICAL ANALYSIS

Discussion

Several studies on the antimicrobial activity of irrigation solutions in endodontics, such as 0.5%,1%, 2.5%, 5% NaOCl are found in literature[16,17] E. faecalis is the most common Enterococcus sp. persisting in treated root canals and are resistant to traditional antibiotics[18] . When E. faecalis grows as a biofilm, the altered genetic and metabolic processes of bacteria along with its complex matrix prevent the entry and action of several antimicrobial agents[19.] The antibiotic resistance has been found to increase up to 1,500 times when compared with planktonic cells. Therefore, testing the effect of an antibacterial irrigant on planktonic cells will not fulfill its effectiveness in vivo conditions[20,21].

Sodium hypochlorite is the gold standard root canal irrigant used in endodontic practice[22].But the drawbacks of sodium hypochlorite includes unpleasant taste, toxicity and causes hypochlorite accidents.

So to overcome this side effect and to meet the requirements of an ideal irrigant, Phytomedicines were discovered by researchers. Several pharmacological activities and medicinal application. Interest on this substance is based on its properties like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, analgesic. Considering all this properties a well-known herbal products like neem, turmeric, aloe vera & honey were used against sodium hypochlorite.

Methodology of this study followed the standard established for agar dilution tests. Study design presented in this paper is more consistent with other studies testing ability of antimicrobial action[23].

The antimicrobial properties of honey related to different factors which lead to antimicrobial effect. This antimicrobial factors represented by Osmotic effect, acidity, hydrogen peroxide and phytochemical factors[24]. In current study honey showed highest antimicrobial activity against E.faecalis. The pure honey contains alkaloids, auterquinone glycosides, cardiac glycosides, flavonoids & reducing Compounds. The antibacterial properties of honey includes, the release of low lives of hydrogen peroxide, some honey have an additional phytochemical antibacterial compounds. The antibacterial property of honey is also due to osmotic effect of its high sugar content as it has an osmolarity sufficient to inhibit the microbial growth[25].

Also several study demonstrated antimicrobial spectrum & efficiency of honey using Staphylococcus aureus, Klebsiella species, Escherichia coli, Streptococcus faecalis, Pseudomonas aeruginosa, proteus mirabilis, Salmonella typhi[26].

Use of neem as an endodontic irrigant might be advantageous because it is a biocompatible antioxidant and thus not likely to cause severe injuries to patients that might occur via NaOCl accidents. Bitter taste associated with this plant can be altered by different formulations due to addition of sweeteners and flavors to increase the patient compliance and acceptability[27,28.] according to Botelho et al and Singhal et al in their experiments concluded that Azadirachta indica is highly efficacious in the treatment of periodontal disease thus exhibiting its biocompatibility with human PDL fibroblasts[27]. In current study neem showed less antimicrobial zone than other studies which were published earlier.

Curcumin, a yellow bioactive pigment, is the major constituent of turmeric which has a wide spectrum of biological actions such as anti-inflammatory, antioxidant, antifungal and antibacterial activities[29]. According to a study by N Niamsa turmeric has shown weak antimicrobial efficacy against E. faecalis. But the aqueous extract of Curcuma longa showed good inhibitory activity against Candida Albicans. On the other hand in present study curcuma longa showed diffused but satisfactory antimicrobial activity against E. faecalis.

Aloe barbadensis Miller (Aloe vera) belongs to the Liliaceae family, of which there are about 360 species. It is a cactus-like plant that grows readily in hot, dry climates and currently, because of demand, is cultivated in large quantities[30].commonly known aloe vera has anti-inflammatory immunostimulatory, antibacterial, antiviral, antifungal and cell growth stimulatory activity[31]. In present study Aloe vera extract had not shown any antimicrobial activity but according to Kithwas etal 2008 , Agarry etal 2005, Cete etal 2005 aloe vera juice shown antimicrobial activity against gram positive bacteria.

Conclusion

- Sodium hypochlorite is the material of choice for irrigation but the herbal product can also provide oral health benefits by inhibiting growth of cariogenic and root canal pathogens. Within the limitations of this study suggested. Honey to be an excellent potential root canal disinfectant against E. Faecalis. But the more clinical trials need to be carried out to see the beneficial effect of this herbal products.

References

- J.Prabhakar, M.Senthilkumar, M.S.Priya, K.Mahalakshmi etal (2010) Evaluation of antimicrobial efficacy of herbal alternatives (triphala and green tea polyphenols), MTAD and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate : An in vitro study JOE ;36:83-86

- Zhongchun Tong, Lin Zhou, Jie Li, Wenkat Jiang etal (2011) In vitro evaluation of the antibacterial activities of MTAD in combination with Nisin against Enterococcus faecalis. JOE ;37:1116-1120

- Qian Xie, Bradford R Johnson, Christopher S Wenckus etal (2012) Efficacy of berberine, an antimicrobial plant alkaloid, as an endodontic irrigant against a mixed culture biofilm in an in vitro tooth model. JOE 38:1114-1117

- Gupta A, Duhan J, Tewari S, Sangwan P etal (2013) Comparative evaluation of Syzygium aromaticum, Ocmium sanctum, and cinnamomum zeylanicum plant extracts against Enterococcus faecalis : a preliminary study. International Endodontic Journal ;46:775-783

- Thilla S Vinothkumar, Mohamed I Rubin, Deivanayagam Kandaswamy etal (2013) Invitro evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. Journal Of Conservative Dentistry ;16:167-170

- Hemanshi Kumar (2013) An in vitro evaluation of the antimicrobial efficacy of Curcuma longa, Tachyspermum ammi, chlorhexidine gluconate and calcium hydroxide on Enterococcus faecalis Journal Of Conservative Dentistry;16:144-147

- Wasudeo Ghonmode etal (2013) Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against E. feacalis – An in vitro study. J Int Oral Health Dec; 5(6): 61–66.

- Mehvish Saleem ,Betty Daniel, Murki K (2011) Antimicrobial Activity Of Three Different Rhizomes Of Curcuma Longa & Curcuma Aromatica On Uropathogens Of Diabetic Patients. Int J Pharm Pharm Sci, Vol 3, Issue 4, 273-279.

- Mithra N Hegde etal (2012) Evaluation of Antimicrobial Activity of Aqueous and Hydro-Alcoholic Curcuma Longa Extracts against Endodontic Pathogens. IOSR Journal of Pharmacy Mar.-Apr, Vol. 2(2) pp: 192-198.

- Renisheya Joy Jeba Malar T etal (2012) Anti-Bacterial And Antifungal Activity Of Aloe Vera Gel Extract. International Journal of Biomedical and Advance Research 03(03) 184-87

- Pugh N, Ross Sa, Elsohly Mh, Pasco Ds (2001) Characterizations Of Aloe Vide, A New High Molecular Weight Polysaccharides From Aloe Vera With Potent Immunostimulatory Activity. J Agri Food Chem; 49(2): 1030-1034.

- N Al Somal (1994) Susceptibility of Helicobacter pylori to the antibacterial activity of manuka honey. Journal of the Royal Society of Medicine Jan Volume 87.

- Litik Mittal (2012) Anti Microbial Activity Of Honey Against Various Endodontic Micro Organisms-An In Vitro Study. Journal of International Dental and Medical Research Nov 1 Volume 5

- Antara Sen (2012) Evaluation Of Antimicrobial Activity Of Different Solvent Extracts Of Medicinal Plant: Melia Azedarach L. Int J Curr Pharm Res, Vol 4, Issue 2, 67-73

- Cleidson Valgas (2007) Screening Methods To Determine Antibacterial Activity Of Natural Products. Brazilian Journal of Microbiology 38:369-380

- RE Hand, ML Smith, JW Harrison (1978) Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod.;4:60–64

- JW Harrison, RE Hand (1981) The effect of dilution and organic matter on the anti-bacterial property of 5.25% sodium hypochlorite. J Endod;7:128–132.

- Pinheiro ET, Gomes BP, Ferraz CC, etal (2003) Microorganisms from canals of root-filled teeth with periapical lesions. Int Endod J;36:1–11.

- Gordon CA, Hodges NA, Marriott C (1988) Antibiotic interaction and diffusion through alginate and exopolysaccharide of cystic fibrosis-derived Pseudomonas aeruginosa. J Antimicrob Chemother;22:667–74.

- Mah TFC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol;9:34–9.

- Socransky SS, Haffajee AD (2002) Dental biofilms: difficult therapeutic targets. Periodontology ;28:12–55, 2000.

- Roger M. Clarkson ,Alex J (1998) Sodium hypochlorite and its use as an endodontic irrigant Australian Dental Journal;43:(4):000-000

- Joshua Davis, Maki J, Babcall J (2007) In vitro comparison of antimicrobial effects of various endodontic medicaments on Enterococcus faecalis, J Endod; 33(5): 567-569

- Molan P C. The Antibacterial Activity of Honey. J Bee World. 1991.

- Dumronglert, E. (1983). A Follow-up Study of Chronic Wound Healing Dressing with Pure Natural Honey. J. Natl Res. Counc. Thail. 15: 39-66

- Carolinie Batista Nobre da cruz etal (2014) Antimicrobial activity of honeys from two stingless honeybee species and Apis mellifera (Hymenoptera: Apidae) against pathogenic microorganisms June Acta Amaz vol.44 no.2 Manaus.

- M Botelho, AD Santos, J Martins, etal (2008) Efficacy of a mouthrinse based on leaves of neem in the treatment of patients with chronic gingivitis. J Med Plants Res;2(11):341–346.

- S Polaquini, T Svidzinski, C Kemmelmeier, A Gasparetto (2006) Effect of aqueous extract from Neem (Azadirachta indica A. Juss) on hydrophobicity, biofilm formation and adhesion in composite resin by Candida albicans. Arch Oral Biol;51(6):482–490.

- Thilla S Vinothkumar etal (2013) In vitro evaluation of five different herbal extracts as a antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction Journal of Conservative Dentistry Mar-Apr Vol 16 Issue 2.

- Newall CA, Anderson LA, Phillipson JD (1996) Herbal medicines, A Guide for Healthcare professionals, Pharmaceutical press, London 2nd edition.

- Kawai K, Beppu H, Shimpo K, Chihara T, Yamamoto N, Aggatsu T, etal (1998) In vivo effects of Aloe arborescens Miller var. natalensis Berger (Kidachi aloe) on experimental Tinea Pedis in guinea pig feet. Phytotherapy Research; 12: 178-182.