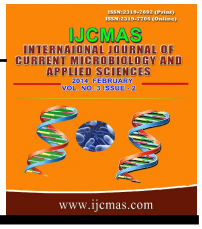
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 2 (2014) pp. 498-504**
http://www.ijcmas.com



Original Research Article

Antibacterial Activity of Turmeric against *Enterococcus faecalis* – An *In vitro* Study

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Keywords

E.faecalis ; Herbal medicines; antibacterial activity; intracanal medicament.

A B S T R A C T

E.faecalis is a facultative anaerobic microorganism commonly detected in asymptomatic, persistent endodontic infections. Herbal medicines like *Curcuma longa* are tried because of its low side effects. Curcumin,(diferuloylmethane) the main yellow bioactive component of turmeric has a wide spectrum of biological actions and this provides a basis for exploring its endodontic applications. The purpose of this in vitro study was to evaluate the antibacterial activity of ethanolic fraction of turmeric powder against *E.faecalis* and to find its efficacy as an intracanal medicament by comparing the activity with calcium hydroxide and 2% chlorhexidine. The concentrations tested were 10microgram/disc, 50 microgm/disc, 100microgm/disc and 1000microgm/disc. Minimum inhibitory concentration of turmeric extract for *E.faecalis* could not be established because it did not show any clear zone around the discs at all the tested concentrations.

Introduction

Although many advances have been made in different aspects of endodontics within the last few years, the main objective remains elimination of microorganisms from the root canal systems and prevention of recontamination after treatment (Endodontics, Colleagues For Excellence, Winter 2011). The principle of treatment to reach favourable outcomes in endodontic infection management requires the recognition of the problem and the removal of the etiological factors. The microenvironment of root canal presents excellent conditions to establish microbial growth.

Reduction of endodontic microbiota has been achieved by a series of antimicrobial strategies that include root canal preparation, irrigating solutions, intracanal dressing, and root canal filling (Carlos *et al*., 2009).

E.faecalis is a facultative anaerobic microorganism commonly detected in asymptomatic, persistent endodontic infections. It can survive by genetic polymorphism and its ability to bind to dentin, invade dentinal tubules, and survive starvation. *E. faecalis* possesses virulence factors including lytic enzymes,

cytolysin, aggregation substance, pheromones, and lipoteichoic acid. It has been shown to adhere to host cells, express proteins that allow it to compete with other bacterial cells, and alter host responses. *E. faecalis* is able to suppress the action of lymphocytes, potentially contributing to endodontic failure (Ravishankar *et al*., 2011).

The most effective method for eliminating *E.faecalis* from the root canal space and dentinal tubules is by the use of sodium hypochlorite(NaOCl) and chlorhexidine in a gel or liquid concentration form. But disadvantages of sodium hypochlorite are unpleasant taste, toxicity, and weakening of the tooth structure by decreasing the hardness and structural integrity of the dentin within the root canal (Ravishankar *et al*., 2011).

Chlorhexidine has a broad-spectrum antibacterial action, sustained action and low toxicity and is recommended as a root canal irrigant. The major advantages of chlorhexidine over NaOCl are its lower cytotoxicity and lack of foul smell and bad taste. However, it cannot dissolve organic substances and necrotic tissues and unable to kill all bacteria and also cannot remove the smear layer (Endodontics, Colleagues For Excellence, Winter 2011).

Calcium Hydroxide($Ca(OH)_2$) inhibits microbial growth in canals due to its antimicrobial, anti-inflammatory and osteogenic potential. Due to its high alkalinity 12.5), it has a high antibacterial activity against most bacteria found in endodontic infection. It has destructive effect over bacteria's cellular membrane and protein structure (Elka *et al*., 2005).

Herbal medicines like *Curcuma longa* are tried because of its low side effects (Bettina *et al*., 2003) *Curcuma Longa* belongs to the family, Zingiberaceae, and is commonly known as turmeric (Endodontics, Colleagues For Excellence, Winter 2011). Turmeric is a natural medicament with a wide spectrum of biologic actions which include anti inflammatory, antioxidant, anti carcinogenic, antimutagenic, anticoagulant, antidiabetic, antifertility, antibacterial and antifungal activities (Ishitha *et al*., 2004). Curcumin,(diferuloylmethane) the main yellow bioactive component of turmeric has a wide spectrum of biological actions and this provides a basis for exploring its endodontic applications. Components of turmeric are named curcuminoids. These components are polyphenols with a strong antioxidant function (Prasanna *et al*., 2011).

The purpose of this in vitro study was to evaluate the antibacterial activity of ethanolic fraction of turmeric powder against *E. faecalis* and to find its efficacy as an intracanal medicament by comparing the activity with calcium hydroxide and 2% chlorhexidine.

Materials and Methods

Materials used were Soxhlet extractor, Mckonkey agar, nutrient broth, Dried turmeric rhizomes, ethanol, sterile petri dishes, *E faecalis*, sterile discs (H media), beakers and incubator.

Preparation of Ethanolic Extract of Turmeric

Commercially available turmeric roots were dried to complete dryness under sunlight and finely powdered using house hold mixer. 40g of turmeric powder was extracted with 500ml ethanol in a soxhlet extractor for 16 hrs. This extract was transferred to a conical flask for drying in

hot air oven (60 degree Celsius+/- 2 degree Celsius)overnight. The extract was then dissolved in dimethyl sulfoxide (DMSO) at 1:2(W/V) ratio. DMSO acts as a solvent without changing any property of the ethanolic extract. Different dilutions were prepared and loaded on to sterile discs.

The concentrations tested were 10microgram/disc, 50 microgm/disc, 100microgm/disc and 1000microgm/disc.

Media and culture conditions for *E.faecalis*

Enterococcus faecalis obtained from the department of microbiology, Yenopoya Medical College was cultured in Mc Conkey's agar media (Hi Media,India). A single colony was picked from the plate and inoculated into a fresh nutrient broth medium and incubated for about 48hrs at room temperature.

Minimum Inhibitory Concentration (MIC) studies

E. faecalis was spread on the agar plate evenly using sterile swabs. Sterile discs of different concentrations of turmeric extract were placed in the agar plates for incubation at 32°Celsius for 48 hrs. 0.2% and 0.5% of chlorhexidine and 0.2% and 0.5% of $Ca(OH)_2$ solutions were prepared in sterile distilled water. 20 microlitre of these samples were loaded to sterile discs and were placed on agar plates inoculated with *E. faecalis*. All the plates in triplicates were incubated for 48 hrs. Formation of inhibitory zones around the discs was observed and recorded.

Results and Discussion

Antibacterial effect of ethanolic extract of *Curcuma longa* powder:

Minimum inhibitory concentration of turmeric extract for *E.faecalis* could not be established because it did not show any clear zone around the discs at all the tested concentrations, (fig1). Chlorhexidine 0.2% solution showed a zone of inhibition of 19.5mm (fig 2). $Ca(OH)_2$, at all tested concentrations did not show any clear zone around the disc indicating no activity against *E. Faecalis* (fig 3).

Complete chemomechanical preparation may be considered an essential step in root canal disinfection (Siqueira and Lopes, 1999).The aim of the root canal treatment is to eliminate bacteria from the infected root canal and to prevent reinfection. Chemomechanical cleaning and shaping of the root canal greatly reduce the number of bacteria, but it has been shown that it is impossible to obtain complete disinfection in all cases. Therefore concern exists about the fate and subsequent activity of the remaining microorganism in the canal (Bettina *et al*., 2004).

Residual pulpal tissue, bacteria, and dentine debris may persist in the irregularities of root canal systems, even after meticulous mechanical preparation. Therefore, several irrigant solutions have been recommended for use in combination

Figure. 1 Antibacterial effect of ethanolic extract of *Curcuma longa* powder

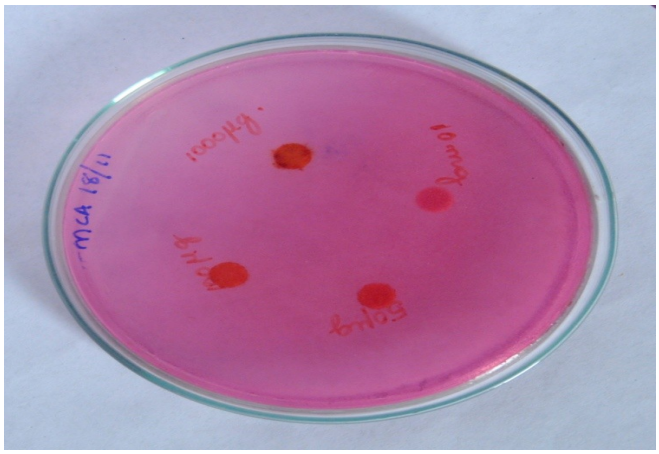


Figure.2 Antibacterial effect of ethanolic extract of *Curcuma longa* powder

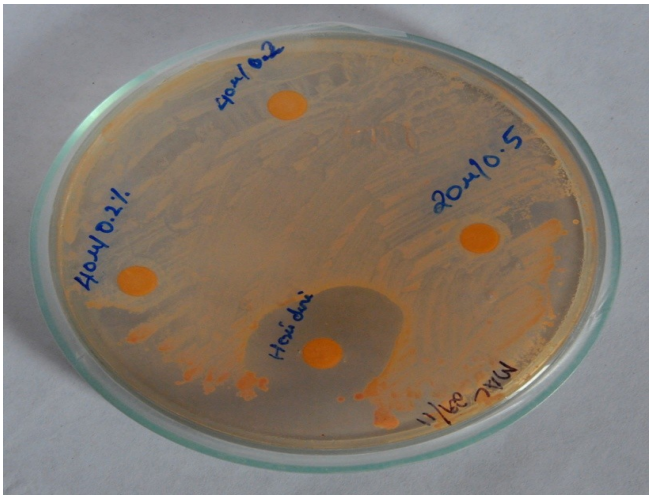
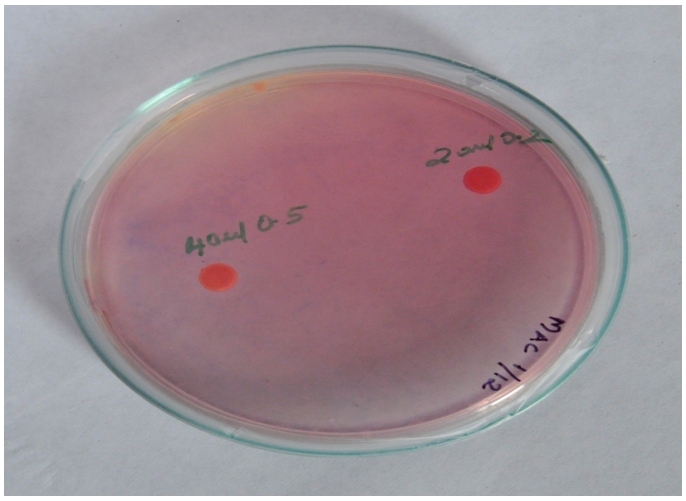


Figure.3 Antibacterial effect of ethanolic extract of *Curcuma longa* powder



with canal preparation. The efficacy of these procedures also depends upon the vulnerability of the involved species. Anaerobic bacteria, especially black-pigmented Gram-negatives, have been linked to the signs and symptoms of endodontic disease but facultative bacteria, such as *Enterococcus faecalis*, have also been isolated from pathologically involved root canals, being considered one of the most resistant species in the oral cavity and a possible cause of failure of root canal treatment (Gomes *et al*., 2001).

Enterococcus Fecalis are resistant to calcium hydroxide and recent studies question the efficacy of this medication in reducing the number of bacteria in the root canals even after a prolonged time of interappointment medication (Bettina *et al*., 2004; Sathorn *et al*., 2007; Bettina *et al*., 2003). With such a complex and dynamic microbial environment in the root canal system, selection of an effective antibacterial agent to use during treatment is critical. Antimicrobial solutions must possess many qualities such as the ability to penetrate the infected site, to suppress or destroy microbial growth, and to avoid the possible development of resistance to the agent (Endodontics, Colleagues For Excellence, Winter 2011).

In this invitro study we have evaluated the antibacterial efficacy of turmeric and compared with that of chlorhexidine and calcium hydroxide. Chlorhexidine a quartenery ammonium compound has a unique feature in that dentine medicated with it acquires antimicrobial substantivity. The positively charged molecules of CHX can adsorb onto dentine and prevent microbial colonization on the dentine surface for some time beyond the actual medication period this may inhibit re-infection of the canal subsequent to treatment during that time period. In a study by Lin *et al* a slow release device containing 5% CHX was placed in root canals for seven days to allow sufficient time for penetration into the dentinal tubules and for CHX to reach its maximum antibacterial effect. This resulted in no bacteria being detected in bovine dentine up to 500μm into the dentinal tubules. When used as an intracanal medicament, CHX was more effective than calcium hydroxide in eliminating *E.faecalis* from inside dentinal tubules. In a study by Almyroudi *et al* , all of the chlorhexidine formulations used, including a CHX/Ca(OH)2 50:50 mix, were efficient in eliminating *E.faecalis* from the dentinal tubules with a 1% CHX gel working slightly better than the other preparations[1].

Calcium hydroxide eliminated *E. faecalis* when they were present in low numbers but not in teeth with previous root fillings where *E. Faecalis* was found in higher numbers[1]. Estrela *et al* evaluated indirect effect of the calcium hydroxide as intracanal dressings in infected dentinal tubules and they were ineffective against *E. faecalis S. aureus P. aeruginosa B. subtilis* and a mixture of these biological indicators, after 7 days' exposure (Estrela *et al*., 2001; Estrela *et al*., 1999).

An in vitro study evaluated the efficacy of three intracanal medicaments 10% and 20% of turmeric, calcium hydroxide, 1% chlorhexidine gel and 1% chlorhexidine gel with 10% calcium hydroxide against E.faeclis in necrotic primary teeth and concluded that chlorhexidine with or without calcium hydroxide was more effective than calcium hydroxide alone (Kumar, Hemanshi. 2013).

Our study showed similar results to above mentioned studies. 0.2%chlorexidine (20 microlitres) of solution showed a clear zone of inhibition(19.5mm) around the disc loaded with *E. faecalis*, whereas calcium hydroxide at all tested concentrations did not show any clear zone around the disc indicating no activity against *E.faecalis.*

A study by Prabhakar *et al* comparing the antibacterial efficacy of turmeric, calcium hydroxide and 2% chlorhexidine against *E .faecalis* showed that complete inhibition was seen with chlorhexidine alone followed by 64% inhibition with calcium hydroxide and 54% with turmeric (Sathorn *et al*., 2007). Singh *et al* observed that inhibition at two depths(of dentin) was 80% for 1% Chlorhexidine, 65% for *Curcuma Longa* and 30% for Calcium hydroxide (Vijay *et al*., 2013).

Neelakantan *et al* found that curcumin had significant antibacterial activity against *E. faecalis*. They concluded that the antibacterial activity of curcumin was similar to sodium hypochlorite and thus herbal medicine can be used in endodontics for root canal failure(Bettina *et al*., 2003). A study by Gul *et al* on the antibacterial efficacy of turmeric found that ionic, resin and ethanolic fractions of turmeric are 100% effective against all tested gram positive organism, which are resistant to most of the broad spectrum antibiotics used (Nadia *et al*., 2004).

In our study the ethanolic fraction of turmeric showed no activity against *E. faecalis* in agar disk diffusion methods at various tested concentrations(10, 50, 100, 1000micrograms/disk). Calcium hydroxide and ethanolic extract of turmeric did not have any observable effectiveness against this bacterium but 0.2% chorhexidine is a viable medicament against *E. faecalis* in vitro. Further studies have to be carried out to establish efficacy of Curcuma Longa as an intracanal medicament at higher concentrations.

Acknowledgement

We would like to thank:Dr. Vidya Bhat, Professor, Department of Prosthodontics with a sense of deep gratitude for helping us sincerely in our research work, Dr. Rekha Bhagwath, Yenepoya Research Center, Yenepoya University for her technical assistance and analysis in the course of this study,and Dr. Vinutha Kamath, for playing a significant role in our study.

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