



Original Research Article

Antibacterial property of allopathic medicine versus phyto medicine: An in-vitro study

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ABSTRACT

A successful endodontic treatment depends on appropriate instrumentation, irrigation, decontamination and obturation. Decontamination of root canal plays a crucial role in the success of endodontic treatment. Therefore, a suitable medicament is essential which can effectively eliminate bacterial contamination. To investigate and compare the antibacterial property of 2% povidone iodine, chlorhexidine, 10% *Curcumin longa*, 50% *Syzygium cumini* and GAM (Gentamycin, amoxicillin and metronidazole) antibiotic against *Enterococcus faecalis*. The inoculae of *E. faecalis* was used to make lawn culture on Mueller-Hinton agar media. Wells were prepared for each sample separately and the plates were then incubated at 37°C overnight. Zone of inhibition for each medicament was measured after 48 hours. The GAM antibiotic demonstrated strongest antibacterial activity, producing the largest zones of inhibition compared with other medicaments, followed by 2% povidone-iodine. There was no statistical difference in the activity of chlorhexidine and *Syzygium cumini*. Distilled water showed no activity. The GAM-antibiotic can be used as an alternative intra-canal medicament which has proved to be the most effective antibacterial agent amongst the various test medicaments used in the present study.

Keywords

Curcumin longa,
E. faecalis, GAM
antibiotic,
Syzygium cumini,
zone of inhibition

Introduction

A successful endodontic treatment depends on appropriate instrumentation, irrigation, decontamination and obturation. Chemomechanical instrumentation removes majority of the infecting bacteria, but this is not achieved completely because of complex root canal anatomy and limitation in accessing the root canal system (Manzur et al., 2007). Persistent endodontic infection

might be attributed to the retention of microorganisms in dentinal tubules (Safavi, 1990). *Enterococcus faecalis* is more likely to be found in cases with post-treatment infection (Siren et al., 1997). Hence an intracanal medicament with good antibacterial action is therefore required to maximize the disinfection of root canal system (Bystrom et al., 1985).

Chlorhexidine gluconate (CHX) is widely used as an irrigant as well as an intra-canal medicament (Delany et al., 1982). It is a broad-spectrum antimicrobial agent (Fardak and Turnbull, 1985) that has substantive antibacterial activity and relatively low toxic effects (Greenstein et al., 1986). However, it cannot be used as a routine medicament as it lacks the property of tissue solubility (Jeansonne and White, 1994) and possess cytotoxic effect. Further, some individuals may be allergic to it (Ferraz et al., 2001).

Povidone iodine (POV-I), which is used widely as a topical antiseptic agent, is an iodophore in which iodine is linked to povidone (polyvinylpyrrolidone), a dextran-like molecule (Fleischer and Reimer, 1997). POV-I appears to be active against all microorganisms, including gram-positive and gram-negative bacteria, spores, mycobacteria, fungi, viruses, and protozoa (Cherry et al., 2007).

Turmeric (*Curcumin longa*) contains a phenolic compound called curcuminoid that possess all the bio-protective properties of turmeric. Crude turmeric extracts have both antioxidant and antimicrobial capacities so that turmeric could be a potent alternative to common antibiotics (Goel, 2009). So in this study we intended to explore the antibacterial property of turmeric to be used as an intra-canal medicament owing to its ease of availability, low cost and lack of adverse effects.

The tropical fruit, *Syzygium cumini* (black plum), is an indigenous plant grown in different parts of India. The plant was demonstrated to contain substantial hypoglycemic, anti-inflammatory and anticancerous activities (Afify et al., 2011). Few authors have reported the antimicrobial property of anthocyanins (Jimenez et al., 2011) (a water-soluble pigment, present in

higher plants imparting color to the plants (Harborne, 1988).

GAM antibiotic solution is a combination of Gentamicin (aminoglycoside), Amoxicillin (penicillin) and Metronidazole. The topical antibiotics can sterilize carious lesions, necrotic pulps, and infected root dentine of primary teeth (Hoshino et al., 1996) where the systemic antibiotics are not much effective due to lack of blood supply (Mohammadi and Abbott, 2009). Repair of damaged tissues can also be expected if the lesions are disinfected (Takushige et al., 2004) based on the concept of lesion sterilization and tissue repair therapy (LSTR).

The present study was undertaken to evaluate, the antibacterial property of 2% povidone iodine, GAM antibiotic solution, 10% *Curcumin longa* and 50% *Syzygium cumini* against *E. faecalis* using agar diffusion test.

Materials and Method

Agar diffusion test

In the present study *Enterococcus faecalis* (ATCC 24212) strain cultured on Brain-heart infusion broth (Sisco Res Lab) was used. Different concentrations of GAM antibiotic (1.25%, 2.5%, 5%, 10% and 20%) were evaluated for their antibacterial property using Agar-diffusion test, and the zones of inhibition were measured and analyzed using one-way ANOVA test. There was no statistical significant difference (p-value = 0.35) in the activity of different concentrations of GAM and hence 1.25% of GAM was chosen for the present study.

The activity of 1.25% GAM solution was then compared with 2% Povidone-iodine, 10% *Curcumin longa* and 50% *Syzygium*

cumini using the same procedure. 2% Chlorhexidine served as positive control and distilled water served as negative control.

E. fecalis strain was incubated under aerobic condition. The Mueller-Hinton agar plates were prepared in sterile glass petridishes and incubated overnight for sterility at 37°C. After ensuring sterility, the inoculae of the strains were prepared using sterile saline. A standardized suspension of the organism was swabbed over the entire surface of the agar plates. Wells of approximately 4 mm depth and 6 mm diameter were then punched in the agar plates with a sterile punch. One well was punched at the centre of each agar plate for all the samples.

Hundred microlitre of each solution was poured in their respective wells and left for diffusion. After 15 minutes the agar plates inoculated with facultative strains were incubated for 48 hours at 37°C, following which the zone of inhibition was measured using a plastic ruler and was recorded for each sample. This procedure was repeated five times for each medicament.

Result and Discussion

The mean value of microbial growth inhibition demonstrated by 2% povidone-iodine, 10% *C. longa*, 50% *S. cumini* and 1.25% GAM are illustrated in Table 1. The results were analyzed using one-way ANOVA test. 1.25% GAM-antibiotic demonstrated strongest antimicrobial action, producing the largest zones of inhibition when compared with other medicaments.

Comparison of antibacterial activity across the group was done using Turkey's multiple comparison test analysis (Table 2). The results concluded that 1.25% GAM was most effective medicament followed by 2% povidone-iodine. There was no statistical difference in the activity of chlorhexidine

and *Syzygium cumini*. *Curcumin longa* showed minimum activity whereas, distilled water showed no activity at all.

The main aim of endodontic treatment of teeth with pulpal and periapical pathology is to eliminate the microorganisms from the root canal system. Microorganisms present within the dentinal tubules can remain unaffected by chemo-mechanical procedures. Since *Enterococcus faecalis* has been recovered in high quantities from failed root-canal treated teeth (Gajan et al., 2009), it has been chosen for the study. Its prevalence in persistent endodontic infections ranges from 24% to 77% (Stuart et al., 2006). Numerous studies have shown another extremely important characteristic of this microorganism: capacity to withstand a wide pH range up to around 11.5 of intracanal medicaments such as calcium hydroxide which is generally a highly potent antimicrobial dressing (Evans et al., 2002). Therefore the use of a biocompatible intracanal medicament possessing better antimicrobial properties in between appointments may reduce or eliminate bacteria in the root canal system with significant increase in the success of root canal treatment.

The present study used Mueller-Hinton Agar as the culture media. Bauer et al., 1966, recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, and provides satisfactory growth of most non-fastidious pathogens along with demonstrating batch-to-batch reproducibility. This agar media complies with requirements of the World Health Organization (World Health Organization, 1961) for procedures commonly performed on aerobic and facultatively anaerobic bacteria (Wood and Washington, 1995).

Table.1 Antibacterial activity of different medicaments against *Enterococcus fecalis*

S.No.	Sample	N	Mean ± SD (in mm)	Significance	
				F-value	P-value
1	2% Povidone-iodine	5	25.60 ± 4.77	60.0854	< 0.001**
2	10% Curcumin longa	5	7.80 ± 5.12		
3	50% <i>Syzygium cumini</i>	5	17.40 ± 2.19		
4	1.25% GAM antibiotic	5	40.80 ± 4.44		
5	2% Chlorhexidine	5	20.20 ± 5.22		
6	Distilled water	5	0		

**Highly significant

Table.2 Comparison of zone of inhibition (in mm) across groups using paired t-test analysis

S.No.	Group I versus Group II		Mean ± SD (in mm)	Significance	
				p-value	Hypothesis
1	POV-I 25.60 ± 4.77	Turmeric	7.80 ± 5.12	< 0.001**	Rejected
		Jamun	17.40 ± 2.19	< 0.05*	Rejected
		GAM	40.80 ± 4.44	< 0.05*	Rejected
		Chlorhexidine	20.20 ± 5.22	0.125	Not rejected
		Distilled water	0	< 0.001**	Rejected
2	Turmeric 7.80 ± 5.12	Jamun	17.40 ± 2.19	< 0.05*	Rejected
		GAM	40.80 ± 4.44	< 0.001**	Rejected
		Chlorhexidine	20.20 ± 5.22	< 0.05*	Rejected
		Distilled water	0	< 0.05*	Rejected
3	Jamun 17.40 ± 2.19	GAM	40.80 ± 4.44	< 0.001**	Rejected
		Chlorhexidine	20.20 ± 5.22	0.194 NS	Not rejected
		Distilled water	0	< 0.001**	Rejected
4	GAM 40.80 ± 4.44	Chlorhexidine	20.20 ± 5.22	< 0.05*	Rejected
		Distilled water	0	< 0.001**	Rejected
5	Chlorhexidine 20.20 ± 5.22	Distilled water	0	< 0.001**	Rejected

* Significant

** Highly Significant

NS = Non Significant

Highest antibacterial property of 1.25% GAM was seen in our study. According to *Nakornchai et al., 2010*, the 3-Mix antibiotic paste containing Ciprofloxacin, Minocycline and Metronidazole is superior to vitapex for root canal treatment of pulpally involved primary molars. But *Kim et al., 2010*, and *Lenherr et al., 2012*, identified the

discolouration caused by Minocycline used in tri-antibiotic paste. Therefore, *Thomson and Kahler, 2010*, substituted Amoxicillin for Minocycline in their case report to avoid this discoloration. Hence in the present study a new combination of antibiotics was used containing Gentamycin, Amoxicillin and Metronidazole and was termed as GAM

paste. Gentamycin has rapid bactericidal activity, and comparatively low levels of resistance, therefore is used for rapid control of infections. Amoxicillin targets gram-positive micro-organisms and metronidazole has a broad spectrum activity against protozoa and anaerobic organisms. The topical antibiotics can sterilize necrotic pulps of primary teeth where the systemic antibiotics are not much effective due to lack of blood supply (Mohammadi and Abbott, 2009).

Curcumin longa has been shown to have a potent antibacterial activity against a number of pathogenic bacteria including *Enterococcus* (Dipti et al., 2008), but in the present study aqueous extract of *Curcumin longa* showed minimum antibacterial activity against *E. faecalis*, which may be due to low concentration of turmeric used.

Also the aqueous extract of *Syzygium cumini* fruit (inhibition zone = 17.40 ± 2.19 mm) was used in our study whereas, methanolic extract of the peel of *Syzygium cumini* was used (inhibition zone = 17.20 ± 0.28 mm), which showed similar results as reported by Priya et al., 2013.

The findings of the present study demonstrated that 1.25% GAM had a good antimicrobial effect against *E. faecalis*. However, the medicament that is effective against single microbe in vitro may not necessarily be effective against the same microbe in vivo because root canal system contains multiple microorganisms. Further studies using the same medicament in failed root canal cases in vivo have to be conducted. The duration of action and the depth of penetration into the dentinal tubules of this medicament also need to be investigated and compared.

Limitations of the Procedure

1. Multiple factors can influence the results of this study like the size of inoculum, growth rate, medium formulation and the pH. Strict adherence to the required protocol ensures reliable results.
2. The inactivation of the medicament may result from the prolonged incubation times required by slow growers.

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