



Research Article

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Antibacterial activity of two herbal extracts and 2% sodium hypochlorite against *Enterococcus faecalis*: An invitro comparative study

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ABSTRACT

Sodium hypochlorite is the most commonly used irrigant but it has disadvantage like high cytotoxicity. Literature have shown that various extracts namely *Acacia catechu*, *Terminalia chebula*, *Propolis*, *Aloe barbadensis*, *Acacia nilotica*, *Curcuma longa*, *Propolis*, *Salvia officinalis* and *Azadirachta indica* have good properties which can be used as a potential endodontic irrigant. So there is a need to find an alternative to sodium hypochlorite against *Enterococcus faecalis*. The aim of the article is to find a natural remedy for treatment of Root canal failure. An attempt was taken to compare the antibacterial effects of *Achillea millifolium* commonly known as Yarrow and *Hemidesmus indicus* known as Indian sarsaparilla with 2% NaOCl against *E. faecalis* using broth dilution method. According to this study yarrow showed moderate antibacterial activity at 50mg/ml, whereas sodium hypochlorite inhibits the growth of *E. faecalis* at 5 mg/ml while *Hemidesmus indicus* did not show antibacterial activity against the tested strain. The study indicates that 2% sodium hypochlorite generally used as a root canal irrigant shows maximum antibacterial activity against *E. faecalis* when compared to *Achillea millifolium* ethanolic leaf extract which showed moderate inhibitory activity against growth of *E. faecalis*. It can be concluded that the tested plant extracts show evidence of anti bacterial activity against *E. faecalis* through enhancing the success of Root canal treatment in a natural way. The results justify the therapeutic applications of the active compound in the indigenous system of medicine, augmenting its therapeutic value in the field of dentistry.

Keywords: *E. faecalis*, *Achillea millifolium*, *Hemidesmus indicus*, Sodium hypochlorite, endodontic irrigant

INTRODUCTION

Pharmacological evaluations proved the value of ethnobotanicals as potential source of bioactive compounds. The constant increase in various side effects caused by synthetic drugs has prompted researchers to go beyond for herbal alternatives. Various phytochemical extracts exhibit antibacterial, anticandidal and therapeutic effects suggesting their efficacy to be used as an endodontic irrigant.[1,2]

Enterococcus faecalis, a facultative anaerobic "gram-positive coccus", and *Candida albicans* are the most commonly isolated species in persistent root canal infections.[3,4]

Sodium hypochlorite (NaOCl) has some undesirable characteristics such as tissue toxicity and allergic potential.⁵ and bad odour and taste; therefore, a trend towards application of herbal drugs has arisen. Many herbal drugs have been evaluated for their ability to inhibit *Candida albicans* and *E. faecalis*.⁶

Previous studies on heartwood, leaf and bark extracts have shown that these agents can inhibit *E. faecalis*.^{7,9}

Another study was conducted on horse chest nut extract against oral microbes. it inhibited all the tested oral microbes that causes dental caries and plaque but unfortunately did not inhibit *E.faecalis*.⁸

Various literature reveals that curcumin, propolis plays a vital role as root canal irrigant.¹⁰

In the present study, we have chosen yarrow extract commonly called *Achillea millifolium* that has immense uses in dentistry.it is used to eradicate the oral microbes that participate in dental plaque formation.

Traditional indications of *Yarrow* use includes digestive problems, liver and gallbladder conditions, menstrual irregularities, cramps, fever, wound healing. *Indian sarasaparila* is used as a tonic, demulcent, diaphoretic, diuretic and blood purifier. The therapeutic action of the herb is due to presence of major chemical constituents like coumarinolignoids hemidesmine and hemidesmin. Both the extracts exhibit anti bacterial and antifungal properties.^{11,12}

EXPERIMENTAL SECTION

Yarrow and *Hemidesmus indicus* are obtained as a gift sample from Green chem herbal extracts and formulations, Bangalore, India. *E.faecalis* ATCC 29212 is used as a bacterial pathogen. Positive control -2 % NaOCl and Negative control-plain broth without herbal extracts is used in the study.Tryptic soy broth is used as a medium to carry out broth dilution method.

Preparation of different concentrations of herbal extracts

200mg of each herbal extract had been weighed aseptically into a sterile tube and dissolved in 2ml of sterile Tryptic soy Broth (TSB).From the stock solution various concentrations were prepared,viz.,0.15mg,0.31 mg,0.62 mg,1.24mg,2.5mg ,5mg,10mg,50mg,100mg/100 μ l respectively in to wells of micro plates. The tested organism was grown in (TSB)Tryptic soy broth medium [MHA-Hi media ,Mumbai] for 24hrs at 37°C and concentration was adjusted to 0.5 Mac farland Standard.¹³ The different concentrations of extracts were taken in 100 μ l quantities in a U bottom micro culture plates. Control well received plain broth without plant extract. 2% sodium hypochlorite was used as positive control. The plates were kept in sealed covers and incubated at 37°C overnight and growth/no growth was detected. All the tests were done in triplicate to minimize the test error.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of herbal extracts against tested microorganisms was determined by macro broth dilution method .¹⁴A series of two- fold dilution of each extract

(0.15mg/100 μ l to 100mg/100 μ l) was made in to which 100 μ l of the standardized bacterial suspension containing 10⁶ organisms was made in Tryptic soy broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990).¹⁵The control well received plain broth without herbal extract .The plates were incubated at 37°C for 24 hours and observed for visible growth. As the extracts were colored, MIC could not be read directly by visual methods. Hence subcultures from all the wells were made and growth/no growth is detected. then the MBC were obtained.

Minimum Bactericidal Concentration (MBC)

The MBCs were determined by selecting wells that showed no growth. The least concentration, at which no growth was observed, was noted as the MBC.

RESULTS

Statistical Analysis

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

(Table 1). One way ANOVA shows that there is significant difference between the growth inhibition of *Achillea millifolium* extract and 2% NaOCl against *E. faecalis* ($p < 0.05$, ANOVA). There is No significant difference in growth inhibition for *Hemidesmus indicus* extract ($p > 0.05$, ANOVA; Fig 5) against *E.faecalis*. The difference was also significant with respect to the solvent control which showed Growth inhibition.

Organism against herbal extract and 2% Naocl	Concentration of Herbal extracts and 2%Naocl(mg/ml)									MIC/MBC
	0.15	0.31	0.62	1.24	2.5	5	10	50	100	
<i>E.faecalis</i>	0.15	0.31	0.62	1.24	2.5	5	10	50	100	
<i>Yarrow</i>	G	G	G	G	G	G	G	NG	NG	50 mg/ml
<i>Hemidesmus indicus</i>	G	G	G	G	G	G	G	G	G	No inhibition
2% Naocl w/v	G	G	G	G	G	NG	NG	NG	NG	5mg/ml

G = Growth

NG= No Growth indicates MIC/MBC.

Table 1: Anti bacterial activity of herbal extracts and NaOcl against *E.faecalis*

DISCUSSION

Recently there seems to be an immense increase in the field of dentistry to opt the use of herbal extracts for eradicating caries, dental plaque and root canal irrigants. Sodium hypochlorite and chlorhexidine is the gold standard root canal irrigant used in endodontal practice.¹⁶ however there are many research studies still investigating the use of herbal extracts in comparison with 0.5%-6% sodium hypochlorite against *E.faecalis*.

Phytochemical extracts such as *Curcuma longa* active ingredient in Turmeric, *Azadiracta indica* in Neem, *Myristica fragrans* in Nutmeg, *Terminalia chebula*, Myrobolan and *Aloe barbadensis* in Aloe vera consists of active ingredients like curcumin, nimbidin, myristic acid, tannins, anthraquinones respectively which have been reported to exert antimicrobial, anti inflammatory and antioxidant properties.^{17,18} Literature supports these extracts showed good antibacterial activity against *E.faecalis*.¹⁹

Total cleaning of the root canal system using mechanical instrumentation is ineffective due to extremely complex root canal anatomy. Irrigants serve several purposes, including tissue solvent, disinfection, flushing of gross debris and lubrication. Proper irrigation of the root canal system during endodontic treatment is essential for successful treatment.²⁰

The most widely used endodontic irrigant is 0.5% to 6.0% sodium hypochlorite (NaOCl), because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue.^{21,22} However, NaOCl solutions exert no effects on inorganic components of smear layer. Chelant and acid solutions have been recommended for removing the smear layer from instrumented root canals, including ethylene diamine tetra acetic acid (EDTA), citric acid, and phosphoric acid.^{23,24}

Essential oils has also been evaluated for antibacterial activity against *E.faecalis*. In a comparative study with chlorhexidine and Naocl, SKJ(*Satureja Khuzistanica* Jamzad) Essential oil with the MIC of 0.31 mg/ml might be considered as an effective antibacterial irrigation solution.²⁵

E. faecalis is found in 4 to 40% of primary endodontic infections. The frequency of *E. faecalis* found in persistent periradicular lesions has been shown to be much higher. In fact, failed root canal treatment cases are nine times more likely to contain *E. faecalis* than primary endodontic infections.²⁶ The most effective method for eliminating *E. faecalis* from the root canal space and dentinal tubules is the use of sodium hypochlorite and 2% chlorhexidine, in a 2% gel or liquid concentration form.²⁷⁻²⁹

Due to the disadvantages of sodium hypochlorite like unpleasant taste, toxicity, and potential weakening of the tooth structure by decreasing the hardness and structural integrity of the dentin within the root canal. To overcome this disadvantage Herbal medicine like Propolis, Curcuma longa, Acacia nilotica, Azadirachta indica, Aloe vera are used because of its low side effects.¹⁸

Statistical analysis shows *Achillea millifolium* extract and 2% NaOCl is found to be significant ($p < 0.05$) by performing one way Analysis of Variance (ANOVA) whereas *Hemidesmus indicus* root extract is not found to be significant ($p > 0.05$)

The present findings suggest that *Achillea millifolium* extract can also be used as a root canal irrigant as sodium hypochlorite and chlorhexidine.

CONCLUSION

Based on the results obtained in this study, it may be concluded that plant extract of *Achillea millifolium* has a moderate spectrum of antibacterial activity against *E. faecalis* that commonly causes root canal failure during endodontic procedure and the extract is equally efficacious to 2% NaOCl which is preferably employed as a root canal irrigant in practice. Thus *Achillea millifolium* ethanolic leaf extract may be used to overcome the problem of increasing Root canal failure in Endodontic field.

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