Original Research Article

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016
ICV: 71.54

A Comparative Evaluation of the Efficacy of Andrographis Paniculata, 2% Chlorhexidine and 5.25% Sodium Hypochlorite Against Enterococcus Faecalis- An Invitro Study

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ABSTRACT

Aim: To evaluate and compare the antimicrobial efficacy of 0.5% phytochemical extracts of Andrographis Paniculata (AP), 2% Chlorhexidine (CHX) and 5.25% Sodium hypochlorite (NaOCl) as an endodontic irrigant against Enterococcus faecalis.

Methodology: Forty freshly extracted human single rooted mandibular premolars were selected, decoronated and standardized to 12mm root length. Cleaning and shaping was done using ProTaper® (Dentsply) up to size F3 and irrigated with 3% NaOCl. Contamination of the specimens was carried out for 21 days at 37ºC. The specimens were then divided into four groups (n=10) for irrigation with 0.5% AP, 2% CHX, 5.25% NaOCl and distilled water as control. Before and after irrigation, microbiological samples were collected using sterilized paper points, and efficacy was expressed as reduction in microbial load using Colony Forming Units. Statistical analysis was performed using One-way Anova/Kruskal-Wallis test with Tukey's Post Hoc test.

Result: In the root canals, the 0.5% herbal extract of AP was found to be equally effective to 2% CHX and highly effective than 5.25% NaOCl against Enterococcus faecalis with a marked decrease in the number of CFU at the 48th hr. from 2.6 x10^3 to 0.9 x10^3. The values were statistically significant with $p<0.5$.

Conclusion: Being a herbal extract with antimicrobial efficacy similar to 2% CHX, 0.5% AP seems to be a better alternative. However further tests and randomised clinical trials are required on AP to use it as an endodontic irrigant in vivo.

Key Words: Enterococcus faecalis, Endodontic irrigant, Leaf extracts, Andrographis paniculata, Colony forming unit

INTRODUCTION

Microorganisms and their toxic metabolic products are responsible for the development and persistence of apical periodontitis of endodontic origin. Enterococcus faecalis, facultative anaerobic gram-positive cocci is the most commonly isolated species in persistent root canal infections[1].

Sodium hypochlorite, the most commonly used irrigant has some undesirable characteristics like tissue toxicity, allergic potential and disagreeable smell and taste. Chlorhexidine used as an irrigant is active against E.faecalis but has less tissue dissolving property[2].

The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives. Various natural plant extracts has antimicrobial and therapeutic effects suggesting its potential to be used as endodontic irrigants. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds[3].

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Received: 02.01.2017 **Revised:** 12.01.2017 **Accepted:** 22.01.2017

*Andrographis Paniculata*or kalmeghis available abundantly in India, Pakistan and Srilanka and also one of the most widely used plants in Ayurvedic formulations. *A.paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations[4]. Andrographis has demonstrated significant activity in fighting common cold, flu and upper respiratory infections. The pharmacological studies suggest its anti-inflammatory, antipyretic, anti-viral, immunostimulatory, potential cancer therapeutic agent, anti-hyperglycaemic and antioxidant properties[5-11]. Researchers have found that the acetone and methanol extracts of leaves and stems of *A.paniculata* showed greater inhibitory effect (30.33±0.88mm) on the growth of *Enterococcus feacalis*[4]. Hence the purpose of this study was to evaluate the antimicrobial efficiency of the leaf extracts of *A.paniculata* against *Enterococcus faecalis* as an endodontic irrigant.

MATERIALS AND METHODS:

Phytochemical extract: Shade dried powder of leaves of *A.paniculata* were obtained (Aravindh herbals, Chennai). The coarse powder of the sample was extracted with 2.5 litres of 95% ethanol by maceration process until the extraction was completed. Then the extract was filtered and the solvent was removed by distillation under reduced pressure.

Specimen preparation:Forty extracted human, permanent straight single-rooted mandibular premolar teeth with no caries, apical fractures and resorption were selected and stored in saline. The teeth samples were decoronated using rotary diamond disc with a standardized length 12 mm below the CEJ. In order to standardize the samples, each canal was prepared up to size 30 with ProTaper rotary nickel-titanium instruments (Dentsply Tulsa Dental, Johnson City, TN) according to manufacturer's instructions. The root apices were coated with nail varnish to seal the apical foramen. The canals were irrigated with 10ml of 5.25% NaOCl and 10ml of 17% EDTA. The specimens to be tested were sterilized at 121°C for 15 minutes at 26 psi and stored in 100% humidity at 30°C until use.

Isolation of micro-organisms: Purestrain of *Enterococcus faecalis* from American Type Culture Collection (ATCC #29212) was used. Respectively cultures were grown overnight at 37°C in brain heart infusion (BHI) broth on a rotary shaker 150 rpm and microbial growth were checked by changes in turbidity at 24 hours.

Antimicrobial activity test: As a preliminary test, the minimum inhibitory concentration(MIC) of *A.paniculata* against *E.faecalis* was checked by well diffusion method. Test pathogens were spread on the test plates- Mueller Hinton Agar (MHA). Sterile well of 6mm diameter was made and 5, 10, 20 µl of the medicament was loaded in the well. The test plates were incubated for 24hr. the zone of inhibition (mm in diameter) were read and taken as activity against the test pathogen. Chlorhexidine was loaded as the positive control and the MIC of *A.paniculata* against *E.faecalis* was found to be 0.5%.

Contamination of the specimens: Contamination of specimens werecarried out for 21 days at 37°C with E.faecalis adjusted to a degree of turbidity 1 according to McFarland scale, which corresponds to a microbial load of 3×10^3 cells/ml. Under laminar flow, the samples were recontaminated every second day with fresh broth containing the microorganism.

Irrigation procedure: The specimens were irrigated with 25-G needle tip was placed to a depth of 1mm short of WL, respectively.

Group 1: Irrigation was done with 2% CHX
Group 2: Irrigation was done with 5.25% NaOCl
Group 3: Irrigation was done with extracts of A.Paniculata
Group 4: Irrigation was done with distilled water.

CFU procedure: After incubation, the tooth canal was drilled and the invaded bacteria was collected and placed in nutrient broth. Then 100µl of the broth was plated on the sterile nutrient agar medium and spread evenly with L-rod and incubated overnight. CFU was evaluated at 0^{th}hr and 48^{th}hr using digital counter.

Results: In the root canals , the 0.5% herbal extract of AP was found to be equally effective to 2%CHX and highly effective than 5.25%NaOcl against Enterococcus faecalis with a marked decrease in the number of CFU at the 48^{th}hr from $2.6x10^3$ to $0.9x10^3$. The mean values of No.of bacterial colonies before and after irrigation are listed in Table [1].

Table 1: Mean values of No.of bacterial colonies before and after irrigation

CFU at 0^{th} hour		CFU at 48^{th} hour	
Samples	**No. of colonies**	**Samples**	**No. of colonies**
GROUP I	$2.3x10^3$	GROUP I	$0.142x10^3$
GROUP II	$2.4x10^3$	GROUP II	$1.88x10^3$
GROUP III	$2.9x10^3$	GROUP III	$0.177x10^3$
GROUP IV	$2.5x10^3$	GROUP IV	$2.5x10^3$

Statistical analysis was performed using One-way Anova/ Kruskal-Wallis test with Tukey's post-hoc test.The values were statistically significant with $p<0.5$

DISCUSSION

The success of endodontic treatment lies in the complete debridement of root canal system with the help of chemi-

cal irrigants and mechanical instruments[1]. Owing to the rising incidence of antimicrobial resistance against various chemotherapeutic and antimicrobial agents, the treatment of bacterial infection requires special consideration which may otherwise lead to grave prognosis. Simultaneously, the evolution of multi drug resistant (MDR) bacterial strains has further aggravated the present situation[12].

In this scenario, herbs have been a valuable source of medication worldwide due to their important antimicrobial principles, phytoconstituents and wider therapeutic potentials. In the present study, the herbal extracts of *A.paniculata* were used due to its wide range of pharmacological effects. Diterpenoids and flavonoids are the main phytochemical constituents which are believed to be responsible for the most biological activities of this plant[13].Andrographolide, a diterpenoid found in leaf extracts is said to possess antimicrobial property and the flavanoids are nature's biological response modifiers. Phenolic compounds are the largest group of phytochemicals that accounts for most of the antioxidant and antimicrobial properties. At low concentration tannins can inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganism[4].

A study conducted by Radha R et al., concluded, extracts of *A.paniculata* showed greater inhibitory effect (30.33±0.88mm) on the growth of Enterococcus faecalis. The herbal extracts can also be used as an endodontic irrigant against E.faecalis, the most common pathogen found in persistent root canal infection.

In the present study, the minimum inhibitory concentration (MIC) of the crude extracts of *A.paniculata* against *E.faecalis* was determined by agar dilution method and it was found to be 0.5%. The root canal specimens were contaminated and incubated for 3 weeks which has been shown to produce a dense infection reaching 200 µm to 400 µm into the dentinal tubules[3]. The antimicrobial activity was evaluated by the number of colony forming units at 0^{th} hour and 48^{th} hour.

The results of the study showed that, 0.5% leaf extracts of *A.paniculata* was highly efficient to 5.25% NaOCl and equally efficient to 2% CHX. *A.paniculata* is biocompatible and severe tissue injuries and adverse effects were less when compared to NaOCl and CHX.Bitter taste associated with this plant can be altered by different formulations due to addition of sweeteners and flavours to increase the patient's compliance and acceptability.

CONCLUSION

Being an herbal extract with several pharmacological activities including its antioxidant property, *A.paniculata* seems to be a better alternative. However further clinical trials and randomised control studies are required to use *A.paniculata* as an endodontic irrigant in vivo.

ACKNOWLEDGEMENT

The authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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Figure 1: Contamination with E.faecalis.

Figure 2: Incubation of tooth samples.

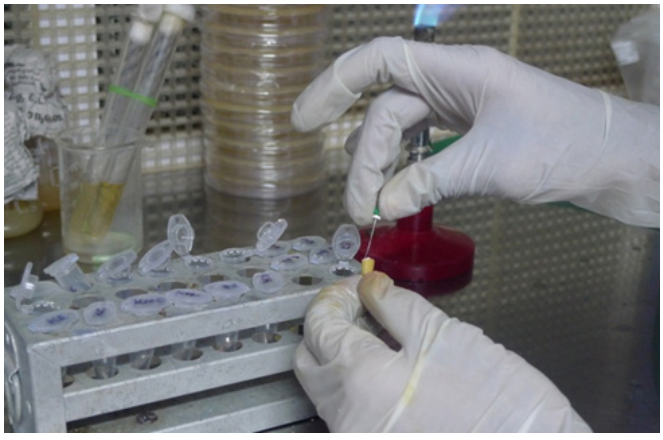

Figure 3: Dentin samples removed for CFU.

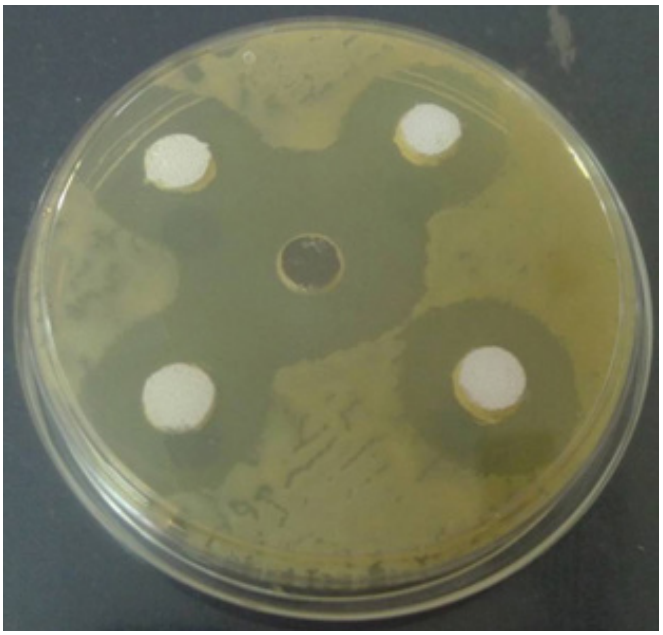

Figure 4: Agar well diffusion method.

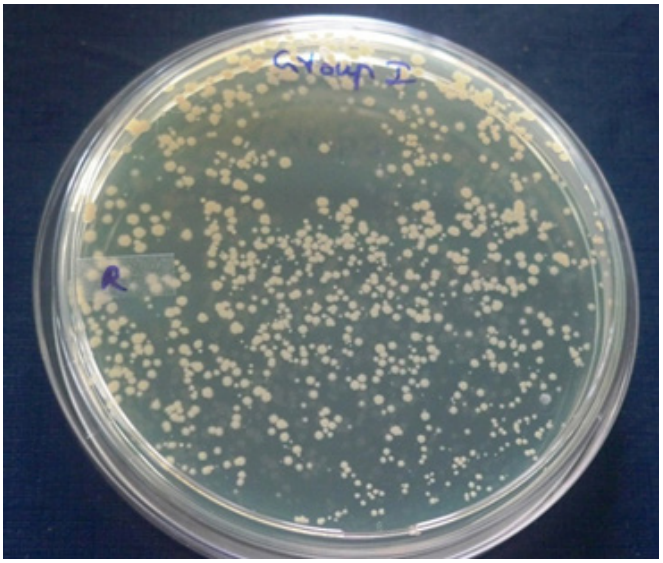

Figure 5: CFU at 0th hr.

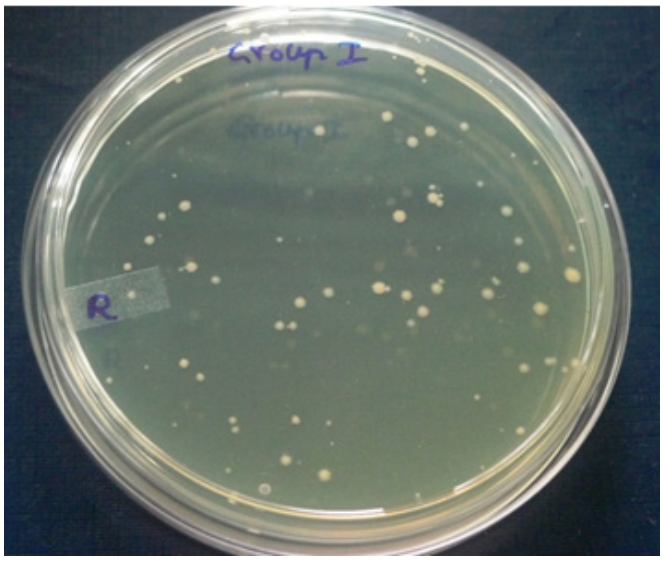

Figure 6: CFU at 48th hr. (2% CHX).

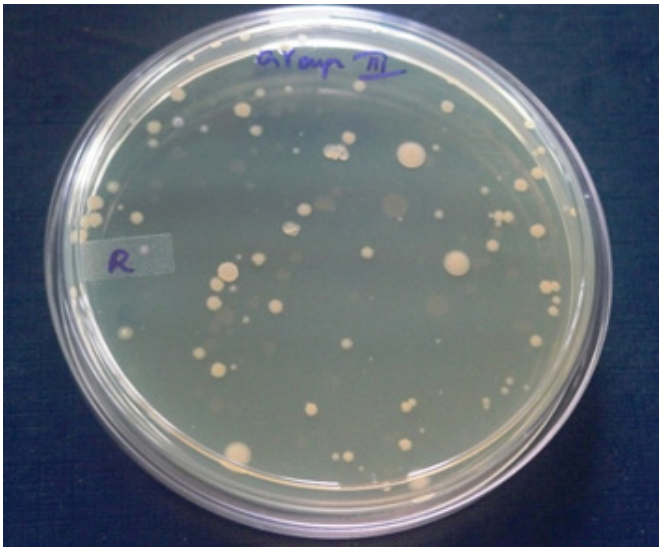

Figure 7: CFU at 48th hr. (0.5% AP).