ORIGINAL ARTICLE

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Comparison of Antimicrobial Efficacy of Herbal Alternatives (Aloe vera extract & Tea tree oil) & 3% Sodium Hypochlorite against Enterococcus Faecalis, Candida Albicans & Mixed Culture: An In-Vitro Study

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ABSTRACT:

Background: Successful endodontic treatment requires complete elimination of micro-organisms from the root canal system. Candida Albicans & Enterococcus Faecalis are the most predominant micro-organisms recovered from teeth requiring retreatment. Sodium hypochlorite has been the most widely used irrigant. The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives in endodontics.

Materials & Methods: Tea tree oil (2% volume), aloe vera extract (1:5), 3% sodium hypochlorite(NaOCl), normal saline (control) & pure cultures of Enterococcus Faecalis(E. Faecalis), Candida Albicans (C.Albicans), & a mixed culture (1:1).

To check the antimicrobial efficacy, agar well diffusion method was performed. Brain heart infusion (BHI) agar plates were prepared & cultures were spread onto them. Wells (7mm diameter) were punched in the agar surface. The solutions were added to the respective wells & the plates were incubated at 37ºC for 24 hours. The diameter of zone of inhibitionwas recorded. The experiment was performed 3 times, mean of the readings was recorded in mm & statistical analysis was performed.

Results: Aloe vera showed significant antimicrobial activity against E. Faecalis, Tea tree oil showed activity against all the three cultures, though significant activity was seen only against C. Albicans. NaOCl,the most commonly used root canal irrigating solution showed significant activity against all the three cultures.

Conclusion: Further preclinical & clinical studies are required to conclusively recommend aloe vera & tea tree oil as root canal irrigants.

Keywords: Candida albicans, Enterococcus faecalis, Sodium hypochlorite.

INTRODUCTION

The major objective in root canal treatment is to disinfect the entire root canal system. Although cleaning, shaping & use of antimicrobial medicaments are effective in reducing the bacterial load, some bacteria do remain behind & multiply, causing re-infection of the canal.[1]

Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in secondary infections are composed of one or a few bacterial species.[2]

Microorganisms and their toxic metabolic products are responsible for the development and persistence of apical periodontitis of endodontic origin. Enterococcus faecalis, a facultative anaerobic gram-positive coccus and Candida albicans are the most commonly isolated species in persistent root canal infections.[3]

Residual pulp tissue, microorganisms, dentin debris may persist in the irregularities of the root canal systems even after meticulous mechanical preparation. Therefore irrigant solutions should be used in combination with canal preparation.[1]

Root canal irrigants are used during chemicomechanical procedures not only as antimicrobial agents but also to flush out loose

debris, to lubricate dentinal walls & to dissolve organic compounds in the canal.[1]

Sodium hypochlorite has been widely recommended as an irrigant for chemicomechanical debridement of root canals because of its tissue dissolution and antimicrobial activity, thus making it an irrigating solution of choice. However, it has some undesirable characteristics like tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste.[4]

Herbal medicine is an increasingly common form of alternative therapy throughout the world. Consequently, herbal medicines are finding their usefulness in the arena of dentistry and their armamentarium. Earlier they were limited to as an important ingredient of tooth pastes, mouthwashes and as pain reliever, but now a days they are increasingly being used in all possible treatments in dentistry like root canals, surgeries, periodontal therapies, anti-plaque agents to name a few.[5]

MATERIALS & METHODS

Tea tree oil (2%), aloe vera extract(1:5), 3% sodium hypochlorite, normal saline & pure cultures of E. Faecalis, C. Albicans, & a mixed culture (i.e. E. Faecalis & C. Albicans in 1:1 ratio) were the materials used. Pure culturesof E. Faecalis & C. Albicans were obtained from the Department of Microbiology, Karamveer Bhaurao Patil college, Vashi, Navi Mumbai.

PREPARATION OF 2% TEA TREE OIL

- Tea tree oil (RYM Exports, Mumbai) was used, prepared to have miscibility in 85% (v/v) ethanol, to get a concentration of 2%.
- The activity of 85%(v/v) ethanol alone was also tested to confirm that it did not have any significantactivity against the microorganisms & thus the readings acquired for tea tree oil.

PREPARATION OF ALOE VERA EXTRACT:

- The leaves of the plant were washed with distilled water & the surface of the leaves was disinfected using alcohol. After cutting & opening the leaves, the fresh pulp was collected (Figure 1) & homogenized.

Figure 1: Fresh pulp

- It was mixed well with liquid media extract (methanol) in 1:5 ratio (Figure 2).

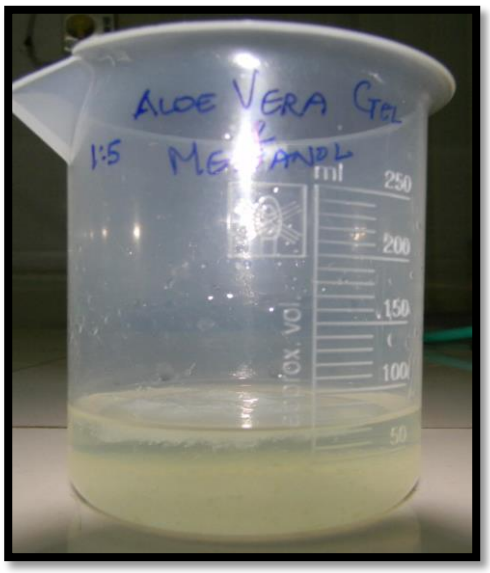

Figure 2: Fresh pulp with methanol

- The mixed solution was placed on a hot water bath for dehydration (Figure 3).

Figure 3: Mixed Solution in hot water bath

- After attaining the precipitate (Figure 4), it was dissolved with the liquid media solvent (methanol).

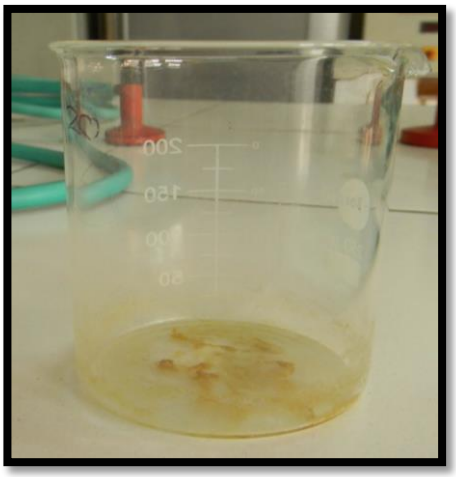

Figure 4: Precipitate mixed with methanol

- The activity of methanol alone was also evaluated to make sure it did not have any significant activity against the cultures & thus, the readings acquired for aloe vera extract.

MICROBIAL CULTURE

E. Faecalis & C. Albicans were grown overnight at 37ºC in Brain Heart Infusion broth & bacterial growth was checked by changes in turbidity at 24hrs.

AGAR DIFFUSION METHOD

- After confirming the growth of respective microorganisms in the broth, 1 ml of each microbial culture suspension was mixed in 20 ml of molten brain heart infusion agar butts, pour plated & allowed for complete solidification at room temperature.
- For mixed culture inhibition, both the cultures (E. Faecalis & C. Albicans) were mixed in 1:1 ratio and 1 ml of the mixed culture suspension was mixed with molten agar, pour plated and solidified.
- After complete solidification 7mm wells were prepared on the surface of the agar and 50ul of respective extracts at predetermined concentrations along with 3% NaOCl were placed.
- Control we had taken was saline.
- The plates were incubated for 24hrs in an incubator at 37ºC & examined for zones of inhibition, the procedure was performed in triplicates & mean was recorded.

The data was statistically evaluated using ANOVA test, Kruskal-Wallis test (non-parametric one way ANOVA test) & Tukey's test (for multiple comparison).

RESULTS

The p-values for ANOVA test for E.faecalis, C.albicans and Mix(1:1) cultures indicates significant difference between the mean zone diameter of 3% NaOCI, tea tree oil (2%), aloe vera and saline.

As data did not pass test of normality, results were also verified using Kruskal-Wallis (non-parametric ANOVA) test. The p-values for E.faecalis, C.albicans and mix(1:1) cultures indicated significant difference between the mean zone diameter of 3% NaOCI, tea tree oil (2%), aloe vera and saline.

Figure 5: Zones of inhibition for 3% sodium hypochlorite (NaOCl)

Figure 6: Zones of inhibition for 2% tea tree oil

Figure 7: Zones of inhibition for Aloe vera (1:5)

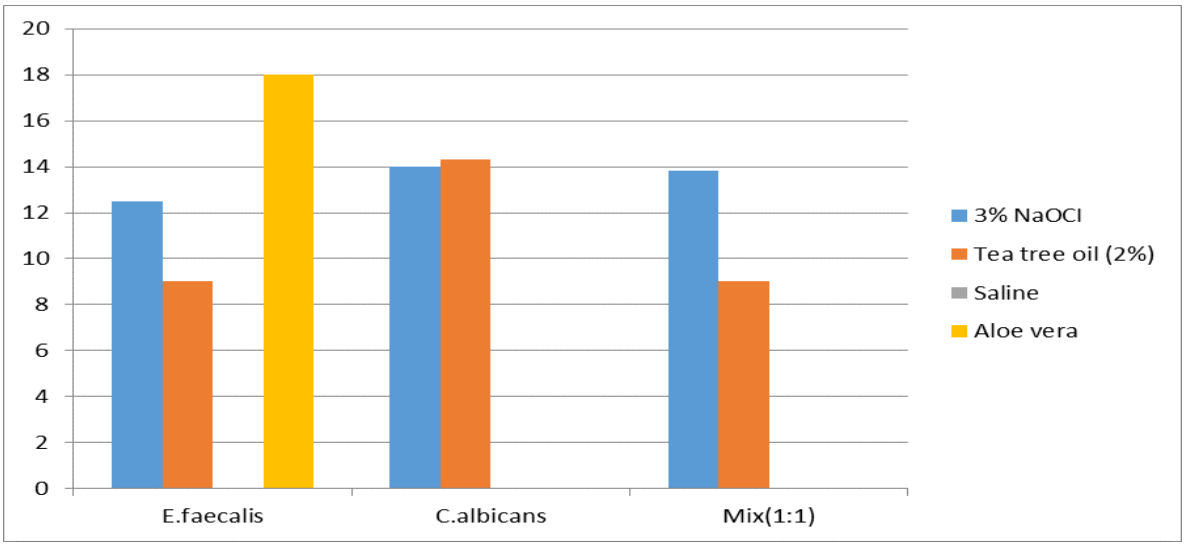

Figure 8: Mean zones of microbial inhibition

Table 1: Mean values of zones of inhibition, p values for ANOVA & Kruskal-Wallis test

	E.faecalis		**C.albicans**		**Mix(1:1)**	
	Mean	SD	Mean	SD	Mean	SD
3% NaOCI	12.50	.50	14.00	.50	13.83	.29
Tea tree oil (2%)	9.00	.00	14.33	.58	9.00	.00
Saline	.00	.00	.00	.00	.00	.00
Aloe vera	18.00	2.18	.00	.00	.00	.00
p value ANOVA Kruskal-Wallis	 p= 0.00<0.05 p= 0.014<0.05		 p= 0.00<0.05 p= 0.021<0.05		 p= 0.00<0.05 p= 0.012<0.05	

Table 2: Tukey's test

Dependent Variable	(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	p-value	Interpretation
E.faecalis	3% NaOCI	Tea tree oil (2%)	3.50000*	.91287	.021	**Significant**
		Saline	12.50000*	.91287	.000	**Significant**
		Aloe vera	-5.50000*	.91287	.001	**Significant**
	Tea tree oil (2%)	3% NaOCI	-3.50000*	.91287	.021	**Significant**
		Saline	9.00000*	.91287	.000	**Significant**
		Aloe vera	-9.00000*	.91287	.000	**Significant**
	Saline	3% NaOCI	-12.50000*	.91287	.000	**Significant**
		Tea tree oil (2%)	-9.00000*	.91287	.000	**Significant**
		Aloe vera	-18.00000*	.91287	.000	**Significant**
	Aloe-Vera	3% NaOCI	5.50000*	.91287	.001	**Significant**
		Tea tree oil (2%)	9.00000*	.91287	.000	**Significant**
		Saline	18.00000*	.91287	.000	**Significant**
C.albicans	3% NaOCI	Tea tree oil (2%)	-.33333	.31180	.717	**Non-Significant**
		Saline	14.00000*	.31180	.000	**Significant**
		Aloe vera	14.00000*	.31180	.000	**Significant**
	Tea tree oil (2%)	3% NaOCI	.33333	.31180	.717	**Non-Significant**
		Saline	14.33333*	.31180	.000	**Significant**
		Aloe vera	14.33333*	.31180	.000	**Significant**
	Saline	3% NaOCI	-14.00000*	.31180	.000	**Significant**
		Tea tree oil (2%)	-14.33333*	.31180	.000	**Significant**
		Aloe vera	.00000	.31180	1.000	**Non-Significant**
	Aloe Vera	3% NaOCI	-14.00000*	.31180	.000	**Significant**
		Tea tree oil (2%)	-14.33333*	.31180	.000	**Significant**
		Saline	.00000	.31180	1.000	**Non-Significant**
Mix(1:1)	3% NaOCI	Tea tree oil (2%)	4.83333*	.11785	.000	**Significant**
		Saline	13.83333*	.11785	.000	**Significant**
		Aloe vera	13.83333*	.11785	.000	**Significant**
	Tea tree oil (2%)	3% NaOCI	-4.83333*	.11785	.000	**Significant**
		Saline	9.00000*	.11785	.000	**Significant**
		Aloe vera	9.00000*	.11785	.000	**Significant**
	Saline	3% NaOCI	-13.83333*	.11785	.000	**Significant**
		Tea tree oil (2%)	-9.00000*	.11785	.000	**Significant**
		Aloe vera	.00000	.11785	1.000	**Non-Significant**
	Aloe Vera	3% NaOCI	-13.83333*	.11785	.000	**Significant**
		Tea tree oil (2%)	-9.00000*	.11785	.000	**Significant**
		Saline	.00000	.11785	1.000	**Non-Significant**
*The mean difference is significant at the 0.05 level.						

The activity of aloe vera was found to be significantly more for E.Faecalis when compared to the other solutions. 3% NaOCl showed significantly more activity against E. Faecalis when compared to tea tree oil (2%). Saline did not show any activity.

When activity against C. Albicans was compared between 3% NaOCl & tea tree oil (2%), their activity was seen to be almost similar. Aloe vera & saline did not show activity against C. Albicans.

Activity of 3% NaOCl is significantly more when compared with other solutions for mixed culture (1:1). Tea tree oil showed minimal activity against mixed culture, whereas aloe vera & saline did not show activity for mixed culture (1:1).

DISCUSSION

The main aim of an endodontic treatment is to remove the diseased tissue, eliminate bacteria from the root canal system and prevent its recontamination. Irrigation is carried out to reduce the number of bacteria in the root canal system and to control the periapical disease.[6]

A wide variety of synthetic antimicrobial agents have been used over the years as endodontic irrigants. Because of the increased antibiotic resistance to these antimicrobial agents, toxic and harmful side effects of few common antibacterial agents, there is a need for alternative agents which are affordable, non-toxic and effective.[6]

It has been found that natural plant extracts could be used as effective endodontic irrigants.[6]

The ideal properties of a root canal irrigant are: it should be systemically nontoxic, should not harm the periodontal tissues, should not cause an anaphylactic reaction, should possess a broad antimicrobial spectrum, should be capable of dissolving necrotic pulp tissue, inactivating endotoxins, and either preventing the formation of a smear layer or dissolving it once it has formed.[7]

Sodium hypochlorite (NaOCl) was chosen for the study as it is one of the most widely used endodontic irrigant because of its ability to destroy a broad spectrum of microbes but it has some undesirable characteristics such as tissue toxicity, allergic potential, and disagreeable taste and inability to remove the smear layer.[6]

The constant increase in antibiotic resistant strains & side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives.[1]Recently, there has been a growing trend to seek natural remedies as part of dental treatment & this approach may be termed phytotherapeutics or ethnopharmacology.[8]

Inclusion of C. Albicans and E. Faecalis in this study was based on the literature that relates these micro-organisms to pulp infections, mainly in recalcitrant infections after endodontic treatment.[4]

E. Faecalis is commonly found in cases of failed endodontic infections.Its resistance is known to increase 1000 to 10,000 fold in the starvation phase. It is probable that the physiologic state of the cells, particularly in retreatment cases, is closest to the starvation phase.[9]

E. Faecalis has been frequently found in obturated root canals exhibiting signs of chronic apical periodontitis, isolated in 23–70% of the positive cultures and often occur in monoculture.[2]

Fungi constitute a small part of the oral microbiota. The largest proportion of the fungal microbiota is made up of Candida species. The incidence of C. Albicans in the oral cavity has been reported to be 30% to 45% in healthy adults and 95% in patients infected with human immunodeficiency virus. The presence C. Albicans is evident in both primary infections (5 – 20 %) and persistent infections (25%) of root canal systems.[2]

The essential oil of Melaleuca alternifolia, known as tea tree oil, has been used in medicine for almost 70 years (Carson and Riley,1995).[10]Tea tree oil can be extracted from leaves & branches of Melaleuca alternifolia through distillation. It has antiseptic activities such as antibacterial, antifungal, antiviral, anti-inflammatory. It has a wide spectrum of antimicrobial activity mainly attributed to terpeno-4-ol. Mechanism of action is due to its hydrocarbon structure & attendant lipophilicity. Hydrocarbons partition preferentially into biological membranes & disrupt their vital functions. This premise is further supported by data showing that TTO causes lysis & the loss of membrane integrity and function manifested by the leakage of ions and the inhibition of respiration.[1]The recommended therapeutic concentration for tea tree oil is 2.5-5%, in which it can retain its antibacterial property without any toxic effect.[10]

In the present study, tea tree oil showed activity against all the three cultures used, though highest activity seen was for C. albicans. In an in-vitro study, it was shown that tea tree oil might disinfect root canal system as effectively as sodium hypochlorite, & its toxicity is less than that of sodium hypochlorite.[12] In another in-vitro study performed by Sinha DJ et al. showed that tea tree oil exhibits good antimicrobial activity but less than that of sodium hypochlorite.[13]

Aloe barbadensis Miller (Aloe Vera) belong to the liliaceal family, of which there are about 360 species. It is a cactus like plant that grows in hot and dry climates. Numerous studies on Aloe vera are being done to demonstrate the antiviral, antibacterial, and not to mention its other use as an analgesic, anti-inflammatory, wound healing properties.[14]

Aloe gel is used to relieve thermal burn, sunburn and promote wound healing & has antimicrobial activity and can help stimulate the body's immune system.[15]

In dentistry Aloe vera is used in cases of Apthous ulcers, Lichen planus , Alveolar osteitis.[14]

Aloe leaves contain clear gel and green part of the leaf that surrounds the gel is used to produce juice or dried substance. It contains alloins and barbadoins as main chemical constituents. Aloe Vera gel has inhibitory effects on S. Pyogens and E. Faecalis because of anthraquinones.[16]

Natural extract of Aloe vera is always better than commercially available aloe vera products, as no preservatives are added in the former one which may give biased results[14], hence freshly extracted aloe vera pulp was used to perform this study.

In the present study aloe vera showed significant activity against E.Faecalis. According to a study performed by S. Datta et al. antimicrobial activity of Aloevera leaf extracts against E.Faecalis and C.Albicans is quite effective and appears to be promising to use as a irrigating solution[2]. In another study performed by M. Anitha et al. depicted the antimicrobial activity of Aloe vera juice against E. Faecalis, while C. Albicans was detected to be the most sensitive strain.[15]

Agar diffusion test is generally accepted procedure, but has some limitations like pH of the substrate, incubation period, and diffusion capacity of the drug having effect on activity of test materials. However evidence also suggests agar diffusion tests shows good correlation with other antimicrobial susceptibility tests.[14]

CONCLUSION

Under the limitations of this study, it was concluded that aloe vera extract has a significant antimicrobial effect against E. Faecalis.

Tea tree oil showed antimicrobial activity against E. Faecalis, C. Albicans & mixed culture, though significant activity was seen only against C. Albicans.

However, further preclinical and clinical trials are needed to evaluate biocompatibility & safety of aloe vera extract & tea tree oil, before they can conclusively be recommended as an intracanal irrigating solutions[13].

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