

COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF VARIOUS HERBAL EXTRACTS AND SODIUM HYPOCHLORITE AGAINST MIXED MICRO -ORGANISMS : AN IN - VITRO STUDY

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ABSTRACT

Bacteria and their products are considered to be the primary etiological agents of pulpal necrosis and periapical lesions as they cause secondary / persistent infections. Their elimination is one of the most important steps in root canal treatment making adequate cleaning and shaping of the root canal system an absolute necessity. Various irrigating solutions have been used to remove debris, necrotic pulp tissue and microorganisms that cannot be reached by mechanical instrumentation.(1)

Failure of root canal treatment occurs when treatment procedures have not met a satisfactory standard for control and elimination of infection. Persisting bacteria in root canals may be those originally present in the necrotic pulp which may be located in missed canals or uninstrumented areas of the canals. Cleaning and disinfection of the root canal system is

one of the major objectives of biomechanical preparation.(2)

Most popular irrigant used is Sodium Hypochlorite which however is very caustic and has deleterious effects on dentine like reduction of the elastic modulus and the flexural strength. It possess few limitations such as tissue toxicity, allergic potential and disagreeable smell and taste (3). To overcome these drawbacks natural plant extracts (Herbal) have been introduced as effective endodontic irrigants. Their advantages being low toxicity, lack of microbial resistance, easy availability and antimicrobial properties. Some of them shown to be effective against various micro-organisms are Green Tea Extracts, Turmeric, Neem, Aloe Vera, Nutmeg, Harad, Tulsi, Cloves, Cinnamon, Coffee, Lemon, Winter Cherry and Mango.(4)

Green Tea Extracts are traditional and most widely consumed beverage of China and Japan, obtained from *Camellia Sinensis*. Catechins and Flavins present in Green Tea Extracts are considered as microbiologically active ingredients.

Triphala consists of three medicinal plants: *Terminalia Chebula*, *Terminalia Bellerica* and *Embellica Officinalis* which are dried and powdered to get an Indian Ayurvedic formulation. It has a

potential of antibacterial activity and anti-inflammatory activity against enteric pathogens.[3] *Withania Somnifera* is a medicinal plant widely used as a home remedy for several diseases and have antimicrobial properties. It exhibits little or no toxicity to cells. Antimicrobial efficacy has been proved by reducing bacterial loads in vital organs of animals. (5)

AIM

To compare the antimicrobial efficacy of Green Tea Extract, Myrobalans (Triphala), *Withania Somnifera* (Ashwagandha), *Ocimum Sanctum* (Tulsi), *Myristica Fragrans* (Jaiphal) and Sodium Hypochlorite against mixed micro - organisms.

METHODOLOGY

Collection of samples

Cultures of bacteria were isolated from patients reporting to I.T.S. Dental College, Hospital and Research Centre. Coronal access was gained to the root canals under rubber dam isolation of 10 involved teeth which were carious with periapical radiolucency. A paper point was inserted into the canal and held for 1-2 mins to obtain culture sample of mixed organisms and was then immediately transferred to BHI broth and incubated for 1 hr. BHI broth was incubated at 37 °C for 24 hrs for growth of culture.

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[6] The grown Culture was mixed in the Peptone water and spread on the Agar plates. Sterile discs of 10 mm diameter were placed on Agar plates to receive the prepared irrigant solution.(3)

PREPARATION OF HERBAL EXTRACTS

HERBAL POWDER USED IN THE STUDY

120 mg/ml pure Green Tea Extracts, Myrobalans, Withania Somnifera, Ocimum Sanctum and MyristicaFragrans were added in 10% DMSO liquid and stirred for 2 min and passed through the filter paper.DMSO was added to improve the herbal products efficiency and it did not interfere with antibacterial properties with disc diffusion method. This solution was stirred for 1-2 minutes then filter through the filter paper.(7)

INTRODUCTION

Accurate working length determination is a prerequisite for successful root canal treatment, thereby reducing the chances of insufficient cleaning of the canal or damaging periapical tissues from overinstrumentation.(1)

Studies have shown that the apical foramen is not always located at the anatomic apex. The deviation between the apical foramen and root tip may be between 0 and 3 mm and occur in 50–98% roots. (2,3) These factors increase the inaccuracy and discrepancy of radiographic canal length determination.

Traditionally, the periapical radiographs has been the primary method of canal length

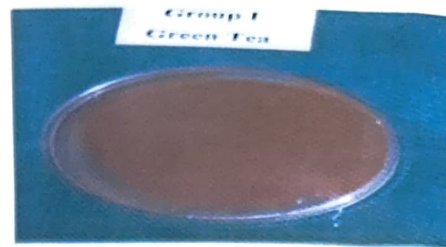
determination, but there are certain technical limitations associated with this approach. Radiographs are two dimensional images of a three dimensional object that do not consistently reveal the root canal portals of exit.(2,4)

To overcome the drawbacks of radiography, the electronic apex locator (EAL) was designed and marketed to determine the terminus of the root canal objectively and accurately. Electronic apex locators have been widely used in endodontics to determine the root length during root canal treatment. In 1962 Sunada reported that when the tip of an endodontic instrument had reached the periodontal membrane through 'apical foramen', the electrical resistance between the instrument and the oral mucous membrane was a constant value of 6.5 KΩ. The major disadvantage with initially introduced Resistance and Impedance based devices, despite considerable developments over the years, was inaccurate and erroneous readings in root canal that contain moisture, vital pulp tissue, blood and other exudates or remnants of intracanal irrigants. (5,6,7) The importance of the irrigating solution in endodontic treatment is well documented. During and after instrumentation, the irrigants facilitate removal of microorganisms, tissue remnants, and dentin chips from the root canal through a flushing mechanism. Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid.

IRRIGANTS DIVIDED INTO SIX

DIFFERENT GROUPS

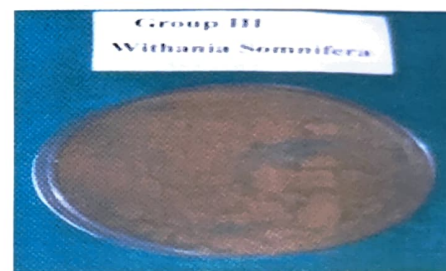
Group I - Green Tea Extracts



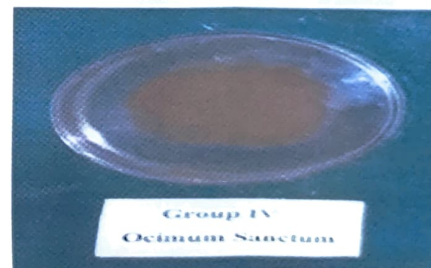
Group II -Myrobalans



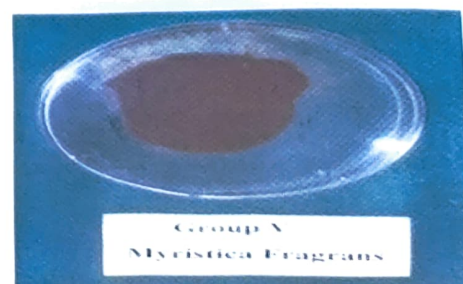
Group III -Withania Somnifera



Group IV - Ocimum Sanctum

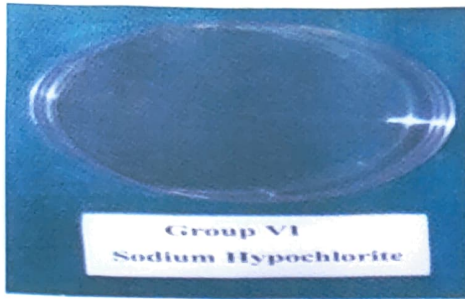


Group V -Myristica Fragrans



research section

Group VI - Sodium Hypochlorite



Agar plates were prepared with cultures and discs were placed on the plates. Then 50 μ l of the respective irrigant solution was pipetted on to the sterile disc group wise. The Agar plates were then incubated for 24 hrs at 37 °C. The Zone of Inhibition was measured and tabulated for all samples of each group.

STATISTICAL ANALYSIS

Statistical analysis was done by ANOVA and POST-HOC TUKEY test. The criteria for statistical significance was $P < 0.05$.

RESULTS

Sodium Hypochlorite showed the maximum antimicrobial activity followed by Green Tea Extracts and Withania Somnifera. There was no statistical difference between Green Tea Extracts and Withania Somnifera. This was followed by Ocimum Sanctum and Myristica Fragrans and there was statistical difference between Sodium Hypochlorite and Ocimum Sanctum and Myristica Fragrans. The efficacy of Green Tea Extracts and Withania Somnifera was significantly more than Ocimum Sanctum and Myristica Fragrans. Myrobalans was least effective on micro-organisms.

Table 1: Zone of inhibition for all samples (IN mm)

Sample No.	GR I	GR II	GR III	GR IV	GR V	GR VI
1	14	10	0	13	10	22
2	15	10	12	16	12	28
3	11	11	13	16	14	27
4	15	10	13	11	14	24
5	10	10	14	12	10	25
6	13	11	15	10	10	26
7	12	11	13	11	12	24
8	11	10	13	10	12	23
9	11	12	11	14	13	25
10	14	10	12	12	10	26

Table 2: Main, SD, Minimum and Maximum Values for all groups.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Means		Minimum	Maximum
					Lower Bound	Upper Bound		
GR I	10	12.90	1.595	.504	11.76	14.04	11	15
GR II	10	5.90	6.280	1.986	1.41	10.39	0	14
GR III	10	11.60	4.222	1.335	8.58	14.62	0	15
GR IV	10	13.10	2.025	.640	11.65	14.55	11	16
GR V	10	13.30	.949	.300	12.62	13.98	12	14
GR VI	10	22.00	1.054	.333	21.25	22.75	21	24
Total	60	13.13	5.715	.738	11.66	14.61	0	24

Table 3: Between and Within Group comparison using anova

	Sum of Squares	df	Mean Square	F	P. Value
Between Group	1333.733	5	266.747	24.282	.000
Within Group	593.200	54	10.985		
Total	1926.933	59			

DISCUSSION

In this study, all the tested irrigants showed antimicrobial activity. Sodium Hypochlorite is both an oxidising and hydrolyzing agent. It is bactericidal and proteolytic. This have been used as an

endodontic irrigants as early as 1920. Irrigation of root canals with Sodium Hypochlorite solutions is now a widely accepted technique. It is not without disadvantages, principally due to its toxicity. It damages all living tissues

except keratinized epithelia. It is extremely corrosive to metals. It is strongly alkaline hypertonic and has a very unpleasant taste. It is most common irrigating solution used during root canal therapy. (6)

Sodium Hypochlorite showed the best antimicrobial activity followed by Green Tea Extract as it contains Catechin which have remarkable anti-inflammatory and antioxidant properties whereas WithaniaSomnifera has same properties. However, there is no statistical difference in these groups. Ocimum Sanctum and Myrobalans was least effective against micro-organisms. These irrigants show effectiveness in various endodontic applications and should be considered as alternative agents.(8) Dimethyl sulphoxide was used as a solvent for Green Tea Extracts, Myrobalans, WithaniaSomnifera, Ocimum Sanctum and MyristicaFragrans. Because it is safe bacterially inert highly polar solvent that helps in bringing out the true properties of the herb being used in the study. (9)

These irrigants showed effectiveness in various endodontic applications and should be considered as alternative agents. (10) The purpose of using herbal alternatives are low toxicity, lack of microbial resistance, easy availability, cost effectiveness and increased shelf life.(3)

CONCLUSION

Within the limitations of this study Sodium hypochlorite remains the gold standard for irrigation in

endodontic infections. Green Tea Extracts and Withania Somnifera showed maximum antibacterial activity against endodontic microflora. Ocimum Sanctum and Myristica Fragrans also showed some efficacy against mixed microflora. Myrobalans solution was not effective as antimicrobial agent. However, further research is required to recommend the use of these herbal alternatives as a root canal irrigant.

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