



COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF THREE HERBAL EXTRACT MIXED WITH ENDODONTIC SEALER: AN IN VITRO STUDY

DR. BIJAY RAJ YADAV¹, DR. POOJA KABRA², DR. EKTA CHOUDHARY³,
DR. SHRUTIKA GOYAL⁴, DR. GARIMA JOSHI⁵

¹Post Graduate 3, Department of Conservative Dentistry& Endodontics, School of dental sciences,
Sharda university, Greater Noida

²Reader, Department of Conservative Dentistry& Endodontics , School of dental sciences , Sharda
university ,Greater Noida

³Professor and Head, Department of Conservative Dentistry& Endodontics , School of dental
sciences , Sharda university, Greater Noida

⁴Post Graduate 3, Department of Conservative Dentistry& Endodontics , School of dental sciences ,
Sharda university, Greater Noida

⁵Post Graduate 3, Department of Conservative Dentistry& Endodontics , School of dental sciences ,
Sharda university , Greater Noida

Corresponding Author:
Dr. BIJAY RAJ YADAV
E-mail- bijayraj17@gmail.com

ABSTRACT- Root canal sealers along with solid core or semi-solid core material aids to fill voids and to seal root canals during obturation and acts for entombment of remaining bacteria and the filling of irregularities in the prepared root canal . Gutta Percha core filling material and root canal sealer . this material alone is not enough to produce and ensure tight seal of the root canal system, it is only adapts to the adjacent dentinal walls. However, root canal sealer is necessary to fill the irregularities and minor discrepancies between gutta percha and canal wall . . A number of herbal products have been tried as root canal sealer for the canal system. . In this Present study Licorice,Guduchi and Bakul were incorporated in a traditional endodontic sealer Endomethasone , and AH Plus to judge their potential of antimicrobial action . The results obtained indicate that the antibacterial effects of herbal solutions are not similar and the difference is attributed to the variation in phytochemical constituents. The major advantages of herbal materials are increased shelf life, costeffectiveness, low toxicity, easy availability and absence of microbial resistance reported.

KEY WORDS- obturating material, root canal sealer, herbal products

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Introduction

In endodontic practice, the success of root canal therapy mainly depend on achieving a compact fluid tight seal of the apical end of the root canal, so as to prevent the

ingress and accumulation of irritants which cause biological breakdown of attachment apparatus leading to failure¹. Root canal sealers along with solid core or semi-solid

core material aids to fill voids and to seal root canals during obturation and acts for entombment of remaining bacteria and the filling of irregularities in the prepared root canal².

Three-dimensional sealing of the entire root canal space with a bio-compatible filling material is a challenging task to be achieved for the success of endodontic treatment³. Filling the root canal space is achieved by two main components: Gutta Percha core filling material and root canal sealer. Gutta Percha is the most favorable bio-compatible filling material that can be used to fill the radicular space. But, this material alone is not enough to produce and ensure tight seal of the root canal system, it is only adapts to the adjacent dentinal walls. However, root canal sealer is necessary to fill the irregularities and minor discrepancies between gutta percha and canal wall⁴. Several types of root canal sealers are used in endodontic practice with each one having its own merits and demerits. Sealers are basically selected based on their sealing ability, adhesive properties, biocompatibility & antimicrobial efficacy⁵. A number of herbal products have been tried as root canal sealer for the canal system (Amla, (Nutmeg, Miswak)

Licorice has been an important herb in Chinese medicine for many years. In addition to being used as flavoring and sweetening agents in candy, tobaccos and beverages, compounds derived from licorice root have been shown to help fight inflammation, viruses, ulcers and even cancer, according to the researchers. Compounds isolated from licorice root may help prevent cavities. Studies have shown that liquorice contains at least two compounds that appear to be potent

inhibitors of *Streptococcus mutans*, a major culprit for dental caries. If further studies show promise, the licorice compounds could eventually be used as cavity-fighting components in mouthwash or toothpaste⁶.

T. cordifolia (Guduchi) is a large, glabrous, perennial, deciduous, climbing shrub of weak and fleshy stem found throughout India. It is a widely used plant in folk and Ayurvedic systems of medicine. The chemical constituents reported from this shrub belong to different classes, such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides⁷.

Mimusops elengi Linn. (bakul) is an evergreen ornamental tree of the family Sapotaceae with pleasant fragrant flowers. It carries a variety of names such as Bakul (Hindi and Bengali), Spanish cherry, West Indian Medlar or Bullet wood tree (English), Bakula (Sanskrit) etc. It has a compact leafy head, and short erect trunk with smooth, scaly, and gray bark. The leaves are elliptical or oblong. It yields white, fragrant flowers, and silky, ovoid fruits which become yellow when ripe. The seeds of this tree are solitary, ovoid, and brown. It is known to have an antimicrobial activity which is also used in Ayurvedic medicine⁸.

Resin-based root canal cements are increasingly gaining popularity because they exhibit long-term dimensional stability and do not contain eugenol. However, the toxicity of resins and specifically the toxicity and the mutagenicity of resin based endodontic sealers are well documented⁹.

The purpose to uptake this study is to overcome the cytotoxic effects of Endodontics sealers without altering the mechanical properties when mixed with herbal extract, So, the preparation of both sealers was done with the Three herbal extract

AIM AND OBJECTIVE-

To compare and evaluate antimicrobial efficacy of three herbal extracts (licorice, Guduchi, Bakul) mixed with two endodontic sealers (Endomethasone, AH-plus)

MATERIAL AND METHODS-

This is an in-vitro study which was conducted in the department of Conservative Dentistry and Endodontics in School of dental sciences, Sharda University, Greater Noida.

Materials-

1-ZOE based root canal selear (Endomethasone- Septodent)

2-Expyo Resin based root canal selear (AH-plus Densply)

3-Herbal plant extract(roots of Licorice, bark of Bakul, and stem of Guduchi (commercially available-Sun pure pvt Ltd)

4- normal saline

Method

Sample selection and sample size: 85 specimens- herbal extracts (Licorice,guduchi, bakul) mixed in endodontics sealers(endomethasone and AH Plus)

Inclusion criteria - Endodontic root canal sealer (ZOE Based And Resin based sealer) Endodontic sealer mixed with Herbal Extract (Licorice, Guduchi, Bakul)

Exclusion criteria- expired material Improper Consistency of mixed sealer

Sample preparation

Roots of Licorice, bark of Bakul, and stem of Guduchi procured, mixed in Endomethasone and AH plus in 1:1 ratio the powder and then paste of the respective sealers was used. To ensure proper proportion, the material was dispensed on the glass slab with the help of cement scoop and mixed with the help of cement spatula. The prepared sealers in their paste forms was then dispensed in each well of agar plate. The inoculated plates with the tested

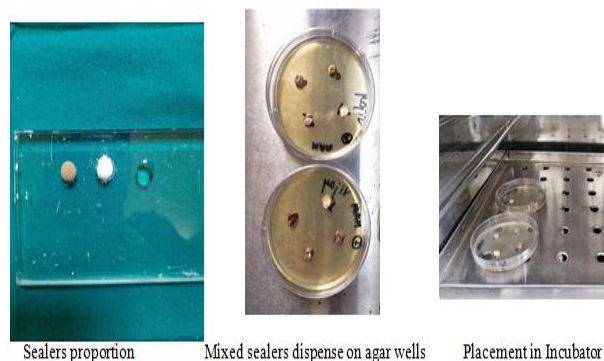
medicaments were kept for 2 h at room temperature to allow the diffusion of the tested groups through the agar. The MH agar plates were incubated at 37°C for 24 h. The plates for facultative anaerobes

Table-1 Sample group

GROUP	COMPOSITION
1	ZINC OXIDE EUGENOL BASED SEALER+E1(BAKUL)
2	ZINC OXIDE EUGENOL BASED SEALER+E2(GUDUCHI)
3	ZINC OXIDE EUGENOL BASED SEALER+E3(LICORICE)
4	RESIN BASED SEALER+E1(BAKUL)
5	RESIN BASED SEALER+E2(GUDUCHI)
6	RESIN BASED SEALER+E3(LICORICE)
7(NEGATIVE CONTROL GROUP)	ZINC OXIDE BASED SEALER
8(NEGATIVE CONTROL GROUP)	RESIN BASED SEALER

Sealer was mixed according to manufacturer's instruction and herbal extracts were incorporated in 1:1 ratio in

were read at 24 h for the size of the zone of inhibition, while readings for strict anaerobes were carried out after 48 h. Growth inhibitory zones around each tested medicaments were evidenced by lack of bacterial colonization (clearing of agar) adjacent to each group. The most uniform diameter segment of the zone of inhibition was measured, and 6 mm (diameter of the well) was extracted from the measurement as the cutoff value. All measurements above this value were considered, indicative of significant bacterial growth inhibition. Wider zones of inhibition were interpreted to indicate greater antimicrobial activity of the involved medicaments.



Depicting zone of inhibition of zinc oxide eugenol group

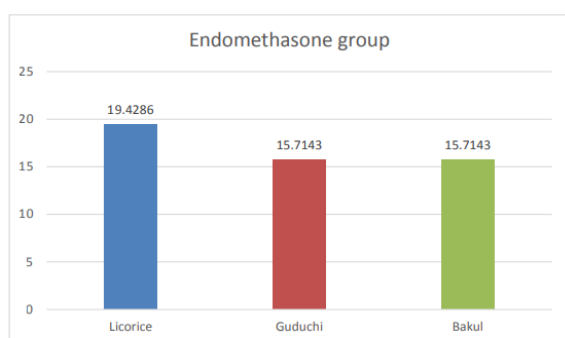


Depicting zone of inhibition of resin based sealers group

RESULTS-

Intragroup comparison of different herbal extracts in Endomethasone group was

done using one way ANOVA test. It was found that overall, there was a statistically significant difference in the ZoI wrt different herbal extracts. The mean ZoI wrt Licorice was the maximum, which was found to be significantly higher than that wrt Guduchi & Bakul. No statistically significant difference was found in the ZoI wrt Guduchi & Bakul extracts.

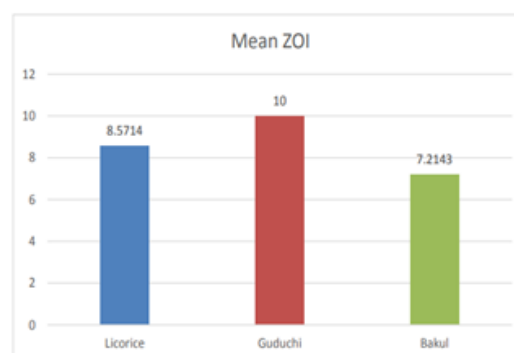


Intragroup comparison of different herbal extracts in AH plus group was done using one way ANOVA test. It was found that overall, there was a statistically significant difference in the ZoI wrt different herbal extracts. The mean ZoI wrt Guduchi was the maximum, which was found to be significantly higher than that wrt Licorice, which was further significantly higher than that wrt Bakul.

Licorice subgroup				
Group	N	Mean	Std. Deviation	P value
Endomethasone	14	19.4286	1.45255	<0.001, S
AH plus	14	8.5714	.75593	

Intergroup comparison of different sealers in Licorice herbal extract subgroup was

done using Independent t test. It was found that the mean ZoI of Endmethasone group was found to be significantly more than that as compared to AH plus group. Intergroup comparison of different sealers in Guduchi herbal extract subgroup was done using Independent t test. It was found that the mean ZoI of Endmethasone group was found to be significantly more than that as compared to AH plus group. Intergroup comparison of different sealers in Bakul herbal extract subgroup was done using Independent t test. It was found that the mean ZoI of Endmethasone group was found to be significantly more than that as compared to AH plus group.



Two way ANOVA statistics to check for interaction between sealer and herbal extract variables showed a statistically significant interaction between the „type of sealer“ and „type of herbal extract“. The „Licorice extract“ produced the maximum ZoI wrt Endomethasone group, while „Guduchi“ herbal extract produced the maximum ZoI wrt AH plus group.

DISCUSSION

The primary objective of the endodontic therapy is to make the root canals free from microbes and debris that are mainly responsible for pulp and periapical infection. This can be achieved by chemical treatment of the root canal system. This treatment comprises of biomechanical preparation, irrigation and inters appointment medicaments. Complete disinfection of the root canal and creating favorable conditions are required for periapical healing thus we will be needing adequate cleaning, shaping and obturation of the canal¹⁰. Siqueira et al. found *E. faecalis* more often in symptom-free teeth than in teeth with acute symptoms¹¹. The prevalence of *E. faecalis* in primary endodontic infections was found to be in the range of 4 to 40%. In persistent peri radicular lesions, the prevalence of *E. faecalis* is much higher. The presence of *E. faecalis* in the root canal treated teeth with peri radicular lesions have shown to be 24 to 77%.¹² Keeping this in mind, Enterococci faecalis strain was selected for the study. The root canal sealers with good sealing ability and antimicrobial activity with low toxic effects on surrounding periapical tissue are desired to kill residual microorganisms inside the canal¹³. However, controversies related to antibacterial effects of sealers on

common isolated bacteria in infected teeth as well as their degree of cytotoxicity, increased antibiotic resistant strains and side effects caused by synthetic drugs has made researchers to look for herbal alternatives¹⁴. In this Present study Licorice, Guduchi and Bakul were incorporated in a traditional endodontic sealer Endomethasone, and AH Plus to judge their potential of antimicrobial action.

Endomethasone (Septodont, France) is an endodontic sealer which was used for decades. It consists of zinc oxide, paraformaldehyde and corticosteroides (hydrocortisone acetate, magnesium stearat, tymol iodide and barium sulfat). Furthermore, Endomethasone is characterized for its antibacterial activity¹⁵.

AH Plus (Dentsply-Maille), a root canal sealer, is characterized with very good mechanical properties. It is delivered into two tubes: Epoxy Paste (diepoxide, calcium tungstate, zirconium oxide, aerosol and pigments) and Amin Paste (1-adamantine amine, N^oN-dibenzyl P-5-ara-nonandiamine-1,9, TCD-diamine, calcium tungstate, Discussion 35 zirconium oxide, aerosol and silicone oil). AH Plus (resin-based sealer) has good sealing ability and good biocompatibility¹⁶.

Licorice, Bakul and Guduchi added to the commercial root canal sealers because of their properties of Significant inhibition of bacterial growth and as well as Efficient antimicrobial.

In determining the antibacterial activity, growth media plays a vital role. Lin et al. reported that the best medium to determine the antibacterial activity was Muller-Hinton agar and the same was utilized in this study¹⁷

After preparation of the samples the Agar plate were incubated at 37°C for 24 hours after that the zone of inhibition was measured with vernier calliper. Growth inhibitory zones around each tested medicaments were seen by lack of bacterial colonization (clearing of agar) adjacent to each group. The most uniform diameter segment of the zone of inhibition was measured, and 6 mm (diameter of the well) was extracted from the measurement as the cut off value .

The results obtained in the present study clearly indicates that the test materials are having significant antimicrobial activity against *E. faecalis*. The licorice have the maximum zone of inhibition when compared to Guduchi and Bakul in endomethasone group. And the results of this study was similar with the study done by, Demizu et al., 1988, Okada et al.,

1989, Haraguchi et al., 1998 documented the antimicrobial activity of Glycyrrhiza¹⁸. Badr et al revealed that when compared to Ca (OH) 2, Liquorice showed good biocompatibility with fibroblast cells and was shown to be effective against *E. faecalis*, when used as an intracanal medicament¹⁹.

The mean ZoI with relation to Guduchi was the maximum, which was found to be significantly higher than that with relation to Licorice, which was further significantly higher than that wrt Bakul in A-H plus group

Intergroup comparison of different sealers in Licorice herbal extract, Guduchi herbal extract and Bakul herbal subgroup was done It was found that the mean ZoI of Endomethasone group was found to be significantly more than that as compared to AH plus group in all three subgroups. The result of this study was with accordance with a study done by Sonali s etal which compared antimicrobial activity of endodontic sealers added to herbal extracts, and concluded that statistically significant zones of bacterial growth inhibition were observed largest with Zinc Oxide Eugenol based sealer when mixed with Glycyrrhiza glabra (Licorice) followed in descending order by zinc oxide eugenol-based sealer mixed with

Tinospora cordifolia (Guduchi) and *Mimusops elengi* (Bakul) respectively²⁰

The results obtained indicate that the antibacterial effects of herbal solutions are not similar and the difference is attributed to the variation in phytochemical constituents. The major advantages of herbal materials are increased shelf life, cost-effectiveness, low toxicity, easy availability and absence of microbial resistance reported.

CONCLUSION

1. Zinc-oxide-eugenol-based root canal sealer (Endomethasone) when mixed with the three tested herbal extracts showed maximum zone of inhibition followed in the descending order by AH plus
2. Among the combination of zinc-oxide-eugenol-based sealer (Endomethasone) with the three herbal extracts, its mixture with liquorice shows the highest zone of inhibition against *E. Fecalis*
3. Epoxy-resin-based sealer (AH plus) when mixed with licorice showed the largest inhibitory zones against *E. Fecalis*
4. Licorice can conclusively be recommended as an endodontic sealer.

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