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Comparative Evaluation of The Antioxidant And Chelating Property of Chemical And Herbal Irrigants on Push out Bond Strength of A Root Canal Endodontic Sealer

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Abstract

Aim and objectives : To evaluate the effect of chemical and herbal agents as final irrigant on producing surface alterations affecting the push out bond strength of an endodontic sealer and the failure pattern in adhesion of an epoxy resin based sealer in dentinal tubules of apical and middle third of root canal using scanning electron microscopy.

Materials and Methods: Single-rooted mandibular premolars (*n* = 80) were prepared and divided into four groups (*n* = 20) based on irrigation regimen used: Group I: 2.5% sodium hypochlorite (NaOCl)-17% ethylenediaminetetraacetic acid (EDTA); Group II: 2.5% NaOCl-10% citric acid (CA); Group III: 2.5% NaOCl-neem extract; and Group IV: 2.5% NaOCl- Alstonia scholaris/ saptaparna extract. After obturation with guttapercha using AH Plus sealer, roots were sectioned at 2 levels – apical and middle third of root canals and push-out test assessed using universal testing machine. One-way ANOVA with *post hoc* Tukey-hoc significant difference tests were applied to assess the significance among various groups.The few samples of groups were examined under SEM to determine the nature of the bond failures.

Results: 17% EDTA showed higher pushout bond strength at the apical and middle third of the root canals which was was statistically significant at $P<0.001$. The herbal extract of neem showed the minimum pushout bondstrength ($p<0.001$).

Conclusions: There was superior efficacy of 17% EDTA on the bond strength of AH Plus sealer at the middle third and apical of the root level when compared with other

irrigants at various root levels.

Keywords: Alstonia scholaris, AH Plus, Citric acid EDTA, NaOCl, Saptaparna; SEM

Introduction

The success, longevity and reliability of any endodontic treatment depends on the effectiveness and sterilization of endodontic files, rotary instrumentation, irrigating solutions, and chelating agents for cleaning and shaping of root canals. The role of microorganisms in the development and perpetuation of pulp and periapical diseases has been clearly studied. The chances of a favourable outcome with root canal treatment are significantly higher if infection is eradicated effectively before the root canal system is obturated, if not may lead to high risk of treatment failure[1].

The most important step in an endodontic treatment is to eliminate the microorganism from the root canal system, which can be done by using the appropriate instruments and effective irrigants during the root canal treatment. However, due to the extremely complex anatomy of the root canal pulp space, these methods are not successful if employed alone. Therefore, ideal endodontic irrigants must have additional properties, such as the ability to dissolve organic and inorganic tissues, antibacterial effects, and biocompatibility with the tissues.[2]

A large number of substances have been used as root canal irrigants, including acids (citric and phosphoric), chelating agent (ethylene diaminetetraacetic acid EDTA), proteolytic enzymes, alkaline solutions (sodium hypochlorite, sodium hydroxide, urea, and potassium hydroxide), oxidative agents (hydrogen peroxide and Gly-Oxide), local anesthetic solutions, and normal saline. 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. However, NaOCl solutions exert no effects on inorganic components of smear layer. Chelants such as EDTA,Citric acid 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. [1]

These irrigants, though effective against the pathogens, have undesirable properties. For example, NaOCl is toxic to the periradicular tissues, causes a reduction in dentin strength due to its proteolytic effect, has an allergic potential and has an unacceptable taste and odour. The search for more biocompatible and dentin friendly irrigants that can overcome the limitations of these chemical antimicrobial irrigants is on the rise. Herbal products are gaining popularity in every field of medicine, mainly due to their biocompatibility. Many herbal extracts have been found to be of potential use in endodontics and also with minimal incidences of complication[3]. The natural products derived from medicinal plants such as neem, tulsi, amla, dhatura, nimbu etc., have proven to be abundant source of biologically active compounds, many of which have become the basis for the development of newer chemicals for pharmaceuticals. [4] According to the results of innumerable studies, authors claim that final irrigating protocols affect the adhesion of sealers to root dentin . The use of these chemical irrigants show decreased microhardness, increased roughness erosion and structural changes of root dentin. They can also hamper the bonding of adhesive systems and affect the dentin fracture toughness.[5] Hence this study makes an attempt to compare the effect of chemical and herbal agent used as a final irrigant on push out bond strength of an endodontic sealer.

Materials and Methods

Eighty single rooted mandibular 1st premolar teeth that are caries free, indicated for extraction due to orthodontic reasons and periodontal problems were collected. Teeth

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were carefully cleaned and stored in 10% buffered formalin for 2 weeks. Teeth were decoronated apical to the cementoenamel junction to standardize the canal length to 16 mm measured from the tip of the root to the cement-enamel junction with a diamond disc under water coolant mounted on a straight micro motor handpiece. The prepared teeth were stored in normal saline solution until use. The samples were then mounted in a putty impression material in order to stabilize the samples for ease of working and to ensure standardization in procedure. The canal patency was determined by passively placing a no. 8 size k-file in narrow canals and 10 k-file in medium sized root canals until the tip of the file was visible at the apical foramen using a magnifying loupe and adjusted to the apical foramen. Those teeth with wider apical patency were discarded and replaced with appropriate teeth. Working lengths were established by subtracting 1mm from the measurement obtained when a size 10 file was placed into the canal until its tip was visible at the apex namely working length of 15mm. Initial negotiation of root canal space was performed using a size 15 manual K-file used in a watch-winding motion to assure the presence of a glide path .In all the experimental groups, the coronal orifice was then sealed with sticky wax. This was done to achieve a close mode of irrigation. During instrumentation of all canals, 2ml of NaOCl (3%) was used as an irrigant during instrumentation of all canals using 30 gauge side vented needle for 1 minute. The samples were prepared up to file size 30/0.06 taper using Protaper Universal (Dentsply Maillefer) Ni-Ti rotary instruments at a rotation of 300 r.p.m .This was followed by rinsing of the canals with 5ml of 0.9% saline to minimize potential interaction of NaOCl with any acidic irrigants that will be employed as a final rinse. The samples were randomly divided into three groups comprising of 20 teeth in each group .

The samples were divided into 4 groups (n=20) according to the endodontic irrigants used and were further be subdivided into two groups according to the area of the slices procured as follows :

GROUP I: 5 ml 3% NaOCl + 17% EDTA (Prime Dental Products,Maharashtra)

GROUP II: 5 ml 3% NaOCl +10% citric acid (Prime Dental Products,Maharashtra)

GROUP III : 5 ml 3% NaOCl+ Azardirachta indica extract (neem) (Ayurveda cart, Gharaunda , Haryana)

GROUP IV: 5 ml 3% NaOCl + Alstonia scholaris Bark extract(saptaparna) (Ayurveda cart, Gharaunda , Haryana)

SUBGROUP A : Apical third of the root canal

SUBGROUP B : Middle third of the root canal.

For each group , 5 ml of irrigating solution will be used for 2 minutes using conventional irrigation method followed by 5 ml 0.9 % saline for 2 minutes using an endodontic irrigating needle. Root canals were dried with paper points and then sealer were mixed according to manufacturer's directions and will be introduced into canal using lentilospiral instrument. All the groups were obturated with gutta percha with single cone technique using AH Plus sealer The obturated teeth were allowed to set for 1 week before push out assessment in 37°C with 100% humidity in an incubator. After setting of the sealer , 2 horizontal sections of 2mm using hard tissue microtome. The slices were stored in bottles filled with 1.5 ml distilled water for 2 days. Afterwards, each section was marked on its apical side and positioned on a base with a central hole in a universal testing machine.

Assessment of Pushout Bondstrength

The materials' dislocation resistance was measured using the push-out strength test with a universal testing machine (Instron, Model 5944 MicroTester Precision Instruments, Norwood, MA,USA)(IISC,BANGALORE) . The push-out test was performed by applying a compressive load to the

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apical side of each slice using a cylindrical plunger attached to the upper portion of the testing machine with a crosshead speed of 1 mm/min. The load upon failure was recorded in Newtons (N) and divided by the bond area (mm2) to express the bond strength in megapascals (MPa). The total bonding area for each slice was calculated using the formula:

π(R+r) [(h2+(R1r)2]0.5 R represents the coronal radius, r is the apical radius, and h is the thickness of the slice.

Failure Mode Analysis

After performing the push-out test, the fractured specimens were evaluated under a Scanning Electron Microscopy. Each of the specimens were sectioned in the bucco-lingual direction with the help of a safe-sided cutting disc under copious irrigation with distilled water. The sectioned slices which retained the obturation material was selected for observing under a scanning electron microscope (LEOVP435, Cambridge, UK). The sectioned slices were soaked in 15% EDTA solution for 10 minutes, followed by soaking in 3% NaOCl solution for 10 minutes, and then washed thoroughly with distilled water. Specimen were dehydrated and silver sputtered

for SEM evaluation at the middle and apical thirds of the root canal. A SEM (NO. S-2400, Hitachi, Omeshi, Tokyo, Japan) was used at 1.3x magnification(CMTI ,BANGALORE). Each sample was categorized according to one of three failure modes: An adhesive failure that occurred at the dentin–material interface, cohesive failure that occurred within the material, or mixed failure, a combination of the two failure modes.

Results

The study data was analysed using Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses. One-way ANOVA test followed by Tukey's post hoc analysis was used to compare the mean push out bond strength at middle & apical region between 4 groups. Student Paired t Test was used to compare the mean push out bond strength between middle & apical region in each study group. Chi square test was used for adhesion failure.The level of significance [P-Value] was set at P<0.05.

Table 1: Comparison of mean Push out bond strength (in Mpa) between 04 groups at middle region using One-way ANOVA Test.

Comparison of mean Push out bond strength (in Mpa) between 04 groups at middle region using One-way ANOVA Test						
Groups	N	Mean	SD	Min	Max	P-Value
Group I	20	7.800	0.799	5.83	8.74	<0.001*
Group II	20	7.755	0.810	6.13	8.74	
Group III	20	3.624	1.265	2.06	5.88	
Group IV	20	4.641	0.628	3.48	5.59	

Table 01 summarizes the comparison of mean Push out bond strength between different groups at middle region. The mean Push out bond strength for group 1 was 7.755 ± 0.810, group 2 was 7.800 ± 0.799, group 3 was 3.624 ± 1.265 and group 4 was 4.641 ± 0.628. This difference in the mean Push out bond strength between 04 groups at middle region was statistically significant at P<0.001

Table 2: Comparison of mean Push out bond strength (in Mpa) between 04 groups at Apical region using One-way ANOVA Test

Comparison of mean Push out bond strength (in Mpa) between 04 groups at Apical region using One-way ANOVA Test						
Groups	N	Mean		Min	Max	P-Value
Group I	20	6.549	0.673	5.83	7.85	<0.001*
Group II	20	6.830	0.605	5.81	8.23	
Group III	20	2.920	0.511	1.99	3.87	
Group IV	20	3.520	0.685	2.45	5.24	

Table 02 the test results demonstrate the comparison of mean Push out bond strength between different groups at apical region. The mean Push out bond strength for group 1 was 6.549 ± 0.673, group 2 was 6.830 ± 0.605, group 3 was 2.920 ± 0.511 and group 4 was 3.520 ± 0.685. This

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difference in the mean Push out bond strength between 04 groups at apical region was statistically significant at P<0.001.

Graph 01: Mean Push out bond strength (in Mpa) between Middle & Apical third region in each study group

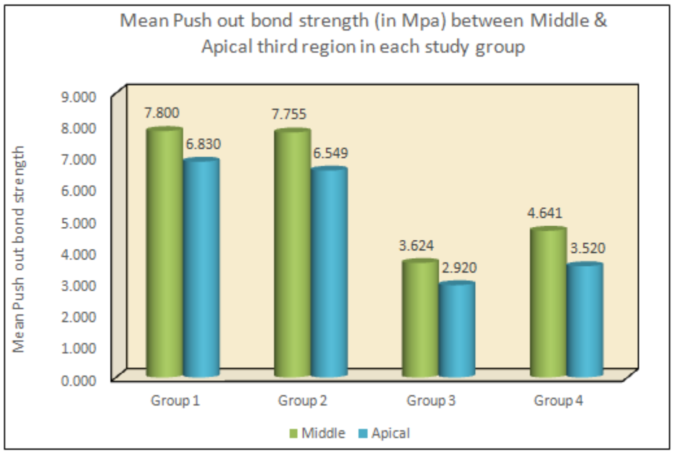
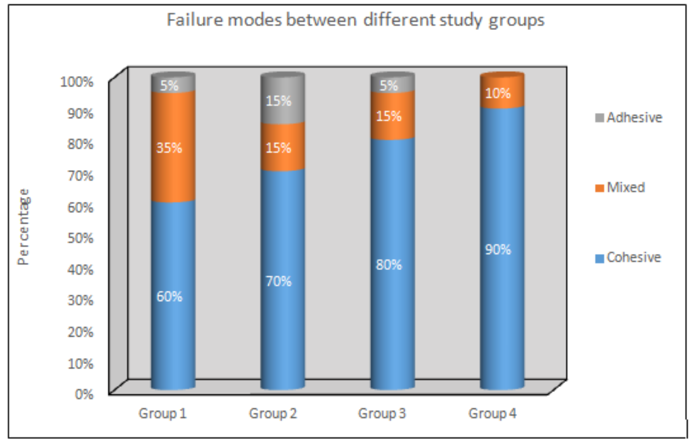


Table 03 : Comparison of failure modes between different study groups using Chi Square Test

Comparison of failure modes between different study groups using Chi Square Test										
Failure Modes	Group 1		Group II		Group III		Group IV		χ^2 Value	P-Value
	n	%	n	%	n	%	n	%		
Cohesive	12	60%	14	70%	16	80%	18	90%	9.067	0.17
Mixed	7	35%	3	15%	3	15%	2	10%		
Adhesive	1	5%	3	15%	1	5%	0	0%		

Table 03: demonstrate the comparison of failure modes between different study group. In all the study groups, Cohesive failure was more predominant [60 – 90%], followed by Mixed failure [10 – 35%] and Adhesive failure [0 – 15%]. There was no significant difference in the modes of failure between different study groups [P=0.17].

Graph 3: Failure modes between different study groups



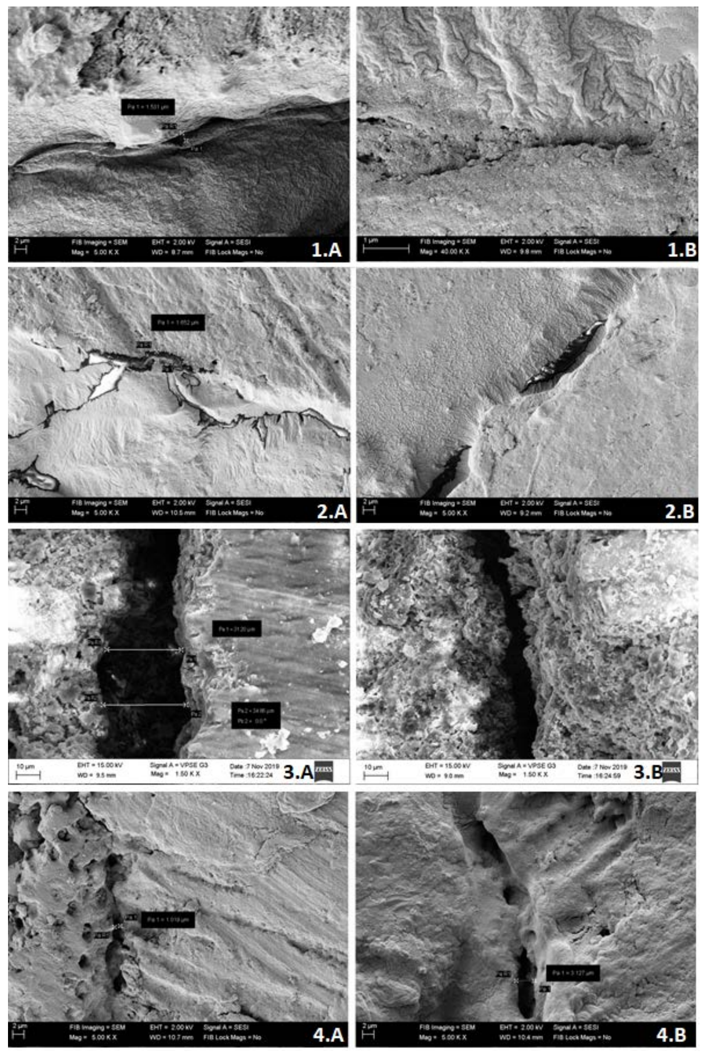


FIG 1A& 1B : Representative debonded surfaces after push out bond strength.AH Plus Sealer with cohesive failure of the sealer close to the borders after irrigating with Group I ; FIG 2A&2B : Representative debonded surfaces after push out bond strength.AH Plus Sealer with cohesive failure of the sealer close to the borders after

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irrigating with Group II; FIG 3B &3C : Representative debonded surfaces after push out bond strength.AH Plus Sealer with cohesive failure of the sealer close to the borders after irrigating with Group III;FIG 4A&4B Representative debonded surfaces after push out bond strength.AH Plus Sealer with cohesive failure of the sealer close to the borders after irrigating with Group IV.

Discussion

Root canal morphology is complex by nature and dealing with such complexities is challenging as it hampers the ability to achieve thorough disinfection of the pulp cavity. Chemo-mechanical debridement of the pulp space with the aid of instruments and irrigating solutions decides the outcome of endodontic therapy.[6] In fact the root canal treatment is affected by clinical factors such as effective biomechanical instrumentation of the root canal to produce a debris free surface, disinfection and dissolution of organic matter to eliminate bacterial pathogens and a three dimensionally sealed and obturated canal. [7]

It is very difficult to create a sterile environment in infected teeth after chemomechanical preparation. The complexity of root canal systems leads to remains of pulp tissue and inorganic debris, mainly in the isthmuses or in areas that instruments did not reach. In this context, irrigants play an indispensable role. They must present with antibacterial activity and an ability to dissolve organic and inorganic tissues.[8] There is no single irrigating solution that alone sufficiently covers all of the functions required from an irrigant. Optimal irrigation is based on the combined use of 2 or several irrigating solutions, in a specific sequence, to predictably obtain the goals of safe and effective irrigation.[9]

To remove the smear layer completely , NaOCl should be mixed with other chelating agents that can remove the inorganic phase of the smear layer. Constant increase in antibiotic resistance and side effects caused by synthetic irrigants shifted the research towards developing herbal alternatives. They have become popular today due to their high antimicrobial activity, biocompatibility, anti-inflammatory and antioxidant properties. A wide variety herbal product has been used in the past in medicine [10] . The use of herbal plant extracts for the eradication of microbes has been the topic of interest due to the drawbacks of sodium hypochlorite and chlorhexidine. Herbal extracts such as Morinda citrifolia, Green tea, Triphala, Azadirachta indica etc.have been used as irrigants in various studies. These studies have proven that herbal plant extracts eliminate microbes causing dental pathologies, thus proving its efficacy as an antimicrobial for oral infections.[11]

EDTA which is a chelating agent normally used in a concentration of 17% and can remove the smear layers when in direct contact with the root canal wall for less than 1 minute according to Doumani et al [12]. Semra Çalt et al on the study on time-dependent effects of EDTA on dentin structures found out that EDTA followed by NaOCl completely removed the smear layer in 1 min. In turn when EDTA is applied for 10 min, excessive erosive effects were observed with dissolution of peritubular and intertubular dentin. According to that study findings, to inhibit the erosion on dentin, EDTA solution must not be applied for longer than 1 min.[13]

Citric acid, which has been proposed as root canal irrigating irrigant because of its properties like the removing capacity of the inorganic component of smear layer and decalcification capacity of dentin. [14]

Azadirachta indica or Neem extract , an antioxidant has proven their efficacy as root canal irrigant due to the antiadherence property of bacterial cells and antimicrobial property.[15]

Alstonia scholaris or saptaparna extract has been studied in dentistry by Khandere et al [16] on efficacy of saptaparna

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leaf and stem latex on krumidanta school (Pain due to dental Caries and found that it contains alkaloids like echitamine, alstonamine, scholarine, picrinine, strictamine, ditamine, vallesamine and scholaricin and antiinflammatory, analgesic activity . Along with it contains polyphenols which accounts for its antioxidant activity and chelating activity. Polyphenols are well-known to chelate pro-oxidant metal ions and prevent free radical formation from this pro-oxidants.Thus it may act as an chelating agent.[17]

The present study proved that in Group I(17% EDTA) was used as a final irrigating solution, showed higher mean value for push out bond strength with a sample size of 20 which is obtained as 7.800 MPa compared with the other 4 groups in the middle third of the root canal. This is in accordance with the study done by Alkhudhairy et al in which EDTA showed higher bond strength values due to the efficacy of EDTA to remove the smear layer and open the dentinal tubules.[18]

A neutral EDTA solution reduces the mineral and noncollagenous proteins (NCPs) component of dentin. Thus, EDTA not only removes calcium ions but also calcium bonded to NCPs. Because the content of NCPs decreases in the apical third of the root canal system, the degree of decalcification of EDTA in this part is low. This is in accordance with several other studies done by Taxeiret et al, Khademi et al and Kandil et al. [19,29,21] Paqueet al.[22] reported that dentin in the apical third of the root canal is sclerosed. Hence, EDTA may not have such a pronounced action on sclerosed dentin in the apical third, it requires an application time of not less than 15 min for optimal results. Thus all this studies suggested that 17% EDTA was able to remove smear layer effectively in the middle third of the root canal thus in turn increase the bondstrength of endodontic sealers.

The present study showed final irrigation with EDTA resulted in slightly higher bond strength values for AH plus than citric acid as EDTA significantly decreasing the wetting ability of dentinal wall (i.e., decreased surface energy) as compared to citric acid .Similar rsults were found by shivanna et al [23]

As far as the herbal irrigants are concerned, neem extract showed least mean pushout bondstrength compared with Alstonia scholaris/saptaparna extract This is due to the higher antioxidant property of saptaparna extract. Chritopher et al evaluated [24] the effect of three different antioxidants on the dentinal tubular penetration of Resilon and Real Seal SE on sodium hypochlorite-treated root canal dentin and concluded that sodium hypochlorite irrigation might interfere with the polymerization of resin cement by leaving residual oxygen in the dentinal tubules after irrigation leading to decrease in bond strength. Thus use of antioxidant may increase the bond strength of resin-obturating materials due to enhanced polymerization and provide a better seal.

The metal chelating property of saptaparna extract was also studied by Pankti et al [25] which states that it has free radical as well as metal chelating property. With this concept saptaparana extract was taken as an endodontic irrigant as it may remove smear layer through the metal chelating property and antioxidant effect thus leading to increased bond strength.

In the present study done it was reported that majority of the specimens in all groups showed cohesive failure followed by the mixed failure. This is in accordance with results from previous studies done by Prado et al [26] , Topcuoglu et al [27] ,that reported a cohesive failure pattern in the AH Plus sealer group . This may be due of the high adhesion capacity of AH Plus sealer to the canal dentin. [28]

The present study showed that AH Plus has high resistance to dislodgement and the use of chelating agents

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and NaOCl has a positive impact on the push out bond strength of AH Plus. The complete exposure of the amino groups of the dentinal collagen due to the removal of the smear layer and debris may increase the number of covalent bonds between the epoxy resin and amino groups resulting in a stronger link of AH Plus to root canal dentin. [29]

Conclusion

Within the limitations of the study it was observed that the chemical irrigants that is 17% EDTA and 10% citric acid showed maximum pushout bond strength compared with the herbal irrigants such as alstonia scholaris/ saptaparna extract and neem extract .However herbal alternatives are easy available, cost-effective, increased shelf-life, low toxicity, and lack of microbial resistance. The in vitro observations of herbal products appear promising, but preclinical and clinical trials are needed to evaluate the biocompatibility and safety factor before they can conclusively be recommended as intracanal irrigating solutions and medicament. Hence, herbs can be used for treatment procedures that have been established to be effective and with minimal risk involved.

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