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ORIGINAL ARTICLE

The Effectiveness of Three Different Plant Extracts Used as Irrigant in Removal of Smear Layer: A Scanning Electron Microscope Study

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ABSTRACT

Aim: In the present study, the role of three plant extracts as irrigant in root canal cleaning after instrumentation was evaluated. The effect of Syzygium aromaticum (S. Aromaticum), Ocimum sanctum (O. Sanctum) and Cinnamomum zeylanicum (C. zeylanicum) plant extracts was evaluated in smear layer removal.

Methods: The study was divided into different groups having 5 teeth each using various irrigating agents to evaluate smear layer removal. Group A: O. Sanctum extract; group A1: O. Sanctum extract with EDTA, group B: S. Aromaticum extract; group B1 S. Aromaticum extract with EDTA, group C: C. zeylanicum extract; group C1 C. zeylanicum extract with EDTA and two control group of 5 teeth each in group D: 3% NaOCl; group D1 3% NaOCl with EDTA (as positive control) and group E: Distilled water (as negative control); group E1 3% Distilled water with EDTA. Each tooth was split longitudinally and prepared for examination by scanning electron microscopy.

Results: The herbal extracts were effective in cleaning root canal walls when combine with EDTA with maximum activity of S. Aromaticum extract with EDTA group.

Conclusion: Under the condition of present study the three herbal plant extracts were ineffective in removal of smear layer when used alone.

Keywords: Cinnamomum zeylanicum, Herbal extracts, Ocimum sanctum, Smear layer, Syzygium aromaticum

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INTRODUCTION

Cleaning and disinfection of the root canal system is one of the major objectives of biomechanical preparation. The effectiveness of endodontic space cleaning depends on both instrumentation and irrigation (1). Therefore, it is necessary to use mechanical, physical, and chemical adjuncts that might act on the organic material, neutralizing and dissolving the toxic products, as well as microorganisms and their by-products (1,2).

During instrumentation of root canal,

a layer of material composed of dentin, remnants of pulp tissue, odontoblastic processes & sometimes bacteria forms on the canal wall which is called as smear layer. This layer must be removed with some chemical agents for the better diffusion of intracanal medicaments and for proper adaptation of sealers to the dentinal walls (3-10).

Sodium hypochlorite and chlorhexidine are the most commonly used irrigants because of their well-known antimicrobial property (11-14). The tissue-dissolving capacity and microbicidal activity of NaOCl make it an excellent irrigating solution (15), but it has only limited effect on the dissolution of smear layer. Similarly chlorhexidine is not able to dissolve tissue & smear layer (16). The use of certain acid solutions have been recommended for removing the smear layer, including: sodium salt of ethylenediaminetetraacetic acid (EDTA) (17), citric acid solutions (18) and orthophosphoric acid (17, 19). However, at the same time the prolonged use of EDTA or its association with sodium hypochlorite in high concentrations might cause excessive dentinal erosion (20-22) and reduction in dentin microhardness (23-24).

NaOCl possess few limitations such as tissue toxicity, allergic potential and disagreeable smell and taste (25). Hence, the requirement of irrigants with low toxicity but high antimicrobial efficiency and ability to remove the smear layer is the need of the hour in dentistry.

The role of natural extracts for endodontic purpose in smear layer removal has been evaluated for plants such as Morinda citrifolia (26) and German chamomile (Marticaria recutita L.) extract and tea tree (Melaleuca alternifolia L.) oil (27) . Therapeutic effects including antibacterial, antifungal, analgesic and anti-inflammatory properties have already been demonstrated for several plants such as Syzygium aromaticum (S. aromaticum)(28) , Ocimum sanctum (O. sanctum)(29) , Cinnamomum zeylanicum (C. zeylanicum)(30).

In the preliminary study, the extracts of Ocimum sanctum (O. sanctum), Cinnamomum zeylanicum (C. zeylanicum), Syzygium aromaticum (S. aromaticum) showed antimicrobial effects against Enterococcus faecalis both in planktonic and biofilm forms (31). In order to use a substance as an irrigant it should also have ability to remove the smear layer from the instrumented root canal walls. Hence the aim of this study was to evaluate by scanning electron microscopy (SEM) analysis the effectiveness of three herbal extracts in biomechanical preparation for removal of the smear layer.

MATERIAL AND METHODS

The medicinal plants used for the experimental purpose were S. aromaticum, O. sanctum and C. zeylanicum at their minimum bactericidal concentration (MBC). In line with the results of previous study (31), the MBC of O. sanctuxm was 200 mg/500μL of 30% dimethyl sulfoxide (DMSO) i.e. 40%, C. zeylanicum was 50mg/500μL of 30% DMSO i.e. 10% and S. aromaticum was 50mg/500μL of 30% DMSO i.e. 10%. The method of preparation of above mentioned plant extracts has been explained in the earlier study (31).

The study was approved by the institutional review board of Pandit Bhagwat Dayal Sharma Institute of Health Sciences, Rohtak, Haryana, India for collection and use of extracted teeth. The teeth were collected from the department of Oral and Maxillofacial Surgery, Rohtak, Haryana with the informed consent of the donor. A total of 50 maxillary and mandibular single rooted non-carious, extracted human teeth with fully developed apices were included in the study. Presence of a single canal was determined by radiographs taken in both the mesiodistal and buccolingual directions. The selected teeth ranged from 21 to 25 mm in length with intact clinical crowns. The teeth were cleaned to remove superficial debris, calculus and tissue tags and were stored in normal saline in order to prevent dehydration before use. The teeth were randomly divided into three experimental groups of 10 teeth each and two control groups of 10 teeth each. Conventional access cavities were prepared using round burs and Endo-Z burs (Dentsply Maillefer, Ballaigues, Switzerland). The working length was established by introducing a K- type file of size 10 or 15 (Dentsply Maillifer, Ballaigues, Switzerland) in the canal until its tip was visualized at the apical foramen.

Testing procedure

The teeth were randomly divided into three experimental groups of plant extracts with 5 teeth each in group A: O. sanctum extract; group A1: O. sanctum extract with EDTA, group B: S. aromaticum extract; group B1 S. aromaticum extract with EDTA, group C: C. zeylanicum extract; group C1 C. zeylanicum extract with EDTA and two control group of 5 teeth each in group D: 3% NaOCl (Neodent, Karol Bagh, New Delhi, India); group D1 3% NaOCl with EDTA (as positive control) and group E: Distilled water (Parenteral Drugs Limited, Baddi, Himachal Pradesh, India) (as negative control) ; group E1 3% Distilled water with EDTA. Root canals were instrumented using the same standardized procedure for all the groups. The coronal and middle segments of the canal were prepared with rotary Protaper Universal instruments ((ProTaper, Dentsply Maillefer, Ballaigues, Switzerland) and finally F2 and F3 were used to prepare the canal up to the working length.

Irrigation protocol

Canals were initially irrigated with 2 mL of experimental extract for 30 s. After each instrument used, the canal was irrigated with 2 mL of tested extract by using a 30-gauge needle (Septodont, Henry Schein, Australia)

adapted to a disposable plastic syringe. The needle was placed up to 3mm short of the working length. After the last instrument was used, experimental extract was left undisturbed for 60 s & then the finally irrigated with 2 mL of experimental extract. In groups A1, B1, C1, D1 and EI final irrigation accompanied by 5 mL of 17% ethylene diamine tetra acetic acid (EDTA) (Neodent, Karol Bagh, New Delhi, India) for 1min & again with 2 mL of experimental extract. Overall, 20 mL of irrigant was used per canal for approximately 6 min 30 s. The same protocol was followed for both the control groups i.e distilled water (negative control) and 3% NaOCl (positive control).

Smear layer evaluation

Presence of smear layer in groups was evaluated by scanning electron microscope. Ten samples from each group were decoronated from which five samples were used to evaluate the smear layer removal through these extracts along with EDTA and other remaining five without using EDTA. Root surfaces were grooved using separation disk. Specimens were split longitudinally in the buccolingual plane, taking care not to contaminate the canal with debris. Specimens were dehydrated in ascending grades of alcohol up to 100% and mounted on aluminum stubs. Gold sputter coating was carried out under reduced pressure in an inert argon gas atmosphere in Agar Sputter Coater P 7340 (Agar Scientific, Essex, U.K.) and specimens were examined under scanning electron microscope (Leo 435, VP, Cambridge, U.K.) operated at 20 KV. Serial photomicrographs were taken of the canal walls at 1500 X magnifications by using digital image analysis software. Each of the root canals was scanned in its entirety to obtain an overview of the general surface topography. SEM micrographs were taken of the coronal, middle, and apical surface topography of each tooth specimen. In total, 150 SEM micrographs were taken from the 50 teeth. The root canal surfaces were assessed for the presence of smear layer by using a modified semiquantitative visual criterion, as described by Madison and Hokett (32) which is as follows:

- Scale 0 - no removal of smear layer and no dentinal tubules visible
- Scale 1 - some removal of smear layer and some dentinal tubules visible
- Scale 2 - complete removal of smear layer and all dentinal tubules visible

Table 1 : The statistical difference of removal of smear layer at various levels of canal along with EDTA between control & experimental groups

Groups	P value(Wilcoxon test)	Results
1. Distilled water with EDTA		
a. Apical vs middle	P = .564	Non-Significant
b. Apical vs coronal	P = .317	Non-Significant
c. Middle vs coronal	P =. 317	Non-significant
2. NaOCl with EDTA		
a. Apical vs middle	P = 1.00	Non-Significant
b. Apical vs coronal	P = .180	Non-Significant
c. Middle vs coronal	P =. 083	Non-significant
3. C. zeylanicum with EDTA		
a. Apical vs middle	P = .102	Non-Significant
b. Apical vs coronal	P = .038	Significant(p<.05)
c. Middle vs coronal	P =. 083	Non-significant
4. S. aromaticum with EDTA		
a. Apical vs middle	P = 1.000	Non-Significant
b. Apical vs coronal	P = .034	Significant (p<.05)
c. Middle vs coronal	P =. 034	Significant(p<.05)
5. O. sanctum with EDTA		
a. Apical vs middle	P = .038	Significant (p<.05)
b. Apical vs coronal	P = .038	Significant(p<.05)
c. Middle vs coronal	P =. 046	Non-significant

Statistical Analysis.

To compare the smear layer removal capacity along with EDTA between apical, middle and coronal aspects of all the groups Freidmann test was applied. Further to evaluate the exact difference in different aspects of root canal Wilcoxon test was used (Table 1 and 2). Intergroup analysis among various aspects of root canal was done by applying Mann whitney test. Additionally the smear layer removal capacity without using EDTA by all the groups was evaluated statistically in the manner as described above.

RESULTS

Intragroup analysis revealed that while using EDTA there was no significant difference in smear layer removal between apical, middle and coronal aspects in both the control groups while experimental groups showed significant difference. Further we found that C. zeylanicum along with EDTA showed significant difference between apical and coronal region. S. aromaticum revealed difference while comparing both apical and middle areas with coronal aspect. In O. sanctum group there was significant difference while comparing apical aspect with that of middle and coronal. Smear layer removal by C. zeylanicum, S. aromaticum, O. sanctum and distilled

Table 2 ; The statistical difference of removal of smear layer at various levels of canal along without EDTA between control & experimental groups

Groups	P value(Wilcoxon test)	Results
1.Distilled water		
a. Apical vs middle	P = 1.00	Non-Significant
b. Apical vs coronal	P = .317	Non-Significant
c. Middle vs coronal	P =. 317	Non-Significant
2. NaOCl		
a. Apical vs middle	P = .317	Non-Significant
b. Apical vs coronal	P = .157	Non-Significant
c. Middle vs coronal	P = .317	Non-Significant
3. C. zeylanicum		
a. Apical vs middle	P = .317	Non-Significant
b. Apical vs coronal	P = .180	Non-Significant
c. Middle vs coronal	P = .317	Non-Significant
4. S. aromaticum		
a. Apical vs middle	P = 1.000	Non-Significant
b. Apical vs coronal	P = .157	Non-Significant
c. Middle vs coronal	P =. 157	Non-Significant
5.O. sanctum		
a. Apical vs middle	P = .317	Non-Significant
b. Apical vs coronal	P = .317	Non-Significant
c. Middle vs coronal	P = 1.000	Non-Significant

water along with EDTA in apical region were at the same level. However, NaOCl along with EDTA showed significant difference while comparing with distilled water, C. zeylanicum and O. sanctum in apical region but S. aromaticum showed comparable results. In middle third all the groups along with EDTA revealed similar results. The results of coronal third simulates with that of middle third except in distilled water and NaOCl groups. The results of intragroup and intergroup analysis excluding EDTA at various aspects of root canal revealed no significant difference between control and experinmental groups. However as a whole there was significant difference between apical and coronal aspects of root canal in removal of smear layer by various agents. Thus the plant extracts used in the present study were effective in removal of smear layer in the presence of EDTA only and moreover, the results were inferior in the apical aspect of root canal as compare to NaOCl along with EDTA group except S. aromaticum which showed comparable results. In middle and coronal aspect including EDTA the results were equivalent to NaOCl.

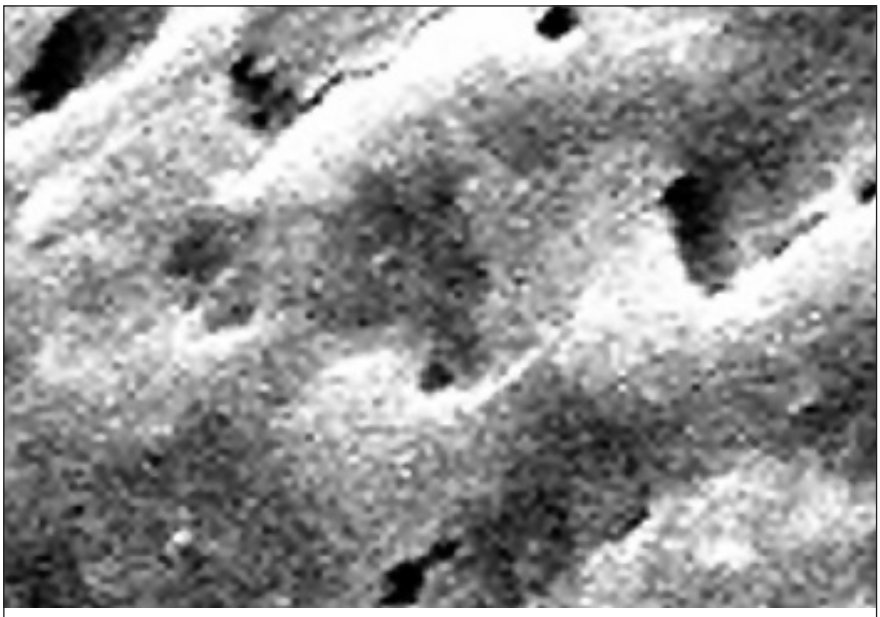

Fig. 1 No smear layer removal by distilled water

INTERPRETATION OF STATISTICAL RESULTS

In control group the use of distilled water with EDTA results in no removal of smear layer in apical region while partial or complete removal in middle and coronal areas. Plain distilled water showed no removal of smear layer in all the regions (Figure 1). NaOCl with EDTA in apical and middle region revealed partial or complete removal of smear layer but complete removal in coronal region (Figure 2). Without EDTA the results were comparable with that of distilled water in all regions.

In experimental group the C. zeylanicum along with EDTA showed no removal of smear layer in apical region while in middle and coronal areas showed partial or complete removal. Without EDTA C. zeylanicum revealed partial removal of smear layer only in few cases. With the use of S. aromaticum along with EDTA partial (Figure 3) or no removal of smear layer was observed in apical and middle regions while partial or complete removal (Figure 4) in coronal areas. Without EDTA S. aromaticum showed similar results with that of C.

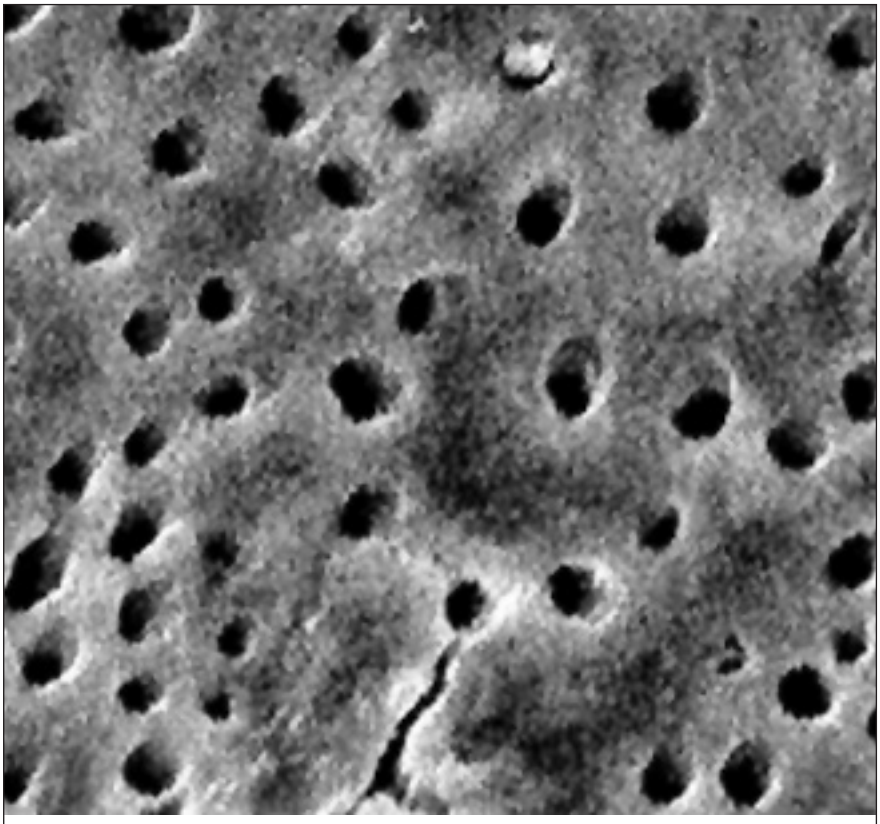

Fig. 2 complete removal of smear layer with NaOCl plus EDTA

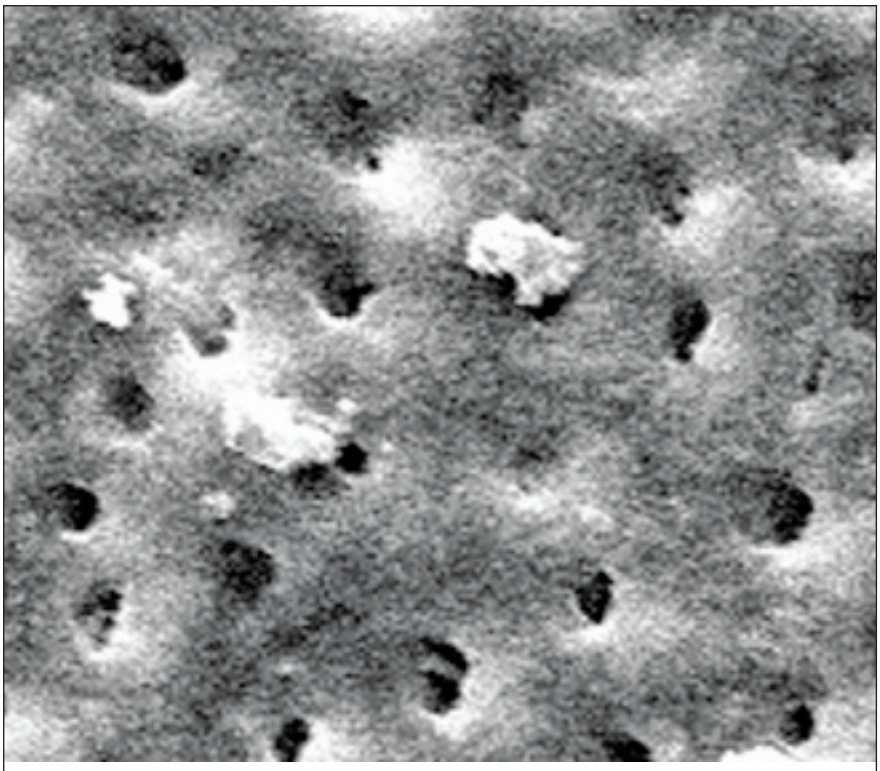

Fig. 3 partial removal of smear layer with S. Aromaticum plus EDTA

Zeylanicum in all segments. The results O. sanctum were exactly comparable with C. zeylanicum no matter the EDTA was used or not. In total the best agent to remove smear layer was found to be NaOCl along with EDTA. In experimental group the decreasing order for removal of smear layer with EDTA was found to be S. Aromaticum > C. Zeylanicum = O. Sanctum. The herbal extracts without the use of EDTA were ineffective in removal of smear layer with some minimal effect only in coronal region.

DISCUSSION

An ideal smear layer removing agent should eliminate both organic and inorganic phases from all canal surfaces without harmful erosive effects on dentin (33). Removal of the smear layer opens the dentinal tubules and allows penetration of irrigants into the tubules (34). It also helps in improving the efficacy of intracanal medications and irrigants and reduces the time needed for canal disinfection (35,36). Lastly smear layer removal also facilitates adaptation of root canal filling to canal walls (37) and reduces apical leakage (38).

bbTo simulate the clinical situation and to test the efficacy of the solutions in all segments of the root canal system the entire root canal wall was utilized. SEM was used to assess the effectiveness of various irrigants in removing the smear layer.

In order to avoid the undesirable effects of NaOCl, three herbal plant extracts which can disinfect the root canal system with less toxicity were selected for the study (31). The data for characterization of some major contents of these plant extracts, as determined by Gas Liquid Chromatography was obtained from the Department of Pharmacy, PGIMS, Rohtak, Haryana, India. (C. zeylanicum contains 89.6 % cinnamaldehyde and 77% of eugenol in essential oil, S. aromaticum contains eugenol, β-caryophylline and eugenol acetate in 71.0%, 23.3 % and 1.5%, respectively in

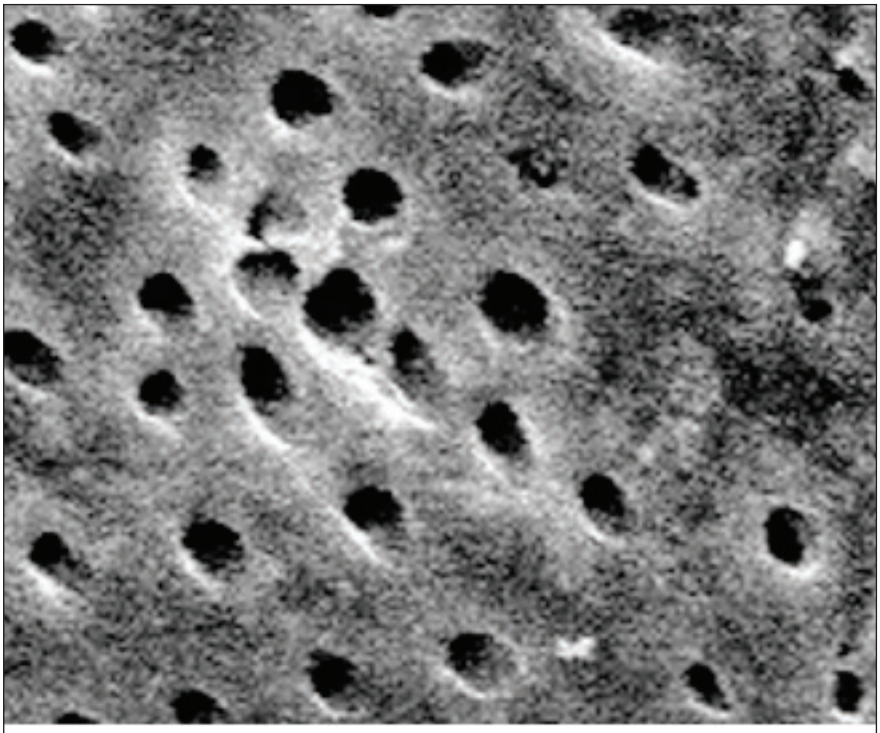

Fig. 4 complete removal of smear layer with S. Aromaticum plus EDTA

essential oil. The major constituents in essential oil of O. sanctum are 53.06% eugenol and 77% methyl eugenol) (31).

In present study Group D1 indicated that irrigation with 3% NaOCl during instrumentation with final flush of 17% EDTA for 1`min was significantly more effective in removing the smear layer compared with distilled water. This result is similar with other studies stated that physio-chemical action of NaOCl is important in removal of organic residue, with EDTA acting mainly on the inorganic residue (39,40). Alone all the plant extracts were ineffective in removal of smear layer from the entire root canal walls with limited action only in the coronal region. This is due to the better flushing action of irrigant in the coronal area due to larger root canal diameter in the coronal region (39,41). The ineffectiveness of these herbal extracts in removal of smear layer may be attributed due to the less acidic nature of secondary metabolites present in each extracts.

The effectiveness of the herbal plant extracts along with EDTA in removing smear layer is only due to EDTA not by the action of extracts. The cleaning effect of all the irrigants was more pronounced in the coronal and middle thirds than in the apical parts of the root canals. The most probable explaination for this is the smaller diameter of the root canal and the consequent decrease in the flow of the irrigant (39, 41,42). The results of S. Aromaticum along with EDTA in removal of smear layer were more as compare to other two groups. This can be due to slight more acidic nature of components (β-caryophylline and eugenol acetate) of S. Aromaticum as compare to other two extracts.

The preliminary study was conducted to evaluate the antimicrobial ability of these plant extracts against E. Faecalis as irrigant and indicated their use more as intracanal medicament rather than as irrigant (31). Further to conclude their endodontic utility their use to remove smear layer was evaluated in the present study. The inability of these extracts in removal of smear layer further highlights the use of these herbal extracts as intra canal medicament. The limitation of present study is that it did not evaluate in vivo effects and the possible interaction of extracts with restorative materials. Although the results of present study were negative but it was necessary to conduct the study for confirmation of clinical utility of these extracts as irrigant or intracanal medicament.

CONCLUSION

The extracts of O. sanctum, C. zeylanicum, S. Aromaticum alone were ineffective in removal of smear layer from the instrumented root canal walls. Along with EDTA the effect of these extracts was comparatively less than NaOCl plus EDTA. Among experimental groups S. Aromaticum with EDTA was most effective.

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