

Comparative Evaluation of Microhardness and Color Change of Root dentin using *Punica granatum* (pomegranate extract), Sodium hypochlorite, Chlorhexidine and Normal saline as an Endodontic irrigant – An *in vitro* study

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ABSTRACT

Aim: To evaluate the microhardness and change in color of root dentin using *Punica granatum*, sodium hypochlorite, chlorhexidine and normal saline as an endodontic irrigant and to implement their use in field of dentistry. **Materials and methods:** 60 single rooted anterior teeth extracted due to periodontal and orthodontic reasons were collected. All the samples were decoronated to achieve constant length of 14mm. Patency of root canal was established using 10k file and working length was determined 1mm short of the file length. Each canal was prepared till F3 protaper. After preparation the samples were randomly divided into 4 groups: Group 1: Saline (Control); Group 2: 5% Sodium Hypochlorite; Group 3: 2% Chlorhexidine; Group 4: 5% *Punica granatum* (pomegranate peel extract). After grouping the samples were longitudinally sectioned. One half of the tooth sample was used for microhardness testing using Vickers microhardness indenter and the other half of sample was used for color change evaluation using spectrophotometer after irrigation with the test solutions on day 1 and day 7th post irrigation. **Results:** The mean microhardness in all four groups decreased comparatively at post as compared to pre and the decrease was evident highest in sodium hypochlorite followed by chlorhexidine, pomegranate and saline the least. In regard to color change sodium hypochlorite, chlorhexidine and pomegranate all showed visible color changes after 7 day of irrigation with pomegranate showing the highest color change. Higher lightness was shown in Group 2 *i.e.*, sodium hypochlorite whereas redness was seen more in Group 4 *i.e.*, pomegranate and yellowish tint was more visible in Group 3. **Conclusion:** 5% *Punica granatum* has showed negligible effect on microhardness of dentin but showed maximum color change when compared to chlorhexidine, sodium hypochlorite and saline.

Key words: *Punica granatum* (pomegranate peel extract), Herbal, Endodontic irrigant, Microhardness, UV-VIS spectrophotometer, Color change, Chlorhexidine.

INTRODUCTION

Endodontic treatment is primarily a debridement procedure that must be completed successfully in order to remove the irritants from the canal and periapical tissue. Depending on the circumstances, debridement may be performed using a variety of techniques, such as instrumenting the canal and placement of medicaments and irrigants.¹

One of the keys to successful root canal procedure is irrigation. Irrigation in endodontics can have several important functions some of which differ depending upon the type of irrigant employed. Irrigants helps to reduce the friction between instrument and dentin and increases the file efficiency. It can also help to reduce the heat generated due to instruments. Additionally, it has the ability to affect the parts of the root canal wall that are challenging for mechanical instruments to access.²

Sodium hypochlorite (NaOCl), Chlorhexidine (CHX), Normal saline are the most commonly used irrigants in daily endodontic practice.

Numerous studies have demonstrated that these endodontic irrigants can change the chemical

constitution of dentin by eliminating the calcium ions found in hydroxyapatite crystals. The modifications in the proportion of organic and inorganic components would then cause variations in the microhardness, permeability, and solubility properties of dentin. The ability of resin-based root canal sealers to adhere to root dentin is also negatively impacted by variations in the calcium ion concentration.³ Measuring microhardness can therefore provide indirect evidence of mineral development or loss in the hard tissues of the oral cavity.³

Aesthetics have become a major priority for both patients and dentists nowadays. Tooth discoloration after endodontic therapy is a common problem that decreases the quality of care and can cause most of patients to be unsatisfied. It is hypothesised that materials entering the dentinal tubules may lead to a gradual discolouration of the tooth.⁴ Sodium hypochlorite and chlorhexidine, which are used as endodontic irrigating solutions, have also been shown in studies to cause noticeable colour changes.

Punica granatum L. (Pomegranate) is a ubiquitous fruit belonging to punicaceae family having its origins from north india. It has been used since long times

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because of its ability to prevents several oral illnesses such as caries and gingivitis. The flavonoids, tannins, and other phenolic chemicals found in pomegranate peel extracts are the active components responsible for these actions.⁵

There haven't been many studies done to examine the colour changes brought on by using pomegranate peel extract as an endodontic irrigant and its effect on the microhardness of the root dentin when used as an endodontic irrigant.

Therefore, this study will be of significance in identifying an alternative herbal irrigant to sodium hypochlorite (NaOCl), and CHX (chlorhexidine).

The objective of this study is to evaluate the microhardness and color change of root dentin using *Punica granatum* (pomegranate peel extract) as an endodontic irrigant in comparison to various endodontic irrigants used such as sodium hypochlorite, chlorhexidine and normal saline.

MATERIALS AND METHODS

Sample selection

A total of 60 single rooted anterior teeth which have been extracted due to periodontal/orthodontic reasons were collected and were cleaned of debris using ultrasonic scaler and stored in distilled water till the time of experiment.

The teeth were collected based on the following inclusion and exclusion criteria (Table 1).

Preparation of pomegranate extract: In a sterile container, 150g of the phytotherapeutic powder (pomegranate peel) was soaked in 1000 ml of sterile distilled water. The sterile container was left undisturbed for 48 hours while undergoing cold maceration which involved intermittent shaking with a sterile glass rod. The preparation was concentrated for 5 days at 110 degrees Celsius using an electrical water bath after being filtered through a sterile muslin clot. The dried extracts were kept in a sterile container. For preparation of stock solution: To achieve 50% of the extract (or 500 mg/ml), 10g of dry extract was diluted in 20 ml of distilled water and kept in an airtight container. Up until its next usage, the solution was kept cooled at 4°C. Figure 1 (Preparation of 5% pomegranate extract solution)

Specimen preparation

The teeth were decoronated at Cementoenamel Junction (CEJ) to achieve a consistent length of 14 mm for all samples using a slow speed straight handpiece (NSK Nakanishi Inc, Tochigi, Japan) and a sterile diamond disc (NTI® Diamond Discs, Axis-sybron endo. Kerr business CA, USA). After preparation of the the canal using protaper rotary till F3. Each sample was irrigated with 2ml of distilled water between each subsequent file size in all groups, delivered using a disposable 2ml, 26-gauge, beveled tip needle irrigation syringe (Unolock) bent at 10mm and placed as far apically as possible into the canal without

binding. The samples were then randomly divided into 4 groups: Figure 2 (Grouping of the samples)

Group 1: Saline (Control)

Group 2: 5% Sodium Hypochlorite

Group 3: 2% Chlorhexidine

Group 4: 5% *Punica granatum* (pomegranate peel extract)

After grouping the samples were sectioned longitudinally starting from cervically till apically into buccal and lingual segments using a diamond disc (Horico, Berlin Germany) making total of 120 segments. The one half of the segment was horizontally embedded in an auto polymerising acrylic resin exposing the dentin part of the root surface. Figure 3 (samples prepared for microhardness testing) In accordance with the procedure provided to each group, each specimen was irrigated with 5 ml of each irrigant by submerging it in the test irrigant solution for 5 min as follows:

Group 1: 5ml of saline (control) for 5 min

Group 2: 5 ml of 5% NaOCl followed by final rinse of saline for 5 min

Group 3: 5 ml of 2% CHX followed by final rinse of saline for 5 min

Group 4: 5 ml of 2% CHX followed by final rinse of saline for 5 min

Microhardness determination

The root dentin microhardness for each specimen was determined using a Vicker's microhardness tester. Using a Vicker's diamond indenter at a 40x magnification, the 3 unique indentations were each made with a 300 g load and a 20-second dwell time interval. Figure 4 (Vicker's microhardness tester machine and indentation seen) The indentations were positioned without overlap at 0.5 mm level to the root canal wall, 100 m from the pulp-dentin interface, at the mid-root level of the root dentin. The microhardness value was calculated using the length of the two diagonals (Vickers Hardness Number [VHN]). The average of the data for the 15 indentations yielded the typical hardness values (VHN).

Color evaluation using spectrophotometer

The other half of the specimen was used for color evaluation using spectrophotometer. The specimens were subjected to irrigation as per the grouping protocol. Figure 5 (specimen irrigated with test solutions)

Group 1: Saline (Control) for 5 min

Group 2: 5% Sodium Hypochlorite for 5min followed by flushing the sample with saline

Group 3: 2% Chlorhexidine for 5 min followed by flusing of the sample with saline

Group 4: 5% *Punica granatum* (pomegranate peel extract) for 5 min followed by flushing of the sample with saline

The specimens were examined at the following stages:

At the day of irrigation

7 days after irrigation

The difference in dL* da* db* scale was used to evaluate the changes in color.

Prior to taking measurements of the samples, the spectrophotometer was calibrated by taking measurements against a standard white backdrop, a black background (provided by the manufacturer), and under illumination of D65, which is comparable to day light. The samples were kept on a 1mm circular jig and was covered with a box to avoid scattering of light. Figure 6 (color evaluation using spectrophotometer)

Table 1: Inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|--------------------------------|--------------------------------------------------|
| • Single rooted anterior teeth | • Vertical fracture teeth |
| • Teeth free of visible cracks | • Horizontal fracture teeth |
| • Caries free teeth | • Caries extending subgingivally |
| | • Open apex cases |
| | • Calcified canals |
| | • Fracture lines extending onto the root surface |
| | • Ankylosed teeth |
| | • Cracked teeth |

Using the provided formula, the total change in colour (ΔE^*) was obtained:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Statistical analysis

Data were summarised in Mean \pm SD (standard deviation). Pre and post data were compared by paired t test. The pre to post change in outcome measures between four independent groups were compared by one factor analysis of variance (ANOVA) and the significance of



Figure 1: Preparation of 5% pomegranate extract solution

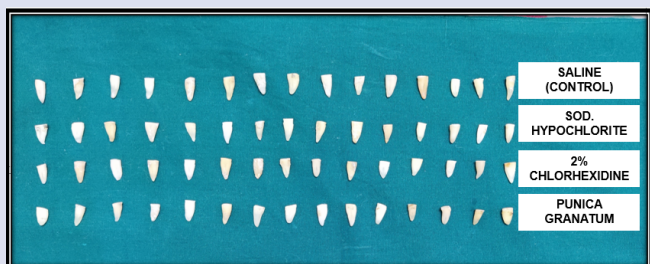


Figure 2: Grouping of the samples

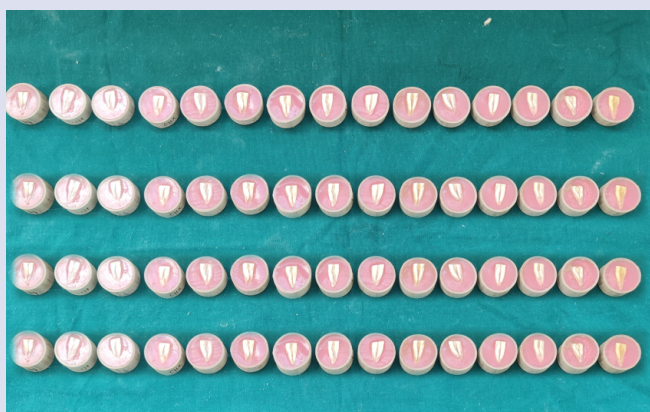


Figure 3: Samples prepared for microhardness testing

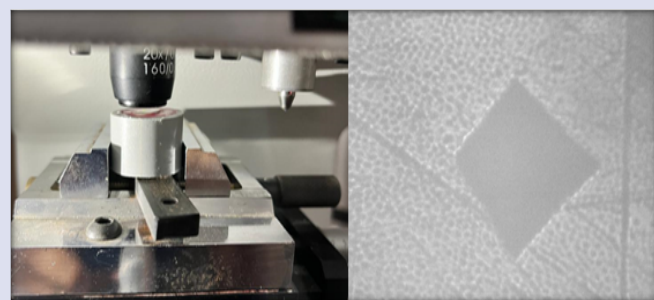


Figure 4: Vicker's microhardness tester machine and indentation seen



Figure 5: Specimen irrigated with test solutions

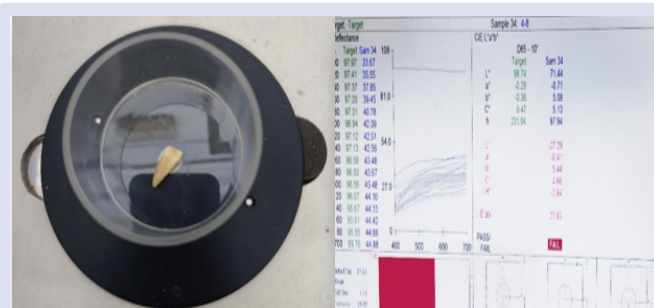


Figure 6: Color evaluation using spectrophotometer

mean difference between (inter) groups was done by Tukey's HSD (honestly significant difference) post hoc test after ascertaining normality by Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. A two-tailed ($\alpha=2$) $P < 0.05$ was considered statistically significant. Analyses were performed on SPSS software (Windows version 22.0).

RESULTS

The pre and post microhardness of all four groups (saline, sodium hypochlorite, chlorhexidine and pomegranate) is summarised in Table 2 and also depicted in Graph 1. The mean microhardness in all four groups decreased comparatively post irrigation as compared to pre and the decrease was evident highest in sodium hypochlorite followed by chlorhexidine, pomegranate and saline the least (saline < pomegranate < chlorhexidine < sodium hypochlorite).

The difference in pre to post mean change or decrease in microhardness of saline, sodium hypochlorite, chlorhexidine and pomegranate was found 4.1, 36.6, 18.9 and 6.4% respectively.

The color change (Δ) of four groups (saline, sodium hypochlorite, chlorhexidine and pomegranate) on the day of irrigation is summarised in Table 3 and also shown in Graph 2. On the day of irrigation, the

mean color change was found highest in pomegranate followed by chlorhexidine, saline and sodium hypochlorite the least (sodium hypochlorite < saline < chlorhexidine < pomegranate).

The color change of four groups on the day of irrigation were summarised in Mean ± SD and compared by ANOVA (F value). The color change of four groups after 7 day of irrigation were summarised in Mean ± SD and compared by ANOVA (F value).

The net mean color change (i.e. after 7 day of irrigation-on the day of irrigation) was found maximum in pomegranate followed by

Table 2: Pre and post microhardness (HV) summary statistics (Mean ± SD, n=15) of four groups.

| Group | Pre-treatment (n=15) | Post treatment (n=15) | Mean change (Post-Pre) | t value | P value |
|---------------------|----------------------|-----------------------|------------------------|---------|---------|
| Saline | 64.49 ± 0.83 | 61.83 ± 0.82 | -2.66 ± 0.71 | 14.51 | < 0.001 |
| Sodium hypochlorite | 64.03 ± 0.92 | 40.59 ± 0.61 | -23.44 ± 1.21 | 74.74 | < 0.001 |
| Chlorhexidine | 64.23 ± 0.85 | 52.09 ± 1.61 | -12.14 ± 1.46 | 32.10 | < 0.001 |
| Pomegranate | 64.43 ± 0.74 | 60.31 ± 0.51 | -4.11 ± 0.94 | 16.93 | < 0.001 |

Table 3: Color change (ΔE) summary statistics (Mean ± SD, n=15) of four groups on the day of irrigation.

| Group | n | Color change (ΔE) | F value | P value |
|---------------------|----|-------------------|---------|---------|
| Saline | 15 | 34.21 ± 0.17 | 212.10 | < 0.001 |
| Sodium hypochlorite | 15 | 30.07 ± 1.12 | | |
| Chlorhexidine | 15 | 39.00 ± 1.95 | | |
| Pomegranate | 15 | 40.56 ± 1.16 | | |

Table 4: Color change (ΔE) summary statistics (Mean ± SD, n=15) of four groups after 7 day of irrigation.

| Group | n | Color change (ΔE) | F value | P value |
|---------------------|----|-------------------|---------|---------|
| Saline | 15 | 34.48 ± 0.28 | 238.10 | < 0.001 |
| Sodium hypochlorite | 15 | 34.17 ± 1.50 | | |
| Chlorhexidine | 15 | 43.33 ± 2.15 | | |
| Pomegranate | 15 | 44.97 ± 1.13 | | |

chlorhexidine, sodium hypochlorite and saline the minimum (saline < sodium hypochlorite < chlorhexidine < pomegranate). Summarised in table 4 and shown in Graph 3.

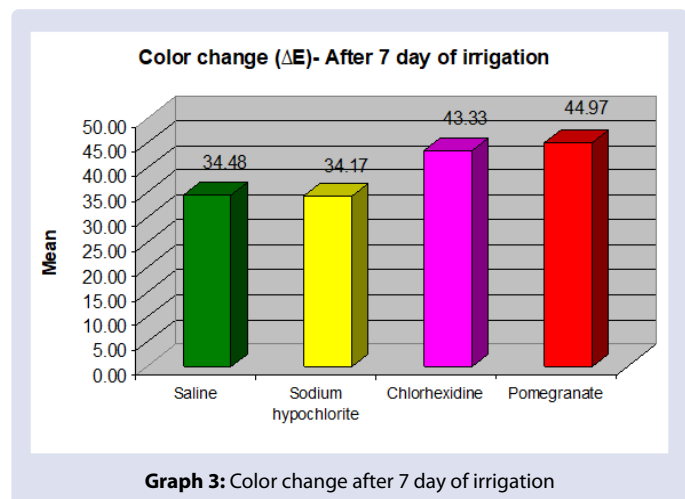
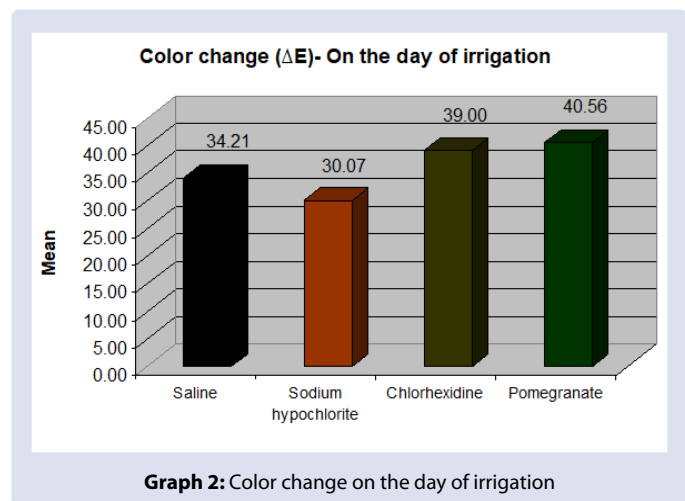
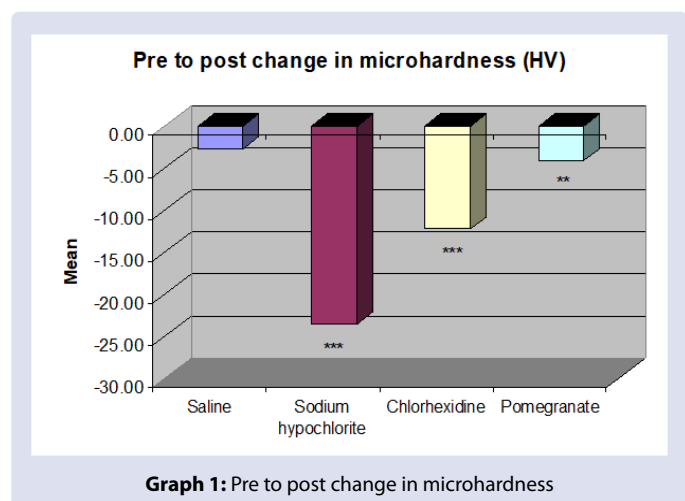
DISCUSSION

Irrigation is the best way for removing dentin and tissue fragments after instrumentation.⁶ It also reduces friction and controls temperature that are important for instrumentation. However, the most important tasks are the lysis of organic and inorganic tissues and the killing of microorganisms.⁷ A variety of solutions can be employed as root canal irrigants in endodontics. NaOCl is routinely used in all endodontic cases⁸ due to its antibacterial effect brought on by HOCl, which predominates at neutral or acid pH. Most frequently, it is employed in concentrations between 0.5% and 5.25%. In contrast to NaOCl, chlorhexidine is another irrigant which is frequently utilised in endodontics because of its excellent antibacterial properties. When compared to NaOCl it lacks tissue dissolving ability.

Normal saline is an isotonic solution that is frequently utilised for irrigation throughout all surgical procedures. It has been used as an endodontic irrigant for decades since it is safe and doesn't have any negative effects, even when extruded into the periapical region. Hence, saline solution was chosen as the negative control for testing microhardness and evaluation of color change in the present study.

In recent years, the issues with chemical agents and its safety concerns have raised the attention toward medicinal plants.⁹ Herbs may be one of the other effective substitutes for modern irrigants, according to *Badole et al.* and it has to be researched more. One or more medicinal qualities, such as antibacterial, anti-inflammatory, astringent, anticarcinogenic, and antiplaque agent, may be present in a plant.¹⁰

This study evaluated the microhardness and color change associated with *Punica granatum* (pomegranate peel extract) when used as an endodontic irrigant and comparing it with conventional irrigants such as sodium hypochlorite, chlorhexidine and normal saline. Vickers



microhardness indenter was used for measuring microhardness of the root dentin in this study.

Microhardness measurements can demonstrate the amount of mineral loss or gain in the dental hard tissues. The chemical and physical characteristics of root dentin may alter as a result of the chemicals used in root canal irrigation.¹¹ Although a reduction in microhardness may make it easier to instrument a root canal, it will also weaken the root structure, which might lead to fracture of the tooth.

There was decrease in microhardness in all the groups after irrigation with all the irrigants however this decrease was greater in sodium hypochlorite. The drop-in microhardness seen in this investigation is consistent with the findings of Oliveira *et al.* (Oliveira *et al.* 2007).¹² The 5% sodium hypochlorite used in this study greatly reduced the microhardness. It was also statistically significant when compared to other groups such as chlorhexidine, normal saline and pomegranate. This action could be brought on by sodium hypochlorite's organic tissue dissolving activity on the collagen component of dentin.¹³ Another possibility is that, NaOCl caused phosphate levels to drop when it came in contact with the dentin. Microhardness can also be impacted by the NaOCl concentration and contact time with dentin.¹⁴ In this study 5% conc. of hypochlorite was used for 5 minutes. Driscoll *et al.*¹⁵ showed that, when dentin was submerged in solutions of 0.5% NaOCl and 5% NaOCl solutions, the weight loss of dentin seen was greater in the latter.

A moderate decrease in microhardness values were noted in 2% chlorhexidine solution which is in contrast to study done by Oliveira *et al* in which considerable reduction in microhardness were seen where they irrigated the root canal dentin for 15 minutes with 2% chlorhexidine solution. The considerable change in dentin after treatment with a high concentration (2% CHX) that was utilised for 5 min. instead of 15 min. indicates powerful direct effects of this chemical on the components of dentin structure, which is how this unique result in our study can be explained. These effects led to the severance of the connections between collagen fibres and hydroxyapatite crystals, causing a reduction in microhardness of dentin that was time dependent.¹⁶

CHX are also known to be potent MMP's inhibitors. When administered at concentrations of 2%, they improve the preservation of the collagen fibrils of dentin by reducing the release of MMPs through their proteinase inhibition effect.¹⁷

In our study *P. granatum* (pomegranate peel extract) when used in 5% concentrations for 5 min showed favourable results for microhardness when compared to chemical adjuncts such as sodium hypochlorite and 2% chlorhexidine. Pomegranate, a natural source of phytochemicals, contains active ingredients like organic acids and bioactive compounds like phenolics and flavonoids, particularly anthocyanins. These bioactive substances have strong antibacterial and antioxidant properties, and they can also prevent ions lipid peroxidation. The catechins present in the peel has the ability to interact with ions present in the dentin. And also, pomegranate peel can help to eliminate smear layer which could be the reason for minimum reduction showed in microhardness of dentin.¹⁸

Currently there are limited studies on use of pomegranate peel extract as an endodontic irrigant. Based on this, color change associated with pomegranate peel extract when used alone as an endodontic irrigant and color alterations associated with NaOCl and CHX upto 7 days of post endodontic irrigation using a spectrophotometer was evaluated. For measuring color CIE L*a*b* (Commission Internationale de l'Eclairage) values were used under D65 illumination which equals to natural day light conditions. In this study pomegranate showed maximum color change on day 1 with a mean value of 40.56. On the day of irrigation, the difference in mean color change of pomegranate

was 15.7, 25.9 and 3.8% higher as compared to saline, sodium hypochlorite and chlorhexidine respectively. This can be explained by the existence of biologically active substances like tannins, which contributes to the colouring of the root dentin. According to research done by Gullon *et al.*, PP (Pomegranate peel) is a fantastic source of healthy biocompounds such as flavonoids (anthocyanins, catechins, and other complex flavonoids), phenolic acids (hydroxycinnamic and hydroxybenzoic acids), and hydrolyzable tannins (ellagic and gallic acids, pedunculagin, punicalin and punicalagin).¹⁹ The presence of anthocyanins and punicalin and punicalagin have color staining ability. Also, dentin is more opaque than enamel. Enamel normally has the tendency to reflect light because of its translucent nature. The degree of light reflection in dentin can be changed because of its composition and the specimen might appear more stained or darker in color.

In terms of 2% chlorhexidine used, it also showed color change with a mean value of 39.00 on day 1 and slight increase was noted on day 7 with a mean value of 43.33 which was less than pomegranate but more than sodium hypochlorite. Furthermore, it was also found significantly ($P < 0.001$) different and higher in chlorhexidine as compared to sodium hypochlorite. The possible explanation could be that chlorhexidine has normally been found to cause staining especially on exposed dentin. The staining seen in terms of chlorhexidine is more prominent in dentin rather in enamel. Although the precise cause of staining is not fully understood, it is thought to be caused by the degradation of the chlorhexidine molecule parachloroaniline, which causes denaturation of proteins and the formation of sulphides which can be difficult to remove. Also, the time utilised for CHX also matters as, higher the concentration and time, higher the staining.²⁰ When it comes to NaOCl, tooth colour changes may be caused by its capacity to destroy organic tissues and bleach teeth. NaOCl, on the other hand, is dissociated into Na⁺ and Cl⁻ ions by organic matter and dentin debris. Stain removal and bleaching are both brought about by the interaction of oxygen derived free radicals and staining compounds like chromophores. Also ΔE is combination of L* a* b* co-ordinates, any change in L* parameter or a* parameter will result in cumulative change in ΔE values.²¹ According to one study done by Zou *et al*, he told that residue of NaOCl can remain behind and tend to crystallize and obstruct the dentinal tubules. These residues can penetrate upto a depth of 300 μ m and can be difficult to excavate from the root canals dentinal walls which also could be one of the reasons for sodium hypochlorite to show visible color change.²² The 5% hypochlorite used in our study showed prominent discolorations but less as compared to chlorhexidine and pomegranate which could be due to bleaching effect associated with use of 5% hypochlorite.

ΔE changes when compared from day 1 to day 7 showed visible perceptible color changes to human eye in sodium hypochlorite, 2% chlorhexidine gluconate and *P. granatum* with more being in *P. granatum*, followed by chlorhexidine and sodium hypochlorite and least in saline which was not clinically visible with values being less than <1.

CONCLUSION

Within the limitations of the study it can be concluded that:

Microhardness determined using Vickers microhardness tester showed maximum reduction in microhardness with 5% sodium hypochlorite, moderate reduction in chlorhexidine and minimum reduction with 5% pomegranate showing favourable results for microhardness in case of *P. granatum*. Saline being isotonic did not change much of microhardness.

Highest color change was seen in pomegranate followed by chlorhexidine, followed by saline and sodium hypochlorite. Not much

significant changes were noted from day 1 to day 7 post irrigation. However, in all groups, the mean color change was slightly higher in after 7 day of irrigation than on the day of irrigation (on the day of irrigation < after 7 day of irrigation).

LIMITATIONS

Since it was an *in-vitro* study which involved use of extracted teeth, the outcomes in real people might differ greatly as a consequence of different body responses. Therefore, long term *in-vivo* investigations are required to assess their effectiveness.

The dark hue of the *Punica granatum* (Pomegranate peel), might discolor the teeth on prolonged usage. Hence, long term clinical trials must be conducted to identify how to minimize their discoloration potential.

Additionally, because it was an aqueous-based extract, its shelf life was constrained, necessitating the need of long-term investigations and trials to improve its shelf life and longevity.

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CONFLICTS OF INTEREST

Nil

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