

## EFFECT OF *MORINDA CITRIFOLIA* JUICE AND TRIPHALA AS ROOT CANAL IRRIGANTS ON SEALER PENETRATION DEPTH INTO THE DENTINAL TUBULES. – A CONFOCAL LASER MICROSCOPE STUDY.

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**ABSTRACT:** **Aim:** The aim of this study was to evaluate the effect of *Morinda citrifolia* juice (MCJ) and Triphala on sealer penetration depth into the dentinal tubules as root canal irrigants. **Materials and Methods:** Forty five single rooted extracted human teeth were collected; decoronated and root canal length were standardized to 16mm. Cleaning and shaping was done with protaper universal rotary instruments till F5. 30 samples were selected randomly and divided into two groups with 15 samples in each, according to the final irrigation regimen. In Group I, 5 ml of MCJ was used for 1min and in Group II; 5 ml of Triphala was used. The remaining 15 samples were irrigated with 5ml of Smear clear which acted as a positive control Group. Obturation was done with corresponding gutta-percha points and AH 26 sealer (Dentsply; DeTrey, Konstanz, Germany) labelled with fluorescent dye and left to set for 48 hrs. Then, the roots were sectioned and subjected to confocal laser microscope. **Results:** Statistical analysis was done by two-way analysis of variance (ANOVA) and Tukey multiple post-hoc procedures. The sealer penetration depth is higher in the MCJ group than the Triphala group at all three levels. **Conclusion:** MCJ showed a significant sealer penetration depth than Triphala.

**KEYWORDS:** Herbal extracts, Sealer penetration depth, Confocal laser microscope, Root canal irrigants, *Morinda citrifolia* juice, Triphala.

### INTRODUCTION

The main goal of root canal treatment is to completely eliminate the different components of pulpal tissue, bacteria, and biofilm and provide a fluid tight seal to prevent reinfection, and to promote healing of the surrounding tissues<sup>1,2</sup>. Mechanical instrumentation alone does not sufficiently disinfect the root canal system properly, especially in the apical third region due to the presence of complex anatomical features such as lateral fins, isthmuses and deltas<sup>3</sup>. Several studies revealed that, with both current nickel titanium instrumentation systems and traditional stainless-steel hand instruments almost half of the root canal walls are left unprepared<sup>4,5</sup>. In addition to this, it produces a layer of organic and inorganic material called the smear layer which contains bacteria and their by-products. It may influence the penetration of obturating materials such as root canal sealers into the dentinal tubules, a desirable outcome<sup>6</sup>.

Therefore, irrigation becomes a foremost factor in achieving the effective root canal debridement by penetrating the mechanically inaccessible areas, flushing debris, and removing the smear layer from the root canal system.<sup>7</sup>

For many days, most commonly used irrigation regimen in root canal treatment is sodium hypochlorite (NaOCl) for dissolving vital and necrotic organic tissue followed by ethylene diaminetetraacetic acid (EDTA), to exert effects on inorganic components of smear layer<sup>8,9,10</sup>. Recently, a new EDTA solution (Smear Clear) has been developed which contains 17% EDTA, cetrimide and surfactants for better smear layer removal than 17% EDTA<sup>9</sup>.

Apart from these desirable properties, these conventional irrigants have several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential, unpleasant smell and taste<sup>11</sup>.

Owing to these potential side effects, safety concerns and ineffectiveness of conventional allopathic formulations, herbal extracts are rejoined and herbal 'Renaissance' is happening all over the globe.<sup>[11]</sup> In medical and dental fields also, herbal or natural products have become more popular today due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties<sup>11-14</sup>. Consequently, several studies have been focused on the feasibility of herbal or

natural products as root canal irrigants and medicaments to be used as an alternative to the conventional chemical products.

*Morinda citrifolia* juice (MCJ), commonly called as Noni juice has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal antihelminthic, analgesic, hypotensive, anti-inflammatory, antitumor and immune-enhancing effects<sup>11-14</sup>. Studies conducted by Murray et al,<sup>15</sup> Madhusudhana K et al,<sup>16</sup> Bhardwaj et al,<sup>17</sup> Rosaline et al,<sup>18</sup> Tyagi et al,<sup>19</sup> Garg P et al<sup>20</sup> proved that the MCJ has significant antimicrobial activity on endodontic biofilms and also effective in smear layer removal similar to conventional irrigants.

Triphala, well-known Indian Ayurvedic herbal formulation consists of dried and powdered fruits of three medicinal plants, namely *Terminalia Bellerica*, *Terminalia chebula* and *Embellica officinalis*. It is biocompatible and safe, apart from its curative properties such as antioxidant, anti-inflammatory and radical scavenging activities. Garg P et al,<sup>20</sup> Prabhakar et al,<sup>21</sup> Shakouie et al,<sup>22</sup> Pujar M et al<sup>23</sup> proved that it has significant antimicrobial effect on E.Feacalis biofilms formed on the tooth substrate<sup>20,21</sup>.

However, to the best of our knowledge, there have been no studies reported on how herbal extracts such as *Morinda citrifolia* juice and Triphala affect the sealer penetration depth into the dentinal tubules when used as final irrigating solutions. Thus the aim of this study was to evaluate and compare the effect of *Morinda citrifolia* juice and Triphala as final irrigating solutions on the maximum depth of sealer penetration into dentinal tubules with Smear Clear.

## Materials and Methods

**Preparation of herbal extracts:** Triphala (Apollo Pharmacy, Chennai, India) solution was made by dissolving 120mg of triphala powder in 2 ml of 10% dimethyl sulfoxide (DMSO) (S.D. Fine Chem Pvt Ltd, Chennai, India). This was stirred for 2 min and then passed through the double filter paper. The strained solution was collected<sup>20,23</sup>. Six percent concentration of pure *Morinda citrifolia* juice (K.S.Varier's Ashtanga Ayurvedics, Trichy, India) was taken.

**Tooth samples preparation:** A total of 45 recently extracted human single-rooted teeth with straight canals were used in this experiment. Care was taken to select teeth that were without caries, cracks, root fissures, fractures or resorption. All the teeth were kept in 3% NaOCl (Prime dental products, Thane, India) for 15 minutes to remove tissue debris. All experimental procedures were performed by the single operator. The root length was standardized to a length of 16 mm from the apex and remaining tooth structure was decoronated.

A size 10-k file (Mani, Tochigi, Japan) was inserted into canal until it could be seen in the apical foramen and length was measured. Working length was determined by subtracting 1 mm from the measured length so that the length of the each sample was 15 mm. Cleaning and shaping was done with ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) rotary instruments up to F5. During this procedure, 1 ml of 3% NaOCl was used after each instrument.

All samples were selected randomly and divided into three groups with 15 samples each, based on final irrigating solutions.

- Group I:** 5 ml of *Morinda citrifolia* juice was used for 1min
- Group II:** 5 ml of Triphala was used for 1min
- Group III:** 5 ml of Smear Clear (Sybron Endo, Orange, CA, USA) for 1min, a standard irrigant for removal of smear layer containing 17% EDTA with surfactants.

These solutions were introduced into the canal with the help of side-vented needle inserted to a depth that was 1mm less than working length. Finally, all root canals were irrigated with 5 ml of distilled water for 1 minute and were then dried with absorbent points (Dentsply Maillefer, Ballaigues, Switzerland). Obturation was done with AH plus sealer (Dentsply; DeTrey, Konstanz, Germany) and corresponding gutta-percha points using the lateral condensation technique. The sealer was mixed with 0.1% fluorescent Rhodamine B isothiocyanate (Sigma-Aldrich, Bengaluru, India) for fluorescence under confocal laser microscopy. All access cavities were sealed with a Cavit G (3M; ESPE, St Paul, MN, USA), after which they were stored in an incubator at 37 °C and 100% humidity for 48 hours to allow the sealer to set.

**Sectioning and confocal microscope analysis of root specimens:** After 48 hours, each tooth was cut into 1 mm thick cross section using slow-speed, water cooled 0.3mm microtome saw (Isomet Buehler, Illinois, USA) from each coronal, middle, and apical section at distances of 2, 5 and 8mm from apex. All sections were polished with silicone carbide abrasive papers. The specimens were mounted onto glass slides and examined with a Leica TCS-SPE confocal microscope (ZeissLeica, Mannheim, Germany) at 10x magnification. The obtained images were evaluated with the help of the Zeiss LSM image browser software. The depth of penetration of the sealer was measured in microns (µm) from the lumen of the canal to the point of maximum sealer penetration depth into the tubules using a Zeiss LSM image browser measuring tool. Confocal microscope was selected in this study to analyze the

sealer penetration as it provides detailed information about the presence and distribution of sealers inside dentinal tubules in the total circumference of the root canal walls through the use of fluorescent rhodamine-marked sealers and artifacts could practically be excluded<sup>3,25</sup>. ( Fig.1)

**Statistical analysis:**

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS version 21). Shapiro-Wilks Normality test results showed that sealer penetration depths among three groups follow the normal distribution. Therefore, parametric methods were applied for the analysis of the data. The effects on the sealer penetration depth of the three final irrigating solutions were analyzed by one way analysis of variance (ANOVA) and Tukey's post-hoc tests. The level of significance was set at  $p < 0.05$  for all tests. ( Table.1)

**Results:**

The analysis of variance (ANOVA) results showed that there was a highly statistically significant difference was observed in the sealer penetration depths among the three experimental groups ( $P < 0.0001$ ). Further analysis within each group showed that there was a significant difference in the sealer ( $P < 0.0001$ ) penetration depths at the coronal, middle, and apical thirds of the teeth.

**Discussion:**

Penetration of sealer in to the dentinal tubules has clinical significance in the long term success of root canal treatment because it can entomb the residual viable bacteria, retents the obturating core material to dentinal wall which prevents further micro leakage and reinfection of the root canal system<sup>1,25,26,27,28</sup>. It may be influenced by many factors such as the obturation technique, the

physical and chemical properties of the sealer, the anatomy of the root canal system and the smear layer produced during instrumentation<sup>3</sup>.

Previously Oksan et al<sup>29</sup> 1993, Kouvas et al<sup>30</sup>,1998, Kokkas et al<sup>28</sup> 2004, Sonu KR et al,<sup>1</sup> 2016, investigated the importance of smear layer removal. They suggested that the effective removal of smear layer may be attributable to the deeper penetration of sealer into dentinal tubules, thereby reduces micro leakage and increases the quality of the endodontic treatment outcome.

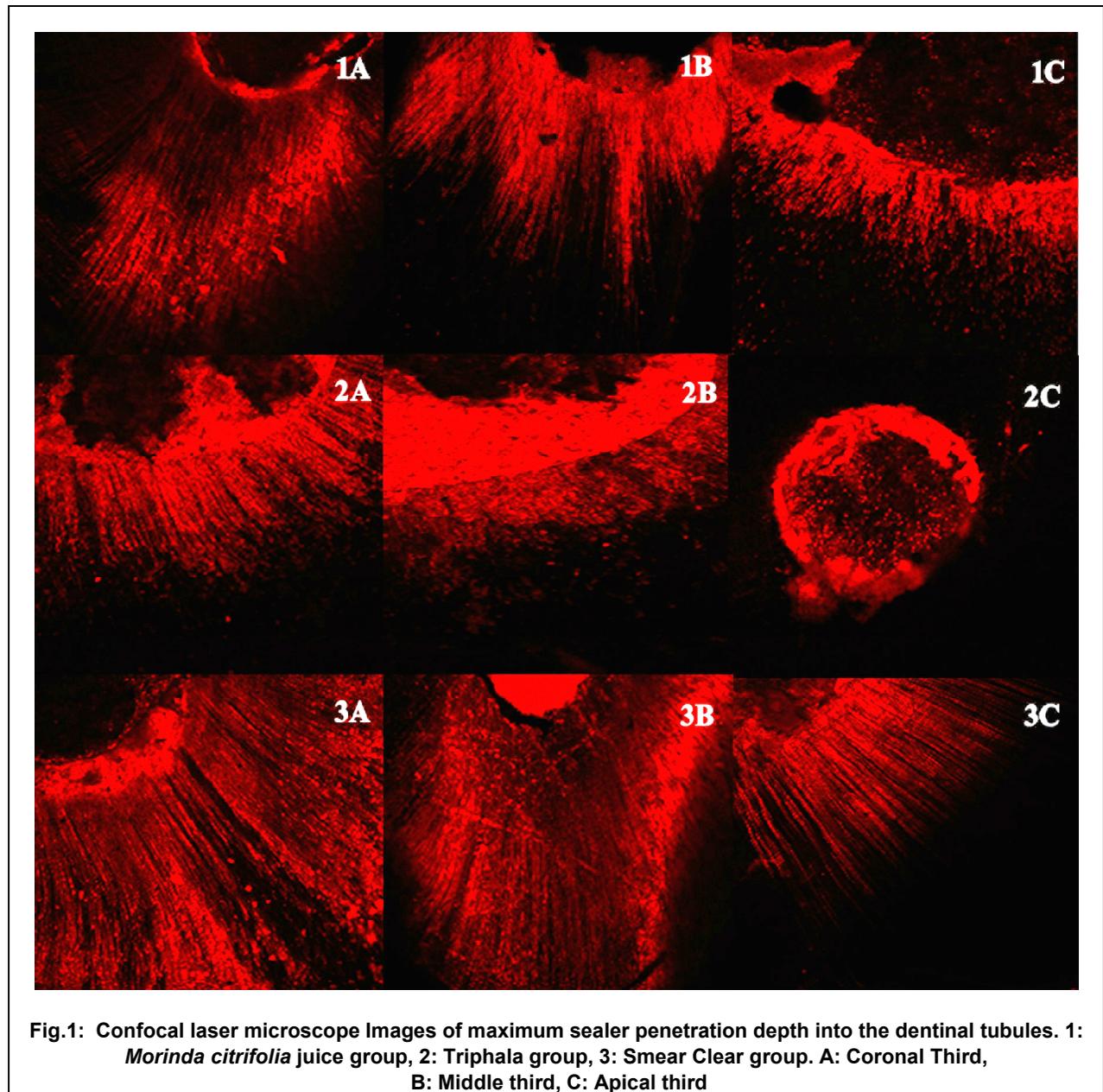
Many authors have recommended the use of a chelating agent such as 17% ethylene diamine tetraacetic acid (EDTA) solution to ensure the removal of the inorganic component, followed by a final irrigation with 1% sodium hypochlorite (NaOCl) solution to dissolve any remaining organic component of the smear layer<sup>8,9,10</sup>. However, the usage of this chemical agents has their own adverse effects<sup>11</sup>. Keeping this in mind, newer irrigating solutions for the elimination of smear layer are being constantly investigated, and in that the forefront and most promising ones are the herbal products such as *Morinda citrifolia* juice and Triphala to be used as an alternative for chemical agents due to their advantages such as easy availability, cost-effectiveness, increased shelf life and low toxicity<sup>11-14</sup>.

The current literature on *Morinda citrifolia* juice and Triphala shown that they have antimicrobial effect and smear layer removal property suggesting their potential to be used as root canal irrigants<sup>15-23</sup>. To supplement that research, the present study was focused on the effects of herbal extracts like *Morinda citrifolia* juice and Triphala on sealer penetration depth as final irrigating solutions by removing the smear layer.

**Table 1: The mean and standard deviation values of the maximum penetration depth of a sealer (in micrometers) into dentinal tubules at the coronal, middle, and apical section of all groups.**

Groups	Coronal third (Mean ± SD)	Middle third (Mean ± SD)	Apical third (Mean ± SD)	p-value
Morinda citrifolia	904.2 ± 249.7	770.9 ± 9.8	384.3 ± 10.0	<0.0001*
Triphala	720.8 ± 20.7	428.3 ± 15.0	254.86 ± 22.0	<0.0001*
Smear Clear	1142.4 ± 14.8	1065.5 ± 26.5	450.04 ± 23.8	<0.0001*
p-value	<0.0001*	<0.0001*	<0.0001*	

ANOVA test, \*P < 0.05 (Significant), \*\*p > 0.05 (Not significant)



**Fig.1: Confocal laser microscope Images of maximum sealer penetration depth into the dentinal tubules. 1: *Morinda citrifolia* juice group, 2: Triphala group, 3: Smear Clear group. A: Coronal Third, B: Middle third, C: Apical third**

The study was performed with utmost care, to avoid all the possible effects of confounding. All the extracted teeth used in the three groups are single-rooted with straight canals, teeth were without caries, cracks, root fissures, fractures or resorption, all procedures were performed by the single operator and the root lengths were standardized (16mm). Moreover, the findings of the present study were interpreted with caution as much amount of literature was not available currently.

In the present study, all the three groups showed the maximum sealer penetration depth better in the coronal and middle thirds than in the apical thirds of root canals. These results are in agreement with those of other studies<sup>3,31-35</sup>. This may be a result of the better removal of the smear layer in coronal thirds than in apical thirds of

root canals. There are more dentinal tubules in the coronal area, and the diameters of the tubules in the coronal area are larger than those in the apical area<sup>3,36</sup>. A larger canal diameter in the coronal and middle third expose the dentin to a higher volume of irrigants, allowing a better flow of the solution and hence further improving the efficiency of smear layer removal<sup>9,37,38,39</sup>.

The study findings also showed that there was a highly statistically significant difference was observed between three groups at all thirds of the teeth. The mean penetration depth of *Morinda citrifolia* juice (904.2 $\mu$ m) is higher than the Triphala group (720.8 $\mu$ m). The higher penetration depth of sealer *Morinda citrifolia* juice in the group may be because it contains some organic acids like caproic acid, ursolic acid, octoanoic acid and linoleic

acid<sup>11,16,40</sup>. The presence of these organic acids might be a probable reason for smear layer removal property of the *Morinda citrifolia* juice which in turn results in significant sealer penetration into the dentinal tubules. However, we found that the penetration depth of the Smear Clear (1142.4µm) is significantly higher compared to the *Morinda citrifolia* juice and triphala. This may be due to the presence of a widely used chelating agent (17% EDTA) with two additional surfactants namely, polyoxyethylene and iso-octylcyclohexyl ether which may result in effective smear layer removal<sup>9,41</sup>.

Other important properties that may contribute to better smear layer removal which in turn promotes more sealer penetration depth into the dentinal tubules are the surface tension and pH of the final irrigating solution. The lower the surface tension values of the irrigant, the more the sealer penetration depth because it improves dentin-wetting ability and can flow into the narrow root canals. This low surface tension value is the probable reason for the better sealer penetration in the Smear Clear (32.268 dyne/cm) followed by *Morinda citrifolia* juice (41.646 dyne/cm) and Triphala (Surface tension value of citric acid is 68.34 dyne/cm which is richer in Triphala that may aid in smear layer removal)<sup>42,43</sup>.

Lower the pH value; the more will be the sealer penetration depth. This is due to the fact that when the pH increases, the availability of calcium ions from hydroxyapatite for chelation decreases. At the same time, a greater dissociation of the acidic irrigant produces an increased attraction for calcium ions which in turn effects in better smear layer removal, which might be more likely for the better sealer penetration in the *Morinda citrifolia* juice (pH = 3.5) than a slightly alkaline pH of Triphala (3-6)<sup>19,44</sup>.

Studies conducted by Ordinola Zapata et al<sup>45</sup>, Rahul Halkai et al<sup>46</sup>, George et al<sup>47</sup>, Berber et al<sup>48</sup>, Peters et al<sup>49</sup>, Haapasalo and Orstovik<sup>50</sup> revealed that resistant bacteria like *E. faecalis* could penetrate to a depth of 400 - 600 µm into the dentinal tubules in an infected root canal system. Though the penetration depth values of both herbal extracts are significantly less than the Smear clear group, but have very clinical significance especially at coronal and middle thirds where values are greater than 400 µm so that it can entomb residual viable *E. Faecalis* present in the dentinal tubules, a most frequently isolated bacterium during endodontic retreatment<sup>51</sup>.

## CONCLUSION

Within the limits of this Invitro study, it could be concluded that,

1. Both herbal extracts such as *Morinda citrifolia* juice and Triphala showed significant sealer penetration at coronal, middle and apical thirds.
2. Comparing between herbal extracts, *Morinda citrifolia* juice showed significant sealer

penetration at coronal, middle and apical thirds than Triphala

3. Comparing with Smear Clear, these herbal extracts showed less sealer penetration at all levels. However, they have clinical significance especially at coronal and middle thirds with values greater than 400 µm.
4. The penetration depth of the sealer at the cervical and middle third of root was significantly more than those at apical third in all groups.

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