

## A Novel combination of Herbs as chelating agents: An in vitro SEM evaluation

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### Abstract

**Background:** Wide range of adverse effects associated with synthetic endodontic irrigants has gradually diverted the dental researchers to explore the possible role of herbal products with similar beneficial effects and with minimal adverse effects. The present research hypothesised that the mixture of natural herbs as irrigants (Lemon and ritha extracts) shall be effective in thoroughly disinfecting the root canal system.

**Methods and material:** Fifty five recently extracted single rooted teeth were gathered, disinfected and stored in sterile water until use. Tooth specimens were then decoronated to achieve a uniform length of 10mm. Working length was determined 1mm short of apex; cleaning and shaping was done using K3 XF rotary files. Tooth specimens were then randomly (computerised randomisation) allocated into groups as per the irrigation protocol: Group A- Distilled water. Group B- 17% EDTA. Group C- Combination of herbal extracts without agitation. Group D- Combination of herbal extracts supplemented with ultrasonic agitation. Group E- Combination of herbal extracts supplemented with hand agitation. Tooth specimens were then sectioned longitudinally and evaluated under SEM for removal of smear layer. Obtained scores were statistical analyzed using Chi-square test, ANOVA and Tukey's test.

**Results:** Group B demonstrated cleaner canal walls with statistically insignificant difference with Group D.

**Conclusion:** Experimental irrigant proves to be an effective alternative to various synthetic irrigants used in endodontic practice especially when supplemented with ultrasonic agitation.

**Keywords:** Agitation; Citrus aurantifolia; Endodontic irrigation; Root canal therapy; Sapindus mukorossi; Smear layer.

### Introduction

Mechanical instrumentation of the canal walls lead to creation of an irregular layer of debris, so called "Smear layer".<sup>1</sup> Scanning electron microscopic (SEM) evaluations reveal that the smear layer is basically made up of two zones, superficial 1-2µm thick layer comprising of organic matter and dentin particles. This superficial layer is termed as 'smear layer'; the deeper zone is termed as 'smear plug' primarily comprising of dentin chips extending up to 40µm into the dentinal tubules.<sup>2</sup> Evidence suggests that it is this smear layer which primarily acts as physical barrier and interferes with proper penetration of various disinfecting agents within the dentinal tubules and ultimately leads to failure of root canal treatment.<sup>3</sup> The presence of smear layer is believed to increase the micro leakage and boost microbial colonization.<sup>4,5</sup> The smear layer should thus be completely eradicated to ensure complete disinfection of the root canal system.

Various chemicals like 17% Ethylene diamine tetra acetic acid (EDTA), citric acid, maleic acid, tetracycline, phytic acid, etc. have been recommended as chelating agents<sup>4</sup>, amongst which 17% EDTA is the most frequently used.<sup>6,7</sup> Various herbal irrigants have been tried as an alternative to synthetic chemicals, owing to the adverse effects associated with these synthetic irrigants. Citrus aurantifolia contains citric acid as its major component and thus has been explored for its probable effect on removal of smear layer removal with mixed results.<sup>8</sup> Saponins comprise of

major component of Sapindus mukorossi which results in superior surfactant action.<sup>9</sup>

Agitation techniques play an essential role in enhancing the efficacy of root canal irrigants. These include hand agitation, sonic and ultrasonic agitation.<sup>10,11</sup> Ultrasonic agitation improves the irrigation dynamics by acting through two fundamental principles of acoustic streaming and cavitations.

Hence the current research hypothesized that the combination of herbal irrigants (Citrus aurantifolia and Sapindus mukorossi extracts) alone as well as in conjunction with passive ultrasonic or hand agitation shall be effective in removal of smear layer in comparison to the 17% EDTA solution.

### Materials and Methods

Ethical approval (SVIEC/ON/Dent/BNPG-13/D14218) was taken from the Ethics committee before commencement of the study. Sample size was determined using:

$$n=3*(3.9202*SD/d)^2;$$

Where, SD = 1.2755

$$d = 2$$

Using the aforementioned formula sample size for this in vitro study was determined to be 55.

Fifty five single rooted teeth having single canal were collected; any soft tissue remnants, calculus were removed; disinfected in 0.1% Thymol solution for 24hrs and subsequently the specimens were stored in sterile water until used. The teeth were then

decoronated below the CEJ to obtain uniform length (10 mm). The apices of all the specimens were coated with cyanoacrylate glue; after which they were embedded in putty impression material to simulate the closed apical system (Fig. 1).



**Fig. 1: a – Tooth apex sealed with cyanoacrylate glue; b – Specimen mounted in putty impression material to mimic closed root canal system**

**Preparation of experimental irrigating solution:** The experimental irrigating solution was prepared by combining the extracts of *Citrus aurantifolia* and *Sapindus mukorossi* in 1:1 ratio following the basic guidelines for preparation of extract suggested by earlier research<sup>12</sup>.

**Specimen Preparation:** Working length was calculated by subtracting 1mm from the total root length. K3™ XF rotary files were sequentially used in crown down manner to enlarge the canal till apical file size #35 and taper 6%. Tooth specimens were irrigated with constant volume (3ml) of irrigating solution using 25-G side vent needles (RC Twents, Prime Dental Products, India). The specimens were then randomly distributed into five groups using computerised randomisation technique as follows:

Group A – Negative Control (n = 5)

Distilled water was used as root canal irrigant during instrumentation as well as final rinse irrigant for 1 minute.

Group B – Positive Control (n = 5)

17% EDTA was used as root canal irrigant during instrumentation as well as final rinse for 15 minutes

Group C – Herbal irrigants without agitation (n = 15)

Combination of *Citrus aurantifolia* & *Sapindus mukorossi* extracts were used as root canal irrigant during instrumentation as well as final rinse irrigant for 15 minutes.

Group D – Herbal irrigants with passive ultrasonic agitation (n = 15)

Combination of *Citrus aurantifolia* & *Sapindus mukorossi* extracts were used as root canal irrigant during instrumentation as well as final wash irrigant for 15 minutes. Passive ultrasonic agitation was performed with the help of 21mm length stainless steel, non-cutting wire having apical size #20 and taper 00 namely Irrisafe™ files. (Acteon, France) These ultrasonic files

were activated by a piezoelectric unit (P5 Newtron™, Acteon, France) at power setting of “Blue 5” (frequency approximately 30 KHz and file tip displacement amplitude 30µm according to the manufacturer) for 10seconds.

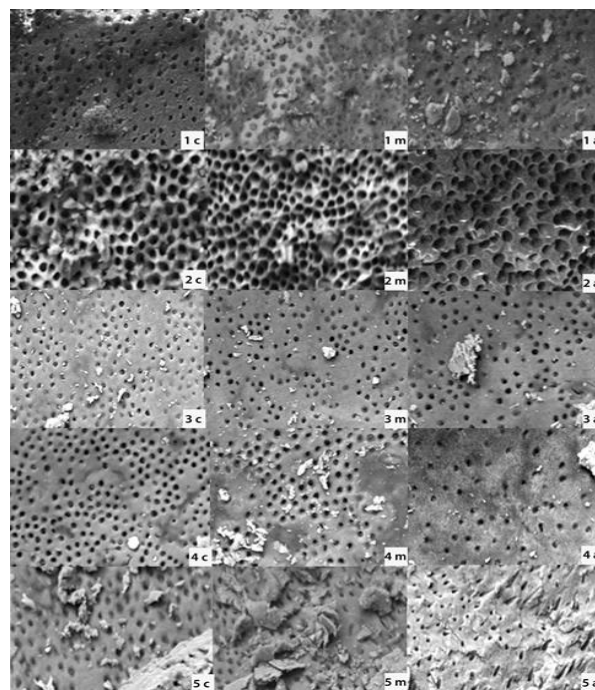
Group E – Hand agitation (n = 15)

Combination of *Citrus aurantifolia* & *Sapindus mukorossi* extracts was used as root canal irrigant during instrumentation as well as final rinse irrigant for 15 minutes. Along with this passive hand agitation was done using V Clean™ Endodontic agitator (SS White, USA) during final rinse for 4 reciprocating circular motions followed by scrubbing along the canal wall as well as during removal of the instrument.

All the specimens were then finally flushed with distilled water.

For all the groups root canal of each specimens were finally rinsed with sterile water.

**Preparation of the Specimens for scanning electron microscopic evaluation:** The tooth specimens were blot dried with the help of sterile paper point and the coronal opening of the root canals were temporarily sealed with modelling wax. The tooth specimens were sectioned the help of chisel and mallet. Three SEM photomicrographs were taken at magnification of × 1000 for evaluation of smear layer and × 1000 for debris at the centre of the coronal, middle, and apical thirds of each specimen (Fig. 1). Cleanliness was evaluated using a criteria as described by Hulsmann et al<sup>13</sup> (Table 2) and the results were tabulated.



**Fig.1: Numerical 1, 2, 3, 4 and 5– represent groups A, B, C, D and E respectively. Alphabet sc, m and a – represent coronal, middle and apical respectively**

**Table 2: Smear layer evaluation criteria**

Score	Description
1	No smear layer and all dentinal tubules open
2	A small amount of smear layer and some dentinal tubules open
3	Homogenous smear layer covering the root canal wall and only a few dentinal tubules open
4	Complete root canal wall covered by a homogeneous smear layer and no open dentinal tubules
5	Heavy homogeneous smear layer covering the complete root canal wall.

**Statistical methods employed:** The collected data was subjected to Krushkal Wallis, Post hoc tukeys test and Pearsons Chi-square test.

### Results

Pearson's Chi- Square test in the coronal third revealed that 60% of the specimens of Group B had score 1 and 40% of the specimens had score 2, indicating cleaner canal walls which was quite similar to Group D having 40% of specimens with score 1 and 60% of specimens having score 2. Group C and Group E showed similar results in the coronal third, however, the results were less efficient than Group B and Group D respectively. Group A was least effective in removal of smear layer. (Table 3)

**Table 3: Pearson's Chi-Square test for smear layer (Coronal third)**

		Group					Total
		Group-A	Group-B	Group-C	Group-D	Group-E	
Coronal	1	0	3	0	6	0	9
		.0%	60.0%	.0%	40.0%	.0%	16.4%
	2	0	2	9	9	9	29
		.0%	40.0%	60.0%	60.0%	60.0%	52.7%
	3	2	0	6	0	6	14
		40.0%	.0%	40.0%	.0%	40.0%	25.5%
4	3	0	0	0	0	3	
	60.0%	.0%	.0%	.0%	.0%	5.5%	
Total		5	5	15	15	15	55
		100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Another significant finding was that Group E performed better than Group C and group A was least effective in removal of smear layer.

### Discussion

Combination of various irrigants either simultaneously or sequentially is essential for meticulous eradication of endodontic biofilm. Available literature suggests that the alternate use of sodium hypochlorite and EDTA as a final rinse is highly effective in complete disinfection of root canal.<sup>14</sup> Although, the combination provides good disinfection; on the darker side, it may cause extensive dentinal erosion<sup>15</sup> and bears highly caustic action on the oral soft tissues. Such adverse effects of synthetic chemical irrigants have created the thrust area for the researchers to develop herbal / natural alternatives as irrigants or medicaments which are more biocompatible.

17% EDTA was opted as the sole irrigant in our test because the experimental combination had only

chelator present and no tissue solvent, hence, the inclusion of NaOCl in the irrigation regimen in conjunction with EDTA could have resulted in biased outcome. Diverse methods are suggested to improve the penetration of irrigating solutions. Addition of surfactant is one of the suggested regimens to improve the effectiveness of irrigating solutions with successful outcomes.<sup>16</sup> The pericarp extract of *Sapindus mukorossi* fruit has detergent like properties attributable to its higher saponins content. It reduces the surface tension of the solution leading to the dissolution of water-insoluble substances/ hydrocarbons. That might be the reason for cleaner canal walls in the groups containing ritha responsible for the better smear layer removal effect when used in combination with *C. aurantifolia* extract in the present research.<sup>17</sup> The results of the previous researches proved this combination to be quite effective in removing the smear layer when used in the ratio of 2:1 respectively.<sup>18</sup> However, the present study

employed the 1:1 combination ratio of the extracts which might be responsible for the differing outcome.

Also, we incorporated the final rinse with distilled water to neutralize the effect of various chemicals. Citrus aurantifolia was quite effective in removing the smear layer as evident in the scanning electron microscopic images which revealed cleaner canal walls in all the thirds of root canal<sup>13</sup>. The optimal concentration of citric acid recommended for effective removal of smear layer is 10%, whereas the available concentration of the citric acid in the study was 6-8%<sup>19</sup> in the prepared extract which further might have been diluted during formulation of the experimental irrigant. This may be the reason for its inferior performance as compared to 17% EDTA group. Sapindus mukorossi being surfactant might only be required in smaller proportion for increasing the wettability of the dentinal surface and citrus aurantifolia being used as chelating agent shall be needed in the greater amount. Thus, 1:1 combination was not as effective due to the substantially reduced amount of available citric acid in the experimental irrigant. Another reason for reduced effectiveness of the experimental irrigant might be the presence of excipients.

To mimic this clinical scenario the apices of the teeth were sealed with help of cyanoacrylate glue and embedded in putty impression material to simulate closed end system and formation of vapour lock.<sup>20</sup>

Various agitation techniques lead to pressure changes and subsequent elimination of the vapour lock which provides replenishment of irrigating solution, thus, improving the cleaning efficacy of the irrigant.<sup>21</sup> Recently various brushes have also been introduced for manual agitation, which are claimed to be effective in cleaning canal walls;<sup>22</sup> hence, V clean™ endodontic agitator was selected. Manual agitation involves the use of syringes with needles having various gauges, vibrated up and down within the root canal space.

The use of ultrasonic energy supplemented with conventional irrigation protocol has shown promising results in the recent researches,<sup>23</sup> also proven true in our in vitro study. This might be attributed to the booster effect in the form of acoustic streaming and cavitations. Acoustic streaming leads to formation of waves around the oscillating file; cavitations implies formation and disruption of bubbles when ultrasonic files come in contact with fluid/ irrigant. Passive ultrasonic irrigation induce powerful acoustic micro streaming within the canal<sup>24</sup>. Irrisafe™ files were chosen for the study because they are composed of non-cutting stainless steel wire, which does not causes any harm to the normal root canal anatomy and are relatively safe. 10 seconds of ultrasonic activation was selected as recommended by the manufacturer; however, recent research reveals that passive ultrasonic activation is effective with the durations of at least 20-30 seconds, therefore, this probably was one of the shortcomings of the study which resulted in inferior performance.<sup>25</sup>

Scanning electron microscope was opted in this study as it is one of the most recommended and commonly available tool for evaluation of smear layer and it provides images at much higher magnification.<sup>26</sup> 0.1% Thymol for disinfection of tooth specimens as it provides adequate disinfection without damaging /altering the properties of dental tissues.<sup>27</sup>

## Conclusion

Experimental irrigant of natural herbs proved to be an effective alternative to various synthetic irrigants used in endodontic practice especially when supplemented with ultrasonic agitation. Further researches shall be carried out after optimising the ratio as well as concentration of herbal products based on the results of this research which might not only improve the cleaning efficacy but also minimise the adverse effects associated with synthetic endodontic irrigants.

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