

## AN IN VITRO COMPARATIVE EVALUATION OF EFFICACY OF DISINFECTING ABILITY OF GARLIC OIL (LASUNA), CLOVE LEAF OIL (LAVANG) AND AUTOCLAVING ON ENDODONTIC K FILES TESTED AGAINST ENTEROCOCCUS FAECALIS

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### ABSTRACT

**Title:** An in vitro comparative evaluation of efficacy of disinfecting ability of garlic oil (*Lasuna*), clover leaf oil (*Lavang*) and autoclaving on endodontic K files tested against *Enterococcus faecalis*. **Background:** The most important step in endodontic treatment is the effective disinfection of cleaning and shaping instruments. Although, autoclaving is the gold standard, but its effects on temper and cutting efficiency of these instruments dilutes its usage. Hence, an attempt is made using garlic oil and clove oil extracts to validate their use in disinfection of endodontic instruments. **Aim:** To comparatively evaluate efficacy of disinfecting ability of garlic oil, clove oil and autoclaving on endodontic K files tested against *Enterococcus faecalis*. **Design:** 30 K files were exposed to the test organism and then were treated with one of the three methods of disinfection. **Results:** All the three methods including autoclaving, garlic oil and clove oil showed considerable effectiveness against the test organism. **Conclusion:** The test chemicals could serve to be valuable dis-infective agents or adjuncts to autoclaving for timely disinfection of endodontic instruments.

**Keywords:** Autoclaving, Clove oil, Disinfection, Endodontic instruments,

### INTRODUCTION

The human oral cavity contains a wide variety of micro-organisms both commensals as well as pathogenic organisms<sup>1</sup>. With such a high of number of bacteria in the oral cavity it is almost impossible to maintain the disinfection in the working area to provide a sterile field for dental treatment. Although the use of rubber dam guarantees a better field, but in cases of paediatric dental patients this consideration is to be given a considerable thought.

Endodontic treatment procedures are second to no routine dental procedure

in terms of infection being dealt with during the procedure. It is during such procedures that the risk of cross infection looms large. Of paramount importance is the need to maintain absolute sterility in the use of endodontic instruments such as files which are frequently used in the dental operatory to routinely perform endodontic treatment. It has been found that these instruments can even be supplied by the manufacturer with a high microbial containing obviating the need to sterilize them even before their first use<sup>2</sup>. The micro-organisms isolated from the root canals of

infected teeth can have serious implications on oral and systemic health of an individual. The endodontic instruments carry the greatest risk of cross infection between individuals due to an extremely high bacterial load and high frequency of use in general dental practice and even in speciality settings including endodontic and pediatric dental practice.

Although various methods have been used to sterilize or disinfect endodontic instruments prior to their next use, every method has its shortcomings. These include the impractical attempt at autoclaving endodontic instruments after each use and the time taken and tediousness of the entire procedure<sup>3</sup>. The effect on the mechanical properties of these instruments on using autoclaving or chemical agents is discouraging<sup>4,5</sup>. Also some methods provide an insufficient level of disinfection for its justified use in a busy practice set up<sup>6</sup>. Finally some methods such as lasers are too expensive an option to pursue its use in sterilization of endodontic instruments. All these considerations can question the thought of endodontic instruments being reusable<sup>7</sup>. Thus, though autoclaving is the gold standard in sterilization of endodontic instruments, the search for an effective time bound practical approach in sterilization/ disinfection of endodontic instruments is far from over, thus implying the need for research in this area.

Of all the endodontically isolated bacteria, it is of particular importance to mention *Enterococcus faecalis*. This organism has been frequently isolated in both primary and persistent endodontic infections in both sets of dentitions. The relatively high frequency of isolation of this bacteria together with its ability to thrive under various conditions since it is facultatively anaerobic giving it the ability

to thrive under aerobic and anaerobic conditions, makes it a difficult bacteria to eradicate<sup>8</sup>. Also, *E. faecalis* in numerous studies has shown to be resistant to various chemicals routinely used in endodontic treatments<sup>9</sup>.

Since, the use of herbal products is on the rise in all avenues of medical and dental setup due to the reliable assurance of inability to develop resistance against these products. The use of these products in endodontic practice is a viable option to consider. *Eugenia carophyllus* (Latin) commonly referred as clove and *Allium sativum* (Latin) commonly referred to as garlic have many health benefits as documented throughout literature. Many studies have outlined the antimicrobial effects of clove (*Lavang*) and garlic (*Lasuna*) extracts against a wide variety of bacteria<sup>10,11</sup>. Thus, the aim of the present study was to comparatively evaluate the efficacy of disinfecting ability of garlic oil, clove oil and autoclaving on endodontic K files tested against *Enterococcus faecalis*.

## METHODS

Endodontic files were used in the present study to test the efficacy of the three disinfection methods namely garlic oil extract, clove oil extract and autoclaving. Thirty #10 K endodontic hand files (Kendo CC Cord, VDW GmbH, Bayerwaldstr, 15 81737 Munich, Germany) 21 mm in length were used in the present study. These files were used in study with the concept that # 10 files are the first endodontic instruments to be inserted into the canal that encounter maximum infection within the canals. All of these K files were pre-sterilized by standard autoclaving protocol at 121°C at 15 lb pressure for 30 minutes. *Enterococcus faecalis* standard strain ATCC (American Type Culture Collection) 21292 was used to infect the en-

endodontic K files. *Enterococcus faecalis* broth was prepared according to standard microbiological protocols and incubated overnight at 37°C for 24 hours. After the broth was obtained the presterilized files were infected by inserting them into a sterile container containing the broth for 30 mins (Figure 1).

The infected files were transferred to a sterile petri dish and incubated at 37°C for 30 mins for drying and ensuring fixation of the microbes on the files. The test chemicals included:

Group I: K Files treated with 0.5% w/w garlic oil extract (Ranbaxy Garlic Pearls) (Figure 2a).

Group II: K Files treated with pure clove leaf oil (Dr. Jain's Forest Herbals Private Limited, Andheri, Mumbai, India) ) (Figure 2b).

Group III: K files treated with autoclaving at 121°C at 15 lb pressure for 30 minutes (Figure 2c).

The endodontic files in group I and II were placed in sterile container with the test chemicals for 5 minutes and group III was autoclaved as described.

To check the disinfecting ability of the test chemicals, three methods were employed. These methods included:

1. Turbidity Method
2. Blood agar plate streaking method
3. Microscopic examination

In the turbidity method, all the infected files were placed in peptone water overnight and checked for turbidity suggesting growth of bacteria (Figure 3a 3b 3c). In case of blood agar streaking plate method, the blood agar plate was divided in such a way that the central portion contained the positive control of standard strain of *E. faecalis* to check against the growth of bacteria after disinfection with test chemicals (Figure 4a 4b 4c). In case of

microscopic examination, the standard strain was stained using Gram's stain and checked in respect to all disinfected files by all the three methods (Figure 5a 5b 5c).

## STATISTICS

All the data was tabulated and tested statistically using one way ANOVA test and Chi square test using SPSS 18 software.

## RESULTS

No statistically significant difference was seen with all the three test methods used for disinfection of hand K endodontic files. All the endodontic instruments used in the study were free of *Enterococcus faecalis* as determined by all the three tests including turbidity test, blood agar plate streaking method and microscopic examination method. The turbidity tests in all cases did not show any turbidity in peptone water. The blood agar plates showed peculiar growth of *E. faecalis* only on positive streaking and no growth on all other test specimens. The microscopic method showed Gram positive cocci in positive control and no bacteria in all test specimens.

## DISCUSSION

Prevention of transmission of infection from one person to another is an important consideration in clinical endodontics. This infection transmission is a curse in endodontic treatment because of all the factors involved in the success of treatment, the most important is asepsis. Legal considerations through use of contaminated instruments further complicate the picture. This is because endodontic instruments play an important role in transmission of various infections including HIV, Hepatitis, Creutzfeld – Jacob's disease, etc. which pose a significant health hazard<sup>12</sup>. Thus, control of this infection is important from the point of view of opera-

tor and patient safely and success of root canal therapy.

*Enterococcus faecalis* is an important micro-organism in the view of its potential to cause significantly resistance pulpal and periapical infection<sup>8</sup>. It is an obligate anaerobe which thrives well under reduced oxygen tension and is significantly resistant to commonly used intracanal medicaments and irrigating regimens as outlined by various studies<sup>9</sup>. Thus, *Enterococcus faecalis* was used in the present study to determine the disinfecting potential of the test chemicals.

Garlic oil has proved its use in infection control as outlined by numerous studies<sup>[13,14]</sup>. In dental scenario, too, various studies have shown its antibacterial potential<sup>10,15</sup>. Tsao and colleagues found that *Pseudomonas* and *Klebsiella* that were resistant to various drugs including Ceftazidime, Gentamycin, Imipenem, Meropenem were sensitive to garlic oil<sup>13</sup>. However, this was at higher concentration than its individual components diallyl monosulphide, diallyl disulphide, diallyl trisulphide and diallyl tetrasulphide in the same order. Fani and coworkers found that multidrug resistant *Streptococcus mutans* including resistance to Penicillin, Tetracycline, Ceftriaxone and Imipenem was significantly sensitive to garlic oil at MIC of 4 – 32 micrograms/ ml<sup>10</sup>. Bakri et al found that many oral bacteria were sensitive to garlic oil with most sensitive ones including *Streptococcus mutans* and *Porphyromonas gingivalis*<sup>15</sup>. Ruddock testified the susceptibility of *Enterococci* to garlic oil as in the present study along with *Neisseria* and *Staphylococci* also being susceptible<sup>14</sup>.

In the present study, garlic oil was found to thoroughly inhibit the persistence of *Enterococcus faecalis* on the endodontic

instruments as similar to autoclaving against the same micro-organism. The mechanism of action of garlic oil is related to the presence of its active component as allicin which is produced by the action of allinase enzyme on alliin. This allicin reacts with thiol groups of enzymes in susceptible bacteria to form S-allylmercaptocysteine thus causing their inhibition. This disrupts the metabolic machinery of the bacteria and causing cell lysis.

Clove oil has been studied extensively since past many centuries. Clove oil is an important component of eugenol which is regularly used in dental practice. Various studies have shown the antibacterial effect of clove oil against various oral and systemic pathogens<sup>11,16,17</sup>. Clove oil has been shown to be effective against *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus* (bacteria), *Candida albicans* and *Saccharomyces cerevisiae* (yeast), with *S. mutans* being the most sensitive<sup>[11]</sup>. Nascimento et al proved the efficacy of clove oil against *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *Candida albicans* when they tested other herbs such as basil, guava, rosemary, sage, pomegranate, yarrow, jambolan, thyme and lemon balm with clove oil being most effective<sup>[16]</sup>. Joseph and Sujatha tested the effectiveness of clove oil against food pathogens and proved its effectiveness against *Enterococcus faecalis*<sup>17</sup>. The only study showing the resistance of *Enterococcus faecalis* against clove oil that was found was one by Bayoub et al where amongst 6 species of bacteria selected for study only *Enterococcus* was resistant<sup>18</sup>. However in their study too it was one of the better herbs as tested against 12 other herbs and was second only to wild thyme.

The mechanism of action of clove oil includes the sensitization of microbial

cell membrane by the phenols and other polycyclic alcohols which affect the phospholipid layer of involved bacteria. The disruption of cell membrane ensues leading to leakage of cellular constituents and finally bacterial cell death.

Autoclaving has been long recommended as an ideal method of sterilization and is the true gold standard for rendering the involved surfaces bacteria and spore free devoid of any chance of infection transmission. The mechanism of action by which, autoclaving works will include denaturation and coagulation of bacterial proteins resulting in cell lysis<sup>19</sup>. However, many authors have scrutinized the use of autoclaving for sterilization procedures because of its effects on the mechanical properties. The present study thus concludes that the disinfection of endodontic instruments can be carried out as effectively as autoclaving by clove oil and garlic oil respectively. However, larger sample size and testing against a wider spectrum of bacteria can help validate and justify the use of these materials as chairside disinfecting agents. Nonetheless, the advantages of these methods include less chairside time, easy process and minimal possibility of developing resistance. Herbs and herbal extracts are a natural and harmless way of controlling infection. Various herbal products can be effectively used in day to day dental practice to prevent cross contamination. Clove oil and garlic oil have excellent antibacterial properties which can have wide applications in endodontic infection control.

## CONCLUSION

Clove oil and garlic oil are strong inhibitors of microbial activity such as demonstrated in the study against a very resilient micro-organism in the form of *Enterococcus faecalis* and thus its poten-

tial as a mainline or adjunct disinfection method for endodontic instruments should be considered.

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- ### FIGURE LEGENDS
1. Figure 1: Endodontic files infected by placing in *Enterococcus faecalis* broth for 30 minutes.
  2. Figure 2a: Disinfection of *E. faecalis* infected K files for 0.5% w/w Garlic oil  
Figure 2b: Disinfection of *E. faecalis* infected K files for Pure Clove oil  
Figure 2c: Disinfection of *E. faecalis* infected K files after autoclaving
  3. Figure 3a: Disinfection of K files checked by turbidity method for control  
Figure 3b: Disinfection of K files checked by turbidity method 0.5% w/w Garlic oil  
Figure 3c: Disinfection of K files checked by turbidity method Pure Clove Oil  
Figure 3d: Disinfection of K files checked by turbidity method after autoclaving
  4. Figure 4a: Disinfection of K files checked by blood agar plate streaking method 0.5% w/w Garlic oil

Figure 4b: Disinfection of K files checked by blood agar plate method for Pure Clove Oil

Figure 4c: Disinfection of K files checked by agar plate method after autoclaving

5. Figure 5a: : Disinfection of K files checked by microscopic examination method for control group

Figure 5b Disinfection of K files checked by microscopic examination method for 0.5% w/w Garlic oil

Figure 5c: Disinfection of K files checked by microscopic examination method for Pure Clove Oil

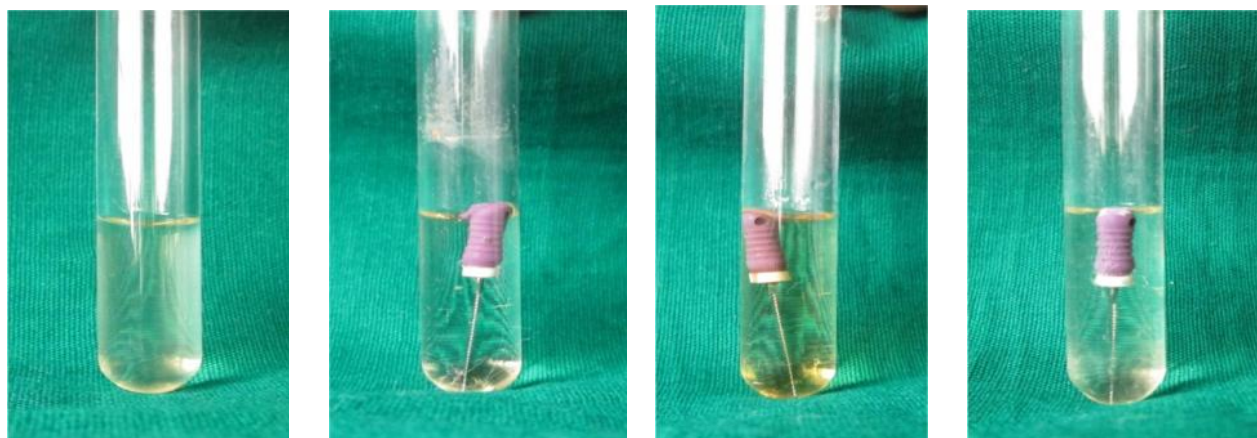
Figure 5d: Disinfection of K files checked by microscopic examination method after autoclaving



**Figure 1: Endodontic files infected by placing in Enterococcus faecalis broth for 30 minutes.**



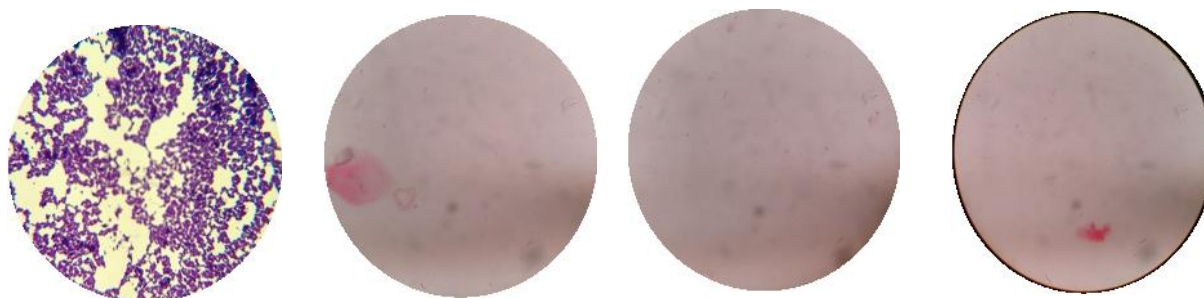
**Fig. 2 a, 2b, 2c: Disinfection of E. faecalis infected K files (a) 0.5% w/w Garlic oil, (b) Pure Clove leaf oil, and (c) Autoclaving**



**Fig.3a, 3b, 3c, 3d: Disinfection of K files checked by turbidity method: (a) Control, (b) 0.5% w/w Garlic oil, (c) Pure Clove leaf oil, and (d) Autoclaving**



**Fig.4a, 4b, 4c: Disinfection of K files checked by blood agar plate streaking method: (a) 0.5% w/w Garlic oil, (b) Pure Clove leaf oil, and (c) Autoclaving**



**Fig.5a, 5b, 5c, 5d: Disinfection of K files checked by microscopic examination method: (a) Control, (b) 0.5% w/w Garlic oil, (c) Pure Clove leaf oil, and (d) Autoclaving**

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