Evaluation of Antibacterial Properties of Organic Gutta-percha Solvents and Synthetic Solvents Against *Enterococcus faecalis*

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**ABSTRACT**

**Background:** The use of solvents is necessary to remove remnants of filling materials within dentinal tubules to allow penetration of irrigating solution within the tubules. **Aim and Objectives:** We aimed at determining the antibacterial effect of various gutta-percha (GP) solvents against *Enterococcus faecalis* (E. faecalis).

**Materials and Methods:** An *in vitro* study was conducted by measuring the zone of inhibition using the disk diffusion method. The test organism used for the study was *E. faecalis*, and the solvents were divided into five groups: eucalyptus oil, chloroform, turpentine oil, xylene, and orange oil. About 500 μL of the suspension was spread over the agar plates, and the empty sterilized disks were impregnated with 10 μL of pure essential oils (EOs). The inoculated plates were incubated at 37°C for 18 to 24h. The antimicrobial activity of each solvent was expressed and measured in terms of the mean diameter of the zone of inhibition (in mm) produced by each solvent at the end of the incubation period. ANOVA was used for intergroup comparison. The P-value of <0.05 was considered significant.

**Results:** The mean zone of inhibition for *E. faecalis* was 24.00 ± 1.21 for eucalyptus oil, 16.30 ± 0.92 for chloroform, 26.50 ± 1.24 for turpentine oil, 13.70 ± 1.26 for xylene, and 19.80 ± 1.32 for orange oil. The difference between the groups was statistically significant (*P* < 0.001).

**Conclusion:** This study demonstrated that the use of turpentine oil during endodontic retreatment significantly reduced the levels of *E. faecalis* as compared with other solvents.

**Keywords:** Antimicrobial property, endodontic retreatment, *Enterococcus faecalis*, essential oil, gutta-percha

**INTRODUCTION**

Successful endodontic treatment is the aim of every endodontist but despite adopting stringent methodology and treatment standards, recurrence of symptoms occurs in a few number of endodontically treated teeth leading to a failure of endodontic treatment. The most common reasons responsible for the failure of endodontic treatment are the poor chemomechanical preparation and persistence of microorganisms in the apical part of the root-filled teeth. The persistence of microorganisms even after the root canal treatment leads to the occurrence of secondary root canal infection. The involvement of microflora is different in primary and secondary root canal infection. *Enterococcus faecalis* (E. faecalis), a facultative anaerobic Gram-positive coccus, is more prevalent in persistent or secondary root canal infection (77%) as compared with primary endodontic infection (40%).[1,2] The procedure of retreatment includes removal of the old obturation, disinfection of the root canal to clean any remnants of contaminated tissue or bacteria responsible for the endodontic infection.
failure, and finally rerestoration of the canals. Schilder introduced the term “retreatodontics” to denote the branch of endodontics responsible for retreatment of an endodontic treatment in either a conservative or surgical way. Several challenges have to be faced by the endodontist during retreatment of the root canal treated tooth. The most common of these is the complete removal of Gutta Percha (GP) from the obturated canal, as it is a time-consuming and tedious procedure that carries a greater risk of iatrogenic injury in the form of microbial infection. The methods employed for complete removal of GP include the use of organic solvents, lasers, thermal methods and mechanical instrumentation using rotary files, ultrasonic instruments, and hand files. A combination of methods should be applied to achieve safe and potentially complete removal of the filling materials from the root canal system.

Organic solvents are a chemical class of compounds that are applied during retreatment to decrease the resistance of obturating materials inside the root canal and to ease in the removal of the material. According to Stabholz and Friedman, the use of solvents is necessary to remove remnants of filling materials within dentinal tubules to allow penetration of irrigating solution and intracanal medication within the tubules. An ideal organic solvent should possess antibacterial properties, be less toxic, clinically safe, and efficient enough to dissolve GP. The antibacterial property of the GP solvent is required for the complete disinfection of the root canal after the removal of the GP. The need to disinfect such canals even during the procedure of GP removal is a vital part in endodontic treatment, as it helps to reduce the duration of the treatment. Varieties of synthetic GP solvents are available in the dental industry. The two most commonly used GP solvents are chloroform and xylene but nowadays their use is limited because of their carcinogenic potential and cytotoxicity, respectively. Hence, alternative GP solvents should be searched; they have a similar efficiency to chloroform and xylene and at the same time should have antibacterial properties, be safe and economical.

An excellent alternative to synthetic GP solvents is the use of essential oils as they are able to dissolve GP cones and endodontic sealers. Essential oils (EOs), a mixture of volatile constituents are biosynthesized by plants. According to the previous literature, essential oils are contained by approximately 3000 plant species, of which 300 plant species are used for the commercial purpose. Some of them were reported of GP solvents such as eucalyptus oil, turpentine oil, and orange oil. Pecora et al. commented that the clinical efficiency of orange oil as a GP solvent is the same as xylene but without the adverse effects of xylene.

Since the microorganism E. faecalis is the most resistant bacteria and is significantly found in secondary root canal infection, its complete eradication with the help of a GP solvent possessing antibacterial properties is the most crucial step of retreatment. Hence, this study was conducted with an aim to determine the antibacterial effect of various GP solvents against E. faecalis.

**Materials and Methods**

An *in vitro* study was conducted in the Department of Endodontics of a dental college.

**Test bacterial isolates**

The test organism used for the study E. faecalis (ATCC 29212) was provided by the pathology laboratory.

**Tested organic solvents**

Five GP solvents were selected to test their antimicrobial activity against E. faecalis. They were divided into five groups: Group A (Eucalyptus Oil), Group B (chloroform), Group C (turpentine oil), Group D (Xylene), and Group E (Orange oil).

**Antimicrobial activities of solvents using disk diffusion method**

An overnight culture for each E. faecalis strain was adjusted to 0.5 McFarland standard (10^8 CFU/ml). About 500 μL of the suspension was spread over the agar plates containing Mueller Hinton Agar (MHA). The experiment was performed and repeated under strict aseptic conditions. The empty sterilized disks (Whatman 6mm in diameter) were impregnated with 10 μL of pure EOs and placed on the agar surface. The inoculated plates were incubated at 37°C for 18 to 24h. The diameter of the zone of inhibition (in mm) produced by each solvent at the end of the incubation period was measured. Each assay was repeated two times.

**Statistical analysis**

The results were tabulated and statistically analyzed by using SPSS version 23.0 (SPSS Inc, Chicago, IL, USA). One-way ANOVA was used to compare between the five groups. *P*-value of <0.05 was considered significant.

**Results**

The mean zone of inhibition for E. faecalis was 24.00 ± 1.21 for eucalyptus oil, 16.30 ± 0.92 for chloroform, 26.30 ± 1.04 for turpentine oil, 13.70 ± 1.26 for xylene, and 19.80 ± 1.32 for orange oil. This difference was statistically significantly different among groups, as tested by ANOVA (*P* < 0.001) [Figure 1, Table 1].
**DISCUSSION**

Organic solvents have been used for a long time for the removal of GP during retreatment of the root canal. A number of synthetic and plant-derived organic solvents are available; however, the choice of an ideal solvent for endodontic retreatment requires a balance between the level of clinical safety, toxicity, and chemical capacity to dissolve GP and when the filling material is unknown, it is very important to have different kinds of solvents at disposal, to use the most effective one. In this study, we have compared the antimicrobial effect of EOs and synthetic GP solvents against *E. faecalis*.

We found that turpentine oil has the maximum effectiveness against Gram-positive *E. faecalis* followed by eucalyptus oil, orange oil, chloroform, and xylene. The findings of Subbiya A *et al.* are almost similar to our study and found greater efficacy of EO (orange oil) over synthetic solvent. They conducted an agar well diffusion assay, which revealed that the orange oil-based solvent (RC Solve) exhibited a greater antibacterial effect than Endosolv-E (tetrachloroethylene based) and xylene against *E. faecalis* ATCC 29212 (*P* = 0.000). In their study, Hunter *et al.* showed that eucalyptus oil and orange oil were statistically similar to chloroform (*P* > 0.05). In contrast to our study, several studies found greater antibacterial efficacy of synthetic solvents. A study conducted by Magalhães *et al.* found that xylene is the most effective for GP dissolution as compared with chloroform, eucalyptol, and orange oil. Yadav *et al.* also concluded that xylene was the more effective solvent followed by the EOs (eucalyptus and orange oil) and distilled water, being the least effective. Gomes *et al.* demonstrated that xylene and chloroform had the highest potential of GP dissolution than that of eucalyptol and orange oil (*P* < 0.01). Oyama *et al.* found that xylene is more effective in dissolving GP as compared with other commonly used solvents.

Synthetic organic solvents possess a major capacity of GP dissolution but they also carry various side effects. Chloroform is an effective organic solvent used for softening GP during endodontic retreatment. Its efficacy as a root canal solvent has been demonstrated in many previous studies. An *in vitro* study conducted by Edgar *et al.* concluded that the use of chloroform during endodontic retreatment significantly reduced intracanal levels of cultivatable *E. faecalis*. According to Wourms *et al.*, chloroform has low cost, better odor, easy availability, and compatibility with zinc oxide-eugenol-based root canal sealers. Nowadays, the use of chloroform is limited as it was banned by the U.S. Food and Drug Administration in 1976 due to its carcinogenic potential and toxicity to the tissues. Therefore, alternatives of chloroform such as halothane, methyl chloroform, ether, and xylene were searched for softening of filling materials.

Another synthetic GP solvent used in this study is xylene. Xylene (dimethylbenzene), an aromatic

![Figure 1: Zone of inhibition for *E. faecalis*](image)

**Table 1: Assessment of antibacterial activity of the solvents against *E. faecalis***

| Bacterial strain       | Group | Solvent  | n  | Mean diameter (mm) | SD   | *P*-value (ANOVA) |
|------------------------|-------|----------|----|--------------------|------|-------------------|
| *Enterococcus faecalis* (ATCC 29212) | Group A | Eucalyptus oil | 20 | 24.00 | 1.21 | <0.001* |
|                        | Group B | Chloroform | 20 | 16.30 | 0.92 |
|                        | Group C | Turpentine oil | 20 | 26.50 | 1.24 |
|                        | Group D | Xylene    | 20 | 13.70 | 1.26 |
|                        | Group E | Orange oil | 20 | 19.80 | 1.32 |

*Bold value signifies statistically significant*
compound, is the most commonly used solvent for removing the root canal obturating materials. Its use is limited due to cytotoxic properties, but the toxicity of xylene is less as compared with benzene and chloroform. Mushthaq et al. stated that xylene, refined orange oil, and tetrachloroethylene can be used for softening GP/resilon during retreatment with various techniques and xylene was found to be the best solvent for both GP and resilon. Magalhaes et al. stated that xylene, refined orange oil, and tetrachloroethylene can be used for softening GP/resilon during retreatment with various techniques and xylene was found to be the best solvent for both GP and resilon.

In our study, turpentine oil was found to be the most effective against Gram-positive Enterococcus faecalis. Turpentine oil has volatile constituents, phenolics (thymol, carvacrol, and eugenol) and oxygenated monoterpenes (α-terpineol, terpinen-4-ol, and linalool). The higher percentages of limonene+β-phellandrene and β-pinene of turpentine oil are responsible for their strong antimicrobial potency as well as for a wide spectrum of antibacterial activity.

Eucalyptus oil is obtained from the leaves of Eucalyptus globulus, which is a plant of the family Myrtaceae. The major constituent is oxygenated monoterpenes (1,8-cineole)(87.32%), and monoterpenes hydrocarbons (12.45%) are present in a minute amount. Most of the antimicrobial activity and anti-inflammatory property of this oil is due to the presence of 1,8-cineole. Eucalyptol oil does not effectively dissolve GP at room temperature, and it has to be heated to act relatively fast. Hence, it is not widely used.

The third EO used in this study is orange oil. Orange oil is an extract of the peel of sweet orange fruit (Citrus sinensis). It is an excellent alternative to synthetic solvents. Orange oil comprises d-limonene (90%), aliphatic hydrocarbon alcohols, and aldehydes such as octana. The probable mechanism of action responsible for its antibacterial effect is through alteration in the permeability of the bacterial cell membrane, cell homeostasis, and reduced ATP synthesis. There is no evidence of carcinogenicity or genotoxicity of orange oil in the literature. Orange oil is an efficient solvent that is clinically safe, less cytotoxic, and more biocompatible; it has a better dissolution capacity and pleasant smell as compared with chloroform.

Hence, it can serve as a replacement for chloroform and xylene for dissolving GP and removing endodontic sealers in cases of retreatment.

More and more natural products with a wide biological activity should be searched, as they have immense potential in treating multidrug-resistant bacteria. However, it is very important to study the interaction of EOs and their constituents in vivo to know their efficacy, as well as toxicity. Therefore, further clinical trials are required in this field to ascertain their use in dentistry.

CONCLUSION

Owing to the limitations of the in vitro study, we concluded that turpentine oil has the maximum effectiveness against Gram-positive Enterococcus faecalis followed by eucalyptus oil, orange oil, chloroform, and xylene. The current study provides additional support to the already available data to use EOs against various strains of bacteria.

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CONFLICTS OF INTEREST
There are no conflicts of interest.

AUTHORS’ CONTRIBUTIONS
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Not applicable.

DATA AVAILABILITY STATEMENT
Not applicable.

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