Original Article

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*

Received : 03-12-20
Revised : 16-12-21
Accepted : 23-01-21
Published : 15-04-21

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

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Access this article online

Quick Response Code:

Website: www.jispcd.org

DOI: 10.4103/jispcd.JISPCD_422_20

How to cite this article: Maria R, Dutta SD, Thete SG, AlAttas MH. Evaluation of antibacterial properties of organic gutta-percha solvents and synthetic solvents against *Enterococcus faecalis*. J Int Soc Prevent Communit Dent 2021;11:179-83.

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.[3] 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,[4] 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.[5] 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.[6,7] 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.[8] Essential oils (EOs), a mixture of volatile constituents are biosynthesized by plants.[9] 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.[10,11] Some of them were reported of GP solvents such as eucalyptus oil, turpentine oil, and orange oil.[12] Pecora *et al*.[12] 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 6 mm 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, 26.50 ± 1.24 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 dissolute 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).[13] In their study, Hunter *et al.* showed that eucalyptus oil and orange oil were statistically similar to chloroform (P > 0.05).[14] In contrast to our study, several studies found greater antibacterial efficacy of synthetic solvents. A study conducted by Magalhães *et al.*[5] found that xylene is the most effective for GP dissolution as compared with chloroform, eucalyptol, and orange oil.[7] Yadav *et al*.[15] 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).[16] Oyama *et al*.[17] found that xylene is more effective in dissolving GP as compared with other commonly used solvents.

Figure 1: Zone of inhibition for *E. faecalis*

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.[18,19] 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*.[20] According to Wourms *et al.*,[7] 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.[21-23] 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

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. Mushtaq *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.[24] Magalhaes *et al*.,[5] Yadav *et al*.,[15] and Oyama *et al*.[16] also also found that xylene is the most effective GP solvent as compared with others.

In recent years, there has been an increasing interest related to the use of natural products even in the field of medicine and dentistry. One of such natural products is EOs obtained from various species of plants. The use of EOs is widely prevalent because of their efficiency, antimicrobial activity, biocompatibility, nontoxicity, and noncarcinogenicity. Owing to the adverse effects of synthetic solvents and growing use of EOs in endodontics, three EOs, turpentine oil, eucalyptus oil, and orange oil, were evaluated in this study for their antibacterial efficacy against *E. faecalis*. The mechanisms of action of EOs depend on their chemical composition. The main component of EO is terpenoids, which, according to Maguna *et al.*, exerts bactericidal action by increasing the membrane permeability and disrupting membrane stability.[25] It was also found in a previous study that the interactions between various volatile constituents of EO produce a synergistic effect that may also enhance the antimicrobial activity.[26]

In our study, turpentine oil was found to be the most effective against Gram-positive *E. 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.[26]

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.[5,27] 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.[28]

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.[29] 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.[28,30,31] There is no evidence of carcinogenicity or genotoxicity of orange oil in the literature.[32] 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.[5,6,17,33,34] 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 *E. 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.

Acknowledgment

Not applicable.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Authors' contributions

Not applicable.

Ethical policy and institutional review board statement

Not applicable.

Patient declaration of consent

Not applicable.

Data availability statement

Not applicable.

References

1. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.

2. Sedgley C, Buck G, Appelbe O. Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR. J Endod 2006;32:104-9.
3. Schilder H. Message from our President: Passage. J Endod 1986;12:177-8.
4. Stabholz A, Friedman S. Endodontic retreatment—case selection and technique. Part 2: Treatment planning for retreatment. J Endod 1988;14:607-14.
5. Magalhães BS, Johann JE, Lund RG, Martos J, Del Pino FA. Dissolving efficacy of some organic solvents on gutta-percha. Braz Oral Res 2007;21:303-7.
6. Zaccaro Scelza MF, Lima Oliveira LR, Carvalho FB, Côrte-Real Faria S. In vitro evaluation of macrophage viability after incubation in orange oil, eucalyptol, and chloroform. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:e24-7.
7. Wourms DJ, Campbell AD, Hicks ML, Pelleu GB Jr. Alternative solvents to chloroform for gutta-percha removal. J Endod 1990;16:224-6.
8. Martos J, Gastal MT, Sommer L, Lund RG, Del Pino FA, Osinaga PW. Dissolving efficacy of organic solvents on root canal sealers. Clin Oral Investig 2006;10:50-4.
9. Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science 2006;311:808-11.
10. Ghribi L, Ben Nejma A, Besbes M, Harzalla-Skhiri F, Flamini G, Ben Jannet H. Chemical composition, cytotoxic and antibacterial activities of the essential oil from the tunisian *Ononis angustissima* L. (Fabaceae). J Oleo Sci 2016;65:339-45.
11. Marković T. Etarska ulja i njihova bezbedna primena. Institut za proučavanje lekovitog bilja „dr Josif Pančić", Beograd, str. 2011; 1-287.
12. Pecora JD, Costa WF, Filho DS, Sarti SJ. Apresentação de um óleo essencial, obtido de *Citrus aurantium*, eficaz na desintegração do cimento de óxido de zinco-eugenol do interior do canal radicular. Odonto 1992;1:130-2.
13. Subbiya A, Padmavathy K, Mahalakshmi K. Evaluation of the antibacterial activity of three gutta-percha solvents against *Enterococcus faecalis*. Int J Artif Organs 2013;5:36358-362.
14. Hunter KR, Doblecki W, Pelleu GB Jr. Halothane and eucalyptol as alternatives to chloroform for softening gutta-percha. J Endod 1991;17:310-1.
15. Yadav HK, Yadav RK, Chandra A, Thakkar RR. The effectiveness of eucalyptus oil, orange oil, and xylene in dissolving different endodontic sealers. J Conserv Dent 2016;19:332-7.
16. Gomes FA, Daniel AD, Nunes RA, Fernandes AN, Ferreira CM, de Matos HM, *et al*. Efficacy of gutta-percha solvents used in endodontic retreatments. RSBO 2013;10:356-61.
17. Oyama KO, Siqueira EL, Santos Md. In vitro study of effect of solvent on root canal retreatment. Braz Dent J 2002;13:208-11.
18. McDonald MN, Vire DE. Chloroform in the endodontic operatory. J Endod 1992;18:301-3.
19. Chutich MJ, Kaminski EJ, Miller DA, Lautenschlager EP. Risk assessment of the toxicity of solvents of gutta-percha used in endodontic retreatment. J Endod 1998;24:213-6.
20. Edgar SW, Marshall JG, Baumgartner JC. The antimicrobial effect of chloroform on *Enterococcus faecalis* after gutta-percha removal. J Endod 2006;32:1185-7.
21. Görduysus MO, Taşman F, Tuncer S, Etikan I. Solubilizing efficiency of different gutta-percha solvents: A comparative study. J Nihon Univ Sch Dent 1997;39:133-5.
22. Whitworth JM, Boursin EM. Dissolution of root canal sealer cements in volatile solvents. Int Endod J 2000;33:19-24.
23. Schäfer E, Zandbiglari T. A comparison of the effectiveness of chloroform and eucalyptus oil in dissolving root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:611-6.
24. Mushtaq M, Farooq R, Ibrahim M, Khan FY. Dissolving efficacy of different organic solvents on gutta-percha and resilon root canal obturating materials at different immersion time intervals. J Conserv Dent 2012;15:141-5.
25. Maguna FP, Romero AM, Garro OA, Okulik NB. Actividad Antimicrobiana de un grupo de Terpenoides. Facultad de Agroindustrias, UNNE, Argentina. Comunicaciones Científicas y Tecnológicas en Internet. Resumen 2006;E-057:1-4.
26. Apetrei CL, Spac A, Brebu M, Tuchilus C, Miron A. Composition and antioxidant and antimicrobial activities of the essential oils of a full-grown *Pinus cembra* L. tree from the calimani mountains (Romania). J Serb Chem Soc 2013;78:27-37.
27. Sharma S, Singh S, Bond J, Singh A, Rustagi A. Evaluation of antibacterial properties of essential oils from clove and eucalyptus. Asian J Pharm Clin Res 2014;7:291-4.
28. Fisher K, Phillips C. The mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*. J Appl Microbiol 2009;106:1343-9.
29. Bauer K, Garbe D, Surburg H. Common Fragrance and Flavor Materials, 4th ed. Weinheim: Wiley-VCH; 2001. p. 189.
30. Dusan F, Marian S, Katarina D, Dobroslava B. Essential oils—their antimicrobial activity against *Escherichia coli* and effect on intestinal cell viability. Toxicol In Vitro 2006;20:1435-45.
31. Di Pasqua R, Betts G, Hoskins N, Edwards M, Ercolini D, Mauriello G. Membrane toxicity of antimicrobial compounds from essential oils. J Agric Food Chem 2007;55:4863-70.
32. Tsuda H, Ohshima Y, Nomoto H, Fujita K, Matsuda E, Iigo M, *et al*. Cancer prevention by natural compounds. Drug Metab Pharmacokinet 2004;19:245-63.
33. Pécora JD, Spanó JC, Barbin EL. In vitro study on the softening of gutta-percha cones in endodontic retreatment. Braz Dent J 1993;4:43-7.
34. Ring J, Murray PE, Namerow KN, Moldauer BI, Garcia-Godoy F. Removing root canal obturation materials: A comparison of rotary file systems and re-treatment agents. J Am Dent Assoc 2009;140:680-8.