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Original Article

Comparative evaluation of *Citrus limetta* peel extract and essential oils as natural solvents for gutta-percha dissolution: An *in vitro* study

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ABSTRACT

Objectives: Giving a successful endodontic retreatment involves getting rid of all gutta-percha (GP) from the root canals. The traditional solvent chloroform is known for being toxic. More attention is being given to finding natural and biocompatible materials. This study hopes to see if sweet lime peel, eucalyptus, and orange oil are good natural alternatives to solvents in removing GP.

Material and Methods: Sixty GP cones were chosen and split into four groups (15 cones in each group), namely control (with chloroform), *Citrus limetta* extract, eucalyptus oil, and orange oil. 5 min of exposure at 37°C was given to every cone by submerging them in 1 mL of solvent. When immersion was done, the cones were taken out, blotted, and weighed once again. The amount a material gained in weight was calculated to see how well it dissolved. Analysis of the data was completed using analysis of variance and Tukey's multiple comparisons test ($P < 0.05$).

Results: Chloroform produced the highest dissolved amount with 45.3 ± 1.5 mg, whereas eucalyptus oil came in second with 38.7 ± 2.1 mg, *C. limetta* extract was third with 34.2 ± 1.9 mg, and orange oil dissolved 31.6 ± 2.3 mg the most. Results from statistical analysis showed that all tested groups were different from one another. Eucalyptus oil demonstrated the highest rate of dissolution, followed by *C. limetta* among all herbal extracts.

Conclusion: Compared to reliably used herbal products, the potential for *C. limetta* peel's extract to dissolve GP is near to as that of eucalyptus oil and even higher than that of orange oil. Yet, more studies are required to show how practical this extract is in retreatment cases.

Keywords: *Citrus limetta*, Endodontic retreatment, Essential oils, Eucalyptus oil, Orange oil

INTRODUCTION

Endodontic retreatment is used to solve problems that have resulted from the failure of the original root canal treatment. The first important step in retreating of a root canal includes taking out the existing gutta-percha (GP) to ensure that the old filling materials have been eliminated and the canal system cleaned and sealed again.^[1] Usually, GP is removed from the root canal using mechanical and chemical methods. Due to their rapid dissolution ability, chloroform and xylene are highly employed chemical solvents.^[2] However, people are worried about their toxicity, possible link to cancer, and effects on the environment, which means safer and compatible alternatives must be developed.^[3,4]

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Lately, more people have shown interest in the use of natural plant-based substances for undertaking endodontic tasks. It has been studied whether eucalyptus oil and orange oil can help soften and dissolve GP, thanks to their useful biological properties, good scent, and pleasant taste.^[5,6] As these oils are not toxic and eco-friendly, they are suitable for use in a medical setting.

Citrus limetta, commonly called sweet lime, is a citrus fruit that has limonene, flavonoids, and essential oils on its peel. The great solvent qualities of phytochemicals have made them useful in both pharmaceutical and dental solutions for their ability to prevent and treat infections and inflammation.^[7] Even though *C. limetta* peel contains similar chemicals to other citrus-based solvents; endodontic retreatment with its extract has not been studied much yet.

Hence, the current study intends to assess and measure the dissolving capabilities of *C. limetta* peel extract versus eucalyptus oil, orange oil, and the conventional chemical solvent, chloroform. The purpose of this research is to find a substitute drug that dissolves effectively and does not endanger the body.

MATERIAL AND METHODS

The present *in vitro* study was designed to compare the efficacy of *C. limetta* peel extract, eucalyptus oil, and orange oil with chloroform in dissolving GP cones. A total of 60 standardized GP cones (International organization for standardization [ISO] size 40) were selected and randomly divided into four groups ($n = 15/\text{group}$) based on the solvent used.

Preparation of chloroform

Chloroform, being volatile, is mixed with ethanol as a solvent to minimize evaporation.

Preparation of *C. limetta* peel extract

Fresh peels of *C. limetta* were collected, washed, shade-dried, and powdered. The powder was subjected to Soxhlet extraction using ethanol as the solvent. The extract was concentrated using a rotary evaporator and stored in an airtight amber container at 4°C until use.

Grouping of samples

- Group I (Control): Chloroform
- Group II: *C. limetta* peel extract
- Group III: Eucalyptus oil
- Group IV: Orange oil

Procedure for dissolution test

Each GP cone was weighed using a precision digital balance (accuracy ± 0.0001 g), and the initial weight was

recorded. The cones were then immersed individually in 1 mL of the respective solvent at 10% concentration^[8] in clean, labeled amber glass vials.^[9] All samples were kept in a thermostatically controlled incubator at 37°C for 5 min to simulate intraoral temperature conditions.^[10]

After the immersion period, the cones were removed using forceps, gently blotted with filter paper for 24 h at room temperature to remove excess solvent, and reweighed immediately [Figure 1]. The amount of GP dissolved was calculated by subtracting the post-immersion weight from the initial weight.^[11]

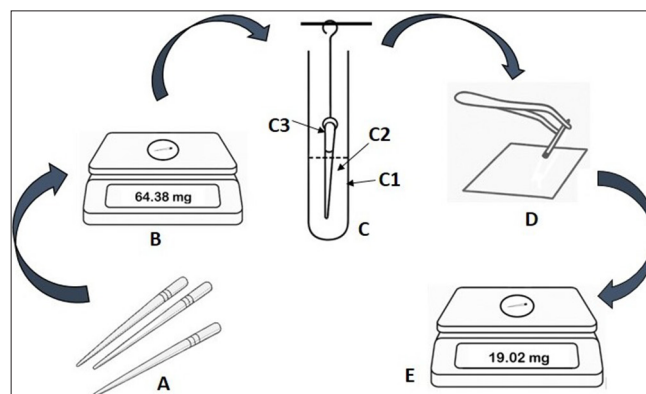


Figure 1: Schematic diagram representing dissolution test methodology. (A) Gutta-percha (GP) cones (B) pre-immersion weight of GP measured (C) immersion of GP into GP solvents at 37°C for 5 min (C1) test tube (C2) GP solvent (C3) GP (D) blotting of GP with filter paper to remove excess solvent (E) post-immersion weight of GP measured and weight difference is calculated.

Statistical analysis

The data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences software (version 25.0; IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to compare the mean dissolution values among the four groups. Tukey's *post hoc* test was applied for pairwise comparison. $P < 0.05$ was considered statistically significant.

RESULTS

The mean amount of GP dissolved by each solvent after 5 min of immersion is presented in Table 1. Among all groups, chloroform (Group I) exhibited the highest dissolution capacity with a mean mass loss of 45.36 ± 1.52 mg. This was followed by eucalyptus oil (Group III) with 38.74 ± 2.10 mg, *C. limetta* peel extract (Group II) with 34.21 ± 1.93 mg, and orange oil (Group IV) with the lowest dissolution at 31.58 ± 2.27 mg. GP was observed to be softer than before as it gets converted to α -phase.

A statistically significant difference was found among the four groups ($P < 0.001$) using one-way ANOVA. *Post hoc* analysis

(Tukey's test) revealed that chloroform was significantly more effective than all natural solvents ($P < 0.05$). Eucalyptus oil showed significantly higher dissolution than orange oil ($P = 0.02$), while *C. limetta* extract exhibited significantly greater solubility than orange oil but was slightly less effective than eucalyptus oil ($P = 0.04$) [Table 2].

These results suggest that while chloroform remains the most effective solvent, *C. limetta* peel extract demonstrates promising dissolving ability comparable to that of eucalyptus oil and superior to orange oil.

Table 1: Mean gutta-percha dissolution (in mg) by different solvents.

Group	Solvent	Mean $\pm$ SD (mg)
I	Chloroform	45.36 $\pm$ 1.52
II	<i>Citrus limetta</i> peel extract	34.21 $\pm$ 1.93
III	Eucalyptus oil	38.74 $\pm$ 2.10
IV	Orange oil	31.58 $\pm$ 2.27

SD: Standard deviation

Table 2: Statistical comparison of mean gutta-percha dissolution between groups.

Comparison	Mean difference (mg)	P-value
Chloroform versus <i>Citrus limetta</i>	11.15	<0.001
Chloroform versus eucalyptus	6.62	0.001
Chloroform versus orange oil	13.78	<0.001
<i>C. limetta</i> versus orange oil	2.63	0.035
<i>C. limetta</i> versus eucalyptus	-4.53	0.042
Eucalyptus versus orange oil	7.16	0.020

$P < 0.05$ is considered statistically significant.

DISCUSSION

To properly disinfect and reshape the root canal, all GP must be removed during retreatment. It has been customary to use chloroform as the preferred chemical for GP dissolution because of how well it works and its fast action.^[1] Still, because it is documented to be toxic to cells, volatile, and possibly cancerous, more studies on safer alternatives are required.^[2,3]

The amount of GP dissolved by *C. limetta* peel extract was assessed and measured against that of eucalyptus oil, orange oil, and chloroform. The dissolution of chloroform was proven to be greatest, which coincides with previous reports.^[4,5] Eucalyptus oil worked best out of all the natural solvents used, and *C. limetta* peel extract and orange oil came in after that. Research done in the past also reveals that eucalyptus oil works better than other essential oils because of its rich eucalyptol, which is able to disperse putty-like filling materials in the mouth.

The first test of *C. limetta* peel extract found that it has good dissolving properties. The peels of many citrus fruits, for example, *C. limetta*, have a lot of d-limonene, a chemical used for cleaning and in medicine and dental medicines.^[12] Even though d-limonene is slower in softening GP than chloroform, it does it effectively.^[13] It has been suggested that the presence of limonene and flavonoids in large amounts in *C. limetta* extract makes it respond similarly to eucalyptus oil by weakening the GP.^[14,15]

Even though orange oil is natural and safe for humans, it dissolved less than the other tested solvents.^[16,17] The reason its efficiency may be weaker is because of differences in limonene, and it could also be due to having extra non-polar substances that weaken the solvent's action.^[18]

According to the results, *C. limetta* peel extract and eucalyptus oil are useful options for endodontic retreatment. Because they are safe, accepted well within the body, and environmentally sustainable, clinicians can choose these as replacements for routine synthetic solvents. Nonetheless, it is necessary to see that what happens in the laboratory may not relate directly to outcomes in patients. Examples of such influences include solvents reaching into root canals, liaisons between endodontic sealers and various materials, and both debris and moisture in the canals.^[19-21]

Researchers should explore more about the toxicity, the ability to kill bacteria, and the long-term traits of these natural solvents when they contact dentin and periapical tissues. The effectiveness and usefulness of *C. limetta* extract might also rise with proper concentration and formulation.

Limitations

The current study is an initial exploration of herbal GP solvents for endodontic use and is not commercially available. Moreover, the toxicity of herbal extracts on human cells used in the present study should be assessed along with any drug interaction, as some medicinal plants are intrinsically toxic by virtue of their constituents and can cause adverse reactions if not used in an appropriate concentration. Further clinical studies are required to assess their efficacy and shelf life, enabling their incorporation into routine dental practice. Additionally, the efficacy of the solvents on the sealers should be tested, as the study only considered the GP.

CONCLUSION

According to the present study, chloroform does the best job of dissolving GP, but eucalyptus oil and *C. limetta* peel extract can be a promising and effective natural alternative. Being both biocompatible and naturally sourced, eucalyptus oil and *C. limetta* extract might be a good alternative when doing endodontic retreatment, calling for more studies.

Ethical approval: The research/study is approved by the Institutional Review Board at Saraswati Dental College and Hospital, Lucknow, number #SD1CE16122024R, dated December 16th, 2024 Ref. SDC&H/IRDC/2024/MDS/G01.

Declaration of patient consent: Patient's consent is not required as there are no patients in this study.

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