Evaluation of the antibacterial effect of gutta percha solvents on enterococcus faecalis in non-surgical root canal retreatment: a systematic review and meta-analysis

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ABSTRACT

Objective: The systematic review and meta-analysis aimed to assess the comparative analysis of antimicrobial effect of gutta percha solvents on E. faecalis in non-surgical root canal retreatement.

Methodology: The PICO strategy guided the electronic search of key terms across PubMed, Science Direct, Google Scholar, Cochrane Library, and Wiley online databases. Bias risk was evaluated utilizing the Cochrane risk of bias tool. Meta-analysis was conducted using RevMan 5.4 software (The Nordic Cochrane Center, Copenhagen).

Results: A total of 5 studies including Chloroform, Orange oil, Saline, xylene, eucalyptol oil, turpentine oil, RC Solve, Endosolv-R gutta percha solvents. Out of 5, 3 studies were selected for the meta- analysis.

Conclusion: In summary, chloroform demonstrated the highest efficacy in non-surgical root canal retreatment among solvents for antibacterial efficacy in relation to E. faecalis, followed by orange oil and eucalyptol, thatshowed comparable effectiveness.

***Keywords*---**Gutta percha solvent, antibacterial effect, non-surgical root canal retreatment.

INTRODUCTION

The recurrent periapical infections constitute a spectrum of histological or microbiological conditions such as intra-radicular infections, extra radicular infections, reactions to foreign bodies, and cysts, particularly those containing healing of fibrous scar tissue, and cholesterol crystals.(1) The main cause of endodontic treatment failures, despite the possibility of several nonmicrobial reasons, persistence of microorganisms in the apical region of the teeth which is previously endodontically treated .(2)Various factors contribute to the failure of root canal treatment, these include the persistence of bacteria within and around the root canal, inadequate cleaning and sealing of the canals, excessive filling material extending beyond the root apex, improper sealing of the tooth's crown, untreated canals within the tooth's root structure, procedural errors during treatment such as flawed access cavity design, and complications arising from instrumentation, such as ledges, perforations, or instrument separation.(2,3)

Treatment failure is more likely occur if microorganisms remains in the root canal during cleaning and shaping or after obturation (4,5). Enterococcus faecalis is a gram-positive, facultative coccus that thrives in anaerobic conditions, known for causing opportunistic infections. It employs various survival mechanism to endure adverse conditions, including growth in low-oxygen environments, high pH levels, a broad temperature range spanning 10° to 60° Celsius, high salinity, and nutrient-deficient surroundings (6).E. faecalis utilizes fluid within the periodontal ligament for sustenance and creates biofilms as a shield against host defenses and disinfectants. Moreover, it demonstrates a capacity to acquire antibiotic resistance, particularly against erythromycin and azithromycin, while also possessing the capability to infiltrate dentinal tubules and adhere to collagen (7).Pinheiro ET primarily attributes the bacterial origins of root canal failure to the presence of E. faecalis (8).These bacteria and their by-products act as an antigen. In defense, host elicit nonspecific anti-inflammatory response and immunological reaction in peri-radicular tissue causes apical periodontitis(9).

The likelihood of successful resolution of apical periodontitis resulting from unsuccessful endodontic treatment is significantly higher when the bacterial load within the root canal is eliminated or significantly diminished to levels conductive to peri-radicular tissue healing(9).Therefore, non-surgical retreatment necessitates the removal of gutta-percha, which can be accomplished through various methods and instruments, including: heat removal using Heated Pluggers; heat and instrument removal employing Headstrom files; rotary instruments such as ProTaper re-treatment kit or Ni-Ti Rotary files; ultrasonic removal utilizing the Piezoelectric ultrasonic effect; and use of hot instruments, manual tools, or microscopes in the cervical third, either alone or in combination with sonic devices or Gates-Gidden drills. All methods for removing gutta percha require the use of solvents. (10). The solvents are: Chloroform, xylene, eucalyptol oil, orange oil, turpentine oil, Endosolv-R, eucalyptol oil, RC solve etc.(11)

Successful retreatment hinges on efficient infection control. Bacteria present in challenging areas like isthmuses, deltas, ramifications, irregularities, and dentinal tubules may remain unaffected by standard endodontic disinfection procedures and require additional treatment. Chemo-mechanical preparation plays a vital role in addressing these areas, as it eliminates residual sealer remnants and bacterial colonies from anatomical complexities inaccessible to mechanical means. Stabholz and Friedman recommend the utilization of solvents to eliminate any remaining filling material in dentinal tubules, aiding the penetration of irrigating solutions and intracanal medication into these structures.(12)

An ideal organic solvent should demonstrate antibacterial properties, possess minimal toxicity, ensure clinical safety, and efficiently dissolve gutta-percha (GP). The antibacterial attribute of the GP solvent is vital for comprehensive root canal disinfection post-GP removal. Disinfecting the canals during GP removal is imperative in endodontic treatment, contributing to shorter treatment durations. A range of synthetic GP solvents is available in the dental field. (8)

Several clinical investigations have been conducted to assess the antimicrobial effectiveness of various gutta-percha solvents against Enterococcus faecalis in nonsurgical root canal retreatment. However, a debate persists regarding the most efficient gutta-percha solvent for such scenarios due to insufficient comparative literature. Therefore, the objective of this systematic review is to compare chloroform with other gutta-percha solvents in the long-term reduction of Enterococcus faecalis bacterial flora, aiming to provide clinicians with a clearer understanding.

MATERIAL AND METHODOLOGY

Protocol and registration:

The PRISMA (Preferred reporting items for systematic review and meta-analysis) 2009 protocol encompasses the rules that the research process follows. PROSPERO CRD42023444449 is the registration reference number.

Focusedquestion:
Is antibacterial effect of chloroform (intervention) as a gutta percha solvent is more effective than that of other gutta percha solvent (comparator) on enterococcus faecalis (population) in non-surgical root canal re-treatment?

Eligibilityand Inclusive Criteria:

1. ArticlesintheEnglishlanguage,performed on non-surgical root canal re-treatment or culture were included, and
2. Articles in which population comprising of permanent mature single rooted teeth, including in-vitro study; ex-vivo study; in-vivo study; comparative study, animal studies, cultural studies were included;
3. Studies that included gutta percha solvents used against enterococcus faecalis bacteria.

Exclusion criteria:

1. Research published in languages other than English, including case series, human histological studies, cohorts, or narrative reviews;
2. Research involving deciduous teeth, immature permanent teeth, or anterior teeth.

Study selection:
Two reviewers independently screened titles identified from the search, and abstract/full-texts retrieved by the electronic search were collected and verified against the eligibility criteria. In addition, a manual review of every citation from the selected studies were conducted in order to find any other research that might be significant. To assess inter-reviewer consistency, Cohen’s kappa (0.80) was employed. Dialogue between the reviewers were used to settle any disagreement. In case of persistent disagreement, the decision determined by third reviewer was considered conclusive.

Literature search:
A thorough search was performed on electronic databases, supplemented by manual searches, to identify all pertinent studies concerning the antibacterial impact of gutta percha solvents on enterococcus faecalis in non-surgical root canal retreatment. In electronic database such as PubMED, Science direct, Chochrane Library, Google Scholar and Wiley Online search terms like “gutta-percha solvents”, “Root Canal Filling Solvents”, “solvent for gutta-percha”, “solvent”, “root canal solvent”, “chloroform”, “gutta-percha removal”, “dissolving organic solvents”, “organic solvents”, “gutta-percha organic solvents”, “gutta-percha dissolving organic solvents”, “Enterococcus faecalis”, “E. faecalis”. Google search strategies were employed using combinations of various parameters as keywords, utilizing "AND," "NOT," and "OR" as conjunctions to refine the search results. The search encompassed articles published up to April 2023.

Contemporary research filters and English language criteria were applied, and duplicate records were eliminated. Subsequently, only abstracts were reviewed, and eligible articles were then accessed in full test. The workflow adhered to the PRISMA checklist.

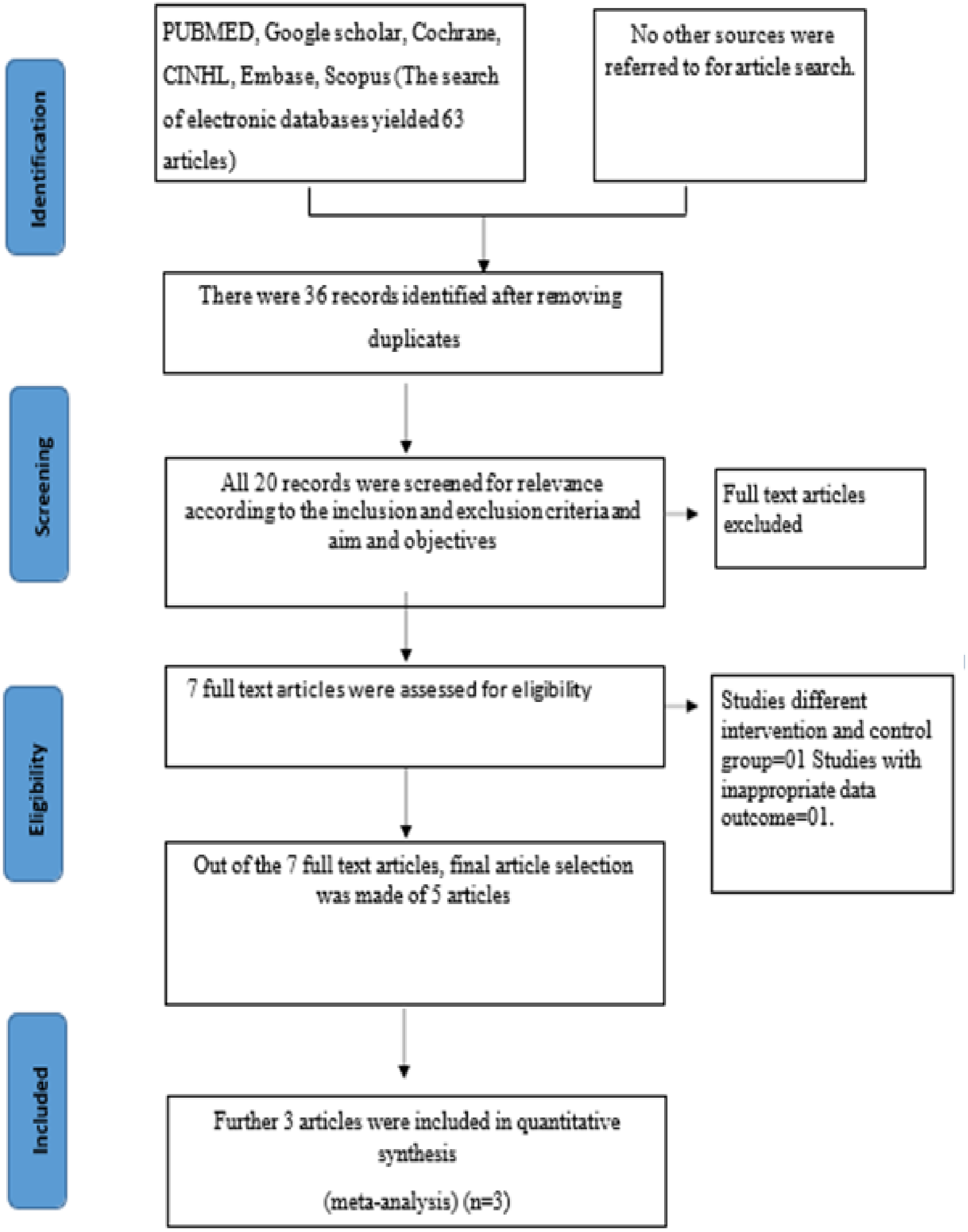

Fig. 1. PRISMA flow chart

Data Extraction and Data Items:

Two reviewers independently extracted information on author's names, publication years, study designs, sample sizes, types of teeth included, intervention groups, control procedures, irrigation protocols, sample preparations, incubation methods, obturation techniques, sealers used, gutta percha removal methods, sample collection procedures, storage methods, incubation for testing, and results. Data from the included studies were entered into a predefined protocol form using Microsoft Office Excel 2007 software.

Table 2: Characteristics of included studies

Sr. No	(A)Author	(B)Year publication	(C)Type of study	(D) Sample size	(E) Type of tooth	(F) Control procedure	(G) Group of intervention	(H) Irrigation	(I)sample preparation
1.	Aminsobhani et al	2022	ex-vivo study	80	single rooted	re-root canal treatment followed by culture	Chloroform, Eucalyptol, Orange oil, xylene	5.25% NaOCl	autoclave, 121 degree, 30min and rehydration 1ml saline, 37 degrees, 2days
2.	Edgar et al	2006	in-vitro	46	single rooted	re-root canal treatment followed by culture	chloroform, saline	5ml of 5.25% NaoCl, 5ml of 25% EDTA	air-dried for 2 days, autoclave, 121 degree, 30min and rehydration 1ml saline, 37 degrees, 2days
3.	Maria et al	2021	in-vitro	00	NR	culture	eucalyptus oil, chloroform, turpentine oil, xylene, orange oil.	NR	culture for each E. faecalis strain was adjusted to 0.5 McFarland standard (108 CFU/ml).
4.	Martos et al	2013	in-vitro	15	single rooted, bovine tooth	culture	orange oil, eucalyptus oil, Chloroform, combine with Cetrimide	NR	section of 6 mm of the middle third, divided into 3, 2-mm slices, each slice was cut into 4 equal sections, submerging them in 17% EDTA for 2 min and then in 2.5% NaOCl for 1 min
5.	Sablok et al	2019	in-vitro	60	single rooted mandibular premolar	re-root canal treatment followed by culture	chloroform, RC solve, Endosolv-R	1ml of 5.25% of NaOCl	autoclave for 15 minutes at 121 degree C

Table 2: Characteristics of included studies

Sr no.	(J) Incubation	(K) obturation	(L) sealer	(M) incubation period	(N) GP removal solvent	(O) gutta percha removal	(P) sample collection	(Q) sample stored	(R) sample incubation for testing	(S) Result
1.	BHI medium containing E faecalis, 37 degrees, 4weeks	Gutta percha using lateral condensation method	gutta flow 2	6 weeks in BHI contain E faecalis	Chloroform, Eucalyptol, Orange oil, xylene	GG files, H files	dried paper points	Eppendorf tubes containing 1 ml BHI	100 µl of each sample dilution was spread plated onto BHI agar plates, incubated at 37°C for 48 hours, and colony-forming units (CFUs) per 1 ml were counted	Chloroform>orange oil> Eucalyptol> xylene
2.	BHI medium containing E faecalis, 37 degrees, 4weeks	Gutta percha using lateral condensation method	no sealer	4 weeks in BHI contain E faecalis	chloroform, saline	GG files, H files, profile files	dried paper points	test tube containing 1.0 ml of saline	Aliquots of 0.1 ml were spread plated onto BHI agar plates, incubated at 37°C for 48 h, and colony-forming units (CFU) per 1 ml were counted.	chloroform> saline
3.	500 µL of the suspension was spread over the agar plates containing Mueller Hinton Agar (MHA)	NR	NR	NR	eucalyptus oil, chloroform, turpentine oil, xylene, orange oil.	NR	NR	inoculated plates were incubated at 37°C for 18 to 24h	Diameter of the zone of inhibition (in mm) produced by each solvent at the end of the incubation period was measured	xylene> chloroform >orange oil >eucalyptus oil > turpentine oil
4.	BHI during 24 h at 37 °C	NR	NR	NR	orange oil, eucalyptus oil, Chloroform, Combinated with Cetrimide	NR	NR	NR	180 µl of each sample dilution was spread plated onto BHI agar plates, incubated for 7 days at 37 °C	orange oil > eucalyptus oil > Chloroform
5.	BHI medium containing E faecalis, 37 degree, 1 weeks	Gutta percha using lateral condensation method	no sealer	24hr to set GP	chloroform, RC solve, Endosolv-R	H files	dried paper points	test tubes containing 5ml of Mueller Hinton broth, 37 C, for 4 days	samples were inoculated in blood agar plates	Endosolv R= RC Solve> Chloroform

Risk of Bias:

Risk of biasin the studies were separately evaluated by two review researchers. The selected studies were categorized as having a low, unclear, or higher risk of bias.
A number of domains were assessed for bias.

Table 3: Risk of bias for included studies

Sr. no	Author (Year)	TYPE OF STUDY	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants (performance bias)	Blinding of outcome (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)
1	Amins obhani et al 2022,	ex-vivo study	Yes	No	No	Yes	No	Yes
2	Edgar et al 2006	in-vitro	Yes	Yes	No	Yes	No	Yes
3	Maria et al 2021	in-vitro	no	No	No	Yes	Yes	Yes
4	Marto s et al 2013	in-vitro	Yes	Yes	Yes	No	Yes	Yes
5	Sablok et al 2019	in-vitro	Yes	Yes	Yes	Yes	Yes	Yes

RESULTS

Study characteristics

After screening, two independent reviewers selected five articles from the mentioned pool of articles. All data pertaining to the selection criteria were extensively deliberated by the reviewer. To resolve any disagreements, third reviewer was involved. The exclusion of studies that were not selected in the inclusion criteria resulted in a notification to the authors asking for further information. The data provided in the selected studies were recorded in excel sheets under the headings: - author's names, year of publications, design of the study, year, sample size, teeth type, groups of intervention, procedure done in control, irrigation protocol, sample preparation, incubation, obturation, sealer used, gutta percha removal, sample collection, sample stored, sample incubation for testing, and the result. (Table 2). The studies encompassed in the review were conducted from 2005 to 2023.

The meta-analysis was performed on three studies that met the necessary criteria for quantitative analysis based on available data outcomes. Other studies were omitted due to incompatible data formats (not in mean ± SD format). The findings are illustrated in the forest plot shown in Figures 2 and 3.

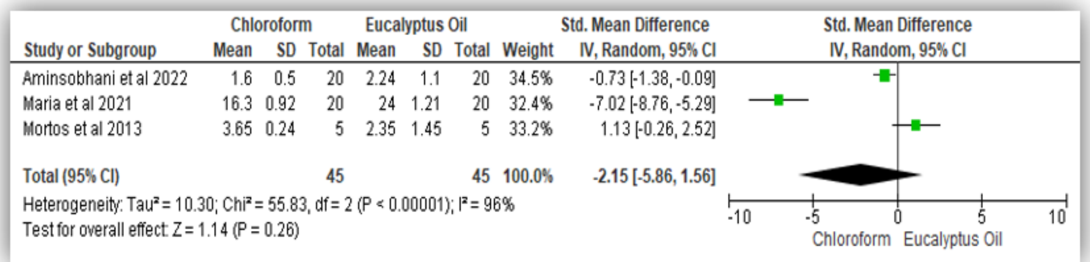

Figure 2: showing Forest plot showing anti-bacterial efficacy of chloroform versus eucalyptus oil with regards to the reduced enterococcus faecalis (EO) in non-surgical root canal treatment as an outcome

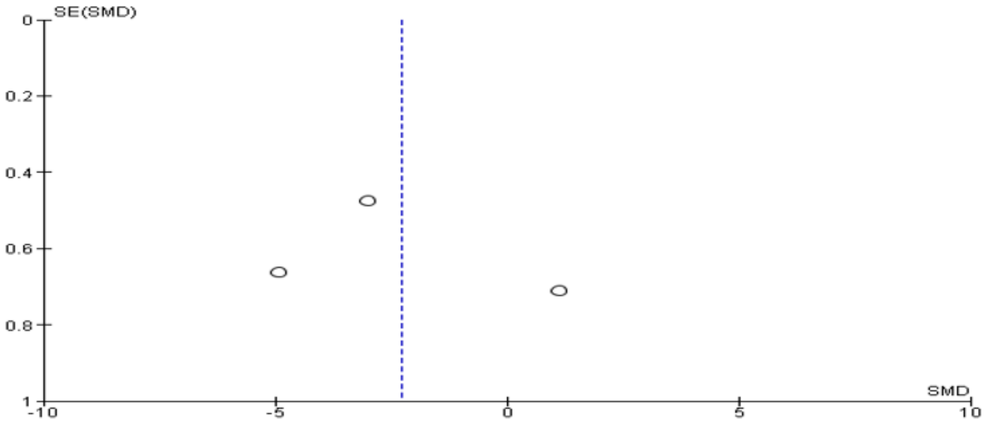

Figure 3: showing Begg's Funnel plot with 95% confidence intervals demonstrating symmetric distribution without systematic heterogeneity of individual study compared with the standard error of each study, indicating an absence of publication bias.

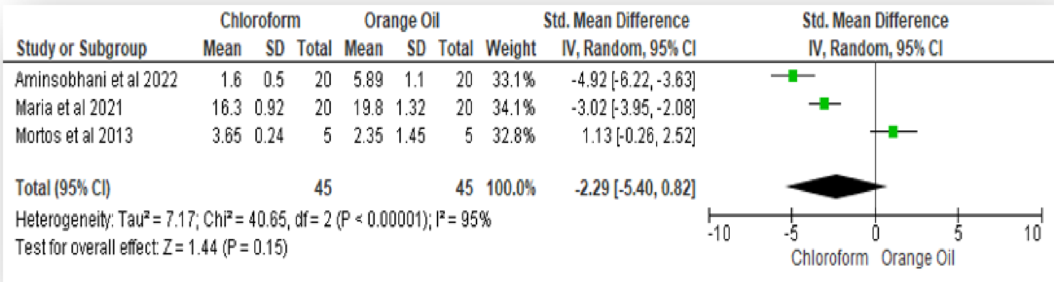

Figure 4: showing Forest plot showing anti-bacterial efficacy of chloroform versus orange oil with regards to the reduced enterococcus faecalis (EO) in non-surgical root canal treatment as an outcome

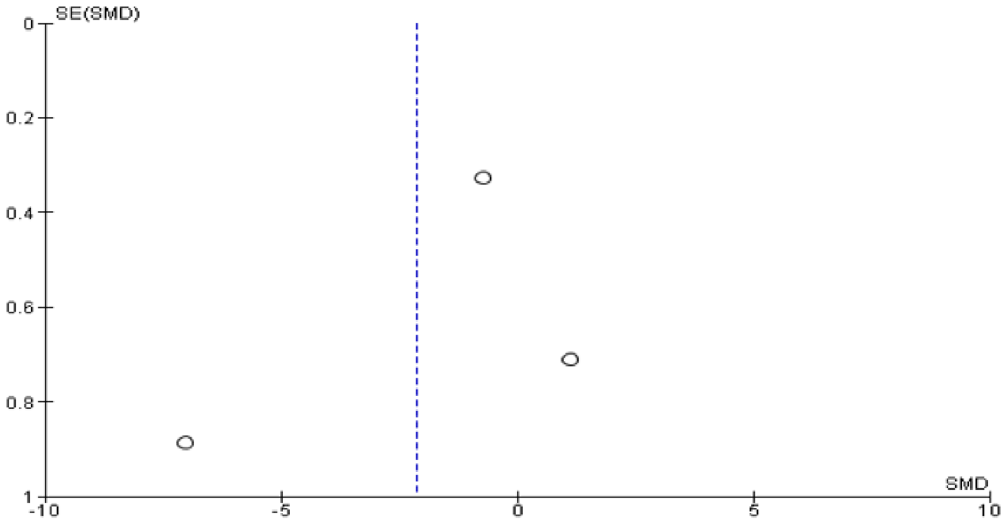

Figure 5: showing Begg's Funnel plot with 95% confidence intervals demonstrating symmetric distribution without systematic heterogeneity of individual study compared with the standard error of each study, indicating an absence of publication bias.

Comparative evaluation between chloroform solvent against eucalyptus and orange oil solvent with regards to the better efficacy in terms reduced enterococcus faecalis (EO) in non-surgical root canal treatment as an outcome. For comparison between chloroform and eucalyptus solvent a standard mean difference(SMD) of 2.15 (-5.86 – 1.56) was seen and the pooled estimates favored chloroform solvent. Chloroform solvent had better anti-bacterial efficacy 2.15 times more as compared to eucalyptus oil solvent.

For comparison between chloroform and orange oil solvent a SMD of 2.29 (-5.40 – 0.80) was seen and the pooled estimates favored chloroform solvent. Chloroform solvent on an average had 2.29 times more anti-bacterial efficacy compared to orange oil solvent. Clinically chloroform solvent had better antibacterial efficacy but statistically a non-significant difference was seen ($p>0.05$). Random effect model was used with I^2 value of 95% with presence of high inconsistency or heterogeneity. To overcome this, further more studies should be carried out with proper reporting guidelines (for RCT or in-vitro studies) during dissemination of result.

Risk of bias

All studies that are included in the review demonstration showed from moderate to high risk of bias across many dimensions. The domains with the highest risk of bias were blinding of participants and personnel (performance bias), allocation concealment (selection bias), and incomplete outcome data (attrition bias). Among the included studies, Aminsobhani et al. (2022), Maria et al, (2021), Edgar et al, (2006), and Martos et al, (2013) had the highest risk of bias compared to others, while Sablok et al, (2019) followed by Martos et al, (2013) reported the lowest risk of bias.

The domains of random sequence generation (selection bias), blinding of outcome assessment (detection bias), selective reporting (reporting bias) assessment of the included studies using the Cochrane risk of bias (ROB) – 2 tool is illustrated in Figure 6 and 7.

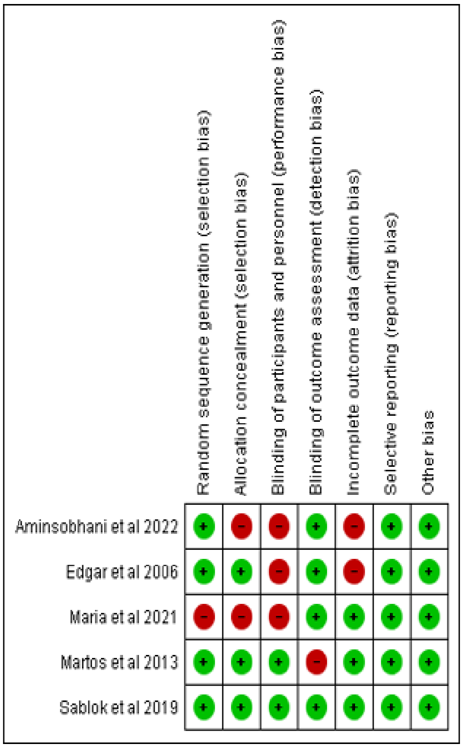

Figure 6: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

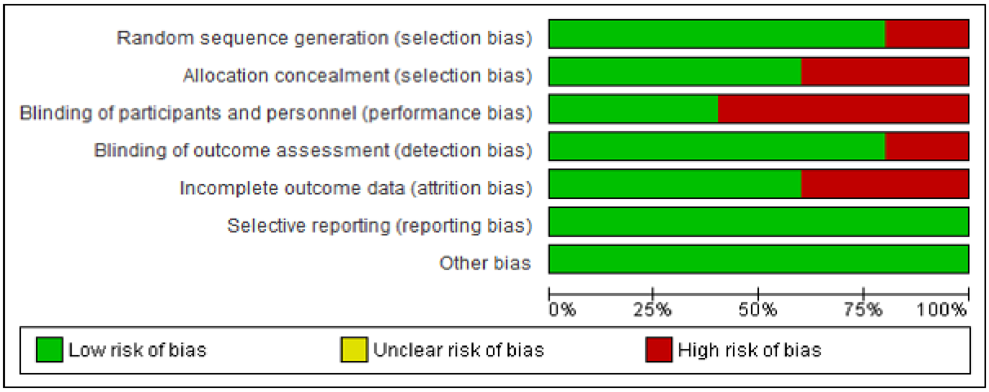

Figure 7: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

DISCUSSION

Various methods that are used to remove gutta percha includes the usage of natural solvents, mechanical instruments (such as hand files, rotary NiTi files), heat of instrument, laser treatments(13). However, the total elimination of gutta percha is typically achieved with the use of solvents such as xylene, orange oil, chloroform, and others. The optimal solvent should have harmony between tissue aggression, toxicity, clinical safety, and dissolving ability(14).

Literature indicates that no single system is universally superior for complete elimination of gutta percha material. Studies advocate for an integration of mechanical and manual techniques, in order to get cleaner root canals devoid of debris and filling material residues. Solvents are considered necessary when gutta-percha and sealer persist in deep isthmus anddentinal wall depressions, particularly in the apical portions of dentinal tubules, indicating the need for additional instrumentation to ensure thorough removal(15).

Chloroform is one of the most effective solvents for gutta percha. However, because of its possiblecarcinogenicity, its use in retreatments is compromised but it has been shown that it's appropriate use in controlled dosage shows no harm. Chloroform has been found in multiple trials to have antibacterial activity against E, faecalis when used for gutta percha dissolution(16,17).

Chloroform's anti-bacterial mechanism clarified a number of discoveries by examining the target bacteria's ATP levels, pyruvic acid content, respiratory metabolism, and cell shape.Scanning electron micrographs revealed bacterial cell destruction and induced plasmolysis. Pyruvic acid concentrations in bacterial solutions significantly increased and bacterial cell ATP level reduces as a result of chloroform's inhibition of tricarboxylic acid cycle. Moreover, it disrupted cell membrane permeability, resulting in metabolic dysfunction, energy synthesis inhibition, and ultimately, cell death(17).

Edgar et al. and Martos et al. found that chloroform exhibited potent antimicrobial effects targeted towards E. faecalis, capable to eradicating it, Xylene and orange oil showed comparable efficacy, both surpassing eucalyptol. However, a prior study by Hunter et al. suggested chloroform had similar effectiveness to eucalyptol and orange oil, Martos et al., noted that the orange oil outperformed eucalyptol, especially with prolonged solvent contact time with bacterial biofilm. (16,17). Solvents demonstrated the highest dissolution percentage within the initial minute. Wax, zinc oxide, resins, gutta percha, and barium sulfate are all components of gutta percha points; however, solvents exclusively target the gutta percha..This rapid dissolution in the first minute may be attributed to the reduced gutta-percha content within the point over subsequent minutes, limiting the solvent's effective area of action, as observed by Almeida GF et al.(18)

Alternative gutta percha solvent characteristic other than antimicrobial activity, like dissolution capability, biocompatibility, damage to cells, cancer causing potential, enhanced odor, and accessibility should be considered when choosing solvent(19). While synthetic solvents are noted for their superior dissolution capacity and antimicrobial properties, they are linked to lower biocompatibility and potential harm to host tissues.

The current systematic review delved into the antimicrobial efficacy of GP solvents against E. faecalis biofilm in non-surgical root canal retreatment, addressing a significant challenge in endodontic practice. By incorporating ex vivo models, which isolate the antimicrobial activity of solvents from other variables, the study aimed to provide clinicians with more clinically relevant insights. Notably, while a specific solvent may exhibit potent antibacterial properties, its limited dissolution capability could compromise the removal of infected gutta-percha, potentially leading to higher residual microbial presence post-treatment. Moreover, E. faecalis, notorious for its treatment resistance and predominance in failed endodontic cases, may exhibit reduced susceptibility in its biofilm form, further complicating treatment outcomes.(16)

The findings of the study revealed that solvents exhibited promising antimicrobial efficacy against the E. faecalis. However, among them, only the xylene and chloroform groups showed significant action against the E. faecalis biofilm.(20)Alternatively, the culture method utilized as a detection technique in this study has limitations in detecting each type of E. faecalis. Specifically, the observed reduction in bacterial load is restricted to culturable species only.

To ensure methodological consistency, the study exclusively included single-rooted teeth as the sample population, as multirooted teeth pose greater complexities in removing gutta percha and cleaning the root canals. However, since this was an ex vivo study where each teeth were assessed independently, without considering its supporting structures, it's important to interpret the results cautiously(20). Further research is needed to explore other organic solvents that exhibit superior dissolution capabilities and effective antimicrobial properties. In vivo studies are essential to assess their results across the short and long terms related to the application of various solvents in non-surgical retreatment.(21)

One study employed a sealer during obturation, while four studies did not utilize a sealer. It is preferable to exclude the sealer as a potential variable. Despite the routine use of sealers in endodontic therapy, many of them possess antimicrobial properties that may influence the outcomes. (20).

Meta analysis was carried for comparative evaluation between chloroform solvent against eucalyptus and orange oil solvent with regards to the better efficacy in terms reduced enterococcus faecalis in non-surgical root canal treatment as an outcome. For comparison between chloroform and eucalyptus solvent a SMD of 2.15 (-5.86 – 1.56) was seen and the pooled estimates favored chloroform solvent. Chloroform solvent had better anti-bacterial efficacy 2.15 times more as compared to eucalyptus oil solvent.

In summary, according to the comprehensive review and meta-analysis, chloroform is the most well researched and efficient solvent, showcasing superior antimicrobial properties against E. faecalis. However, it's crucial to acknowledge potential drawbacks such as its impact on the bond strength of root fillings during re-obturation and its toxicity if not used under clinically controlled condition.

LIMITATIONS

Additional research is warranted to evaluated the effectiveness of gutta percha solvents and antimicrobial agents to identify optimal combinations that offer sufficient solvent capacity without compromising antimicrobial activity. Investigations into the impact of gutta-percha solvents on sealers post-gutta-percha removal are also warranted. Furthermore, exploration of natural products with broad biological activity holds promise for addressing multidrug-resistant bacteria. However, in-depth in vivo studies are essential to assess their efficacy and potential toxicity. Therefore, further clinical trials are necessary to determine their applicability in dentistry.

CONCLUSION

Despite the study's limitations, chloroform emerged as the most effective solvent against E. faecalis in non-surgical root canal retreatment, followed by orange oil and xylene, that showed comparable effectiveness. Eucalyptol exhibited less efficacy in comparison. Chloroform and xylene demonstrated favorable antibiofilm activity. These solvents play a crucial role in root canal disinfection, addressing a key challenge in non-surgical root canal retreatment, while also aiding in the elimination of gutta percha remaining components. This systematic review, encompassing five articles with a moderate risk of bias, supports these findings. Furthermore, a meta-analysis of three articles confirmed chloroform's superior antimicrobial activity against E. faecalis, as illustrated in the forest plot.

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