Review Article

PROTOCOL FOR A SYSTEMATIC REVIEW OF THE *IN VITRO* ANTIMICROBIAL EFFECT OF CINNAMON ESSENTIAL OIL ON ORAL MICROBIOME

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ABSTRACT

The technique for a comprehensive review aimed at assessing the antibacterial properties of cinnamon essential oil (CEO) on the oral microbiota through in vitro investigations is presented in this publication. This evaluation is registered with PROSPERO using registration number CRD42024577599 in accordance with PRISMA criteria. The review aims to address the effectiveness of CEO in modulating both bacterial and fungal populations in the oral cavity. The focus will be on how different types of cinnamon (Cinnamomum verum, Cinnamomum zeylanicum, Cinnamomum cassia) affect microbial concentrations, with primary outcomes including minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC). Secondary outcomes will involve comparing the efficacy of CEO against various pathogens and determining methodological variations across studies. A thorough literature search will be carried out using a number of databases, including PubMed, SCOPUS, DOAJ, and Google Scholar, with each author assigned a distinct search task. The selection of articles will be managed using established assessment criteria, and data will be synthesized narratively, focusing on both antibacterial and antifungal effects of CEO. The review will also assess clinical and methodological heterogeneity, evaluating the risk of bias using the ROBINS-E tool and the certainty of evidence with the GRADE approach. The anticipated results aim to clarify CEO's role in oral health, highlight methodological inconsistencies, and offer recommendations for future research. By synthesizing evidence from various studies, this review will contribute to understanding the potential applications of CEO in preventing and managing oral diseases, addressing gaps in current research, and providing clearer guidance for clinical practice.

Key words: Cinnamon essential oil, Oral microbiome, Systematic review, Antimicrobial effect.

Introduction

Bacteria, fungus, viruses, and other microorganisms are all part of the complex community known as the oral microbiome [1-3]. Numerous parts of the oral cavity, including the tongue, gums, teeth, tonsils, and saliva, are home to these organisms [4]. The oral microbiota is essential to oral health because it helps with the first stages of food digestion, guards against infections, and contributes to the immune system [5-7].

Oral disorders, including caries, periodontitis, halitosis, and other associated systemic problems, can be exacerbated by dysbiosis, an imbalance in the oral microbiota. Awareness of how to preserve oral health and fend against illnesses requires an awareness of the oral microbiota. Furthermore, for a person's general health, the oral microbiome is crucial, being pivotal in pathogen protection and maintaining microbial homeostasis in the oral cavity [8]. In this context, there is growing interest in the role of natural compounds, such as essential oils, in controlling oral microbiota and how it affects dental health [9].

The increasing popularity of essential oils (EOs), specifically Cinnamon Essential Oil (CEO), as natural antimicrobial agents in the treatment and prevention of oral diseases [10, 11] has generated a significant volume of *in vitro* research [9]. However, variability in experimental methods and reported results complicates the interpretation of their true effectiveness. Therefore, there is a need to clarify methods that enable the scientific community to conduct systematic reviews based on scientific evidence, providing the most comprehensive, high-quality, and clear information available.

In this publication, a procedure for a systematic review that describes the synthesis techniques is presented and critically evaluates the existing evidence on the antimicrobial effect of cinnamon essential oil on the oral microbiome in *in vitro* studies [12, 13].

Materials and Methods

Protocol and registration

It was made sure that the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) declaration [14] was followed, and a prior protocol will be registered in PROSPERO under registration number

CRD42024577599, which can be accessed at the following link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024577599.

Focus question

What will be the effect of cinnamon essential oil on the oral microbiome?

Eligibility criteria

The PICOTS tool—which stands for population, intervention, comparison, outcome, time, and study type—was used to define eligibility criteria [14]. Detailed information on inclusion, exclusion, and author assumptions will be provided in **Table 1**.

Table 1. Inclusion and Exclusion Criteria with Data Assumptions by Authors

Data Item	Inclusion Criteria	Exclusion Criteria	Definition
Population	Oral microbiome	Human microbiome not including the oral cavity	Specific environment targeted by the study object
Intervention	Application of cinnamon essential oil (any variety)	Use of any essential oil other than cinnamon	Non-pharmacological treatment employed
Outcome	Therapeutic effect at the oral level	Effect not reported	Reported effect of cinnamon essential oil on the oral microbiome
Study Type	Original articles and websites with references. Languages included were English and Spanish.	Review articles	Types of studies and characteristics of included websites
Time	No restriction on publication date	Not applicable	Age of the information sources used

Information sources

From February to May 2025, an exhaustive literature search will be conducted in PubMed, Google Scholar, SCOPUS, DOAJ, and Google websites. D.Z.C will conduct searches in PubMed and Google, G.C.V will search in SCOPUS and DOAJ, while M.A.G.R will perform searches on Google websites. Article and website inclusion will be managed by M.A.C.M. and C.P.S., who will employ the assessment criteria for *in vitro* studies [15] in their decision-making. In cases of discrepancy, external support will be sought. Mendeley will be used as the reference management tool [16, 17]. The search strategy for each database and its coverage will be presented in **Table 2**.

Table 2. Search Strategy Employed

Database	Search Strategy	Coverage
PubMed	("cinnamomum zeylanicum"[MeSH Terms] OR ("cinnamomum"[All Fields] AND "zeylanicum"[All Fields]) OR "cinnamomum zeylanicum"[All Fields] OR "cinnamon"[All Fields]) AND ("oils, volatile"[MeSH Terms] OR ("oils"[All Fields] AND "volatile"[All Fields]) OR "volatile oils"[All Fields])	1987 to present
Google Scholar	"cinnamon essential oil" AND "oral"	1996 to present
Scopus	"cinnamon essential oil" AND "oral"	2002 to present
DOAJ	"cinnamon essential oil" AND "oral"	2011 to present
Google Website	"cinnamon essential oil" AND "oral microbiome"	1993 to present

Data collection process and synthesis methods

Titles will be reviewed first, then abstracts and keywords, and lastly entire texts, before manuscripts are chosen. The obtained information will be transferred to an Excel table including the following data: Database, Author-Country, Bacterial Concentration Before Treatment [18, 19],

Bacterial Concentration After Treatment, Objective, Procedures, Outcomes, MIC, MFC, Conclusions, Target Microorganism, Microorganism Type, Findings, and Cinnamon Type Employed. PRISMA selection flow chart will be employed for illustrate this process **(Figure 1)**.

A narrative synthesis of the obtained data will be performed, presented according to the antibacterial effect of EO and the antifungal effect of EO. In cases of missing information in the manuscripts, supplementary material will first be consulted, and subsequently, the corresponding author will be contacted via ResearchGate.

Our primary outcomes will be the target microorganism measured on a nominal scale (bacteria or fungus), and the type of EO used, reported as Cinnamomum verum, Cinnamomum zeylanicum, Cinnamomum cassia, or Cinnamomum zeylanicum. Our secondary outcomes will be the minimum inhibitory concentration (MIC) reported before and after treatment, expressed in terms of antimicrobial agent concentration per unit volume.

Establishment of heterogeneity

Clinical heterogeneity will be analyzed concerning the type of microorganism treated (bacteria or fungus) and the type of EO used. Methodological heterogeneity will be assessed concerning the incubation time of microorganisms expressed in hours. This process will be conducted by an external reviewer.

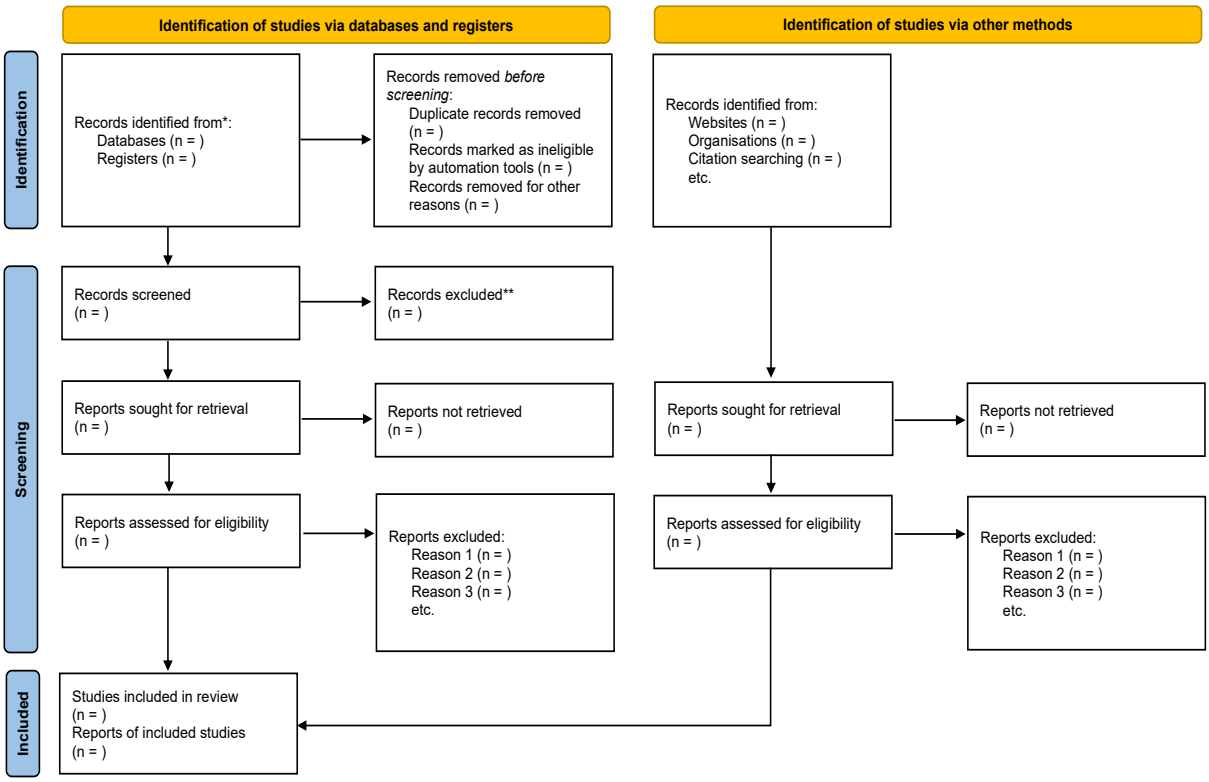

Figure 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources

Study risk of bias and certainty assessment

The included studies will be evaluated for bias using the ROBINS-E (Risk Of Bias In Non-randomized Studies of Exposures) tool [20]. With many important areas in mind, ROBINS-E will be developed to assess the risk of bias in non-randomized studies looking at exposures. These domains will include participant selection, assessing whether the sample is representative and whether confounding factors have been controlled [21, 22]; exposure measurement, evaluating whether the cinnamon essential oil will be administered consistently and accurately; and outcome assessment, reviewing whether the methods used to measure antimicrobial effects are adequate and valid. ROBINS-E will also examine information bias and reporting bias, ensuring that studies report their findings comprehensively and transparently.

The GRADE technique, which is applied for evaluating the strength of recommendations and the quality of proof in systematic reviews, will be utilized for the assurance evaluation [23]. First, the evidence quality will be categorized as high, moderate, low, or extremely poor based on the research design, bias risk, consistency, and accuracy of the findings. Then, the strength of recommendations will be determined based on the quality of evidence, the benefits and risks of the intervention, and patient [24-26] preferences. Both processes will be carried out by M.A.G.R. and C.P.S. simultaneously, and discrepancies will be

addressed with support from the other authors.

Results and Discussion

The systematic review will summarize the antimicrobial effects of Cinnamon Essential Oil (CEO) on the oral microbiome [27, 28] based on in vitro studies. The primary outcomes will focus on the Minimum Inhibitory Concentrations (MIC) for bacterial strains and Minimum Fungicidal Concentrations (MFC) for fungal strains treated with CEO. These values will be compared across different types of cinnamon (Cinnamomum verum, Cinnamomum zeylanicum, Cinnamomum cassia) to evaluate their effectiveness. Secondary outcomes will include a comparative analysis of CEO's efficacy against various pathogens and an examination of methodological variations across studies. The review will present a narrative synthesis of the data, highlighting variations in experimental protocols, such as incubation times and concentrations used, that may affect the results. Additionally, clinical and methodological heterogeneity will be assessed to understand the impact of different study designs on the overall findings. Visual aids, including tables and figures, will illustrate key results, with the PRISMA flow diagram depicting the study selection process.

The systematic review will analyze the effectiveness of Cinnamon Essential Oil (CEO) in modulating the oral microbiome, focusing on both antibacterial and antifungal properties. It is anticipated that CEO will show variable effectiveness depending on the type of cinnamon used, with potential differences in MIC values reported across studies. The discussion will address the implications of these findings for oral health, taking into account the potential impact of CEO on oral conditions such as halitosis, periodontitis[29, 30], and cavities.

The review will also explore methodological differences across studies, including variations in experimental protocols, which may contribute to inconsistencies in reported results. The potential benefits of using CEO as a natural antimicrobial agent will be weighed against these methodological challenges. The discussion will highlight the need for standardized experimental approaches to improve the reliability of results and provide clearer recommendations for clinical practice.

Conclusion

In accordance with in vitro research, this systematic review will offer a thorough assessment of cinnamon essential oil's (CEO) antimicrobial properties on the oral flora. By synthesizing evidence from various studies, it will offer insights into the effectiveness of CEO in managing oral pathogens and maintaining oral health.

The review will aim to clarify the role of different types of CEO and their concentration levels in inhibiting microbial growth. In order to more fully comprehend the possible therapeutic uses of CEO in oral health care, it will also point out gaps in the existing research and recommend topics for further study. The findings will contribute to the evidence base supporting the use of natural antimicrobial agents in preventing and treating oral diseases.

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Ethics statement: None

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