RESEARCH ARTICLE

Synergistic antibacterial activity of *Salvia officinalis* and *Rosmarinus officinalis L.* extracts against oral pathogens: An In Vitro Study

[version 1; peer review: 1 approved with reservations, 2 not approved]

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v1 **First published:** 18 Feb 2026, **15**:295
https://doi.org/10.12688/f1000research.177155.1
Latest published: 18 Feb 2026, **15**:295
https://doi.org/10.12688/f1000research.177155.1

Abstract

Background

Recent attention has shifted towards alternative herbal mouthwashes due to the long-term unwanted side effects associated with Chlorhexidine, a common oral hygiene agent. These herbal options aim to serve as preventive therapies against dental caries, which are primarily caused by Streptococcus mutans.

Methods

This study evaluated the in vitro antimicrobial activity of ethanolic extracts from Salvia officinalis and Rosmarinus officinalis L. against Streptococcus mutans using the disk agar diffusion method to measure the zone of inhibition. The study assessed both the individual and synergistic effects of the extracts and determined their minimum inhibitory concentration (MIC).

Results

The findings indicated that the 50% ethanolic extracts of both *S. officinalis* and *R. officinalis L.*, as well as their combined application, exhibited significant antimicrobial activity against *Streptococcus*

Open Peer Review

Approval Status ✗ ? ✗

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mutans. The MIC values were 200 µg/mL for *S. officinalis* and 400 µg/mL for *R. officinalis L.* Additionally, higher concentrations of 1600 µg/mL for *S. officinalis* and 3200 µg/mL for *R. officinalis L.* resulted in a 10 mm inhibition of bacterial growth.

Conclusion

These findings suggest that these extracts are promising candidates as natural preventive agents for managing dental caries and promoting oral health.

Keywords
Saliva officinalis, Rosmarinus officinalis, Chlorhexidine, Streptococcus mutans.

This article is included in the Plant Science gateway.

This article is included in the Pathogens gateway.

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Author roles: Kuraitby S: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – Original Draft Preparation; **Nasr M**: Data Curation, Methodology; **Al Hamwi M**: Data Curation, Methodology; **A. Aljoujou A**: Conceptualization, Project Administration, Supervision, Writing – Review & Editing; **Daoud N**: Conceptualization, Writing – Review & Editing; **Hamdan Al Zarzour R**: Conceptualization, Writing – Review & Editing; **Khazem M**: Conceptualization, Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

How to cite this article: Kuraitby S, Nasr M, Al Hamwi M *et al.* **Synergistic antibacterial activity of *Salvia officinalis* and *Rosmarinus officinalis L.* extracts against oral pathogens: An In Vitro Study [version 1; peer review: 1 approved with reservations, 2 not approved]** F1000Research 2026, **15**:295 https://doi.org/10.12688/f1000research.177155.1

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Introduction

Oral health is one of the major global health challenges. Dental caries remains one of the most widespread oral diseases, affecting more than two billion people of all ages.[1] It is attributed to oral infections caused by primary oral pathogens in dental caries, such as *Streptococcus sobrinus* and *Streptococcus mutans.*[2]

The main etiological process in dental caries starts with the biotransformation of fermentable carbohydrates like fructose, glucose, and sucrose by glucosyltransferases, producing dental plaque or biofilm that adheres to the oral surfaces, protects itself within an extracellular environment, and leads to the gradual progression of carious lesions.[3]

This microbial biofilm is composed of various virulence factors, microbial communities, and extracellular polymeric substances (EPS), supported by the acidic microenvironment in addition to the physical barrier of the biofilm to enhance the persistence of the invading bacteria, and augmenting the aggressive development of dental caries.[4] This matrix is considered a shield, preventing the penetration of antimicrobials and stimulating biofilm-associated bacteria over than 1000 times resistant to antibiotics compared with their planktonic counterparts.[5] Therefore, consistent physical methods are generally considered vital for a healthy routine across all ages to control biofilms, mainly through tooth brushing and chemical antimicrobial agents, which are the primary key players in oral disease prevention and treatment. An ideal antibiofilm agent should possess the efficacy to inhibit biofilm formation, minimise the risk of microbial resistance, be safe for oral tissues, and be free of tooth staining, and preferably have an acceptable taste.[6] Nevertheless, to date, no conventional product satisfies all these standards, raising the crucial need for novel agents that might safely and effectively inhibit oral pathogens.[7]

Chlorhexidine (CHX) interacts with the negatively charged microbial membrane, leading to effective cell disruption and preventing biofilm formation.[8] It is the gold standard antiseptic mouthwash. However, despite its efficacy, long-term use of CHX is linked to undesirable side effects, including increased calculus formation, alterations in mucosal tissues, tooth discolouration, and temporary taste disturbances,[9] as well as its non-selective toxicity, which might negatively affect bone and mucosal cells.[10] These drawbacks justify the search for alternative antimicrobial agents. In addition, the increasing microbial resistance of pathogens associated with oral diseases highlights the need for innovative antimicrobial strategies[11]

For centuries, herbal products have been used to prevent and manage dental diseases. One promising approach involves the use of extracts from medicinal plants, which have gained significant attention due to their safe and effective analgesic, antimicrobial, and anti-inflammatory properties. These benefits are attributed to the phytochemical content found in these plants.[12]

Rosmarinus officinalis, from the family Lamiaceae, is an aromatic evergreen plant widely distributed and extensively considered for its phytochemical ingredients.[13] Used in Traditional medicine, it also serves as a favourable food spice, seasoning, and food preserving agent. It exhibits many pharmacological activities, including anti-inflammatory, anti-tumor, antibacterial, antiviral, antithrombotic, antidiabetic, diuretic, and antioxidant effects.[14]

Sage or *Salvia officinalis* from the Lamiaceae family, native to Asia and North Africa, naturally grows in dry stony grounds. Its remarkable therapeutic effects include anti-inflammatory, relaxant, antispasmodic, antiperspirant, and antidiabetic effects.[15]

Considering the increasing popularity of herbal products due to their minimal side effects when used properly, and the complications associated with synthetic mouthwashes, this study aimed to investigate the individual and synergistic effects of 50% ethanolic extracts of Salvia officinalis and Rosmarinus officinalis L. by evaluating their antimicrobial effects.

The null hypothesis of this study is that the synergistic antibacterial activity of Salvia officinalis and Rosmarinus officinalis L. extracts does not significantly influence oral pathogens.

Materials and methods

Study design

The *in vitro* tests were conducted at the Faculty of Dental Medicine, Damascus University.

Ethical approval was obtained from the Damascus University Ethics Committee (Date 2025/No. DN-210224-11-H9). The study adhered to the ethical guidelines outlined in the Declaration of Helsinki.[16] The patient who obtained a human swab was provided written informed consent before enrollment.

Extracts preparation

The aerial parts of *Salvia officinalis* and *Rosmarinus officinalis L.* were collected from the Medicinal Plants Garden at the Faculty of Pharmacy, Damascus University. These plant materials were taxonomically identified by Dr Essa Assaf, Faculty of Pharmacy, Damascus University, Damascus, Syria. Voucher specimens of these plants are deposited in the herbarium of the Faculty of Pharmacy, Damascus University, under the voucher numbers S142 and R170.

After drying in the shade, the plant materials were ground into a fine powder. Next, the extraction was performed using the maceration technique, which involved soaking 100 g of each plant species powder in Ethanol (50%, v/v) obtained from Al-Farabi Pharmaceutical Company (Damascus, Syria). The mixture was placed in a covered flask, shaken at 90 rpm for 72 hours. Afterwards, the resulting solutions were filtered through a 0.5-mm Whatman filter paper. The solvents were then removed under vacuum using a rotary evaporator, and the dried extracts were stored in sterile containers in the freezer at -18 °C.

Isolation and identification of *Streptococcal* bacteria

Swab samples were collected from various regions of the oral cavity of a patient diagnosed with Grade II dental plaque accumulation, as assessed by the Plaque Index (PI), with particular focus on areas with evident plaque buildup.

The samples were inoculated into broth and then streaked onto laboratory-prepared blood agar. Plates were incubated under facultative anaerobic conditions at 37°C for 24 hours.

Identification of isolated bacteria

After incubation, the resulting colonies were subcultured and subjected to a series of identification tests to isolate streptococcal species. The procedures included microscopic inspection, which revealed Gram-positive cocci organized in chains, consistent with the morphological characteristics of streptococci. Catalase testing was performed using hydrogen peroxide (3%). Alpha-hemolysis was observed on the laboratory-prepared blood agar.

To confirm the bacterial identity, API 20 Strep identification strips (bioMérieux, Marcy-l'Étoile, France; catalogue number: 20600) were used according to the manufacturer's guidelines. The results indicated that the isolated strain belongs to *Streptococcus mutans*. The confirmed isolate was presented on tryptic soy agar (TSA) (HiMedia Laboratories, Mumbai, India; catalogue number: M290) for further use (Figure 1).

Determination of the Minimum Inhibitory Concentration (MIC)

To determine the MIC, specific concentrations of *Salvia officinalis* and *Rosmarinus officinalis L.* extracts were prepared by dissolving 300 mg of each extract in 3 mL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA; catalogue number: D2650). The solution was placed in a shaker within a water bath set to 36 °C to ensure complete dissolution. Following this, the extracts were diluted using saline phosphate buffer (HiMedia Laboratories, Mumbai, India; catalogue number: AL007) to achieve the following serial dilutions: 12.5, 25, 50, 100, 200, 400, and 800 µg/mL.

A 0.5 McFarland standard bacterial suspension (bioMérieux, Marcy-l'Étoile, France; catalogue number: 25543) was used. Subsequently, 4 ml of the 0.5 McFarland suspension was added to each test tube, followed by 1 ml of the prepared double-concentration plant extracts.

For the controls, a positive control was established using 1 mL of 0.12% chlorhexidine with fluoride (Sigma-Aldrich, St. Louis, USA; catalogue number: C9394), while a negative control consisted of DMSO mixed with the 0.5 McFarland suspension.

Antibacterial efficacy testing using the diffusion method on Müller-Hinton agar

The agar diffusion method was used to evaluate the antibacterial activity of each extract on Müller-Hinton agar (HiMedia Laboratories, Mumbai, India; catalogue number: M173). Sterile filter paper discs (Whatman No. 1, Sigma-Aldrich, St. Louis, USA; catalogue number: Z375301) were soaked in 50 ml of each extract at concentrations near the minimum inhibitory concentration (MIC) and then placed on the agar surface. The plates were incubated for 48 hours at 37°C. After incubation, the diameters of the inhibition zones (in mm) were measured and recorded. The activity of the mixtures was compared to that of each extract and the positive control (Figure 2).

Results

1. Minimum Inhibitory Concentration (MIC) test

The effect of *Salvia officinalis* and *Rosmarinus officinalis L.* extracts on *Streptococcus mutans* was evaluated using the MIC method. Visual inspection revealed no bacterial turbidity at 200 µg/mL for Salvia officinalis extract and at

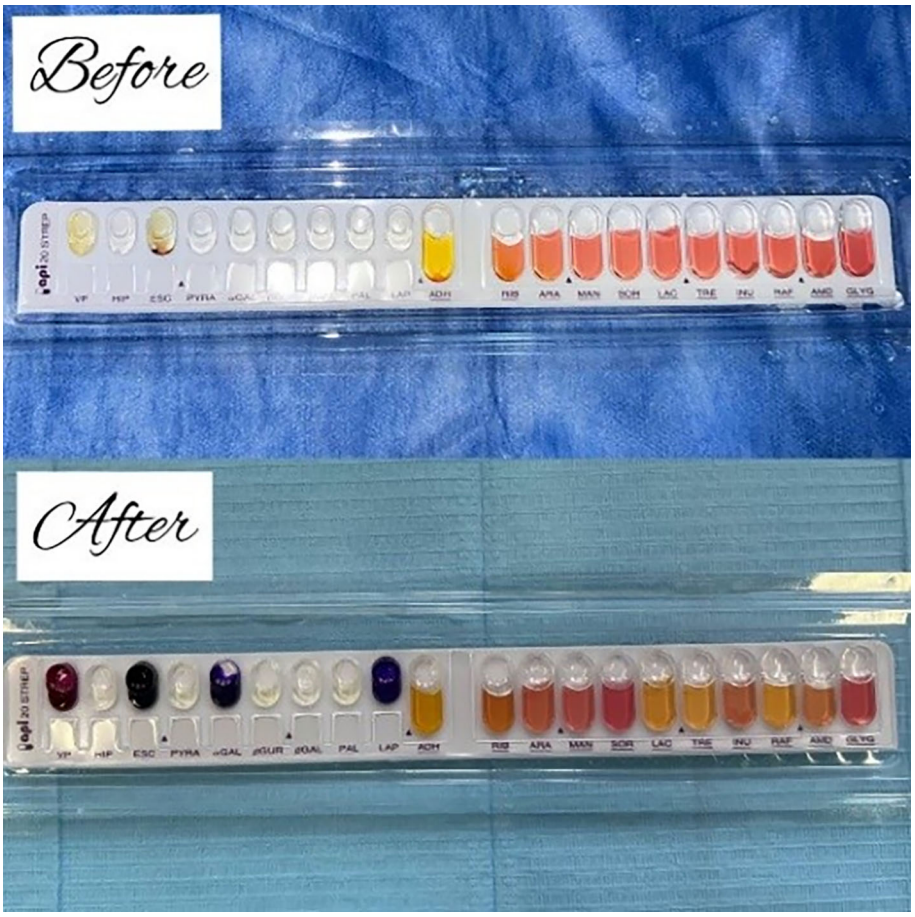

Figure 1. **Identification of bacteria using API 20 Strep biochemical tests.**

Figure 2. **Antibacterial efficacy testing using the diffusion method on Müller-Hinton agar.**

400 µg/mL for *Rosmarinus officinalis* extract. The positive control showed no turbidity, confirming inhibition of bacterial growth, while the negative control exhibited clear turbidity, indicating active bacterial growth.

2. Antibacterial efficacy test

The diameter of the inhibition zones increased proportionally with concentration for both extracts. The results were compared with the positive control containing a standard antibiotic (Tables 1 and 2).

3. Synergistic activity test of *Saliva* and *Rosmarinus officinalis*

The results indicated an enhanced antibacterial effect when both extracts were used together, demonstrating synergistic efficacy against *Streptococcus mutans* (Table 3).

Discussion

Streptococcus mutans is a facultative anaerobic, Gram-positive bacterium recognized for its strong ability to adhere to tooth surfaces. This bacterium metabolizes sucrose, producing lactic acid as a byproduct. The lactic acid lowers the pH in the oral cavity, which erodes tooth enamel. This aciduric nature of *Streptococcus mutans* allows it to thrive in low pH environments. Additionally, S. mutans produces a sticky, insoluble polysaccharide made of dextran, which promotes plaque formation.[17] A significant feature of *Streptococcus mutans* virulence is its capacity to create biofilms on tooth surfaces. This ability is enhanced by the production of exopolysaccharides (EPS), which are generated by glucosyltransferases (Gtfs).[18] Furthermore, two-component systems (TCSs) in S. mutans are essential for detecting and reacting to environmental changes, including the presence of antimicrobial substances. The TCSs comprise two primary components: the Histidine Kinase (HK) and the Response Regulator (RR). The HK, found in the cell membrane, identifies specific environmental cues, such as the presence of antimicrobials, while the RR relays the response within the cell.[19]

Table 1. The diameters of inhibition zones for each concentration of Salvia officinalis.

Results of a diffusion test on Muller-Hinton medium for the effectiveness on *Streptococcus mutans*				**Positive control**
Concentration used of *Salvia officinalis* (µg/mL)				chlorhexidine
200µg/mL	400µg/mL	600µg/mL	800µg/mL	0.12%
The diameters of the inhibition zones (in mm) for each concentration				
-	-	1mm	8mm	15mm

Table 2. The diameters of inhibition zones for each concentration of Rosmarinus officinalis.

Results of a diffusion test on Muller-Hinton medium for the effectiveness on *Streptococcus mutans*				**Positive control**
Concentration used of *Rosmarinus officinalis L* (µg/mL)				chlorhexidine
400µg/mL	800µg/mL	1600µg/mL	3200µg/mL	0.12%
The diameters of the inhibition zones (in mm) for each concentration				
-	-	2mm	3mm	15mm

Table 3. The synergistic activity on Streptococcus mutans.

Results of a diffusion test on Muller-Hinton medium for the Synergistic Activity Test on Streptococcus spp					**Positive control**
Concentration used of *Salvia officinalis* (µg/mL)					Chlorhexidine 0.12%
400	800	800	1600	1600	
Concentration used of *Rosmarinus officinalis L.* (µg/mL)					
800	800	1600	1600	3200	
The diameters of the inhibition zones (in mm) for each concentration					
-	2mm	4mm	6mm	10mm	15mm

Streptococcus mutans plays a significant role in the development of dental caries by forming harmful bacterial biofilms. These bacteria can produce acid within the biofilm, which poses a risk to dental health.[20] Recent research has indicated that infections caused by members of the oral viridans group, such as S. mutans, are responsible for almost 20% of infective endocarditis cases. These bacteria form intricate biofilms that can enter the bloodstream. Furthermore, different therapeutic approaches for addressing dental problems can unintentionally facilitate the transfer of these bacterial pathogens from the mouth into the bloodstream, leading to bacteremia.[21]

Consequently, the growth of streptococci needed to be limited through the use of antimicrobials. Chlorhexidine is regarded as safe for oral use when applied at suitably low concentrations and has become the benchmark for antibacterial mouth rinses, proving effective in diminishing oral biofilm. Chlorhexidine contains positively charged groups that bind to the negatively charged surfaces of bacterial cells, damaging the cell wall and leading to significant leakage of intracellular components. While mild damage to the cell wall and leakage of intracellular material won't result in immediate cell death, such damage will lead to a loss of intracellular pressure, accompanied by slight deformation of the cell wall in adhering bacteria. However, the long-term use of CHX is linked to a lot of undesirable side effects.[22]

Given the ongoing global rise in antimicrobial resistance and the adverse effects associated with long-term CHX use, this study aimed to introduce a natural, plant-based antimicrobial agent for preventing oral diseases. Specifically, the study investigated the effects of extracts from *Salvia officinalis* and *Rosmarinus officinalis L.* on *Streptococcus mutans.* To the best of our knowledge, this study is the first to examine the synergistic antibacterial efficacy of *Salvia officinalis* and *Rosmarinus* officinalis extracts on *Streptococcus mutans* isolated from oral plaque.

In this study, at a concentration of 800 µg/mL, *Salvia officinalis* demonstrated significant effectiveness against streptococcal bacteria, as indicated by an inhibition zone diameter of 8 mm. This antibacterial effect is likely attributed to the essential oil of *Salvia officinalis*, which contains active monoterpenoid compounds such as α-thujone, camphor, 1,8-cineole (eucalyptol), β-myrcene, α-pinene, borneol, and carvacrol—compounds known for their antimicrobial properties.[23]

Previous studies on *Salvia officinalis* have shown that it possesses antimicrobial activity, though it exhibits lower effectiveness against *Candida* species compared to other oils.[24] Another study has shown that Salvia officinalis exhibits antimicrobial effects against bacterial pathogens, including *Staphylococcus aureus* and *Providencia stuartii.* The presence of camphor and camphene contributes to its notable antifungal and antibacterial properties.[25]

On the other hand, at concentrations ranging from 1600 to 3200 µg/mL, *Rosmarinus* officinalis demonstrated significant antibacterial activity, indicated by an inhibition zone diameter of 2-3 mm.

This effect can be linked to the presence of important phenolic compounds in rosemary extracts, such as diterpene derivatives 1,8-Cineole (Eucalyptol), camphor, urosolic acid, carnosic acid, rosmanol, and rosmarinic acid. The differences in phenolic profiles are what contribute to the antimicrobial effects of rosemary extracts.[14] This study concurs with the research conducted by Tsai T-H et al., which showed that a methanolic crude extract of R. officinalis can inhibit the growth of *Streptococcus mutans.* Furthermore, it has been reported that both aqueous and methanolic extracts of R. officinalis possess antimicrobial activity against Streptococcus sobrinus and Streptococcus sanguinis.[26]

To achieve maximum effectiveness and minimize potential side effects from the use of plant extracts in high concentrations, the synergistic action of two extracts at half their typical concentrations was studied. The results indicated that saliva officinalis at a concentration of 1600 µg/mL, combined with rosemary officinalis at a concentration of 3200 µg/mL, resulted in an inhibition halo diameter of 10 mm for bacterial growth. This was a more effective outcome than using each extract individually. Furthermore, this result was comparable to the commercial chlorhexidine solution used as a positive control, which produced an inhibition halo diameter of 15 mm.

The combined antibacterial effects prevent the proliferation of *Streptococcus mutans* by disrupting bacterial cell membranes, leading to leakage of cellular contents and subsequent cell death. Additionally, it inhibits glucosyltransferase activity, reducing the production of extracellular polysaccharides essential for biofilm formation. Furthermore, *Salvia officinalis* destabilises bacterial membranes, disrupts energy metabolism, and reduces acid production in *S. mutans.*[27,28]

Conclusion

Laboratory results from this study indicate that extracts of Salvia officinalis and Rosmarinus officinalis exhibit vigorous synergistic antibacterial activity against Streptococcus mutans. When used together, these extracts are as effective as chlorhexidine. This finding suggests that these plants may serve as viable and safe alternatives for controlling dental

biofilm. However, further clinical evidence is necessary to evaluate the effectiveness of these extracts at appropriate, secure, and effective concentrations.

Ethics approval statement

The study protocol received approval from the Damascus University Ethics Committee (Date 2025/No. DN-210224-11-H9). The patient who obtained a human swab was provided written informed consent before enrollment.

Data availability statement

All datasets required to reproduce the results reported in this article are included.

Zenodo: Synergistic antibacterial activity of Salvia officinalis and Rosmarinus officinalis L. extracts against oral pathogens: An in vitro Study. https://doi.org/10.5281/zenodo.18444254.[29]

The datasets include:

- Minimum Inhibitory Concentration (MIC) values for Salvia officinalis and Rosmarinus officinalis L. against Streptococcus mutans.
- Diameters of inhibition zones (in mm) for each extract at all tested concentrations, as reported in Tables 1 and 2.
- Results of the synergistic activity test combining both extracts, as reported in Table 3.
- Any replicates performed for each measurement.

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Publisher Full Text

Open Peer Review

Current Peer Review Status: ✗ ? ✗

Version 1

Reviewer Report 24 April 2026

https://doi.org/10.5256/f1000research.195328.r473714

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Overall assessment

The authors have chosen a worthwhile question. Replacing chlorhexidine with a safer, plant-based alternative is a genuinely useful aim, and an in-vitro screen of *Salvia officinalis* and *Rosmarinus officinalis* L. against *Streptococcus mutans* is a reasonable place to start. Unfortunately, several weaknesses in how the study was designed, executed, and reported undermine the conclusions the authors wish to draw.

The first difficulty is that the work rests on a single clinical isolate from a single patient. There is no reference strain (such as ATCC 25175 or UA159) to anchor the results, no replicate isolates to gauge strain-to-strain variation, and no mention of how many biological or technical replicates were performed. As a consequence, neither reproducibility nor generalizability can be judged from what is presented.

The second concern is statistical. A null hypothesis is stated at the end of the Introduction, but it is never actually tested. Tables 1–3 give single point values for zone diameters, with no standard deviations, no confidence intervals, and no comparative tests across concentrations or between extracts. A stated hypothesis that receives no formal evaluation is a gap the revision will need to close.

A third problem is more fundamental, because it touches the central claim. The word "synergistic" appears in the title, the abstract, and the conclusion, yet synergy itself is never operationalized. No checkerboard assay, no Fractional Inhibitory Concentration Index (FICI), and no time-kill experiment were performed. What the data actually show is that a combination of the two extracts produces a larger inhibition zone than either extract alone a result that is fully compatible with simple additivity, or even indifference, and that does not meet the accepted pharmacological definition of synergy.

There is also a passage in the Methods that needs clarification. The disk-diffusion protocol states that paper disks were "soaked in 50 mL" of extract. For a standard 6 mm Whatman disk the physical uptake is on the order of 10–20 µL, so 50 mL is physically implausible. This is very likely a unit typographical error (µL for mL), but as written the procedure departs sharply from CLSI M02 and cannot be reproduced.

A related, material-level issue is that the extracts themselves are not characterized. No extraction

yield, no total phenolic content, no HPLC or GC-MS fingerprint is reported. Without at least a minimal phytochemical profile, the active material being tested is effectively undefined, and any future laboratory would be unable to prepare a comparable preparation.
Finally, the comparison with chlorhexidine which is the rhetorical anchor of the paper does not survive close reading. The 0.12 % CHX positive control (roughly 1,200 µg/mL of active agent) produced a 15 mm zone; the combined herbal treatment produced a 10 mm zone at a total concentration exceeding 4,000 µg/mL. The herbal combination is therefore both less potent and requires several-fold more material, which does not support the manuscript's claim that the extracts are "as effective as chlorhexidine."
These concerns overlap in places with the two previously published reviewer reports, but they extend further in several respects: in pressing for a formal FICI-based synergy analysis, in quantifying the asymmetry of the chlorhexidine comparison, in flagging the absence of replicates and dispersion statistics, and in noting internal numerical inconsistencies between the Abstract, Results text, and Tables 1–3. None of these concerns is fatal in principle they can be addressed in a revision but collectively they presently prevent the central claims of the paper from being considered scientifically sound.

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1. Ajdić D, McShan W, McLaughlin R, Savić G, et al.: Genome sequence ofStreptococcus mutans UA159, a cariogenic dental pathogen. *Proceedings of the National Academy of Sciences*. 2002; **99** (22): 14434-14439 Publisher Full Text
2. Balouiri M, Sadiki M, Ibnsouda S: Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 2016; **6** (2): 71-79 Publisher Full Text
3. Heinrich M, Jalil B, Abdel-Tawab M, Echeverria J, et al.: Best Practice in the chemical characterisation of extracts used in pharmacological and toxicological research—The ConPhyMP—Guidelines12. *Frontiers in Pharmacology*. 2022; **13**. Publisher Full Text
4. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Disk Susceptibility Tests. *CLSI standard M02, 14th ed., Wayne, PA: CLSI*. 2024.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral microbiology, antimicrobial susceptibility testing methodology, phytochemistry reporting standards , biostatistics for in-vitro comparative assays, Orthodontics, nanotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 20 April 2026

https://doi.org/10.5256/f1000research.195328.r473718

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Sarmad S. Salih Al Qassar
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Reviewer Report

The manuscript presents an interesting and clinically relevant investigation into the synergistic antibacterial effects of *Salvia officinalis* and *Rosmarinus officinalis* extracts against *Streptococcus mutans*. The topic is timely, particularly in light of the increasing demand for natural alternatives to conventional antimicrobial agents such as chlorhexidine. The introduction is generally well-organized and provides a solid background on dental caries, biofilm formation, and the limitations of existing chemical agents. However, the research gap should be more explicitly articulated, particularly emphasizing what distinguishes this study from prior work on herbal antimicrobials beyond the concept of synergy.

From a methodological standpoint, the study design is acceptable for an in vitro investigation, but several important limitations must be addressed. The use of a **single clinical isolate** of *S. mutans* significantly limits the generalizability of the findings; inclusion of multiple isolates or a standard reference strain is strongly recommended. Additionally, the manuscript does not clearly report the number of experimental replicates, which raises concerns about reproducibility and statistical reliability. The description of the agar diffusion method also requires clarification, particularly regarding the reported volume of extract used for disc impregnation, which appears inconsistent with standard protocols.

The results demonstrate a dose-dependent antibacterial effect and suggest a synergistic interaction between the two plant extracts. While this is a strength of the study, the **lack of statistical analysis** for inhibition zone comparisons and MIC values is a significant weakness. The inclusion of appropriate statistical tests (e.g., ANOVA or non-parametric equivalents) is necessary to support the conclusions. Furthermore, while the comparison with chlorhexidine is relevant, the statement that the herbal combination is “as effective” as chlorhexidine appears overstated and should be interpreted more cautiously.

The discussion provides a reasonable explanation of the antimicrobial mechanisms based on

phytochemical constituents; however, it would benefit from deeper critical analysis rather than descriptive repetition of known mechanisms. Importantly, the manuscript would be strengthened by integrating additional relevant literature to better contextualize the findings. In this regard, the authors are encouraged to incorporate the following studies:

- Evaluation of antiseptic mouthwashes protocol against SARS-CoV-2 on orthodontic appliances (2024), which provides valuable insight into the effectiveness and limitations of conventional antimicrobial mouthwashes in controlling oral biofilms. This reference can support the rationale for exploring alternative antimicrobial agents.
- Assessment of the mechanical properties and antimicrobial efficiency of orthodontic adhesive modified with Salvadora Persica oil (2024), which is particularly relevant as it highlights the potential of plant-derived compounds in enhancing antimicrobial performance in dental applications, thereby strengthening the justification for the current study.
- Durability of different types of mouthwashes on the salivary buffering system in orthodontic patients (2021), which may be used to support the discussion on oral environmental factors such as salivary pH and their influence on antimicrobial efficacy.

Several limitations should also be more explicitly acknowledged, including the in vitro nature of the study, the absence of cytotoxicity or biocompatibility assessment, the lack of extract standardization, and the use of a single-species biofilm model rather than a multispecies oral biofilm. Addressing these points will improve the scientific rigor and clinical relevance of the manuscript.

In conclusion, the study presents promising preliminary findings; however, **revisions are required**. Strengthening the methodology, incorporating statistical analysis, refining the discussion, and integrating relevant literature will significantly enhance the quality and impact of the manuscript.

Suggested References (Full Citations)

Refer to reference no. 1-3

References

1. Alkasso I.R., Salih Al Qassar S.S., Taqa G.A.: Durability of different types of mouthwashes on the salivary buffering system in orthodontic patients. *Dentistry 3000, 9(1), A1*. 2021.

2. Abdulhaddi A., Salih Al Qassar S.S., Mohammed A.M.: Assessment of the mechanical properties and antimicrobial efficiency of orthodontic adhesive modified with Salvadora Persica oil. *Romanian Journal of Stomatology, 70(2), 153–159*. 2024.

3. Al Qassar S.S.S., Qibi L.H., Qasim A.A., Dawood H.M.: Evaluation of antiseptic mouthwashes protocol against SARS-CoV-2 on orthodontic appliances (an in vitro study). *Brazilian Dental Science, 27(2), e4196*. 2024.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Orthodontics, biomaterial,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 17 March 2026

https://doi.org/10.5256/f1000research.195328.r460748

Santi Chismirina
Universitas Syiah Kuala, Banda Aceh, Indonesia

General comment
Overall, this study is noteworthy since it combines two plants from the same genus (Lamiaceae) that may work in concert to prevent dental caries. This article discusses the potential of natural materials as herbal mouthwash substitutes, which is relevant to current oral health research trends. However, the variables being studied are really simple. The statistical analysis and extraction method must also be enhanced to ensure the accuracy of the data supplied. As such, the recommendations are for evaluation and improvement. Overall, this work has potential; nevertheless, it requires a more robust methodology, more research variables, and a more comprehensive data analysis.

Abstract
The material is already described in the abstract, which includes a succinct problem description (side effects of CHX) and a remedy (herbs). It is necessary to include the extraction method type, though. Additionally, if the quantities of secondary metabolites (flavonoids, tannins, and terpenoids) that are responsible for antibacterial activity are examined, the extract should be characterized. Provide a more detailed list of the quantitative results, particularly the test material's MIC value in comparison to the positive control. Describe the primary implications of your research findings in the conclusion. Herb and bacterial names should be italicized.

Introduction

This structured background provides a comprehensive and accurate overview of the urgency of developing new oral care agents, supported by pharmacological evidence regarding the antimicrobial activity of both plants. In the introduction, the authors also balance the evidence of CHX's efficacy as a gold standard with its limitations (side effects and potential resistance). Suggestions for the authors include ensuring prevalence figures using the latest WHO data.

Methode
In the extract preparation process, explain in detail:

- The drying process includes the time and procedure for drying the herbal ingredients.
- The procedure for grounding the plant materials into a fine powder (amount of plant material, equipment used, equipment speed, duration).
- The amount of solvent, duration, and temperature used for soaking the herbs.
- The duration and temperature used during evaporation.

In the Isolation and Identification of Streptococcal Bacteria process, clarify the name of the broth media used.

In the identification process of isolated bacteria, the catalase test only states "Catalase testing was performed..."; it would be better to add the result (e.g., "...catalase negative..."). Furthermore, the API 20 Strep is a good tool, but *S. mutans* requires additional confirmation (such as PCR for the gtfB gene) to definitively differentiate it from *S. sobrinus* within the mutans streptococci group. If this is only a preliminary study, the API 20 Strep is sufficient.

The methodology for determining the Minimum Inhibitory Concentration (MIC) is comprehensive; nonetheless, the descriptions of dilution and control require technical enhancement. To confirm that DMSO has no effect on bacterial growth, add a "inoculum only" treatment as a growth control (without DMSO) and make sure the total volume calculation in the tube is consistent (the double-concentration dilution step needs to be clarified). The description of the negative control is not specific enough. It should be written as: "The negative control consists of a solvent (DMSO) and media/bacteria (without extract)". Ensure that the DMSO used does not inhibit bacterial growth at the highest concentration used. Ensure that the volume of the positive (Chlorhexidine) and negative control is the same as the treatment volume (1 mL extract + 4 mL bacteria). There is no explanation regarding how the combination of Salvia and Rosmarinus was prepared? (1:1, 1:2 ratio, or vice versa), also the Synergy Analysis Method used. Does it use the Fractional Inhibitory Concentration Index (FICI) to determine synergism, additivity, or antagonism?

In antibacterial efficacy testing using the Müller-Hinton agar diffusion method, the method is valid in principle, but the immersion volume (to microliters/µl) and incubation time (reduced) need to be improved to meet microbiological standards. The description written by the authors states "soaked in 50 ml". This is too much. Standard disk diffusion methods usually use small volumes (e.g., 10–20 µl) dripped onto the disk or immerse the disk in very small volumes to ensure the disk is not damaged. In terms of incubation time, for Mueller-Hinton agar and fast-growing bacteria (such as Streptococcus mutans), CLSI standards typically require 16-18 hours, or a maximum of 24 hours for certain microorganisms, rather than 48 hours. Excessive incubation can produce inaccurate inhibition zones due to overgrowth or degradation of the active ingredient. If incubation is carried out for 48 hours, explain the reason in the results.

Result

To make it simpler for readers to see the variations, please increase the resolution of Figures 1 and 2, particularly Figure 1, before and after they are arranged sequentially (parallel) without large spaces or gaps. There are more subtitles for Figure 2. A, B, and C (A....., B...., and C...., respectively)

Discussion
To ensure that the inhibitory zone results are adjusted for the disc diameter (usually 6 mm), Tables 1 and 2 are transferred to the results. There is no comparison of the findings with earlier research on the active ingredients in the two plants in the discussion. Additionally, an explanation of the biological pathway processes underpinning the synergy between the two herbs, leading to larger therapeutic effects, is required, as is a description of the effects of S. officinalis and R. officinalis separately and in combination. It is also recommended that the authors make clear the limitations of the study in the discussion.

Conclusion
The conclusion concisely addresses the research objectives and does not repeat the discussion.

Reference
Some references are outdated (more than 10 years old); please update with more recent studies.

Recommendation
Revised and reviewed. Overall, this article has potential but requires strengthening the methodology, more detailed data analysis, and additional research variables.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Microbiology and Immunology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.