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Original Article

Antimicrobial synergy of *Salvadora persica*, clove, and propolis against oral pathogens: Relevance to dental applications

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ABSTRACT

Background: The use of natural plant extracts to combat pathogenic bacteria offers a promising approach to preventing microbial resistance. This study explores the synergistic antimicrobial effects of *Salvadora persica*, propolis, and clove extracts against *Candida albicans* and *Streptococcus mutans*.
Materials and Methods: This was an *in vitro* experimental study designed to evaluate the antimicrobial activity and synergistic interactions of ethanol extracts of *S. persica*, clove, and propolis against *S. mutans* and *C. albicans* using agar dilution and checkerboard assays. The chemical composition of the extracts was analyzed using Soxhlet and accelerated solvent extraction methods, followed by gas chromatography–mass spectrometry analysis.
Results: The minimum inhibitory concentration values for *S. persica*, clove, and propolis against *S. mutans* were 4.5 mg/ml, 9 mg/ml, and 2.2 mg/ml, respectively, and for *C. albicans*, 4.5 mg/ml for *S. persica* and clove, and 1.1 mg/ml for propolis. Combinations of these extracts demonstrated synergistic effects with fractional inhibitory concentration indexes ranging from 0.3 to 1.5 against both pathogens.
Conclusion: These results suggest that combining plant extracts with propolis may offer an effective strategy for treating oral infections. Given the role of *S. mutans* in dental caries, these findings support the potential use of these natural compounds in preventive and therapeutic dental applications.

Key Words: Antibacterial effect, *Candida albicans*, medicinal plants, *Streptococcus mutans*, synergistic effects

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INTRODUCTION

Dental infections pose significant challenges for health care, commonly originating from bacterial invasion of the dental pulp and potentially spreading to surrounding tissues.[1] These infections also affect the gums, causing conditions such as gingivitis, which can progress to periodontal disease. Dental plaque, a complex biofilm formed on tooth surfaces, plays a central role in plaque-induced gingivitis by harboring bacteria.[2-6] Key contributors to dental caries include Gram-positive bacteria such as *Streptococcus mutans* and the opportunistic yeast *Candida albicans*, which can demineralize tooth enamel. *C. albicans* is notable for its ability to switch between yeast and hyphal forms and form antifungal-resistant biofilms.[7,8]

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S. mutans is a key contributor to dental caries, forming biofilms on tooth surfaces and metabolizing dietary carbohydrates into lactic acid. This acid lowers the pH of dental plaque, leading to enamel demineralization and the onset of tooth decay.[9,10] Effective prevention and management of plaque-induced gingivitis requires controlling the pathogenic microorganisms within dental plaque, typically through mechanical cleaning methods such as brushing and flossing, combined with antimicrobial agents in oral hygiene products. However, current treatments can be costly and less accessible, especially in developing regions, and may cause side effects.[11,12] The growing resistance among bacteria and fungi to conventional drugs highlights the urgent need for alternative therapies, including the use of medicinal plants and natural substances that offer safer, more affordable antimicrobial options.[11,12]

Antimicrobial resistance poses a critical global health threat, potentially leading to 10 million deaths annually by 2050 and substantial economic costs.[13] This crisis has driven the search for new therapeutic agents with fewer side effects, including medicinal plants and natural compounds known for their antimicrobial, anticancer, and anti-inflammatory properties.[14] Phytochemicals such as flavonoids, phenols, saponins, tannins, and terpenoids disrupt microbial cell structures and inhibit fungal growth.[15,16]

Propolis, a resinous substance produced by honeybees, is notable for its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative pathogens, alongside immunomodulatory, antitumor, anti-inflammatory, antioxidant, antiviral, antifungal, and antiparasitic effects. It is generally considered safe and less toxic than synthetic drugs.[17,18]

Salvadora persica (miswak) is recognized for preventing dental caries and gum inflammation, with the World Health Organization endorsing its use for oral hygiene. Clove extract, derived from *Syzygium aromaticum*, is traditionally used in dental care for its antiseptic and analgesic properties and shows efficacy against oral bacteria, as well as antifungal, antioxidant, anticancer, and other health benefits. Eugenol, clove's main active compound, contributes to these effects.[19]

Although the individual antimicrobial activities of *S. persica*, propolis, and clove extracts are well-studied, the combined antimicrobial effects have not been explored. Interactions between bioactive compounds can result in synergistic, additive, neutral, or antagonistic effects, warranting investigation.[19]

Based on recent findings regarding the individual effects of these extracts, the present study aimed to investigate the combined antimicrobial activity of *S. persica*, clove, and propolis against *C. albicans* and *S. mutans*. The study also sought to determine the optimal concentration of these extracts for the formulation of a mouthwash with the most potent antimicrobial properties.

MATERIALS AND METHODS

Study design

This research was conducted as an *in vitro* experimental study aimed at evaluating the antimicrobial properties and synergistic effects of ethanol-based extracts of *S. persica*, clove, and propolis against two oral pathogens: *S. mutans* and *C. albicans*. The study employed agar dilution methods to determine minimum inhibitory concentrations (MICs) and a three-dimensional checkerboard assay to assess synergistic interactions.

Materials

S. persica and clove were obtained from the Tehran medicinal plants market and subsequently taken to the herbarium at the Faculty of Pharmacy, Tehran University of Medical Sciences, for identification. The specimens of *S. persica* and *S. aromaticum* were assigned herbarium codes of PMP-1375 and PMP-571, respectively. Propolis was collected from the Alborz Beekeepers' Cooperative Company and authenticated with a high-quality code (PMP-1819) from the Iranian Plant Protection Research Institute.

Bacterial strains

Two reference strains, *C. albicans* ATCC 10231 and *S. mutans* ATCC 35668, were used in this study. *S. mutans* was cultured on brain–heart infusion (BHI) agar at 37°C for 24 h, while *C. albicans* was cultured on Sabouraud dextrose agar (SDA) at 25°C for 48 h. All culture media were obtained from Merck Co. (Germany).

Preparation of extracts

To prepare the ethanol extract of *S. persica*, 300 g of powdered stems were subjected to Soxhlet extraction with 70% v/v ethyl alcohol using a Soxhlet extractor for 10 h or until the solvent turned clear and colorless. The extract was concentrated using a rotary vacuum evaporator at 40°C to obtain

a concentrated extract, which was freeze-dried for further use.[20] The gallic acid content in the extract of *S. persica* was quantified using a ultraviolet (UV) spectrophotometer (Shimadzu, Japan) and compared to a standard gallic acid solution.

Clove fruits (250 g) were ground into smaller particles using a grinder and subsequently transferred to a percolator for extraction with 70% ethanol at room temperature. The plant powder was subjected to multiple rounds of exposure to the solvent, with each exposure lasting 24, 48, or 72 h. The extracts were then concentrated using a rotary evaporator (Heidolph, Germany) at a temperature of 30°C under low vacuum pressure. The concentrated extract was completely dried, and the final weight of the total extract was recorded.[21] The eugenol content in the extract of clove was quantified using gas chromatography-flame ionization detector (GC-FID), and the results were standardized against an eugenol standard chart.

In order to increase the yield of bioactive compounds from *S. persica* and clove, the accelerated solvent extraction (ASE) method was employed for the extraction. The extraction was performed using an ASE (Thermo Fisher Scientific, USA) system, which is a solid-liquid extraction method designed to efficiently extract analytes from solid matrices under elevated temperature and pressure. Extraction was carried out at a temperature of 25°C and a pressure of 10–15 MPa. The extraction solvent ethanol (70%) was used in accordance with the standardized procedure for optimal compound recovery.[22]

The propolis was submerged in 96% ethyl alcohol in a sealed glass container for 12 days at 25°C with occasional shaking. The ethanolic extract was filtered through Whatman filter paper no. 4 every 4 days and then fresh 70% ethyl alcohol was added for 4 days at 25°C with occasional shaking. The extract was then filtered and evaporated under a laminar hood. The quercetin content in the extract of propolis was quantified using a UV spectrophotometer and compared to a standard quercetin solution.

Quantitative determination of free gallic acid, eugenol, and quercetin

GC-FID (Santa Clara, CA, USA) was employed to standardize clove extract using an eugenol standard. The analysis was performed on an Agilent 7890 GC equipped with an HP-5 GC column (30 m × 0.32 mm × 0.25 µm) from Agilent Company. The oven temperature was shown in Table 1. Nitrogen was used as the carrier gas. Injection was performed in the split mode ratio of 1:60. The injector temperature was set to 250°C. To assess the accuracy and precision of the proposed method and to validate the test method, the International Council for Harmonisation of Technical Requirements (ICH) analytical validation guidelines were employed.[23] A calibration curve for the eugenol standard substance was drawn over the concentration range of 100–10000 µg/ml. The calibration equation was determined by analyzing the relationship between the area under the curve and the concentration of the eugenol standard.

The standardization of the propolis ethanolic extract was performed using quercetin as a standard, with the assistance of a UV spectrophotometer at λmax of 510 nm. The concentration range of quercetin was 100–1000 µg/ml.

Gallic acid standard was used to standardize the *S. persica* extract. To draw a calibration curve using a UV spectrophotometer, concentrations of gallic acid ranging from 8 to 75 mg/ml at a wavelength of 756 nm were used.

In vitro evaluation of antibacterial activity by agar dilution method

Determination of minimum inhibitory concentration of propolis, clove, and Salvadora persica extracts

The agar dilution method was used for the determination of MIC of propolis, *S. persica*, and clove ethanolic extracts. The bacterial suspensions of *S. mutans* and *C. albicans* were diluted with normal saline and adjusted photometrically to a uniform suspension equivalent to 1×10^7 CFU/ml.

Two-fold dilution of the extracts was prepared in 1 ml of a solution containing dimethyl sulfoxide (DMSO) at a ratio of 5:2:3 with ethanol and water. Each dilute was added to 9 ml of molten SDA for *C. albicans* and 9 ml of BHI agar for *S. mutans* to give the final concentrations of 18–0.5 mg/ml. The SDA plates were spot-inoculated with 3 µL of *C. albicans* suspension, and BHI agar plates were spot-inoculated with 3 µL

Table 1: The oven temperature of gas chromatography-flame ionization detector to standardize clove extract

	Rate (°C/min)	Value (°C)	Hold time (min)	Run time (min)
Initial	-	100	0.5	0.5
1 ramp	5	150	5	15.5
2 ramp	10	200	5	25.5

of *S. mutans* suspension, including a control plate containing 1 ml DMSO (5):ethanol (2):water (3), without any antibacterial agent. The plates containing bacteria were incubated at 30°C–35°C for 24 h and those containing fungi were incubated at 20°C–25°C for 48 h. The MIC was determined as the lowest concentration of the agent that completely inhibits visible growth of the microorganisms. All experiments were repeated three times on different days.

Screening for synergistic interactions of three extracts

Synergistic effects of ethanolic extract combinations were evaluated with a three-dimensional checkerboard assay. The concentration range of the extracts utilized in the checkerboard assay was such that the dilution range encompassed the sub-MIC concentrations for each extract. For the checkerboard assay, the agar dilution method was employed using standard Petri dishes (10 cm diameter) and Mueller-Hinton (MH) agar medium for *S. mutans* and SDA for *C. albicans*. A series of two-fold dilution of each extract in sub-MIC range was prepared in DMSO (5):ethanol (2):water (3). Then, 0.1 ml aliquots of each sub-MIC concentration of three extracts were transferred to an empty sterile Petri dish and mixed thoroughly with 9.7 ml of a suitable molten agar medium to final volume of 10 ml. Therefore, different combinations of three extracts were prepared in petri dishes. The SDA plates were spot-inoculated with 3 µL of *C. albicans* suspension, and BHI agar plates were spot-inoculated with 3 µL of *S. mutans* suspension. The plates containing bacteria were incubated at 30°C–35°C for 24 h, and those containing fungi were incubated at 20°C–25°C for 48 h. The fractional inhibitory concentration (FIC) was determined for the plates in which visible growth of *S. mutans* in BHI agar and *C. albicans* in SDA agar was inhibited using the given equation, which involves summing the individual FICs for each extract in the plate:

FIC index = (MIC of clove extract in combination/MIC of extract alone) + (MIC of *S. persica* in combination/MIC of extract alone) + (MIC of propolis extract in combination/MIC of extract alone). Synergy was defined as ∑FIC ≤0.5; partial synergism: 0.5< ∑FIC ≤0.75; additivity as 0.75< ∑FIC ≤1, indifference as 1< ∑FIC ≤4, and antagonism as ∑FIC >4.[24-26]

Statistical analysis

Differences between groups were analyzed using one-way analysis of variance, followed by an appropriate *post hoc* test, performed with GraphPad Prism software version 5.4 (GraphPad Software, Inc. La Jolla, California, United States). $P < 0.05$ was considered statistically significant.

RESULTS

Quantitative determination of free gallic acid, eugenol, and quercetin

The yield obtained from clove extraction via the percolation method was found to be 22%. In comparison, the extraction efficiency achieved with the ASE method was calculated to be 24.04%, which is relatively similar to that of the percolation method ($P < 0.05$). The efficiency of the percolation method for extracting the extract from *S. persica* was calculated to be 7.6%, which was adopted due to the low efficiency of the ASE method for extraction. The efficiency of this extraction method was calculated to be 12.61%, which was a better efficiency value than that of the percolation method. The extraction efficiency of propolis was determined to be 82.85%, indicating a high level of efficiency.

A calibration curve for the eugenol standard substance was drawn over the concentration range of 1000–10000 µg/ml, and standard curves were obtained by plotting the data in Supplementary Figure 1. The average results obtained from the calibration curve showed that the amount of eugenol in 1 ml of clove extract is equal to 608.97 µg.

The calibration curve for gallic acid at a wavelength of 765 nm [Supplementary Figure 1] indicated that each ml of *S. persica* extract contains 61.41 µg of gallic acid. The standard calibration curve for quercetin was plotted at a λmax of 510 nm, with concentrations ranging from 100 to 1000 µg/ml [Supplementary Figure 1]. The findings revealed that each ml of propolis extract contains 1.125 mg of quercetin.

Minimum inhibitory concentrations of single antimicrobial substances

The MICs of propolis, *S. persica*, and clove extracts against *C. albicans* and *S. mutans* are shown in Table 2. The MIC of propolis against *C. albicans* was 1.1 mg/ml, indicating strong antimicrobial activity. The MICs of *S. persica* and clove against *C. albicans* were 4.5 mg/ml. The MIC of propolis against *S. mutans* was 2.2 mg/ml, while those for *S. persica* and clove were 4.5 and 9 mg/ml, respectively.

Investigating the effect of different concentrations of *Salvadora persica*, clove, and propolis extracts against *Streptococcus mutans*

The antibacterial activity of individual and combined ethanolic extracts was evaluated by agar dilution assay. Concentrations resulting in no bacterial growth are shown in Figure 1.

Supplementary Table 1 presents the total FIC values for combinations tested against *S. mutans*. The best combination for inhibition was clove at MIC/16 + propolis at MIC/4 + *S. persica* at MIC/8, which showed the lowest total FIC.

Figure 2 illustrates the synergistic and partial synergistic effects of the extract combinations against *S. mutans*.

Investigating the effect of different concentrations of *Salvadora persica*, clove, and propolis extracts against *Candida albicans*

The possible synergistic antifungal interactions of these extracts were evaluated by broth microdilution assay [Figure 3]. Supplementary Table 2 shows the FICs of their combinations against *C. albicans*.

Figure 4 depicts synergistic and partial synergistic combinations against *C. albicans*. The most effective combination was clove MIC/8 + propolis MIC/8 + *S. persica* MIC/8 with the lowest total FIC.

Table 2: Minimum inhibitory concentration values of *Salvadora persica*, clove, and propolis against *Candida albicans* and *Streptococcus mutans*

Extracts	MIC values (mg/mL); Microbial strains	
	Streptococcus mutans	*Candida albicans*
Clove	9	4.5
Salvadora persica	4.5	4.5
Propolis	2.2	1.1

MIC: Minimum inhibitory concentration

DISCUSSION

The extraction yields indicated comparable efficiencies between percolation and ASE for clove, while ASE performed better than percolation for *S. persica*, likely due to its optimized temperature and pressure. Propolis demonstrated the highest extraction efficiency, consistent with its complex resinous matrix.

Eugenol quantification by GC-MS confirms its role as the main active compound in clove extract and justifies its use as a standard. Similarly, gallic acid and quercetin served as reliable marker compounds for *S. persica* and propolis, respectively, reflecting phenolic content and biological activity.

MIC results align with prior studies showing propolis's potent antifungal and antibacterial effects. Its MIC values against *C. albicans* and *S. mutans* were lower compared to *S. persica* and clove, demonstrating superior efficacy. Clove and *S. persica* exhibited moderate activity, supporting their traditional antimicrobial usage.

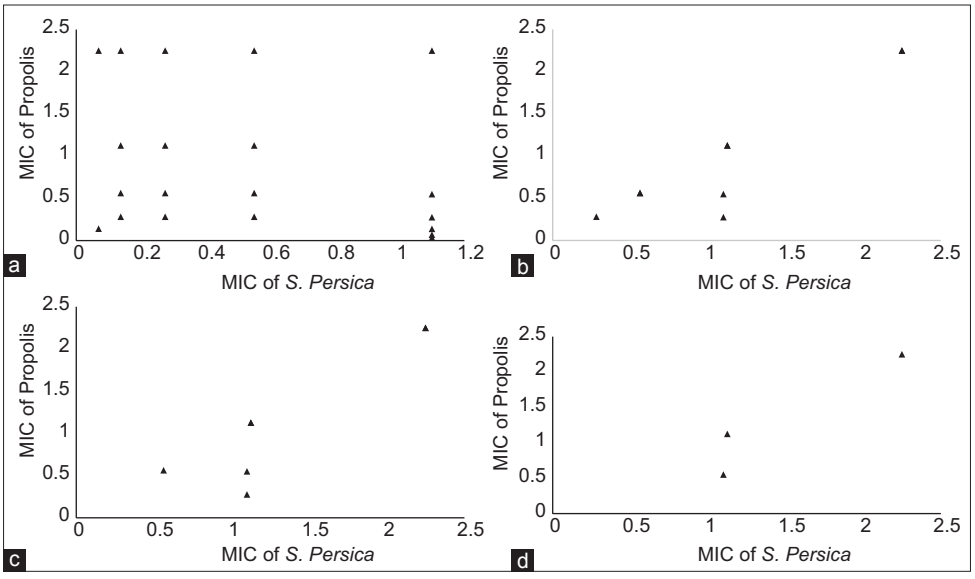

Figure 1: Various sub-minimum inhibitory concentration (MIC) concentrations of *Salvadora persica*, propolis, and clove extracts inhibited the growth of *Streptococcus mutans* bacteria. (a) The concentration of clove extract is constant (MIC/2). (b) The concentration of clove extract is constant (MIC/4). (c) The concentration of clove extract is constant (MIC/8). (d) The concentration of clove extract is constant (MIC/16). MIC: Minimum inhibitory concentration, *S. persica: Salvadora persica.*

The synergistic antibacterial effects observed in the combination assays against *S. mutans* likely result from complementary mechanisms of action of individual extracts. Clove essential oil, predominantly eugenol (70%–90%), disrupts bacterial membranes and shows broad-spectrum antimicrobial properties, including against antibiotic-resistant strains.[27] *S. persica*, known for its antimicrobial secondary metabolites, may enhance these effects via synergistic interaction with chitosan nanoparticles or other extracts.

Propolis exhibits broad-spectrum antibacterial effects, particularly against Gram-positive bacteria like *S. mutans*, attributed to its flavonoids and phenolic acids which disrupt microbial membranes and cellular functions.[27] Geographical variation affects propolis efficacy, with samples from the Middle East demonstrating robust activity.[28]

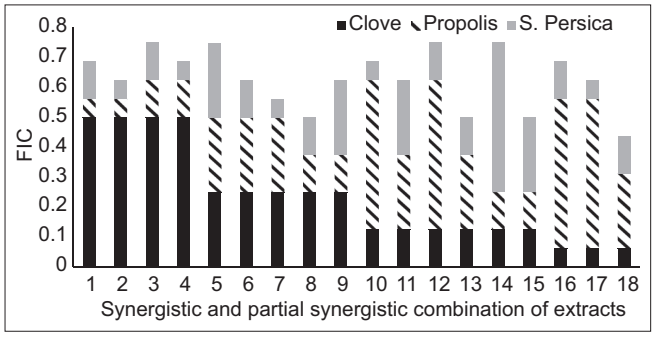

Figure 2: Total fractional inhibitory concentration of combination of *Salvadora persica*, clove, and propolis extracts against *Streptococcus mutans*. FIC: Fractional inhibitory concentration.

Against *C. albicans*, propolis showed significant antifungal activity, as supported by its bioactive compounds inducing fungal cell death.[27] *S. persica* also exerted antifungal effects, although more pronounced against *Aspergillus* species than Candida.[27] Clove extract's antifungal properties are primarily due to eugenol, which damages fungal membranes, reduces ergosterol synthesis, and, when combined with other essential oils, may overcome resistance.[28,29]

The observed synergistic antifungal activity among the extracts may be leveraged to develop novel, natural therapeutic agents for oral fungal infections.[28]

CONCLUSION

Natural plant-derived compounds have emerged as promising substitutes for traditional antibiotics, particularly in managing infections caused by antibiotic-resistant bacterial strains.[28,29] The findings of this research demonstrated the antibacterial potency of all the natural substances examined. Furthermore, the synergistic interaction observed

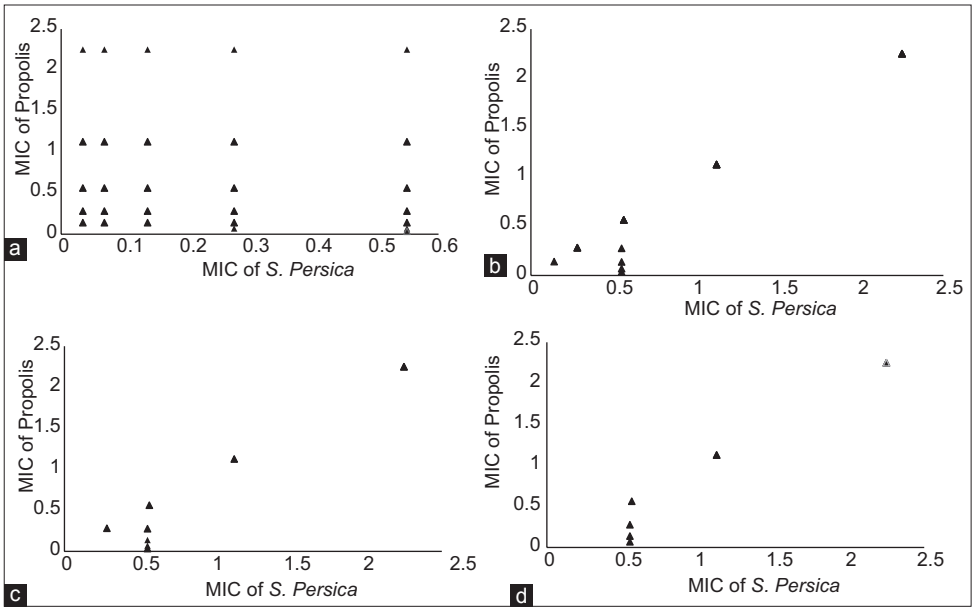

Figure 3: Various sub-minimum inhibitory concentration (MIC) concentrations of *Salvadora persica*, propolis, and clove extracts inhibited the growth of *Candida albicans*. (a) The concentration of clove extract is constant (MIC/2). (b) The concentration of clove extract is constant (MIC/4). (c) The concentration of clove extract is constant (MIC/8). (d) The concentration of clove extract is constant (MIC/16). MIC: Minimum inhibitory concentration, *S. persica: Salvadora persica*.

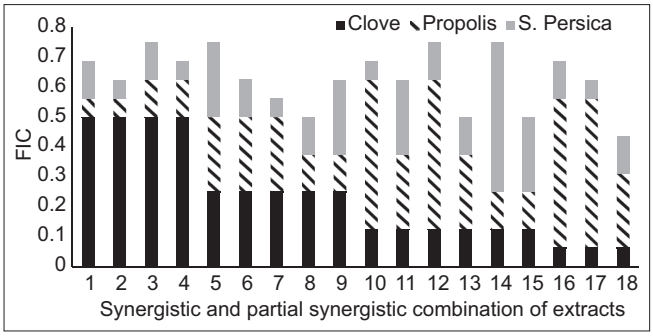

Figure 4: Total fractional inhibitory concentration of combination of three antimicrobial extracts of *Salvadora persica*, clove, and propolis against *Candida albicans*. FIC: Fractional inhibitory concentration.

between propolis, *S. persica*, and cloves suggests the potential for developing herbal remedies with enhanced antimicrobial properties capable of combating microorganisms and oral infections, including those caused by *S. mutans* and *C. albicans* species. Furthermore, given the increasing global trend toward developing herbal medicine formulations by combining various natural components, with a primary objective of standardizing and optimizing the active ingredients in these products, this study has demonstrated the efficacy of concurrently utilizing alcoholic extracts of propolis, *S. persica*, and cloves in antibacterial mouthwashes against oral infections. Considering the key role of *S. mutans* in dental plaque formation and caries development, these findings underscore the potential of such natural combinations in the prevention and adjunctive management of dental diseases.

Data availability

Data will be made available on request.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

1. Erazo, D., Brizuela, M., & Whetstone, D. R. Dental Infections. StatPearls. 2025
2. Samaranayake LP. General microbiology and Microbes of relevance to dentistry. Essential Microbiology for Dentistry 2nd ed. Churchill Livingstone, 2002; 1-53.
3. Madigan MT, Martinko JM, Parker J. Brock biology of microorganisms (Vol. 11). Upper Saddle River, NJ: Prentice hall. 1997.
4. Devine DA, Cosseau C. Host defense peptides in the oral cavity. Adv Appl Microbiol 2008;63:281-322.
5. Khalil MA, El-Sabbagh MS, El Naggar EB, El-Erian RH. Antibacterial activity of *Salvadora persica* against oral pathogenic bacterial isolates. Niger J Clin Pract 2019;22:1378-87.
6. Prakash SK. Dental abscess: A microbiological review. Dental research journal 2013;10:585.
7. Hussain AS, Ali M, Javed NK. Phytoconstituent from the stem bark of *Mangifera indica* variety "SAFEDA." Int J Pharm 2011;2:103-5.
8. Soliman S, Alnajdy D, El-Keblawy AA, Mosa KA, Khoder G, Noreddin AM. Plants' natural products as alternative promising anti-*Candida* drugs. Pharmacogn Rev 2017;11:104-22.
9. Doomra R, Goyal A. Azadirachta indica–Therapeutic Potential in Oro Dental Conditions. 2018.
10. Smith EG, Spatafora GA. Gene regulation in *S. mutans*: Complex control in a complex environment. J Dent Res 2012;91:133-41.
11. Sahin F, Karaman I, Güllüce M, Oğütçü H, Sengül M, Adigüzel A, *et al.* Evaluation of antimicrobial activities of *Satureja hortensis* L. J Ethnopharmacol 2003;87:61-5.
12. Stoeken JE, Paraskevas S, van der Weijden GA. The long-term effect of a mouthrinse containing essential oils on dental plaque and gingivitis: A systematic review. J Periodontol 2007;78:1218-28.
13. Shrivastava SR, Shrivastava PS, Ramasamy J. World Health Organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. J Med Soc 2018;32:76-7.
14. Mahizan NA, Yang SK, Moo CL, Song AA, Chong CM, Chong CW, *et al.* Terpene derivatives as a potential agent against antimicrobial resistance (AMR) pathogens. Molecules 2019;24:2631.
15. Suurbaar J, Mosobil R, Donkor AM. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens. BMC Res Notes 2017;10:660.
16. Silva Junior IF, Raimondi M, Zacchino S, Cechinel Filho V, Noldin VF, Rao VS, *et al.* Evaluation of the antifungal activity and mode of action of *Lafoensia pacari* A. St.-Hil., Lythraceae, stem-bark extracts, fractions and ellagic acid. Rev Bras Farmacogn 2010;20:422-8.
17. Búfalo MC, Figueiredo AS, de Sousa JP, Candeias JM, Bastos JK, Sforcin JM. Anti-poliovirus activity of *Baccharis dracunculifolia* and propolis by cell viability determination and real-time PCR. J Appl Microbiol 2009;107:1669-80.
18. Ayad AS, Benchaabane S, Daas T, Smagghe G, Loucif-Ayad, W. (2025). Propolis Stands out as a Multifaceted Natural Product:

Meta-Analysis on Its Sources, Bioactivities, Applications, and Future Perspectives. Life, 15(5), 764.
19. Akca AE, Akca G, Topçu FT, Macit E, Pikdöken L, Özgen IŞ. The comparative evaluation of the antimicrobial effect of propolis with chlorhexidine against oral pathogens: An *in vitro* study. Biomed Res Int 2016;2016:3627463.
20. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ 2014;15:45-6.
21. Pandey VK, Srivastava S, Dash KK, Singh R, Dar AH, Singh T, *et al*. Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. Heliyon, 2024;10(1).
22. Hassali HA, Abd Aziz AH, Razali R. Accelerated Solvent Extraction: An Innovative Sample Extraction Technique for Natural Products (No. INIS-MY--2016-089). 2015.
23. Athar MD, Tamboli ET, Ansari SH, Ahmad S. Quantification of eugenol in hydro-distilled clove oil (*Eugenia caryophyllus*) and its marketed products by validated GC-MS method. J Herbs Spices Med Plants 2013;19:365-76.
24. Sopirala MM, Mangino JE, Gebreyes WA, Biller B, Bannerman T, Balada-Llasat JM, *et al.* Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2010;54:4678-83.
25. Didry N, Dubreuil L, Pinkas M. Antimicrobial activity of naphtoquinones and *Allium* extracts combined with antibiotics. Pharm Acta Helv 1992;67:148-51.
26. Vazirian M, Hamidian K, Noorollah M, Manayi A. Enhancement of antibiotic activity and reversal of resistance in clinically isolated methicillin-resistant *Staphylococcus aureus* by *Trachyspermum ammi* essential oil. Res J Pharmacogn 2019;6:1-10.
27. Gebara EC, Lima LA, Mayer M. Atividade antimicrobiana da própolis contra bactérias periodontopatogênicas. Brazilian Journal of Microbiology 2002;33:365-9.
28. Al-Ani I, Zimmermann S, Reichling J, Wink M. Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. Medicines (Basel) 2018;5:2.
29. Kaushik H, Sharma R, Kumar A. Natural products against resistant bacterial infections: A systematic literature review. The Microbe, 2025;100247.

Supplementary Table 1: Total fractional inhibitory concentration of the concentrations of the extracts that were able to prevent the growth of *Streptococcus mutans* bacteria

FIC clove	FIC *propolis*	FIC *Salvadora persica*	ΣFIC index	Interpretation
0.5	0.03125	0.5	1.03125	Indifference
0.5	0.0625	0.5	1.0625	Indifference
0.5	0.0625	0.25	0.8125	Additive
0.5	0.0625	0.125	0.6875	Partial synergism
0.5	0.0625	0.0625	0.625	Partial synergism
0.5	0.125	0.5	1.125	Indifference
0.5	0.125	0.25	0.875	Additive
0.5	0.125	0.125	0.75	Partial synergism
0.5	0.25	0.5	1.25	Indifference
0.5	0.25	0.25	1	Additive
0.5	0.25	0.125	0.875	Additive
0.5	0.25	0.0625	0.8125	Additive
0.5	0.5	0.5	1.5	Indifference
0.5	0.5	0.25	1.25	Indifference
0.5	0.125	0.0625	0.6875	partial synergism
0.5	0.5	0.125	1.125	Indifference
0.5	0.5	0.0625	1.0625	Indifference
0.5	0.5	0.03125	1.03125	Indifference
0.5	0.5	0.015625	1.015625	Indifference
0.25	0.5	0.125	0.875	Additive
0.25	0.25	0.5	1	Additive
0.25	0.25	0.25	0.75	Partial synergism
0.25	0.5	0.25	1	Additive
0.25	0.5	0.25	1	Additive
0.25	0.25	0.125	0.625	Partial synergism
0.25	0.25	0.062	0.562	Partial synergism
0.25	0.125	0.5	0.875	Additive
0.25	0.125	0.125	0.5	Synergistic
0.25	0.5	0.062	0.812	Additive
0.25	0.125	0.25	0.625	Partial synergism
0.125	0.5	0.062	0.687	Partial synergism
0.125	0.25	0.25	0.625	Partial synergism
0.125	0.25	0.5	0.875	Additive
0.125	0.5	0.5	1.125	Indifference
0.125	0.5	0.25	0.875	Additive
0.125	0.5	0.125	0.75	Partial synergism
0.125	0.25	0.125	0.5	Synergistic
0.125	0.125	0.5	0.75	Partial synergism
0.125	0.125	0.25	0.5	Synergistic
0.0625	0.25	0.5	0.8125	Additive
0.0625	0.25	0.25	0.5625	Additive
0.0625	0.5	0.5	1.0625	Indifference
0.0625	0.5	0.25	0.8125	Additive
0.0625	0.5	0.125	0.6875	Partial synergism
0.0625	0.5	0.0625	0.625	Partial synergism
0.0625	0.25	0.125	0.4375	Synergistic

FIC: Fractional inhibitory concentration

Supplementary Table 2: Fractional inhibitory concentrations of combined propolis, *Salvadora persica*, and clove ethanolic extracts against *Candida albicans*: Results indicating synergistic, additive, or no effect

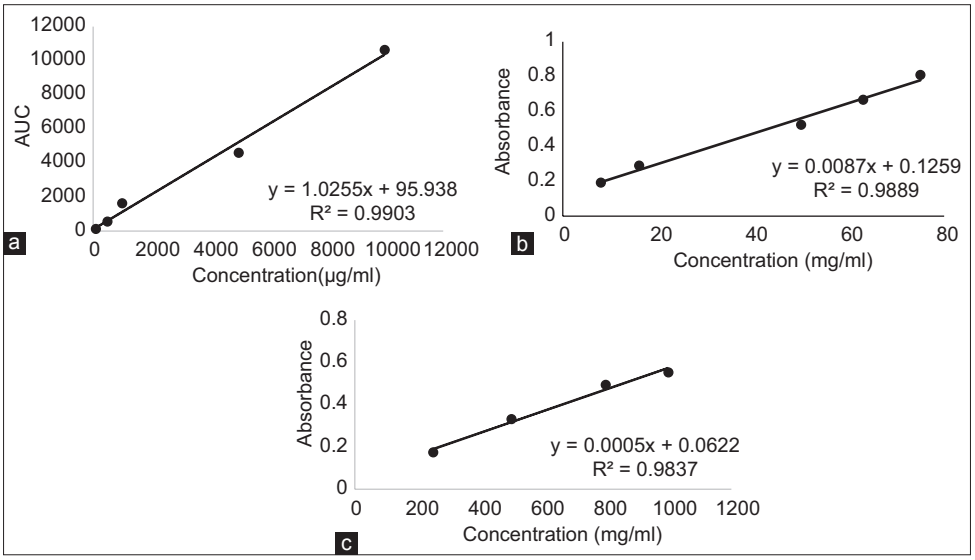
FIC clove	FIC *propolis*	FIC *Salvadora persica*	ΣFIC index	Interpretation
0.5	0.03125	0.03125	0.5625	Partial synergism
0.5	0.0625	0.3125	0.593	Partial synergism
0.5	0.03125	0.0625	0.593	Partial synergism
0.5	0.0625	0.0625	0.625	Partial synergism
0.5	0.03125	0.125	0.656	Partial synergism
0.5	0.0625	0.125	0.687	Partial synergism
0.5	0.125	0.125	0.75	Partial synergism
0.5	0.03125	0.25	0.781	Additive
0.5	0.25	0.0625	0.8125	Additive
0.5	0.0625	0.25	0.8125	Additive
0.5	0.25	0.125	0.875	Additive
0.5	0.125	0.25	0.875	Additive
0.5	0.25	0.25	1	Additive
0.5	0.5	0.01562	1.01562	Indifference
0.5	0.5	0.0312	1.0312	Indifference
0.5	0.03125	0.5	1.03125	Indifference
0.5	0.5	0.0625	1.0625	Indifference
0.5	0.0625	0.5	1.0625	Indifference
0.5	0.5	0.125	1.125	Indifference
0.5	0.125	0.5	1.125	Indifference
0.5	0.5	0.25	1.25	Indifference
0.5	0.25	0.5	1.25	Indifference
0.5	0.5	0.5	1.5	Indifference
0.5	0.5	0.007	1.007	Indifference
0.5	0.25	0.0312	0.7812	Additive
0.5	0.25	0.156	0.906	Additive
0.5	0.125	0.0625	0.6875	Partial synergism
0.5	0.125	0.0312	0.6562	Partial synergism
0.25	0.25	0.125	0.625	Partial synergism
0.25	0.25	0.0625	0.5625	Partial synergism
0.25	0.25	0.0312	0.5312	Partial synergism
0.25	0.125	0.5	0.875	Additive
0.25	0.125	0.25	0.625	Partial synergism
0.25	0.125	0.125	0.5	Synergistic
0.25	0.125	0.0625	0.4375	Synergistic
0.25	0.0625	0.5	0.8125	Additive
0.25	0.0625	0.25	0.5625	Partial synergism
0.25	0.0625	0.125	0.4375	Synergistic
0.25	0.0625	0.0625	0.375	Synergistic
0.25	0.0312	0.5	0.7812	Additive
0.25	0.0312	0.25	0.5312	Partial synergism
0.25	0.0312	0.125	0.4062	Synergistic
0.25	0.25	0.25	0.75	Partial synergism
0.25	0.25	0.5	1	Additive
0.25	0.5	0.0078125	0.7578125	Additive
0.25	0.5	0.015625	0.765625	Additive
0.25	0.5	0.03125	0.78125	Additive
0.25	0.5	0.0625	0.8125	Additive
0.25	0.5	0.125	0.875	Additive
0.25	0.5	0.25	1	Additive
0.25	0.5	0.5	1.25	Indifference
0.125	0.5	0.5	1.125	Indifference
0.125	0.5	0.25	0.875	Additive
0.125	0.5	0.125	0.75	Partial synergism

Contd...

Supplementary Table 2: Contd...

FIC clove	FIC *propolis*	FIC *Salvadora persica*	ΣFIC index	Interpretation
0.125	0.5	0.0625	0.6875	Partial synergism
0.125	0.5	0.03125	0.65625	Partial synergism
0.125	0.5	0.015625	0.640625	Partial synergism
0.125	0.5	0.0078125	0.6328125	Partial synergism
0.125	0.25	0.5	0.875	Additive
0.125	0.25	0.25	0.625	Partial synergism
0.125	0.25	0.125	0.5	Synergistic
0.125	0.25	0.0625	0.4375	Synergistic
0.125	0.125	0.5	0.75	Partial synergism
0.125	0.125	0.25	0.5	Synergistic
0.125	0.125	0.125	0.375	Synergistic
0.125	0.0625	0.5	0.6875	Additive
0.125	0.0625	0.25	0.4375	Synergistic
0.125	0.0312	0.5	0.6562	Partial synergism
0.0625	0.5	0.0625	0.625	Partial synergism
0.0625	0.25	0.5	0.8125	Additive
0.0625	0.25	0.25	0.5625	Partial synergism
0.0625	0.25	0.125	0.4375	Synergistic
0.0625	0.125	0.5	0.6875	Partial synergism
0.0625	0.125	0.25	0.4375	Synergistic
0.0625	0.0625	0.5	0.625	Partial synergism
0.0625	0.5	0.5	1.0625	Indifference
0.0625	0.5	0.25	0.8125	Additive
0.0625	0.5	0.125	0.6875	Partial synergism

FIC: Fractional inhibitory concentration



Supplementary Figure 1: Standard curve of (a) Eugenol, (b) Gallic acid, (c) Quercetin. AUC: Area under the curve.