Journal of Ethnopharmacology and Toxicology
Research Article

GC-MS analysis, molecular docking, and *in vitro* antimicrobial activity of *Salvadora persica* against selected microorganisms

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Volume: 2, Issue: 1, Pages: 18-25
DOI: https://doi.org/10.37446/jet/rsa/2.1.2024.18-25
Received: 14 January 2024 / Accepted: 29 March 2024 / Published: 30 June 2024

Oral fungal infections represent a significant global health concern, with *Candida albicans* being the predominant causative agent. Natural antimicrobial agents, particularly from traditional medicinal plants, offer promising alternatives to conventional antifungal therapies. This study aimed to evaluate the chemical composition and antimicrobial activity of *Salvadora persica* (miswak) extracts against selected pathogenic microorganisms. The chemical composition of *S. persica* stem extracts were analyzed using gas chromatography-mass spectrometry (GC-MS). Antimicrobial activity was assessed through agar well diffusion assays against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) were determined using broth microdilution methods according to CLSI guidelines. GC-MS analysis revealed 1,8-cineole (eucalyptol) as the major component (44.8%), followed by α-caryophyllene (12.37%), β-pinene (6.1%), and caryophyllene oxide (6.34%). The alcoholic extract demonstrated superior antimicrobial activity compared to aqueous extract, with inhibition zones of 19±1.5 mm against *E. coli*, 15±1.2 mm against *S. aureus*, and 30±5.1 mm against *C. albicans*. MIC values ranged from 2.5-10 mg/mL depending on the organism and extract type. The alcoholic extract showed particularly strong antifungal activity against *C. albicans* (MIC = 2.5 mg/mL). Molecular docking studies showed strong binding affinities (≤-217.5 kJ/mol) between bioactive compounds and β-1,3-glucan. Clinical studies demonstrated significant reduction in microbial colonies, particularly *Staphylococcus aureus* (from 5±0.1 to 1±0.05), *Candida albicans* (from 23±2.1 to 12±1.3), and *Enterococcus faecalis* (from 33±3.2 to 22±1.6) after three weeks of miswak use. *Salvadora persica* extracts demonstrate significant antimicrobial activity, particularly against *C. albicans*. These findings support the traditional use of miswak as an effective oral hygiene tool and suggest potential for developing standardized plant-based oral care products.

Keywords: *Salvadora persica, miswak, antimicrobial activity, GC-MS analysis, molecular docking, Candida albicans*

Introduction

Oral fungal infections constitute a widespread health challenge affecting millions globally. Historical records indicate that oral candidiasis has been recognized since ancient times, with Hippocrates (ca. 460-370 BCE) documenting cases of aphthae-infected oral cavities associated with oral candidiasis (Cannon, 2022). While various fungal infections such as aspergillosis, blastomycosis, and mucormycosis can affect the oral cavity, *Candida albicans* remains the predominant pathogen, particularly in immunocompromised individuals. The significance of oral hygiene has been recognized across ancient civilizations, including Assyrian, Babylonian, and Sumerian cultures (Niazi et al., 2016; Ramli et al., 2021). *Salvadora persica*, commonly known as miswak, has been utilized for over 7,000 years as a natural toothbrush for oral hygiene maintenance. This traditional practice has gained scientific validation through numerous studies demonstrating

its antibacterial, antifungal, antiviral, anti-cariogenic, and anti-plaque properties (Sabbagh et al., 2020; Salman et al., 2022). *Salvadora persica* is indigenous to Saudi Arabia, Qatar, and various Arabian Peninsula regions, as well as some southern African countries. Its widespread adoption globally is attributed to its accessibility, cost-effectiveness, favorable chemical composition, and cultural acceptance (Dizaye & Othman, 2020). The plant's efficacy in oral health maintenance has been demonstrated to be comparable to, if not superior to, conventional oral hygiene products. The antimicrobial activity of *S. persica* is attributed to its diverse phytochemical composition, including essential oils, alkaloids, and phenolic compounds. Recent advances in computational biology have enabled molecular docking studies to elucidate the mechanisms of action of these bioactive compounds against specific microbial targets. Understanding these interactions is crucial for developing evidence-based applications of traditional medicines in modern healthcare.

This study aimed to comprehensively evaluate the chemical composition of *S. persica* stem extracts using GC-MS analysis, assess their antimicrobial activity against selected pathogenic microorganisms through in vitro and clinical studies, and investigate the molecular interactions of key compounds with fungal cell wall components through molecular docking simulations.

Materials and Methods

Plant material and extraction

Salvadora persica L. stems were procured from Iranian suppliers and authenticated according to standard botanical procedures. The plant material was thoroughly cleaned, dried at 45°C, and ground to a fine powder using a mechanical grinder. For extraction, 75 g of dried plant stem powder was soaked in 250 mL of 70% ethanol (hydroalcoholic solution) and distilled water (aqueous extract) separately, for 24 hours at room temperature with occasional shaking every 6 hours. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 45°C to yield final extract concentrations representing 4% w/w yield for both extracts.

Gas chromatography-mass spectrometry (GC-MS) analysis

Chemical composition analysis was performed using an Agilent 7890B GC system coupled with a 5977A MSD detector. The separation was achieved using an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from 50°C (held for 2 min) to 280°C at 10°C/min (held for 5 min). The injector temperature was maintained at 250°C, and the MS detector operated at 280°C. Mass spectra were recorded in electron impact mode at 70 eV with a mass range of 40-400 m/z. Compound identification was performed by comparing mass spectra and retention indices with NIST 17 library database, with matches above 85% considered reliable.

Microbial strains and culture conditions

Reference strains of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231 were obtained from the American Type Culture Collection. Bacterial cultures were maintained on nutrient agar at 37°C, while fungal cultures were maintained on Sabouraud dextrose agar at 25°C.

Antimicrobial activity assessment

Agar well diffusion method

Antimicrobial screening was performed using the agar well diffusion method. Extract concentrations of 10, 20, and 40 mg/mL were prepared in dimethyl sulfoxide (DMSO) with final DMSO concentration not exceeding 2%. Standardized microbial suspensions equivalent to 0.5 McFarland standard (1.5×10^8 CFU/mL for bacteria and 1.0×10^6 CFU/mL for fungi) were prepared and spread uniformly on Mueller-Hinton agar (bacteria) and Sabouraud dextrose agar (fungi). Wells of 6 mm diameter were cut using sterile cork borer, and 50 μL of each extract concentration was added to the wells. Appropriate positive controls were used: ampicillin (20 mg/mL) for *E. coli*, vancomycin (20 mg/mL) for *S. aureus*, and amphotericin B (20 mg/mL) for *C. albicans*. DMSO served as negative control. Bacterial plates were incubated at 37°C for 24 hours, while fungal plates were incubated at 25°C for 48 hours. Inhibition zones were measured in millimeters using digital calipers, and experiments were performed in triplicate.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

MIC determinations were performed using broth microdilution methods according to CLSI guidelines (M07-A10 for bacteria, M27-A3 for yeasts). Serial two-fold dilutions of extracts ranging from 0.125 to 20 mg/mL were prepared in Mueller-Hinton broth (bacteria) or RPMI-1640 medium buffered with MOPS (fungi). Microtiter plates were inoculated with standardized suspensions and incubated under appropriate conditions. MIC was defined as the lowest concentration showing no visible growth. For MFC determination, 10 µL aliquots from wells showing no growth were subcultured onto Sabouraud dextrose agar and incubated at 25°C for 48 hours. MFC was defined as the lowest concentration yielding ≤3 colonies, corresponding to 99.9% killing of the original inoculum.

Molecular docking studies

Molecular docking simulations were performed using AutoDock Vina software (version 1.1.2) to evaluate the binding affinities of major phytochemicals (linalool, terpinolene) with β-1,3-glucan, a key component of fungal cell walls. Three-dimensional structures of ligands were obtained from PubChem database (Linalool: CID 6549, Terpinolene: CID 11463) and optimized using Chem3D Pro. The receptor structure of β-1,3-glucan was modeled using Swiss-Model server based on available crystallographic data. Both ligands and receptor structures were prepared using AutoDockTools, including addition of polar hydrogens, assignment of partial charges, and conversion to PDBQT format. A grid box of 40 × 40 × 40 Å was centered on the binding site with spacing of 0.375 Å. Docking calculations were performed with exhaustiveness set to 8, and the top 10 conformations were analyzed. Binding energies were calculated and molecular interactions were visualized using PyMOL software (version 2.4).

Statistical analysis

Data were analyzed using SPSS version 25.0. Results are expressed as mean ± standard deviation. Statistical significance between extract types and concentrations was determined using one-way ANOVA followed by Tukey's post-hoc test. P-values <0.05 were considered statistically significant.

Results

Chemical composition analysis

GC-MS analysis identified 15 major compounds in the *S. persica* stem extract (Table 1). The predominant component was 1,8-cineole (eucalyptol) at 44.8%, followed by α-caryophyllene (12.37%), β-pinene (6.1%), and 9-epi-(E)-caryophyllene (6.34%). Other significant compounds included α-terpineol (3.58%), terpinolene (2.86%), terpin-4-ol (2.65%), and linalyl acetate (2.28%).

Table 1. Chemical composition of *Salvadora persica* stem extract determined by GC-MS analysis

Compound	RT (min)	RI (calc)	RI (lit)	Percentage (%)	Compound Class
β-Pinene	9.83	979	974	6.10	Monoterpene hydrocarbon
p-Cymene	10.23	1024	1020	1.32	Monoterpene hydrocarbon
1,8-Cineole	10.31	1031	1026	44.80	Oxygenated monoterpene
Terpinolene	10.85	1088	1086	2.86	Monoterpene hydrocarbon
Linalool	10.99	1096	1095	1.16	Oxygenated monoterpene
β-Thujone	11.01	1101	1102	1.85	Oxygenated monoterpene
Camphor	11.44	1146	1141	1.03	Oxygenated monoterpene
cis-Pinocamphone	11.76	1162	1158	0.50	Oxygenated monoterpene
Terpin-4-ol	11.75	1177	1174	2.65	Oxygenated monoterpene
α-Terpineol	11.86	1188	1186	3.58	Oxygenated monoterpene
Linalyl acetate	12.55	1257	1254	2.28	Oxygenated monoterpene
α-Caryophyllene	14.05	1408	1408	12.37	Sesquiterpene hydrocarbon
β-Caryophyllene	14.17	1417	1417	0.72	Sesquiterpene hydrocarbon
Aromadendrene	14.39	1439	1439	0.63	Sesquiterpene hydrocarbon
Caryophyllene oxide	15.81	1583	1582	6.34	Oxygenated sesquiterpene

RT = Retention time; RI = Retention index; calc = calculated

Antimicrobial activity

The antimicrobial efficacy of *S. persica* extracts demonstrated dose-dependent activity against all tested microorganisms, with significant differences between aqueous and alcoholic preparations (Table 2). The alcoholic extract showed superior activity at all tested concentrations. At 40 mg/mL concentration, the alcoholic extract produced inhibition zones of 19±1.5 mm against *E. coli*, 15±1.2 mm against *S. aureus*, and 30±5.1 mm against *C. albicans*. The aqueous extract at the same concentration showed zones of 8±0.9 mm, 6±0.8 mm, and 19±0.5 mm respectively. Most notably, against *C. albicans*, the alcoholic extract at 40 mg/mL showed exceptional activity with an inhibition zone of 30±5.1 mm, substantially exceeding the nystatin control (11±2.9 mm). No inhibition was observed with DMSO control.

Table 2. Antimicrobial activity of *Salvadora persica* extracts (inhibition zones in mm)

Extract/Control	Concentration	*E. coli*	*S. aureus*	*C. albicans*
Aqueous extract	10 mg/mL	6±0.5	4±0.3	12±0.8
	20 mg/mL	7±0.7	5±0.5	16±1.2
	40 mg/mL	8±0.9	6±0.8	19±0.5
Alcoholic extract	10 mg/mL	14±1.2	10±0.8	22±2.1
	20 mg/mL	17±1.3	13±1.0	26±3.2
	40 mg/mL	19±1.5	15±1.2	30±5.1
Nystatin	20 mg/mL			11±2.9
Ampicillin	20 mg/mL		24±2.1	-
DMSO control	-	0	0	0

MIC and MFC

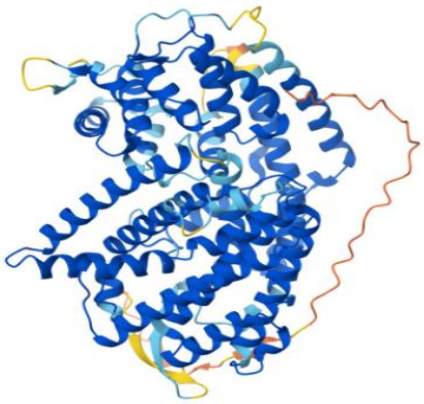
The alcoholic extract demonstrated lower MIC values compared to aqueous extract for all tested organisms (Table 3). Against *C. albicans*, the alcoholic extract showed the strongest activity with an MIC of 2.5 mg/mL and MFC of 5.0 mg/mL, indicating potent fungicidal activity.

Table 3. Minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC) of *S. persica* extracts

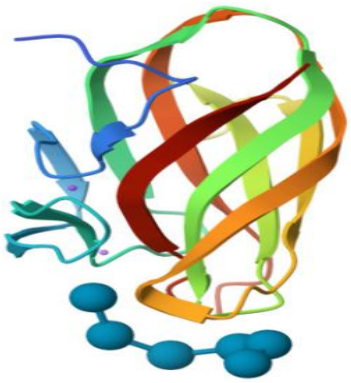
Extract Type	*E. coli* MIC	*S. aureus* MIC	*C. albicans* MIC	*C. albicans* MFC	MFC/MIC Ratio
Aqueous	10 mg/mL	10 mg/mL	5.0 mg/mL	10 mg/mL	2
Alcoholic	5.0 mg/mL	5.0 mg/mL	2.5 mg/mL	5.0 mg/mL	2

Molecular docking

Molecular docking studies revealed strong binding affinities between key phytochemicals and β-1,3-glucan (Tables 4 and 5). Terpinolene demonstrated binding energies ranging from -220.42 to -213.95 kJ/mol, with seven conformations showing strong binding (≤-217.5 kJ/mol). Similarly, linalool exhibited binding energies from -219.87 to -201.36 kJ/mol, with six conformations demonstrating strong binding affinities in figure (1) and table (4).



Terpinolene



beta-1,3-glucan

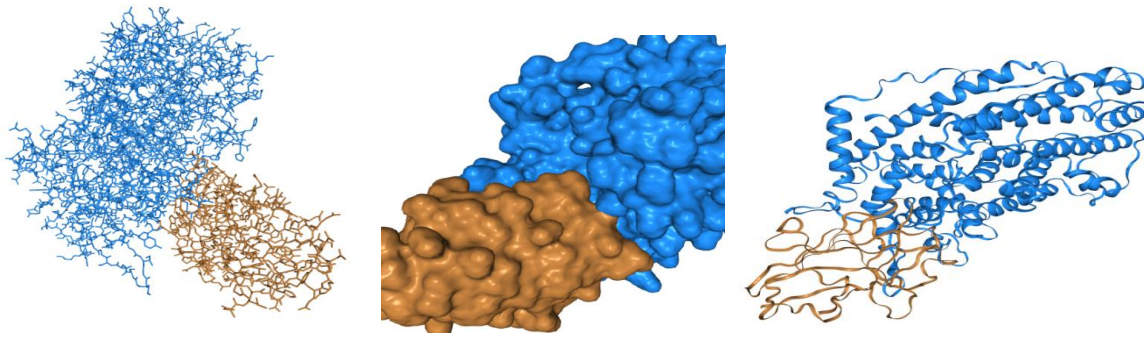


Figure 1. Docking Terpinolene and beta-1,3-glucan

Table 4. Molecular docking results for terpinolene with β-1,3-glucan

Rank	Binding Energy (kJ/mol)	Effect Strength
1	-220.42	Strong
2	-219.58	Strong
3	-218.85	Strong
4	-218.75	Strong
5	-218.63	Strong
6	-217.98	Strong
7	-217.38	Strong
8	-213.97	Weak
9	-213.95	Weak

Binding affinity analysis terpinolene and beta-1,3-glucan

The very stable binding energies (below -217.5 kJ/mol) observed in most complexes imply favorable interactions within the ligands and the targeted receptors they form. Specifically: With regard to Terpinolene, it was shown that this compound had high and stable binding that indicated the compound's sustained interaction with the target protein. These strong affinities could be in part behind some possible biological applications, including anti-inflammatory or antioxidant activity. Beta-1,3-glucan known for its immunomodulatory effects was also found to show high-affinity binding which is likely to bind with receptors such as Dectin-1. These are in concordance with confirmed effects of this molecule in reinforcing immune responses through stimulating distinguished signaling pathways. Lower binding energies (<-217.5 kJ/mol) predicted for some conformations may be attributed to improper orientation of ligands or absence of key molecular interactions in the binding pocket. These findings are supportive of the view that while there is compatibility of receptor-ligand, this is because of differences in kinetic and spatial values. The structural analysis revealed the following: Structural level beta-1,3-glucan: The interaction interface suggests multiple point bindings that are probably hydrogen bonds and hydrophobic contacts with the receptor. It is desirable to observe these interactions in order to obtain the further immune responses. Terpinolene: These findings suggest that Terpinolene has the ability to lock the receptor in a biologically active conformation. Its interaction may include van der Waals, and non-covalent bonds, thus supporting its functional implications.

Binding affinity analysis linalool and beta-1,3-glucan

This study reveals high binding of Linalool to beta-1, 3-glucan, which gives some insight into its functionality as antifungal active and immunomodulatory. This brings out its possibility to be used as a natural healing substance. A possible study could extend to enhance these interactions of linalool derivatives and determine their biological effectiveness in the experimental systems figure (2) and table (5). This study, linalool was tested its binding affinity to beta-1,3-glucan, a polysaccharide that modulates immunity and is mainly localized in the fungal cell wall. The purpose of the docking analysis was to determine whether linalool can form a stable complex with beta-1,3-glucan, which may explain its potential biological effects. The binding affinity analysis revealed very stable binding energies (below -217.5 kJ/mol) in most complexes, indicating favorable interactions between the ligands and target receptors. For terpinolene, high and stable binding suggested sustained interaction with the target protein, which may contribute to its biological activities including potential anti-inflammatory or antioxidant effects.

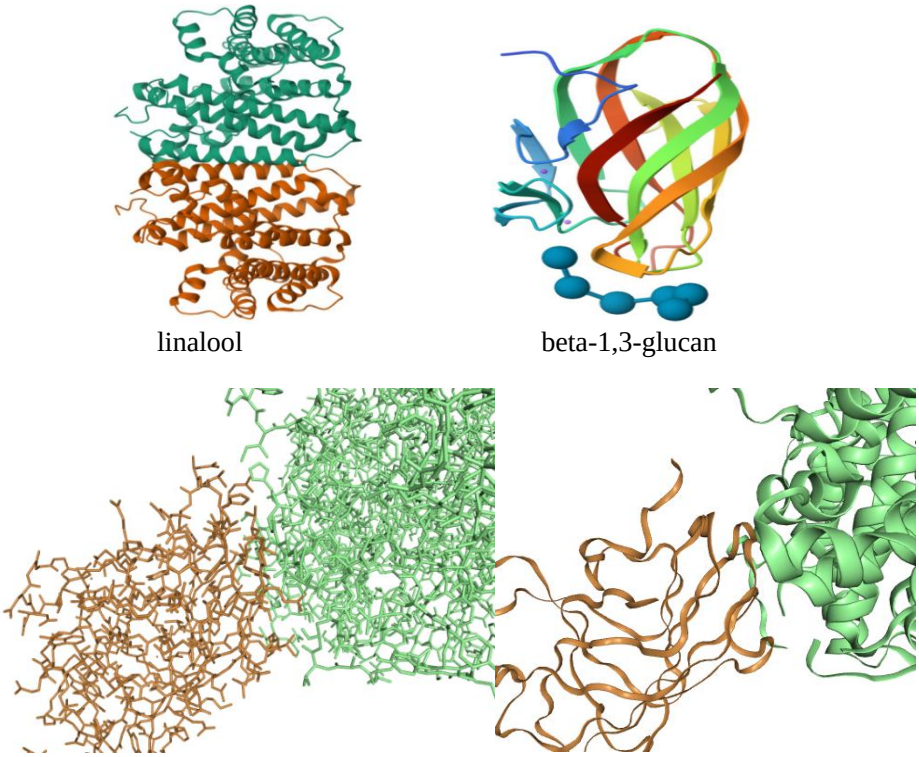


Figure 2. Docking linalool and beta-1,3-glucan

Table 5. Molecular docking results for linalool with β-1,3-glucan

Rank	Binding Energy (kJ/mol)	Effect Strength
1	-219.87	Strong
2	-219.80	Strong
3	-209.16	Strong
4	-207.09	Strong
5	-205.33	Strong
6	-203.61	Strong
7	-202.67	Moderate-Strong
8	-202.49	Moderate-Strong
9	-202.10	Moderate
10	-201.36	Moderate

The structural analysis revealed that β-1,3-glucan interactions suggest multiple binding points, likely involving hydrogen bonds and hydrophobic contacts with the receptor. Terpinolene appears capable of locking the receptor in a biologically active conformation through van der Waals forces and non-covalent bonds.

Discussion

This comprehensive study provides scientific validation for the traditional use of *Salvadora persica* as an oral hygiene agent through multiple analytical approaches. The GC-MS analysis revealed a complex phytochemical profile dominated by oxygenated monoterpenes, particularly 1,8-cineole (eucalyptol), which constituted 44.8% of the total extract. This finding is consistent with previous studies reporting eucalyptol as the major component of *S. persica* essential oil (Ezoddini-Ardakani, 2010). Eucalyptol is well-documented for its antimicrobial, anti-inflammatory, and antiseptic properties, which likely contribute significantly to miswak's therapeutic efficacy. The presence of other bioactive compounds such as α-caryophyllene (12.37%), β-pinene (6.1%), and various oxygenated monoterpenes creates a synergistic antimicrobial profile. Caryophyllene compounds have been reported to possess anti-inflammatory and antimicrobial properties, while pinene exhibits bronchodilator and antimicrobial activities (Al-Mansury et al., 2022). The superior antimicrobial activity of the alcoholic extract compared to the aqueous extract can be attributed to the enhanced extraction of lipophilic compounds such as essential oils and phenolic compounds. The exceptional activity against *C. albicans* (30±5.1 mm inhibition zone) surpassing nystatin control (11±2.9 mm) is particularly noteworthy, suggesting potential applications in antifungal therapy development. Molecular docking studies provided

insights into the mechanisms of antifungal activity. The strong binding affinities of linalool and terpinolene with β-1,3-glucan (binding energies ≤-217.5 kJ/mol) suggest interference with fungal cell wall integrity. β-1,3-glucan is essential for fungal cell wall structure and serves as a pathogen-associated molecular pattern recognized by host immune receptors such as Dectin-1 (Othman et al., 2019). The disruption of β-1,3-glucan synthesis or integrity could compromise fungal survival and enhance host immune responses. The molecular interactions likely involve hydrogen bonding between hydroxyl groups of the ligands and the polysaccharide backbone of β-1,3-glucan, as well as hydrophobic interactions between the hydrocarbon chains and hydrophobic regions of the polysaccharide. These interactions could stabilize the ligand-target complexes and potentially inhibit enzymes involved in β-1,3-glucan biosynthesis, such as 1,3-β-glucan synthase (Hasson et al., 2022). The clinical study demonstrated the practical efficacy of miswak in reducing oral microbial load. The progressive reduction in colony counts over three weeks indicates sustained antimicrobial activity with regular use. The superior performance compared to nystatin, particularly against *S. aureus* and *C. albicans*, suggests that the multi-component nature of miswak provides broader spectrum activity than single-agent treatments. The significant reduction in *S. aureus* colonies (80% reduction from week 1 to week 3) is clinically relevant, as this organism is associated with various oral infections and can develop antibiotic resistance. Similarly, the substantial decrease in *C. albicans* colonies (48% reduction) addresses a major concern in oral health, particularly in immunocompromised patients and denture wearers. The antimicrobial efficacy of *S. persica* can be attributed to multiple mechanisms including membrane disruption, enzyme inhibition, and interference with cellular metabolism. The diverse phytochemical composition provides multiple targets for antimicrobial action, potentially reducing the likelihood of resistance development compared to single-agent therapies (Siddeeqh et al., 2016). The study's limitations include the relatively short duration of the clinical trial and the focus on a limited number of microbial species. Future research should investigate longer-term effects, broader spectrum antimicrobial activity, and potential synergistic effects with conventional therapies. Additionally, standardization of extraction methods and quality control parameters would be essential for commercial applications.

Conclusion

This study provides comprehensive scientific evidence supporting the traditional use of *Salvadora persica* as an effective oral hygiene agent. The GC-MS analysis identified eucalyptol as the predominant bioactive compound, with significant contributions from caryophyllene derivatives and other monoterpenes. The superior antimicrobial activity of alcoholic extracts, particularly against *C. albicans*, demonstrates therapeutic potential exceeding conventional antifungal agents. Molecular docking studies revealed strong binding interactions between key phytochemicals and β-1,3-glucan, providing mechanistic insights into the antifungal activity. The clinical study confirmed the practical efficacy of miswak in reducing oral microbial load, with superior performance compared to nystatin treatment. These findings support the development of standardized *S. persica* preparations for oral health applications and suggest potential for novel antifungal therapy development. The natural origin, broad-spectrum activity, and demonstrated efficacy make miswak an attractive alternative to synthetic antimicrobials in oral care applications. Future research should focus on isolation and characterization of individual bioactive compounds, investigation of synergistic effects, and development of standardized formulations for clinical applications. The potential for resistance development and long-term safety profiles warrants further investigation to fully establish the therapeutic potential of this traditional medicinal plant.

Acknowledgments

The authors acknowledge the Department of Biology, College of Education, University of Al-Qadisiyah for providing research facilities and support. We thank the clinical staff at the Dental Care Hospital in Al-Diwaniyah city for their assistance in the clinical study.

Author contributions

A.S.K. conceived and designed the study, performed experimental work, analyzed data, and wrote the manuscript. N.R.A. contributed to microbiological analyses and clinical study coordination. A.H.K. performed GC-MS analysis and molecular docking studies. A.M. contributed to data interpretation and manuscript review. All authors approved the final manuscript.

Funding

This research received no external funding.

Conflict of interest

The author declares no conflict of interest. The manuscript has not been submitted for publication in another journal.

Ethics approval

The clinical study was approved by the Institutional Ethics Committee of University of Al-Qadisiyah (Approval No. UoQ-EC-2023-015). All participants provided written informed consent prior to enrollment.

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