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In vitro antibacterial activity of *Salvadora persica L.* (Miswak) against bacteria associated with dental caries in Shendi, Sudan

Abstract

Background: There is an increasing demand to identify naturally occurring plant-derived substances with antimicrobial activity as alternatives to conventional antibiotics. Miswak, obtained from the Arak plant (*Salvadora persica*), is traditionally used by Muslim communities as a natural oral hygiene product to clean teeth and maintain dental health.

Objective: This study aimed to assess *in vitro* the antibacterial activity of different concentrations of ethanolic extracts of *S. persica* Linn. against bacterial strains associated with dental caries using the agar well diffusion method.

Methodology: A cross-sectional study was conducted in Shendi town, Sudan, between January and March 2025, at the Microbiology Laboratory, Faculty of Medical Laboratory Sciences, Shendi University. A total of 50 oral swabs were collected from patients suffering from dental caries. Bacterial isolates were identified using Gram staining and biochemical tests. The ethanolic extract of *S. persica* was tested at concentrations of 100%, 50%, 25%, 12.5%, and 6.25% (v/v).

Results: Among the 50 isolates, the identified bacteria were: *Staphylococcus aureus* (20%), *Streptococcus pyogenes* (2%), *Streptococcus* group C or G (4%), undifferentiated Gram-negative bacilli (2%), *Streptococcus viridans* (52%), and *Staphylococcus epidermidis* (18%). The ethanolic extract of *S. persica* showed no antibacterial activity against any of the tested bacterial strains. However, a consistent zone of inhibition of approximately 7 mm was observed for all extracts and all bacterial strains using the agar well diffusion method. This value is considered negligible and does not represent a true antibacterial effect, especially since the well diameter itself was 6 mm, meaning the actual inhibition halo around the well is only 0.5 mm per side, which is generally classified as indicating resistance according to standard criteria. The observed zone likely represents a diffusion artefact of the solvent rather than a true antibacterial effect, indicating resistance across all tested strains.

Conclusion: The ethanolic extract of *S. persica* demonstrated no significant antibacterial effect against the bacterial isolates associated with dental caries, indicating resistance across all tested strains.

Keywords: antimicrobial, *salvadora persica*, *streptococcus*, *streptococcus viridans*, herbal medicine

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Introduction

Efforts to control and manage human infections caused by bacterial pathogens have been increasingly challenged by the emergence of multidrug-resistant (MDR) organisms since the 1990s, as well as the more recent rise of extensively drug-resistant clinical isolates.[1] The misuse and overuse of antibiotics have significantly contributed to the acceleration of antimicrobial resistance, posing a major global health concern.[2] Furthermore, the limited development of new antimicrobial agents has exacerbated this issue, particularly in the face of increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), and carbapenem-resistant Gram-negative bacilli, which present serious therapeutic challenges.[2-4] Medicinal plants play a vital role in human life, serving as sources of food, medicine, cosmetics, flavoring agents, and other daily necessities. Owing to their potential health benefits, plant-derived compounds have gained considerable attention in complementary and alternative medicine worldwide. Various plant extracts have demonstrated promising therapeutic properties, particularly against infectious pathogens.[5,6] Among the 182 plant species traditionally used as chewing sticks, *Salvadora persica L.,* commonly known as miswak, is one of the most important. It belongs to the family Salvadoraceae and has been widely used across the Middle East, Asia, and Africa for oral hygiene purposes. Different parts of the plant, including roots, twigs, and stems, are commonly used as natural toothbrushes to maintain oral health.[7,8] Previous studies have reported that aqueous and methanolic extracts of miswak possess a wide range of biological activities, particularly antimicrobial effects against organisms implicated in the development of dental plaque and periodontal diseases.[9] Several *in vitro* studies have demonstrated its antibacterial and antifungal activities against various cariogenic and periodontal pathogens, including *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Haemophilus influenzae*, and *Candida albicans*.[10] Moreover, evidence from controlled clinical trials suggests that *Salvadora persica* extracts may serve as effective antimicrobial agents when used as irrigants during endodontic treatment of teeth with necrotic pulps.[11] Despite extensive research on its antimicrobial effects against oral

microorganisms, there remains limited information regarding its activity against other human pathogens, particularly multidrug-resistant (MDR) strains.[12] Miswak is primarily obtained from the slender branches and, to a lesser extent, the roots of the Arak tree (*Salvadora persica*) (Figure 1).[13] Recent studies have also highlighted its diverse pharmacological properties, including antibacterial and antifungal activities.[14] Therefore, the present study was designed to evaluate the *in vitro* antibacterial activity of *Salvadora persica L.* extracts against bacteria associated with dental caries among individuals in Shendi Town, Sudan.

Figure 1 Miswak, which is mainly obtained from slim branches of the Arak plant.

Methodology

This cross-sectional, laboratory-based study was conducted in Shendi Town, located in northern Sudan on the southeastern bank of the Nile River, approximately 150 km northeast of Khartoum. The town lies between latitude 18.17°N and longitude 24.23°E, with geographical coordinates 16°41′N and 33°26′S, and covers a total area of 14,596 km². Shendi is characterized by a hot desert climate and is situated about 45 km southwest of the ancient city of Meroe. It is also recognized as an important historic trading center. The study was carried out from January to March 2025 in the laboratories of the Faculty of Science and Technology and the Microbiology Laboratory of Shendi University, where all experimental procedures were performed. The study population included participants residing in Shendi Town.

Sampling and sample size

Participants included in this study were adults aged 18 years or older, residing in Shendi Town, who had not used any systemic or topical antibiotics within one month before sample collection. Individuals with recent antibiotic use or those who had taken other herbal remedies for oral health were excluded. A total of 50 oral swab samples were collected from participants presenting with dental caries. Data were obtained through direct interviews using a structured questionnaire designed to collect all relevant information related to the study.

Miswak collection

Fresh twigs of *Salvadora persica* were collected from Shendi Town on January 29, 2025. They were thoroughly washed several times with water, then shade-dried for three days. The dried twigs were subsequently ground into a fine powder using a household electric blender.

Extract preparation

The extraction was performed at the Department of Botany, Faculty of Science and Technology, University of Shendi, using the hot extraction method with a Soxhlet apparatus. Twenty grams of the plant powder were placed in the thimble of the Soxhlet apparatus, which was connected to a suitably sized round-bottom flask containing 200 mL of absolute ethanol. The extraction process was carried out for 3 hours. Following extraction, ethanol was evaporated by heating the extract at 40 °C on the same day. The residual solvent was completely removed by leaving the extract in an open glass beaker at room temperature for 24 hours. The dried extract was then stored in an airtight container under refrigeration until further use.

Bacterial clinical isolates

Each participant was first asked to rinse the mouth thoroughly to minimize contamination. Oral swabs were then collected under aseptic conditions using sterile disposable swabs. The swabs were cultured on chocolate agar and blood agar plates, which were incubated for 18–24 hours. Subsequently, bacterial isolates were sub-cultured on Mueller-Hinton agar, before sensitivity testing, the isolates were confirmed based on colonial morphology, Gram staining, and standard biochemical tests (e.g., catalase and coagulase tests for staphylococci; bacitracin sensitivity and sugar fermentation tests for streptococci; and API 20E for Gram-negative bacilli).

Preservation of organisms

After identification, pure cultures of the bacterial isolates were incubated for 24 hours and subsequently preserved at 4 °C in a refrigerator for further use.

Preparation of serial dilutions

The ethanolic extract of *Salvadora persica* was dissolved in sterile distilled water to obtain different concentrations: 100%, 50%, 25%, 12.5%, and 6.25% (v/v).

Preparation of bacterial suspension

Following primary culture, bacterial isolates were sub-cultured to ensure purity. Two milliliters of normal saline were dispensed into sterile test tubes and autoclaved at 121 °C for 15 minutes. A loopful of purified bacterial colony was inoculated into the sterile saline. The turbidity of the bacterial suspension was then adjusted to match the McFarland standard (0.5) to ensure uniform inoculum density.

Antibacterial assay

The antibacterial activity of *S. persica* extract was evaluated using the agar well diffusion method as described by Lino and Deogracious, with slight modifications. A freshly prepared bacterial suspension was swabbed uniformly across the surface of Mueller-Hinton agar plates using sterile cotton swabs. Wells of 6 mm diameter were aseptically bored into the agar medium using a sterile cork-borer. Each well was filled with 50 µL of the different extract concentrations. As a negative control, one well was filled with the extraction solvent (ethanol diluted in sterile distilled water to the same percentage as the highest test concentration). As a positive control, a standard antibiotic (e.g., 0.2% chlorhexidine) was used to confirm the susceptibility of the test organisms. After allowing diffusion at room temperature, the plates were incubated at 37 °C for 24 hours. The antibacterial activity was determined by measuring the diameter of the inhibition zones around the wells in millimeters.

Data collection and analysis

Data were entered, verified, and analyzed using the Statistical Package for the Social Sciences (SPSS), version 26. Descriptive

statistics were applied, and categorical variables were summarized as frequencies and percentages.

Results

The demographic characteristics of the study participants are presented in Table 1. Out of the 50 participants included in this study, females constituted the majority (58%), while males accounted for 42%. The distribution of bacterial isolates obtained from oral swabs is shown in Table 2. The most predominant organism identified was *Streptococcus viridans* (52%), followed by *Staphylococcus aureus* (20%) and *Staphylococcus epidermidis* (18%). Other isolates included *Streptococcus pyogenes* (2%), Lancefield group C or G streptococci (4%), and undifferentiated Gram-negative bacilli (4%). The antibacterial activity of 100% ethanolic extract of *Salvadora persica* against all isolated bacteria is summarized in Table 3. The results demonstrated a uniform inhibition zone of 7 mm for all tested organisms, with no variation observed between different bacterial species (mean ± SD: 7 ± 0 mm). Similarly, the effect of different concentrations of the extract (100%, 50%, 25%, and 12.5%) on bacterial isolates is illustrated in Table 4. No differences in inhibition zone diameters were observed across all concentrations and bacterial species, as all recorded values remained constant at 7 ± 0 mm. Importantly, the observed inhibition zone (7 mm) is only marginally greater than the well diameter (6 mm), indicating a negligible inhibition halo (approximately 0.5 mm on each side). Moreover, this value was comparable to that obtained with the negative control (solvent), suggesting that the observed effect may be attributed to solvent diffusion rather than true antibacterial activity.

Table 1 Shows population distribution according to gender

Gender	Frequency	Percent %
Male	21	42%
Female	29	58%
Total	50	100%

Table 2 The frequency and percentage of isolated bacteria

Organisms	Frequency	Percent %
S. aureus	10	20%
S. epidermidis	9	18%
S. pyogenes	1	2%
Lancefield grouping C or G	2	4%
S. viridans	26	52%
Undifferentiated Gram-negative bacilli	2	4%
Total	50	100%

Table 3 Antibacterial activity of 100% *S. persica* ethanolic extract against isolated bacteria

Organisms	Mean inhibition zone (100%)
S. aureus	7 ± 0
S. epidermidis	7 ± 0
S. pyogenes	7 ± 0
Lancefield grouping C or G	7 ± 0
S. viridans	7 ± 0
Undifferentiated Gram-negative bacilli	7 ± 0

Table 4 Effect of *S. persica L.* ethanolic extract at different concentrations on isolated bacteria

Organisms	Mean inhibition zone according to concentrations			
	100%	50%	25%	12.5%
S. aureus	7 ± 0	7 ± 0	7 ± 0	7 ± 0
S. epidermidis	7 ± 0	7 ± 0	7 ± 0	7 ± 0
S. pyogenes	7 ± 0	7 ± 0	7 ± 0	7 ± 0
Lancefield grouping C or G	7 ± 0	7 ± 0	7 ± 0	7 ± 0
S. viridans	7 ± 0	7 ± 0	7 ± 0	7 ± 0
Undifferentiated Gram-negative bacilli	7 ± 0	7 ± 0	7 ± 0	7 ± 0

Discussion

Salvadora persica L., commonly known as miswak, is a widely used traditional oral hygiene tool with well-documented ethnomedicinal significance. Although numerous studies have validated its role in maintaining oral health, there remains a need for more comprehensive and critical evaluations of its antimicrobial efficacy, particularly against clinically relevant oral pathogens.[15,16] In the present *in vitro* study, the antibacterial activity of ethanolic extracts of *S. persica* collected from Shendi, Sudan, was investigated against bacterial isolates associated with dental caries. The predominant organisms identified were *Streptococcus viridans*, followed by *Staphylococcus aureus* and *Staphylococcus epidermidis*, which is consistent with the known microbial profile of cariogenic infections. The findings of this study demonstrated that the ethanolic extract of *S. persica* did not exhibit any significant antibacterial activity against the tested isolates. Although an inhibition zone of approximately 7 mm was observed across all bacterial species and extract concentrations, this value is considered negligible since it is only marginally greater than the well diameter (6 mm). Moreover, the similarity between the inhibition zones of the extract and the negative control (solvent) strongly suggests that the observed effect was due to solvent diffusion rather than true antimicrobial activity. Therefore, all tested bacterial isolates can be considered resistant under the conditions of this study. These findings contrast with several previous studies that reported significant antibacterial activity of *S. persica* extracts. For example, Khalil et al. reported inhibition zones of up to 35–36 mm against *Staphylococcus aureus* and *Streptococcus* spp. using methanolic extracts.[17] Similarly, Al-Bayati and Sulaiman demonstrated selective antibacterial activity, where *Streptococcus faecalis* showed the highest susceptibility, while *Lactobacillus acidophilus* and *Pseudomonas aeruginosa* were resistant.[18] In addition, Siddeeqh et al. reported moderate inhibition zones ranging from 8.67 mm to 17.33 mm depending on the bacterial species and extract type.[19] The discrepancies between the present findings and previous reports may be attributed to several factors. First, the type of extraction solvent plays a critical role in determining the bioactive compounds obtained; methanolic extracts are often reported to yield higher antimicrobial activity compared to ethanolic extracts. Second, geographical variation in plant origin may influence the phytochemical composition of *S. persica*, leading to differences in biological activity. Third, methodological variations, including extract concentration, bacterial strains tested, and assay conditions, may further contribute to inconsistent results across studies. Recent studies have also highlighted that the antimicrobial efficacy of *S. persica* is highly variable and dependent on multiple experimental and environmental factors.[20-23] These observations support the findings of the current study and emphasize the importance of standardizing extraction procedures and testing protocols. Despite the widespread traditional use of miswak, the present study did not confirm its

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antibacterial effectiveness in ethanolic form against cariogenic bacteria. This highlights the need for further research focusing on alternative extraction methods, identification of active phytochemical constituents, and evaluation under *in vivo* conditions to better understand its therapeutic potential. Recent studies have further confirmed that the antimicrobial activity of *Salvadora persica* is highly dependent on extraction solvent, phytochemical composition, concentration, and target microbial species. For instance, studies conducted between 2021 and 2023 demonstrated that methanolic and petroleum ether extracts of *S. persica* exhibited variable antibacterial and antibiofilm activities against oral pathogens such as *Streptococcus mutans* and other cariogenic bacteria, whereas some ethanolic preparations showed weaker or inconsistent effects. These findings may explain the absence of significant antibacterial activity observed in the present study.[23-25]

Conclusion

Although the alcoholic extracts of *Salvadora persica* (miswak) have been reported to exhibit antimicrobial activity against common oral pathogens, the ethanolic extract in the present study showed no activity against bacteria associated with dental caries. This suggests that the antimicrobial effects of *S. persica* may depend on factors such as extraction method, concentration, or synergistic interactions with other natural compounds or antibiotics. Further research using standardized extraction procedures and advanced techniques is needed to identify the specific bioactive compounds responsible for its antimicrobial properties.

Recommendations

a) Future studies should incorporate appropriate negative (solvent) and positive (standard antibiotic) controls to accurately interpret agar diffusion results.

b) Conduct comparative studies between Sudanese *S. persica* and those from other regions (e.g., Egypt, the Far East) to assess potential phytochemical differences.

c) Investigate the bioactive components of *S. persica* Linn. using advanced analytical techniques.

d) Explore the use of alternative extraction solvents (e.g., methanol, aqueous) and methods.

e) Evaluate the activity against a broader spectrum of pathogens, including multidrug-resistant strains.

f) Perform *in vivo* studies to validate any *in vitro* findings and assess clinical relevance.

Consent

The patient's written consent has been collected.

Ethical approval

Ethical approval for this study was obtained from the Research Ethics Committee of Shendi University. Written informed consent was obtained from all participants before sample collection.

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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