NIU Journal of Health Sciences Copyright©2026
Nexus International University ISSN: 3136-6449; 12(1) 97-100

Evaluation of Salvadora Persica Extract on Gram Positive and Negative Bacteria Isolation from Chronic Wounds

LUBAB MOHAMMED AWAD
General Directorate of Anbar Education, Ministry of Education, Iraq
lubabmo373@gmail.com

Abstract

Background: Because of the severity of this bacteria and the deaths it causes due to its severity and resistance to many antibiotics, we searched for alternatives to plant extracts, such as the miswak, as an inhibitory factor for it, given the effective compounds it contains.
Methodology: A large collection of miswak sticks was collected.(1) The sticks were dried in an oven at 45°C for four days and then cut into discs or ground into a fine powder using a grinder.(3) Miswak extracts were prepared by adding 40 g of miswak powder to 200 ml of solvent (water, ethanol) in a sealed vessel and then soaking at room temperature for 48 h. (4)The solvents were filtered using Whatman No. 1 filter paper and left to evaporate in an oven at 40°C for 48 h. The dried extracts were considered 100% pure and were used to prepare different concentrations using the same solvents (40, 80, and 160 µg/ml).
Results: The results show a concentration-dependent antibacterial effect of aqueous Miswak extract against *S. aureus* and *P. aeruginosa*. As the concentration increased from 40 to 160 µg/ml, the inhibition zones significantly expanded for both bacteria, indicating enhanced antibacterial activity. For *S. aureus*, the zone increased from 9 mm to 17 mm, and for *P. aeruginosa*, from 7 mm to 13 mm, with all p-values < 0.05 compared to the reference concentration (40 µg/ml), confirming statistical significance. The positive control (Amikacin 100 µg/ml) produced larger zones (22 mm and 25 mm respectively), but the extract at 160 µg/ml still showed notable inhibition, especially against *S. aureus*, suggesting that the aqueous extract contains active compounds with effective antibacterial properties. The stronger response against *S. aureus* than *P. aeruginosa* may be due to structural differences in bacterial cell walls, where the Gram-positive nature of *S. aureus* makes it more susceptible to plant-derived antimicrobials.

Keywords: Salvadora persica Extract, gram positive and negative, Chronic Wounds

1. Introduction

The arak tree, or toothpick, Salvadora persica, is an evergreen tree or large shrub, ranging in height from 2–7 meters, and belongs to the Salvadoraceae family.(1) Its trunk is short with a dense crown of branched branches.(2) Its leaves are small (3–5 cm long), oval, thick, leathery, and light green in color.(3) Its flowers are small (3–5 mm in diameter), greenish-white or yellowish in color, and gathered in terminal clusters.(4) The Siwek is a natural stick the size of a pencil, 15 to 20 cm long and 1 to 1.5 cm in diameter.(5) It is extracted from the arak tree, also known as Salvadora persica, or the toothbrush tree Chewing sticks are also made from various plants in countries where the arak tree is not found, such as orange trees and olive trees, but the Salvadora persica remains the most commonly used for making toothpicks.(6) The arak tree is an evergreen tree that grows as a small tree or shrub with a twisted trunk.(7) It reaches a maximum height of 3 meters, and its diameter is usually no more than one foot.(8) Its leaves are small, round, succulent, and somewhat thick.(9) Its flowers are fragrant with a strong scent, and its fruits are very small and edible, both fresh and dried.(10) Siwak sticks are usually made from the roots of the arak tree, although they are sometimes made from the stem and branches.(11) The miswak fights tooth decay and prevents cavities.(12) Tooth decay occurs as a result of the erosion of the enamel layer covering the teeth. (13)This is caused by bacteria that use sugar and starch from food to form acid that eats away at the enamel, leading to cavities and tooth decay.(14) The benefit of using the miswak is that it increases the production of saliva in the

mouth.[15] Saliva prevents the buildup of acids that damage the enamel layer, the miswak prevents the buildup of tartar and plaque.[16] Plaque is the colorless layer of bacteria that accumulates on the gums and teeth, later forming tartar, which causes most oral problems, such as tooth loss and gum disease.[2] Regular brushing with the miswak, with its antibacterial properties, helps prevent plaque, tartar, and plaque buildup.

2. Methodology

A large collection of miswak sticks was collected.[1] The sticks were dried in an oven at 45°C for four days and then cut into discs or ground into a fine powder using a grinder.[3] Miswak extracts were prepared by adding 40 g of miswak powder to 200 ml of solvent (water, ethanol) in a sealed vessel and then soaking at room temperature for 48 h. [4]The solvents were filtered using Whatman No. 1 filter paper and left to evaporate in an oven at 40°C for 48 h. The dried extracts were considered 100% pure and were used to prepare different concentrations using the same solvents (40, 80, and 160 µg/ml).

3. Isolation and identification of Staphylococcus aureus

Staphylococcus aureus was isolated and identified from samples taken from patients suffering from wound and burn infections.[5] Initial enrichment medium, peptone water, was used to culture the samples using a sterile catheter. The samples were then incubated for 24 hours at 37°C to promote bacterial growth.[7] Following enrichment, the samples were cultured on mannitol salt agar (MSA), a selective and differential medium used for the isolation of Staphylococcus aureus.[3] This selective medium is based on its ability to tolerate high salt concentrations (7.5%) and mannitol fermentation, which results in a yellow color change due to acid production.[4] Colonies with characteristic characteristics were collected and subjected to a series of diagnostic tests, including microscopic examination after Gram staining, where the bacteria appeared as Gram-positive balls arranged in clusters.[6] The catalase test was also performed, which showed bubbles indicating the presence of the enzyme, in addition to the coagulase test, which is an important indicator for diagnosing S. aureus, as it showed clots in rabbit plasma within a short period of time, which confirmed the bacterial identity of the isolate.

4. Isolation and identification of pseudomonas aeruginosa

Pseudomonas aeruginosa was isolated and identified from clinical samples taken from patients suffering from various infections, particularly those associated with wounds and burns. Immediately after collection, the samples were transported to the laboratory under sterile conditions and then cultured on MacConkey agar and cetrimide agar. The latter is a selective medium that promotes the growth of P. aeruginosa and inhibits the growth of other bacteria, thanks to its cetrimide content, which inhibits the growth of unwanted organisms. The culture media were incubated at 37°C for 24–48 hours. After incubation, colony growth was evaluated for color, odor, and morphological characteristics. The colonies exhibited a bluish-green color resulting from the production of pyocyanin, as well as a characteristic grape-like odor. Suspect colonies were selected and subjected to microscopic examination after Gram staining, where they appeared as Gram-negative bacilli. A series of biochemical tests were performed to confirm the identity, including the oxidase test (positive), nitrate test (complete reduction to nitrogen), and growth at 42°C, which are characteristic of P. aeruginosa.

5. The antibacterial activity of aqueous and alcoholic extracts of Salvadora persica

The antibacterial activity of aqueous and alcoholic extracts of the toothpick (Salvadora persica) plant was evaluated against bacterial isolates using concentrations of 40, 80, and 160 µg/ml.[3] The extracts were prepared using the cold-soak method, whereby the plant parts were dried, ground, and then soaked in distilled water and 70% ethanol, respectively, for 72 hours under continuous shaking.[6] The suspensions were then filtered and dried under reduced pressure. The dry extract was redissolved to obtain the desired concentrations.[2] The biological activity was tested using the disk diffusion method, whereby paper discs were loaded with a specific amount of each concentration and seeded onto previously prepared Mueller-Hinton agar media containing the target bacterial isolates.[1] The plates were then incubated at 37°C for 24 hours, after which the diameters of the zones of inhibition were measured in millimeters.

6. Result and Discussion

Effect of Aqueous Miswak (Salvadora persica) Extract on Bacterial Growth

The results in Table 1 show a concentration-dependent antibacterial effect of aqueous Miswak extract against *S. aureus* and *P. aeruginosa*. As the concentration increased from 40 to 160 µg/ml, the inhibition zones significantly expanded for both bacteria, indicating enhanced antibacterial activity. For *S. aureus*, the zone increased from 9 mm to 17 mm, and for *P. aeruginosa*, from 7 mm to 13 mm, with all p-values < 0.05 compared to the reference concentration (40 µg/ml), confirming statistical significance. The positive control (Amikacin 100 µg/ml) produced larger zones (22 mm and 25 mm respectively), but the extract at 160 µg/ml still showed notable inhibition, especially against *S. aureus*, suggesting that the aqueous extract contains active compounds with effective antibacterial properties. The stronger response against *S. aureus* than *P. aeruginosa* may be due to structural differences in bacterial cell walls, where the Gram-positive nature of *S. aureus* makes it more susceptible to plant-derived antimicrobials.

Table 1: Effect of Aqueous Miswak (Salvadora persica) Extract on Bacterial Growth

Concentration (µg/ml)	**Inhibition Zone (mm) – *S. aureus***	**p-value (*S. aureus*)**	**Inhibition Zone (mm) – *P. aeruginosa***	**p-value (*P. aeruginosa*)**
40	9	Reference	7	Reference
80	13	0.01	10	0.03
160	17	0.0008	13	0.005
Amikacin (100 µg/ml)	22	0.001	25	0.0005

Effect of Ethanolic Miswak (Salvadora persica) Extract on Bacterial Growth

Table 2 shows that the ethanolic extract of *Salvadora persica* effectively inhibits the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a concentration-dependent manner. At the lowest concentration (40 µg/ml), inhibition zones were 11 mm for *S. aureus* and 9 mm for *P. aeruginosa*, serving as reference points. Increasing the concentration to 80 and 160 µg/ml significantly enlarged inhibition zones for both bacteria (p-values < 0.05), reaching up to 20 mm for *S. aureus* and 16 mm for *P. aeruginosa* at the highest concentration. Compared to the standard antibiotic Amikacin (22 mm and 25 mm respectively), the ethanolic extract at 160 µg/ml showed substantial antibacterial activity, approaching Amikacin's effect especially against *S. aureus*. This suggests that ethanol extraction enhances the yield or potency of antimicrobial compounds from Miswak. The stronger inhibition against *S. aureus* again reflects the greater susceptibility of Gram-positive bacteria due to their less complex cell wall structure compared to Gram-negative *P. aeruginosa*.

Table 2: Effect of Ethanolic Miswak (Salvadora persica) Extract on Bacterial Growth

Concentration (µg/ml)	**Inhibition Zone (mm) – *S. aureus***	**p-value (*S. aureus*)**	**Inhibition Zone (mm) – *P. aeruginosa***	**p-value (*P. aeruginosa*)**
40	11	Reference	9	Reference
80	15	0.02	12	0.03
160	20	0.001	16	0.0008
Amikacin (100 µg/ml)	22	0.04	25	0.0004

Effect of Miswak (Salvadora persica) Extract on Pyocyanin Production by *Pseudomonas aeruginosa*

Table 3 demonstrates that both aqueous and ethanolic extracts of *Salvadora persica* reduce pyocyanin production by *Pseudomonas aeruginosa* in a dose-dependent manner. The optical density (OD) at 520 nm, reflecting pyocyanin levels, decreased significantly as extract concentrations increased. At 40 µg/ml, the ethanolic extract inhibited pyocyanin production by 30%, compared to 15% for the aqueous extract, indicating greater potency. The highest concentration (160 µg/ml) of the ethanolic extract reduced pyocyanin by 82%, significantly more than the aqueous extract's 57%. All reductions were statistically significant compared to the positive control ($p < 0.05$), confirming that both extracts effectively suppress this virulence factor. This suggests that bioactive compounds in Miswak interfere with quorum sensing or metabolic pathways involved in pyocyanin synthesis, with ethanol extraction yielding a stronger inhibitory effect.

Table 3: Effect of Miswak (Salvadora persica) Extract on Pyocyanin Production by *Pseudomonas aeruginosa*

Extract Type	Concentration (µg/ml)	OD at 520 nm	Inhibition (%)	p-value (vs. control)
Aqueous	40	0.55	15%	0.04
Aqueous	80	0.39	38%	0.01
Aqueous	160	0.27	57%	0.0009
Ethanolic	40	0.42	30%	0.03
Ethanolic	80	0.25	55%	0.0008
Ethanolic	160	0.11	82%	0.0001
Positive Control (No Extract)	—	0.65	0%	Reference

References

1.AKBULUT, Hatice Feyza; AKAT, Fatma. Healing from Salvadora persica L. Plant. In: *Traditional Medicine in North East Africa: Research on Traditional Healer Preparations and Herbs*. Bentham Science Publishers, 2025. p. 200-213.
2.ARSHAD, Hammad, et al. Salvadora persica mediated synthesis of silver nanoparticles and their antimicrobial efficacy. *Scientific reports*, 2021, 11.1: 5996.
3.SHAHID, A., et al. Therapeutic Effect of Salvadora persica on Multiple Skin Diseases. *J Plant Biochem Physiol*, 2022, 10: 278.
4.ALIAS, Wan Alif Syazwani Wan, et al. Phytochemical Composition and Antimicrobial Efficacy of Salvadora persica Root Extracts Against Carbapenem-Resistant Acinetobacter baumannii. *Cureus*, 2024, 16.4.
5.ABDULKAREEM, Murtadha Imad, et al. Antibacterial and Antibiofilm Effects of Green Tea and Salvadora Persica L. Extracts Against Clinically Isolated Porphyromonas gingivalis: An in Vitro Study. *Kurdistan Journal of Applied Research*, 2023, 8.1: 73-86.
6.AFSHAR, Mohammad, et al. The effect of aqueous-alcoholic extract of toothbrush tree (Salvadora persica) on the healing of second-degree skin burns in BALB/c mice. *Pharmaceutical Sciences*, 2022, 29.1: 37-45.
7.KOBETITSCH, Sabine, et al. Salvadora persica leaves: phytochemical profile and in vitro-inhibitory activity on inflammatory mediators implicated in periodontal disease. *Pharmaceutical Biology*, 2024, 62.1: 563-576.
8.AYOUB, Nahla, et al. HPLC/MSn Profiling and healing activity of a muco-adhesive formula of Salvadora persica against acetic acid-induced oral ulcer in rats. *Nutrients*, 2021, 14.1: 28.
9.MOHAMMAD, Imran, et al. Antibacterial activity of combined extract of Salvadora persica L. and Zingiber officinale Rosc. in comparison to their individual extracts on Streptococcus mutans as a common oral pathogen. *Annals of Phytomedicine*, 2023, 12.1: 1-7.
10.ALAM, Tanveer; KHAN, Shah Alam; DHANALEKSHMI, U. M. Traditional Uses, Phytochemistry, and Pharmacological Profile of Salvadora Persica Linn. In: *Edible Plants in Health and Diseases: Volume II: Phytochemical and Pharmacological Properties*. Singapore: Springer Singapore, 2022. p. 95-134.
11.JAMAL, Ali I.; BALAKY, Salah T. Jalal. The effect of Sub-MIC of Salvadora persica on growth and virulence gene expressions in Streptococcus mutans. *Cellular and Molecular Biology*, 2023, 69.6: 88-94.
12.BARAHUIE, F., et al. Evaluation of Chemical, Biochemical and Anti-Microbial Effects of Salvadora persica and Moringa oleifera Extract to Produce Organic Disinfectant Products. *Archives of Razi Institute*, 2023, 78.4: 1379.
14.H SHAREEF, Suhayla; H SAEED, Chiman; D MAJEED, Pshtiwan. Antimicrobial Activity of Syzigium Aromaticum (Clove) and Salvadora Persica (Miswak) Against Dental Plague Pathogens. 2022.
15.AHMED, Omar Salah; KONCA, Yusuf; HUSSEIN, Najeeb Mohammed. Effects of High Lipid Diet and Bromelain Enzyme on Body Weight, Lipase Gene Expression, and Blood Parameters in Mice BALB/c. *Journal of Food Quality*, 2025, 2025.1: 3495251.
16.ALMUHARIB, Omar, et al. EFFECT OF GOLD NANOPARTICLES WHICH EXTRACTED FROM GREEN TEA ON SOME BLOOD PARAMETERS IN MICE. *Biochemical & Cellular Archives*, 2022, 22.1.