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Investigation Of Salvadora Persica Extract's Inhibitory Activity On Specific Pathogenic Bacteria

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Abstract
*Twelve organic extracts from Salvadora persica (S. persica) were tested for their antibacterial properties against Gram⁺ *Staphylococcus aureus and Gram⁻ Escherichia coli*. Disk diffusion assays revealed dose-dependent inhibitory activity, with concentrations of 3%, 5%, and 7% (w/v) producing significant inhibition zones of 35 ± 0.5 mm and 30 ± 0.5 mm for E. coli and S. aureus, respectively. The extracts have shown superior activity against Gram⁻ bacteria relative to Gram⁺ strains ($p < 0.05$). Comparative analysis demonstrated that the antibacterial potency of S. persica extracts either matched or exceeded that of standard antibiotics, particularly against multidrug-resistant (MDR) variants. The results underscore the potential of S. persica as a natural, cost-effective substitute for traditional antibiotics, presenting a promising solution for addressing antibiotic-resistant bacteria. Additional in vivo and experimental research are necessary to confirm these findings and identify the bioactive chemicals responsible for the reported effects.*
Keywords: *Salvadora persica, antibacterial properties, antibiotic alternative inhibition zone*

INTRODUCTION

Salvadora persica, commonly known as Miswak or Arak, belongs to the familySalvadoraceae.[1] It is an evergreen shrub or small tree native to arid regions, particularly in India, where it thrives in saline soils. Traditionally, its fresh leaves have been consumed as salad and employed in folk medicine for treating conditions such as cough, asthma, rheumatism, scurvy, and pilesFor centuries, Miswak has held cultural and medicinal significance, especially in oral hygiene practices. Its use predates Islam, with ancient Arab populations utilizing it to clean, whiten, and polish teeth. The oral health benefits of S. persica are attributed to both its mechanical action (from brushing) and its pharmacological effects, thanks to a rich composition of bioactive compounds. These include benzyl isothiocyanate, saponins, tannins, silica, and alkaloids, which collectively contribute to its antimicrobial and therapeutic properties.s.[2] Salvadora persica is widely distributed across the arid regions of India, often thriving in saline soils. Its fresh leaves are consumed as salads and are also used in traditional medicine to treat various conditions such as scurvy, rheumatism, piles, and other ailments. The ancient Arabs used the miswak, derived from this plant, to whiten and polish their teeth—an established practice that predates Islam. The beneficial effects of S. persica on dental health and oral hygiene are attributed to both mechanical and pharmacological actions. Numerous studies have investigated the diverse chemical constituents responsible for these therapeutic properties..[3][4] Research has identified benzyl isothiocyanate as a major compound extracted from the roots of Salvadora persica, along with other constituents such as saponins, tannins, silica, a small amount of resin, and trimethylamine., and a substantial amount of alkaloidal components. Compounds like β-sitosterol, m-anisic acid, and salvadorine [1,3-Bis-(3-methoxy-benzyl)-urea] have also been identified. The plant, native to India, has been found to have a high mineral content in its roots, with minerals constituting 27.06% of its total composition. Other chemical components include elemental sulfur, sulfur-containing mustard oil, sodium bicarbonate (known for its high sulfur content), trimethylamine, chlorides, fluoride, silica (SiO_2), vitamin C, flavonoids, and sterols.[5][6][7] E. coli are highly adaptable microbes and integral constituents of the normal gut microbiota in humans and animals. Although generally innocuous, these commensal organisms can obtain mobile genetic elements that harbor virulence factor genes, thereby evolving into new human pathogens capable of inducing a variety of intestinal and extra-intestinal illnesses.[8] S. aureus is among the most prevalent infectious organisms globally, contributing to considerable morbidity and mortality. This pathogen can induce ailments varying from benign dermal infections to critical, life-threatening disorders such as pneumonia and sepsis. The

management of S. aureus infections is further hindered by antibiotic resistance, and an effective vaccination is presently unavailable.[9]

MATERIALS AND METHODS

1. Study extracts (S. persica)
2. Isolates used in the study: The bacterial isolates were obtained from the Public Health Laboratory. These isolates were previously identified and characterized for use in research
3. Extraction of Active Compounds from S. persica
4. S. persica L. was collected from Saudi Arabia. The active compounds were extracted using a continuous Soxhlet extraction method with four organic solvents of varying polarity: petroleum ether, ethyl acetate, methanol, and ethanol. This process yielded four types of crude extracts. The solvents were subsequently removed using a rotary evaporator at temperatures below 40 °C. Each crude extract was then subjected to column chromatography for the purification and isolation of individual compounds. Structural identification of the isolated components was further supported by various analytical techniques.
5. Testing the Biological Activity of the Extracts

The bacterial isolates used in the study were reactivated in Brain Heart Infusion (BHI) medium, prepared according to the manufacturer's instructions. The agar diffusion method was employed as described in... [11], The agar diffusion method was employed to assess the biological activity of the extracts. Mueller Hinton Agar (MHA) was prepared according to the manufacturer's instructions and poured into Petri dishes. Once solidified, the medium was inoculated with 1.0 mL of bacterial suspension at a concentration of 1×10^6 cells/mL. The bacterial concentration was determined using a spectrophotometer at a wavelength of 450 nm [11]. The bacterial suspension was uniformly spread over the surface of the agar using sterile cotton swabs. The plates were then left undisturbed for 15 minutes to allow the suspension to be fully absorbed into the medium. Subsequently, wells measuring 8 mm in diameter were created in each plate using a sterile cork borer.. Subsequently, 10 µL of each extract at concentrations of 3%, 5%, and 7% were added to the wells using a micropipette. The dishes were incubated at 36°C for 24 hours. Subsequent to the incubation, the outcomes were documented by quantifying the widths of the inhibitory zones in millimeters. The presence of an inhibitory halo (a clear zone surrounding the wells) indicated a favorable outcome, and the diameter of the halo was assessed. The extract's name and the bacteria cultivated in the dish were recorded. Bacterial growth surrounding the wells and the lack of an inhibitory halo indicated a negative outcome (negative control).

6. **Testing the Sensitivity of Bacteria to Antibiotics**

The bacterial sensitivity to antibiotics was assessed utilizing Mueller Hinton Agar (MHA) media. Nine antibiotics, prepared by Bioanalyse Company, were evaluated for their biological effectiveness. Sterile cotton swabs evenly spread 1 mL of Tryptic Soy Broth (TSB) culture onto the MHA medium. The inoculated dishes were left for 30 minutes to allow the suspension to absorb into the medium. Antibiotic discs, pre-saturated with the respective antibiotics, were placed onto the agar surface using sterile forceps. The dishes were incubated at 36°C for 24 hours 12. After incubation, the results were recorded by measuring the diameters of the inhibition zones in millimeters [10], as shown in Table 2. Additionally, representative images of the results are provided in Pictures (1, 2, 3).

Results

Inhibitory Activity of the Extract S. persica

S. persica extract demonstrated significant inhibitory activity against $Gram^-$ and $Gram^+$ bacteria, including E. coli and S. aureus. The study results revealed a notable inhibitory effect of the S. persica extract on bacterial growth. The average diameter of the inhibition zone for E. coli ($Gram^-$ bacteria) was 35 mm, while for *Staphylococcus aureus* ($Gram^+$ bacteria), it was 30 mm. The most effective extracts against E. coli ($Gram^-$ bacteria), in descending order of effectiveness, are listed in Table 1

- Miswak aqueous extract (7%)
- Dodecane (3%)
- Cyclohexane amine N, N-dimethyl (5%)
- 6-Octadecenoic acid (Z) (3%)
- *cis*-10-Nonadecenoic acid (7%)
- Trifluoroacetic acid, pentadecyl ester (5%)
- *cis*-Vaccenic acid (5%)
- Silver nanoparticles (7%)
- (+)-Ascorbic acid 2,6-dihexadecanoate (5%)
- Tetracosane (3%)
- 5H-Cyclopenta[b]pyridine (7%)

Similarly, the most effective extracts against *Staphylococcus aureus* (Gram^{+} bacteria), in descending order of effectiveness, are listed in Table 1:

- Silver nanoparticles (7%)
- 9-Octadecenoic acid, ethyl ester (7%)
- *cis*-Vaccenic acid (5%)
- Trifluoroacetic acid, pentadecyl ester (5%)
- (+)-Ascorbic acid 2,6-dihexadecanoate (5%)
- *cis*-10-Nonadecenoic acid (7%)
- Tetracosane (3%)
- Cyclohexane amine N, N-dimethyl (5%)
- Dodecane (3%)

The detailed results are summarized in Table (1).

No	Extract	Conce. of extract	Inhibition zone diameter mm against *E.coli*	Inhibition zone diameter mm against *Staphylococcus aureus*
1	Cyclohexan amine N, N-di methyl	5%	24	13
2	Dodecane	3%	30	11
3	Trifluoroacctic acid, pcntadecyl ester	5%	22	18
4	5H-cyclopenta[b] pyridine	7%	16	15
5	6-octadecenoic acid(z)	3%	23	12
6	-(+)-Asorbic acid 2,6dihexadecanoate	5%	18	17
7	Cis-10–nanodecenoic acid	7 %	23	15
8	Tetracosane	3%	17	14
9	Cis Vaccenic acid	5 %	22	25
10	9-octadecenoic acid, ethyl ester	7%	15	28
11	Miswak aqueous extract	7%	35	30
12	Silver nanoparticles	7%	20	15
13	control	-----	Zero	Zero

Table (1) shows The effectiveness of the extracts against bacterial isolates.

Images below show the extract's effectiveness against *E.coli* bacteria and *Staphylococcus aureus*.

Figure (1)

Figure (2)

Figure (3)

Figure (4)

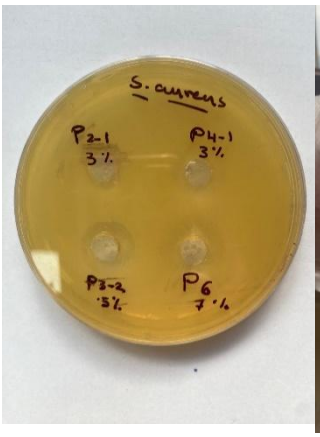

Figure (5)

Figure (6)

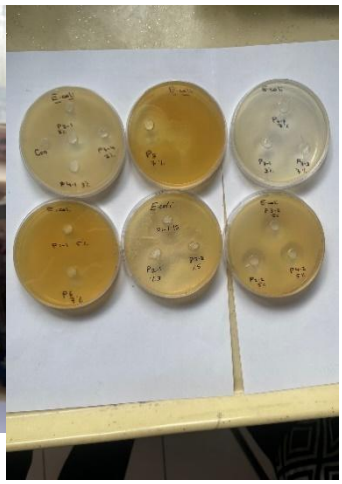

Figure (7)

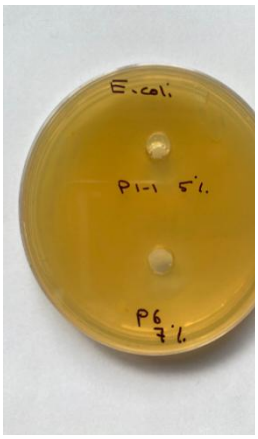

Figure (8)

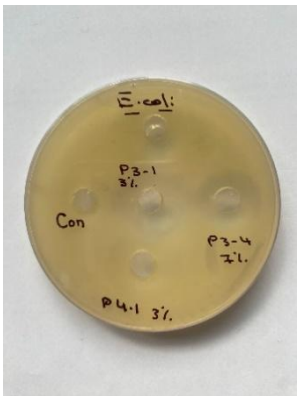

Figure (9)

Figure (10)

Figure (11)

Figure (12)

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Figure (13)

Figure (14)

Figure (15)

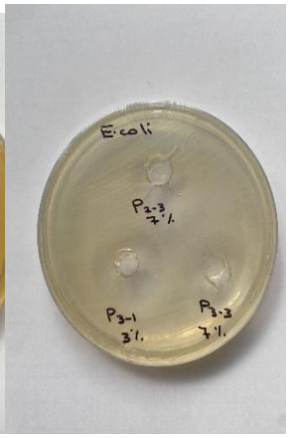

Figure (16)

Figure (17)

Figure (18)

Figure (19)

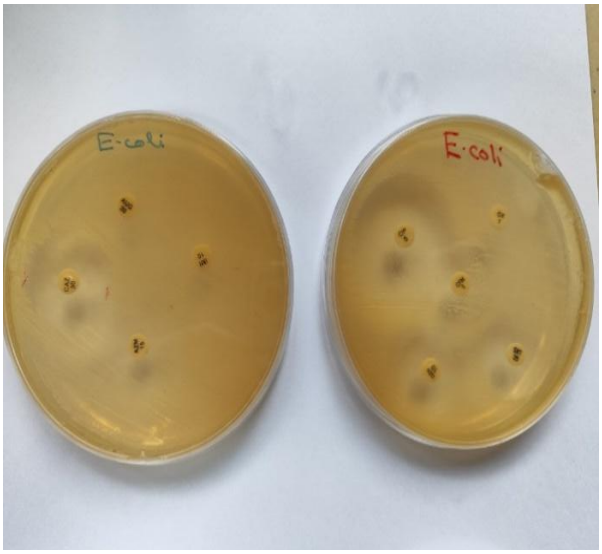

Figure (20)

- **Sensitivity of the bacteria to antibiotics**

Table 2 illustrates the susceptibility of E. coli and S. aureus isolates to antibiotics.

Antibiotics	**Abbreviation**	**Conce.**	**Diameter of the inhibition zone (mm)**			
			E.coli	**Result**	***Staphylococcus aureus***	**Result**
Amoxicillin-clavulanate	AUG	(20/10 µ g)	Zero	R	Zero	R
Ciprofloxacin	TIP	5µ g	28	S	30	S
Ceftazidime	CAZ	30 µ g	22	S	Zero	R
CeFexime	CFM	30 µ g	Zero	R	Zero	R
Gentamicin	GEM	30 µ g	17	I	17	S
Trimethoprim Sulfamethoxazole	SXT	5µ g	21	S	17	S
Imepenem	IMP	10µ g	Zero	R	Zero	R
Azithromycin	AZM	15µ g	9	R	17	I
Oxacillin	OX	1µ g	Zero	R	Zero	R

[R =resistant, S = sensitive, I =intermediate]

The antibiotic susceptibility testing for the nine antibiotics indicated that E. coli exhibited resistance to oxacillin, cefixime, amoxicillin-clavulanic acid, azithromycin, and imipenem. However, they were sensitive to ciprofloxacin and trimethoprim-sulfamethoxazole and showed intermediate sensitivity to gentamicin. Similarly, the *Staphylococcus aureus* isolates were resistant to oxacillin, cefixime, amoxicillin-clavulanic acid, gentamicin, and imipenem. In contrast, they were sensitive to ciprofloxacin and trimethoprim and exhibited intermediate sensitivity to azithromycin.

Table (3): for compared

Antibiotics	Introbacteriales (E. Coli)			Staphylococcus aureus		
Amoxicillin Clavulanate (AMC)	≥18	14-17	13≤	S	I	R
Cefixime (CFM)	≥19	16-18	15≤	N	N	N
Imipenem IMP	≥23	20-22	19≤	N	N	N
Gentamicin GEN	≥18	15-17	14≤	≥15	13-14	12≤
Azithromycin AZM	≥13	_____	12≤	≥18	14-17	13≤
Tetracycline	≥14	11-13	10≤	≥19	18-15	14≤
Ciprofloxacin	≥26	22-25	21≤	≥21	16-20	15≤

SXT	≥16	11-15	10≤	≥16	11-15	10≤
Oxacillin $_{CX}$	≥18	15-17	14≤	2≤ Mic	_____	Mic ≥4
Ceftazidime $_{CHZ}$	≥21	20-18	17≤	N	N	N

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DISCUSSION:

Table (1) presents the results of the effectiveness of the S. persica extract at a concentration of 7%. The table demonstrates significant inhibitory activity against the bacterial isolates under study. This inhibitory effect is attributed to active compounds in S. persica, such as fluoride, alkaloids, essential oils, and tannins. These compounds are known for their potent antimicrobial properties, particularly against Gram$^-$ bacteria. Table (2) illustrates the sensitivity of bacterial isolates to antibiotics. Some isolates resisted certain antibiotics, likely due to genetic mutations or the transfer of resistance genes via plasmids, transduction, or transformation. However, other antibiotics showed effectiveness against the microbes. Notably, the S. persica* extract exhibited an antimicrobial effect comparable to that of antibiotics, and in some cases, it was even more effective. The findings indicate that S. persica extract may function as a viable alternative to antibiotics, effectively suppressing both Gram$^-$ bacteria (e.g., E. coli) and Gram$^+$ bacteria (e.g., S. aureus).

REFERENCES

[1] H. Y. Jasim and H. M. Habeeb, "Effect of biotic and abiotic elicitors on Salvadora persica callus in vitro," *Baghdad Sci. J.*, vol. 21, no. 9, p. 2829, 2024.

[2] M. V Prakash and M. Anbarashan, "The Ukāy, Salvadora persica (Salvadoraceae): Historical and literary evidence from stone inscriptions, copper plates, and literature," *Indian J. Hist. Sci.*, pp. 1–6, 2024.

[3] D. Kumar and P. K. Sharma, "Traditional use, phytochemicals and pharmacological activity of Salvadora persica: A Review," *Curr. Nutr. Food Sci.*, vol. 17, no. 3, pp. 302–309, 2021.

[4] M. K. M. Elbestawy, G. M. El-Sherbiny, and S. A. Moghannem, "Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant Helicobacter pylori," *Molecules*, vol. 28, no. 6, p. 2448, 2023.

[5] S. C. L. Lim *et al.*, "Efficacy of ivermectin treatment on disease progression among adults with mild to moderate COVID-19 and comorbidities: the I-TECH randomized clinical trial," *JAMA Intern. Med.*, vol. 182, no. 4, pp. 426–435, 2022.

[6] P. Kumar and P. K. Sharma, "Soil salinity and food security in India," *Front. Sustain. Food Syst.*, vol. 4, p. 533781, 2020.

[7] F. Aljarbou, A. Almobarak, A. Binrayes, and H. M. Alamri, "Salvadora Persica's Biological Properties and Applications in different Dental specialties: a narrative review," *Evidence-Based Complement. Altern. Med.*, vol. 2022, no. 1, p. 8667687, 2022.

[8] B. Pakbin, W. M. Brück, and J. W. A. Rossen, "Virulence factors of enteric pathogenic Escherichia coli: A review," *Int. J. Mol. Sci.*, vol. 22, no. 18, p. 9922, 2021.

[9] G. Y. C. Cheung, J. S. Bae, and M. Otto, "Pathogenicity and virulence of Staphylococcus aureus," *Virulence*, vol. 12, no. 1, pp. 547–569, 2021.

[10] P. Gaur, V. Hada, R. S. Rath, A. Mohanty, P. Singh, and A. Rukadikar, "Interpretation of antimicrobial susceptibility testing using European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) breakpoints: analysis of agreement," *Cureus*, vol. 15, no. 3, 2023.

[11] World Health Organization. Manual for Laboratory Investigation of acute enteric infection CCD, 183: 83 –3(1987) Standards Methods for dilution.

[12] National Committee for Clinical Laboratory antimicrobial susceptibility tests For bacteria that grow aerobically. Approved standard M7-A4.Wayne. PA. The USA. 1997.

[13] https://www.tdlpathology.com/media/5tqplkvb/tap5358_tdl_lab_guide_2024_np_web.pdfLaboratory Guide 2024