Cytotoxicity and Antimicrobial Potential of Salvodora Persica

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Salvadora persica Miswak is a halophytic shrub renowned for its traditional medicinal uses, particularly in oral hygiene. This study was carried out to investigate the antimicrobial and cytotoxic potential of the ethanolic extract of the whole plant of S. persica. The extract was evaluated for its antibacterial and antifungal potential against a range of bacterial (Gram-positive and Gram-negative) and fungal strains using the disc and agar well diffusion methods, respectively. Cytotoxicity was assessed using the Brine Shrimp Lethality Assay (BSLA). The results exhibited significant, dose-dependent antimicrobial activity, with the extract showing greater efficacy against Gram-positive bacterium Staphylococcus aureus, with the inhibition zone of 31.0 mm at 300 mg mL^{-1} extract concentration. It also showed notable inhibition against several fungal pathogens with a maximum inhibition zone of 32 mm for Candida albicans and Trichophyton longifusus at 300 mg mL^{-1} concentration. Furthermore, the extract exhibited potent cytotoxicity in the BSLA, with a lethal concentration (LC_{50}) value of 10 µg mL^{-1}, indicating the presence of bioactive compounds with toxic potential. The findings confirmed that S. persica possesses substantial antimicrobial and cytotoxic properties, supporting its traditional use and suggesting its potential as a source for developing natural therapeutic agents.

Keywords: Antibacterial, Antifungal, Brine Shrimp Lethality Assay, Cytotoxicity, Ethanolic Extract, Salvadora Persica.

Introduction:

Natural products have long been recognized as structurally important bioactive therapeutic agents. Over the past decade, the compounds derived from plants have been widely recognized for their diverse biological activities, including antibacterial [1][2], antifungal [3][4], antioxidant [5], anticancer [6], and other health-related and pharmaceutical properties [7][8]. Salvadora persica L., commonly known as the toothbrush tree or Miswak, is an evergreen halophytic shrub. It is native to the arid and semi-arid regions of Africa, the Middle East, and Asia, including Pakistan and India [9]. This multipurpose plant is widely known for its use as a natural toothbrush (chewing stick) across various cultures, particularly within the Muslim community [10]. The plant is highly resilient, tolerating extreme saline and drought

conditions, which is why it thrives in harsh environments where few other medicinal plants persist [11]. Beyond its mechanical cleaning action, its therapeutic value is attributed to a rich repertoire of bioactive compounds released upon chewing, including trimethylamine, chloride, fluoride, saponins, flavonoids, and alkaloids, which immediately alter saliva composition [12]. Phytochemical studies have revealed that S. persica contains a complex array of secondary metabolites, which collectively contribute to its therapeutic efficacy.

Different parts of S. persica have been traditionally used to treat numerous ailments. The leaves are reported to be analgesic, anthelmintic, and anti-inflammatory and are used for asthma, scurvy, and rheumatism [13]. The fruits are considered diuretic and lithontriptic, while the root bark is used for chest complaints, stomach-aches, and gonorrhoea [14]. Stem bark, the primary source of S. persica, is well-documented for alleviating toothache, preventing plaque, and exhibiting antibacterial activity [15]. Furthermore, the seeds and flowers are traditionally used in the management of diabetes, rheumatic pain, and gastric disorders [16][17].

Despite extensive ethnomedicinal usage and emerging pharmacological evidence, most studies on S. persica have focused primarily on its role in oral care or on isolated plant parts such as the roots or stem bark. Only limited research has investigated the biological activity of whole-plant extracts, particularly regarding their cytotoxic and antimicrobial potential against a broader panel of microorganisms. Given the increasing demand for natural antimicrobial and anticancer agents, there is a critical need to explore plant extracts that may provide multi-target therapeutic benefits.

In view of the extensive pharmacological potential of different parts of S. persica and the gap in existing literature, the present study was designed to investigate the therapeutic potential of the ethanolic extract of the whole plant.

Objectives:

The objectives of this study are

To prepare the ethanolic extract of S. percica

To analyse the antibacterial potential of the ethanolic extract of S. percica,

To investigate the antifungal activity of the ethanolic extract of S. percica and

To study the cytotoxicity of the ethanolic extract of S. percica.

Materials and Methods:

Plant Material Collection and Extract Preparation:

The whole plant of S. persica was collected from the Botanical Garden of the University of the Punjab, Lahore, Pakistan, and taxonomically identified by Dr. Abdur Rehman Niazi, Institute of Botany, University of the Punjab, Lahore. The collected plant material, particularly the larger twigs, was cut into small pieces and thoroughly washed with distilled water to remove debris. The material was air-dried at room temperature for four days and subsequently ground into a fine powder using a grinder. The powdered sample was macerated in ethanol for seven days to obtain the extract. The resulting extract was filtered and stored in a refrigerator for further use.

Agar Well Diffusion Assay for Antifungal Activity:

The antifungal potential of the ethanolic extract of S. persica against various fungal strains was determined using the agar well diffusion method. Briefly, 30 mL of potato dextrose agar was sterilized by autoclaving and subsequently dispensed into sterile Petri dishes, which were set to solidify at room temperature. A sterile cork borer was used to create a 5-mm-diameter well at the centre of each Petri plate. Different concentrations of the plant extract (100, 200, and 300 mg mL^{-1}) were introduced into the wells using a sterile spatula, followed by the inoculation of fungal spores. The plates were sealed with a thin layer of paraffin and then incubated at 28 °C for three days. Miconazole served as the standard reference drug.

After incubation, the Petri plates were examined for inhibition zones, and the diameters were measured in triplicate [18].

Disc Diffusion Assay for Antibacterial Activity:

S. persica ethanolic extract was assessed for its antibacterial activity using a range of Gram-positive and Gram-negative bacterial strains. For the disc diffusion assay, firstly, agar plates were prepared by mixing 100 µL bacterial suspension and 100 mL liquid nutrient agar. Different plant extract doses, viz. 100, 200, and 300 mg mL^{-1} were loaded on separate sterilized filter paper discs (6 mm diameter) and laid on agar plates. Imipenem was used as the reference standard. All plates were incubated for 24 h at 28 °C. The inhibition zone diameter was determined in mm [19].

Brine Shrimp Lethality Assay (BSLA):

BSLA was used to examine the cytotoxicity potential of the ethanolic extract of S. persica. To conduct this bioassay, different concentration of plant extract was prepared from a stock solution of extract dilution method using the dilution method 1, 10, 100, and 1000 µg mL^{-1}. Triplicate test tubes were prepared for each plant extract concentration. Artificial seawater was prepared by dissolving 27 g of sea salt in 3 L of distilled water. This solution was used to hatch shrimp eggs, which were placed in a plastic container serving as a hatching chamber partitioned into light and dark (covered) compartments. About 15 g of shrimp eggs were placed in the dark compartment and incubated for 24 h to enable hatching into nauplii. Following incubation, the nauplii were separated from any unhatched eggs. Subsequently, test tubes containing the plant extracts were treated with a prepared solution of 10 nauplii in 1 mL of seawater. After 24 h, the number of dead nauplii was recorded [20].

Mortality (%) = [Number of dead nauplii/Total number of nauplii (dead + live)]×100

Statistical Analysis:

All the data about antifungal, antibacterial, and toxicity activities were subjected to ANOVA followed by application of LSD to separate the treatment means at $P \leq 0.05$ using STATISTIX 8.1.

Results and Discussion:

Antifungal Activity:

Table 1 illustrates the antifungal activity of the ethanolic extract of S. persica against different fungal strains. Three concentrations (100, 200, and 300 mg mL^{-1}) of the plant extract were used, and the results are represented by zone of inhibition measurement. The data clearly show that the antifungal activity of the extract increased with higher concentrations. At a concentration of 300 mg/mL, the antifungal activity of the extract was comparable to that of Miconazole, the standard reference, across all tested fungal strains. The S. persica extract showed excellent antifungal potential against F. solani, C. glabrata, and C. albicans, with maximum zone of inhibition of 7, 21, and 32 mm, respectively. It was least active against M. canis and T. longifusus.

Table 1. Zone of inhibition values at different concentrations of alcoholic Salvadora persica extract against various fungal strains.

Fungal Strain	Zone of inhibition (mm)			
	Concentration of plant extract (mg mL^{-1})			Standard drugs
	100	200	300	Miconazole
Microsporum canis	11 d	14 c	19 b	27 a
Candida albicans	25 d	29 c	32 b	36 a
Fusarium solani	2 d	4 c	7 b	9 a
Tricophyton longifusus	18 d	25 c	32 b	40.7 a
Candida glabrata	15 c	17 c	21 b	24.5 a

Aspergillus flavus	09 c	12 b	14 b	20 a

Values with different letters in a row show a significant difference as determined by the LSD test at P≤0.05.

Antibacterial Potential:

The significance of antibacterial drugs lies in their selective toxicity, meaning they specifically target bacterial cells while causing minimal or no harm to the human host. As prokaryotes, bacteria offer a wider range of unique targets for selective toxicity compared to viruses and fungi [21]. The results of the antibacterial potential of S. persica extract are shown in Table 2. The zone of inhibition data indicate that the ethanolic extract of S. persica exhibited greater antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. Additionally, the antibacterial effect was concentration-dependent. At the highest concentration (300 mg mL^{-1}), the antibacterial activity of S. persica was comparable to that of Imipenem, as indicated by the zone of inhibition values. Among Gram-positive bacteria, the highest inhibition was seen against S. aureus, while for Gram-negative bacteria, S. persica extract was highly active against S. typhi, showing zone of inhibition values of 31 and 21, respectively.

Table 2. Zone of inhibition values at different concentrations of alcoholic Salvadora persica extract against various bacterial strains.

Bacterial Strains	**Zone of inhibition (mm)**			
	Concentration of plant extract (mg/mL)			**Standard drugs**
	100	**200**	**300**	**Imipenem**
Gram negative				
Pseudomonas aeruginosa	16.0 c	19.4 b	18.2 b	24.2 a
Escherichia coli	15.6 c	23.0 b	24.5 b	30.5 a
Salomella typhi	17.3 c	20.0 b	21.0 b	25.7 a
Gram positive				
Bacillus subtilis	23.7 d	26.4 c	29.1 b	33.8 a
Staphylococcus aureus	24.3 d	27.3 c	31.0 b	33.1 a
Shigella flexneri	19.5 c	21.2 c	24.6 b	27.4 a

Values with different letters in a row show a significant difference as determined by the LSD test at P≤0.05.

Brine Shrimp Lethality Bioassay:

The brine shrimp test is a critical way to examine the cytotoxicity potential of bioactive ingredients of natural products. It is a primary toxicity screening of plant extract, fungal toxins, pesticides, and many other materials like heavy metals, dental material, etc. [22][23][24]. The nauplii, measuring approximately 2–2.2 mm in length, could be easily observed without a magnifier and were small enough to be hatched in large numbers within a limited laboratory space. Experimental results demonstrated that the ethanolic extract of the plant exhibited notable brine shrimp larvicidal activity. The lethality concentration (LC50) of S. persica extract was 10 µg mL-1 as presented in Table 3. The mortality of nauplii was found to be in direct relationship with the plant extract concentration. At 100 µg mL-1 concentration, there was 53% mortality, and 100% mortalities of nauplii was recorded at a concentration of 1000 µg mL-1 S. persica extract.

Table 3. The survival counts of brine shrimp nauplii after treatment with the ethanolic extract of S. persica

Extract Conc. (µg mL^{-1})	**Total survivors count after 24 h**	**Mortality (%)**
1	23 a	23%

10	16 b	47%
100	14 b	53%
1000	0 c	100%

Values with different letters in a column show a significant difference as determined by the LSD test at P≤0.05.

Discussion:

The present study demonstrated that the ethanolic extract of S. persica possesses significant cytotoxicity and antimicrobial activity. The results of antifungal activity clearly indicated concentration-dependent response, and maximum inhibition was observed against C. albicans, C. glabrata, and F. solani. The results were in accordance with the results reported in the literature. Korejo et al. [25] reported Salvadora species showed 6-10 mm of zone of inhibition against F. solani. It is also reported in literature that S. persica extract is potentially active against Aspergillus flavus and Candida albicans [26]. The comparatively lower activity against M. canis and T. longifusus suggests differential sensitivity among fungal groups, possibly linked to variations in cell wall composition.

The antibacterial study further supports the antimicrobial potential of S. persica. The ethanolic extract of S. persica exhibited stronger inhibition against gram-positive bacteria, which is in accordance with the previous findings of Al-Bayati and Sulaiman, who reported significant activity against S. aureus with 9.2–13.6 mm zone of inhibition at various concentrations [27]. Another study reported that S. persica is moderately active against Escherichia coli, as also represented by our results [28].

The ethanolic S. persica extract exhibited dose-dependent brine shrimp lethality. The lethality of S. persica to brine shrimps demonstrated the potent cytotoxic potential and the presence of antitumor components in the ethanolic extract of S. persica. According to the literature, an LC_{50} value of less than 1000 µg mL^{-1} is considered toxic, whereas greater than 1000 µg mL^{-1} is thought to be non-toxic [29]. These results indicate that the observed nauplii mortality can be attributed to the bioactive compounds present in the plant extract.

Conclusion:

Salvadora persica is an important medicinal plant, especially for oral health. Its ethanolic extract was checked for its antimicrobial and anticancer potential. It showed potent activity against various bacterial and fungal strains. It also showed significant results in the brine shrimp lethality bioassay, exhibiting good cytotoxicity. It was concluded from the results that S. persica may serve as an effective antibacterial agent against both gram-positive and gram-negative bacteria, as well as various fungal strains. It could also be tested for other bacterial and fungal strains, which could cause oral diseases.

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