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Antibacterial Activity of Methanol Extract of *Salvadora Persica* (Linn) Stem against Gram-Positive Bacteria Isolated from Oral Infections among the Patients Attending the University Health Clinic of Ahmadu Bello University Zaria.

***[1]Garba S., [1]Sulaiman M. T., [1]Muhammad S., [1]Sani A. and [1]Mohammed R. B.**

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria, Kaduna, Nigeria

*Correspondence author: mira0668@gmail.com, +234 803 484 2392

Abstract

Salvadora persica L., commonly referred to as Miswak, is a chewing stick commonly used to maintain dental hygiene. The aim was to determine the antibacterial effectiveness of the methanol crude extract of S. persica against gram-positive bacteria obtained from orally infected individuals who were registered at the University Health Clinic of Ahmadu Bello University. A total of twenty (20) samples were collected from patients who were diagnosed with oral infections. These samples underwent traditional biochemical and microbiological testing. The antibiotic susceptibility test was conducted using the Kirby-Bauer disc diffusion method, and the findings were interpreted following the CLSI standards. The extraction of Salvadora persica L. was carried out using cold maceration with methanol. The crude extract was evaluated using the agar well diffusion and broth dilution techniques. Phytochemical screens were conducted using established procedures. The findings revealed that Staphylococcus aureus had the highest prevalence rate of 60%, whereas Streptococcus sp. had a prevalence rate of 10%. A high level of resistance was detected for amoxicillin (100%), chloramphenicol (85%), ciprofloxacin (60%), ceftriaxone (50%), and gentamycin (47.5%). The cold maceration extraction process yielded in % crude extract yield of 11.59%. The phytochemical elements were saponin, tannin, alkaloid, flavonoid, phenol, heart glycoside, carbohydrate, steroid, and terpenoid. An area of inhibition measuring 18-22mm and a minimum inhibitory concentration (MIC) of 25mg/ml were employed to detect a significant degree of activity. The methanolic extract of Salvadora persica L. has shown significant therapeutic efficacy against gram-positive bacteria recovered from orally infected patients.

Key terms: Gram-positive bacteria, infection of the oropharynx, antibiotic

INTRODUCTION

Oral infections and dental lesions are important global health issues that impact individuals globally. Maintaining good hygiene is the most effective way to prevent periodontal disease and dental infections. A variety of equipment and substances are accessible to uphold and safeguard dental and oral well-being, such as toothbrushes, mouthwash, and toothpaste (Kheddouma *et al.*, 2021). Oral health conditions have been widespread since ancient times, and their frequency has notably risen due to the heightened consumption of processed sugar (Bifulco et al., 2016). W.D. Miller was the first to propose the presence of oral microorganisms in the human oral cavity that can convert carbohydrates into acid, leading to the formation of tooth decay. In 1949, J.K. Clarke successfully isolated the initial strain of *Streptococcus* mutans (Ferdpus et al., 2019).

Different studies have linked several species to oral infections (Ferdpus *et al.*, 2019). These species include *Streptococcus mutans, Prevotella intermedia, Bacteroides forsythus, ampylobacter rectus, Actinobacillus actinomycetemcomitans, Eikenella corrodens, Porphyromonas gingivalis, Eikenella corrodens, Eubacterium species, Fusobacterium nucleatum, Fusobacterium nucleatum, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus mitis, Eubacterium species* and oral spirochetes like Treponema denticola.

Tooth infections, such as dental caries and periodontal disorders, occur when pathogenic bacteria multiply and spread in the oral cavity.

Without treatment, these infections can cause significant discomfort, agony, and even problems that affect the entire body. An abscessed tooth, also known as a periapical abscess, occurs when an infected tooth develops a cavity filled with pus in its root (Rasouli *et al.*, 2014). This disorder can potentially spread to the adjacent bone and teeth. As a result of the emergence of antibiotic resistance in microorganisms, scientists have been motivated to explore new antibacterial chemicals from various sources, such as medicinal plants. Kheddouma*et al.* (2021) have shown that many plant extracts have substantial therapeutic efficacy against infectious pathogens. Using natural plant extracts for mouthwash and oral hygiene has gained considerable attention in the last twenty years. *Salvadora persica*, also called Miswak, Arak, or toothbrush tree, is an extensively utilized chewing tool within the Muslim community. The World Health Organisation (WHO) has endorsed using the fibrous branches of *S. persica* for oral hygiene, given that they have been found to protect against specific oral pathogens(Rasouli *et al.*, 2014).

MATERIALS AND METHODS

Sample Collection and Identification

The Salvadora Stem Collection and Identification involved acquiring *Salvadora persica* stems from Ahsan Islamic chemist Gwammaja Kano specifically to use them as chewing sticks. The samples were initially gathered and treated in Pakistan by M SHAN and subsequently recognized in the herbarium of the Department of Botany, Ahmadu Bello University, Zaria, by DrNamadiSunusi, with the specific identification number: ABU026607.

Preparation for Extraction:

The stick was rinsed with distilled water, divided into small segments, and left to air-dry at room temperature for two weeks. It was then diminished in size into smaller components. The methanol extract was obtained by soaking 500g of fragmented Salvadora persica in 2.5L of pure methanol for 72 hours. After filtration, the filtrate was evaporated using a vacuum evaporator at a temperature of 40°C. The extract was preserved in a hygienic plastic container and refrigerated until needed. Before testing, the miswak extract was reconstituted in distilled water to achieve a final 200mg/ml concentration. Subsequently, the same method was used to generate a series of dilutions ranging from 200mg/mL to 6.25mg/mL.

Preliminary Phytochemical Screening:

Evaluation of phytochemical constituents such as cardiac glycosides, steroids, flavonoids, tannins, alkaloids, anthraquinones, saponins, etc. The results were determined using conventional techniques outlined by (Musa, 2005; Sofowora, 2008; Evans, 2009).

Collection of Samples:

Twenty-three (23) samples were collected from oral-infected patients who visited the Ahmadu Bello University Medical Center's Dental Clinic using antiseptic swab sticks. The samples were conveyed to the Department of Pharmaceutical Microbiology microbiology laboratory, where they were inoculated into a sterile nutrient broth and incubated at a temperature of 37°C for 24 hours.

Procedure for isolation:

The isolation procedure involved pouring freshly made nutrient agar into a sterile petri plate and allowing it to harden. Afterward, the inoculating loop was sterilized, and a loopful of the 24-hour cultured nutrient broth was evenly streaked over the nutrient agar. The plate was incubated at 37°C for 24 hours (Supriya, 2023).

Differential isolation

Fresh blood agar plates were prepared. A single colony of 24-hour agar culture was picked and streaked onto the sterile blood agar plate using a sterilized loop, which was then incubated at 37°C for 24 hours.

Identification of gram-positive bacterial strains:

These were identified using microbiological standard protocols, microscopic examination of cell shape and structure (Gram's Staining), and biochemical assays, such as the slide and tube reaction test for catalase and coagulase, as well as growth on mannitol salt agar (Cheesebrough, 2010).

Test for determining the sensitivity of bacteria to antibiotics:

Modified Kirby-Bauer disc agar diffusion tests were conducted on each of the previously identified isolates using the Clinical Laboratory Standard Institute suggested technique. The

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experiment utilized a disc that included the following antibiotics: ciprofloxacin (5µg), gentamicin (10µg), chloramphenicol (30µg), amoxicillin (30µg), and ceftriaxone (30µg) (Oxoid Ltd. Basingstoke, London).

***Salvadora persica* extract susceptibility test:**

The antibacterial activity of Salvadora Stem extracts was assessed using the Agar well Diffusion Method. The antimicrobial efficacy of the extract derived from *Salvadora persica* was assessed using the cup plate method on Mueller Hinton agar. The agar plate was pre-saturated with the isolated *Staphylococcus aureus*. A total of six perforations were made on the agar plate using a sterilized cork borer. Afterward, a small amount of liquefied agar was placed into the well to seal the bottom. Five drilled holes with a diameter of 6mm were utilized to introduce the *Salvadora persica* extract at five separate concentrations: 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml. A conventional antibiotic, Ciprofloxacin 5µg/ml, was placed into the sixth opening to serve as a control. The plate was left unattended for one hour to ensure proper diffusion of the pre-diffusion period into the matrix. Afterward, it was placed in an incubator at 37°C for 24 hours. The zone of inhibition was identified following the identical process conducted using the isolated Streptococcus as the inundated organism.

Statistical analysis.

The data obtained in this investigation was displayed as mean values and proportions in the relevant tables and figures.

RESULTS

S. aureus was determined to be the most common pathogen, with a prevalence rate of 60%, followed by *Streptococcus* species at 10%, as depicted in Figure 1. Amoxicillin demonstrated complete resistance (100%), while chloramphenicol exhibited a resistance rate of 85%. Ciprofloxacin showed a resistance rate of 60%, ceftriaxone had a resistance rate of 50%, and gentamycin had a resistance rate of 47.5%. These resistance rates are depicted in Figure 2. The cold maceration extraction resulted in a crude extract yield of 11.59%. The following phytochemical compounds were identified: saponin, tannin, alkaloid, flavonoid, phenol, cardiac glycoside, carbohydrate, steroid, and terpenoid (see Table 1). The 18-22mm Zone of Inhibition (Figure 3) and 25mg/ml Minimum Inhibitory Concentration (MIC) (Figure 4) showed significant effectiveness.

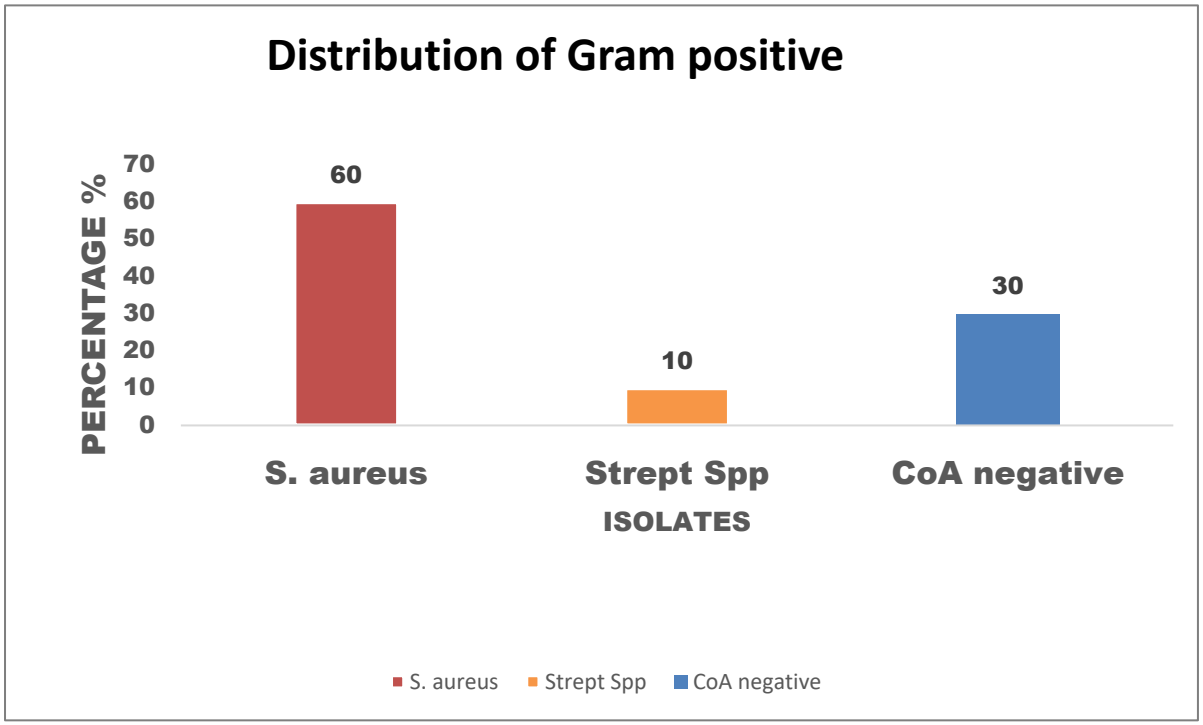

Figure 1: Distribution of Gram-positive

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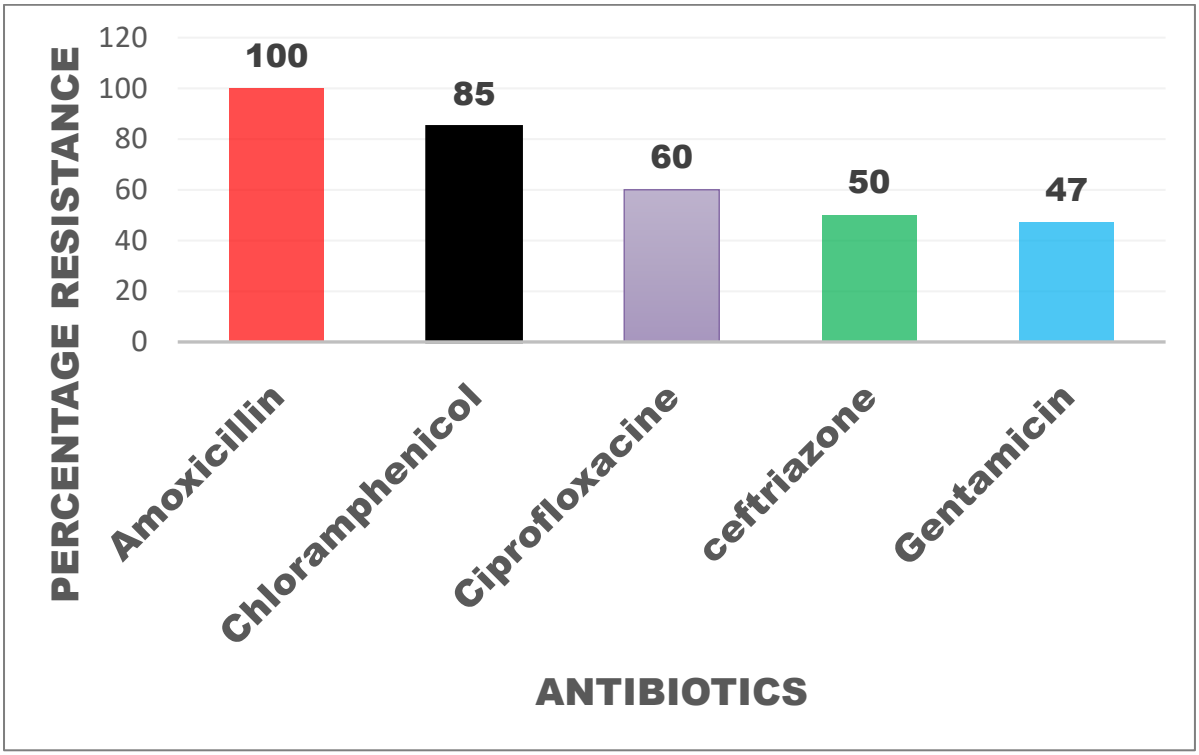

Figure 2: Antibiotic resistant profile of Gram-positive isolates.

Table 1: Preliminary Phytochemical Screening of *Salvadora persica* extract

Phytochemicals	Salvadora persica MeOH
Carbohydrate	+
Flavonoids	+
Steroids/	+
Terpenoids	+
Saponins	+
Cardiac glycoside	+
Phenols	+
Tannins	+
Alkaloids	+

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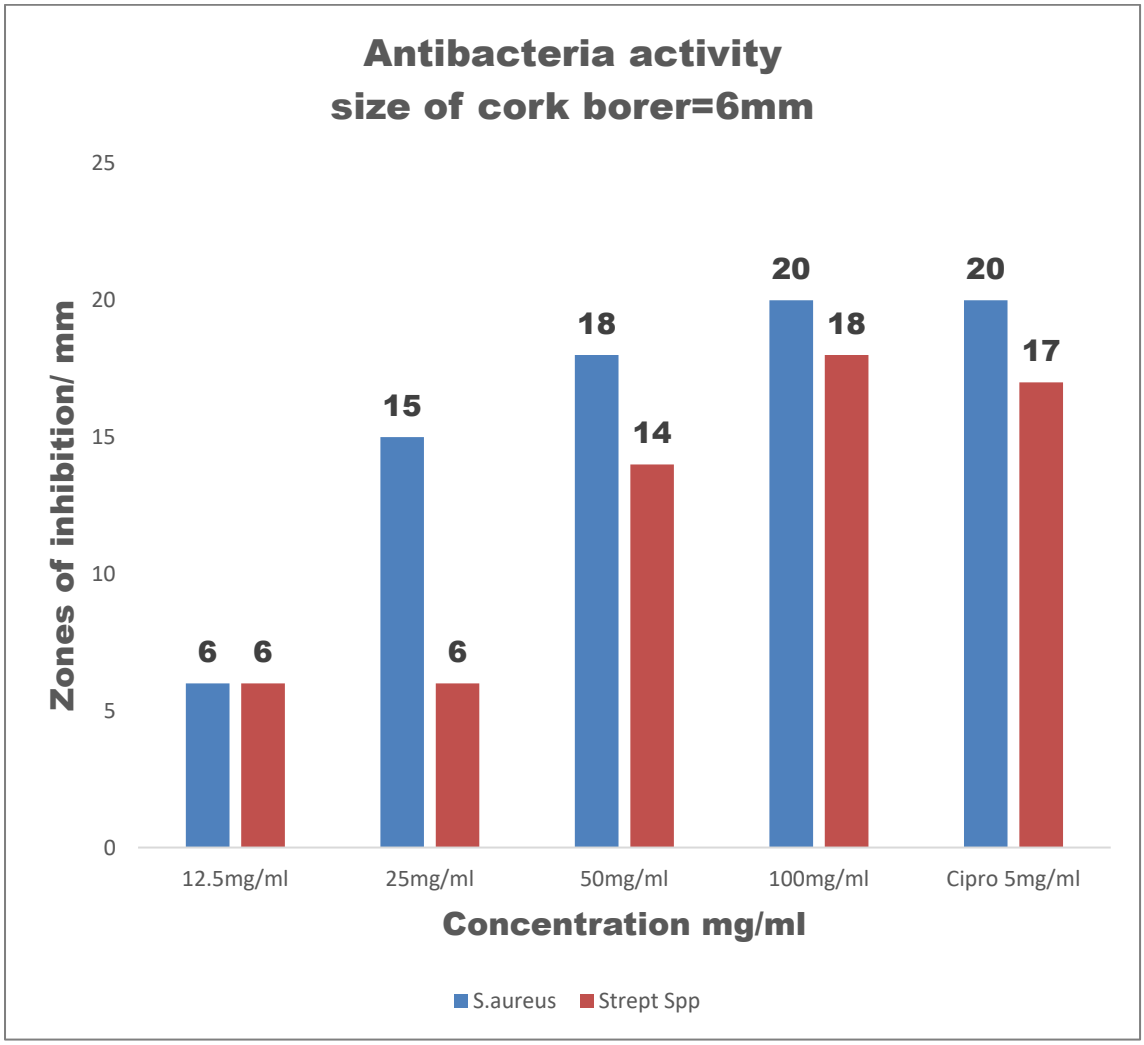

Figure 3: In vitro antibacterial activity of ***Salvadora persica*** extract Gram-positive

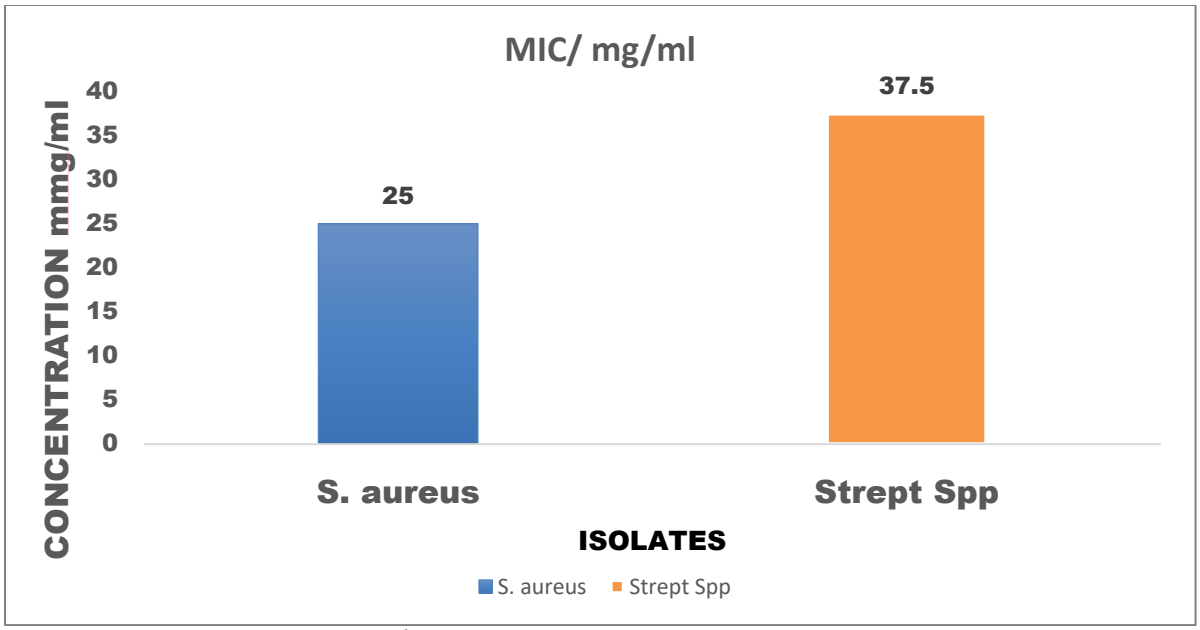

Figure 4: MIC OF ***Salvadora persica*** Extract against of Gram-positive

DISCUSSION

Gram-positive bacteria such as *Streptococcus* spp, *S. aureus*, *Bacillus* spp, *Enterococcus* spp, and *Actinobacillus* are commonly found in the oral microbiota. This study found that *S. aureus* was the most common gram-positive bacteria, followed by *Streptococcus* species. This agrees with the study carried out in Minna, Northern Nigeria, as reported by Daniyan *et al.* in 2011. Also, India (Umesheppa *et al.*, 2021) and Pakistan (Jamil *et al.*, 2020) reported the same. On the contrary, studies in southern Nigeria (Enitan *et al.*, 2021; Philips *et al.*, 2021) and Cameroon (Bissong *et al.*, 2014) revealed that *Streptococcus* spp were the most predominant gram-positive bacteria isolated. Others indicated *Bacillus* spp (Sakthivel *et al.* 2016) and *Enterococcus* spp (Zawadzki *et al.* 2016). However, the differences in organisms can be ascribed to various factors, such as the sample site and size, the duration of the investigation, the severity of the infections, and the geographical location in which the research was conducted. High resistance observed in this study with amoxicillin, chloramphenicol, and ciprofloxacin was also reported in other studies carried out in Nigeria (Daniyan *et al.*, 2011; Philips *et al.*, 2021). Furthermore, this is similar to the findings in Ghana (Donkar *et al.*, 2020), India (Batabyel *et al.*, 2012), and Korea (Kim *et al.*, 2015). However, this is also dissimilar to the results of previous studies conducted in Ogun, Nigeria (Enitan *et al.*, 2021), Pakistan (Jamil *et al.*, 2020), and Poland (Garbacz *et al.*, 2021), which showed that the isolates were susceptible to the same antibiotics. The high prevalence of antibiotic resistance can be ascribed to the selective pressure caused by frequent improper usage, excessive administration of antibiotics, and insufficient sanitary conditions in hospitals (Abdulaziz *et al.*, 2022). In addition, the phytochemical contents discovered in this study were similar to those found by Adigun *et al.* (2023) and in ethanol and aqueous extracts (Abdallah *et al.*, 2015). However, the therapeutic and physiological qualities of the plants are ultimately dictated by the presence of these phytochemicals. The antibacterial susceptibility test revealed activity against the isolates at different concentrations. These findings align with the results of previous studies conducted by Al-Ayed *et al.* in 2016 and Adigun *et al.* in 2023, which reported efficacy against multidrug-resistant *S. aureus* and *Streptococcus*. Adigun *et al.* (2023) reported high activity against *S. aureus*, with a similar minimum inhibitory concentration (MIC) of 25 mg/ml as seen in our study, compared to *Streptococcus*. Kumar *et al.* (2016) reported the efficacy of the same extract against *Bacillus subtillis*. In addition, other researchers (El-sherbiny *et al.*, 2023) have reported that the petroleum ether extract of the same plant exhibits antibiofilm action and is effective against beta-lactam-resistant *Streptococcus*.

CONCLUSION

The methanolic extract of *Salvadora persica L. has* shown significant efficacy against gram-positive oral pathogens, making it a promising option for treating oral infections and promoting oral hygiene.

RECOMMENDATIONS

The extract should undergo further examination, including HPLC-MS and column chromatography fractionation, to discover active chemicals and isolate their constituents.

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CONFLICT OF INTEREST

The authors confirm that they do not have any conflicts of interest.

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