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Investigation of the antimicrobial activity of water and methanol extracts of *Salvadora persica L.* (Miswak) plant against some pathogenic microorganisms

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Abstract

The toothbrush tree *Salvadora persica L*, also called miswak, belonging to the family *Salvadoraceae*, is one of the most important of the 182 plant species used as chewing sticks. It is widely used in many Asian, African and Middle Eastern countries. The roots, branches and stems of this plant have been used for oral hygiene and small miswak sticks have been used as toothpicks for oral hygiene. In this study, commercially purchased *Salvadora persica*, L. (Miswak) plant used in oral hygiene were tested against seven pathogenic bacteria (*Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853) and two fungi (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) at eight different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12. 5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml) were determined. While the aqueous extract did not show any antimicrobial activity against seven pathogens, the methanol extract showed activity against three pathogens. The methanol extract of *S. persica* showed antimicrobial activity against *Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623 and *Klebsiella pneumoniae* ATCC 70060 strains.

Keywords: *Salvadora persica*, Methanol, Antimicrobial Activity, Micro-dilution, Disc Diffusion

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INTRODUCTION

The emergence of multidrug-resistant human bacterial pathogens in the 1990s, and more recently of widely resistant clinical isolates, has hampered efforts to control and manage human infections caused by these organisms (Magiorakos et al., 2012). The development of antimicrobial resistance due to the misuse of antibiotics has become a matter of concern (WHO, 2015). Furthermore, the continuous increase in global isolation rates of clinical isolates such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE) and carbapenem-resistant Gram-negative bacilli poses a serious therapeutic challenge, as no new antimicrobial agents are currently available for the treatment of infected patients (Elabd et al., 2015; Asaad et al., 2013; Al-Ayed et al., 2016).

Studies on the antibacterial properties of *Salvadora persica* (miswak) show that especially water and methanol extracts are effective against various pathogens. For example, one study found that miswak extracts exhibited moderate to strong antibacterial activity against multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (Al-Ayed et al., 2016).

Similarly, there are studies on the antibacterial activities of different plant and fungal species. For example, the antimicrobial effects of methanol and ethanol extracts of *Ramaria aurea* mushroom against MRSA and other pathogens were investigated and found to be effective against some strains (Güneş and Alkan, 2024).

Plants are important in human life and meet the daily needs of people. They are used as cosmetics, food, flavourings, ornaments and medicines. Medicinal plants have become part of complementary medicine worldwide

due to their potential health benefits. Various plant extracts have great potential against infectious agents and can be used for therapeutic purposes (Upadhyay et al., 2010; Gomez-Flor et al., 2006).

The toothbrush tree *Salvadora persica L*, also called miswak, is widely used in many Asian, African and Middle Eastern countries. In studies, it has been determined that the roots, branches and stems of *Salvadora persica L* plant are used for oral hygiene and small miswak sticks are used as toothpicks to ensure oral hygiene (Sher et al., 2011). Water and methanol extracts of miswak have been reported to 4have various biological properties against organisms thought to be important in the development of dental plaque and periodontitis (Sofrata et al., 2008).

Previous in vitro studies have shown that miswak is effective against *Staphylococcus aureus, Streptococcus mutans, Streptococcus faecalis, Streptococcus pyogenes, Lactobacillus acidophilus, Pseudomonas aeruginosa, Aggregatibacter actinomycetemcomitans,* They reported antibacterial and antifungal effects on cariogenic bacteria and periodontal pathogens including *Porphyromonas gingivalis, Haemophilus influenzae* and *Candida albicans* (Naseem et al. , 2014; Al-Sieni, 2014; Al-Bayati and Sulaiman, 2008; Alireza et al., 2014; Fallah et al., 2015; Alili et al., 2014; Mohammed, 2013; Al-Ayed et al., 2016).

A literature survey shows that much effort has been devoted to the study of the inhibitory effect of *Salvadora persica* on oral organisms, but there is little information on the antibacterial and antifungal activity of *Salvadora persica* against other human pathogens. In this project, the antibacterial and antifungal activity of water and methanol extracts of *Salvadora persica* against seven pathogenic bacteria and two pathogenic fungal species was investigated. While the water extract did not show any antimicrobial activity against seven pathogens, the methanol extract showed activity against three pathogens.

MATERIALS AND METHODS

Commercial Supply of *Salvadora persica L.* (Miswak) Plant

The powder form of *Salvadora persica* plant was purchased from Aktarloji Natural Products company and stored at room temperature in a cool and dry place until the study.

Preparation of *Salvadora persica* Extracts

Aqueous Extracts (H_2O)

40 g of *S. persica* (miswak) plant powder was taken, 200 ml of distilled water was added and homogeneous mixing of water and plant extract was ensured in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuum, dried at 60°C with a rotary evaporator and diluted with 25% Dimethyl sulfoxide (DMSO) for antimicrobial activity study (Kandil et al., 1994).

Methanol Extracts (MeOH)

200 ml of distilled water was added to 40 g of finely powdered *S. persica* (miswak) and a homogeneous mixture of water and plant extract was provided in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the solvent was removed using a rotary vacuum evaporator at 40°C, the concentrated extract was obtained and the dilution required for the antimicrobial activity study was made with 25% Dimethyl sulfoxide (DMSO) (Hassanin et al., 2020, Saad et al., 2020).

Determination of In Vitro Antimicrobial Activity

Microorganism

Pathogenic microorganisms used in this study were obtained from the American Type Culture Collection. Gram-positive bacteria (*Bacillus cereus* ATCC 10987, *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70060, *Pseudomonas aeruginosa* ATCC 27853), Fungi (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) were used in the antimicrobial activity study.

Disc Diffusion Method

The antimicrobial activity of *S. persica* (miswak) was evaluated using the disc diffusion method (Bauer et al., 1966). Muller Hinton Agar (MHA) was used to activate bacterial cultures and Sabouraud Dextrose Agar (SDA-Difco) was used for fungi. Before the study, microorganisms were transferred to Muller Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB-Difco) for fungi and allowed to grow overnight at 37°C (28°C for fungi). The turbidity of the prepared suspensions of the test strains was adjusted to 0.5 McFarland equivalent (1.5×10^8 cfu/ml) and 100 µl of pathogenic microorganisms were spread on the surface of the agar plate with sterile swabs and allowed to dry in Laminar Flow for five minutes. *S. persica* (miswak) extracts (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml) obtained using solvents (water and methanol) were mixed with 1 ml of 25% Dimethyl sulphoxide (DMSO). Filter paper discs (6 mm) were impregnated with 25 µl of the extracts to check their antimicrobial activity and allowed to dry in Laminar Flow. After drying, the plates with bacteria were incubated overnight at 37°C and the plates with fungi were incubated at 28°C. 25% DMSO was used as a blind control, Amphicillin (AM10), Polymyxin B (PB300) and Nystatin (Cyc) (for fungi) were used as positive controls. The zone of inhibition was observed and measured in millimetres. The study was repeated three times and the results were averaged.

RESULTS AND DISCUSSION

Commercially available powdered form of *S. persica* plant was taken 40 g, 200 ml of distilled water was added and homogeneous mixing of the plant extract with water and methanol was achieved in a shaking incubator at 180 rpm overnight. The extract was then filtered using Whatman No. 1 filter paper and the filtrate was evaporated in vacuo and dried with a rotary evaporator at 60°C for water and 40°C for methanol and the necessary dilution for antimicrobial activity study was made with 25% Dimethyl sulfoxide (DMSO) (Kandil et al., 1994). A schematic visualisation of the extraction process is given in Figure 1.

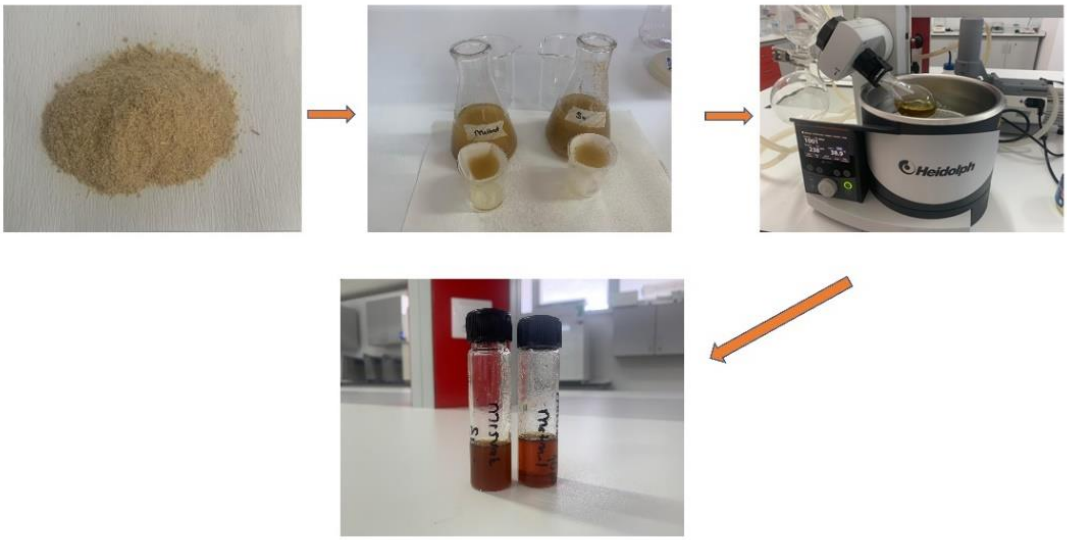

Figure 1. Schematic image of the preparation of *Salvadora persica* extracts

Water and methanol extracts of *S. persica* (miswak) were inoculated on discs at eight different concentrations: 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6 mg/ml, 3 mg/ml and 1 mg/ml.

The antibacterial activities of water and methanol extracts of *S. persica* against 4 gram-positive, 3 gram-negative and 2 pathogenic fungi are listed in Table 1. In general, the methanol extract of miswak showed activity against three of the tested pathogenic microorganisms (Figure 2), while no concentration of water extracts showed growth inhibitory effect (Figure 3).

Table 1. Antimicrobial activity of *Salvadora persica* extracts prepared with water and methanol solvents.

Microorganisms	Concentrations of *Salvadora persica* extracts (mg/mL) and inhibition zones (mm)																		
	Water								Methanol								Positive control		
	200	100	50	25	12.5	6	3	1	200	100	50	25	12.5	6	3	1	Ampicillin (AM10)	Polymixin B (PM300)	Nystatin (NS100)
Bacillus cereus	-	-	-	-	-	-	-	-	10	10	9	9	9	-	-	-	30	10	-
Bacillus subtilis	-	-	-	-	-	-	-	-	12	10	10	9	9	8	-	-	30	10	-
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	10	-
Enterococcus faecalis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	10	-
Escherichia coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22	15	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	10	9	9	-	-	-	-	-	40	20	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30	15	-
Candida albicans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30

When the inhibition zone diameters are evaluated, it is seen that the methanol extract of *S. persica* is effective against members of the genus *Bacillus* from gram positive bacteria. It formed an inhibition zone between 10 mm and 9 mm on *B. cereus* bacteria. The lowest effective concentration is 12.5 mg/ml. *B.subtilis* bacteria formed an inhibition zone between 12 mm and 8 mm. The lowest effective concentration was 6 mg/ml. *K. pneumoniae*, another pathogenic bacterium, formed a zone of inhibition between 10 mm and 9 mm and showed the lowest effect at a concentration of 50 mg/ml (Figure 2).

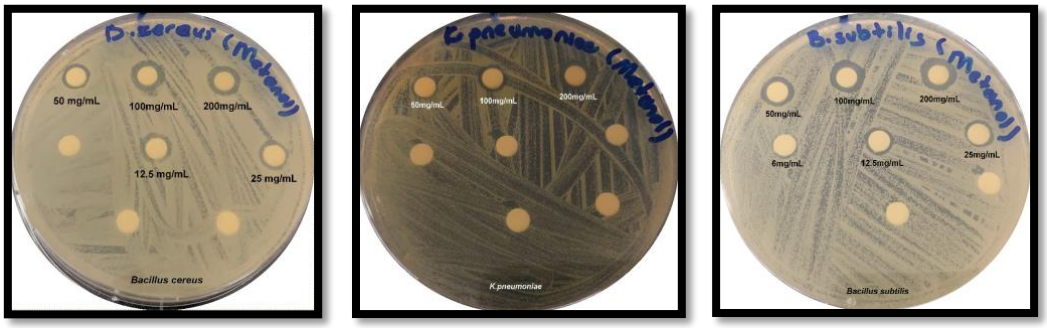

Figure 2. Inhibition zones created by the extract obtained from *Salvadora persica* with methanol solvent against *Bacillus* genus members and *K.pneumoniae* pathogen

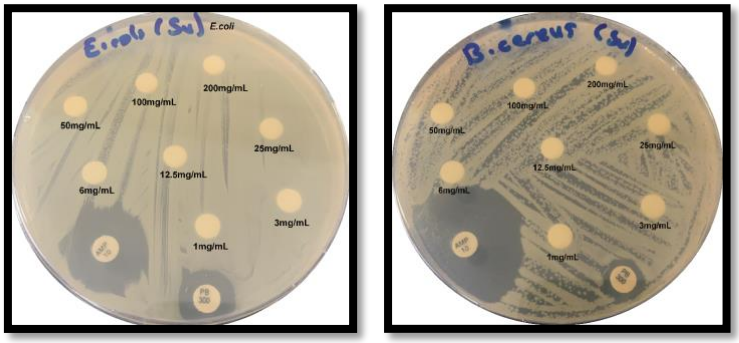

Figure 3. Petri dish image in which the extract obtained from *Salvadora persica* with water solvent has no effect on *E.coli* and *B.cereus* pathogens

When compared with the standard antibiotics used, the extract obtained from methanol exhibited antimicrobial activity lower than amphicillin and polymyxin B standards at a concentration of 200 mg/ml against members of the genus *Bacillus*. The methanol extract showed lower activity against *K. pneumoniae* pathogenic bacteria than polymyxin B antibiotic.

Al-Ayed et al. (2016) evaluated the in vitro antibacterial activity of *S. persica L.* extracts against 10 MDR (MRSA, MRSE, *Streptococcus pyogenes*, *E. faecalis*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. marcescens*, *A. Baumannii* and *S. maltophilia*) bacterial clinical isolates other than oral pathogens. The antibacterial activity of water and methanol miswak extracts was evaluated using agar dilution and minimum inhibitory concentration (MIC) methods. In general, 400 mg/mL miswak extracts were found to be the most effective in all strains. Methanol extract showed a stronger antibacterial activity against Gram-negative (3.3-13.6 mm) bacteria than Gram-positive (1.8-8.3 mm) bacteria. The lowest MIC value was observed for *E. coli* (0.39, 1.56 μg/mL), followed by *Streptococcus pyogenes* (1.56 μg/mL). The highest MIC values (6.25, 12.5 μg/mL) were recorded for methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*.

Apaydın (2018) investigated the antioxidant and antimicrobial effects of essential oil obtained from *S. persica* (miswak) plant. For this purpose, 250 grams of powdered miswak sample was extracted by hydrodistillation method. Antimicrobial properties of essential oils were determined by disc diffusion method according to the method of Parvathy et al. They found that miswak oil showed antimicrobial activity against *S. aureus* and *E. coli* bacteria by forming a zone of 4 and 18 mm in diameter, respectively.

Another study was conducted by Al-Sieni in 2014. He collected and dried *Salvadora persica* (miswak) and *Commiphora gileadensis* plants and evaluated the extracts extracted with methanol and warm water for their antibacterial activity against 5 different bacterial genera (*Fusobacterium nucleatum, Lactobacillus casei, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans* and *Streptococcus salivarius*) using agar well diffusion method. Some human pathogens were among the tested bacteria. The obtained extracts showed significant inhibitory effects against all tested bacteria with various degrees of growth inhibition. Methanol extracts were more effective than water extracts. The minimum inhibitory concentrations (MIC) of the methanol extracts

ranged from 50-100 µg/ml. In conclusion, *S. persica* and *C. gileadensis* showed moderate to high inhibitory activity on pathogenic bacteria and can be used traditionally in alternative medicine.

Among the reasons for the difference between the results obtained in this study and the results of other researchers, miswak extracts may exhibit different antibacterial and antifungal activity due to reasons such as the method of obtaining the extract, the components in the chemical content of the extract, solvent diversity, the type of pathogenic microorganism used or the use of different strains of the same species (Elnabris et al., 2013; Ramalingam and Amutha, 2013; Gümüş and Ünlüsayın, 2016).

CONCLUSION

Until now, many different studies have been carried out on extracts and oil of *S.persica* plant and it has been observed that they show various activities against Gram-positive, Gram-negative and fungi. This makes *S. persica* plant especially important in terms of having the potential to be used in the field of health.

In conclusion, the methanol extract of *S. persica* exhibited antibacterial activity against three pathogens and further in-depth studies should be carried out to isolate the active constituents involved in this activity.

Some studies show that plant-based antimicrobials may offer potential treatment options, especially against pathogens that show antibiotic resistance. Therefore, the development and use of plant-based antimicrobials is considered as an important step in the fight against antimicrobial resistance.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and table are original and that they have not been published before.

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