RESEARCH ARTICLE

The Effectiveness of *Salvadora persica* Extracted Miswak and ZnO NPs on Pathogenic Bacteria

Muntaha R. Ibraheem

Department of Biomedical Sciences, Alkawarizmi College of Engineering, University of Baghdad, Baghdad, Iraq

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ABSTRACT

Salvadora persica, (Miswak) deem oral hygiene essential biological and chemical activities. The current study aimed to study the biological activity of *S. persica* of extract using Fourier transform infrared spectroscopy (FTIR) show that contains analysis of the FTIR test results of miswak extract. While the analysis using UV-vis displays that the extract contains analysis of UV-vis test for miswak extract. The extract shows higher activity against bacteria than fungi. The inhibition zone of *Escherichia coli, Staphylococus aureus,* and the ZnO nanoparticles show high biological activity against (bacteria and fungi). The chewing stick which is commonly known as the Miswak tree and its scientific name is *S. persica* L, occurs in most of the countries of Asia and Africa. The plant has long been prized in the Middle East for its vital biological and chemical properties and its usage in mouth hygiene, as mentioned in ethnobotanical records. This research aims to summarize this species' biological effects (antibacterial and antifungal) by testing the effect of different concentrations of extracted Miswak (aqueous extracts) in *E. coli* as gram-negative In addition to use different measurement devices, FTIR, UV-vis spectroscopy, and gas chromatography (GC) to analyze the extracted Miswak. We found that *S. persica* has beneficial therapeutic effects and has the potential to be used as a useful adapt genic herbal treatment based on its chemical and pharmacological characteristics.

Keywords: Antimicrobial activity, Fourier transform infrared spectroscopy, Miswak extracted, Ultraviolet spectroscopy

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INTRODUCTION

Multidrug-resistant (MDR) human bacterial infections and highly resistant clinical isolates have hampered efforts to manage and control human illnesses since the 1990s. *S. persica* L., often known as miswak, is one of the most popular of the 182 plant species used as chewing sticks. It belongs to the Salvadoraceae family. Plant extracts with antiviral and antifungal activities can be used medicinally. Antibacterial agents can be found naturally in this plant.[1-3] Plant-based medications have long been used in traditional medicine. *S. persica*, a member is a 4–6 m tall evergreen shrub with a short stem, white bark, and smooth green leaves that has been demonstrated to have substantial activity for dental disorders. It belongs to the Salvadoraceae family. The miswak tree is another name because its teeth have been cleaned with roots and twinges for centuries. In the muslin world, it is one of the most commonly used medicinal plants for oral hygiene.[1] Almost all marketplaces sell miswak, a root preparation. It is produced by chopping roots or stings into 10 to 25 cm pieces. *Candida albican's* growth and acid production are inhibited by miswak. Miswak extract has a strong flavor that may encourage salivation while attracting customers with its pleasant aroma and flavor.[4-6] On both aerobic and anaerobic bacteria isolated from dental samples, Miswak demonstrated a significant antibacterial effect. Antiviral, wound-healing, and antidepressant activities have been attributed to miswak tree extracts. Miswak is made from a tree called *S. persica*, which is mostly found in Egypt, Saudi Arabia, and India. Additionally, this plant has flavonoids, silica, fluoride, sulfated material, vitamin C, tannic acid, calcium oxalate, and saponin. In most Asian, African, and Middle Eastern countries, using herbal toothbrushes is a popular practice in maintaining oral hygiene. Many herbs have been used as tooth sticks, including *S. persica*, *Aegles marmelos*, *Azadirachta indica*, *Acacia arabica,*[1] and many others. Of all the herbs, *S. persica* has the most widespread use worldwide. Its strings and roots are used to make miswak, a chewing stick. Miswak, on the other hand, is a root preparation that is widely sold in markets. It's made by chopping the stings or roots into 10 to 25 cm pieces. It's cheap and effective oral hygiene equipment used 3 to 10 times daily. Miswak extract may increase saliva production and appeal to consumers with a pleasant aroma and flavor.

**Author for Correspondence: muntaha@kecbu.uobaghdad.edu.iq*

In a global study on oral hygiene, the World Health Organization (WHO) recommended the usage of miswak. Miswak works as an abrasive agent to remove stains and whiten teeth. Its effectiveness is defined by its ability to moisten tooth enamel and reach caries-prone regions such pits, fissures, and interproximal zones.[7-10]

MATERIALS AND METHOD

In this research, the miswak was extracted with alcohol (ethanol). So in the beginning, we milled the miswak, which was about 7.415 gm. For 4 days, absolute ethanol 99% free of water, was used by mixing milled miswak and ethanol in the soxhlet device. Miswak extract was prepared after 16 hours then concentrated by distillation under a vacuum (rotary evaporator) at 45°C. The evaporation of alcohol is done under a vacuum at a temperature less than the boiling point of alcohol. Plant material was cut into small pieces and dried in the shade according to the Herbarium's regular technique before being crushed. In a soxhlet extractor, the crushed miswak root powder (500 g) was extracted separately with water and methyl alcohol. After extraction, the solvent was filtered through a filter paper (Whatman No. 1) to remove large particles. The solvent was removed using a Buchi B-491 evaporator, a vacuum controller V-850, and a vacuum pump V-700. After freeze drying, the semi-solid extract was transferred to sterile labeled vials and stored at -20°C until utilized.[11] After that, numerous concentrations were made in order to determine the ideal concentration for killing bacterial and fungal growth.[12]

Bacterial Clinical Isolates

Six gram-negative microscopic animals and all strains of MRSA, MRSE, *Streptococcus pyogenes,* and *Enterococcus faecalis*, that are penicillin-safe were chosen. Among the microorganisms that have been identified are *Stenotrophomonas maltophilia, Acinetobacter baumannii, Pseudomonas aeruginosa, Serratia marcescens,* and *E. coli.* was used to check them as demonstrated by the maker's bearings B-20. Urinary part infections cause seven nosocomial strains. Two of the cases were a direct result of flow framework sicknesses, while one was a result of ventilator-related pneumonia. According to the Clinical and Laboratory Standards Institute (CLSI) standards, all isolates' antimicrobial hindrance plans were analyzed using the reference stock microdilution system.[21,22] PCR upgrade of the mecA quality recognized methicillin impediment in *S. aureus* and *S. epidermidis* strains.[23] The revised Hodge test[21] was used to confirm carbapenem resistance in gram-negative bacteria. The disengages were tested for multidrug resistance (MDR) employing assurance from three kinds of enemy of contamination (penicillins, cephalosporins, aminoglycosides, carbapenems, and quinolones).[21]

Miswak Collection

In the spring of 2015, a neighborhood market in the Saudi Arabian region of Jazan gave biting sticks of *S. persica*. The sticks were washed with unadulterated water prior to being slashed into little species and dried at room temperature for quite a while. After that, an electrical blinder was employed to entirely convert them to powder.

Extracts Preparation

To make a liquid concentrate, 1-L of cleansed water and 95% methanol were combined with *S. persica* powder. At 60 and 40°C; at non-liquid temperatures, the filtrate was evaporated in a vacuum evaporator.

Inoculum Preparation

Antibacterial movement was stopped using agar dispersion and least inhibitory concentration methods on miswak separates. Each bacterial solution was tested using the McFarland Standard, which contains 1.5–108 CFU per mL of clean saline (0.84% NaCl).

Agar Diffusion Method

In general, 100 L of bacterial suspension were evenly distributed throughout the agar plates. A sterile glass fine was used to remove the expected number of wells from the agar, each measuring 3 mm in width, ensuring that each agar plate had a sufficient dispersion of gaps on the edge and one in the middle. After that, the wells were filled with 50 L of sterile concentrate (watery or methanol) that had been prepared using *S. persica* stock (400, 200, 100, and 50 mg/mL). The plant extricate was then pre-hatched at room temperature for 2 hours to guarantee legitimate medium dispersion. The plates were then hatched at 37°C for 24 hours.[25] The restraint zone was determined as the normal distance across of the entire development hindrance zone (in mm), overlooking the well's width. Positive controls were anti-infection circles from Difco Laboratories in Detroit (30 g gram-positive isolates get vancomycin, while gram-negative microbes get 10 g tobramycin.), while negative controls were water (for fluid concentrate) or methanol (for methanol remove). Every microorganism was tried multiple times to ensure consistency. The typical zone measurement information from three cycles was used to lay out the last restraint zones. This was finished to guarantee that the restraint zones for each trial were accomplished under similar circumstances.

The Minimal Inhibitory Concentration (MIC)

The MIC of *S. persica* not entirely settled in 96 multi-well mL plates utilizing a changed rendition of the typical microdilution technique.[26] To sum up, the broke up separates each microtiter plate line had 50 mL of each liquid and methanol remove pipetted into the essential well, followed by 50 mL of TSB pipetted from the first to the twelfth well of each segment. Two fold consecutive debilitating was achieved by moving 50 L of scalar debilitating from each line's first to second wells. The concentrates were tried at fixations going from 25 to 0.003 mg/mL to quantify bactericidal movement. At last, each was loaded with 10 liters of bacterial suspension. Two column lines were utilized as controls in each plate: In a 32 to 0.015 g/mL consecutive debilitating, one with vancomycin as a gram-positive segregates positive control, and another with tobramycin as a gram-negative segregates negative control.

Plates were incubated at 37°C for 18 to 24 hours. The MIC was determined as the least focus at which no turbidity was noticed. Plates were checked separately to lay out MIC, and last MIC values for each concentrate were figured utilizing the typical MIC values from three imitates to guarantee exactness and consistency.

RESULTS AND DISCUSSION

The release of compounds from the extract into the medium when they were mixed was most likely the cause of their impacts on growth. The various reactivity of each strain to extracts suggested that different chemical components of Miswak were extracted by solvent. The intensity of antimicrobial action may also be affected by the pH of the extract, since the water extract had the greatest pH, while the other solvents had the lowest pH. The chemical content of the aqueous miswak extract is attributed to which attaches to the cell membrane, separates it from the bacteria, and kills the bacterial cell.

The results of FTIR analysis showed the similarity of the spectrum types of miswak. Compounds containing many compound and phosphorus atoms as an active group within the 2540–1924 cm^{-1} wave.

Similarities were found on the spectrum of miswak sample. Figure 1, shows the active compound as in Table 1.

Spectrum cuts were only taken in 100–1000 nm areas for miswak types. Whereas concentrations of 190 and 500 nm did absorb compounds containing phosphorus atoms, as shown in Figure 2 of UV analysis. It is attributed as a result of the chemical content of the aqueous miswak extract. This substance is effective against the genetic material in the bacterial cell.

According to the findings, nanoparticles exhibited a different antimicrobial effect on gram-negative bacteria. (*E. coli*,) and positive bacteria (*S. aureus*) with 3 mg/mL of ZnO nanoparticles had significant effect on both bacteria with inhibition zone 10–15 mm and miswak concentration 5 mg/mL with inhibition zone 20–25 mm for both bacteria.

The antibacterial impact of zinc oxide nanoparticles on the studied bacteria may be attributed to interaction with the bacteria's cell wall, which leads to creation of pores that aggregate ZnO nanoparticles in the pits, induced the permeability of the cell membrane. The influence of nanoparticles on proteins in the cytoplasm of the cells, which leads to the regulation of functions in the cells, could be another reason for the killing of bacterial cells. The surface area in contact with the bacteria is known to affect the antimicrobial activity of nanoparticles.

The antibacterial efficacy of *S. persica* aqueous and methanol extracts against ten MDR pathogenic pathogens is shown in Table 2. Miswak extracts with a 400 mg/mL concentration were the most effective against all strains. The methanol extract of miswak suppressed the development of the pathogens studied more than the aqueous extract. Gram-negative bacteria (with diameters ranging from 3.3–13.6 mm) were found to be more resistant to the methanol extract than gram-positive bacteria (with diameters ranging from 1.8 to 8.3 mm). *E. coli* (4.8–13.6 mm), *K. pneumoniae* (4.6–12.7 mm), and *Serratia marcescens* (4.5–12.5 mm) were the bacteria that showed the largest growth inhibition. With an inhibitory zone diameter of 2.2–7.4 mm, *Streptococcus pyogenes* was shown to be the gram-positive pathogen most vulnerable to methanol extract (Figure 3-6).

Table 1: The results of FTIR analysis showed the similarity of the spectrum types of miswak.

No.	*Wavelength range (1/cm)*	*Description of vibration*	*Infrared absorption*
1	3436-3380	phenol OH-strains	Compound containing Oxygen atom
2	2980-2900	Aldehyde C-H	Compound containing Oxygen atom
3	2540-1924	phosphoric acid and p-H strain	Compound containing phosphorus
4	1652-1450	C-C strain	aromatic compound
5	1380-1276	C-H pending strain	aromatic compound
6	1084-1044	P-O strains	Compound containing phosphorus atom
7	884-500	P-C strains	Compound containing phosphorus atom

Table 2: The effect of different concentrations of ZnO nanoparticles on bacterial growth.

Concentration of ZnO NPs (mg\mL)	*Bacterial growth*		*Concentration of miswak (mg\mL)*
	E. coli	*S. aureus*	
10	-	-	10
5	-	-	5
1.5	-	-	1.5
0.5	-	-	0.5
0.05	+	-	0.05
0.005	+	+	0.005
0.0005	++	++	0.0005

(-) No growth, (+) Slightly growth, (++) Moderate growth, (+++) Good growth.

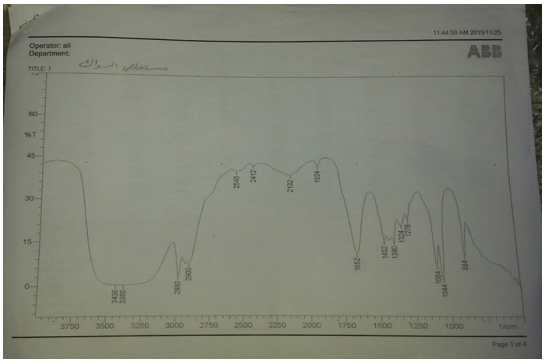

Figure 1: Analysis of the FTIR test results of miswak extract

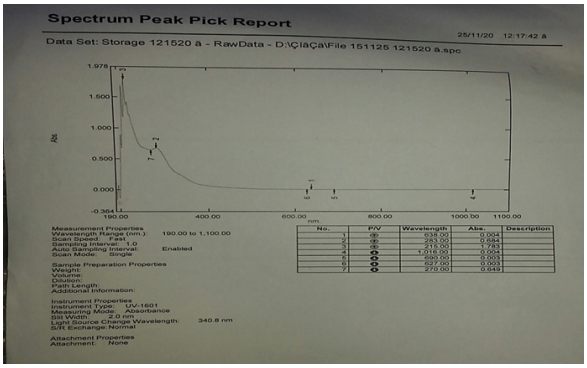

Figure 2: Analysis of UV–vis test for miswak extract

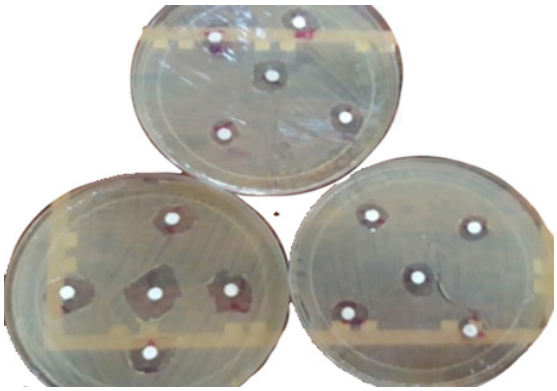

Figure 3: The effect of *S. persica* on *E. coli* using agar well diffusion method for 1-day, at 37°C, in N.A.

Figure 4: The effect of *S. persica* on *S. aureus* using agar well diffusion method for 1-day, at 37°C, in N.A.

Figure 5: The effect of *S. persica* and nanoparticles on *E. coli* using agar well diffusion method for 1-day, at 37°C, in N.A.

Figure 6: The effect of *S. persica* and nanoparticles on *S. aureus* using agar well diffusion method for 1-day, at 37°C, in N.A.

The MDR-microscopic organisms diseases are hard to treat because of antimicrobial opposition, which has been an overall well-being worry for quite a while.[1] Conventional medication's usage of regular plant removal has ignited a whirlwind of exploration. The antibacterial viability of *S. persica* watery and methanol extricates against various MDR gram-positive and gram-negative microorganisms was concentrated on in this review. Miswak extricates at 400 mg/mL were demonstrated to be the most proficient against all microbes in this review. The methanol separate was more powerful against gram-negative microscopic organisms than gram-positive microorganisms. Other examinations[10,11,13,16,17] have yielded comparative outcomes. Al-Bayati and Sulaiman concentrated on the antibacterial properties of *S. persica* fluid and methanol separates against seven oral contaminations, and their discoveries go against one another.[12] Inhibition of all bacteria was found to be more effective with the aqueous extract than with the methanol extract. *S. persica* extracts contain a variety of phytochemicals that have been shown to have antibacterial activities. *S. persica* demonstrated good MIC values against gram-positive and gram-negative bacteria, including *E. coli, K. pneumoniae, P. aeruginosa,* and *S. marcescens*. The exterior bacterial layer may be invaded by BITC, disrupting bacterial redox workouts and limiting their capacity to maintain awareness of film potential.[9-13,16,17] *E. faecalis, S. aureus, S. mutans, S. pyogenes, E. faecalis,* and *P. aeruginosa*.[9-13,16,17] Regardless, this overview revealed high MIC values for MRSA, *A. baumannii*, and *S. maltophilia*. This is the fundamental review we're aware of, which explores the antibacterial suitability of miswak withdraws against MDR microorganisms.[18,19] Our disclosure is very disturbing. Due to their capacity to evade normal guards, secure assurance from

many antimicrobial classes, and their proclivity for getting by in nosocomial settings, these arising microorganisms represent a serious danger to human well-being. Periodontitis is a complex etiology characterized by periodontal tissue inflammation. The main periodontitis pathogens were once considered to be members of the well-known "red bacterial complex" (*Treponema denticola, Porphyromonas gingivalis*, and *Tannerella forsythia*). The presence of the nanomaterial at a certain concentration in the miswak extract as an antibiotic improves strength of miswak extract as an antibiotic.[20,22]

CONCLUSION

The chemical analysis of *S. persica* aqueous extract using FTIR and UV–vis shows it contains different active compound used in a chemical and pharmacological application and also the aqueous extract contains high antibacterial and antifungal activity. The activity increased with increasing concentration, also the ZnO nanoparticles shows high biological activity against (bacteria) for the first time. This study shows that methanol and aqueous miswak extracts had a strong to moderate antibacterial activity against all MDR-pathogens and methanol extract. Using *S. persica* (Miswak) root extract in an aqueous and methanolic dispersion media has been suggested as a green strategy for the ZnO NPs synthesis. When the medium dispersion water is used, ZnO NPs are formed, however, when the medium dispersion methanol is used, ZnO nanorods are formed. Phytochemicals with embedded ZnO NPs and ZnO nanorods are readily visible in SEM pictures. The FTIR was used to investigate the functional groups of phytochemicals responsible for NP synthesis and their reducing and stabilizing properties. This bio-fabricated approach to *S. persica*-mediated ZnO NPs synthesis is both environmentally benign and cost effective, making it economically viable for use in bactericidal, wound healing, antibacterial, and other medicinal applications, as well as a variety of electrical applications. The presence of nanomaterial at certain concentration in miswak extract as an antibiotic improves strength of the miswak extract as an antibiotic.

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