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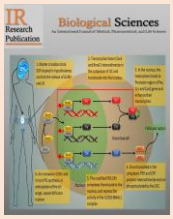

Antibacterial Effect of *Salvadora persica* Extract on Staphylococcus aureus Isolated from Gingivitis Patients

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ABSTRACT

Salvadora persica (miswak) is traditionally used as a natural oral hygiene tool. This study investigates the antibacterial activity of hot aqueous miswak extract against *Staphylococcus aureus* isolated from patients with gingivitis. Fifty samples were collected from patients diagnosed with gingivitis at a primary health center in Babylon Governorate between January and June 2025. Bacterial isolates were identified using standard microbiological methods. Miswak extract was prepared in concentrations of 20%, 30%, 40%, and 50%. The antimicrobial activity was assessed using the agar well diffusion method and compared with conventional antibiotics: vancomycin, carbenicillin, and piperacillin. The *Staphylococcus aureus* isolates were gotten, 4 isolates from males (26.66%) and 11 isolates from females (73.33%). There was no significant difference between the infection rate of bacterial isolates and the use of miswak (P=0.37), the occurrence of bleeding in the gums (P=0.36), and smoking (P=0.37). The miswak extract at a concentration of (50%) showed a greater effect than the other concentrations, as the inhibition zone was (9.86) mm with highly significant differences at the probability level concentrations of extract. (P<0.05), in addition to the presence of significant differences between the effect of Miswak at concentrations (30%, 40%, 50%) and the sensitivity of the isolates to vancomycin, Pipracillin and carbenicillin. This study was limited by its small sample size, single-center design, and possible variability in miswak chemical composition due to plant source and storage conditions. Future multi-center studies with larger populations are recommended. The higher the concentration of the aqueous extract of Miswak, the greater the effect on *Staphylococcus aureus* isolated from gingivitis compared to some types of antibiotics.

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Introduction

The human mouth is inhabited from birth by a large number and variety of microbes, both beneficial and harmful to oral and dental health. Several factors in the oral environment contribute to the

proliferation and diversity of microbes, including, in particular, the regularity of natural teeth, the cleanliness and maintenance of dentures, the integrity and vitality of gum tissue and periodontal ligaments, as well as an important factor related to the quality of food (1). Saliva deposits protein-sugar compounds that form thin, transparent layers called plaque on the tooth surfaces (enamel). This helps oral microbes adhere to these layers and then multiply and accumulate in large quantities, forming thin organic layers known as microbial plaque on the tooth surfaces, above and below the gum margin. It is scientifically proven that if teeth are not cleaned well or regularly daily, and if the accumulation of microbial plaque continues, it leads to gingivitis, which is characterized by reddening, swelling, and easy bleeding of the gums. It may develop into periodontal disease (periodontitis). However, microbial plaque can be removed and protection from periodontal disease can be ensured only with good daily care and regular professional attention (2,3). For centuries, many peoples have been using the stems or roots of some plants to make sticks that can clean teeth, called chewing sticks. Many centuries ago, the Babylonians, Greeks, Romans, and ancient Egyptians, Muslims, and various parts of Africa and Asia, especially the Middle East, use the Miswak plant, which is the stem or root of a small shrub called Arrak, with the scientific name *Salvdora persica*, for health, social, and religious purposes (4). The use of Miswak also leads to a decrease in the need for treatment of diseases of the tissues surrounding the tooth, and Miswak sticks have mechanical and chemical properties that are antimicrobial plaque, antidental erosion, and reduce bleeding gums. Miswak extract has been used in toothpastes and mouthwashes to control microbial plaque and the health of the tissues surrounding the tooth (5).

Scientific studies have proven that the miswak has a significant effect on stopping the growth of oral bacteria because it contains active ingredients against bacteria, including sulfur and trimethylamine, which work to lower the pH of the mouth and thus prevent the growth of bacteria. In addition, it contains vitamin C and citrulline, and these two substances play a role in protecting the gums from infections. As for chloride, fluoride, and silica, these substances are known to increase the whiteness of teeth (6). In addition to these compounds, chemical analysis of the extract showed that it contains sulfur compounds and isothiocyanate, which are responsible for the inhibitory effect of the extract against bacteria, and trimethylamine reduces adhesion to surfaces and removes accumulated bacterial plaque. Tannins, Tannic acid, and Benzyl isothocyanate compounds play a major role in the inhibitory effect against microorganisms and in treating gum infections (7). Nitrate ions (NO_3^-), which naturally occur in *Salvadora persica* (miswak) extract, have been theorized to account for its antimicrobial action. The ions can play a significant role in the inhibitory activity of the extract against various pathogenic bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. Nitrate has been shown to be involved in oxidative inhibition processes that can inhibit bacterial survival and virulence.

Particularly, the miswak extract has been shown to exhibit antimicrobial properties against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (8). *S. aureus* has also been listed as one of the most clinically important opportunistic pathogens due to its ubiquitous exposition on human skin, nasal mucosa, pharynx, gastrointestinal tract, and reproductive tract. It is also frequently isolated from environmental sources such as hands, air, soil, foods, and textiles. *S. aureus* has a wide range of virulence factors, including the excretion of exotoxins (e.g., enterotoxins), and some enzymes like DNase, gelatinase, hemolysins, lipase, leucocidin, β-lactamase, and coagulase. These help it become resistant to phagocytosis and enable it to invade host tissue (9).

There has been increasing scientific interest in recent years regarding exploring the antimicrobial properties of medicinal plants. *Salvadora persica* has particularly been in the limelight given its natural antibacterial constituents and potential as an alternative or addition to conventional antimicrobial therapy, especially in combating resistant and widespread bacterial strains. This study aims to investigate the effect of a hot water extract of the miswak plant. Different concentrations were applied to *S. aureus* bacteria isolated from patients with gingivitis and the effect of the extract was compared with the effect of the antibiotics used.

Materials and Methods

Sample collection and isolation

Fifty gingival swabs were collected using sterile moistened cotton swabs. Samples were cultured on blood and MacConkey agars and incubated at 37°C for 24 hours. Identification was based on colony morphology, Gram staining, and biochemical tests (catalase, coagulase, DNase, and fermentation assays).

Demographic details

The study included 50 patients aged between 18 and 55 years (mean age ± SD = 34.2 ± 9.1 years), with 22 males and 28 females. All participants were clinically diagnosed with gingivitis, systemically

healthy, and had not received antibiotic treatment within the previous three months.

Extract preparation

Miswak samples (*Salvadora persica*) were sourced locally and authenticated. Fifty grams were ground and mixed with 500 ml boiling distilled water at approximately 100°C and maintained at that temperature for 30 minutes. The mixture was then cooled to room temperature, filtered through Whatman No.1 paper, and centrifuged at 300 rpm. Extracts were diluted to 20%, 30%, 40%, and 50% concentrations based on preliminary tests showing measurable antibacterial activity within this range. The prepared extracts were stored at 4°C in sterile, airtight containers for no longer than 7 days before use. Sterility was confirmed by culture.

Antibacterial testing

The well diffusion method was used. Bacterial suspensions were adjusted to 0.5 McFarland and spread on Mueller-Hinton agar. Wells of 5 mm (were filled with) 50 µL (of extract or control distilled water. Plates were incubated at 37°C for 48 hours. Zones of inhibition were measured in mm. Each assay was performed in triplicate.

Antibiotic sensitivity comparison

Sensitivity to vancomycin (VA), carbenicillin (PY), and piperacillin (PRL) was tested using the disc diffusion method.

Statistical analysis

Data were analyzed using ANOVA followed by Tukey's post hoc test. Chi-square test was used for categorical variables. Significance was set at $p < 0.05$.

Results

Out of 50 samples, 15 (30%) tested positive for *S. aureus*. No significant associations were found with age, sex, smoking, miswak use, and bleeding ($p > 0.05$).

The 50% concentration of miswak extract had the largest inhibition zone (9.86 ± 2.98 mm), significantly greater than 40%, 30%, and 20% ($p < 0.05$). Extracts at 50%, 40%, and 30% concentrations had significantly higher antibacterial activity compared to vancomycin and carbenicillin ($p < 0.05$), but piperacillin remained more effective than miswak. This indicates that while miswak shows substantial antibacterial activity, it may serve best as an adjunctive therapy alongside conventional antibiotics, especially in regions with limited access to synthetic antimicrobial agents.

The hot aqueous extract of Miswak showed highly significant differences at the probability level ($P < 0.05$) on *S. aureus* bacteria, as the inhibition diameters were high, ranging between (3.53 ± 2.69) mm at the concentration of 20%, and (9.86 ± 2.98) mm at the concentration of 50%, meaning there were significant differences between the highest and lowest concentrations. It is clear that the 50% concentration was more effective than the concentrations (40%, 30%, 20%), as shown in figure (2). These results are consistent with other studies that found that the higher the concentration, the greater the effect on these bacteria (9, 10). While the results differed from the study by researcher Almas (2001), who found no effect of this extract on *S. aureus* bacteria. The researcher attributed this to the fact that the extract was not fresh and was used a month after its preparation.

Discussion

In fact the study successfully evaluated the antibacterial potential of Salvadora persica extracts through minimum inhibitory concentration (MIC) assays, it must be noted that the lack of comprehensive chemical characterization of the selected plant extracts may limit both reproducibility and interpretability of the findings. Furthermore, the cytotoxicity assessment was omitted (10); however, this might be considered acceptable, as multiple previous studies have consistently reported no significant toxicity associated with Salvadora persica extracts. Indeed, this plant has been widely used in traditional medicine for centuries without any confirmed adverse effects (11).

Staph. aureus that was Gram dye positive may respond to the activity of the extract due to its bacterial wall composition, as these bacteria lack an outer membrane layer that enhances the permeability of materials to the interior of the cell (12). In addition, various studies have found that the miswak plant is a very effective one in reducing the percentage of bacteria in saliva fresh (Miswak) and essential (oil Miswak extract) possessed high antibacterial activity against Gram negative bacteria that include some of oral pathogens Porphyromonas gingivalis and *Aggregatibacter actinomycetemcomitans*. Benzyl isothiocyanate (BITC) was the major antimicrobial compound of Miswak (13) and plaque bacteria in the gum, since it has antibacterial groups like (Cl_2, SO_4^{-2}, SCN^-, NO_3^-) which stop the growth of an array of bacteria,

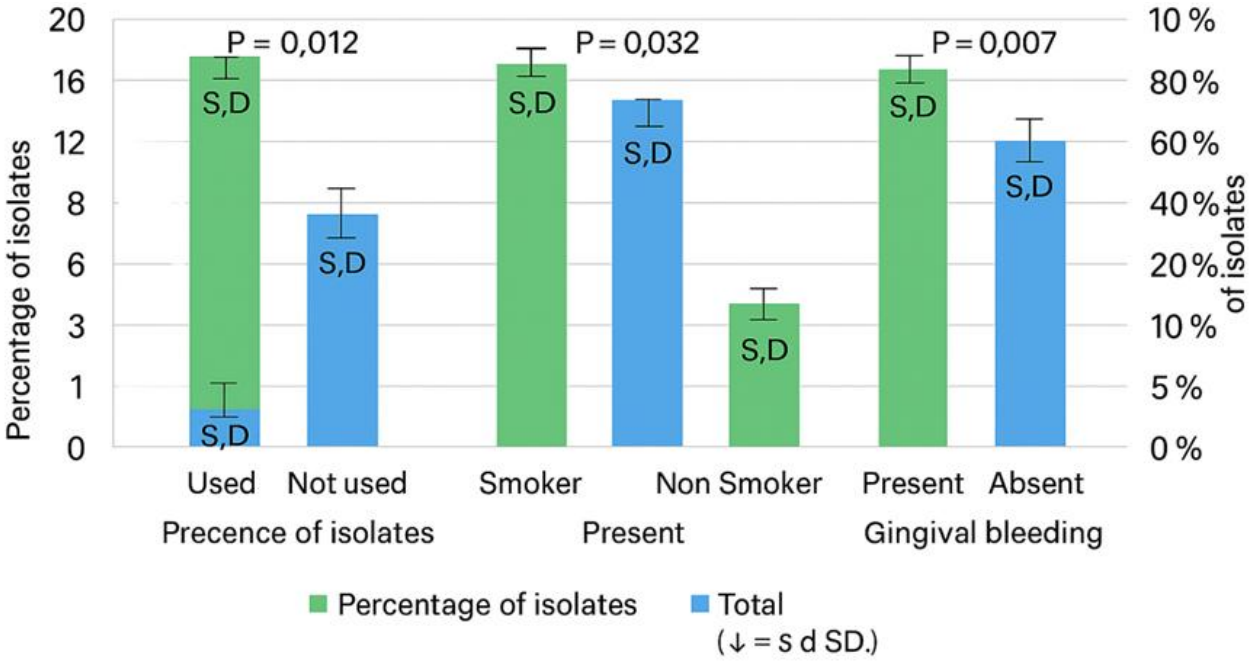

Figure 1. Relationship between the incidence of *Staphylococcus aureus* isolates and the use of miswak, smoking, and bleeding gums. Error bars represent standard deviation; *p < 0.05 considered significant.

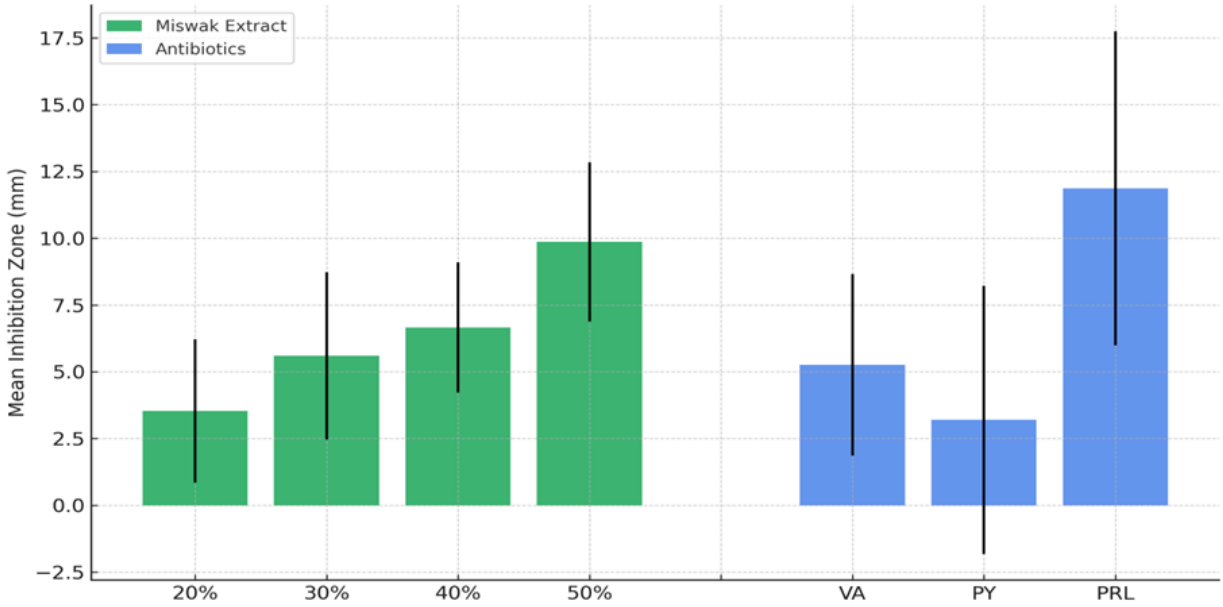

Figure 2. Effect of miswak extract (20%–50%) and antibiotics (vancomycin, carbenicillin, piperacillin) on bacterial growth. Bars represent mean inhibition zone diameters ± standard deviation. Asterisks indicate statistically significant differences compared to control (p < 0.05).

including *Staph. aureus* (14). The findings also indicated that the variations of the effect which the toothpick exerted at concentrations (50%, 40%, 30%) were responsible for the sensitivity of the isolates to vancomycin and carbenicillin antibiotics due to the emergence of enhanced resistance of bacteria towards the aforementioned antibiotics (15,16).

While it was less resistant to the antibiotic piperacillin (third-generation penicillin), and this may be due to the effectiveness of this antibiotic as well as the few number of resistant isolates that were isolated from gum infections (17).

Thus, this study validated that there is a positive relation between the concentration of miswak extract and its effectiveness against the bacteria in question. Additionally, the effect of the extract on bacterial isolates was significantly different from the effect of some antibiotics.

Conclusion

Based upon the significant effects of the *Salvadora persica* extracts, against the oral cavity pathogens in our study, we recommend that Miswak could be used as a dental hygiene method to prevent tooth caries.

Contribution of authors

Murtadha K. Ibraheim and Ghanim Aboud jabber Al-Mola conceptualized and wrote the initial draft of the manuscript. Rafah Hady Lateef and Diana Jalal Albiaty supervised data interpretation and revised the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of Interest

The author declares these no Conflict of interest

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Data Availability Statements

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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