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Antimicrobial Activity of Synthesized Nanoparticles Using *Prosopis farcta* Extract Against *Pseudomonas aeruginosa* Isolated From Patients

Saeide Saeidi, Elham Javadian, Moslem Birjandi, Fereshteh Javadian*

Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran

***Corresponding author:** Fereshteh Javadian, Javadian@yahoo.com

Abstract

Introduction: The aim of this study was to evaluate the antimicrobial activity of synthesized nanoparticles using *Prosopis farcta* extract against *Pseudomonas aeruginosa* isolated from patients.

Methods: The formation and characterization of silver nanoparticles (AgNPs) were confirmed by UV-Vis spectroscopy, energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and transmission electron microscope (TEM). All 12 strains of *P. aeruginosa* were isolated from urine cultures of hospitalized patients (Amir Al-Momenin hospital, Zabol, South-Eastern Iran) with urinary tract infection, during 2015-2016. The minimum inhibitory concentrations (MIC) were investigated by microdilution method.

Results: The analysis of nanoparticle synthesis showed that the nanotube diameter was 11 mm. The results of this study showed that the MIC of AgNPs was 6.25 ppm, and 2 strains were inhibited in this concentration, while the maximum inhibitory concentration was 100 ppm and 1 strain was inhibited. The maximum bactericidal concentration was 100 ppm, at which 3 strains were lost.

Conclusion: According to the results, nanoparticles produced by *P. farcta* extract are a good inhibitor of *P. aeruginosa* and can be used for treating infections caused by *P. aeruginosa*.

Keywords: Silver nanoparticles, Biological synthesis, *Prosopis farcta, Pseudomonas aeruginosa*

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Introduction

Antimicrobial properties of silver have been known for many years.[1] But recently due to its nanoparticle construction, the contact surface has increased and its antimicrobial properties have raised to more than 99%.[2] Nano-sized silver particles are clusters of silver atoms ranging from 1 to 100 nm in diameter[3] which are inserted into the membrane surface of the bacteria by binding to sulfur-containing proteins and by changes in morphology and permeability of the membrane. The effect on the respiratory chain and cell division is susceptible to lead to cell death.[4] In addition to antibacterial properties, they also have strong anti-fungal and anti-viral properties and are therefore of high clinical interest.

Prosopis farcta, the Syrian mesquite, is a species of the genus *Prosopis*, which grows in and around the Middle East. *P. farcta* is a below-ground tree. Above ground, it looks like a shrub with a height of 20–100 cm (in rare cases up to 4 m high).

The medicinal properties of this plant include the treatment of gastric ulcer, abortion, bloody diarrhea, rheumatism, laryngeal inflammation, heart disease and shortness of breath.[5] In other studies, also, anti-diabetic, antispasmodic, and anti-inflammatory properties of *P. farcta* have been mentioned.

The aim of this study was to investigate the antimicrobial activity of synthesized nanoparticles using *P. farcta* extract against *Pseudomonas aeruginosa* isolated from patients.

Methods

Isolation of *Pseudomonas aeruginosa*

Different strains of *P. aeruginosa* collected in this study were isolated from the patients admitted to Amir Al-Momenin hospital in Zabol, Iran. To identify the genus *P. aeruginosa*, hot caring catalase, oxidase and confirmation tests of sugars were used.

Plant Materials

Prosopis farcta leaves were collected from a region in Iran (Zabol, South-Eastern, Iran), planted in a herbarium in Kerman Azad University, approved, and finally dried at the room temperature. Samples were crashed and transferred into glass containers and preserved until extraction procedure was performed in the laboratory.

Preparation of Leaf Extract

Leaf samples (50 g) were sterilized using 30% sodium hypochlorite for 5 minutes and then rinsed 3 times with

sterile distilled water. The process was followed by soaking the samples in 70% alcohol for 2 minutes and then rinsing 5 times with sterile distilled water. Sterile water was added to disinfected seeds (with the proportional volume 2:1) and incubated at 25°C for 7 days. The prepared seed extract was filtered through No. 40 Whatman filter papers and kept in a refrigerator for further studies.

Synthesis of Silver Nanoparticles

Silver nitrate (AgNO3) was used as the source of silver nanoparticles (AgNPs). A volume of 5 mL of the obtained seed extract was diluted by 15 mL sterile water and added to 2 mM concentrated silver nitrate solution, for the reduction of Ag+ to Ag0. Formation of AgNPs from 2 mM solution of silver nitrate was confirmed by UV–Vis spectral and transmission electron microscopy (TEM) analysis.

Minimum Inhibitory Concentration

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the procedure designed by Yu et al. Briefly, serial doubling dilutions of the AgNPs produced in the plant *P. farcta* seed extract were prepared in a 96-well plate, ranged from 12.5 µL/mL to 200 µL/mL. To each well, 10 µL of indicator solution and 10 µL of Mueller-Hinton broth were added. Finally, 10 µL of bacterial suspension (10^6 CFU/mL) was added to each well, to achieve a concentration of 104 CFU/mL. The plates were then wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. They were prepared in triplicate and then placed in an incubator at 37°C, for 18-24 hours. The color change was assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The MIC is defined as the lowest concentration of the extract, at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

Results

During the nanoparticle biosynthesis, using the extract, the color of *P. farcta* solution changed rapidly from light greenish to dark yellowish brown, due to surface plasmon resonance (SPR). The absorption spectrum of yellowish brown solution containing AgNPs showed a SPR with a peak at 430 nm (Figure 1).

The size of nano silver was 11 nm. The results of the study showed that the minimum and maximum antibiotic inhibitory concentrations of imipenem against bacteria were 2 and 128 µg/mL. While the minimum and maximum inhibitory concentrations of ceftazidime were 128 and 1024 µg/mL, and the minimum and maximum inhibitory concentrations of ampicillin were 32 and 512 µg/mL. In addition, the minimum and maximum inhibitory concentrations of cefazolin were 16 and 512 µg/mlL (Table 1). The results of this study showed that the MIC of AgNPs was 6.25 ppm, at which 2 strains were inhibited, while the maximum inhibitory concentration was 100 ppm, at which one strain was inhibited. The highest bactericidal concentration was 100 ppm and 3 strains were lost in this concentration (Table 1).

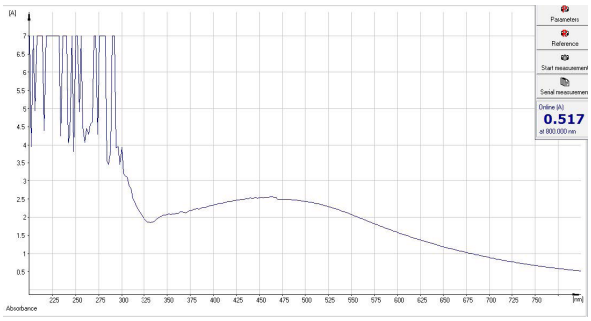

Figure 1. UV-Vis Spectra of AgNPs Biosynthesized From Aqueous Extract of *Prosopis farcta*.

Discussion

The results of this study showed that the MIC of AgNPs was 6.25 ppm, at which 2 strains were inhibited, while the maximum inhibitory concentration was 100 ppm, and 1 strain was inhibited. The highest bactericidal concentration was 100 ppm and 3 strains were lost in this concentration.

In a study by Miri et al, AgNPs were synthesized from extract of *P. farcta* at room temperature. Formation of AgNPs at 1 mM concentration of $AgNO_3$ produced spherical shape nanoparticles with mean diameter about 10.8 nm. The results showed that strong bacterial inhibitors were resistant to several antibiotics.[6] In the study of Miri et al, AgNPs were synthesized using aqueous extract of *Salvadora persica* bark. Moreover, the MIC values of AgNPs were 100 and 400 µg/mL on *Escherichia coli and Staphylococcus aureus*, respectively. The MBC of AgNPs was also 200 µg/mL on *E. coli* and no result was observed for *S. aureus* bacteria. The results showed that synthesized nanoparticles had favorable antibacterial properties.[7]

In a study by Kim et al, on the antimicrobial activity of AgNPs against *S. aureus* and *E. coli*, the results indicated that the MIC of these two bacteria was 100 mg/mL.[8]

In a study by Yasin et al, who carried out the biological synthesis of AgNPs in a bamboo plant extract, the results showed that the diameter of the AgNPs synthesized in this plant was 50 nm. The results of antimicrobial activity against *E. coli* and *S. aureus* showed that by increasing the concentration of AgNPs, the diameter of the inhibition zone was increased.[9]

In a study by Shameli et al, who performed the biological synthesis of AgNPs, the results showed that the inhibition zone diameter was 11.23 mm, and the diameter of the inhibition zone against *S. aureus* and *S. typhimurium* was

Table 1. MIC and MBC of Nanosilver Against Bacteria

Bacterial Code	Imipenem	Ceftazidim	Ampicillin	Cefazolin	MIC	MBC
1	64	512	32	256	50	100
2	64	512	128	256	25	50
3	64	256	128	256	25	50
4	64	512	128	512	12.5	25
5	64	128	256	16	100	100
6	64	512	256	Non grow	12.5	25
7	2	512	256	Non grow	12.5	25
8	2	512	128	128	6.25	12.5
9	2	1024	64	256	50	100
10	2	1024	512	256	25	50
11	128	1024	256	128	6.25	12.5
12	2	256	32	256	12.5	25

1.65 ± 0.56 and 9.67 ± 0.33 mm, respectively.[10]

The study of Sondi et al confirmed the antimicrobial activity of AgNPs against *E. coli* .[11]

In the study of Guzman et al who synthesized nanoparticles, synthetic nanoparticles were obtained with a diameter of 24 nm and the diameter of the inhibition zone of nanoparticles against *S. aureus,* methicillin-resistant *S. aureus* (MRSA), *E. coli and P. aeruginosa* was 12, 12, 10, and 10 mm respectively.[12]

Escherichia coli, S. aureus, Staphylococcus epidermis, Leuconostoc mesenteroides, Bacillus subtilis, Klebsiella mobilis, and *Klebsiella pneumonia* among others [13-24]; (b) fungi such as *Aspergillus niger, Candida albicans, Saccharomyces cerevisiae, Trichophyton mentagrophytes, and Penicillium citrinum*[25-27]; and (c) virii such as hepatitis B, HIV-1, syncytial virus were capable of synthesizing nanosilver.[28-31]

The results of the study by Gopinath et al, who synthesized nanoparticles using *Pterocarpus santalinus*, showed that the inhibition zone against *B. subtilis* lactation, *S. aureus, Streptococcus pyogenes, P. aeruginosa, Proteus virginia* and *Shigella dysenteriae* was 2, 3.3, 3, 2 and 3 mm, respectively.[32]

In the study of Das et al, who synthesized nanoparticles, the diameter of the synthetic nanoparticles was 12 nm, which inhibited *S. aureus, Bacillus* and *P. aeruginosa*.[33]

Conflict of Interest

None.

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