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Antibacterial Effects of Silver Nanoparticles Produced by *Satureja hortensis* Extract on Isolated *Bacillus cereus* from Soil of Sistan Plain

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Background: Drug resistance of microorganisms to antimicrobial agents is on the rise. Therefore, an alternative route to overcome drug resistance of various microorganisms is needed.
Objectives: This study was conducted to assess antibacterial activity of silver nanoparticles (AgNps) produced by *Satureja hortensis* extract against *Bacillus cereus* isolated from soil of Sistan plain (Zabol, Southeastern Iran).
Materials and Methods: *Bacillus cereus* was isolated from collected soil samples by serial dilution and agar plating method. The minimum inhibitory concentrations (MIC) of silver nanoparticles produced by *Satureja hortensis* extract were evaluated by microdilution method.
Results: AgNPs display Gaussian dispensation with a mean diameter of 17.58 nm with some deviations. The highest MIC value was 25 ppm against one *Bacillus cereus* and the least MIC value was against two *Bacillus cereus* strains (12.5 ppm).
Conclusions: The silver nanoparticles produced by *Satureja hortensis* extract owed antibacterial activity against *Bacillus cereus*. Furthermore, antimicrobial activity was based on the concentration of silver NPs to produce the most significant effect against *Bacillus cereus*.

Keywords: Nanoparticles; *Bacillus cereus*; Antibacterial Activity

1. Background

Bacillus cereus is an endemic, soil-dwelling, Gram-positive, rod-shaped, and beta hemolytic bacterium. Some strains are hazardous to humans and able to cause food-borne disease, while some harmless strains are used as probiotics to diminish *Salmonella* in the intestines and cecum (1). *B. cereus* emulates with microorganisms such as *Salmonella* and *Campylobacter* in the gut and reduces the number of these organisms in body. Temperature less than 100 °C allows some *B. cereus* spores to survive. This problem is aggravated when food is not refrigerated, leading to germination of the endospores. Germination and growth usually occur between 10°C and 50°C (50 and 122°F). Bacterial growth and consequently production of enterotoxins, which is highly resistant to heat and ingestion of contaminated food lead to diarrhea and emetic syndrome (2). *Satureja* is a genus of aromatic plants of the Lamiaceae family, related to rosemary and thyme. *Satureja* species are native to warm temperate regions and may be annual or perennial. There are about 30 species called savories; summer savory and winter savory are the most important types cultivated in the Eastern Mediterranean region. It is widely distributed in different parts of Iran (3). Due to the rich chemical compound, raw material is used in pharmaceutical and food industries. It is widely used with black tea leaves in Azerbaijan. Savory plays an important role in Bulgarian and Italian cuisine, particularly when cooking beans. In traditional medicine, *Satureja hortensis* is used as an appetizing antispasmodic, expectorant, antidiarrheal agent and stimulates sexual tendency (3). Essential oils from different *Satureja* species have different effects both qualitatively and quantitatively (4). Essential oil of cultivated summer *S. hortensis* is rich in ç-terpinene and carvacrol (5).

2. Objectives

The present study was performed to determine the antibacterial activity of silver nanoparticles produced by *Satureja hortensis* seed extract against *Bacillus cereus* isolated from soil of Sistan plain, southeastern Iran.

3. Materials and Methods

3.1. Sample Collection

Soil samples were collected from Sistan plain (Zabol, Southeastern Iran). Soil was collected randomly 0-20 cm beneath the surface using spatula and packed in sterile polybags and transferred to laboratories (6).

3.2. Isolation of Bacteria From soil Sample

Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method where in the soil sample was diluted from 10-1 to 10-5 dilutions, and the diluted soil samples were spread on sterile Nutrient agar plates. Then inoculated plates were incubated at 37°C for 24 hours.

3.3. Isolation of Bacillus cereus

Three bacteria isolation from soil of Sistan plain and their growth characteristics were recorded. These elements included morphology of colony after overnight incubation at 30°C and presence or absence of precipitation zone. *Bacillus cereus* colony appears pink when growing on mannitol-egg yolk-polymyxin (MYP), peacock blue on polymyxin-egg yolk-mannitol-bromothymol as blue agar (PEMBA), and orange-pink on Bacara. All three formulations include egg yolk. If the bacterium produces lecithinase, the colony is surrounded by a halo or precipitation zone. *Bacillus cereus* colonies grown on Brilliance and BCM (a new chromogenic plating media) are seen as turquoise green.

3.4. Plant Materials

Satureja hortensis seed was collected from Zahedan and Kerman (Southeastern, Iran) and dried at room temperature. Samples were break down and transferred into glass container and preserved until extraction procedure in laboratory.

3.5. Preparation of Seed Extract

Fifty grams of seed sample was sterilized using 30% sodium hypochlorite for five minutes and then washed three times with sterile distilled water. The procedure was followed by drenching in 70% alcohol for two minutes and then washing five times with sterile distilled water. Sterile water was added to disinfected seeds (2:1 V/V) and incubated at 25°C temperature for seven days. Prepared seed extract was filtered through 40 Whatman filter paper and kept in a refrigerator for further investigations.

3.6. Synthesis of Silver Nanoparticles

Silver nitrate (AgNO3) was used as the source to synthetize silver nanoparticles. Five milliliters of the obtained seed extract was diluted in 15 mL sterile water and added to 2mM silver nitrate (NO3Ag) for the reduction of Ag+ to Ag0. Formation of silver nanoparticles from 2mM solution of silver nitrate was confirmed by using UV-vis spectral analysis.

3.7. Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used to determine MIC according to Yu (7). Briefly, serial doubling dilutions of silver nanoparticles produced in *Satureja hortensis* seed extract were gathered in a 96-well microtiter plate ranged from 12.5 ppm to 200 ppm. To each well, 10 μL of indicator solution and 10 μL of Mueller Hinton Broth were added. Finally, 10 μL of bacterial suspension (106 CFU/mL) was added to each well to achieve the concentration of 104 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and placed in incubator at 37°C for 18-24 hours. Then color change was defined visually. The lowest concentration at which the color change occurred was considered as the MIC value. MIC is specified as the lowest concentration of extract at which the microorganism does not display a visible growth. Microorganism growth was detected by turbidity.

4. Results

Silver nanoparticles detected Gaussian distributions with an average diameter of 17.58 nm with some deviations. The highest MIC value was 25 ppm against one *Bacillus cereus* and the least values were observed against two *Bacillus cereus* strains (12.5 ppm).

5. Discussion

Nanotechnology or nanotech is the manipulation of matter on an atomic, molecular, and supramolecular scale. Nanotechnology could make many new materials and devices with different applications in electronics, biomaterials, medicine, and energy production. On the other hand, nanotechnology brings concerns about the toxicity and environmental impact of nanomaterials (8). Recently, biosynthetic methods using microorganism such as bacteria and fungus or plants extract have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nanomaterials (9-11). In this study, the highest MIC value was 25ppm against one *Bacillus cereus* and the least values were observed against two *Bacillus cereus* (12.5 ppm). In the study of Sunkar, the AgNPs had an antibacterial activity against a few pathogenic bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. typhi*, and *Escherichia coli* (12). In Awwad study, carob leaf extract was obtained by boiling dried small pieces of carob leaves in sterile distilled water and silver nanoparticles were synthetized. They observed that microbial growth of *E. coli* was independent of AgNP concentration and the inhibition zone ranged from 8 to 12 mm (13). Silver nanoparticles were also prepared using orange peel extracts. The resulting systems were in the size of 10 nm and exhibited promising antibacterial properties against bacteria including *Escherichia coli*, *P. aeruginosa*, and *S. aureus* (14). In the study of Mubayi, the maximum zone of Inhibition of AgNPs was 12 mm for *E. coli* (Clinical isolate) and there was no zone of inhibition for *P. aeruginosa* (15). In Kora research, Gum-silver nanoparticles exerted growth inhibition effects around the wells against

examined bacteria. Inhibition zone of around 12.25 mm diameter was determined for the Gram positive bacterial strain *S. aureus* ATCC 25923. In the case of Gram-negative bacterial strains *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 27853, detected inhibition zones were 9.0, 8.0, and 11.0 mm, respectively (16). In the study of Kasraei, Composites containing nano zinc-oxide particles or silver nanoparticles displayed higher antibacterial activity against *Streptococcus mutants* and *Lactobacillus* compared to the control group (17). In the study of El Kassas et al. silver nanoparticles (AgNPs) were biosynthesized with an aqueous extract of Pterocladiella (Pterocladia) capillacea; biosynthesized AgNPs were 11.4 ± 3.52 nm in diameter. Their results also revealed that biosynthesized AgNPs inhibited the entire panel of examined bacteria with a marked specificity towards *Bacillus subtilis* (18). In the study of Thirunavoukkarasu, an aqueous leaf extract of *Desmodium gangeticum* was used to synthesize silver nanoparticles. These biologically synthesized nanoparticles had a highly toxic effect against pathogenic bacteria like *Escherichia coli* (19). In the study of Sudha, synthesized nanosilver particles had antibacterial effects on different organisms causing various diseases in human. Cellular metabolites of Microcoleus species created nanosilver particles, which showed antibacterial activity against pathogenic bacteria including *Proteus vulgaris*, *Salmonella* typhi, *Vibrio cholera*, *Bacillus subtilis*, and *Escherichia coli* (20). Other report by Bhati-Kushwaha showed the antimicrobial effect against pathogens such as *Escherichia coli*, *Vibrio cholerae* and *Aspergillus Niger* (21). In the study of Mariselvam, silver nanoparticles (AgNPs) were synthesized using ethyl acetate and methanol (EA: M 40:60) extracts of inflorescence of Cocos nucifera tree. Qualitative assessment of inflorescence extract indicated its inhibitory effects. Synthesized AgNPs exhibited significant antimicrobial activity against *Klebsiella pneumoniae*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* (22). The present study indicated that antimicrobial activity depends on the concentration of AgNPs to produce the most significant impacts against *Bacillus cereus*. This green-synthesized method is rapid, facile, convenient, less time consuming, environmentally safe, and can be applied in a variety of existing applications. This plant leaf extract compounds can be extended to the synthesis of other metal and metal oxide nanoparticles. Based on our research, silver nanoparticle had antibacterial activity against *Bacillus cereus* and the least MIC value against *Bacillus cereus* was 12.5 ppm.

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Authors' Contributions

Ebrahim Shirmohammadi, Saeide Saeidi, Taher Mohasseli, and Ali Rahimian-Boogar wrote the paper. All authors had equal role in design, work, statistical analysis and manuscript writing.

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