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The Antibacterial Activity of Silver Nanoparticles Produced in the Plant *Sesamum indicum* Seed Extract: A Green Method Against Multi-Drug Resistant *Escherichia coli*

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Background: The nanoparticles synthesis through biological processes is evolving a new era of research interests in nanotechnology. In usual methods silver nanoparticles are synthesized through chemical methods, having extremely toxic and flammable natures.
Objectives: The aim of the present study was to synthesize silver nanoparticles, through the green method of utilizing *Sesamum indicum (S. indicum)* extract and to determine the potential antibacterial effects of the product against multi-drug resistant *Escherichia coli (E. coli)*.
Materials and Methods: The formation and characterization of AgNPs (silver nanoparticles) were confirmed by UV-vis spectroscopy, energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and transmission electron microscope (TEM). All 30 strains of *E. coli* were isolated from urine cultures of hospitalized patients (Amir Al-Momenin Hospital, Zabol, South-Eastern Iran) with urinary tract infection, 2011-2012. The minimum inhibitory (MIC) concentrations were investigated by microdilution method.
Results: The results showed that isolated *E. coli* were resistant to four different antimicrobial agents including ceftazidime (26.6%), cefixime (40%), tetracycline (63.3%) and erythromycin (56.6%). The highest MIC value for produced nano silver in *S. indicum* seed extract, was 200 ppm, against five isolates of *E. coli*.
Conclusions: Considering the sufficient antimicrobial activities of nanoparticles tested in this study, they are suggested for enterobacterial infection treatment, especially in hospital environment.

Keywords: Anti-Bacterial Agents; Escherichia coli; Tuberculosis, Multidrug-Resistance; Nanoparticles

1. Background

Human beings are often infected by different microorganisms like bacteria, molds, yeasts, and viruses, present in their living environments. Recently, the development of resistant or even multi-drug resistant pathogens has become a major problem. These problems and needs have led to a resurgence in the use of silver-based antiseptics which have broad-spectrum activity and considerably lower propensity to induce microbial resistance, compared to the antibiotics (1, 2). Silver ions and silver-based compounds high toxicity to microorganisms have been known for a long time, therefore, silver ions have been used in many formulations. Recently it was shown that hybrids of silver nanoparticles, with amphiphilic hyper branched macromolecules exhibit effective antimicrobial surface coating (3, 4). Accordingly, using a non-chemical process for the synthesis of silver nanoparticles is of importance. Plant extract solutions and bio-organisms have been now in spot light for their considerable capability of synthesizing nanoparticles, including silver and gold nanoparticles. Sesame belongs to the family Pedaliaceae and genus *Sesamum* (5). This genus consists of about 36 species, of which 19 species are indigenous to Africa (6, 7). Sesame (*S. indicum*) is one of the oldest cultivated plants in the world, mainly grown for its oil rich edible seeds. Sesame seed contains 40-50% oil, 20-25% protein, 20-25% carbohydrate and 5-6% ash (8). Sesame seed oil is used in cooking, salad preparation and margarine and is the raw material for some industrial products including paints, varnishes, soaps, perfumes, pharmaceuticals and insecticides, while sesame seeds are used in baking, candy production and other food industries.

Implication for health policy/practice/research/medical education:
The medicinal plants have recently attracted considerable attention, due to their use for therapeutic purposes, against enteric pathogens.

2. Objectives

The aims of the present study was to synthesize silver nanoparticles by using green method, on *S. indicum* extract and to determine the production potential antibacterial effects, against multi-drug resistant *E. coli*.

3. Materials and Methods

3.1. Isolation of Bacteria

All 30 *E. coli* strains were isolated from urine cultures of hospitalized patients (Amir Al-Momenin Hospital, Zabol, South-Eastern Iran) with urinary tract infection, 2011-2012. Isolated bacteria were identified by Gram's stain and standard biochemical tests (9).

3.2. Agar Disk Diffusion Assay

The susceptibility of all antibiotics was carried out using disc diffusion method on Muller-Hinton agar, as recommended by CLSI (10). The procedure is briefly described here: *E. coli* isolated plates were grown overnight on blood agar and nutrient agar and colony suspension was prepared using the sterile saline water, equivalent to 0.5 McFarland standard. Suspension (10 μL) was spread over the media plate and antibiotic disc was transferred aseptically on the surface of inoculated media. Isolated plates were tested with different antibiotics and their concentration shown in parenthesis: ceftazidim (30 μg), tetracyclin (30 μg), erythromycin (15 μg) and ceftazidime (30 μg).

3.3. Plant Materials

The *S. indicum* seeds were collected from a region in Iran (Kerman, South-Eastern, Iran), planted in Kerman Azad University Herbarium, 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.

3.4. Preparation of Seed Extract

Seed samples (50 gr) were sterilized, using 30% sodium hypochlorite for five minutes and then rinsed three times with sterile distilled water. The process was followed by soaking the samples in 70% alcohol for two minutes and then rinsed five times with sterile distilled water. Sterile water was added to disinfected seeds (with the proportional volume 2:1) and incubated at temperature 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.

3.5. Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was used as the source for silver nanoparticles. 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 Ag^0. Formations of silver nanoparticles from 2 mM solution of silver nitrate were confirmed by using UV–vis spectral and transmission clectron microscopy (TEM) analysis.

3.6. 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. (11). Briefly, serial doubling dilutions of the silver nanoparticles produced in the plant *S. indicum* seed extract were prepared in a 96-well mL 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 10^4 CFU/mL. The plates were then wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. They were prepared in triplicates 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.

4. Results

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

Figure 2 show the TEM image of *S. indicum* seed extract, containing 2 mM $AgNO_3$ solution at 30°C. It way shown that *S. indicum* seed extract often produces semi spherical silver nanoparticles. The silver nanoparticles showed Gaussian distributions with the average diameter of 13 nm, with some deviations. Antibiotic susceptibility of *E. coli* isolates was evaluated in four antimicrobial agents. However, *E. coli* were resistant to all four agents namely ceftazidime (26.6%), cefixime (40 %), tetracycline (63.3%) and erythromycin (56.6%) (Table 1).

The antimicrobial activities of the *S. indicum* seed extract is due to silver nanoparticle production and their antimicrobial potency, which is quantitatively assessed by the presence or absence of inhibition. The *S. indicum* seed extract produced silver nanoparticle showed inhibitory activity against bacteria with varying magnitudes, having dose dependent effects. The highest and lowest MIC values against five isolates of *E. coli* were found to be 200 ppm and 50 ppm, respectively (Table 2).

5. Discussion

Plants and microorganisms provide unexplored natural sources for development of potentially new drugs. Our results show that *E. coli* were resistant to four antimicrobial agents including ceftazidime (26.6%) cefixime (40%), tetracycline (63.3%) and erythromycin (56.6%) and the highest MIC value was found to be 200µL/mL against five isolates of *E. coli*. Duran et al. showed that cotton fabrics incorporated with silver nanoparticles, displayed a significant antibacterial activity against *S. aureus* (12). Kim et al. confirmed that *S. aureus* and *E. coli* are substantially inhibited by Ag-NPs. They also found that the antibacterial activity of Ag-NPs did not fluctuate with temperature or pH, showing that the MIC of Ag- NPs against *S. aureus* and *E. coli* was 100 µg/mL (13).

Yasina et al. (14) examined the antibacterial activity of nano silver against the bacterial strains (*E. coli* and *S. aureus*). The bacteria cultured were with different concentrations of AgNPs (20, 40, 60 and 80 µg/mL) in agar plates. Their results showed that the lowest concentration of AgNPs, with which the cell growth on agar plate continues with the rate of 1×10^8 CFU/mL, is 20 µg/mL. The cell growth gradually decreased to 0, as the concentrations increased, ranging from 1×10^7 to 1×10^3 CFU/mL. In a MIC/MBC study, the lowest concentration of AgNPs to be effective in killing the bacteria in up to 1×10^7 CFU/mL concentrations, was 20 µg/mL, as there was no cell growth seen on plating after incubation at 37°C for 24 hours (14). The ability of ZnO NPs to induce frame shift mutation is dependent on the presence of S9 fraction. It is possible that the S9 fraction increases the internalization of NPs and then increases the generation of ROS that induce frame shift mutation in the bacteria. However, TiO_2 NPs induce frame shift mutation in *S. Typhimurium* (TA98 and TA1537), independent of S9 fraction. TiO_2 NPs are toxic to *Pseudomonas aeruginosa* (-), *Enterococcus hire* (+), *E. coli* (-), *S. aureus* (+) and *Bacteroides fragilis* (-) (15). Shittu et al. showed that both methanolic and ethanolic extracts of sesame have broad spectrum antimicrobial effect against all tested microorganisms, except *Streptococcus pneumoniae*, *Candida albicans* and *S. aureus*, while the aqueous extract exhibited no inhibitory effect on *S. aureus* and *S. pneumoniae* (16).

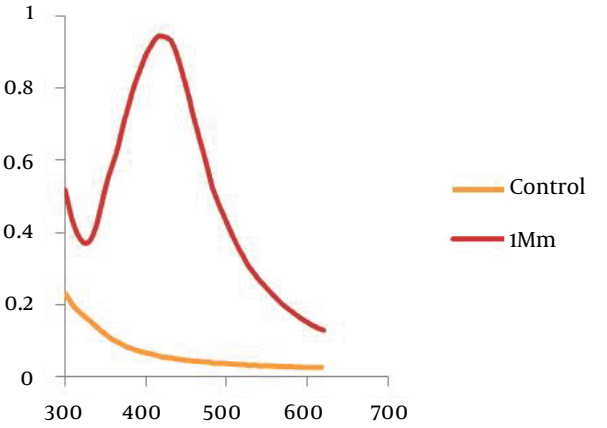

Figure 1. UV-Vis Spectrum of Ag Nanoparticles

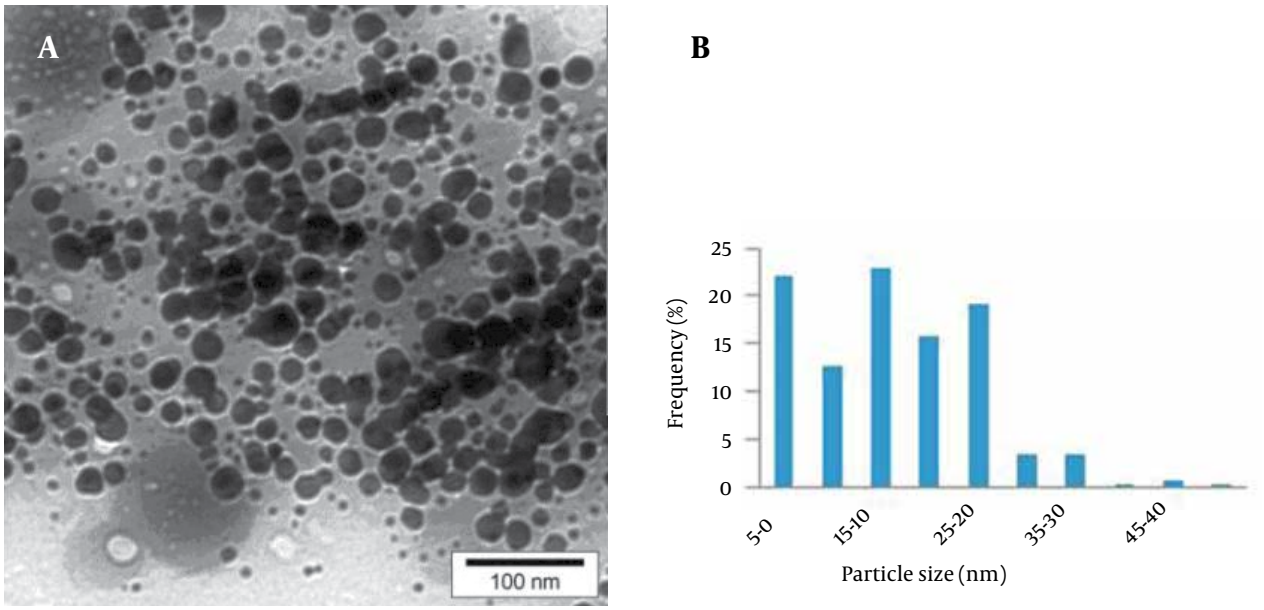

Figure 2. (A) TEM Image and (B) Particle Size Distribution of Ag Nanoparticles Synthesized by Seed Extract

Table 1. Antimicrobial Susceptibility of 30 Isolated Strains of *E. coli* (%) [a]

	CAZ	E	CN	TE
S	56.6	16.6	43.3	30
I	16.6	26.6	16.6	6.6
R	26.6	56.6	40	63.3

[a] Abbreviations: S, sensitive; I, intermediate; R, resistant; CAZ, ceftazidime, TE, Tetracyclin, E, erythromycin, CN, cefixime.

Table 2. Antimicrobial Susceptibility and Minimum Inhibitory Concentration of Nano Silver in the *Sesamum Indicum* Seed Extract [a,b]

Isolated Bacterial	MIC of Nano silver, μL/mL	Antibiotic-Resistance Pattern	Isolated Bacteria	MIC of Nano silver, μL/mL	Antibiotic-Resistance Pattern
1	50	A_1, A_2, A_3, A_4	16	100	-
2	50	A_1, A_3	17	100	A_1, A_2, A_4
3	5	A_2, A_3, A_4	18	100	A_4
4	50	A_1, A_2, A_3, A_4	19	100	-
5	50	A_1, A_2, A_3, A_4	20	100	A_1, A_2
6	200	-	21	100	A_1, A_2, A_4
7	200	-	22	100	A_1, A_2, A_4
8	100	A_4	23	100	A_1, A_2, A_3, A_4
9	100	A_1, A_4	24	100	-
10	100	A_1, A_4	25	100	A_1
11	100	A_4	26	100	A_1, A_2, A_4
12	100	A_1, A_2, A_3, A_4	27	200	A_1, A_2, A_4
13	100	A_3, A_4	28	100	A_1, A_2
14	100	A_1, A_4	29	200	-
15	100	A_4	30	200	-

[a] Abbreviation: MIC, minimum inhibitory concentration.
[b] A_1, erythromycin; A_2, cefixime; A_3, ceftazidime; A_4, Tetracyclin.

The ethanolic extract had no inhibitory effect against *S. aureus* despite having both antibacterial and antifungal activities (17). The seed oil of *Sesame* sp was found to contain certain natural antibacterial agents, effective against common skin pathogens, like *Staphylococcus* and *Streptococcus*, as well as common skin fungi including the athlete's foot fungus (18). Medicinal plants could be sources of compounds with useful bacteria managing functions. However, further studies about the isolation of active compounds and the absence of toxicity of plant extracts are necessary to propose using these plant derived productions as alternative treatments for managing drug resistant pathogens.

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Authors' Contribution

Mohammad Bokaeian, Mousa Sheikh, Mehdi Hassansahian, Saeide Saeidi and Shahla Sahraei had equal roles in designing the study, gathering data, statistical analysis and manuscript writing.

Financial Disclosure

The authors declare no conflict of interest.

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