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The antibacterial activity of silver nanoparticles produced in the plant *Sesamum indicum* seed extract, green method against multi-drug resistant *Klebsiella pneumoniae*

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ABSTRACT

The present study was the synthesis of silver nanoparticles by using green method on extract from Sesamum indicum and determined the potential antibacterial against K. pneumonia isolation of urinary tract infection. The formation and characterization of AgNPs were confirmed by UV-Vis spectroscopy, energy-dispersive spectroscopy (EDX), X-ray diraction (XRD) and transmission electron microscope (TEM). All 14 strains of K. pneumonia isolated from urine culture of hospitalized patients Hospital(Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011- 2012 and the minimum inhibitory concentrations were investigated by microdulition method. The synthesized silver nanoparticles showed inhibitory K. pneumonia. Significance of the above in the light of existing literature is discussed in the present communication.

Key words: Silver nanoparticles, *Sesamum indicum* seed powder, UV- visible spectroscopy, Antibacterial activity, *Klebsiella pneumoniae*

INTRODUCTION

Recently, metal nanoparticles have gained a lot of attention due to their unique chemical, optical, magnetic, mechanical, and electric magnetic properties. Thus metallic nanoparticles are used in different applications such as electronics, catalysis and photonic [1]. Silver nanoparticles (Ag-NPs) have been known for its inhibitory and bactericidal effects in the past decades [2]. Antibacterial activity of silver containing materials can be applied in medicine for reduction of infections on the burn treatment [3, 4] prevention of bacteria colonization on catheters [5, 6] and elimination of microorganisms on textile fabrics [7,8] as well as disinfection in water treatment [2]. Silver ions cause the release of K+ ions from bacteria; thus, the bacterial plasma or cytoplasm membrane, which is associated with many important enzymes and DNA is an important target site of silver ions [9, 10, 11, 12]. When bacterial growth was inhibited, silver ions were deposited into the vacuole and cell walls as granules. They inhibited cell division and damaged the cell envelope and cellular contents of the bacteria [13]. Plant extract solutions and

bio-organisms have been in spot light for their extreme ability to synthesis nanoparticles, including silver and gold nanoparticles. Sesame belongs to the family *Pedaliaceae* and genus *Sesamum* [14] .The genus consists of about 36 species of which 19 species are indigenous to Africa [15]. Sesame (*Sesamum indicum*) is one of the oldest cultivated plants in the world that mainly grown for its oil rich edible seeds. Sesame seed contains 40-50% oil, 20-25% protein, 20-25% carbohydrate and 5-6% ash [16]. The present study was, the Synthesis of silver nanoparticles by using green method on extract from *S. indicum* and determine the potential antibacterial against *K. pneumonia* isolation of urinary tract infection.

MATERIALS AND METHODS

Isolation of bacteria: All 14strains *K. pneumonia* isolated from urine culture of hospitalized patients Hospital(Zabol, southeast of Iran) suffered from urinary tract infections during the years 2011- 2012 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests [17].

Antibiotics susceptibility

The susceptibility of for antibiotics was carried out using disc-diffusion method on Muller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (CLSI, 2002) with minor modification. Briefly, *K. pneumoniae* isolates were grow overnight on nutrient agar and colony suspension was prepared using a sterile saline water equivalent to a 0.5-Mc Farland standard. Suspension (100 µl) was spread over the media plate and antibiotic disc aseptically transferred on the surface of inoculated media plate. Then the plates were incubated for 24 h at 37°C and zone of grow inhibition recorded. Isolates were tested with different antibiotics with different concentration as shown in parenthesis viz. ceftazidim (30 µg), tetracyclin (30 µg), erythromycin (15 µg), cefixime (30 µg), penicillin (10 µg), ampicillin (25 µg), nalidixic acid (30 µg).

Plant materials

The seed of *Sesamum indicum* was collected in SE of Iran (Kerman, Iran) and thespecimens were deposited in Zabol University Herbarium and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of seed extract

Seed samples sterilized using 30% sodium hypochlorite for 5 minutes and then were rinsed, three times with sterile distilled. The process was followed by soaking in 70% alcohol for two minutes and then rinsed five times with sterile distilled water. Sterile water was added to disinfected seeds (2:1 V/V) and incubated 25°C temperature for 7 days. The prepared seed extract was filtered through 40 whatman filter paper and was kept in a refrigerator for further studies.

Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was used as the source of the synthesis of silver nanoparticles. 5mL of the obtained seed extract was diluted by 15mL sterile water and was added to concentration of 2mM silver nitrate for the reduction of Ag+ to Ag0. Formation of silver nanoparticles from 2mM solution of silver nitrate was confirmed by using UV–Vis spectral and Transmission Electron Microscopy (TEM) analysis.

Minimum Inhibitory Concentration (MIC)

The broth microdilution method was used to determine MIC. Briefly, serial doubling dilutions of the silver nanoparticles produced in the plant *Sesamum indicum* seed extract were prepared in a 96-well microtiterplate ranged from 12.5ppm to 200ppm. 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 wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37ºC for 18-24 hours. The color change was then 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 [18].

Statistical assessment

All experiments and measurement were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010 software. All experimental results were analyzed using mean descriptive statistics and the correlation-coefficient. A value of $P<0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Silver nanoparticles were synthesized from silver nitrate solution using the seed powder extract of *S. indicum* using domestic microwave within two minutes of time. Color of silver colloid is attributed to Surface Plasmon Resonance (SPR) arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field [19]. UV–Vis spectroscopy is an important technique to confirm the formation of silver nanoparticles in aqueous solution. The synthesized microwave assisted seed powder mediated silver nanoparticles were subjected to optical measurements by UV–Vis spectrophotometer. Absorption spectra of silver nanoparticles formed in the reaction media showed an absorbance peak at 430 nm. The spectra are shown in the fig.2. The figure also shows the histogram of particles size versus number of particles observed on TEM grid. It is clear from the histogram that more number of nanoparticles has the diameter within the range of 13nm. In our study, the AgNPs synthesized using microwave assisted seed powder extracts of *S. indicum* has exerted a fairly significant antimicrobial action on the tested micro organisms. This is evident by the values of diameter of MIC obtained during assessment of antimicrobial activity (Table1). Silver is said to be a universal antimicrobial substance for centuries. Though, silver ions or salts have limited usefulness as an antimicrobial agent. Such as, the interfering effects of salts and antimicrobial mechanism of continuous release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using silver nanoparticles. Some researchers have reported that the antimicrobial effect of the Ag-NPs on Gram-negative bacteria was dependent on the concentration of Ag in the nanoparticles and was closely related to the formation of “pits” in the cell walls [20, 21]. The study of Yasin showed evident that around the paper soaked in AgNPs with different concentrations has a significant inhibition zone against *E. coli* and *S. aureus* . The Ag NP uptake by marine biofilms and reduction of marine biofilms are dependent on the concentration of Ag NPs [22]. Exposure to Ag NPs may prevent colonization of new bacteria onto the biofilm and decrease the development and succession of the biofilm. MgF2 NPs have antimicrobial activity and are able to prevent the biofilm formation of common pathogens such as *E. coli* and *S. aureus* [23]. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms [24]. Spadaro showed strong biocidal effects on as many as 16 species of bacteria including E. coli [25]. Ag+ inhibits phosphate uptake and exchange in Escherichia coli and causes efflux of accumulated phosphate as well as of mannitol, succinate, glutamine, and proline [26]. According to the study of Guzman nanoparticles of silver showed high antimicrobial and bactericidal activity against gram positive bacteria such as *Escherichia Coli*, *Pseudimonas aureginosa* and *staphylococcus aureus* which is a highly methicillin resistant strain[27]. Additionally, MICs ranging from 13.5 to 1.69 lg/mL were reported for bacterial strains such as S. aureus CCM 3953, *Enterococcus faecalis* CCM 4224, E. coli CCM 3954, and P. aeruginosa CCM 3955, and for strains isolated from human clinical material like *P. aeruginosa*, methicillin-susceptible S. epidermidis, methicillin-resistant S. epidermidis, methicillin-resistant S. aureus, vancomycin-resistant Enterococcus faecium, and K. pneumonia when exposed to 26-nm silver nanoparticles prepared by the reduction of [Ag(NH3)2] with D-maltose (Kvitek et al. 2008).

Table1: Results of the anti -bacterial activity of the synthesized silver nanoparticles

Bacterial isolate No.	MIC(ppm)	Antibiotic-resistance pattern
1	100	$A_2,A_3,A_4,A_5,A_6,A_7,A_8$
2	100	A_2,A_3,A_6,A_7,A_8
3	100	A_3,A_4,A_5
4	50	A_5,A_6,A_7
5	200	A_3,A_5,A_6
6	200	A_1,A_2,A_3,A_4,A_5
7	100	A_1,A_2,A_3,A_4,A_5,A_6
8	100	$A_1,A_2,A_3,A_4,A_5,A_6,A_7,A_8$
9	50	A_5,A_6,A_7,A_8
10	50	$A_1,A_2,A_3,A_4,A_5,A_6,A_7,A_8$
11	100	A_3,A_4,A_5
12	100	A_2,A_5,A_6,A_8
13	100	A_3,A_5,A_7
14	100	A_3,A_4,A_5,A_6

CONCLUSION

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of silver nanoparticles using aqueous *S. indicum* seed extracts. The silver ions in an aqueous olution were exposed to the *S. indicum* seed extracts, the biosynthesis of AgNPs were confirmed by the rapid colour change of plant extracts. The natural benign AgNPs were confirmed further by using UV-Vis spectroscopy. AgNPs biosynthesized from bamboo leaves also exhibits great antimicrobial activities against sample bacteria cultures. These biosynthesis silver nanoparticles can potentially be used for different medical applications.

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