Antibacterial activity of silver nanoparticles produced in the plant sesame indicum seed extract by the green method against bacteria Staphylococcus epidermidis and Salmonella typhi

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ABSTRACT: The application of nanoscale materials and structures, usually ranging from 1 to 100 nanometers (nm), is an emerging area of nanoscience and nanotechnology. Nanosilver has developed as a potent antibacterial, antifungal, anti-viral and anti-inflammatory agent. The result show that Several factors have been reported to influence silver nanoparticle toxicity like particle size, shape, crystallinity, surface chemistry, capping agents, as well, as for environmental factors such as pH, ionic strength, and the presence of ligands, divalent cations, and macromolecules. In this paper Silver nanoparticles were synthesized using Sesamum indicum. Synthesized nano particles were characterized using UV-visible spectrophotometer and Transmission Electron Microscopy (TEM). The TEM image of the nanoparticles is confirmed that the synthesis of silver nanoparticles by Sesamum indicum seed extract. The antibacterial activity of AgNPs(synthesized by the Green method) was investigated against Gram-positive bacteria, i.e., Staphylococcus epidermidis and Gram-negative bacteria, i.e., Salmonella typhi by the serial dilution method using nutrient broth at different sizes of AgNPs. MIC (Minimum inhibitory concentration of growth) and MBC (Minimum bacteria concentration) were determined. The antimicrobial activity of silver nanoparticles were demonstrated that the bacteria have antibacterial effects. The total effect of nanoparticle antibacterial was dependent on nanoparticle concentration, physiology, metabolism and intracellular, Selective permeability of membranes and the bacterial cell wall.

Keywords: green synthesis, Silver nanoparticles antibacterial activity, Staphylococcus epidermidis, Salmonella typhi, Sesamum indicum

INTRODUCTION

Silver has known to be a metal that came into use even before Neolithic revolution. Even the Greeks used it for cooking and to keep water safe. The first recorded medicinal use of silver was reported during 8th century (Moyer, 1965). Nanotechnology, is the powerful tool for the creation of new objects in nanoscale dimensions, is a cutting edge technology having important applications in modern biomedical research (Parak, 2003; Gao, 2005; Alivisatos, 2004; Salata, 2004). The intrigue in nanomaterial research for regenerative medicine is easy to see and is wide spread. The potential benefits of nanomaterials in biomedical and industrial applications for human health and environment are now accepted in the literature (David et al., 2005; Lanone and Boczkowski, 2006). The search for components with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms (Davis, 1982). Silver nanoparticles also reported to possess anti-fungal, 15 anti-inflammatory, (Nadworny et al., 2010) anti-viral, (Vaidyanathan et al., 2009), anti-angiogenesis (Kalishwaralal et al., 2009) and anti-platelet activity (Shrivastava et al., 2009). Sesame belongs to the family Pedaliaceae and genus Sesamum (Purseglove, 1974). The genus consists of about 36 species of which 19 species are indigenous to Africa (Weiss, 1998; Uzo, 1998). Sesame plays an important role in human nutrition. Its seeds are used essentially for the production of oil, but also in the production of the paste (tehineh) and in food formulations such as Halaweh (sweetened tehineh), java beans and salads Abou-Gharbia et al., 2000; Abu-Jdayil, Al-Malah, &Asoud, 2002; Namiki, 1995). The present study was the Synthesis of silver nanoparticles by
using tea seed extract from Sesamum indicum and determine the potential antibacterial against Staphylococcus epidermidis and Salmonella typhi.

MATERIALS AND METHODS

Bacterial strains and culture conditions
The bacterial strains such as Staphylococcus epidermidis PTCC1114 and Salmonella typhi PTCC1609 were used for antimicrobial assay. All the strains were grown in Nutrient agar medium contains beef extract, peptone, sodium, yeast, distilled water at pH 7.2 and incubated at 37°C for overnight.

Plant material
The seed Sesamum indicum was purchased from selling companies seeds at Kerman, Iran and kept in sterilized screw-cap glass container. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Preparation of extracts
Preparation of seed extract
First, 50g of Sesamum indicum seeds disinfected with sodium 30% hypochlorite for 5min and then washed with distilled water 3times and each time was 1 minute. The seeds are disinfected with 70% alcohol for 2 minutes and then washed with distilled water 3times and each time was 2 minutes. 2times the volume of sterile water was added to the seeds and then at 25°C for one week boarding were dark environment. After a week of extracts from the Whatman paper No 40 was smooth and it was used for sample preparation extracts was obtained and then stored at 4°C in air tight screw-cap tube. The tube dilution test is the standard method for determining levels of resistance and sensitive and ranged from 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256ml. Serial dilutions of the extract plant are made in a liquid medium which is inoculated with a standardized number of organisms and incubated for a prescribed time. The lowest concentration (highest dilution) of extract plant preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC). Additionally, the minimal bactericidal concentration (MBC) can be determined by subculturing the contents of the tubes on to antibiotic-free solid medium and examining for bacterial growth. 1. Number sterile capped test tubes 1 through 8. All of the following steps are carried out using aseptic technique. 2. Add 1.0 ml of silver nanoparticles solution to the first tube. 3. Add 1.0 ml of sterile nutrient broth to all tubes. 4. Transfer 1.0 ml from the first tube to the second tube. 5. Using a separate pipette, mix the contents of this tube and transfer 1.0 ml to the third tube. 6. Continue dilutions in this manner to tube number 8, being certain to change pipettes between tubes to prevent carryover of antibiotic on the external surface of the pipette. 7. Remove 1.0 ml from tube 8 and discard it. 8. The final concentration of silver nanoparticles is now one-half of the original concentration in each tube. 9. Add 100 µl of bacteria (cfu/ml 108) to all tubes. 10. Incubate all tubes at 37°C overnight. 10. Examine tubes for visible signs of bacterial growth. The highest dilution without growth is the minimal inhibitory concentration (MIC).

RESULTS

Synthesis of silver nanoparticles
Silver nitrate (AgNO3) was used as the source for the synthesis of silver nanoparticles. Value 5ml seed extracts obtained by 15ml sterile water and diluted to concentrations of the order 1mM For the reduction of silver nitrate Ag+ to Ag0 was added. Formation of silver nanoparticles from 1mM solution of silver nitrate was confirmed by using UV–vis spectral analysis. During the biosynthesis using the extract the color of the reaction medium changed rapidly from light greenish to dark yellowish brown due to Surface Plasmon Resonance. The absorption spectra of yellowish brown silver nanoparticle solution showed a Surface Plasmon Resonance with a peak at 420nm. (Figure 1) Figure 2 is TEM images obtained with 5ml seed extract of sesame seed and 1mM AgNO3 solution at 30°C. It is shown that relatively Spherical nanoparticles are formed. The silver nanoparticles showed Gaussian distributions with average diameter of 14 nanometer with some deviations. From the image, it is evident that the morphology of silver nanoparticles is spherical which is in agreement with the shape of SPR band in the UV–vis spectrum.
**Antibacterial property of silver nanoparticles produced in the plant Sesamum indicum seed extract against bacteria**

The antimicrobial activity of the extract and their potency was quantitatively assessed by the presence or absence of inhibition. The plants extract showed inhibitory activity against Staphylococcus epidermidis and Salmonella typhi bacteria with varying magnitudes and these effects were dose dependent manner. The levels of MIC and MBC were observed ranges from 1.128 to 1.32 and 1.64 to 1.16 ml in radius respectively (Table 1). The least MIC value was observed against Staphylococcus epidermidis (1.32 ml) and The least MBC value was observed against Staphylococcus epidermidis (1.16 ml).

**Table 1. The MIC and MBC value**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Concentration</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>1/256</th>
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<tbody>
<tr>
<td>S.typhi</td>
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<td>-</td>
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<tr>
<td>S.epidermidis</td>
<td></td>
<td>-</td>
<td>+</td>
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</table>

-= MBC
+= MIC

**DISCUSSION**

Silver nanoparticles have attracted intensive research interest because of their important applications in antimicrobial, catalysis, and surface-enhanced Raman scattering (Li et al., 2006; Setua et al., 2007). For centuries, silver has been used as an antimicrobial agent. The recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics (Panaek et al., 2006; Sandbhy et al., 2006). Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death (Feng et al., 2000). In the study, the levels of MIC and MBC were observed ranges from 1.128 to 1.32 and 1.64 to 1.16 ml radius respectively (Table 1). The least MIC and MBC value was observed against Staphylococcus epidermidis. Silver nanoparticles of size 8 nm from leaves of Nicotiana tabacum inhibits Pseudomonas putida, P. vulgaris, Escherichia coli DH5, B. subtilis, P. aeruginosa and Salmonella typhi (Suranjit et al., 2011).
study of Ramteke, AgNPs stabilized by Tulsi leaf extract were found to have enhanced antimicrobial activity against well-known pathogenic strains, namely Staphylococcus aureus and E.coli(Ramteke et al., 2013). silver nanoparticles showed growth inhibition around the wells against the tested bacteria. ZOI of around 12.25 mm was observed for the Gram positive bacterial strain S. aureusATCC 25923. In the case of Gram-negative bacterial strains E. coli ATCC 25922, E.coli ATCC 35218, and P. aeruginosa ATCC 27853, the detected ZOI were 9.0, 8.0, and 11.0 mm, respectively (Kora et al., 2012). Recently, the authors reported the synthesis of green AgNPs using thecell biomass of Cochliobolus lunatus(Salunkhe et al., 2011). Similarly, other studies have shown that exposure of silver ions to Trichoderma viride(Fayaz et al., 2010) and Escherichia coli(Shahverdi et al., 2007) filtrate resulted in the reduction of silver ions and the formation of extremely stable silver particles.bacteria. The study of Jena, the result showed distinct differences in the susceptibility of bacteria to CS-AgNPs. P. aeruginosa, S. typhi, and S. aureuswere found to be more susceptible to the action of CS-AgNPs. In contrast, the inhibitory effect of nanoparticles was moderate in M. smegmatis(Jena et al., 2012). The study of Gergios et al., the enhanced growth inhibition of AgNPs was investigated by monitoring the fluorescence intensity of suspensions of E. coli cells at 37 °C which encode the green fluorescent protein(GFP). Thus, the fluorescence intensity directly correlates with the E. coli population (Gergios et al., 2006) and the initial fluorescence corresponds to approximately 10^7 CFU/mL. Several studies on the usage of metal nanoparticles in the water filter have been carried out due to its antibacterial and as pesticide removal properties(Jain et al., 2005). In an earlier report, the enhanced antibacterial potency of AgNPs was analyzed (Shrivastava et al., 2007). Recent report has demonstrated the therapeutic potential of AgNPs against leishmaniasis, another parasitic disease caused by Leishmania tropica(Allahverdyiev et al., 2011). In view of this, further studies are envisaged to explore the other potential applications of this extant and nanoparticles.

REFERENCES


