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Antibacterial Activity of Silver Nanoparticles Produced by *Plantago Ovata* Seed Extract Against Antibiotic Resistant *Staphylococcus aureus*

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Background: Silver nanoparticles can be applied to control infections.
Objectives: The current study aimed to detect antibacterial activity of silver nanoparticles produced by *Plantago ovata* seed extract against antibiotic resistant *Staphylococcus aureus*.
Patients and Methods: All the antibiotic resistant *Staphylococcus aureus* strains isolated from the patients with urinary tract infection admitted to the hospitals in Zabol (South-eastern Iran) from January 2011 to January 2012 were studied. Then, minimum inhibitory concentration (MIC) of the isolates against the prepared extract was evaluated by the microdulition method.
Results: The silver nanoparticles revealed Gaussian distributions with the average diameter of 13 nm with some deviations, and results showed that the highest and the lowest MIC of *P. ovata* seed extract were 100 and 12.5 mg/mL, respectively.
Conclusions: The current study results showed that silver nanoparticles produced by *Plantago ovata* seed extract had an antibacterial effect on *Staphylococcus aureus* which was resistant against cefixime, trimethoprim-sulfamethoxazole and penicillin.

*Keywords:*Antibiotic Resistance; Anti-Bacterial Agents; *Staphylococcus aureus*

1. Background

Development of microbial resistance against antibiotics is a major worldwide issue. *Staphylococcus aureus* is the normal flora of human skin and the anterior nares, but frequently causes severe infections such as endocarditis, meningitis, septicemia, gastroenteritis, toxic shock syndrome, and certain food intoxications in humans (1). Rapid identification of *S. aureus* is crucial for proper management in patients with severe infections (2, 3). Today, nanoparticles are being used as fundamental building blocks of nanotechnology and caused antibacterial effect by some plants or seeds (4, 5). The major important and special feature of nanoparticles is that they exhibit larger surface area to volume ratio (4, 5). In recent years, plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. In recent decades, researches showed that many seeds and plants such as *Plantago ovata* have anti-bacterial effects (6-10). *Plantago ovata* can reduce the level of cholesterol in blood. Also, it has anti-diarrheal effect that can set blood sugar. Although, it has been used as a laxative, it is more used as a true dietary fiber that can reduce the symptoms of both constipation and mild diarrhea. Also, its effect is reported in the treatment of ulcerative colitis (UC) and Crohn's disease (CD) (9, 10). The genus Plantago contains over 200 species. *Plantago ovata* and *P. psyllium* are produced commercially in several European countries such as Spain, France, and also in Iran and India. Plantago seed, known commercially as black French or Spanish *psyllium*, is obtained from *P. psyllium*. The seed produced from *P. ovata* is commercially known as white or blonde psyllium, Indian plantago, or Isabgol. India dominates the world market in the production and export of psyllium.

2. Objectives

The current study aimed to determine the antibacterial activity of silver nanoparticles produced by *Plantago ovata* seed extract against antibiotic resistant *Staphylococcus aureus*.

3. Patients and Methods

A cross-sectional study was carried out on the bacteria isolated from the patients with urinary tract infection hospitalized in Zabol hospitals (South-eastern Iran) from January 2011 to January 2012.

3.1. Isolation of Bacteria

All samples were collected aseptically from 50 patients.

Identification of all causative microorganisms was performed by standard microbiological methods.

3.2. Plant Materials

Seeds of *Plantago ovata* were collected from some regions of Zabol, Iran. The seeds were dried at room temperature, then transferred into glass containers, and stored until the conduction of extraction process.

3.3. MIC Determination of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was used as the source for silver synthesis. The biosynthesis of silver nanoparticles using the extract of *P. ovata* was confirmed by the change in the color of the solution from yellow to brown, and then the MIC was detected as the lowest concentration of the extract when bacteria did not demonstrate the visible growth. The broth micro-dilution method was used to detect MIC. Serial doubling dilutions of the silver nanoparticles produced in the plant *P. ovata* seed extract were prepared in a 96-well micro-titer plate ranging from 12.5 ppm to 200 ppm to each well, 10 µL of the indicator solution and 10 µL of Mueller Hinton Broth were added. Then, 10 µL of bacterial suspension (106 CFU/mL) was added to each well to achieve a concentration of 104 CFU/mL. The plates were covered slackly with cling film to ensure that the bacteria were not dehydrated. The plates were prepared in triplicates and then maintained in an incubator at 37°C for 18 to 24 hours. Then, the color change was determined. The microorganism growth was detected by turbidity.

4. Results

The current study, results showed that 12 samples of *S. aureus* which infected the urinary tract system were resistant to cefixime (33%), trimethoprim-sulfamethoxazole (41. 66%), and penicillin (50%). The MIC of *P. ovata* against these drug resistant *S. aureus* is shown in Table 1, and it indicates that the highest and the lowest MIC values of *P. ovata* against *S. aureus* were 100 and 12.5 mg/mL, respectively. The silver nanoparticles revealed Gaussian distributions with the average diameter of 13 nm with some deviations.

Table 1. Drug Resistant *Staphylococcus aureus* isolates and Their MIC Values

Bacterial	MIC, ppm
***S. aureus* 1**	25
***S. aureus* 2**	50
***S. aureus* 3**	25
***S. aureus* 4**	12.5
***S. aureus* 5**	50
***S. aureus* 6**	50
***S. aureus* 7**	25
***S. aureus* 8**	25
***S. aureus* 9**	50
***S. aureus* 10**	25
***S. aureus* 11**	100
***S. aureus* 12**	100

5. Discussion

Based on the results of the current study, all samples of *S. aureus* showed resistance to cefixime, trimethoprim-sulfamethoxazole, and penicillin by silver nanoparticles produced by *Plantago ovata* seed extract. Silver nanoparticles have attracted intensive interests because of their important applications in antimicrobial catalysis (11, 12). The results of the current study showed that the highest MIC of *P. ovata* seed extract was 100 mg/mL and the lowest MIC was 12.5. In the survey by Bokaeian, the highest and the lowest MIC of *P. ovata* seed extract values were 200 and 12.5 ppm, respectively. They also observed that at a specific dose of AgNPs killed the bacteria without harming the host cells. They reported that silver nanoparticles have a potent antimicrobial activity against the antibiotic resistant *Klebsiella pneumoniae* strains (7). Motamedi et al. reported that even the high concentration of Plantago ovata extract did not have antibacterial effect against Brucella melitensis; while Oliveria decumbens and Crocus sativus extracts were effective even at the lowest concentrations (13). In the research performed by Krishnaraj C, silver nanoparticles of 20-30 nm extracted from the leaves of *Acalypha indica* showed antimicrobial activity against *Escherichia coli* and *Vibrio cholera*. In their study, silver nanoparticles of 10 µg/mL were recorded as the minimal inhibitory concentration (MIC) against *E. coli and V. cholera* (14). In the study by Konwarh et al. silver nanoparticles of 3-12 nm extracted from the peels *of Citrus sinensis* were reported to show activity against *Bacillus subtilis* (15). The study by Saxena A et al. showed that 33. 67 nm particles extracted from *Allium cepa* stem had an antimicrobial activity against *E. coli* and *Salmonella typhimurium* (16). In the research performed by Ramteke et al. AgNPs stabilized by Tulsi leaf extract. Antimicrobial activity of this extract was evaluated against the well-known pathogenic strains such as *Staphylococcus aureus* and *E. coli*, and the inhibitory zones of 11mm and 10mm were observed for *E. coli* and *S. aureus*, respectively (17). In the study by Prokopovich, the results showed that only the nanoparticles with the highest amount of inorganic fraction showed antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA) at concentrations as low as 0.1% (w/w) (18). In the study by Duran, the results detected that cotton fabrics incorporated with silver nanoparticles had a significant antibacterial activity against *S. aureus* (19). Soo-Hwan et al. reported that the MIC of Ag-NPs against *S. aureus* and *E. coli* was 100 µg/mL (20). Considering the mentioned studies and their results, it can be concluded that silver nanoparticles have a potent antimicrobial effect against antibiotic resistant bacteria such as *S. aureus*. However, more researches are required to evaluate the practical value of these particles before the therapeutic usage.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

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References

1. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev.* 1997;**10**(3):505–20.
2. Durack DT, Lukes AS, Bright DK. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Duke Endocarditis Service. *Am J Med.* 1994;**96**(3):200–9.
3. Martineau F, Picard FJ, Roy PH, Ouellette M, Bergeron MG. Species-specific and ubiquitous-DNA-based assays for rapid identification of Staphylococcus aureus. *J Clin Microbiol.* 1998;**36**(3):618–23.
4. Daniel MC, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev.* 2004;**104**(1):293–346.
5. Kato H. In vitro assays: Tracking nanoparticles inside cells. *Nat Nanotechnol.* 2011;**6**(3):139–40.
6. Sharifi-Mood B, Shafaghat M, Metanat M, Saeidi S, Sepehri N. The antibacterial effect of Ajowan essential oil on bacteril growth. *Int J Infect.* 2014;**1**(20):19394.
7. Bokaeian M, Mohasseli T, Saeidi S, Sephri M. Antibacterial activity of silver nanoparticles produced by Plantago ovata seed extract against antibiotic resistant Klebsiella pneumonia. *J Bio &Env Sci.* 2014;**4**(5):125–31.
8. Bokaeian M, Saeidi S, Shahi Z, Sahraei S, Zarei H, S., Baig G. Evaluation of Antibacterial Activity of Withania somnifera Leaf Extracts against Antibiotic-Resistant Isolates of Klebsiella pneumoniae. *Int J Infect.* 2014;**1**(20):21085.
9. Salas-Salvado J, Farres X, Luque X, Narejos S, Borrell M, Basora J, et al. Effect of two doses of a mixture of soluble fibres on body weight and metabolic variables in overweight or obese patients: a randomised trial. *Br J Nutr.* 2008;**99**(6):1380–7.
10. Hannan JM, Ali L, Khaleque J, Akhter M, Flatt PR, Abdel-Wahab YH. Aqueous extracts of husks of Plantago ovata reduce hyperglycaemia in type 1 and type 2 diabetes by inhibition of intestinal glucose absorption. *Br J Nutr.* 2006;**96**(1):131–7.
11. Li Z, Lee D, Sheng X, Cohen RE, Rubner MF. Two-level antibacterial coating with both release-killing and contact-killing capabilities. *Langmuir.* 2006;**22**(24):9820–3.
12. Setua P, Chakraborty A, Seth D, Bhatta MU, Satyam PV, Sarkar N. Synthesis, optical properties, and surface enhanced Raman scattering of silver nanoparticles in nonaqueous methanol reverse micelles. *J Phys Chem C.* 2007;**111**(1):3901–7.
13. Motamedi H, Darabpour E, Gholipour M, Seyyed Nejad SM. In vitro assay for the anti-Brucella activity of medicinal plants against tetracycline-resistant Brucella melitensis. *J Zhejiang Univ Sci B.* 2010;**11**(7):506–11.
14. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surf B Biointerfaces.* 2010;**76**(1):50–6.
15. Konwarh R, Gogoi B, Philip R, Laskar MA, Karak N. Biomimetic preparation of polymer-supported free radical scavenging, cytocompatible and antimicrobial "green" silver nanoparticles using aqueous extract of Citrus sinensis peel. *Colloids Surf B Biointerfaces.* 2011;**84**(2):338–45.
16. Saxena A, Tripathi RM, Singh RP. Biological synthesis of silver nanoparticles by using onion (Allium cepa) extract and their antibacterial activity. *Dig J Nanomater Bios.* 2010;**5**(1):427–32.
17. Ramteke C, Chakrabarti T, Ketan Sarangi B, Pandy RA. Synthesis of Silver Nanoparticles from the Aqueous Extract of Leave of Ocimum sanctum for Enhanced Antibacterial Activity. *J Chemistry.* 2013.
18. Prokopovich P, Leech R, Carmalt CJ, Parkin IP, Perni S. A novel bone cement impregnated with silver-tiopronin nanoparticles: its antimicrobial, cytotoxic, and mechanical properties. *Int J Nanomedicine.* 2013;**8**:2227–37.
19. Duran ND, Marcoto P IH, De Souza G L, Aves O,, Esposito E. Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment. *J Biomed Nanotechnol.* 2007;**3**(1):203–8.
20. Soo-Hwan K, Lee HS, Ryu DS, Choi SJ, Lee DS. Antibacterial Activity of Silver-nanoparticles Against Staphylococcus aureus and Escherichia coli Korean. *Microbiol. Biotechnol J.* 2011;**39**(1):77–85.