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Synthesis of Silver Nanoparticles Using *Verbena officinalis* Plant Extract and Investigation of the Antimicrobial Activity Against *Acinetobacter baumannii* Bacteria

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Abstract

Introduction: The aim of this study was to synthesize silver nanoparticles (AgNPs) using *Verbena officinalis* plant extract and to investigate the antimicrobial activity against *Acinetobacter baumannii* bacteria isolated from patients in Zabol.

Methods: AgNPs were obtained through reacting silver nitrate solution 2 mM and *V. officinalis* leaf extract. The AgNPs were characterized by ultraviolet–visible (UV-Vis) spectrophotometer, scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). To determine minimum inhibitory concentration and to test antibiogram of nanoparticle synthesized, broth micro dilution methods were used, respectively.

Results: Nanoparticles were formed with an average size of 42.57 ± 5.34 nm. The results of this study showed that synthetic nanoparticles in the *V. officinalis* plant extract were good inhibitors of antibiotics resistant *A. baumannii* so that the lowest inhibitory concentration was 3.1 µL/mL.

Conclusion: The results clearly indicated that *V. officinalis* AgNPs had a potential antimicrobial activity against *A. baumannii*.

Keywords: Viability synthesis, *V. officinalis*, antimicrobial activity, *A. baumannii*

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Introduction

Since nanoparticles are the mediator between bulk material and atomic or molecular states, they are very important.[1] In the 21st century, nanotechnology is expected to significantly affect science, economics, and everyday life and become one of the driving forces behind the next industrial revolution. Different aspects of this new technology include the production, properties, and manipulation of nanoscale structures. In the past decade, the attention of science and industry has focused on the production of nanoparticles. Solid particles can be nanocrystals, crystalline, or monocrystalline aggregates in a range of 1-100 nm. There are many methods for synthesizing nanoparticles. Many techniques are inefficient in terms of material and energy use.[2]

Chemical synthesis methods of particle stability are controversial and difficult to produce on a large scale. For this reason, there is a demand for the production of nanoparticles through environmentally friendly methods. An alternative production method is the production of nanoparticles using biological methods.[3]

Another benefit of biosynthesis of nanoparticles is the lack of high pressure, energy, temperature, and toxic chemicals. Silver nanoparticles (AgNPs) have strong antimicrobial, antifungal, and antiviral properties.

With the development of new materials or methods, people's concern for environmental pollution has doubled by producing nanoparticles from chemical methods and generating hazardous side-products. Therefore, there is a need for clean, non-toxic, and environmentally friendly green chemistry. Safe biological methods can be considered as an alternative to conventional chemical methods for the preparation of nanoparticles.[4]

The use of green plants for the bioavailability of nanoparticles is an exciting and largely unknown possibility.[5]

Silver and gold nanoparticles have a variety of bio applications due to their biocompatibility. Chemical methods typically result in the presence of some toxic agents on nanoparticles. For this reason, the use of plants as sources of sustainability and availability in the preparation of biocompatible nanoparticles has attracted many researchers in recent years.

Aloysia citrodora is one of the indigenous plants of Iran, which nowadays is cultivated in northern part of Iran. In addition, there are native species of *Lippia* in the tropical

and subtropical regions of our country. This plant is from the Vervain family and has 200 species.[6] It is very important for traditional medicine and has long been used to treat gastro-intestinal and respiratory diseases. The *A. citrodora*'s species has anti-malarial and antiviral properties.[7]

Acinetobacter coco is a gram-negative, non-mobile, capsular, aerobic, and spore free bacillus that does not have the ability to ferment glucose.[8]

This bacterium is widely spread in soil and water, and *Acinetobacter baumannii* grows at different temperatures and pHs. Acinetobacter is often isolated not only from soil and water in nature but also from animals.[9]

Acinetobacter is an opportunistic pathogen that causes a wide range of infections including pneumonia, meningitis, endocarditis, skin and soft tissue infections, conjunctivitis, infection of burn wounds, and bacteremia.[10]

The aim of this study was to synthesize AgNPs using *A. citrodora* extract and to investigate the antimicrobial activity against *A. baumannii* in Zabol.

Methods

After sampling, the swab in a tube Falcon 50 mL that contained 20 mL of sterile saline was transferred to the laboratory. In addition, the swabs in the Falcon tube were vortex several times, then the Falcon tube was removed into blood agar containing 5% sheep blood and MacConkey Agar environments containing antifungals amphotericin B (2 µg/mL) awere cultured on agar. Falcon saline remained in the tubes for 20 minutes at 4000 rounds (rpm) and then centrifuged in BHI broth with antifungals and a part of the environment was taken and located, 24 hours at 30°C in a shaker incubator group. After that period, the broth on solid media-rich blood agar and agar medium containing antifungal MacConkey were moved again and incubated at 30°C. Slide preparation and also growing ability on the MacConkey were studied. Oxidase test, DNase, TSI (triple sugar iron), O-F containing 10% glucose, SIM and citrate were conducted for them.

Preparing Plant Extracts and Synthesis of Silver Nanoparticle

To prepare aqueous extracts of *V. officinalis*, first heaves of *V. officinalis* were washed 2 times (once with purified water and once with distilled water). The heaves were air dried under shade and powdered using a disintegrator. About 2 g of the sample was added to 20 mL sterilized distilled water and was placed in a shaker incubator for 2 hours. The extract was cooled and filtered through Whatman No. 1 filter paper.[11] For the synthesis of AgNP, the stocked silver nitrate solution 0.1 M (0.169 g in 10 mL of distilled water) was prepared. Synthesis of AgNPs was carried out by mixing and reaction of silver nitrate solution of 2 mM and extract of *V. officinalis* (1 mL of the extract obtained with 19 mL distilled water was combined and then 400 µL from silver nitrate solution of 0.1 M was added). Then, the mixture was placed into the incubator at a temperature of 37° C for 24 hours.

Characterization of Silver Nanoparticles

UV-Vis Spectra Analysis

Sample (1 mL) of the suspension was collected periodically to monitor the completion of bioreduction of Ag+ in aqueous solution, 1 mL of sample was diluted with 2 mL of distilled water. Then, the ultraviolet–visible (UV–Vis) spectrum of solution was measured between wavelengths 280 to 700 nm in a spectrophotometer (Rayleigh, UV-2100, China), with a resolution of 1 nm.

Fourier-Transform Infrared Spectroscopy Analysis

Fourier-Transform Infrared Spectroscopy (FTIR) analysis of the dried AgNPs was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Bruker optics Ft Tensor, 27, Germany.

Scanning Electron Microscopy Analysis

AgNPs were centrifuged at 10 000 rpm for 15 minutes. Supernatant was collected and poured nanoparticles deposited on glass slides and dried at room temperature. The images of AgNPs were obtained using a scanning electron microscopy (SEM) (KYKY, Model No. EM-3200).

Evaluation of Antibacterial Effects of Silver Nanoparticles Synthesized by Leaf Extract of *Acinetobacter baumannii* Bacteria

Minimal Inhibitory Concentration

The *A. baumannii* bacteria were grown in nutrient broth (NB) medium. Concentrations of 10, 5, 2.5, 1.25, 0.62, and 0.31 mg/mL synthesized AgNPs were used for evaluation of antibacterial effects on the *A. baumannii.* Concentrations were determined by broth micro-dilution technique in sterile 96 well plate. A volume of 100 µL of nanoparticles synthesized at concentration of 20 mg/mL was placed into the first well of the plate microtiter that contained 100 µL NB medium to obtain a concentration of 10 mg/mL. Serial dilution was performed by pumping the contents of the first well and removal of 100 µL from it and adding it to the second well. This operation was carried out to the last well. Then, 100 µL of bacterial suspension of equivalent to 0.5 McFarland (1.5×10^6 CFU/mL) was added to the wells. Each plate was prepared with a set of controls. Plates were placed in an incubator (Binder, USA) at 37°C for *A. baumannii*. The lowest concentration at which no visible bacterial growth can be found was taken as the minimum inhibitory concentration (MIC) value.[12]

Minimal Bactericidal Concentration

The method used and described below is an amended version of the procedure described in the BSAC (British

Society for Antimicrobial Chemotherapy) Guide to Sensitivity Testing and can be adapted for determining the minimal bactericidal concentration (MBC) of AgNPs synthesized by biological method. After determining the MIC of AgNPs synthesized, 10 µL from all wells that had no visible bacterial growth were removed and then were cultured on the nutrient agar (NA) media. The MBC is the lowest concentration of antimicrobial agent that openly kills >99.9% of the initial bacterial population where no visible growth of the bacteria is observed on NA medium.[12]

Results

AgNPs Characterization

UV-Vis Spectra Analysis

As the *V. officinalis* leaf aqueous extract was added to silver nitrate solution, the color of the solution changed from light yellow to reddish brown after the process of reduction of Ag+ to AgNP which indicated AgNPs formation (Figure 1). Figure 2 shows the UV–Vis spectra of AgNP formation using constant $AgNO_3$ concentration (2 mM) with *V. officinalis* leaf extract at 37°C after 24 hours between wavelengths of 280 to 700 nm. The results of optical density showed that the maximum absorption measuring solution containing the nanoparticles was around 420 nm.

Fourier-Transform Infrared Spectroscopy Analysis

FTIR spectrum of Ag nanoparticles synthesized using *V. officinalis* leaf extract is shown in Figure 3. Prominent bands of absorbance were observed at around 1075.31, 1384.48, 1626.65, 2925.17, and 3422.64 cm^{-1}. The observed peaks at 1075.31, 1384.48 cm^{-1} denote the stretching vibration of aliphatic and aromatic amines, respectively.[13] Strong peak in 1626.65 was related to stretching vibration of the C=O that usually exists in proteins and indicates the presence of protein in the plant extract as a reducing agent and a stabilizer.[13] Relatively broad peak in 3422.64 cm^{-1} shows the presence of hydroxyl functional groups (O-H). These peaks demonstrate the compounds of plant extract. C=H aliphatic bonds are intense peaks in the ranges of 2850 to 3000 cm^{-1} and the presence of these peaks are observed in the frequency range of 2925.17 cm^{-1} in the structure of the plant extract.

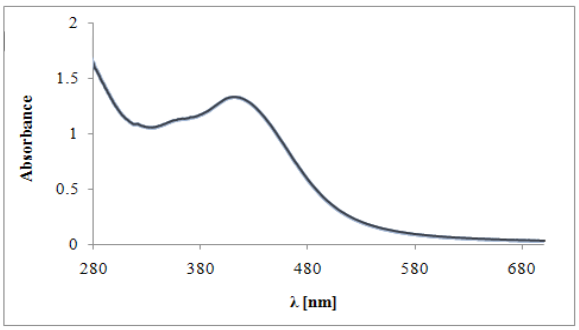

Figure 1. UV-Vis Spectra of AgNPs Biosynthesized From Aqueous Extract of *Verbena officinalis*.

Scanning Electron Microscopy Analysis

The SEM image of the AgNPs is shown in Figure 3. Nanoparticles were formed with an average size of 42.57 ±5.34 nm.

The results of this study showed that the MIC against antibiotic resistant *Acinetobacter* was 3.1 µL/mL, in which 2 strains were inhibited in this concentration. While the MIC was 50 µL/ml and one strain was inhibited in this concentration. The MBC was 100 µL/mL (Table 1).

Discussion

Since nanoparticles are the interface between bulk material and atomic or molecular states, they are very important.[14]

Metallic nanoparticles have been extensively studied for their outstanding electrical, optical, chemical, and magnetic properties.[15]

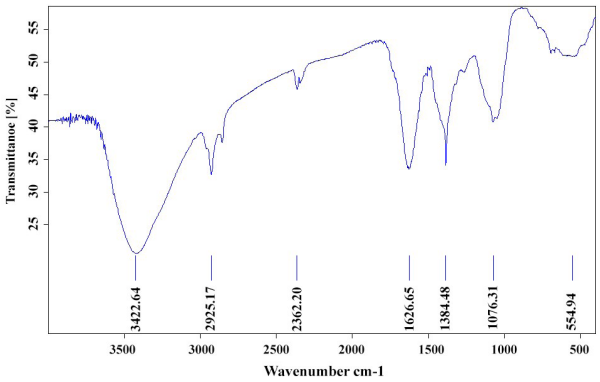

Figure 2. FTIR Analysis of AgNPs Obtained From *Verbena officinalis* Leaf Extract.

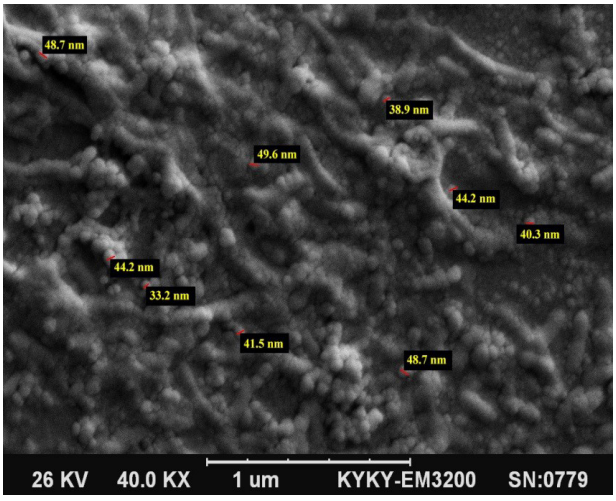

Figure 3. SEM Images of Silver Nanoparticles Formed by the Reaction of 2 mM Silver Nitrate and 1 ml Leaf Extract of *Verbena officinalis*.

Table 1. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Silver Nanoparticles Against *Acinetobacter Baumannii*

Bacterial Strain	MIC	MBC
1	12.5	25
2	12.5	25
3	3.1	6.25
4	25	50
5	12.5	25
6	3.1	6.25
7	25	50
8	6.25	12.5
9	6.25	12.5
10	50	100

The use of microorganisms, plants, herbal extracts, or herbal biomass can be a good alternative to the physical and chemical methods of this process. The bioavailability of nanoparticles has very low dangers for humans, the air, and, in general, the ecosystem. The synthesis of nanoparticles using biological materials has been favored by many researchers due to their new chemical and physical properties and their applications in various sciences of medicine, agriculture, optics, electronics, and mechanics.

In 2002, Gardea-Torresdey et al synthesized gold nanoparticles from alfalfa extract in a rich medium.[16] So far, biological production of AgNPs has been synthesized by plants such as *Piper longum*,[17] *Azadirachta*,[18] *Acalypha indica*,[19] *Ocimum sanctum*, and *Catharanthus roseus*.[20]

In a study by Koohsari et al, it was found that ethanolic extract of *A. citrodora* leaves can prevent the growth of many bacteria, especially gram-positive bacteria such as *S. aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, and *Enterococcus faecalis*, while gram-negative bacteria were resistant to the extract. Besides, antibacterial activity of ethanolic extract of this plant was more than its aqueous extracts.[21]

In another study by Oskay et al, the MIC of ethanolic extract of *A. citrodora* leaves against *S. aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Candida albicans* were reported as 10, 30, 22, and 6 µg/mL, respectively and the MBC was reported in the range of 10 to 50 µg/mL.[22]

In the study carried out by Tabarsa et al, the antimicrobial activity of lemon leaf extract on *E. coli* and *S. aureus* was investigated. The results of this study on *E. coli* showed that there was a significant difference between the number of bacteria and the control group in all concentrations and that it had the highest effect at a concentration of 100 mg/mL on days 3-7 and 14. In the case of *S. aureus*, there was a significant difference in concentration of 50 mg/mL, and on the 21st day, the largest decrease in the microbial population was observed.[23]

In another study, *Adenophora triphylla* essential oil exhibited an interesting antibacterial activity against *Bacillus subtilis* and *S. aureus*. No antibacterial activity was observed against *Listeria monocytogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *E. coli*. *A. triphylla* essential oil partially inhibited the growth of the fungal strains and the pathogenic yeast was observed against *Phanerochaete chrysosporium*, *Trichoderma reesei,* and *Candida albicans*.[24]

Perez Zamora et al concluded that *Aloysia*, *Lantana*, *Lippia*, *Phyla*, and *Stachytarpheta genera*, and their main essential oils were monoterpenes and sesquiterpenes including β-caryophyllene, thymol, citral, 1,8-cineole, carvone, and limonene. These compounds have been found to possess antimicrobial activities. The synergism of these essential oils with antibiotics is being studied by several research groups.[25]

Abuhamdah et al highlighted that *A. citriodora* was also active against bacterial strains *S. aureus* and *B. subtilis* but inactive against *E. coli*.[26]

The results of these researchers were confirmed by Ansari et al and MIC of the essential oil was reported for *S. aureus* as 15 µg/mL.[27]

The results clearly indicated that *V. officinalis* AgNPs had a potential antimicrobial activity against gram-positive and gram-negative bacteria.

Conclusion

The results clearly indicated that *V. officinalis* AgNPs have potential antimicrobial activity against gram positive and gram negative bacteria.

Conflict of Interest

None.

References

1. Kumar Das R, Laxman Pachapur V, Lonappan L, et al. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. Nanotechnology for Environmental Engineering. 2017;2:18.
2. Klaus-Joerger T, Joerger R, Olsson E, Granqvist C. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends Biotechnol. 2001;19(1):15-20.
3. Sintubin L, De Windt W, Dick J, et al. Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. Appl Microbiol Biotechnol. 2009;84(4):741-749. doi:10.1007/s00253-009-2032-6
4. Shankar SS, Rai A, Ankamwar B, Singh A, Ahmad A, Sastry M. Biological synthesis of triangular gold nanoprisms. Nat Mater. 2004;3(7):482-488. doi:10.1038/nmat1152
5. Mukherjee P, Ahmad A, Mandal D, et al. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano Lett. 2001;1(10):515-519. doi:10.1021/nl0155274
6. Shahhoseini R, Ghorbani H, Saleh R, Omidbaigi R. Identification of essential oil content and composition of *Lippia citroidora* seed. Journal of Plant Production. 2011;18(4):91-96.

7. Pascual ME, Slowing K, Carretero E, Sanchez Mata D, Villar A. Lippia: traditional uses, chemistry and pharmacology: a review. J Ethnopharmacol. 2001;76(3):201-214.
8. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities. J Infect Dev Ctries. 2009;3(5):335-341.
9. Abbas Poor S, Mardaneh J, Dehbashi S, Jasemi SS. Profile of antimicrobial susceptibility isolated microorganisms from hospitalized patients in PICU ward and detection of Methicillin-resistant *Staphylococcus aureus* and ESBL-producing bacteria by phenotypic methods. Iran South Med J. 2014;17(4):647-657.
10. Michalopoulos A, Falagas ME. Treatment of Acinetobacter infections. Expert Opin Pharmacother. 2010;11(5):779-788. doi:10.1517/14656561003596350
11. Kumarasamyraja D, Jeganathan NS. Green synthesis of silver nanoparticle using aqueous extract of *Acalypha indica* and its antimicrobial activity. Int J Pharma Bio Sci. 2013;4(3):P469-476.
12. Petrus EM, Tinakumari S, Chai LC, et al. A study on the minimum inhibitory concentration and minimum bactericidal concentration of Nano Colloidal Silver on food-borne pathogens. Int Food Res J. 2011;18(1):55-66.
13. Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. Arab J Chem. 2014;7(6):1131-1139. doi:10.1016/j.arabjc.2013.04.007
14. Thakkar KN, Mhatre SS, Parikh RY. Biological synthesis of metallic nanoparticles. Nanomedicine. 2010;6(2):257-262. doi:10.1016/j.nano.2009.07.002
15. Azizian Shermeh O, Valizadeh J, Noroozifar M, Ghasemi A, Valizadeh M. Optimization, characterization and anti microbial activity of gold nano particles biosynthesized using aqueous extract of *Sambucus ebulus* L. Eco-Phytochemical Journal of Medicinal Plants. 2016;4(1):1-18. (Persian)
16. Gardea-Torresdey JL, Parsons JG, Gomez E, et al. Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants. Nano Lett. 2002;2(4):397-401. doi:10.1021/nl015673+
17. Jacob SJ, Finub JS, Narayanan A. Synthesis of silver nanoparticles using *Piper longum* leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloids Surf B Biointerfaces. 2012;91:212-214. doi:10.1016/j.colsurfb.2011.11.001
18. Sokmen M, Serkedjieva J, Daferera D, et al. In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. J Agric Food Chem. 2004;52(11):3309-3312. doi:10.1021/jf049859g
19. 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):50-56. doi:10.1016/j.colsurfb.2009.10.008
20. Preuss HG, Echard B, Enig M, Brook I, Elliott TB. Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Mol Cell Biochem. 2005;272(1-2):29-34.
21. Koohsari H, Ghaemi E, Sadegh Sheshpoli M, Sadegh A. Evaluation of antibacterial activity of lemon verbena (*Lippia citriodora*) leaves. Ann Biol Res. 2013;4(10):52-55.
22. Oskay M, Tamer AU, Ay G, Sari D, Akta K. Antimicrobial activity of the leaves of *Lippia triphylla* (L`Her) O. Kuntze (Verbenaceae) against on bacteria and yeasts. J Biol Sci. 2005;5(5):620-622. doi:10.3923/jbs.2005.620.622
23. Tabarsa HA, Koohsari H, Fadavi A. Antibacterial effect of lemon verbena leaf extract against *Escherichia coli* and *Staphylococcus aureus* in traditional ice cream. Journal of Food Industry Research. 20167;26(4):737-747. (Persian)
24. Ali H, El-Beltagi H, Nasr NF. Evaluation of antioxidant and antimicrobial activity of *Aloysia triphylla*. Electron J Environ Agric Food Chem. 2011;10(11):3044-3053.
25. Perez Zamora CM, Torres CA, Nunez MB. Antimicrobial activity and chemical composition of essential oils from Verbenaceae species growing in South America. Molecules. 2018;23(3). doi:10.3390/molecules23030544
26. Abuhamdah S, Abuhamdah R, Al-Olimat S, Chazot P. Phytochemical investigations and antibacterial activity of selected medicinal plants from Jordan. European J Med Plants. 2013;3(3):394-404. doi:10.9734/EJMP/2013/3540
27. Ansari M, Larijani K, Tehrani MS. Antibacterial activity of *Lippa citriodora* herb essence against MRSA *Staphylococcus aureaus*. Afr J Microbiol Res. 2012;6(1):16-19.