

Green synthesis of silver nanoparticles from seed extract of *Brassica nigra* and its antibacterial activity

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Abstract. Pandit R. 2015. Green synthesis of silver nanoparticles from seed extract of *Brassica nigra* and its antibacterial activity. *Nusantara Bioscience* 7: 15-19. We report the green synthesis of silver nanoparticles using seed extract of *Brassica nigra*. UV-visible spectroscopic analysis showed the absorbance peak at 432 nm which indicated the synthesis of silver nanoparticles. Nanoparticles Tracking and Analysis (NTA) was used to determine the size of synthesized silver nanoparticles. Zeta potential analysis was carried out to study the stability of nanoparticles while FTIR analysis confirmed the presence of proteins as capping agents that provided stability to nanoparticles in colloid. Antibacterial activity of silver nanoparticles was evaluated against *Propionibacterium acnes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. The activity of Vancomycin was significantly increased in combination with silver nanoparticles showing synergistic activity against all bacteria while the maximum activity was noted against *P. acnes*.

Keywords: Antibacterial, *Brassica nigra*, nanoparticles, phytosynthesis.

INTRODUCTION

Nanotechnology is a versatile subject, which deals with biology, chemistry, physics, and engineering. The term "Nano" is derived from the Greek word which means dwarf and size of particle is around 1 to 100 nm (Singh et al. 2011). The word 'nano' is used to designate one billionth of a meter (Brigger et al. 2002; Rai et al. 2009). Nanotechnology involves the synthesis of nanoparticles which exhibit different sizes, shapes, and morphology (Singh et al. 2011). Nanoparticles being very small in size possess large surface area to volume ratio due to which nanoparticles exhibit very different properties such as electrical, magnetic and optical properties than its bulk material (Kim et al. 2007). Biofabrication of nanoparticles is the most flourishing area of interest in the field of nanoscience and technology (Kalaiarasi et al. 2013).

Various chemical and physical methods are known for preparation of silver and other metal nanoparticles. These methods are very costly and toxic to the environment (Kalaiarasi et al. 2013). Silver nanoparticles are fabricated by the reduction of silver ions to neutral silver atoms. Silver ions are reduced by the use of reducing agents (Kaushik et al. 2010). Biosynthesis of nanoparticles is nothing but the bottom-up approach of nanoparticles synthesis. Phytochemicals present in the plants possess anti-oxidant or reducing properties which are responsible for reduction of metal compounds. Methods used for the biosynthesis of metal nanoparticles are eco-friendly, biocompatible, nontoxic and clean (Sharma and Yangard 2009).

Phytosynthesis of silver nanoparticles has been reported from actinomycetes (Ahmad et al. 2003), fungi (Gade et al. 2008; Ingle et al. 2008; Gajbhiye et al. 2009; Gaikwad et

al. 2013) *Carica papaya* (Mude et al. 2009), *Opuntia ficus-indica* (Gade et al. 2010), *Allium cepa* (Saxena et al. 2010), *Argemone mexicana* (Singh et al. 2010), *Ocimum* (Mallikarjun et al. 2011), *Foeniculum vulgare* (Bonde 2011), *Iresine herbstii* (Dipankar and Murugan 2012), *Murraya koenigii* (Bonde et al. 2012), *Hydrilla verticillata* (Sable et al. 2012), *Rauvolfia tetraphylla* (Kalaiarasi et al. 2013), *Lawsonia inermis* (Gupta et al. 2014). Biological synthesis of metal nanoparticles was also reported from bacteria (Drzewieck et al. 2014). Nanoparticles possess activity against wide range of Gram positive and Gram-negative bacteria as well as it possesses antifungal (Gupta et al. 2013) and antiviral activity (Gaikwad et al. 2013). From the prior studies, it was reported that silver nanoparticles could be used in several antimicrobial preparations (Rai et al. 2009). As silver nanoparticles possess antibacterial activity, it could be used for increasing shelf life of fruits (Gudadhe et al. 2013), in dental materials (Chladek et al. 2011), cosmetics (Kokura et al. 2010), water treatment (Sheng and Liu 2011) and in the coating of stainless steel which is used in medical devices (Knetsch and Koole 2011).

Brassica nigra is an important medicinal plant which belongs to the Brassicaceae family. Seeds of *B. nigra* are black, globular and near about 1mm in diameter. They possess pungent taste and nutty like odor. Seeds are used as a spice and also as a flavoring component. Due to medicinal properties, the seeds of *B. nigra* are used in the treatment of joint pains, tooth pain, throat tumors (Erdogru 2002). In the present study, we report, biological synthesis of silver nanoparticles from seeds of *B. nigra* and its antibacterial efficacy against *P. acnes*, *P. aeruginosa* and *K. pneumoniae*.

MATERIALS AND METHODS

Brassica nigra seeds were purchased from local market of Amravati, Maharashtra, India.

Preparation of seed extract and synthesis of silver nanoparticles (Ag-NPs)

Five gram of *B. nigra* seeds were crushed in 100 ml distilled water with the help of mortar and pestle. It was filtered with Whatman filter paper no. 1 and centrifuged at 4000 rpm for 20 min. Then it was again passed through membrane filter. The membrane filtered extract was treated with 1mM silver nitrate solution.

Detection and characterization of Ag-NPs

Visual observation

The primary detection of silver nanoparticles synthesis was observed by visual color change. The color change indicates the formation of Ag-NPs.

UV-Vis Spectroscopy

Synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometer (Shimadzu UV-1700 Japan) by analyzing sample in the range of 200-800nm.

Nanoparticles tracking and Analysis system (NTA)

The Ag-NPs synthesized by the extract was characterized by NTA to find out the average size of the particles. NTA is a laser-based light scattering system in which particles are suspended in the liquid medium are injected into LM viewing unit and viewed in close proximity to the optical element. NTA depends upon the Brownian movement of the nanoparticles. For the analysis, samples were diluted with the nuclease-free water, and 0.5 ml of diluted sample was injected into the sample chamber and observed through CCD camera attached to LM 20 (Nanosight Ltd).

Measurement of zeta potential

Zeta potential was measured by using a Zetasizer Nano ZS 90 (Malvern Instrument Ltd, UK). 1000 μ l of sample was transferred in the clear disposable zeta cell for the measurement of zeta potential. Measurements were made by means of Dynamic Light Scattering (DLS) in the range of 0.1-1000 μ m.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR reveals the biomolecules responsible for the reduction of silver ions and stabilization of Ag-NPs in the solution. The FTIR (Perkin-Elmer FTIR-1600, USA) analysis in the range of 500-4000 cm^{-1} was performed to determine the presence of capping agent and role of molecules involved in the synthesis of Ag-NPs.

Assessment of antimicrobial activity of Ag-NPs

Test pathogens

Antimicrobial activity of synthesized silver nanoparticles was tested against *P. acnes* (MTCC 1951), *P. aeruginosa* (MTCC 4676) and *K. pneumoniae* (MTCC 4030). The pure cultures were procured from Microbial

Type Culture Collections (MTCC) center, Institute of Microbial Technology (IMTECH), Chandigarh.

In vitro evaluation of antibacterial activity

Kirby-Bauer disc diffusion method was used to evaluate the antibacterial potential of AgNPs alone and its combined effect along with antibiotic vancomycin against *P. acnes*, *P. aeruginosa* and *K. pneumoniae*. Standard antibiotic discs of vancomycin were purchased from Hi-Media, Mumbai. To evaluate the combined effects, each standard antibiotic disc impregnated with 20 μ L solution of silver nanoparticles was placed on to the agar surface inoculated with test bacteria. The plates were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured.

RESULTS AND DISCUSSION

Brassica nigra seed extract was used for the fabrication of silver nanoparticles. The color of the extract changes from yellowish to brick red color after addition of 1mM AgNO_3 (Figure 1). The color change was because of the reduction of silver ions into silver nanoparticles. These results corroborate with the result obtained by researchers who worked on the synthesis of silver nanoparticles from plants (Bonde 2011; Mallikarjun et al. 2011; Gupta et al. 2014).

UV-Vis spectroscopy is the significant method which gives the preliminary confirmation of silver nanoparticles. The absorption spectra of the fabricated silver nanoparticles showed absorbance spectra at 432 nm. (Figure 2). The synthesized silver nanoparticles showed absorbance at specific wavelength because of the surface Plasmon resonance phenomenon of silver nanoparticles (Gaikwad et al. 2013). The results showed resemblance with the results of Gade et al. (2010) and Gupta et al. (2014) who reported the synthesis of silver nanoparticles from *Opuntia ficus-indica* and *Lawsonia inermis*.

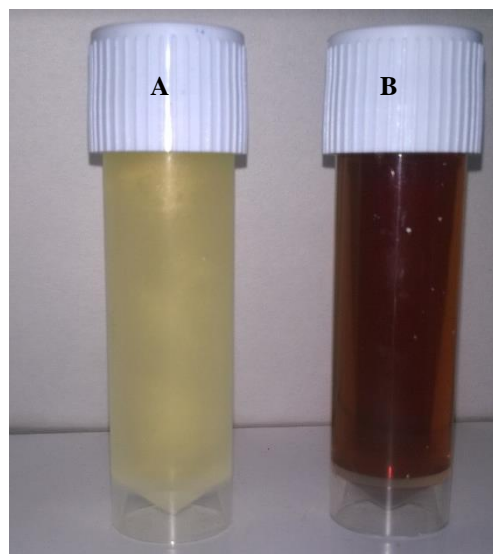


Figure 1. Synthesis of silver nanoparticles: A. *B. nigra* seed extract, B. Seed extract after treatment of 1mM AgNO_3 .

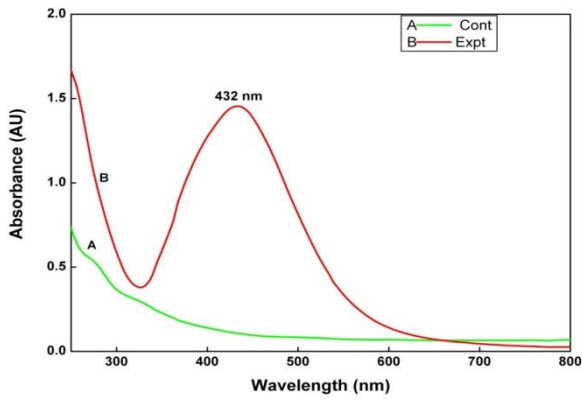


Figure 2. UV-Vis spectra of the synthesized silver nanoparticles showing absorbance at 432 nm. A. Control (seed extract), B. Experimental (AgNPs)

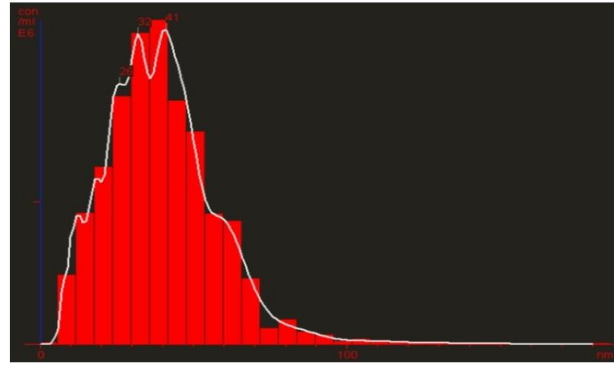


Figure 3. Nanoparticle tracking analysis NTA (NanoSight-LM 20) histogram showing particle size distribution and the average size of silver nanoparticles (41 nm).

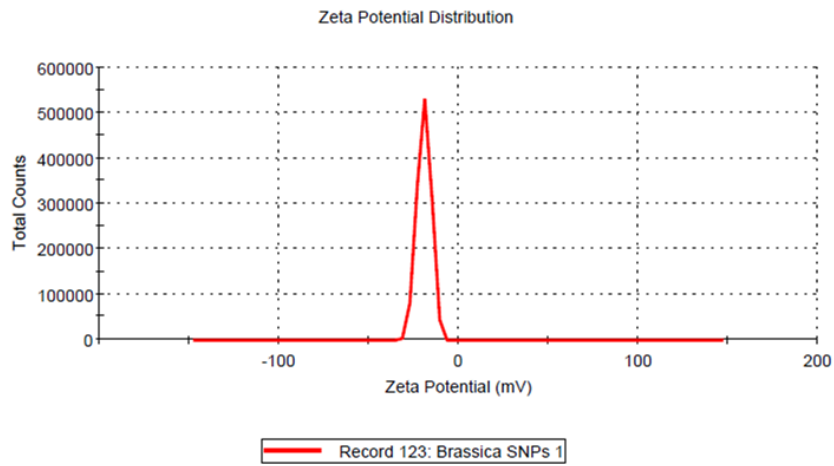


Figure 4. Zeta potential of silver nanoparticles (-19.3 mV)

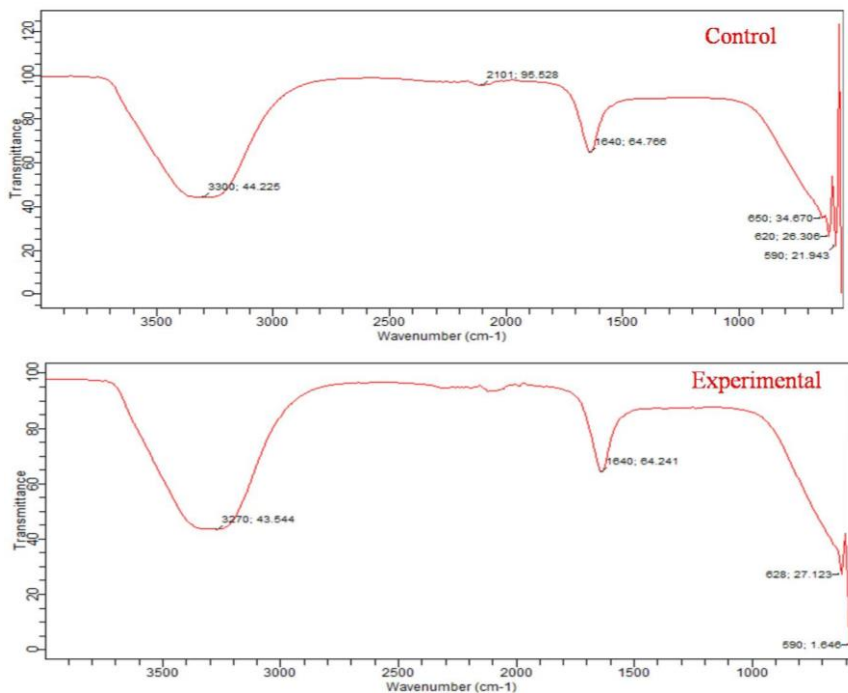


Figure 5. FTIR spectra of seed extract (control) and silver nanoparticles (experimental) synthesized by seed extract of *B. nigra*

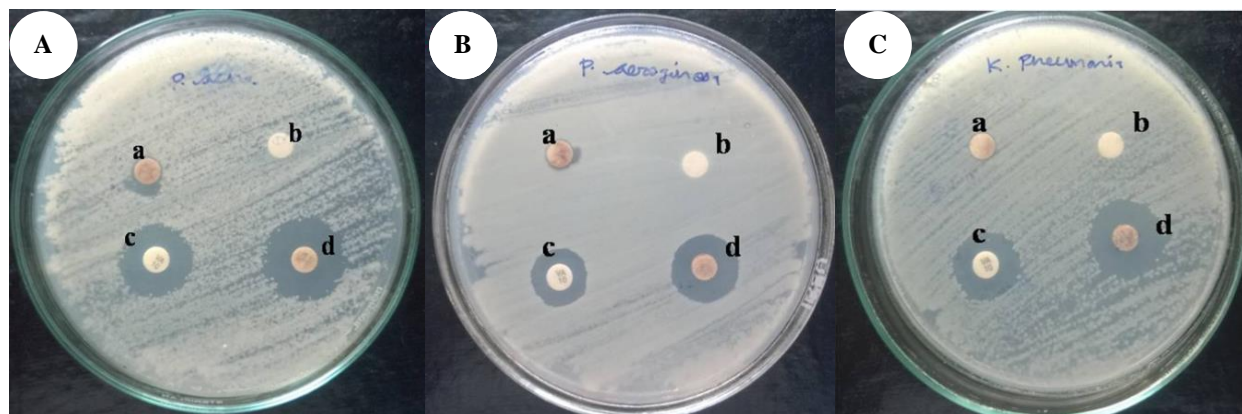


Figure 6. Antibacterial activity of silver AgNPs and its synergistic activity against A. *P. acnes*, B. *P. aeruginosa*, C. *K. pneumoniae*, where: a. AgNPs, b. Control (Seed extract, c. antibiotic, d. Antibiotic + AgNPs.

Table 1. Antibacterial activity of silver nanoparticles and its synergistic activity against different microorganisms by disc diffusion method.

Name of test micro-organism	Zone of inhibition in mm				
	AgNPs	Seed extract (control)	1mM AgNO ₃	Vancomycin	Vancomycin + AgNPs
<i>P. acnes</i>	8±0.50	0	0	17±0.65	21±0.56
<i>P. aeruginosa</i>	6±0.71	0	0	15±0.21	18±0.11
<i>K. pneumoniae</i>	5±0.21	0	0	17±0.54	19±0.12

Note: ± Standard deviation

Nanoparticle tracking analysis (NTA) of silver nanoparticles is based on tracking the Brownian motion of each particle. The average size of synthesized nanoparticles was found to be 41 nm. The result of NTA correlates with results of obtained by Gupta et al. (2013) for determining the size of Lawsonia-mediated synthesized AgNP. Stability of fabricated nanoparticles was measured by zeta potential analysis. Zeta potential was found to be -19.3mV (Figure 4). It indicates that the nanoparticles synthesized were moderately stable.

FTIR spectroscopy is performed to get the idea regarding various functional groups and their interactions with silver, which may be accountable for fabrication and stabilization of silver nanoparticles. In FTIR spectrum of control (Figure 5.A) peaks were observed at 590, 620, 650, 1640, 2101 cm⁻¹ and in experimental (Figure 5.B) peaks were observed at 590, 628, 1640, 3270 cm⁻¹. The bands at 590, 620, 628, 650 cm⁻¹ correspond to the bonding vibrations of alkyl halide (Anarkali et al. 2012). The bands obtained at 1640cm⁻¹ is due to presence of C-N stretching vibrations of amines (Raheman et al. 2011; Bharathi et al. 2014). The bands obtained at 3270 corresponds to the bonding vibration of Secondary alcohol (Anarkali et al. 2012).

The *in vitro* activity of nanoparticles synthesized from seed extract of *B. nigra* and combination of silver nanoparticles along with antibiotics were evaluated against *P. acnes*, *P. aeruginosa* and *K. pneumoniae* (Figure 6). The antibiotic Vancomycin was used as standard antibacterial agent against all microbes. 1 mM AgNO₃ was also tested against all the above-mentioned organisms, but it does not show any zone of inhibition. Fabricated silver nanoparticles alone and in combination with antibiotics showed

significant activity against all tested bacteria. Aqueous seed extract and 1mM AgNO₃ did not show activity against any of the bacteria (Table 1). *P. acnes* showed maximum antibacterial and synergistic activity as compared to other two bacteria. The antibacterial activity of the synthesized nanoparticles showed similarity with Anarkali et al. (2012) and Raheman et al. (2011). They have reported that the efficiency of silver nanoparticles enhanced in combination of standard antibiotics. The results corroborate with the finding of Gupta et al. (2014) who reported the antibacterial activity of silver nanoparticles against *Candida albicans*, *Microsporum canis*, *P. acne*, and *Trichophyton mentagrophytes*.

From the prior result, it was considered that the free radicals which are formed during synthesis of silver nanoparticles may be responsible for the bacterial death. It was suggested from electron spin resonance study silver nanoparticles may possess the ability to synthesize free radicals when it comes in contact with the bacteria. These free radicals may damage the cell membrane of bacteria, and it may lead to cell death (Danilcauk et al. 2006; Kim et al. 2007). Rajeskumar and Malarkodi (2014) proposed that the silver nanoparticles may have the ability to interact with the bacterial cell wall and the silver ions may interfere in the respiratory chain reaction. It may result in the damage of the bacteria. It was proposed that silver nanoparticles may inhibit the biofilm formation of the bacteria and it may be responsible for cell death (Percival et al. 2007). DNA is composed of sulfur and phosphorous, and silver nanoparticles may react with DNA, and it may cause the problem in the replication process of bacteria and may cause cell death (Prabhu and Poulouse 2012).

In conclusion, rapid biosynthesis of silver nanoparticles is possible with seed extract of *B. nigra*. The fabricated nanoparticles showed antimicrobial activity against all the test pathogens *P. acnes*, *P. aeruginosa*, and *K. pneumoniae*. It is clear from the synergistic activity that silver nanoparticles enhanced the activity of antibiotics. Phytosynthesis of silver nanoparticles is most suitable, easily scale up and eco-friendly method.

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