

A biogenic approach for green synthesis of silver nanoparticles using extract of *Foeniculum vulgare* and its activity against *Staphylococcus aureus* and *Escherichia coli*

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Abstract. Bonde S. 2011. A biogenic approach for green synthesis of silver nanoparticles using extract of *Foeniculum vulgare* and its activity against *Staphylococcus aureus* and *Escherichia coli*. *Nusantara Bioscience* 3: 59-63. We report green synthesis of silver nanoparticles from extract of *Foeniculum vulgare* (fennel, saunf). The synthesis of silver nanoparticles was detected by changing color from green to brown after treatment with AgNO₃ (1mM) and the UV-visible spectrophotometer analysis showed the absorbance peak at about 427 nm, which indicates the synthesis of silver nanoparticles. Nanoparticle Tracking and Analysis (NTA) by LM-20 was used for multi-parameter analysis, allowing for characterization of particle size and particle distribution of silver nanoparticles synthesized from extract of *F. vulgare*. NTA revealed the polydispersed nanoparticles in the range of 18-83 nm. Phytosynthesized silver nanoparticles showed antibacterial activity against the *Staphylococcus aureus* (ATCC-25923) and *Escherichia coli* (ATCC-39403). The silver nanoparticles also demonstrated remarkable antibacterial activity against two human pathogenic bacteria when used in combination with commercially available antibiotics. The bactericidal activity of the standard antibiotics was significantly enhanced in presence of silver nanoparticles against pathogenic bacteria, viz. *E. coli*-JM-103 (ATCC-39403) and *S. aureus* (ATCC-25923). Silver nanoparticles in combination with vancomycin showed maximum activity against *E. coli* (increase in fold area 5.76. and followed by *S. aureus* (1.08) and Gentamicin showed the maximum activity *S. aureus* (2.6) while *E. coli* (0.96). The approach of phytosynthesized silver nanoparticles using *F. vulgare* appears to be cost-efficient, eco-friendly and easy alternative to conventional methods of synthesis.

Keywords: *Foeniculum vulgare*, silver nanoparticles, LM20, antibacterial.

Abstrak. Bonde S. 2011. Pendekatan biogenik untuk sintesis nanopartikel perak menggunakan ekstrak *Foeniculum vulgare* dan aktivitasnya terhadap *Staphylococcus aureus* dan *Escherichia coli*. *Nusantara Bioscience* 3: 59-63. Kami melaporkan sintesis nanopartikel perak dari ekstrak *Foeniculum vulgare* (adas). Sintesis nanopartikel perak terdeteksi dengan mengubah warna dari hijau sampai coklat setelah perlakuan dengan AgNO₃ (1mm); dan analisis spektrofotometer UV-vis menunjukkan puncak absorbansi pada sekitar 427 nm, yang menunjukkan sintesis nanopartikel perak. Analisis Pelacakan Nanopartikel (NTA) oleh LM-20 digunakan untuk analisis multi-parameter, memungkinkan untuk karakterisasi ukuran partikel dan distribusi partikel nanopartikel perak yang disintesis dari ekstrak *F. vulgare*. NTA mengungkapkan nanopartikel tersebar di kisaran 18-83 nm. Fitosintesis nanopartikel perak menunjukkan aktivitas antibakteri terhadap *Staphylococcus aureus* (ATCC-25923) dan *Escherichia coli* (ATCC-39403). Perak nanopartikel juga menunjukkan aktivitas antibakteri yang luar biasa terhadap dua bakteri patogen manusia apabila digunakan dalam kombinasi dengan antibiotik yang tersedia secara komersial. Aktivitas bakterisida antibiotik standar secara signifikan ditingkatkan dengan adanya nanopartikel perak terhadap bakteri patogen, yaitu: *E. coli*-JM-103 (ATCC-39403) and *S. aureus* (ATCC-25923). Nanopartikel perak yang dikombinasi dengan vankomisin menunjukkan aktivitas maksimal terhadap *E. coli* (kenaikan berlipat 5,76) dan diikuti oleh *S. aureus* (1,08); dan gentamisin menunjukkan aktivitas maksimum *S. aureus* (2,6) sedangkan *E. coli* (0,96). Pendekatan fitosintesis nanopartikel perak menggunakan *F. vulgare* tampaknya memerlukan biaya yang efisien, ramah lingkungan dan merupakan alternatif mudah untuk metode sintesis konvensional.

Kata kunci: *Foeniculum vulgare*, nanopartikel perak, LM20, antibakteri.

INTRODUCTION

Phytosynthesis at present seems to be the biological method of much interest (Safaepour et al. 2009). Phytosynthesis is better compared to microorganisms because the later suffer from various problems like culture maintenance and cost-effectiveness during the scale-up process. Various plants have been successfully used for the synthesis of biogenic metal nanoparticles (Singh et al. 2011).

The rapid synthesis of silver nanoparticles by biological method using plant extracts of *Pinus*, *Persimmon*, *Ginkgo*, *Magnolia*, and *Platanus* were used and compared for their extracellularly metallic silver nanoparticles (Song et al. 2008) and the utilization of *Azadirachta indica* (neem). (Shankar et al. 2004), *Medicago sativa* (alfalfa), *Aloe vera* (Chandran et al. 2006), *Embllica officinalis* (amla). (Amkamwar et al. 2005), *Capsicum annuum* (Li et al. 2007), *Cinnamomum*

camphora (Huang et al. 2007), *Gliricidia sepium* Jacq. (Raut et al. 2009), *Carica papaya* (Mude et al. 2009), *Opuntia ficus-indica* (Gade et al. 2010), *Murraya koenigii* (Bonde et al. 2010), *Ocimum sanctum* (Mallikarjuna et al. 2011), *Saururus chinensis* (Nagajyoti et al. 2011), and microorganisms (Duran et al. 2005; Bhainsa et al. 2006) has been reported fennel (*Foeniculum vulgare*) is an important crop plant with medicinal value being carminative. Its fruits are used as a digestive adjuvant having antimicrobial activity (He and Huang 2011). The multi-drug resistant pathogens are responsible for causing death worldwide (Bandow et al. 2003; Wright et al. 2005) and hence there is a pressing need for the development of novel antimicrobial agents. Reports suggest that silver nanoparticles can be used effectively against multi-drug resistant bacteria (Ingle et al. 2008) due to their small size and relatively large surface area in comparison to their volume makes easy to interact with substances and increases their antibacterial efficacy. Silver nanoparticles are the new generation of antimicrobials (Rai et al. 2009) and it can be used in many antimicrobial preparations. Gade et al. (2008) and Ingle et al. (2008) reported the antibacterial activity of silver nanoparticles synthesized by fungi. Antibacterial activity of silver nanoparticles synthesized by leaf broth of *Gliricidia sepium* was reported by Raut et al. (2009). Duran et al. (2007) successfully developed silver nanoparticle impregnated wound dressings and textile fabrics which can be used for burnt patients. Silver nanoparticles are also used for the preparation of surgical masks (Li et al. 2006).

In the present study, *F. vulgare* was used for the synthesis of silver nanoparticles and the activity of synthesized silver nanoparticles was evaluated against *S. aureus* and *E. coli* in combination with commercially available five antibiotics viz. gentamicin, oxacillin, vancomycin, ampicillin, and amoxicillin, to study synergistic effect, if any.

MATERIALS AND METHODS

The test plant

The young and healthy leaves of *Foeniculum vulgare* were collected from the field of Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, Maharashtra State, India.

Test bacteria

Escherichia coli-JM-103 (ATCC-39403) and *Staphylococcus aureus* (ATCC-25923) were used to evaluate the activity of silver nanoparticles in combination with standard antibiotics (viz. gentamicin, oxacillin, vancomycin, ampicillin, and amoxicillin, purchased from Himedia).

Extraction

The leaves of *F. vulgare* (20 g) were washed twice in tap water and rinsed thrice in distilled water. Then surface sterilized by HgCl₂ (0.1%) for 1 min, cut into small pieces and crushed with 100 mL of sterilized distilled water in an Omni mixer. Later, crude extract was filtered through muslin cloth and centrifuged at 10,000 rpm for 15 min to

obtain clear leaf extract which was later used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, leaf extract was challenged with AgNO₃ (1mM) solution and incubated at room temperature. Control (without treatment with AgNO₃ (1mM), i.e. only leaf extract) was also maintained. After the reduction of aqueous silver ions into silver nanoparticles, residual silver ions (unreacted silver ions) were removed from the reaction mixture by centrifugation and presence of unreacted silver ions were detected by the treatment with sodium chloride (NaCl) as a result white precipitate of silver chloride (AgCl) was formed after reacting with Ag⁺ ion. Triplicates of each treatment were maintained.

Detection and characterization of silver nanoparticles

Visual observation. After treatment of leaf extract with AgNO₃ (1mM), the color change of the reaction mixture was visually observed.

UV-Vis spectrophotometric analysis. The aliquots of reaction mixture were subjected to the measurement of absorbance by UV-visible spectrophotometer (Perkin Elmer, Lambda-25, at a resolution of 1 nm from 250 to 800 nm for the detection of silver nanoparticles.

NanoSight LM-20 analysis. Liquid sample of silver nanoparticles at the concentration range of 10⁷-10⁹/mL was introduced into a scattering cell through which a laser beam (approx. 40 mW at $k = 635$ nm) was passed. Particles present within the path of the laser beam were observed via a dedicated non-microscope optical instrument (LM-20, NanoSight Pvt. Ltd., UK, having CCD camera. The motion of the particles in the field of view (approx. 100 X 100 μ m) was recorded (at 30 fps) and the subsequent video and images were analyzed. Each particle visible in the image is individually but simultaneously traced from frame to frame, the mean square displacement is determined by analytical programme.

Particle size measurement. Particle sizing experiments were carried out by means of laser diffractometry, using Zetasizer nano series (Malvern) Measurements were taken in the range between 0.1-1000 μ m.

Assessment of antibacterial activity

The disc diffusion method was used to evaluate the antibacterial potential of silver nanoparticles and combined effect of silver nanoparticles with five antibiotics against two human pathogenic bacteria: *E. coli*-JM-103 (ATCC-39403) and *S. aureus* (ATCC-25923) grown on nutrient agar plates. The overnight grew bacterial culture having 10⁵ CFU/mL was used to assess the activity. The test bacterial cultures were inoculated on to solidified agar plates. The different standard antibiotic discs (viz. gentamicin, oxacillin, vancomycin, ampicillin, and amoxicillin) purchased from Hi-Media, Mumbai was used. 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 and its activities were evaluated by calculating the increase in fold-area. The assays were performed in triplicate.

Assessment of increase in fold area

The increase in fold area was assessed by calculating the mean surface area of the inhibition zone of each tested antibiotic. The fold increase area of different antibiotics was calculated by using the equation calculated as $(b^2 - a^2) / a^2$, where a and b are the inhibition zones for A and B, respectively. In the same way, c and d.

RESULTS AND DISCUSSION

The synthesis of silver nanoparticles by leaf extract of *F. vulgare* was performed in the present study. On treatment of leaf extract with AgNO_3 (1mM) and incubated in dark at room temperature, within 1 hour of the reaction, color changes from green to brown (Figure 1) indicating the synthesis of silver nanoparticles. It is an efficient and rapid method of synthesis which corroborate with the results obtained by other researchers who worked with different plant systems (Shankar et al. 2004; Chandran et al. 2006; Li et al. 2007; Mallikarjun et al. 2011). Colour change was due to the excitation of surface plasmon vibrations in the metal nanoparticles (Ahmad et al. 2003).

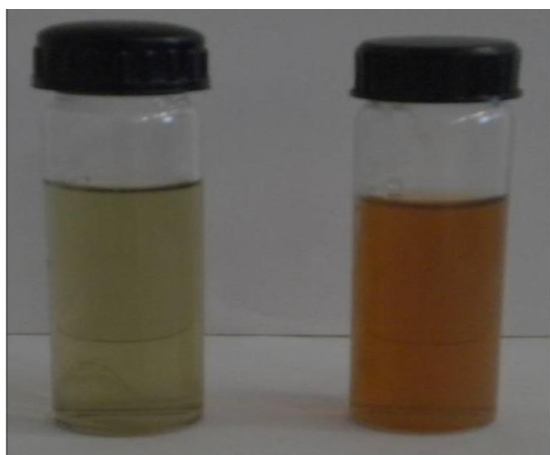


Figure 1. Control (left) and silver (right) nanoparticles synthesized from *Foeniculum vulgare*

UV-Vis spectrophotometric analysis has proved to be a very useful technique for the analysis of nanoparticles. In order to verify the synthesis of silver nanoparticles, the test samples were subjected to UV-Vis spectrophotometric analysis. The test samples (leaf extract treated with 1mM silver nitrate. were collected in aliquots from the reaction mixture and analyzed to record their absorbance by UV-Vis spectrophotometer. This analysis showed the sharp absorbance at around 427 nm in the form of peak (Figure 2), which was specific for silver nanoparticles (Shankar et

al. 2004; Chandran et al. 2006; Elumalai et al. 2010). Huang et al. (2007) and Li et al. (2007) reported similar results, observed that when sun-dried leaf extract of *Cinnamomum camphora* and *Capsicum annum* were challenged with aqueous silver ions, the reaction mixture containing silver nanoparticles showed the absorption peak at about 427 nm due to the excitation of plasmon resonance vibration.

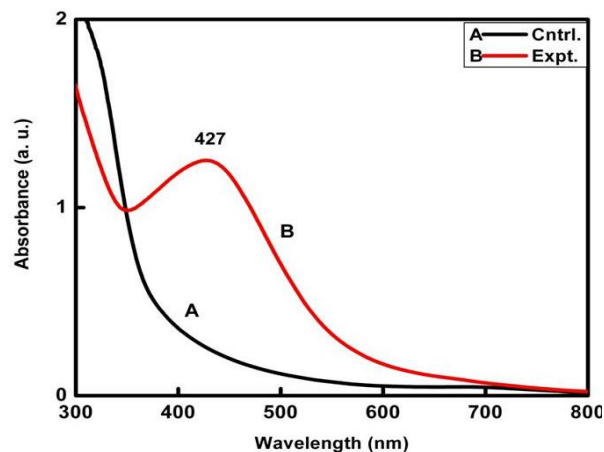


Figure 2. UV-Visible spectroscopy of synthesized silver nanoparticles from *Foeniculum vulgare*

Here we demonstrated Nanoparticle Tracking and Analysis (NTA) to measure the dispersion characteristics i.e. size and size distribution on their brownian motion in suspension. NTA allows individual nanoparticles in a suspension to be microscopically visualized and their brownian motion to be separately but simultaneously analyzed and from which the particle size distribution can be obtained on a particle-by-particle basis which enables separation of particle population by size and intensity. The NTA showed particle populations by size and intensity. These results correlate the results obtained by Montes-Burgos and group (Montes-Burgos et al. 2010). Total Concentration of silver nanoparticles synthesized by leaf extract of *F. vulgare* was found to be 7.6 particles/frame, 3.35×10^8 particles/mL. The size of silver particle analyzed by NTA was in the range of 18-83 nm. Statistical distribution of silver nanoparticles using LM 20, mean: 82nm, mode: 83 nm. SD: 25 nm. Distribution of Particle Size/Concentration of Ag NPs was showed in Figure 3a and the particle populations of Ag NPs using NanoSight LM-20 were studied (Figure 3a and 3b).

Particle size determination of the formulated nanoparticles was shown under different categories like size distribution by volume, by intensity (Figure 4). First and second peaks, the average diameter of the particles were found to be 127, 100% and width 37.25 nm. The formed silver nanoparticles are well distributed with respect to volume and intensity is an indication of the formation of well built silver nanoparticles and their monodispersity.

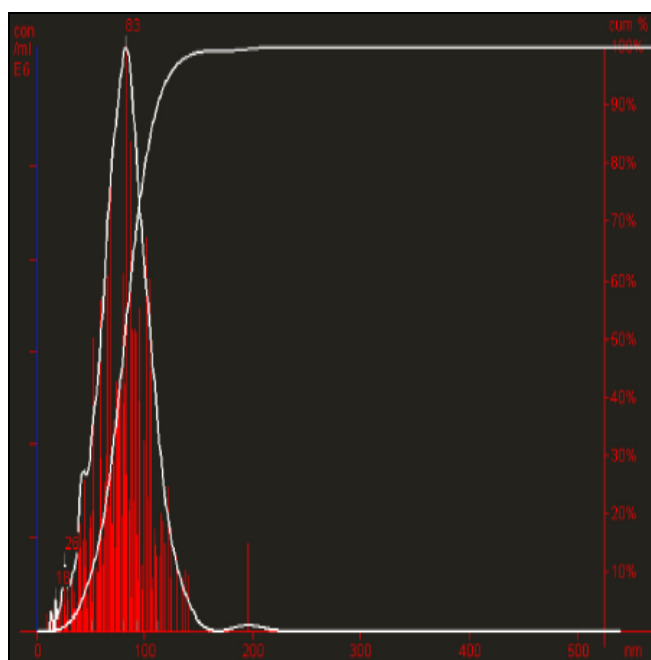


Figure 3a. Particle Size / Concentration of Ag NPs

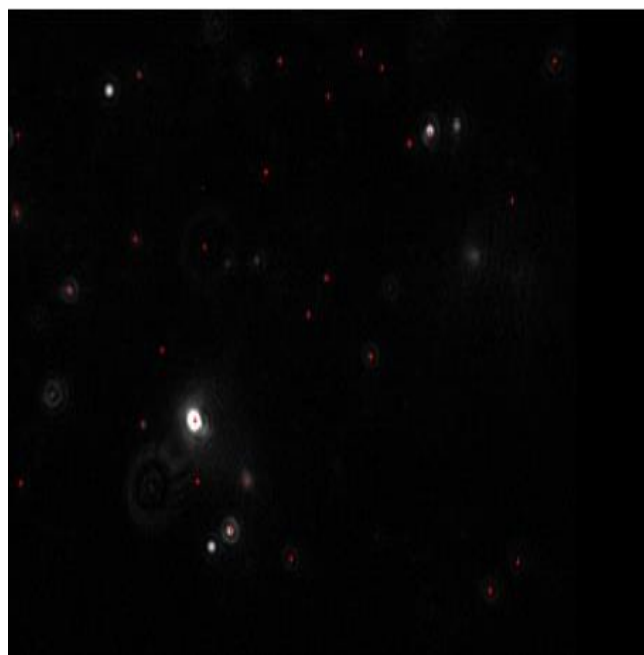


Figure 3b. Particle populations of Ag NPs using NanoSight LM-20.

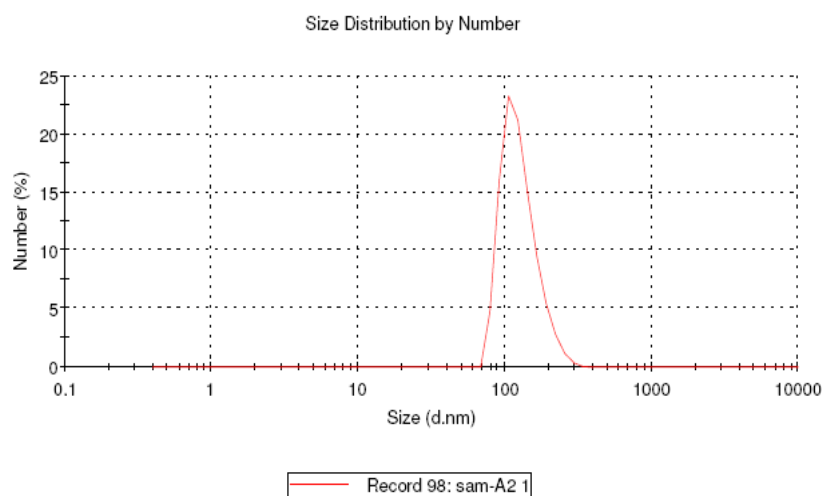


Figure 4. Particle size distribution of Ag-NPs by intensity with Zeta Analyzer.

Table 1. Comparison in increase in fold area zone of activity of different antibiotics against *S. aureus*, and *E. coli* (in absence and in presence of silver nanoparticles (Ag-NPs) at concentration of 20 μ l/disc. (Where, Ab= Antibiotics, Ab+ Ag-NPs= Antibiotics + silver nanoparticles)

Antibiotics	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>		
	Ab (A)	Ab+ Ag-NPs (B)	*Increase in fold area	Ab (C)	Ab+ Ag-NPs (D)	*Increase in fold area
Gentamicin	19 \pm 0.3	36 \pm 0.1	2.6 \pm 0.2	20 \pm 0.3	28 \pm 0.4	0.96
Oxacillin	24 \pm 0.2	29 \pm 0.3	0.32 \pm 0.3	28 \pm 0.5	30 \pm 0.1	0.14
Vancomycin	18 \pm 0.4	26 \pm 0.4	1.08 \pm 0.2	10 \pm 0.1	26 \pm 0.2	5.76
Ampicillin	27 \pm 0.1	31 \pm 0.2	0.31 \pm 0.1	21 \pm 0.2	24 \pm 0.4	0.31
Amoxycillin	29 \pm 0.2	32 \pm 0.1	0.21 \pm 0.3	19 \pm 0.2	25 \pm 0.3	0.73

Note: Inhibition zone in mm

We report synthesis of silver nanoparticles by *F. vulgare* for the first time as there is no report of synthesis of silver nanoparticles by this plant. Moreover, the phytosynthesized silver nanoparticles were used for the evaluation of their antibacterial efficacy in combination with commercially available antibiotics. From the present study, it was observed that the efficacy of silver nanoparticles against test bacteria (*E. coli* and *S. aureus*) was increased when assessed in combination of antibiotics. It is evidenced by the data provided in Table 1, which showed increase in activity fold area for each antibiotic. The bactericidal activity of the standard antibiotics was significantly increased in presence of silver nanoparticles against pathogenic bacteria, viz. *E. coli*-JM-103 (ATCC-39403) and *S. aureus* (ATCC-25923). Silver nanoparticles in combination with Vancomycin showed maximum activity against *E. coli* (increase in fold area 5.76) and *S. aureus* (1.08) and gentamicin showed the maximum activity *S. aureus* (2.6) while *E. coli* (0.96). While other antibiotics did not show significant inhibitory activity against the test bacteria. These findings support the report by Birla et al. 2009 year, who reported that the activity of commercially available antibiotics with silver nanoparticles synthesized by a

fungus *Phoma glomerata* was more against Gram-negative bacteria compared to the Gram-positive bacteria and also Savithramma et al. (2011) report the silver nanoparticles of *Boswellia ovalifoliata* showed maximum inhibition of *E. coli*. The green method of synthesis is safer than others. There are three types of antimicrobial mechanisms observed by Song et al. (2006), i.e. (i) Plasmolysis, cytoplasm of bacteria separated from bacterial cell wall, was observed in Gram-negative bacteria and Gram-positive bacteria, (ii) inhibited cell wall synthesis and (iii) induces metabolic disturbances to pathogenic bacteria.

CONCLUSION

The present study included the bioreduction Ag⁺ ions by plant *F. vulgare* and its antibacterial activity. The study reveals that plant species are good and fast rate source of synthesis and the antibacterial efficacy against *S. aureus* and *E. coli* confirmed that the silver nanoparticles are capable of rendering antibacterial efficacy and strengthen the medicinal value of plants. Phytosynthesis of silver nanoparticles are most convenient, easily scale up and eco-friendly.

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