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Green Synthesis of Silver Nanoparticles from *Pimenta dioica* Berries and its Antimicrobial Potential

Poonam Kaithal*, Rajiv Kant, Rohit Lall, Archana and Preetam Verma

Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology & Sciences, Prayagraj, Allahabad, U.P., India

**Corresponding author*

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A B S T R A C T

This present study for the first time reports a facile and ecofriendly route for synthesis of Silver nanoparticles by using the extract of berries of *Pimenta dioica*. Extract of berries was used for reduction of silver nitrate into silver nanoparticles (AgNPs). Silver nanoparticles have unique phytochemical properties and activities compared to their whole parent material. Due to multiple applications of Silver nanoparticles (AgNPs) various biological methods are gaining recognition for their production. The silver nanoparticles were irregular spherical in shape. In the reaction mixture brown color appeared which confirmed the primary indication of Ag NPs formation and it was confirmed by using UV-visible spectroscopy which shows maximum peak intensity at 425 nm. Sample was characterized by using UV-viz Spectrophotometer, SEM, and TEM. The synthesized silver nanoparticles were spherical in clusters. Crystalline size calculated was found to be 40 nm; and TEM shows that synthesized nanoparticles having size of 94±29 nm. The Ag NPs were evaluated for their antimicrobial activity on Gram negative bacteria *Escherichia coli* and Gram positive *Staphylococcus aureus* bacteria by qualitative methods and it was confirmed.

Introduction

Nanobiotechnology has made an appearance as an important division in nanotechnology. In nano scale the materials show dramatic changes in their various properties such as optical, catalytic, electrical etc. Numerous methods for synthesis of nanoparticles are present however non toxic and ecofriendly biological methods are most attractive. Especially when nanoparticles are intended to be in medicinal use. The biological methods to synthesis nanoparticles provide large quantities of nanoparcticles along with the reduced environmental impact compare to chemical methods. It is well

known since 1900s that plants are capable to reduce metal ions. Nanoparticle synthesis based on plant extract is an important topic for researchers.

The *Pimenta dioica* plant is member of family Mytracea and native of Caribbean island. It is also known as allspice, pimento or new spice (Zhang and Lokeshwar, 2012). Pimenta is a typical 22 ft long evergreen tree with light gray bark and dark green leaves. Its white flowers blossoms in summers and later they produce berries. The berries of pimento are green in color and after sun drying they appear like peppercorns. Dried unripe berries of pimento called allspice. British gave the name "Allspice" because of the strong aromatic fragrance like the combined fragrance of pepper, nutmeg, clove and cinnamon (Ashton, 2002 & Vaughan and Geissler, 1997). Various parts of *P. dioica* has been used for many ethnomedicinal uses all over the world such as diabetes, sore joints, myalgia (muscle ache), assorted odontalgia (toothache), dysmenorrheal (menstrual cramps), dyspepsia and anti-inflammatory (Zhang and Lokeshwar, 2012**).** It has other importance to use as spices in seasoning, pickling, Caribbean cuisines and baking. Apart from these uses the extracted oil from berries have been used in manufacturing of candle industries, perfumery and cosmetics (Zhang and Lokeshwar, 2012).

Metal/metal oxide nanoparticles has attracted a lot of researchers due to their versatility in functions in the field of chemistry, medicine, physics and biological sciences (Yokohama and Welchons, 2007). Out of many metal nanoparticles, silver nanoparticles gained special interest due to many properties like catalytic strength, chemical stability, electrical conductive and antibacterial activity (Sharma *et al.*, 2009). Many studies have been carried out with silver nanoparticles due to their high antimicrobial activities.

One can synthesize nanoparticles from various approaches like chemical, physical and biological. But "green nanotechnology" based nanoparticles synthesis is always in demand (Singhal, 2011). Plants are free from chemical toxins and can provide great platform for the synthesis of nanoparticles. Plant bases nanoparticle synthesis is better than microorganism's based synthesis (Singhal, 2011). Some examples of naturally occurring sources which gave promising results for silver nanoparticles synthesis are neem (*Azadirachta indica*), green tea (*Camellia sinesis*), leguminous shrub (*Sesbania drummondii*), starch, Aloe vera plant extract, leaf broths and natural rubber (Vijayaraghavan *et al.,* 2012).

Moreover the antimicrobial potency is dependent on the nanoparticles size. Smaller the particles gave higher antimicrobial strength because of the equivalent silver mass content.

Materials and Methods

AR grade Silver nitrate ($AgNO_3$) was purchased from Merck India Limited (Mumbai, India). Dried berries of *Pimenta dioica* were obtained from Paraman food works, Panruti (Tamil Nadu, India). Mueller-Hinton Broth/Agar was used for culture growth to test antimicrobial effects.

Preparation of extract

The dried berries of *Pimenta dioica* were grounded in electric mixer to obtain the fine powder for extraction. The dried powder of the berries was extracted with methanol and aqueous using soxhlet apparatus.

Synthesis of AgNPs (Silver Nanoparticles)

1 mM silver nitrate ($AgNO_3$) solution was mixed with aqueous extract of *Pimenta dioica*. Solution of plant extract and silver nitrate was mixed in concentration of different ratio eg: 30:1, 60:1, 120:1 & 240:1). It is important to avoid photo-oxidation of silver nitrate for which conical flask was covered with aluminum foil. Mixture in the conical flask was placed on stirrer for continuous stirring at 60 ^{0} C for approximately an hour (Banerjee *et al.,* 2014; Mata *et al.,* 2015).

Characterization of *Pimenta dioica*-AgNPs

Primary observation of the AgNPs is the change in color then UV-vis spectrophotometer is used to observe the information about synthesis of AgNPs within the range of 300-900 nm. Then *Pimenta dioica* berry extract mediated silver nanoparticles were analyzed and information obtained by using various other techniques. SEM and TEM were used to monitor the morphology of synthesized AgNPs. For TEM, freshly synthesized AgNPs were washed and after washing a drop was placed on copper disk along with carbon grid to dry with hot air and used for analysis. For SEM, powdered form of AgNPs was used.

Antimicrobial Effect of PD-AgNPs

Pathogenic bacterial culture of Gram-negative bacteria (*E.coli* MTCC687) and Gram-positive bacteria *S.aureus* MTCC3160) were used for the antimicrobial study. The agar well diffusion method was used as explained by Patil *et al.,* 2017. Bacterial suspension (100µl) of McFarland-standard was added on MHA agar plate. Wells were prepared with the help of 8mm Cork-borer on agar plate and 100µl of AgNPs were dropped on each well (Concentration range-50-200µg/ml). Plates kept incubating for 24 hrs at 37^0C. After incubation, plates showed clear zone around each well called Zone of Inhibition (ZOI). ZOI denoted inhibition in bacterial growth and measured in mm.

Results and Discussion

UV-vis Spectroscopy

For structural characterization of Silver nanoparticles, UV spectroscopy is one of the most widely used techniques. Figure 1 represents UV visible spectrum ranges from 200–800 nm. It can be observed that the peak occurs at 425 nm indicates the presence of silver nanoparticles; and it is due to the excitation of surface plasmon.

TEM and SEM analysis of PD-AgNPs

The surface morphology and size of silver nanoparticles was calculated by SEM and TEM techniques. SEM images (Figure 3 A and B) show individual particles as well as aggregates. TEM and SEM study reveals irregular spherical shape of nanoparticles. The aggregation is might be due to the presence of secondary metabolites in plant extract. Figure 2 (C and D) represents TEM images of Ag nanoparticles. Silver nanoparticles exhibit size 94±29 nm.

Antimicrobial activity test of Ag nanoparticles

Antibacterial activity of Ag nanoparticles was tested against two strains of pathogenic bacteria such as *E. coli* and *S. aureus*. Silver nanoparticles are reported as potential antimicrobial agent against pathogenic bacteria. Here is the study and confirmation of antibacterial efficiency by standard agar well diffusion method for Ag nanoparticles synthesized by *Pimenta dioica* berries; and with a green synthesis route. Parameters used and readings are mentioned in Table 1. Well number 4 and 5 corresponding to negative and positive control respectively. As negative control contains methanol no zone of inhibition was detected; and positive control contains antibiotics Hence zone of inhibitions measuring 25 mm and 24 mm for *E. coli* and *S. aureus* respectively.

Silver nanoparticles were prepared by green synthesis using *Pimenta dioca* as capping agent. The prepared PD-AgNPs *(Pimenta dioica* silver nanopartciles) were then characterized by using techniques such as SEM, TEM, UV-viz spectroscopy. The surface plasma resonance was confirmed by using UV-vis spectroscopy. The peaks of synthesized *Pimenta dioica* silver nanoparticles was recorded at 425 nm, which confrms the formation of production of AgNPs. SEM (Scanning electron microscopy) images of AgNPs shows the size and morphology of the particles.

Spherical shape of nanoparticles were observed in the images. Observed aggregation in SEM images was might be due to the presence of secondary metabolite in the plant extract. The TEM images of AgNPs exhibit the size range from 94±29 nm.

After confirming the formation of *Pimenta dioca* medicated silver nanoparticels, they subjected to study the antimicrobial effects bacterial culture. For antimicrobial assay the disc diffusion methods was used. The Gram-positive bacteria *S.aueus* and Gram-negative bacteria *E.coli* were studied to check the antimicrobial efficiency of PD-AgNPs.

Green synthesis of nanoparticles using plant extract is a simple, cost effective, eco-friendly approach and capable to synthesize antibacterial nanoparticles. Here, the extract of berries of *Pimenta dioica* was used for reduction of silver nitrate into silver nanoparticles (AgNPs).

Ag NPs were characterized by SEM, TEM and UV-visible spectroscopy. The surface plasma resonance of green synthesized Ag NPs was confirmed by UV visible spectroscopy. The biosynthesized AgNPs found to have significant antimicrobial effect against pathogenic bacteria: *Escherichia coli* and *Staphylococcus aureus*.

Table.1 Antibacterial activity study.

S. No.	Sample	Zone of Inhibition in mm	
		E.coli	***S. aureus***
1	50µg/ml NP	Nil	Nil
2	100µg/ml NP	11	10
3	150µg/ml NP	13	12
4	Negative control	Nil	Nil
5	Positive control	25	24

Table showing above the Zone of Inhibition obtained for the naoparticle against selected bacterial species

Fig.1 UV visible spectrum of Ag nanoparticles.

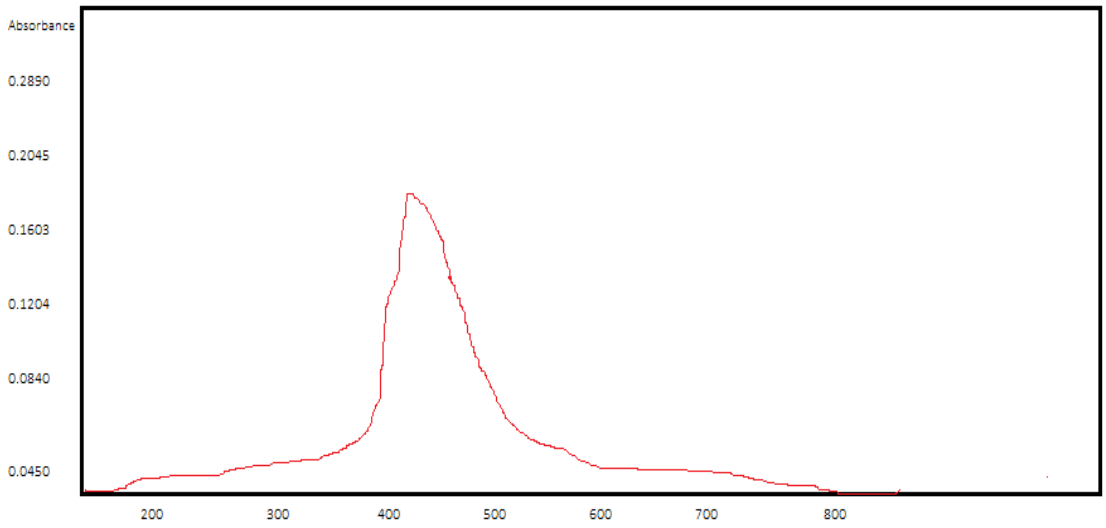


Fig.2 SEM images (A) and (B), TEM images (C), (D) of Silver nanoparticles, and E is the particle size distribution.

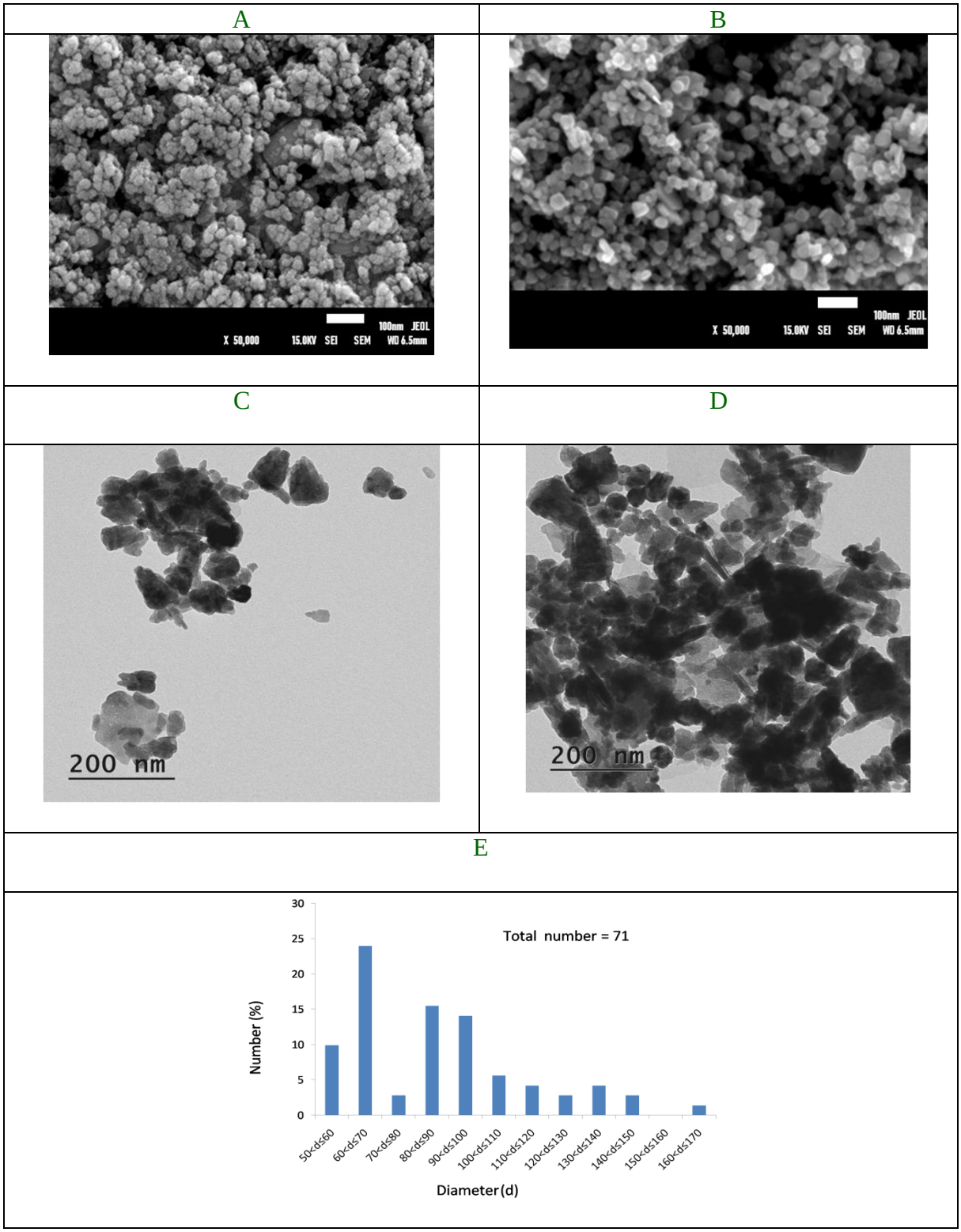
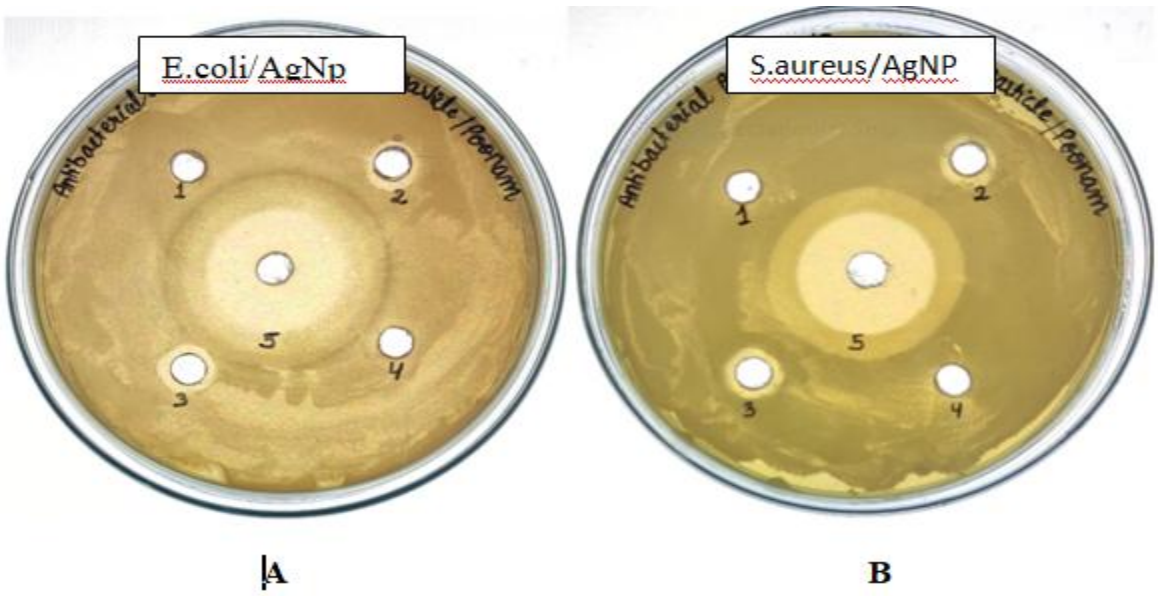


Fig.3 Antibacterial activity test of nanoparticle against A- *P.aerugenosa* and B- *S. aureus*



In the figure above wells are labelled as, well 1- 50µg/ml NP; well 2-100µg/ml NP; well 3-150µg/ml NP; well 4- negative control (methanol); well 5- positive control (antibiotic)

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