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SHORT COMMNICATION

Biosynthesis of silver nanoparticle using citrus plants and its antimicrobial activity

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ABSTRACT

Plant extract from *C. aurantium* and *Punica granatum* was used for the synthesis of silver nano-particles (AgNPs) from silver nitrate solution. AgNPs were characterized by UV–visible spectrophotometer, change in colour was visually observed in the silver nitrate solution added to the biological extracts. The bio reduction of precursor silver ions was monitored by sampling of aliquots (1ml) at different time intervals. Absorption measurements were carried out on UV–visible spectrophotometer at a resolution of 1 nm. UV Visible analysis of several weeks old samples was also carried out to check the stability of Silver nano-particle. Among inorganic antibacterial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage. The antibacterial activity of biologically produced was tested on different *E.coli, P. aeruginosa, S. aureus, B. subtilis* bacteria. The inhibition tests were carried out by the disc diffusion method. Growth inhibition of bacteria by AgNPs were compared with $AgNO_3$ solution and found that AgNPs were competent enough with $AgNO_3$ solution. The Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. Of these, silver nano-particles are playing a major role in the field of nanotechnology and nanomedicine.

Keywords: biosynthesis, silver, nano particle, citrus plants

1. INTRODUCTION

Nanotechnology is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometers (nm). Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering.

Recently, biosynthetic methods employing biological microorganisms such as bacteria [1], fungus [2] and plants extract [3-5] have emerged as a simple and viable alternative to more complex chemical synthetic procedures to obtain nanomaterial. Different types of nanomaterial like copper, zinc, titanium [6], magnesium, gold [7], alginate [8] are successfully biosynthesised but out of all these silver nanoparticles are more effective with antimicrobial potential against bacteria, viruses and other eukaryotic microorganisms [9]. Silver nanoparticles have also gained significance due to their broad-spectrum activity against bacterial infections. Thus, biosynthesised silver nanoparticles are playing a major role in the field of nanotechnology and nanomedicine. Over the decades, use of plants for the synthesis of silver nanoparticles has drawn attention of researchers because of its rapid, economical, eco-friendly protocol and it provides a single step technique for the biosynthesis. Therefore this study aims to biosynthesis the silver nanoparticle from traditional citrus plant and evaluates its antimicrobial efficacy on some universal microorganisms.

2. MATERIALS AND METHODS

2.1. Collection of plant sample

Two different natural plants were selected for the silver nanoparticles synthesis. The fruits *Citrus aurantium* and *Punica granatum* were collected from a garden at Himayat Bagh (19.8974° N, 75.3325° E), Aurangabad city in India. The fruits were washed, cut and its extract was made with the help of a blender. A mesh strainer was then used to strain the pomegranate liquid into a container.

2.2. Biosynthesis of silver nanoparticles

Extract of fresh fruits like *C. aurantium* and *P. granatum* were taken. This extract was then filtered using Whitman filter paper no 1. The filtered mixture was then boiled for 10 minutes and decanted. The prepared mixture is taken in 5 separate conical flasks 10 ml each. 50 ml of aqueous $AgNO_3$ solution (1mM, 3mM, and 5mM) was added to the respective flasks drop by drop with continuous stirring for reduction of Ag^+ ions at room temperature under dark condition. 3mL of 8% SDS (w/v) was added to the solution. The conical flasks were sealed with cotton plugs and observed for any color change.

2.3. Effect Of Temperature

In two separate flasks A and B, 5 ml of AgNP solution were taken. Solution of flask A was prepared at room temperature while the solution of flask B was prepared at 40^{0}C. 3 ml of 8% w/v SDS was added to each flask. The conical flasks were then sealed using cotton plugs and observed for any color change.

2.4. Effect of pH

10 ml of sample extract was taken in 8 separate conical flasks. pH of the extract was set ranging from 3 to 10. In each conical flask of sample extract, 50 ml of 1mM $AgNO_3$ solution was added drop by drop with constant stirring at 40^{0}C. 3 ml of 8% w/v SDS was added for stabilization. The conical flasks were then sealed using cotton plugs and observed for any color change.

2.5. Characterization of silver nanoparticles

The characterization of silver nanoparticle was first determined by UV–vis spectrophotometer, the absorption spectra of the samples were taken at 400-430 nm. The de-ionized water was used as the blank and wavelength was observed.

2.6. Antibiotic activity

The antibacterial activity of biosynthesized AgNPs was tested on different 4 different microorganisms *E.coli, P. aeruginosa, S. aureus and B. subtilis*. The inhibition tests were carried out by the well diffusion method [10]. Growth inhibition of bacteria by AgNPs was compared with $AgNO_3$ solution and the observation was recorded.

3. RESULTS AND DISCUSSION

3.1. Formation and Characterization:

The detailed study on biosynthesis of AgNP by citrus plants extract such as *C. aurantium* and *P. granatum* were employed and is reported in this work. The production of AgNPs was indicated by change in color from colorless to golden yellow color. The yellow colour formation during the synthesis of AgNPs is also previously reported [12]. The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–vis spectrophotometer analysis. The colour and absorbance spectrum at 430 nm is due to the excitation of surface plasmon vibrations (SPV) of silver atoms [13].

3.2. Effect of pH and Temperature:

It was observed that minimum 3 ml of (8% w/v) of SDS solution is needed to 5mM of $AgNO_3$ solution to avoid clumping of AgNPs. Production was tried at two different temperatures one at room temperature and the other at 40^0C. It was observed that, the extract show best result at 40^0C. The smaller particles coagulate to form AgNPs. From previous studies it is seen that the particle size get

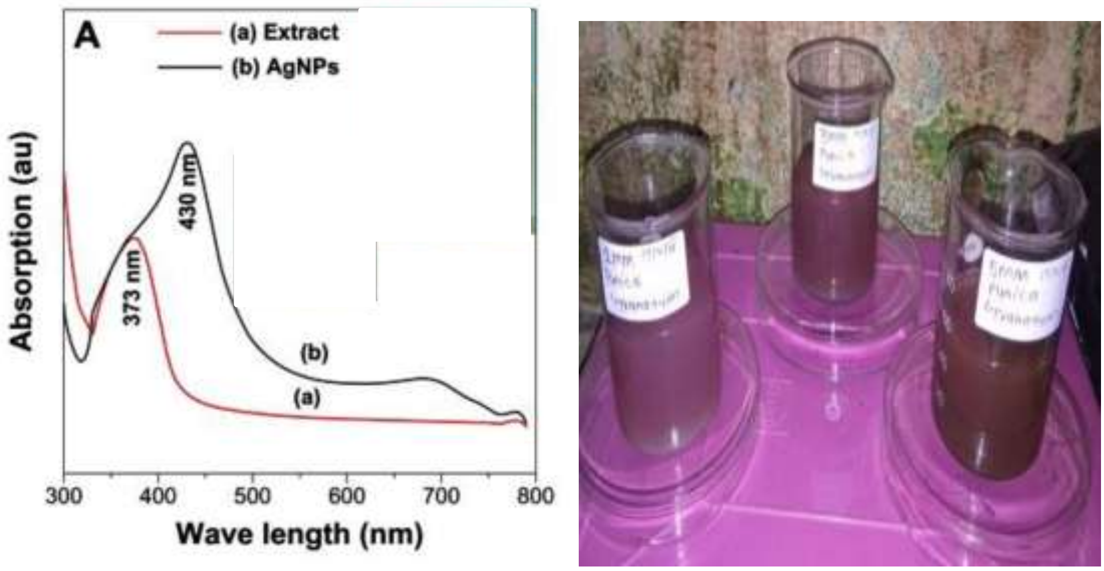

Figure 1. UV-vis spectrophotometer analysis of synthesized nano-particle along with its % transmission

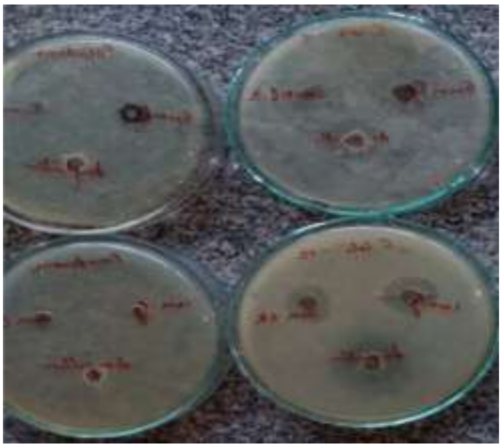

Figure 2. *E.coli* treated with 1mM, 3mM, and 5mM (A) $AgNO_3$ (1mM, 3mM, and 5mM) , (B) + *E.coli* with AgNPs (1mM, 3mM, and 5mM). *P. aeruginosa* treated with 1mM, 3mM, and 5mM (A) $AgNO_3$ (1mM, 3mM, and 5mM) , (B) – *P. aeruginosa* with AgNPs (1mM, 3mM, and 5mM.

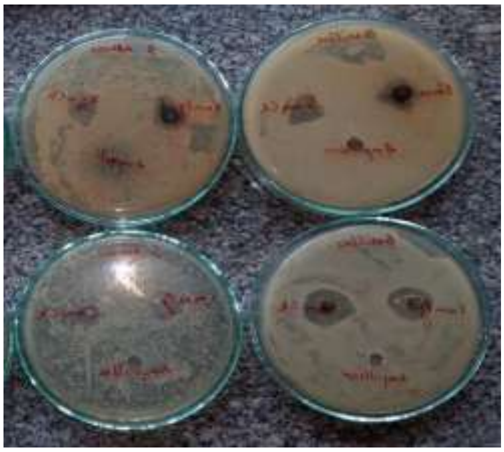

Figure 3. *S. aureus* treated with 1mM, 3mM, and 5mM (A) $AgNO_3$ (1mM, 3mM, and 5mM), (B) - *S. aureus* with AgNPs discs (1mM, 3mM, and 5mM). *B. substilis* treated with 1mM, 3mM, and 5mM (A) $AgNO_3$ (1mM, 3mM, and 5mM), (B)- *B. substilis* with AgNPs (1mM, 3mM, and 5mM).

smaller when exposed to higher reactive temperature [14]. Therefore, AgNPs with larger size can be obtained at relatively lower temperature and vice versa. pH was varied with constant volume of $AgNO_3$ with lemon as the plant extract. It was observed that the pH below 4 – 7 the AgNPs peak was obtained at 430nm. This can be concluded that the spherical shaped nanoparticle was formed at this pH [15].

3.3. Antimicrobial activity:

Four bacteria were used to test the antibacterial activity by well diffusion method. It was found effective for reducing the growth of bacteria. Out of all the organisms the synthesized AgNPs from flask B was most effective on *E.coli* followed by *B. subtilis*. The AgNP can easy degrade the gram negative bacteria may be due to no presence of cell wall unlike gram positive bacteria [16] (figure 2 & 3). The AgNPs from flask A did not show effective response against the four test organisms. Among inorganic antibacterial agents, silver has been employed most extensively since ancient times to fight infections and control spoilage.

4. CONCLUSION

The silver nano-particles have been produced by *C. aurantium* and *P. granatum* extracts, which is an economical, efficient and eco-friendly process. UV–visible spectrophotometer have confirmed the reduction of silver nitrate to silver nanoparticles. The zones of inhibition were formed in the antimicrobial screening test indicated, that the Ag NPs synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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