Biosynthesis of silver nanoparticles using plant seeds and their antimicrobial activity

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ABSTRACT :

Biological synthesis of nanoparticles has attracted attention due to its inherent advantages over the physical and chemical methods of nanoparticle synthesis. The greener way of the synthesis attributes to avoid use of the hazardous chemicals and their subsequent effects. In this investigation, we have used plant seed extract of Pisum sativum to develop safe, reliable and eco-friendly process for the synthesis of silver nanoparticles. This approach showed that seed extract of Pisum sativum reduced silver nitrate into the formation of silver nanoparticles. The generated silver nanoparticles were characterized by using UV–vis spectroscopy, Transmission electron microscopy (TEM) and Fourier transform spectroscopy (FTIR). The formation of silver nanoparticles was confirmed by UV-vis spectrophotometer. The UV-vis spectra showed peak at 423.02 nm. TEM image showed that generated nanoparticles were spherical in shape with size range of 3-36 nm. The generated silver nanoparticles revealed antimicrobial activity by well diffusion method against Escherichia coli and yeast Candida albicans.

Keywords: Silver nanoparticles, antimicrobial activity, biological synthesis, TEM.

INTRODUCTION

Nanotechnology is an emerging field of science with its increasing applications in diverse areas for the development of new materials at nanoscale levels [1]. Various chemical and physical methods are being explored for silver nanoparticles production but most of the techniques involved are capital intensive. It needs use of hazardous chemicals, stabilizing agents, capping agents [2-3]. Sustainable initiatives have been taken to use green chemistry approach to improve and protect environmental issues. Development of cost effective and ecofriendly methods to synthesize nanomaterials seems to be a challenge for researchers [4-5]. Some Simpler techniques add the valuation to the synthesis productivity and operability of the nanoparticles.

The biological systems have positioned themselves as one of the best candidature for the nanoparticle generation. Being safe and ecofriendly they have grabbed enormous attention in the field of nanotechnology [6-9]. Plant mediated synthesis of nanoparticles is advantageous over other methods such as microorganism by eliminating the elaborative and complicated processes for maintaining microorganism cultures [10].

Metal nanoparticles have grabbed the attention of researchers due to their unique as well as dynamic properties which do not exist in its bulk form [11-12]. In recent times, efficient nanosilver materials are progressively used in diverse fields of biotechnology and medicine; having major focus of researchers over their biological applications [13-14]. Different types of nanoparticles available but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy [15].

Due to the increase in microorganisms resistance to multiple antibiotics and rising healthcare costs [16-17], there is a need to develop new and efficient antimicrobial agents. This is the gray area where we can introduce the biologically synthesized silver nanoparticles against the pathogenic and drug resistant microorganisms. Keeping all these views in mind, in present investigation we used local variety of *Pisum sativum* for the synthesis of silver nanoparticles and their antimicrobial activity evaluated against the pathogenic microorganisms such as *Escherichia coli* and *Candida albicans.*

Materials and Methods

Preparation of seed extract

The fresh seeds of local variety of *Pisum sativum* (Black pea) were purchased from local grocery of Navi Mumbai. The seeds were washed thoroughly twice with sterile double distilled water (DDW). 20 grams of seeds were crushed and mixed with 100 ml of sterile distilled water. The mixture was boiled at 100^0C for 10 mins. Then it was filtered to obtain aqueous extract. The extract was stored at 4^0C.

Synthesis of silver nanoparticles

$AgNO_3$ was purchased from Sigma-Aldrich, prepared fresh 3mM aqueous solution of silver nitrate. 2 ml of the seed extract was added to 4 ml of 3mM solution. This system was kept at 70^0C boiling water bath for the synthesis of silver nanoparticles. Color change was observed. Mixture

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was then centrifuged and UV-vis spectrophotometer readings were taken.

Characterization of Silver nanoparticles:

UV-visible spectroscopy analysis

The change in color of the reaction mixture was recorded by visual observation. The reduction of silver nitrate to silver nanoparticles was analyzed by measuring UV-vis spectra of the solution. The absorption spectrum of the sample was taken in the range of 300-500 nm using Thermo scientific UV–vis spectrophotometer.

TEM analysis

Prior to the sample preparation for TEM analysis, sonication of sample was done for 10 min. The thin film of the sample was prepared on a small copper grid and allowed to dry. TEM analysis was performed on a PHILIPS- Model No-CM200 instrument at IIT-SAIF, Bombay.

FT-IR measurement:

FTIR analysis was performed using Bruker, Germany, Model-3000 Hyperion Microscope with Vertex 80 FTIR System. Sample was prepared on KBr pellet and it was allowed to dry. Then used for the characterization.

Antimicrobial activity

Silver nanoparticles generated by seed extract of *Pisum sativum* were tested for their antimicrobial activity against *Escherichia coli* and *Candida albicans* using well diffusion method. A strain of Escherichia coli and Candida albicans was swabbed uniformly on the individual plates (Brain Heart Infusion agar plates) using sterile cotton swab. Wells were prepared by using cork borer of size 8 mm. Extract, 50µl and 100µl sample of nanoparticles solution were poured into wells by using micropipette. After incubation at 37°C for 24 hrs, the zones of inhibition were measured.

Result and Discussion:

Visible Observation of silver nanoparticles:

A color change from light pale to brown was observed on mixing of seed extract of *Pisum sativum* with 3 mM silver nitrate solution (Fig.1). The visible color change indicates the formation of silver nanoparticles. This may be as a result of $AgNO_3$ reduction and stimulation of surface plasmon resonance. No precipitation was observed. The color change was stable even after completion of the reaction.

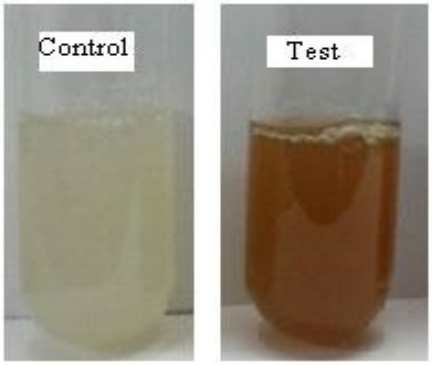

Figure 1: Visual observation silver nanoparticles synthesized by using local variety of *Pisum sativum* seeds. Control showed no change in color but test reaction mixture showed brownish color that indicating synthesis of silver nanoparticles.

U.V. Spectrophotometer analysis

The UV-vis spectroscopy is one of the most widely used simple and sensitive technique for the analysis of nanoparticle synthesis. The color exhibited by the samples is due to the excitation of electrons of the transition metals which affects the absorbance in the ultraviolet region. The silver nanoparticles synthesized by *Pisum sativum* seed extract were confirmed by UV-vis spectrophotometer, it showed the peak at 423.02 nm which corresponds to the absorbance of silver nanoparticles. (Fig.2).

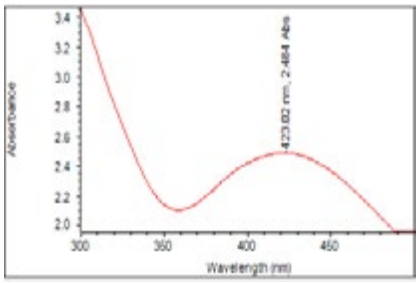

Figure 2: UV-Vis spectrophotometer analysis of generated silver nanoparticles by *Pisum sativum* seed extract

TEM analysis

The unique morphology and size distribution of the prepared nanoparticles were elucidated by using Transmission electron microscopy. Figure-3 exhibits the TEM micrograph of the prepared silver nanoparticles at magnification 50 nm. It was observed that the generated silver nanoparticles were spherical in shape with size range from 3 to 36 nm.

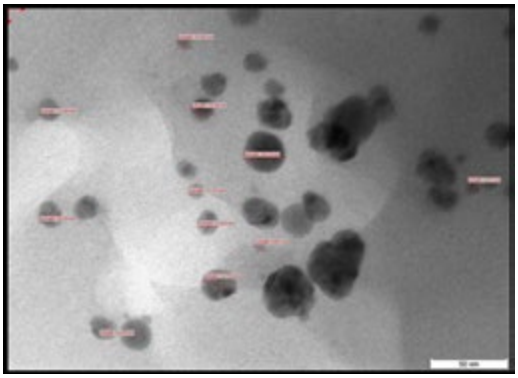

Figure 3: TEM micrograph illustrating the size and shape of silver nanoparticles generated by using seed extract of *Pisum sativum*. These synthesized nanoparticles were spherical in shape with size range 3-36 nm

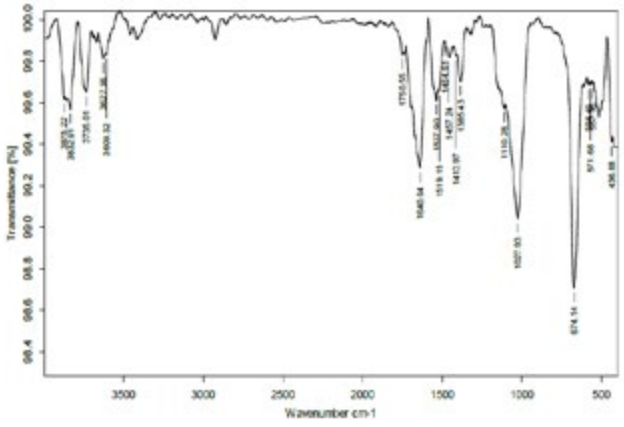

Figure 4: FTIR analysis of silver nanoparticles synthesized by using seed extract of *Pisum sativum*.

It was found that the nanoparticle possessed definite surface morphology. FTIR spectrum reveals prominent bands at 3875.22 cm^{-1}, 3735.01 cm^{-1}, 3609.32 cm^{-1}, 1750.55 cm^{-1}, 1647 cm^{-1}, 1543 cm^{-1}, 1027.93 cm^{-1} and 674.14 cm^{-1}. These groups might be responsible for synthesis and

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stabilization of generated silver nanoparticles.

Antibacterial activity

The antimicrobial activity of generated silver nanoparticles was evaluated against pathogenic microorganisms such as *Escherichia coli* and *Candida albicans* using well diffusion method. The antibacterial activity was evaluated by zone of inhibition. Results in Table-1 clearly indicate the greater zone of inhibition with increasing concentration of silver nanoparticles. It has been observed that the generated silver nanoparticles were able to inhibit growth of *Escherichia coli* and *Candida albicans*. However, as compared to *E. coli* greater inhibition was observed in fungal isolate *Candida albicans* at both the concentrations (50µl & 100µl). The results indicate that silver nanoparticles synthesized from seed extract of *Pisum sativum* show potential antimicrobial activity against the *Candida albicans*.

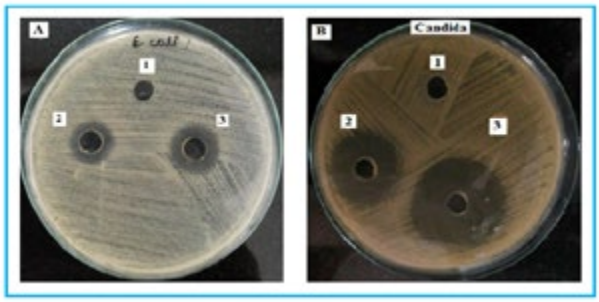

Figure 5: Antimicrobial activity of silver nanoparticles synthesized by *Pisum sativum* seed extract. (A) *Escherichia coli* and (B) *Candida albicans*. 1- Control, 2- nanoparticle (50 µl) and 3- nanoparticle (100 µl).

Sr. No.	Organisms	Diameter of zone of inhibition		
		Control	50 µl	100 µl
1.	*Escherichia coli*	-	16 mm	20 mm
2.	*Candida albicans*	-	25 mm	38 mm

Table 1: Antimicrobial activity as zone of inhibition for microorganisms *E. coli* and *Candida albicans* against generated silver nanoparticle by *Pisum sativum* seed extract.

CONCLUSION

Biological approach has been successfully demonstrated for the synthesis of silver nanoparticles using seed extract of *Pisum sativum*. The generated silver nanoparticles showed the antimicrobial activity against *Escherichia coli* and more efficient in case of *Candida albicans*. This method is simple, reliable and ecofriendly for reduction of silver nitrate to silver nanoparticles. Therefore, the expansion and execution of such newer technologies may unfold the role of plants in green synthesis of nanoparticles.

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