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ORIGINAL ARTICLE

Biological Synthesis of Antimicrobial Silver Nanoparticles by *Phaseolus vulgaris* Seed Extract

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ABSTRACT

Biological synthesis of silver nanoparticles is generally a time-consuming process in comparison to chemical process. Despite voluminous reports on biological synthesis of silver nanoparticles, there is still a challenge to develop fast synthesis of nanoparticles in the range of minutes/seconds through biological route. Several disadvantages are generally being posed by slow biological synthesis of silver nanoparticles including cost of operation. To overcome this difficulty, fast and simple method has been developed for the synthesis of silver nanoparticles, using *Phaseolus vulgaris* seed extract simply by increasing the temperature. The method is very quick and the color change of the reaction can be observed within 20 seconds. This process was able to synthesize silver nanoparticles within 80 seconds at 100°C which was confirmed by absorption peak at 413.79 nm in UV-visible spectrum. Initially, it was observed that *P. vulgaris* seed extract was unable to synthesize silver nanoparticles at 37°C even after 24 hours. The silver nanoparticles generated by this method were predominantly spherical in shape and in the range of approximately 4 to 30 nm in size, as characterized by transmission electron microscopy (TEM). On FTIR analysis, it was found that the nanoparticles possessed definite surface exposed groups. Generated silver nanoparticles showed antimicrobial activity against clinical isolates, *Escherichia coli* and *Candida albicans*. Thus, this biological process offers a simple, ecofriendly and very fast synthesis of antimicrobial silver nanoparticles.

Keywords: Silver nanoparticles, Very fast synthesis, Biological synthesis, Antimicrobial, *Phaseolus vulgaris* seed.

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INTRODUCTION

In the last few years, 'nanotechnology' has become a rapidly spreading accelerated technology and it has received enormous attention all over the globe, setting a landmark in scientific revolution.[1] The environment-friendly nanoparticles with low cost and easy procedures are a growing concern nowadays.[2] A unique and remarkable property of nanoparticles is to effectively arch between bulk materials and atomic or molecular structures; therefore, it has a great scientific interest.[3] Nanoparticles exhibit a number of significant properties including antibacterial activity.[4]

Recently, there is special interest in the study of nanoparticle synthesis using biological systems and its various applications.[5-7] Although, nanoparticles produced through biogenic process are ecofriendly, it is generally a very slow process which needs hours for the synthesis. Nanoparticle synthesis mediated by biological systems (bacteria, fungi, yeast and plant extracts) has attracted researchers, as the known synthesis of nanoparticles by chemical methods are mostly capital-intensive, inefficient in material and energy use and often cause health hazards due to usage of toxic chemicals.[8-11] Various studies on the synthesis of nanoparticles by different types of plant extracts have clearly showed their vast potential in green synthesis. In this direction, many plants have been described and reported for the synthesis of nanoparticles.

Silver nanoparticles are some of the most extensively studied materials with varied applications including application in nano medicine and evaluated for their antimicrobial activities against a wide range of pathogenic organisms.[12-14] Still the main concern areas of research are to identify new antimicrobial agents from natural substances.[15,16] Generally, biological synthesis of silver nanoparticle is slow and time-consuming process.[17] Therefore, demonstration of biological based fast synthesis for nanoparticle is an interesting area of research. Thus, the present study aims to develop very fast and simple method for the synthesis of antimicrobial silver nanoparticles through plant derived aqueous extract. To the best of our knowledge, this is the first report where *Phaseolus vulgaris* aqueous seed extract has been explored for very fast synthesis of silver nanoparticles.

MATERIALS AND METHODS

Extract Preparation

P. vulgaris seeds were purchased from local grocery of Navi Mumbai. Seeds were washed thoroughly twice with sterile distilled water. Twenty-five gram of crushed seeds were added to 125 ml of sterile distilled water in 250 ml conical flask and boiled at 100ºC for 10 minutes. It was filtered with Whatman filter paper no. 1, to obtain an aqueous extract.

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was purchased from Sigma and fresh 3 mM of silver nitrate solution was prepared. The reaction mixture contains the prepared seed extract and 3 mM $AgNO_3$ aqueous solution in 1:2 ratio and incubated at temperature 37°C, 60°C and 100°C. The change in color was observed and time was recorded at 100°C. Mixture was then centrifuged and used for the characterization.

Characterization of Nanoparticles

UV-visible Spectroscopy Analysis

The color change during silver nanoparticle synthesis was recorded through visual observation. The reduction of silver ions was also recorded by UV-vis spectra of the solution. UV-vis spectra of synthesis were monitored with the help of thermo scientific UV-vis spectrophotometer.

TEM Analysis

The TEM analysis was performed on a Philips instrument of IIT, Bombay, Model No. CM200, operating voltages: 20 to 200 kV. Prior to analysis, silver nanoparticles (AgNPs) were sonicated for 10 minutes. The thin film of the sample was prepared on a small copper grid by just dropping a very small amount of the sample on the grid, extra solution were removed using a blotting paper. The liquid fraction was allowed to evaporate at room temperature. Nanoparticles were observed at 50 nm and 100 nm scale.

FTIR Measurement

Fourier transform infrared spectrometer (FTIR) measurement of sample 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 Assay

The silver nanoparticles synthesized were tested for their antimicrobial activity by well diffusion method against clinical isolates *Escherichia coli* and *Candida albicans*. Sterile cotton swab was used to incubate organism on respective culture plates. Wells of size 8 mm have been made on brain heart infusion (BHI) agar plates using cork borer. Different concentrations (25, 50, 75 and 100 µl) of silver nanoparticles solution were poured into wells. After incubation at 35°C for 24 hours, the different levels of zone of inhibition were measured. The experiment was done in triplicate.

RESULTS AND DISCUSSION

In present study, we have used *P. vulgaris* seed extract for the generation of silver nanoparticles in a very rapid and simple protocol which can be even demonstrated at laboratory level. *P. vulgaris* is a herbaceous annual plant and its leaves and pods were reported earlier for slow synthesis of silver nanoparticles in respect to different aspects.[18,19] However, present study has demonstrated the very fast synthesis of silver nanoparticles and the time consumed in the process was lesser than earlier reported in *P. vulgaris* and other plants system.[20] The principle of the method for the synthesis of silver nanoparticle was based on combinational approach toward the use of reduction ability of *P. vulgaris* seed extract in the presence of elevated temperature in a well defined system. Temperature is known to affect the speed of synthesis of nanoparticles.[21,22]

Formation of AgNPs by reduction of silver nitrate during exposure to *P. vulgaris* extract can be easily monitored by the change in color of the reaction mixture from colorless to yellow brown color. It is probably due to the excitation of surface plasmon vibrations. It indirectly indicates the conversion of silver ions into the silver with the size of nanometric range. It is also evident that the change in color of the reaction medium is probably due to the presence of reducing substance. Finally, synthesized Ag NPs have been characterized by UV-vis spectroscopy, transmission electron microscopy (TEM) and FTIR.

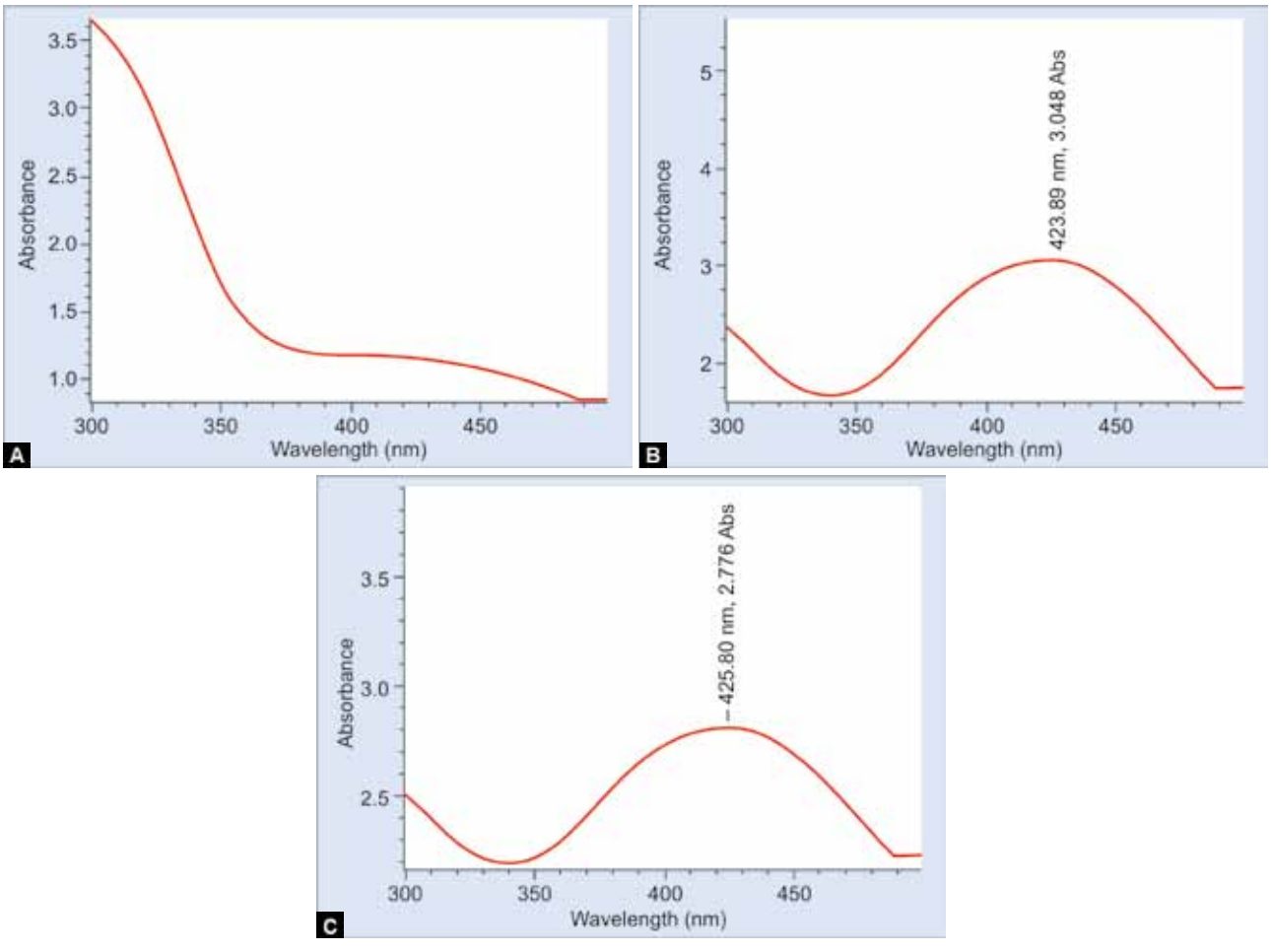
On the trial basis to understand the role of incubation temperature on the synthesis of silver nanoparticles, seed extract was mixed with 3 mM $AgNO_3$ aqueous solution and incubated at temperature 37°C and 60°C and the change in color was observed. Although, these temperatures supported reasonable color change during reaction, the time taken by the synthesis of silver nanoparticle was very long as it was recorded (data not shown). The time taken for conversion of silver nitrate to silver nanoparticle was less at 60ºC in comparison to 37°C. Result showed that there is significant role of temperature on speedy synthesis of silver nanoparticle using *P. vulgaris* in presence of proper control reaction sample. UV-vis spectroscopic analysis was also carried out for synthesized nanoparticle on thermo scientific UV-vis

spectrophotometer. The UV-visible spectrum of nanoparticle was recorded by spectrophotometer in the range of 300 to 500 nm and it was confirmed by appearance of peak of absorbance (Figs 1A to C). Extending our experiment for achieving very rapid synthesis of silver nanoparticle, the reaction was also carried out at 100°C by keeping reaction mixture in water bath in the ratio of 1:2 ml of seed extract and 3 mM $AgNO_3$ aqueous solution. Interestingly, very fast color change was observed. During the course of extensive experiment on determination of time required for synthesis of silver nanoparticles at 100°C, it has been explored the potential of heat stable aqueous extract of *P. vulgaris* for reduction ability of silver nitrate solution. For this various reaction, samples were evaluated at regular interval of 20 seconds up to 80 seconds during the synthesis of silver nanoparticles. Color change was observed in each reaction with varied amplitude. Intensity of color increased with time (Fig. 2).

Although color changes were observed only after 20 seconds but peak initiation was observed at 60 seconds (Fig. 3). The reduction of silver ions recorded was quite rapid. Generally, biogenic methods are considered as very slow when compared with chemical methods. To the best of our knowledge, the time taken in the present study for the synthesis of silver nanoparticle is lesser than reported earlier. This is very rapid method and color change can be easily observed during progression of reaction and reasonable color change can be recorded only after 20 seconds.

Nanoparticle synthesis by this method is also confirmed by transmission electron microscope. Heterogeneous synthesis of silver nanoparticles was observed with varied size ranging from 4 to 30 nm (Figs 4A to C). Transmission electron microscopy images of AgNPs at different magnifications revealed that the AgNPs are predominantly spherical in shape and are not generally in physical contact with each other. Lower magnification image of nanoparticles denote the embedded presence of these particles in a dense matrix of reaction environment which may be due to the stabilizing components contributed by *P. vulgaris* seed extract.

To understand the possible groups exposed on the surface of silver nanoparticle generated by *P. vulgaris* seed extract and there by interaction with each other FTIR has been taken (Fig. 5). The binding properties of synthesized AgNPs were also investigated by FTIR spectroscopy analysis. FTIR measurements were taken using Bruker,



Figs 1A to C: UV-vis absorption spectrum of silver nanoparticles synthesized at: (A) 37°C recorded after 24 hours, (B) 37°C recorded after 48 hours and (C) 60°C recorded after 24 hours

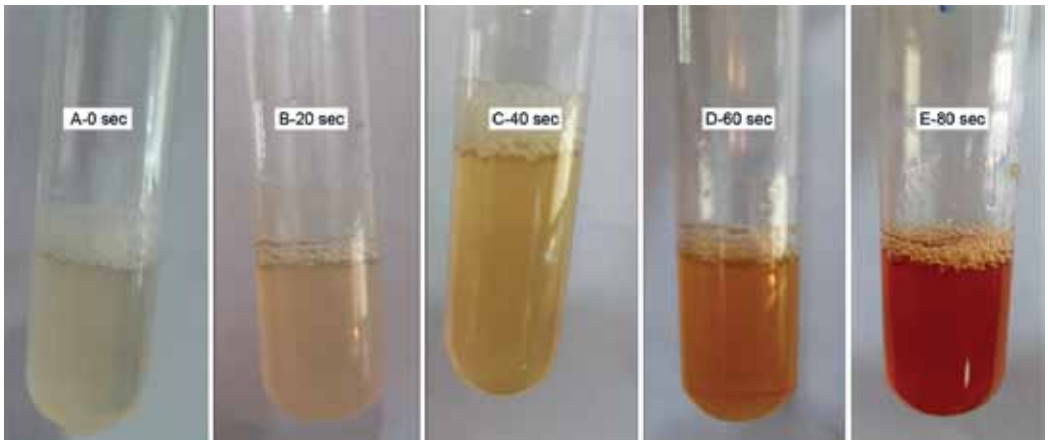


Fig. 2: Visual observation of color change after 80 seconds

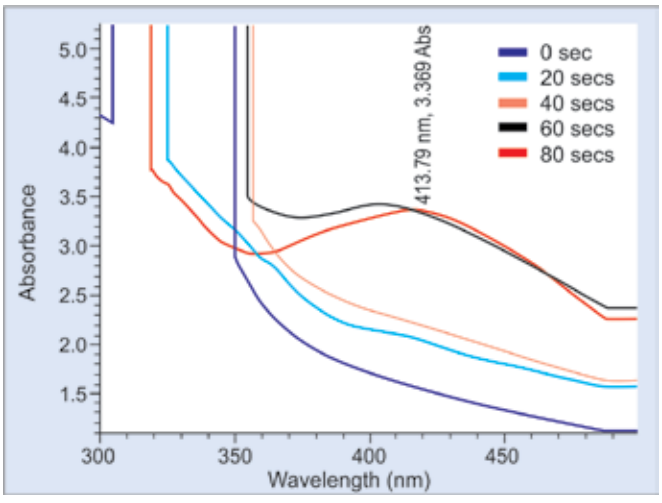
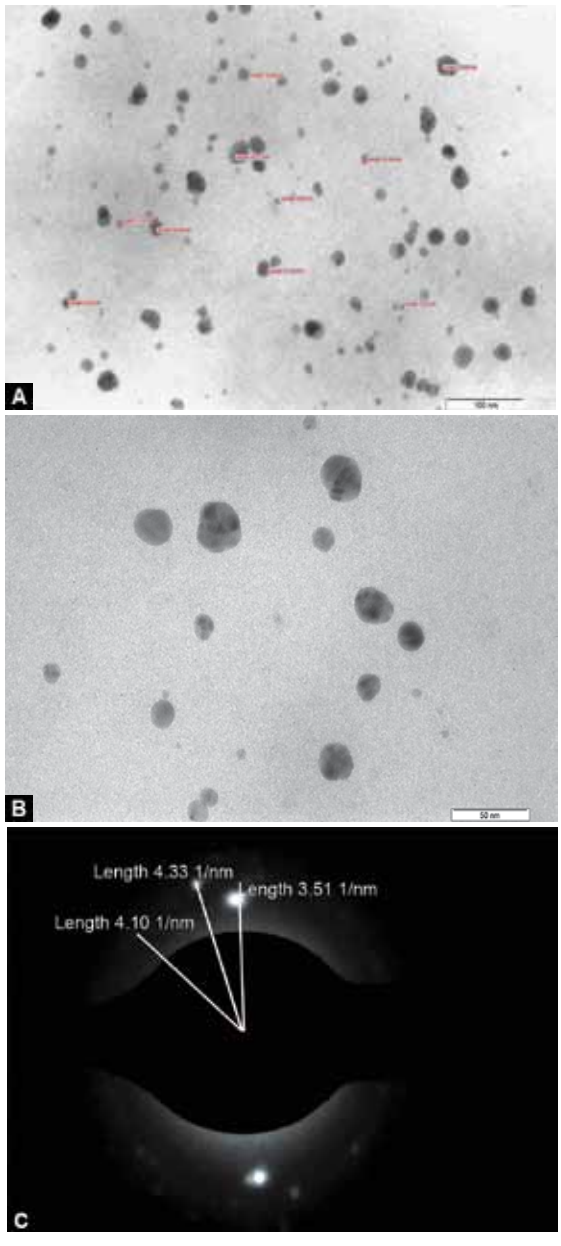


Fig. 3: UV-vis absorption spectrum of AgNPs recorded after every 20 seconds of regular interval

Germany, Model-3000 Hyperion microscope with vertex 80 FT-IR system. Sample was prepared on KBr pellet. The spectra were recorded in the wave number range of 450 to 4000 (Fig. 5). It was found that the nanoparticle possessed definite surface morphology. Number of peaks were observed under FTIR but three prominent peaks were observed at 673.43, 1039.75 and 1390 cm^{-1}. The peak at 673.43 cm^{-1} might be alkene or halo group, the peak at 1039.75 cm^{-1} represents the sulfoxide and peak at 1390.37 represents aldehyde group. Result presented in Figure 5 showed that there is stabilization of silver nanoparticles by this *P. vulgaris* seed extract.

Silver nanoparticles are generally known to produce antibacterial activity to a wide range of bacteria by operating various mechanisms. In some cases it also showed antifungal activity. Therefore, the antimicrobial activity of synthesized silver nanoparticles was also investigated against clinical isolates *E. coli* and *C. albicans*. Arresting of microbial growth in the form of zone of inhibition was measured using various concentration of silver nanoparticle solution (25, 50, 75 and 100 µl per/well). The



Figs 4A to C: TEM micrograph of silver nanoparticles synthesized by *P. vulgaris* seed extract: (A) at 100 nm scale, (B) at 50 nm scale and (C) diffraction pattern of silver nanoparticle. It is observed that most of the nanoparticles shown in the micrograph are in the range of 4 to 30 nm in size

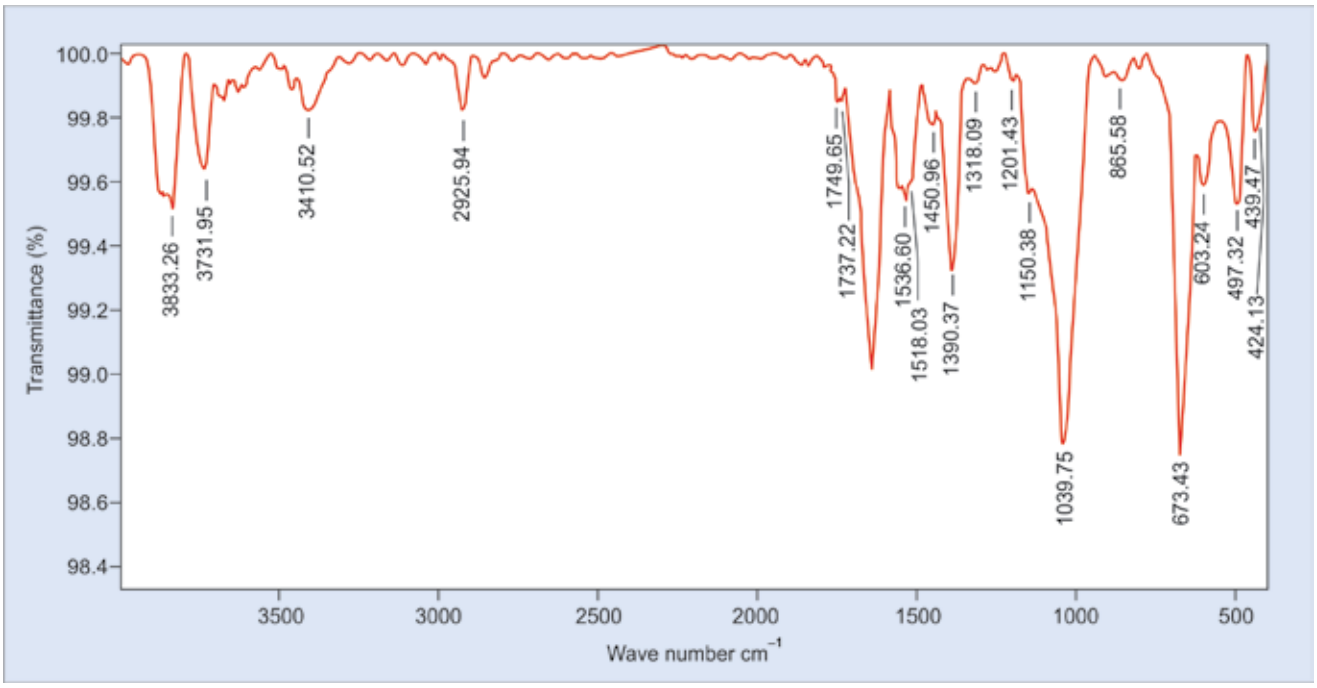


Fig. 5: Fourier transform infrared spectrometer analysis of generated silver nanoparticles

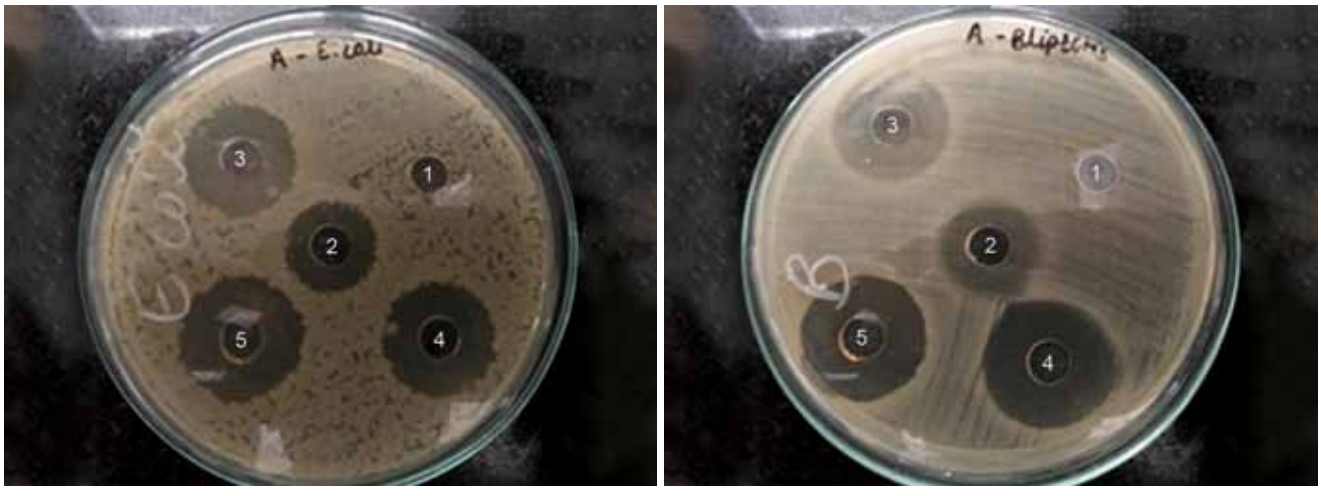


Fig. 6: Antimicrobial activity against microorganisms *E. coli* and *C. albicans:* (1) control, (2) 25 µl nanoparticles, (3) 50 µl nanoparticles, (4) 75 µl nanoparticles and (5) 100 µl nanoparticles

synthesized nanoparticles exhibited different degrees of antimicrobial activity against microorganisms tested probably due to different mechanisms involved in killing of microorganisms (Fig. 6). Inhibition of microbial growth was observed as the zone of inhibition whose diameters are estimated (Table 1).

Although, number of mechanisms has been proposed for antibacterial activity of silver nanoparticles, Lin et al explained and believed that silver nanoparticle bind with negatively charged cell wall which leads into cell death.[23] Interestingly, it was observed that synthesized nanoparticles showed potential antimicrobial activity with gram negative *E. coli* and *C. albicans* with more activity toward *C. albicans*. Thus, biological synthesis of silver nanoparticle is able to generate biologically active silver nanoparticle with antimicrobial activity.

Table 1: Antimicrobial activity as zone of inhibition for various microorganisms *E. coli* and *C. albicans* against generated silver nanoparticle

S. no.	*Organisms*	*Diameter of zone of inhibition—mm (Cork borer diameter 8 mm)*				
		Control	*25 µl*	*50 µl*	*75 µl*	*100 µl*
1.	*E. coli*	—	15 mm	18 mm	20 mm	23 mm
2.	*C. albicans*	—	16 mm	19 mm	25 mm	27 mm

CONCLUSION

Thus, the present method is very fast, simple, and efficient for the synthesis of antimicrobial silver nanoparticle to

E. coli and *C. albicans* which can be achieved without any use of chemicals. It confirms the ability of *P. vulgaris* seed extract for the rapid synthesis of silver into silver nanoparticle. This method eliminates the difficulties posed during biological synthesis, as in most of the cases, it was reported as a very slow process. Moreover, the process is cost effective too. The process can be explored for large scale synthesis of silver nanoparticle because of lesser specificity of the reaction parameters. This type of method can also be used for the demonstration purpose at basic laboratory setup. Hence, the present method can be used for rapid bioactive silver nanoparticle synthesis in a very simple protocol.

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