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Research article

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One Pot Green Synthesis and Structural Characterisation of Bio-Silver Nanoparticles Using Prunus Persica L.

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Abstract: The Plant mediated green synthesis of nanoparticles has attracted more attention among the researchers due to their physicochemical and biological properties and their immense applications in environmental, biomedical, agriculture, textile, and energy storage. The biosynthesis of nanoparticles using plants provides more advantages such as easy operation, eco-friendly, utilization of non-hazardous chemicals, less temperature or energy usage, etc. when compared to physical and chemical synthesis methods. In the present study, we propose aqueous extracts from the endemic-medicinal fruit *Prunus persica L.* as an efficient bioproduct for the green synthesis of silver - nanoparticles (AgNPs). The biosynthesized AgNPs were characterized using UV-Visible spectroscopy, Fourier transforms Infrared spectroscopy (FTIR) High-Resolution Transmission electron microscopy (HR-TEM), Scanning electron microscopy, Atomic Force Microscopy (AFM), and X-ray diffractometer (XRD). HR-TEM gives the formation of monodispersed spherical shape with a mean diameter of 12.6 ± 3.8 nm. The SAED pattern of the nanoparticles showed that the particles were highly crystalline in nature. The purity of the synthesized AgNPs samples was studied by EDS and confirmed the presence of Ag elements corresponding to TEM images. The 2D and 3D structure images of the nanoparticle were studied and the average particle size calculation has been performed using NOVA-TX software in AFM. X-ray diffraction analysis showed that the particles were crystalline in nature with face - centered cubic structure. The antimicrobial efficacy was evaluated using the resazurin assay against both *Escherichia coli* and *Bacillus subtilis* bacteria. The antimicrobial assay of the silver nanoparticles showed higher activity against *Bacillus subtilis* than *Escherichia coli.* Results confirmed this protocol as rapid, simple, eco-friendly and alternative to conventional physical, and chemical methods.

Keywords: *Prunus persica L*, bio-silver, nanoparticles, antibacterial, resazurin assay.

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1. INTRODUCTION

In the recent years, plants mediated biosynthesis of nanoparticles shows potential role and has been a preferred choice for synthesizing various types of nanoparticles in the modern era. Currently, bio nanoparticles with unparalleled characteristics are widely studied due to their immense potential applications in medical, environmental, food industries, packaging and energy storage devices.[1] There are various approaches for the synthesis of nanoparticles including physical, chemical and even biological. Conventional physical and chemical methods lead to many environmental and biological risks by releasing toxic chemicals and energy consumptions during the synthesis process. Hence the utilization of biological or green approach methods for developing nanomaterials play a vital role.[2] The biological synthesis incorporates use of plants, microbes, biomolecules and biopolymers etc. among the different strategies of biosynthesis, and plant mediated approaches have many potential applications due to eco-friendly, cost-effective, high stability, controlled nucleation and easy process. Numerous studies report the use of stable silver nanoparticle synthesized by fruit extract with their notable antimicrobial activities: *Tiliacora acuminate*, [3] *Phyllanthus emblica,*[4] *Solanum torvum*, [5] *Silybummarianum*, [6] *Alpinia nigra,*[7] and *Cornelian cherry*[8]. In these studies, fruit flavonoids, triterpenoids, polyphenols, proteins, and pigments serve as reducing and capping agents when synthesized with silver nanoparticles.[9] *Prunus persica is a* commonly known as peach in *English*. It is consumed worldwide for its antiscorbutic properties.[10] It is an economically essential crop. The overall worldwide production is over 22 million tons.[11] It is cultivated in the mid hills of The Himalayas that extends from Jammu and Kashmir in the west to Khasi hills in the east. For about 1000–2000 m above sea level, *Prunus persica*[12] has a wide range of biologically active compounds that are rich in Phenolic acids, flavonoids, vitamins (significantly C and A), organic acids, antholyanim, antioxidants, dietary fibers and natural sugars. [10,13,14] Prunus persica was chosen because it has many anti-disease properties like anti-cancer, anti-allergic, anti-tumor, anti- bacterial, anti-microbial and anti-inflammatory.[14] Moreover, the usage of natural, renewable and also low cost material, viz. *Prunus persica,* would be able to produce metal silver nanoparticles with aqueous medium by avoiding the presence of hazardous substances including toxic solvents. There are only a few reports which describe synthesis of silver nanoparticles from *Prunuspersica.* The aim of the work is to investigate the role of the fruit extract that is displayed in the stabilization, and also the formation of silver nanoparticles and their synthesis. The crystal structure and the surface morphology were characterized by UV-vis spectrophotometer, Fourier transform infrared (FT-IR), energy dispersive analysis (EDX), AFM, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The antibacterial activity of the silver nanoparticles has been evaluated by the agar diffusion method against gram positive and the gram negative bacteria.

2. MATERIALS AND METHODS

2.1 *Preparation of aqueous fruit extract of Prunus persica L.*

Fresh fruits of *Prunuspersica* were selected for the biosynthesis of AgNPs because of its medicinal properties, easy availability and cost effectiveness. The Fruits were collected from Kallatty village, Nilgiris, India (Latitude: 11° 29' 29.7744" N and Longitude 76° 44' 1.1400" E). The collected fruits were washed using tap water followed by the double distilled water. Around 100 g of washed fruits were chopped and grinded with 500 ml of distilled water. The fruit juice has been filtered by using Whatman No. 1 filter paper and collected final extract was used for further experiments.

2.2 *Synthesis of the Bio-silver nanoparticles*

Bio-AgNPs was synthesized by adding 5.0 ml of the prepared fruit extract to 100 ml of 1 mm $AgNO_3$ in a 250 ml conical flask by continuous stirring at room temperature. There was an observation of color change from pale yellow to brown which indicates the formation of Bio-AgNPs in the solution (Fig A). The Bio-AgNPs was separated by centrifugation at 5000 rpm for 30 min, then it was washed and dried at room temperature and it was used for further studies.

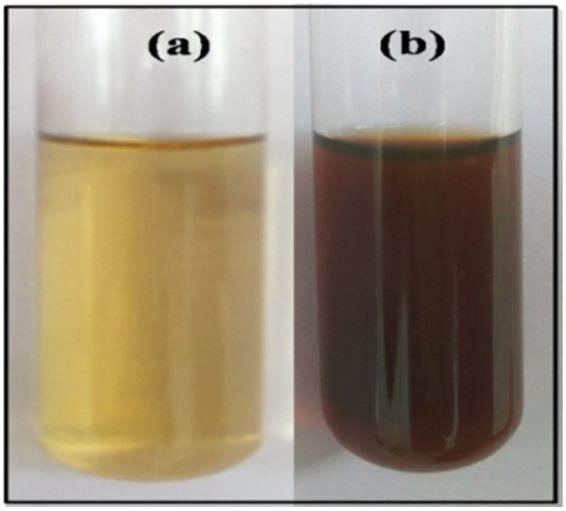


Fig A: Biosynthesis of Bio-AgNPs using before (a) and after (b) adding silver nitrate

2.3 *Characterization*

The FTIR spectroscopic analysis was being carried out by using a Nicolet Avatar-320 FTIR spectrometer (Nicolet Instruments, Madison) at a scan range of about 400–4000 cm^{-1} with a scanning speed of 2 mm/S. Entirely the dried samples were treated with spectral grade KBr for pelleting at a ratio of 1:50 and it was used for FTIR analysis. The phase identification and crystal structures of the Bio-AgNPs are characterized by XRD technique using X-ray diffractometer (XRD- 600, Shimadzu, Japan) having about CuKα radiation, α = 1.54 Å with generator settings of 30 mA; 40 kV; step size 0.05 (2θ) with a scan step time of 10.16 seconds in about continuous mode High resolution transmission electron microscopic (HR-TEM) images that were taken in JEOL- JEM 2100 (Japan) with an accelerating voltage of 200 kV. The selected area electron diffraction (SAED) of the nanocomposite was performed. In order to evaluate the elemental composition of the adsorbent material, Energy Dispersive X-Ray Spectroscopy (EDS) technique was being applied to the corresponding HR-TEM image. The morphological features of the sample were studied by using a scanning electron microscope (SEM), Carl Zeiss Evo 18 Germany and the images were also captured at an accelerating voltage of 20 kV. UV–visible absorption spectroscopic analysis was being performed by using the V-650 UV-Vis Spectrophotometer, JASCO, USA.

2.4 *Resazurin assay for assessing antibacterial activity of Bio-AgNP*

E. coli and *B. subtilis* were grown in Luria Bertani broth and were incubated at about 37°C with shaking at 100 rpm. Cells were harvested and were centrifuged at 5000 rpm for about 15 minutes to obtain pellets. 1x phosphate buffer saline was used to wash the pellet and to remove traces of media. The optical density of suspension was measured at 600 nm and was compared with McFarland standard to enumerate cell number in the pellet. Approximately $1x10^6$ cells were inoculated per well with 200 µl of LB medium. Different concentrations (5, 10, 15, 20, 40, 60, 80, 100 ppm) of AgNP were added into the wells along with 10 µL of resazurin indicator solution. Resazurin dye (7-hydroxy-3H-phenoxazine-3-one 10-oxide) was broadly used as an indicator of cell viability in many cytotoxicity assays. The absorbance is recorded at 570 nm, and the IC50 value is determined.[15]

3. RESULTS AND DISCUSSION

3.1 *UV–Visible spectra analysis of the Bio-AgNPs*

The appearance of brownish color of the synthesized PE-AgCINPs and also the increase in the intensity of peak was because of the excitation of the surface plasmon resonance, that indicates the typical for AgNPs having ƛmax values that are in the visible range of 400 – 500 nm. The results were confirmed that the Ag+ ions have reduced into silver nanoparticles due to the fruit extract (Fig B). Similarly, several researchers have observed that the absorption spectrum of Ag nanoparticles is between the range of 425 and 460 nm due to surface plasmon resonance of the AgNPs.A.[16-18] There are many similar reports for UV-Visible spectra analysis of the silver nanoparticles.[9]

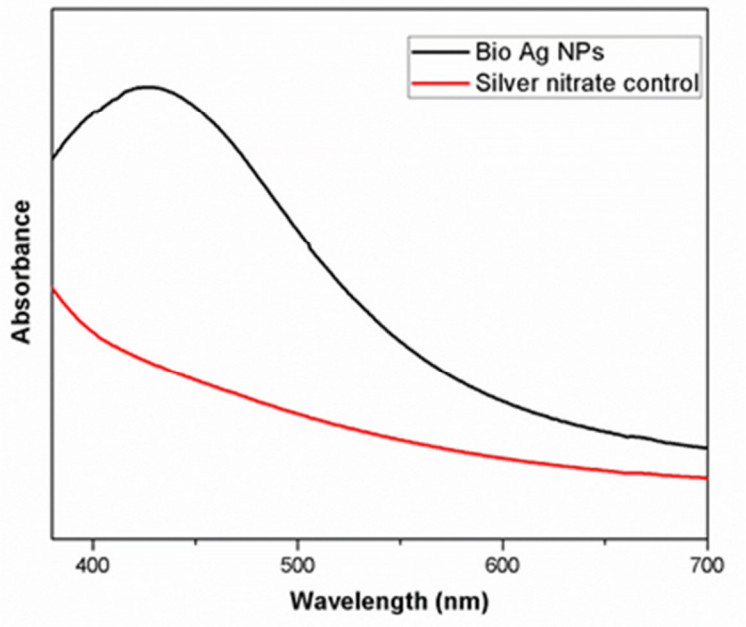


Fig B: UV – Vis Spectroscopic analysis of Bio-AgNPs and control Silver nitrate solution

3.2 *Fourier Transform Infrared (FTIR) Spectra analysis of Bio-AgNPs*

In the present work, FTIR spectral measurements were carried out in order to identify the potential biomolecules that were in the cannonball leaf extract and which was responsible for reducing and capping the bio-reduced silver nanoparticles. The figure shows the peak close to 3333 cm^{-1} because of the stretching vibrations of the –NH and –OH groups, also an

absorption band was at 2,910 cm^{-1} due to the –CH group. The weaker band that was at 1629 cm^{-1} corresponds to the amide I that was arising due to carbonyl stretch in the proteins. The spectra also showed a sharp and strong absorption band at the 1,631 cm^{-1} that assigned to the stretching vibration of the (NH) C=O group. The band at 670 cm-1 for AgNPs has been observed to be associated with bioreduction of the silver ions (Fig C). Further, it is observed that the flavonoids, polyphenols and other secondary metabolites present in the fruit extract acts as a reducing agent, which reduces Ag^{+} to Ag^{0} and the amino group as a stabilizing agent in the biosynthesis of AgNPs. Similar observations were reported by.[19,18] There are many similar reports for FTIR analysis of the silver nanoparticles.

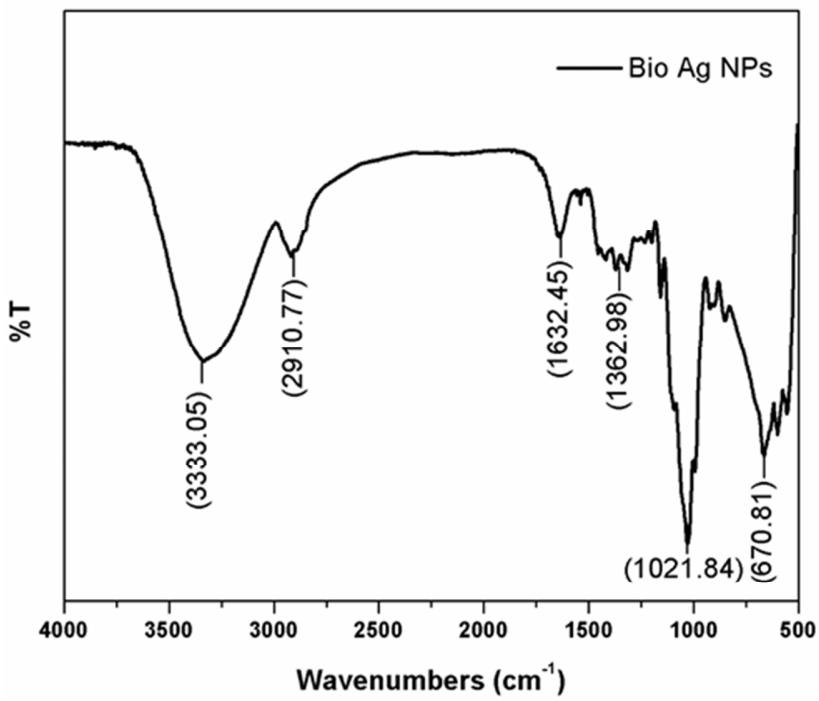
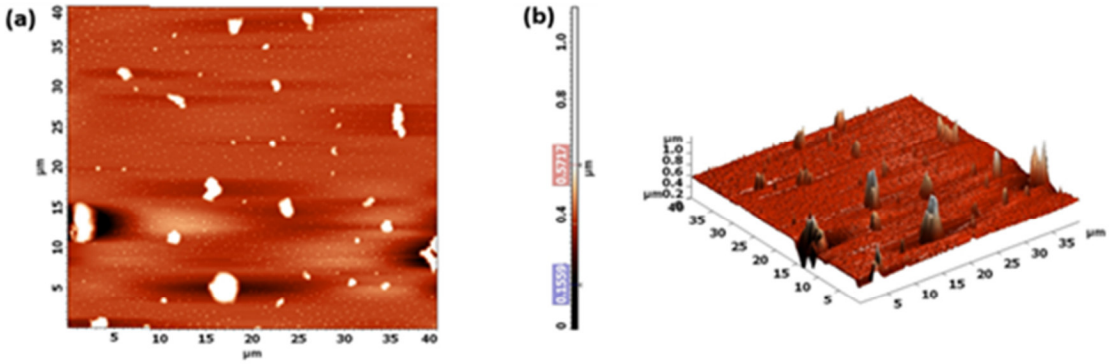


Fig C : FTIR analysis of Bio-AgNPs

3.3 *Atomic Force Microscopy (AFM) analysis of Bio-AgNPs*

The surface topology of the biologically synthesized SNPs was studied by AFM analysis. The AFM image of the Bio-AgNPs reveal the particles are being poly dispersed, having the size ranges from 40 to 70 nm (Fig D(a)). The raw data obtained from this AFM microscope is treated with a designed image processing software (NOVA-TX) to further exploit the three-dimensional (3D) image of the nanoparticles (Fig D (b)). The average size of the nanoparticles from the particular magnified area is analyzed by the NOVA-TX software i.e., 55 nm. Here the X-axis shows the size of the particles and Y-axis shows the number of particles (Fig D (c)). Similar results were reported for the synthesis of the silver nanoparticles by[20] *Punicagrantum* and *Carica* papaya fruits extracts respectively.



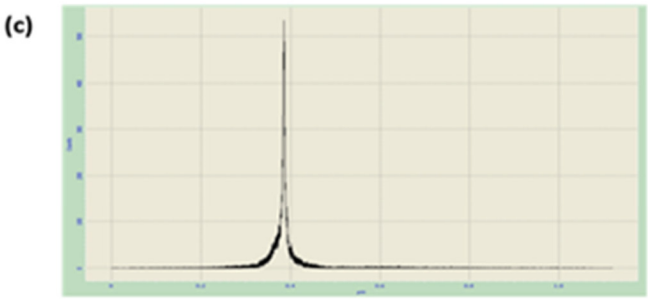


Fig D: Atomic force microscopy micrograph of Biosynthesized silver nanoparticles (AgNPs) (a), three-dimensional image and average particle size index of AgNPs analyzed by NOVA-TX software (b and C).

3.4 *X-ray diffraction (XRD) analysis of Bio-AgNPs*

The XRD pattern indicates that the structure of Ag-NPs is face-centered cubic. The XRD peaks were at 2θ of about 38.18°, 44.25°, 64.72°, and 77.40° and it could be attributed to the 111, 200, 220, and 311 crystallographic planes of face-centered cubic silver crystals, respectively. Diffractogram (Fig. E) is compared with the standard powder diffraction card of JCPDS, with that of silver file no. 04-0783. Further peaks at 2θ values in Bio-AgNPs pattern can be attributed to the residues of the organic contents of the fruit extract. From the XRD image it is clearly evident that the crystallization of some fruit extract metabolite moieties on the surface of the Ag NPs, which is in agreement with.[21] This is acceptable evidence to confirm the involvement of the fruit extract compositions in the formation of the silver nanoparticles. There are many similar reports for XRD analysis of the silver nanoparticles[9].

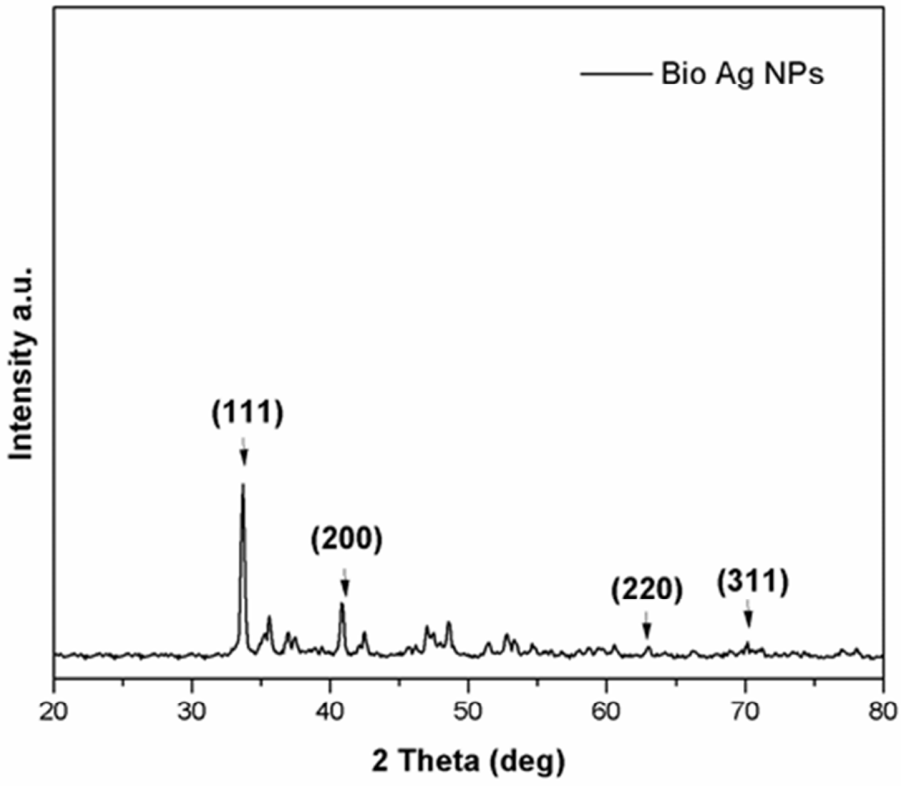


Fig E: X-ray diffraction pattern of Bio-AgNPs

3.5 *The High Resolution Transmission Electron Microscopy (HRTEM) analysis of Bio-AgNPs*

Size and the morphology of Bio-AgNPs were confirmed by HRTEM analysis. The morphology of synthesized particles were spherical in shape (Figure F a). Mean diameter of the crystalline C-Ag NPs was 12.6 ± 3.8 nm. The high magnification image shows the clear distribution and nucleation of the silver nanoparticles (FIG F b). The SAED pattern of the silver nanoparticles clearly shows that the particles were crystalline in nature and the results are in good agreement with the XRD results (Figure F c). Figure d shows the energy dispersive spectrum of the synthesized nanoparticles, that suggests the presence of silver as the ingredient element. The presence of elements such as Ag, C, and Cu are shown in the inset of Fig. F d. The presence of carbon and copper are due to the carbon

copper grid (sample holder) of the HR-TEM. The results were similar that was reported for the synthesis of the silver nanoparticles by[22] and [23] from *Prosopis farcta* and *Solanum mammosum* l fruits extracts respectively[9].

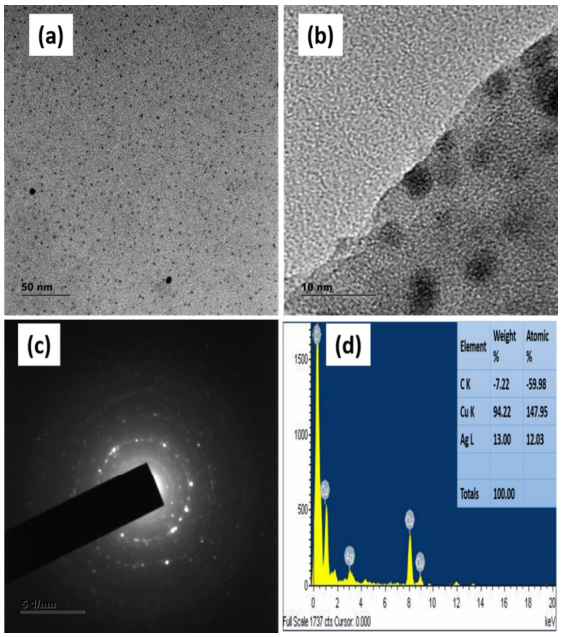


Fig F. The HRTEM image of Bio-AgNPs (a), its high magnification image (b), corresponding SAED (c) and EDS spectrum (d)

3.6 *Scanning Electron Microscopy (SEM) analysis of Bio-AgNPs*

Scanning electron microscopy of Bio-AgNPs were carried out to analyze the surface and the shape. The clusters of the nanoparticles were formed while performing the analysis and spherical NPs are being observed in SEM image (Fig G and b). The nanoparticles were found aggregated, and few individual particles were also observed. The elemental mapping of the AgNPs by SEM-EDS shows the presence of Ag as shown in Fig. G c. Strong signal of the peak was being observed and that proves typical for absorption of metallic silver nanoparticles. Thus, EDS results confirm the purity of the prepared nanoparticles. There are many similar reports for SEM analysis of the silver nanoparticles[9].

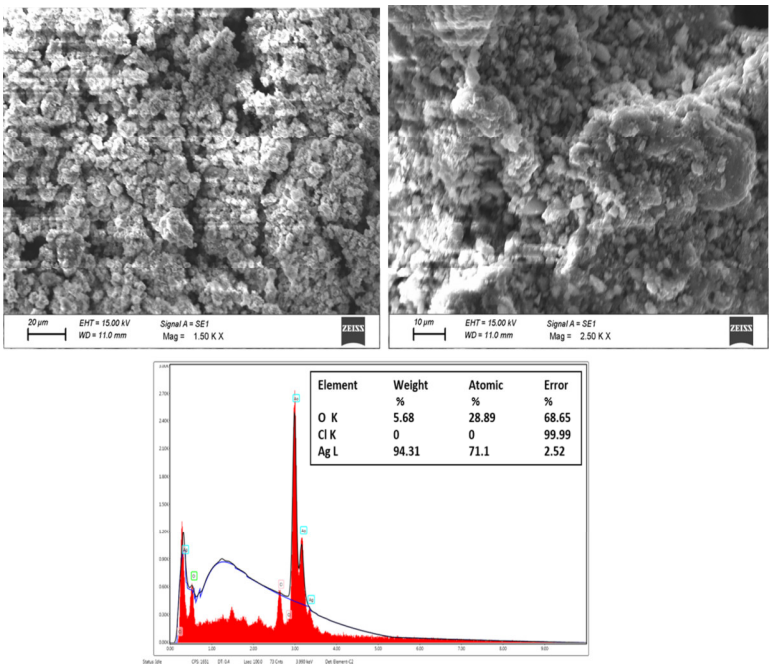


Fig G. SEM images of Bio-AgNPs (a and b) and its EDS spectrum (c)

3.7 *Antibacterial activity*

The results also show that the silver nanoparticles when treated with *E.coli* and *B. subtilis* inhibit cell growth. As given in the graph (Fig. H) with increase in the concentration of Bio-AgNPs from 5 to 100 ppm and there is a decrease in cell viability for both E. *coli* and B. *subtilis* which is a concentration dependent phenomenon. It was observed that more inhibition was observed for B. subtilis when compared to that of *E. coli.* The IC50 value of synthesized silver nanoparticles are found to be 15 ppm for *B.subtilis* and 20 ppm for *E.coli*. It is proposed that the reactive oxygen species by the silver particles can induce apoptotic pathways in bacteria which could ultimately kill the bacteria. There are many similar reports for the antibacterial activity of the silver nanoparticles[15]

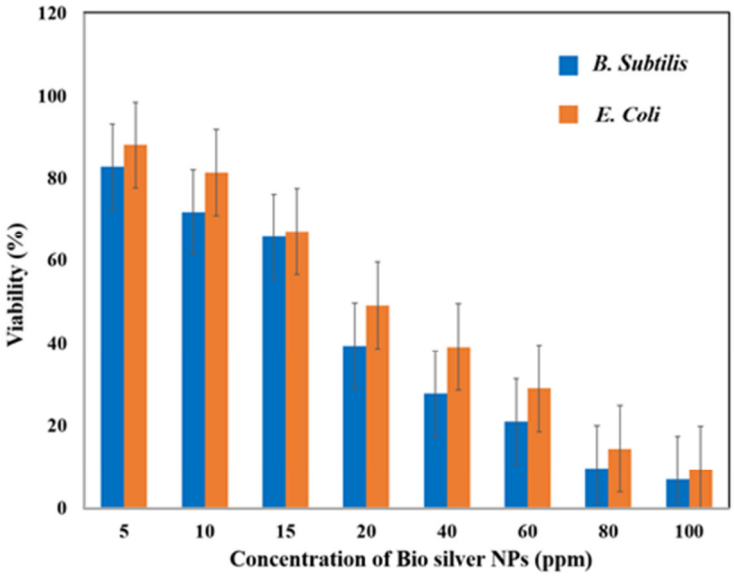


Fig H: Resazurin assay for antibacterial activity of *Bio-AgNPs*

The green synthesis of Bio-AgNPs using *Prunus persica* fruit extract was shown to be rapid, eco-friendly and cost-effective method. The synthesized AgNPs exhibited strong absorption between a maximum of 400–500 nm depending on the size and morphology of the resultant nanoparticles. The average particle size of synthesized Bio-AgNPs obtained using the green approach was found to be 12.6 ± 3.8 nm. XRD results confirmed that the AgNPs is a face-centered cubic crystalline structure. FTIR showed that the main possible absorbed compounds on the surface of the silver nanoparticles can be polyphenolic, flavonoids, and other secondary metabolites. Fruit extract of *prunus persica L* exhibited high antibacterial activity against B.subilis more than E-coli. Moreover, *prunus persica L* fruit extract mediated synthesized AgNPs have a valuable potential in the pharmaceutical application and these AgNPs could become an alternative to chemically synthesized silver nanoparticles.

4. CONCLUSION

In the summary, the study has demonstrated an eco-friendly, rapid, green approach to the synthesis of functional silver nanoparticles by the use of *Prunus persica* fruit extract, which supplies a simple, ecological and cost-effective method that lead to the synthesis of AgNPs.

5. AUTHOR CONTRIBUTION STATEMENT

Mrs.Shamina S conceptualized and gathered the data with regard to this work. Mrs.Shamina S, Dr.S.P.Suriyaraj and Mr.V.Sengiskhan, analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

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7. CONFLIT OF INTEREST

Conflict of interest declared none.

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