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Green Synthesis of PS-Ag/AgCl Nanomaterials and their Antibacterial Activity

Anil Kumar Gautam[1] and Gajanan Pandey[1,*]

[1]Department of Chemistry, Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India.
*Correspondence: pandeygajana@rediffmail.com

1.Abstract： **Last** few decade the nanomaterials were synthesized by green approach applied. It is a rapid, simple,one step, cost effective, eco-friendly and nonhazardous approach. Silver nanomaterials (PS-Ag/AgCl) using *Pistia stratiotes* aqueous leaves extract. The leaves extract acts as a reducing,stablising and capping agent. the compounds responsible for the reduction of silver salt, the functional groups present in leaves extract were explored by FT-IR (Fourier-Transform Infrared Spectroscopy) and aslo Various techniques used to characterize synthesized nanomaterials are spherical surface morphology and materials size (15nm-50nm) explored by HR-TEM (High-resolution Transmission Electron Microscopy) and UV-visible spectrophotometer showed an absorbance peak in the range of 420 nm, surface morphology explored by SEM (Scanning Electron Microscopy), elemental analyzed by EDX (Energy-dispersive X-ray spectroscopy), and Crystallinity and crystal lattice and nanomaterials are Ag/AgCl also explored by XRD (X-ray Powder Diffraction). The silver nanomaterials showed antibacterial activity against gram-negative (Escherichia-coli) Bacteria.As a result the nanomaterials (PS-Ag/AgCl) are safe and nontoxic.

Keywords: Nanomaterials; Anti-bactrial,Green Synthesis

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1. Introduction

In the last few decades, the green synthesis of nanomaterials achieved a pathway of an alternative approach to the synthesis of metallic nanomaterials with negligible formation of toxic waste products[1]. The noble-metal nanomaterial(e.g. gold, platinum, silver, and palladium, etc.)[2–5] have been synthesized by the green method which gives tremendous results regarding the product[6]. Synthesis approaches to the nanomaterials pathway are mainly divided into three types. First is the chemical, second, is physical and third is green approach methods. In the chemical and physical approach methods used high pressure, temperature, and hazardous chemicals. which affects nature as well as increases the cost of the process [7–9]To overcome these negative impacts researchers adopted the green approach method and used plant extract for the synthesis of metal nanomaterials[]. The green synthetic approach of metal nanomaterials utilizing micro-organisms bacteria, yeast, and fungi has been known but there are many demerits, including more purification steps and an intricate process of maintaining microbial growth [4,10–11].To remove these shortcomings, we used the *Pistia stratiotes* extract for the synthesis of AgNPs.

Metal nanomaterials have wide applications in different biological fields and are used in such as biomedicine, pharmaceutical industries, cosmetics industries, electronics, biosensors, catalysis, and optics. Many of the researchers have been reported especially in biomedical applications[12] like anticancer[13], antibacterial[14], and antioxidant[15–17]. These properties are due to the size (1-100nm) and Shape of metallic nanomaterials[18]. The shape and size of green synthesis metallic nanoparticles depend on the amount of salt, pH value, Temperature, process of timing, and contents in plant extract and are highly sensitive to the environment under which the process is going.

In the view of chemistry, The plant extract has functional groups such as hydroxyl (-OH), amine(-NH), etc, and many plant derivatives such as tannins, terpenoid saponins, starches, and polypeptides[19] and other heterocyclic compounds[20,21]. These functional groups of plant derivatives bind with metals and act as reducing and capping/stabilizing agents for the metal atom[6]. Capping agents also prevent agglomeration of the nanomaterials and reduce toxicity]. Green synthetic PS-AgNps are environment-friendly, cost-effective, negligible toxic, and negligible maintained in-process and enhance the application of the synthesis of metallic nanomaterials by using plant extract.

Many plants including *Azadirachtaindica*,[22] *Berberisvulgaris*[23], *Jatrophacurcas*[24] *Hypericumperforatum*[25], *Eriobotrya japonica*[26] and *Fumaricaparviflora*[27] has been used to synthesize silver nanoparticles for different pharmacological properties have been reported. The leaf and root of *Pistia stratiotes* could be used as a source of nutrients with antibacterial, antifungal,antidermatophytic, and toxicology [28]. It belongs to the Araceae family commonly known as jalkumbhi, water cabbage, and water lettuce which is a free-floating aquatic plant[29] and widespread in lakes and ponds in the tropic and subtropic regions. In this work, we synthesized PS-Ag/AgCl Nps by utilizing the plant extract of *Pistia stratiotes*. The capping enhances biochemical activity such as antibacterial activity, antioxidant, anticancer, and anti-inflammatory. Herein, confirmation of synthesized PS-Ag/AgCl Nps with the help of X-ray powder diffraction and UV-vis. analysis. Furthermore the shape and size with the help of HR-TEM, SEM, and functional groups with the FT-IR.

2. Experimental

2.1. Material

The silver salt ($AgNO_3$) analytical grade was bought from Sigma-Aldrich. *Pistia stratiotes* plant was collected from the university campus pound during February. During the synthesis and other studies, we used deionized water and ethanol for washing.

Escherichia coli strain AKS-1 16S ribosomal RNA gene, partial sequence GenBank: MK478816 was obtained from the Department of Environmental Microbiology, BabasahebBhimraoAmbedkar University Lucknow. The culture was maintained in their appropriate agar slants at 4^0C and used as stock culture.

2.2. Characterization of PS-AgNps

The growth of PS-Ag/AgCl Nps nanoparticles was synthesized by *Pistia stratiotes* leaves extract at room temperature and in normal conditions. High-resolution transmission electron microscope (HR-TEM) and scanning electron microscope (SEM) measure and characterize surface morphology, size, and crystalline nature of PS-Ag/AgCl Nps. Ultraviolet-visible spectroscopy (UV-Vis), and X-ray diffraction spectroscopy (XRD) data are used to evaluate the crystalline nature of PS-Ag/AgCl Nps and Fourier transform infrared spectroscopy (FTIR) is used to Explored the preparation of PS-Ag/AgCl Nps by the possible present functional group in plant extract to the reduction, stabilization, and capping of silver nanomaterials and EDS is used for the confirmation of contamination of Ag and other elements present in PS-AgNps.

2.3. Anti-bacterial Activity of PS-AgNps

To test the toxic effect of PS-Ag/AgCl Nps prepared by *Pistia stratiotes* leaves extract on Gram-negative (E. coli) bacteria, the growth inhibition analysis was conducted in Luria–Bertani (LB) broth media. The experiments were performed as last reported methods with few changes (M. Sathishkumar et al., 2009)[36]**.** For growth inhibition analysis, three well-plates, each containing 50 mL LB broth media and a sufficient amount of PS-Ag/AgCl Nps were inoculated with 50 mL of the freshly growed bacterial suspension to maintain essentially bacterial concentration in the same range in the well. The three well-plates were then incubated in a rotary shaker at 165 rpm at 37 ^{0}C. The growth of the pathogen was supervised every hour for 24 hours by the absorbance at 600 nm. A control experiment containing only media and bacteria bare of PS-Ag/AgCl Nps were also included.

3. Result and discussion

3.1. Green synthesis parameter

The leaves of the plant *Pistia stratiotes* were collected and washed three times with tap water and deionized water. The washed plant leaves were dried in sunlight for 2 hours to prepare the extract. A solution was made by boiling 20 g of dried leaves in 300 ml of deionized water for 2 hours, which was then filtered using Whatman filter paper. The synthesis of PS-Ag/AgCl Nps started with 150 ml of plant extract and 1.7 grams of silver nitrate salt. The plant extract solution was mixed drop by drop with silver salt in an Erlenmeyer flask and kept at room temperature until the color changed from colorless to black at 1200 rpm. The change in color within one hour indicated the reduction of Ag+ to Ag. The solution was sonicated for half an hour and rinsed with ethanol. The collected nanomaterials were placed on a petri plate and dried in a vacuum oven at 60°C for 24 hours. The dried silver nanomaterial (Ag/AgCl) was utilized for further study.

3.2. Characterization of PS-Ag/AgCl Nps

FTIR study

Plant extract of *Pistia stratiotes* playing as reducing and capping of silver metal and different functional groups of phytoconstituents compounds play an important role in the synthesis of silver nanomaterials which were characterized by FTIR spectroscope. A sharp peak at 3398 cm^{-1} is owing to the N-H stretching vibration of functional groups $-NH_2$ and O-H the overlapping of the stretching vibration of attribution for water and *pistia stratiotes* leaf extract[22]. 2933 cm^{-1} peak shows the primary and secondary amine of C-H stretching vibration[30]. The peak at 1630cm^{-1} agree with to amide C=O stretching[22]. The peak at 1066 cm^{-1} and 600 cm^{-1} shows the stretching of the phenol group [31]. Furthermore, peaks at 1376 cm^{-1} and 823 cm^{-1} agree with the -C-O- stretching involve phenol or tertiary alcohol. The explore peaks are mainly attributed to flavonoids, terpenoids, and polyphenols. phytoconstituents of plant extract have a strong affinity towards metal ions and a protective coat-like shell which prevents their further aggregation and stabilization of silver nanomaterials **Fig.1(A)**

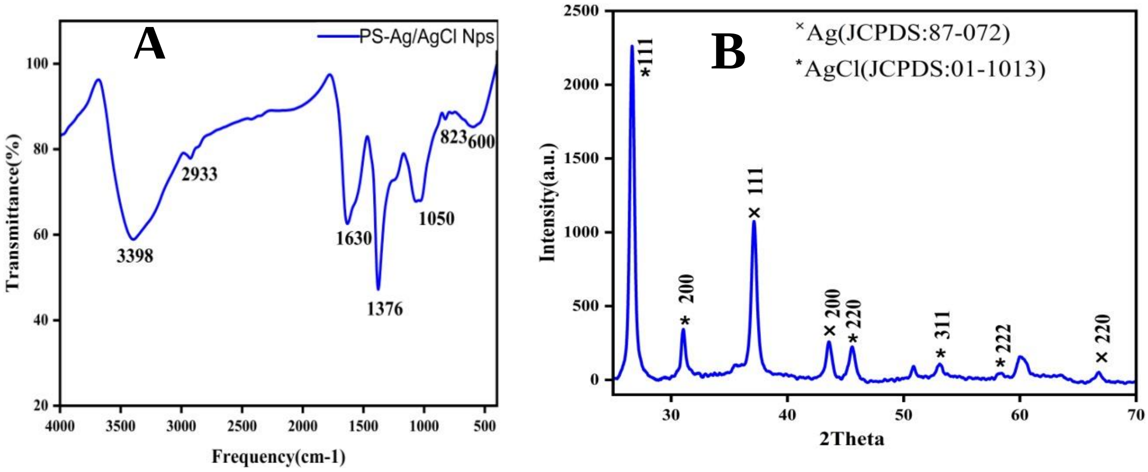


Figure 1. (A) FTIR and (B) XRD Data of Nanomaterials (PS-Ag/AgCl Nps).

XRD study.

XRD patterns of PS-Ag/AgCl Nps synthesized from the leaves extract of *Pistia stratiotes*. Several Bragg reflections with 2θ values of 26.63, 37.04, 37.11, 43.57, 45.57, 53.10, 58.36, 66.79, angle[32] corresponding to (111), (200), (111), (200), (220), (311), (222), and (220) planes respectively for PS-Ag/AgCl Np at 25˚C. Ag and AgCl nanomaterial peaks are present in **Fig.1(B).** It means Ag is present on the surface of the AgCl and also shows a face-centered cubic structure. The sharp peaks revealed the crystalline nature of PS-Ag/AgCl Nps were formed by leaves extract.

UV-Visible study

The formation of silver nanoparticles in colloidal solution has been confirmed by the using UV-visible analysis technique. Color change of silver nanoparticles from colorless aqueous solution to reddish-brown with the bioreduction. The change of color is due to surface Plasmon vibration in PS-Ag/AgCl Nps.The UV-visible spectra showed the appearance of different absorption maxima between 392nm-460nm [25]. figure no- shows the peak of approx 420nm this intense peak is shown to the formation of uniform spherical PS-Ag/AgCl Nps[25].

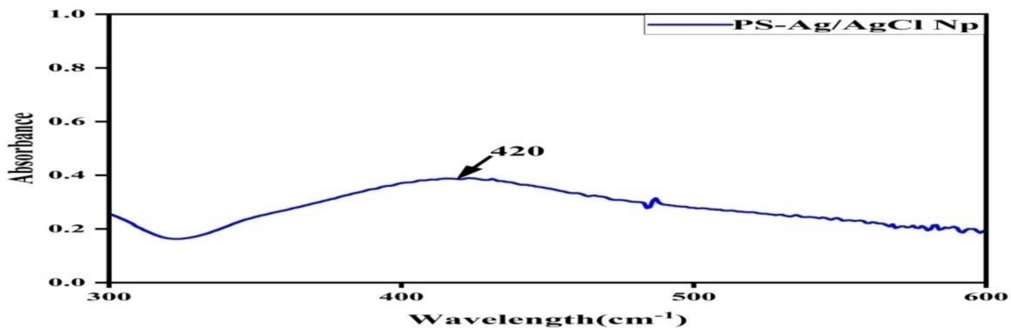


Figure 2. Electronic microscopy study(HR-TEM,EDX).

The HRTEM analysis has been used to Explore the size, shape, and surface morphology of silver nanomaterials[22]. The images are present in **Fig.3:(A,B,C)** . the image of biochemical synthesized nanomaterials (PS-Ag/AgCl Nps) represented that the particles are nanoscale and uniform. size range of nanoparticles is 15nm to 50nm With spherical shape. these findings are also confirmed by the other researchers[33–35]. Crystanillity and lattice pattern were explored by Saed image in **Fig.3(D)** .SEM image also explain the surface morphology of nanomaterials and EDX analysis explored the composition, purity and percentage of nanomaterials in **Fig.3: (E,F**) respectively. Silver matels more in compared to Chlorine . It is also explore that AgCl surface was covered by the Silver matels .

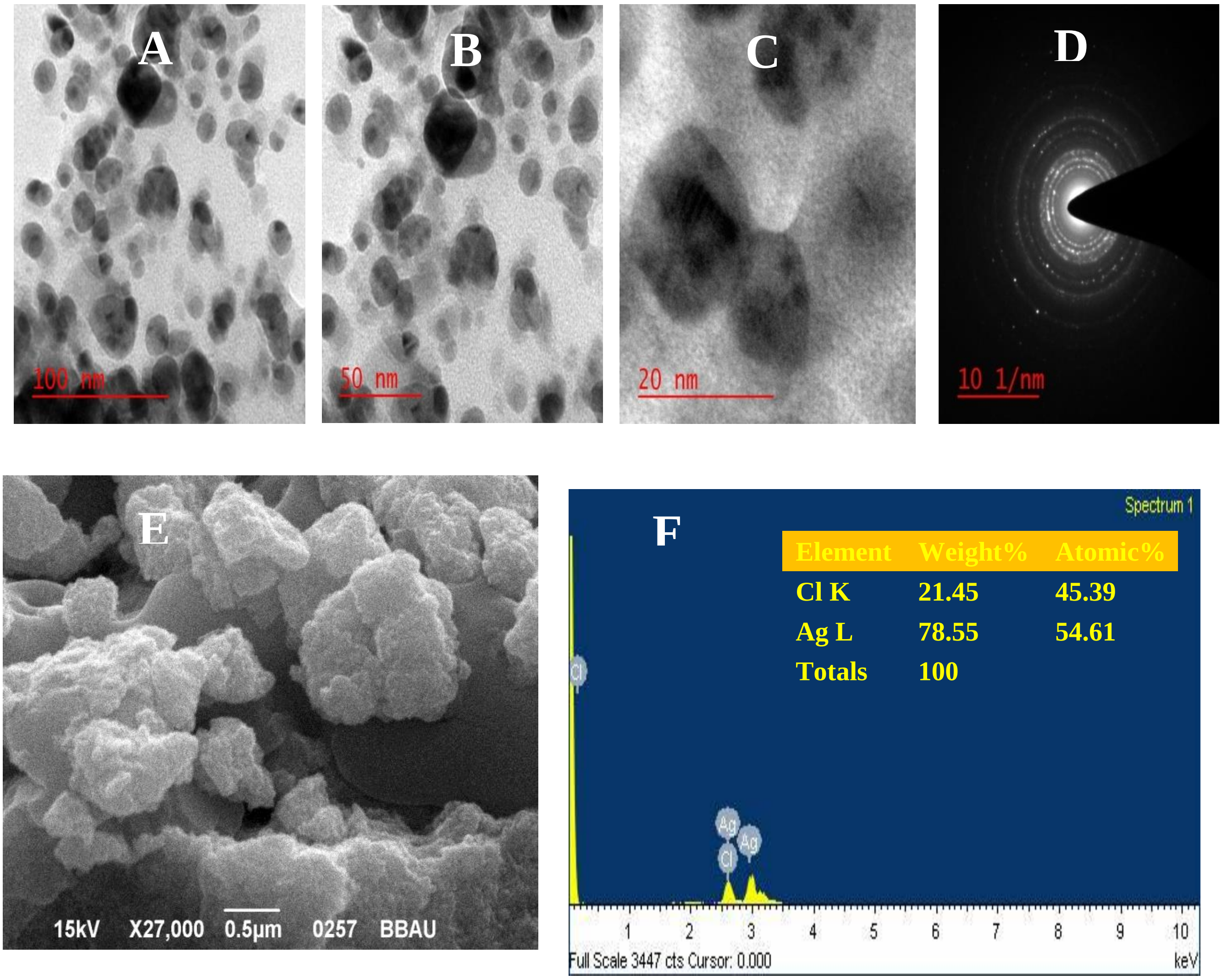
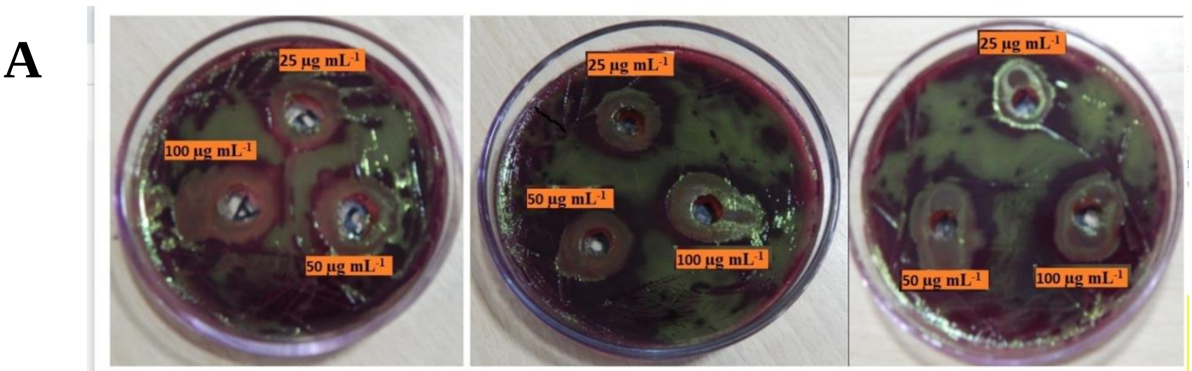


Figure 3. (A,B and C) HR-TEM Images and D Saed pattern of PS-Ag/AgCl Nps and (E) SEM image ,(F) EDX data.



B

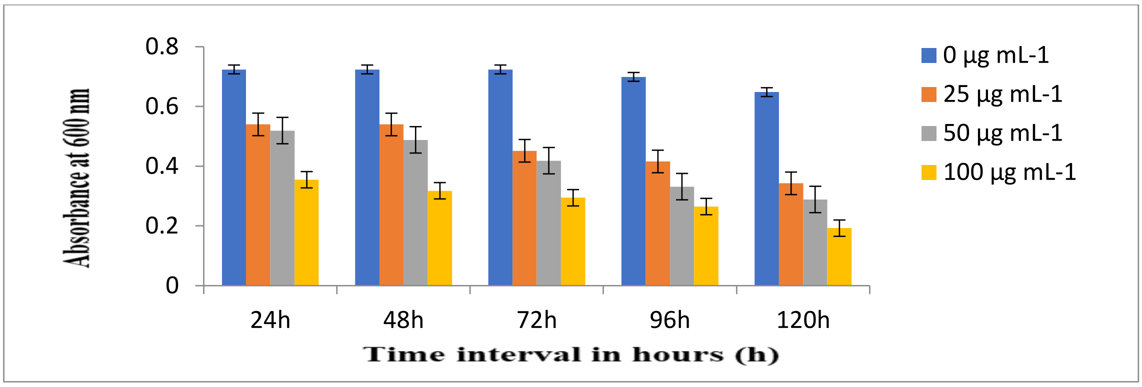


Figure 4. A) The antibacterial activity test of green synthesized PS-AgNps for *E. coli* and Graph **(B)**representing the growth inhibition of the *E. coli* bacteria at absorbance 600 nm during the period of 24 h to 120 h picture.

4. Anti-bacterial activities of Green synthesized PS-Ag/AgCl Nps

The gram-negative bacteria *E. coli* were finalized for evaluating the antibacterial activity of green synthesized silver nanomaterials(PS-AgNps). As shown in **Fig.4:(A),** the absorbance at 600 nm for the pathogen with different doses of silver nanomaterials was plotted over 120 hours. The anti-bacterial activity of silver nanomaterials against Bacteria Expored a potential dose-dependent manner. The growth inhibition was observed even in the minimum range of concentration at 25 $\mu g/ml^{-1}$ of silver nanomaterials. However, the maximum inhibition concentration (MIC) of as-prepared silver nanomaterials was at 100 $\mu g/ml^{-1}$ for *E. coli* bacteria that could be effectively inhibited within 120 h. After 50 $\mu g/\ ml^{-1}$ concentrations at 72hrs periods, the stationary phase was observed and after 120hrs bacterial cultures declined, so spectrophotometer reading was taken up to 120hrs. it has occurred due to cell wall damage or unfavorable conditions due to the *Pistia stratiotes* leaves extract nanomaterials anti-bacterial activity. However, it may be due to the silver nanomaterials being spherical with an average particle size of 15nm to 50nm. In addition, as per the report [37]. the silver nanomaterials synthesized by K¨UP and his coworkers11 using the leaves extract of *Aesculuship pocastanum* had a bigger average size of 50.0 -5.0 nm but Show stronger anti-bacterial effects for a Bacteria with MIC of 1.56 $\mu g/ mL^{-1}$ (Wei et al., 2020)[38]. It seems that the toxic effects of green synthesized silver nanomaterials for bacterial strains have an ambiguous relationship with the average size.

We have to observe different antibacterial activity in a result may be due to the capped constituent of the biosynthesized silver nanomaterials, it may lead to various mechanisms for the cell wall damage and toxic effect of the bacterial cells. It has been represented in **Fig.4 (B)** with absorbance at 600nm with different time intervals.The densities of the cell culture after the 120h time interval were also measured via scanning spectrophotometry analysis at 600 nm. As per the scanning results, in control (0$\mu g\ mL^{-1}$) flask has higher absorbance and decreased continuously by increasing the concentration of the silver nanomaterials in the flask in **Fig.5** It has represented the viability of the bacterial cultures at different stress conditions due to silver nanomaterials. However, the anti-bacterial activity of silver nanomaterials was also measured by Eosin methylene blue (EMB) agar petri plates via well diffusion assay has been represented in **Fig.4 (A)** The clear zone of inhibition has been noted in triplicate experiments with different concentrations of silver nanomaterials.

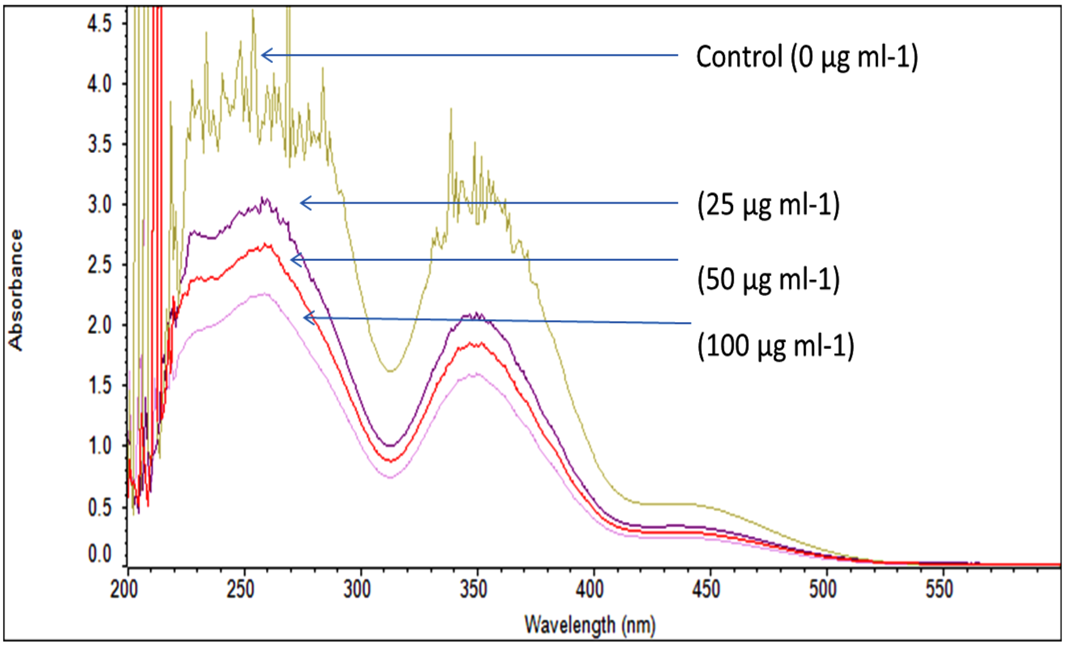


Figure 5. pectrophotometer scanning graph representing the decreased in absorbance by increasing wavelength after the treatment of PS-Ag/AgCl Nps at different concentration after 120 h of the exposure at 600 nm wavelength.

5. Conclusion

This study based on revealing the PS-Ag/AgCl Nps are eco-friendly, cost-effective, and comparatively less toxic was synthesized with the green approach by using *Pistia stratiotes* plant leaves. furthermore, the biological process is simpler and easier for downstream processing. these nanomaterials are spherical. No chemical reagent or any surfactant in this method to stabilize nanoparticles.PS-Ag/AgCl Nps have been confirmed by using FT-IR, UVis, HRTEM, SEM, EDX, and XRD analysis. the PS-Ag/AgCl Nps so prepared expressed effective antibacterial and suggested PS-Ag/AgCl Nps might be useful as a silver dressing for wounds or as an alternative material. PS-Ag/AgCl Nps are further studied to fully characterize the cytotoxicity.

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