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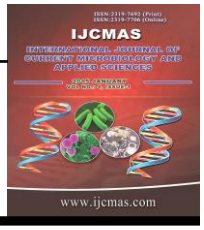

Original Research Article

Antibacterial activity and toxicological evaluation of sliver nanoparticles through toxtrak toxicity test

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Keywords

Antibacterial activity, *B. subtilis*, *E. coli*, Bio-AgNPs, CH-AgNPs, toxtrak toxicity test, resazurin dye and percentage inhibition (PI)

A B S T R A C T

The antibacterial activity of extracted biological synthesized sliver nanoparticles (Bio-AgNPs) were observed 0.027, 0.024, 0.018, 0.028 against *B. subtilis* and 0.023, 0.020, 0.015, 0.025 against *E. coli* for garlic, onion, papaya and apple extract respectively. For chemically synthesized sliver nanoparticles (CH-AgNPs) used as reference/control sample, the zone of inhibition was observed 0.033 and 0.029 against pathogens *E. coli* and *B. subtilis*. The above observation of antimicrobial activities was clearly indicated that the CH-AgNPs are suitable for inhabit the growth of photogenic bacteria with greater disadvantage of higher toxicity. On the other hand the Bio-AgNPs extract, apple on average equally inhabited the growth of pathogenic bacteria with lesser toxicity then garlic>onion>papaya. Bio-AgNPs was proved under UV-VIS absorption spectroscopy and observed plasmon peak maximum at 399nm, 400nm, 420nm and 410nm for garlic, onion, papaya and apple respectively. Toxicity of both Bio-AgNPs and CH-AgNPs was tested using toxtrak test to calculate toxic effect value percentage inhibition (PI). The PI of CH-AgNPs was much greater (85.45%) than the Bio-AgNPs synthesized from apple (55.70%), onion (51.39%), garlic (46.35%) and followed by papaya (33.59%). Hence, Bio-AgNPs is the best alternate of CH-AgNPs for AgNPs coating on drugs of pharmaceutical companies.

Introduction

Since the beginning of the twenty-first century, nanosilver has been gaining popularity and is now being used in almost every field, most importantly the medical field. However, there have been reports of how nanosilver cannot discriminate between different strains of bacteria and can hence destroy microbes beneficial to the ecology Allsopp et al., (2007). There are only very few studies conducted to assess the toxicity of nanosilver. *In vitro* toxicity assay of silver nanoparticles in rat liver cells has shown that even low-level exposure to silver nanoparticles resulted in oxidative stress and

impaired mitochondrial function Hussain et al., (2005). Previous research evidenced in *vivo* genotoxicity of nano-TiO2 in normalized after CHL co-administration which supports the oxidative stress as the possible mechanism for titanium toxicity Akmal et al., (2014).

The present study is based on synthesis and evaluating the percentage toxicity of chemically and biologically synthesized silver nanoparticles. Biologically synthesized silver nanoparticles are more biocompatible and friendlier to the nominal human body and own micro flora. They do not severely disturb it during the ingestion of drugs coated with nanoparticles such as sliver, titanium dioxide and gold etc. Micro flora of a human body normally contains *B. subtilis* which is a Gram-positive, catalase-positive and *E. coli* is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms) and constitute about 0.1% of gut flora Eckburg et al., (2005) . These gut flora of humans work as a probiotic in healthy individuals which, rarely causes food poisoning. It has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions, but still nanoparticles of sliver, gold and titanium dioxide etc. destroyed them and make own immunity week to weaker day by day.

Materials and Methods

Sample and Pathogens Collection

All chemicals used in this experiment were of the highest purity and obtained from Sigma and Merck. Garlic (*Allium* sativum) and Onion (*Allium cepa*) in vegetables and papaya (*Carica papaya)* and apple *(Malus domestica)* was used in fruits for the Bio-AgNPs collected from local market in 2013. The cultures of *B. subtilis* (gram positive) and *E. coli* (gram negative) were used to demonstrate the toxic effects of silver nanoparticles on gut microbial community probiotic collected from the microbiology laboratory of this Institute.

Preparation of the Extracts

100 gm of onion and garlic for vegetables and papaya and apple for fruits were ground separately to obtain the extracts. The extract was then mixed with 100ml deionized water in a conical flask and the mixture was boiled for 10 min. Extracts were filtered using Whatmann no-1 filter paper and filtrates were collected and centrifuged at 10000 rpm for 15 min at RT.

Biologically Synthesized Silver Nanoparticles (Bio-AgNPs)

$AgNO_3$ was used as precursor for synthesis of AgNPs 5 ml of 0.75 mM $AgNO_3$ aqueous solution was added to 100 ml of clear vegetable and fruit extract (Supernatant). Then, the conical flask containing the solution was put into a shaker (150 rpm) at 30°C for 72 h. In this process, the vegetable and fruit extracts acts as the reducing and stabilizing agent P.K. Tyagi et al., (2012). Silver nanoparticles were obtained gradually by the erosion and chemical degradation of plant extracts.

Chemically Synthesized Silver Nanoparticles (CH-AgNPs)

Thirty-eight millimeter tri sodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 0.75 mm $AgNO_3$ were used for CH-AgNPs. 50 ml aqueous $AgNO_3$ was taken and boiled up to 70 to 80°C and was mix to 10 ml tri sodium citrate in a drop wise method. The solution was continuously stirred through magnetic stirrer for 4 to 5 min. After proper mixing, the solution was then incubated at 30°C for

45 min. Silver nanoparticles were obtained gradually by the erosion and chemical degradation.

Characterization techniques of Ag Nanoparticles

In this research the UV-Vis Spectroscopy, FTIR analysis, SEM and TEM analysis characterization tool and techniques were used.

UV-VIS Spectroscopy

The Ag nanoparticle was characterized in a JASCO-V-530, UV-VIS spectrophotometer, to know the kinetic behavior of silver nanoparticles. The scanning range for the samples was 280-700 nm at a scan speed of 400 nm/min. Base line correction of the spectrophotometer was carried out by using a blank reference.

The UV-Vis spectra analysis of AgNPs of all the samples was recorded. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 h after diluting a small aliquot of the sample into distilled water. The color change in reaction mixture solution was recorded through visual observation which showed bioreduction of silver ions in aqueous solution.

FTIR analysis

Freeze-drying or lyophilized samples was used for FTIR studies of AgNPs in which performed to characterize the chemical structure of nanoparticles. FTIR spectra of AgNPs synthesized from Papaya and Apple extract only.

SEM analysis

ZEISS EVO series scanning electron microscope EVO 50 machine was used to characterize morphology of nanoparticles. The ZEISS EVO 50 is a versatile analytical microscope with a large specimen chamber. Freeze dried samples of Ag NPs solution were sonicated with distilled water; small drop of this sample was placed on glass slide allowed to dry. The accelerating voltage of the microscope was kept in the range 0.2 - 30 kV.

TEM analysis

Transmission electron microscopy (TEM) analysis of the sample was done in IIT Delhi. A drop of the solution was placed on carbon-coated copper grid and later exposed to infrared light (45 min) for solvent evaporation.

Toxicity estimation

Toxicity of sliver nanoparticles was tested using toxtrak test to calculate percentage inhibition (PI). PI is only a relative measure and since there is toxic substances that increase respiration, to give result to a negative number. The PI of both chemically and biologically synthesized silver nanoparticles was compared in order to evaluate toxic effect value.

Toxtrak test and Resazurin dye

Toxtrak test was used to estimate the toxicity of chemically and biologically synthesized silver nanoparticles. Take a two group of six test tubes of broths for 48 hr containing *B. subtilis* and *E. coli* culture respectively. For both groups the first one test tube was marked as control, second test tube for both groups were incubated with 1 ml of each chemically synthesized silver nanoparticle and rest of four test tube were incubated with 1 ml of each biologically synthesized silver nanoparticles synthesized from apple, onion, garlic and papaya

respectively. The concentration of the silver nanoparticles ranges from 25 to 50 µg/ml in both solutions. Resazurin dye was added in the volume of 40 µl per test tube and incubated for 0 to 4 hr. The absorption was recorded just after adding the dye (0 h) in all the six test tubes of both groups. Absorption was recorded after every 1 hr intervals up to 4 hr. The presence of toxicity of both in CH-AgNPs and Bio-AgNPs estimated to calculate the decreasing rate of absorbance (degradation) this decreases is indicated the reduction of resazurin dye. These changes/differences (decrease) in absorbance (Last absorbance / initial absorbance) are measured by the changes in absorbance of the sample as compared to a control sample.

The Resazurin offers a simple, rapid and sensitive measurement for the viability of mammalian cells and bacteria. Living cells are metabolically active and are able to reduce the non-fluorescent dye resazurin to the strongly-fluorescent dye resorufin. The fluorescence output is proportional to the number of viable cells over a wide concentration range. This also allows the calculation of the proliferation rate for cells capable of consecutive cell division. Early scientist used resazurin dye to quantify bacterial content in milk Pesch et al., (1929). It was introduced commercially initially under Alamar Blue trademark (Trek Diagnostic Systems, Inc), and now also available under other names such as AB assay, Vybrant (Molecular Probes) and UptiBlue (Interchim). Usually, resazurin dye is used for bacterial toxicity in milk quality, but in this research we used resazurin to estimate the toxicity of sliver nanoparticles.

Antimicrobial Activity

The antibacterial activity assays were done on human gut microflora *B. subtilis E. coli* by standard disc diffusion method V. Sarsar et al., (2013). Muller Hinton Agar plates were prepared onto which antimicrobial assays were monitored. Fresh overnight cultures of inoculums of each culture were spread on to Muller Hinton agar plate. The sterile discs approximately 5mm in diameter were placed on Mueller Hinton agar plates treated with biosynthesized nanoparticles and chemically synthesized nanoparticles. The discs were then placed over the swabbed Muller Hinton agar plates and incubated at 37°C for overnight. After 24 h of incubation the zone of inhibition was investigated.

Results and Discussion

Reduction of Ag ion into silver particles during exposure to the plant extracts from garlic and onion supernatants could be followed by color change. The plant extract from garlic and onion supernatants were pale yellow before the addition of silver ions and this changed to a brownish color on completion of the reaction with ions. The result obtained in this investigation is very interesting in terms of identification of potential extract for synthesizing the sliver nanoparticles.

UV-Vis Spectroscopy characterization of AgNPs

UV Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. A surface plasmon peak of Bio-AgNPs was located at 399-nm with absorption of 1.16, 400-nm with absorption of 0.23, 420-nm with absorption of 0.90, 410-nm with absorption of 2.66 indicates the presence of nanoparticles in garlic, onion, papaya and apple respectively (Figure 2 & 3). On the other hand the CH-AgNPs, the solution turns transparent golden brownish which

shows the presence of silver nanoparticles and on performing UV Vis spectrophotometer, the absorption peak was observed at 398 nm with absorption at 0.24 (Figure 1).

FTIR analysis of the nanoparticles samples

FTIR analysis of the freeze-dried samples (apple & papaya) was carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of silver nanoparticles. The amide linkages between amino acid residues in proteins give rise to well known indications in the infrared region of the electromagnetic spectrum. FTIR spectrum reveals two bands at 1394 and 1326 cm_1 that correspond to the bending vibrations of the amide group bands of the proteins respectively. The amide group bands corresponding stretching vibrations were seen at 3517 and 3149 cm_1 respectively (Figure 4 & 5). The presence of these indications of peaks of amino acids supports the presence of proteins in cell-free filtrate as observed in UV-Vis spectra. It is well known that protein nanoparticles interactions can occur either through free amine groups or cysteine residues in proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes Alt et al., (2005). The two bands observed at 1500 and 1255 cm_1 can be assigned to the C-N stretching vibrations of the aromatic and aliphatic amines, respectively Morley et al., (2007). These observations indicate the presence and binding of proteins with silver nanoparticles which can lead to their possible stabilization. FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with silver nanoparticles. The finding of FTIR indicates that it is not just the size and shape of proteins, but the conformation of protein molecules that plays an important role for the formation of nanoparticles.

Scanning Electron Microscope (SEM) analysis of the nanoparticles samples

Freeze dried samples of Ag NPs solution were sonicated with distilled water; small drop of this sample was placed on glass slide allowed to dry. The accelerating voltage of the microscope was kept in the range 0.2 - 30 kV. The SEM micrograph shows biosynthesized silver nanoparticles aggregates. In this micrograph observed spherical nanoparticles in the size range 50-150-nm with highly dense silver nanoparticles. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Figure 6&7).

Transmission Electron Microscopy (TEM) analysis of the nanoparticles samples

A drop of the solution was placed on carbon-coated copper grid and later exposed to infrared light (45 min) for solvent evaporation. TEM provide further insight into the morphology and particle size distribution profile of the AgNPs and revealed pattern similar to the biosynthesized AgNPs characterized using TEM. The data obtained from transmission electron- micrograph showed distinct shape and size of nanoparticles. The particle were spherical in shape in the range of 5~100nm and uniformly distributed without significant agglomeration. The largest size found was 80nm (Figure 8&9).

Toxicity Estimation

Toxicity estimation of both in CH-AgNPs and Bio-AgNPs via toxtrak test and the

absorbance of control, CH-AgNPs and Bio-AgNPs synthesized from apple, onion, garlic and papaya is carried out at a wavelength of 603 nm, which is specific for the blue color. The percentage inhibition (PI) is expressed equation is as follow:

$$PI = [1- (\Delta As / \Delta Ac)] \times 100$$

In this PI equation, the ΔAs and ΔAc represent the changes/differences (decrease) in absorbance for the sample and the control, respectively. In this case, Δ is the initial-final value. The PI is a relative measure only, in which the presence of toxic substances that increase the respiration and the results of PI equation were observed in a negative number.

Table 1 contains five groups of values in which one PI of CH-AgNPs sample and four PI of Bio-AgNPs samples. Absorptions of control and various broths samples were treated with chemically (A_{CH}) and biologically (A_{BIO}) synthesized silver nanoparticles. To determine PI value, first the changes/differences (decrease) in absorbance for the control (ΔAc), CH-AgNPs (ΔA_{CH}) and Bio-AgNPs (ΔA_{BIO}) value of decrease were calculated. The value of decrease was substituted in PI Equation, to finally get the toxicity percentage.

Calculate changes/differences (decrease) in absorbance

a) For control sample: **ΔAc** = 0.2163-2.9314 = **-2.7151**

b) For **CH-AgNPs** sample: **ΔA_{CH}** = 1.5962 - 1.9914 = **-0.3952**

c) For **Bio-AgNPs** sample 1 (**Garlic**): **ΔA_{BIO1}** = 1.2823 - 2.6021 = **-1.3198**

d) For **Bio-AgNPs** sample 2(**Onion**): **ΔA_{BIO2}** = 1.6401 – 3.0969 = **-1.4568**

e) For **Bio-AgNPs** sample 3(**Papaya**): **ΔA_{BIO3}** = 1.1502 - 2.9631 = **-1.8029**

f) For **Bio-AgNPs** sample 4 (**Apple**): **ΔA_{BIO4}** = 1.2930 - 2.4956 = **-1.2026**

Calculate the toxicity percentage

To estimate the sliver nanoparticles toxicity by putting the final value of changes/differences (decrease) of ΔAcs, ΔA_{BS} and ΔAc in PI Equation ($PI = [1- (\Delta As / \Delta Ac)] \times 100$)

Toxicity of CH-AgNPs sample

This is to calculate the **CH-AgNPs** toxicity for putting the final changes/differences (decrease) of ΔA_{CH} and ΔAc in PI Equation. Here, ΔAs is A_{CH}, so the PI Equation is:

$PI = [1- (\Delta A_{CH} / \Delta Ac)] \times 100$
$PI = [1-(-0.3952 / -2.7151)] \times 100$
PI = 85.45%

Toxicity of Bio-AgNPs sample

This is to calculate the **Bio-AgNPs** toxicity for putting the final changes/differences (decrease) of ΔA_{BIO} and ΔAc in PI Equation. Here, ΔAs are ΔA_{BIO}, (ΔA_{BIO1} for **Garlic,** ΔA_{BIO2} for **Onion**, ΔA_{BIO3} for **Papaya** and ΔA_{BIO4} for **Apple**).

PI Equation for Bio-AgNPs sample 1 **(**ΔA_{BIO1} **Garlic)**:
$PI = [1- (\Delta A_{BIO1} / \Delta Ac)] \times 100$
$PI = [1-(-1.3198/-2.7151)] \times 100$
PI = 51.39%
PI Equation for Bio-AgNPs sample 2 **(**ΔA_{BIO2} **Onion)**:
$PI = [1- (\Delta A_{BIO2} / \Delta Ac)] \times 100$

PI = [1-(-1.4568/-2.7151)] × 100
PI = 46.34%
PI Equation for Bio-AgNPs sample 3 **(**ΔA_{BIO3} **Papaya**):
PI = [1- (ΔA_{BIO3} / ΔAc)] ×100
PI = [1-(-1.8029/-2.7151)] × 100
PI = 33.59%
PI Equation for Bio-AgNPs sample 4 **(**ΔA_{BIO4} **Apple**):
PI = [1- (ΔA_{BIO4} / ΔAc)] ×100
PI = [1-(-1.2026/-2.7151)] × 100
PI = 55.70%

The aforementioned data clearly indicates that the toxic effect value, PI of CH-AgNPs is much greater (85.45%) than the Bio-AgNPs synthesized from apple (55.70%), onion (51.39%), garlic (46.35%) and followed by papaya (33.59%). These observations show that the human gut microbial community probiotic bacteria *B. subtilis* killed by CH-AgNPs in a maximum percentage as compare to Bio-AgNPs. Toxicity percentage of Bio-AgNPs are observed in decreasing order apple > onion >garlic> papaya.

Antimicrobial activity

The antimicrobial activity as observed for Bio-AgNPs from both the samples of vegetables (garlic and onion) and fruits (papaya and apple) and their comparison with CH-AgNPs (reference/control sample) to confirm the presence/absence of silver nanoparticles. Antimicrobial activities of the synthesized Ag nanoparticles were determined, using the disc diffusion method [21]. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. (Table 2) were used as positive controls. The plates containing the test organism and Bio-AgNPs/ CH-AgNPs were incubated at 370C for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimetre.

The anti microbial activity of extracted Bio-AgNPs from garlic, onion, papaya and apple of concentration 50mg/ml were checked against pathogens *E. coli* and *B. subtilis* using disk diffusion method and the zone of inhibition (m diameter) was observed 0.027, 0.024, 0.018, 0.028 against *B. subtilis* and 0.023, 0.020, 0.015, 0.025 against *E. coli* for garlic, onion, papaya and apple extract respectively. For CH-AgNPs (reference/control sample) the zone of inhibition was observed 0.033 and 0.029 against pathogens *E. coli* and *B. subtilis*.

The above observation of antimicrobial activities was clearly indicated that the CH-AgNPs are more suitable for inhabit the growth of photogenic bacteria with disadvantage of higher toxicity. On the other hand the Bio-AgNPs apple extract on average equally inhabit the growth of pathogenic bacteria with very less toxicity then garlic>onion>papaya.

On the basis of percentage inhibition results that Bio-AgNPs are more friendly and biocompatible to human gut microbial community probiotic as compare to CH-AgNPs. On the other hands we can say that the CH-AgNPs are more suitable for inhabit the growth of photogenic bacteria but greater disadvantage of their toxicity while, Bio-AgNPs on average equally or slightly lesser inhabited the growth of pathogenic bacteria with less toxicity. Our findings fulfill the results of previous studies in which clearly indicated, that the CH-AgNPs

are more toxic as compare to Bio-AgNPs from plants of *Allium spp.* P.K. Tyagi et al., (2013). The biologically synthesized AgNPs was less toxic, biocompatible and friendlier to the nominal human gut micro flora and do not severely disturbs it during the ingestion of drugs containing silver nanoparticles as compare to chemically synthesized AgNPs P.K. Tyagi et al., (2013).

Hence, Bio-AgNPs is the best option for pharmaceutical company for sliver coating on drugs as compare to CH-AgNPs due to their less toxicity and friendly behavior of probiotic present in our gut in the form of microbial gut flora. The unique physical and chemical properties of silver nanoparticles make them excellent candidates for a number of day-to-day activities, and also the antimicrobial and anti-inflammatory properties make them excellent candidates for many purposes in the medical field. However, there are studies and reports that suggest that nanosilver can allegedly cause adverse effects on humans as well as the environment. It is estimated that tonnes of silver are released into the environment from industrial wastes, and it is believed that the toxicity of silver in the environment is majorly due to free silver ions in the aqueous phase.

The adverse effects of these free silver ions on humans and all living beings include permanent bluish-gray discoloration of the skin (argyria) or the eyes (argyrosis), and exposure to soluble silver compounds may produce toxic effects like liver and kidney damage; eye, skin, respiratory, and intestinal tract irritations; and untoward changes in blood cells Panyala et al., (2008). This investigation provides evidence that plant extract-stabilized nanoparticles may be ideal candidates for future studies exploring their use in biomedical and pharmacy applications. This synthesis procedure offers a less cost-effective and green alternative to traditional protocols that may be readily scaled up for industry as a result of the low synthesis temperatures and time required.

Table.1 Absorption readings of control, various broths treated with chemically and biologically synthesized (garlic, onion, papaya and apple) silver nanoparticles

Incubation period with dye (h)	**Test tube 1 Control (ΔAc)**	**Test tube 2 CH-AgNPs (ΔA_{CH})**	**Test tube 3 BIO-AgNPs Garlic (ΔA_{BIO1})**	**Test tube 4 BIO-AgNPs Onion (ΔA_{BIO2})**	**Test tube 5 BIO-AgNPs Papaya (ΔA_{BIO3})**	**Test tube 6 BIO-AgNPs Apple (ΔA_{BIO4})**
0	2.9314	1.9914	3.0969	2.6021	2.9531	2.4956
1	2.5528	1.8327	3.0000	2.5686	2.7356	2.2560
2	2.0489	1.7375	2.7447	2.2518	2.3040	1.9502
3	1.0041	1.6049	1.8794	2.0757	1.7620	1.4625
4	0.2163	1.5962	1.6401	1.2823	1.1502	1.2930

Table.2 Antibacterial activities zone of inhibition (m diamenter) of B. subtilis and E. coli against CH-AgNPs and BIO-AgNPs

Bioactive agent	Concentration	Zone of Inhibition (m diameter)	
		B. subtilis	*E. coli*
CH-AgNPs	**1 mM**	**0.033**	**0.029**
BIO-AgNPs Garlic (ΔA_{BIO1})	1 mM	0.027	0.023
BIO-AgNPs Onion (ΔA_{BIO2})	1 mM	0.024	0.020
BIO-AgNPs Papaya (ΔA_{BIO3})	1 mM	0.018	0.015
BIO-AgNPs Apple (ΔA_{BIO4})	1 mM	0.028	0.025

Figure.1 Ultraviolet-visible spectrum (UV-Vis) of chemically synthesized sliver nano particles (CH-AgNPs)

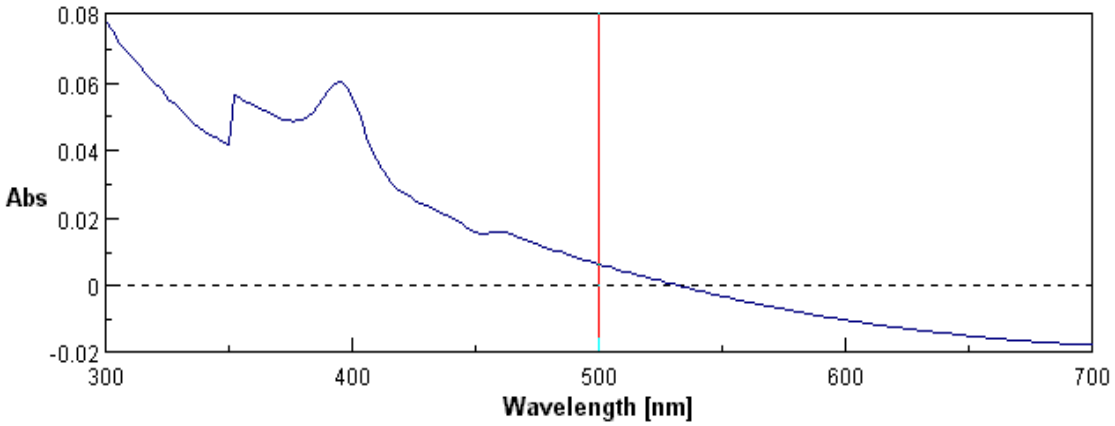
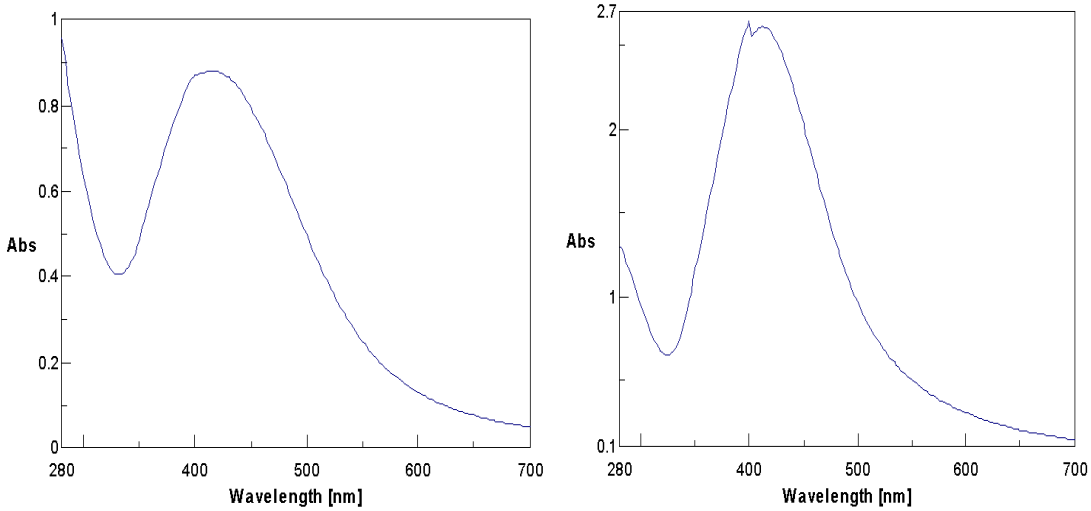


Figure.2 Ultraviolet-visible spectrum (UV-Vis) of biologically synthesized sliver nano particles (Bio-AgNPs) synthesized from apple and Papaya extract after 6 hours.



Apple **Papaya**

Figure.3 Ultraviolet-visible spectrum (UV-Vis) of silver nanoparticles synthesized from Garlic and Onion extract after 6 hours

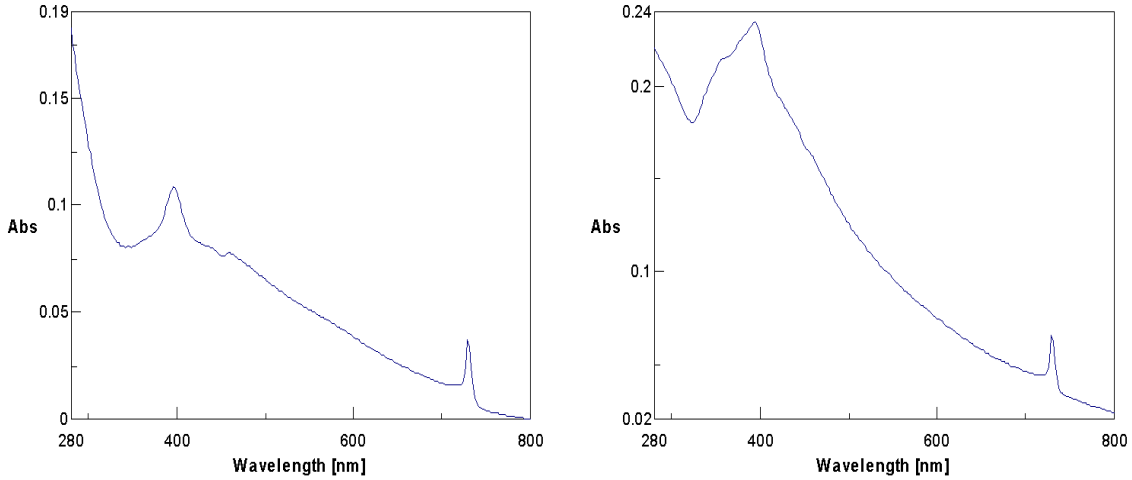


Figure.4 FTIR spectra recorded from powder of silver nanoparticles synthesis from Papaya

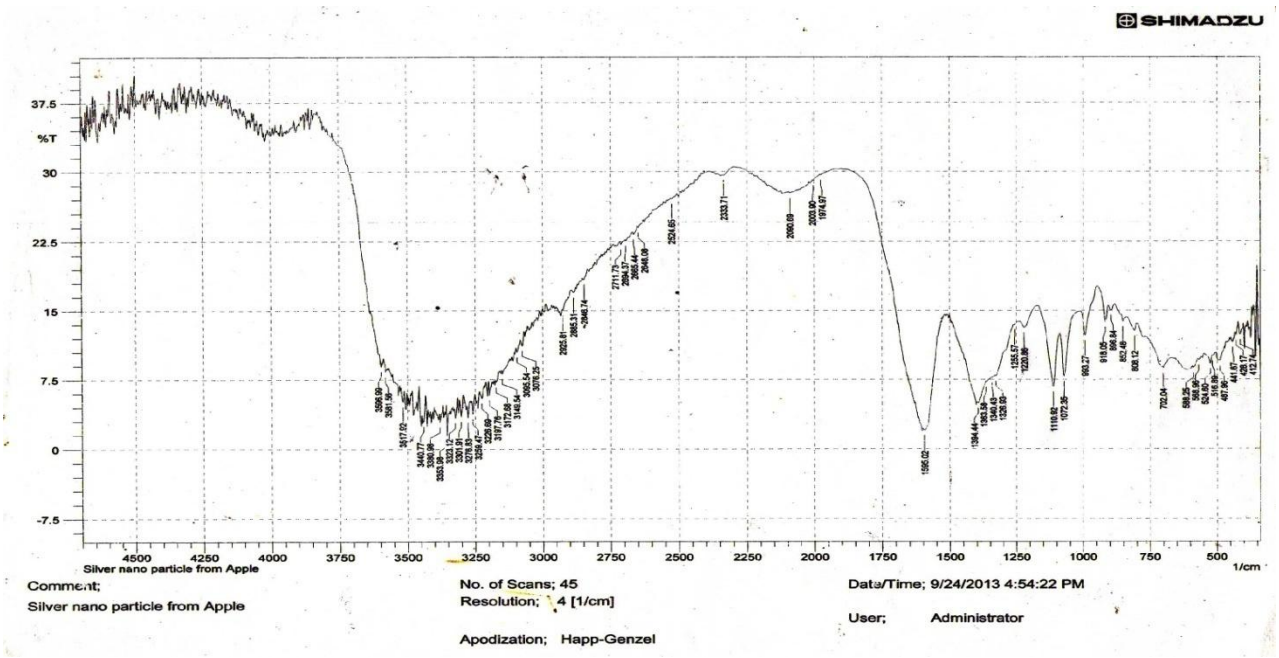


Figure.5 FTIR spectra recorded from powder of silver nanoparticles synthesis from apple

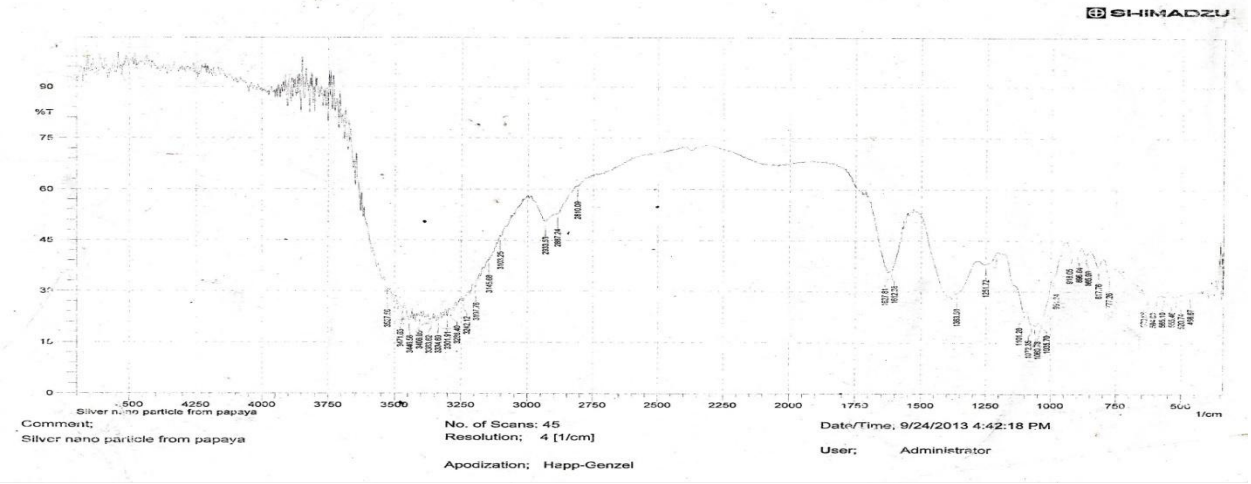


Figure.6 Scanning electron micrograph of silver nanoparticles synthesis from apple

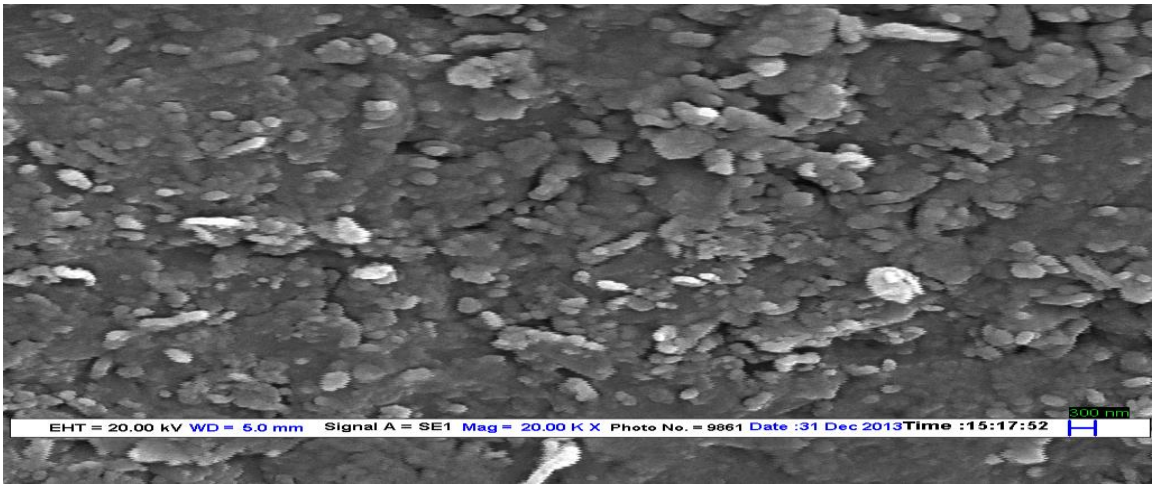


Figure.7 Scanning electron micrograph of silver nanoparticles synthesis from papaya

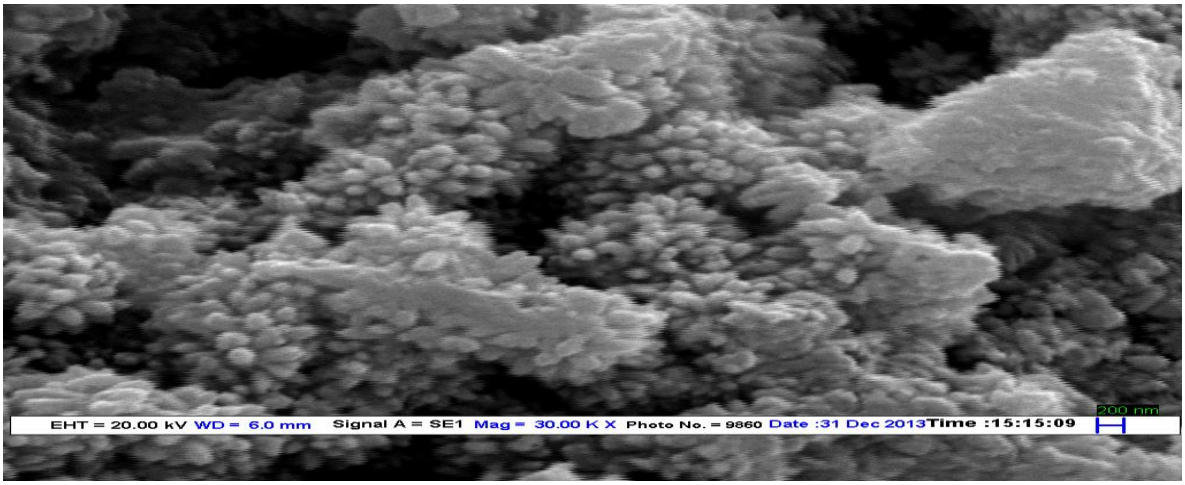


Figure.8 Transmission electron micrograph of the silver nanoparticles synthesized by apple

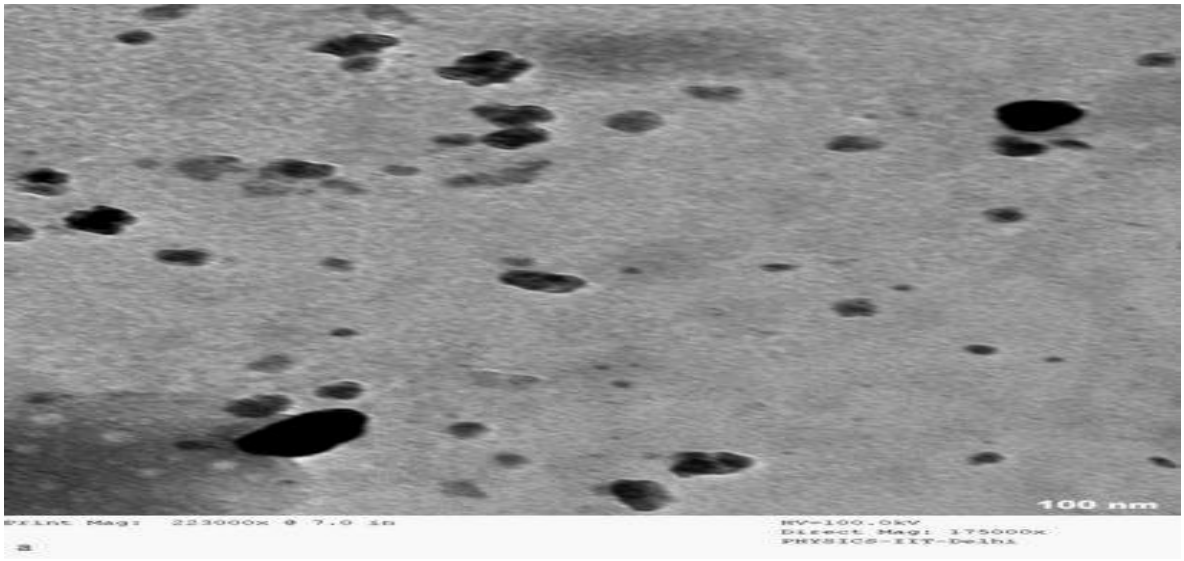
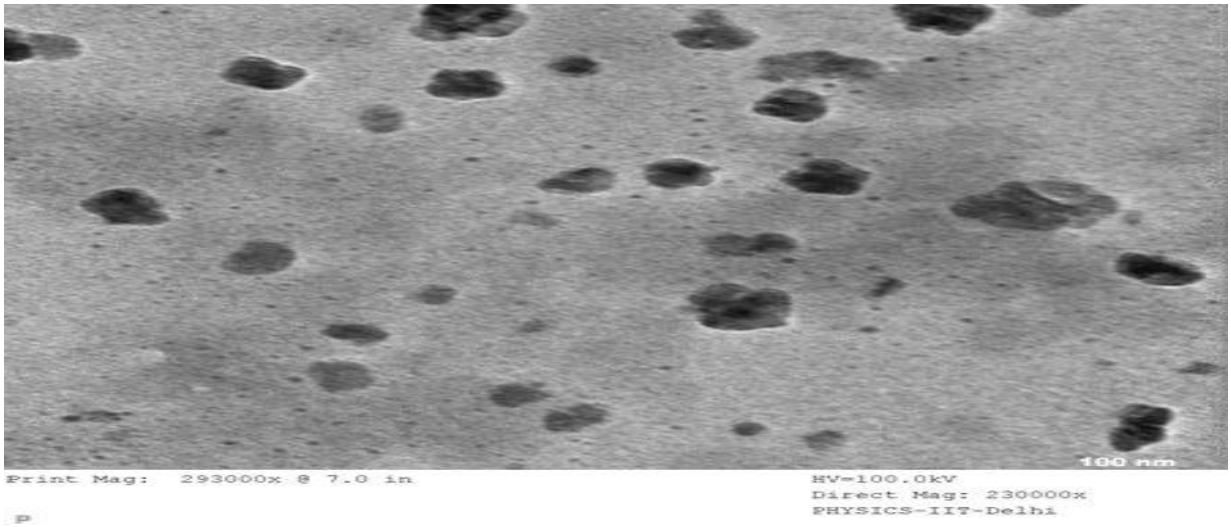


Figure.9 Transmission electron micrograph of the silver nanoparticles synthesized by papaya



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