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Synthesis, analysis and application of noble metal nanoparticles by *Cucurbita pepo* using different solvents

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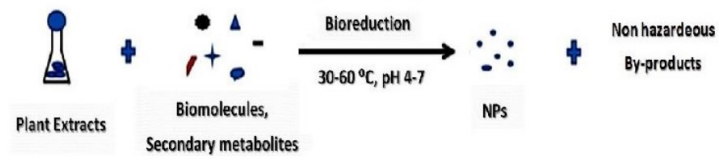
ABSTRACT

The synthesis of metal nanoparticles through biological approach is an important aspect of biotechnology. The biological method provides a feasible alternative as compared to chemical and physical methods. The synthesis of metal nanoparticles using plant derived materials is an effective method for the production of metal nanoparticles.This work reports the rapid biosynthesis of silver nanoparticles from plant extract *Cucurbita pepo*. The plant extract was prepared using two different solvents i.e. double distilled water and 70% ethanol by hot percolation method. The sample was subjected to different reaction conditions i.e. pH (3, 7, 9) and temperature (0 °C, r.t., 37 °C, 60 °C, 100 °C). The preliminary characterization of nanoparticles was done by using UV-VIS spectrophotometer at different wavelengths on the basis of color of the sample obtained from different solvents. Confirmatory analysis of the synthesized silver nanoparticles were done by energy dispersion X-ray spectrometer (EDS) and transmission electron microscopy (TEM). These biosynthesized silver nanoparticles were used in the evaluation of antimicrobial activity that was done by Minimum Inhibitory concentration method against different pathogenic strains. The detection, analysis of presence of metal ions in the synthesized silver nanoparticles by using UV-VIS spectrophotometer at 630 nm.

Graphical Abstract



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Introduction

The tantalizing potential of nanotechnology is to fabricate and combine nanoscale approaches and building blocks to make useful tools and ultimate inventions for medical sciences.

Nowadays, the use of microorganisms to synthesize functional nanoparticles has been of great interest. There microbial processes have opened up many new opportunities to explore novel things like biosynthesis of metal nanoparticles. Nanoparticle synthesis, a process which has been conducted empirically for thousands of years, is an essential component of nanotechnology because specific properties are realized at the nanoparticle, nanocrystal or monolayer level and manufacturing process aim to take advantage of four kinds of effects [1]:

- New physical, chemical or biological properties that are caused by size scaling.
- New phenomenon that occur due to size reduction to the point where interaction length scales of physical, chemical and biological phenomenon become comparable to the size of the particle, crystal or respective microstructure gain.
- Generation of new atomic, molecular and macromolecular structures of materials.
- Significant increase in the degree of complexity and speed of processes in particular systems.

Synthesis of Nanoparticles is intensively studied by using chemical, physical and biological methods. Thus, synthesis of Nanoparticles through biological approach is an important aspect of nanotechnology. Recently, the use of biological molecules as templates for green technology is increasing and plants, plant waste & bacteria have frequently been used for the synthesis of nanoparticles. Plants are the better option for the nanoparticle synthesis because they are mostly nontoxic, provide natural capping agents, reduce the cost of microorganism isolation and culture media [2], ample availability and wide array of reducing metabolites.

The biological reduction of metals by plant extracts has been known since 1900s. The rapid biosynthesis of nanoparticles is done from plant extracts by using different solvents double distilled water and Ethanol at certain reaction conditions using Hot Percolation Method.

The study, synthesis and utilization of nanoparticles entail in it a variety of application scopes, some of which are as follows [3]:

- Targeted drug delivery.
- Remedy of ovarian cancer.
- Extend shelf life of container food.
- Improvement in stem cell therapy.

The objectives of our study are as follows:

- To study the biological synthesis of silver nanoparticles using plant material *Cucurbita pepo.*
- To prepare raw extract from plant *Cucurbita pepo* using Hot Percolation Method.
- To study and analyze the biological synthesis of silver nanoparticles using double distilled water and ethanol as a solvent.
- To study the biological synthesis of silver nanoparticles from plant extract of *Cucurbita pepo* subjected to different reaction conditions i.e. pH and temperature.
- To preliminary characterize silver nanoparticles obtained from plant extract using UV-VIS spectrophotometer.
- To confirm the synthesized nanoparticles as being silver nanoparticles through Energy Dispersion X-ray spectrometer.
- To confirm the characterization of the synthesized silver nanoparticles using Transmission Electron Microscopy (TEM).
- To analyze the Antimicrobial activity using Minimum Inhibition Concentration method against pathogenic bacteria.
- To detect, study and analyze the presence of metal ions in the synthesized silver nanoparticles.

Review of literature

Tools for the characterization of nanoparticles

To evaluate the synthesized nanomaterials, many analytical techniques have been used, including ultraviolet visible spectroscopy (UV-VIS spectroscopy), X-ray diffractometry (XRD), fourier transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron icroscopy (TEM), atomic force microscopy (AFM), energy dispersion X-ray spectrophotometer (EDS) [4]. These techniques are used for determination of different parameters such as particle size, shape, crystallinity, fractal dimensions, pore size and surface area. Moreover, orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques. The tools used in this study are:

- UV-VIS spectrophotometer
- Energy Dispersion X-ray Spectrometer
- Transmission Electron Microscopy

The plant extract used for this study is *Cucurbita pepo*, a cultivated plant of the genus *Cucurbita*. It yields varieties of winter squash and pumpkin, but the most widespread varieties belong to *Cucurbita pepo* subsp. *pepo*, called summer squash. It has been domesticated in the New World for thousands of years. Some authors maintain that *C. pepo* is derived from *C. texana*, while others suggest that *C. texana* is merely feral*C. pepo*. They have a wide variety of uses, especially as a food source and for medical conditions. *C. pepo*.

Evaluation of antimicrobial activity

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibiotics are used against bacteria and antifungals are used against fungi. For the treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bactericides (killing bacteria), algicides (killing algae) or fungicides (killing fungi). Evaluation of Antimicrobial is done by various methods including diffusion methods i.e. agar well diffusion methods and disc methods and minimum inhibitory concentration method. This method used in this study is the Minimum Inhibitory Concentration Method.

Metal ion detection

Heavy metals like Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Cr^{6+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+}, Hg^{2+}, and Mn^{2+} are reported to be potential environmental pollutants as many of them are toxic even at trace ppm level concentrations [5]. Therefore, determination of toxic metals in the biological system and aquatic environment has become a vital need for remedial processes. So far, there are several reports available for the detection of heavy metal ions using various analytical instruments. The analytical instrument used for metal ion detection in this study is Spectrophotometer.

Materials and Methods

Plant material

The *Cucurbita pepo* was collected from the local market of Ludhiana. It was properly

cleaned with running tap water and was used for experimental purposes (Figure 1).

Figure 1. Showing Vegetable of *Cucurbita pepo*

Preparation of 1M $AgNO_3$ stock solution

For each time experiment set, fresh stock of $AgNO_3$ solution was prepared. 1 mM $AgNO_3$ (MW 169.88) 0.169 gm was dissolved in 1000 ml of double distilled water resulting in 1000 mL $AgNO_3$ solution.

Biosynthesis of silver nanoparticles from Cucurbita pepo using double distilled water as a solvent

Preparation of cucurbita pepo raw extract

[A] Sample of *Cucurbita pepo with peel*:
Cucurbita pepo was grinded in distilled water to form fine paste. 25 g paste was diluted 5 times in double distilled water to get the final volume of about 125 mL and then was subjected to hot percolation method. In method, the material is heated up to 40-50 °C for 2-3 h till the resultant mixture boils completely and then kept undisturbed for 10 min. The filtrate so obtained was kept in the water bath at 60 °C till reduced volume of filtrate was obtained and was used as raw extract for the synthesis of silver nanoparticles. The resultant mixture was then filtered out using Whatman filter paper no.1 in conical flask (Figure 2).

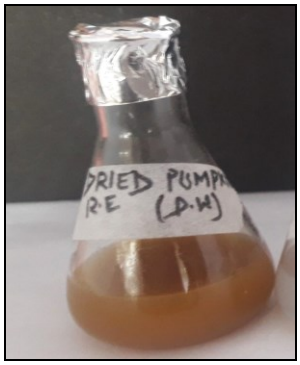


Figure 2. Showing Reduced volume of raw extract of *Cucurbita pepo* after hot percolation and water bath

- Different reaction factor analysis silver nanoparticles

[A] Sample of *Cucurbita Pepo* with peel:

- pH: 2.5 mL raw extract was augmented with 50 mL of $AgNO_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. The incubation temperature of 37 °C was maintained for each flask. Change in color was observed as preliminary observation. The optical density of sample at 630 nm on regular interval of 1 hour was recorded using UV-VIS spectrophotometer. Sample with maximum optical density at defined pH (3, 7, and 9) was further used (Figure 3).

Figure 3. Showing various pH of the Cucurbita pepo augumented with $AgNO_3$

Temperature**:** sample at pH 7 with maximum optical density at 630 nm was observed and further subjected to different temperature conditions i.e. 0 °C, RT (22 °C), 37 °C, 60 °C, and 100 °C. Change in color was observed as

preliminary observation. The Optical density of sample was observed at 630 nm using UV-VIS Spectrophotometer (Figure 4).

Figure 4. Showing various temperature of Cucurbita pepo

Energy dispersion x-ray spectrometer and transmission electron microscopy characterization

Preparation of sample of *Cucurbita pepo* (with peel) using double distilled water as a solvent:

- Sample at pH 7 with maximum optical density at 630 nm of sample of *Cucurbita pepo* with peel and 670 nm of sample without peel was subjected at temperature 60 °C and was selected for the characterization of synthesized nanoparticles.
- The synthesized nanoparticles at pH 7 and temperature 60 °C was centrifuged twice at 10,000 rpm for 20 min.
- Clear pellet was observed after centrifugation of sample.
- Washing of the pellet was done by double distilled water and the process was repeated two times.
- The pellet so obtained was preserved in solvent used in the sample.

Sample Preparation (with peel) using ethanol as a solvent:

- Sample at pH 7 with maximum optical density at 630 nm of sample without peel and 430 nm of sample of *Cucurbita pepo* with peel was subjected at temperature 60 °C and was selected for the characterization of synthesized nanoparticles.
- The synthesized nanoparticles at pH 7 and temperature 60 °C was centrifuged twice at 10,000 rpm for 20 min.
- Clear pellet was observed after centrifugation of sample.
- Washing of the pellet was done by ethanol and the process was repeated two times.
- The pellet so obtained was preserved in solvent used in the sample.

(A) Energy dispersion x-ray spectrometer (EDS)

Procedure of Energy Dispersion X-ray Spectrometer (EDS)

- At the time of characterization, pellet dissolved in a particular solvent was again centrifuged and dissolved in double distilled water.
- Aliquots of nanoparticles dissolved in solvent used were placed on a carbon coated copper grid and were allowed to dry under ambient conditions.

• Then this carbon coated grid of nanoparticles were placed inside a partly evacuated chamber connected to power supply.
• High electron beam bombarded the sample placed on a grid, depending on the amount of energy absorbed by the sample. TEM and EDS detector is usually located at an angle of 10-20° with regard to sample.
• The sample was surrounded by the furnace without any direct line of sight from the sample to the EDS detector.
• Then the EDS spectrum was shown on TEM's peripheral monitor.

Transmission electron microscopy (TEM)

It is a microscopy technique whereby a beam electrons transmitted through a sample interacting with the silver nanoparticles as it passes through. An image is formed from the interaction of electrons transmitted through the sample; the image is magnified and focuses onto an imaging device such as fluorescent screen, on a layer photographic film, or to be detected by sensor such as a CCD camera.

Procedure of transmission electron microscopy (tem) analysis

• At the time of characterization, pellet dissolved in a particular solvent was again centrifuged then again dissolved in double distilled water. Aliquots of nanoparticles dissolved in solvent used were placed on a carbon coated copper grid and allow to dry under ambient conditions. Then this carbon coated grid of nanoparticles are placed inside a partly evacuated chamber connected to power supply. Preparing TEM samples achieved the dilution ratio of nanoparticles to achieve a monolayer of nanoparticles visible on the sample grid when viewing in the microscope.

Nanoparticles were identified at areas of highest particle density to be viewed as images in order to collect more information possible from each image.

Antimicrobial activity of synthesized silver nanoparticles against pathogenic strains

Collection and maintenance of test organism

Three strains of pathogenic bacteria i.e. Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were obtained from Christian Medical College and hospitality, Ludhiana.

Preparation of medium for revival of bacterial strains

The three pathogenic trains (Escherichia coli, Pseudomonas aeruginase, Staphylococus aureus) was revived in peptone water under laminar air flow.

Table 1. Showing Composition of peptone water

Ingredients	Gms/litre
Distilled water	100 mL
Peptic digestion of animal issue	10,000
Sodium chloride	5,000
pH	7 ± 2

Figure 5. Showing the revival medium peptone water

Peptone water to be used for culturing bacteria can prepared from dehydrated or powdered form of peptone water. For this, 15 g of powder is mixed in distilled to form 1 L of

peptone water solution it is warmed slightly with frequent agitation to dissolve it completely. The medium then autoclaved at 15 psi at 121 °C for 15-20 min for sterilizate before using (Table 1 and Figure 5).

Reviving of bacterial strains

The strains to be revived were taken out of refrigerator & thrown at room temperature. The strain was inoculated into peptone water. The inoculations was transferred using sterilized cotton swab. Bacterial Strains were revived in peptone water. Strains were kept in incubator at 37±1 degree celsius for 15 days for complete revival of the cultures (Figure 6).

Figure 6. Showing different pathogenic strains

[A] Determination of antibacterial activity by minimum inhibition concentration method using double distilled water/ethanol as a solvent (with peel)

Antibacterial activity was determined using the different *cucurbita pepo* plant against three pathogenic bacteria (Escherichia coli, pseudomonas aeruginosa and staphylococcus aureus) using minimum inhibitory concentration (MIC) method.

MIC is the lowest concentration of an antimicrobial (like an antifungal, antibiotic and bacteriostatic) drug that will inhibit the visible growth of a microorganisms after incubation.

MIC can be determined by culturing microorganisms in liquid media i.e. Muller Hinton Broth.

A lower MIC value indicates that less amount of sample is required for inhibiting the growth of microorganism; therefore sample with lower MIC scores are more effective antimicrobial agents (Table 2 and Figure 7).

Media preparation for minimum inhibitory concentration (MIC):

Muller Hinton Broth was prepared. The bacterial strain were maintained by subculturing them in Muller Hinton Broth. In this 2-3 mL of bacterial strain is mixed in 12 mL of Muller Hinton Broth (Figure 8).

Composition

Table 2. Showing composition of Muller Hinton Broth

Ingredients	Gms/litre
Beef, infusion from	100 mL
Casein acid hydrolysate	10,000
Starch	5,000
pH (at 25 °C)	7 ± 2

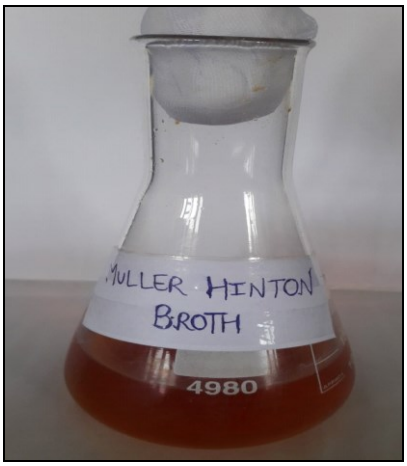


Figure 7. Showing the medium Muller Hinton Broth

Procedure of minimum inhibitory concentration method

Microbial strain and synthesized silver nanoparticles at 60 °C, pH 7 were mixed/diluted together in 3 ratios i.e. 1:1, 1:2, 1:3 i.e. 3 mL of microbial strain was mixed with 3 mL of silver nanoparticles, 3 mL of microbial strain was mixed with 6 mL silver nanoparticles and 3 mL of microbial strain was mixed with 9 mL silver nanoparticle.

Sample test tubes so prepared were then incubated at 37 °C. At interval of 1 h, MIC based on turbidity of sample in test tubes was determined using UV-VIS spectrophotometer at 630 nm. Plot determining antimicrobial activity of AgNps against pathogenic strains were examined. Test tubes with lowest microbial growth i.e. test tubes with less of either ratio (i.e. 1:1, 1:2, 1:3) were observed after 1 hour incubation.

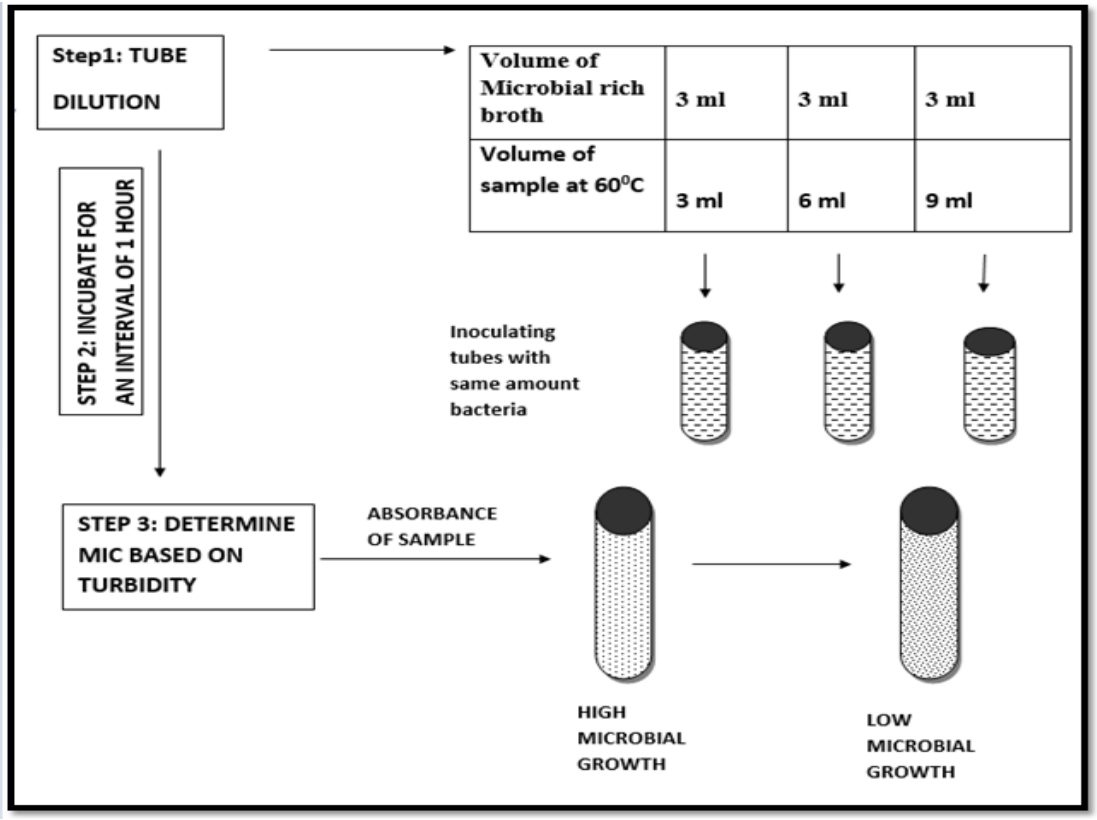


Figure 8. Procedure of the MIC method

[A] Metal ion detection using double distilled water/ethanol as solvent (with peel)

Noble metal nanoparticles have drawn remarkable interest in past few years. A small change in the Nanoparticles size, shape, surface nature, and distance between particles leads to tunable changes in their optical properties. Nanoparticles are found to be sensitive to heavy metals like Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{3+}, Cd^{2+}, Cr^{3+}, and Mn^{2+} are reported to be potential environmental pollutants as many of them are toxic even at trace ppm level concentration.

Procedure

I) Dirty or contaminated water was taken from the industrial waste in Ludhiana. *II*) To detect the presence of metal ions in silver nanoparticles, contaminated water of 3ml and 1ml of silver nanoparticles was mixed and then

the metal ion solution of varied concentrations was added in the above mixture i.e. 50 µL, 45 µL, 40 µL, and 35 µL in each tube. *III*) The optical density at 630 nm was measured and analyzed in dirty water with silver nanoparticles, using UV-VIS spectrophotometer. *IIII*) Incubation was given to each test tubes at room temperature for 2 h. *V*) After incubation, the optical density at 670 nm (with peel) of sample was observed.

Results and Discussion

Synthesis of silver nanoparticles from Cucurbita pepo

In the present study, silver nanoparticles were synthesized from *Cucurbita pepo*. Bioreduction of Ag^{+} to Ag^{0} was observed when the aqueous extract was augmented with $AgNO_3$ at different experimental conditions including, pH and temperature.

Using double distilled water as a solvent

Effect of pH on the biosynthesis of silver nanoparticles using paste (with peel) of Cucurbita pepo

In this, 2.5 mL raw extract was augmented with 50 ml of $AgNO_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. Change in color i.e. yellow to white at pH 3, yellow to white cream at pH 7, yellow to black at pH 9 was observed as preliminary observation. The incubation temperature of 37 °C was maintained for each flask on regular intervals of 1 hour. The optical density of sample was recorded at 630 nm using UV-VIS spectrophotometer. Sample with maximum optical density at pH 7 was recorded and further used (Table 3).

Table 3. Showing the effect of pH on optical density observed at interval of 1hr at 630nm using UV-VIS spectrophotometer

pH	Optical density	Optical density			Mean	Standard deviation
	Initial	After 1 hour	After 2 hour	After 3 hour		
3	1.28	1.52	1.09	1.03	1.23	0.220756
7	1.62	1.41	1.06	1.69	1.445	0.282902
9	1.05	1.26	1.43	1.22	1.24	0.155991

Figure 9. Showing change in color was observed at different pH i.e. 3, 7, 9 and maximum optical density was observed at pH 7 at 630 nm

In above Tables and Figure 9, it was concluded that with increase in pH at 630 nm, the concentration of nanoparticles increases. Alkaline pH 9 and neutral pH 7 contain maximum number of nanoparticles, there is a rare production of nanoparticles at pH 3. Maximum concentration of nanoparticles was observed at neutral pH 7 based on the its maximum optical density using UV-VIS spectrophotometer at 630 nm.

Effect of temperature on silver nanoparticles using paste (with peel) of cucurbita pepo

Sample with pH 7 having maximum optical density at 630 nm was further subjected to different temperature conditions i.e. 0 °C, RT (22 °C), 37 °C, 60 °C, 100 °C. Change in color was observed at different temperatures i.e. yellow to brown red at 0 °C, yellow to red at RT, yellow to dark brown at 37 °C, yellow to black red at 60 °C, yellow to black at 100 °C as preliminary. The maximum optical density of the sample *Cucurbita pepo* was observed with pH 7 at temperature 60 °C at 630 nm using UV-VIS spectrophotometer (Table 4).

Table 4. Showing change in color Cucurbita pepo paste extract augmented with silver nitrate after one hour of incubation at different temperatures 0 °C, RT (22 °C), 37 °C, 60 °C, 100 °C indicating silver nanoparticles synthesis at 630 nm

Temperature	Optical density			Mean	Standard deviation
	Initial	After 1 hour	After 2 hour		
0 °C	1.19	1.15	1.24	1.193333	0.045092
RT (22 °C)	1.35	1.27	1.31	1.31	0.04
37 °C	1.55	1.52	1.41	1.493333	0.073711
60 °C	1.45	1.57	1.53	1.516667	0.061101
100 °C	0.75	0.91	1	0.886667	0.126623

Figure 10. Showing the maximum absorbance was observed with pH 7 at temperature 600 °C at 630

Figure 10 demonstrates the effect of plant extract augmented with 2.5 mL extract to silver nitrate solution at different temperature. It was observed that with increase in temperature at optimum pH 7, the concentration of nanoparticles increases. Temperature 60 °C

selected as optimum temperature for synthesis of silver nanoparticles because maximum number of nanoparticles was observed but after temperature 60 °C, rare nanoparticles were observed at temperature at 100 °C, therefore 60 °C temperature were selected as optimum temperature for the synthesis of silver nanoparticles from the plant extract of *Cucurbita pepo*.

Using 70% ethanol as a solvent

Effect of pH on the biosynthesis of silver nanoparticles using paste (with peel) of Cucurbita pepo

In this, 2.5 mL raw extract was augmented with 50 mL of $AgNO_3$ solution. This reaction mixture was subjected to varied pH conditions i.e. pH 3, 7, 9. Change in color was observed. The incubation temperature of 37 °C was maintained for each flask on regular intervals of 1 h. The optical density of sample was recorded at 430 nm using UV-VIS spectrophotometer. Sample with maximum optical density at pH 7 was used further for the experiment (Table 5).

Table 5. Showing the effect of pH on optical density observed at interval of 1 h at 430 nm using UV-VIS spectrophotometer

pH	Optical density				Mean	Standard deviation
	Initial	After 1 hour	After 2 hour	After 3 hour		
3	0.44	0.24	0.24	0.25	0.2925	0.098446
7	0.92	0.79	0.79	0.88	0.845	0.065574
9	0.48	0.69	0.5	0.42	0.5225	0.116726

In above tables and figure, it was concluded that with increase in pH at 630 nm, the concentration of nanoparticles increases. Alkaline pH 9 and neutral pH 7 contain maximum number of nanoparticles, there is a rare production of nanoparticles at pH 3. Maximum concentration of nanoparticles was observed at neutral pH 7 based on the its maximum optical density using UV-VIS spectrophotometer at 430 nm.

Effect of temperature on silver nanoparticles using paste (with peel) of Cucurbita pepo Sample

with pH 7 at maximum optical density at 430 nm was further subjected to different temperature conditions i.e. 0 °C, RT (22 °C), 37 °C, 60 °C, 100 °C. Change in color was observed at different temperatures i.e. white to brown at 0 °C, white to dark red at RT, white to dark brown at 37 °C, white to black red at 60 °C, white to yellow at 100 °C as preliminary. The Optical density of the sample at 430 nm using UV-VIS Spectrophotometer. Maximum absorbance of *Cucubita pepo* was observed at temperature 60 °C at pH 7 (Table 6).

Table 6. Change in color Cucurbita pepo paste extract augmented with silver nitrate after one hour of incubation at different temperatures 0 °C, RT (22 °C), 37 °C, 60 °C, 100 °C indicating silver nanoparticles synthesis at 430 nm

Temperature	Optical density			Mean	Standard deviation
	Initial	After 1 hour	After 2 hour		
0 °C	0.21	0.07	0.23	0.17	0.087178
RT (22 °C)	0.09	0.34	0.88	0.436667	0.403774
37 °C	0.14	0.38	0.27	0.263333	0.120139
60 °C	0.06	0.22	1.52	0.6	0.80075
100 °C	0.0	0.25	0.39	0.216667	0.19218

Figure 11. Showing maximum absorbance observed with pH 7 at temperature 600 °C at 430 nm using UV-VIS spectrophotometer

Figure 11 reveals the effect of plant extract augmented with 2.5 mL extract to silver nitrate solution at different temperature. It was observed that with increase in temperature at optimum pH 7, the concentration of nanoparticles increases. Temperature 60 °C selected as optimum temperature for synthesis of silver nanoparticles because maximum number of nanoparticles was observed but after temperature 60 °C, rare nanoparticles were observed at temperature at 100 °C, therefore 60 °C temperature were selected as optimum temperature for the synthesis of silver nanoparticles from the plant extract of *Cucurbita pepo.*

Characterization of silver nanoparticles using energy dispersion x-ray spectrometer (EDS) and transmission electron microscopy (TEM)

Double distilled water as a solvent

Energy dispersion x-ray spectrometer (EDS) analysis

Using double distilled water as a solvent, maximum absorbance with pH 7 at temperature 60 °C at 630 nm using UV-VIS spectrophotometer observed in plant material *Cucurbita pepo* was selected for the confirmation of presence of silver (Ag) as a true

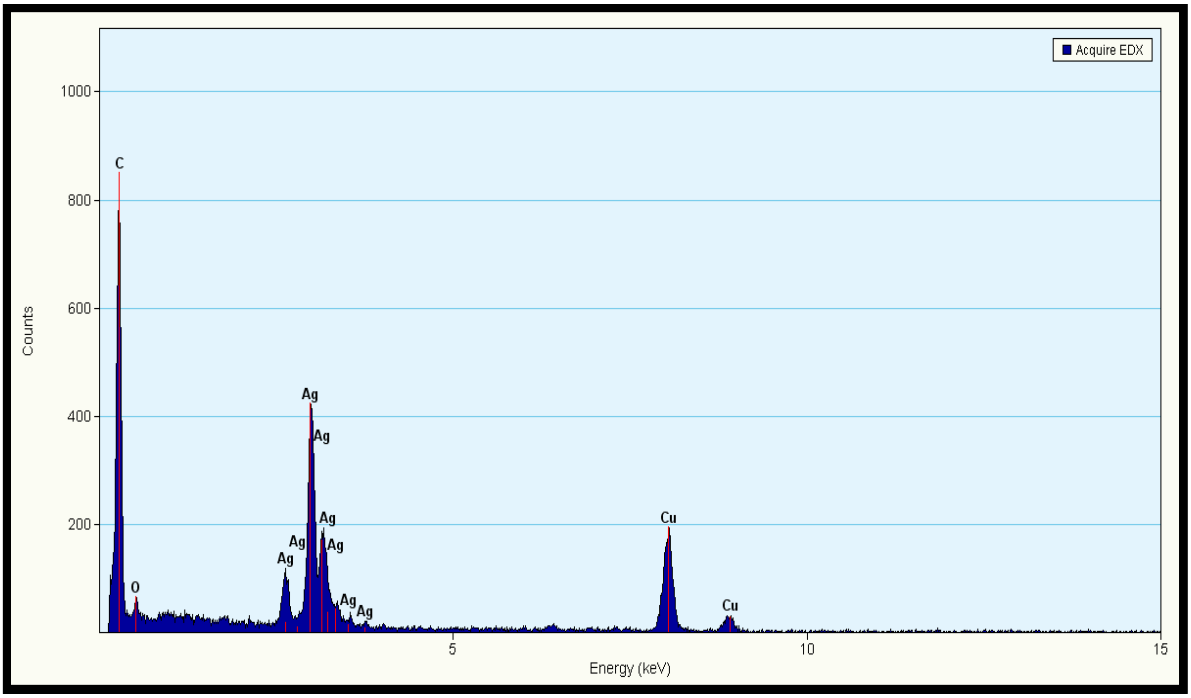


Figure 12. Showing Confirmatory analysis of silver as a true metal ion

Above picture (Figure 12) shows the confirmatory analysis for presence of silver being as a metal ion in the biological source *Cucurbita pepo* sample. Copper in the above image shown, comes from as the grid is made from copper so when the sample is placed on grid the copper gets mixed with sample containing particles and oxygen comes from the external environment. The peaks in the image shows the in this amount silver particles are formed in the sample.

Transmission electron microscopy (TEM) analysis

Using double distilled water as a solvent, maximum absorbance pH 7 at temperature 60 °C was observed in plant material *Cucurbita pepo* with peel sample was selected for the confirmation analysis of synthesized silver nanoparticles.

For the confirmatory analysis of synthesized silver nanoparticles from *Cucurbita pepo*, TEM was performed (Figure 13). So, the shape and size of the resultant nanoparticles in plant material *Cucurbita pepo* with peel were elucidated with the help of transmission electron imcroscopy. Aliquots of nanoparticles solution were placed on a carbon coated copper grid and allowed to dry under ambient conditions and washing of the sample was given by triple distilled water and TEM images were recordedwhich confirms the synthesis of nanoparticles in the plant extract. TEM micrographs showed that nanoparticles produced are mostly spherical in shape and sizes were around 50 (±5) nm. Silver nanoparticles formed as confirmed from the characterization of energy dispersion X-ray spectrometer. The Silver nanoparticles so formed were shown in the Figure 14.

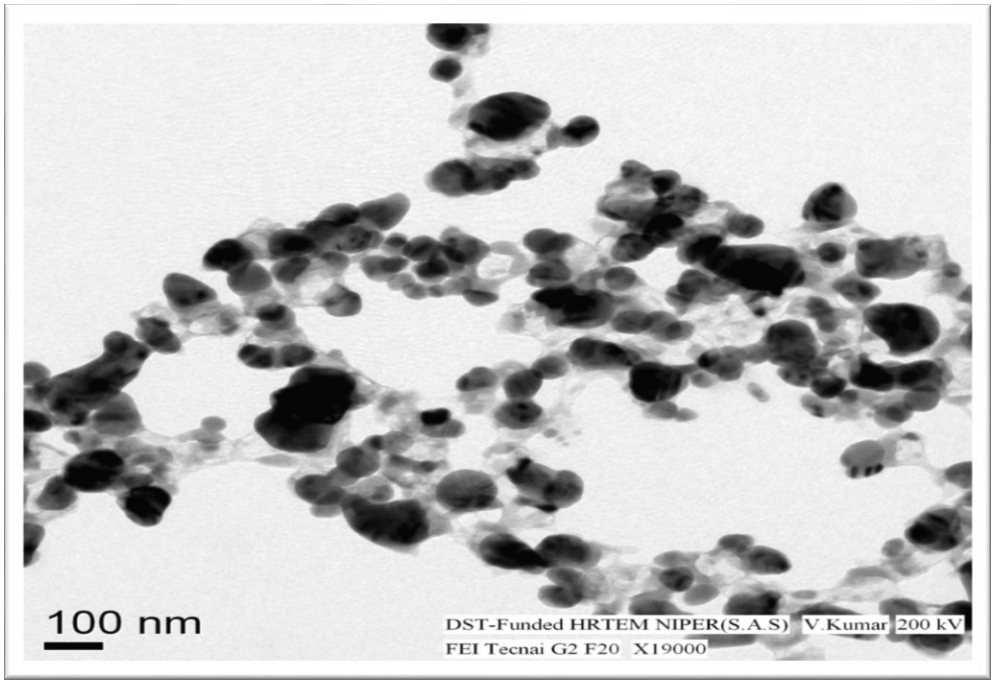


Figure 13. TEM image of the nanoparticles with the particle size of 50 nm

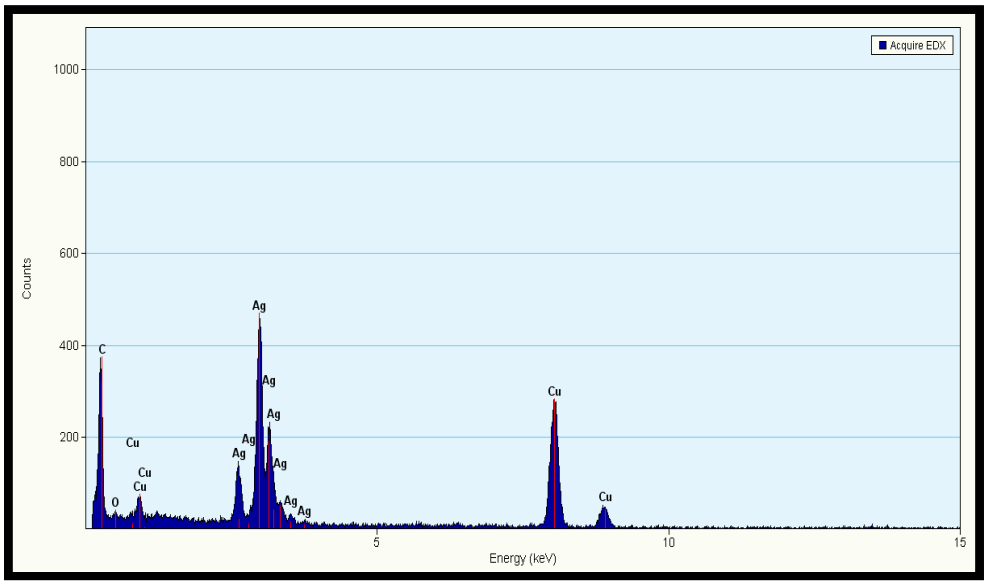


Figure 14. Showing the confirmatory analysis of silver as a metal ion

70% Ethanol as a solvent

Energy dispersion x-ray spectrometer

Using 70% ethanol as a solvent, maximum pH 7 at temperature 60 °C at 430 nm was observed in plant material *Cucurbita pepo* with peel for the confirmation of presence of silver (Ag) as a true metal ion. Above picture shows the confirmatory analysis for presence of silver being as a metal ion in the biological source *Cucurbita pepo* sample. Copper in the above

image shown, comes from as the grid is made from copper so when the sample is placed on grid the copper gets mixed with sample containing particles and oxygen comes from the external environment as sample. The peaks in the image shows the in this amount silver particles are formed in the sample.

Transmission electron microscopy

Using 70% ethanol as a solvent, maximum pH 7 at temperature 60 °C at 430 nm was observed in plant material *Cucurbita pepo* with peel sample was selected for the confirmation analysis of synthesized silver nanoparticles.

For the confirmatory analysis of synthesized silver nanoparticles from *Cucurbita pepo*, TEM was performed. So the shape and size of the resultant nanoparticles in plant material *Cucurbita pepo* with peel were elucidated with the help of Transmission Electron Microscopy. Aliquots of nanoparticles solution were placed on a carbon coated copper grid and allowed to dry under ambient conditions and washing of the sample was given by triple distilled water and TEM images were recorded which confirms the synthesis of nanoparticles in the plant extract. TEM micrographs showed that nanoparticles produced are mostly spherical in shape and sizes were around 35 (±5) nm. Silver nanoparticles formed as confirmed by the EDS analysis. The Silver nanoparticles so formed were shown in the Figure 15.

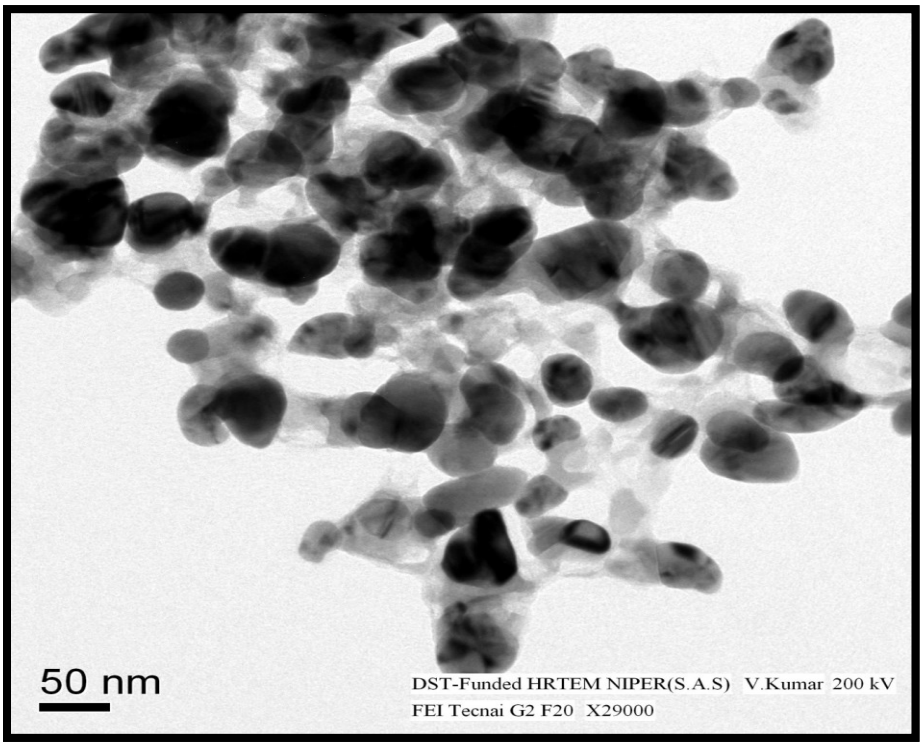


Figure 15. Showing Confirmatory analysis of nanoparticles of size 35 nm using TEM

Antimicrobial activity of synthesized nanoparticles against pathogenic strain by minimum inhibitory concentration Double distilled water as a solvent

Table 7. shows the optical density of different pathogenic strains (Staphylococcus aureus, Pseudomonas aeruginus, Escherichia coli) at different concentrations

Minimum inhibitory concentration method			
	Antimicrobial effect		
Pathogenic strains	Optical density of Concentration of Nanoparticles+Muller Hinton broth at 630 nm		
	1:1 (3 mL of MHB+ 3mL of Nanoparticles)	1:2 (3 mL of MHB+ 6 mL of Nanoparticles)	1:3 (3 mL of MHB+9 mL of Nanoparticles
Staphylococcus aureus	0.78	0.68	0.55
Pseudomonas aeruginosa	0.63	0.58	0.53
Escherichia coli	0.70	0.61	0.54

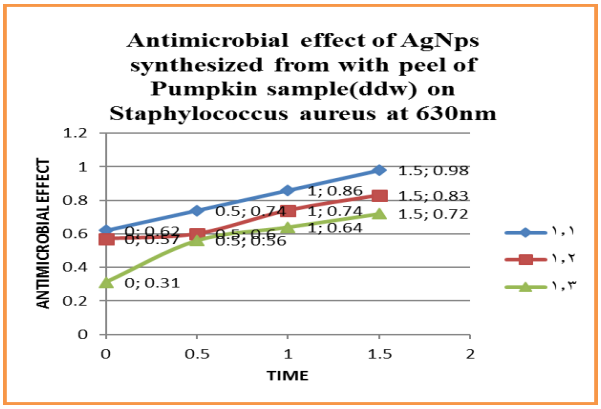


Figure 16. a) Antimicrobial effect of AgNps synthesized from with peel of Pumpkin sample (ddw) on Staphylococcus aureus at 630 nm

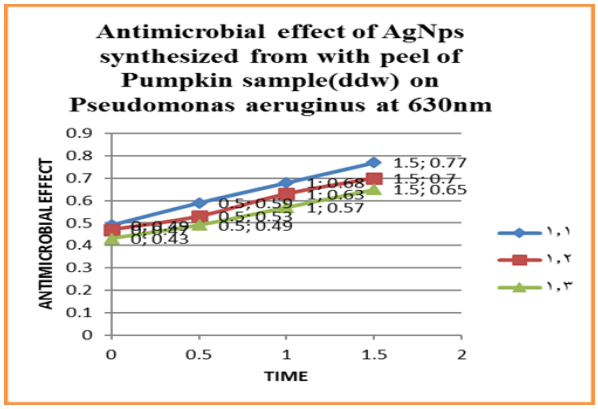
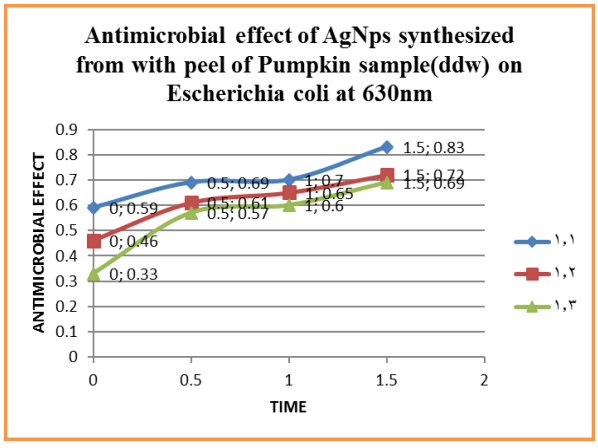


Figure 16. b) Antimicrobial effect of AgNps synthesized from with peel of Pumpkin sample (ddw) on Pseudomonas aeruginus at 630 nm

Figure 16. c) Antimicrobial effect of AgNps synthesized from with peel of Pumpkin sample (ddw) on Escherichia coli at 630 nm



Graphs (Figures 16a, b and c) showing the antimicrobial effect of AgNps synthesized of pumpkin with peel on different microbial strains i.e. *Staphylococcus aureus* (Figure 17), *Pseudomonas aeruginosa* (Figure 18), *Escherichia coli* (Figure 19) using triple distilled

water as a solvent. The antimicrobial effect depends on the MIC values of sample. The mean MIC values obtained was the highest for 1:1 sample and was the lowest 1:3. A lower MIC value indicates that less amount of sample is required for inhibiting the growth of microorganism; therefore, sample with lower MIC concentration i.e. at 1:3 of sample is more effective antimicrobial agent (Table 7).

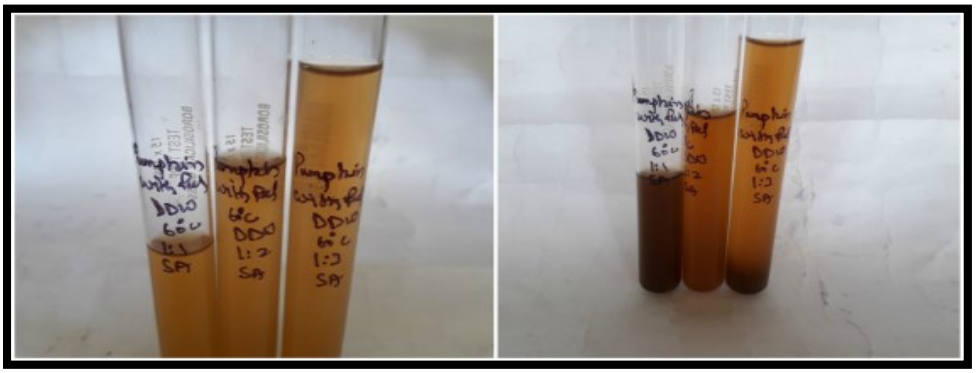


Figure 17. Antimicrobial effect of silver nanoparticles using Cucurbita pepo in Staphylococcus aureus

Figure 18. shows Antimicrobial effect of silver nanoparticles using Cucurbita pepo in Pseudomonas aeruginosacoli

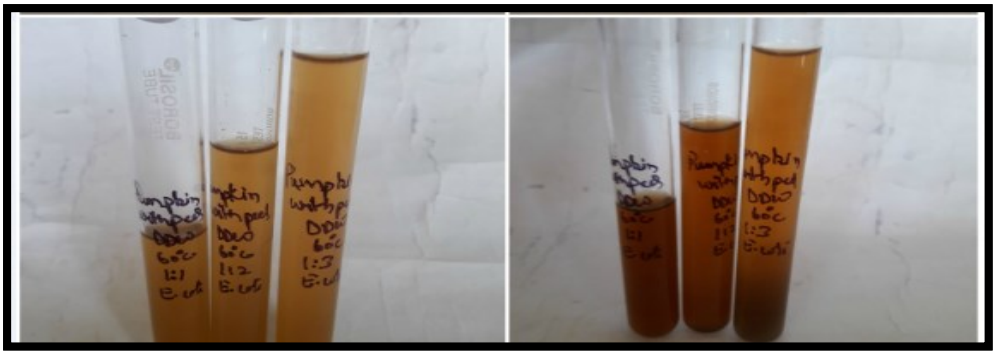


Figure 19. shows Antimicrobial effect of silver nanoparticles using Cucurbita pepo in Escherichia coli

Table. 8 shows the optical density of different pathogenic strains (Staphylococcus aureus, Pseudomonas aeruginus, Escherichia coli) depends at different concentrations

Minimum Inhibitory Concentration Method			
Pathogenic strains	Antimicrobial Effect Optical density of Concentration of Nanoparticles + Muller Hinton broth containing microbial strain At 670 nm		
	1:1 (3 mL of MHB+3 mL of Nanoparticles)	1:2 (3 mL of MHB+6 mL of Nanoparticles)	1:3 (3 mL of MHB+9 mL of Nanoparticles
Staphylococcus aureus	1.52	1.39	0.9
Pseudomonas aeruginosa	1.09	0.97	0.93
Escherichia coli	1.04	1.02	0.87

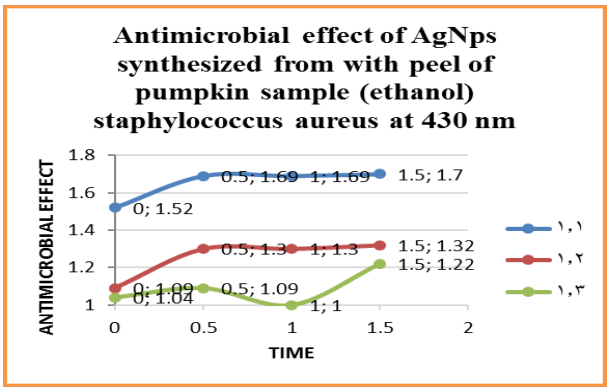


Figure 20. a) Antimicrobial effect of AgNps synthesized from with peel of pumpkin sample (ethanol) staphylococcus aureus at 430 nm

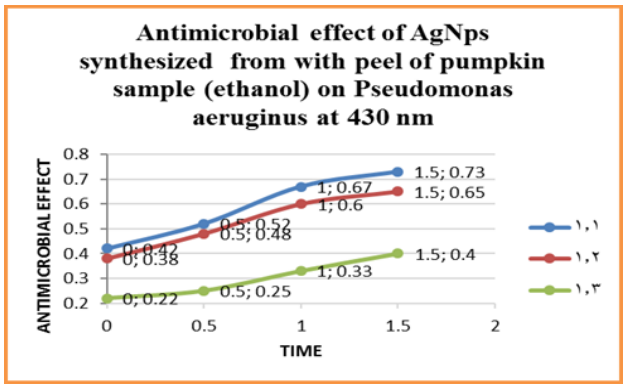
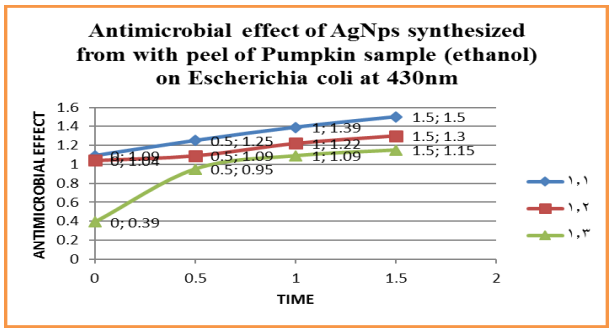


Figure 20. b) Antimicrobial effect of AgNps synthesized from with peel of pumpkin sample (ethanol) on Pseudomonas aeruginus at 430 nm

Figure 20. c) Antimicrobial effect of AgNps synthesized from with peel of Pumpkin sample (ethanol) on Escherichia coli at 430 nm



Graphs (Figures 20**a**, **b** and **c**) showing the Antimicrobial effect of AgNps synthesized of pumpkin with peel on different microbial strains i.e. *Staphylococcus aureus* (Figure 21), *Pseudomonas aeruginus* (Figure 22), *Escherichia coli* (Figure 23) using triple distilled water as a solvent. The antimicrobial effect depends on the MIC values of sample. The mean MIC values obtained was the highest for 1:1 sample and was the lowest 1:3. A lower MIC value indicates that less amount of sample is required for inhibiting the growth of microorganism; therefore, sample with lower MIC concentration i.e. at 1:3 of sample is more effective antimicrobial agent (Table 8).

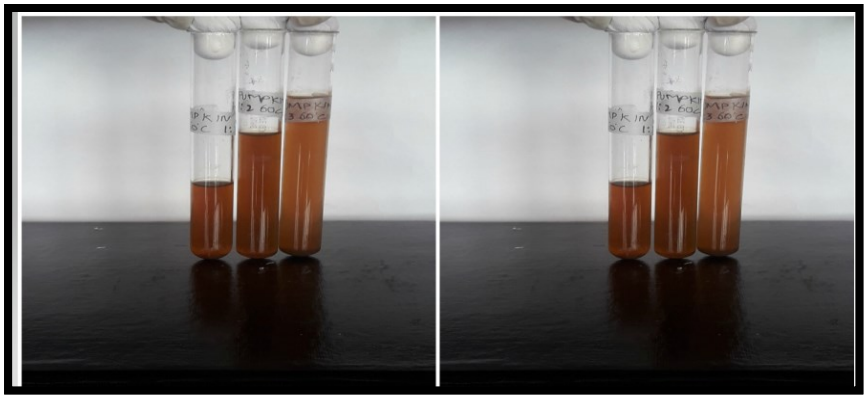


Figure 21. shows Antimicrobial effect of silver nanoparticles using Cucurbita pepo in Staphylococcus aureus

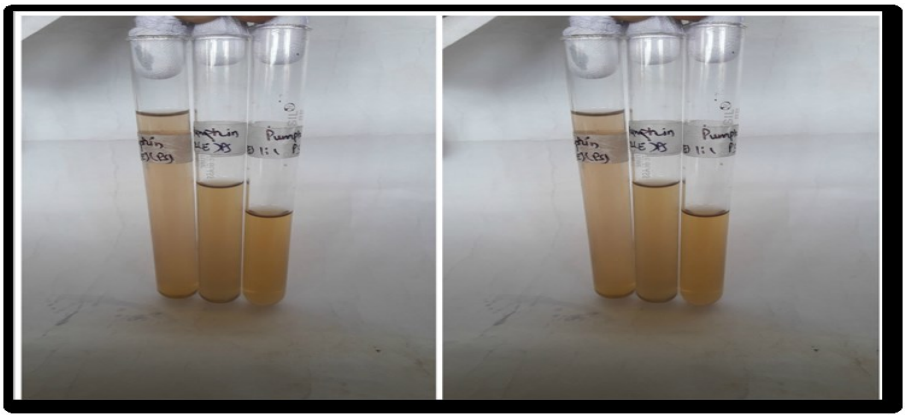


Figure 22. Antimicrobial effect of silver nanoparticles using Cucurbita pepoin Pseudomonas aeruginosa

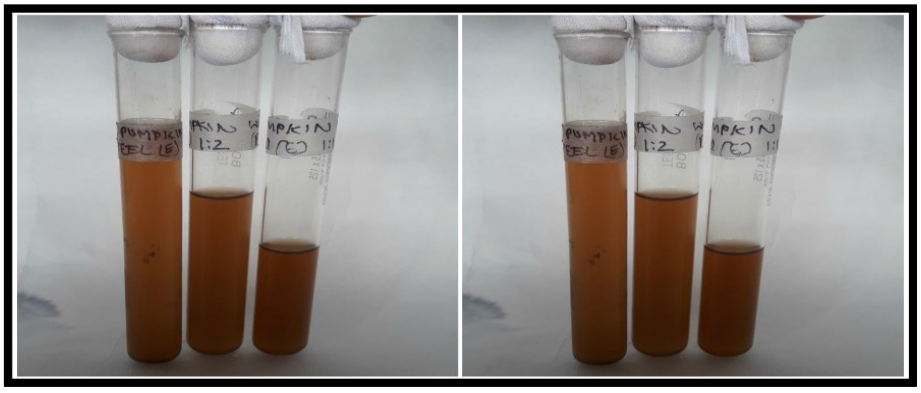


Figure 23. Antimicrobial effect of silver nanoparticles using Cucurbita pepo in Escherichia coli

Determination of metal ions in nanoparticles

Determination of metal ion using double distilled water (with peel) as a solvent.

Table 9. The optical density for the presence of metal ion at 630 nm

Optical density at 630 nm		
	Initial	After 2 hours
C.W+AgNps	0.24	0.27
C.W+AgNps+50 µl metal ion	0.24	0.54
C.W+AgNps+45 µl metal ion	0.10	0.02
C.W+AgNps+4 0 µl metal ion	0.48	0.24
C.W+AgNps+35 µl metal ion	0.16	0.29

The Table 9 shows that the change in optical density when silver nanoparticles were added to just contaminate water was from 0.23 to 2.00. This means that silver nanoparticles alone can be used to detect metal ions in contaminated water but with the addition of even the smallest amount (35 µL) of meatal ion, this change in optical density was affected as it went from 0.20 to 0.25, indicating an increase in the ability of nanoparticles to detect the presence of metal ions in contaminated water.

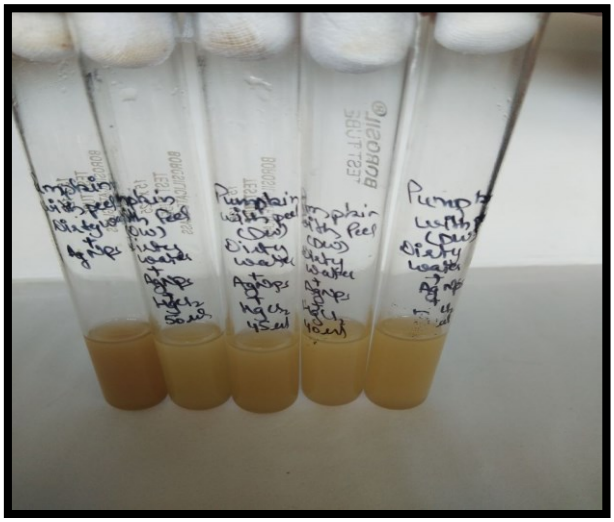


Figure 24. The detection of metal ion in the synthesized silver nanoparticles

As we know contaminated water consist of heavy metals like Hg^{2+}, Zn^{2+}. The addition of synthesized silver nanoparticles to this contaminated can be used to detect the presence of added metal ions like Hg^{2+}, Zn^{2+}. The optical density of sample containing contaminated water with added metal ions and silver nanoparticles was noted using UV-VIS spectrophotometer at 630 nm before incubating the mixture for 2 h. Upon incubating the mixture at room temperature, a significant change in optical density was observed hence the presence of metal ions i.e. Hg^{2+} was confirmed in Figure 24.

Summary

Nano-biotechnology has emerged up as integration between biotechnology and nanotechnology for developing biosynthetic and environment friendly technology for the synthesis of nanomaterial. In this study, silver nanoparticles were synthesized from the plant extract of *Cucurbita pepo.* This plant has been used extensively in curing wide variety of health

problems. Biological synthesis of nanoparticles involves natural phenomenon that takes place in the biological systems. Biological method is cheap, fast and ecofriendly method as compared to the physical and chemical method and evaluated as very good choice of antimicrobial agents due to continuous increase in emergence and re-emergence of multidrug resistant pathogens. In the present study, sample or extract was prepared by treating it by Hot Percolation Method and compared at different reaction conditions i.e. pH and temperature. Bio reduction of Ag^{+} to Ag° was observed when 2.5 mL of extract was augmented with $AgNO_3$ kept at different pH (3, 7, and 9) and different temperatures (0 °C, RT, 37 °C, 60 °C, 100 °C).

It was concluded that plant extract of *Cucurbita pepo* with peel sample of *Cucurbita pepo*, uniform number of silver nanoparticles were synthesized at pH 7 at optical density of 1.44 and temperature 60 °C at optical density of 1.51 as compared to those synthesized with other reaction conditions of temperature and pH. These synthesized silver nanoparticles were first preliminary characterized by UV-VIS spectrophotometer due to change in color at 670 nm for with peel sample [6].

It was concluded that for the plant extract of *Cucurbita pepo* with peel sample, uniform number of silver nanoparticles were synthesized at pH 7 an optical density of 0.845 and temperature 60 °C and optical density of 0.203 at temperature 60 °C as compared with other reaction conditions of temperature and pH. These synthesized silver nanoparticles were first preliminary characterized by UV-VIS spectrophotometer due to change in color at 430 nm for with peel sample.

It was concluded that with increase in temperature conditions number of silver nanoparticles synthesized increases due to surface Plasmon resonance but after boiling the number of nanoparticles starts decreasing with further increase in temperature.

Since the optical density of samples with peel was high with double distilled water and 70% ethanol as a solvent; these samples were used for the confirmatory analysis for the presence of silver being a metal ion and silver nanoparticles was performed with the help of pellets developed from extract suspensions and was performed using EDS and TEM that showed silver as a true metal ion and size of silver nanoparticles with extract respectively. As confirmed the TEM test the size of the nanoparticles synthesized was 50 (±5) nm for with peel sample of DDW and 35 (±5) nm of 70% ethanol.

The optimum reaction condition of silver nanoparticles at Ph 7 and 60 °C was selected for synthesis of silver nanoparticles. At this pH and temperature synthesized silver nanoparticles further used for antimicrobial activity and metal ion detection.

To determine the antimicrobial activity by minimum inhibition concentration method against three different pathogenic strains i.e. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* mixed with Muller Hinton broth at different concentrations in the synthesized silver nanoparticles of ratio 1:1 (3 mL MHB mixed and 3 mL of nanoparticles), 1:2 (3 mL MHB mixed and 6ml of nanoparticles), 1:3 (3 mL MHB mixed and 9 mL of nanoparticles); the optical densities were obtained for all these strains at different concentrations.

In order to determine antimicrobial activity using minimum inhibition concentration method against different strains of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* mixed in Muller Hinton broth at different concentrations with all the synthesized nanoparticles (of 70% ethanol and double distilled water with peel) at ratios 1:1 (3 mL MHB mixed with strains and 3ml of nanoparticles), 1:2 (3 mL MHB mixed with strains and 6 mL of nanoparticles), 1:3 (3 mL

MHB mixed with strains and 9 mL of nanoparticles); the turbidity on the basis of optical density of each of the strains was obtained.

Since for the ratio 1:3, the optical density was the lowest in all the samples, hence the turbidity was highest at 630 nm, it was concluded that 1:3 sample was the best microbial agent i.e. 1:3 sample shows an antagonism towards the microbial strain.

To confirm the detection of metal ions using nanoparticles, 1 mL of the synthesized nanoparticles (of 70% ethanol and DDW with peel) were mixed in 3 mL of contaminated water with $HgCl_2$ added in minute amount at four different concentrations; 50 µL, 45 µL, 40 µL, and 35 µL.

The optical densities of these different mixtures were observed initially and after two hours, from extent of change in the observed optical density when we add minimum quantity of $HgCl_2$ i.e. 35 µL, this shows that even the small amount of metal added, the presence of metal ions was detected.

Most of the scientist use different plants for the synthesis of silver nanoparticles and their medicinal activity are under investigation in research. This environmentally friendly method of biological silver nanoparticles synthesis can potentially be applied in various products that directly come in contact with human body such as cosmetics, food and consumer goods, besides medical applications.

Conclusion

The present study that concluded on the *Cucurbita pepo* can be used as a good source for biosynthesis of the silver nanoparticles in different solvents. The reduction of metal ions through plant extracts leading to the formation of silver nanoparticles of fairy well-defined dimensions. The major advantage of synthesizing silver nanoparticles using *Cucurbia pepo* is that they are easily available, safe and nontoxic.

The optical density of pH and temperature of the nanoparticles synthesized from *Cucurbita pepo* with peel was high for both double distilled water and 70% ethanol as solvent. Hence it was concluded that DDW and 70% ethanol as solvent and *Cucurbita pepo* with peel is a good source for the synthesis of silver nanoparticles.

The TEM test confirmed that nanoparticles synthesized from *Cucurbita pepo* with peel and 70% ethanol as solvent were smaller (35±5) in size as compared to other combinations of source and solvents. Hence these synthesized nanoparticles were of the highest quality.

From the Minimum inhibitory concentration method for antimicrobial the following results were observed:

- The antimicrobial activity of sample with peel was for *Pseudomonas aeruginosa* at 1:3 concentration using double distilled water as a solvent, 1:3 sample was an antagonist for *Pseudomonas aeruginosa strain.*
- The antimicrobial activity of sample with peel was the highest for *Escherichia coli* at 1:3 concentration using 70% ethanol as a solvent, 1:3 sample was an antagonist for *Escherichia coli strain.*

Hence it can be concluded that the 1:3 sample shows most antagonism towards the gram-positive bacteria.

From metal ion detection following results were obtained:

- The change in optical density when silver nanoparticles were added to just contaminated water was from 0.69 to 0.59. This means that silver nanoparticles alone can be used to detect metal ions in contaminated water but with the addition of even the smallest amount (35 µl) of meatal ion, this change in optical density increase from 0.16 to 0.52, indicating an increase in the ability of nanoparticles to detect

the presence of metal ions in contaminated water.

- The change in optical density when silver nanoparticles were added to just contaminated water was from 0.24 to 0.27. This means that silver nanoparticles alone can be used to detect metal ions in contaminated water but with the addition of even the smallest amount (35 µl) of meatal ion, this change in optical density increase from 0.16 to 0.29, indicating an increase in the ability of nanoparticles to detect the presence of metal ions in contaminated water.
- The change in optical density when silver nanoparticles were added to just contaminated water was from 1.3 to 0.35. This means that silver nanoparticles alone can be used to detect metal ions in contaminated water but with the addition of even the smallest amount (35 µL) of meatal ion, this change in optical density was affected as it went from 0.13 to 0.27, indicating an increase in the ability of nanoparticles to detect the presence of metal ions in contaminated water.
- The change in optical density when silver nanoparticles were added to just contaminated water was from 0.23 to 2.00. This means that silver nanoparticles alone can be used to detect metal ions in contaminated water but with the addition of even the smallest amount (35 µL) of metal ion, this change in optical density was affected as it went from 0.20 to 0.25, indicating an increase in the ability of nanoparticles to detect the presence of metal ions in contaminated water.

The presence of the metal ion was detected even for the minimum concentration of metal added in the sample. Hence, the nanoparticles synthesized from the plant extract of sample *Cucurbita pepo* with peel using 70% ethanol as a solvent delivered the better results as compared to other combinations of source plant extract and solvent. These inferences confirm that ethanol is better than water because it is polar due to OH group and with high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules and can easily dissolve both polar and non-polar solvents.

Disclosure statement

No potential conflict of interest was reported by the authors.

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