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DESIGN AND CHARACTERIZATION OF SILVER NANOPARTICLES OF *MOMORDICA CHARANTIA* LINN. BY GREEN SYNTHESIS

Sunil T. Galatage [1] and Digambar S. Parpolkar [*2]

Sant Gajanan Maharaj College of Pharmacy [1], Mahagaon, Kolhapur - 416503, Maharashtra, India.
Quality Assurance, Zydus Cadila [2], Kundaim Industrial Estate Ponda - 403115, Goa, India.

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Correspondence to Author:
Digambar S. Parpolkar

Sr. Executive,
Quality Assurance, Zydus Cadila,
Kundaim Industrial Estate Ponda - 403115, Goa, India.

E-mail: digambarparpolkar@gmail.com

ABSTRACT: Objective: Present research work describes an eco-friendly and quick method for the development of silver nanoparticles (AgNP) using *Momordica charantia* Linn. extract and silver nitrate solution. Our investigation reports the antimicrobial activity of developed nanoparticles against *Staphylococcus aureus*, *Escherichia coli*. **Method:** Silver nanoparticle developed by reduction of silver nitrate solution by *Momordica charantia* Linn. extract. Extract of *Momordica charantia* Linn. acts as a reducing agent. Ulta Violet Visible spectroscopy used as a confirmatory test for the development of silver nanoparticles. **Result:** The silver nanoparticles synthesized were confirmed by their change of color to dark brown due to the phenomenon of surface plasmon resonance. The characterization studied was done by UV-visible spectroscopy, Scanning electron microscopy (SEM), X-Ray diffraction (XRD), Fourier Transmission infrared spectroscopy (FTIR), and study antibacterial activity. FTIR gives information about functional groups present in the synthesized silver nanoparticles. X-ray diffraction studies indicate that developed silver nanoparticles are highly crystalline in nature. SEM of synthesized AgNP shows that particles are spherical in shape and uniformly distributed. Synthesized AgNP shows good antibacterial activity against clinically important pathogens *Staphylococcus aureus, Escherichia coli.* **Conclusion:** The outcome of this research work suggests that green synthesized AgNP of *Momordica charantia* Linn. hold the promise of prominent antibacterial activity against *Staphylococcus aureus, Escherichia coli*.

INTRODUCTION: The need for the biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, the chemical synthesis method leads to the presence of some of the toxic chemical absorbed on the surface that may have an adverse effect on the medical applications.

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This is not an issue when it comes to biosynthesized nanoparticles *via* the green synthesis route. So, in search of cheaper pathways for nanoparticle synthesis, scientists used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties, they are usually responsible for the reduction of metal compounds into their respective nanoparticles [1].

Green synthesis provides advancement over chemical and physical methods as it is cost-effective, environment friendly, easily scaled up for large scale synthesis, and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals. One of the substances used in nano-formulation is silver (nanosilver). Due to its antimicrobial properties, silver has also been incorporated in filters to purify drinking water and clean swimming pool water. To generate nanosilver, metallic silver has been engineered into ultrafine particles by several methods; include spark discharging, electrochemical reduction, solution irradiation, and cryo-chemical synthesis [2].

This is due to the higher surface area per mass, allowing a larger amount of atoms to interact with their surroundings. Due to the properties of silver at the nanoscale, nanosilver is nowadays used in an increasing number of consumer and medical products. Because silver is a soft white, lustrous element, an important use of silver nanoparticles is to give a product a silver finish.

Still, the remarkably strong antimicrobial activity is the major direction for the development of nano-silver products. Examples are food packaging materials and food supplements, odor-resistant textiles, electronics, household appliances, cosmetics, and medical devices, water disinfectants, and room sprays [3]. The silver nanoparticles are of interest because of the unique properties (*e.g.,* size and shape depending optical, electrical, and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. Green synthesis approaches include mixed-valence polyoxo-metalates, polysaccharides, Tollens, biological, and irradiation method, which have advantages over conventional methods involving chemical agents associated with environmental toxicity [4].

The antimicrobial property of silver is related to the amount of silver and the rate of silver released. Silver in its metallic state is inert, but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as it binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane leading to cell distortion and death. Silver also binds to bacterial DNA and RNA by denaturing and inhibits bacterial replication [5].

In present research work, we have undertaken the silver nanoparticles formulation by herbs, which are having anti-oxidant activity, and they are reducing agents. The developed silver nanoparticles will be compared for their various characteristics which will give truthful information regarding the performance of silver nanoparticles developed by green synthesis.

MATERIALS AND METHODS:

Materials: Silver Nitrate – Sigma Aldrich chemical Mumbai, Nutrient Agar Powder- Unique Biological Kolhapur. The plant of *Momordica charantia* Linn. specimen was identified and authenticated by Dr. S. G. Killedar Shivaji University, Kolhapur, Maharashtra (India), and the voucher specimen no. SGMCP/PH.COG/HERB/ 01-2018 herbarium was deposited in Pharmacognosy Department.

Synthesis of Silver Nanoparticles by using Momordica *charantia* Linn.: Fresh fruit of *Momordica charantia* Linn. were collected from Parpolkar greenhouse, Ajara, Maharashtra, India. The *Momordica charantia* Linn. fresh fruit extract used for the reduction of Ag^+ ions to Ag° was prepared by taking 20 g of fruit taken into 500 ml borosil flask and washed several times with water to remove the dust particles and then dried to remove the residual moisture and grinded. It taken in 500 ml borosil flask along with 100 ml of distilled water and then boiling the mixture for 15 min. Then, the solution was incubated for 15 min and subjected to centrifuge for 30 min at room temperature with 10000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of a vacuum filter. Then the solution was used for the reduction of silver ions Ag+ to silver nanoparticles (AgNps) [6].

Synthesis of Silver Nanoparticles: Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich chemicals and preparation of an aqueous solution of silver nitrate ($AgNo_3$) at a concentration of 0.1mMol/ml was prepared and used for the synthesis of silver nanoparticles.

Four concentration ratios of plant and metal ions were prepared (10:1, 20:1, 30:1 & 40:1) by increasing the concentration of plant extract concentration in the solution. 1 mM $AgNO_3$ metal ion was added to the prepared plant extract. Then the bio reduced aqueous component was used to further characterization [6].

Characterization of the Prepared Silver Nanoparticles:

Visual and UV-Visible Study: To study the formation of silver nanoparticles (AgNPs), Visual and UV-Vis spectrum of the reaction solution were carried out. The figure shows the visual calorimetric pattern of samples before and after the reaction at different time intervals. It clearly shows that $AgNO_3$ appears colorless, and the extract is with color before the reaction. The colorless aqueous $AgNO_3$ solution became yellowish brown within 1 h when treated with the extract at room temperature, and it is a preliminary indication of the formation of AgNPs.

The intensity of color changes the presence of AgNPs in the medium was confirmed by the Surface Plasmon Resonance band (SPR) observed by UV–Vis absorption spectroscopy. For spherical AgNPs, the characteristic surface plasmon resonance (SPR) absorption band of AgNPs is observed in the range of 400-475 nm [7].

FTIR Analysis: FTIR spectroscopy was used to characterize the presence of different functional groups/phytochemical constituents. The chemical composition of the synthesized silver nanoparticles was studied. The characterization of functional groups on the surface of AgNPs by plant extracts was investigated by FTIR analysis (Alginate), and the spectra was scanned in the range of 4000–400 cm^{-1} range at a resolution of 4 cm^{-1}. [8]

SEM Analysis: The morphological features of synthesized silver nanoparticles from plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24 h of the addition of $AgNO_3$, the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV [9].

Evaluation of Antibacterial Activity of Silver Nanoparticles: Antibacterial activity of silver nanoparticles was tested against both Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria *Staphylococcus aureus* obtained from the Department of Microbiology. The minimum inhibitory concentration (MIC) values were determined. Serial dilutions of the stock solutions of the plant extracts in broth medium were prepared in a microliter plate, and the microbial suspensions were added in the microwells. The MIC values were determined as the lowest concentrations preventing visible growth. Streptomycin was used as a positive control [10].

X-ray Diffraction (XRD) Analysis: The particle size of silver nanoparticle was determined using X-Rayy Diffraction. This was carried out using Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with Cu kα Radiation at 2θ angle. X-rayy diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and cell dimension. The analyzed material is finely ground, and the average bulk composition is determined. The particle size of the particle on the silver nanoparticle was determined using Debye Sherrer's equation $0.94\lambda / B \cos\theta$ [11].

Antibacterial Activity: Nutrient agar medium (25 ml) was taken in two 100-ml Erlenmeyer flasks and sterilized. After cooling the medium at about 45 °C, freshly grown liquid culture (0.25 ml) of *Staphylococcus aureus* and *Escherichia coli* was added in either of the flasks, mixed thoroughly but quickly, and poured in four (two for each bacterium) Petri dishes equally. The dishes were kept at room temperature in a laminar hood. After the medium was solidified, cups of ~0.5 cm diameter were made by a cork-borer each Petri dish at about 1.5 cm away from the disk-wall. Occasionally some liquid may come out from the medium in the cups, which is removed by aspiration by a Pasteur pipette or a micro-pipette. Thereafter samples were added in each well of each Petri dish.

After adding the samples in the cups, the dishes were kept in a refrigerator for an hour for proper absorption of the samples into the surrounding medium from the well. The plates were then transferred into an incubator set at 37 °C to allow bacterial growth on the medium. After 24 h the plates were taken out of the incubator and observed for the zone of bacterial growth inhibition around the cups [12].

RESULTS AND DISCUSSION:

Visual Analysis: In the visual analysis, the reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the formation of silver nanoparticle.

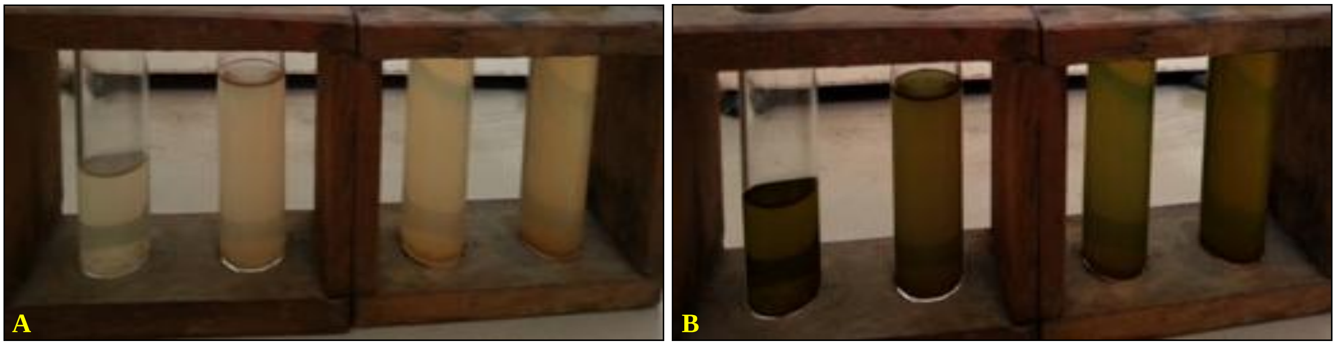

FIG. 1: VISUAL ANALYSIS *MOMORDICA CHARANTIA* LINN. FRUIT EXTRACT (A) BEFORE AND (B) AFTER ADDITION OF SILVER NITRATE

UV-Visible Study of Silver Nanoparticles of *Momordica charantia* Linn.: The intensity of absorption peak increases with an increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles the insets to **Table 1, 2, 3, 4** represent the absorbance at λ_{max} (*i.e.*, at 430 nm) versus time of reaction. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag^+ ions is complete within 24 h. after the addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 2 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time.

Ultraviolet and visible light is energetic enough to promote outer electrons to higher energy levels, and UV-Vis spectroscopy is usually applied to molecules in solution. The UV-Visible spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements.

TABLE 1: UV-VISIBLE SPECTRA OF *MOMORDICA CHARANTIA* LINN. 10:1 AT DIFFERENT TIME INTERVAL

Abs. at nm	extract	0 min	10 min	1 h	24 h	48 h
400	0.451	0.555	0.561	0.609	0.655	0.693
410	0.45	0.559	0.567	0.615	0.663	0.697
420	0.45	0.579	0.591	0.651	0.671	0.706
430	0.448	0.605	0.621	0.676	0.713	0.739
440	0.447	0.576	0.591	0.655	0.685	0.729
450	0.446	0.57	0.589	0.643	0.679	0.716
460	0.446	0.567	0.573	0.641	0.668	0.699
470	0.445	0.568	0.569	0.64	0.651	0.695
480	0.445	0.567	0.565	0.639	0.65	0.687
490	0.444	0.569	0.55	0.639	0.648	0.675
500	0.443	0.567	0.555	0.635	0.649	0.665

TABLE 2: UV-VISIBLE SPECTRA OF *MOMORDICA CHARANTIA* LINN. 20:1 AT DIFFERENT TIME INTERVAL

Abs. at nm	0 min	10 min	1 h	24 h	48 h
400	0.595	0.645	0.701	0.743	0.757
410	0.599	0.657	0.715	0.753	0.767
420	0.609	0.661	0.731	0.761	0.786
430	0.642	0.687	0.756	0.783	0.805
440	0.623	0.657	0.725	0.765	0.786
450	0.603	0.619	0.713	0.769	0.779
460	0.587	0.613	0.711	0.758	0.763
470	0.588	0.61	0.714	0.742	0.765
480	0.577	0.609	0.709	0.735	0.757
490	0.577	0.605	0.709	0.735	0.755
500	0.577	0.605	0.708	0.735	0.753

TABLE 3: UV-VISIBLE SPECTRA OF *MOMORDICA CHARANTIA* LINN. 30:1 AT DIFFERENT TIME INTERVAL

Abs. at nm	0 min	10 min	1 h	24 h	48 h
400	0.609	0.671	0.731	0.773	0.787
410	0.625	0.683	0.735	0.784	0.797
420	0.639	0.696	0.751	0.789	0.799
430	0.662	0.725	0.769	0.805	0.821
440	0.653	0.699	0.745	0.791	0.794
450	0.643	0.683	0.733	0.783	0.783
460	0.627	0.675	0.728	0.775	0.775
470	0.615	0.669	0.727	0.772	0.774
480	0.617	0.657	0.725	0.765	0.775
490	0.615	0.655	0.724	0.761	0.779
500	0.614	0.655	0.727	0.762	0.771

TABLE 4: UV-VISIBLE SPECTRA OF *MOMORDICA CHARANTIA* LINN. 40:1 AT DIFFERENT TIME INTERVAL

Abs. at nm	0 min	10 min	1 h	24 h	48 h
400	0.599	0.637	0.723	0.773	0.751
410	0.61	0.643	0.737	0.797	0.759
420	0.617	0.649	0.749	0.811	0.767
430	0.636	0.668	0.781	0.838	0.799
440	0.623	0.659	0.757	0.799	0.775
450	0.619	0.642	0.756	0.788	0.759
460	0.608	0.644	0.736	0.791	0.747
470	0.598	0.636	0.732	0.778	0.745
480	0.597	0.627	0.729	0.787	0.743
490	0.589	0.625	0.721	0.785	0.743
500	0.588	0.625	0.722	0.783	0.746

FTIR Graph of Silver Nanoparticles of *Momordica charantia* Linn.: FTIR spectrum of *Momordica charantia* Linn. mediated AgNPs, the silver nitrate salt and dried *Momordica charantia* Linn. extract, in $AgNO_3$ peaks were observed at 430 nm.

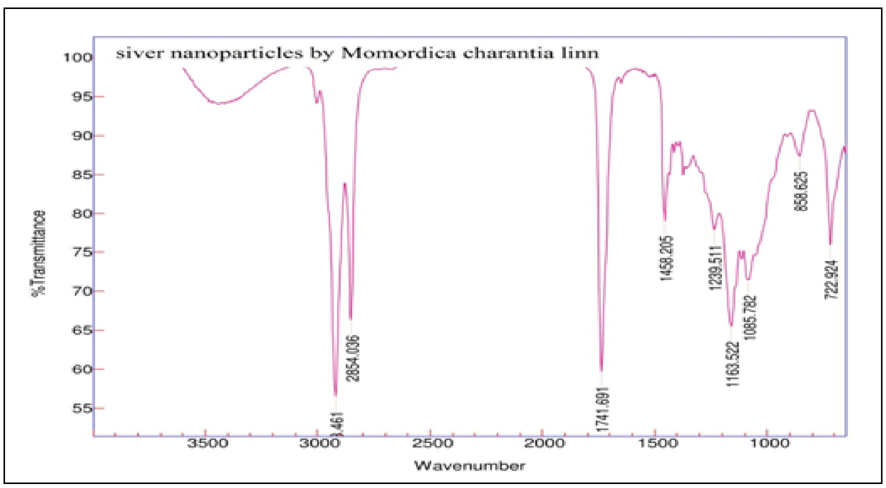

FIG. 2: FTIR SPECTRA OF SILVER NANOPARTICLE OF *MOMORDICA CHARANTIA* LINN.

SEM Analysis of Silver Nanoparticles of *Momordica Charantia* Linn.: Scanning electron microscopy (SEM) is a technique that uses electrons instead of light to form an output image. The SEM is an instrument that produces a largely magnified image by using electrons instead of light to form an image. A beam of electrons is produced at the top of the microscope by an electron gun. The electron beam follows a vertical path through the microscope, which is held within a vacuum. The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample. Once the beam hits a sample, electrons and X-rays are ejected from the sample. Detectors collect these X-rays, backscattered electrons, and secondary electrons and convert them into a signal that is sent to a screen similar to a television screen.

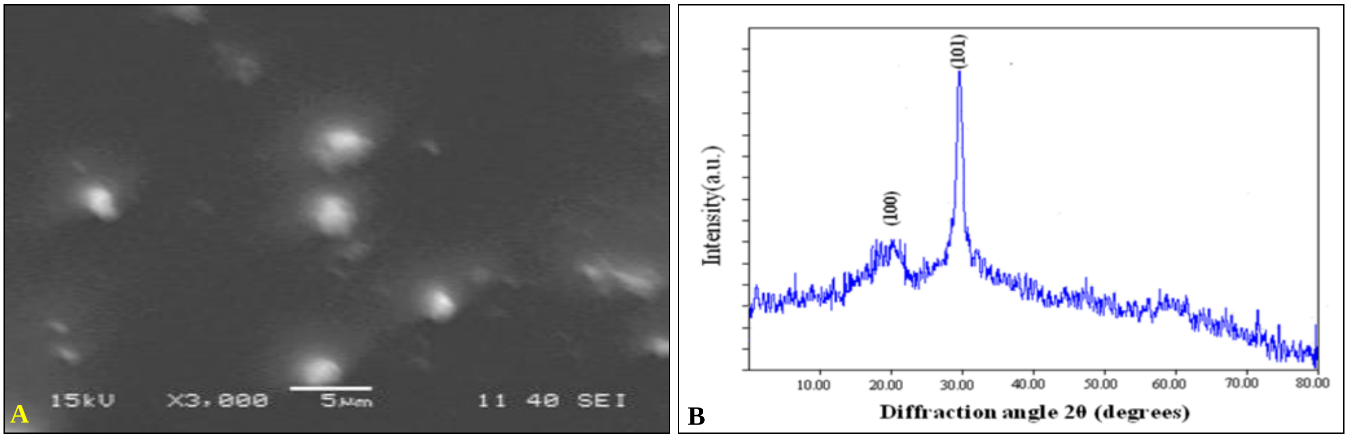

FIG. 3: A) SEM B) XRD OF SILVER NANOPARTICLES OF *MOMORDICA CHARANTIA* LINN.

X-ray Diffraction (XRD) Analysis of Silver Nanoparticles of *Momordica charantia* Linn.: The suspension of silver nanoparticles was dried inside a vacuum chamber for 24 h so that a small amount of dry silver nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The XRD curve **Fig. 3B** confirmed that the nanoparticles are nothing but silver.

The interpretation of this XRD pattern reveals the existence of diffraction lines at low angles (5° to 75°). The silver nanoparticles of *Momordica charantia* Linn. showed the two peaks of silver at 20°, 30° that can be assigned to the (100) and (101) facets of silver, respectively.

Antibacterial Activity of Silver Nanoparticles of *Momordica charantia* Linn.: After 24 h of incubation at 37 °C, a distinct zone of bacterial growth inhibition was observed around all the experimental and positive control cups. The results strongly suggest that silver nanoparticles of plant extract possess antibacterial activity. The particles show activity against both gram-positive and gram-negative bacterial strains used in this study through the inhibition zone appears shown in **Fig. 4**.

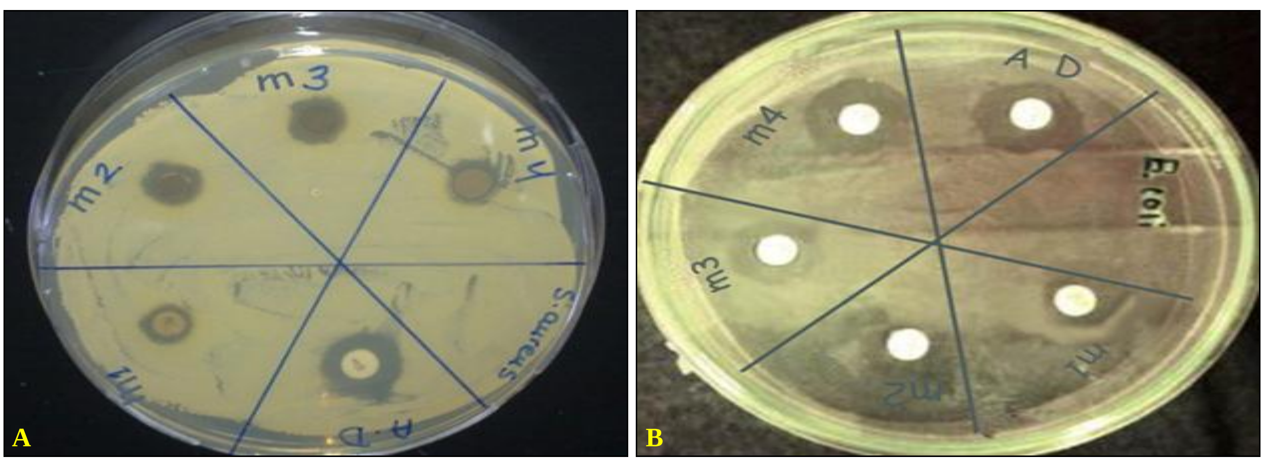

FIG. 4: THE DISK INHIBITION ZONE FOR AgNPs OF *MOMORDICACHARANTIA* LINN. (A) *STAPHYLOCOCCUS AUREUS* (B) *ESCHERICHIA COLI*

DISCUSSION: The aim of the study is to synthesize the non-toxic environmental nano-particles leads to the developing interest in biological approaches which are free from the use of toxic chemicals as by-products. Thus, there is an increasing demand for "green nanotechnology". Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents.

Nanoparticles show completely new or improved properties, such as size, distribution, and morphology of the particles, *etc*. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields.

Silver nanoparticles are widely used in drug carrier, anti-microbial studies, nanotechnology, and biotechnology and in cancer treatment.

This study reports on the anti-bacterial activities of AgNPs prepared from *Momordica charantia* Linn. extract, which has been a green and cost-effective synthetic method. The present study characterized by the different evaluation parameters like Visual and UV–visible study, Fourier Transform Infrared spectroscopy (FTIR). Scanning Electron Microscopy (SEM), Antibacterial activity of Ag-nanoparticles, X-ray diffraction (XRD) analysis *etc*.

Particle size is an important indicator of product quality and performance. It is also crucial for nanoparticle accumulations in the bacterial tissues. This phenomenon called enhanced permeability and retention (EPR) effect, and provides higher accumulations of nanoparticles with smaller particle size. Particle sizes of AgNPs were found 4-25 nm size with narrow distribution. With this green synthesis method, it was possible to obtain particle sizes lower than even 100 nm. It can contribute to higher drug internalization into the bacterial cells.

The intensity of color changes the presence of AgNPs in the medium was confirmed by the Surface Plasmon Resonance phenomenon (SPR). The metal nanoparticles have free electrons, which give the SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with a light wave. The sharp bands of silver nanoparticles were observed around 430 nm in case of *Momordica charantia* Linn. From different literatures it was characteristic surface plasmon resonance (SPR) absorption band of AgNPs is observed in the range of 400-475 nm. From our studies we found the SPR peak for *Momordica charantia* Linn. at 430 nm.

FTIR gives the information about functional groups present in the synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals which would act simultaneously as reducing, stabilizing and capping agent. FTIR spectrum clearly illustrates the bio-fabrication of silver nanoparticles mediated by the plant extracts. UV-Visible spectrophotometer, XRD, FTIR and SEM techniques have confirmed the reduction of silver nitrate to silver nano-particles.

X-ray diffraction is an analytical technique is generally used for phase identification of a crystalline material and can provide information on unit cell dimensions as well. X-ray diffraction is now a common technique for studying crystal structures and atomic spacing. X-ray diffraction is based on the constructive interference of mono-chromatic X-rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The XRD curve confirmed that the nanoparticles are nothing but silver.

A scanning electron microscopy analyzes the shape of the silver nanoparticles that were synthesized by the green method. SEM analysis shows silver nanoparticles were roughly spherical in shape and were uniformly distributed.

This eco-friendly synthesized AgNPs of *Momordica charantia* Linn. could be a competitive alternative to the conventional physical/chemical methods and have the potential to use in bacterial gram-positive and gram negative infections in the near future. The zones of inhibition were formed in the antibacterial test indicated that the Ag NPs synthesized in this process has an efficient antibacterial activity against pathogenic bacteria. The biologically synthesized silver nanoparticles could be of immense use in the medical field for their efficient antibacterial activity.

CONCLUSION: The UV visible shows surface Plasmon resonance band metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with lightwave. So, we confirmed that *Momordica charantia* Linn. fruit extract has more potential to reduce Ag ions into Ag nanoparticles. FTIR gives the information about functional groups present in both synthesized silver nanoparticles for understanding their transformation from simple inorganic $AgNO_3$ to elemental silver by the action of the different phytochemicals, which would act as a reducing agent. SEM confirms the shape of synthesized silver nanoparticles. SEM analysis shows synthesized silver nanoparticles were roughly spherical in shape. X-ray diffraction used for phase identification of crystalline material and can provide information on unit cell dimensions as well. It is now a common technique for studying crystal structures and the atomic spacing of nanoparticles. Biologically synthesized Ag-nano-particles, showed antibacterial activity against *Staphylococcus aureus* (Gram-positive bacterium) and *Escherichia coli* (Gram-negative bacterium).

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