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Green Synthesis and Characterization of Silver Nanoparticles from *Momordica charantia* Fruit Extract: Study of Antimicrobial Activities

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ABSTRACT

The use of environmentally benign materials such as plant extract for the synthesis of silver nanoparticles (AgNPs) offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications. In this study, we have used Momordica charantia fruit extract as a reducing agent for the synthesis of AgNPs. Characterization of the AgNPs was done by UV-Visible Spectroscopy, Scanning Electron Microscopy (HR-SEM), X-ray diffraction (XRD), Dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FT-IR). An intense surface plasmon resonance band at 450nm in the UV–visible spectrum clearly revealed the formation of AgNPs. SAED and XRD patterns confirmed the presence of highly crystalline and face-centered cubic structure AgNPs and they were generally found to be spherical in shape with variable size ranging from 10 to 50nm, as evident by SEM. Phenolic compounds present in Momordica charantia fruit extract were mainly responsible for the reduction and the stabilization of AgNPs. The biosynthesized AgNPs exhibited a strong antimicrobial activity against gram positive and negative bacteria, Fungi.

Key words: *Green Synthesis, Silver Nanoparticles, Momordica Charantia Fruit Extract, Antimicrobial Activity.*

INTRODUCTION

Synthesis of silver nanoparticles (AgNPs) is a growing area for research due to its potentiality in the application and development of advanced technologies. AgNPs are known to have electrical conducting, magnetic, catalytic, sensing and optical properties[6], used in coating or embedding for medical purposes and found to be effective as antibacterial, antiviral, anti- inflammatory, anti-angiogenesis, anti-platelet activity etc[8]. In addition to their medical uses, AgNPs are also used in clothing, catalysis, biosensing, bio-labeling, food industry, paints, optics, electronics, imaging, water treatment, selective coatings for solar energy absorption, sunscreens and cosmetics[3].

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Nanotechnology is a broad-based science involving manipulation of atoms, electrons, protons and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in medicine, engineering, agriculture, surface science, marine science, and geology. It involves in the dimensions at nanoscale size ranging up to 100nm[11]. Nanoparticle has potential applications in various fields such as healthcare, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics, and space industries. It also have been achieved extensively in the drug delivery system for the treatments of cancer, diabetes, allergy, infection and inflammation. Nanoparticles are grouped into organic, inorganic, metal and semi-conductor nanoparticles due to their superior material properties. There are many ways to synthesize nanoparticles such as solid reaction, co-precipitation, chemical reaction, and sol gel method, microwave irradiation etc. The various nanoparticles like gold, silver, copper, iron, palladium, zinc, quantum dots (CdS, ZnS), among these Silver nanoparticles are known as excellent antimicrobial agents, and therefore they could be used as alternative disinfectant agents. On the other hand, released silver nanoparticles could pose a threat to naturally occurring microorganisms[1].

Momordica charantia is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is extremely bitter. The Latin name Momordica means "to bite," referring to the jagged edges of the leaves, which appear as if they have been bitten. It has shown antibacterial, anticancerous, antileukemic, antiprotozoal, antitumorous, antiviral, antiparasitic, antifungal, anti-obesity, anti-ulcer, hypoglycemi and, immune stimulant activities[10]. It has been used by natural health practitioners for diabetes, cancer, high cholesterol, viral infections and bacterial infections. The main constituents of *Momordica charantia* responsible for the medicinal properties are triterpenes, proteins, steroids, alkaloids and other phenolic compounds[4,7].

Several techniques have been demonstrated that AgNPs can be synthesized using chemical and physical methods, but due to the fact of usage of a huge amount of toxic chemicals, expensive reagent, longer time and high temperature conditions, it becomes a mandate to find an alternative method. Green chemistry approach emphasizes that the usage of natural plants has offered a reliable, simple, nontoxic and eco-friendly method.

Here, we have used convenient and environment-friendly method for the synthesis of AgNPs by reducing the silver ions presents in the solution of silver nitrate with the leaf extract of *Momordica charantia* (bitter gourd). The fruit extract of *Momordica charantia* acts as reducing and capping agents for AgNPs.

MATERIALS AND METHODS

Materials

Silver nitrate (>99% pure) was purchased from Sigma Aldrich, India. Potato dextrose broth, Potato dextrose agar, Nutrient broth, Nutrient agar plate, was supplied by Hi-media, India.

Sample collection and preparation (*Momordica charantia*)

The *Momordica charantia* were collected from the Acharya n g Ranga agricultural University, Tirupathi, India (Fig. 1) and were brought to the nanotechnology laboratory and washed with distilled water several times to remove the impurities. The clean leaves were dried at room temperature in the shade for a week and powdered using a mortar and pestle.

Preparation of *Momordica charantia* extract

Dried powdered *Momordica charantia* (5g) was mixed with 100ml distilled water then the solution was kept for continues heating at 80°C for 1hour at room temperature with frequent shaking. After that the extract were filtered by using Whatmann No1 filter paper. The extract was collected and stored at 4°C for further use.

Synthesis of silver nanoparticles from *Momordica charantia*

10 ml of the aqueous extract of *Momordica charantia* was added into 90ml of aqueous solution of 1mM Silver nitrate. The mixture was exposed to a range of controlled temperatures for 24h. Appearance of brown color in solution indicated the formation of AgNPs. The solution was then kept in dark for further analysis collected and stored at 4°C for further use.

Collection of microbes (Bacteria and fungi)

The microbes (Bacteria and fungi) samples were collected from Nanotechnology laboratory, Regional Agricultural Research Station, Tirupathi, (Chittoor District) Andhra Pradesh, India. These samples were stored in an ice box and transported to the laboratory for microbiological characterization. Through serial dilution pour plate technique, fungal sp. was isolated using potato dextrose agar (PDA) medium, and Gram negative and Gram-positive bacteria were isolated from nutrient agar medium. Further, it is maintained in potato dextrose agar slants (fungi) and nutrient agar slants (bacteria) for onward analysis.

Antimicrobial activity

Antibacterial activity of *Momordica charantia* produced AgNPs

The antibacterial activity of AgNPs was evaluated against the following pathogenic strains *E. coli*, *Pseudomonas Fluorescence*, *Staphylococcus aureus* and *Bacillus subtilis*. These cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator. Disc diffusion method disc of 5Mm was made for nutrient agar medium and each disc was dipped at different concentration (170, 100, 50ppm) efficiency of prepared AgNPs. The pure cultures of bacterial pathogens were sub-cultured on an appropriate medium. For comparison, plate of the same diameter with 5Mm Cefmetazole (30mcg) was used. After incubation at 37°C for 24h the zones of bacterial inhibition were measured. The assays were performed triplicate.

Antifungal activity of *Momordica charantia* produced AgNPs

The antifungal activity of AgNPs was evaluated against the following pathogenic strains *Aspergilus niger*, *Aspergilus flavus*, *Schelorosium rolfsii*, *Rhizopus oligosporus*, and these cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator. Disc diffusion method disc of 5Mm was made on nutrient agar medium and each disc was dipped at different concentration (170, 100, 50ppm) efficiency of prepared AgNPs. The pure cultures of fungal pathogens were sub-cultured on an appropriate medium. Discs of 5 mm diameter were made on potato dextrose agar medium. Each strain was swabbed uniformly onto the individual plate. For comparison, plate of the same diameter with 5Mm itraconazole (30 mcg) was used. After incubation at 37°C for 24h the zones of fungal inhibition were measured. The assays were performed triplicate.

Characterization of Ag nanoparticles

UV – Visible spectrum for synthesized nanoparticles

The nanoparticles were monitored by UV–visible spectrum at various time intervals. The UV – Visible spectra of this solution was recorded in spectra 50 ANALYTIKJENA Spectrophotometer, from 250 to 400nm.

FTIR Analysis for synthesized nanoparticles

The nanoparticles were harvested and characterized by FTIR. The FTIR spectrum was taken in the mid IR region of 400–4000 cm^{-1}. The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

X-ray Diffraction analysis for synthesized nanoparticles

The nanoparticles were harvested and characterized by XRD and TEM. The XRD pattern was recorded using computer controlled XRD-system, JEOL, and Model: JPX-8030 with CuK radiation (Ni filtered = 13418 A^{o}) at the range of 40kV, 20A. The 'peak search' and 'search match' program built in software (syn master 7935) was used

to identify the peak table and ultimately for the identification of XRD peak.

Particle Size and Zeta potential analyzer for synthesized nanoparticles

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 µm syringe driven filter unit and the size of the distributed nanoparticles were measured by using the principle of Dynamic Light Scattering (DLS) technique made in a Nanopartica (HORIBA, SZ-100) compact scattering spectrometer.

High Resolution Scanning Electron Microscope

The structural morphological characteristics of the bacterial sample were observed under scanning electron microscope (HR-SEM) Hitachi's SU6600 at magnification ranging from 10X to 600,000X operated at accelerating voltage of 30 kv.

RESULTS AND DISCUSSION

UV-Visible spectral analysis

It is well- known that silver nanoparticles exhibit brown color, which arises due to excitation of surface Plasmon vibrations of the silver nanoparticles. After addition of 1mM silver nitrate solution to the aqueous extract, the colour of the composition has been changed to dark brown colour. The maximum absorbance peak is observed at 450nm for *Momordica charantia* (Fig. 2). The overall observations suggest that the bio reduction of (silver ions) Ag^{+} to $Ag^{(0)}$ was confirmed by UV-Visible spectroscopy

FT-IR analysis

FT-IR spectrum of the biosynthesized silver nanoparticles using *Momordica charantia*. (Fig. 3) shows the absorption peaks at 3361, 2885, 2097, 1637, 1406, 577 and 567cm^{-1}. The peak at 3361cm^{-1} reveals the presence of N-H stretching vibration, indicating the primary and secondary amines group of proteins, 2885cm^{-1} reveals the presence of C-H stretching vibration, indicating the presence of alkenes, 2097cm^{-1} 1637cm^{-1} reveals the presence of N-H bend stretching vibration, indicating the presence of primary amines, 1406cm^{-1} reveals the presence of C-C stretching vibration, indicating the presence of aromatics, 577 and 567cm^{-1}indicating C-Br stretching vibration of alkyl halides The position of these bands were comparable to phenols, flavonoids and tannins. Thus, we can confirm that the nano capping of the *Momordica carantia* extract is responsible for the reduction and subsequent stabilization of the AgNPs. *Momordica carantia* extract were coated by phyto compounds and secondary metabolites such as saponins, terpenoids containing the functional groups of amines, aldehydes, carboxylic acids and alcohols, Particularly the extract of *Momordica carantia* contained the chemical constituents of phenolic compounds Alkaloids, flavonoids, steroids , glycosides, reducing sugar, saponins are acting as a capping and reducing agents. The reduction of silver ions to silver nanoparticles was confirmed from the peak value of Bitter gourd extract.

XRD analysis

XRD analysis of AgNPs represented in (Fig. 4) shows several size dependent features leading to regular peak position, height and width. XRD was mainly carried out to study the crystalline nature of the green synthesized *Momordica carantia* silver nanoparticles. The diffraction intensities were recorded from 10°-80° at 2θ angles. Four different and important characteristic peaks were observed at the 2θ of 38.6°, 45.8°, 64.8° and 78.6° that correspond to (111), (200), (220) and (311) planes indicating that are the SNPs are highly crystalline respectively

Dynamic light scattering analysis

The particle size distribution spectra for the silver nanoparticles were recorded as diameter (nm) verses frequency (%/nm) spectra with diameter (nm) on x-axis and frequency (%/nm) on y-axis. The zeta potential spectra for the silver nanoparticles were recorded zeta potential verses intensity spectra with zeta potential (mV) on x-axis and intensity (a.u) on y-axis. Dynamic light scattering technique has been used to measure hydrodynamic diameter of the hydrosol (particle suspension). *Momordica charantia* AgNPs was found to be 100.5nm (Fig. 5a) the recorded value of zeta potential of the silver nanoparticles was -

22.9mV (Fig. 5b) which resulted in the agglomerated state of the formed AgNPs.

High resolution Scanning Electron Microscopy analysis

Momordica charantia silver nanoparticles were characterized from the SEM micrograph, it is evident that AgNPs were spherical in shape and were poly-dispersed. The measured average size of AgNPs was 35.0nm and the nanoparticles ranges from 24.4nm to 35.0nm occasional agglomeration of the AgNPs has been observed (Fig. 6). The more stable spherical shape and isotropic nanoparticles was formed by the action of large number of bimolecules ranged in the solution.

Antimicrobial activity of *Momordica charantia* extracts mediated silver nanoparticles

It is well- known that silver nanoparticles exhibit brown color, arising due to excitation of surface Plasmon vibrations in the silver nanoparticles. Silver nanoparticles obtained from *Momordica charantia* shown have very strong inhibitory action against fungal sp, Gram-positive and Gram-negative bacteria. These isolates were collected from nanotechnology laboratory, Acharya N G Ranga Agricultural University, Tirupathi. Three concentrations of NPs (170, 100, 50ppm) were prepared and were applied against an array of bacterial species *viz.*, *Escherichia coli, Staphylococcus aureus, Pseudomonas fluorescence* and *Bacillus subtilis*, fungal species *viz.*, *Aspergillus flavus, Sclerotium rolfsii, Aspergillus niger* and *Rhizopus oligosporus*. The higher concentration (170ppm) of AgNPs showed significant antimicrobial effect compared with other concentrations (100 and 50ppm).

The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when bacteria were treated with silver nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane[2], leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death[9]. It is observed that silver nanoparticles have penetrated inside the bacteria and have caused damage by interacting with phosphorus- and sulfur-containing compounds such as DNA[5]. Moreover, *Momordica charantia* AgNPs showed good antibacterial and antifungal activity (Fig. 7a and 7b), (Table. 1 and 2). The findings in this study may lead to the development of AgNPs-based new antimicrobial systems for medical applications.

Fig: 1: *Momordica charantia*

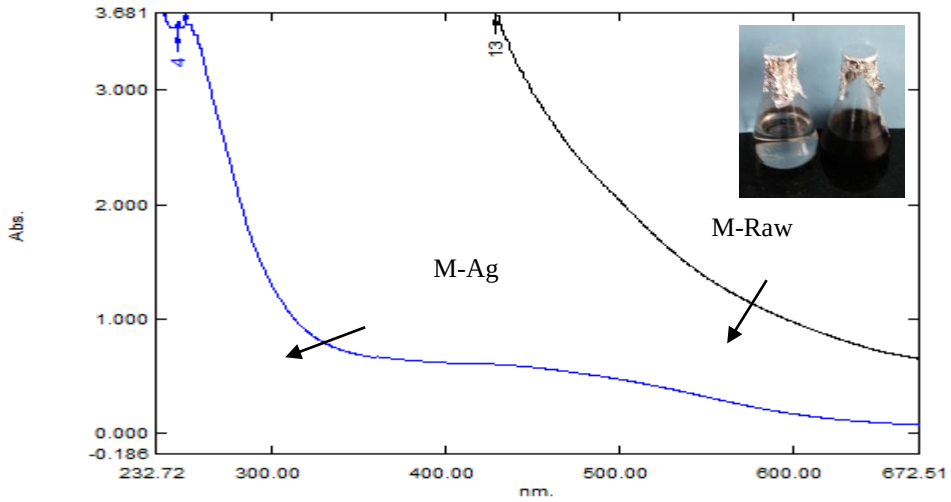


Fig. 2: UV/Visible absorption spectrum of synthesized silver nanoparticles from *Momordica charantia*

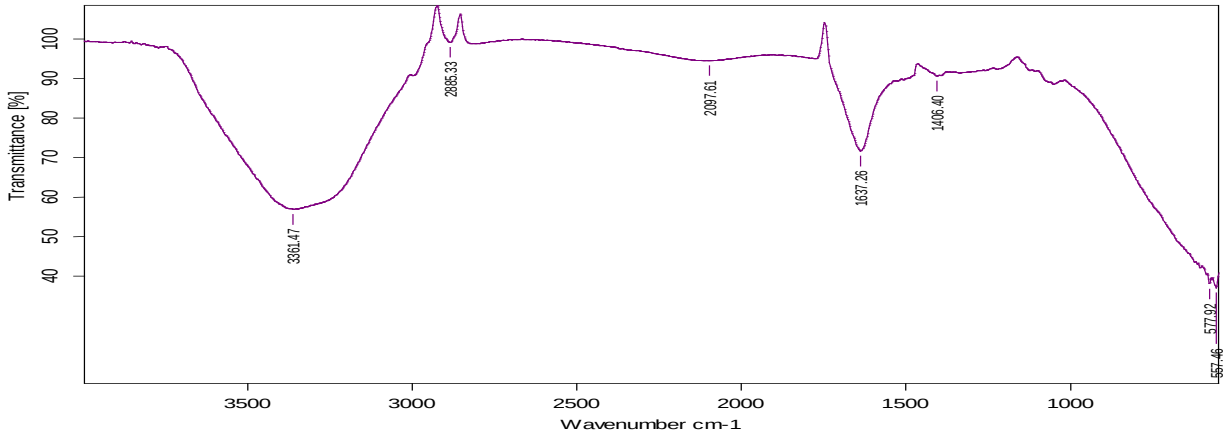


Fig. 3: FT-IR spectrum of synthesized silver nanoparticles from *Momordica charantia*

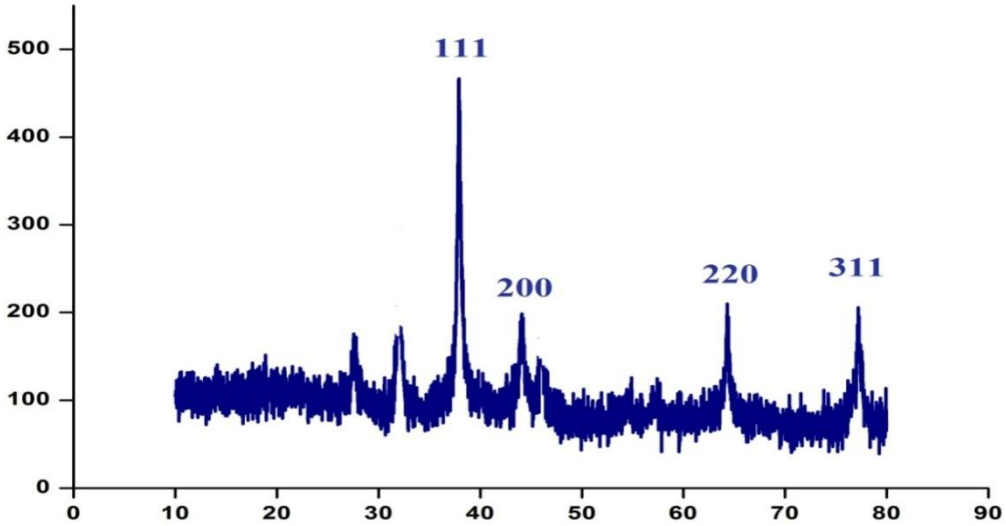


Fig. 4: XRD analyses of synthesized silver nanoparticles from *Momordica charantia*

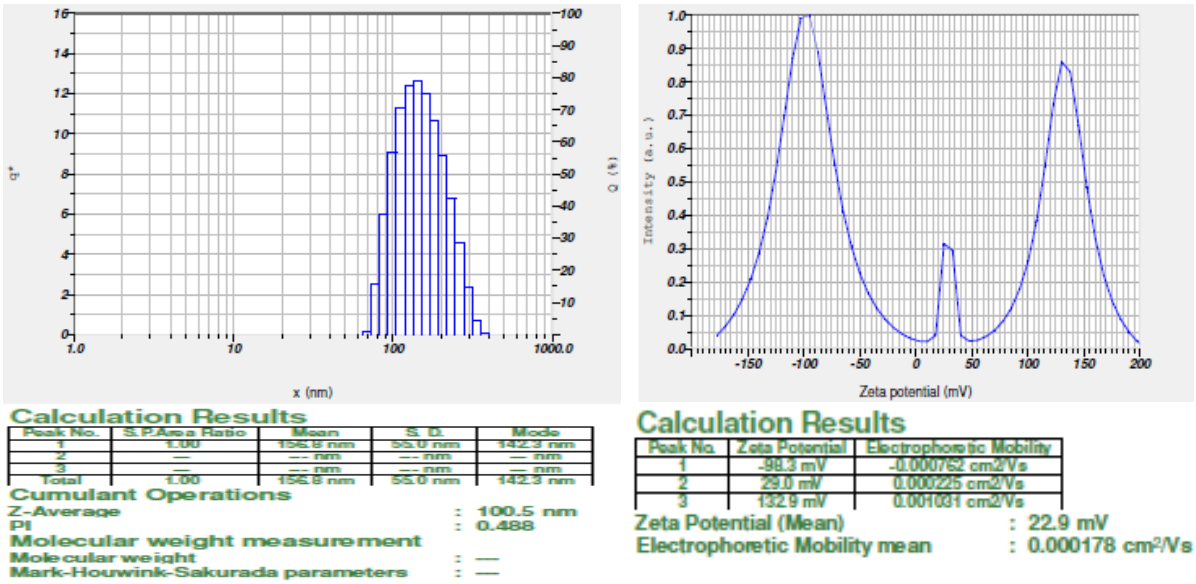


Fig. 5a (Particle size) 5b (Zeta potential) DLS analysis of synthesized silver nanoparticles from *Momordica charantia*

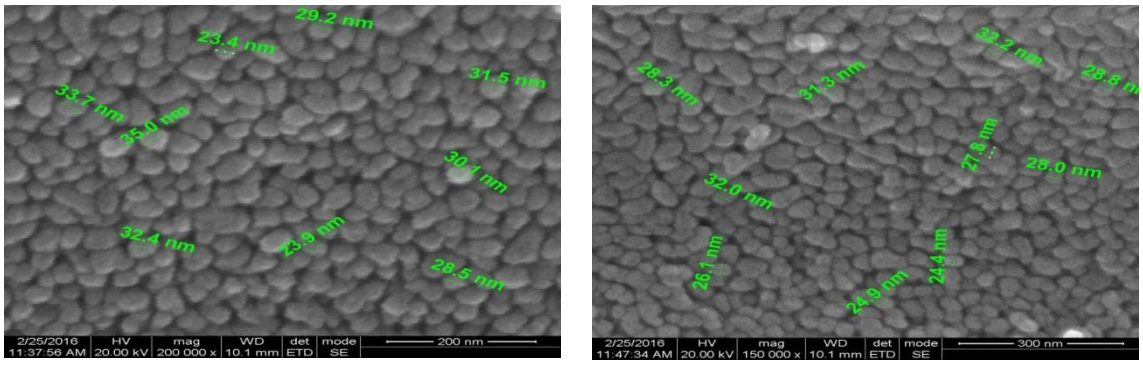
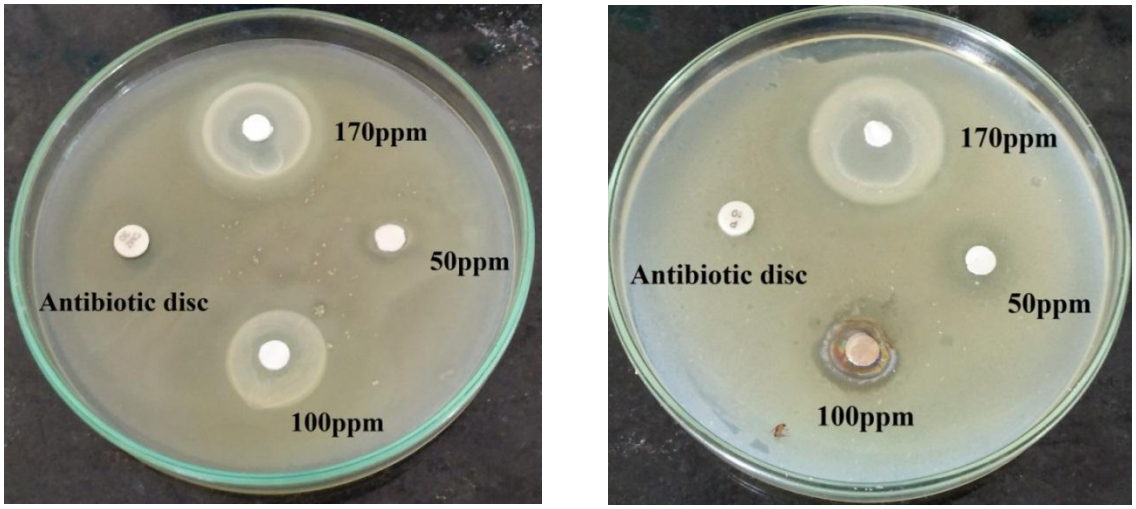
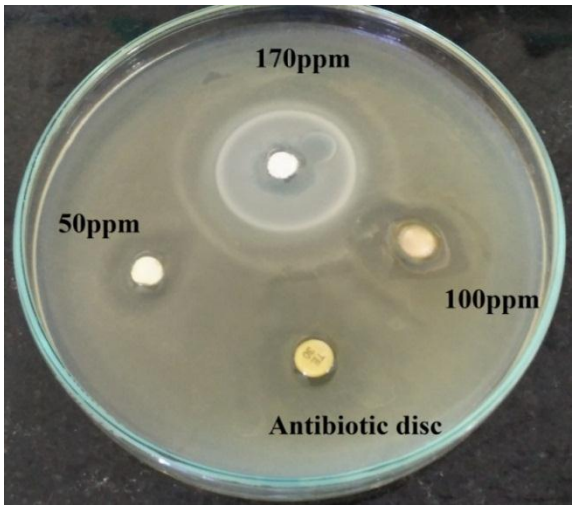


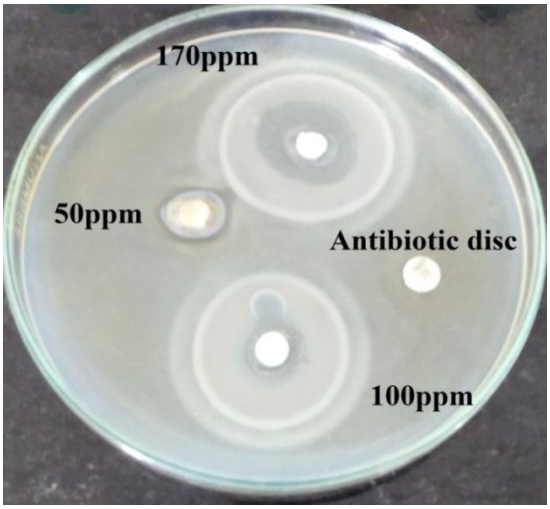
Fig. 6: HR-SEM analysis of synthesized silver nanoparticles from *Momordica charantia*



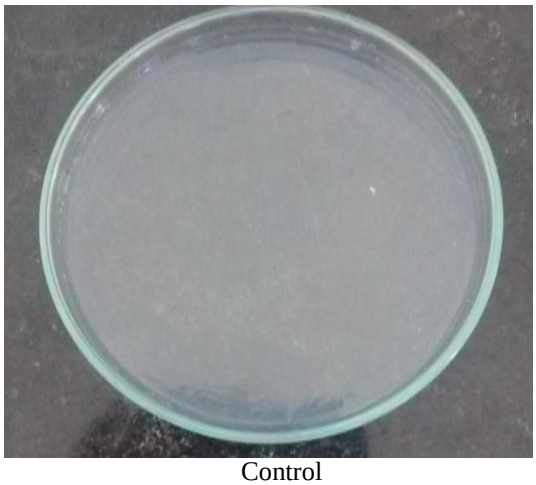
Escherichia coli *Staphylococcus aureus*



Pseudomonas fluorescence

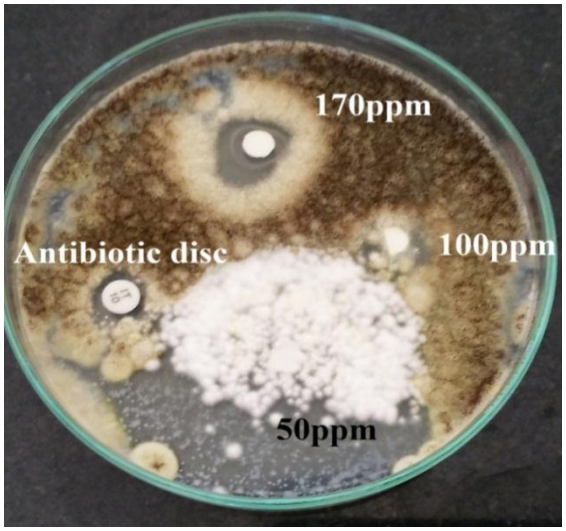


Bacillus subtilis

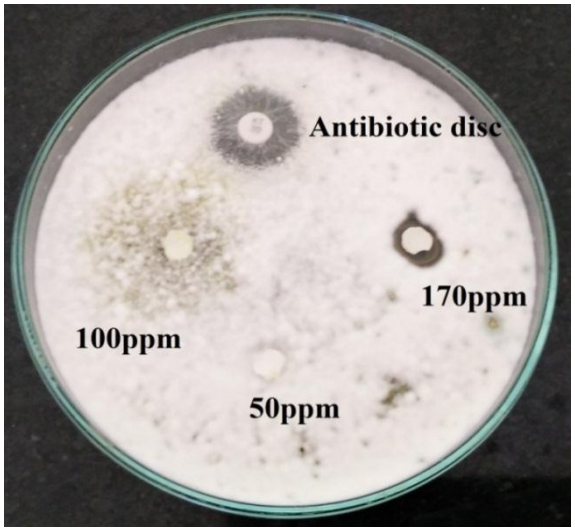


Control

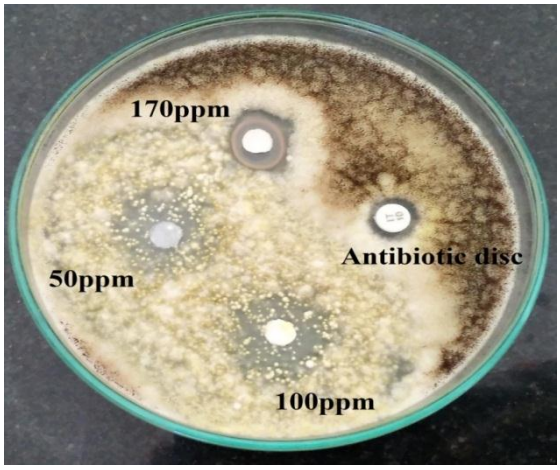
Fig. 7a Anti bacterial activity of synthesized silver nanoparticles from *Momordica charantia*



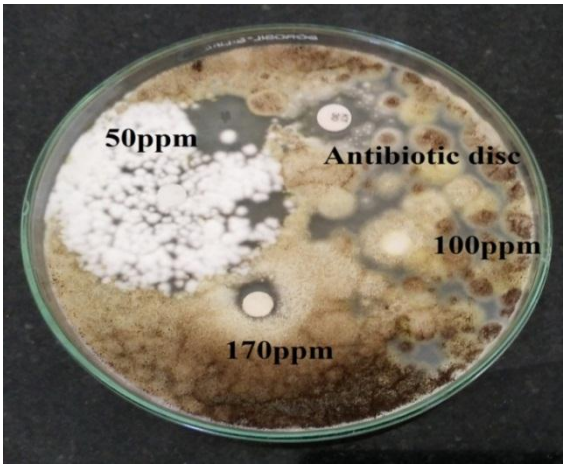
Aspergillus flavus



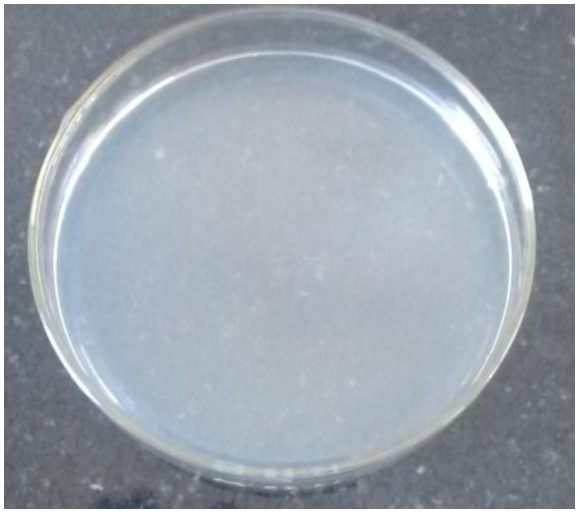
Sclerotium rolfsii



Aspergillus niger



Rhizopus oligosporus



Control

Fig. 7b Anti fungal activity of synthesized silver nanoparticles from *Momordica charantia*

Table: 1 *In vitro* antibacterial studies against bacteria using *Momordica charantia* extract mediated silver nanoparticles as inhibitors

S.No.	BACTERIA	*MOMORDICA CHARANTIA* AQUEOUS EXTRACT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES			
		170±1.4ppm	**100±1.1ppm**	**50±0.8ppm**	**PENCILLIN-G 30mcg**
1.	*Escherichia Coli*	3.0±1.2	2.0±0.2	0.8±0.06	0.2±0.02
2.	*Staphylococcus aureus*	3.0±1.4	2.8±0.5	1.9±0.2	0.2±0.05
3.	*Pseudomonas fluorescence*	3.0±1.6	2.8±0.3	2.1±0.5	1.1±0.2
4.	*Bacillus subtilis*	3.2±0.2	2.9±0.6	2.8±0.4	0.5±0.06

*****The presented data are the mean (n=3) ± standard error of three replicates

Table: 2 *In vitro* antifungal studies against fungi using *Momordica charantia* extract mediated silver nanoparticles as inhibitors

S.No.	FUNGI	*MOMORDICA CHARANTIA* AQUEOUS EXTRACT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES			
		170±1.4ppm	**100±1.1ppm**	**50±0.8ppm**	**ITRACONAZOLE 30mcg**
1.	*Aspergillus flavus*	1.2±0.5	0.7±0.02	0.4±0.04	1.3±0.3
2.	*Sclerotium rolfsii*	1.8 ±0.6	1.0±0.3	0.6±0.02	0.9±0.2
3.	*Aspergillus niger*	1.8±0.5	1.3±0.3	1.1±0.2	1.2±0.4
4.	*Rhizopus oligosporus*	1.0±0.6	0.8±0.02	0.5±0.03	1.1±0.4

*****The presented data are the mean (n=3) ± standard error of three replicates

CONCLUSION

Bio synthesis of silver nanoparticles using the *Momordica charantia* fruit extract is demonstrated with possible role of phenolic compounds as reducing and stabilizing agent. Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of AgNPs synthesis and would be suitable for developing a biological process for large-scale production. AgNPs exhibit excellent antimicrobial activity against gram negative and positive bacteria when compared to fungi. Thus, this biosynthesized AgNPs can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices, industrial, agricultural, biotechnological and environmental applications.

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