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***J. Nat. Prod. Plant Resour*., 2014, 4 (6):1-8**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Biosynthesis of silver nanoparticles by *Momordica charantia* leaf extract: Characterization and their 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 leaf extract as a reducing agent for the synthesis of AgNPs. Characterization of the AgNPs was done by UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). An intense surface plasmon resonance band at 418 nm 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 50 nm, as evident by TEM. Phenolic compounds present in Momordica charantia leaf extract were mainly responsible for the reduction and the stabilization of AgNPs. The biosynthesized AgNPs exhibited a strong antibacterial activity against Kelbsilla pneumonia bacteria.

Keywords: Green synthesis, silver nanoparticles, *Momordica charantia* leaf extract, characterization, 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 [1-3], used in coating or embedding for medical purposes [4] and found to be effective as antibacterial, antiviral, anti- infammatory, anti-angiogenesis, anti-platelet activity etc.[5,6]. 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 [7-15].

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 [16-20]. It has been used by natural health practitioners for diabetes, cancer, high cholesterol, viral infections and bacterial infections [21, 22]. The main constituents of *Momordica charantia* responsible for the medicinal properties are triterpenes, proteins, steroids, alkaloids and other phenolic compounds [23, 24].

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 leaf extract of *Momordica charantia* acts as reducing and capping agents for AgNPs.

MATERIALS AND METHODS

2.1. Chemicals used

Silver nitrate used for the synthesis of AgNPs was purchased from E. Merck, Germany. *Momordica charantia* leaves used in this work were collected from the local area (Thoothukudi, Tamilnadu, India).

2.2. Preparation of *Momordica charantia* leaf extract

About 10 g of fresh leaves of *Momordica charantia* were taken and washed thoroughly with distilled water to remove dust particles. These washed leaves were cut into very small pieces and boiled in 100 mL of distilled water for 1 hr in a round-bottom flask with a condenser. The leaf extract was filtered using Whatman No. 41 filter paper to obtain the pure leaf extract (Figure 1).

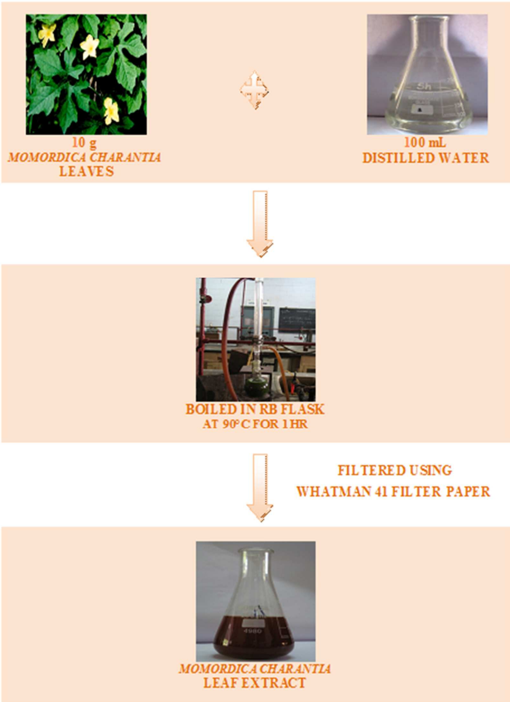

Figure 1 - Preparation of *Momordica charantia* leaf extract

2.3. Biosynthesis of silver nanoparticles

About 10 mL of freshly prepared *Momordica charantia* leaf extract was added to 100 mL of 1 mM silver nitrate solution. This mixture was heated at 60°C for 1 hr and then AgNPs were collected by centrifugation and dried (Figure 2).

2.4. Characterization

The UV-Visible spectra of the AgNPs and *Momordica charantia* leaf extract were recorded on JASCO V-530 UV-Visible spectrometer. FTIR measurements were performed on a Thermo Scientific Nicolet iS5 instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. The average particle size of AgNPs was determined by using XPERT-PRO X-ray diffractometer operating at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation. Philips-CM200 Transmission Electron Microscopy (TEM) was used to study the shape, particle size and lattice image of the silver nanoparticles. The grid for TEM analysis was prepared by placing a drop of the AgNPs suspension on a carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer.

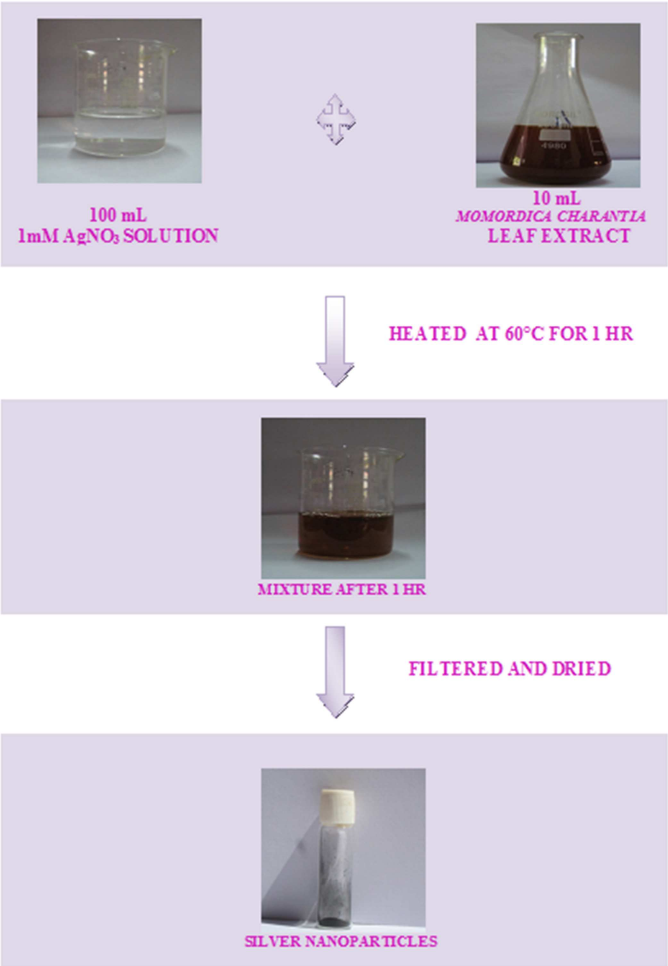

Figure 2 - Biosynthesis of AgNPs

2.5. Antimicrobial study

AgNPs biosynthesized from *Momordica charantia* leaf extract were tested for antimicrobial activity against *Kelbsilla pneumonia* bacteria (Gram-negative) by using Agar-well diffusion method. The bacteria culture was spread evenly on the nutrient agar plate using sterile glass rods. Wells were prepared on agar plates. To these wells nanoparticles solution (1mg/mL) and standard antibiotic *Streptomycin* solution (1mg/mL) were added. After incubation at 37 °C for 24 hr, the diameter of inhibition zones around AgNPs were measured and compared with the diameter of inhibition zone around commercial standard antibiotic *Streptomycin*.

RESULTS AND DISCUSSION

3.1. UV-Visible Spectroscopic analysis

As shown in UV-Visible spectra (Figure 3), the SPR bands centered at 418 nm confirms the formation of AgNPs in the solution. The appearance of the peak is due to the size dependant quantum mechanical phenomenon called Surface Plasmon Resonance (SPR). This effect becomes influential when the De-Broglie wavelength of the valence electrons becomes equal to or less than the size of the particle [25]. The UV-Visible spectrum of the leaf extract shows a band at 270 nm arising due to n-π^* transition.

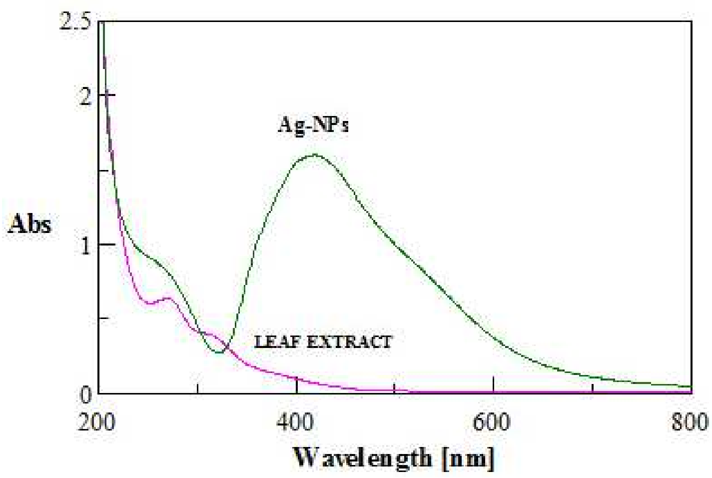

Figure 3 - UV-Visible spectra of AgNPs and *Momordica charantia* leaf extract.

3.2. X-ray diffraction analysis

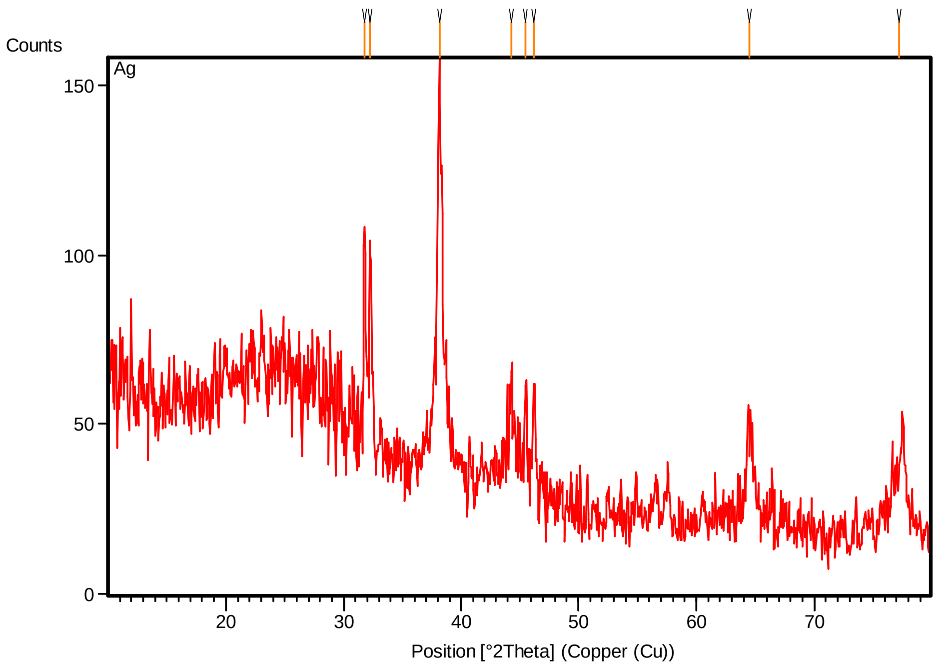

Figure 4 - XRD pattern of AgNPs

The XRD peaks at 2θ values of 38.13° ,45.50°, 64.50° and 77.19° can be attributed to the (111), (200), (220), and (311) crystalline planes of face-centered-cubic (FCC) structure of AgNPs, respectively. XRD pattern (Figure 4) clearly illustrates that the AgNPs are crystalline in nature [26]. The peak corresponding to the (111) plane is more intense than the other planes. This indicates that the nanoparticles are abundant in (111) plane. The size of the AgNPs as estimated from the FWHM of the (111) peak (i.e.100%) using the Scherrer formula is 18.27 nm. The average size of the AgNPs as estimated using the Scherrer formula is 36.44 nm. In addition to the Bragg peaks representative of face-centered-cubic structure of AgNPs, additional as yet unassigned peaks are also observed suggesting that the crystallization of bioorganic phase occurs on the surface of the AgNPs [27, 28].

3.3. Transmission electron microscopic analysis

TEM images (Figure 5A-C) reveal that AgNPs are chiefly spherical in shape and non uniform in size ranging from 10-50 nm and in good agreement with XRD measurements. AgNPs are surrounded by a faint thin layer of other materials (indicated by arrow marks in Figure 5A and 5B), which may be the capping organic materials from *Momordica charantia* leaf extract. Under careful observation, it is noted that the nanoparticles are not in direct contact even within the aggregates indicating stabilization of the nanoparticles by a capping agent. The ring-like diffraction pattern and the approximately circular nature of the selected area electron diffraction (SAED) spots indicated in Figure 5(D) reflects that the particles are crystalline in nature.

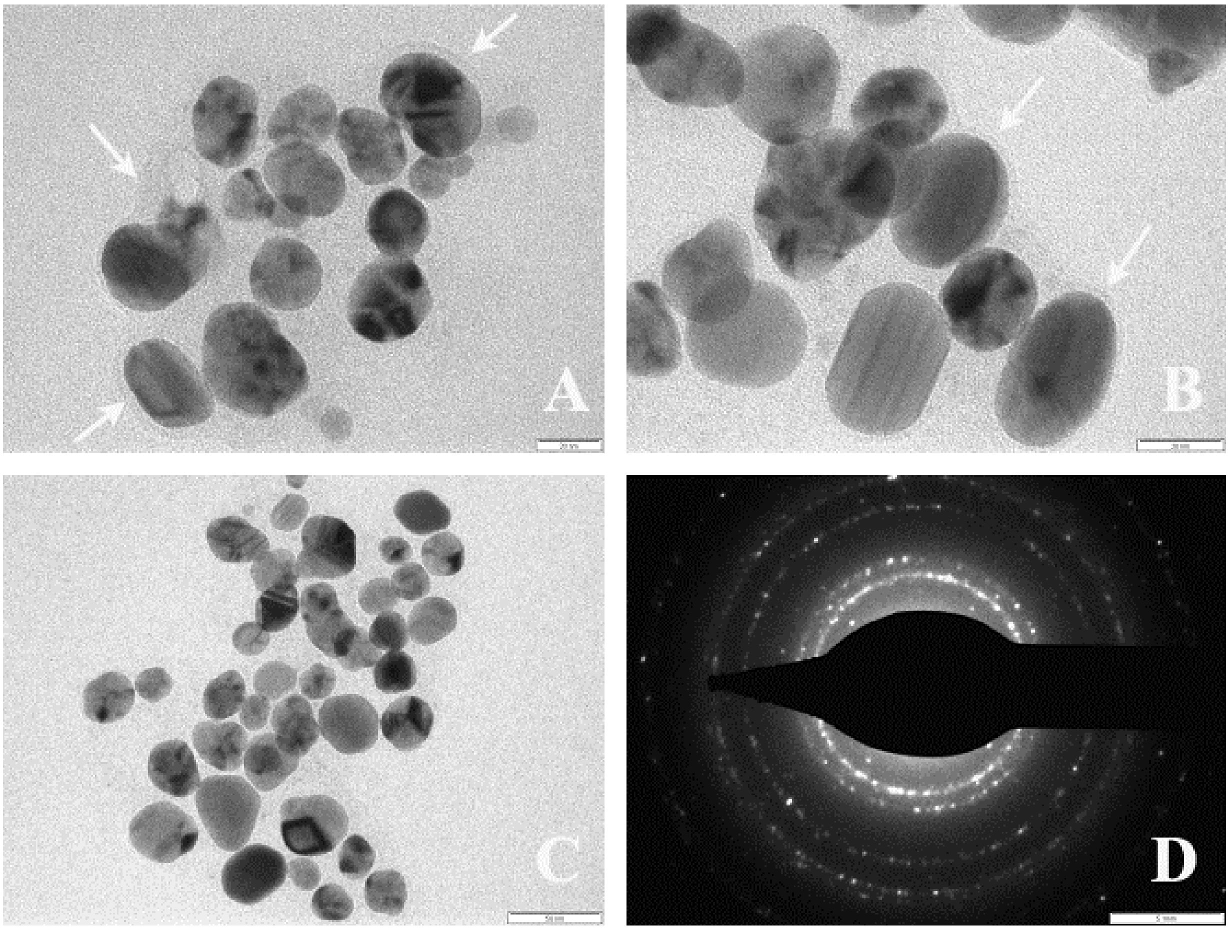

Figure 5 -TEM images of AgNPs in (A) 20nm (B) 20 nm (C) 50 nm scale (D) SAED pattern of AgNPs

3.4. FTIR analysis

The FTIR spectroscopy of *Momordica charantia* leaf extract (Figure 6) shows prominent peaks at 1050, 1385, 1597 and 3445 cm^{-1} due to C–N stretching (aliphatic amines) [29], C-H group(aromatic) [30], C=O stretching [31] and O–H stretching [32] respectively. The majority of the IR bands are characteristic of triterpenes, proteins, steroids, alkaloids and other phenolic compounds present in the leaf extract. The FTIR spectroscopy of AgNPs (Figure 6) shows prominent peaks at 1020, 1384, 1630 and 3424 cm^{-1} due to C–N stretching (aliphatic amines) [29], C-H group (aromatic) [30], C=O stretching [32] and O–H stretching [32] respectively. In particular, the broad and intense absorption peak at around 3445 cm^{-1} corresponds to the OH stretching vibrations of phenolic compound like galic acid. The shift from 3445 to 3424 cm^{-1} may indicate the involvement of OH functional group in the reduction of Ag^+ ions [33]. The IR absorption spectra indicate that the stretching vibration peak of carbonyl group shifted from 1597 to 1630 cm^{-1}. Since phenolic compounds are easily oxidized to form quinones, the shift from 1597 to 1630 cm^{-1}, may indicate that the product of gallic acid reduction of Ag^+ may be a quinoid compound [34]. The absorption bands that appear in the IR spectrum of the leaf extract could also be seen in the IR spectra of phytocapped AgNPs. This shows that the phytoconstituents protect the AgNPs from aggregation [35].

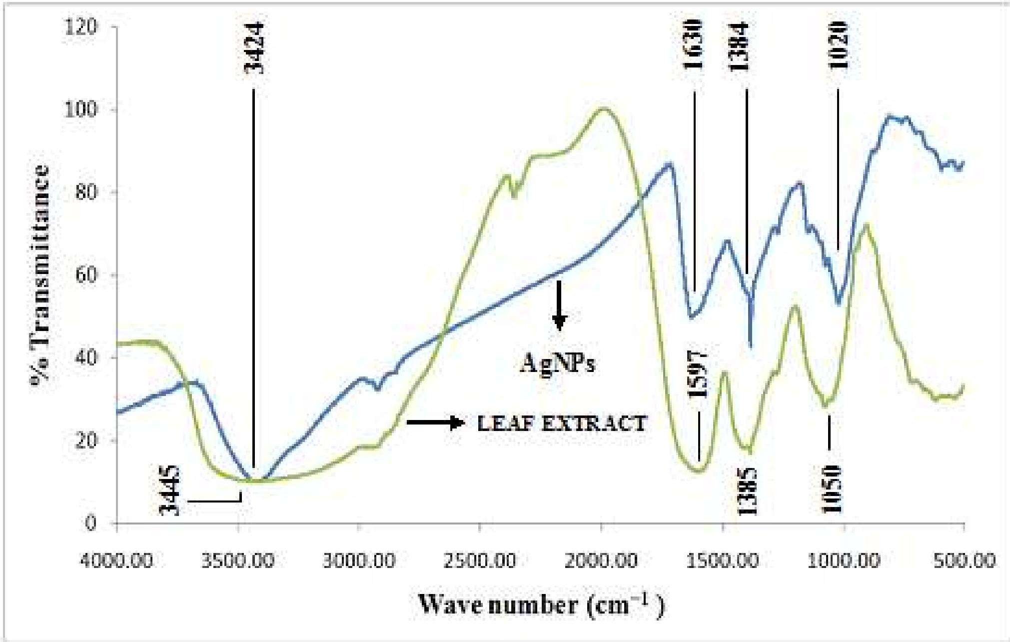

Figure 6 - FTIR spectra of AgNPs and *Momordica charantia* leaf extract

3.5. Mechanism

The main constituents of *Momordica charantia* are triterpenes, proteins, steroids, alkaloids and other phenolic compounds [23, 24, 36 and 37]. In the current study, the mechanism of AgNPs synthesis may be explained by the higher total phenolics content in the *Momordica charantia* leaf extract. These phenolics are strong antioxidants with high reducing capacity. Phenolic content in *Momordica charantia* leaf extract facilitates the reduction of silver ions to nanoscale-sized silver particles due to the electron donating ability of these phenolic compounds like gallic acid [38].

$$AgNO_3 \longrightarrow Ag^+ + NO_3^-$$

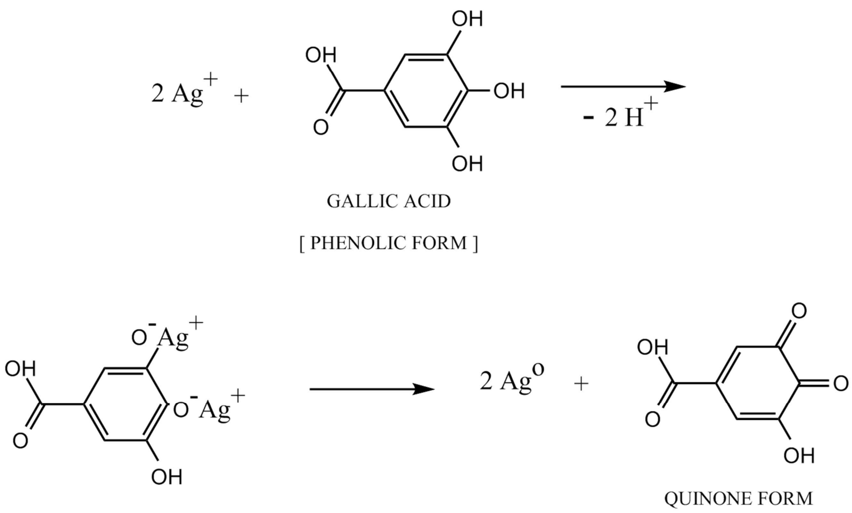

Figure 7 Mechanism of AgNPs synthesis

The possible mechanism for the reduction of Ag^+ is proposed and presented in Figure 7. In this scheme, Ag^+ ions can form intermediate complexes with phenolic OH groups present in Gallic acid which subsequently undergo oxidation to quinone form with consequent reduction of Ag^+ to AgNPs [39]. Moreover, the quinoid compound produced due to the oxidation of the phenol group in phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension stabilization [40].

3.6. Antimicrobial activity

Silver is said to be a universal antimicrobial substance for centuries. Though, silver ions or salts have limited usefulness as an antimicrobial agent. Such as, the interfering effects of salts and antimicrobial mechanism of continuous release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using AgNPs.

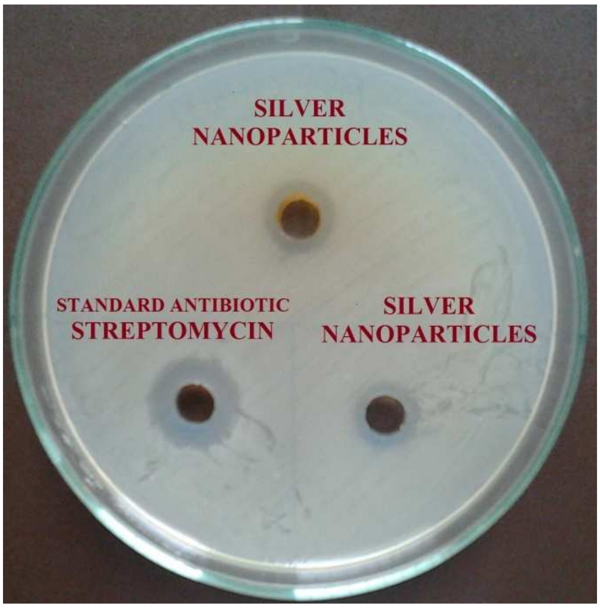

Figure 8 - Antibacterial activity of AgNPs

The antibacterial activity of biosynthesized AgNPs against *Kelbsilla pneumonia* bacteria (gram negative) has been studied and shown in Figure 8. AgNPs and standard antibiotic *Streptomycin* show zone of inhibition of 10 mm and 20 mm respectively. The result given in Table 1 indicates that biologically synthesized AgNPs possess tremendous antimicrobial properties.

Table 1 - Antibacterial activity data of AgNPs

Name of Species	Zone of Inhibition (mm)	
	Silver nanoparticles	*Streptomycin*
Kelbsilla pneumonia	10	20

CONCLUSION

Bio synthesis of silver nanoparticles using the *Momordica charantia* leaf 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 *Kelbsilla pneumonia* bacteria. Thus, this biosynthesized AgNPs can be used as effective growth inhibitors in various microorganisms, making them applicable to diverse medical devices and antimicrobial control systems.

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