

## Synthesis of AgNPs of *Momordica charantia* Leaf Extract, Characterization and Antimicrobial Activity

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### Abstract

The physicochemical and optoelectronic properties of metallic nanoparticles are strongly dependent on the size and size-distribution of the nanoparticles. In this study the silver nanoparticles were synthesized from leaf extract of *Momordica charantia* at room temperature as well as stirred at 60°C and their antibacterial activity was studied by Disc Diffusion Method. The effects of different leaf extract concentrations, metal ions concentration, reaction times and reaction temperatures on the synthesis of silver nanoparticles were evaluated. The Nanoparticles were characterized with UV-Visible, XRD, SEM, and FTIR. The UV-Vis spectra showed that the Surface Plasmon Resonance peak of silver colloids synthesized from *M.charantia* leaf extract were observed at 404 nm and 424 nm for stirred at 60°C and room temperature condition. X-ray diffraction (XRD) analysis confirmed that the nanoparticles were crystalline in nature with Face Centered Cubic structure. Scanning Electron Microscopy (SEM) analysis showed that silver nanoparticles were spherical in shape with an average size of 20-50 nm. The FTIR measurement was carried out to identify the possible functional groups responsible for efficient stabilization of silver nanoparticles. The inhibitory activity of prepared silver nanoparticles were tested against the pathogen *Staphylococcus aureus* at different AgNPs concentration which showed moderate inhibitory action against *S. aureus* and *B. subtilis*.

**Keywords:** Silver nanoparticles; *Momordica charantia*; Surface plasmon resonance; Antimicrobial activity

### Introduction

Nanoparticle research is currently an area of intense scientific interest due to a wide variety of applications in biomedical, optical and electronic fields [1]. Metal nanoparticles are very important due to their potential applications in emerging areas of Nanoscience and technology. Size, shape, and surface morphology plays a vital role in controlling the physical, chemical, optical, and electronic properties of these nanoscopic materials [2]. Noble metals such as Ru, Pd, Ag, Pt and Au are exhibiting a wide range of material behavior along the atomic to bulk transition [3]. Silver in colloidal state exhibits distinctive properties, such as good conductivity, chemical stability, catalytic and antibacterial activity. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures.

The major methods used for silver nanoparticles synthesis are the physical and chemical methods. The problem with the chemical and physical methods is that the synthesis is expensive and can also have toxic substances which can be absorbed by the nanoparticles. The biological method is one of the alternative methods which involve bacteria, fungi, and plant extracts [4]. Plants have flavonoids, alkaloids and polyphenolic compounds which may reduce the silver ions to silver nanoparticles and acts as capping and stabilizing agent [3]. The biosynthesis of silver nanoparticles using several plant extracts such as *Moringa oleifera* [5,6], *Solanum xanthocarpum* [7], *Azadirachta indica* [8], *Eucalyptus chapmaniana* [9], *Bacopa monniera* [10], *Tinospora cordifolia* [11], *Brassica oleracea* [12], *Arbutus unedo* [13] have been already demonstrated in various approaches.

*Momordica Charantia* or Bitter Melon is a Tropical vegetable, is a common food in Indian cuisine and is used extensively in folk medicine as a remedy for diabetes. Bitter melon has been used in various Asian traditional medicines for a long time. The major parts used are leaves, fruits and flowers. Leaves are simple; usually palmately 5-7 lobed, tendrils unbranched or 2 branched. The herbaceous, tendril bearing

vine grows to 5 m. It bears simple, alternate leaves 4-12 cm across, with 3-7 deeply separated lobes. The main constituents of *M. Charantia* are Alkaloids, charantin, Momordicin, ascorbic acid, phenol and protein [14].

In the present study, the biosynthesis of silver nanoparticles using the leaf extract of *Momordica charantia* has been reported which belongs to the family Cucurbitaceae. Synthesized nanoparticles were characterized with UV-Visible spectroscopy, XRD, FTIR, and SEM and the antimicrobial activity of synthesized silver nanoparticles against *S. aureus* and *E. coli* were also analyzed (Table 1).

### Experimental

#### Materials

Silver nitrate, AgNO<sub>3</sub> (99.8%) purity was purchased from Sigma-Aldrich chemical Pvt.Ltd. Leaves of *Momordica charantia* was collected from sivagangai district, Tamil Nadu. Double Distilled water was used throughout the experiments.

#### Preparation of leaf extract

Leaves of *M. charantia* were rinsed with tap water thrice followed by Double distilled water to remove the dust and other contaminants. 5 gms of green fresh leaves were weighed and sliced into small pieces. Then 100 ml of Double Distilled Water was added and boiled in a 500

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Diffraction Angle (2θ)	d-spacing(A°)		Diffraction Planes	Crystallite Size (nm)	Lattice constant (a) (A°)
	Experimental values	JCPDS values			
38.18°	2.360	2.3587	(111)	17	4.0876
44.33°	2.0447	2.0427	(200)	12	4.0894
64°	1.4430	1.4444	(220)	7	4.0814

**Table 1:** XRD data for AgNPs synthesized using *M. charantia* leaf extract.

ml Erlenmeyer flask. The mixture was heated at 60°C for 30 minutes. After cooling the extract, it was filtered using whatman No.1 filter paper and finally a yellow colour extract was collected and stored in 4°C for further synthesis process.

### Biosynthesis of silver nanoparticles

Silver Nitrate and leaf extract was taken in different molar ratios (1:1, 1:2, and 1:3). Silver nanoparticles were prepared by adding 10 ml of 1 mM AgNO<sub>3</sub> aqueous solution to 10 ml, 20 ml, and 30 ml of *M. charantia* extract. The solution were stirred at room temperature as well as heated at 60°C at constant stirring for 90 minutes (Figure 1). The colour change of reaction mixture was observed from yellow to dark brown colour which indicates the formation of silver nanoparticles. The optimum factors for silver nanoparticles synthesis were carried out in different conditions such as differing silver ion concentration (0.5, 1 and 2 mM) (Figure 2), Temperature (70°C, 80°C and 90°C) (Figure 3), Reaction Time (2 hrs to 30<sup>th</sup> day). The influence of these parameters on silver nanoparticle synthesis was analyzed by UV-Vis spectrophotometer.

### Characterization

A Double beamed spectrophotometer (Helios alpha) was used for absorption spectra measurements at different wavelength (200-800 nm). The graph of wavelength on X-axis and Absorbance on Y-axis was plotted. In every case double distilled water was taken as the blank solution. The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 30 minutes and repeated the centrifugation process for 3 to 4 times and filtered the resulting solution and dried the pellet in hot air oven at 60°C for 10-15 min. Crystalline nature of metallic silver was examined by X-ray diffraction analysis using X'Pert PRO Analytical-PW 3040/60 X- ray diffractometer with a Cu Kα radiation monochromatic filter in the range 10-90°. Morphology and size of silver nanoparticles were analyzed by JEOL JSM-6701F Field Emission Scanning Electron Microscope. FTIR measurements were done using Nicolet 380 FT-IR spectrophotometer.

### Evaluation of antibacterial activity

The synthesized silver nanoparticles were tested for its potential antibacterial activity against bacterial pathogens *Staphylococcus aureus* by disc diffusion method which involves swabbing the cultures in pre-sterilized Muller Hinton agar plates and four wells were cut in the same using sterile cork borer. Each well was loaded with 10 µl of the solutions in the following order: water as negative control, aqueous leaves extract of *Momordica charantia*, solution of silver nanoparticles, and standard Amikacin. Then the sample loaded Muller Hinton agar plates and sabouraud dextrose agar plates were incubated at 37°C for 24 hrs respectively. Then the formation of Zone of Inhibition was observed.

## Results and Discussion

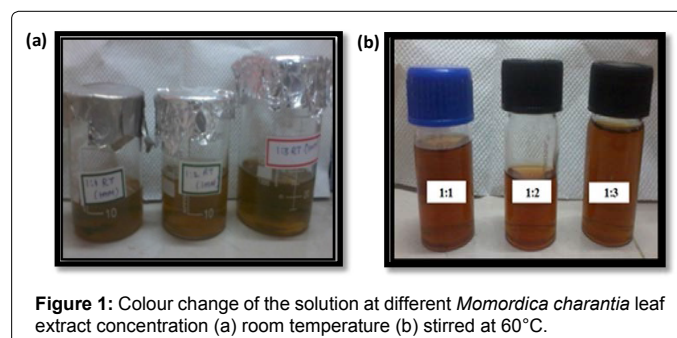
### UV-Visible spectral analysis

**Effect of leaf extracts concentration:** The formation of silver nanoparticles and the stability in aqueous solution were confirmed

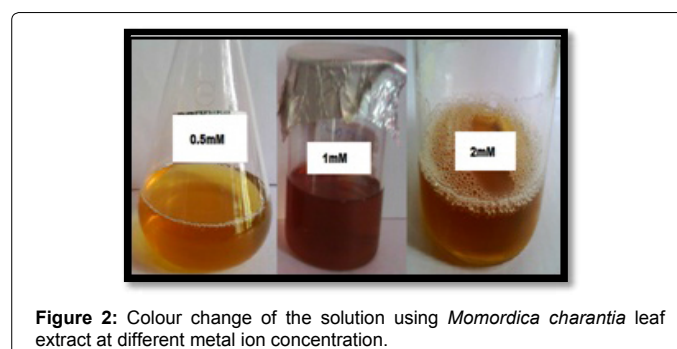
by UV-Vis absorption spectroscopy. The SPR band arises when nanoparticles are irradiated with visible light, because of the collective oscillations of the conduction electrons [15]. The optical absorption spectra of Silver Nanoparticles of three different molar ratios 1:1, 1:2, 1:3 have been analyzed at room temperature and also stirred at 60°C. The UV-Vis spectra of silver nanoparticles at different concentration of *M. charantia* leaf extract was shown in Figure 1. Silver colloids exhibited dark brown colour in aqueous solution due to the surface plasmon resonance. As increasing the leaf extract concentration, the peak shifted towards the blue end from 428 nm to 424 nm for room temperature and from 412 nm to 404 nm for stirred at 60°C. When the particles decrease in size, the absorption peak usually shifts towards the blue wavelength [15]. It was observed that absorbance increases as concentration of leaf extract increase which was due to higher reduction of silver nitrate into silver nanoparticles. Since, the peak was broader, the particles were polydispersed. The single band indicated that the particles were spherical in shape.

When comparing the silver nanoparticles synthesized at room temperature and stirred at 60°C for molar ratio 1:3 of extract, the reduction of the silver nanoparticles was higher while heating (Figure 2). The maximum intensity was observed at 404 nm synthesized while heating. This proved that quantitatively greater reduction occurs with stirring at 60°C. Whereas in the case of room temperature, the intensity was decreased and the wavelength was longer with 424nm. This was due to large, polydispersed and anisotropic nanoparticles [16].

**Effect of reaction time:** The effect of reaction time for silver nanoparticles at room temperature and stirred at 60°C was studied for 30 ml of leaf extract. UV-Vis spectra were recorded as function of reaction time as shown in Figure 3. It was exposed at different time interval such as 2 hrs, 1<sup>st</sup> day upto 30<sup>th</sup> day. Figure 3 shows the SPR peak at 424 nm for room temperature and 404nm for stirred at 60°C indicated the formation of silver nanoparticles and was found that it steadily increasing with the time of reaction without change in peak wavelength. The absorption peak intensity increased rapidly



**Figure 1:** Colour change of the solution at different *Momordica charantia* leaf extract concentration (a) room temperature (b) stirred at 60°C.



**Figure 2:** Colour change of the solution using *Momordica charantia* leaf extract at different metal ion concentration.

with increase in reaction time which was due to the formation of silver nanoparticles. It was observed that increase in intensity can be correlated with an enhancement in the number of nanoparticles in the reaction medium [17]. The formation of AgNPs was found within 1hr. when comparing both room temperature and heating, the absorbance was higher while heating. This confirmed that higher temperature favours the formation of silver nanoparticles.

**Effect of metal ion concentration:** The UV-Vis spectrum (Figure 4) shows the effect of silver nitrate concentration in the synthesis of silver nanoparticles by *M.charantia* extract. The silver nitrate molarity was increased from 0.5 mM to 2 mM. By increasing the concentration, there was a blue shift in SPR band of silver nanoparticles, because the particles are smaller in size and also there was a fall in absorbance. When the SPR band shifts to blue end, the electrons are donated to the particles [18]. But, as the concentration increased the peak became broader. Hence it was polydispersed. The study of Neran Ali Thamer et al. [19] has been found that the maximum peak was observed at 1mM. In the present study the optimum concentration of Silver Nitrate was found to be 1mM which was shown in Figure 4.

**Effect of reaction temperatures:** The UV-Vis spectrum (Figure 5) shows the effect of temperature in the silver nanoparticles synthesis at concentration of 30 ml of leaf extract with silver nitrate solution. The temperature was varied from 60°C to 90°C in steps of 10°C. The size of silver nanoparticles was affected by varying the temperature. When increasing the temperature, the SPR band became broader and also the peak shifted towards the red end from 404 nm to 452 nm. This increase in wavelength was due to the aggregation of nanoparticles. When the particles increase in size, the absorption peak usually shifts towards the red wavelength [17]. It can be observed that an optimum temperature is required for the completion of reaction due to the instability of formed silver nanoparticles. The optimum temperature required for this reaction to complete was obtained to be 60°C (Figures 6-8).

### X-ray diffraction analysis

Figure 9 shows the XRD pattern of dried silver nanoparticles synthesized using 30 ml of *M. charantia* leaf extract. Three diffraction peaks were observed at 38.18°, 44.33°, 64° in the 2θ range 10-90°

which was due to the reflections of (111), (200), (220) planes which corresponds to face centered cubic (fcc) structure of silver (JCPDS file no.65-2871). Thus, XRD pattern clearly showed that the silver nanoparticles formed in this synthesis were crystalline in nature. The average crystalline size calculated by Debye-Scherrer equation was 22 nm. The unassigned peaks at 2θ=27.56° and 32° was due to ascorbic acid present in the leaf extract and was verified using JCPDS file no. 22-1536.

### SEM Analysis

Figure 10 shows the SEM image of AgNPs synthesized with 30 ml of *M. charantia* leaf extract. The average size of the synthesized silver nanoparticles was found to be in the range of 20-50 nm with the crystallite size estimated from the XRD analysis shown in Figure 9. The silver nanoparticles were found to be spherical in shape and were aggregated. This aggregation may be due to secondary metabolites in the leaf extracts.

### FTIR analysis

The FTIR spectra (Figure 11) of silver nanoparticles synthesized at room temperature showed a strong band at 3292.3 cm<sup>-1</sup> which corresponds to O-H Stretching which was due to the presence of double distilled water. The band at 1636.1 cm<sup>-1</sup> reveals the presence of C=C group which was due to the presence of Momordicin compound

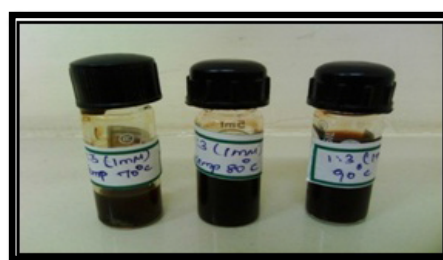


Figure 3: Colour change of the solution using *Momordica charantia* leaf extract at different temperatures.

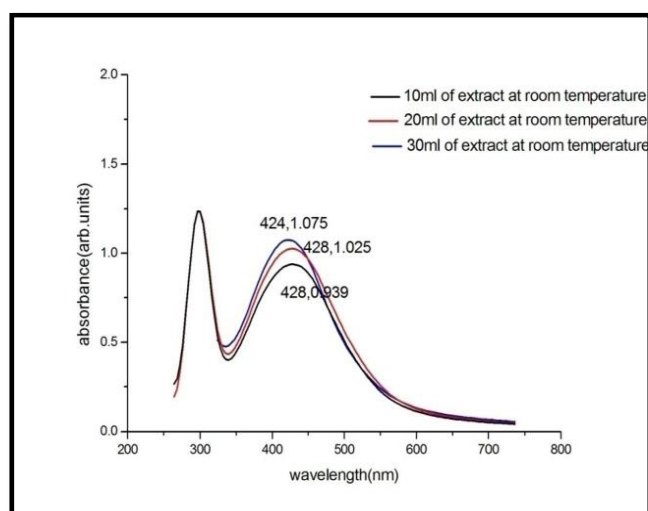
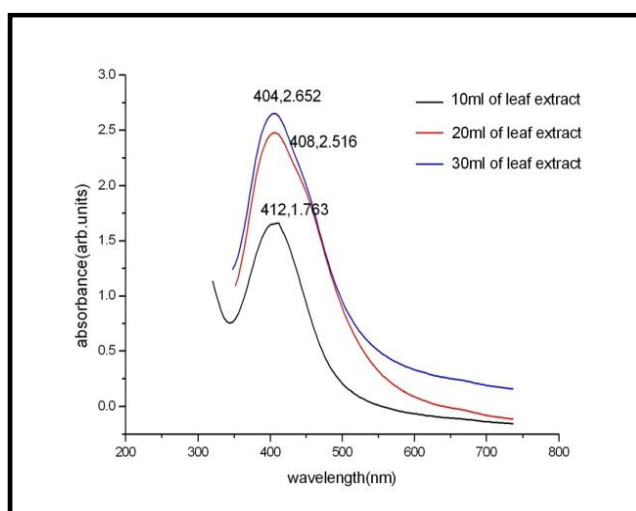


Figure 4: UV-Vis spectra of silver nanoparticles at different concentration of *M. charantia* leaf extract (room temperature and stirred at 60°C).



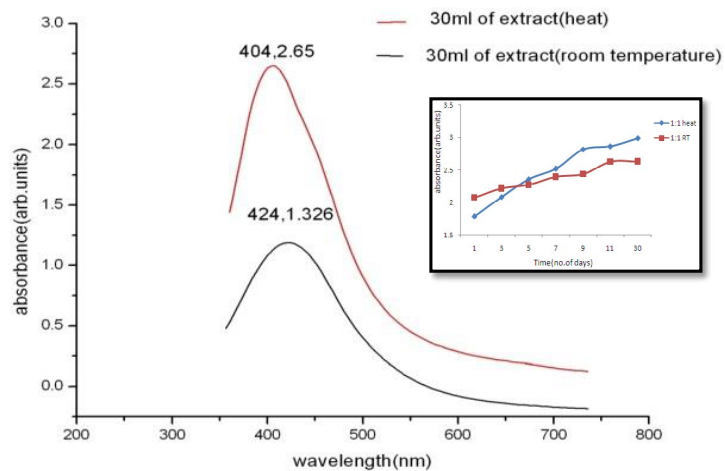


Figure 5: comparison of UV-Vis spectra of silver nanoparticles using *M. charantia* (room temperature vs. stirred at 60°C).

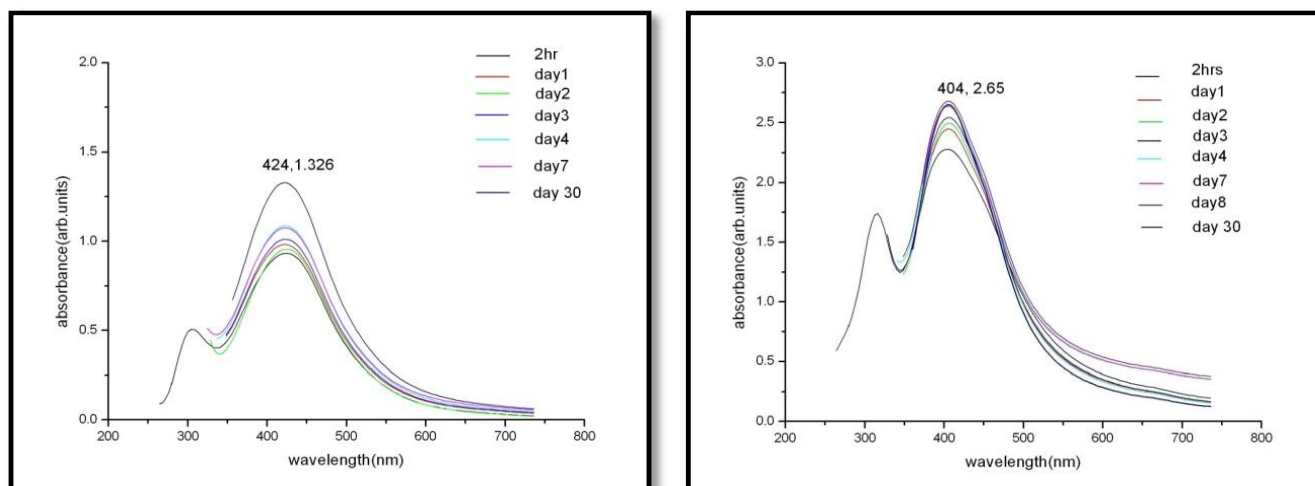


Figure 6: UV-Vis spectra of silver nanoparticles at different reaction time of *M. charantia* leaf extract (room temperature and stirred at 60°C).

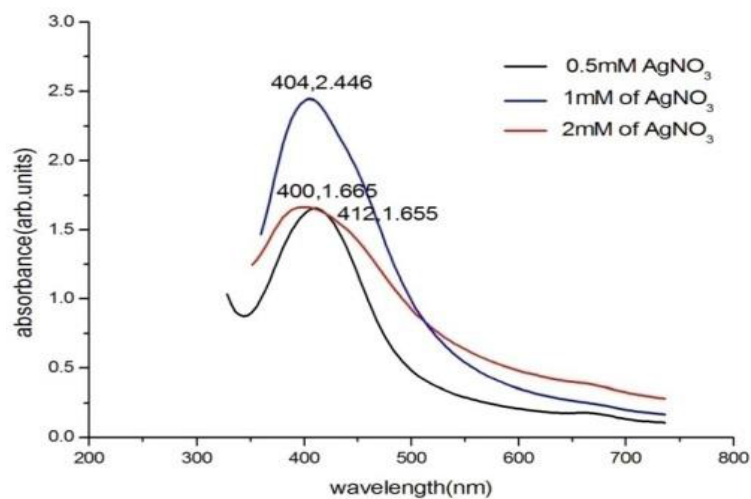


Figure 7: UV-Vis spectra of silver nanoparticles at different concentration.

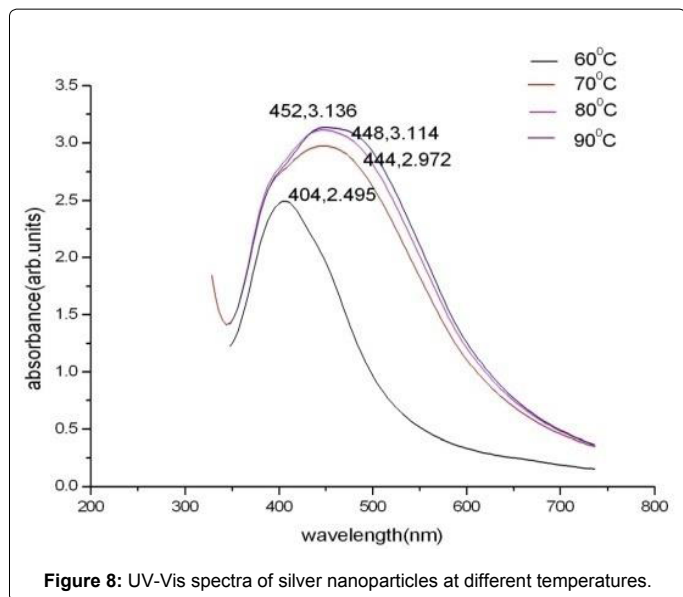


Figure 8: UV-Vis spectra of silver nanoparticles at different temperatures.

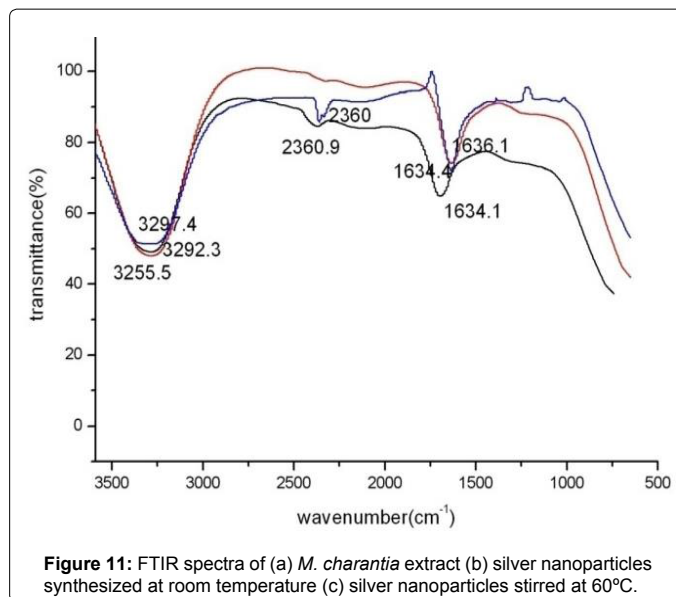


Figure 11: FTIR spectra of (a) *M. charantia* extract (b) silver nanoparticles synthesized at room temperature (c) silver nanoparticles stirred at 60°C.

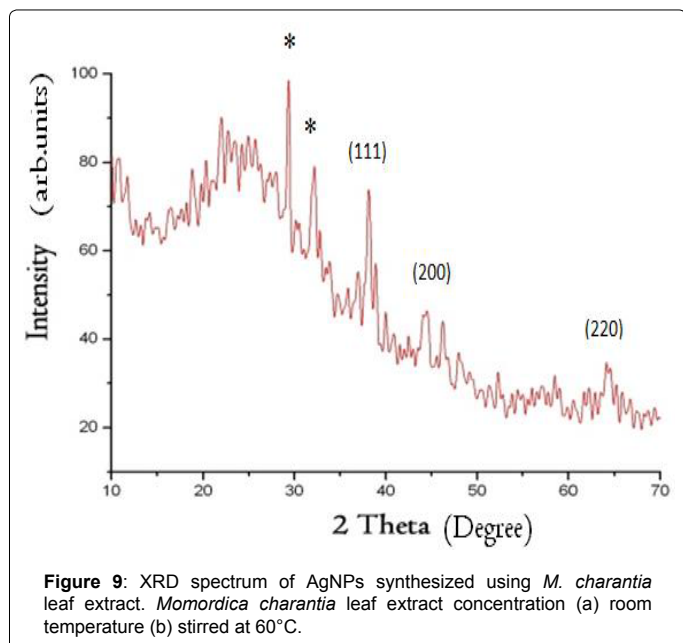


Figure 9: XRD spectrum of AgNPs synthesized using *M. charantia* leaf extract. *Momordica charantia* leaf extract concentration (a) room temperature (b) stirred at 60°C.

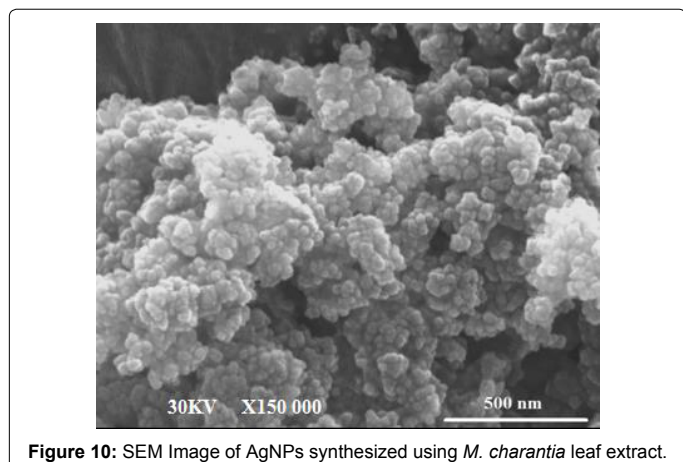


Figure 10: SEM Image of AgNPs synthesized using *M. charantia* leaf extract.

present in the leaf extract. For silver nanoparticles stirred at 60°C, the C=C ring stretching and O-H stretching were observed at 1634.1 cm<sup>-1</sup> and 3255.5 cm<sup>-1</sup>. This peaks were due to the presents of Momordicin which was one of the major phytochemical present in the leaf extract. When comparing the spectrum of both room temperature and heating, a weak peak was observed at 2360.9 cm<sup>-1</sup> in the heating process which may be due to the vibration of -CH<sub>3</sub> Stretching of charantin compound. The Momordicin and charantin compound present in bitter melon was responsible for the reduction of silver nanoparticles and also used as stabilizing agent.

### Antibacterial activity

Silver nanoparticles have been known to have inhibitory and bactericidal effects. The antibacterial activity of phyto-synthesized silver nanoparticles was analyzed against gram positive bacteria namely *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria namely *E. coli*. The results of antimicrobial activity of each concentration were shown in Table 2. Figure 12 shows the diameter of the zone of inhibition (ZOI) for leaf extract and for different concentrations of AgNPs with *M. charantia* extract. For gram positive *Staphylococcus aureus*, the moderate ZOI at 8 mm in 20 µl and 6 mm in 10 µl for AgNPs with *M. charantia* extract was observed. For *Bacillus subtilis*, the moderate ZOI at 8 mm in 20 µl for AgNPs with *M. charantia* extract was observed. As increasing the concentration of the nanoparticles, the zone of inhibition was also increased in both gram positive bacteria. When comparing the ZOI of leaf extract and AgNPs with *M. charantia* extract, silver nanoparticles inhibited better than leaf extract. The cell wall of Gram positive bacteria composed of a multiple layer of peptidoglycan forms more rigid structure leading to complicated diffusion and penetration of the AgNPs. The microbicidal activity is due to the silver cations released from AgNPs pertaining to changes in the membrane structure of microbes, which lead to the increased membrane permeability of the bacteria and finally cell death [5]. In case of gram negative bacteria, there was a less ZOI obtained.

### Conclusion

The phytosynthesis of AgNPs using leaf extract were rapid, eco-friendly and conventional method when compared to physic-chemical synthesis. In the present study, silver nanoparticles of three different

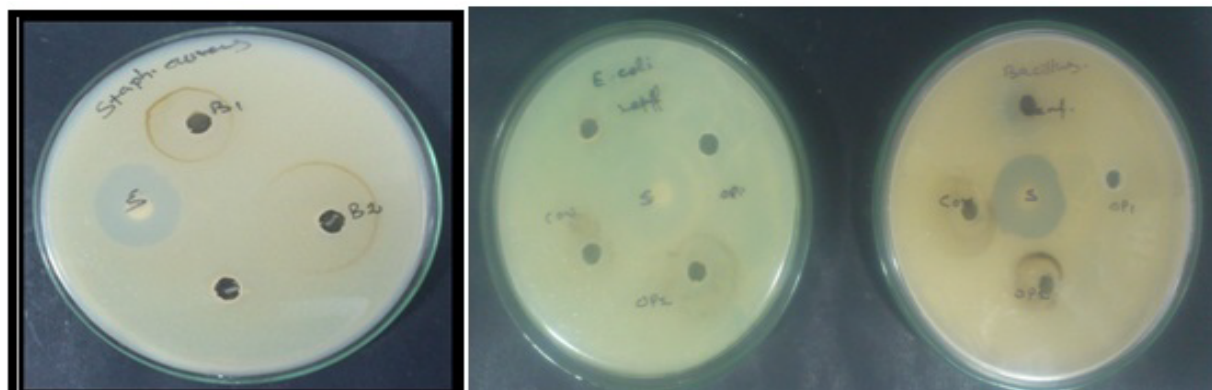


Figure 12: Antibacterial activity of silver nanoparticles against (a) *S. aureus* (b) *E. coli* (c) *B. subtilis*

Name of the bacteria	Concentration of silver nanoparticles using leaf extract ( $\mu\text{l}$ )	Zone of inhibition (mm in diameter)
<i>Staphylococcus aureus</i>	B1 (10 $\mu\text{l}$ )	06
	B2 (20 $\mu\text{l}$ )	08
	Control	NIL
	standard	14
<i>Bacillus subtilis</i>	OP1 (10 $\mu\text{l}$ )	NIL
	OP2 (20 $\mu\text{l}$ )	8
	Leaf	6
	Control	NIL
<i>E. coli</i>	Standard	14
	OP1 (10 $\mu\text{l}$ )	NIL
	OP2 (20 $\mu\text{l}$ )	3
	Leaf	NIL
	Control	NIL
	Standard	17

Table 2: Antibacterial activity of silver nanoparticles synthesized from *M. charantia* leaf extract.

molar ratios were synthesized at room temperature and stirred at 60°C using *M. charantia* extract. The Effect of different leaf extract concentrations, reaction times, metal ion concentrations, reaction temperatures on the formation of nanoparticles were analyzed by the changes in absorbance and wavelength of surface plasmon resonance of the silver nanoparticles. The XRD study showed the crystalline nature of the nanoparticles and the average size of the nanoparticles was calculated by Debye-Scherrer's formula which was found to be 22 nm. The SEM analysis showed that silver nanoparticles were spherical shape with an average size of about 20-50 nm. The compounds responsible for the bioreduction of  $\text{Ag}^+$  ion and the functional groups present in *M. charantia* extract were investigated using FTIR. The synthesized silver nanoparticles using aqueous leaf extracts showed better antibacterial efficacy against *Staphylococcus aureus* and *Bacillus subtilis*.

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#### References

- Abhilash M (2010) Potential applications of Nanoparticles. International Journal of Pharma and Bio Sciences 1: 1-12.

- Poovathinthodiyil Raveendran, Jie Fu, Scott L Wallen (2003) Completely Green Synthesis and Stabilization of Metal Nanoparticles. Journal of American Chemical Society 125: 13940-13941
- Vanaja M, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Malarkodi C, et al. (2013) Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract. Advances in Applied Science Research 4: 50-55.
- Sukumaran Prabhu, Eldho K Poullose (2012) Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. International Nano Letters 2: 1-10.
- Prakash P, Gnanaprakasam P, Emmanuel R, Arokiyaraj S, Saravanan M (2013) Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi*, Linn. for enhanced antibacterial activity against multi drug resistant clinical isolates. Colloids Surf B Biointerfaces 108: 255-259.
- Prasad TNKV, Elumalai EK (2011) Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity, Asian Pac J Trop Biomed 1: 439-442.
- Amin M, Anwar F, Janjua MR, Iqbal MA, Rashid U (2012), Green Synthesis of Silver Nanoparticles through Reduction with *Solanum xanthocarpum* L. Berry Extract: Characterization, Antimicrobial and Urease Inhibitory Activities against *Helicobacter pylori*, Int J Mol Sci 13: 9923-9941.
- Lalitha A, Subbaiya R, Ponmurugan P (2013) Green synthesis of silver nanoparticles from leaf extract *Azadirachta indica* and to study its anti-bacterial and antioxidant property, International Journal Of Current Microbiology And Applied Sciences 2: 228-235.
- Sulaiman GM, Mohammed WH, Marzoog TR, Al-Amiery AA, Kadhum AA (2013) Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. Asian Pac J Trop Biomed 3: 58-63.
- Mahitha B, Deva Prasad Raju B, Dillip GR, Madhukar Reddy C, Mallikarjuna K (2011) Biosynthesis, Characterization And Antimicrobial Studies Of AgNPs extract From *Bacopa monniera* Whole Plant, Digest Journal of Nanomaterials and Biostructures 6: 135-142.
- Rajathi K, Vijaya Raj D, Anarkali J, Sridhar S (2012) Green Synthesis, Characterization And *In-Vitro* Antibacterial Activity Of Silver Nanoparticles By Using *Tinospora cordifolia* Leaf Extract. International Journal of Green Chemistry and Bioprocess 2: 15-19.
- Mercy Ranjitham A, Suja R, Caroling G, Sunita Tiwari (2013) Invitro Evaluation of Antioxidant, Antimicrobial, Anticancer Activities and Characterization of *Brassica oleracea*. Var. *bortrytis*. L Synthesized Silver Nanoparticles. Int J Pharm Pharm Sci 5: 239-251.
- Srinivas Naik L, Paul Marx K, Sree vennela P, Venkata Ramana Devi Ch (2013) Green synthesis of silver nanoparticles using Strawberry leaf extract (*Arbutus unedo*) and evaluation of its antimicrobial activity-a Novel study. International Journal of Nanomaterials and Biostructures 3: 47-50.
- Sathish Kumar D, Vamshi Sharathnath K, Yogeswaran P, Harani A, Sudhakar K (2010) International Journal of Pharmaceutical Sciences Review and Research 1.

15. Kiruba Daniel SCG, Mahalakshmi N, Sandhiya J, Kasi Nehru, Muthusamy Sivakumar (2013) Rapid synthesis of Ag nanoparticles using Henna extract for the fabrication of Photoabsorption Enhanced Dye Sensitized Solar Cell (PEDSSC). *Advanced Materials Research* 678: 349-360.
16. Pala Rajasekharreddy, Pathipati Usha Rani, Bojja Sreedhar (2011) *J Nanopart Res* 12: 1711-1721.
17. Umadevi M, Shalini S, Bindhu MR (2012) Synthesis Of Silver Nanoparticles Using D.Carota Extract. *Adv Nat Sci Nanosci Nanotechnol* 3: 1-6
18. Bindhu MR, Umadevi M (2013) Synthesis of monodispersed silver nanoparticles using *Hibiscus cannabinus* leaf extract and its antimicrobial activity. *Spectrochimica Acta part A: Molecular and Biomolecular Spectroscopy* 101: 184-190.
19. Neran Ali Thamer, Lamia A Almashhedy (2014) Green Synthesis Optimization And Characterization Of Silver Nanoparticle using Aqueous Extract Of *Crocus sativus* L. *Int J Pharm Bio Sci* 5: 759-770