International Journal of Pharmacy Research and Technology
2013, Volume 3, Issue 2, 11-16
ISSN 2250 – 0944 (Online)
ISSN 2250 – 1150 (Print)

Research Article

Green Synthesis of Silver Nanoparticles by Using *Tinospora Cordifolia* Stem Powder, Characterization and Its Antibacterial Activity Against Antibiotics Resistant Bacteria

Samir A. Anuj
Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New V.V. Nagar
***Corresponding Author* Email: samiranuj@gmail.com Contact:** +91 9427975819
Received: 15/06/2013, Revised: 30/09/2013 Accepted: 09/10/2013

ABSTRACT
Silver nanoparticles were synthesized from 1mM aqueous silver nitrate through an ecofriendly and cost effective method by using *Tinospora cordifolia* stem powder. The bioreduction behavior of stem powder of *Tinospora cordifolia* is responsible for this biosynthesis. The stem powder extracts mixed with silver nitrate showed gradual change in the color of the extracts from yellow to dark brown. The formation of silver nanoparticles was confirmed by UV-Visible spectrophotometer, X-Ray diffraction (XRD), Fourier transform infrared (FTIR), Energy dispersive spectroscopy (EDAX) and Transmission electron microscopy (TEM). Silver nanoparticles have been known to have bactericidal and inhibitory effects. Resistance to antibiotics by pathogenic bacteria has emerged in recent years and is a major health problem. The effects of silver nanoparticles and different antibiotics on antibiotic resistant bacteria have not been studied.

Key Words: Silver nanoparticles, *Tinospora cordifolia*, antibacterial activity, Energy dispersive spectroscopy (EDAX), X-Ray diffraction (XRD), Fourier transform infrared (FTIR), Antibiotic resistance bacteria.

INTRODUCTION

Nanoparticles are the basic essential elements in the field of nanotechnology and it exhibits fabulous advanced characteristic features based on their properties such as size, morphology and other size dependent properties[1]. Nanotechnology is the most advanced field of study in the modern material sciences[2]. Nanoparticles can one of the nearly everyone sought materials for the future significant in many of the fields. Nano particles can only be formed in chemical reactions, either by combustion or by condensation. They cannot be produced by mechanical processes such as brushing or grinding. This is because of the strong Van der waals forces that keep them attached to one or other surface and prevent them from being released into the air when two particles collide those forces will keep them irreversibly together. Noble metals like silver, gold and platinum exhibit a particularly wide range of material behavior along the atomic to bulk transition[3]. Among these noble metals silver have wide applications in jewellery, dental and health additive in traditional Chinese and Indian Ayurvedic medicine[4]. Silver nanoparticles exhibit tremendous applications in drug delivery[5], wound healing[6], sensor applications[7] and also used antimicrobial agent in paint[8]. Silver nanoparticles were actively involved in the medical sciences due to their antimicrobial actions in food pathogens *Staphylococcus aureus* and *Escherichia coli*[9], *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*[10], *Streptococcus pyogenes* and *Salmonella typhi* and having good antifungal activities againts *Aspergillus niger*, *Candida albicans* and *Penicillium citrium* [12].

Synthesis of silver nanoparticles by biological method using fungi, bacteria, enzymes, algae and plant extracts has more advantages due to their environment being process and ability of large scale production over physical and chemical methods [13]. Biological methods of synthesis have smooth way for the "greener synthesis" of nanoparticles and these have confirmed to be better methods due to slower kinetics, they offer enhanced manipulation and control over crystal growth and their stabilization. Green synthesis of silver nanoparticles shows more advantageous over other biological processes are bacteria and fungi, because it eliminates the cell culture maintaining process and also it more suitable for large scale production of silver nanoparticles [14]. The synthesized silver nanoparticles were characterized using UV-Visible spectrophotometer, XRD, FTIR, EDAX and TEM.

The antimicrobial activity against human pathogenic bacteria is significant. Comparison between silver nanoparticles and antibiotics provides efficiency of silver nanoparticles. Antibiotics inhibits growth of only prokaryotic micro-organisms, while silver nanoparticles inhibits growth of fungi also which indicates that the silver nanoparticles inhibits growth of both prokaryotes and eukaryotes. Another interesting characteristic of silver nanoparticles is that they kill the antibiotics resistance bacteria also.

Materials and Methods

Preparation of *Tinospora Cordifolia's* Stems Powder

Fresh *T. cordifolia* plants were collected from surroundings of Ashok and Rita Patel Institute of Integrated Study and Research In Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Anand, Gujarat, India. Dried finely cut stems in hot air oven at 50^{o} C to 55^{o} C for one week. Taking 10g of *T. cordifolia's* dried stem powder add in flask with 150ml of distilled water and then boiling the mixture for 8-10 min. and cooled that mixture. This cooled mixture was centrifuged at 5000 rpm for 10 min. and collected yellow supernatant. This supernatant used for further experiments.

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was purchased from Himedia chemicals, Ahmedabad, Gujarat, India. In the typically synthesis process of silver nanoparticles, add 40ml supernatant of boiled stem powder into the 200ml of 1mM of silver nitrate solution in stirring at room temperature.
The bioreduced component was monitored by using UV-Visible spectrophotometer periodically.

Characterization of Silver Nanoparticles
BY COLOR CHANGE

The color change in reaction mixture was recorded through visual observation. The color change of the supernatant from light yellow to dark brown indicated that the silver nanoparticles were synthesized.

UV-Visible Spectral Analysis

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of at different time intervals and the absorption maxima was scanned by UV-Visible spectrophotometer at the wavelength of 300-700 nm on UV-Visible spectrophotometer (Perkin Elmer Lambda 25 spectrophotometer), using deionized water as the reference.

X-Ray Diffraction Studies (XRD)

The synthesized silver nanoparticles were centrifuged at 10,000 rpm for 15 min. and collect the pellet. The pellet was washed with distilled water to remove impurities and dried to get powder. The X-Ray diffraction assay was performed for the detection of crystalline nature of the metal nanoparticles was done by X-Ray diffractometer (Phillips, Holland model: X" Pert), operating at 40 kV and current of 30 mA with Cu Kα radiation ($\lambda = 1.5404^o$) and the 2θ scanning range was 0-90^o at 2^o min^{-1}. The colloidal suspension containing metal nanoparticles was dried on a small glass slab.

Fourier Transform Infrared Spectroscopy (FT-IR)

To identify the bio-molecules associated with the synthesis of nanoparticles by plant mediated was performed by using FT-IR (Perkin Elmer, Spectrum GX). The dried silver nanoparticles were grinded with KBr pellets and measured at the wavelength range from 4000 to 400 cm^{-1}.

Transmission Electron Microscopic Examination (TEM)

Transmission electron microscopic examination was done to know the morphology of silver nanoparticles, using high-resolution analytical transmission electron microscope (Phillips, Netherland Model: Technai20). In this examination we used centrifuged powder of the solution of silver nanoparticles. For sample preparation, 2-3 drops of the colloidal silver solution were dispensed onto a carbon coated 200-mesh copper grid and dried under ambient condition before examination.

Energy Dispersive Spectroscopy (EDAX)

The presence of elemental silver was carried out by using Scanning Electron Microscope (make Philips, Netherlands) equipped with Energy Dispersive X-ray system EDAX XL-30 operating at 15-25 kV.

Antimicrobial Activity Against Antibiotics Resistance Bacteria

The silver nanoparticles synthesized by this method were tested for antimicrobial activity against antibiotics resistance bacteria by agar well diffusion method against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Wells were made on nutrient agar plates using gel puncture. Using a micropipette, 100 µL of antibiotics Tetracycline, Penicillin G and Ag NPs were added to different wells at a same concentration around 0.0011mg/0.1ml against *Staphylococcus aureus*, *Pseudomonas aerogenosa* and *Escherichia coli*. After incubation at 37^o c for 24 hours, the diameter of zone of inhibition was measured.

RESULTS AND DISCUSSION
Characterization Of Silver Nanoparticles

The presence of Ag NPs was checked by following methods. These methods provided the evidence that the reaction between silver nitrate and plant's stem powder produced Ag NPs.

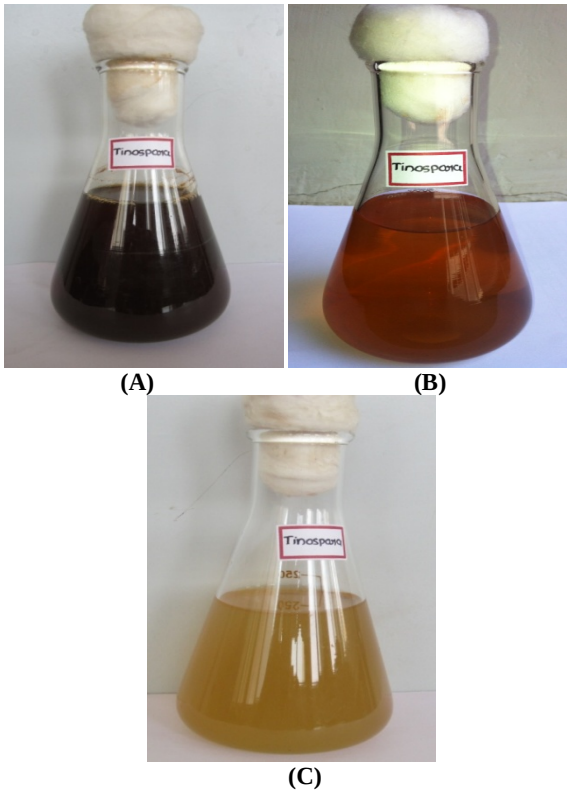


Figure 1 A) Color was changed from yellow to **B)** light brown after 30-35 minutes and **C)** After18-24hrs color was changed into dark brown.

BY COLOUR CHANGE

The sequential color change indicates the formation of Ag NPs by our plant materials. This is primary test for the checking of formation of Ag NPs.

The color reduction of $AgNO_3$ into nanoparticles was visibly evident from the color change. Stem extract was added into silver nitrate solution. Within few minutes the appearance of brown color was observed and it indicates the formation of Ag NPs. The color was changed from yellow (A) to light brown (B) after 30-35minutes. After18-24hrs color was changed into dark brown (C). This color change indicates the formation of Ag NPs. It is indicate that

formation of Ag NPs. The *Azadiracta indica* plant from silver nanoparticles. After few minutes the appearance of brown color was observed and it indicates the formation of Ag NPs[15]. Similar type of result also observed in our plant of *Tinospora cordifolia.* It is synthesis of silver nanoparticles by within few minutes the appearance of brown color was observed and it indicates the formation of Ag NPs.

By UV- Visible Spectroscopy

By UV-Visible spectroscopy we got the λ_{max} at 430nm which is strong evidence for the formation of Ag NPs.

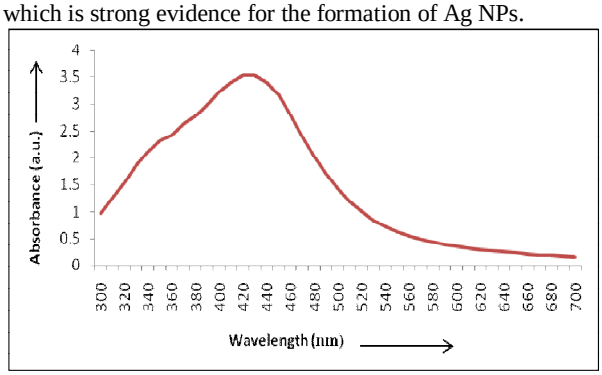


Figure 2 UV spectra of silver nanoparticles synthesized by plant mediated method

Silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300 nm to 700 nm. Figure shows the UV absorption spectra of the synthesized Ag NPs using the extract of *Tinospora cordifolia* stem. The reduction of $AgNO_3$ into NPs was showed an absorbance peak at around 430 nm, which is characteristics Ag NPs, due to its surface Plasmon resonance absorption. Metal nanoparticles have free electrons, which gives surface Plasmon resonance (SPR) absorption band due to the combined vibration of electrons of metal NPs in resonance with light wave. During initial reaction time the band was broad and the peak positioned at 380 nm due to the formation of large size of NPs in the initial time. After incubation the band shifts into 430 nm. As increasing the reaction time, the reaction rate was gradually increased. In this study stem extract mediated synthesized Ag NPs were rapid process and stable for several months due to the presence of stabilizing agent in the stem extract. In the biogenic synthesis of silver nanoparticles from the leaf extract of *Syzygium cumini* (L.). UV absorption spectra of the synthesized Ag NPs by using plant mediated method [16].

X-Ray Diffraction

The biosynthesized silver nanoparticles were confirmed by the characteristic peaks observed in the XRD image. The control of plant extract did not show the characteristic peaks Fig. 3, while in Fig. 4 we observed peaks which indicates the presence of crystalline materials.

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed numbers of Bragg's reflections that may be indexed on the basis of the face cantered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at the 2θ values of the XRD pattern was ranging from 30 to 80 and six strong peaks were observed at 32.21, 38.15, 44.47, 46.22, 64.38 and 77.53 were corresponds to the planes (54.36), (89.13), (52.54), (100), (58.95) and (52.77) respectively (Fig. 6), which are indexed to the face centered cubic structures of silver nanoparticles. The XRD pattern of these peaks indicates the silver nanoparticles is crystalline in nature and some of the unassigned peaks were observed, it may be due to the fewer bio-molecules of stabilizing agents are enzymes or proteins in the plant extract (Fig. 4). The green synthesis of silver nanoparticles by *Mulberry* leaves extract. The XRD patterns displayed in this work are in good agreement with the earlier research reported for green synthesis of silver nanoparticles.

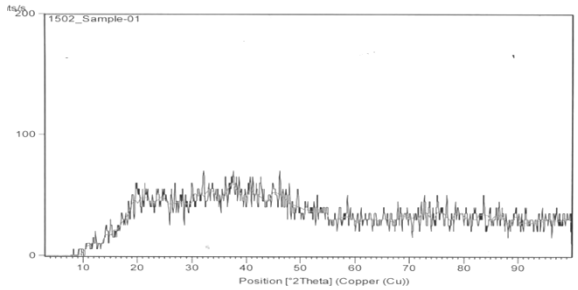


Figure 3 Control of plant of *Tinospora cordifolia* stem extract

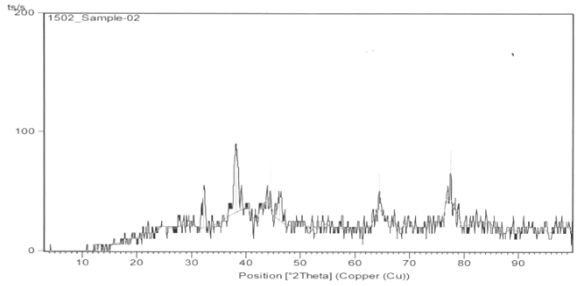


Figure 4 XRD patterns of Ag NP_S synthesized by of *Tinospora cordifolia* stem extract

It was found that the average size from XRD data using Debye- Scherer equation was 60.89 nm.

Debye-Scherrer's equation,

$D = K \lambda / \beta \cos\theta$

Where,

$\beta = \pi / 180 \times FWHM$

(FWHM= Full Width Half Maximum)

K= 0.94

$\lambda = 1.54059$ Å

$K\lambda = 0.94 \times 1.54059$ Å

$= 1.4482$

For example, in our result we got six peaks. We take 3rd peak for calculation by Debye- Scherer equation,

$D = K \lambda / \beta \cos\theta$

$K\lambda = 0.94 \times 1.54059$ Å

$= 1.4482$

$\beta = \pi / 180 \times FWHM$

$= 3.14 / 180 \times 0.4833$

$= 0.03609$

$2\theta = 44.475$, So, $\theta = 22.2375$
And $\cos\theta = 0.9256$
Now,
$D = K \lambda / \beta \cos\theta$
$= 1.4482 / 0.03609$
$= 43.35$ nm.

The presence of structural peaks in XRD patterns and average crystalline size around 60.89 nm clearly illustrates that Ag NPs synthesized by our green method were nanocrystalline in nature. The size of Ag NPs found by TEM and found by XRD is different in size due to aggregation which is common in XRD.

As mentioned in the method section, the silver nanoparticles once formed were repeatedly centrifuge and redispersed in sterile distilled water prior to XRD analysis, thus ruling out the presence of any free biological materials that might independently crystallize and giving rise to Bragg reflection.

The mean size of Ag NPs was calculated using the Debye-Scherrer's equation. An average size of the Ag NPs synthesized by our plant extract was 60.89nm with size ranging from 43.35nm to 108.59nm (Table 2).

FT-IR SPECTROSCOPY

Fig. 5 shows the peaks, which associated with the specific functional groups which participates in the bioreduction process of silver nanoparticles.

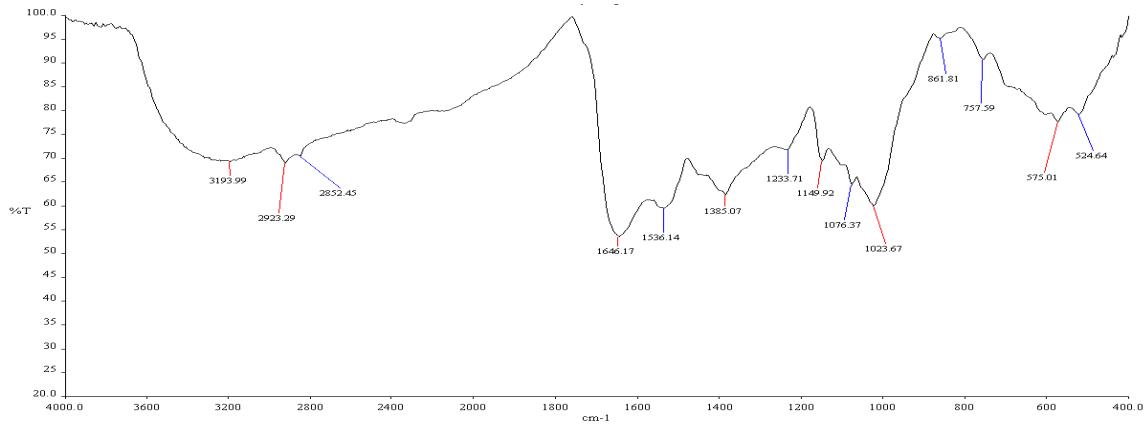


Figure 5 FT-IR spectrum of the stem extract after adding into Silver nitrate

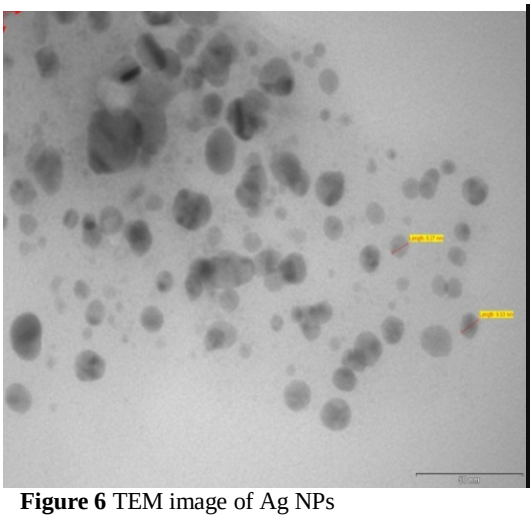


Figure 6 TEM image of Ag NPs

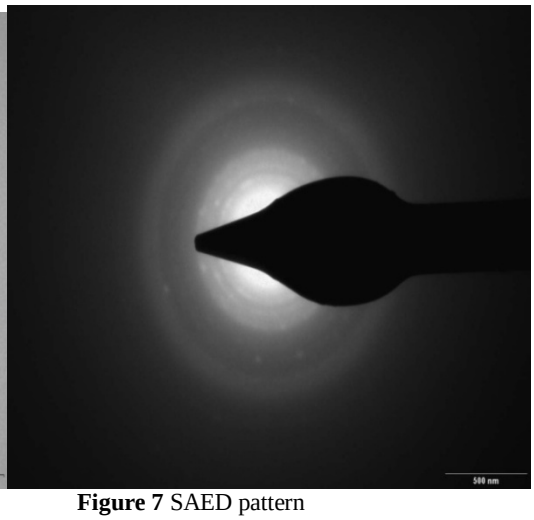


Figure 7 SAED pattern

Figure 5 shows peaks situated at 3193.99cm-1 (O-H bond), 2923.29cm-1 (C-H bond), 2852.45cm-1 (C-H bond), 1646.17cm-1 (C=C), 1536.14cm-1 (N-H), 1385.07cm-1 (NO_2), 1233.71cm-1 (C-O), 1149.92cm-1 (C-O), 1076.37cm-1 (C-N), 1023.67cm-1(C-N), 861.81cm-1 (C-H), 757.59cm-1(C-H), 575.01cm-1, 524.64cm-1.These peaks are known to associated with the- OH, -CH, C=C, C-O.

The hydroxyl groups of these compound have a stronger ability to bind silver ions and may be involve in the biosynthesis of Ag NPs and act as reducing agent for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^o). The biological molecules such as secondary metabolites could possibly play major role in the synthesis and stabilization of the metal nanoparticles was proved [18]. The functional groups present in the figure 7 are actively participates in the biosynthesis of silver nanoparticles.

Transmission Electron Microscope (TEM)

By this analysis we got the spherical Ag NPs produced by our plant. It is observed that most of the nanoparticles shown in the Fig. 6 is in the range of 4-20nm and few particles are agglomerated.

A TEM image recorded from the silver was coated on carbon coated copper TEM grid is shown in Figure 8. The morphology of the nanoparticles was spherical in nature. TEM image constitutes large no. of non uniform NPs revealed that the Ag NPs produced by reaction of Ag+ with the stem extract of *Tinospora cordifolia.*

Under careful observation, it is evident that the silver nanoparticles surrounded by a faint thin layer of other materials, which we suppose are capping organic material from *Tinospora cordifolia* stem extract. Agglomeration of SNPs may be due destabilization of electric double layer of silver ions .The microscopic observation is in agreement with the UV-Vis spectroscopic studies. Silver nanoparticles synthesized were highly uniform in size ranging 4 to 20nm. Extracellular fabrication of silver nanoparticles using *Pseudomonas aeruginosa bacteria* [19]. Fig. 7 shows the SAED pattern recorded from the synthesized Ag NPs. The electron diffraction pattern gives evidences that the Ag NPs seem to be clearly crystalline in nature.

Energy-Dispersive Analysis of X-Ray Spectroscopy (EDAX)

EDAX analysis gives full elemental profile of sample and indicates the amount of any element present in term of percentage. The strong signals of silver correspond to the peaks in the graph confirming presence of Ag NPs. EDAX study reveals that the elemental Ag is present in concentration 47.21%. It is followed by carbon 59.02% and oxygen 28.41%.

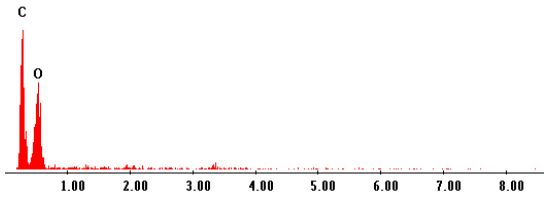


Figure 8 Stem extracts without Ag NPs

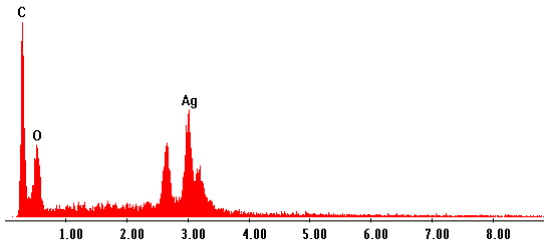


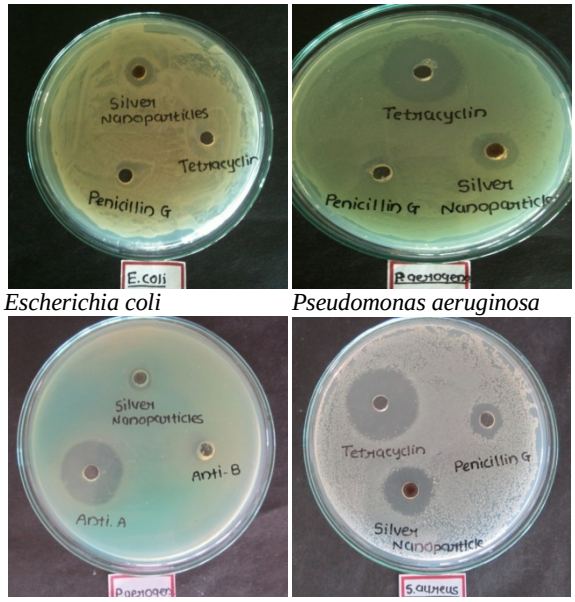
Figure 9 Stem extract with Ag NPs

The vertical axis shows the counts of the X-ray and the horizontal axis shows energy in Kev. It is also indicates the presence of bio-organic and bio-inorganic interference, which is naturally occurred in the stem extract of *Tinospora cordifolia* thus the analysis confirms the bio-reduction of silver from ionic silver to elemental silver i.e. Ag^{+} to Ag^{0}. The XRD pattern of these peaks indicates the silver nanoparticles is crystalline in nature and some of the unassigned peaks were observed, it may be due to the fewer biomolecules of stabilizing agents are enzymes or proteins in the mushroom extract [20].

Antimicrobial Activity of Ag Nps Againts Antibiotics Resistance Micro-Organisms

Interesting characteristic of Ag NPs is that its antimicrobial activity against antibiotics resistance micro-organisms.

In this studied we were observed the zone of inhibition by Ag NPs against antibiotics resistance micro-organisms. We were taken 2 antibiotics Tetracycline, Penicillin G and Ag NPs in same concentration around 0.0011mg/0.1ml against *Staphylococcus aureus, Pseudomonas aerogenosa* and *Escherichia coli.*



Escherichia coli *Pseudomonas aeruginosa*

Pseudomonas aeruginosa *Staphylococcus aureus*

Figure 10 Antimicrobial Activity of Silver Nanoparticles Against Antibiotics Resistance Micro-Organisms (Here, Anti- A (Tetracycline), Anti- B (Roxithromycin).)

Now a day, the main problem of antibiotics usage is that many micro-organisms like *Staphylococcus aureus, Pseudomonas aerogenosa, Escherichia coli* etc. are become resistance against antibiotics. The modes of action of antibiotics are specific for particular antibiotics. For example, some antibiotics like Penicillin inhibits cell wall synthesis only, after cell division they can't affect cells. Some antibiotics like Tetracycline inhibit protein synthesis only. So, micro-organisms easily developed resistance capacity against antibiotics. But in case of Ag NPs, it is very difficult to develop resistance capacity due to their wide range of its antimicrobial activities.

Another drawback of antibiotics is that they have antimicrobial activity against prokaryotes only, while Ag NPs affects both prokaryotes and eukaryotes. These micro-organisms are sensitive against Tetracycline and Ag NPs but resist against Penicillin G. So, we can say that Ag NPs are better than some antibiotics like Penicillin G. This result indicates our interest in Ag NPs due to their antimicrobial activity, which is very important now a day.

CONCLUSION

Tinospora cordifolia is widely used in Ayurvedic medicine because of its multipotent bioactive molecules and most of their pharmacological evaluations by modern test have been reported. Based on the present study of this plant stem extracts used for the biogenic synthesis of silver

nanoparticles and characteristics. Biogenic synthesis of silver nanoparticles is better than physico-chemical methods because biological methods are eco-friendly, easy scale up of process, etc. While green synthesis of silver nanoparticles is better than other biological methods. Bacteria and fungi mediated synthesis of silver nanoparticles requires long time period. UV-Vis studies effectively monitored bio-reduction of silver ions. XRD analysis provides information about crystalline nature of silver nanoparticles. The silver nanoparticles synthesized by *Tinospora cordifolia* stem powder have size ranging from 4-20nm and they are having uniform spherical shape and evenly separated. TEM study has determined particle size and shape. EDAX analysis provides evidences of presence of elemental silver in stem powder. FT-IR provides information about functional groups which participates in the synthesis of silver nanoparticles. Antibiotics inhibit growth of only prokaryotic micro-organisms, while silver nanoparticles inhibit growth of fungi also which indicates that the silver nanoparticles inhibits growth of both prokaryotes and eukaryotes. Another interesting characteristic of silver nanoparticles is that they kill the antibiotics resistance bacteria also.

Due to above special characteristics, silver nanoparticles have many application in medical field.

ACKNOWLEDGEMENT

This project would be incomplete without thanking the people who made it possible. It is my pleasure and honors in expressing a few words of gratitude and respect to one and all those who supported me in completing this work. Firstly, I would like to appreciate the kind support and encouragement provided by Charutar Vidhyamandal (CVM). I would like to thank **Dr. Nilanjan Roy**, Director, ARIBAS, **Dr. Kirti Pawar** (Ex Director) and **Dr. P S Patel** (Ex Director) ARIBAS to fulfill all requirements during my project work. With profound sense of gratitude, I sincerely thank to my Guide **Dr. Kalpesh B Ishnava, Assistant Professor in Plant Biotechnology** who gave me opportunity to work under his guidance. I will always be grateful to him for all his suggestions and correction, care, moral support and for giving friendly environment to work.

I express my regards to Dr. Anju Kunjadia, Dr. Arif Khan, Dr. Mukund Thakur, Dr. Hemul Patel and Pragna mam for their valuable support. I am also thankful to Haribhai for helping throughout the work. Finally, I would express my strong feeling of gratitude to my loving parents for their love, care, moral support and blessings.

REFERENCES

1. Singh A, Jain D, Upadhyay MK, Khandelwal N,Verma HN, Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities, Dig. J. Nanomater. Biostruct, 5(2):483- 489, (2010).
2. Jain Devendra, Daima Hemant Kumar, Kachhwaha Sumita, Kothari SL, Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities, Dig. J. Nanomater. Biostruct,
3. Simi CK and Abraham TE, Hydrophobic grafted and cross-linked starch nanoparticles for drug delivery, Bioprocess Biosyst Eng, 30 (3):173 (2007).
4. Smith AM, Duan H, Rhyner MN, Ruan G and Nie SA, A systematic examination of surface coatings on the optical and chemical properties of semiconductor quantum dots. Phys Chem Chem Phys, (8): 3895-3903, (2006).
5. Mohanpuria P, Nisha K Rana and Yadav SK, Biosynthesis of nanoparticles: technological concepts and future applications, J Nanopart Res, 10 (3):507–517, (2008).
6. Vaidyanathan R, Kalishwaralal K, Gopalram S and Gurunathan S, Nanosilver The burgeoning therapeutic molecule and its green synthesis, Biotechnology Advances, 27 (6): 924–937, (2009).
7. Maneerung T, Tokura S, Rujiravanit R, Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing, Carbohydrate Polymers, 72 (1): 43–51, (2008).
8. Smitha SL, Nissamudeen KM, Philip D and Gopchandran KG, Studies on surface Plasmon resonance and photoluminescence silver nanoparticles, Spectrochim. Acta A, 71 (1): 186– 190, (2008).
9. Kalimuthu K, Babu RS, Venkataraman D, Bilal M and Gurunathan S, Biosynthesis of silver nanocrystals by *Bacillus licheniformis* , Colloid Surf. B, 65 (1):150–153, (2008).
10. Kowshik M, Ashtaputre S, Kharazi S, Vogel W, Urban J, Kulkarni SK and Paknikar KM, Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3**,** Nanotechnology, 14 (1): 95–100, (2003).
11. Kokura S, Handa D, Takagi T, Ishikawa T, Naito Y and Yoshikawa T, Silver nanoparticles as a safe preservative for use in cosmetics, Nanomedicine 6(4): 570 574, (2010).
12. Kumar A, Vemula PK, Ajayan PM and John G, Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil, Nat. Mater, 7(3): 236–241, (2008).
13. Kyung HC, Park JE, Osaka T and Park SG, The study of antimicrobial activity and preservative effects of nanosilver ingredient, Electrochimica Acta 51 (5): 956–960, (2005).
14. Zhang Y, Peng H, Huang W, Zhou Y and Yan D, Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles, Journal of Colloid and Interface Science, 325 (2): 371–376, (2008).
15. Elavarasi Natarajan, Bharathi Purushothaman, Poongodi Palanisamy, Divya Somasundaram And Shalu Subathra.2012. Studies On Morphological Characterization And Antimicrobial Activity Of Silver Nanoparticles Synthesized By Bio And Chemoreductive Methods. Int J Pharm Bio Sci; 3(4): (P) 264 – 273 (2012).
16. Ram Prasad,V. Satyanarayana Swamy, Kumar Suranjit Prasad And Ajit Varma.2012. Biogenic Synthesis Of Silver Nanoparticles From The Leaf Extract Of *Syzygium Cumini* (L.)And Its Antibacterial Activity. Int J Pharm Bio Sci; 3(4): (P) 745 – 752 (2012).
17. Akl M. Awwad And Nidà M. Salem.2012.Green Synthesis Of Silver Nanoparticles Bymulberry Leavesextract. Nanoscience Nanotechnology, 2(4): 125-128 (2012).
18. Inbakandan, Venkatesan and Ajmal Khan, Biosynthesis of gold nanoparticles utilizing marinesponge *Acanthella elongate* (Dendy, 1905), Colloids and Surfaces B: Biointerfaces, 81(2):634–639, (2010).
19. Goldie Oza, Sunil Pandey, Ritu Shah, Madhuri Sharon.2012. Extracellular Fabrication of Silver Nanoparticles using *Pseudomonas aeruginosa* and its Antimicrobial Assay. Adv. Applied Sci. Res., 3 (3): 1776-1783 (2012).
20. Daizy P, Biosynthesis of Au, Ag and Au–Ag nanoparticles using edible mushroom extract.Danilcauk, M, Lund, A, Saldo, J, Yamada, H, Michalik, J: Conduction electron spin resonance of small silver particles. Spectrochimaca. Acta. Part A. 63, 189–191 (2006).