INTERNATIONAL JOURNAL
OF PHARMA AND BIO SCIENCES

Internationally indexed journal

Indexed in Chemical Abstract Services (USA), Index coppernicus, Ulrichs Directory of Periodicals, Google scholar, CABI ,DOAJ , PSOAR, EBSCO , Open J gate , Proquest , SCOPUS , EMBASE ,etc.

Rapid and Easy Publishing

The "International Journal of Pharma and Bio Sciences" (IJPBS) is an international journal in English published quarterly. The aim of IJPBS is to publish peer reviewed research and review articles rapidly without delay in the developing field of pharmaceutical and biological sciences

Pharmaceutical Sciences

- Pharmaceutics
- Novel drug delivery system
- Nanotechnology
- Pharmacology
- Pharmacognosy
- Analytical chemistry
- Pharmacy practice
- Pharmacogenomics
- Polymer sciences
- Biomaterial sciences
- Medicinal chemistry
- Natural chemistry
- Biotechnology
- Pharmacoinformatics
- Biopharmaceutics

Biological Sciences

- Biochemistry
- Biotechnology
- Bioinformatics
- Cell biology
- Microbiology
- Molecular biology
- Neurobiology
- Cytology
- Pathology
- Immunobiology

Indexed in Elsevier Bibliographic Database (Scopus and EMBASE)
SCImago Journal Rank 0.129
Impact factor 0.67*

*Instruction to Authors visit **www.ijpbs.net**

For any Queries, visit "contact" of www.ijpbs.net

Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 849 - 863

Research Article **Nanotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES BY USING DRIED STEM POWDER OF *TINOSPORA CORDIFOLIA*, ITS ANTIBACTERIAL ACTIVITY AND COMPARISON WITH ANTIBIOTICS

SAMIR A. ANUJ* AND KALPESH B. ISHNAVA

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New V.V. Nagar, Anand, Gujarat – 388121.

ABSTRACT

It is a well-known fact that silver nanoparticles are highly toxic to microorganisms. So, nowadays the synthesis and antimicrobial activity of silver nanoparticles is a major area of research in the field of nanobiotechnology. The problem with most of the chemical and physical methods of silver nanoparticles is that they are expensive and can also involve the use of toxic, hazardous chemicals, which may pose biological and environmental risks. To overcome this, the biological method provides a feasible alternative. But a major drawback of using bacteria, algae and fungi to synthesize silver nanoparticles is that it is very slow and the cell culture maintaining process when in comparison with plant extracts. Hence, the use of plant materials to synthesize silver nanoparticles becomes an option that is feasible. The *Tinospora cordifolia* is an important medicinal plant. Recently, this plant also used in the synthesis of silver nanoparticles. In this present study, we have synthesized silver nanoparticles using *Tinospora cordifolia* dried stem powder from 1mM aqueous silver nitrate. Utilizing the reduced property of stem powder, silver nanoparticles were synthesized at room temperature. The stem powder extracts mixed with silver nitrate showed a 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). The antibacterial activity of silver nanoparticles against antibiotic resistant bacteria is very important characteristic of silver nanoparticles. When we compare the silver nanoparticles with the antibiotics, it provides an idea about the efficiency of silver nanoparticles.

KEY WORDS: Silver nanoparticles, *Tinospora cordifolia,* Characterization, Energy dispersive spectroscopy (EDAX), X-Ray diffraction (XRD), Fourier transform infrared (FTIR), Antibacterial activity, Antibiotics.

SAMIR A. ANUJ

Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New V.V. Nagar, Anand, Gujarat – 388121.

***Corresponding author**

1. INTRODUCTION

The antibiotics are very good antibacterial agents, but the developments of resistant pathogens are major problem nowadays. Some bacteria like *Staphylococcus aureus* and *Candida albicans* become resistance to antibiotics[1]. Therefore, the new antibacterial agent required against resistant pathogens. Micro molar doses (1 to 10 µM) of silver ions are sufficient to kill bacteria[2], while silver can be toxic at high doses to mammals[3, 4]. It is well known that silver nanoparticles are highly toxic to microorganisms like *Bacillus subtilis* and *Klebsiella mobilis*[5], *Staphylococcus aureus* and *Escherichia coli*[6], *Pseudomonas aeruginosa* and *Klebsiella pneumonia*[7], *Streptococcus pyogenes*, and *Salmonella typhi*[8] and having good antifungal activities againts *Candida albicans*, *Penicillium citrium* and *Aspergillus niger*[9]. There are however various theories on the action of silver nanoparticles on microbes to cause the antibacterial effect. The exact mechanism of action of Ag NPs as antimicrobial agent is not well known but it seems that the NPs interfere in the respiratory metabolism of the organisms. The bacterial cell membrane contains abundance of sulphur containing proteins. Ag NPs can react with these proteins, leading to the inhibition of enzyme functions or interact with phosphorus moieties in DNA resulting in inactivation of DNA replication[10, 11]. The formation of free radicals from the surface of the Ag NPs may be considered to be another mechanism by which the cells die. These free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death[12, 13]. The inhibition variation was occurred due to the differences of cell wall composition in Gram positive and Gram negative bacteria. Gram positive bacteria is made up of thick cell wall contain peptidoglycon, so that nanoparticles did not affect easily. But NPs is easily penetrates into Gram negative bacteria due to structure of cell wall contain thin lipid layer, so NPs easily enter into the cell and disturb it. Researchers suggest that antimicrobial activity also depends upon the size and shape of Ag NPs. 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[14]. There are also many more techniques of synthesizing silver nanoparticles, such as thermal decomposition in organic solvents[15], chemical and photoreduction in reverse micelles[16, 17], spark discharge[18], and cryochemical synthesis[19] which yielded nanoparticles between the ranges of 5 to 80 nm in diameter. The problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals. Biological methods for the synthesis of silver nanoparticles are better methods due to slower kinetics, they offer enhanced manipulation and control over crystal growth and their stabilization. Plant mediated 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[20]. Comparison between silver nanoparticles and antibiotics provides the efficiency of silver nanoparticles. The antimicrobial activity of silver nanoparticles and antibiotics is almost same. In this studied we showed that the antibacterial activity of silver nanoparticles and antibiotics were almost same. The synthesized silver nanoparticles were characterized using UV-Visible spectrophotometer, XRD, FTIR, EDAX and TEM.

2. MATERIALS AND METHODS

2.1 Preparation of Tinospora cordifolia's stems powder

Fresh *T. cord folia* 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° C to 55° C for one week. Taking 10g of *T. cordifolia's* dried stem powder add in a 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.

2.2 Synthesis of silver nanoparticles

Silver nitrate ($AgNO_3$) was purchased from Hi-media chemicals, Ahmadabad, 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.

2.3 Characterization of silver nanoparticles

2.3.1 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.

2.3.2 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.

2.3.3 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 the 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 (λ = 1.5404°) and the 2θ scanning range was 0-90° at 2° min^{-1}. The colloidal suspension containing metal nanoparticles was dried on a small glass slab.

2.3.4 Fourier transforms 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}.

2.3.5 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 the examination.

2.3.6 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.

2.4 Determination of antibacterial activity

The silver nanoparticles synthesized by this method was tested for antimicrobial activity by agar well diffusion method against human pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumillus*, *Streptococcus pysogens*, *Serratia marcescens* and *Escherichia coli*. Approximately 20 ml of nutrient agar media was poured in sterilized Petri dishes. Each bacterial culture was grown in nutrient broth for 24 h. 100µl of each overnight grown culture (1×10^5 cfu/ml) of the

strain was swabbed uniformly onto the individual plates. With a sterile cup borer two wells were made on nutrient agar plates. Using a micropipette, 100µl of the sample of nanoparticles solution was poured onto each of the two wells on all plates. 100µl distilled water added in second well of each plates as a control. All the plates were incubated at 37^0 for 24 hours. The next day, zone of inhibition was measured.

2.5 Comparisons between antibiotics and silver nanoparticles

Comparisons between antibiotics and silver nanoparticles were carried out by well diffusion method against *Bacillus pumillus, Serettiamarceneus, Streptococcus pyogenes* and *Staphylococcus aureus.* Each bacterial culture was grown in nutrient broth for 24 h. 100µl of each overnight grown culture (1×10^5 cfu/ml) of the strain was swabbed uniformly onto the individual plates. 2.24gms of nutrient agar powder (Hi-Media) was mixed with 80ml distilled water and autoclave at 121º C, 15lbs for 15minutes. The sterilized media were poured into petriplates. Under sterile condition, 100µl of 4 pathogenic bacterial culture taken and spread over 4 different solidified N-agar plates uniformly. Three wells were prepared in each plate which containing pathogenic bacteria. Add same concentration of silver nanoparticles and antibiotics about 0.0011mg/100µl silver nanoparticles in 1st well, Tetracycline in 2nd well and Roxithromycin in 3rd well respectively in each plate. All the plates were incubated in incubator at 37º C and the next day observed the zone of inhibition and measured the zone size.

3. RESULTS AND DISCUSSION

3.1 Characterization of silver nanoparticles

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

3.1.1 By color change

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

Figure 1

Figure 2

Figure 3

Color was changed from yellow (Fig. 1) to light brown (Fig. 2) after 30-35 minutes and After18-24hrs color was changed into dark brown (Fig. 3)

The color reduction of $AgNO_3$ into nanoparticles was visibly evident from the color change. Stem powder was added into a 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 (Fig. 1) to light brown (Fig. 2) after 30-35minutes. After18-24hrs color was changed into dark

brown (Fig. 3). This color change indicates the formation of Ag NPs. Earlier this type of color change was observed in plant extracts of *Moringa oleifera*[21].

3.1.2 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.

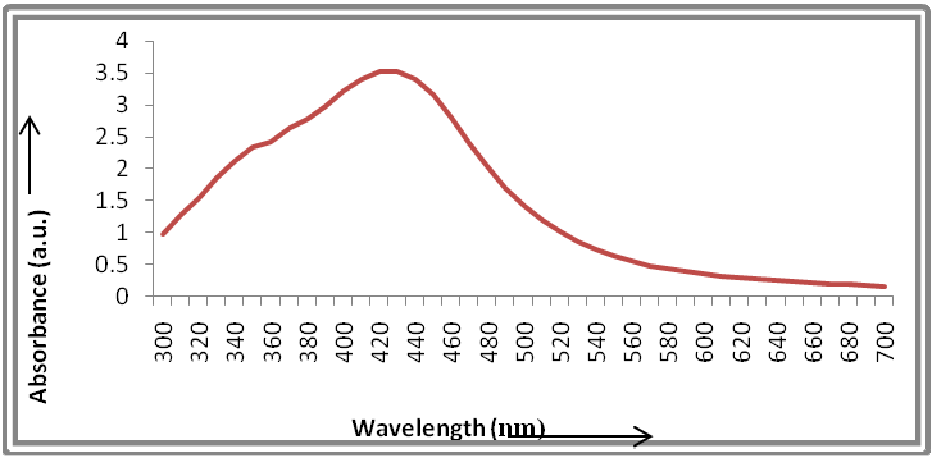


Figure 4
UV spectra of silver nanoparticles synthesized by plant mediated method

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 to700 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. Same type of result observed in the case of leaves of Euphorbia hirta[22]. 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.

3.1.3 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 in Fig. 5, while in Fig. 6 we observed peaks which indicates the presence of crystalline materials.

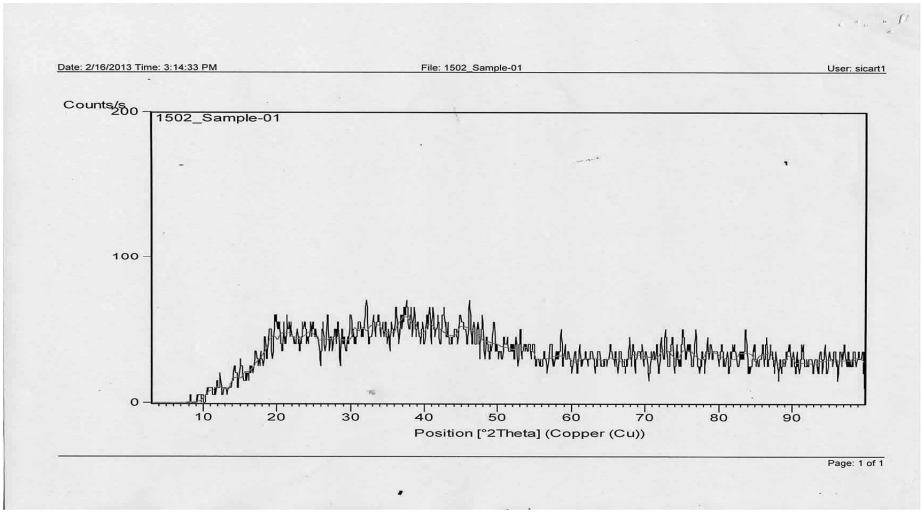


Figure 5
Control of plant of Tinospora cord folia stem extract

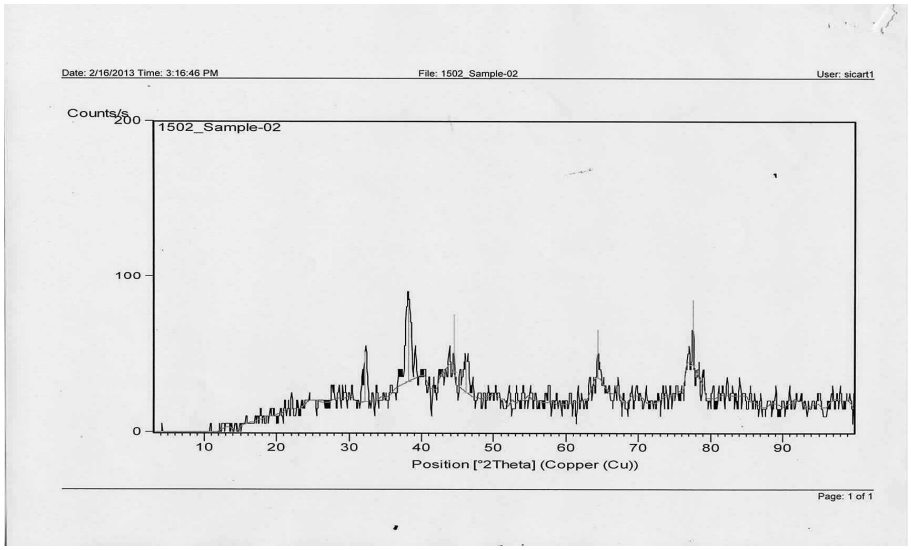


Figure 6
XRD pattern of Ag NP$_S$ synthesized by of Tinospora cord folia stem extract

The crystalline nature of the silver nanoparticles was carried out by XRD. 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. 6). The synthesis of silver nanoparticles with sharp bands of Bragg peaks, and this might be due to the stabilization of the synthesized nanoparticles by the various reducing agents of the *T. cordifolia* dried stem, and thus provides the crystallization nature of the silver nanoparticles[23]. 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

λ = 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.

Table 1

Measurement of the size of AgNPs of Tinospora cord folia stems extract by using Debye-Scherrer's equation

Sr. No.	2θ	FWHM	β = π/ 180*FWHM	Cosθ	D = K λ / β Cosθ
1	32.2152	0.5904	0.02954	0.9607	51.09 nm
2	38.1575	0.5904	0.02954	0.9450	51.94 nm
3	44.475	0.4833	0.03609	0.9256	43.35 nm
4	46.2219	1.2000	0.01453	0.9197	108.59 nm
5	64.386	0.6536	0.02643	0.8462	64.75 nm
6	77.5334	0.4287	0.04069	0.7797	45.64 nm

Average= 51.09 nm + 51.94 nm + 43.35 nm + 108.59 nm + 64.75 nm + 45.64 nm / 6 = 60.89 nm. The presence of structural peaks in XRD patterns and average crystalline size around 60.89 nm clearly illustrates that Ag NPs were crystalline 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. 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 1).

3.1.4. FT-IR spectroscopy

Fig. 7 shows the peaks, which associated with the specific functional groups which participates in the bioreduction process of Ag NPs.

Figure 7
FT-IR spectrum of the stem extract after adding into Silver nitrate

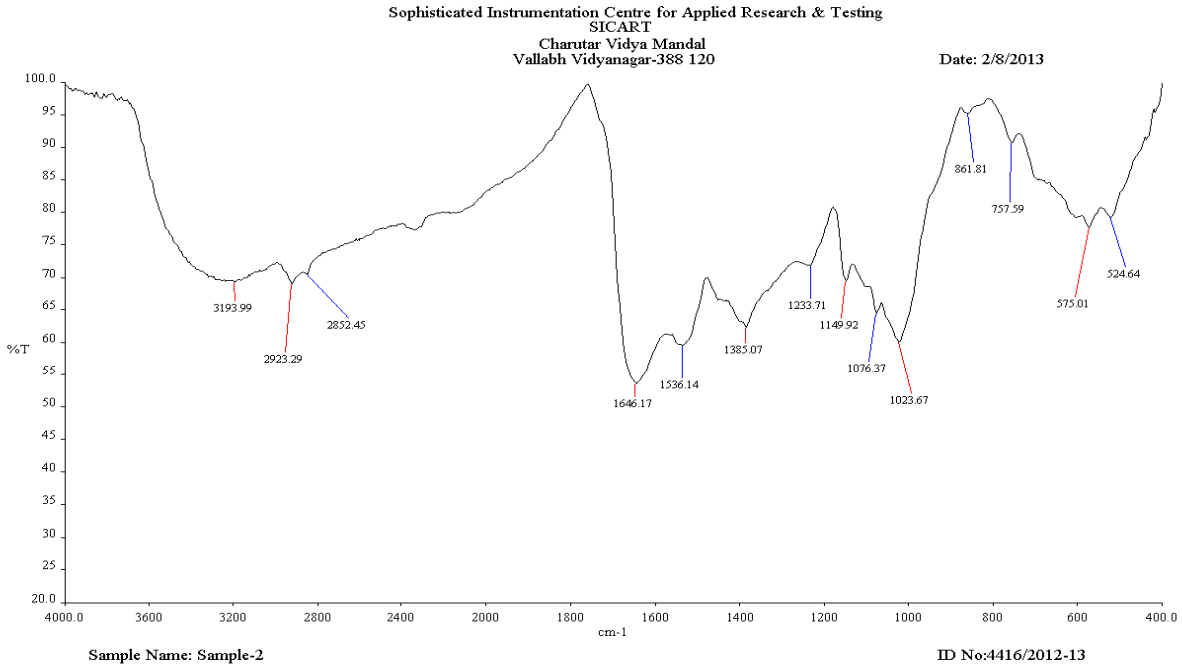


Figure 7 shows peaks situated at 3193.99cm^{-1} (O-H bond), 2923.29cm^{-1} (C-H bond), 2852.45cm^{-1} (C-H bond), 1646.17cm^{-1} (C=C), 1536.14cm^{-1} (N-H), 1385.07cm^{-1} (NO_2), 1233.71cm^{-1} (C-O), 1149.92cm^{-1} (C-O), 1076.37cm^{-1} (C-N), 1023.67cm^{-1}(C-N), 861.81cm^{-1} (C-H), 757.59cm^{-1}(C-H), 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 compounds 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 may possibly play a major role in the synthesis and stabilization of the metal nanoparticles. The biological molecules such as secondary metabolites could possibly play a major role in the synthesis and stabilization of the metal nanoparticles was proved[24]. The functional groups present in the figure 7 are actively participates in the biosynthesis of silver nanoparticles.

3.1.5 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 figure 8 is in the range of 4-20nm and few particles are agglomerated.

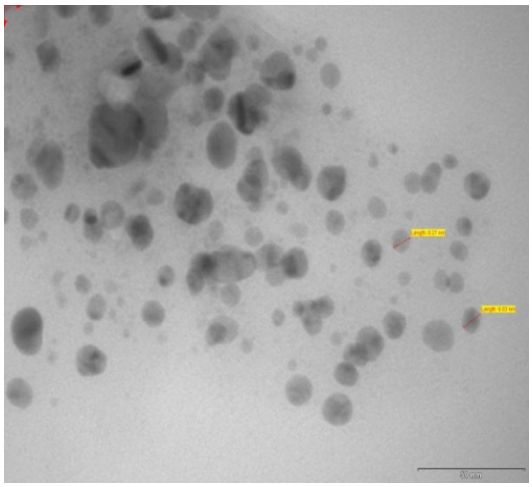


Figure 8: TEM image of Ag NPs

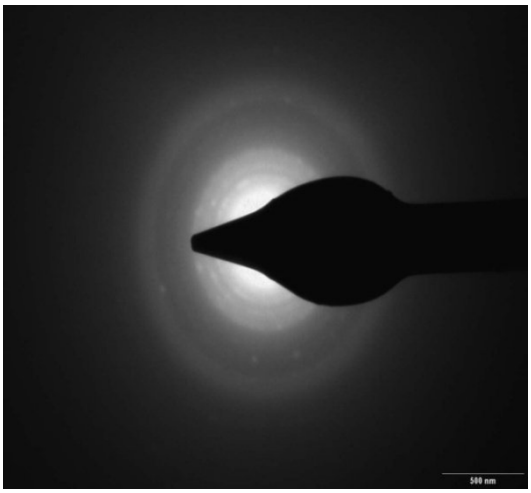


Figure 9: SAED pattern

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[25]. 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 Ag NPs may be due destabilization of electric double layer of silver ions .The microscopic observation is in agreement with the UV-Vis spectroscopic studies. Fig. 9 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.

3.1.6 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 AgNPs.

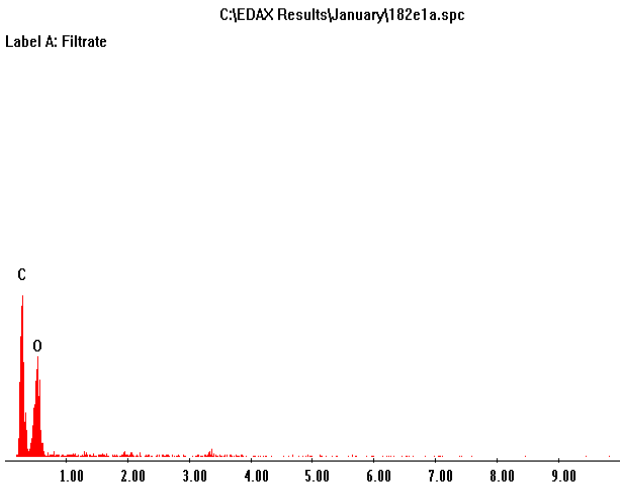


Figure 10: Stem extracts without Ag NPs

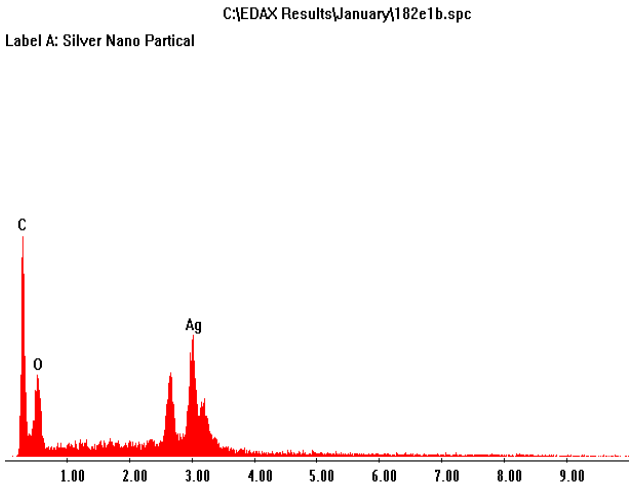


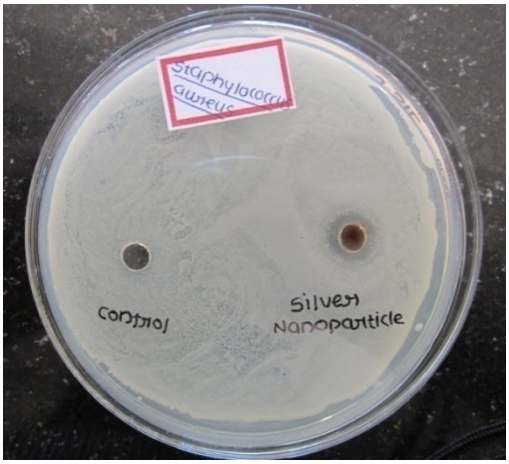
Figure 11: Stem extract with Ag NPs

EDAX study reveals that the elemental Ag is present in concentration 47.21%. It is followed by carbon 59.02% and oxygen 28.41%. 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}. EDAX studied of elemental silver by bio and chemoreductive methods supports this result[26]. 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[27].

3.2 Antibacterial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was evident from the clear zone of inhibition. The bactericidal effect of Ag NPs causes the zone of clearance. The antibacterial activity of Ag NPs was studied by well diffusion method and zone of inhibition measured against human pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumillus, Streptococcus pysogens, Serratia marcescens* and *Escherichia coli.*

Figure 12
Antibacterial activity of Ag Nps against Human pathogenic bacteria



Staphylococcus aureus

Pseudomonas aeruginosa

Bacillus pumillus

Streptococcus pysogens

Serratia marcescens

Escherichia coli

Table 2
Antibacterial activity of Ag NPs against Human pathogenic micro-organisms

Sr. No.	Microorganisms	Zone of inhibition (in mm)
1	*Staphylococcus aureus*	4 mm
2	*Pseudomonas aeruginosa*	3 mm
3	*Bacillus pumillus*	3 mm
4	*Streptococcus pysogens*	4 mm
5	*Serratia marcescens*	5 mm
6	*Escherichia coli*	5 mm

In Gram positive bacteria the action of Ag NPs was very less as compared to Gram negative bacteria. This variation was occurs due to the differences of cell wall composition of Gram positive and Gram negative bacteria. The cell wall of Gram positive bacteria was made up of thick peptidoglycon layers so, that nanoparticles did not penetrate easily. But in case of Gram negative bacteria nanoparticles easily penetrated due to thin layer of peptidoglycon. So, nanoparticles easily enter into cell and affect the permeability of the membrane. The formation of free radicals by the Ag NPs may affect the cell viability. The Ag NPs can interact with the phosphorus of the DNA which can lead to problems in the replication of the bacteria. Ag NPs also interacts with the sulfur containing proteins and affects their functions. However the exact mechanism of inhibitory action of Ag NPs is not well known. Researchers suggest that antibacterial activity also depends upon the size and shape of Ag NPs.

3.3 Comparison between silver nanoparticles and antibiotics

This is very important parameter for the checking of the efficiency of silver nanoparticles against antibiotics. The antimicrobial activity of Ag NPs on different micro-organisms is well known. We were taken same concentration of silver nanoparticles and antibiotics about 0.0011mg/100µl.

TABLE 3

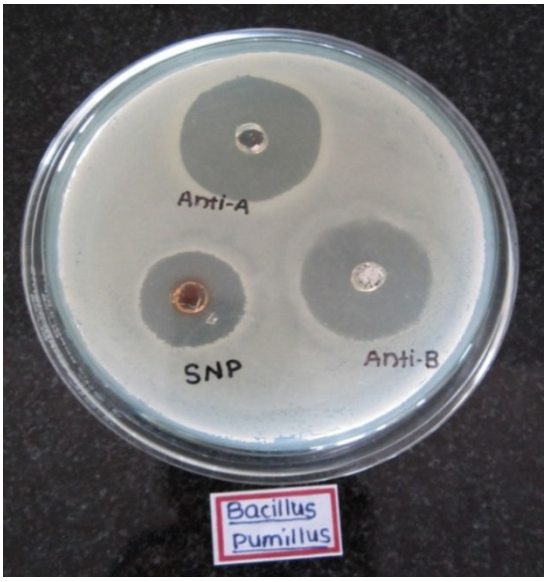
Silver nanoparticles of T. cordifolia stem powder efficiency against antibiotics

SR. NO.	MICROORGANISMS	ZONE OF INHIBITION(in mm)		
		Anti- A	Anti- B	Silver nanoparticles
1	*Bacillus pumillus*	12 mm	10.5 mm	8 mm
2	*Streptococcus pyogenes*	12 mm	10 mm	12 mm
3	*Serettiamarceneus*	13.5 mm	14 mm	12 mm
4	*Staphylococcus aureus*	13 mm	10.1 mm	8 mm

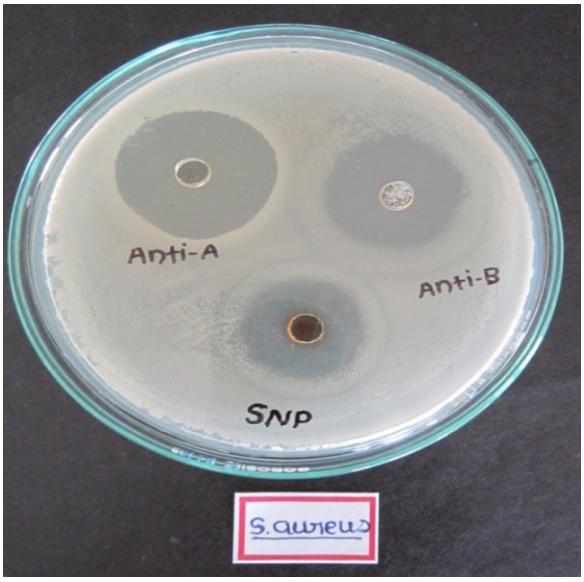
Here, Anti- A (Tetracycline), Anti- B (Roxithromycin).

Figure 13

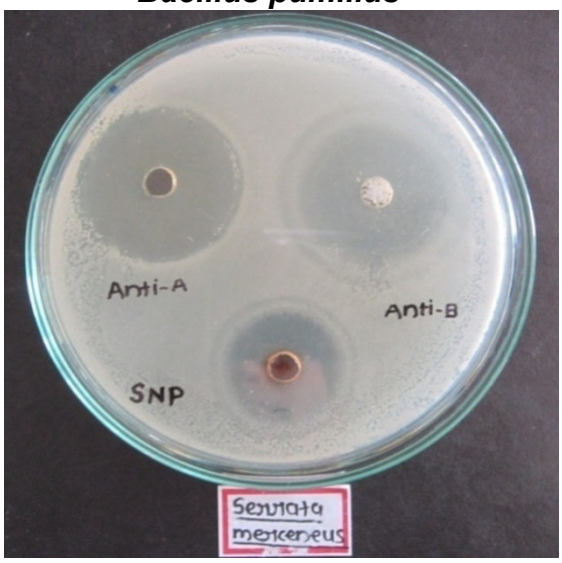
Antibacterial activity of silver nanoparticles and antibiotics



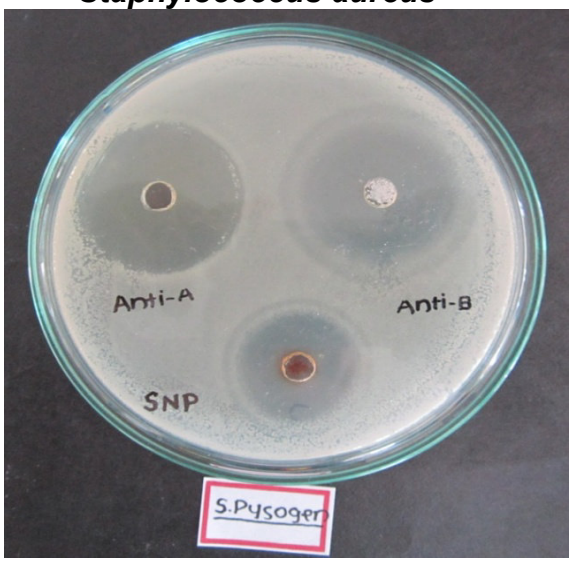
Bacillus pumillus



Staphylococcus aureus



Serettia marceneus



Streptococcus pyogenes

From the table, we showed that the antimicrobial action is approximately same to the antibiotics. So, it is clear that the Ag NPs are good candidates for their usage as antimicrobial agent instead of antibiotics. It is fact that Ag NPs not gives more antimicrobial activity than antibiotics

but it is another fact that antibiotic resistance micro-organisms easily killed by Ag NPs.

CONCLUSION

Plant mediated synthesis of silver nano particles 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 a long time period. The antimicrobial activity of silver nanoparticles and antibiotics is almost same. Antibiotics inhibit 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. Synthesized silver nanoparticles were characterized by UV-Vis spectrophotometer, XRD, TEM and EDAX analysis. FT-IR provides information about functional groups which participates in the synthesis of silver nanoparticles.

ACKNOWLEDGEMENT

This project would be incomplete without thanking the people who made it possible. I would like to appreciate the kind support and encouragement provided by 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 the 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 for their valuable support. Finally, I would express my strong feeling of gratitude to my loving parents for their love, care, moral support and blessings.

REFERENCES

1. M.Schaller , J.Laude, H.Bode waldt, G.Hamm, and H.C.K orting. Toxicity and antimicrobial activity of a hydrocolloid dressing containing silver particles in an *ex vivo* model of cutaneous infection. Skin Pharmacol. Physiol. 17-31 (2004).
2. Liu, Z., Stout, J. E., Tedesco, L., Boldin, M., Hwang, C., Diven, W. F., and Yu, V. L. 1994. Controlled evaluation of copper-silver ionization in eradicating *Legionella pneumophila* from a hospital water distribution system. J. Infect. Dis. 169:919-922.
3. Conrad, A. H., Tramp, C. R., Long, C. J., Wells, D. C., Paulsen, A. Q., and Conrad, G. W. 1999. Ag+ alters cell growth, neurite extension, cardiomyocyte beating, and fertilized egg constriction. Aviat. Space Environ. Med. 70:1096-1105.
4. Hirasawa, F., Kawarada, Y., Sato, M., Suzuki, S., Terada, K., Miura, N., Fujii, M., Kato, K., Takizawa, Y., and Sugiyama, T. 1997. The effect of silver administration on the biosynthesis and the molecular properties of rat ceruloplasmin. Biochim. Biophys. Acta 1336:195-201.
5. 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).
6. 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).
7. Won KS, Youk HJ and Park WH, Antimicrobial cellulose acetate nanofibers containing silver nanoparticles, Carbohydrate Polymers, 6(5): 430 434, (2006).

8. Anima N and Saravanan M, Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE, Nanomedicine: Nanotechnology, Biology, and Medicine, 5 (4): 452–456, (2009).

9. Singh M, Kalaivani R, Manikandan S, Sangeetha N and Kumaraguru AK, Facile green synthesis of variable metallic gold nanoparticle using *Padina gymnospora*, a brown marine macroalga, Appl Nanosci, 1-7, (2012).
10. Morones, JR, Elechiguerra, JL, Camacho, A, Holt, K, Kouri, JB, Ramirez, JT, Yacaman, MJ: The bactericidal effect of silver nanoparticles. Nanotechnology 16, 2346–2353 (2005)
11. Hatchett, DW, Henry, S: Electrochemistry of sulfur adlayers on low-index faces of silver. J. Phys. Chem. 100, 9854–9859 (1996)
12. 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)
13. Kim, JS, Kuk, E, Yu, K, Kim, JH, Park, SJ, Lee, HJ, Kim, SH, Park, YK, Park, YH, Hwang, C-Y, Kim, YK, Lee, YS, Jeong, DH, Cho, MH: Antimicrobial effects of silver nanoparticles. Nanomedicine 3, 95–101 (2007)
14. Gnanadesigan M, Anand M, Ravikumar S, Maruthupandy M, Syed Ali M, Vijayakumar V and Kumaragu AK, Antibacterial potential of biosynthesised silver nanoparticles using *Avicennia marina* mangrove plant, Applied Nanoscience, 2 (2):143–147, (2012).
15. Esumi, K, Tano, T, Torigue, K, Meguro, K: Preparation and characterization of bimetallic Pd-Cu colloids by thermal decomposition of their acetate compounds in organic solvents. Chem. Mater. 2, 564–56 (1990)
16. Pileni, MP: Fabrication and physical properties of self-organized silver nanocrystals. Pure Appl. Chem. 72, 53–65 (2000)
17. Sun, YP, Atorngitjawat, P, Meziani, MJ: Preparation of silver nanoparticles via rapid expansion of water in carbon dioxide microemulsion into reductant solution. Langmuir 17, 5707–5710 (2001)
18. Tien, DC, Tseng, KH, Liao, CY, Tsung, TT: Colloidal silver fabrication using the spark discharge system and its antimicrobial effect on Staphylococcus aureus. Med. Eng. Phys. 30, 948–952 (2007)
19. Sergeev, MB, Kasaikin, AV, Litmanovich, AE: Cryochemical synthesis and properties of silver nanoparticle dispersions stabilised by poly(2- dimethylaminoethyl methacrylate). Mendeleev. Commun. 9, 130–132 (1999)
20. Singh M, Kalaivani R, Manikandan S, Sangeetha N and Kumaraguru AK, Facile green synthesis of variable metallic gold nanoparticle using *Padina gymnospora*, a brown marine
macroalga, Appl Nanosci, 1-7, (2012).
21. M. Shivashankar and Garvit Sisodia, Biosynthesis of silver nanoparticles obtained from plant extracts of Moringa oleifera. Int. J. LifeSc. Bt and Pharma. Res., Vol. 1, No. 3, July 2012.
22. J. hemachandran, T. Thirumalai, S. Viviyan Therasa and EK. Elumalai, Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities.
23. Asmathunisha N, Kathiresan K, Anburaj R, Nabeel MA (2010) synthesis of antimicrobial silver nanoparticles by callus leaf extracts from saltmarsh plant Sesuvium portulacastrum L. Coll Surf B Biointer 79:488-493
24. 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).
25. Goldie Oza, Sunil Pandey, Ritu Shah and Madhuri Sharon, Extracellular Fabrication of silver nanoparticles using *Pseudomonas aeruginosa* and its antimicrobial assay, N.S.N. Research centre for nanotechnology and Bionanotechnology, Jambhul Phata, Ambernath (W), Maharashtra, India.

Advances in Applied Science Research, 2012, 3 (3):1776-1783

26. Elavarasi Natarajan et. Al., Studies on morphological characterization and antimicrobial activity of silver nanoparticles synthesized by bio and chemoreductive methods, Int J Pharma Bio Sci 2012 Oct; 3(4):(P)264-273
27. 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).