

RTBCE 2014[12th August 2014]
Recent Trends in Biotechnology and Chemical Engineering

A Rapid and Green Route to Synthesis Of Silver Nanoparticles from *Plectranthus Barbatus* (*Coleus Forskohlii*) Root Extract for Antimicrobial Activity

D. Manikandan^{1*}, D. Gnana Prakash² N. Nagendra Gandhi^{1*}

¹Department of Chemical Engineering, A.C. College of Technology, Anna University, India

²Department of Chemical Engineering, SSN College of Engineering, Anna University, India

*Corres.author: geomani.kandan@gmail.com, n_nagendra2002@yahoo.com

Abstract: To develop simple rapid procedure for bio manufacturing of silver nanoparticles (Ag NPs) are under exploration is due to wide biomedical applications in nanotechnology. A green rapid biogenic synthesis of AgNPs utilizing the aqueous extract of *Plectranthus barbatus* (*coleus forskohlii*) root extracts. These biosynthesized nanoparticles were characterized by using UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscope (SEM) and (HRTEM) analysis proved that the spherical shape and average size of the particle was 20 nm. The results showed that the root extract of *coleus forskohlii* is a very good bioreductant for the synthesis of AgNPs. So obtained silver nanoparticles were found to exhibit antibacterial activity against two human bacterial pathogens such as gram positive (*Bacillus subtilis*) and gram negative bacteria (*Alcaligenes faecalis*).

Keywords: Green -synthesis, *Plectranthus barbatus*, Silver nanoparticles (AgNPs), *Bacillus subtilis*, *Alcaligenes faecalis*.

Introduction:

In modern era in the world today, nanotechnology has been played major role in modern research field. Silver nanoparticles have been demonstrated to exhibit anticancer and antimicrobial properties against bacteria with close attachment of the nanoparticles themselves with the microbial cell¹⁻⁵. Their biological effectiveness can also increase on the account of a rise in surface energy. A number of approaches are available for the synthesis of silver nanoparticles such as, thermal decomposition of silver compound, microwave assisted process, electrochemical method and now recently via green chemistry route. However, and synthesis of the nanoparticles involves the use of hazardous chemicals, low material convention, high energy requirements, and wasteful purification. Therefore, there is growing need to develop environmental friendly process for the synthesis of nanoparticles without using toxic chemicals.

Nanoparticles exhibit completely new or improved properties compared with large particles, for good example silver it has been widely used or utilized for thousands of years in human history. Its applications include jewels, utensils, currency, dental alloy, photography and explosives. Among silver's many applications its disinfectant property is being exploited for hygienic and medicinal purpose, such as treatment of mental

illness. Nicotine addiction and infection of disease like syphilis and gonorrhoea⁶⁻¹¹. Silver nanoparticles have been demonstrated to exhibit anticancer and antimicrobial properties against bacteria with close attachment of the nanoparticles themselves with the microbial cell and the activity being size dependent and nanoparticles present a higher surface area to volume ratio to decrease in the size of the particles. Specific surface area is relevant to catalytic activity and other related properties such as antimicrobial activity of AgNPs^{3,5}, as the specific area of nanoparticles is increased. Their biological effectiveness can also increase on the account of a rise in surface energy

Biological approaches using microorganisms and plant extract for metal nanoparticles synthesis have been suggested as valuable alternative to chemical method¹²⁻¹⁹. The use of plant materials for the synthesis of nanoparticles could be more advantageous, because it does not require elaborate process such as intracellular synthesis and multiple purification steps (or) maintenance of microbial cell cultures. In this work, *Plectranthus barbatus* is a medicinal plant, commonly known as *Coleus forskohlii* and Indian Coleus. It is a tropical perennial plant related to the typical coleus species.

Materials and methods

Materials

All chemicals used in this experiment were of high level purity.

Plant extraction and synthesis of silver nanoparticles

Lamiaceae family *C. forskohlii* roots were harvested from Salem district, Tamilnadu, India for silver nanoparticles. Synthesis of plant root extract was prepared by mixing 2.5 g of dried powder with 100 ml of deionized water in 250 ml conical flask and boiled for 80 °C for 1hour. For reduction of Ag⁺ ions, 10 ml of plant root extract was mixed with 90 ml of 1 mM concentration of aqueous of AgNO₃ and then heated at 80 °C for 15 min. A change from brown to reddish color was observed.

Characterization of AgNPs

UV-vis spectroscopy measurements (Shimadzu UV 1800) were carried out as a function of time of the reaction at room temperature operated at a resolution of 1 nm, FTIR spectroscopy analysis were carried out to identify the biomolecules responsible for the reduction of Ag⁺ ions and capping of the bio-reduced silver nanoparticles synthesized by using plant extract in the region 500–4000cm⁻¹. X-ray diffraction (XRD) measurement of the bio-reduced silver nitrate solution using an X'pert pro P analytical X-ray diffractometer instrument at a voltage of 45 kV and a current of 40 mA with Cu K α radiation. Scanning electron microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. TEM micrograph of silver nanoparticles, Histogram of particle size from TEM.

Antibacterial activity study

The antimicrobial activity of silver nanoparticles (AgNPs) was determined using the well diffusion assay method. Approximately, 5 ml of molten and cooled media (nutrient agar) was poured in sterilized Petri dishes. A 100ml nutrient broth culture of each bacterial organism (1×10⁵cfu/ml) was used to prepare bacterial lawns. Two wells were prepared in the agar plates. The wells were labeled as A, B well was loaded with 30 μ L of Ag nanoparticles suspended 'hydrosol' and 'B' well loaded with 30 μ L of positive control drugs (chloramphenicol) was used in the positive control. The plates containing the bacterial and Ag nanoparticles were incubated at 37 °C. The plates were examined for evidence of zones of inhibition, which appeared as a clear area around the wells.

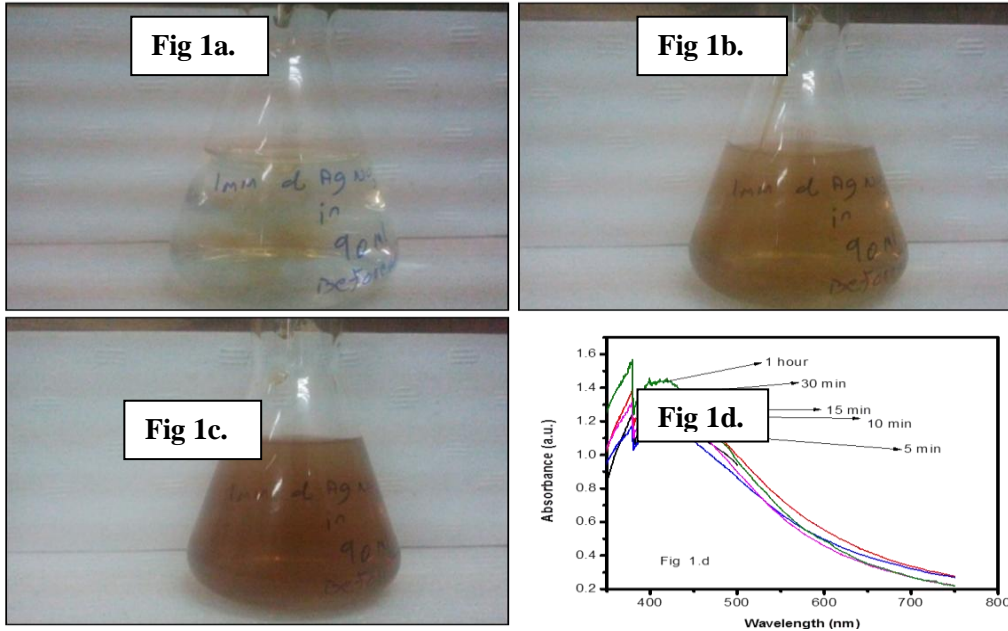
Results and discussion

UV-VIS spectra analysis

The reaction mixture, *C. forskohlii* roots extract with aqueous solution of the silver nitrate, started to change its color from brown to reddish brown color (Figure- 1(a,b,c)). It indicated the formation of silver nanoparticles with reduction of silver ion. The characteristic surface Plasmon absorption bands were observed at 440 nm. (Figure- 1d). It indicates the effect of contact time on NPs synthesis (root extract/metal ion

concentration ration 1:30; contact time 5,10,15,30 min and 1 hour) Extinction spectra of silver synthesized from AgNO₃ were shown in (Figure- 1d).

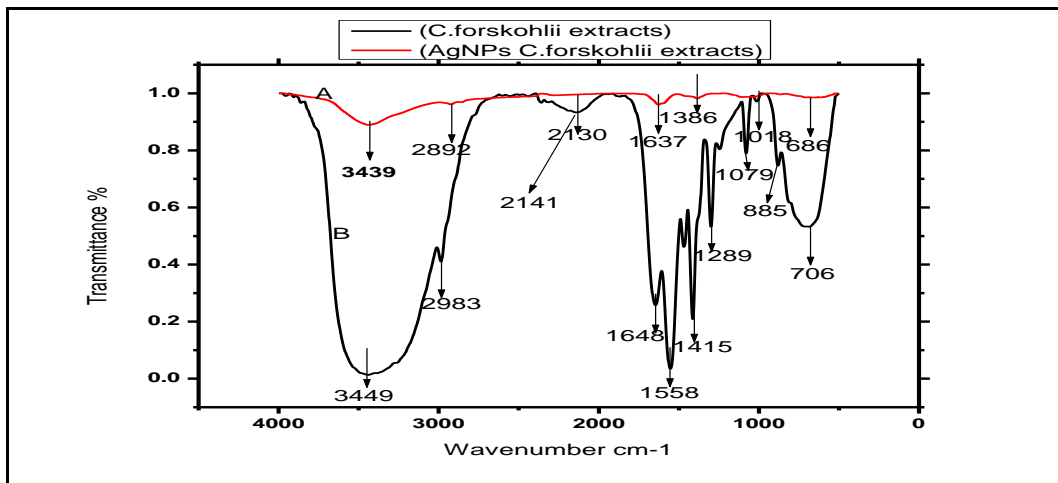
Figure 1(a,b,c): Formation of silver nanoparticles with reduction of silver ion and Figure 1d: Plasmon absorption bands



Fourier transforms infrared spectroscopy (FTIR) Studies

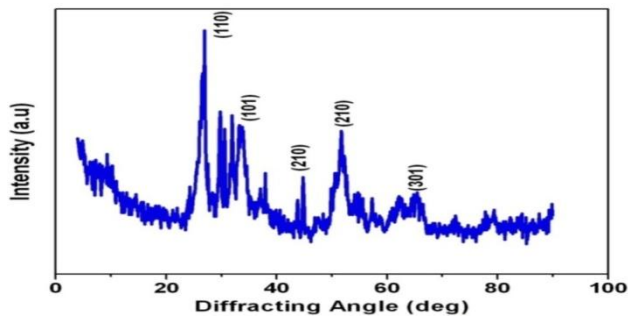
FTIR spectroscopy analysis were carried out to identify the biomolecules responsible for the reduction of Ag⁺ ions and capping of the bioreduced silver nanoparticles synthesized by using plant extract in the region 500–4000^{cm-1}. Fig 2 The black peaks corresponding to presence of fatty acids, carbonyl groups, flavanones and amide I band of proteins, peak located at 3449 ^{cm-1}, 2983 ^{cm-1}, 2141 ^{cm-1}, 1648 ^{cm-1}, 1558 ^{cm-1}, 1415 ^{cm-1}, 1289 ^{cm-1}, 1079 ^{cm-1}, 885 ^{cm-1} and 706 ^{cm-1}. Shows the synthesized AgNPs using *Coleus forskohlii* roots aqueous extract where the absorption reduction of red peaks were located at 3439 ^{cm-1}, 2892 ^{cm-1}, 2130 ^{cm-1}, 1637 ^{cm-1}, 1386 ^{cm-1}, 1018 ^{cm-1}, 886 ^{cm-1}, 1386 ^{cm-1}, 1018 ^{cm-1} and 686 ^{cm-1}.

Figure 2: Comparison of FTIR for (a) a AgNPs – *C.forskohlii* extracts sample and (b) Pure *C.forskohlii* extracts



X-ray Diffraction (XRD) Studies

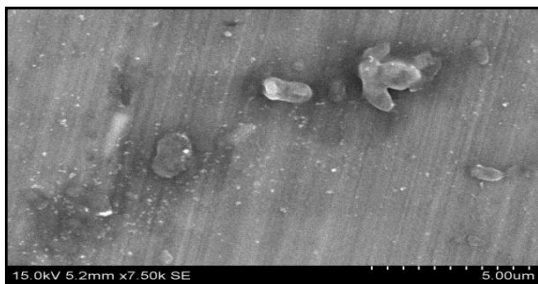
Figure 3: X-ray diffraction analysis of silver nanoparticles



The XRD patterns of synthesized silver nanoparticles (Figure- 3). There are intense peaks in the whole spectrum of 2θ values ranging from 20° to 85° . The peaks at 29.74° , 38.26° , 42.33° , 47.33° and 64.37° and their corresponding (hkl) values are (1 1 0), (101), (2 1 0) and (3 0 1) which confirmed to the presence of silver nanoparticles. It suggests that the prepared silver nanoparticles are biphasic in nature. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is a characteristic of nanocrystallites.

Scanning electron microscopy (SEM)

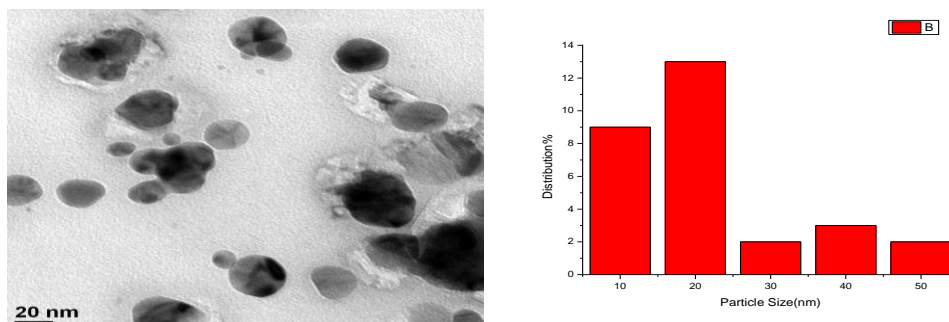
Figure 4: SEM micrograph of silver nanoparticles



Scanning electron microscopy (SEM) analysis shows uniformly distributed silver nanoparticles on the surfaces of the cells (Figure- 4). The silver nanoparticles were capsules in shape with particle size range $5.00\ \mu\text{m}$. The larger silver particles may be due to the aggregation of the smaller ones, due to the SEM measurements. (Figure- 4) represents the view of the sample at 15.0 KV 5.2mm \times 7. 50k magnification. Around the examined area, one can notice the presence of objects of sizes within $400\ \mu\text{m}$ to $500\ \mu\text{m}$. Those objects having of tiny particles, as can be proved by SEM studies results gathered on one of the particles which was seems as agglomerates and group of silver nanoparticles.

Transmission Electron Microscopy (TEM)

Figure 5: (a)TEM micrograph of silver nanoparticles (b) Histogram of particle size from TEM



TEM of the silver nanoparticles is as shown in the (Figure- 5a). The Spherical like morphology is observed. The histogram of particle size which is obtained from TEM (Figure- 5b) and revealed the distribution

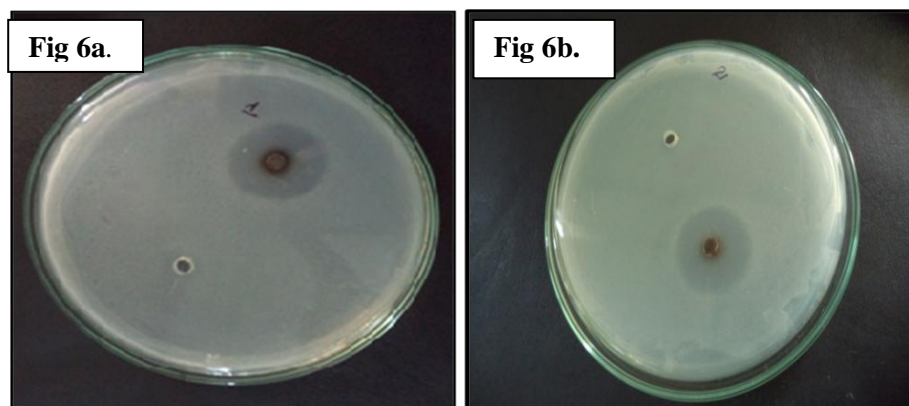
of particle size is in the range 10 nm to 100 nm. However, the high distribution of particle is at 20 nm in comparison with other particle

Antibacterial studies

Antimicrobial Activity by well diffusion method:

Biosynthesis of silver nanoparticles from *C. forskohlii* roots were tested for their antimicrobial activity by well diffusion method against pathogenic organisms like two different groups of bacteria *Bacillus subtilis* (gram positive bacteria) and *Alcaligenes faecalis* (gram negative bacteria). Nutrient Agar medium were used as media to grow bacteria. The bacterial strains were stored at 4°C. The pure cultures of organisms were sub cultured on Nutrient broth at 37°C rotary shaker at 150 RPM. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm have been made on Nutrient agar plates using gel puncture. Using micropipette 25µl of the sample of nanoparticles solution were poured into wells on all plates. After incubation at 37°C for 24 h, the different levels of zone of inhibition were measured. The results of antimicrobial activity with zone of inhibition were tabulated in the (Table and Figure- 6) respectively

Fig 6. Antimicrobial activity of silver nanoparticles against *Bacillus subtilis* (Gram positive) and *Alcaligenes faecalis* (Gram negative) human bacterial pathogens by using *Coleus forskohlii* root extract as reducing agent



S.No	Strain	Zone of inhibition	
1.	<i>Bacillus subtilis</i>	Control	25 µl
		0 mm	26 mm
2.	<i>Alcaligenes faecalis</i>	0 mm	23 mm

Conclusion

A rapid route for the preparation of silver nanoparticles by the reduction of silver nitrate with a bio-reduction method using *Coleus forskohlii* aqueous root extract as the reducing agent. The XRD and FTIR studies confirmed the formation of silver nanoparticles. Synthesized silver nanoparticles was done by color change from pale green to dark brown color and was determined by UV-visible spectroscopy at 440 nm. SEM and TEM analysis were employed for the morphology analysis of silver nanoparticles and high distribution of particle size was around 20nm. The antibacterial activity of biologically synthesized silver nanoparticles was evaluated against *Bacillus subtilis* and *Alcaligenes faecalis* human bacterial pathogen. Further research is needed in this area to explore the pathways and mode of action for silver nanoparticles on bacterial cell surface and metabolism. Hence, this technique is a green, rapid and novel route to synthesize of silver nanoparticles which could be used in biomedical applications.

References

1. Mukunthan KS, Elumalai EK, Trupti NP, Ramachandra Murty V. Catharanthusroseus: a natural source for the synthesis of silver nanoparticles, Asian Pac J Trop Biomed, 2011; 1(4); 270-274.
2. Abu Bakar NHH, Ismail J, Abu Bakar M. Synthesis and characterization of silver nanoparticles in natural rubber, Mater Chem Phys ,2007;104:276–283.
3. Bae E, Park HJ, Lee J, Kim Y, Yoon J, Park K, Choi K ,Yi J .Bacterial cytotoxicity of the silver nanoparticle related to physicochemical metrics and agglomeration properties, Environ Toxicol Chem,2010;29(10); 2154-2160.
4. Sangiliyandi G, Kalimuthu K, Ramanathan V, Deepak V, Sureshbabu R, Jeyaraj M, Nellaiah H, Soo HE. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli, Colloids and Surfaces B: Biointerfaces, 2009; 1; 328-335.
5. Pal S, Tak YK, Song JM .Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticles. A study of the gram-negative bacterium Escherichia coli, Appl Environ Microbiol, 2007; 73; 1712–1720.
6. Mukunthan KS, Elumalai EK , Patel Trupti N, Ramachandra Murty V .Catharanthus roseus: a natural source for the synthesis of silver nanoparticles, Asian Pac J Trop Biomed 2011; 1(4); 270–274.
7. Navaladian S, Viswanathan B, Viswanath RP ,Varadarajan TK .Thermal decomposition as route for silver nanoparticles, Nanoscale Research Letters,2007; 2 (1);44-48 .
8. Maria S. Barbara S. Jacek B. Electrochemical synthesis of silver nanoparticles, Electrochemistry Communications, 2006; 8(2); 227–230.
9. Gulbranson SH, Hud JA, Hansen RC. Argyria following the use of dietary supplements containing colloidal silver protein, Cutis ,2000; 66(5);373–374.
10. Sreeram KJ, Nidin M, Nair BU. Microwave assisted template synthesis of silver nanoparticles, Bull Mater Sci,2008; 31(7); 937–942.
11. Begum NA, Mondal S, Basu S, Laskar RA,Mandal D. Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of black tea leaf extracts, Colloids Surf B Biointerfaces , 2009 ;71 (1);113–115.
12. Bhattacharya D, Gupta RK. Nanotechnology and potential of microorganisms, Crit Rev Biotechnol, 2005; 25; 199–204.
13. Mohanpuria P, Rana NK, Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications, J Nanopart Res, 2007; 10; 507–517.
14. Rajasekhar Reddy G, Antony BM, Nagendra Gandhi N. Green Synthesis, Characterization and *in vitro* Antibacterial Studies of Gold Nanoparticles by Using *Senna siamea* plant seed aqueous extract at ambient conditions, Asian J. Chem ,2013; 25(15) ;8541-8544.
15. Begum NA, Mondal S, Basu S, Laskar RA, Mandal D. Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of black tea leaf extracts, Colloids Surf. B, 2009;71(1); 113–118 .
16. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphoraleaf, Nanotech2007;18;105104–105115.
17. Shankar SS, Ahmad A, Sastry M. Geranium leaf assisted biosynthesis of silver nanoparticles, Biotechnol. Prog, 2003; 19; 1627–1631.
18. Jha KA, Prasad K. Green synthesis of silver nanoparticles using Cycas leaf, Inter. J. Green. Nanotech, 2010; 1(2); 110–117.
19. Rajasekhar Reddy G, Antony BM, Nagendra Gandhi N. 2,2-Diphenyl-1-Picrylhydrazyl free radical scavenging assay and bacterial toxicity of protein capped silver nanoparticles for antioxidant and antibacterial applications, Asian J. Chem ,2013;25(16); 9249-9254.
