International Journal of Drug Development & Research
| January-March 2013 | Vol. 5 | Issue 1 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.13 & H index 2

Full Length Research Manuscript

Green Synthesis of silver nanoparticles using *Coleus forskohlii* roots extract and its antimicrobial activity against Bacteria and Fungus

C. Baskaran[1]*, V.Ratha bai
PG & Research Department of zoology, Presidency College, Chennai-600 005.

Abstract

Biosynthesis of nanoparticles is under exploration is due to wide biomedical applications and research interest in nanotechnology. Bioreduction of silver nitrate ($AgNO_3$) used for the synthesis of silver nanoparticles respectively with the plant extract; *Coleus forskohlii* roots extract (Lamiaceae). The plant extract is mixed with$AgNO_3$, incubated and studied synthesis of nanoparticles using UV–Vis spectroscopy. The nanoparticles were characterized by X-ray diffraction (XRD), FTIR, SEM equipped with EDS. The silver nanoparticles synthesized were generally found to be needle in shape with 82.46 nm. The results showed that the roots extract of *Coleus forskohlii* is very good bioreductant for the synthesis of silver nanoparticles and synthesized nanoparticles active against clinically isolated human pathogens 7 Bacteria and 5 fungus.

*Corresponding author, Mailing address:
C. Baskaran
Email: basphd@gmail.com

Key words:
Nanoparticles, Bacteria, fungus, *Coleus forskohlii* roots extract.

How to Cite this Paper:
C. Baskaran, V.Ratha bai "Green Synthesis of silver nanoparticles using *Coleus forskohlii* roots extract and its antimicrobial activity against Bacteria and Fungus" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 114-119.

Article History:----------
Date of Submission: 24-11-2012
Date of Acceptance: 31-01-2013
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

For time immemorial, nature has made noble metals part of our daily life. Recently there has been considerable interest in the development of techniques for the controlled synthesis of metal-nanoparticles of well-defined size, shape and composition, as they find applications in biomedical field and areas such as optics and electronics [1-4]. The application of noble metal nanoparticle based chemistry for drinking water purification has been summarized for different types of contaminants very recently[5].Among metal-nanoparticles, silver nanoparticles exhibit tremendous applications in spectrally selective coatings for solar energy

absorption, optical receptors, bio-labeling, intercalation materials for electrical batteries, filters, antimicrobial agents and sensors[6].Silver nanoparticle-embedded antimicrobial paint[7] is a promising area of environmentally friendly applications. Hence, a variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. The physical methods [8] are highly expensive and chemical methods are harmful to the environment [9]. Therefore, there is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocols [7].

The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material. Sondi et al. studied the antimicrobial activity of silver nanoparticles against *Escherichia coli* as a model of Gram-negative bacteria [10]. Interdisciplinary research has widened the horizons of material research, drawing new inspirations from biological systems. The towering environmental concerns had triggered the researchers to device novel methods of synthesizing the nanomaterials in biological systems such as bacteria, fungi and plants, termed as "green chemistry" approaches. Biosynthesis of silver nanoparticles using bacteria [11], fungi [12], yeast [13] and plants [14] were well documented. However, exploration of the plant systems as the potential nanofactories, has heightened interest in the biological synthesis of nanoparticles.

Coleus forskohlii roots extract (Lamiaceae), a traditional medicinal plant of South India, has the source of bio-reductant and stabilizers. The present study was aimed to rapid synthesis of silver nanoparticles using aqueous roots extract of C. forskohlii and evaluates its antimicrobial activity against Disease causes pathogens such as Bacteria and fungus.

MATERIALS

Chemicals

The silver nitrate ($AgNO_3$) and other components were purchased from HiMedia (Mumbai, India).

Preparation of plant extract

The roots of *Coleus forskohlii* were washed thoroughly thrice with distilled water and were shade dried for 5 days. The fine powder was obtained from the dried roots by using kitchen blender. The roots powder was sterilized at 121 ◦C for 15 min. 20 g of powder was taken and mixed with 200 ml of Milli Q water and kept in boiling water bath at 60 ◦C for 10 min. The extracts were filtered with Whatman filter paper No 1. The filtered extract was stored in refrigerator at 4 ◦C for further studies.

Biosynthesis of silver nanoparticles

For the biosynthesis silver nanoparticles, 1.5 ml of plant extracts is mixed with 30 ml of $AgNO_3$ solution (1 mM/ml) and incubated at 28 ◦C for 24 h. Small aliquot of solution is used for the UV–Vis spectroscopy and FTIR is performed to the extract which was exposed before and after addition to the silver nitrate solution. The reactions mixture is centrifuged at 6000rpm for 10 min and the pellet was resuspended in small amount of sterilized double distilled water and then small amount of suspension was sprayed on glass slide to make thin film. The thin film was kept in hot air oven to dry and then the thin film was used for the SEM analysis equipped with EDS (Model JEOL/EO, JSM-6390).

Screening of microbial property in synthesized nanoparticles

The bacterial spp. used for the test were *Escherichia coli (E. coli), Bacillus cereus (B. cereus), Pseudomonas aeruginosa (P. aeruginosa), Micrococcus luteus,(M.luteus), Klebsiella pneumonia(K.pneumonia), Proteus mirabilis(P. mirabilis), Staphylococcus aureus (S. aureus*), The fungus spp used for the test were *Aspergillus niger (A.niger), Aspergillus flavus(A.flavus), Candida albicans(C.albicans), Candida tropicalis(C.*

tropicalis) and candida kefyr. All the stock cultures were obtained from Microlab, Institute of Research and Technology, Vellore, Tamilnadu, India. The microorganisms were grown overnight at 37°C in Mueller-Hinton Broth at pH 7.4.

Culture media and inoculums preparation

Nutrient agar /broth (Himedia, India.) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37° C for 72 hrs and Potato dextrose agar /and potato dextrose broth (Himedia, India)were used as the media for the culturing of fungal strains. Loops full of all the fungus cultures were inoculated in the potato dextrose broth (PDA) and incubated at room temperature for 72 hrs.

Testing for antibacterial activity

The synthesized particles were inoculated to the well obtained above were screened for their antibacterial activity in comparison with standard antibiotics in-vitro by well diffusion method. The cup-plate agar well diffusion method was employed to assess the antibacterial activity of the prepared synthesized particles. 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates, 5 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. Alternate cups were filled with 20 µl of each synthesized particles using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours. The respective solvents were used as controls. The diameters of the growth inhibition zones were measured at 24 hours of incubation averaged and the mean values were tabulated.

Antifungal activity

The synthesized particles were also screened for their antifungal activity in comparison with standard antibiotics in-vitro by well diffusion method. Lawn culture was prepared using the test organism on potato dextrose broth (PDA). The inoculated plates were kept aside for a few minutes. Using well cutter, wells were made in those plates at required distance. Using sterilized micropipettes 25µl of synthesized particles of selected *Coleus forskohlii* roots extract was added in to the well. The plates with yeast like fungi were incubated at 37°C for overnight. The plates with mold were incubated at room temperature for 48 hrs. The activity of the extract was determined by measuring the diameters of zone of inhibition.

RESULTS

Biosynthesis of silver nanoparticles

Silver nanoparticles were synthesized using roots extract of *Coleus forskohlii* Interestingly, silver nanoparticles were synthesized rapidly within 1 h of incubation period. The aqueous silver nitrate solution was turned to yellowish brown color within 1 h, with the addition of roots extract in Fig. 1. Intensity of brown color increased in direct proportion to the incubation period. It was due to the excitation of surface plasmon resonance (SPR) effect and reduction of AgNO3. The silver surface plasmon resonance was observed at 420nm which steadily increases in intensity as a function of time of reaction (ranging from 30 min to 5 h) without showing any shift of the wavelength maximum in Fig.2.

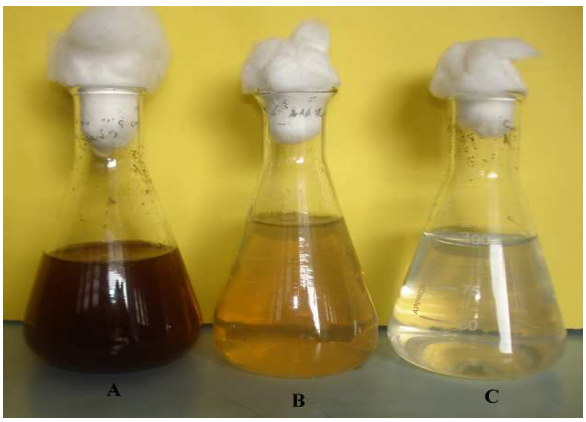

Fig. 1: Synthesis of silver nanoparticles (A) synthesized silver nanoparticles in brown color solution (B) Before synthesis silver nanoparticles in yellow color solution. (c) AgNO3 solution.

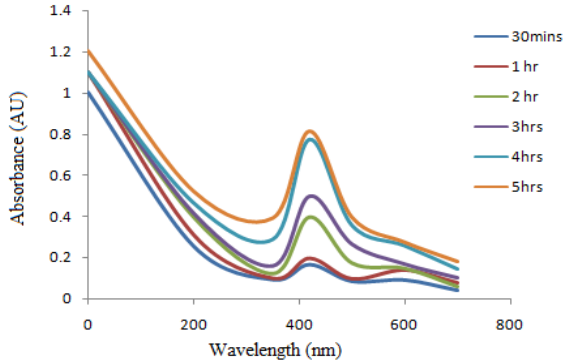

Fig. 2: UV–vis spectra of aqueous silver nitrate with *Coleus forskohlii* roots extract at different time intervals.

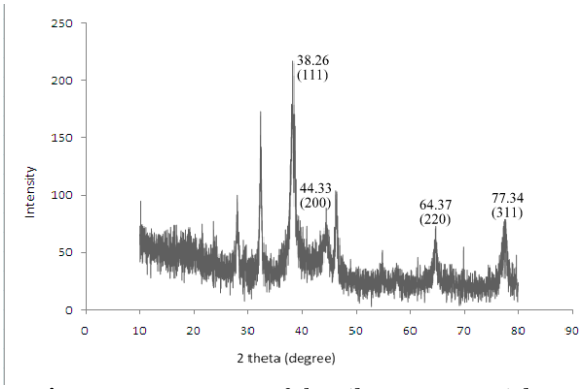

Fig 3: XRD patterns of the silver nanoparticles synthesized from aqueous root extract of *Coleus forskohlii*.

The XRD patterns of vacuum dried silver nanoparticles synthesized using root extract of Coleus forskohlii. A number of Bragg reflections with 2θ values of 38.26°,44.33°, 64.37°,and 77.34° sets of lattice planes are observed which may be indexed to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) facts of silver respectively in Fig. 3. 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. According to the scanning electron micrograph, the morphology of the silver nanoparticles was observed and approximately needle. This reveals that the powder particles are slightly agglomerated but its size range of 82.46nm and the closed view of needle nanoparticle has showed and Fig. 4a. Above results suggested that the silver nanoparticles are synthesized due to the action of plant extract *Coleus forskohlii* roots, which act as good bioreductant for biosynthesis. In the analysis by energy dispersive spectroscopy (EDS) of the silver nanoparticles the presence of elemental metal signal was confirmed. Although silver signal are present, the presence of silicon signal may be due to the thin film made on the glass slide taken for the EDS (Figs. 4b). Thus the XRD pattern proves to be strong evidence in favor of the UV–vis spectra and SEM images for the presence of silver nanocrystals.

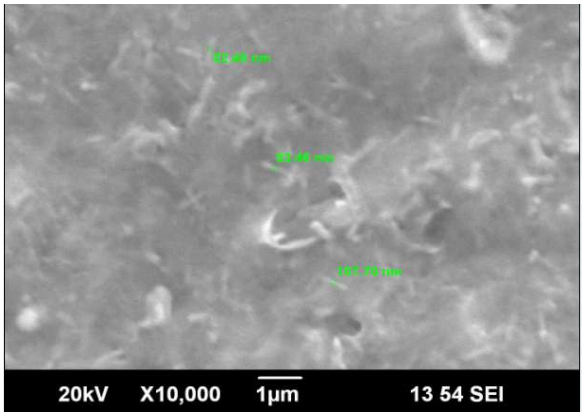

Fig. 4a

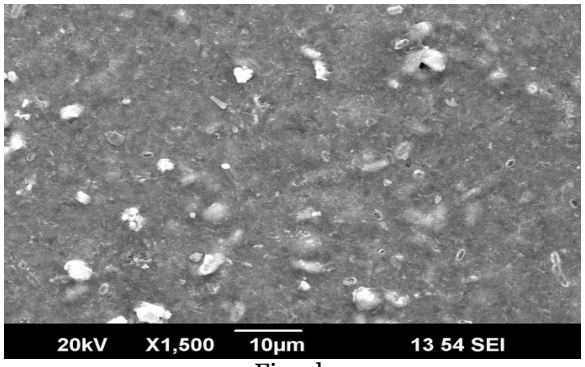

Fig. 4b

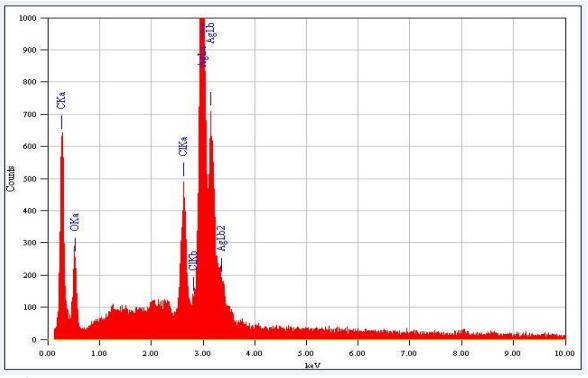

Fig 4. SEM images of silver nanoparticles. (4a) The needle shaped nanoparticles in range of 82.46 nm synthesized from *Coleus forskohlii* roots extract and

also showed closed view of silver nanoparticle which is needle in shape. (4b) EDS spectrum showed silver signal.

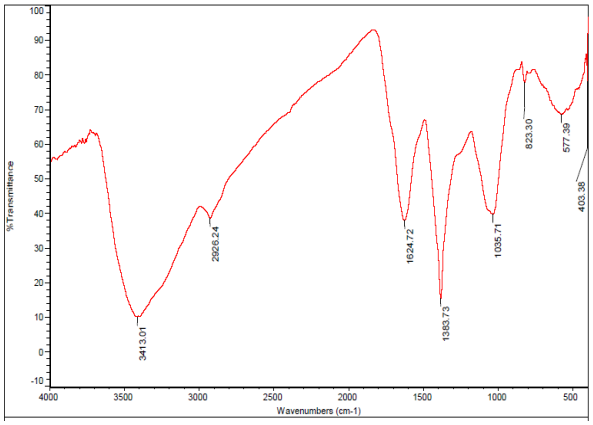

Fig. 5: FTIR spectrum of silver nanoparticles synthesized by *Coleus forskohlii* roots extract.

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. Fig 5 shows the synthesized AgNPs using *Coleus forskohlii* roots aqueous extract where the absorption peaks were located at 1035.71, 1383.73 and 1624.72 in the region 500–4000cm–1. The peaks corresponding to presence of fatty acids, carbonyl groups, flavanones and amide I band of proteins.

Comparative analysis of antimicrobial effects among nanoparticles, antibiotics.

The comparative analysis was focused on the sensitivity of the microorganisms towards silver nanoparticles, antibiotics. The Silver nanoparticles synthesized via green route are highly toxic to multidrug resistant bacteria and fungai hence has a great potential in biomedical applications. Table 1 shows that the antimicrobial potency of nanoparticle and the five broad spectrum antibiotics against seven bacteria and five fungus pathogens which clearly show that silver nanoparticles have shown antibacterial activity equivalent to that of standards against the entire tested organisms. The antibacterial activity of silver nanoparticles against *E. coli* is higher than those others are due to the variation in the cell wall composition between Gram positive and negative bacteria. The antifungus activity of silver nanoparticles against *C.albicans* is higher than that others.

Organism	silvernano	*Ciprofloxacin*	*Erythromycin*	*Cefoxitin*	*Vancomycin*	Rifamycin
E.coli	18	20	12	-	-	25
B.cerus	10	25	15	-	14	22
P.aeruginosa	13	24	-	-	-	25
M. luteus	10	18	10	-	5	20
K pneumonia	9	16	12	7	6	18
P.mirabilis	8	23	-	9	-	23
S.aureus	15	27	11	20	18	19

Organism	silvernano	*Ciprofloxacin*	*Erythromycin*	*Cefoxitin*	*Vancomycin*	Rifamycin
A.niger	5	7	-	-	-	11
A.flavus	-	6	-	-	-	8
C.albicans	8	6	-	-	-	8
C.tropicalis	7	-	12	9	20	32
C.kefyr	5	9	-	-	27	13

Table 1: Shows that the antimicrobial potency of nanoparticle and the five broad spectrum antibiotics against seven bacteria and five fungus pathogens.

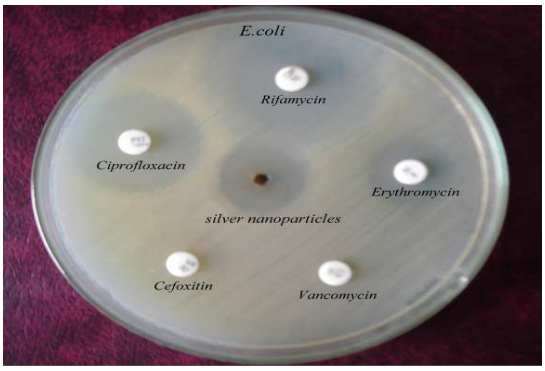

Fig 6: Image of antibacterial activity of silver nanoparticles

CONCLUSION

To summarize, we succeeded in the biological reduction of silver by *Coleus forskohlii* roots extract. Silver nanoparticles were synthesized in ambient conditions and characterization of synthesized nanoparticles was carried out by UV–Vis spectroscopy, XRD, FT IR and SEM equipped with EDS. It is believed that phytochemicals present in the extract of *Coleus forskohlii* roots extract has reduced the silver ions into metallic nanoparticles. This may be a first report that root had been sterilized before the extraction, we assumed to decant surface inhabitant microorganisms. The synthesized silver exhibited a strong antibacterial activity against E. coli. The process for the synthesis of nanoparticles in large scale using these readily available plant extract may have commercial viability and to develop studies in the interface between biology and material science. By using such plant extracts to develop nanomedicine against various human and veterinary pathogens.

REFERENCE

1) M. Gericke, A. Pinches, Hydrometallurgy 83 (2006) 132–140.
2) J.R. Morones, J.l. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramirez, M.J.Yacaman, Nanotechnology 16 (2005) 2346–3543.
3) S. Pal, Y.K. Tak, J.M. Song, Appl. Environ. Microbiol. 73 (2007) 1712–1720.
4) A. Pal, S. Shah, S. Devi, Mater. Chem. Phys. 114 (2009) 530–532.
5) T. Pradeep, Anshup, Thin Solid Films 517 (2009) 6441–6478.
6) S.L. Smitha, K.M. Nissamudeen, D. Philip, K.G. Gopchandran, Spectrochim. Acta A 71 (2008) 186–190.
7) A. Kumar, P.K. Vemula, P.M. Ajayan, G. John, Nat. Mater. 7 (2008) 236–241.
8) M. Raffi, A.K. Rumaiz, M.M. Hasan, S.I. Shah, J. Mater. Res. 22 (2007) 3378–3384.
9) K.J. Lee, B.H. Jun, J. Choi, Y. Lee, J. Joung, Y.S. Oh, Nanotechnology 18 (2007) 335601.
10) I. Sondi, B. Salopek-Sondi, J. Colloid Interf. Sci. 275 (2004) 177–182.
11) A.R. Shahverdi, S. Minaeian, H.R. Shahverdi, H. Jamalifar, A.S. Nohi, Process. Biochem. 42 (2007) 919–923.
12) R. Varshney, A.N. Mishra, S. Bhadauria, M.S. Gaur, Digest J. Nanomater. Biostruct. (2009) 349–355.
13) M. Kowshik, S. Ashtaputre, S. Kharraz, W. Vogel, J. Urban, S.K. Kulkarni, K.M. Paknikar, Nanotechnology 14 (2003) 95–100.
14) R.G. Haverkamp, A.T. Marshall, J. Nanoparticle Res. 11 (2009) 1453–1463.