Phytochemical screening and Green Synthesis of Silver Nanoparticles Using Aqueous Extract of *Catharanthus roseus* Stem Bark

Dr. A. Leema Rose[2] and F. Janeeta Priya*[1]

[1,2] Department of Chemistry, Holy Cross College, Tiruchirappalli – 620002,Tamilnadu ,india.

*Corresponding Author: Email janeetapriya@gmail.com

Abstract

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. The phytochemical screening of stem bark of *catharanthus roseus* revealed the presence of bioactive compounds. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of *catharanthus roseus* stem bark extract as reducing agent as well as capping agent. A change in color from yellowish brown to reddish brown and a strong peak at 440 nm under UV spectral analysis confirmed the formation of silver nanoparticles. Nanoparticles were characterized using UV–Vis absorption spectroscopy, EDAX and SEM. SEM analysis showed the average particle size as well as revealed their structure. The antibacterial activities of the formed silver nanoparticles have also been discussed.

Keywords: Silver nanoparticles, green synthesis, UV, EDAX and SEM.

INTRODUCTION

"Nanotechnology is the application of science to control matter at the molecular level" [1].Nanoparticles have unique properties as a consequence of their size, distribution and morphology and, therefore, these are a very important component in the rapidly developing field of nanotechnology. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, biology, biomedical science, and water treatment. Nanoparticles possess very high surface to volume ratios. This property can be utilized in the scientific fields, where high surface area is needed. As an example, in the catalytic industry, some nanoparticles have actually proven to be good catalysts [2]. Moreover, the nanoparticles show bactericidal effects. In recent years, the use of biological methods for the synthesis of metallic nanoparticles has received considerable attention, because these are inexpensive and eco–friendly; also, they can be carried out in one step. Silver ions are highly toxic for microorganisms and, therefore, have multiple roles in the medical field [3].

Biological methods of nanoparticle synthesis using microorganisms [4]–[6], enzymes, fungus, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods [7]. Sometimes the synthesis of nanoparticles using plants [8] or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures [9]. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process [10]. Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use [11].

Catharanthus roseus (*C.roseus*) (L.) G. Don. (Apocynaceae) is one of the important medicinal plants, due to the presence of the indispensable anti-cancer drugs, *i.e.*,vincristine and vinblastine. Roots of this plant are the main source of the anti-hypertension alkaloid ajmalicine[12].It possesses known antibacterial, antifungal, antibiotic, antioxidant, wound healing and antiviral activities[13-15]. Silver nanoparticles show potential antimicrobial effects [16-17]. Herein, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of C. roseus stem bark extract. Further, these biologically synthesized nanoparticles are found highly toxic against different pathogenic bacteria.

2. Materials and methods

2.1. Materials

All chemicals used in this experiment were of highest purity and obtained from Eswar scientific (trichy, India). C. roseus leaves were harvested from T. Valavanur, Tamilnadu for silver nanoparticle synthesis.

2.2 Preliminary investigation for the presence of phytochemicals

Aqueous extracts of the stem bark of *Catharanthus roseus* were investigated for the presence of phytochemicals viz. polyphenols, alkaloids, terpenoids, flavonoids, carbohydrates, steroids and steroids by following standard methods [18]

2.3. Plant extract and synthesis of silver nanoparticles

Plant leaf extract was prepared by mixing 5 g of dried powder with 50 mL deionized water in 250 mL of Erlenmeyer flask and boiled for 10 min. For the reduction of Ag^+ ions, 5 mL of leaf extract was mixed to 40 mL of 1 mM aqueous of $AgNO_3$ and then, heated at 60 °C for 15 min. A change from yellowish brown to reddish brown color was observed.

2.4 UV-Vis spectra analysis

The reduction of pure Ag^+ ion was monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting a small aliquot of the sample with distilled water after regular interval of time. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1700 (Shimadzu).

2.5. SEM and EDX analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.In order to carry out EDX analysis, the leaves extracts reduced silver nanoparticles were dried and drop coated onto carbon film and performed on Hitachi S-4500 SEM instrument equipped with a Thermo EDX attachments.

2.6. ANTIMICROBIAL ACTIVITY

2.6.1. DETERMINATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity was performed by disc diffusion method. Composition of Nutrient Agar (NA-Himedia) Media for Bacteria shown in **table 1**

Animal's tissue	5.00g
Sodium chloride	5.00g
Beef extract	1.50g
Yeast extract	1.50g
Agar	15.0g

Table 1: Composition of Nutrient Agar (NA-Himedia) Media for Bacteria

2.6.2. Microorganisms

The microbial strains employed in the biological assays were Gram – **positive** bacteria: *Staphylococcus aureus* (MTCC 3160), and Gram – **negative** bacteria: *Escherichia coli* (MTCC 732) and Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

2.6.2. Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8ºC until use.

2.6.3. Preparation of sample solutions for the experiment

The Nanoparticle was weighed (10mg/10ml) and dissolved in deionized water. Different doses of 50µl, 100 µl and 150 µl used for the experiment.

2.6.4. Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization After sterilization, the discs were loaded with sample solutions and again kept under refrigeration for 24 hrs. Standard solution as Chloromphenical for bacteria used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

3.RESULTS AND DISCUSSION

3.1.Phytochemical analysis

The phytochemical screening of Aqueous extracts of the stem bark of *Catharanthus roseus* revealed positive result for carbohydrates, terpenoids, alkaloids, flavanoids, carbohydrates, tannins,proteins, phenol and anthocyanins while negative result for steroids, anthraquinones and glycosides as showed in Table 2.

Phytochemical compounds	**Plant name**	**Water**
Terpenoids	*Catharanthus roseus*	+
Flavanoids	*Catharanthus roseus*	+
Alkaloids	*Catharanthus roseus*	+
Steroids	*Catharanthus roseus*	+
Glycosides	*Catharanthus roseus*	-
Carbohydrates	*Catharanthus roseus*	+
Saponins	*Catharanthus roseus*	+
Tannins	*Catharanthus roseus*	+
Proteins	*Catharanthus roseus*	-
Saponins	*Catharanthus roseus*	+
Emodins	*Catharanthus roseus*	-
Anthraquinones	*Catharanthus roseus*	-
Anthocyanins	*Catharanthus roseus*	+
Sterols	*Catharanthus roseus*	-

Table: 2 photochemical screening of Aqueous extracts of the stem bark of *Catharanthus roseus*

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilities their qualitative separation of pharmacologically active chemical compounds [19]

Characterization of the Ag nanoparticles

3.2. UV-VIS spectra analysis

The reaction mixture, C. roseus stem bark extract with aqueous solution of the silver nitrate, started to change its color from yellowish brown to reddish brown. It indicated the formation of silver nanoparticle with the reduction of silver ion. The characteristic surface Plasmon absorption bands were observed at 440 nm. Fig. 1shows the UV-Vis spectra of silver nanoparticles synthesized by using C. roseus stem bark extract.

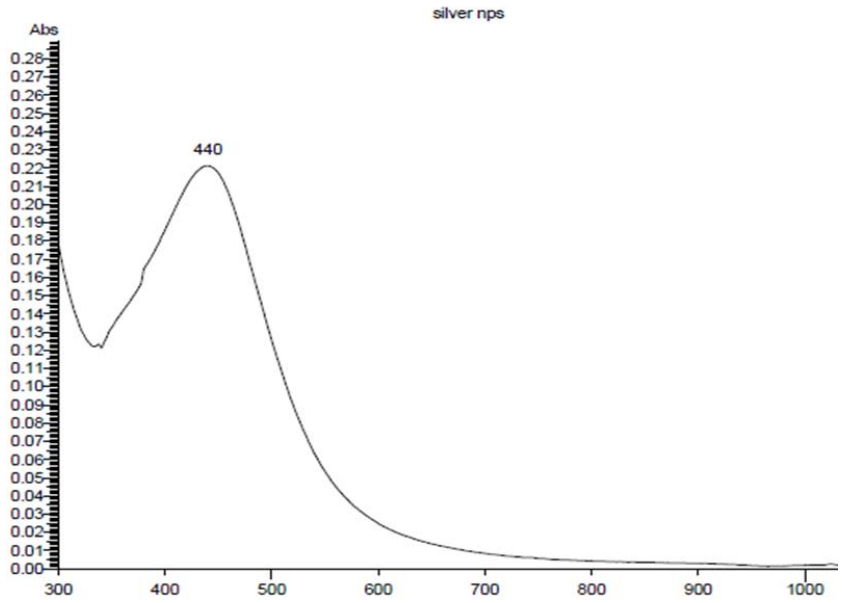

Fig 1 : UV-Visible spectroscopy for silver nanoparticles.

3.3.SEM and EDAX Analysis

The SEM image of silver nanoparticles synthesized by using is shown in C. roseus stem bark extract. Fig. 2 which shows distinct and clear image of synthesized silver nanoparticles having spherical shapes. This image further indicates that the silver nanoparticles are not aggregated i.e monodisperse in nature. Analysis through EDX spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles (Figure 3). The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in KeV.

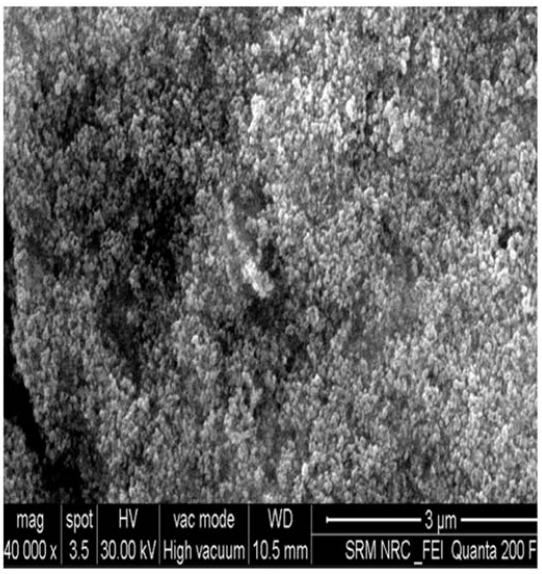

Fig.2 : SEM images of synthesized silver nano particles

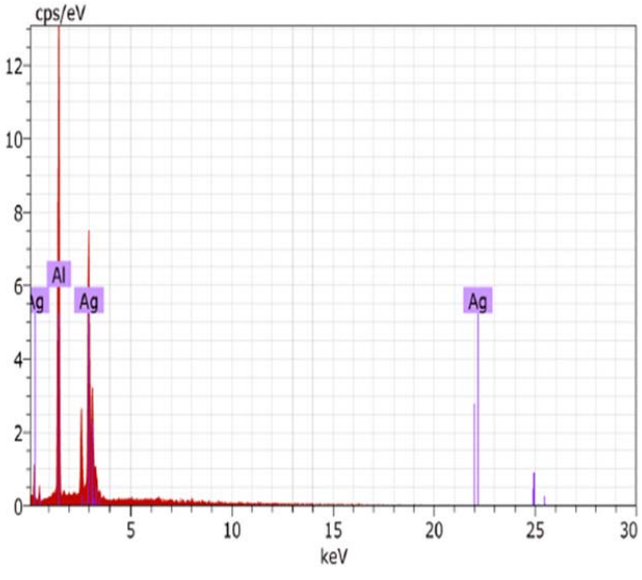

Fig 3: EDAX analysis for silver nanoparticles Spectrum: sample

El AN	Series	unn.c[wt.%]	norm.c[wt.%]	Atom.c[at.%]	Error	(1 Sigma) [wt.%]
Ag 47	L-series	48.04	60.37	27.59		1.60
Al 13	K-series	31.54	39.63	72.41		1.65
	Total	79.58	100	100		

Table 4: Elemental composition of silver nanoparticle

3.4. Anti-bacterial activity:

Antibiogram was done by disc diffusion method using nanoparticle. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar. Briefly, inoculums containing *Staphylococcus aureus* and *Escherichia coli* specie of bacteria were spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

3.4.1. Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganism by the samples were measured using a millimeter scale.

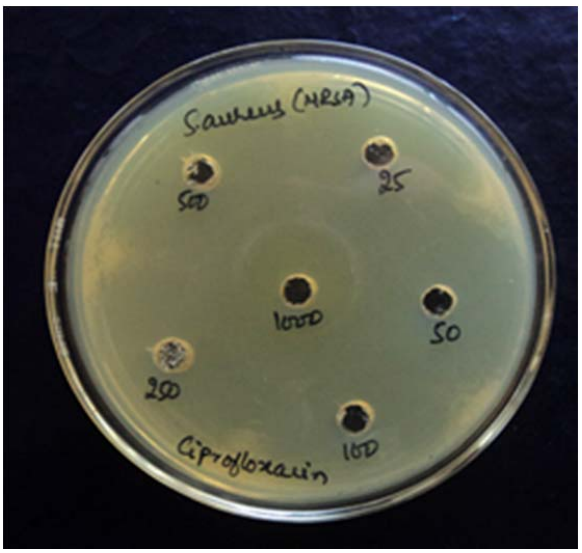

Figure. 4 Antimicrobial activity of silver nanoparticle

Table: 5 \

Sample	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
AgNps	0	0	0	0	10	12	500
	25 µg	**50µg**	**100µg**	**250µg**	**500µg**	**1000µg**	**MIC µg**
Ciprofloxacin	30	32	34	35	38	*	25

Antibacterial activity for staphylococcus aureus

4. CONCLUSION

It can be concluded that the extract C. roseus stem bark extract contains various phytochemicals. The present study confirmed the silver nanoparticle formation using the C. roseus stem bark extract and its antimicrobial properties against selected bacterial species, some of which are human pathogens. Further study required to use these nanoparticles for the treatment of disease using animal models.

5. ACKNOWLEDGEMENT

The authors would like to thank the Department of chemistry, holy cross college, trichy for providing logistic support and their continuous encouragement and advice.

REFERENCES

[1] S. Senapati, PhD thesis, University of Pune, 2005.
[2] Jiang, Z.J.; Liu, C.Y.; Sun, L.W. Catalytic properties of silver nanoparticles supported on silica spheres. J. Am. Chem. Soc. 2004, 71, 2341–2343.
[3] S.Galdiero, A.Falanga, M.Vitiello, V.Marra, M.Galdiero, "Silver nanoparticles as potential antiviral agents", Molecules, vol.16, pp.8894-8918, 2011.
[4] Klaus T, Joerger R, Olsson E, Granqvist CG. Silver-based crystalline nanoparticles, microbially fabricated. J Proc Natl Acad Sci USA. 1999;96:13611–13614.
[5] Nair B, Pradeep T. Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. Cryst Growth Des. 2002;2:293–298.
[6] Konishi Y, Uruga T. Bioreductive deposition of platinum nanoparticles on the bacterium *Shewanella* algae. J Biotechnol. 2007;128:648–653.
[7] Willner I, Baron R, Willner B. Growing metal nanoparticles by enzymes. J Adv Mater. 2006;18:1109–1120.
[8] Vigneshwaran N, Ashtaputre NM, Varadarajan PV, Nachane RP, Paraliker KM, Balasubramanya RH. Biological synthesis of silver nanoparticles using the fungus *Aspergillus flavus*. Mater Lett. 2007;61:1413–1418.
[9] Shankar SS, Ahmed A, Akkamwar B, Sastry M, Rai A, Singh A. Biological synthesis of triangular gold nanoprism. Nature. 2004;3:482.
[10] Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, et al. et al. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomumcamphora* leaf. Nanotechnology. 2007;18:105104.
[11] Kumar V, Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol. 2009;84:151–157.
[12] Jaleel CA, Gopi R, Alagu Lakshmanan GM, Panneerselvam R. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Sci. 2006a;171:271–276.
[13] Jaleel CA, Gopi R, Paneerselvam R. Alterations in non-enzymatic antioxidant components of Catharanthus roseus exposed to paclobutrazol, gibberellic acid and Pseudomonas fluorescens. Plant Omics J 2009; 2: 30-40.
[14] Jayanthi M, Sowbala N, Rajalakshmi G, Kanagavalli, Sivakumar V. Study of antihyerglycemic effect of Catharanthus roseus in alloxan induced diabetic rats. Int J Pharm & Pharm Sci 2010; 2(4): 114-116.
[15] Nayak BS, Pinto Pereira LM. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med 2006; 6: 41.
[16] K.-H. Cho, J.-E. Park, T. Osaka and S. G. Park, *Electrochim. Acta*,2005, **51**, 956–960.
[17] N.Dura'n, P.D.Marcato, S.De, I. H. Gabriel, O. L. Alves and E.Esposito, *J. Biomed. Nanotechnol.*, 2007, **3**, 203–208.
[18] Kardong D, Upadhyaya S, Saikia LR. Screening of phytochemicals, antioxidant and antibacterial activity of crude extract of pteridium aquilinum Kuhn. J. Pharmacy Research. 2013; 6: 179-182.
[19] Suganya RS, Priya K, and Roxy B, Phytochemical screening and antibacterial activity from *Nerium oleander* and evaluate their plant mediated nanoparticles synthesis; International Research Journal of Pharmacy, 2012; 3(5): 285-288.