

Green Synthesis Of Silver Nanoparticles From *Cynodon Dactylon* Leaf Extract

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Abstract: There is an increasing commercial demand for nanoparticles (NPs) due to their wide applicability in various areas. Metallic nanoparticles are traditionally synthesized by wet chemical synthesis techniques where the chemicals used are quite often toxic and flammable. Hence the development of reliable and eco-friendly processes for synthesis of metallic nanoparticles is an important step in the field of nanotechnology. Plant extracts are eco-friendly and thus can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. In the present study, we used a novel approach to synthesize stable silver (Ag) NPs using *Cynodon dactylon* leaf extract and observed a rapid reduction of silver ions leading to the formation of stable silver nanoparticles in solution when exposed to sun light compared to other modes of synthesis viz, microwave synthesis, microwave, UV, direct boiling and room temperature. The UV-Vis spectrum of Ag NP in aqueous solution shows an absorbance peak around 450 nm due to Surface plasmon resonance. Silver nanoparticles were confirmed with XRD which exhibited intense peak. Besides the above, the FTIR analysis showed the presence of difference functional groups involved in capping the silver nanoparticles. Again, silver nanoparticles size was confirmed by SEM analysis which shows that particles were in the range of 30 to 50 nm in size. This study, to the best of our knowledge, is the first attempt to perform eco-friendly synthesis of Ag NPs using *Cynodon dactylon* and sun light and produced the maximum yield which may benefit various industries with wide range of applications, including renewable clean energy, clean water, medical advances, cosmetics, food and food packaging, paints, coatings, information, technologies and aerospace developments.

Keywords: Silver nanoparticles, *Cynodon dactylon*, XRD, FTIR, SEM.

1. Introduction

In recent years, like other technology developments, nanotechnology also expected to grow based on their demand and its wider applications and the number of research being conducted in this field is rapidly growing throughout the world.[1]. Nanotechnology deals with the development of nanometer sized materials. [2]. In the field of nanotechnology different concepts of engineering, electronics, and material science are applied in molecular or submicron level [3]. Particles with a size up to 100 nm are usually referred as nanoparticles and they exhibit completely new properties based on their size, distribution and morphology [4]. In the nanoscale level the properties of the materials are different from that of their bulk materials and the increased surface area of these nanoparticles is mainly responsible for their different chemical, optical, and mechanical properties (5) .A characteristic feature of the metallic nanoparticles is the presence of surface plasmon resonance. A strong surface plasmon resonance absorption is exhibited in the UV–Visible region by the metallic nanoparticles. Surface plasmon resonance arises due to existence of free electrons in the conduction band because of the small size of the particles [6].Metallic nanoparticles (NPs) possess unique optical, electrical and photothermal properties and the Silver Nanoparticles are exploited widely because of their excellent antibacterial properties. [7]. Silver nanoparticles are used in the development of new technologies in the areas of electronics, material sciences and medicine and because of their extensive applications in various areas more research is being conducted on the silver nanoparticles by the scientists throughout the world. [8]. Methods which are available presently for nanoparticles synthesis are chemical, physical and Biomimetics. Biomimetics method uses biological systems such as plants, bacteria and fungi for the synthesis of nanoparticles. Biological methods for the production of nanopartices are considered as a safe and environment friendly alternative to the conventional physical and chemical methods as it is a cost effective method and the usage of high pressure, energy, temperature and toxic chemicals is completely eliminated [9]. Therefore the synthesis of nanoparticles using biological method is preferred over the physical and chemical methods and the term “Green nano synthesis” has been proposed for the synthesis of nanoparticles through biological route [10].Using plants for the nanoparticle synthesis can be advantageous over the other biological methods as the process of maintaining cell cultures can be eliminated and nanoparticle synthesis using plant leaf extracts can be more economical when compared to the whole plants because of the

feasibility in the downstream processing steps [11].Although the biosynthesis of silver nanoparticles from many plants such as *Euphorbia hirta L* [12], *Coriandrum Sativum* [13] , *Solanum torvum* [14] have been reported, the potential plants as the biological source for nanoparticle synthesis is yet to be explored fully. In this paper, we report a low-cost, convenient, green synthesis approach to obtain large quantities of silver nanoparticles by reduction of silver ions with using *Cynodon dactylon* leaf extract.

2. Materials and methods

2.1. Preparation of leaf extract by Homogenization method

The *Cynodon dactylon* leaf extract was washed several times with de-ionized water. The extract used for the synthesis of silver nanoparticles was prepared by taking 20 g of thoroughly washed and finely cut *Cynodon dactylon* leaves with 200 ml of distilled water. The suspension was homogenized .The homogenized suspension was centrifuged and the supernatant was collected. The extract obtained was filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use.

2.2 Preparation of leaf extract by boiling method

The *Cynodon dactylon* leaves were washed several times with deionised water. 20 g of finely cut *Cynodon dactylon* leaves were taken and boiled in 200 ml of double distilled water for 3min and filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use.

2.3Optimization and synthesis of silver nanoparticles

Sunlight Irradiation

1mM Silver Nitrate solution was prepared. To the homogenized leaf extract solution 10 ml of 1 mM AgNO₃ solution was added with constant stirring. The conical flask was exposed to the sunlight. The color of the solution gradually changed from yellow to brown color. The reduction of pure silver ions were monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water. U-V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer. The same procedure was repeated for the leaf extract prepared using boiling method.[30]

Microwave irradiation

1mM Silver Nitrate solution was prepared. To the homogenized leaf extract solution 10 ml of 1

mM AgNO₃ solution was added with constant stirring. The conical flask was placed in a domestic microwave oven (National Model N N-GD 576M). The sample was subjected to several short burst of microwave irradiation at frequency of 2.45 GHz, at power output of about 100W in a cyclic mode (on 15 s, off 15 s) to prevent overheating as well as aggregation of metals. The irradiation process was conducted for a minimum of 15 cycles. At the end of 15 cycles the reduction of pure silver ions were monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water U.V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer. The same procedure was repeated for the leaf extract prepared using boiling method.[29]

UV irradiation

1mM Silver Nitrate solution was prepared. To the homogenized leaf extract solution 10 ml of 1 mM AgNO₃ solution was added with constant stirring. The conical flasks was kept under and exposed under UV radiation until the color change was observed. The reduction of pure silver were monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water U.V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer.. The same procedure was repeated for the leaf extract prepared using boiling method.[27]

Room temperature

1mM Silver Nitrate solution was prepared. To the homogenized leaf extract solution 10 ml of 1 mM AgNO₃ solution was added with constant stirring. 10 ml of 1 mM AgNO₃ solution was added to the conical flask with constant stirring, and was incubated under Room temperature until the color change was observed. The reduction of pure silver were monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water U.V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer.. The same procedure was repeated for the leaf extract prepared using boiling method.[28]

Direct Boiling

1mM Silver Nitrate solution was prepared. To the homogenized leaf extract solution 10 ml of 1 mM AgNO₃ solution was added with constant stirring and boiled until the color change was observed. The reduction of pure silver were monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of

the sample in distilled water U.V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer.. The same procedure was repeated for the leaf extract prepared using boiling method [27].

2.4 Production and Recovery of silver nanoparticles by centrifugation

Among various methods used, sunlight irradiation method was very effective and homogenized leaf extract had shown more synthesis of nanoparticles. Thus homogenized leaf extract and sunlight exposure method was chosen for the bulk production of nanoparticles. For the bulk production of silver nanoparticles 100ml of 1mM Silver nitrate was added to the homogenized leaf extract in a conical flask and exposed to sunlight. The solution consisting silver nanoparticles was subjected to centrifugation at 10,000rpm for 15 minutes. The pellet formed was dissolved in 0.1 ml of toluene water and air dried.

2.5 Characterization of Silver nanoparticles

2.5.1. UV-Vis Spectral Analysis

The reduction of pure Ag²⁺ ions was monitored by measuring the U-V Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water. U.V visible spectroscopy was carried out on a ELCO 150 U-Visible spectrophotometer.

2.5.2. XRD analysis

The Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 mins. the pellet was washed three times with 20 ml of de-ionized water. The dried mixture of silver nanoparticles was collected for the determination of formation of Silver nanoparticles by X' Pert PRO X-ray diffractometer operated at a voltage of 40 kv and a current of 30 mA with Cu K radiation.

2.5.3. FTIR analysis

For FTIR measurements, Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 minutes. the pellet was washed three times with 20 ml of de-ionized water. The samples were dried and analyzed on IR-Prestige-21 [SHIMADZU] operating at a resolution of 2cm⁻¹.

2.5.4. SEM analysis

Scanning electron Microscopic [SEM] analysis was done using Hitachi S-4500 machine. Thin films of samples were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid. Extra solution was removed using blotting paper and then films on SEM grid were allowed to dry by putting it under mercury lamp for 5 minutes.

3. Results

3.1 Optimization Studies

Among the different exposure studies Sunlight irradiation method and homogenized extract showed better production of silver nanoparticles.

3.1. UV Vis Analysis

Reduction of silver into silver nanoparticles are marked by color change. Silver nanoparticles exhibit dark yellowish brown colour due to surface plasmon resonance. The strong surface plasmon resonance band appears at the range of 440-480nm. Sunlight irradiated reaction mixture showed a strong characteristic absorbance peak at around 450 nm.



Control After reaction

Figure.1 shows the silver nitrate solution before and after reaction.

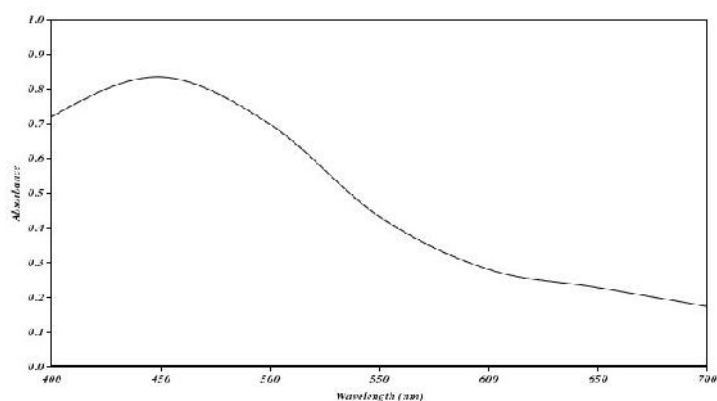


Figure.2 shows UV-Visible Absorption spectra of Silver Nanoparticles synthesized using *Cynodon dactylon* leaf extract. Absorbance peak is observed at 450 nm.

3.2. SEM Analysis

SEM image showed relatively spherical shaped particles in the range of 30 -50 nm.

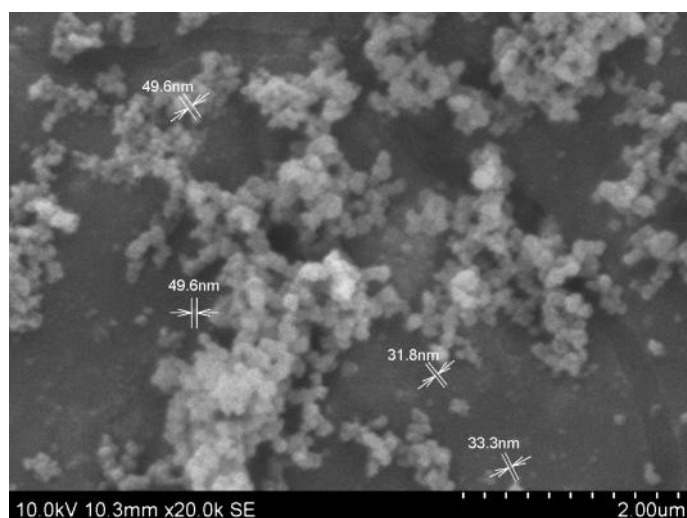


Figure.3. shows the SEM micrograph of the Silver Nanoparticles . The size of the particle ranged from 30 to 50 nm.

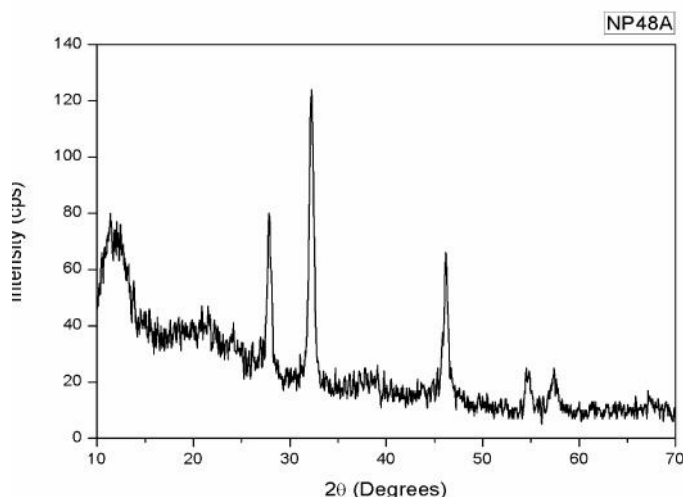


Figure 4 shows XRD pattern of silver nanoparticles. The XRD pattern showed 3 intense peaks in the whole spectrum of 2θ value ranging from 10 to 70.

3.3. XRD analysis

Structural characterization was performed using XRD analysis. The XRD pattern of the silver nanoparticles is shown in Fig 3. XRD analysis showed distinct diffraction peaks which can be indexed the angle values of (111),(200),(220)and (240).

3.4. FTIR

FTIR measurement was carried out to identify possible biomolecules of *Cynodon Dactylon* leaf extract responsible for the formation and stabilization of nanoparticles. Prominent IR bands are observed at 3273, 3232, 2956, 2917, 2848, 1648, 1542, 1457,1383,1238,1074 and 1036 cm^{-1} . The peak at 2956 and 2917 indicates C-H stretching due to alkanes. The peak at 3273 indicates C-H stretching due to alkynes. The peak at 3273, 3232, 2956 and 2917 correspond to the OH stretching vibrations of alkanes, phenols and hydrogen bonded carboxylic acid. Peak at 1648 indicate C=C stretching vibrations to alkanes, NO₂ asymmetric stretching vibrations to nitro compounds and NH bending vibrations due to amines. Peak at 1542 indicates NO₂ asymmetric stretching vibrations to nitro compounds. Peak at 1457 indicates CH scissoring and bending due to umbrella deformation of methyl group. Peak at 1383 indicates CH scissoring and bending due to umbrella deformation of methyl group and NO₂ symmetric stretching vibrations to nitro compounds. Peak at 1238 indicates C-O stretching due to alcohols, ethers, carboxylic acids, ethers and esters, NO₂ symmetric stretching vibrations to nitro compounds and C-N stretching due to amines. Peak at 1074 and 1036 indicates stretching due to alcohols, ethers, carboxylic acids, ethers and esters and C-N stretching due to amines.

4. Discussion

Cynodon Dactylon leaf extract was used for the synthesis of silver nanoparticles. Different optimization studies were carried out for the synthesis of silver nanoparticles from *Cynodon dactylon* leaf extract. The optimization studies indicated that sunlight exposure method and homogenized extract solution were best for the production of silver nanoparticles. The reaction medium confirmed the presence of the silver nanoparticles. The color of the reaction medium gradually changed to dark brown because of the surface plasmon resonance. The color change was observed after adding Silver nitrate solution to the homogenized extract. The visible range of UV spectra for silver is between 400 -600 nm.

The presence of silver nanoparticles was confirmed by UV spectra analysis. UV spectra analysis showed absorbance peak at 450 nm which confirms the presence of the silver nanoparticles.

The morphology of the silver nanoparticles was obtained through characterization using SEM. SEM image showed relatively spherical shaped particles in the range of 30 to 60 nm.

The Silver nanoparticle solution was centrifuged at 20,000 rpm for 20 minutes and the dried powder was used for XRD analysis and FTIR analysis.

The biosynthesized silver nanoparticle was further confirmed by the characteristic peaks observed XRD. The XRD pattern showed three intense diffraction peaks in the whole spectrum of 2θ values ranging from 10 to 70.

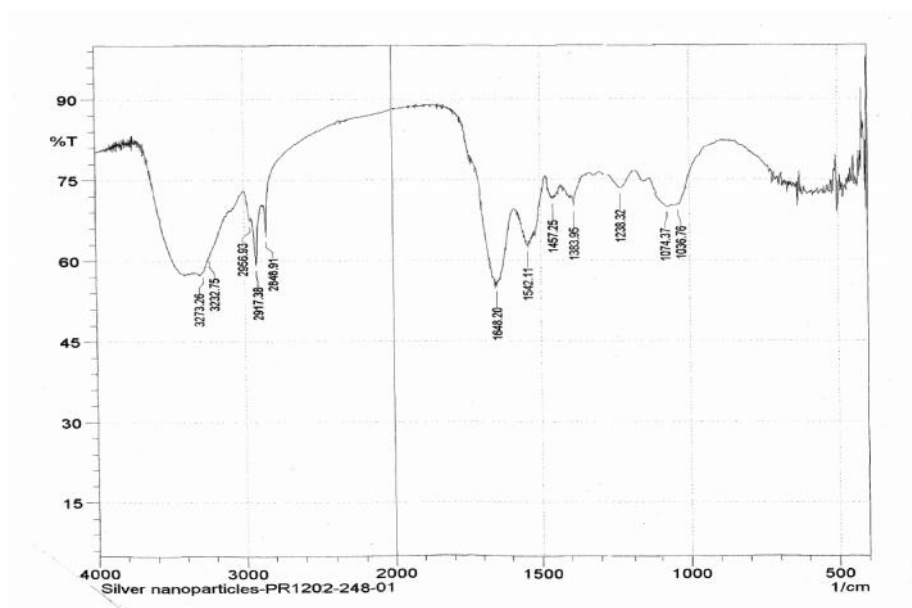


Figure. 5. shows the FTIR spectra of silver nanoparticles. The presence of functional groups is revealed by FTIR analysis .Different peaks obtained in the FTIR analysis indicates the presence of alkanes, phenols, carboxylic acid groups, nitro compounds, alcohol, ethers and esters.

In the FTIR analysis bands were present at 3273, 3232, 2956, 2917, 2848, 1648, 1542, 1457, 1383, 1238, 1074 and 1036 cm^{-1} which indicates the presence of alkanes, phenols, carboxylic acid groups, nitro compounds, alcohol, ethers and esters. FTIR analysis confirmed that the bioreduction of Ag^+ ions to silver nanoparticles are due to the reduction by capping material of plant extract.

The present study showed a simple green route for rapid synthesis of silver nanoparticles using *Cynodon dactylon* leaf extract.

5. Conclusion

In the present study green synthetic route is used for the synthesis of silver nanoparticles using

Cynodon dactylon leaf extract. This green synthesis method is rapid, less time consuming and environmentally safe can be used in various biotechnological applications.

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References

1. Mukunthan K S, Elumalai E K, Patel T N and Ramachandramurty V, *Asian Pacific Journal of Tropical Biomedicine*, 2011, 270-274.
2. Salata O V, *J. Nanobiotechnology*, 2004, 2:3.
3. Lu J M, Wang X, Muller C M, Wang H, Lin P H, Yao Q, and Chen C, *Expert Rev Mol Diagn*, 2009, 9(4) 325–341.
4. S atyavani K, Gurudeeban S and Balasubramanian T R, *J. Nanobiotechnology*, 2011, 9:43.
5. Vithiya K and Sen S, *IJPSR*, 2011, 2(11) 2781-2785.
6. Sharma V K, Yngard R A and Lin Y, *Advances in colloid and Interface Science*, 2009, 145, 83-96.
7. Kemp M M, Kumar A, Clement D, Ajayan P, Mousa S and Linhard R J, 2009 *Nanomedicine* 4(4) 421–429.
8. Safaepour M, Shahverdi A R, Khorramizadeh M R and Gohari AR, *Avicenna Journal of Medical biotechnology*, 2009, 1(2) 111-115.

9. Prashar U K ,Saxena P S and Srivatsava A, *Digest Journal of Nanomaterials and Biostructures*,2009,4(1) 159-166.
10. Banu A and Rathod V,*International Journal of Biomedical and Advance Research* 2011 ,2(5) 148-158.
11. Annamalai A S ,Babu S T, Rose N A , Sudha D and Liza C V,*World Applied Sciences Journal*, 2011, 13 (8) ,1833-1840.
12. Elumalai E K , Prasad T.N.V.K.V , Kambala V, Nagajyothi P C and David E, *Archives of Applied Science Research*, 2010, 2 (6), 76-81.
13. Sathyavathi R, Balamurali Krishna M , Venugopal Rao S, Saritha R, and Narayana Rao D,*Advanced Science Letters* , 2010 ,3 ,138–143.
14. Govindaraju K, Tamilselvan S, Kiruthiga V and Singaravelu G. *Journal of Biopesticides*,2010, 3 (1), 394 - 399.
15. Morones J R, Elechiguerra J L, Camacho A , Holt K , Kouri J, Ramirez J T and Yacaman M J ,*Nanotechnology*, 2005,16 ,2346-2350.
16. Kalimuthu K, Babu R S ,Venkatataraman D , Bilal M and Gurunathan S, *Journal Colloids and Surfaces B: Biointerfaces*,2008,65,150-153.
17. Safaepour M, Shahverdi A R, Shahverdi H R ,Khorramizadeh M R and Gohari AR *Avicenna J Med Biotech*,2009,1(2) 111-115.
18. Panacek A, Kvitek L, Pucek R, Kolar M, Vecerova R, Pizurova N, Sharma V K, Nevecan T and Zboril R , *J Phys Chem B*, 2006,110(33) 16248-16253.
19. Shankar S S, Ahmad A and Sastry M. *Biotechnol Prog* , 2003,19(6) 1627-1631.
20. Dadosh T,*Materials letters* , 2009 ,63,2236-2238.
21. Shahverdi A R, Fakhimi A, Shahverdi H R and Minaian S ,*Nanomedicine* , 2007, 3,168-171.
22. Christensen L, Vivekanandhan S, Misra M and Mohanty A K, *Adv. Mat. Lett* 2011 2(6), 429-434.
23. Gardea-Torresdey J L, Gomez E, Peralta-Videa J R, Parsons J G, Troiani H and Jose-Yacaman M, *Langmuir* , 2003,19,1357–1361.
24. Duran N, Marcato P D, Alves O L, De Souza G I H and Esposito E, *J Nanobiotechnol*, 2005, 3, 8–14.
25. Marambio-Jones C and Hoek E M V, *Journal of Nanoparticle Research*, 2010, 12(5),1531–1551.
26. Nadagouda M N, Hoag G, Collins J, Varma R S ,*Cryst Growth Des*, 2009,9(11) 4979–4983.
27. Jancy Mary E and Imbathamizh L, *Asian J Pharm Clin Res*,2012, 5, 159-162.
28. Sivakumar P,Nethradevi C and Renganathan S, *Asian J Pharm Clin Res*,2012, 5(3), 97-101.
29. Renukadevi K and Aswini RV, *International Journal of Nanomaterials and Biostructures*, 2012; 2(2) 5-10.
30. Rastogi L and Arunachalam J, *Materials Chemistry and Physics*, 2011,129 (1-2), 558-563.
