Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

PHYTOSYNTHESIS OF SILVER NANOPARTICLES USING MEDICINAL AND DYE YIELDING PLANT OF *BIXA ORELLANA* L. LEAF EXTRACT

M. Thilagam[1], A. Tamilselvi[2]*, B. Chandrasekeran[3] and C. Rose[4]
[1]Research scholar, Department of Plant Biology & Plant Biotechnology, Presidency College, Chennai,Tamilnadu, India
[2]Scientist, CHORD, Central Leather Research Institute, Adyar, Chennai, Tamilnadu, India
[3]Head of the Department of CHORD, Central Leather Research Institute, Adyar, Chennai, Tamilnadu, India
[4]Head of the Department of Biotechnology, Central Leather Research Institute, Adyar, Chennai, Tamilnadu, India
*Corresponding Author Email: selviala@yahoo.co.uk
DOI: 10.7897/2277-4572.02441
Published by Moksha Publishing House. Website www.mokshaph.com

Received on: 20/05/13 Revised on: 20/06/13 Accepted on: 28/06/13

ABSTRACT
Here in, we report an environmentally friendly approach to synthesize silver nanoparticles. The bio active compounds found in plants induce the reduction of silver ions from silver nitrate to silver nanoparticles. We report the synthesis of silver nanoparticles and also phytochemical analysis of the leaf extract using dye yielding plant *Bixa orellana*. The synthesis of silver nanoparticles was detected by changing colour from yellowish green to brown after treatment with silver nitrate (1mM). The synthesized particles were characterized by UV-vis Spectroscopy, scanning electron microscopy, FTIR, and X-ray diffractometry. UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 439 nm corresponding to the plasmon absorbance of silver nanoparticles. FT-IR analysis indicated the involvement of carboxyl, hydroxyl, phenol and amine functional groups. The phytochemical analysis of the extract has also been carried out which indicated presence of alkaloids, terpenoids, steroids, phenols and tannins. X-ray diffraction (XRD) spectrum of the silver nanoparticles exhibited 2θ values corresponding to the silver nano crystal and scanning electron microscopy revealed the size of silver nanoparticles in the range of 35-65nm.
Keywords: Silver nanoparticles, *Bixa orellana,* phytochemicals, XRD, SEM

INTRODUCTION

Various metals have been used in a synthesis of nanoparticles which are useful in the field of medicine, catalysis, electronics, optics, photonics, biolabelling, optoelectronics, photography and surfaced enhanced Raman scattering (SERS) detection[1]. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations without any side effect[2], however biocompatible inert nanomaterials have been find their way in cancer diagnosis and delivery of anticancer drugs[3]. In small concentrations, silver is safe for human cells, but lethal for micro organisms[4]. As most of pathogenic microorganisms developed resistance against antibiotics there is need for an alternative antibacterial resistance. Silver nanoparticles have an important advantage over conventional antibiotics and no organism has ever been reported to readily develop resistance to it.

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles[5]. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles. Green synthesis of nanoparticles includes biological, polysaccharide, tollens, irradiation and mixed valence polyoxometallates [6]. Off these plants mediated synthesis is cost effective, environmentally friendly, and safe for human therapeutic use[7].

Many reports are available on the biogenesis of silvernano particles using several plant extracts, Pinus, Persimmon, Ginkgo, Magnolia and Platanus[8], *Azadirachta indica* (neem)[9], *Medicago sativa* (alfalfa), *Aloe vera*[10], *Gliricidia sepium* Jacq.[11], *Carrica papaya*[12], *Opuntiaficus indica*[13], *Murrayako enigii*[14], *Osmium sanctum*[15], *Saururus chinenis* [16], *Eucalyptus hybrida*[17], *Coriandrum sativum*[18], *Jatropha cruces* (Seed), *Jatropha curcas* (Latex), Clove extract, *Brassica junco*, Morganella sp., *Cinnamon zeylanicum* [Bark extract & Powder], *Cinnamomum camphora* [Dried Leaf], *Helianthus annus* (Asteraceae) [Leaf Extract], *Basella alba* (Basellaceae), *Oryza sativa*, *Saccharum officinarum*, Sorghumbi colour, Zeamays (Poaceae), *Emblica officinalis*, Alfalfa, Geranium (*Pelargonium graveolens*), *Hibiscus sabdariffa*, *Phomaglo merata*, *Cinnamomum camphor* and *Capsicum annuum* L.[19]. *Bixa orellana* is a small evergreen tree native of Northern South America but it is widely cultivated in tropical countries for its seeds for food colourants and medicinal value. Seeds and leaves of the tree are used to prepare remedies for a variety of illnesses such as tonsillitis, asthma, pleurisy, rectal disorders, headache, jaundice, sunstroke, and burns[20]. It has not been reported on the synthesis of silver nanoparticles using Bol (*Bixa orellana* leaves). The present study describes the process of synthesis of silver nanoparticles using aqueous Bol extract followed by their characterization. This paper demonstrates that the reaction of aqueous silver ions with Bol extract resulted in the extracellular formation of silvernano particles at room temperature.

MATERIALS AND METHODS

Collection of the Plant

The leaves of *Bixa orellana* L. were collected from the forest area of Dharmapui district of Tamilnadu during the month of October.

Preparation of the Extract

Fresh leaves of Bol were washed several times with tap water and later with deionised water. 10g of the leaves were macerated in mortal and pestle with 20ml of de-ionized water and filtered. Then the filtrate was centrifuged for 15min at

10,000rpm, supernatant was collected and stored at 4°C. The solid content of the leaf extract was found to be 10%. 1mM of silver nitrate solution was prepared in 200ml of deionised water. To the silver nitrate silver nitrate solution 10ml of leaf extract was added and kept in dark condition at room temperature for period of 72 hours. After 72 hours colour change is noted from light green to reddish brown. Bio reduction of silver ions in the solution was monitored using UV-Vis spectrophotometer.

Phytochemical analysis of Bol leaf extract

The qualitative chemical tests for various phytoconstituents were carried out for water extract of Bol.[21].

Characterization of Silver Nanoparticles by UV-Visible spectroscopy:

UV-Visible spectroscopy was used for monitoring the synthesis of silver nanoparticles and it is a powerful tool for the characterization of colloidal particles. Noble metal particles are ideal candidates for study with UV- Vis spectroscopy, since they exhibit strong surface Plasmon resonance absorption in the visible region and are highly sensitive to the surface modification. The reduction of silver ions was monitored by measuring the UV-Vis spectrum of the solution at 24hours of interval after diluting a small aliquot of the sample into de ionised water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer model UV Cary 100.

Characterization of silver nanoparticles by Scanning Electron Microscopy (SEM)

This study was undertaken to know the size and shape of the silver nanoparticles using JOEL-JFC 6360 SEM. Prior to analysis, thin films of the samples 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. Then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken.

Characterization of silver nanoparticles by FTIR analysis

After 72 hours of incubation the silver nanoparticles were isolated by repeated centrifugation (3-4times) of the reaction mixture at 10 000 rpm for 15 min. The supernatant was replaced by de ionized water and the pellet was stored as lyophilized powder. The dried silver nanoparticles were subjected to FTIR analysis by potassium bromide pellet (FTIR grade) method in 1: 100 ratios and spectrum was recorded in FT-IR Nicolet impact 400 Spectrophotometer using diffuse reflectance mode.

Characterization of silver nanoparticles by X-Ray Diffraction (XRD)

For XRD measurements the silvernano particles were dried in oven at 60 ◦C and such dried powder was further analyzed on XRD for their phase structure and material identification. XRD measurements were recorded on Philips PW 1830. The crystalline silver nanoparticle was calculated from the width of the XRD peaks, using the Debye-Scherer formula $D= 0.94\lambda/\beta\cos\theta$ Where D is the average crystallite domain size perpendicular to the reflecting planes. λ is the X ray wave length, β is the full width at half maximum and θ is the diffraction angle.

Table 1. Phytochemical analysis of aqueous leaf extract of *Bixa orellana*

Alkaloids	Present
Glycosides	Absent
Flavonoids	Present
Steroids	Present
Terpenoids	Present
Tannins	Present
Reducing sugars	Present
Fats and oils	Absent

(a) (b)

Figure 1. Colour change in reaction mixture (a) control (only leaf extract) (b) silver nitrate + Bixa leaves extract)

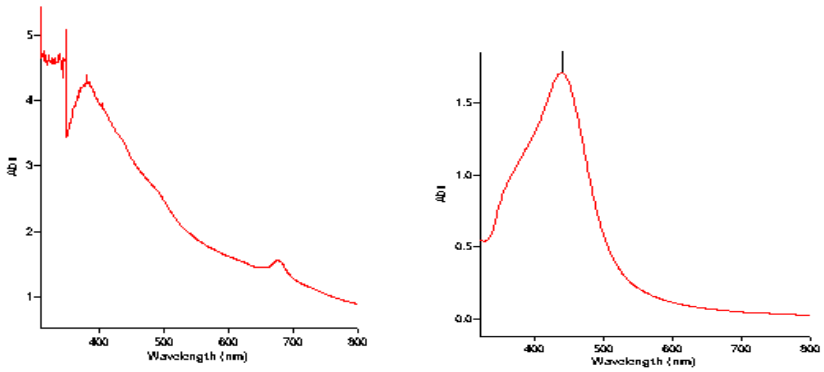

Figure 2. UV-Vis absorption spectrum of leaf extract and reaction mixture after 48 hours of incubation

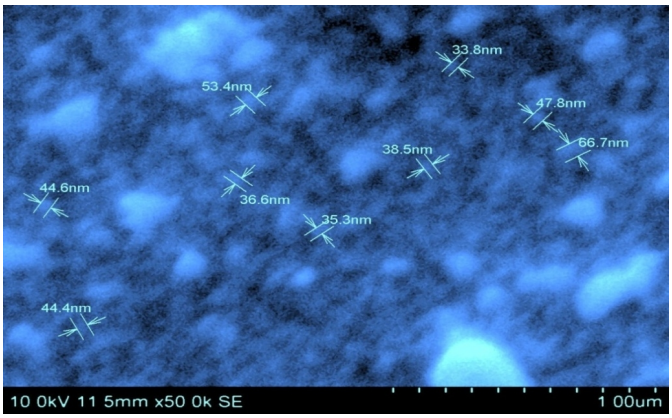

Figure 3. SEM image of silver nano particles

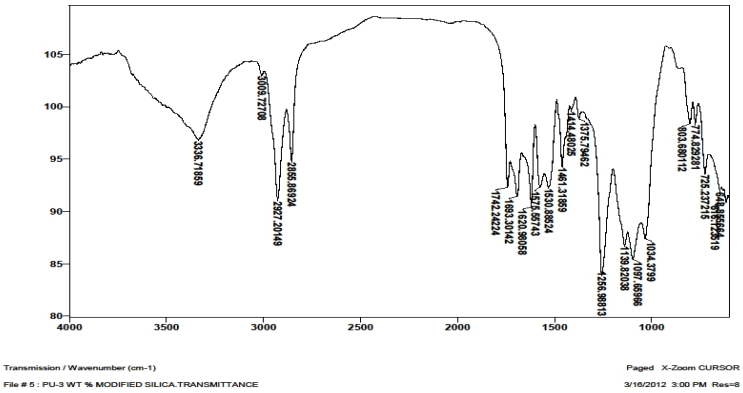

Figure 4. FTIR spectrum of Bol leaf

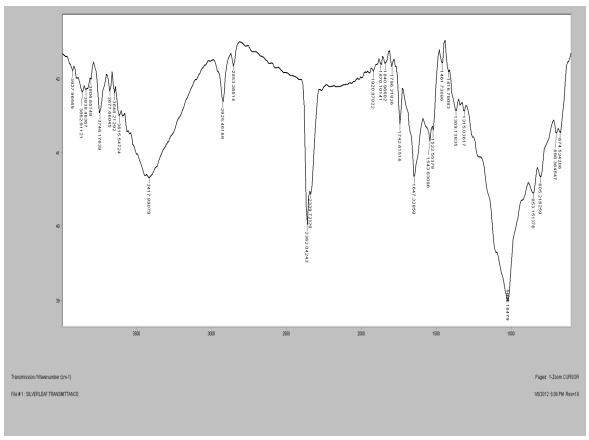

Figure 5. FTIR spectrum of silver nano particles

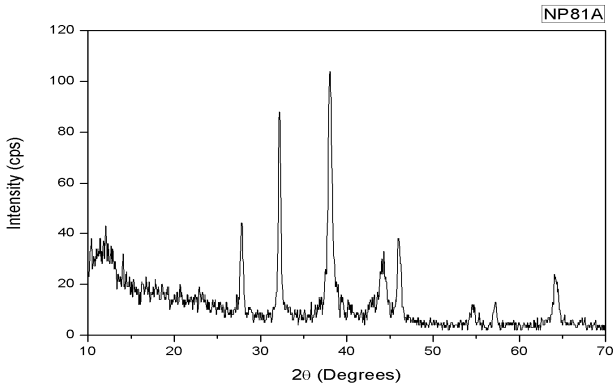

Figure 6. XRD pattern of silvernano particles synthesized by Bol leaf extract

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles using Bol leaf extract can be easily monitored from the change in colour of reaction mixture. The colour of the reaction mixture changed to reddish brown after addition of silver nitrate in 72 hours (figure 1). This resulted due to excitation of surface plasmon vibrations in the silver nanoparticles[22]. The change in colour of the reaction mixture after 72 hours is presented in Figure 1which indicated the formation of silver nanoparticles. This colour formation indicates that silver ion in reaction mixture have been converted into elemental silver.

The qualitative assessment of phytochemical analysis is presented in Table 1 which revealed the presence of alkaloids, steroids, tannins, flavonoids, reducing sugars and terpenoids. Previously published studies of preliminary phytochemical analysis reported the presence of glycosides including other phytochemicals[23, 24]. But Glycosides and saponins found to be absent[25]. The results of the phytochemical analysis indicated that plant extract components involved in the reduction of silver nitrate to elemental silver. Natural antioxidants have been reported to have strong reducing ability [26]. As Bol possess potent antioxidant activity[27], we attribute the reduction process to their presence of high quantity of antioxidant compounds in the leaf extract.

Figure 2 shows UV absorption spectrum of silvernano particles before and after synthesis. Control flasks maintained without silver nitrate solution did not show any change of colour and its absorbance maximum was found to be at 388nm, 404nm 675nm etc. and no peak was observed in the range of 410 – 450nm . Figure 3 shows the SEM image of silvernano particles. The scanning electron microscopic (SEM) image shows high density Ag nanoparticles synthesized by Bol plant extracts further confirmed the presence of Ag nanoparticles. It was shown that relatively spherical and cubic Ag nanoparticles were formed with diameter of 35 to 65 nm. Similar spherical nanoparticles with less than 100 nm was observed using Morinda leaf extract[28].

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. FTIR absorption spectrum of Bol extract and silver ions is shown in the Figure 4 and 5. FTIR measurements were carried out to identify the possible bio molecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by leaf broth. The Peaks at 3740 cm-1, 3862cm-1 and 3417-1 assigned to OH stretching and H- bonded stretching with respective functional groups of alcohols and phenols. The peaks at 2926, 2852cm-1 assigned to O-H stretch of functional group of carboxylic acids. The peak found at 2362 and 2339cm-1 corresponds to C triple bond stretch. The peaks at 1543 and 1641cm-1 can be attributed to the presence of N-H bend and peaks at1401 and 1416 signify the presence of C-C stretch in aromatic ring [29]. The peaks near 1047cm-1 corresponds C-N stretch and peaks at 853 cm-1and 805cm-1 signifies either N-Wag or C-H “oop”. The complete disappearance of C=O stretch band indicates after the bioreduction may be responsible for the reduction of Ag ions. In the present study the peaks are more significant of phenols tannins and terpenoids which attributes to the synthesis of silvernano particles.

Figure 6 shows the X-ray diffracted image of silver nano particles. The diffracted intensities were recorded from 20◦ to 80◦ at 2 theta angles. The diffraction pattern showed peaks at 27.82, 32.2, 38.05, 44.30, 46.01, 64.12 correspond to silver nanoparticles confirming the existence of silver planes in the sample. The Braggs reflections were observed at 2θ = 32.2, 46.01 and 27.82. Hence XRD pattern thus clearly illustrated the formation of silver nanoparticle. Average size of the particles synthesized was 61.1nm with cubic and hexagonal shape[30]. The obtained results illustrate that silver ions had indeed been reduced to Ag0 by Bol plant extract under reaction conditions.

CONCLUSION

Bio-reduction of silver ions by the Bol extract has been demonstrated. The phytochemicals which are present in Bol extract are the active compounds involved in the formation of silver nanoparticles. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and the average size of synthesized silver nanoparticles was 61.1nm. As this plant possess rich source of antioxidant compounds and phytochemicals, silvernano particles synthesized by it would have potent bactericidal, wound healing and other medical applications.

ACKNOWLEDGEMENT

The authors are very thankful to the Director of CLRI, Adyar for executing the work at CHORD Department.

REFERENCES

1. Ponarulselvam, Panneerselvam, Murugan, Aarthi, Kalimuthu, Thangamani. Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. Asian Pacific journal of Tropical Biomedicine 2012; 7: 574-580.
2. Jeong SH, Yeo SY, Yi SC. The effect of filler particle size on the antibacterial properties of compounded polymer/ silver fibers. J. Mat. Sci. 2005; 40: 5407-5411. http://dx.doi.org/10.1007/s10853-005-4339-8
3. Sengupta S., Eavarone D., Capila I., Zhao GL., Watson N, Kizitepe T. Nature 2005; 24: 436-568.
4. Sharma VK, Yngard RA and Lin Y. Silver nanoparticles: Green synthesis and antimicrobial activities. Adv. Coll. Int. Sci, 2009; 145: 83-88. http://dx.doi.org/10.1016/j.cis.2008.09.002 PMid:18945421
5. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. Colloids Surf. B Biointerf 2007; 28: 313–318. http://dx.doi.org/10.1016/S0927-7765(02)00174-1
6. Abishek Kaler, Navin Pael, Uttam Chand Banerjee. Green synthesis of silver nanoparticles CRIPS 2010; 11: 1-4.
7. Kumar V and Yadav SK. Plant-mediated synthesis of silver and gold nanoparticles and their applications. J Chem Technol Biotechnol 2009; 84: 151 – 157. http://dx.doi.org/10.1002/jctb.2023
8. Song JY and Beom SK. Rapid biological synthesis of silver nanoparticles using plant leaf extract. Bioproc Biosyst Engineer 2008; 32:79-84. http://dx.doi.org/10.1007/s00449-008-0224-6 PMid:18438688
9. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J. Colloid Interface Sci. 2004; 275: 496-502. http://dx.doi.org/10.1016/j.jcis.2004.03.003 PMid:15178278
10. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of Gold. Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract. Biotechnol. Prog 2006; 22: 577-583. http://dx.doi.org/10.1021/bp0501423 PMid:16599579
11. Raut RW, Lakkakula JR, Kolekar NS, Mendhulkar VD and Kashid SB. Phytosynthesis of silver nanoparticle using Gliricidia sepium (Jacq.). Curr Nanosci 2009; 5: 117-122. http://dx.doi.org/10.2174/15734130978731467 4
12. Mude N, Ingle A, Gade A, Rai M. Synthesis of silver nanoparticles using callus extract of Carica papaya-A first report. J Pl Biochem Biotechnol 2009; 18: 83-86. http://dx.doi.org/10.1007/BF03263300
13. Gade AK, Gaikwad SC, Tiwari V, Yadav A, Ingle AP and Rai MK. Biofabrication of silver nanoparticles by Opuntia ficus-indica: In vitro antibacterial activity and study of the mechanism involved in the synthesis. Curr Nanosci 2010; 6: 370-375. http://dx.doi.org/10.2174/15734131079165902 6
14. Bonde SR, Rathod DP, Ingle AP, Ade RB, Gade AK, Rai MK. Murraya koenigii mediated synthesis of silver nanoparticles and its activity against three human pathogenic bacteria. J Exp Nanosci 2010 (in press).

15. Manish Dubey, Seema Bhadauria and Kushwah BS.Green synthesis of Nanosilver particles from extract of Eucalyptus hybrid leaf. Digest Journal of Nanomaterials and Biostructures 2009; 18: 537-543.
16. Mallikarjun K, Narsimha G, Dillip GR, Praveen B, Shreedhar B, Lakshmi S, Reddy VSand Raju DP. Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. Digest J Nanomat Biostruct 2011; 6: 181-186.
17. Nagajyoti PC, Prasad TN, Shreekanth VM, Lee KD. Biofabrication of silver nanoparticles using leaf of Saururus chinenis. Digest J. Nanomat Biostruct 2011; 6: 121-133.
18. Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R, Narayana Ra D. Biosynthesis of Silver Nanoparticles Using Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics, Advanced Science Letters. 2010; 3: 138–143. http://dx.doi.org/10.1166/asl.2010.1099
19. Krishnan Vijayaraghavan and Kamala Nalini SP. Biotemplates in the green synthesis of silver nanoparticles Biotechnol J 2010; 5: 1098–1110. http://dx.doi.org/10.1002/biot.201000167 PMid:20669257
20. De Oliveira AC, Silva IB, Manhaes-Rocha DA, Paumgartten FJ. Induction of liver Monooxygenases by annatto and bixin in female rat. Braz J Med Biol Res 2003; 36:113- 118. http://dx.doi.org/10.1590/S0100-879X2003000100015 PMid:12532234
21. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphaphallus paeoniifolilus (Araceae). International journal on pharmaceutical and Biomedical Research 2010; 5: 150-157.
22. Arangasamy Leela1 and Munusamy Vivekanandan. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. African Journal of Biotechnology 2008; 3: 3162-3165.
23. Radhika, Nasreen Begum SK, Srisailam K, Reddy VM. Diuretic activity of Bixa Orellana Linn. Leaves extracts. Indian journal of Natural Products and Resources September 2010; 9: 353-355.
24. Sanjeet kumar, Kabi M, Kumari M. Study on phytochemical analysis from leaves of Bixa orellana (Kumkum). Emerging Science 2010; 2: 1-4.
25. Tamil selvi A, Dinesh M. G., Satyan R. S., Chandrasekaran B. And Rose C. Leaf and Seed extracts of Bixa orellana L. Exert anti-microbial activity against bacterial pathogens. Journal of Applied Pharmaceutical Science.2011; 9: 1-7.
26. Y. Zhou, W. Lin, J. Huang. Biosynthesis of gold nanoparticles by foliar broths: roles of biocompounds and other attributes of the extracts. Nanoscale Research Letters 2010; 5: 1351-1359. http://dx.doi.org/10.1007/s11671-010-9652-8 PMid:20676207 PMCid:2897042
27. Jamil Ahmad Shilpi, Md. Taufiq-Ur-Rahmanb, Shaikh Jamal Uddin c, Md. Shahanur Alamc, Samir Kumar Sadhu d, V'eronique Seidel . A Preliminary pharmacological screening of Bixa orellana L. leaves Journal of Ethno pharmacology 2006; 54: 264–271.
28. Gnanasekar Sathishkumar, Chandrakasasn Gobinath, Karuppiah Karpagam, Vedagiri Hemamalini, Kumpati Premkumar, Sivaperumal Sivaramakrishnan . Phytosynthesis of silver nanoscale particles using Morinda citrifolia and its inhibitory activity against human pathogens. Colloids and Surfaces B: Biointerfaces 2012; 95: 235-240 http://dx.doi.org/10.1016/j.colsurfb.2012.03.001 PMid:22483838
29. Harekrishna Bar, Dipak Kr. Bhui, Gobinda. P, Sahoo, Priyanka Sarkar, Santanu Pyne, Ajay Misra. Green synthesis of silver nanoparticles using seed extract of Jatropha curcas, Colloids and Surfaces A: Physicochemical and Engineering Aspects 2009; 348: 212-216. http://dx.doi.org/10.1016/j.colsurfa.2009.07.021
30. Jancy Mary E and Inbathamizh. Green synthesis and characterization of nano silver using leaf extract of Morinds pubescens, Asian journal of Pharmaceutical and Clinical Research 2012; 5: 1-5.

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
M. Thilagam, A. Tamilselvi, B. Chandrasekeran and C. Rose. Phytosynthesis of silver nanoparticles using medicinal and dye yielding plant of Bixa orellana L. leaf extract. *J Pharm Sci Innov*. 2013; 2(4):9-13.