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

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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 20 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) detection¹. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations without any side effect²; however biocompatible inert nanomaterials have been found their way in cancer diagnosis and delivery of anticancer drugs³. In small concentrations, silver is safe for human cells, but lethal for micro organisms⁴. 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 nanoparticles⁵. However, there is still need for economic, commercially viable as well as 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 use⁷. Many reports are available on the biogenesis of silver nanomaterials using several plant extracts, Pinus, Persimmon, Ginkgo, Magnolia and Platanus⁸, Azadirachta indica (neem³), Medicago sativa (alfalfa), Aloe vera⁹, Gliricidia sepium Jaqu.¹⁰, Carrica papaya¹¹, Opuntia ficus-indica¹², Murrayako enigii¹³, Osimum sanctum¹⁴, Saururus chinesis¹⁵, Eucalyptus hybrida¹⁶, Coriandrum sativum¹⁷, Jatropha curcas (Latex), Clove extract, Brassica junco, Morganella sp., Cinnamomum 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, Alalfà, Geranium (Pelargonium graveolens), Hibiscus sabdariffa, Phomaglo merata, Cinnamomum camphor and Capsicum annuum L.¹⁸. 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 burns²⁰. 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 silvernanoparticles at room temperature.

MATERIALS AND METHODS

Collection of the Plant
The leaves of Bixa orellana L. were collected from the forest area of Dharmapuzhi 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

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10,000 rpm, supernatant was collected and stored at 4°C. The solid content of the leaf extract was found to be 10%. 1 mM of silver nitrate solution was prepared in 200 ml of deionized water. To the silver nitrate silver nitrate solution 10 ml 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 24 hours of interval after diluting a small aliquot of the sample into deionized 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 (3-4 times) of the reaction mixture at 10,000 rpm for 15 min. The supernatant was replaced by deionized 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 silver nano 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 = 0.94λ/βcosθ 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           |

Figure 1. Colour change in reaction mixture (a) control (only leaf extract) (b) silver nitrate + Bixa leaves extract
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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 silver nano 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. The change in colour of the reaction mixture after 72 hours is presented in Figure 1 which 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. But Glycosides and saponins found to be absent. 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. As Bol possess potent antioxidant activity, 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 silver nano 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 and 675nm etc. and no peak was observed in the range of 410 – 450nm. Figure 3 shows the SEM image of silver nano 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. 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 cm⁻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 at 1401 and 1416 signify the presence of C-C stretch in aromatic ring. The peaks near 1047cm⁻¹ corresponds C-N stretch and peaks at 853 cm⁻¹ and 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 silver nano particles.

Figure 6 shows the X-ray diffraction image of silver nano particles. The diffraction 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 Bragg’s 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. 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, silver nanoparticles synthesized by it would have potent bactericidal, wound healing and other medical applications.

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REFERENCES


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