Research Article

Synthesis and Characterization of Silver Nanoparticles Using *Persea americana* (Avocado) and its Anti-inflammatory Effects on Human Blood Cells

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ABSTRACT

Green Synthesis and characterization of nanoparticles is under exploration due to its wide medical applications and various research interests in nanotechnology. In the current study, the plant extract of Persea Americana (Avocado)(Family-Lauraceae) is used for the synthesis of silver nanoparticles (AgNPs). This study investigates an efficient and sustainable route of AgNPs preparation from 1 mM aqueous $AgNO_3$ using leaf extracts. The complete reduction of silver ions was observed after 12 hrs of reaction at 40^{0}C under shaking condition. The colour changes in reaction mixture (pale yellow to dark brown colour) was observed during the incubation period, because of the formation of silver nanoparticles (AgNPs) in the reaction mixture enables to produce particular colour due to their specific properties (Surface Plasmon Resonance). The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy, Fourier Transform Infra-Red (FT-IR) spectroscopy analysis, X-Ray Diffraction (XRD) pattern, Transmission electron microscopy (TEM). The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.42 nm using XRD data by Scherrer's formula, which is approximately similar as the size obtained in TEM Analysis (27.58nm). In total, the AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in Anti-inflammatory activity of Pharmaceutical research to obtain better result of plant as shown by our study.

Keywords: Silver Nitrate, Nanoparticles, UV, FT-IR, XRD, TEM, Anti-inflammatory activity, Persea americana leaf extract, etc.

INTRODUCTION

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles, generate a large amount of hazardous byproducts. Thus, there is a need for green method that includes a clean, non-toxic and environment friendly method of nanoparticles synthesis.[1] As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration.[2] Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extra celluarly using plants or their extracts in a controlled manner according to their size, shape and dispersity.[3] The aqueous silver nitrate solution, after reacting with geranium leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm).[4]

Various approaches available for the synthesis of silver NPs include chemical[5], electrochemical[6], radiation[7], photochemical methods[8] and Langmuir-Blodget[9,10] and biological techniques.[11] Here we have developed a rapid, eco-friendly and convenient green method for the synthesis of silver nanoparticles from silver nitrate using leaf extracts of three Indian medicinal, namely (black tulsi), by microwave radiation method. In this research, the plant mediated synthesized AgNPs were characterized and studied in details with all of their properties significant to current science and prevailing technologies. Therefore the objective of this present study was to synthesis and characterizes the biologically active nanoparticles from the leaf extracts of Persea americana.

MATERIALS AND METHODS

Collection of leaf

Fresh leaf of Persea americana were collected from Salem, during the month of May and identified by Dr. John Britto, The Director, Rabinat Herbarium and Center for Molecular Systematics, St. Joseph's College (Campus), Trichirappalli-2, Tamilnadu, India.

(**Plant authentication no: PN009)**

Preparation of leaf extract

The fresh and young leaf samples of Persea americana was collected, washed thoroughly with sterile double distilled water (DDW). Twenty gram of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW. After that the mixture was boiled for 5mins and filtered. The extract was stored in 4 ^{0}C.

Synthesis of silver nanoparticles

Silver nitrate was used as precursor in the synthesis of silver nanoparticles. 100 ml of Persea americana leaf extract was added to 100 ml of 0.1N $AgNO_3$ aqueous solution in conical flask of 250 ml content at room temperature.

The flask was thereafter put into shaker (100 rpm) at 40° C and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. The mixture was completely dried after a period of 20 minutes and hence nanoparticles in form of powders were obtained.

Figure 1: Optical photograph of Persea Americana A- 0.1 N $AgNO_3$ solution B- Leaf extract C- Leaf extract + $AgNO_3$ D- Leaf extract + $AgNO_3$(After 30mins) E- Leaf extract + $AgNO_3$(After 1 hr) F- Leaf extract + $AgNO_3$(After 2 hrs) G- Leaf extract + $AgNO_3$(After 24 hrs)

UV-visible spectroscopy analysis

The colour change in reaction mixture (metal ion solution + leaf extract) was recorded through visual observation.

The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of solid and subsequently measuring UV-visible spectra of the solid sample.

UV-visible spectra of sample were monitored as a function of time of reaction on the UV-visible spectroscopy. The investigations were carried out using Perkin Elemer (Lambda 35 model) spectrometer in the range of 190nm to 1100nm.

FT-IR measurement

The Fourier transform infrared (FTIR) investigations were carried out using PERKIN ELEMER (Spectrum RXI) spectrometer in the range of 400 cm^{-1} to 4000 cm^{-1}. The functional groups were identified using the peak assignments.

XRD measurement

The sample was drop-coated onto Nickel plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample was prepared.

The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Rigaku miniflex-3 model with 40 kV, 15 mA with Cukα radians at 2θ angle with a wavelength of 1.5418A^{o}.

TEM analysis

Sample is dispersed with acetone and exposed in ultra sonics for 5 minutes. Take a drop of a solution from the samples and drop it on the grid, leave it until it dries. After drying the samples is inserted into TEM instruments using model Tecnai T20 Making in FEI, Netherlands operating at 200KeV Tungsten Filament.

Anti-Inflammatory Activity

The human red blood cell (HRBC) membrane stabilization method

The method as prescribed (Gopalkrishnan; Sakat) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectro photometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula,

Percentage of Protection (%) =(100- OD of drug treated sample/OD of Control) X 100

Albumin denaturation method

The method as prescribed (Sakat) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows,

Percentage of inhibition (%) =(OD of Control- OD of Sample/ OD of Control) X 100

RESULTS

UV-visible spectroscopy analysis

UV-Vis spectroscopy analysis showed that absorbance band of silver nanoparticles synthesized using Persea americana leaf extract absorption band at 218.91 nm as

characteristic poly-unsaturated and aromatic compound present (Isoquinoline) (Advanced strategies in food analysis, UV/VIS spectrometry by Richard Koplík)

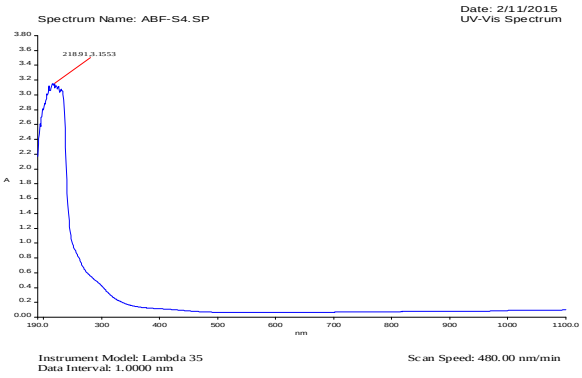

Figure 2: UV-Visible spectrum of synthesized silver nanoparticles using leaf extracts of Persea americana.

FT-IR measurement

The functional groups of Persea americana were identified using the peak assignments. A strong peak at 3752.39 cm^{-1} was assigned to the C-H stretching in aromatic group. The strong and broad band at 3431.46 cm^{-1} was assigned to O-H stretching alcohol and phenol group, The medium peak at 2948.08cm^{-1} was assigned to –C-H stretching in alkenes, medium peak at 2818.86 cm^{-1}, 2782.76 cm^{-1} and 2715.08 cm^{-1} was assigned to H-C=O and C-H aldehyde group. The medium peak at 2373.40 cm^{-1} was assigned to N-H stretching in amines. The medium peak at 1611.15 cm^{-1} was assigned to N-H stretching in 1° amines. The medium peak at 1362.99 cm^{-1} was assigned to C-H stretching in alkanes. The medium peak at 1063.49 cm^{-1} was assigned to C-N stretching in aliphatic amines. The strong peak at 880.05 cm^{-1} was assigned to C-H “opp” in aromatic. The medium peak at 770.21 cm^{-1} was assigned to C-H rock in alkanes, and also the medium peak at 571.52 cm^{-1} was assigned to C-Br stretching in alkyl halides are observed.

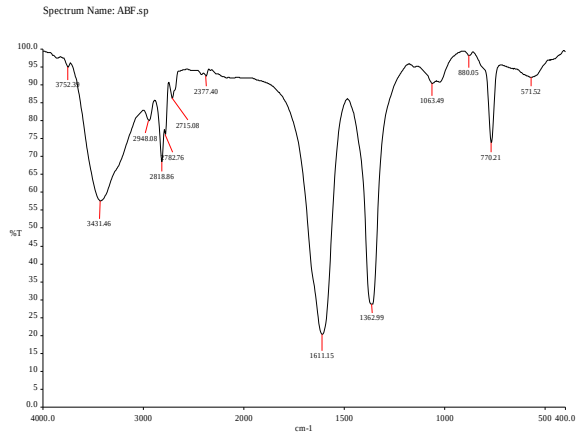

Figure 3: FT-IR spectrum of synthesized silver nanoparticles using leaf extracts of Persea americana.

XRD measurement

Determination of crystalline size

Average crystallite size of silver was calculated using the Scherrer’s formula,

$D = k\lambda / \beta\cos\theta$

D- Average crystallite size: K- Constant: λ- X- ray Wavelength: β- Angular FWHM of the XRD peak at the diffraction angle: θ- Diffraction angle. By using Scherrer’s formula in XRD data, the size of the particle is approximately found to be 27.42nm.

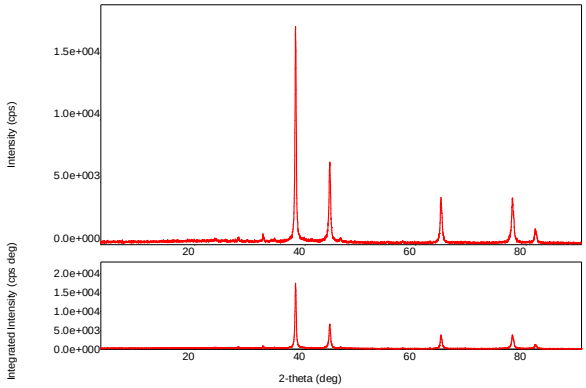

Figure 4: XRD spectrum of synthesized silver nanoparticles using leaf extracts of Persea americana.

TEM analysis

The figure shows the TEM image obtained by the reaction of Persea americana leaf extract and 0.1N silver nitrate solution separately. This Persea americana Ag-NPs was found to be 27.58 nm.

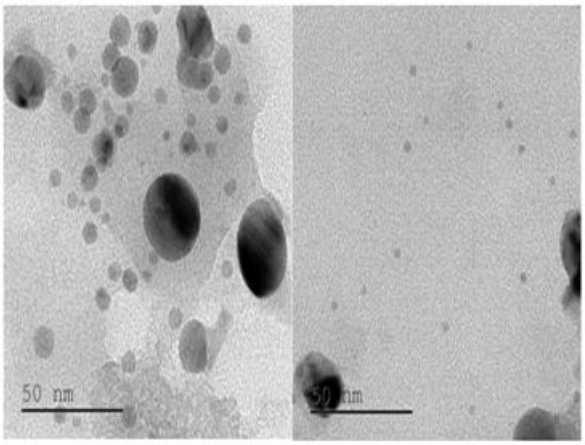

Figure 5: TEM image of synthesized silver nanoparticles using leaf extracts of Persea americana.

Anti-Inflammatory Activity

Anti-inflammatory study like human red blood cell (HRBC), membrane stabilization, inhibition of albumin de nutrition indicated that anti-inflammatory activity. The medical use of Persea americana has a good anti-inflammatory activity. As the concentration of the sample increases, the percentage of inhibition also increases.

Table 1: The human red blood cell (HRBC) membrane Stabilization activity of synthesized silver nanoparticles using leaf extracts of Persea americana.

S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	18.56 ± 0.17
2	200	21.48 ± 0.26
3	400	40.13 ± 0.31
4	600	49.73 ± 1.86
5	800	56.15 ± 1.74

Table 2: Inhibition of Albumin Denaturation activity of synthesized silver nanoparticles using leaf extracts of Persea americana.

S.No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M
1	100	19.13 ± 0.49
2	200	25.42 ± 0.61
3	400	44.23 ± 0.52
4	600	57.09 ± 1.37
5	800	62.81 ± 1.83

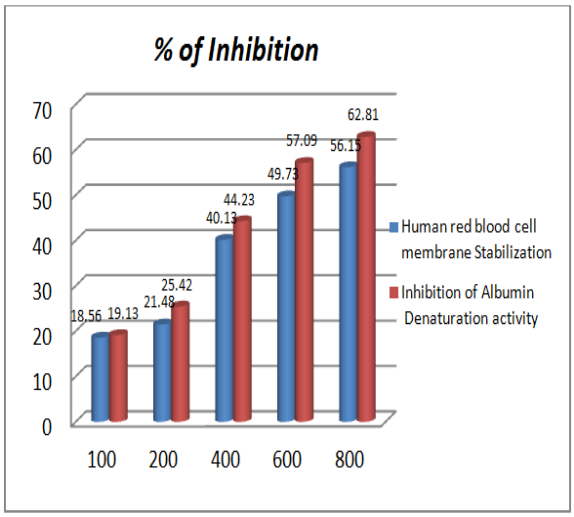

Figure 6: The human red blood cell (HRBC) membrane Stabilization and Inhibition of Albumin Denaturation activity of synthesized silver nanoparticles using leaf extracts of Persea americana.

DISCUSSION

In this race of AgNPs preparation, plant-mediated green bio-synthesis of silver nanoparticle is considered as a widely acceptable technology for rapid production of silver nanoparticles for successfully meeting the excessive needs current market demands. As a result, there is a reduction in the employment or generation of hazardous substances to human health and the environment. Studies have shown that Alfalfa roots can absorb Ag (0) from agar medium and are able to transport it to the plant shoot in the same state of oxidation.[12] Existing literature also reports successful synthesis of silver nanoparticles through a green route where the reducing and capping agent selected was the latex obtained from Jatropha curcas[13]. AgNPs were also obtained using Aloe vera[14], Acalyphaindica[15], Garcinia mangostana[16] leaf extracts. Crataegusdouglasii fruit extract as well as various other plant extracts[17,18] as reducing agent.

CONCLUSION

In conclusion, the bio-reduction of aqueous silver ions by the leaf extract of the Persea Americana has been established. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles and the synthesized nanoparticles are relatively stable in solution. The size of silver nanoparticles was determined by using XRD & TEM analysis. The results showed that the leaf extract is optimum for the synthesis of silver nanoparticles and it is also known to have the ability to inhibit the growth of various pathogenic microorganisms. The average size of synthesized silver nanoparticles is found to be 27.42 nm using XRD data by Scherrer's formula (Fig-4), which is approximately similar as the size obtained in TEM Analysis (27.58nm) (Fig-5). In addition to that, anti-inflammatory study like human red blood cell (HRBC), membrane stabilization, inhibition of albumin de nutrition indicates the anti-inflammatory activity, which shows the medicinal use of the plant. As the concentration of the sample increases, the percentage of inhibition also increases (Table-1&2) and (Fig-6), which confirms that AgNPs has a good anti-inflammatory activity. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green Environment' route.

This green environment approach towards the synthesis of nanoparticles has many advantages such as, process scaling up, economic viability and safe way to produce nanoparticles.

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