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Biological synthesis and characterization of silver nanoparticles using *Eclipta alba* leaf extract and evaluation of its cytotoxic and antimicrobial potential

PARAMASIVAM PREMASUDHA[1], MUDILI VENKATARAMANA[2,*], MARRIAPPAN ABIRAMI[3], PERIYASAMY VANATHI[4], KADIRVELU KRISHNA[2] and RAMASAMY RAJENDRAN[3]

[1]Department of Nanoscience and Technology, Bharathiar University, Coimbatore 641 046, India
[2]DRDO-BU CLS, Bharathiar University Campus, Coimbatore 641 046, India
[3]Department of Microbiology, PSG College of Arts and Science, Coimbatore 641 014, India
[4]Department of Microbiology, Maharaja Co-education College, Perundurai 638 052, India

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Abstract. **With increasing global competitions there is a growing need to develop environmentally benevolent nanoparticles without the use of toxic chemicals. The biosynthesis of silver nanoparticles (AgNPs) using plant extracts became one of the potential areas of research. The bioreduction of metal ion is quite rapid, readily perform at room temperature and easily scale up. The present study describes a rapid and eco-friendly synthesis of AgNPs using *Eclipta alba* plant extract in a single pot process. The efficiency and the influence of various process variables in the biosynthesis of AgNPs analysed include redundant concentration, temperature and time. AgNPs were rapidly synthesized using aqueous leaf extract of *E. alba* and was observed when the medium turned to brown colour with the addition of silver ion. Biosynthesized AgNPs were characterized by the help of UV–visible spectroscopy for their stability and physicochemical parameters were studied by dynamic light scattering, Fourier transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy. The obtained results confirmed that recorded UV spectra show the characteristic surface plasmon resonance band for AgNPs in the range of 400–440 nm and physiochemical structural analysis shown that obtained AgNPs were crystalline in nature. Further, cytotoxic and antimicrobial activities of biosynthesized AgNPs against RAW 254.7, MCF-7 and Caco-2 cells as well as Gram positive and Gram negative bacteria were assessed. *In-vitro* cytotoxicity activity of characterized AgNPs against tested cell lines showed significant anti-cell-proliferation effect in nanomolar concentrations. The antibacterial activity of synthesized AgNPs showed effective inhibitory activity against human pathogens, including, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Thus, the significant outcome of this study would help to formulate value added herbal-based nano-materials in biomedical and nanotechnology industries.**

Keywords. ***Eclipta alba*; silver nanoparticles; cytotoxicity; antimicrobial activity.**

1. Introduction

Nanotechnology is being used in diverse areas like chemistry, biology, catalysis, medicine, photonics, electronics, bio-labelling and information storage.[1] Due to their unique physicochemical characteristics of nanoparticles, including catalytic activity,[2] optical and electronic properties[3,4] as well as cytotoxic and antimicrobial properties[5] in recent year's scientists showed gaining interest towards the development of novel methods for synthesis of nanoparticles. Nanoparticles comprising of one or more dimensions with 100 nm or less have engrossed great attention due to their unusual and captivating properties.[6,7] Silver was recognized as a disinfecting agent; in its nanoparticle forms induce their ability in functions from medicine to culinary items.[8] Human beings are frequently infected by microorganisms such as bacteria, yeast, mold, virus, etc. Silver and silver ion-based materials are usually used for their bactericidal and fungicidal activities.[9] Their antimicrobial effect is appropriate to blockage of respiratory enzyme pathways, interacting with the sulphur-containing proteins and modification of microbial DNA.[10,11] The antimicrobial activity of silver is much superior to other metals, such as mercury, copper, lead, chromium and tin.[12] Hence, silver and silver ion-containing materials are used as prostheses, catheters, vascular grafts and as wound dressings in several biomedical applications.[2,3] As a result, the usage of silver-based viable products including topical ointments, bandages, augmentation devices; tissue scaffolds, antimicrobial filters, water purification systems and gels have been used in improving the public health care.[13,14]

Various methods, including physical and chemical methods were developed to synthesize metal nanoparticles, such as chemical reduction,[15] electrochemical reduction,[16] photochemical reduction,[17] etc. Nanoparticles have a tendency

*Author for correspondence (ramana.micro@gmail.com)

to aggregate in most cases, surface passivator reagents are needed to prevent aggregation.[18] Unluckily many of these organic passivators like thiourea[19] and mercaptoacetate[20] are toxic to the environment when used in large quantities. In contrast to the chemical and physical methods, biological method of nanoparticles synthesis using microorganisms, enzyme and plant or plant extract offers numerous benefits to offer a valuable contribution as eco-friendly technologies into nano-material science.[21] On the other hand, biological methods are free from the use of toxic solvents for synthesis of nanoparticles which are hazardous to the environment.[18] The utility of plant-based phytochemicals in the overall synthesis and architecture of nanoparticles and various nanoparticles embedded products is highly attractive as it brings an important symbiosis between natural/plant sciences and nanotechnology. This connection between plant sciences and nanotechnology provides an inherently green approach to nanotechnology referred to as green nanotechnology.[22] Among the various known biosynthesis methods, plant-mediated nanoparticle synthesis is preferred as it is cost-effective, eco-friendly and safe for human therapeutic applications. Many reports are available on the biogenesis of AgNPs using several plant extracts, particularly *Lantana camara*, *Moringa oleifera*, *Catharanthus roseus*, *Eucalyptus hybrid*, *Cassia auriculata*.[23] However, potential of the plants as biological materials for the synthesis of nanoparticles is still under exploitation.

In the present study, we developed an optimized method for syntheses of silver nanoparticles (AgNPs) using aqueous leaf extracts of *E. alba*. Various reaction conditions were optimized to obtain mono-dispersed AgNPs and were characterized using ultraviolet (UV), dynamic light scattering (DLS), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM) imaging. Cytotoxic and antimicrobial activities of biosynthesized AgNPs were evaluated against RAW 254.7, MCF-7 and Caco-2 cells as well as Gram positive and Gram negative bacteria to reveal the functional utility of synthesized AgNPs in biomedical applications.

2. Materials and methods

2.1 *Materials*

The fresh leaves of *E. alba* were collected from Western Ghats, India. Silver nitrate ($AgNO_3$), Muller Hinton Agar (MHA), nutrient broth, MTT (methyl thiozolyldiphenyltetrazolium bromide) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. The bacterial cultures of *Escherichia coli* (MTCC-1652), *Staphylococcus aureus* (MTCC-96) was obtained from Microbial Type Culture Collection, Chandigarh, India and pathogenic cultures of *Pseudomonas aeruginosa* were obtained from Kovai Medical Centre and Hospital, Coimbatore, India.

2.2 *Methods*

2.2a *Preparation plant extract and biosynthesis of AgNPs*: To improve the reproducibility of the investigational results and to optimize the biosynthesis method as a complete, the factorial aim of research, the 'one factor-at-a time' method, was utilized in this study. Now, the investigational features are diverse one at an occasion among the residual factors held stable. The leaf extract was prepared using freshly collected leaves of *E. alba*. They were surface cleaned thoroughly with running tap water, followed by distilled water. Then the leaves were cut into small pieces and boiled with sterile deionized water (ddH_2O) at 60°C for 5 min. The prepared leaf extract was filtered through Whatman No. 1 filter paper to attain clear solution and was used to synthesize AgNPs. Biological reduction of $AgNO_3$ was carried out initially as follows: 1 mM $AgNO_3$ solution was added to different concentrations of plant extract including 1 : 3, 1 : 4, 1 : 5, 1 : 7 (1 : 9 for example 1 : 9 ratio—1 part of 10% extract and 9 parts of sterile ddH_2O and incubated for 30 min in rotary shaker at 110 rpm at 37°C. To observe the kinetics of the AgNPs biosynthesis, in order to obtain the monodispersed and uniform sized AgNPs, several parameter reaction conditions were optimized including *E. alba* aqueous extract (10%), time (20–24 min) and temperatures (28, 32, 37, 45 and 60°C) to 1 mM $AgNO_3$ solution.

2.2b *Separation and purification of AgNPs from crude matrix*: The AgNPs were separated by centrifugation at 10,000 rpm for 15 min to dispose some of unwanted biological molecules; subsequently the pellet was redispersed in sterile ddH_2O. The purification of nanoparticles by centrifugation and re-dispersion in sterile ddH_2O was constantly carried out to ensure the better elimination of free entities. The purified pellet was freeze dried and lyophilized using a lyophilizer.

2.2c *Characterization of nanoparticles*: *UV–vis spectroscopy:* The formation of AgNPs was primarily observed by monitoring the change in colour of the extract after treated with $AgNO_3$ (1 mM). The bio-reduction of Ag ions in aqueous extract was monitored with the UV–visible spectra of the solutions after diluted a small aliquot (0.1 ml) of the sample to 10 times with ddH_2O. UV–visible spectra were recorded with Hitachi double beam spectrophotometer (Hitachi, Japan) from 300 to 700 nm wavelength at room temperature. Double distilled water was used as reaction blank.

DLS: Size distribution and zeta potential of bio-reduced AgNPs were measured using DLS (Zetasizer Nano ZS, ZEN3600 and Malvern, UK). The mean size and its zeta potential of the particles were obtained.

FT-IR: Crude AgNPs were purified and washed with ddH_2O three times and dried. After drying AgNPs were grinded with KBr pellets and were subjected to FT-IR

spectroscopy in the range of 450–4000 cm^{-1} at a resolution of 4 cm^{-1}.

XRD measurements: XRD analysis of the biologically synthesized AgNPs cast onto glass slides were done as per the previous report of Singhal *et al.*[9] Briefly, PAN ANALYTICAL X-ray diffractometer machine operating at a voltage of 40 kV and current of 20 mA with Cu K(α) radiation of 1.54187 nm wavelength. The scanning as carried out with 2θ angle from 20° to 80° at 0.02 deg min^{-1}, with 2θ time constant.

SEM: SEM experiments were performed to characterize size and shape of bio-reduced AgNPs. Purified AgNPs were sonicated for 15 min to make it uniform distribution and a drop of this solution was loaded on carbon-coated copper grids and solvent was allowed to evaporate under infrared light for 30 min. SEM measurements were performed on Icon Analytical, FEI Quanta 200.

2.2d *In-vitro cytotoxic potential of AgNPs*: *MTT assay*: Cell viability and IC50 values of the compounds were determined by MTT assay.[24] Briefly, RAW 254.7, MCF-7 and Caco-2 cells (5×10^3) were seeded into individual 96-well plates and incubated at humidified environment with 5% CO_2 at 37°C. After 24 h of incubation, cells were treated with different concentrations of AgNPs ranging from 0 to 1000 ng. After 48 h of incubation media was removed and 10 µl of MTT (5 mg ml^{-1}) dye was added along with 90 µl of serum-free medium and incubated at 37°C. Four hours later the medium containing MTT was aspirated and replaced by solubilization solution DMSO for 30 min. Following this incubation, the absorbance was measured in an ELISA reader at 570 nm. IC50 values were determined as per the previous reports.[24]

2.2e *Screening of antibacterial activity of AgNPs*: The synthesized AgNPs of *E. alba* was tested for antibacterial activity by well diffusion method and minimum inhibitory concentration (MIC) against clinically isolated Gram positive and Gram negative microorganisms like *S. aureus, E. coli* and *P. aeruginosa*. The pathogenic cultures were subculture into peptone broth and incubated at 37°C to attain 10^5–10^6 CFU ml^{-1} using MacFarland's standard and were used in further experiments.

Agar well-diffusion method: Muller Hinton Agar plates were swabbed with fresh cultures of pathogenic organisms using sterile cotton swab. Approximately 6-mm diameter of well was made with the help of sterile gel puncture. Using a micropipette, the synthesized AgNPs (mg ml^{-1}) were loaded at different concentrations including 25, 50, 70 and 100 µl. Chloramphenicol was used as a positive control, followed by the plates was incubated at 37°C for 18–24 h to measure the zone of inhibition.

MIC: The MIC was determined by the microdilution method. The synthesized AgNPs was suspended in ddH_2O water. The culture broths of the test organisms were diluted to contain approximately 10^5–10^6 CFU ml^{-1}. The MIC was defined as the lowest concentration of the nanoparticles that inhibited the visual growth of the test cultures on the test tubes.

2.2f *Statistical analysis*: Data are expressed as mean ± SE of a minimum of four replicates and all the experiments were repeated twice. Statistical differences between control and target groups for all experiments were determined using Student's *t*-test with two-way Anova was set at $p \leq 0.05$.

3. Result and discussion

3.1 *Syntheses of AgNPs: process optimization and UV characterization*

In order to prepare mono-dispersed and uniform shaped AgNPs by bio-reduction methods different conditions were optimized including extract concentration, reaction time and temperature. Synthesis of metal nanoparticles using various plant extracts were reported previously.[9,23] Based on these reports, initial reaction was carried out with a 10% extract concentration (W/V) of 100 ml in deionized water was used for the reduction of silver ions (1 mM $AgNO_3$) to nanoparticles. When the plant leaf extract of *E. alba* was subjected to $AgNO_3$, the biosynthesis reaction started within few minutes and the colour reaction was observed visually in which clear $AgNO_3$ solution changed into brown colour which indicates the formation of AgNPs (figure 1) as reported earlier.[9,25] The formation of the reduced AgNPs ($\lambda_{maximum}$) was observed by UV–visible spectrophotometer at 300–700 nm range. The UV–visible spectra showed distinct maximum absorbance at 433 nm and it was corresponding to the surface plasmon resonance (SPR) of AgNPs (figure 2). The absorption band of metal nanoparticles is conquered by the SPR, which displays a shift in the direction of the red end or blue end depending upon the particle size, shape, aggregation state and the adjacent dielectric medium.[26]

When the reaction was carried out with varied extract concentrations, time intervals and temperatures with constant $AgNO_3$ (1 mM) concentration, the intensity of the colour varies with directly proportional to the extract concentration (figure 3a), reaction time (figure 3b) and reaction temperature (figure 3c). In present study synthesized nanoparticles with different extract ratios (W/V) such as 1 : 3, 1 : 4 and 1 : 5 to 1 mM $AgNO_3$ observed very weak plasmon resonance band (absorbance value 0.31, 0.32, 0.53) at 349, 337, 520 nm, respectively. The presence of the minor and major absorption bands could have been due to polydispersity in the size or shape of the nanoparticles. Upon increasing the extract ratio to 1 : 7 and 1 : 9, $\lambda_{maximum}$ increased to 411 and 432 nm, respectively, with an absorbance 1.08 and 1.09. On other hand, increasing the ratio to 1 : 19 a significant change in $\lambda_{maximum}$ to 457 nm was distinguished however absorbance declined to 0.769. This could be explained

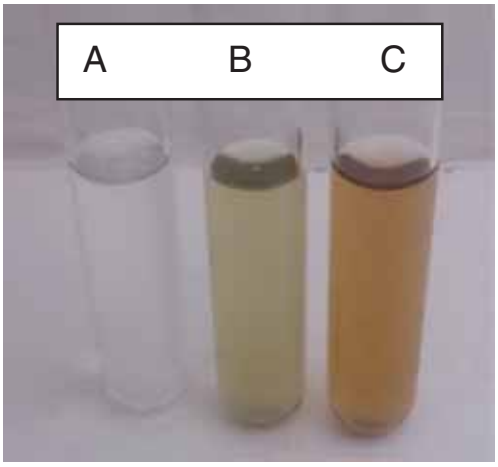

Figure 1. Synthesis of silver nanoparticles using aqueous extract of *Eclipta alba*: (A) silver nitrate ($AgNO_3$), (B) aqueous extract of *E. alba* and (C) aqueous extract with silver nitrate.

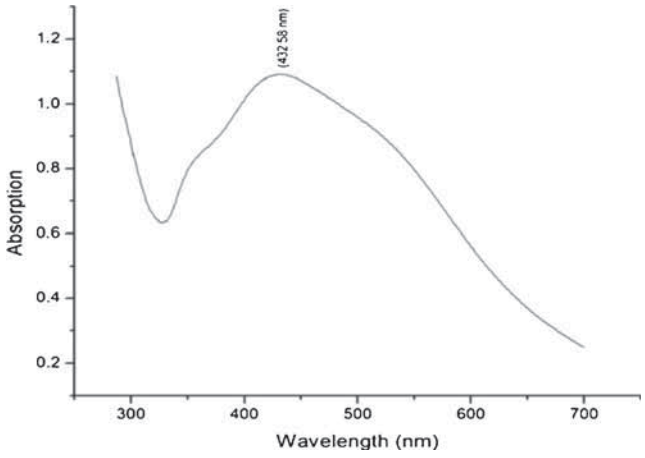

Figure 2. UV–visible spectra analysis of silver nanoparticles by *E. alba.*

by the reduction of silver ions to atoms by means of a reducing agent. The obtained atoms then nucleate in small clusters that grow into particles. Depending on the availability of atoms, which in turn depends on the silver salt to reducing agent concentration ratio, the size and shape of the nanoparticles can be controlled.[27] In present study, the optimized extract concentration was determined as 1 : 9 (W/V) ratio of 10% *E. alba* extract in deionized water. Figure 3a shows the 1 : 9 (W/V) ratio of UV–visible spectrum of the silver nanoparticles attained from diverse interaction time tests.

On the other hand, among the interaction time points studied from 30 min to 72 h, 24 h of incubation showed maximum intensity of the colour change and the change in $\lambda_{maximum}$ from 428 to 433 nm was observed. Upon increasing the interaction time up to 48 h, the $\lambda_{maximum}$ value did not vary significantly. The highest absorbance was observed at 24 h at 1.09. Further experiments were followed with this extract concentration. Among the studied temperatures (28–60°C) on bioreduction of AgNPs, 32°C showed the significant increase in AgNPs formation. As shown in table 1, at this temperature the change in $\lambda_{maximum}$ to 433 nm was distinguished with an absorbance of 1.09. Table 1 represents the optimization of different reaction conditions.

3.2 *Characterization of AgNPs*

Several characterization techniques have been reported worldwide for the characterization of herbal and medicinal plants with silver-based nanoparticles which includes visual examination, UV–vis, FT-IR, DLS, XRD TEM and SEM analysis.[9,28]

3.2a *UV–Vis spectral characterization*: UV–vis characterization of bio-reduced AgNPs was followed as per the previous reports of Singhal *et al.*[9] After the optimization of bio-reduction of $AgNO_3$ to AgNPs in the presence of *E. alba* aqueous leaf extract UV spectral characterization was undertaken. UV spectral readings an! obtained $\lambda_{maximum}$ values indicated that the presence of AgNPs in the solution after the addition of extract (1 : 9) to 1 mM $AgNO_3$ for 24 h reaction time with 32°C (figure 3). The present study results are in line with the previous reports of Singhal *et al*,[9] Nayak *et al*[18] and Kaler *et al*[28] for the biosynthesis of AgNPs from cell-free extracts of microorganisms as well as plant extracts.

3.2b *DLS analysis of particle size and zeta potential*: The particle size analysis showed that the AgNPs synthesized in the bioreduction process using plant leaf extract were extensively distributed in the solution. The particle size of the AgNPs was approximately 422 nm and zeta potential of the nanoparticles was 34 mV and it showed good stability (figure 4). The DLS measured size is slightly bigger as compared to the particle size measured from SEM micrographs, it could be explained by the DLS method which measures the hydrodynamic radius.

3.2c *XRD characterization*: The XRD pattern of biosynthesized AgNPs was confirmed by the characteristics peak observed in XRD image (figure 5). In the XRD pattern, four prominent diffraction peaks were observed at $2\theta =$ 37.85°, 44.0°, 64.2° and 77.2°, which correspond to (111), (200), (220) and (311) Bragg's reflections of the face-centred cubic (fcc) structure of metallic silver, respectively. Peaks observed in the pattern are well agreed with the previously reported values (JCPDS card no. 04-0783). The sharp as well as broad diffraction pattern infers that the synthesized system possess nanodimensional state. The multiple peaks possessed by the particles refer the multiple faceted growth orientation of the particles.[29] It is also presumed that the

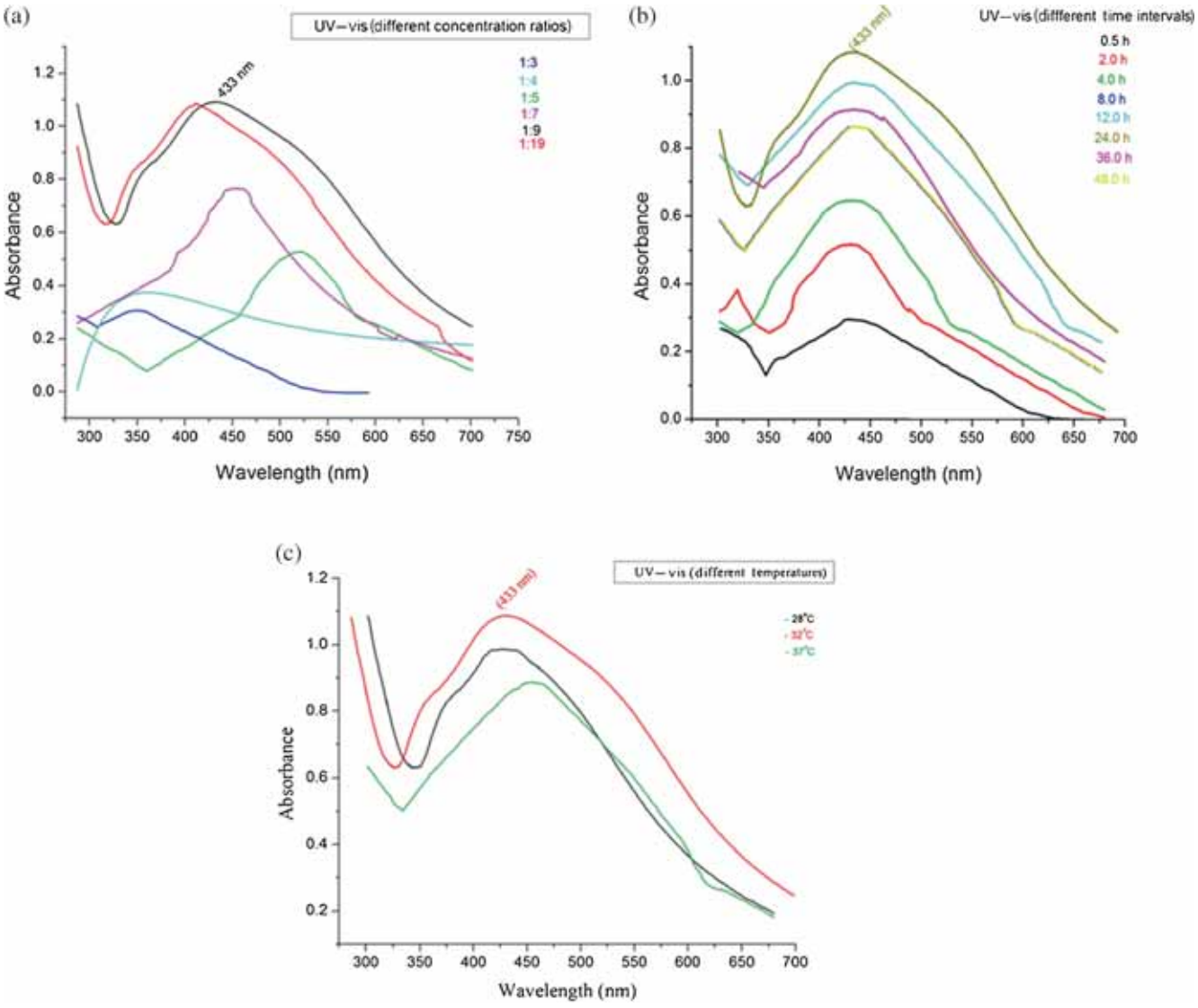

Figure 3. UV–visible spectra analysis of bioreduced silver nanoparticles syntheses optimization by *E. alba*. (**a**) UV–vis analysis of different concentration ratios, (**b**) different time intervals and (**c**) different temperatures.

broadness in the peak may also arise from the local crystal defects (elongational strain/compressional stress) in the nanocrystals.

3.2d *FT-IR spectroscopy*: FT-IR spectroscopic studies were carried out to find out possible bio-reducing agents present in the extract used (figure 6). It is confirmed the fact that to identify the bio-molecules for reduction and efficient stabilization of the metal nanoparticles, the band at 3603 and 3471 cm^{-1} of O–H stretch as also the H-bonded shifted to 3541 and 3495 cm^{-1}, alcohols and phenols. The peak at 3325 cm^{-1} of N–H stretch shifted to 3379 and 3278 cm^{-1}, indicating primary and secondary amines and amides. The band at 2931 cm^{-1} of C–H stretch shifted to 2924 and 2854 corresponding to alkanes. The band at 2777 cm^{-1} of C–H stretch represents aldehydes have only present in the plant leaf extract. The band at 2167 cm^{-1} corresponding to –C ≡ C– stretching represents alkynes have both samples, while the peak at 1689 cm^{-1} stating C=O represents carbonyl groups. The bands at 1527 cm^{-1} of asymmetric bond represents nitrocompound restrain in both samples. The bands at 1265 and 1180 cm^{-1} indicating the presence of C–H stretching alkyl halides has shifted to 1172 and 1018 cm^{-1}. The band at 1064 cm^{-1} corresponding to C–N stretch represents aliphatic amines has only present in the plant leaf extract.

3.2e *SEM image analysis*: SEM analysis was used to measure the size and shape of the AgNPs formed and the images of the SEM visualization are shown in figure 5. Synthesized nanoparticles showed face centred cubic form and showed a large distribution of sized with ranging from 310 to 400 nm. There was no visible agglomeration was observed between the nanoparticles (figure 7).

3.3 *In-vitro cytotoxic potential of AgNPs*

In consideration with possible biomedical applications of AgNPs, in the present study was undertaken with different cancerous cell line models including RAW 264.7 (mouse macrophage cells), MCF-7 (human breast cancer cells) and Caco-2 (human adenocarcinoma cells). Results of the present study reveals that, bioreduced AgNPs shown significant cytotoxic effects against tested *in-vitro* cancerous cell line models (figure 8). These results are in agreement with the earlier reports of Kaler *et al*.[28] MCF-7 cells more susceptible to AgNPs (IC50 5 μM) followed by RAW 264.7 (7 μM) and Caco-2 cells (10 μM).

Table 1. UV–visible spectra of silver colloid solution produced under different conditions.

S. no.	Variable conditions	Wavelength (nm)	Absorbance (λ_{max})
Effect of concentration (ml)			
1	1 : 3	349	0.31
2	1 : 4	337	0.32
3	1 : 5	520	0.53
4	1 : 7	411	1.08
5	1 : 9	433	1.09
6	1 : 19	457	0.76
Effect of temperature (°C)			
7	28°C	428	0.99
8	32°C	433	1.09
9	37°C	454	0.89
10	45°C	ND	ND
11	60°C	ND	ND
Effect of time intervals (h)			
12	0.5	428	0.29
13	1.0	428	0.4
14	2.0	430	0.52
15	4.0	430	0.65
16	6.0	431	0.73
17	8.0	432	0.87
18	12.0	433	1.0
19	24.0	433	1.09
20	36.0	433	0.92
21	48.0	433	0.87

3.4 *Antibacterial activity of synthesized AgNPs*

3.4a *Agar well-diffusion method*: The antibacterial activity of AgNPs synthesized by plant leaf extract was examined against Gram negative and Gram positive pathogenic bacteria including *S. aureus*, *E. coli* and *P. aeruginosa* using the agar well-diffusion method at different concentrations such as 25, 50, 75, 100 μl and control (30 μl) (figure 9).The antibacterial effect of AgNPs was determined on the basis of zone of inhibition (mm) (table 2). Obtained results were in line with previous reports of Venkata Subbaiah and Savithramma,[30] for the antimicrobial properties of AgNPs against tested pathogenic bacteria.

3.4b *MIC*: The MIC of the AgNPs against pathogenic bacteria is shown in table 3. It was found that the MIC value of *E. coli* showed lesser activity followed by *S. aureus* and *P. aeruginosa*. The percentages of inhibition in the growth of pathogenic microorganisms in different concentrations of biosynthesized AgNPs are shown in figure 10. Present study observed results reveal that bioreduced AgNPs shows significant antibacterial property compared with positive drug control, it could be explained by their large surface area, which gives better contact with microorganisms thus alter the microbial metabolism. The nanoparticles attached to the cell membrane and penetrated inside the microorganisms. The sulphur-containing proteins were present in bacterial membrane and phosphorus-containing compounds present in DNA that was interacted with AgNPs

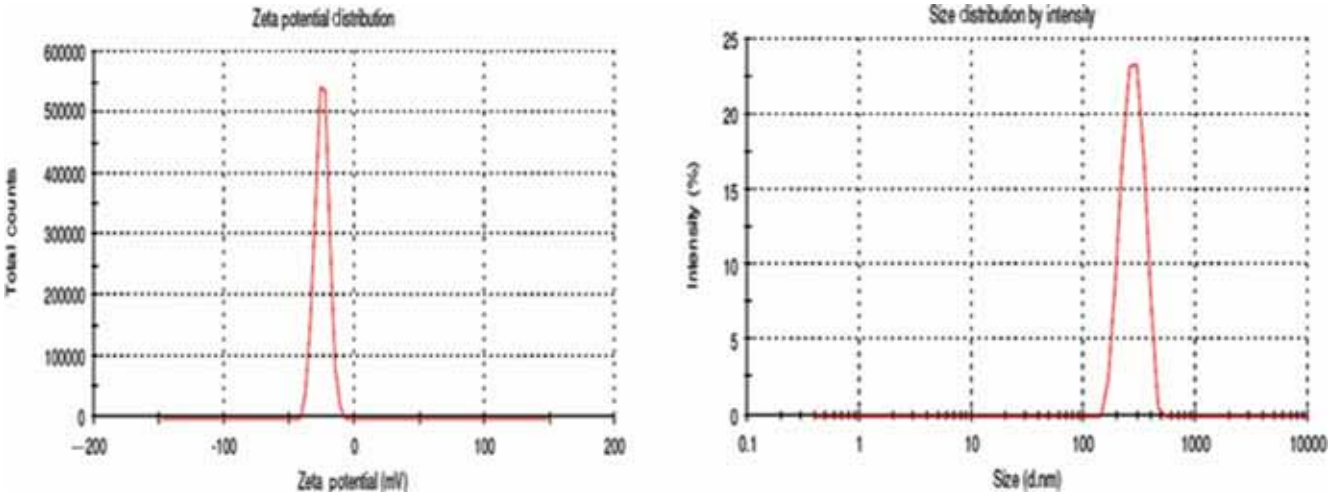

Figure 4. Dynamic light scattering analysis to determine size and zeta potential of bioreduced silver nanoparticles synthesized by *E. alba*.

and mainly affect the respiratory chain, cell division finally leading to death. The silver ions from nanoparticles released into the bacterial cells which improved their bactericidal activity.[31,32]

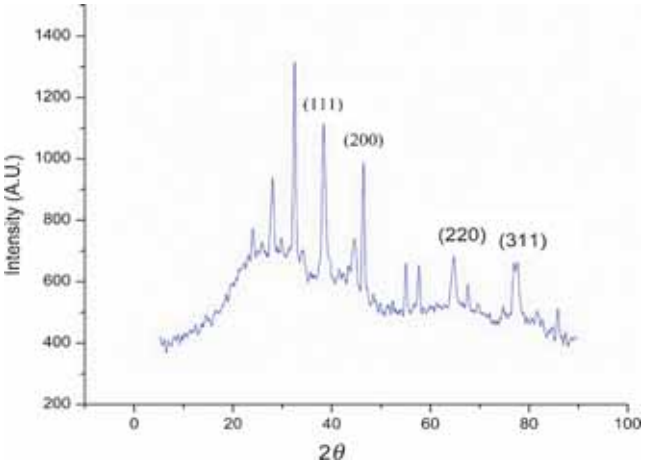

Figure 5. X-ray diffraction pattern of the bioreduced silver nanoparticles synthesized by *E. alba*.

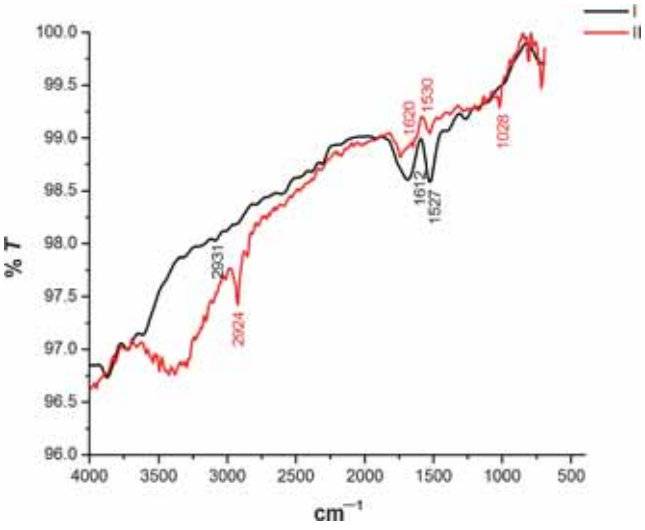

Figure 6. FT-IR analysis of bioreduced silver nanoparticles synthesized by *E. alba*. (I) FT-IR analysis leaf extract of *E. alba* and (II) silver nanoparticles of *E. alba*.

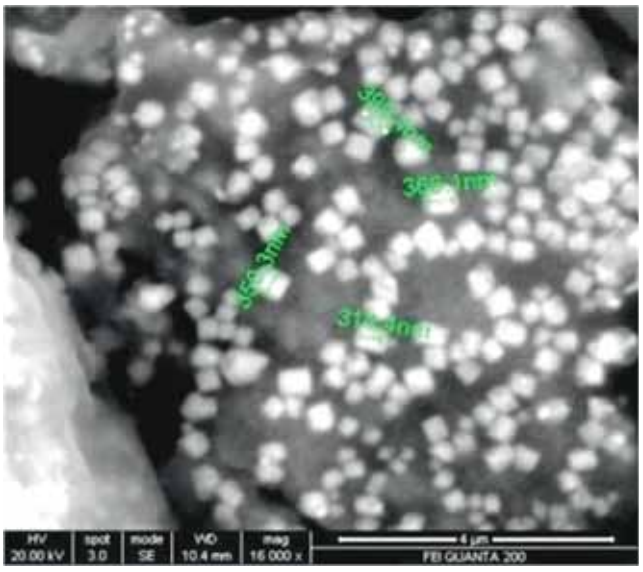

Figure 7. SEM images of bioreduced silver nanoparticles synthesized by *E. alba*.

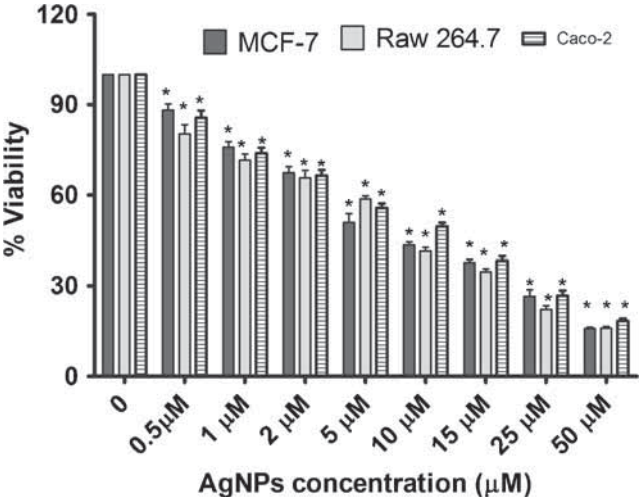

Figure 8. *In-vitro* cytotoxic potential of bioreduced silver nanoparticles synthesized by *E. alba*. MTT assay was carried out to know the anticancer activity (IC50) of the bioreduced AgNPs against MCF-7; RAW 264.7 and Caco-2 cells. Experiments were conducted in triplicates. Data are represented mean ± SEM. $^{*}p < 0.05$ compared with controlled group, calculated by Dunetts test.

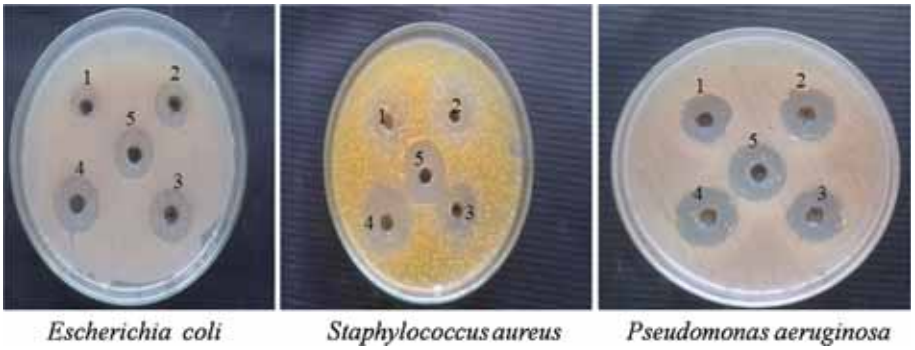

Figure 9. Antibacterial activity of bio-reduced silver nanoparticles synthesized by *E. alba* at different concentrations 1—25 μl, 2—50 μl, 3—75 μl, 4—100 μl, 5—control.

Table 2. Antibacterial activity of synthesized silver nanoparticles of *Eclipta alba* against various microorganisms by well diffusion method.

	Zone of inhibition (mm)				
Pathogenic organisms	25 µl	50 µl	75 µl	100 µl	C
Escherichia coli	5	7	9	11	13
Staphylococcus aureus	8	10	11	13	15
Pseudomonas aeruginosa	7	9	11	12	13

Table 3. Minimum inhibitory concentration of synthesized silver nanoparticles of *Eclipta alba* against various microorganisms.

Pathogenic microorganisms	Blank	Concentration of synthesized silver nanoparticles O.D. at 450 nm							
		10 µl	20 µl	30 µl	40 µl	50 µl	60 µl	70 µl	80 µl
E. coli	1.195	0.982	0.752	0.642	0.532	0.419	0.341	0.252	0.109
S. aureus	1.160	0.720	0.612	0.525	0.431	0.315	0.220	0.118	0.082
P. aeruginosa	1.172	0.792	0.653	0.542	0.449	0.321	0.247	0.135	0.096

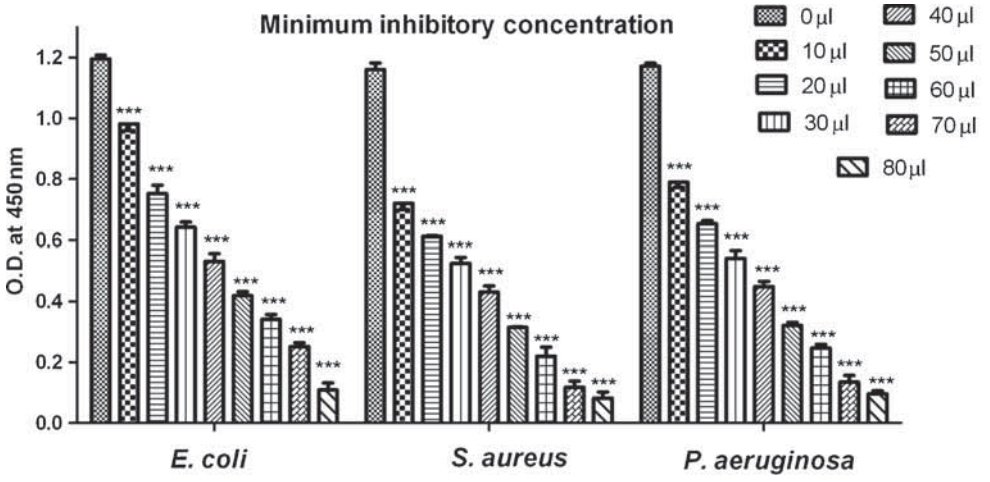

Figure 10. Minimum inhibitory concentration of bioreduced silver nanoparticles synthesized by *E. alba* against tested microorganisms. MIC test was carried out to know the minimal inhibitory concentration (MIC) of the bioreduced AgNPs against pathogenic bacterial cultures including *E. coli*, *S. aureus* and *P. aeruginosa*. Experiments were conducted in six independent replicates. Data are represented mean ± SEM. *** $p < 0.001$ compared with controlled group, calculated by two-way ANOVA.

4. Conclusion

The present study reports the facile approach of biosynthesizing AgNPs from $AgNO_3$ using the aqueous extract of *E. alba*. The adopted method is well suited with green chemistry principles as the plant extract serves as a dual functional molecule as reductant and a stabilizing agent for the synthesis of AgNPs. The efficiency and the influence of various process variables in the biosynthesis of AgNPs analysed include reductant concentration, temperature and time. The interesting conclusion arrived from the study is that the shape and size of the nanoparticles synthesized have the direct and strong influence and dependent on process variables used in the experiment. Although, highly monodispersed AgNPs were attained at concentrations 1 : 9 (V/V) at 32°C in 24 h. The UV–visible spectra confirmed the reduction of silver ions at 433 nm on 24 h of incubation time. XRD analysis confirmed the crystalline fcc structure of AgNPs. From FTIR and XRD analyses it was observed that *E. alba* leaf extract acted as apparent stabilizer for the synthesis of AgNPs. The stability of the nanoparticles also recorded as stable in zeta potential estimation by DLS. The size and morphology of particles were characterized using SEM and the images showed the AgNPs in the range of 310–400 nm. Further, the biosynthesized AgNPs showed significant antibacterial action on tested pathogenic microorganisms. As a result it is observed that a fine tuning of process variables may give the end product with typical physical characteristics.

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Conflict of interest

None.

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