Chapter-III

GREEN SYNTHESIS OF SILVER NANOPARTICLES BY GLORIOSA SUPERBA L AND THEIR CATALYTIC ACTIVITY

ABSTRACT

The effect of the leaves extract of Gloriosa superba Linn. (Glory Lily) on silver nanoparticles (AgNPs) synthesis from silver nitrate aqueous solution also analysed the photochemical in the plant extract. The plant extracts contain the following phytochemical such as alkaloid, amino acids, carbohydrates and proteins. These phytochemicals are involved in the silver nanoparticles synthesis as a reducing and capping agents for converting silver nitrate to silver nanoparticles. The catalytic activity of AgNPs is tested on the reduction of methylene blue by UV–visible spectrophotometer. AgNPs have shown the significant catalytic activity on reduction of methylene blue absorbance. Moreover, the silver nanoparticles have a good antibacterial activity.
Chapter-III

GREEN SYNTHESIS OF SILVER NANOPARTICLES BY GLORIOSA SUPERBA.L AND THEIR CATALYTIC ACTIVITY

3.1 Introduction

Metallic nanoparticles have attracted scientists tremendously due to its unique optoelectronic and physicochemical properties. Physiochemical properties in biological applications of metal nanoparticles such as Ag, Au, Pt and Pd are widely exploited. Their applications include biosensing, media recording, optics, catalysis and environmental remediation (Moreno-Manas and Pleixats 2003; Ren et al., 2005; Sun et al., 2000; Kamat, 2002; Kanel et al., 2005; Cao et al., 2005; Chen, et al., 2004; Gillham and Hannesin 1994; Lin et al., 2008; Wang and Zhang 1997). Specific sizes and morphologies of metallic nanoparticles can be synthesized by chemical and physical methods (Sun et al., 2003; Srikanth et al., 2001; Valle-Orta et al., 2008; Guo et al., 2001; Alquudami, Annapoomi, 2007).

The biosynthesis of nanoparticles has been proposed as a cost effective, environmental friendly and an alternative method to the chemical and physical methods. Consequently, nanomaterials have been synthesized by microorganisms (Ahmad et al., 2002; Ahmad et al., 2003; Shahverdi et al., 2007; Jha et al., 2009; Bharde et al., 2008; Saravanakumar, 2012; Kathiresan et al., 2011) and synthesis of AgNPs by plant leaf extracts such as Crossandra infundibuliformis (Kaviya, et al., 2012), Acalypha indica (Krishnaraj et al., 2010), Rhizophora mucronata (Gnanadesigan et al., 2011), Mentha piperita (MubarakAli, et al., 2011),
**Azadirachta indica** (Shankar et al., 2004), **lemongrass** plant extract (Rai, et al., 2009), **Stevia rebaudiana** (Yılmaz et al., 2011), **Chenopodium album** (Amarendra and Krishna, 2010), **Cassia fistula** (Li Qin et al., 2010) and banana peel extract (Ashok et al., 2010), mangroves (Amathunisha et al., 2012).

Synthesis of nanoparticles by plant extracts is potentially advantageous over microorganisms due to the ease of scale up, less biohazards and less elaborate process of maintaining cell cultures (Bar et al., 2009; Sathishkumar et al., 2009). Generally, the plant materials have the remarkable application in the medicinal and therapeutic uses especially in wound, cuts and an antidote to snake bite and for skin diseases. It also contains the various chemical constituents such as alkaloids, flavanoids, phenols, fatty acids, proteins in *G. superba* (Qin et al., 2010, Chandrasekharan et al., 2011). This work was first time describes the, effect of the *G. superba* derived leaf-mediated biosynthesis of silver nanoparticles and biocatalytic activity of silver nano particles on reduction of methylene blue (MB). The formation of AgNPs was studied by UV-visible spectrophotometer. AgNPs was further characterized by FT-IR, XRD, TEM and SEM with EDX.

### 3.2 Materials and methods

Silver nitrate and methylene blue are obtained from Sigma–Aldrich Chemicals.

**3.2.1 Preparation of leaf extract**

Fresh leaves of *G. superba* are collected from Jayankondam, Ariyalur Dist, Tamil Nadu, India. The fresh leaves are washed several times with running tape water, followed by distilled water. 20g of leaves are weighed and boiled for 15 minutes in
100 ml of Milli-Q water and then the extracts are filtered through Whatman filter paper No. 1. The filtered extract is stored in refrigerator at 4 °C. This extract is used as reducing as well as stabilizing agent.

### 3.2.2 Synthesis of Silver nanoparticles

30 ml of $10^{-2}$ M aqueous solution of silver nitrate is taken in Erlenmeyer flask and different concentrations (2.0, 2.5, 3.0 and 3.5 ml) of *G. superba* leaf extract of is added in Erlenmeyer flask separately at room temperature. After 30 min the solution turns yellow to dark brown indicating the formation of silver nanoparticles.

### 3.2.3 Effect of synthesized AgNPs on the reduction of methylene blue

The catalytic activity of synthesized AgNPs, two reactions are carried out in a 3.5 ml capacity quartz cuvette and absorbance values are monitored using UV-visible spectrophotometer. In the first reaction, 1 ml of methylene blue ($1 \times 10^{-4}$ M) is mixed with 0.2 ml of aqueous leaf extract and 1.8 ml of water, this reaction was monitored after 30 min (I). In second reaction, 1 ml of methylene blue ($1 \times 10^{-4}$ M) is mixed with 0.2 ml extract and 1.8 ml of synthesized AgNPs and this reaction is monitored at three different time intervals viz., 30 min, 45 min and 60 min (II). In all the reactions total volume of the mixture is made up to 3 ml. The values of absorption maxima ($\lambda_{\text{max}}$) are compared, with that of methylene blue (Jebakumar Immanuel Edison and Sethuraman 2012). The schematic representations of the effect of synthesized AgNPs on the reduction of methylene blue by leaf extract are shown in Fig. 3.1.
Fig. 3.1: Schematic representations of the effect of synthesized AgNPs on the reduction of methylene blue by leaf extract.
3.2.4 Antimicrobial assay

Antimicrobial activity of the synthesized silver nano particles are tested against the human pathogenic bacteria. Antimicrobial assay are carried out by disc diffusion technique followed by Kelman et al., 2001. Pathogenic bacterial strains are inoculated in sterile nutrient broth and incubated at 37 °C for 24h. Pathogens are swabbed on the surface of the Muller Hinton Agar plates and discs (Whatman No.1 filter paper with 6 mm diameter) are impregnated with the 50 µl of plant leave extracts on the surface. Control discs are placed with AgNO₃, DMSO and distilled water to assess the effect of AgNO₃ on pathogens. The plates are incubated at 37 °C for 24 h and the antibacterial activity is measured based on the inhibition zone around the disc impregnated with synthesized compounds.

3.3. Results and discussion

3.3.1 UV-Vis analysis

Effect of the leaves extract of G. superba on the silver nanoparticles synthesis is tested. The Fig.3.2 represents the UV–Vis absorption spectra for colloidal silver nanoparticles synthesized by different concentrations of G. superba leaves extract varying from 2.0 to 3.5 ml. It is clearly observable that there is no absorption in visible region for G. superba extract sample.

However, a small absorption band at 435 nm starts appearing in the absorption spectra of the prepared sample (2.0 ml sample). This band grew and blue shifted from 435 nm to 400 nm with increase in the extract amount. This band corresponds to the absorption by colloidal silver nanoparticles in the visible region (380-450 nm) due to the excitation of surface Plasmon vibrations (Njagi et al., 2011).
Fig. 3.2: UV–Vis absorption spectra analysis of silver nanoparticles synthesized by G. superba leaf extract.
The increase of the peaks intensity indicates that the concentration of silver nanoparticles increases (W.Z. Zhang, et al., 2006). The symmetric and narrow absorption peak implies the narrow size distribution of the silver nanoparticles at higher G. superba quantity. The blue shift of maximum absorption wavelength indicates that the size of silver nanoparticles decreases with increasing G. superba quantity.

This observation clearly indicates the successful reduction of silver nanoparticles by G. superba leaf extract. The G. superba content increases, the rate of spontaneous nucleation increases, and a higher number of nuclei are formed during the nucleation burst. Thus, the number of final particles increases as well, and the mean particle size therefore decreases. This observation clearly indicates the successful reduction of Ag nanoparticles by G. superba extract.

It is found that SPR wavelength has a small shift to shorter wavelength and consequently FWHM decreases showing decrease in particle size. Below 2 ml of the extract, no SPR band is observed due to insufficient quantity of reducing agent. More symmetrical SPR band is observed with higher amounts of leaf extract. These results show that the quantity of plant material is a key factor determining the formation and size distribution of nanoparticles (Sheny et al., 2011).

3.3.2. PL analysis

The synthesized colloidal silver nanoparticles of different sizes are found to be photoluminescent spectra Fig. 3.3. It is observed that the photoemission wavelength is independent of the particle size while the intensity increases sharply
with decrease of particle size. This visible luminescence of Ag is due to excitation of electrons from occupied d bands into states above the Fermi level.

Subsequent electron–phonon and hole–phonon scattering process leads to an energy loss and finally photoluminescent radiative recombination of an electron from an occupied sp band with the hole (Zhao et al., 2006; Wilcoxon et al., 1998; Mooradian 1969; Boyd et al., 1986; Beversluis et al., 2003; Lin et al., 2003; Wang et al., 2005).

The intensity of photoluminescence peak is increased with increase of extract quantity and is almost doubled on increase of extract quantity to 3.5 ml. Show the photoluminescence spectra of Ag nanoparticles prepared by reduction, at 435, 450, and 460 nm, respectively. The position and shape of the photoluminescence peak is almost independent of the excitation wavelength of 260 nm. From the spectra, it can be seen that the photoluminescence spectra differ each other, which shifts to the higher energy with decreasing the size of Ag nanoparticles in the colloid. Besides the difference among the photoluminescence spectra, three peaks: at 435, 450, and 460 nm on excitation wavelength was observed from three silver colloids of different size. Similar to the analogy of photoluminescence from noble metals, these three visible PL peaks can be assigned to radiative recombination of Fermi level electrons and sp- or d-band holes (Wang et al., 2005).
Fig 3.3: Photoluminescence emission spectra of silver nanoparticles synthesised by *G. superba* leaf extract.
3.3.3 FT-IR analysis

The synthesis solutions of AgNPs in each case contained many molecules and some of these become adsorbed on the surface of AgNPs. FT-IR analysis have been conducted to further demonstrate the successful conjugation of some such molecules associated with AgNPs (Tripathy et al., 2010).

FT-IR spectra of G. superba leaf extract and AgNPs are shown in Fig. (3.4 a, b). FT-IR spectra is carried out to identify the potential biomolecules in the G. superba leaves extracts to responsible for the reduction and capping of the AgNPs.

Some pronounced absorbance bands are observed at around 3400-3450 cm\(^{-1}\) (N–H and O-H stretching), The absorption peak at 3427 cm\(^{-1}\) observed in control extract, which is due to OH stretching vibration, get narrower and shifted to higher frequency regions (3447 cm\(^{-1}\)). 2920–2930 cm\(^{-1}\) (CH (Sym/Asym) aliphatic) and 1620–1650 cm\(^{-1}\) (aromatic rings), suggest the presence of proteins on the surface of Ag particles (Rajakumar, et al., 2011; Tripathy et al., 2010).

The absorption peaks are located at 1026, 1381, 1633, and 1742, 2854, 2924, 3427, 3447 cm\(^{-1}\) the peaks corresponding to presence of fatty acids, carbonyl groups, flavonones and amide I band of proteins (Shankar et al., 2004). These structural changes indicated that the reduction and stabilization of silver nanoparticles proceed via the coordination between the amide group and silver ions.
Fig. 3.4: FT-IR spectra of (a) leaf extract and (b) synthesised Ag nanoparticle.
The FT-IR studies is confirmed the fact that the amide group form proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium.

3.3.4 XRD analysis

XRD patterns of Ag nanoparticle reveal the character as function of increasing concentration of G. superba leaf extract with fixed concentration of AgNO₃. The synthesized silver nanoparticles are highly crystalline with diffraction peaks corresponding to the face-centred cubic (fcc) phase of metallic silver. The X-ray diffraction (XRD) has proven to be a valuable research tool to prove the formation of silver nanoparticles, determining the crystal structure of the as prepared silver nanoparticles and to calculate the crystalline particle size. Fig. 3.5 shows the XRD pattern of the silver nanoparticles prepared using different 2.0, 2.5, 3.0 and 3.5 ml of G. superba leaf extract concentration.

XRD analysis showed three distinct diffraction peaks at 38.1°, 44.37° and 64.95°, which corresponds to the planes 111, 200, and 220 of face cantered cubic crystal structure (Eisa et al., 2011; Sheny et al., 2011). No diffraction peaks corresponding to the precursor AgNO₃ and/or bi-products (such as silver oxide) are observed, which confirms that only metallic Ag is formed in situ by G. superba extract treatment. The intensity of peaks reflects the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks are broad which indicates that small crystallite size is obtained Wani et al. (2011) was matched with database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783.
The average grain size of the silver nanoparticles formed in the bioreduction process was determined using Scherr’s formula, 

\[ D = \frac{k\lambda}{\beta \cos \theta} \]

where ‘D’ is particle diameter size, \( k \) is a constant equal 1, ‘\( \lambda \)’ is wavelength of X-ray source (0.1541 nm), ‘\( \beta \)’ is the full width at half maximum (FWHM) and ‘\( \theta \)’ is the diffraction angle corresponds to lattice plane (111). The average size of particles is estimated as in the range between 10-25 nm. The calculated particle sizes of the silver nanoparticles as a function of \( G.\) superba extract quantity is shown in Table 3.1.

Table-3.1
The calculated particle sizes of the silver nanoparticles as a function of \( G.\) superba extract quantity

<table>
<thead>
<tr>
<th>S.No</th>
<th>( G.) superba leaf extract (ml)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.0</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>2.5</td>
<td>23</td>
</tr>
<tr>
<td>3.</td>
<td>3.0</td>
<td>15.2</td>
</tr>
<tr>
<td>4.</td>
<td>3.5</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Fig. 3.5: XRD patterns of AgNPs synthesised by *G. superba* leaf extract.
3.3.5 FE-SEM analysis

FE-SEM analyses of synthesized silver nanoparticles are clearly distinguished owing to their size difference. The presence of the elemental silver is observed in the graph obtained from EDX analysis, which also supports the XRD results. This indicates the reduction of silver ions to elemental silver. In addition, the rapid biosynthesis of silver nanoparticles of different shapes was observed and the sizes of nanoparticles are increased by high concentrations of \textit{G. superba} leaf extract.

Representative FE-SEM micrographs (Fig. 3.6 a, b, c, d of 2.0 ml - 3.5 ml extract) of synthesized nanoparticles have cubic structures with size range of 10-25 nm. The energy-dispersive X-ray spectroscopy (EDX) attachment present with the FE-SEM is known to provide information on the chemical analysis of the fields being investigated or the composition at specific locations (spot EDX).

Fig 3.7 shows a representative profile of the spot EDX analysis and obtained by focusing on AgNPs. The silver nanoparticles formed are predominantly spherical with uniform shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Rajakumar \textit{et al}, 2011).
Fig. 3.6: FE-SEM images of AgNPs synthesised by *G. Superba* leaf extract (a-d).
Fig. 3.7: EDX spectrum of AgNPs.

The particle shape of plant-mediated AgNPs is mostly spherical with the exception of neem (Azadirachta indica) which yielded polydispersed particles both with spherical and flat platelike morphology and are 5-35 nm in size (Shankar et al., 2004).

Silver NPs are synthesized using leaf extract of Acalypha indica; from the SEM image, the size of the control silver nitrate obtained is greater than 1,000 nm, whereas synthesized silver nanoparticles measured 20-30 nm in size (Krishnaraj et al., 2010). Silver nanoparticles formed are predominantly cubical with uniform shape. It is known that the shape of metal nanoparticles considerably changes their optical and electronic properties (Xu and Kall, 2002).
3.3.6 TEM analysis

The application of TEM in nanosciences is significant to view the particles in nanoscale. The shape and size of the particles are elucidated with the help of TEM. TEM images of green synthesized AgNPs at different concentration of leaf extract quantities were observed (Fig.3.8 a-d of 2.0 ml- 3.5 ml extract) that particles are mostly spherical in shape.

TEM images of AgNPs at different leaf extract concentration are taken and it is noted that at highest concentrated leaf extract solution, small particles are found varying from 10-25 nm in case of AgNPs, respectively (Figs. 3.8 a-d). It is evident from the TEM images that the shape and size of the silver nanoparticles is controlled by concentrated leaf extract. It is noticed that already the lowest amount of leaf extract in the reaction mixture is effective for the generation of nanoparticle.

Based on the UV–Vis spectra the sharpness of the absorption peak is dependent on the concentration of leaf extract, which gets further sharpened at still higher concentrations (Fig.3.2). Correlation between the peak sharpness and formation of nanoparticles is noted by TEM images of leaf extract mixed samples (a, b, c and d). With increase in leaf extract quantity there is decrease in the particle size of the silver.
Fig. 3.8. TEM images of AgNPs synthesised by *G. Superba* leaf extracts (a-d).
3.3.7 *Catalytic activity of AgNPs on reduction of methylene blue by G. superba extract*

It is a well known fact that AgNPs and their composites show greater catalytic activity in the area of dye reduction and removal (Kundu *et al.*, 2002) the reduction of methylene blue by arsine in the presence of silver nano particles (Kundu *et al.*, 2002), and the present study aims to the reduction of methylene blue by the natural green aqueous extract of *G. superba* containing AgNPs.

Pure methylene blue dye has a $\lambda_{\text{max}}$ value of 664 nm. 30 minutes after the addition of the extract to the dye, the absorbance is gradually decreased and is shifted to higher wavelength. The decrease of absorbance is indicative of the ability of phyto extract to reduction of methylene blue. AgNPs and the extract at the end of 30 min time interval showed a remarkable decrease in the absorbance of methylene blue and increase of SPR peak of AgNPs (Fig. 3.9).
Fig. 3.9: UV–Visible spectrum of methylene blue reduction by *G. superba* in the presence of AgNPs.
3.3.8 *Antibacterial activity*

Antibacterial activity of the silver nanoparticles is tested by disc diffusion method against gram positive and negative pathogenic bacteria. Antimicrobial activity of NPs is significantly reported against human pathogenic bacteria, mainly *S. aureus*, *B. subtilis* *S. typhi*, and *E. coli* and proved effective Fig. 3.10. Results of antimicrobial studies show higher sensitivity of AgNPs for *S. aureus*, *B.subtilis* compared to that of *S. typhi* and *E. coli*. Table 3.2 and 3.11.

Silver nanoparticles synthesized by *G.superba.L* extracts have been found to possess highest antimicrobial activity against *S. aureus*, *B. subtilis*, *S.typhi* and *E. coli* respectively, Distilled water and DMSO have no antibacterial activity. This differential antimicrobial activity of silver nanoparticles can be attributed to their differential sizes and shape: the antimicrobial activity increases with decreasing size of the silver nanoparticles (Baker *et al.*, 2005).

**Table-3.2**

*Diameter zone of inhibition by AgNPs, extract and AgNO₃ against human pathogenic bacteria*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogenic bacteria</th>
<th>Inhibition zone (mm)</th>
<th>AgNPs</th>
<th>Extract</th>
<th>AgNO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td></td>
<td>14.3</td>
<td>10.7</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus subtilis</em></td>
<td></td>
<td>16.5</td>
<td>8.7</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhi</em></td>
<td></td>
<td>12.7</td>
<td>6.9</td>
<td>4.3</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td></td>
<td>15</td>
<td>10.4</td>
<td>7.2</td>
</tr>
</tbody>
</table>

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Fig. 3.10: Antimicrobial activity of AgNPs synthesized by *G. superba* against *S. aureus, B. subtilis, S. typhi* and *E. coli.*
Fig. 3.11: Size of inhibition zone diameter.
The effect of antibacterial activity is higher in the case of silver nanoparticles synthesized at less concentration of AgNO₃ (0.01 M) compared to high silver nanoparticles concentrations. Smaller particles have a larger surface area for interaction to give more bactericidal effect than the larger particles (Baker et al., 2005). Recent research reports revealed that Silver NPs (AgNPs) have good antibacterial activity. Bacteria usually are incapable of developing resistance against Ag-NPs,
Chapter - IV

SYNTHESIS, CHARACTERIZATION AND CATALYTIC ACTIVITY OF SILVER NANOPARTICLES USING TRIBULUS TERRESTRIS LEAF EXTRACT

ABSTRACT

Biomediated silver nanoparticles are synthesized with the aid of an eco-friendly biomaterial, namely, aqueous Tribulus terrestris extract. Silver nanoparticles are synthesized using a rapid, single step, and completely green biosynthetic method employing aqueous Tribulus terrestris leaf extracts as both the reducing and capping agent. Silver ions are rapidly reduced by aqueous Tribulus terrestris leaf extracts, leading to the formation of highly crystalline silver nanoparticles. The formatted of the silver nanoparticles are analysed by surface plasmon spectra using an UV–Vis (Ultra violet), spectrophotometer. Morphology and crystalline structure of the prepared silver nanoparticles are characterized by TEM (Transmission Electron Microscope) and XRD (X-ray Diffraction), techniques, respectively. FT–IR (Fourier Transform Infrared), analysis suggests that the obtained silver nanoparticles might be stabilized through the interactions of carboxylic groups, carbonyl groups and the flavonoids present in the *Tribulus terrestris* extract. AgNPs have shown the significant catalytic activity on reduction of methylene blue dye. Moreover, the silver nanoparticles have a good antibacterial activity.