Chapter-V

BIOREDUCTION OF METHYLENE BLUE AND ANTIBACTERIAL ACTIVITY

5.1 Introduction

A significant area of research in nanotechnology is synthesis of nanoparticles (NPs) of different chemical compositions, sizes, and shapes. There is a growing need to develop environmentally NPs synthesis processes that do not use toxic chemicals in the synthesis protocol. As a result, researchers in the field of NPs synthesis have turned to biological systems (Ahmad et al., 2003). Since some noble metal NPs such as gold, silver and platinum NPs are widely applied to human contacting areas, there is a growing need to develop environment-friendly processes of NPs synthesis that do not use toxic chemicals (Song and Kim 2009). In addition, synthesis of silver nanoparticles using chemical methods could still lead to the presence of some toxic chemical species being adsorbed onto the surface of nanoparticles which may reason adverse effects in their applications. Because of this, the bio-inspired synthesis of silver nanoparticles has become significant in the recent years.

Several biological systems including bacteria, fungi and algae have been used for this purpose (Pugazhenthiran et al., 2008; Fayaz et al., 2009; Xie et al., 2007; Mohanpuria et al., 2008) had reviewed the synthesis of plant mediated silver and gold nanoparticles. Plant extracts, like Magnolia kobus, Diopyros kaki, Ficus benghalensis and Citrus limon extracts, have been shown to produce nanoparticles with good stability (Song et al., 2009; Saxena et al., 2012;
Prathnaa et al., 2011) due to the presence of reducing agents like alkaloids, polyphenols and flavonoids which are the major phytoconstituents.

Among the noble metals, silver nanoparticles (AgNPs) have become the focus of intensive research, because of low cost and emerging applications. Use of biogenic nanoparticles, being an environmentally benign greener option, is very much preferred in a variety of applications. Metal nanoparticles have received great attention due to their catalytic role in the reduction and degradation of dyes. Ag NPs find wider applications as catalyst in the reactions such as heterocyclizations, cycloaddition of imines, oxidation of ethylene to ethylene oxide and methanol to formaldehyde which are industrially important (Nadagouda et al., 2011).

Moreover, AgNPs act as an oxidoreduction catalyst in the degradation of dyes by electron relay effect between donor and acceptor molecules (Mallick et al., 2006). In the present study, the reduction of methylene blue was carried out and electron source with the help of biogenically synthesized Ag NPs as a green catalyst. Additionally, we have also examined the antibacterial activity of these biosynthesized AgNPs since AgNPs has been widely used as an antibacterial agent. Using aqueous leaf extract of Coccinia indica the AgNPs are synthesized. The formation of Ag NPs has been characterized by visible observation, UV–Vis Spectrophotometer, FT-IR, XRD, TEM, and SEM with EDX measurements. Further, the reduction of methylene blue is monitored by UV–Vis Spectrophotometer.
5.2 Materials and methods

Silver nitrate and methylene blue are purchased from Sigma–Aldrich Chemicals for this study. All glassware’s were washed with HNO₃ and distilled water and dried in oven. Coccinia indica leaves are collected from this Chidambaram rural area.

5.2.1 Preparation of leaf extract

Method (3.2.1) is the same

5.2.2 Synthesis of Silver nanoparticles

Method (3.2.2) is the same

5.2.3 Catalytic activity

Method (3.2.3) is the same

5.2.4 Antimicrobial assay

Method (3.2.3) is the same

5.3 Result and discussions

5.3.1 UV–Vis analysis

UV–Vis absorbance spectroscopy has been proved to be a very useful technique for metal nanoparticle study to the peak positions and shapes are sensitive to particle size. Fig. 5.1 shows the UV–Vis absorption spectra of the Ag nanoparticles with different concentration of leaf extract addition amount of 2.0, 2.5, 3.0 and 3.5 ml. Upon the addition of aqueous extract of Coccinia indica to the silver nitrate solution, the solution turned to yellowish brown. This indicated the formation of AgNPs (Mukherjee et al., 2001). The Surface Plasmon Resonance

114
(SPR) peak that appeared initially leaf extract 2.0 ml added at the wavelength 436 nm shifted to 420 nm in addition of leaf extract 3.5 ml (Fig. 6.1). The symmetric and narrow absorption peak involves the narrow size distribution of the silver nanoparticles at higher Coccinia indica quantity.

The blue shift of maximum absorption wavelength indicates that the size of silver nanoparticles decreases with increasing Coccinia indica L quantity (Zayed et al., 2012).
Fig. 5.1: UV-Vis spectra of silver nanoparticles using *C. indica* leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
5.3.2 PL analysis

The photoluminescence spectra obtained from the synthesized silver nanoparticles are shown in Fig. 5.2. The PL emission has been obtained within the visible range, from 400 to 600 nm, with peak positions at 560, 520, 490 and 424 nm for a, b, c, d samples respectively. The PL emission peaks have been found to be gradually decreased 600 nm got shifted to 424 nm addition of leaf extract 3.5 ml (Fig. 5.2), while the intensity increases sharply with decrease of particle size.

In the other words this emission behaviour supports the involvement of Coccinia indica extract in stabilizing the silver nanoparticles. Subsequent relaxation by the electron-phonon scattering process leads to an energy loss and finally, the photoluminescent radiative recombination of an electron from an occupied sp band with the hole takes place. The optical properties of silver nanoparticles depend on both interband and intraband transitions between electronic states. The PL emission displayed here are consistent with earlier reports (Vasireddy et al., 2012; Sarkar et al., 2010; Bar et al., 2009).
Fig. 5.2. PL spectra of silver nanoparticles using *C. indica* leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
5.3.3 XRD analysis

An XRD profile of biosynthesized silver nanoparticles is shown in Fig. 5.3. The diffraction peaks at 38.26°, 44.38°, 64.72° and 77.50° [assigned to the (111), (200), (220) and (311) planes of a faced centre cubic (fcc) lattice of silver] were obtained. The XRD patterns displayed here are consistent with earlier reports (Bar et al., 2009; Satishkumar et al., 2009).

The mean particle diameter of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, refraction peak using the following Scherrer’s equation.

\[ D = \frac{K\lambda}{\beta\cos\theta} \] (5.1)

Where D is the size of the particles, K is the shape dependent Scherrer’s constant, \( \lambda \) is the wavelength of radiation and \( \beta \) is the full peak width and \( \theta \) is the diffraction angle. For natural plant extract synthesized silver nanoparticles the calculated average particle size of the silver is found to be 15-50 nm table 1. It can be seen that the size of the silver nanoparticles could be manipulated by controlling the amount of the Coccinial indica extract used in such way that as more extract is used, the smaller nanoparticles are obtained Table 5.1. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is a characteristic of nanocrystallites.
Fig. 5.3: XRD spectra of silver nanoparticles using C. indica leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
Table 5.1

The calculated particle sizes of the silver nanoparticles as a function of

*C. indica* extracts quantity

<table>
<thead>
<tr>
<th>S.No</th>
<th><em>C.indica</em> leaf extract (ml)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.0</td>
<td>41.1</td>
</tr>
<tr>
<td>2.</td>
<td>2.5</td>
<td>37.0</td>
</tr>
<tr>
<td>3.</td>
<td>3.0</td>
<td>22.6</td>
</tr>
<tr>
<td>4.</td>
<td>3.5</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Thus the XRD pattern proves to be strong evidence in favour of the UV–Vis spectra and TEM images for the presence of silver nanoparticles.

5.3.4 *FT-IR* analysis

FTIR measurements are carried out to identify the potential biomolecules inhibiscus leaf responsible for reduction and capping of the bioreduced silver nanoparticles. The IR bands (Fig.5.4) observed the band at 3388 cm\(^{-1}\) corresponding to O-H stretching of high concentration of alcohols or phenols. The band at 2854 cm\(^{-1}\) assigned to aldehydic C-H stretching this suggest the presence of saturated molecules secondary metabolites, proteins and lipids are present in the plant leaves (Mano Priya *et al.*, 2011).
Fig. 5.4: FT-IR spectra of silver nanoparticles using *C. indica* leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
The band at 2354 cm\(^{-1}\) is N-H bending vibrations of amino acids. The bands at 1650 cm\(^{-1}\) are characteristic of amide I band amide II (Caruso et al., 1998). The peak at 1047 cm\(^{-1}\) corresponds to C-N stretching vibration of the amine. The band positions from 765 to 895 cm\(^{-1}\) are due to C–C and C–H phenyl ring substitution as expected for this plant. These compounds may be responsible for production of Ag NPs from leaves of *Coccinia indica* leaf extract.

### 5.3.5 SEM analysis

The surface morphology of nanoparticles was investigated using FE-SEM. Formation of AgNPs and its agglomeration was clearly observed in Fig.5.5.

The FE-SEM micrographs showed spherical and crystalline shape with particle size of the nanoparticles ranges from 15 to 50 nm. The micrograph observation showed synthesized AgNPs are not in direct contact even within the aggregates, indicating stabilization of the nanoparticles. The nanoparticles were distributed uniformly on the surface with formation of aggregated and smooth surface. SEM micrograph shows the synthesized AgNPs using aqueous leaf extract of Manilkara zapota which are spherical and oval in shape and size of 15-50 nm (Kundu et al., 2002).

SEM analysis showed the particle size between 25 and 110 nm as well as the cubic structure of the nanoparticles (Mallick et al., 2006). The particle shape of plant-mediated AgNPs were mostly spherical with exception of neem (Azadirachta indica) which yielded polydisperse particles both with spherical and flat platelike morphology 5–35 nm in size (Jebakumar Immanuel Edison, and Sethuraman 2013).
Fig. 5.5: FE-SEM images of silver nanoparticles using *C. indica* leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
Fig. 5.6: EDX spectrum of silver nanoparticles.

The results of EDX analysis is shown in Fig. 5.6. Elemental silver can be seen in the graph presented by the EDX analysis in support of XRD results, which indicated the reduction of silver ions to elemental silver.

5.3.6 TEM analysis

The separation between the silver nanoparticles seen in the TEM image could be due to capping effect of *Coccinia indica* extract. The TEM images are recorded at different leaf extract concentration to find the individual particles.

It is observed that most of the silver nanoparticles are spherical in shape. A few agglomerated silver nanoparticles were also observed in some places. The biologically synthesized silver nanoparticles using the extract of *Coccinia indica* of synthesized silver nanoparticles showed the average size of 22 nm spherical shaped particles. Structural morphology and crystallinity are further confirmed by TEM micrograph images.
Fig.5.7: TEM images of silver nanoparticles using *C. indica* leaf extract at different concentrations (a) 2.0 ml (b) 2.5 ml (c) 3.0 ml (d) 3.5 ml.
Chapter-V

Bioreduction of methylene blue and antibacterial activity......

The aliquot of silver nanoparticle solution was placed into a drop coated copper grid and the sample is allowed to dry. The synthesized silver nanoparticles were observed in spherical shape and average size of the particles is 22 nm (Fig. 5.7). The variation in the particle sizes such as 16, 22, 37 and 41 nm difference in size is possibly due to the fact that the nanoparticles are being formed at different concentration of leaf extract.

The leaf extracts mixed samples at (2.0, 2.5, 3.0 and 3.5 ml). With increase in leaf extract quantity there is decrease in the particle size of the silver results suggest the increase in formation of nanoparticles at higher leaf extract quantity as well (Table 5.1). The amount of extract used for the synthesis of metal nanoparticles was also found less among many reported research (Kelman et al., 2001; Khandelwal et al., 2010). These results mean that, the size of the prepared particles gets smaller and the particle size distribution is improved with increasing of extract quantity.

5.3.7 Effect of leaf extract

For silver nanoparticles, with an increase in the extract quantity an increase in the peak absorbance was found in UV–Vis spectrum (Fig.5.1). The solution showed a colour change from yellowish to reddish brown indicating increase in particle size. For the generation of small NPs, lowest amount of leaf extract is preferred which is found to be lesser among previously reported ones (Dwivedi and Gopal, 2010), (Smitha et al., 2009; Dubey et al., 2009). Below 2.0 ml of leaf extract, the colour of the colloid changes from yellowish to reddish brown with a decrease in UV-vis absorbance (Fig. 5.1). The UV–Vis spectra of AgNPs (Fig. 5.1)
show a linear relationship between absorbance and quantity of extract. Using leaf extract, 3.5 ml is found to be more suitable for the preparation of small and stable AgNPs confirmed for these techniques. UV-Vis Spectrophotometer, XRD, TEM, and SEM measurements.

5.3.8 Catalytic activity of silver nanoparticles for the reduction of methylene blue

In order to investigate the potential of the silver nanoparticles as a catalyst for the reduction of methylene blue, the catalytic reaction was monitored by UV-Vis spectrometer. The details of the catalytic experiment have been described in the Fig. 5.8 shows the typical UV–Vis spectra for the reduction of methylene blue measured at different time.

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) studied the reduction of methylene blue by arsine in the presence of silver nanoparticles (Shankar et al., 2004)], while (Bar et al. 2009) studied the catalytic activity of AgNPs on the reduction of phenosaflarin dye. Jebakumar and Sethuraman, (2012) studied catalytic activity of AgNPs on the reduction of methylene blue and 4-nitrophenol (Parashar et al., 2009; Smitha et al., 2009). Pure methylene blue 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.
Fig. 5.8: UV-Vis spectra of silver nanoparticles reduction of methylene blue.
The decrease of absorbance is indicative of the ability of phytoextract to degrade methylene blue. Moreover, the silver nanoparticles are very effective for the catalytic reduction of methylene blue and only a small amount of the catalyst could strikingly promote the reduction reaction.

5.3.9 **Antibacterial activity of the silver nanoparticles**

The biologically synthesized silver nanoparticles showed excellent antimicrobial activity against human pathogenic bacteria such as Gram positive bacteria *S. aureus, B. subtilis* and Gram negative bacteria *S. typhi, and E. coli*. Fig. 5.9.

In all the figures, the light zones around the filter paper represented the inhibition zones. The silver nanoparticles synthesized by *Coccinia indica* extract were found to have highest antimicrobial activity against *B. subtilis* (13 mm) and *E. coli* (14.7 mm) respectively and the lesser antimicrobial activity of silver nanoparticles is found against *S. aureus* (12.5 mm) and *S. typhi* (11.4 mm).

The silver nanoparticles showed efficient antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms. The AgNPs exhibited antibacterial activity against broad range of Gram positive and Gram negative bacteria by interacting with bacterial cell wall or plasma membrane, bacterial DNA and bacterial proteins (Chaloupka *et al.*, 2009). However, 1 M AgNO₃ solution showed less antibacterial activity, while the distilled water and DMSO show antibacterial effect against any of the test pathogens. The antibacterial effect of the AgNPs is comparable to that of AgNO₃ as observed from the zones of inhibition mentioned in Fig. 5.10 and Table 5.2.
Fig. 5.9. Antibacterial activity of silver nanoparticles against human pathogen

(A) AgNps (B) Leaf extract (C) AgNO₃ (D) Distilled water (E) DMSO.
Fig. 5.10: Size of inhibition zone diameter.
Table-5.2

Diameter zone of inhibition by AgNPs, Extract and AgNO$_3$ against Human pathogenic bacteria

<table>
<thead>
<tr>
<th>S.No</th>
<th>Pathogenic bacteria</th>
<th>Inhibition zone (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AgNPs</td>
<td>Extract</td>
<td>AgNO$_3$</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>12.5</td>
<td>7</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus subtilis</em></td>
<td>13</td>
<td>8.3</td>
<td>6.8</td>
</tr>
<tr>
<td>3</td>
<td><em>Salmonella typhi</em></td>
<td>11.4</td>
<td>5</td>
<td>4.3</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>14.7</td>
<td>9.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

However, it is more convenient to use silver as nanoparticles than AgNO$_3$, since the latter shows acute toxicity to human and animals and is also irritating to the skin, eyes and mucous membrane. Moreover, AgNPs and other nanoparticles can be used to prepare and develop nanomedicine, new generation of antimicrobials, drug delivery systems, biosensors and various other applications such as silver based dressings, silver-coated medicinal devices (Sahu *et al.*, 2013).
Chapter - VI

RAPID BIOLOGICAL SYNTHESIS OF SILVER NANOPARTICLES USING LEUCAS MARTINICENSIS LEAF EXTRACT FOR CATALYTIC AND ANTIBACTERIAL ACTIVITY

ABSTRACT

A novel green approach for the synthesis and stabilization of silver nanoparticles (AgNPs) using water extract of Leucas martinicensis leaf. As obtained the nanoparticles are characterized by UV–Vis, Transmission Electron Microscope (TEM) and X-ray Diffraction (XRD). Crystalline nature of the Ag nanoparticles is confirmed by the prominent peaks in the XRD pattern. The FT-IR spectra suggest that the possible biomolecules are responsible for efficient stabilization of the sample. The effects of leaf quantity on biosynthesis of AgNPs are investigated by UV–Vis spectrophotometer. The synthesized AgNPs are observed to have a good catalytic activity on the reduction of methylene blue by Leucas martinicensis leaf. This is confirmed by the decrease in absorbance maximum values of methylene blue with respect to time through UV–Visible spectrophotometer. Moreover the antibacterial activity of synthesized Ag NPs against Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Escherichia coli are screened.