Chapter-IV

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

4.1. Introduction

Nanoparticles synthesis is an evergreen research field of 21st century in which the connotation of the biomediated experimental process is highly important. The biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increasing attention due to a growing need to develop rapid, clean, nontoxic, simple and environmentally friendly synthetic technologies.

The significance of such a synthetic protocol has been well demonstrated (Mandal et al., 2006; Narayanan et al., 2010; Krumov et al., 2009). Among nanoparticles, silver nanoparticles have potential applications in the area of life sciences especially in food chemistry (Li et al., 2009), biomedicine (Chaloupka et al., 2010), agriculture (Park et al., 2006) and cosmetics (Kokura et al., 2010). They have also been used in catalytic, optical and electrical properties (Cao 2004). A number of synthetic methods have been employed for the synthesis of silver-based nanoparticles involving physical, chemical (Sinha et al., 2009) and biochemical techniques (Huang et al., 2007).

With increasing focus on green chemistry, natural compounds like glucose (Raveendran et al., 2003), chitosan (Li et al., 2011), soluble starch (El-Rafie et al., 2011) and some microorganisms (Vigneshwaran et al., 2007; Shahverdi et al., 2007; Vigneshwaran et al., 2006), etc., have attracted
considerable research interest as safer alternatives, reducing and stabilizing agents to synthesize the silver nanosphere. Synthesis of nanoparticles through biochemical routes, using plant extracts as reducing and capping agents, has received special attention among others, due to maintaining an aseptic environment during the process (Basavaraja et al., 2008; Gardea-Torresdey et al., 2002; Prathna et al., 2011; Rastogi and Anunachalam 2011; Liu et al., 2010; Ali et al., 2011; Vidhu et al., 2011). Therefore, medicinal plants having well established therapeutic importance are being widely used for the size and shape-controlled synthesis of silver nanoparticles (Zhan et al., 2011; Dubey et al., 2010; Kumar et al., 2011; Smitha et al., 2009).

Gopinath et al. (2009) reported the synthesis of silver nanoparticles using fruit body Tribulus terrestris and its antimicrobial activity Tribulus terrestris is a flowering plant of the Zygophyllaceae family, native to warm temperate and tropical region of the old world in Europe, Southern Asia, Africa and Northern Australia (Firas Al-Bayati et al., 2008). It has been widely used in the Ayurvedic system of medicine for the treatment of various urinary disorders. Tribulus terrestris is used to increase the hormones.

Tribulus terrestris has become increasingly popular among athletes because it reportedly increases strength and stamina (Arpandirajan et al., 2008). The medicinal value of Tribulus terrestris plants lies in some chemical substances that produce a definite physiological action in a human body. This plant contains both organic and inorganic constituents. The bioactive compounds like alkaloids, flavonoids, tannins and phenolic compounds are present in the plant. In the present study, the synthesis of AgNPs using water extract of Tribulus terrestris leaf by the reduction of Ag⁺ ions is reported. The formation of AgNPs was also studied
using UV-Visible spectrophotometer. AgNPs were further characterized by FT-IR, XRD, TEM and SEM studies.

Moreover, its catalytic activity on reduction of Methylene Blue (MB) in the presence of *Tribulus terrestris* extract is also studied. MB is a thiazine dye, used in the analysis of trace levels of sulphide ions in aquatic samples. The cationic form of MB is used as an antimalarial agent and chemotherapeutic agent in the aquaculture industry. Moreover, it is used in microbiology, surgery and diagnostic field (Small et al., 2007; Burhenne et al., 2008; Xu et al., 2009).

### 4.2 Materials and methods

#### 4.2.1 Materials

Silver nitrate and methylene blue are purchased from Sigma–Aldrich Chemicals for this study. All glassware’s are washed with HNO₃ and distilled water and dried in oven. *T. terrestris* leaves are collected from this institute garden.

#### 4.2.2 Preparation of leaf extract

Preparation method (3.2.2) is the same

#### 4.2.3 Synthesis of Silver nanoparticles

Synthesis method (3.2.3) is the same

#### 4.2.4 Catalytic activity

Catalytic method (3.2.4) is the same

#### 4.2.5 Antimicrobial assay

Method (3.2.5) is the same
4.3. Results and Discussion

4.3.1 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. 4.1 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. The spectroscopic UV-Vis observations, confirm silver nanoparticles presence with peak maximum around 320-350 nm.

It can be seen that the appearance of four peaks at 436, 422, 410, 405 nm clearly indicates the formation of silver nanoparticles. The characteristic silver SPR bands are detected around 400-450 nm (Njagi et al., 2011). These absorption bands are assumed to correspond to the silver nanoparticles. This observation clearly indicates the successful reduction of silver nanoparticles using Tribulus terrestris extract.

Surface Plasmon peak observed confirms the influence of aqueous Tribulus terrestris leaf extract in reducing Ag+ ions to AgNPs from aqueous AgNO₃ solution. Absorbance intensity increases increasing extract quantity and it is observed that the surface plasmon peak occurs at 436 nm with a slight shift in the peak towards shorter wavelength or blue shift. This shift may be attributed to slight modification in the size and shape of nanoparticles (Raghunandan Bedre et al., 2011).
Fig. 4.1: UV–Vis absorption spectra of silver nanoparticles various concentration of leaf extract (A) 2.0 ml (B) 2.5 ml (C) 3.0 ml (D) 3.5 ml.
Reduction of silver ions present in the aqueous solution of silver composite during the reaction with the ingredients present in the leaves of *T. terrestris* extract observed by the UV-Vis spectroscopy revealed the presence of AgNPs which may be correlated with the UV-Vis spectra.

4.3.2 PL analysis

In this article, photoluminescence properties of different silver nanoparticles have been studied. The intensity of photoluminescence peak is increased with an increase in extract quantity and is almost doubled on the increase of the extract quantity to 3.5 ml. The position and shape of the photoluminescence peaks are almost independent of the excitation wavelength. From the spectra, Fig. 4.2, it can be seen that the photoluminescence spectra differ greatly from each other, which shifts to the higher energy with decreasing the size of silver nanoparticles.

The photoluminescence spectrum, the reduction of silver nanoparticles display clearly blue shift, and it includes the strong peak of 438 nm and the band centred at 700 nm which includes five peaks: 438, 496, 590 and 594 nm and can be observed from three silver nanoparticles of different size (Zhao and Fang, 2004). The synthesized silver nanoparticles of different sizes are found to be photoluminescent. It is observed that the photoemission wavelength is dependent of the particle size while the intensity increases sharply with decrease of particle 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 (Mooradian 1969; Zhaoa *et al.*, 2006).
Fig. 4.2: Photoluminescence spectra of silver nanoparticles various
Concentrations of leaf extract (A) 2.0 ml (B) 2.5 ml
(C) 3.0 ml (D) 3.5 ml.
4.3.3 XRD analysis

Fig. 4.3 shows the XRD pattern of AgNPs obtained in the present study. After reaction, the diffraction peaks at 2θ=38.27°, 46.27°, 64.63°, and 77.66° assigned to the (111), (200), (220), and (311) planes of a faced centre cubic lattice of silver are obtained (Eisa et al., 2011; Sheny et al., 2011).

The intensity of peaks reflects the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks are broad which indicate that small crystallite size (Wani et al., 2011) obtained is 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 is determined using Debye–Scherrer formula, D=Kλ/βcosθ where ‘D’ is particle diameter size, K is a constant equal 1, ‘λ’ is wavelength of X-ray source (0.1541 nm), ‘β’ is the full width at half maximum (FWHM) and ‘θ’ is the diffraction angle corresponds to lattice plane (111). The average size of particles is estimated at 15-40 nm. It can be seen that the size of the silver nanoparticles can be manipulated by controlling the amount of the Tribulus terrestris extract used in such way that as more extract is used and the smaller the nanoparticles are obtained (Table 4.1).

Table 4.1
The calculated particle sizes of the silver nanoparticles as a function of Tribulus terrestris extract quantity

<table>
<thead>
<tr>
<th>S.No</th>
<th>T. terrestris leaf extract (ml)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.0</td>
<td>36</td>
</tr>
<tr>
<td>2.</td>
<td>2.5</td>
<td>32</td>
</tr>
<tr>
<td>3.</td>
<td>3.0</td>
<td>27</td>
</tr>
<tr>
<td>4.</td>
<td>3.5</td>
<td>18</td>
</tr>
</tbody>
</table>
Fig. 4.3: XRD patterns of AgNPs various concentration of leaf extract (A) 2.0 ml (B) 2.5 ml (C) 3.0 ml (D) 3.5 ml.
4.3.4 FT-IR analysis

FT-IR measurements are carried out to identify the possible biomolecules in *T. terrestris* leaf responsible for capping leading to efficient stabilization of the silver nanoparticles. Fig.4.4 the bands at 1016 and 1020 cm\(^{-1}\) (C-OH stretching) are observed, which are typical for polysaccharides (Shankar et al., 2004). The bands at 1654 and 1535 cm\(^{-1}\) are characteristic of amide I and II band (Caruso et al., 1998) respectively.

The amide band I is assigned to the stretch mode of the carbonyl group united to the amide linkage while the amide II band arises as a result of the N-H stretching modes of vibration in the amide linkage. The band at 1454 cm\(^{-1}\) is assigned to the methylene scissoring vibrations from the proteins. It is well known that proteins can bind to silver nanoparticle through either free amine groups or residues in the proteins (Gole et al., 2001) and therefore stabilization of silver nanoparticles by the surface bound proteins is possible in the present green synthesis. The strong broad peak at 3000-3600 cm\(^{-1}\) is characteristic of the N-H stretching vibration. Also, the hydroxyl peak at 3396 and 3468 cm\(^{-1}\) decrease in the presence of nanoparticles, which indicates that Ag\(^+\) ions reduced with the hydroxyl groups of the flavanoid; and polyphenol and the hydroxyl groups, are oxidized to carbonyl groups (Jilie and Shaoning 2007).
Fig. 4.4: FT-IR spectra of synthesised silver nanoparticles various concentration of leaf extract (A) 2.0 ml (B) 2.5 ml (C) 3.0 ml (D) 3.5 ml.
Two bands at 1653 and 1531 cm\(^{-1}\) are assigned to the amide I and II bands of proteins (Burt \textit{et al}., 2004), respectively, and the band observed at 1460 cm\(^{-1}\) can be assigned to the C-N stretching vibrations of the amines (Sanghi and Verma 2009). The bands at 2914 and 2847 cm\(^{-1}\) arise from C-H stretching modes. In particular, the 1232 cm\(^{-1}\) band arises most probably from the C-O group of polyols such as hydroxyl flavones (Begum \textit{et al}., 2009).

### 4.3.5 FE-SEM analysis

Morphological and structural studies are investigated using Field Emission Scanning Electron Microscopy (FE-SEM). It is observed that they are spherical in shape with smooth surface (Fig.4.5). The shape of the particles has correlated with SPR band at 430 nm for silver nanoparticles. The resulting AgNPs are mostly uniform size.

SEM images of biologically synthesised typical silver nanoparticles are obtained from 2.0 ml to 3.5 ml of leaf extract, although the exact shape of the nanoparticles is clearly predicted. In addition, the rapid biosynthesis of silver nanoparticles of different sizes is observed. The nanoparticles increased by high concentrations of \textit{Tribulus terrestris} leaf extract.

The particle shape of plant-mediated AgNPs are mostly spherical with an exception of neem (Azaddirachita indica) which yielded polydisperse particles both with spherical and flat plate like morphology 5–35 nm in size (Shankar \textit{et al}., 2004). SEM images of AgNPs from Emblica officinalis are also predominantly spherical with an average size of 16.8 nm ranging from 7.5 to 25 nm (Ankamwar \textit{et al}., 2005).
Fig. 4.5: FE-SEM images of silver nanoparticles various concentration of leaf extract (A) 2.0 ml (B) 2.5 ml (C) 3.0 ml (D) 3.5 ml.
Using leaf extract, 3.5 ml is found to be more suitable for the preparation of small and stable AgNPs confirmed for these techniques. Fig 4.6 shows a representative profile of the spot EDX analysis and obtained by focusing on AgNPs.

![EDX spectrum of AgNPs](image)

**Fig.4.6: EDX spectrum of AgNPs.**

**4.3.6 TEM analysis**

The biologically synthesized silver nanoparticles using the leaf extract of *Tribulus terrestris* structural morphology and crystallinity are further confirmed by TEM micrograph images. The TEM images are recorded at different concentration to find the individual particles. The synthesized silver nanoparticles are observed in spherical shape; and the average size of the particles was 28 nm (Fig. 4.7). The variation in the particle sizes such as 18, 27, 32 and 36 nm are different in size possibly due to the fact that the nanoparticles are being formed at different extract (Kasthuri *et al.*, 2009).
Fig. 4.7: TEM images of Ag nanoparticles various concentration of leaf extract

(A) 2.0 ml (B) 2.5 ml (C) 3.0 ml (D) 3.5 ml.
It can be seen that the particles range in size from 40 nm to 15 nm, with increasing extract amount. In addition, the size distribution gets narrower as the quantity of *Tribulus terrestris* extract increases. These results mean that, the size of the prepared particles gets smaller and the particle size distribution is improved with an increase of extract quantity.

4.3.7 Effect of leaf concentration

We further investigated the possibility of controlling the particle size and shape by changing the composition of the reaction mixture. With increasing amount of leaf extract, consequent colour changes are observed from yellow to dark brown for AgNPs. For AgNPs, with an increase in the extract quantity an increase in the peak absorbance is found in UV–Vis spectrum (Fig. 4.1). SEM and TEM images of the silver nanoparticles synthesized using different *Tribulus terrestris* leaf extract concentrations (A: 2.0 ml, B: 2.5 ml, and C: 3.0 ml, D: 3.5 ml) with 30 ml of 0.01 M of AgNO₃ for 15 min. The particle size is observed to decrease with an increase in the leaf concentration. Control of the shape and size of metallic nanoparticles enables tuning of their optical, electronic, magnetic, and catalytic properties (Dwivedi and Gopal 2010).

4.3.8 Catalytic activity of AgNPs on reduction of methylene blue by *Tribulus terrestris* 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. studied the reduction of methylene blue by arsine in the presence of silver nanoparticles (Kundu et al., 2002), while Mallick et al. studied the catalytic activity of AgNPs on the reduction of phenosaffarin dye (Mallick et al., 2006).
Fig. 4.8: UV–Visible spectra of methylene blue reduction by *Tribulus terrestris* in the presence of AgNPs.
The present study aims at the reduction of methylene blue by the natural green aqueous extract of *Tribulus terrestris* containing AgNPs. Pure methylene blue dye has a λ_{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 potential of phytoextract to degrade methylene blue. System containing dye, AgNPs and the extract at the end of 30 min time interval showed a marked decrease in the absorbance of methylene blue and increase of SPR peak of AgNPs (Fig. 4.8).

4.3.9 *Antibacterial activity*

The zone of inhibition in different bacterial strains against AgNPs, AgNO₃ and leaf extract is shown in (Fig. 4.9). The biologically synthesized silver nanoparticles are found to be highly toxic against human pathogenic bacteria such as *S. aureus*, *B. subtilis*, *S. typhi* and *E. coli* by disc diffusion method. The silver nanoparticles synthesized by *Tribulus terrestris* extracts are found to have highest antimicrobial activity against *E. coli* (12.5 mm) and *B. subtilius* (12.3 mm), respectively and the lesser antimicrobial activity of silver nanoparticles is found against *S. aureus* (11.6mm) and *S. typhi* (9.8 mm) and. The negative control (DMSO and Distilled water) has shown activity against all the microbial strains tested. It is no activity has been observed against pathogens. The positive control (AgNO₃) was showed activity against all the microbial strains tested the maximum activity against *S. aureus* and *B. subtilis* (6.4 mm and 6.8 mm). Silver nanoparticles are very effective against micro-organisms because of their enormously high surface area.
Fig. 4.9: Antimicrobial activity of Ag NPs synthesized by T. terrestris

S. aureus, B. subtilis, S. typhi and E. coli.
Fig. 4.10: Size of inhibition zone diameter.
Table 4.2

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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AgNPs</td>
</tr>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>11.6</td>
</tr>
<tr>
<td>2</td>
<td>Bacillus subtilis</td>
<td>12.3</td>
</tr>
<tr>
<td>3</td>
<td>Salmonella typhi</td>
<td>9.8</td>
</tr>
<tr>
<td>4</td>
<td>Escherichia coli</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Several studies proposed that Ag NPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. 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 4.9. Results of antimicrobial studies show higher sensitivity of AgNPs for *S. aureus*, *B. subtilis* compared to that of *S. typhi* and *E. coli*. Table 4.2 and Fig 4.10. Smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs (Kvitek et al., 2008). It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria (Morones et al., 2005).
Chapter - V

BIOREDUCTION OF METHYLENE BLUE AND ANTIBACTERIAL ACTIVITY

ABSTRACT

Green synthesis of silver nanoparticles (AgNPs) using *Coccinia indica* leaf extract is studied, the biogenic synthesis of metal nanomaterials offers an environmentally benign alternative to the conventional chemical synthesis routes. Colloidal silver (Ag) nanoparticles are synthesized by reacting aqueous AgNO₃ with *Coccinia indica* L. leaf extract. Upon contact, rapid reduction of Ag⁺ ions is observed in <1 min with Ag nanoparticle formation reaching 90% completion in <20 min. Effect of quantity of extract and the particle size and shape are investigated. The results obtained from UV–Vis spectrum, X-ray diffraction (XRD), and Transmission electron microscope (TEM). The biosynthesis of silver nanoparticles is in the size range of 15-50 nm and is crystallized in face centred cubic symmetry. The possible biochemical mechanism leading to the formation of silver nanoparticles is studied using FT-IR. The Ag NPs showed high performance in the catalytic reduction of methylene blue. Furthermore, the Ag nanoparticles thus obtained showed highly potent antibacterial activity toward Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*S. typhi* and *E. coli*) microorganisms.