Chapter 6

Plant Mediated Synthesis of Silver Nanoparticle and their Antimicrobial Activity

“Design in nature is but a concentration of accidents, culled by natural selection until the result is so beautiful or effective as to seem a miracle of purpose.”
- Michael Pollan
“Plants are nature’s alchemists, expert at transforming water, soil and sunlight into an array of precious substances, many of them beyond the ability of human beings to conceive, much less manufacture.”

- Michael Pollan

6.1. Introduction

The noble metals have attracted major attention due to their application in the nanobiology field, which have proven to be highly versatile and tunable materials for a range of bioapplications including biophysical studies, biological sensing, imaging, medical diagnostics, and cancer therapy. Among these noble metals, AgNPs have been studied extensively which has been known since ancient times as effective antimicrobial agent for the treatment of diseases, for food preservation and water purification. In recent advancement, AgNPs are now being used in medical devices, optical devices, electronics, biotechnologies, biosensors and catalysis [1]. Green nanoscience involves the application of green chemistry principles which reduces or eliminates the use or generation of hazardous substances to the design of nanoscale products, the development of nanomaterial production methods, and the application of nanomaterials [2]. A number of chemical and physical methods have been used to synthesize metal nanoparticles are described in chapter 2. The chemicals used for these synthesis processes are often costly, toxic and non-ecofriendly which may become organic pollutants when the large production is concern [3]. Therefore recently, biological synthesis of nanoparticles has been studied extensively as it is cost effective, clean and eco-friendly and non-toxic where biological entities as well as the non hazardous chemicals are used for the synthesis methods to minimize or avoid undesirable consequences [4].

6.1.1. Importance of phytosynthesis over microbial synthesis processes

As quoted in chapter 2, a great deal of effort has been devoted toward the biosynthesis of metal nanoparticles using bacteria, fungi, actinomycetes, yeast, and viruses. In comparison to microorganisms, the phyto-synthesis method is devoid of complex and multistep processes like microbial isolation, culturing, maintenance etc., and also is a very rapid and cost-effective approach
that can be easily scaled up for bulk production of nanoparticles. Moreover, it has been shown that the rate of nanoparticle synthesis is faster using plants than microorganisms, and the produced nanoparticles are more stable [5]. In addition, phytosynthesis is truly a “green” synthesis route in comparison to other known methods of nanoparticle synthesis.

6.1.2. Plausible mechanism of phyto reduction of nanoparticles

Reduction of silver ions to nanoparticles using extract of Desmodium trifolium was ascribed to the presence of H+ ions, NAD+ and ascorbic acid in the extract [1]. Synthesis of highly stable AgNPs (16–40 nm) using leaf extract of Datura metel has been reported [6]. The extract contained alkaloids, proteins, enzymes, amino acids, alcoholic compounds, and polysaccharides which were said to be responsible for the reduction of the silver ions to nanoparticles [6] (Kesharwani et al., 2009). Quinol and chlorophyll pigments present in the extract also contributed to the reduction of silver ions and stabilization of the nanoparticles.

Here this chapter deals with phyto synthesis of AgNPs using two plants Canna edulis Ker-Gawl. and Manilkara zapota (L.). The aqueous leaf extract of C.edulis and seed extract of M. zapota have been used for the reduction of the Ag+ ions to nanoparticles. The as-synthesized nanoparticles were characterized by UV-Vis spectroscopy, XRD, FT-IR, DLS and Zeta potential analyzer.

6.2. Synthesis of AgNPs using leaf extract of Canna edulis Ker-Gawl.

In the present chapter, the biosynthesis of AgNPs using leaf extract of flowering plant, C. edulis Ker-Gawl. The Canna species is a tropical herb grown from a rhizome, with banana-like leaves and multicolor flowers. Many parts of Canna are used in traditional medicine as diaphoretic and diuretic in fevers and dropsy, as a demulcent, to stimulate menstruation, treat suppuration, rheumatism and to regain energy [7]. The extractions of C. indica L. also showed antitumor activity in rats, weak cytotoxic activity in cell culture and
antibacterial activity [8]. The Canna species also have been very much useful in constructed wetland for removal of organic pollutants, phosphorous and heavy metals [9]. Even though plants of Canna species have medicinal as well as environmental applications, they are not explored for synthesis of AgNPs. Herein the aqueous leaf extracts of Canna species are used for the reduction of aqueous AgNO₃ to AgNPs. The formed AgNPs were characterized by various analytical techniques. AgNPs were demonstrated for the antimicrobial activity against human pathogenic microorganism.

6.3. Experimental Details

6.3.1. Chemicals and plant material

The chemicals of GR grade and media components were purchased from Loba Chemie and Hi-media, India respectively. These chemicals are used without further purification for experiments. Plants of Canna species were obtained from the local garden in Kolhapur, India.

6.3.2. Microorganisms used

Gram positive species viz Staphylococcus aureus NCIM 2654, Bacillus cereus NCIM 2155, Enterococcus faecalis NCIM 2403, Gram negative species viz Escherichia coli NCIM 2066, Salmonella typhimurium NCIM 2501, fungi viz Candida albicans NCIM 3466, Candida tropicalis NCIM 3118, Candida krusei NCIM 3515, Candida guilliemondii NCIM 3126, Candida lusitaniae NCIM 3484 and Penicillium chrysogenum NCIM 924 used for the antimicrobial study were purchased from NCIM (National Collection of Industrial Microorganisms), National Chemical Laboratory (NCL), Pune, India. The cultures were maintained on their respective media at 4 °C.

6.3.3. Preparation of plant extracts

The leaves C. edulis Ker-Gawl. were washed properly under tap water. 15 g of leaves were chopped in small pieces and kept in 100 ml double distilled water for 10 min at 50 °C. The leaf extract obtained from C. edulis Ker-Gawl. was labeled as CELE. The leaf extract was filtered through nylon cloths and
then through Whatman no. 1 filter papers. The extract was kept at 4 °C for 24 h and then used for the synthesis experiments.

6.3.4. Synthesis of AgNPs

10 mL CELE, in 500 mL Erlenmeyer flasks, was added in the 90 mL of 2 mM AgNO₃ solution and incubated for 24 h at room temperature in dark condition. For the optimization of the synthesis method, the effect of different parameters like AgNO₃ concentration, leaf extracts concentration and temperature was studied. To observe effect of AgNO₃ concentration on AgNPs synthesis, 10 mL of CELE was added in 500 mL Erlenmeyer flasks containing 90 mL of 2 mM, 4 mM and 6 mM aqueous AgNO₃. To 100 ml of 10% CELE, different concentration (2 mM, 4 mM and 6 mM) AgNO₃ was added to observe effect of aqueous AgNO₃ on synthesis process. To demonstrate effect of leaf extract concentrations on AgNPs synthesis, 10 mL, 30 mL and 50 mL CELE were added in 500 mL Erlenmeyer flasks containing 90 mL of 2 mM aqueous AgNO₃ solution. Also the effect of variation temperature range from 30 to 60 °C and pH range from 5 to 9 was analyzed on synthesis of AgNPs using 10 mL CELE and 90 mL of 2 mM aqueous AgNO₃. The pH was adjusted using 1 N NaOH for alkaline pH and 1 N acetic acid for acidic pH. The change in the color was observed after 24 h of incubation and AgNPs synthesis was analyzed using UV-Vis spectrophotometer.

6.3.5. Characterizations of AgNPs

The formation of the AgNPs was monitored by UV–visible (UV-Vis) spectroscopy of the leaf extracts by recording spectra between wavelength 300 to 600 nm and simultaneously monitoring the appearance of the characteristic peak at 400–500 nm using a Schimadzu (Model No.UV1800) double beam spectrophotometer. XRD pattern of AgNPs drop coated and air-dried on the glass substrate was recorded to study the structural and phase analysis by Philips PW-3710 diffractometer using Cu Kα radiation in the 2θ range from 20° to 80°. The X-Ray diffraction (XRD) patterns were evaluated by X’pert
high score software and compared with JCPDS card No.04-0783. Energy-dispersive analysis of X-ray spectroscopy (EDX, JEOL JSM 6360) was recorded by focusing on freeze dried leaf extracts. The Fourier transform infrared (FTIR) spectra of were recorded in transmittance mode with Alpha ATR Bruker (Eco ATR 500-400 cm$^{-1}$) spectrum of freeze dried CELE-AgNPs. TEM study was used to determine the morphology and size of the AgNPs. For this purpose, the colloidal solution of the CELE-AgNPs was transferred on to a carbon coated copper grid and allowed to air dry. The grid was then scanned using Philips CM200 model Transmission Electron Microscopy, operating voltage 20-200 kV with resolution 2.4 Å. DLS measurements and zeta potential of AgNPs in colloidal solution were performed using a NICOMP™ 380 ZIS (Santa Barbara, California, USA) for the determination of hydrodynamic diameter (HDD). The Scanning electron microscopy (SEM) was used to observe the effect of AgNPs on pathogenic microorganisms using a JEOL-JSM-6360.

6.3.6. Antimicrobial activity

The antimicrobial activity of AgNPs, prepared using 10 mL CELE and 90 mL of aqueous 2 mM AgNO$_3$, was evaluated using agar diffusion method against Gram positive, Gram negative and fungal human pathogens. Log phase bacterial cultures containing $10^8$ cfu ml$^{-1}$ of each bacterial strain grown in nutrient broth were swabbed on the surface of Mueller–Hinton agar plates. *Candida* species were grown in MGYP (malt extract- 3%, glucose- 1%, yeast extract- 3%, and peptone- 5%) broth and for the antimicrobial test were spread on the MGYP agar plates. The 300 µL of 10 µg mL$^{-1}$ CELE-AgNPs were used for the antimicrobial activity. For comparison, the CELE and 2 mM AgNO$_3$ were used for the antimicrobial activity. The zone of inhibition was measured in milli meter (mm) after 24 h of incubation. The effect of the AgNPs on microorganisms was observed using SEM where *Salmonella typhimurium* and *P. chrysogenum* were grown in presence (test) and absence (control) of AgNPs.
To visualize the effect of AgNPs, the test and control microbial cells were fixed using 2.5% glutaraldehyde and observed under SEM.

6.4. Results and Discussion

6.4.1. UV-Vis Spectroscopy studies

After addition of 10 mL CILE and CELE to 90 mL of 2 mM aqueous AgNO₃ and incubating further for 24 h, the change in the color from light brown to dark brown was prominently observable. The change in color was due to the surface plasmon resonance (SPR) of the formed AgNPs in the medium [10]. The absorbance peak around 410 nm in UV-Vis spectrophotometric analysis confirmed the formation of AgNPs using both leaf extract (figure 6.1) corresponds to the spherical nanoparticles [11]. The formed AgNPs were tagged by their respective leaf extract sources as CILE-AgNPs and CELE-AgNPs. From the figure 6.1, it is clear that there is no absorption of light in both leaf extracts in the range 400-500 nm. Interestingly the amount of AgNPs formed using CILE is very less as compared to that formed using CELE. The active reduction of aqueous AgNO₃ to AgNPs was found using CELE in 24 h of incubation. So the CELE was used for our further experiments.

Figure 6.1. UV-Vis spectra of the CELE-AgNPs
To study the effect of concentration of CELE, different concentrations as 10 mL, 30 mL and 50 mL of CELE were added to the 2 mM aqueous AgNO₃ in different experiments. From figure 6.2(a), it can be seen that as concentration of CELE increased from 10-50 mL, there is an increase in the intensity of the peak around 410 nm due to increase in formation of nanoparticles. At 50 mL of CELE, there is behavioral change of the UV-Vis spectroscopy analysis. This may be due to presence of large number of biomolecules present on AgNPs which have created the hindrance in absorption of light. The effect of AgNO₃ concentration was studied using 10mL of CELE on synthesis of AgNPs. There was red shift towards 420 nm form 410 as the concentration of AgNO₃ increased from 2 to 6 mM (figure 6.2 (b)).

**Figure 6.2.** UV-Vis spectroscopy of AgNPs synthesised using (a) different concentrations of CELE with 2 mM AgNO₃, (b) different concentrations of AgNO₃ with 10% CELE, (c) at different pH rage from 5-9, (d) at different temperatures rage from 30-60 °C
This phenomenon is attributed to formation of large nanoparticles with large number of nanoparticles. There is no color change observed in AgNO₃ aqueous solution, confirming absence abiotic formation of AgNPs. The effect of pH on the synthesis of AgNPs was studied by varying pH from 5 to 9. From the figure 6.2(c), it is evident that the synthesis of AgNPs using CELE is mainly depends on pH of the synthesis media. There was no synthesis occurred in the CELE of pH 5 and 6 as there was no absorption peak around 410 nm. The SPR intensity went on increasing as pH of extract increased from pH 7 to 9. So it is clear that the synthesis of AgNPs using CELE requires neutral or basic pH than acidic pH. Ionization of the phenolic groups present in the extract at neutral and basic pH may be the reason for the formation of AgNPs at these pH conditions [12]. The slight acidic pH may inactivate the reducing functional groups. The reason for no synthesis at acidic pH is not clearly understood. At high alkaline condition there may be formation of silver hydroxide. So for the synthesis of AgNPs the neutral conditions are most favorable. For the rapid synthesis of the AgNPs using CELE, temperature may play crucial role. The effect of temperature was clearly observable in figure 6.2(d). As temperature of the synthesis rose from 30-60 °C, the SPR intensity also showed rise with the temperature and also there was shift towards shorter wavelength which is attributed to formation of smaller nanoparticles. The high temperature induced the CELE for rapid reduction of the AgNO₃ to form smaller AgNPs.

6.4.2. Crystallography study

XRD pattern taken using Cu Kα target in the range 30–80 ° of AgNPs is shown in figure 6.3. The all peaks were well matched with JCPDF Card No. 04-0783 which exhibits the characteristic peaks of silver crystallites observed at 2θ values of 37.8, 44.1, 62.9 and 75.9°. The obtained pattern is for fcc cubic crystal structure. The crystallite size was calculated from the full-width at half-maximum (FWHM) of the diffraction peaks using the Debye–Sherrer formula; $D = \frac{0.9}{\beta \cos \theta}$, where D is the mean grain size, λ is the X-ray wavelength for Cu
target, $\beta$ is FWHM of diffraction peak and $\theta$ is diffraction angle. In order to measure the size of nanoparticles accurately each peak was Gaussian fitted and also the instrumental broadening was subtracted using Si standard sample broadening. The size of nanoparticles from value measured for (111) plane of reflection is approximately of 15 nm which approximately similar to actual size by TEM.

![XRD pattern of CELE AgNPs](image1)

**Figure 6.3.** XRD pattern of CELE AgNPs

### 6.4.3. Compositional analysis and TEM

![EDX spectra and TEM micrograph](image2)

**Figure 6.4.** (a) EDX spectra CELE AgNPs, (b) TEM micrograph CELE AgNPs (Inset: SAED pattern of CELE AgNPs)
The compositional analysis was carried out by EDX, showed characteristic signals of crystalline AgNPs approximately at 3 keV (figure 6.4(a)) [13]. The TEM analysis confirmed the spherical AgNPs (figure 6.4(b)) with average size of 25 nm. The rings observed in SAED are corresponded to planes, (111), (200), (220) and (311) of face-centered cubic (fcc) structure of elemental silver (Inset: figure 6.4(b)).

6.4.4. FT-IR study

The interaction of biomolecules present in CELE with AgNPs was evaluated using FT-IR measurement of the freeze dried CELE-AgNPs which may be responsible for the reduction and the stability (capping material) of nanoparticles in colloidal solution.

![FTIR spectrum](image)

**Figure 6.5.** FT-IR spectrum of CELE-AgNPs

The biomolecules may be the peptides, proteins, carbohydrates, flavonides, polyphenols etc present in leaf extract. The amide linkages between amino acid residues in proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. FTIR spectrum reveals two bands at 1650.5 and 1550 cm$^{-1}$ that corresponds to bending vibrations of the
amide I and amide II bands of the proteins respectively; while their corresponding stretching vibrations were seen at 3310 and 2903.6 cm\(^{-1}\) respectively (figure 6.5). The protein–nanoparticle interactions can occur either through free amine groups or cysteine residues in proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes [14]. The two bands observed around 1381 and 1038 cm\(^{-1}\) can be assigned to the C–N stretching vibrations of the aromatic and aliphatic amines, respectively [15]. These results indicated that the carbonyl group of proteins adsorbed strongly to metals, demonstrating that proteins could have also formed a layer along with other bio-organic molecules, which would have played important role in reducing Ag\(^+\) ions to Ag\(^0\) ions and secured nanoparticles from aggregation and subsequently added advantage for the stabilization of AgNPs.

**6.4.5. Particle size distribution and Zeta potential studies**

Hydrodynamic diameter (HDD) distribution of AgNPs in colloidal solution was found to be 40±5 nm (figure 6.6(a)).

![Figure 6.6](image)

**Figure 6.6.** (a) HDD of CELE AgNPs, (b) Zeta potential of CELE AgNPs

The zeta potential is an indication of the stability of colloidal aqueous dispersions and usually particles with zeta potential ($\xi > \pm 30$ mV) are considered to be stabilized due to electrostatic repulsion. Here the zeta potential
of as-formed AgNPs in colloidal solution was -33.7 mV which shows the excellent stability of the AgNPs in the colloidal solution (figure 6.6(b)).

CELE contains high concentration polyphenols, flavonoids, antioxidants and proteins [7]. The phytochemicals such as flavonoids, polyphenols or phytochelatins/ glutathiones/ metallothioneins present in the parenchyma of cycas leaf were extracted for the synthesis of spherical AgNPs in the size range of 2–6 nm [10, 16]. Whereas the proteins present in bell pepper, Capsicum annuum L. reduced and stabilized silver ions to AgNPs [17]. The phytochemicals present in the CELE contributed for the active reduction of aqueous AgNO$_3$ to CELE-AgNPs. The exact mechanism behind the difference in activity of both cell free extract in reduction of AgNO$_3$ was not clearly understood but it has created area of interest for next investigation.

6.4.6. Antimicrobial Activity

Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications. The different mechanisms of the bactericidal effect of AgNPs have been proposed from the several studies but the exact mechanism remains to be understood. Many literatures stated that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell [17]. It is also possible that AgNPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [17]. Smaller AgNPs having the large surface area available for interaction would give more bactericidal effect than the larger AgNPs [18]. The shape of AgNPs also has been shown very essential for the effective antibacterial activity [19].

Although CELE has mild antimicrobial activity, there was no observable antimicrobial activity of CELE against the test human pathogenic microorganisms. For the comparison, the antimicrobial activity of aqueous AgNO$_3$ was analyzed with as-prepared AgNPs. CELE-AgNPs showed prominent antimicrobial activity against all test microorganisms including
Gram negative, Gram positive and fungal species which was more that of AgNO₃ (figure 6.7).

![Graph showing antimicrobial activity of CELE, AgNO₃, and CELE-AgNPs against pathogenic microorganisms in zone of inhibition (mm)](image)

**Figure 6.7.** The antimicrobial activity of CELE, AgNO₃ and CELE-AgNPs against pathogenic microorganisms in zone of inhibition (mm)

Here the Gram positive species showed little resistance than that of Gram negative species. The architectural difference in both Gram species adds major key factor for the resistance. The fungal species has shown resistant to aqueous AgNO₃ but found more susceptible to CELE-AgNPs. To visualize the toxicity of AgNPs to the microorganisms, the two microorganisms *S. typhimurium* and *P. chrysogenum* were grown in presence and absence of AgNPs. Figure 6.8 (a) is showing SEM images of intact *S. typhimurium* cells in absence of AgNPs. The disturbed cell membrane of *S. typhimurium* can be seen in figure 6.8(b) in presence of AgNPs. The intact mycelium of fungus *P. chrysogenum* (figure 6.9 (a)) was completely damaged by the AgNPs (figure 6.9 (b)). The possible mechanism of antimicrobial activity of the AgNPs is discussed in the chapter 1.
Figure 6.8. SEM images of a) *S. typhimurium* in absence, b) *S. typhimurium* in presence of CELE AgNPs

Figure 6.9. SEM images of (a) *P. chrysogenum* in absence, (d) *P. chrysogenum* in presence, of CELE-AgNPs.
6.5. Synthesis of AgNPs using aqueous seed extract of Manilkara zapota (L.)

In the present section of the chapter, biological route for the synthesis AgNPs using aqueous extract of *Manilkara zapota* (L.) (MZSE) is demonstrated. *Manilkara zapota* (L.) (*M. zapota*) belonging to the family *Sapotaceae*, is an evergreen, glabrous tree, 8-15m in height. It is native to Mexico and Central America but it is also cultivated throughout Indian subcontinent including Bangladesh. The seeds of *M. zapota* (L.) are aperients, diuretic tonic and febrifuge. Stem bark is astringent and febrifuge [21]. The leaves and bark are used to treat cough, cold, dysentery and diarrhea [22]. The leaf extract of the *M. zapota* (L.) has been utilized for the synthesis of AgNPs [23]. As the seeds of *M. zapota* in general are considered as a waste by product in urban areas, the use of these seeds for the synthesis of AgNPs can be a cost effective and robust biosynthesis process. The nanoparticles have been characterized by UV–Vis spectroscopy, EDX, DLS, TEM and XRD analysis. The anti-candidal activity of AgNPs against four *Candida* species was tested using agar diffusion method. The outline of the study is given in the figure 6.10.

![Figure 6.10. The outline of the synthesis method and application](image-url)
6.6. Experimental Details

6.6.1. Materials, Chemical and Microorganisms

AgNO$_3$ and microbial culture media components were purchased from Hi-Media, India. *Manilkara zapota* seeds were collected from local market, Kolhapur, India. *Candida albicans* NCIM 3466, *Candida tropicalis* NCIM 3118, *Candida krusei* NCIM 3515, *Candida guilliermondii* NCIM 3126, *Candida lusitaniae* NCIM 3484 purchased from NCIM (National Collection of Industrial Microorganisms), National Chemical Laboratory (NCL), Pune, India were used for the antimicrobial study. The cultures were maintained on MGYP media at 4 °C.

6.6.2. Preparation of seed extracts and synthesis of AgNPs

*M. zapota* seeds were washed with tap water to remove adhering impurities. Seeds were then air dried under sunlight to remove the moisture completely. The seeds were chopped and powdered in a ball mill. The final sieved powder was used for all the further studies. The 10% w/v concentration of seed extract was prepared using double distilled water and filtered through filter paper to remove undissolved matters of extract. The 10% concentration of the MZSE was added to 0.01 M AgNO$_3$ in 250 ml Erlenmeyer flask. The mixture was then heated at 80 °C in the water bath. The change in the color of mixture was observed with time interval.

6.6.3. Characterization

The formation of the AgNPs was monitored by UV–visible (UV-Vis) spectroscopy of the MZSE by recording spectra between wavelength 200 to 800 nm and simultaneously monitoring the appearance of the characteristic peak at 400–500 nm using a Schimadzu (Model No. UV 1800) double beam spectrophotometer. XRD pattern of AgNPs drop coated and air- dried on the glass substrate was recorded to study the structural and phase analysis by Philips PW-3710 diffractometer using Cu K$_\alpha$ radiation in the 2θ range from 20° to 80°. The X-Ray diffraction (XRD) patterns were evaluated by X’pert
high score software and compared with JCPDS card No.04-0783. Energy-dispersive analysis of X-ray spectroscopy (EDX, JEOL JSM 6360) of air-dried on the glass substrate was recorded for compositional analysis. TEM study was used to determine the morphology and size of the AgNPs. For this purpose, the colloidal solution of the AgNPs was transferred on to a carbon coated copper grid and allowed to air dry. The grid was then scanned using Philips CM200 model Transmission Electron Microscopy (TEM), operating voltage 20-200 kV with resolution 2.4 Å. DLS measurement of AgNPs in colloidal solution was performed using a NICOMP™ 380 ZIS (Santa Barbara, California, USA) for the determination of hydrodynamic diameter (HDD).

6.6.4. Antimicrobial activity of AgNPs

The as-prepared AgNPs were tested for the anti-candidal activity using agar diffusion method (Cos et al., 2006). Candida species were grown in MGYP broth and for the antimicrobial test were spread on the MGYP agar plates. The freshly prepared AgNPs and the seed extract were used for the antimicrobial activity. After 24 h of incubation of microorganisms with AgNPs and seed extract, the zone of inhibition (ZOI) in mm were recorded.

6.7. Results and discussions

6.7.1. UV-Vis spectroscopy studies

The heating of MZSE with 0.01 M aqueous AgNO₃ for about 30 min led to change in the color from milky white to yellowish orange (figure 6.11(B)). This color change observed due to a characteristic surface plasmon resonance (SPR) showed by AgNPs. [24] The UV-Vis spectroscopy record (figure 6.11(A)) showed the absorbance around 435 nm [25] which is a typical absorbance of the AgNPs, confirming the reduction of AgNO₃ to AgNPs by using seed extract.
6.7.2. XRD study

The crystalline nature of the AgNPs was studied using XRD. XRD pattern taken using Cu Kα target in the range 30–80° of AgNPs is shown in figure 6.12. The all peaks were well matched with JCPDF Card No- 89-3722 which exhibits the characteristic peaks of silver crystallites observed at 2θ values of 38.04, 44.74, 64.76 and 77.68°. The obtained pattern is for fcc cubic crystal structure. The crystallite size was calculated from the full-width at half-maximum (FWHM) of the diffraction peaks using the Debye–Sherrer formula, 

$$D = \frac{0.9 \lambda}{\beta \cos \theta}$$

where D is the mean grain size, λ is the X-ray wavelength for Cu target, β is FWHM of diffraction peak and θ is diffraction angle. The crystallite size of nanoparticles from value measured for (111) plane of reflection is approximately of 15 nm.

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Figure 6.11. (A) UV-Vis spectra of a) MZSE and b) MZSE AgNPs, (B) photographic images of a) MZSE and b) MZSE AgNPs
Figure 6.12. XRD pattern of the MZSE AgNPs

6.7.3. Compositional analysis

The compositional analysis was carried out by EDX, showed characteristic signals of crystalline AgNPs approximately at 3 keV (figure 6.13) [12]. The presence of C, N and O confirmed the existence of the biomolecules on the AgNPs.

Figure 6.13. The EDX spectra of MZSE AgNPs
6.7.4. TEM and particle size distribution studies

The TEM analysis confirmed the spherical AgNPs with average size of 20 nm (figure 6.13(a)) which is in agreement with crystallite size calculated by the Debye–Sherrer formula. Hydrodynamic diameter (HDD) distribution of AgNPs in colloidal solution was found in range of 40-100 nm; measured using DLS (figure 6.13(b)).

![Figure 6.13](image)

**Figure 6.13.** (a) TEM of MZSE AgNPs, (b) DLS of MZSE AgNPs colloidal solution.

In many literatures it has been demonstrated that the phenolics, terpenoids, sesquiterpenes, and flavonoids are involved in formation of AgNPs in phytosynthesis of metal nanoparticles [26]. There is abundant presence of flavonoids and phenolic compounds in MZSE [27]. So there is a possible role of these molecules in formation of AgNPs using MZSE.

6.7.5. Antimicrobial studies

The as-synthesized AgNPs demonstrated prominent anti-candidal activity against all *Candida* species, corresponding to their zone of inhibition (table 6.1). Though the seed extract has demonstrated for antimicrobial activity [27], it showed no antimicrobial activity against *Candida* species. The AgNPs exhibited antibacterial activity against broad range of Gram positive, Gram negative bacteria and fungus by interacting with microbial cell wall or plasma...
membrane, DNA proteins and spores [28]. However, exact mechanism behind the antimicrobial effect of AgNPs is still unclear. So the AgNPs synthesized using MZSE can be used for candidal infections with further cytocompatibility studies.

**Table 6.1.** The zone of inhibition by MZSE AgNPs against *Candida* species

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Microorganisms</th>
<th>Zone of Inhibition (ZOI) in mm</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MZSE</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida albicans</em> NCIM 3466</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td><em>Candida tropicalis</em> NCIM 3118</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida krusei</em> NCIM 3515</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td><em>Candida guilliermondii</em> NCIM 3126</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td><em>Candida lusitaniae</em> NCIM 3484</td>
<td>0</td>
</tr>
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</table>

**6.8. Summary**

Herein this chapter the leaf extract of *Canna edulis* Ker-Gawl. and seed extract of *Minlka zapota* (L.) are used for the synthesis of AgNPs. TEM showed average 25 nm nanoparticles and HDD was found 40±5 nm with zeta potential of -33.7 mV which demonstrate excellent stability of AgNPs in colloidal solution. As-prepared AgNPs were also analyzed for antimicrobial activity. The synthesis of AgNPs was also obtained from aqueous seed extract of *Mailkara zapota* (L.) within 3 h of incubation. Size of nanoparticles formed using seed extract was found of average 20 nm from TEM and HDD was in range of 40-100 nm. The as-prepared AgNPs were specifically studied for antimicrobial activity against pathogenic *Candida* sp.
Chapter 6

Phytosynthesis of AgNPs

References