Potential of Biosynthesized Silver and Zinc Oxide Nanoparticles from *Carissa opaca* Extracts for Antimicrobial Activity and Wastewater Treatment

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Abstract: The present study focuses on biosynthesis of stable silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnO NPs) from the leaf and stem extract of a therapeutic plant *Carissa opaca*. The visual observation, Fourier Transformed Infrared Spectroscopy (FTIR), Inductively Coupled Plasma analysis (ICP), High Resolution Transmission Electron Microscopy (HRTEM), and Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) were used to characterize and confirm the synthesized AgNPs and ZnO NPs. Afterwards, the synthesized nanoparticles were used to analyze their antimicrobial activity via in-vitro disk diffusion method against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus niger*, and *Candida albican*. Both the nanoparticles showed maximum zone of inhibition against *Pseudomonas aeruginosa* (bacterial strain), whereas in the case of fungi, higher zone of inhibition was observed using ZnONPs against *Candida albican* and AgNPs against *Aspergillus niger*. The biosynthesized AgNPs was also used for degradation of methylene blue under visible-light irradiation and found dye removal efficiency of 97.4% within 1 h.

Keywords: nanoparticles; biosynthesis; secondary metabolites; redox reaction; antimicrobial activity; dye degradation

1. Introduction

The nanoparticles are of keen interest due to their novel properties in the fields of agriculture, cosmetics, medical diagnostics, wastewater treatment, electronics, pharmaceuticals, healthcare industry, etc. The demand for metallic as well as non-metallic nanoparticles has increased over the past few years [1] which ultimately led to the development of physical and chemical procedures to synthesize nanoparticles. These conventional techniques involve the use of harsh conditions/harmful chemicals which release of toxic byproducts, thus affecting the environment as well as human health. Therefore, the biological approach of synthesizing nanoparticles came into existence with the intention to reduce or even eliminate compounds that are hazardous to human health and environment. It involves the use of microbes, plants, seaweeds, microalgae and organic waste products for the synthesis of nanoparticles [2,3]. Plant extracts have intrigued the interest of researchers because of their simplicity, low cost, and quick reaction time, as well as their capacity to reduce metal ions to metal nanoparticles [4]. Also, employing plant extracts to synthesize nanoparticles is more advantageous than using microorganisms or culture specimens because it eliminates the complicated procedure of maintaining cell cultures which may be time-consuming and expensive [5]. Plants naturally confer numerous secondary metabolites such as alkaloids, terpenoids, saponins, phenolic acids, flavonoids, etc. which exhibit a huge potential in medicinal and health sector [6]. The secondary metabolites play a critical role in the redox reaction by acting as reducing, capping as well as a stabilizing agent during nanoparticles
biosynthesis [7]. However, due to several convolutions in identifying the precise chemical constituents responsible for the synthesis of metallic nanoparticles, the green synthesis of nanoparticles becomes difficult [8].

Due to the efficiency of various metal nanoparticles against pathogenic microorganisms such as bacteria, fungi, yeast, viruses, etc., nanotechnology has been recognized as having a wide potential throughout this decade [9]. A high surface area to volume ratio makes metallic nanoparticles effective against a variety of pathogenic microbes, which has attracted scientists’ attention in recent years due to the growing microbial resistance to metal ions and antibiotics [5]. A variety of bacteria, including *Escherichia coli* (*E.coli*) [10], *Staphylococcus*, and *Streptococcus* mutants [11], are highly cytotoxic to AgNPs and ZnONPs. Food packaging systems [12], dental materials [13], severe burn therapy [14], and water filtration [15], all use AgNPs and ZnONPs to treat bacterial illnesses [16–18]. Nanoparticles such as silver, zinc oxide have demonstrated their potential against various fungal as well as bacterial strains by generating reactive oxygen species (ROS) thereby inhibiting DNA replication and ATP synthesis, hence stopping the biochemical pathway of the cell, eventually leading to microbial death (Figure 1) [18]. Adsorption and photocatalysis are regarded as the best and most efficient methods for eliminating harmful dyes [19]. Recently, hybrid solutions enabled by nanotechnology have emerged, giving researchers a hope for treating wastewater instead of using traditional techniques. An exceptional characteristic of metals like silver, gold, zinc oxide, and titania at the nanoscale, such as their high photocatalytic activity, rapid oxidation, lack of generation of polycyclic by-products, and antibacterial properties, make them an ideal choice for the effective treatment of polluted water [20].

![Figure 1. Schematic diagram displaying generalized mechanism of antimicrobial potential of biosynthesized nanoparticles.](image)

Methylene blue (MB) is a cationic dye widely used in important domains such as the medical, chemical, pharmaceutical and aquaculture industries and is known to be a hazardous cationic colourant. Long-term contact with MB could harm the eyes permanently and cause burning. Additionally, it may make breathing difficult and result in certain common symptoms such as vomiting, nausea, excessive perspiration, gastritis, diarrhoea, and mental confusion [20]. Therefore, it is essential to treat wastewater containing the dye.

In the present study, simple and eco-friendly method for the biosynthesis of AgNPs and ZnONPs using leaf and stem extract of *Carissa opaca* under ideal environmental conditions was used. Keeping in view the crises of drinking water, a cost-effective and environmentally friendly method was developed in which biosynthesized nanoparticles (AgNPs...
and ZnONPs) have been used to degrade the dyes from the industrial wastewater, especially from textile industries, which are challenging to treat using other biological processes.

The biosynthesized AgNPs and ZnONPs were also used to evaluate their antimicrobial potential against opportunistic human pathogens such as *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Bacillus subtilis* (BS), *Aspergillus niger* (AN), and *Candida albican* (CA). The zone of inhibition was analyzed to determine the biocidal characteristics of nanoparticles for strain-specific biocides. Thus, it can be concluded that biosynthesized AgNPs and ZnONPs using leaf and stem of *Carissa opaca* would not only be economically viable but also have potential towards pharmaceutical and environmental applications.

### 2. Material and Methods

#### 2.1. Collection of Plant Material

The leaves and stem of *Carissa opaca* belonging to the *Apocynaceae* family were collected from the campus of Shri Mata Vaishno Devi University, Katra, Jammu and Kashmir (32.9418° N and 74.9541° E, elevation 754 m), India. Silver nitrate (AgNO₃) and zinc nitrate [Zn(NO₃)₂] was purchased from S D fine—Chem Limited, Mumbai, India. Throughout the study, deionized water was used for the synthesis and purification of nanoparticles. All the chemicals and reagents used were of analytical grade.

#### 2.2. Preparation of Plant Extract

Freshly plucked leaves and stem of *Carissa opaca* were collected, washed under running water and twice with deionized water to remove the impurities. They were airdried for 2–3 days before being ground into powder. Now take 5 gm of powder and mix it with 100 mL of deionized water in 500mL Erlenmeyer flask and kept on heating for 30 min. The mixture was cooled, filtered using Whatmann filter paper no. 1 and further centrifuged at 5000 rpm for 10 min. The supernatant was collected in 250 mL Erlenmeyer flask as the final extract. The extract was stored at 4°C for later experiments.

#### 2.3. Biosynthesis of Silver (AgNPs) and Zinc Oxide (ZnONPs) Nanoparticles

The biosynthesis of silver nanoparticles (AgNPs) was performed under dark conditions in order to minimize the photo activation of silver nitrate (AgNO₃). The optimized volume of plant extract (30 mL) was mixed slowly with 70 mL of 1mM AgNO₃ (pH 9) with constant stirring at 40°C under dark conditions. Likewise for ZnONPs, optimized volume of plant extract (30 mL) was mixed slowly with 70 mL of 1mM Zn(NO₃)₂ at pH 9 with constant stirring at 70°C temperature. The biosynthesized AgNPS and ZnNPs were separated from the reaction mixture by centrifugation at 9000 rpm for 10 min, followed by a rinse with distilled water. Finally, the pellets were lyophilized in order to obtain powdered nanoparticles.

#### 2.4. Antimicrobial Activity of Biosynthesized Silver and Zinc Oxide Nanoparticles

The antimicrobial potential of biogenic AgNPs and ZnONPs was assessed using the disk diffusion method [21–23] against pathogenic microbial strains such as *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Bacillus subtilis* (BS), *Aspergillus niger* (AN), and *Candida albican* (CA). The bacterial strains were cultured in nutrient broth and the overnight grown cultures were swabbed evenly onto separate plates containing sterile Muller Hington Agar after an overnight culture. The 6mm sterile discs were placed on Muller Hington Agar plates and 20 µL synthesized nanoparticles (10 mg/mL) were poured onto the discs. These plates were then incubated at 37°C for 24 h and zones of inhibition were examined. Three separate trials were conducted, and the mean results for zone diameter were considered as final observation. Whereas in the case of fungus, the fungal strains were first cultured in Potato dextrose broth for a period of 1–2 weeks and then swabbed onto petri plates containing Potato Dextrose Agar (PDA). Each 6 mm disc received 20 µL synthesized nanoparticles (10 mg/mL) which were further incubated for a period of 5–7 days to check the zone of inhibition. As a control, Ampicillin, chloramphenicol and amphotericin b were
used. The antimicrobial activity was performed in triplicates, and the mean result of the zone of inhibition was determined.

2.5. Photocatalytic Dye Degradation on Methylene Blue Dye

The photocatalytic activity of the silver nanoparticles was evaluated by analyzing the degradation profile of methylene blue (MB) dye under visible light.

In this study, upon optimizing the various factors such as dose of nanoparticles, pH, concentration of dye solution and time, the reaction was run for 90 min. in which 50mL AgNPs was added to 100 mL MB dye solution (10 ppm). The reaction was stirred in dark for 30 min. to achieve equilibrium between adsorption and desorption. It was then allowed to stir under visible light for 2 h and the percentage of dye decolourization was analysed by taking 2 mL sample of the reaction mixture after every 15 min. The sample was centrifuged and supernatant obtained used to measure the absorbance using UV-VIS spectrophotometer at 660 nm.

The percentage decolourisation was calculated further using Equation (1):

\[
\text{Decolourisation} (\%) = \frac{C_0 - C}{C_0} \times 100
\]

where \(C_0\) and \(C\) (both in mgL\(^{-1}\)) are the initial (\(t = 0\)) and remaining concentration (after any irradiation time \(t\) of dye in solution, respectively.

Further, under optimal circumstances, the catalyst’s potential to be reused for the breakdown of the malachite green dye was studied. Centrifugation was done to separate the catalyst from the first cycle, and it was then cleaned twice with distilled water and dried in an oven at 80 °C to remove any remaining water before being utilised in the second cycle. Likewise, The catalyst was reused for consecutive four cycles.

2.6. Characterization of Green Synthesised Nanoparticles

2.6.1. UV-Visible Spectroscopy

The synthesis of silver nanoparticles and the reduction of the silver cation (Ag\(^+\)) ions by test plant extracts like Carissa opaca in the solutions were both studied using UV-Visible spectroscopy. Using a double beam UV-VIS Spectrophotometer (Shimadzu-UV1800) with a resolution of 1 nm in the range of 200–700 nm, the UV-VIS spectra of these materials were measured. To change the baseline, double-distilled water was employed.

2.6.2. Fourier-Transform Infrared Spectroscopy (FTIR)

The dried AgNPs were analyzed using FT-IR utilizing a Model: Spectrum 400, Perkin Elmer, USAFT-IR in the 4000–400 cm\(^{-1}\) transmittance mode with a resolution of 4 cm\(^{-1}\). The functional moieties were studied based on distinctive spectral bands.

2.6.3. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS)

A SEM-EDS (Carl Zeiss, Model: Sigma 500 and Bruker, Quantax 200) was used to examine the silver nanoparticles’ size, aggregation, and magnification, and with the help of EDS, energy spectrum and abundance of certain components were determined.

2.6.4. X-Ray Diffraction (XRD)

The size, shape, and crystalline quality of the biosynthesized silver nanoparticles were examined using X-ray Diffractometer (Bruker D8 focus, Billerica, MA, USA). All the XRD data was collected and further studied using OriginPro v.8.5 software.

2.6.5. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

ICP-OES was done in order to examine the elemental compositions, size distributions, and aggregation of nanoparticles, colloids, and their constituents in the sample.
2.6.6. High Resolution Transmission Electron Microscopy (HR-TEM)

High resolution Transmission electron microscopy (JEOL, Model: JEM 2100 Plus, Peabody, MA, USA) was used to study the shape and size of biosynthesized nanoparticles.

3. Result and Discussion

3.1. Visual Observation

In this study, the change of color during biosynthesis of nanoparticles was visually observed as shown in Figure 2. This happened due to the reduction of Ag$^+$ and Zn$^{2+}$ ions by secondary metabolites present in the plant extracts, which acts as reducing agent, capping agent as well as stabilizing agent during the nanoparticles’ biosynthesis [7]. This change is further monitored with a UV-VIS spectrophotometer in a range of wavelengths from 200 to 800 nm.

The pH influences the shape, size, and rate of synthesis of nanoparticles which may be due to the fact that, with an increase in pH, there is an increase in the formation of nucleation centers which increases the reduction rate leading to change in color from pale yellow to brown, indicating the bio reduction of silver ions to create silver nanoparticles [24]. It was observed that at pH 5, there was no color change. The color change was observed after 10 min of the reaction at pH 5, whereas at pH 7, the change in color was observed after 5 min and at pH 9, the change in color was observed within seconds. The lower pH (acidic) suppresses the formation of the nanoparticles due to the low availability of functional groups in the leaf extract [25].

3.2. UV-VIS Spectrophotometer

To verify the biosynthesis of AgNPs and ZnONPs, nanoparticles were scanned at 200–800 nm in a UV-VIS spectrophotometer (LAB INDIA UV-3000+). The colour of AgNPs changed from pale yellow to reddish-brown due to the electron excitation. In the present study, maximum absorption was observed at 371 nm in case of AgNPs synthesized using leaf extract and at 332 nm in the case of AgNPs synthesized using stem extract of
Carissa opaca. The absorption spectrum near 350 nm of AgNPs corresponds to transverse plasmon vibration in the silver nanoparticles [26]. In case of ZnONPs synthesis using leaf and stem extract of Carissa opaca, the maximum absorption at 327 and 338 nm was observed in case of ZnONPs synthesized using the leaf and stem extract of Carissa opaca. Numerous variables, like the reaction medium’s dielectric constant and the particle’s size, affect the precise position of absorption.

In order to determine the material’s band gap, the diffuse reflectance spectrum is transformed into the absorption spectrum which is done using Kubelka- Munk Theory given in Equation (2).

\[ F(R) = \frac{(1 - R)^2}{2R} = \frac{K}{S} \]  \hspace{1cm} (2)

where R denotes diffuse reflectance, K denotes molar absorption coefficient, and S denotes scattering coefficient. Using the Tauc relation provided by Equation (3), the material’s optical band gap is determined. This relationship explains the connection between a material’s band gap (Eg) and linear absorption coefficient (\( \alpha \)) of a material.

\[ (\alpha h\nu)^2 = A(h\nu - E_g) \]  \hspace{1cm} (3)

where \( h\nu \) is the photon’s energy (1239.7/eV), \( E_g \) is a material’s band gap, and A is a constant of proportionality. By replacing \( \alpha \) with \( F(R) \), the above equation can be modified to

\[ [F(R)h\nu]^2 = A(h\nu - E_g) \]  \hspace{1cm} (4)

As seen in the Figure 3A–D, the graph is formed by placing energy (hv) across the x-axis and \([F(R)h\nu]^2\) across the y-axis. Calculating the linearly fitted region \([F(R)h\nu]^2\) on the x-axis, the band gap value is calculated. In the present study, it is calculated that the biosynthesized silver nanoparticle’s band gap is 3.0 and 3.2 eV whereas zinc oxide nanoparticles showed band gap of 3.0 eV.

**UV-Visible spectrophotometry:**

![Graph](image)

*Figure 3. Cont.*
Figure 3. Cont.
3.3. FTIR

FTIR (Model: Spectrum 400, Perkin Elmer, Waltham, MA, USA) analysis detects various peaks that indicate different characteristics of functional groups associated with the biosynthesized nanoparticles. Both organic and inorganic compounds can be analyzed using this analytical approach. According to graphical representations, the spectrum for this study was obtained between 4000–400 cm\(^{-1}\). The sample was analyzed using FTIR in solid form. The results of the FTIR studies indicated the involvement of alcohol, phenol, aromatic compounds, alkynes, and amine functional groups in the synthesis of silver and zinc oxide nanoparticles at different ranges of wave number as shown in Figure 4A,B. These FTIR findings demonstrated that different functional groups in the Carissa opaca extract may be adsorbed on the surface of AgNPs and ZnONPs and may be responsible for both stabilizing and reducing the generated nanoparticles.

In this study the samples were analyzed using FTIR in solid form. FTIR spectrum of AgNPs synthesized using leaf and stem extracts of Carissa opaca shown in Figure 4A demonstrates intense IR peaks at 3440, 3432.89 characterized the N-H, O-H and H-bonded phenols and alcohols stretching vibrations of amide groups respectively, 2924.22, 2919.44, 2854.62, 2854.13 denotes methylene C-H asym./sym. Stretch, 2013.10, 2087.86 explains isothiocyanates (-NCS), 1634, 1634.59 corresponds to Alkenyl C=C stretch, 1383.98, 1383.65 corresponds to methyl group (-CH\(_3\)), 1129.47, 1111.47 denotes C-O stretching of alkyl ethers and cyclic ethers, 611.20 and 601.89 appears in the range 720–590 denoting OH bending of alcohol and hydroxyl compounds.
Figure 4. FTIR spectra of (A) silver and (B) zinc oxide nanoparticles synthesized using the leaf and stem extract of *Carissa opaca* displaying various peaks.

The FTIR spectrum of ZnONPs synthesized using leaf and stem extracts of *Carissa opaca* shown in Figure 4B demonstrates intense IR peaks at 3434.21, 3433.45 which denotes O-H and H-bonded phenols and alcohols stretching vibrations, 2924.96, 2925.54 denotes C-H stretch of methylene, 1630.91, 1624.97 corresponds to N-H bend of primary and secondary amines, 1384.94, 1384.21 are allotted to CH₃ group, 1108.61, 1078.61 corresponds to aromatic C-H bend and primary amine, C-N stretch, 536.54, 547.03 peaks falls between 400–600 cm⁻¹ range which corresponds to Zn-O stretching vibrations [27].
The FTIR spectrum of ZnONPs synthesized using leaf and stem extracts of *Carissa opaca* shown in Figure 4B demonstrates intense IR peaks at 3434.21, 3433.45 which denotes O-H and H-bonded phenols and alcohols stretching vibrations, 2924.96, 2925.54 denotes C-H stretch of methylene, 1630.91, 1624.97 corresponds to N-H bend of primary and secondary amines, 1384.94, 1384.21 are allotted to CH$_3$ group, 1108.61, 1078.61 corresponds to aromatic C-H bend and primary amine, C-N stretch, 536.54, 547.03 peaks falls between 400–600 cm$^{-1}$ range which corresponds to Zn-O stretching vibrations [27]. These band assignments indicating different functional groups are responsible for both stabilizing and reducing the metal ions to nanoparticles [28].

3.4. Inductively Coupled Plasma Optical Emission Spectrometry

To estimate the presence of silver in AgNPs and ZnO in ZnONPs biosynthesized from leaf as well as stem extracts of *Carissa opaca*, inductively coupled plasma optical emission spectrometry (ICP-OES) was performed in triplicates, which is an analytical technique that can be used to measure elements at trace levels in biological fluids. The nanoparticle sample was first acid digested using HNO$_3$ and HCl in 3:1 ratio. The average concentration of silver using ICP-OES was detected to be 180.513 mg/L of biosynthesized silver nanoparticles from leaf extract whereas 126.37 mg/L from stem extract whereas, the average concentration of zinc oxide using ICP-OES was detected to be 233.6 mg/L of biosynthesized zinc oxide nanoparticles from leaf extract whereas 195.7 mg/L from stem extract.

3.5. SEM-EDX

Scattering electron microscopy (SEM)(Carl Zeiss Sigma 500) studies were performed in order to examine the surface morphology, size, and shape of biosynthesized nanoparticles. Figures 5a and 6a shows the SEM image showing morphology and topology of silver nanoparticles, which are almost spherical in shape with agglomeration. The average size of nanoparticles is less than 100 nm. Likewise, Figures 7a and 8a indicates the SEM image showing morphology and topology of zinc oxide nanoparticles, which are almost spherical in shape with agglomeration. This agglomeration is due to the polarity and electrostatic attraction of silver nanoparticles.

Further, the confirmation of silver in the synthesized product was further confirmed by Energy-dispersive X-ray spectroscopy (EDX) (Bruker, Quantax 200) that depicts the strong signal represents the elemental silver and zinc oxide, whereas the weaker signal represents traces of oxygen, zirconium, and other atoms which are from proteins/ enzymes present in the leaf extracts as shown in Figures 5–8b. These weaker signals can also be due to the possibility of elemental transfer from tool to work piece. The EDX also indicated the weight percentage as well as atomic percentage of elemental silver and zinc along with other elements that have weak signals.

3.6. High Resolution Transmission Electron Microscopy (HRTEM)

HRTEM (JEOL model, JEM 2100 Plus, Peabody, MA, USA) analysis of biosynthesized silver nanoparticles from the leaf and stem extract of *Carissa opaca* shown was performed to analyze the surface morphology and shape of biosynthesized silver nanoparticles from the leaf and stem extract of *Carissa opaca*. The HRTEM micrograph in Figure 9a,b is depicted at 50 nm, displaying the spherical morphology of biosynthesized silver nanoparticles from the leaf and stem extract of *Carissa opaca*. The HRTEM micrograph in Figure 10a,b is depicted at 200 nm, displaying the spherical morphology of biosynthesized zinc oxide nanoparticles from the leaf and stem extract of *Carissa opaca* with an average particle size of 27.8 nm and 24.9 nm. The HRTEM micrograph in Figure 10a,b is depicted at 200 nm, displaying the spherical morphology of biosynthesized zinc oxide nanoparticles from the leaf and stem extract of *Carissa opaca* with an average particle size of 18.3 nm and 15.4 nm.
Figure 5. (a) SEM image of silver nanoparticles synthesised from the leaf extract of Carissa opaca; (b) EDX image of silver nanoparticles biosynthesised using the leaf extract of Carissa opaca depicting weight percentage and atomic percentage.
Figure 6. (a) SEM image of silver nanoparticles biosynthesised using the stem extract of *Carissa opaca*. (b) EDX image of silver nanoparticles biosynthesised using the stem extract of *Carissa opaca* depicting weight percentage and atomic percentage.
Figure 7. SEM-EDX image of ZnONPs biosynthesized using the leaf extract of *Carissa opaca* depicting the (a) morphology as well as (b) weight and atomic percentage of biosynthesized ZnONPs.
Figure 8. SEM-EDX image of ZnONPs biosynthesized using the stem extract of *Carissa opaca* depicting the (a) morphology as well as (b) weight and atomic percentage of biosynthesized ZnONPs.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mass%</th>
<th>Atomic%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>21.27</td>
<td>46.09</td>
</tr>
<tr>
<td>O</td>
<td>17.33</td>
<td>28.55</td>
</tr>
<tr>
<td>Zn</td>
<td>61.41</td>
<td>24.76</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
were identified in case of silver nanoparticles synthesized using stem extract of *Carissa opaca* with 2°, 27.8°, 32.2°, 38.1°, 44.3°, and 46.2°. The observed peak broadening and noise were probably macromolecules present in the plant extract which may be responsible for the reduction of silver ions to nanoparticles [29].

The ZnONPs peaks were observed at 31.8°, 34.5°, 36.3°, 47.5°, 56.6°, 62.9°, and 67° in case of ZnONPs synthesized using leaf extract whereas the peaks observed at 31.8°, 34.5°. values at various peaks of 38.2°, 44.4°, 64.5°, and 77.5° were identified in case of silver nanoparticles synthesized using stem extract of *Carissa opaca* are shown in Figure 11A. These peaks correspond to (111), (200), (220) and (311) crystal planes which are in accordance with the standard JCPSD file no. 101-1259. The lattice parameters biosynthesized of silver and zinc oxide nanoparticles are shown in Table 1.

By employing X-ray diffraction (XRD) spectroscopy, the crystalline characteristic of biosynthesized silver nanoparticles was observed. The characteristics Bragg reflections with 2θ values at various peaks of 38.2°, 44.4°, 64.5°, and 77.5° were identified in case of silver nanoparticles synthesized using leaf extract and 27.8°, 32.2°, 38.1°, 44.3°, and 46.2° were identified in case of silver nanoparticles synthesized using stem extract of *Carissa opaca*.

3.7. X-ray Diffraction (XRD)

By employing X-ray diffraction (XRD) spectroscopy, the crystalline characteristic of biosynthesized silver nanoparticles was observed. The characteristics Bragg reflections with 2θ values at various peaks of 38.2°, 44.4°, 64.5°, and 77.5° were identified in case of silver nanoparticles synthesized using leaf extract and 27.8°, 32.2°, 38.1°, 44.3°, and 46.2° were identified in case of silver nanoparticles synthesized using stem extract of *Carissa opaca* are shown in Figure 11A. These peaks correspond to (111), (200), (220) and (311) crystal planes which are in accordance with the standard JCPSD file no. 110-0136. The bioorganic phase crystallisation that takes place on the surface of the nanoparticles may be the cause of the unassigned peaks (marked as star). The observed peak broadening and noise were probably macromolecules present in the plant extract which may be responsible for the reduction of silver ions to nanoparticles [29].
Figure 11. XRD data showing its peak X-ray diffraction patterns of (A) silver whereas depicts patterns of (B) zinc oxide nanoparticles synthesized from leaf and stem extract of *Carissa opaca*. (*) indicates unassigned peaks which may be due to bioorganic phase crystallization on the surface of nanoparticles.

The ZnONPs peaks were observed at 31.8°, 34.5°, 36.3°, 47.5°, 56.6°, 62.9°, and 67° in case of ZnONPs synthesized using leaf extract whereas the peaks observed at 31.8°, 34.5°, 36.2°, 47.7°, 56.6°, 62.9° and 68.1° represents diffraction angles in case of ZnONPs synthesized using stems extract of *Carissa opaca* are shown in Figure 11B and they matches up with the (100), (002), (101), (210) and (103) crystal planes of hexagonal zinc oxide (JCPDS File No. 101-1259). The lattice parameters biosynthesized of silver and zinc oxide nanoparticles are shown in Table 1.
Table 1. Lattice parameters of biosynthesized silver and zinc oxide nanoparticles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cell Parameters A (Å)</th>
<th>C (Å)</th>
<th>Vol (Å³)</th>
<th>Crystalline Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNPs (leaf)</td>
<td>4.08</td>
<td>-</td>
<td>67.91</td>
<td>19.07</td>
</tr>
<tr>
<td>AgNPs (stem)</td>
<td>4.09</td>
<td>-</td>
<td>68.41</td>
<td>21.57</td>
</tr>
<tr>
<td>ZnONPs (leaf)</td>
<td>3.24</td>
<td>5.19</td>
<td>66.68</td>
<td>12.1</td>
</tr>
<tr>
<td>ZnONPs (stem)</td>
<td>3.24</td>
<td>5.19</td>
<td>66.68</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Further, the crystallite size was estimated from full width half maxima (FWHM) of the intense peaks using Scherrer equation (Equation (5))

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

where, the wavelength of monochromatic incident Cu-k\(\alpha^{-1}\) radiations is depicted to \(\lambda\) (1.5406 Å), Full Width Half Maximum (FWHM) is denoted as \(\beta\) (in radians), \(D\) is average crystalline size and \(\theta\) is the Bragg angle [30].

Considering the diffraction data, the lattice parameter shown in table of the biosynthesized ZnONPs and AgNPs has been determined using the Equations (6) and (7).

\[
\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2 + k^2 + hk}{a^2} \right) + \left( \frac{1}{c} \right)^2
\]

\[
\frac{1}{d^2} = \left( \frac{h + k + l}{a} \right)^2
\]

where \(d\) is the inter spacing and \(hkl\) are miller indices.

The volumes of unit cells were also calculated using formula given in Equations (8) and (9).

Volume of ZnONPs

\[
V = \sqrt{\frac{3}{2}}a^2c
\]

Volume of AgNPs

\[
V = a^3
\]

3.8. Antibacterial and Antifungal Activity

The antimicrobial activity of silver and zinc oxide nanoparticles, was analyzed using disk diffusion method against SA, KP, BS, AN, and CA as shown in Figures 12 and 13, in which zones of inhibition shows the activity of nanoparticles against said microbes. The Figure 14a,b and Figure 15a,b showed the sized of zone produced by the biosynthesized nanoparticles. From the experiment, it was inferred that biosynthesized nanoparticles were effective for both types of microbes. The antibacterial activity shown was due to the accumulation of biosynthesized nanoparticles in cell walls that form pits in the cell walls and eventually lead to the death of the bacterial cells. In the case of fungal cells also, the walls of fungal cells are damaged by nanoparticles, changing the potential of those membranes [31]. The nanoparticles trigger morphological alterations in the cellular membrane after adhesion, resulting in membrane permeability and respiratory function distortion via membrane depolarization, and eventually cell morphology distortion and the induction of apoptosis [32].
Figure 12. The figures represent antibacterial potential of biosynthesized silver nanoparticles against different bacterial and fungal strains like *Bacillus subtilis* (BS), *Klebsiella pneumoniae* (KP), *Pseudomonas aeruginosa* (PA), *Candida albican* (CA), and *Aspergillus niger* (AN). In the plates Ab and AF represents antibiotic and antifungal drug (positive control), NP1 represents AgNPs from Leaf extract, NP2 represents AgNPs from stem extract, PE1 represents leaf extract, PE2 represents stem extract and DW represents distilled water (Blank).

Figure 13. The figures represent antibacterial potential of biosynthesized silver nanoparticles against different bacterial strains like *Bacillus subtilis* (BS), *Klebsiella pneumoniae* (KP), *Pseudomonas aeruginosa* (PA), *Candida albican* (CA), and *Aspergillus niger* (AN). In the plates Ab and AF represents antibiotic and antifungal drug (positive control), NP1 represents AgNPs from Leaf extract, NP2 represents AgNPs from stem extract, PE1 represents leaf extract, PE2 represents stem extract and DW represents distilled water (Blank).
Figure 14. Graph displaying antimicrobial potential of biosynthesized silver nanoparticles from the leaf and stem extract of *Carissa opaca* against (a) bacterial and (b) fungal strains.
Figure 15. Graph displaying antibacterial potential of biosynthesized zinc oxide nanoparticles from the leaf and stem extract of *Carissa opaca* against (a) bacterial and (b) fungal strains.

It was observed that the larger inhibitory zones (16.3 ± 3.2 mm) and (19.3 ± 4.9 mm) against *Pseudomonas aeruginosa* was obtained using biosynthesized silver and zinc oxide nanoparticles respectively. Whereas, in the case of fungi, higher zone of inhibition was observed using ZnONPs against *Candida albican* (16.0 ± 1 mm) and AgNPs against *Aspergillus niger* (11.3 ± 1.5 mm).

Nanoparticles’ ability to electrostatically adsorb positive ions in the structure of metal nanoparticles and negative ions in the structure of bacteria is their antimicrobial mechanism.
The cell membrane oxidation and oxidative stress responses are brought on by this binding’s action on the thiol proteins of bacterial cell membranes [33]. The mechanism by which nanoparticles exert their antimicrobial properties is that they gradually release metal ions, which inhibit ATP and DNA transcription and cause direct damage to the cell membrane as well as the production of reactive oxygen radicals and eventually killing the microbe [33].

Silver nanoparticles have antibacterial efficacy against microorganisms, according to research by Patra et al. The disruption of the cell membrane by Ag\(^+\) ions, which can also impact DNA replication, may be the cause of the antibacterial capabilities seen in this work and earlier studies. Additionally, the reactive oxygen species (ROS) produced by silver nanoparticles, such as hydrogen peroxide (H\(_2\)O\(_2\)), can drive the production of free radicals, causing oxidative stress and the toxicity of bacterial cells [15,34]. The effectiveness of zinc oxide nanoparticles against bacteria has been assessed using a variety of techniques. Zn\(^{+2}\) ion-cell membrane compound interactions are among these interactions [35,36]. Ions are capable of chemically reacting with intracellular molecules after crossing the membrane and entering the cell, which can lead to intracellular component damage [37]. H\(_2\)O\(_2\) can be produced in the environment by zinc oxide nanoparticles as well, and once it is created, it can interact with the chemical constituents of cell membranes. Zinc oxide nanoparticles also physically obstruct cell membrane transmission channels, disrupt electron transport, and physically harm the coating and membrane through abrasion and corrosion [37]. These effects all have a negative impact on the cell.

3.9. Photocatalytic Dye Degradation of Methylene Blue

Using synthesized silver nanoparticles and visible light, the photo catalytic decolorization of the methylene blue (MB) dye was carried out. 50 mg of AgNPs were added to MB (10 ppm) and exposed to visible light under constant stirring for a period of 1–5 h. Dye degradation percentage grew with time. The rate of photocatalytic degradation was high in the early hours and gradually decreased as shown in Figure 16. Around 660 nm, the MB’s distinctive absorption peak was seen.

Upon optimization, 50 mg AgNPs was added to 100mL MB dye (10 ppm) having pH 9 and it was found that after 1 h the percentage dye degradation was increased to 97.4%. Surface Plasmon Resonance is a peculiar phenomenon that only silver nanoparticles display where electrons collectively oscillate from the outermost band to a higher energy state as a result of intense photons striking nanoparticles surfaces. The molecular oxygen found in water absorbs this plasmonic excitation of surface electrons and changes it into the free radical species O\(_2^*\) [38]. Although the electrons from the methylene blue dye molecules adsorbed on the surface of the silver nanoparticles fill the positive charge (holes) which oxidize the dye. Additionally, the produced free radical species react with the H\(^+\) ion, which is created when water molecules break, to produce other active radicals like OH\(^*\) and HO\(_2^*\). The breakdown of intricate organic structures and the breakdown of MB dye into azo dye intermediates are caused by the production of these free radicals. The primary cause of degradation and color fading from dark to light blue is the breakdown of complex organic structures into smaller, more oxidizable intermediate molecules [39].

The key element for integration into industrial applications is the photocatalyst’s stability or reusability. In the current work, optimal circumstances (visible light irradiation at a dye concentration of 10 ppm at pH 9, 50 mg AgNPs for 105 min) have been achieved for the reusability of green synthesised AgNPs in the photodegradation of MB dye. The fourth cycle’s reusability test was measured and it was observed that the catalyst’s activity diminished after every cycle. MB dye’s photodegradation was 97.4% after the first cycle and dropped to 71% after the fourth cycle as shown in Figure 17.
the free radical species $O_2^*$ [38]. Although the electrons from the methylene blue dye 
were transferred to the silver nanoparticles, which resulted in the formation of reactive 
species like $OH^*$ and $HO_2^-$. The breakdown of intricate organic structures and the 
breakdown of dye molecules adsorbed on the surface of the silver nanoparticles fill the positive charge 
and generate oxidative stress and the toxicity of bacterial cells [15,34]. The effectiveness of zinc oxide nanoparticles 
was studied by exposing them to visible light under constant stirring for a period of 1–5 h. Dye degradation of the 
methylene blue (MB) dye was carried out. 50 mg of AgNPs were added to MB (10 ppm) and exposed to visible light 
under constant stirring for a period of 1–5 h. Dye degradation percentage increased with time. The rate of photocatalytic 
degradation was high in the early stages, but it gradually decreased as shown in Figure 16. Around 660 nm, the MB 
dye's distinctive absorption peak was observed. The absorbance of the MB dye was measured and found to decrease 
with time, indicating the degradation of the dye. The percentage degradation increased with the number of cycles, 
and after the fourth cycle, it dropped to 71% as shown in Figure 17. The reusability assessment of AgNPs for decolorization of 
methylene blue dye was carried out, and it was observed that the catalyst's activity diminished after every cycle. MB dye's 
photodegradation was 97.4% after the first cycle and dropped to 71% after the fourth cycle as shown in Figure 17.

Figure 16. Graph demonstrates the decrease in absorbance of methylene blue dye with time under visible light source upon optimization of various factors such as dose of nanoparticles, pH, concentration of dye solution and time.

Figure 17. The reusability assessment of AgNPs for decolorization of methylene blue dye.

4. Conclusions

As a practical, economical, and ecofriendly alternative to chemical synthesis, which yields size-controlled, monodispersed nanoparticles with exceptionally high stability, the biogenic synthesis of AgNPs and ZnONPs from leaf and stem extract of Carissa opaca has been developed. The synthesis of nanoparticles took less time in case of the present plant which is a novel criterion used in this study. Our investigation on their antimicrobial as well as dye degradation potential demonstrated that biosynthesized nanoparticles were effective against several strains of bacteria and fungi and also degraded the dye. The one-pot green
synthesis using plant extracts is expected to result in enhanced antimicrobial benefits by amplifying the particular plant extract’s ability to combat microbes. This opens the door for these NPs to be used in a variety of applications, including the production of effective antibacterial agents for the management of emerging multidrug-resistant pathogenic bacteria. Moreover, nanoparticles also displayed their potential as promising catalyst for the effective photo catalytic degradation of methylene blue from aqueous solution. The application of AgNPs and ZnONPs in the field of health care sector and to treat wastewater has a lot of potential to create a sustainable environment.

5. Future Aspects

Biosynthesized nanoparticles have a great potential as antimicrobial agent and for the wastewater treatment. This study aims to optimize the production of biosynthesized nanoparticles with target to achieve the novel nanoparticles (more stable, efficient and have wide range of industrial applications). Nanoparticles that are biosynthesized can be designed to selectively target different toxins in wastewater and more effectively adsorb or breakdown particular contaminants such heavy metals, colors, chemical compounds, or microbes. Future studies will examine the antimicrobial potential of these nanoparticles against antibiotic resistant microbes. They can be widely used as a result of improvements in synthesis methodologies, scalability, targeted pollutant removal, and synergistic approaches, together with an emphasis on sustainability and safety.

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