

Article

Biosynthesis, Characterisation, and Antimicrobial Activities of Nickel-Doped Silver Nanoparticles Using *Caralluma umbellata* Plant Root Extract

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Abstract

Greenly synthesised Ni-doped Ag nanoparticles utilising *Caralluma umbellata* root extracts, and an investigation into their optical properties, biological properties, and characterisation, is the focus of the study. Characterisation was performed using FTIR analysis, UV-Vis, X-ray diffraction, and field emission scanning electron microscopy. The synthesis of Ni-doped Ag nanoparticles was confirmed through UV-Vis spectroscopy, revealing a peak at 396 nm and a band gap energy of 3.24 eV. XRD analysis revealed a face-centred cubic structure with a crystallite size of 55.22 nm (as-prepared) and 18.56 nm (annealed at 200 °C). Reduction and capping were demonstrated by FTIR, as evidenced by the presence of phytochemicals. The Ag NPs demonstrated potent antibacterial activity against both Gram-positive and Gram-negative bacteria, with a minimal inhibitory concentration of 1.25 µg/mL observed against *Streptococcus mutans*. Their vigorous anti-oxidant activity, as well as in vitro anti-diabetic potential through alpha-amylase and alpha-glucosidase inhibition, also proves suitable for biomedical applications.

Keywords: *Caralluma umbellata*; biological activities; characterisations; nickel-doped silver nanoparticles

1. Introduction

Chemical compounds with possible medicinal properties are mostly found in medicinal plants. Nowadays, herbal remedies are often used to treat illnesses instead of pharmaceutical chemicals, which might cause adverse effects. “Medicinal plants are a rich source of diverse chemical compounds with potential therapeutic properties [1].” Traditional medicinal plant research remains an effective and rational avenue for the discovery of novel anti-inflammatory drugs. Roughly three-quarters of the global population depends on traditional treatments, especially herbs, for their health. One of the most important uses for *Caralluma umbellata* in medicine is to treat a wide range of ailments due to its various therapeutic characteristics. Plants of the genus *Caralluma*, which are Asclepiadaceae, may reach a height of 30 cm and produce terminal umbels of flowers. This flora has 200 species and 2500 genera, and it is native to hilly regions in the Indian states of Andhra Pradesh, Odisha, Kerala, Tamil Nadu, and Karnataka [2]. Its fruit paired follicles are cylindrical, green, and sometimes marked with tiny black dots. The yearly market



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for pharmaceuticals produced from plants is around \$20 billion in the US. Additionally, only around 5–15% of plants with medicinal properties have been thoroughly investigated for their chemical potential. As a result, growing a wide variety of useful compounds in plant cultures has enormous promise. Consequently, plant-based cultures hold enormous promise for the sustainable production of diverse and valuable bioactive compounds [3]. Patients with diabetes often experience the dangerous condition known as hyperglycemia. Due to their effectiveness, few side effects, and relatively low prices, herbal remedies are gaining popularity as an alternative to oral anti-hyperglycemic pharmaceuticals for treating hyperglycemia [4]. The Indian subcontinent has a long history of using the medicinal herb *Caralluma umbellata* Haw [5]. This research aims to examine its anti-hyperglycaemic effect by in vitro tests. Investigations into the pharmacological effects of Ni-doped Ag NPs produced from *Caralluma*'s water-based extract are the focus of the current study. Evidence suggests that *Caralluma umbellata* root extract may have antibacterial properties when used in the manufacture of Ag nanocrystallites smaller than 50 nm [6]. These days, biogenic Ag nanoparticles are not just used to fight bacteria; they are also being investigated for potential cancer treatments [7]. Ag nanoparticles produced by Meliazedara Chand showed extraordinary cytotoxicity when tested on HeLa cells from humans and an in vivo model of Dalton's ascites lymphoma in mice [8]. The nanotechnology study has shown that there may be a green chemical way to make nanoparticles that are crucial for technology. A new and serious threat to public health is the widespread development of microbes that are resistant to antimicrobial drugs [9]. The eco-friendly process of biosynthesising metallic nanoparticles is important in the field of applied nanotechnology. Because of their many uses in fields as diverse as electronics, health, chemistry, energy, and catalysis, nanoparticles are in high demand in the commercial sector [10]. Recent advances in nanoparticle synthesis have explored various plant and microbial sources for producing silver nanocrystallites [11]. Plants such as *Morinda citrifolia*, *Rauvolfia tetraphylla*, and *Terminalia catappa* have been identified as valuable bio-factories, offering a rapid, ecological, and controlled method for the production of Ag nanocrystallites. The green production of metal and metal oxide nanoparticles has been actively researched for environmental and photocatalytic applications. TiO₂ nanoparticles have received special attention due to their durability and catalytic performance [12]. For instance, *Crataegus Mexicana*-mediated ZnO nanoparticles demonstrated the efficacy of plant-assisted synthesis routes with their hexagonal zincite structure, quasi-spherical morphology (18–37 nm), strong visible photoluminescence, and effective photocatalytic degradation of dyes like methylene blue and Congo red within 180 min [13]. Recent research has focused on Ni-doped Ag nanoparticles to take advantage of synergistic electronic effects, building on similar developments in TiO₂ and ZnO systems. The current study focuses on their green production utilising plant extracts for multifunctional environmental and medicinal applications. In this study, silver nitrate (AgNO₃) doped with Ni (NO₃)₂ served as the precursor for the green synthesis of Ag nanocrystallites. All these reports demonstrate clearly that numerous plant systems could easily and quickly reduce silver ions and result in stable and biologically active nanoparticles [14]. Nonetheless, in spite of this development, there has been no methodical study introduced based on the extraction of *Caralluma umbellata* root extract. However, the plant has been traditionally known as having strong medicinal effects and abundant secondary metabolites that might be effective reducing and stabilising agents. Moreover, although extensive literature exists on silver nanoparticles produced by using several different botanical systems, very few studies have been performed to produce nickel-doped silver nanoparticles (Ni-doped Ag NPs) via a green route using plants, and none of the previous literature has used the plant *Caralluma umbellata* to produce doped nano-structures. This is a major gap, since it has been known that metal doping can be used to increase nanoparticle

stability, antimicrobial activity, and reactivity on surfaces. Hence, it is evident that there is a necessity to explore *Caralluma umbellata* as a new biogenic precursor to the production of Ni–Ag nanoparticles, and evaluate their structure, functions, and antimicrobial properties. Using several types of synthesised Ni-doped crystallites, this research aimed to assess *C. umbellata*'s antimicrobial potential in vitro. UV-Visible Spectrophotometry, FT-IR, and FE-SEM were used to characterise these crystallites. The goal of investigating Ni-doped Ag nanoparticles in diabetes treatment is to find supplemental nanomaterial-based enzyme inhibitors that can lessen post-prandial glucose rises rather than replace current medication. Such nanoparticles may help to multi-target metabolic control by concurrently reducing oxidative stress and blocking α -amylase and α -glucosidase at low doses. This is especially important for oxidative stress-mediated insulin resistance.

2. Materials and Methods

2.1. Materials Required

Tirupathi, Andhra Pradesh, India, is the site of plant collection for *Caralluma umbellata* roots. The chemicals, such as nickel nitrate ($\text{Ni}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$), and silver nitrate (AgNO_3), were purchased from Sigma Aldrich, Mumbai, India. The entire process was conducted using distilled water to ensure purity, and the chemicals were obtained as analytical grade (AR), requiring no further purification.

2.2. Synthesis Process of Ni-Doped Ag Nanoparticles Using *Caralluma umbellata* Roots

The synthesis of Ni-doped Ag nanoparticles using *Caralluma umbellata* root extract is shown in Figure 1. About 50 g of the *Caralluma umbellata* roots collected were air-dried and ground into a powder. 12 h soaking in 500 cc of distilled water was followed by a 6 to 8 h magnetic stirred extraction at a temperature of 70 °C. Once removed from the stirrer, a brownish semi-solid was obtained, filtered using Whatman No.1 filter paper, and then concentrated in a water bath. The moisture was evaporated entirely by drying the concentrated extracts at 37 °C for 3 to 4 days. Because of the plant extract's ability to function as a buffer, the synthesis was carried out at a pH that was close to neutral (6.8–7.2). Neutral pH was chosen for the best nucleation and stability, since preliminary experiments showed that extremely acidic or alkaline environments caused particle aggregation. In order to guarantee adequate reduction and prevent excessive organic over-capping that can impede particle formation, the extract concentration was set based on initial optimisation.



Figure 1. Extraction of Ni-doped Ag Nanoparticles using *Caralluma umbellata* root extract.

For the production of nanoparticles, 10 mL of root broth with 90 mL of a solution of Ag(NO₃) (0.5 mM) and 10 mL of Ni (NO₃)₂ (0.5 mM) were combined in a 250 mL Borosil conical flask to reduce the Ag⁺ ions. By conducting the reaction in a water bath at several temperatures (between 40 °C and 90 °C), the impact of temperature on Ag NPs was investigated. The apparatus was maintained at 37 °C in the dark to prevent the silver nitrate from becoming photoactivated. The synthesis of silver nanoparticles was analysed, and the product was subsequently filtered with Whatman I filter paper to eliminate micro-particles and centrifuged for final purification. The filtrate was centrifuged, washed repeatedly with distilled water, dried, and annealed at 200 °C for 2 h to improve the properties. The dried Ni-doped Ag nanoparticles were validated by the use of FT-IR, FESEM, and UV-Visible Spectrophotometry [15].

2.3. Characterisation Methods

A Shimadzu UV-1800 UV-Visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan) operating in the 200–800 nm wavelength range was employed to assess the optical properties and validate the synthesised Ni-doped Ag nanoparticles. After dispersing the nanoparticles with distilled water, they were sonicated to prevent agglomeration and studied in quartz cuvettes. The optical band gap and electronic transitions were computed using Tauc plots of the surface plasmon resonance peak and absorption profile [16]. The identification of functional groups that are involved in bio-reduction and capping was accomplished by the utilisation of Fourier Transform Infrared Spectroscopy (FTIR) on a Bruker Alpha II spectrometer (Bruker Optics GmbH, Ettlingen, Germany) [17]. To find typical vibrations that were indicative of phenols, proteins, polysaccharides, and metal oxygen linkages, samples of dried nanoparticles were mixed with KBr, pressed into transparent pellets, and scanned (4000–400 cm^{−1}). A PANalytical X'Pert PRO diffractometer (PANalytical B.V., Almelo, The Netherlands), possessing radiation of Cuα (1.5406 Å), was used for powder XRD investigation [18]. The instrument was used with 40 kV and 30 mA, and patterns of diffraction were obtained under 2θ in the range between 10° and 80°. The identification of the crystalline phases was performed with the help of matching the observed peaks with JCPDS standard files, and the crystallite size was calculated by means of the (111) reflection with the help of the Debye–Scherrer equation shown below.

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

where λ is the wavelength (Cu Kα is 1.5406), D is the crystallite size (nm), K represents the Scherrer constant (shape factor, typically set at 0.9), and θ is the full width at half maximum (FWHM) of the Bragg diffraction angle of a chosen peak. Field Emission Scanning Electron Microscopy (FE-SEM) on a JEOL JSM-7610F (JEOL Ltd., Tokyo, Japan) was utilised for the examination of the surface morphology, particle distribution, and microstructural characteristics of the produced Ni-doped Ag nanoparticles [19]. A Quorum Q150R ES coater (Quorum Technologies Ltd., Laughton, East Sussex, UK) was used to sputter-coat samples with a small layer of gold before imaging to make them more conductive.

Sensitivity was evaluated through the agar well diffusion technique. Bacteria were cultured utilising sterile cotton pads and Muller–Hinton agar (MHA) Petri dishes. The MHA plates were bored with holes using an 8.0 mm sterile metallic cork borer [20]. The microbial samples were cultivated once more after a day. There were 1.5 × 10¹ CFU/mL of microorganisms, which is the same as half a McFarland's turbidity standard tube. For this experiment, Ni-doped Ag NPs were used at 30, 25, 20, 15, 10, and 5 mg concentrations. Approximately 100 mL of each concentration was introduced into the sterile Muller–Hinton and plates within their respective designated wells. As an oppressive parameter, 100 µL of

purified plant extract was subsequently utilised. The subsequent antibiotic dosages were administered: 30 µg of vancomycin for Gram-positive bacteria and 5 µg of gatifloxacin for Gram-negative bacteria. Then, the plates were incubated at 37 °C for 24 h. Using a zone measuring scale (Himedia, Mumbai, India), the inhibitory zones were measured in millimetres (mm) for each concentration [21]. An inhibitory concentration of Ni-doped Ag NPs was determined by finding the concentration that resulted in the smallest growth-inhibition zone. The techniques followed were in accordance with the criteria set forth by the Clinical and Laboratory Standards Institute, and each test was performed three times [7]. Ni-doped Ag NP stock solutions were prepared in sterile distilled water and serially diluted (two-fold) to achieve concentrations ranging from 0.625 to 80 µg/mL. Fresh cultures were standardised to the 0.5 McFarland standard (approximately 1×10^8 CFU/mL for bacteria and 1×10^8 CFU/mL for fungi) to prepare uniform microbiological suspensions. A total of 100 µL of nutritional broth (for bacteria) or Sabouraud dextrose broth (for fungi) was added to each well of a sterile 96-well microtiter plate, and then 100 µL of the Ni-doped Ag NP dilution was added. Lastly, each well received 10 µL of microbial inoculum.

To evaluate the anti-oxidant capabilities and compare the efficacies of different substances, the FRAP test is used. In this experiment, a possible anti-oxidant interacts with a colourless Fe^{3+} complex, resulting in the reduction of the complex to a very blue Fe^{2+} complex [22]. It is a well-liked, simple, appropriate, and repeatable way to measure anti-oxidant effectiveness [22]. FRAP and DPPH were used to determine anti-oxidant activity by spectrophotometrically monitoring the reduction of ferric ions and scavenging of DPPH radicals, respectively. Finally, all tests were triplicated, and the mean SD was used to describe the results. A one-way ANOVA was used to check for statistical significance; $p < 0.05$ was regarded as significant [23].

The α -amylase inhibitory property of the prepared Ni-doped Ag NPs was determined by the analysis described with minor alterations [24]. In a typical assay, 0.1 mL of Ni-doped Ag NPs (10–100 µg/mL) was mixed with 0.1 mL of 0.02 M sodium phosphate buffer (pH 6.9) and 0.1 mL of α -amylase solution (0.5 mg/mL) to obtain a final reaction volume of 0.3 mL [25]. A substrate of 500 µL of 1 percent starch solution was pre-incubated at 37 °C. The reaction mixture was incubated at 37 °C for an additional 10 min, after which 1.0 mL of dinitrosalicylic acid (DNS) reagent was incorporated to terminate the reaction. The solution was boiled in a water bath for 5 min, then chilled. It was subsequently diluted with distilled water, and the absorbance of the resulting solution was measured at 540 nm using a UV-Vis spectrophotometer. Acarbose was taken as a positive control. The percentage inhibition was determined by the formula:

$$\% \text{ Inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad (2)$$

where Abs are absorbance readings, indicating how much a substance blocks the enzyme from breaking down starch.

Ni-doped Ag nanoparticles (at various concentrations) were introduced into 100 µL of phosphate buffer (0.1 M, pH 6.8) and 50 µL of a 0.1 U/mL solution of 0.1 M α -glucosidase. The solution was incubated at 37 °C for a duration of 10 min. Subsequently, 50 µL of 5 mM p-nitrophenyl- α -D-glucopyranoside (pNPG) substrate was added and incubated once more at 37 °C. The reaction was concluded by the addition of 2 mL of 0.1 M Na_2CO_3 . The released p-nitrophenol was measured at 405 nm. The conventional inhibitor utilised was acarbose. The percentage of inhibition was determined using the same methodology as employed in the α -amylase assay. All the tests were conducted three times. The results were presented as mean and standard deviation. Nonlinear regression analysis was employed to ascertain the half-maximal inhibitory concentration (IC_{50}).

One-way ANOVA and post hoc Tukey tests demonstrated statistical significance and were applied accordingly. $p < 0.05$ is regarded as statistically significant [26].

3. Results and Discussion

3.1. Powder X-Ray Diffraction (PXRD)

The powder X-ray diffraction (PXRD) outlines of Ni-doped Ag nanoparticles displayed unique diffraction peaks indexed to the (210), (122), (111), (200), (220), (311), and (222) planes, as shown in Figure 2. These reflections resemble the characteristic face-centred cubic (FCC) lattice structure, confirming the crystalline nature of the Ni-doped Ag NPs. Consistent with previous results and JCPDS card number 04-0783, all of the detected peaks are identical. To determine the size of the crystallites in Ag powders that have been doped, the central diffraction peak of (111) is used. The crystalline structure of the Ag nanoparticles generated by the extract is clearly shown by the XRD results. The size of the Ag nanoparticles in two samples was determined to be about 55.22 nm and 18.56 nm, based on the full width at half maximum (FWHM) of the (111) diffraction peak, respectively, by measuring the width of the (111) reflection. In contrast, Ag nanoparticles produced using *Morinda citrifolia* leaf extract typically show larger crystallite sizes in the range of 28–35 nm, indicating comparatively weaker nucleation control [27]. This suggests that the phytochemicals in *C. umbellata* provide more effective reduction and capping, resulting in smaller, more crystalline nanoparticles.

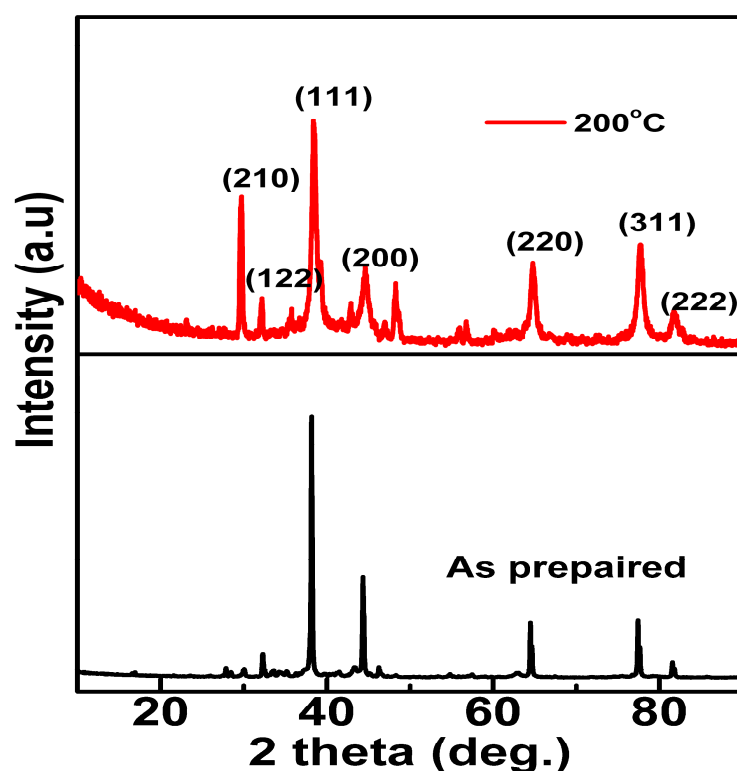


Figure 2. XRD pattern of the as-prepared and 200 °C annealed sample.

The synthesis conditions used in this study favour the primary reduction of Ag^+ ions, followed by the incorporation of Ni species into the growing Ag lattice and surface defect sites, even though the standard reduction potential of the $\text{Ni}^{2+}/\text{Ni}^0$ couple (-0.257 V) is significantly lower than that of Ag^+/Ag^0 ($+0.80$ V). Ni^{2+} ions are partly reduced and stabilised in phytochemical-rich environments by coordination with oxygen-containing functional groups, resulting in the formation of Ni–O–Ag interfacial connections instead of discrete metallic nickel nanoparticles.

The lack of secondary Ni or NiO diffraction peaks in the XRD patterns indicates that nickel either exists as highly distributed amorphous surface species below XRD detection limits or is substitutionally doped inside the Ag lattice at low concentrations. This behaviour is in line with earlier studies on heterometallic Ag–Ni complexes produced using environmentally friendly methods, where phytochemical ligands provide kinetically stable coordination environments to prevent nickel from oxidising and dissolving quickly.

Moreover, increased atomic diffusion and redistribution of Ni species within the Ag framework are indicated by the improved crystallinity and decreased crystallite size seen during annealing, which contribute to structural stability rather than phase segregation.

3.2. SEM Analysis

FE-SEM analysis was employed to observe the microstructure, morphology, and grain size distribution of the samples synthesised using NaOH and sintered at 200 °C (Figures 3 and 4). The micrographs revealed noticeable agglomeration, which can be ascribed to the ultrafine grain size of the nanoparticles. In addition, the morphology does not exhibit any discernible pattern or structure. The large volume of gases produced during the reaction is responsible for the fluffy texture and voids. The particles appeared polydisperse, with several clusters formed due to insufficient stabilisation by phytochemicals at lower temperatures. In contrast, the annealed sample displayed a markedly improved morphology, characterised by well-defined, predominantly spherical particles with smoother surfaces and reduced agglomeration. According to a quantitative analysis of FE-SEM micrographs, the as-synthesised Ag nanoparticles mostly had particle sizes between 40 and 80 nm, with observable agglomeration. The particle size range considerably shrank to 15–30 nm after annealing, which is consistent with an XRD study showing a smaller average size of around 18.56 nm. Following annealing, SEM examination shows a strong correlation with XRD-derived crystallite sizes, which also demonstrate better peak sharpness and a decrease in coherent domain size, indicating improved crystallinity. The fact that XRD reflects primary crystallographic domains while SEM catches secondary particle assemblies is the reason for the observed disparity between SEM and XRD sizes. Refined grain structure, less agglomeration, and a more uniform particle size distribution are the outcomes of annealing, which simplifies the elimination of organic residues and encourages atomic rearrangement.

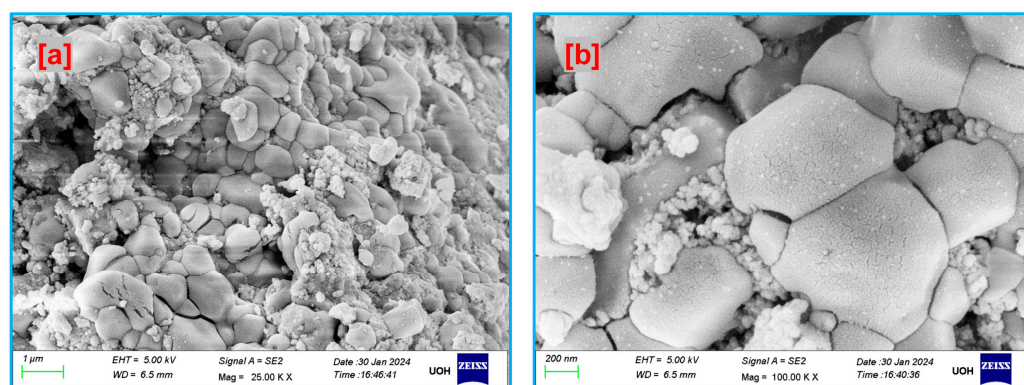


Figure 3. (a) Low magnification (b) high magnification FE-SEM images of the as-prepared sample with different magnifications.

The increased thermal energy facilitated the elimination of structural defects present in the prepared sample, leading to denser, compact nano-structures with more precise grain boundaries.

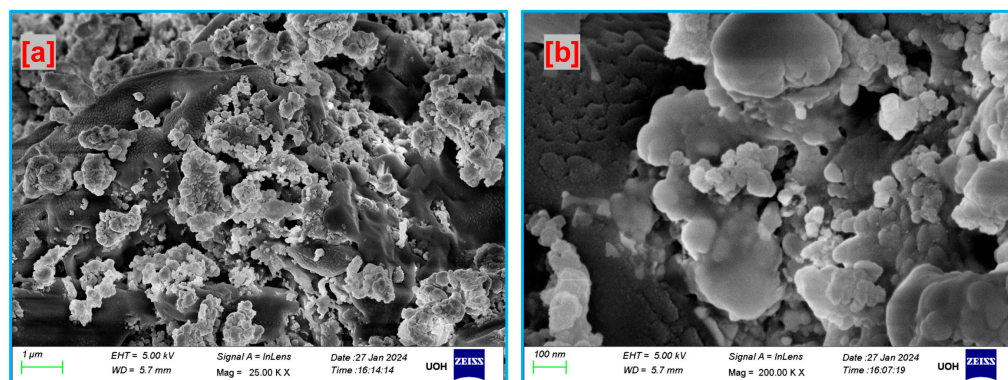


Figure 4. (a) Low magnification (b) high magnification FE-SEM images of a 200 °C annealed sample with different magnifications.

3.3. UV-Vis Absorbance Analysis

When characterising the structure of metal NPs, ultraviolet-visible spectroscopy (UV) is the method of choice. Figure 5 illustrates that at approximately 400 nm, the surface plasmon resonance (SPR) of the Ni-doped Ag NPs becomes evident. The peak seen here verifies that Ag NPs were produced by reducing silver nitrate. Perhaps because these Ag NPs are spherical, they exhibit an SPR peak in this region. Figure 5 also shows the direct optical band gap that was determined from the Tauc plot. The Ag NPs that were produced have a direct optical bandgap energy of 3.24 eV. The reported direct optical bandgap values for all samples showed a reduction in bandgap energy. Reduced particle size, increased crystallinity, and electrical interaction between Ag and Ni dopant states are all responsible for the modest blue shift in SPR that occurs after annealing. Instead of relying solely on agglomeration-driven effects, nickel inclusion modifies local electron density, which in turn affects plasmon oscillation frequency. Samples of Ni doped with Ag nanoparticles exhibit absorption peaks at various wavelengths in the UV-vis spectra. A heated Ni-doped Ag sample displays minor shifts in peak location, namely at 304 and 396 nm, in contrast to the untreated Ni-doped Ag sample, which exhibits an absorption edge at 306 and 398 nm, respectively.

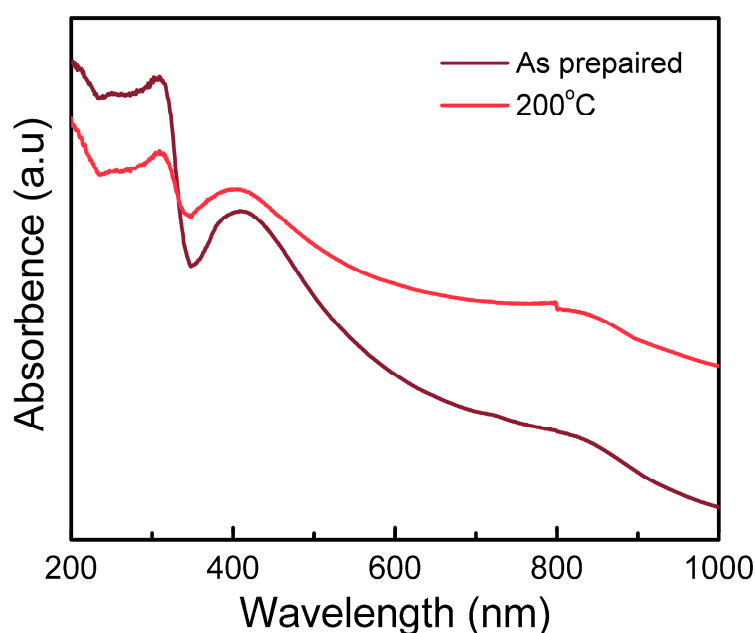


Figure 5. UV-Vis absorbance spectra of the as-synthesised and sintered at 200 °C samples.

Figure 6 shows the results of a Tauc plot using $h\nu$ against $(\alpha h\nu)^2$ to find the direct optical band gaps for all samples. The optical band gap values of the samples were determined to be 3.5 eV for Ni-doped Ag in the untreated state and 3.7 eV after heating. These results are in good agreement with the reported energy gap values for Ni-doped Ag nanoparticles. The optical behaviour of Ag nanoparticles is size dependent, as their band gaps vary from 2.51 eV to 3.51 eV depending on the production technique. Specifically, bandgap energies rose to 3.5–3.7 eV as a consequence of Nickel substitution.

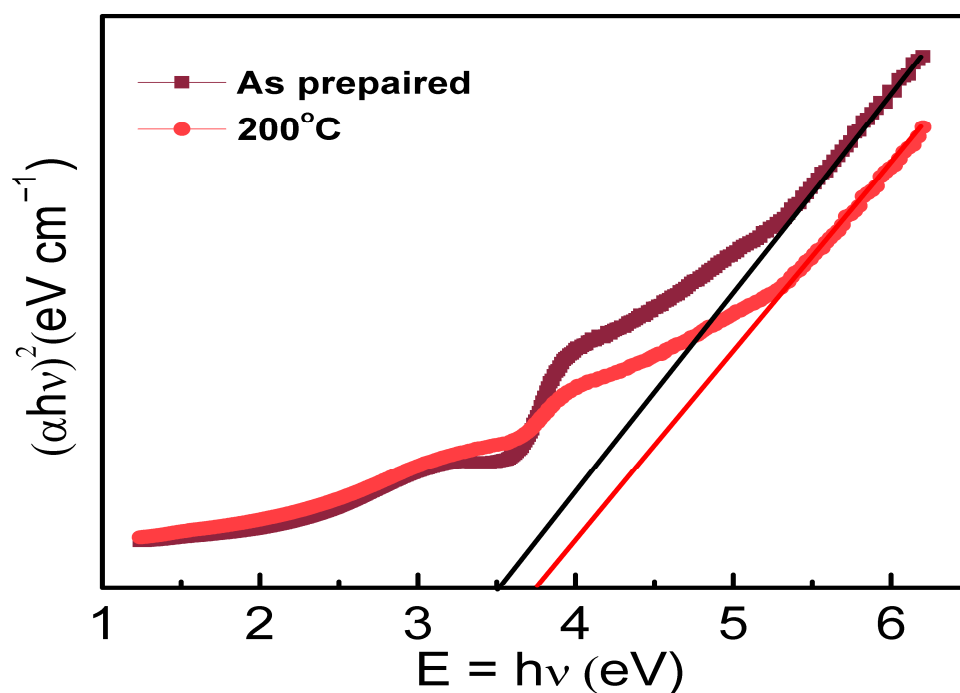


Figure 6. Tauc plot: Variation of $(h\nu)^2$ versus photon energy of the as-synthesised and sintered at 200 °C samples.

3.4. Structural and Optical Analysis

3.4.1. FTIR Analysis

FTIR spectra of the synthesised Ni-doped Ag NPs on *Caralluma umbellata* root extract, when freshly prepared and after annealing (200 °C), indicate that there are distinguishable vibrational characteristics corresponding to the phytochemical capping of the nanoparticles and metal–ligand interactions (Figure 7). At 3337.21 and 3288.53 cm^{-1} , there is a broad band, which can be identified as O–H stretching of polyphenols and flavonoids, and proves their participation in the bio-reduction of Ag^+ and Ni^{2+} ions. The C–H stretching of aliphatic groups causes the peaks of 2939.94 and 2934.94 cm^{-1} , which confirms the existence of organic residues of the extract. The strong absorptions at 1597.83 and 1596.77 cm^{-1} indicate aromatic C=C stretching and/or amide I vibrations, indicating that the presence of proteinaceous or phenolic elements serves as stabilising ligands about the nanoparticle surface. The C–N stretching and C–H bending bands of phenolic components can be observed between 1377.89 and 1309.42 cm^{-1} , which contributes to the additional evidence of the plant metabolic nanoparticle functionalisation. Peaks at 1056.07 and 1040.04 cm^{-1} are very strong and are indicative of the C–O stretching of alcohols, esters, and polysaccharides, which are common for the capping process during green synthesis. Significantly, the low-frequency band at 817.55 cm^{-1} implies that there is metal–oxygen (Ni–O and Ag–O) stretching vibrations, which confirms nickel ions have been successfully incorporated into the silver nanoparticle scaffold. The FTIR peaks of the Ni-doped Ag NPs prepared using *Caralluma umbellata* were very similar to the previously reported *Moringa oleifera* Ag

NPs. The general OH band at $3337\text{--}3288\text{ cm}^{-1}$ indicated the presence of phenolics in the reduction in *M. oleifera*. The aromatic/amide peak ($1597\text{--}1596\text{ cm}^{-1}$) corresponded to the $1590\text{--}1605\text{ cm}^{-1}$ band of *M. oleifera*. Similarly, there was a close agreement between the C-N/C-O region at $1377\text{--}1309\text{ cm}^{-1}$ and $1377\text{--}1309\text{ cm}^{-1}$, which was a signal of similar phytochemical capping activity [28].

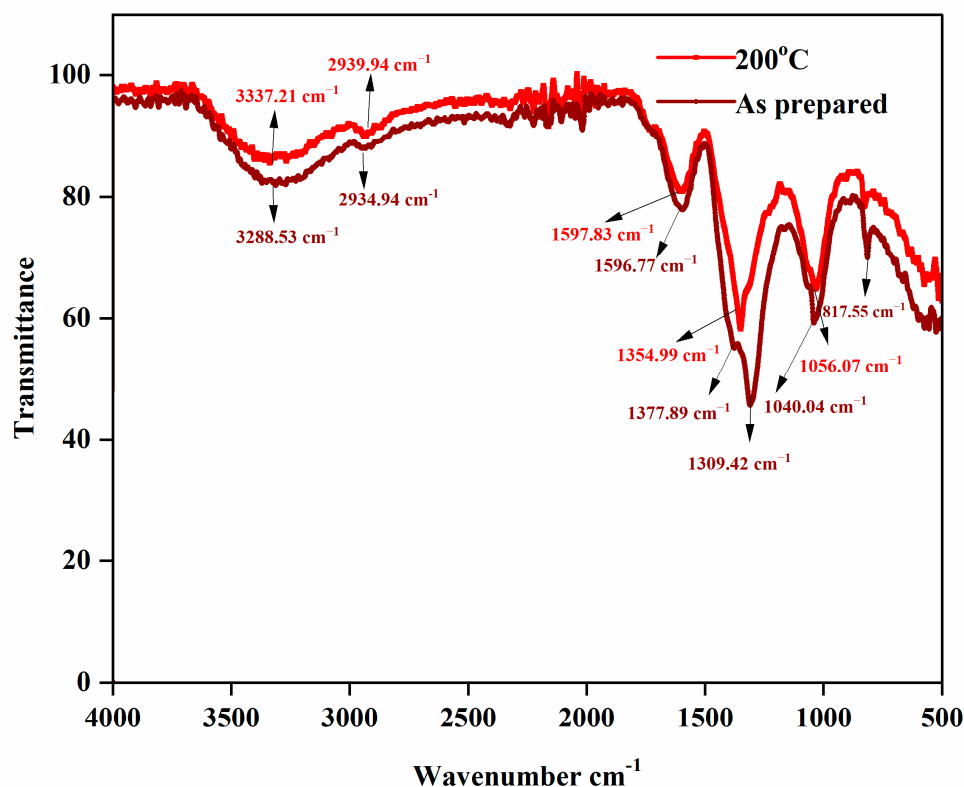


Figure 7. FTIR transmission spectra of the as-synthesised and sintered at $200\text{ }^{\circ}\text{C}$ samples.

Comparative analysis of the two spectra indicates that annealing at $200\text{ }^{\circ}\text{C}$ decreases the intensity of the OH and CH vibrational bands, which is indicative of thermal degradation of the organic capping layers, and enhanced purity of the metallic core. At the same time, the sharpness and intensity of the metal–oxygen peak increase after heating, which signifies the increased crystallinity and the improvement of the metal–ligand coordination. This spectral development is consistent with thermally treated, plant-mediated Ag NPs observed in the literature, which again proves the structural integrity and compositional stability of the Ni-doped Ag NPs prepared using *C. umbellata* [29]. Under atmospheric circumstances, nickel nanoparticles are known to experience fast surface oxidation. But in the current system, the phytochemical capping made from *C. umbellata* root extract is essential for stabilising the system and preventing nickel species from being directly exposed to air. The production of persistent Ni–O–Ag or Ni–O–organic interfacial contacts is suggested by FTIR analysis, which verifies the persistence of metal–oxygen coordination bands even after annealing.

The chemical permanence of Ni inclusion rather than its quick dissolution is further supported by the preservation of antibacterial and anti-oxidant activities following heat treatment. As a result, nickel in the current nanoparticles behaves as a stable dopant inside a silver-dominant nano-structure rather than as free nanonickel, significantly reducing concerns about quick environmental oxidation.

3.4.2. Optical Properties

Impedance spectroscopy is an effective method for investigating the conductivity characteristics of nanocomposites. An observed trend reveals Z' values decreasing with frequency, ultimately converging at higher frequencies. Particularly notable are the elevated Z' values in the lower frequency range, followed by a decline at higher frequencies (Figure 8), indicative of polarisation effects. This pattern confirms the insulating behaviour attributed to the formed nanoparticles.

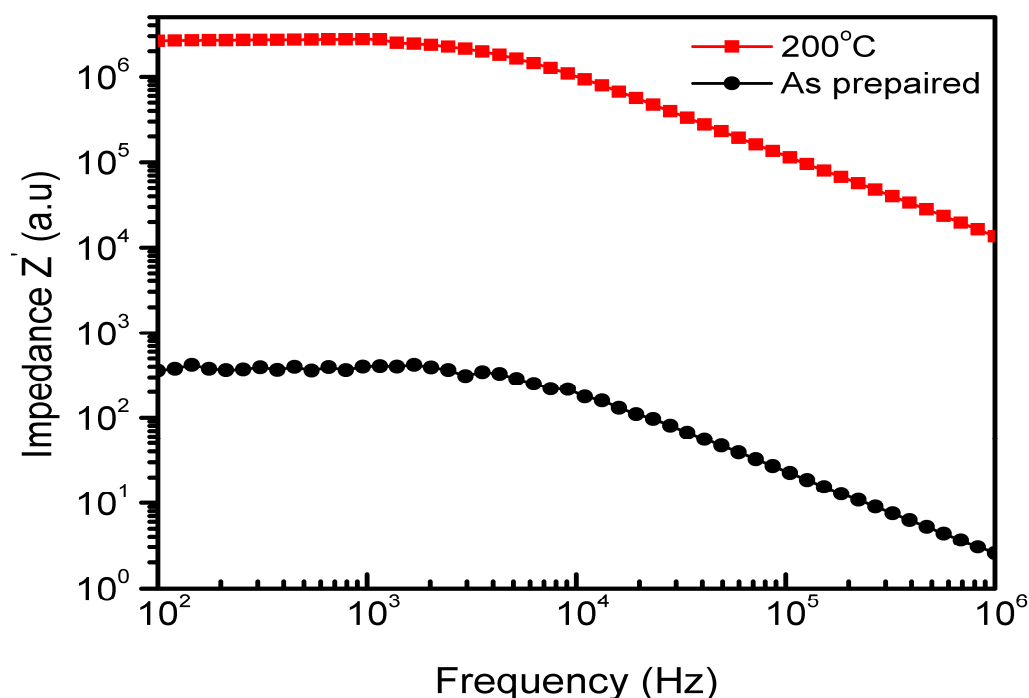


Figure 8. Impedance vs. frequency for the as-prepared and 200 °C sample.

The relative permittivity resistance serves as a crucial parameter delineating the polarisation characteristics of a medium. As depicted in Figure 8, the resistance of nanocomposites with and without annealing and doping contents is explored across the frequency range of 100–1,000,000 Hz. In this visualisation, it is notable that the resistance of all samples diminishes with escalating measurement frequency, aligning with the fundamental principles of dielectric physics. At lower frequencies, polarisation is readily established, while at higher frequencies, dipole polarisation gradually fails to synchronise with the frequency variations. Additionally, within the nanocomposites, an increase in doping content correlates with a decrease in relative permittivity. Orientation polarisation and interfacial polarisation emerge as primary determinants influencing relative permittivity. Following the Potential Barrier Model, the decline in relative permittivity is attributed to the constraint imposed on dipole movement within the interaction zone.

Figure 9 illustrates the loss tangent of Ni-doped Ag NPs at room temperature. The dielectric loss in these composites comprises conductivity loss and polarisation loss. The loss tangent of the composites exhibits minimal variation at low frequencies due to weak interfacial polarisation. However, as the frequency increases, the loss tangent of the composites gradually rises, attributed to the inability of interface polarisation to match the changing frequency of the electric field at high frequencies. This increase in loss tangent is driven by the augmented polarisation loss from dipole reorientation, leading to an amplified disparity in the loss tangent with increasing frequency.

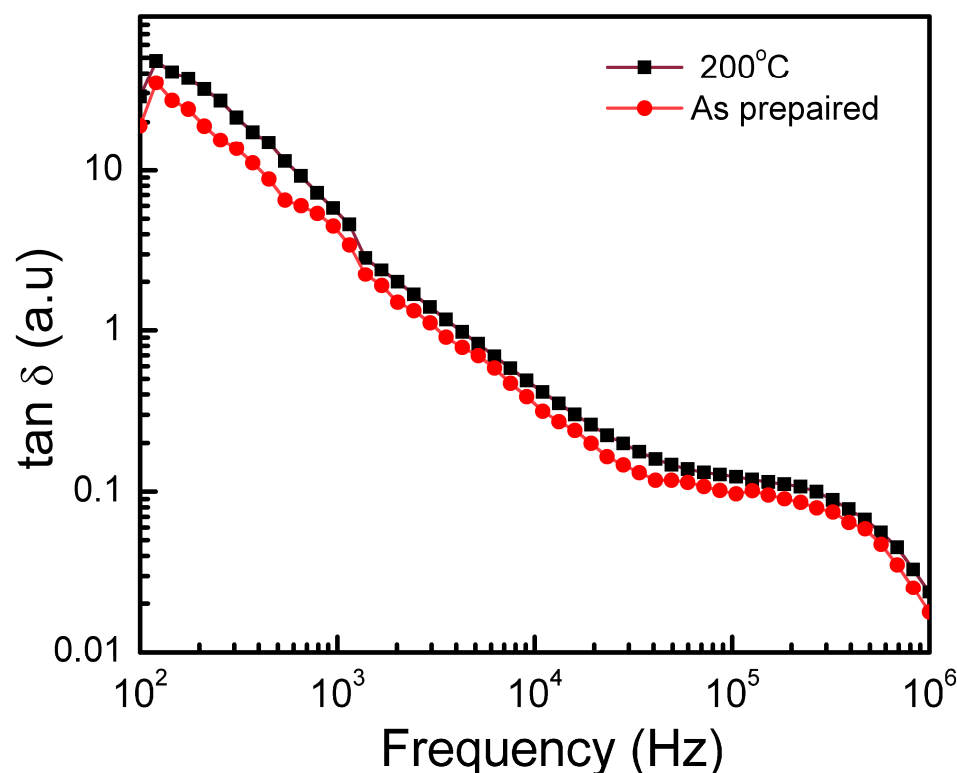


Figure 9. $\tan\delta$ vs. frequency for the as-prepared and 200 °C sample.

3.5. Antimicrobial

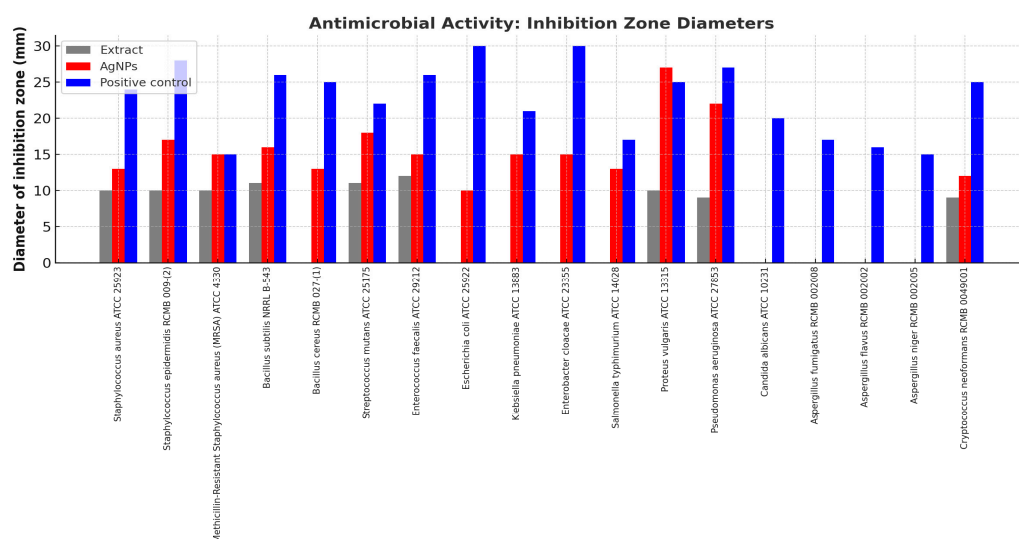
3.5.1. Antimicrobial Sensitivity Testing

The agar well diffusion method was used to test the antimicrobial activity of Ni-doped Ag nanoparticles made from the root extract of the *Caralluma umbellata* plant. The results showed that the nanoparticles were very effective at killing Gram-positive bacteria, Gram-negative bacteria, and fungal isolates. According to Table 1 and Figure 10, Ni-doped Ag NPs were always able to produce bigger circles of inhibition as compared to the crude extract, thus proving the increase in antimicrobial potency by metal doping and nanoparticle formation. The nanoparticles had a pronounced activity against *Staphylococcus epidermidis* (17 ± 1 mm), *Streptococcus mutans* (18 ± 0.58 mm), and MRSA ATCC 43300 (15 ± 1 mm) among Gram-positive bacteria, suggesting a substantial disruption of the thick peptidoglycan layers and interference with cell wall integrity. Even with the more resistant Gram-negative bacteria (which in most cases have an outer membrane that prevents this type of activity), with Ni-doped Ag NPs, the inhibitory effect was quite significant, with *Proteus vulgaris* (27 ± 0.58 mm) and *Pseudomonas aeruginosa* (22 ± 1 mm) being most susceptible. This implies that the nanoparticles are reactive enough on their surfaces to enter or destabilise lipid-rich outer membranes [30]. Fungal pathogens were relatively less sensitive, but *Cryptococcus neoformans* exhibited a detectable zone of inhibition of 12 ± 1 mm, which was indicative of the reaction of nanoparticles with ergosterol-containing fungal membranes. Overall, it was discovered that the Ni-doped Ag nanoparticles are significantly more effective than the plant extract alone and show notable antimicrobial efficacy at much lower concentrations than crude plant extracts, providing a supplementary rather than a direct replacement for traditional antibiotics. Thus, it proves that *C. umbellata* root phytochemicals are effective reducing and stabilising agents, and that Ni doping has a significant effect on antimicrobial activity.

Table 1. Inhibition zone diameters of Ni-doped Ag NPs from *Caralluma umbellata* plant root extract using the agar plate diffusion assay.

Selected Strain	Zone Inhibition Diameter (mm)		
	Root Extract	Ni-Doped Ag NPs	Positive Control
Bacteria (Gram-positive)			
<i>Bacillus subtilis</i> NRRL B-543	11 ± 0.58	16 ± 0.58	26 ± 1
Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA) ATCC 4330	10 ± 0	15 ± 1	15 ± 0.58
<i>Staphylococcus epidermidis</i> RCMB 009-(2)	10 ± 1	17 ± 1	28 ± 1
<i>Staphylococcus aureus</i> ATCC 25923	10 ± 1	13 ± 1	24 ± 0
<i>Bacillus cereus</i> RCMB 027-(1)	NA	13 ± 1	25 ± 0
<i>Enterococcus faecalis</i> ATCC 29212	12 ± 0.58	15 ± 0.58	26 ± 1
<i>Streptococcus mutants</i> ATCC 25175	11 ± 0.58	18 ± 0.58	22 ± 0.58
Bacteria (Gram-negative)			
<i>Proteus vulgaris</i> ATCC 13315	10 ± 0	27 ± 0.58	25 ± 1
<i>Enterobacter cloacae</i> ATCC 23355	NA	15 ± 0.58	30 ± 0.58
<i>Klebsiella pneumoniae</i> ATCC 13883	NA	15 ± 1	21 ± 0.58
<i>Escherichia coli</i> ATCC 25922	NA	10 ± 0.58	30 ± 1
<i>Pseudomonas aeruginosa</i> ATCC 27853	9 ± 0	22 ± 1	27 ± 0
<i>Salmonella typhimurium</i> ATCC 14028	NA	13 ± 0.58	17 ± 1
Fungi			
<i>Aspergillus niger</i> RCMB 002005	NA	NA	15 ± 0
<i>Aspergillus flavus</i> RCMB 002002	NA	NA	16 ± 1
<i>Aspergillus fumigatus</i> RCMB 002008	NA	NA	17 ± 1
<i>Cryptococcus neoformans</i> RCMB 0049001	9 ± 0.58	12 ± 1	0
<i>Candida albicans</i> ATCC 10231	NA	NA	20 ± 0.58

NA, No antimicrobial activity. The result shown in bolt undergoes further testing to determine minimum inhibitory concentration (MIC).

**Figure 10.** Agar well diffusion assay inhibition zone diameter graphical chart of Ni-doped Ag NPs.

3.5.2. MIC Analysis

Agar well-diffusion testing was used to conduct AST. It was shown that Ag-CS NPs inhibited all of the tested isolates. The obtained MIC results of the Ni-doped Ag nanoparticles prepared through *Caralluma umbellata* root extract are similar to the inhibition patterns on the agar diffusion plates. The Ni-doped Ag nanoparticles prepared by the *Caralluma umbellata* root extract indicated a high dose-effective antimicrobial concentration of the root extract against the clinically significant pathogenic microorganisms. The MIC values, as Table 2 and Figure 11 show, were in the range of 1.25 through to 10 µg/mL, which means that very low levels of nanoparticles were enough to prevent microbial growth. The greatest sensitivity was observed with *Streptococcus mutans* with an MIC of 1.25 g/mL, giving the impression that the nanoparticles have a close association with the thick peptidoglycan matrix of Gram-positive cocci. By making reactive oxygen species (ROS) and attaching to membrane proteins, the Ni-doped Ag NPs probably damage the structure of cells, killing bacteria quickly. *Proteus vulgaris* (MIC: 2.5 µg/mL) and *Pseudomonas aeruginosa* (MIC: 5 µg/mL) were not inferior in terms of susceptibility as well, and showed that the nanoparticles can penetrate or destabilise the outer LPS-rich membrane of Gram-negative species, which is more resistant because of the complex structure. A small increment in concentration was necessary to overcome those strains that are more resistant, like MRSAATCC 43300, *Klebsiella pneumoniae*, and *Salmonella typhimurium* (MIC: 10 g/mL), though still lower levels compared to most of the plant-derived or chemically synthesised Ag NPs, which further reinforces the claim of improved antimicrobial activity through doping with Ni. The overall low values of MIC confirm the notion that the nanoparticles have high levels of bactericidal activity at low concentrations, which can be explained by the fact that the Ag⁺ release, Ni-induced catalytic stress, and phytochemical capping agents present in *C. umbellata* are beneficial to stability and bioavailability. All these results combine to support the potential of Ni-doped Ag nanoparticles as a promising next-generation antimicrobial agent with the potential to act on a wide range of pathogens of high potency and low dosage. Ag⁺ ion release, Ni-induced catalytic stress, and phytochemical surface functionalisation work in concert to increase the antibacterial efficiency of Ni-doped Ag NPs as compared to the plant extract alone. Increased production of reactive oxygen species (ROS) at the nanoparticle–cell interface is made possible by nickel inclusion, which encourages localised electronic disturbances inside the Ag lattice.

Table 2. The agar plate diffusion test of a fold change in the diameters of the inhibition zones of Ni-doped Ag NPs.

Selected Strain	MIC (µg/mL)
<i>Pseudomonas aeruginosa</i> ATCC 27853	5
<i>Streptococcus mutans</i> ATCC 25175	1.25
<i>Salmonella typhimurium</i> ATCC 14028	10
<i>Klebsiella pneumoniae</i> ATCC 13883	10
Methicillin-Resistant <i>Staphylococcus aureus</i> (MRSA) ATCC 4330	10

Crucially, nickel is added as a structurally bound dopant rather than a freely diffusible hazardous metal, reducing uncontrolled environmental discharge. The antibacterial activity is obtained at sub-toxic doses at the concentrations used (MIC values as low as 1.25 µg/mL), which is consistent with green nanomedicine concepts aiming at dose reduction and improved effectiveness.

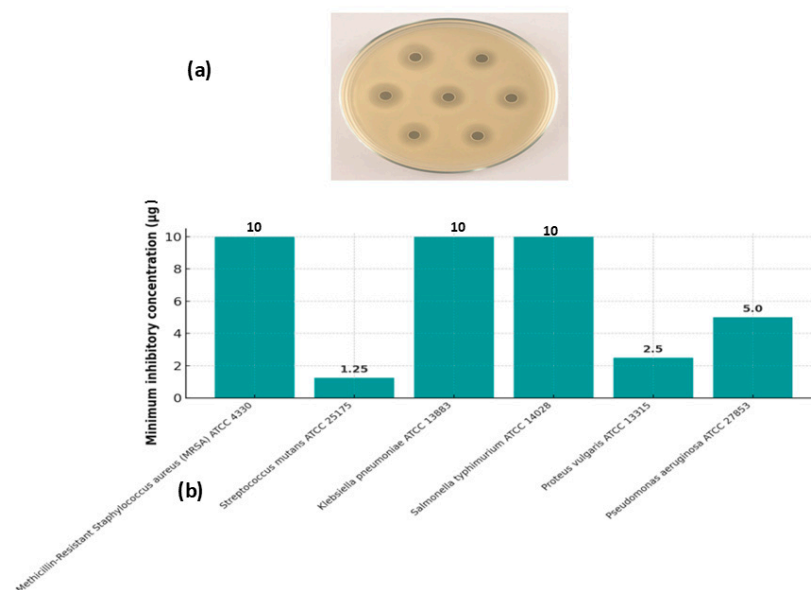


Figure 11. (a) Minimum inhibitory doses of Ni-doped Ag NPs (1 mg/mL) determined via the agar plate diffusion assay (b) MIC concentration analysis in different bacterial stains.

3.6. Anti-Oxidant Activities

FRAP and DPPH assays were conducted to determine the anti-oxidant properties of the Ni-doped Ag NPs that had been synthesised, and the data are shown in Figure 12a,b. In the FRAP test (Figure 12a), the RSA was rising continuously between 31.15% at 10 ppm and 37.38% at 50 ppm, which proved that the reduction potential is strongly concentration-dependent. In all the concentrations tested, the Ni-doped Ag NPs had a higher reducing power than the ascorbic acid standard, which means that the nanoparticles have a more efficient reducing power. This excellent performance is mainly owed to the phytochemical-enriched capping layer that is deposited on the surface of the Ag NPs during the green synthesis. Flavonoid, phenolic, and aromatic compounds contain abundant electron-donating hydroxyl groups, which participate in hydrogen atom transfer and contribute to the reduction of Fe^{3+} to Fe^{2+} . The radical intermediates, which are stabilised by resonance during this process, further amplify the redox cycling of the nanoparticles, which increases the FRAP response of the nanoparticles in comparison to traditional anti-oxidants. This high reducing action is in line with previous reports on plant-derived Ag NPs synthesised with *Azadirachta indica*, *Rhus coriaria*, and *Moringa oleifera*, where FRAP values would generally be between 25 and 36% at comparable concentrations, thus showing that the present Ni-doped Ag NPs would exhibit anti-oxidant action at the highest level of performance range of reported phytochemical Ag NPs.

Similarly, in the DPPH assay (Figure 12b), there was a strong concentration-dependent radical quenching effect of the nanoparticles. The significant reduction in absorbance at 517 nm was found with an increase in the concentration until the 4 mL mark, which indicated very effective scavenging of the nitrogen-centred free radicals. Ni-doped Ag NPs were obtained, which consistently exhibited higher DPPH inhibition when compared to the ascorbic acid reference at concentrations of up to 55–58% RSA, which is indicative of robust hydrogen atom transfer (HAT) and single-electron transfer (SET) activity. This sharp increase in scavenging efficiency as the volume increased indicates a smaller IC_{50} value of the nanoparticles, which is a reflection of the higher anti-oxidant activity than traditional plant extracts and most systems based on Ag NPs. As an illustration, DPPH RSA values of Ag NPs synthesised with *Curcuma longa* [31] and *Allium sativum* [32] normally report around 40–48% in similar concentrations, but the current Ni-doped Ag NPs show about

10–15% in terms of the enriching influence of nickel introduction and phytochemical-rich surface passivation.

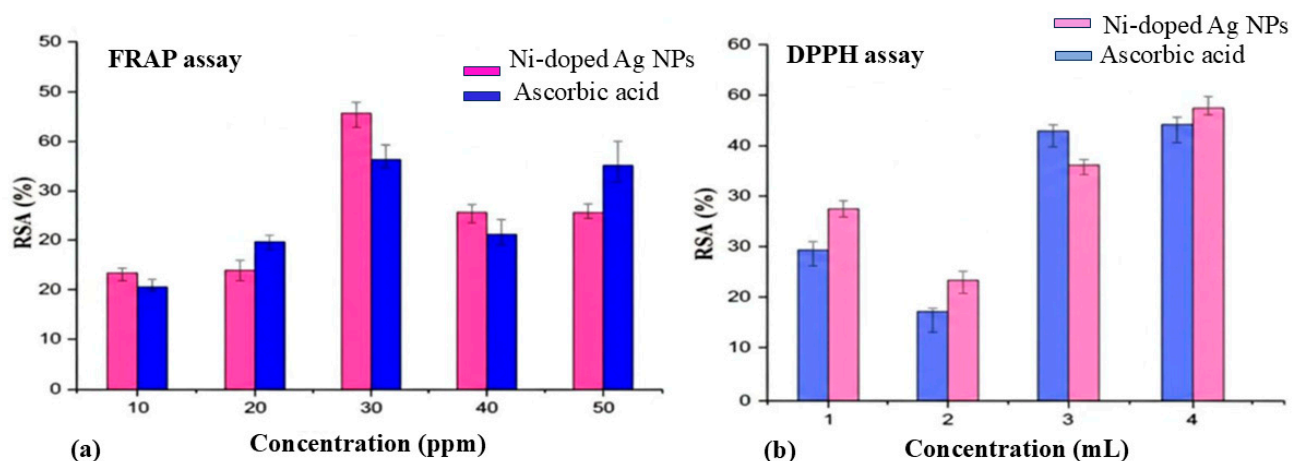


Figure 12. (a) FRAP assay; (b) DPPH radical-scavenging capacity assay of Ni-doped Ag NPs.

The fact that the DPPH response is stronger than the FRAP values indicates that the nanoparticles are more effective in direct radical neutralisation as compared to ferric ion reduction. This is in line with the availability of phenolic compounds, in which OH groups will easily donate hydrogen to DPPH radicals, to produce stable phenoxy groups. When compared to other doped nanoparticle systems reported in the literature, such as Cu-doped Ag, Zn-doped Ag, and Fe-doped metal nanoparticles, the present Ni-doped Ag NPs exhibit comparable or superior anti-oxidant activity at lower concentrations. For instance, Cu- and Zn-doped Ag nanoparticles typically report DPPH scavenging efficiencies of 40–55% at comparable doses; however, the current system reaches up to ~58%, indicating the beneficial redox contribution of nickel doping in combination with phytochemical surface functionalisation. Moreover, the metabolites bound to the surface facilitate electron transfer and stabilise the resulting nanoparticle radical complexes, which avoids the further progression of oxidative reactions. The synergistic effect of redox-active surface biomolecules, increased electron density with incorporation of nickel, and the inherent catalytic action of silver nano-structures, therefore leads to the combined FRAP and DPPH results. Thus, the anti-oxidant properties of the nanoparticles can be compared to those of plant-mediated Ag NPs, and in most instances, they are even greater, which indicates their high potential in the future as natural anti-oxidant decoagulants.

3.7. Anti-Diabetic Activities

Table 3 demonstrates that the Ni Ag NPs had a strong inhibitory effect that was dose-dependent, with a 68.1% and 82.4% inhibition of α -amylase and α -glucosidase, respectively, in the presence of 50 and 100 $\mu\text{g/mL}$. The values of IC_{50} of the Ni-doped Ag NPs were similar to those of acarbose (42.1 $\mu\text{g/mL}$ and 36.5 $\mu\text{g/mL}$) and indicate their strong enzyme-inhibitory activity. This inhibition is a direct indication of the capability of the nanoparticles to delay the hydrolysis of complex carbohydrates into glucose and hence assist in regulating post-prandial hyperglycemia. The improved capability of inhibition is explained by the synergistic effect of the metallic nanoparticle core, the catalytic effect of nickel doping, and the bioactive functional groups (detected by FTIR) present on the capping layer formed by the roots of *C. umbellata*, which have insulin-sensitising or insulin-mimetic capabilities [33]. Though the experiments with certain molecular pathways were not within the frames of the present work, nanoparticles of the same profile have been reported to target insulin receptors and can affect the metabolic signalling pathways, including AMPK and PI3K/AKT.

Translocation of GLUT-4 transporters to the cell surfaces, which are linked to the activation of these pathways, boosts glucose uptake in insulin-sensitive tissues. The potential mechanistic action is represented in Figure 13. In addition, the proven anti-oxidant activity of the Ni Ag NPs through the FRAP test and DPPH test boosts its anti-diabetic applicability to a great extent. The nanoparticles can provide protection to pancreatic 6-cells and enhance general glucose homeostasis at least by scavenging reactive oxygen species (ROS), which is one of the causes of insulin resistance and 6-cell dysfunction [34]. This anti-oxidant activity is probably the focus of the phytochemical capping of *C. umbellata*. To summarise, the results indicate that the Ni-doped Ag NPs prepared with *C. umbellata* root extract have a multi-modal anti-diabetic activity, which includes effective digestive enzyme inhibition with a possible insulin pathway regulation and effective anti-oxidant cell protection [35]. Although these in vitro findings are very promising, more molecular, cellular, and in vivo analysis is required to confirm their therapeutic usefulness and to completely understand the mechanism pathways.

Table 3. α -Amylase and α -Glucosidase inhibitory activity of *C. umbellata* extract and Ni-doped Ag NPs.

Sample	Concentration ($\mu\text{g/mL}$)	α -Amylase Inhibition (% $\pm$ SD)	α -Glucosidase Inhibition (% $\pm$ SD)
<i>C. umbellata</i> root extract	50	32.5 ± 1.8	28.4 ± 2.1
	100	45.2 ± 2.3	38.7 ± 1.9
Ni-doped Ag NPs	50	68.1 ± 1.5	72.3 ± 2.0
	100	82.4 ± 1.2	88.6 ± 1.4
Acarbose (Standard)	50	74.5 ± 0.9	79.8 ± 1.1

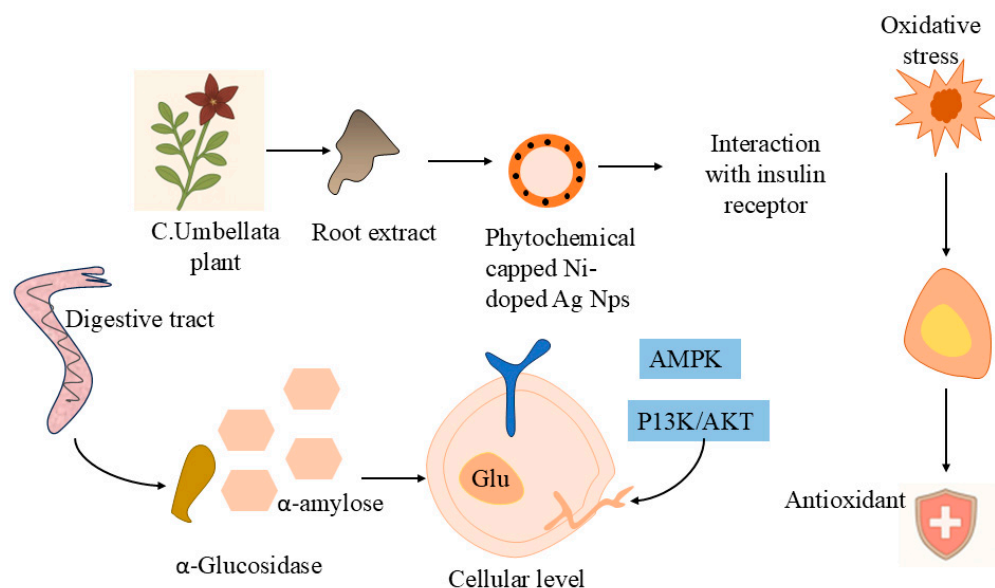


Figure 13. Assumed mechanism of the anti-diabetic property of Ni-doped Ag NPs using *C. umbellata* root extract.

4. Conclusions

This research was able to show the green synthesis of nickel-doped silver nanoparticles (Ni-doped Ag NPs) with the reducing and capping agent being a root extract of *Caralluma umbellata* in aqueous solution. The synthesis of the nanoparticles was thoroughly characterised, and confirmed that the crystallites were spherical and face-centred cubic. XRD analysis showed crystallite sizes of about 55.22 nm in the as-prepared sample, and 18.56 nm

in the one annealed at 200 °C, whereby the annealing process increased the crystallinity and decreased the agglomeration process as measured through FE-SEM. UV-Vis spectroscopy presented a peak of a surface plasmon resonance at 396 nm and a direct optical band gap of 3.24 eV, as calculated. FTIR showed the presence of major phytochemical functional groups (O-H, C=O, and C-O) with bio-reduction and stabilisation, as well as the presence of metal–oxygen links, which showed successful Ni doping. The Ni-doped Ag NPs had a potent and broad-spectrum antimicrobial action with a high inhibitory effect on *Proteus vulgaris* (27 ± 0.58 mm zone) and *Streptococcus mutans* (MIC = 1.25 µg/mL). In addition, the nanoparticles exhibited strong in vitro anti-oxidant activity in FRAP and DPPH studies and had a preliminary anti-diabetic effect in the form of α -amylase and α -glucosidase enzyme inhibition. All these results highlight the potential of *Caralluma umbellata* root extract to develop stable, multifunctional Ni-doped Ag NPs with potential use in antimicrobial therapy, anti-oxidant delivery, and diabetes management. While the present study demonstrates promising antimicrobial and anti-diabetic activity, cytotoxicity evaluation in non-pathogenic mammalian cell lines was beyond the scope of this work. It will be addressed in future investigations before clinical translation.

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