Green Synthesis of Silver Nanoparticles using *Randia dumetorum* seed, Characterization and Antioxidant activity

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Abstract

New trend of an eco-friendly process for the synthesis of silver nanoparticles (AgNPs) is an important aspect in the field of nanotechnology. In recent years, the utilization of secondary metabolites from plant leaf broth has emerged as a novel technology for the synthesis of various nanoparticles. In this report, silver nanoparticles were synthesized by the leaf extract of Randia dumetorum. Effect of different reaction parameters such as precursor salt concentration and leaf broth percentage morphology of the (AgNPs) were analyzed. The plant biomolecules induce the reduction of Ag^{1+} ions to Ag^{0} nanoparticles and also act as a capping and stabilizing agent. The formation of (AgNPs) was monitored by absorbance spectra of UV-visible spectrophotometer at different stages during the synthesis process.

The biosynthesized (AgNPs) were characterized by different instrumental techniques and the particles obtained are crystalline having spherical shape with the average size 25 nm and are highly stable. The optimum conditions for synthesis are as follows: percentage of seed broth 5%, $[AgNO_3]$ = 1 milimole and temperature 85 °C. The present study could prove to have an enormous impact in the immediate future to synthesize metallic nanoparticles on an industrial scale, biological activities, etc.

Keywords: Green synthesis, *Randia dumetorum* seed, Silver nanoparticles Antioxidant activity.

Introduction

Nanotechnology is most critical in science and technology due to which excellent properties are more important. Nanoparticles are widely used in biological, chemical, environmental and pharmaceutical industries etc.[1] In addition, nanoparticles are also used in packaging. Nanotechnology is the most crucial in science and technology, due to which excellent properties are of great importance. Nanoparticles are widely used in technology in biological, chemical, environmental and pharmaceutical industries etc.[2]

In addition, nanoparticles are also used in packaging and food preservation processes, drug delivery, water purification, storage devices, tissue engineering etc. They are used as antimicrobial agents, as cytotoxic agents, in bone regeneration, cell adhesion capabilities and in bio-labeling etc.[3]

However, the synthestie approaches are both expensive and harmful to the environment, so in the new era, to overcome these problems, we have developed green synthesis methods that are environmentally friendly, simple and cheap. We can synthesize nanoparticles using inorganic metals such as platinum, gold, silver, copper, zinc, iron etc. which can be used in numerous industrial applications. Many researchers suggest using silver nanoparticles to synthesize those using biological, physical and chemical methods as effective antimicrobial agents and with low toxicity to humans.[4]

The process of producing nanoparticles through physical methods of silver nanoparticles is very expensive. It requires very high vacuum and the chemical process uses toxic chemicals like sodium citrate and lead oxide. This produces toxic by-products during chemical processing[5] due to which it became very necessary to adopt a new method. Biological process was used instead of chemical process and its synthesis process and increased adverse effects have been seen in medical application. In the green synthesis process, we use plants, fungi, bacteria, algae etc.[6] as reducing agents, capping agents and stabilizing agents. Green synthesis has led to great progress in nanotechnology and its by-products are also not harmful to the environment and are a safe process. Various functional groups such as carboxylate, carbonyl and amine can bind the surface of the formed NPs to avoid their aggregation.[7]

Randia dumetorum (family Rubiaceae) is highly reputed Ayurveda medicinal tree commonly known as the Madanphal, Mindhal. It is seen in Gujarat, Tamilnadu, forest of Dehradun, Suralik range, Bengal, Bihar, Orrisa, South Maharashtra and costal districts of south India. Extracts of plant leaves, branches, roots, fruits, seeds etc. are made in water or alcohol. Plants contain many organic compounds that are used in various medical applications such as analgesic, antitumor, hepatoprotective, antipyretic, anti-inflammatory, antioxidant etc. Using this extract, metal nanoparticles are made which are used in many industries such as dairy degradation, textile industry, increasing drug reactivity, making metal machines etc.[24]

A review of existing literature on AgNPs highlights their synthesis, functionalization and extensive use across various fields with a strong emphasis on biomedical applications. Researchers have explored various synthetic methods

aiming to optimize particle size, shape and surface properties as these factors influence their effectiveness in biological systems. The antimicrobial and anticancer properties of AgNPs are well-documented with studies investigating their interactions with pathogens and cancer cells to understand their mechanisms of action and enhance therapeutic outcomes.[8] The vast body of research underscores the versatility of AgNPs and their potential for continued development in targeted therapies, diagnostics and environmental applications.[9]

The biological activity of AgNPs, notably their antibacterial and anticancer properties, has been widely researched, demonstrating their potential to break microbial cell membranes, create reactive oxygen species (ROS) and trigger apoptosis in cancer cells.[10] Their selective cytotoxicity makes them valuable for treating infections and targeting cancer cells while sparing healthy cells in lower concentrations. These properties also enable their use in drug delivery systems, where AgNPs can be engineered to deliver drugs precisely to diseased cells or tissues.[11-14] This study focuses on synthesizing AgNPs using *Randia dumetorum* seed extract to create NPs with improved stability, bioavailability and efficacy for antimicrobial and anticancer applications.

Material and Methods

Materials and reagents: Silver nitrate was used as a precursor salt and mindhal (*Randia dumetorum*) seeds were collected from Visnagar market, Gujarat, India. Double distilled water was used throughout the study. Seeds of mindhal were rinsed thoroughly 2-3 times with double distilled water to remove dust and unwanted particles. Seed were dried for 2 days at room temperature and then ground to powder with a mortar and pestle. 250 mL conical flask containing 5 gm. of powder of seed with 100 mL double distilled water was heated at 85°C temperature for 30 min with stirring. Obtained seed powder broth was filtered and stored at 4 °C temperature for further experiments.

Preparation of an aqueous extract of Randia Dumetorum seed: In a synthetic procedure, AgNPs were obtained via a green reduction route. The flask containing an aqueous solution of salt $AgNO_3$ (1 mili mole) was heated to 85°C with magnetic stirring, then mindhal seeds broth (5%) were added drop wise to this solution. Silver nitrate solution is sensitive to light, so flask was totally covered by aluminum foil for whole reaction. After 2 hours, the color of dispersion gradually changed to yellow, orange, brown and finally to dark reddish brown. The resulting dark reddish brown color solution was centrifuged for 20 min at 10000 rpm. Nanoparticles settled down. Techniques like UV, TEM, XRD, FTIR and Zetasizer analysis were used for investigating the morphology, crystalline nature, functional group and stability of synthesized AgNPs.[15]

Green synthesis of AgNPs using *Randia dumetorum* seed: The green synthesis of AgNPs using *Randia dumetorum* extract begins with the manufacture of an aqueous extract from plant seed, which acts as a natural reducing and stabilizing agent. The silver ion precursor was produced as a 1 mM solution of silver nitrate ($AgNO_3$). The *Randia dumetorum* extract was added drop wise to the $AgNO_3$ solution, stirring continuously at room temperature. After 2-3 hours at ambient conditions, the reaction mixture gradually changed color to yellowish-brown, indicating the reduction of Ag^+ ions and production of AgNPs via surface plasmon resonance. The AgNPs were purified by centrifuging the mixture at 10,000 rpm for 20 minutes to separate the NPs, then washing with distilled water to remove any remaining extract or unreacted ions. The purified AgNPs were then dried and kept for further characterization and applications.[16]

Characterization: The synthesized AgNPs were characterized using various analytical methods to understand their optical, morphological and structural properties. UV-Visible spectroscopy (Shimadzu-1800) was used to assess optical characteristics over a wavelength range of 300 to 800 nm, identifying specific absorbance peaks indicative of NPs formation. Transmission electron microscopy (TEM) was used using a JEM1010-JEOL model to undertake morphological analysis including size and shape. A tiny droplet of re-dispersed AgNPs in water was put on a carbon-coated copper grid and air-dried to ensure imaging stability.

Crystallinity was determined by X-ray diffraction (XRD) on a Miniflex Rigaku-600 at 30 kV and 2 mA, scanning at 10° per minute across a 2θ range of 3° to 80°. Using Cu-Kα radiation and a graphite monochromator revealed good diffraction patterns, confirming the crystalline structure. FT-IR spectroscopy (Shimadzu-8400) was utilized to identify functional groups on Gymnema sylvestre extract and the surface of AgNPs. The spectra ranged from 4000 to 500 cm^{-1}, providing insights into molecular interactions and NP stabilization.

Antioxidant activity test: The antioxidant activity was measured with the DPPH test. For this, 3 mL of the test extract was combined with 1 mL of a 0.1 mmol/L DPPH solution in methanol. The combination was incubated at 37°C for 30 minutes before being measured at 517 nm with a spectrophotometer, using ascorbic acid as a reference. The radical scavenging activity, given as the percentage of inhibition (I), was estimated by comparing the absorbance of the test sample (At) with that of the control (Ac).[17]

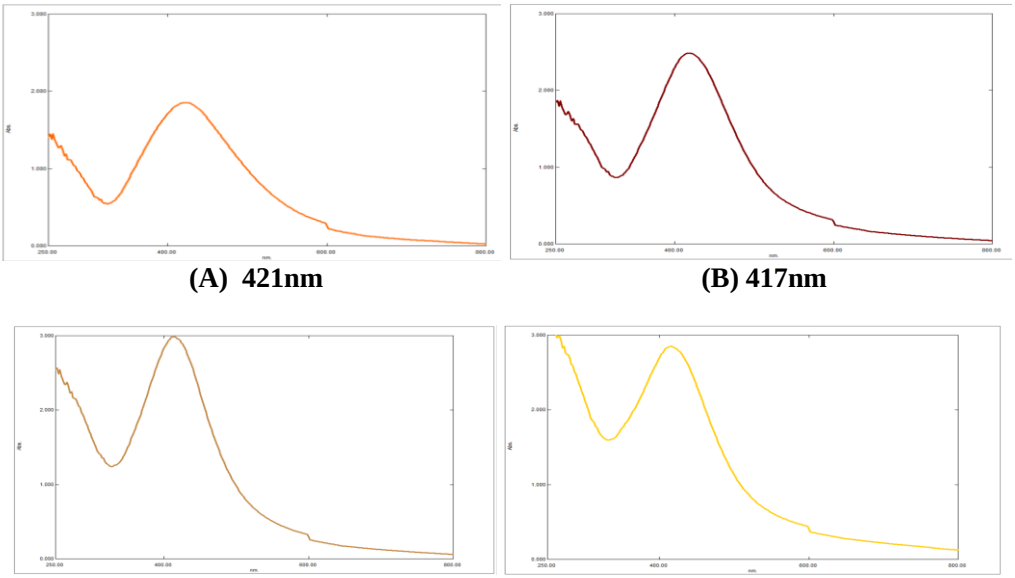
$$I = \frac{At}{Ac} \times 100 \quad (1)$$

Results and Discussion

UV-Visible Spectroscopy: Figure 1 displays the UV-visible spectrum with different absorbance peaks for AgNPs, *Randia dumetorum* seed extract and AgNPs. The spectrum of each component provides information about its optical characteristics and how it interacts with the composite structure. The presence of flavonoids and phenolic

chemicals, which contain conjugated structures that cause π-π transitions, is responsible for the plant extract's distinctive absorbance peak at about 412 nm. These substances provide the plant extract its reducing power, which enables it to use a green synthesis method to transform Ag ions into AgNPs.

The AgNPs exhibit a strong absorbance peak around 412 nm a feature attributed to the SPR effect. This peak occurs due to the collective oscillation of electrons on the NPs surface when exposed to light. The exact position of the SPR peak can vary slightly depending on particle size, shape and surrounding medium, but it typically lies within the 400-450 nm range for small AgNPs. This peak confirms the successful synthesis of AgNPs through reduction by the plant extract.[18]



(A) 421nm **(B) 417nm**

(C) 412 nm **(D) 415nm**

Figure 1: UV-visible absorption spectra of silver nanoparticles as a function of wavelength versus absorbance at a different ratio of plant extract and silver nitrate solution.

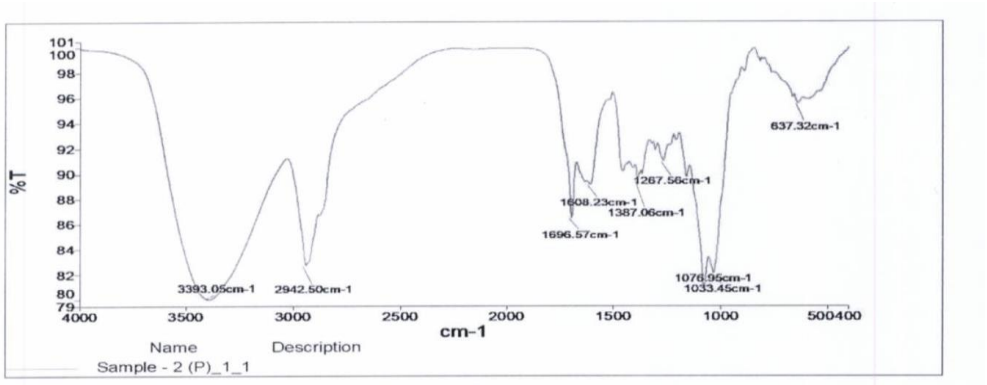


Figure 2(A): *Randia Dumetorum* seed extract FTIR

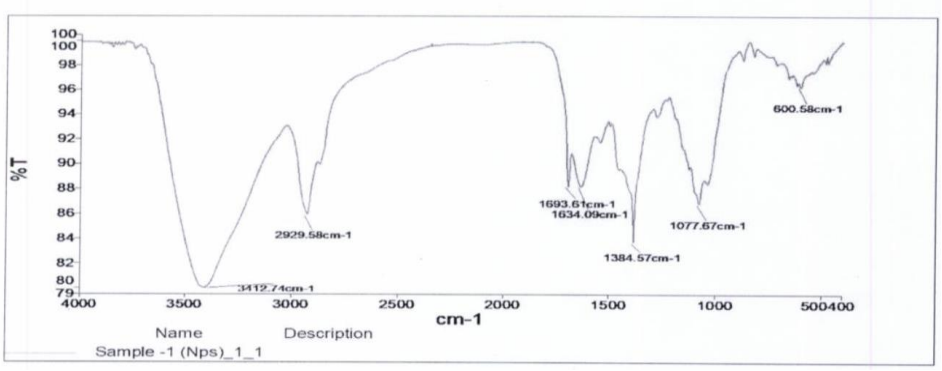


Figure 2(B): AgNPs FTIR

Figure 2: FTIR Spectrum of *Randia Dumetorum* seed extract and AgNPs

Fourier transforms infrared spectroscopy (FTIR): FTIR analysis was carried out to know the number of phytoconstituents in leaf extracts of *Randia dumetorum* plants that act as stabilizing and capping agents in the present green synthesis. The IR spectrum for *Randia dumetorum* shows a band at 3393.05 cm^{-1} which corresponds to OH stretching of hydrogen bonded alcohols or phenol as in fig. 2A. 2942 cm^{-1} indicates the presence of aliphatic C-H bonds from methylene (–CH_2–) and methyl (–CH_3) groups. These peaks suggest the presence of organic molecules such as proteins, terpenoids, or other biomolecules. The peak corresponding to 1608 cm^{-1} indicates N—H bending vibrations of 1 amines and 637 cm^{-1} corresponds to aromatic stretching vibrations 1696 cm^{-1}. A sharp peak in this region indicates the presence of carbonyl groups, which could be from ketones, aldehydes, carboxylic acids, or amides.[19]

The IR spectrum for *Randia dumetorum* synthesized of silver nanoparticles shows a band at 3412.05 cm^{-1} which corresponds to OH stretching of hydrogen bonded alcohols or phenol as in fig. 2B. 2929 cm^{-1} indicates the presence of aliphatic C-H bonds from methylene (–CH_2–) and methyl (–CH_3) groups. These peaks suggest the presence of organic molecules such as proteins, terpenoids, or other biomolecules. The peak corresponding to 1634 cm^{-1} indicates N-H bending vibrations of 1"amines and 600 cm^{-1} corresponds to aromatic stretching vibrations. The peak at the range of 1077 cm^{-1} reveals the confirmation of C-O stretching vibrations in alcohols, carboxylic acids, esters, ethers.

FT-IR study indicates that presence of hydroxyl (—OH) and nitro (NH) groups in a leaf extract is primarily involved in reduction of Ag^+ ions to Ag^0 nanoparticles. FTIR analysis confirms the presence of functional groups from natural compounds that contribute to the reduction of silver ions and stabilization of the resulting silver nanoparticles.

X-ray Diffraction Spectroscopy (XRD): The XRD spectrum of AgNPs, as shown in figure 3, confirms their crystalline nature through distinct Bragg reflection peaks observed at 2θ values of 27.86°, 32.208°, 38.25° and 45.53°. These peaks correspond to the (111), (200), (220) and (311) lattice planes of a face-centered cubic (FCC) silver crystal structure, matching the JCPDS Card No. 04-0783. The highest intensity of the (220) plane indicates it as the dominant orientation, a common feature in AgNPs synthesis due to the plane's inherent stability.[20]

Transmission Electron Microscopy (TEM): The TEM analysis of AgNPs revealed valuable insights into the morphology, distribution and average size of the NPs. As shown in the figure 4, the NPs were well-dispersed and predominantly spherical, which is indicative of uniform synthesis. The analysis confirmed an average particle size of approximately 25 nm, as reflected in the histogram depicting the size distribution, thus reducing surface energy and minimizing the likelihood of particle aggregation. The uniformity of the NPs observed in the TEM micrographs aligns with the Gaussian distribution seen in the size analysis, suggesting that the synthesis process was controlled and reproducible. This characteristic is essential for applications that demand consistent NPs properties such as biomedical and optical fields.[21]

TEM results highlight the efficiency of synthesizing AgNPs which are 25 nm in size on average. The observed morphology and size distribution suggest that the AgNPs are suitable for applications where stable and dispersed NPs are needed. The uniform particle size, confirmed through TEM analysis, corroborates the findings from UV-Visible and FTIR spectroscopy, providing a comprehensive understanding of the synthesized AgNPs' characteristics.

Zetapotantial of AgNPs: Zeta potential mean -16.2mV is moderately negative potential value suggesting that the silver nanoparticles have moderate level of electrostatic repulsion. This repulsion helps to stabilize the nanoparticles and prevent aggregation. This negative value indicates that the silver nanoparticles have a net negative surface charge, which is likely due to the adsorption of negative charged biomolecules (proteins, polysaccharide) from the mindhal seed.[22]

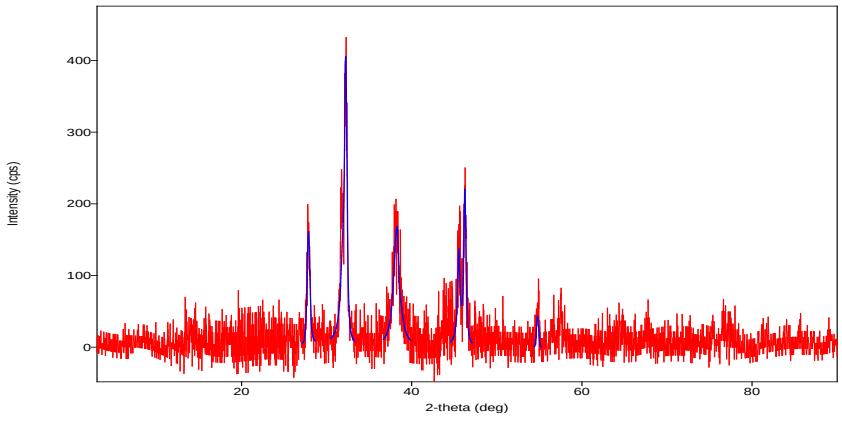
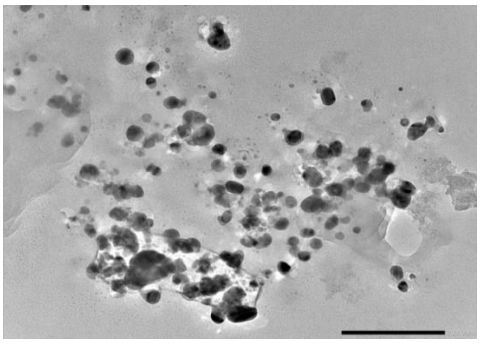
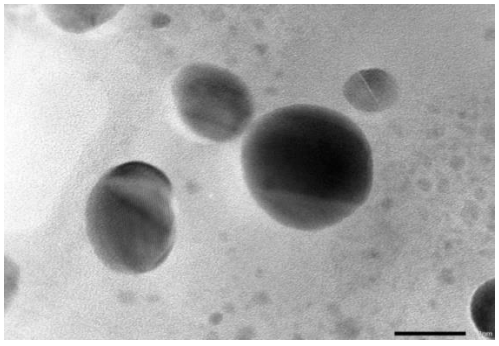


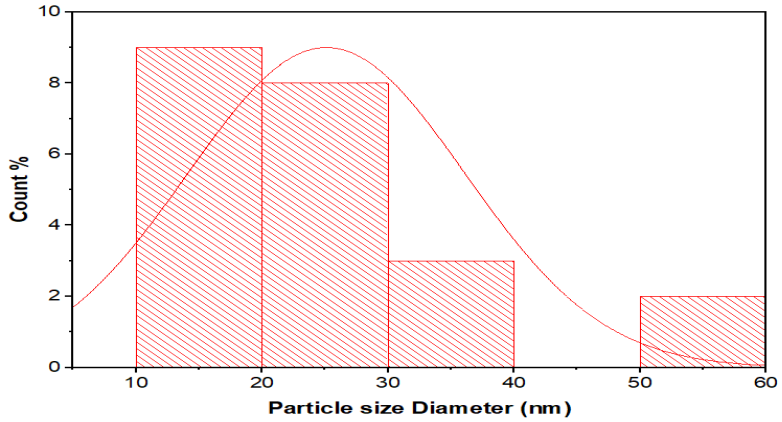
Figure 3: XRD of biosynthesized Silver Nanoparticle



(A) HRTEM (image scale is 200 nm)



(A) HRTEM (image scale is 20 nm)



Particle Size Distribution Curve (B)

Figure 4: (A)TEM Images of AgNPs and (B) Particle Size Distribution Curve

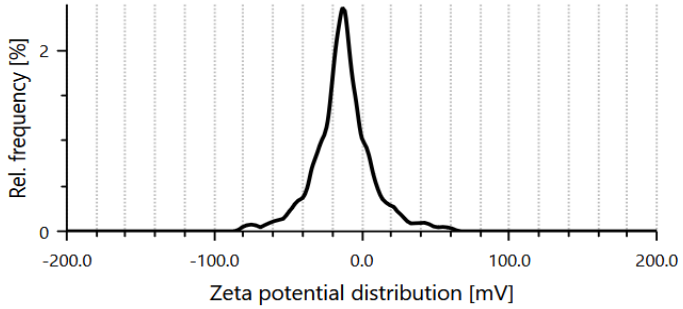


Figure 5: Zeta potential of AgNPs

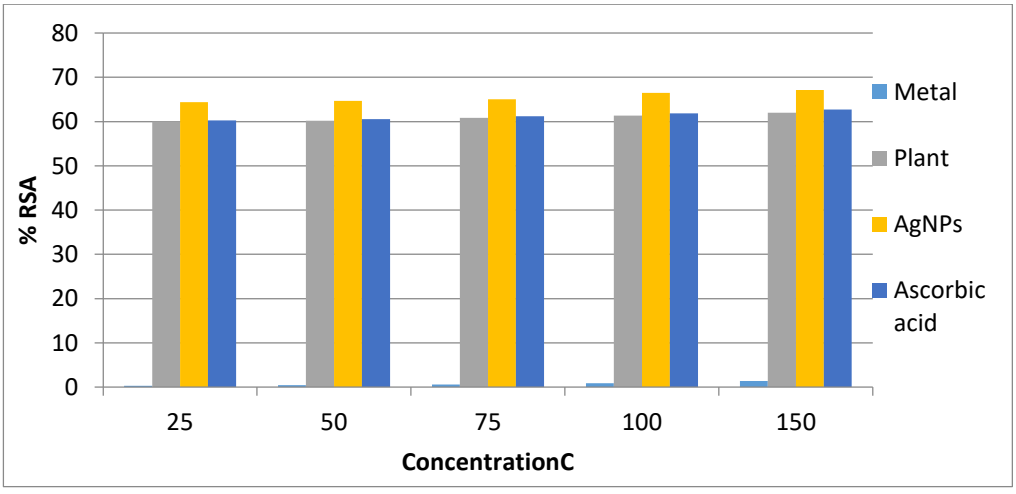


Figure 6: Plot of Antioxidant Efficacy of Various Concentrations of AgNPs against DPPH

Table 1
XRD data table

S.N.	2-theta	theta	FWHM	Crystallite size D (nm)	D nm (Average)	hlk
1	27.86	13.93	0.38	21.53	25.92 ± 3.89	111
2	32.208	16.104	0.38	21.75		200
3	38.25	19.125	0.76	11.06		220
4	45.53	22.765	0.28	30.76		311
5	46.24	23.12	0.24	35.99		222
6	54.76	27.38	0.26	34.40		112

Table 2
Antioxidant Efficacy of Various Concentrations of AgNPs against DPPH

Sample Number	Concentration of AgNPs (µg/mL)	DPPH Scavenging Effect (%) $AgNO_3$	DPPH Scavenging Effect (%) AgNPs	DPPH Scavenging Effect (%) Aqueous seed extract	DPPH Scavenging Effect (%) Ascorbic acid
1	25	0.32	64.36 ± 0.02	60.01	60.251
2	50	0.44	64.68 ± 0.04	60.2	60.54
3	75	0.56	65.04 ± 0.03	60.87	61.223
4	100	0.88	66.45 ± 0.02	61.34	61.883
5	150	1.38	67.10 ± 0.03	61.98	62.725

Antioxidant Activity: The antioxidant activity of synthesized AgNPs was evaluated using the DPPH assay where AgNPs served as radical scavengers and DPPH provided a radical source. Upon addition of AgNPs, the DPPH solution color shifted from deep violet to pale yellow, indicating a reduction in DPPH radicals. This visual change, along with a gradual decrease in absorbance at 517 nm, confirms the free radical scavenging ability of AgNPs and suggests a dose-dependent antioxidant effect. This graph illustrates the antioxidant activity of AgNPs as measured by the DPPH scavenging effect shows in figure 6. As the concentration of AgNPs increases, there is a corresponding increase in the DPPH scavenging percentage, reaching a maximum of 66.45% at 100 ug/mL. This trend confirms the dose-dependent nature of AgNPs' antioxidant activity, highlighting their potential for free radical scavenging.

As illustrated in table 2, the antioxidant efficacy of AgNPs increased with concentration. Specifically, at a 100 µg/mL concentration, the DPPH scavenging effect reached 66.45± 0.02%, indicating strong antioxidant activity at higher doses. This data supports the dose-dependent antioxidant potential of AgNPs, with an evident increase in DPPH scavenging at higher concentrations. This trend underscores the potential application of AgNPs as effective antioxidants, particularly at elevated doses.[23]

Conclusion

In conclusion, the successful green synthesis of silver nanoparticles (AgNPs) using *Randia dumetorum* seed extract, UV-Visible spectroscopy identified characteristic peaks, with AgNPs showing a surface plasmon resonance at 412 nm. FTIR analysis revealed interactions of AgNPs preventing aggregation, while XRD confirmed a face-centered cubic (FCC) structure with high crystallinity.

Additionally, DPPH assay results showed strong antioxidant activity, with a dose-dependent free radical scavenging effect. These findings highlight the potential of AgNPs synthesized from *Randia dumetorum* seed for biomedical applications, particularly in antioxidant therapies.

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