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Synthesis and Biological Activities of Cactus-Mediated Silver Nanoparticles

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ABSTRACT

The field of nanotechnology is expanding swiftly along with the diversified antioxidant and antibacterial applications of silver nanoparticles. **Objectives:** To articulate AgNPs using pigmented extract from two cactus varieties i.e., Rattail cactus (*Disocactus flagelliformis*) and Moon Cactus (*Gymnocalycium mihanovichii*). **Methods:** Using a green synthesis methodology, silver nitrate solution and both cactus extracts were mixed and incubated, followed by a color variation detection. UV-visible, and FTIR analysis were performed to validate the synthesis of silver nanoparticles. Antioxidant activity was assessed using DPPH assay, whereas well diffusion methods evaluated the antibacterial ability of cactus nanoparticles. **Results:** Blending of aceto-water extract of cactus plant with silver nitrate resulted a discrete color change from yellow to blackish brown. UV-Visible spectroscopy demonstrated surface plasmon peaks at 250 nm as well as at 400-450 nm. Fourier Transform Infrared (FTIR) spectroscopy identified interactions in nanoparticle samples due to specific functional groups in cactus pigmented extract. Antioxidant capacity was appraised via DPPH assay at 500 ppm and 1000 ppm, enlightening enhanced radical scavenging (31-48%) with increased concentration, exclusively at 1000 ppm. Antibacterial efficacy was verified by agar well diffusion against *Escherichia coli* and *Staphylococcus aureus*. It revealed distinct zones of inhibition, with greater activity against *E. coli* (17mm zones of inhibition) versus *Staphylococcus aureus*. **Conclusions:** The results of our study place Moon and Rattail Cactus pigment extracts as active green assets for AgNP fabrication, emphasizing their value in biomedical advances.

INTRODUCTION

Microscopic particles ranging from 1 to 100 nanometers (nm) in size are called nanoparticles. The main factors that determine the properties of nanoparticles (NPs) are their sizes and shapes. It is important to synthesize nanoparticles with an appropriate size, structure, monodispersed, and morphology to achieve the particular uses [1]. In nanotechnology, green technology has come forward that is environmentally friendly and can be utilized to reliably produce nanomaterials and nanoparticles from non-toxic green plants. The presence plays a crucial role in plant-aided reduction of metal ions. Plants' flavonoids, terpenoids, and alkaloids are directly involved in the reduction and thus design of silver nanoparticles. These phytochemicals act as reducing agents by reducing metal ions into their metallic form and also serve as stabilizing and capping agents, preventing the aggregation of nanoparticles [2]. Various researchers have explored the potential of medicinal plants for synthesizing nanoparticles with unique properties [3]. However, there still exists the need for exploring more plants for the cost-effective synthesis of nanoparticles. Moon cactus (*Gymnocalycium mihanovichii*) is primarily a decorative plant; however, its fever-reducing property, anti-inflammatory properties, and antioxidant activities (due to its betalain pigments) have been reported [4, 5]. Another variety of cactus, i.e., Rattail cactus (*Disocactus*

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flagelliformis), is another member of the family Cactaceae, which has been depicted to have anticancer and anti-inflammatory properties that urged us to explore its potential for nanoparticles synthesis [6]. In recent years, silver nanoparticles (AgNPs) have emerged as the most popular nanostructures with promising properties suitable for various biological applications. They exhibit broad-spectrum antimicrobial activity and thus can be employed for anticancer and antimicrobial therapy, wound repair, and bone healing. The role of silver nanoparticles as biosensors is also well documented [7, 8]. Silver particles are well known as drug carriers, enhancing bioavailability and targeting specific cells or tissues [9]. A variety of Cactus species (*Euphorbia cactus*, *Ethiopian cactus*, and *Opuntia ficus-indica* Cactus plant) have shown their silver nanoparticle synthesis potential.

However, two important cactus varieties, like moon and rattail cactus, were seen to have specific betalain pigments with antioxidant power, and these species have not been explored yet for their reducing potential or nanoparticle synthesis. To fill this gap, this research aimed at exploring and comparing the potential of, i.e., moon and rattail cactus, to synthesize silver nanoparticles, to characterize the silver nanoparticles via UV-Visible spectroscopy and FTIR analysis. Moreover, to study the antimicrobial and antioxidant activities of formed cactus mediated silver nanoparticles.

METHODS

This experimental study was performed at the Institute of Biochemistry, University of Sindh, Jamshoro, during August-December 2025. Red tail cactus flowers and Moon Cactus colored parts were collected from a local nursery and recognized by the Department of Plant Sciences, University of Sindh. Plants were rinsed with distilled water and ground using a mortar and pestle to obtain the paste. This mixture was soaked in acetone and water in a 1:1 ratio to fully immerse the material, and the bottle was stored in a dark place for 24 hours. After 24 hours, the mixture was centrifuged to obtain a clear plant extract. 1 mM silver nitrate solution was prepared and used as the metal ion source. For the synthesis reaction, the plant extract was slowly introduced into the silver nitrate solution in a 1:9 ratio under continuous stirring. The reaction mixture was kept at room temperature in dark conditions [10]. The reduction of silver ions was indicated by a gradual color transition of the reaction mixture from transparent to brown, confirming the synthesis of silver nanoparticles. The mixture was allowed to react for 24 hours to achieve maximum nanoparticle formation. The synthesized nanoparticles were separated by centrifugation at 10,000 rpm for 15 minutes. The collected pellets were washed repeatedly with deionized water to eliminate residual plant compounds. The purified silver nanoparticles were re-suspended in deionized water and stored at 4 °C in light-protected containers for subsequent analysis [11]. For UV-visible analysis, three dilutions of each extract were prepared: 1 mL, 2 mL, and 4 mL. These dilutions were scanned across the UV-Visible range of 400-800 nm. Absorbance values and λmax were recorded for each scan and graphs were generated using Origin 2020 software. FTIR analysis included the direct placement of nanoparticle powder on a PerkinElmer Spectrophotometer over the wavenumber range of 4000–400 cm^{-1}. Antioxidant potential of synthesized nanoparticles was evaluated via DPPH Assay. For this, fresh 0.1mM DPPH dissolved in methanol, and 1ml of this prepared DPPH was allowed to mix with 1ml of nanoparticles suspension (500 and 1000 ppm). The test tubes were incubated in the dark for 30 minutes at 37°C, and then absorbance was recorded at 517 nm along with three replicates [3]. All antioxidant and antibacterial assays were performed in triplicate technical replicates using three independently synthesized nanoparticle batches (biological replicates) to ensure reproducibility and reliability of results. The synthesis of silver nanoparticles using each cactus extract was performed independently three times (n=3 biological replicates). For each biological replicate, all subsequent assays (UV-Vis, FTIR, DPPH, antibacterial) were conducted in technical triplicate. Results from the three independent syntheses were pooled for statistical analysis. Cactus nanoparticles in 500ppm and 1000ppm concentration were dissolved in methanol for antibacterial analysis. Firstly, bacterial cultures were developed on nutrient agar media for 24hrs at 37°C. *E. coli* and *S. aureus* bacterial suspensions were swabbed evenly on the entire autoclaved agar surface. Using the well diffusion method, equal-diameter wells were formed in the agar using a sterile corn borer. About 50µl of cactus silver nanoparticles was mixed in each well, and this was followed by incubating the plates at 37ºC for 24 hours. This was repeated thrice for the same sample to achieve accuracy [12]. The next day, Zones of inhibition were measured to assess antibacterial activity. . To avoid observer bias, all measurements (zone of inhibition diameters, UV-Visible absorbance readings, DPPH absorbance values) were performed by a researcher blinded to the sample identities. Samples coding was done by independent person and codes were not revealed.

All experiments were performed with three independent nanoparticle syntheses (biological replicates, n=3), each measured in technical triplicate. Results are presented as mean ± standard deviation (SD). Blinding was applied during all measurements to avoid observer bias. For DPPH data, one-way ANOVA and regression were used. For antibacterial data, unpaired two-tailed t-tests compared zones of inhibition between bacterial species and between

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concentrations. A p-value<0.005 was considered statistically significant. 95% confidence intervals (CIs) are reported. Statistical analyses were performed using GraphPad Prism 9.0.

RESULTS

Cactus extract and metal salt were mixed and incubated, then a color change was observed, confirming the synthesis of nanoparticles. Absorbance intensity was seen to be increased with volume, highest for 4 mL and lowest for 1mL. However, the 4 ml sample showed much noise, which needs to be corrected by diluting the sample (Figure 1).

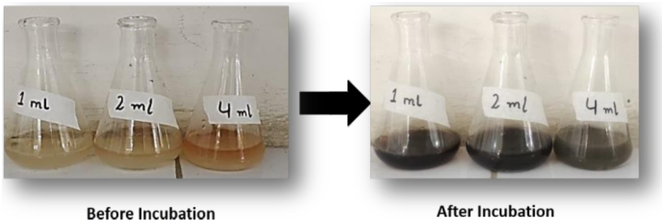

Figure 1: Color Change Showing the Synthesis of Nanoparticles

The UV-Visible spectroscopy analysis confirms the successful green synthesis of silver nanoparticles (AgNPs) using moon cactus (a) and rat-tail cactus plant extract (b), as evidenced by the characteristic surface plasmon resonance (SPR) peaks in 250-300 nm, as well as peaks were also seen at 350 nm and 400-450 nm, particularly with the use of 4 ml cactus extract (Figure 2).

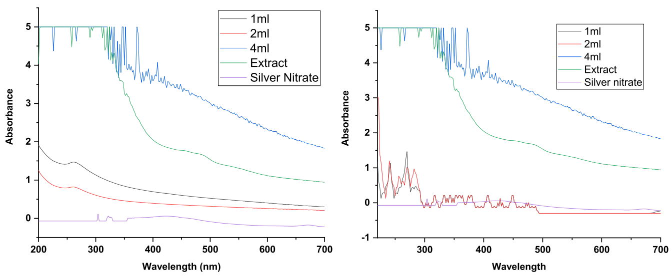

Figure 2: UV-Visible Absorbance Spectra of Silver Nanoparticles (AgNPs) Biosynthesized Using Moon Cactus Extract (a) and Rat-tail Cactus Extract (b)

FTIR analysis demonstrated a broad band at 3000–3500 cm ^{1}, suggestive of O-H stretching vibrations, which indicate hydroxyl-rich cactus biomolecules' contribution to nanoparticle synthesis/ stabilization. Other peaks at 1000 cm ^{1}and 1500–1800 cm ^{1} were seen to be involved in nanoparticle synthesis (Figure 3).

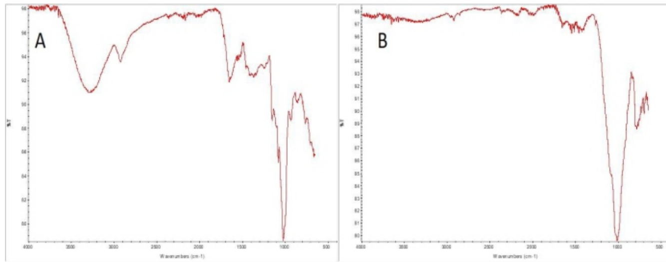

Figure 3: FTIR Spectra of (A) Rattail Cactus Nanoparticles and (B) Moon Cactus Nanoparticles

In an antibacterial assay, the presence of well-defined clear zones indicates suppression of bacterial growth in the surrounding area (Figure 4).

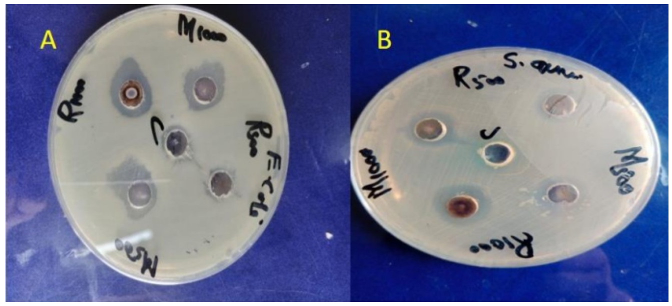

Figure 4: Zones of Inhibition of Moon and Rattail Cactus Silver Nanoparticles Against (a) *E. coli* and (b) *S. aureus*

In contrast, the negative control did not show any zone of inhibition, confirming that the observed antibacterial activity was solely due to the nanoparticle treatment. The overall efficacy of the current AgNPs against Gram-negative *E. Coli* was visually confirmed as potent, i.e., 17 mm zone of inhibition. Rattail cactus was found to inhibit S. aureus with the highest zone of inhibition (i.e., 16 mm) at 1000ppm concentration. Negative control (Distilled water) showed negligible zones, while the highest zones of inhibition were shown by ciprofloxacin (positive control) (Table 1).

Table 1: Zones of Inhibition (mm)

Samples	Zones of Inhibition (mm)	
	E. Coli	***S. aureus***
M.C. 500 ppm	13	12
M.C 1000 ppm	17	14
R.C 500 ppm	11	09
R.C 1000 ppm	17	16
Distilled Water (-ve control)	00	00
Ciprofloxacin	21	19

The antibacterial assay revealed concentration-dependent activity (Table 1). Moon cactus AgNPs at 1000 ppm showed significantly greater inhibition against *E. coli* (17.0 ± 0.58 mm) compared to *S. aureus* (14.0 ± 0.58 mm, p = 0.01, 95% CI: 1.48–4.52), confirming statistically superior activity against the Gram-negative bacterium. Similarly, Rattail cactus AgNPs at 500 ppm showed a significant difference (*E. coli*: 11.0 ± 0.58 mm vs *S. aureus*: 9.0 ± 0.58

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mm, p = 0.03, 95% CI: 0.48–3.52). Positive control (ciprofloxacin) produced expected inhibition zones (21.0 ± 0.58 mm for *E. coli*, 19.0 ± 0.58 mm for *S. aureus*), validating the assay (Table 2).

Table 2: Zones of Inhibition against *E. coli* and *S. aureus* (n=3)

Samples	*E. coli* (Mean ± SD)	*S. aureus* (Mean ± SD)	p-value*	95% CI
Moon Cactus (500 ppm)	13.0 ± 0.58	12.0 ± 0.58	0.080	(-0.52, 2.52)
Moon Cactus (1000 ppm)	17.0 ± 0.58	14.0 ± 0.58	0.010	(1.48, 4.52)
Rattail Cactus (500 ppm)	11.0 ± 0.58	9.0 ± 0.58	0.030	(0.48, 3.52)
Rattail Cactus (1000 ppm)	17.0 ± 0.58	16.0 ± 0.58	0.080	(-0.52, 2.52)
Distilled Water (-ve)	0.0 ± 0.00	0.0 ± 0.00	–	–
Ciprofloxacin (+ve)	21.0 ± 0.58	19.0 ± 0.58	0.020	(0.48, 3.52)

*Unpaired t-test

In the DPPH Assay, as we added the DPPH Solution to the sample solution, a clear change in color was observed from purple to yellowish brown. A color change showing transition towards a pale-yellow shows good DPPH scavenging (Figure 5).

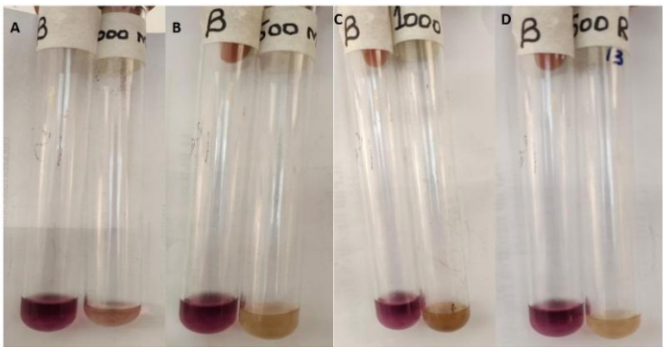

Figure 5: Color Change Showing DPPH Scavenging by (a) Moon Cactus NPs1000ppm, (b) Moon Cactus NPs 500ppm, (c) Rattail Cactus NPs 1000ppm, (d) Rattail Cactus NPs 500ppm

When calculated by the mentioned formula, DPPH percent inhibition by cactus nanoparticles was found to be increased in a concentration-dependent manner, i.e., 48% for 1000 ppm and 31% for 500 ppm. The IC_{50} value of Moon cactus AgNPs (1052 ppm) was lower than that of Rattail cactus AgNPs (1587 ppm), indicating the superior antioxidant potency of Moon cactus-derived nanoparticles. However, both values are considerably higher than ascorbic acid (10 ppm), suggesting that further optimization of synthesis conditions (e.g., reducing aggregation, improving capping efficiency) could enhance radical scavenging activity (Table 3).

Table 3: DPPH Percent Scavenging of Silver Nanoparticles

Samples	Scavenging Activity (%)	IC (ppm)	95% CI (IC_{50})	p-value
Moon Cactus (1000 ppm)	47.9 ± 0.60	1052	(987–1123)	<0.001
Moon Cactus (500 ppm)	31.46 ± 0.06	1052	(987–1123)	–
Rattail Cactus (1000 ppm)	31.71 ± 0.01	1587	(1492–1689)	–
Rattail Cactus (500 ppm)	11.17 ± 0.005	1587	(1492–1689)	<0.001
Blank (DPPH Only)	0.07 ± 0.00	–	–	–
Ascorbic Acid (Positive Control)	–	10	(8.5–11.8)	<0.001

The high R^2 values (>0.94) and significant p-values (<0.005) confirm a strong, statistically significant positive linear relationship between cactus extract volume and absorbance intensity, validating the concentration-dependent increase in nanoparticle yield (Table 4).

Table 4: Linear Regression Parameters for Absorbance Intensity Versus Cactus Extract Volume

Parameters	Moon Cactus AgNPs	Rat-tail Cactus AgNPs
R^2	0.964	0.941
p-value (Slope ≠ 0)	0.008	0.011
95% CI for Slope	(0.048, 0.126)	(0.036, 0.122)

DISCUSSION

A visible color change as well as UV visible analysis confirmed that the nanoparticle samples (with sample volumes of 1 and 2 ml) exhibited a sharp SPR peak around 250-300 nm, different from the usual SPR at 400-450 nm. Although such absorption peaks are not considered as primary SPR for silver nanoparticles synthesis, when 250 nm is present with a 450 nm peak, then it may indicate the formation of some silver nanoclusters. It may suggest that phyto-organic compounds involved in silver reduction are still present in the current sample [13]. This may be due to residual phytochemicals of Cactus in nanoparticles samples, clumping or agglomeration of synthesized AgNPs, as when nanoparticles clump, then the SPR peak may disappear when they are oxidized to silver. It may be corrected by using strategies such as dilution of the UV-visible sample, increasing reaction incubation time [14], and characterizing the nanoparticles via SEM and DLS. Absorbance intensity was increased in the case of 4 ml, which reflects more silver reduction and nanoparticle yield from phytochemicals in the cactus extract [15, 16]. The 4 ml sample depicts sharp peaks around 380 nm and 420 nm, but due to noise, it may not reflect the actual SPR band, so it needs to be diluted and repeated again. These results validate that the rat-tail cactus acts as an efficient bio-reductant and stabilizer for eco-friendly AgNP production. FTIR analysis showed sharp peaks at ~1000 cm^{-1}, which is suggestive of the C-O stretching vibration of either alcohol or phenolic compounds from cactus extract. It indicates that phenols or flavonoids in cactus extract have actively participated in reducing silver ions as well as capping them. Sharp peaks in the 1500–1560 cm^{-1} range were observed in the rattail cactus nanoparticles sample, which shows amide II band or aromatic vibrations often came from flavonoids [17]. The synthesized nanoparticles displayed visible inhibitory effects against both Gram-negative and Gram-positive bacteria, i.e., *E. Coli* and *S. aureus*,

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respectively. The Antibacterial efficacy of plant-mediated nanoparticles is possible due to their large surface area and nanosize, which helps them to strongly interact with the bacterial cell membrane [18]. Reported results of such membrane interactions include membrane damage, enhanced permeability, and intracellular contents leakage, eventually causing cell death. Additionally, induction of reactive oxygen species (ROS) is also reported, which leads to oxidative stress and can cause damage to vital cellular constituents such as proteins or DNA. The higher susceptibility of *Escherichia coli* compared to *Staphylococcus aureus* may be due to the presence of an outer membrane in its structure and cell wall differences [19]. DPPH analysis suggested that moon cactus-derived silver nanoparticles were more efficient in scavenging DPPH free radicals (i.e., 48%) as compared to rattail cactus at 1000 ppm concentration. This difference in antioxidant potential may be due to differences in pigment composition and varied phytochemicals that possess free radical scavenging ability [20, 21]. Current results are different from the already reported Euphorbia Cactus nanoparticles' DPPH potential of 96.12% at a concentration of 80 µg/ml [22]. It may be improved after correcting the problems of aggregation and correcting UV-visible surface plasmon readings.

Some limitations of this study include a lack of funding, due to which advanced characterization techniques could not be performed. Moreover, IC50 values of cactus nanoparticles need to be calculated via GraphPad Prism. Future studies should focus on characterizing these nanoparticles via SEM, XRD, and zeta potential to know their specific properties. Stability studies and in vivo assays would further help us to determine the toxicity of cactus nanoparticles before their use in biomedical applications.

CONCLUSION

Moon cactus and rattail cactus were explored for their potential to synthesize silver nanoparticles. Blending of both cactus extract with silver salt resulted in a visible color change from yellow to blackish brown. Specific SPR peaks were absent while using 1 ml and 2 ml cactus extract, whereas 4 ml cactus extract was shown to exhibit peaks around 420 nm, although its noise needs to be corrected by using diluted samples. FTIR analysis depicted phenols and flavonoids of Cactus as major reducers and capping partners. Fabricated silver nanoparticles showed 31-48% DPPH percent scavenging and a pronounced zone of inhibition (17mm) against E. coli at 1000 ppm concentration. Therefore, these nanoparticles need to be further characterized and explored for their use as strong antimicrobials and robust green antioxidants.

Authors' Contribution

Conceptualization: BK
Methodology: SN, AAK
Formal analysis: AAK, AR
Writing and Drafting: BK, AAK, MW, AR
Review and Editing: BK, SN, AAK, MW, AR

All authors approved the final manuscript and take responsibility for the integrity of the work

Conflicts of Interest

All the authors declare no conflict of interest.

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