

Synthesis of silver nanoparticles via eco-friendly green chemistry from *Fragaria nubicola*

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Abstract: Background: Nanotechnology offers innovative and sustainable solutions across diverse scientific fields, with green synthesis gaining attention as a safer and eco-friendly alternative to conventional nanoparticle production methods. Plant-mediated synthesis offers a safe alternative for producing biologically active nanoparticles suitable for biomedical applications. *Fragaria nubicola*, a medicinally important plant rich in bioactive compounds, represents a promising biological source for green nanoparticle synthesis. Leveraging such plant extracts not only minimizes environmental impact but also enhances the functional properties of nanoparticles. **Objectives:** In this study, silver nanoparticles (AgNPs) were synthesized using *F. nubicola* extract and systematically characterized to elucidate their structural and functional properties. **Methods:** The synthesized AgNPs were characterized by UV-visible spectroscopy, scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectrometry (EDS), Fourier transform infrared (FTIR) spectrometry and X-ray diffraction (XRD). Furthermore, the antiradical scavenging activity of plant-mediated nanoparticles was evaluated using the DPPH assay. **Results:** UV-visible analysis confirmed nanoparticle formation through a characteristic surface plasmon resonance peak at 420 nm. SEM images revealed predominantly crystalline particles with a size ranging from 74 to 107 nm, while EDS analysis confirmed the presence of elemental silver, indicated by a strong signal at 3 keV. XRD analysis further validated the crystalline nature of the AgNPs, with an average particle size of 69.5 nm. The phylogenically synthesized nanoparticles demonstrated significant antioxidant activity, exhibiting an 84.64% radical scavenging potential at a concentration of 80 µg/ml, which was comparable to that of ascorbic acid. **Conclusion:** Collectively, this study establishes a robust, cost-effective, and eco-friendly strategy for the synthesis of silver nanoparticles using *F. nubicola*. The notable antioxidant activity and green synthesis approach underscore the potential applicability of these AgNPs in nanomedicine, particularly in diagnostic and therapeutic applications.

Keywords: Antioxidant; Eco-friendly; *Fragaria nubicola*; Silver nanoparticles; Nanomedicine

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INTRODUCTION

In recent years, nanotechnology has profoundly influenced advancements in science and technology. It offers innovative strategies to address complex challenges across multiple disciplines, including materials science, environmental science and medicine (Safari *et al.*, 2025). Nanotechnology is an emerging field focused on the design and creation of materials and structures with unique molecular arrangements by precisely controlling atoms and molecules to develop materials with novel properties for practical applications. This technology facilitates the fabrication of highly precise materials at both micro and macro scales (Firdous *et al.*, 2025).

Within this context, the green synthesis of nanoparticles (NPs) has emerged as a prominent area of research, due to its sustainability, environmental compatibility and economic feasibility (Safari *et al.*, 2025). Although

traditional physical and chemical methods for nanoparticle synthesis are effective, they often involve toxic reagents and harsh reaction conditions that can negatively impact the biocompatibility and environmental safety of the resulting nanoparticles. In contrast, green synthesis approaches, particularly those employing plant extracts, have gained significant attention as sustainable and environmentally benign alternatives for nanoparticle production. This eco-friendly method not only enhances the biocompatibility of nanoparticles but also aligns with the principles of sustainable development, making it a promising strategy for the large-scale synthesis of silver and other metallic nanoparticles with potential application in biomedicine and industrial sectors (Miu and Dinischiotu, 2022; Dewi *et al.*, 2025).

Several techniques have been developed for the fabrication of nanoparticles, including microwave-based, sono-electrochemical, chemical reduction, radiation, photochemical reduction, hybrid approaches, and microemulsion methods. Among these, green synthesis

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has emerged as the most preferred due to its environmentally benign nature (Masum *et al.*, 2019; Kakakhel *et al.*, 2021). Initially, during the early 20th century, physical and chemical methods were predominantly employed for nanoparticle synthesis. However, these conventional approaches are often expensive and release hazardous chemicals into the environment. Consequently, researchers have shifted towards the use of metals and their oxides to produce stable, biocompatible, affordable and eco-friendly nanoparticles (Vanlalveni *et al.*, 2021). Increasingly, green synthesis approaches utilizing plant extracts are being adopted for nanoparticle fabrication and manipulation. These environmentally friendly methods minimize the adverse effects associated with traditional nanotechnological processes, thereby enhancing the suitability of the resulting nanoparticles for application in biomedicine, agriculture, and environmental remediation (Rashid *et al.*, 2024).

Nanoparticles are now being synthesized from various plant sources and exhibit significant anticancer, antibacterial, and antioxidant activities, making them valuable in diverse biomedical applications such as drug delivery, tissue engineering, imaging, and regenerative medicine. These nanoparticles are commonly synthesized using noble metals like silver (Ag), gold (Au) and platinum (Pt), as well as transition metals including iron (Fe), copper (Cu), cobalt (Co), Zinc (Zn) and manganese (Mn), along with their respective metal oxides (Hano and Abbasi, 2021; Khan *et al.*, 2025). Metallic nanoparticles have gained considerable interest due to their potent medicinal properties and can be synthesized in various sizes and shapes. They are generally considered non-toxic, chemically inert, and offer versatile surface functionalization (Khan *et al.*, 2025). In recent years, noble metals have also been immobilized on semiconductor materials to enhance the photocatalytic activity, demonstrating superior plasmon resonance, improved enzymatic stability, reusability, and enhanced biological performance (Pu *et al.*, 2018; Wang *et al.*, 2025).

Furthermore, these fabricated materials present innovative solutions for environmental and ecological restoration by contributing to effective and sustainable remediation outcomes (Zhao *et al.*, 2025). Numerous studies have also reported the activation of stress-tolerant defense mechanisms in various plant species exposed to air pollutants, emphasizing their potential role in phytoremediation (Rabbani *et al.*, 2024). In addition, plants act as highly effective natural capping and reducing agents during nanoparticle synthesis, serving as reservoirs of bioactive compounds such as phenols, terpenoids, alkaloids, carbohydrates, and flavonoids, which facilitate the reduction of silver ions (Ag⁺) to silver nanoparticles (AgNPs) (Balciunaitiene *et al.*, 2021; Kakakhel *et al.*, 2021).

Antioxidants play a vital role in protecting biological systems from oxidative damage caused by free radicals—highly reactive and unstable molecules generated during metabolic processes. When produced in excess, free radicals lead to oxidative stress, which can damage cellular components such as lipids, proteins, and DNA, contributing to the development of various diseases, including cancer, cardiovascular disorders, and various neurodegenerative conditions. Antioxidants counteract this damage by donating electrons to stabilize free radicals. Although endogenous antioxidant defense mechanisms exist within the body, exogenous sources are often required to maintain redox balance. However, long-term exposure to some synthetic antioxidants has been associated with adverse health effects, increasing the risk of chronic diseases. Consequently, natural antioxidants from plant sources are considered safer alternatives. Recent investigations further suggest that green-synthesized silver nanoparticles exhibit significant antioxidant activity comparable to plant-derived compounds, making them a promising and eco-friendly substitute for conventional synthetic antioxidants (Khan *et al.*, 2025; De Mel *et al.*, 2025).

Fragaria nubicola (Hook. f.), a wild strawberry of the *Rosaceae* family, is widely distributed across the northern regions of Pakistan, Afghanistan, China, Nepal, and India at elevations ranging from 1500 to 3600 meters. The plant is traditionally valued both nutritionally and medicinally. Its fruits are consumed for their high nutritional content, while various parts of the plant, including leaves, fruits, and roots, are used in traditional remedies in the form of decoctions and crude extracts to treat wounds, digestive disorders, fever, inflammation, diabetes, and hypertension (Roshan *et al.*, 2019). Phytochemical investigations of *Fragaria* species reveal a rich composition of bioactive compounds such as phenols, organic acids, tannins, flavonoids and minerals. These constituents contribute to a wide spectrum of pharmacological properties, including antioxidant, antibacterial, anti-inflammatory, wound healing, and antidiabetic effects (Fierascu *et al.*, 2020). In particular, wild *Fragaria* fruits are known to be abundant in proteins, polyphenols, flavonoids, flavanols, tannins, and proanthocyanidins, which play a significant role in combating oxidative stress and promoting overall health (Bahukhandi *et al.*, 2020). Recent studies have also reported the neuroprotective (Hu *et al.*, 2022) and anti-inflammatory (Mashaal *et al.*, 2023) activities of *F. nubicola*, highlighting its therapeutic potential. In parallel, green synthesis of metal nanoparticles using medicinal plants has emerged as an advanced, biocompatible, cost-effective, and environmentally friendly approach. Although strawberries are known for their potent antioxidant capacity attributed to phenolic and flavonoids, no study to date has reported the green synthesis of silver nanoparticles using the whole plant extract of *F. nubicola*. Furthermore, the plant is abundant and easily accessible and exhibits strong stress tolerance

under harsh environmental conditions. Its extensive medicinal properties provide an additional advantage, as the plant's bioactive constituents may facilitate the synthesis of nanoparticles with improved stability and therapeutic potential. Collectively, these attributes make *F. nubicola* an excellent candidate for nanoparticle synthesis with enhanced biological activity. Therefore, *F. nubicola* was selected for the synthesis of AgNPs to evaluate the antioxidant potential of the resultant nanostructures.

MATERIALS AND METHODS

Collection and identification of plant materials

Whole plants of *F. nubicola* were collected from the hilly areas of Muzaffarabad, Azad Jammu and Kashmir. Botanical identification was confirmed by an expert, and a voucher specimen (RR-3181) was prepared and deposited at the herbarium of the University of Azad Jammu and Kashmir. The collected plants were dried and stored for subsequent use.

Extraction of plant

To prepare the extract of *F. nubicola*, the dried powder (200 g) and 500 ml of pure ethanol were mixed by stirring for 2 hours. The mixture was left to sit for 4 days to allow maximum extraction. After this period, it was filtered to remove solid residues, and the resulting mixture was concentrated on a rotary evaporator (Buchi R-200) at a controlled temperature of 40-45 °C. The concentrated extract was further air-dried to obtain a semi-solid crude form, which was stored at 4°C for future analysis and experimentation.

Silver nanoparticles synthesis

This process was carried out with slight modifications to the method outlined by (Feng *et al.*, 2018). A stock solution in 100 ml of distilled water was prepared by adding 1 mM silver nitrate. In parallel, 30 mg of extract was dissolved in 60 ml of distilled water in a conical flask, stirring at 40 °C for 10 min using a magnetic hot plate stirrer, to ensure complete solubility. To initiate nanoparticle synthesis, the extract solution and AgNO₃ solution were combined in a 1:10 ratio, followed by stirring at 28 °C for 30 minutes, and the temperature was further increased to 75 °C for 1 hour until the reaction started appearing by a change in color. The reaction mixture was left for incubation for 24 hours at ambient temperature. Throughout the experiment, the pH of the solution was monitored and maintained at 7 by adding sodium hydroxide solution to ensure the synthesis of stable NPs (Liaquat *et al.*, 2022; Tesfaye *et al.*, 2023). The generation and collection of synthesized particles was first confirmed by noting a change from light to dark color, then by ultraviolet (UV) spectra taken at 24 hours over a wavelength range of 300-800 nm. After confirmation, centrifugation was carried out at 8000 rpm for ten minutes. Finally, the purified nanoparticles were obtained by washing with water and ethanol, followed by drying and

storage at 4 °C for characterization and antioxidant assays (Feng *et al.*, 2018; Sivalingam and Pandian, 2024).

Characterization of silver nanoparticles

The bio-reduction of silver ions to silver nanoparticles was evaluated at 0 hours and 24 hours to check the stability of particles, by measuring the absorbance of the reaction mixture using a UV-Vis spectrophotometer (SP-UV 500, spectrum Instruments, Thailand) (Jalilian *et al.*, 2020). The morphology, encompassing shape and size along with elemental composition, was determined by scanning electron microscopy/energy dispersive X-ray spectrometry SEM/EDX (Model 700, Shimadzu, Japan). In this procedure, a droplet of nano suspension was placed on the copper grid surface coated with carbon, which was then allowed to dry before microscopy (Krithiga *et al.*, 2015). High-resolution images were recorded at 20 kV at different magnifications.

The functional groups involved in the synthesis of silver nanoparticles were identified and analyzed by FTIR using the potassium bromide (KBr) disc method on a Fourier infrared spectrometer (Agilent Technologies, USA), covering a range of 400 to 4000 cm⁻¹ (Jalilian *et al.*, 2020). In XRD analytical technique, a glass plate was dipped into the AgNP solution to form a thin layer of silver nanoparticles and the sample was analyzed using X-ray diffractometer (JEOL, JDX-3532), operating at 40 kV and 30 mA, using K α radiation in $\theta - 2\theta$ mode, using Debye-Scherrer equation, observing the peak widths to evaluate the crystallinity of the produced nanoparticles (Krithiga *et al.*, 2015).

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

Where $k = 0.94$, λ = x-rays wavelength, β = width at half maxima, θ = Bragg angle

Antioxidant activity

The antioxidant potential of phyto-synthesized silver nanoparticles from *F. nubicola* was determined using the commonly followed 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, with a slightly modified version of the procedure outlined by (Singh *et al.*, 2021). Fresh solutions of both AgNPs and DPPH (1mM) were prepared, and 1 ml of each concentration (20, 40, 60, and 80 µg/ml) and 1 ml of DPPH were reacted together. After incubation in the dark for half an hour, the absorbance was measured at 517 nm using a spectrophotometer, by running samples in triplicate. Ascorbic acid was used as the positive control, while methanol served as the negative control. The percent inhibition by nanoparticles was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

All data expressed was the mean \pm standard deviation of triplicate values, performed by two-way ANOVA followed by post hoc comparison (at $p < 0.05$ significant level).

RESULTS

Synthesis of nanoparticles

The development of plant-mediated AgNPs was monitored periodically upon adding the extract to AgNO₃ solution. Initially, the solution exhibited a light yellowish-brown color. Over time, as the synthesis progressed, a noticeable shift in coloration from light-dark brown occurred, indicating a successful reduction of ions. The process was completed after overnight incubation with significant changes in coloration of the reaction material serving as a clear visual confirmation of nanoparticle formation.

UV-Vis analysis

After visual confirmation of synthesis, the nanoparticles were further validated by assessing their absorbance using a spectrophotometer, which revealed a characteristic surface plasmon resonance (SPR) peak for *F.nubicola* fabricated silver nanoparticles at 420 nm, depicting the reduction of Ag⁺ to Ag⁰ as shown in Fig.1.

Scanning electron microscopy/ Energy dispersive X-ray spectrometry (SEM-EDX)

The size and morphological features of nanoparticles were investigated using SEM/EDX. This analysis provided detailed insights into the shape and structure. SEM images of the phyto-synthesized AgNPs are shown in Fig. 2A and 2 B along with the EDX spectrum displaying elemental data is shown in Fig. 3. The nanoparticles synthesized from *F. nubicola* had particle sizes ranging from 74 to 107 nm in diameter at 33,000 magnifications, comprising both small and large spherical as well as irregularly shaped particles. These results are also consistent with those reported for nanoparticles synthesized from various other plants (Mavaei *et al.*, 2020).

Fourier transform infrared analysis

The interactions between bioactive compounds in AgNPs of *F.nubicola* extract, explored by FTIR analysis contributed to the reduction of silver ions and capping, displaying multiple peaks, as illustrated by Fig.4, each associated with different functional groups. Major peaks included 3227.9 cm⁻¹ for O-H corresponding to phenol, 2922.23 cm⁻¹ and 2855.14 cm⁻¹ for C-H attributing to alkyl groups, 2113.40 cm⁻¹ for C≡C indicating alkynes, 1610.20 cm⁻¹ for C=O corresponding to amide groups, 1517.02 cm⁻¹ for N-H or N-O suggesting presence of amines or nitro compounds, 1237.47 and 1037. 47 cm⁻¹ for C-O stretching of ethers or alcoholic compounds and 451.0 cm⁻¹ for C-Cl of chloroalkanes, respectively.

X-ray diffraction

Table 1 and Fig. 5 represent the diffraction pattern of silver nanoparticles of *F. nubicola*. The average crystalline size of AgNPs determined by previously discussed Scherrer formula was found to be 69.5 nm, with particle sizes varying between 30.07 nm and 109 nm. The XRD reflections at 2θ values of 32.28°, 38.22°, 44.4°, 64.46° and

77.26° correspond to 110, 111, 200, 220 and 311 planes, respectively. Additionally, several unidentified peaks at specific 2 θ values may be ascribed to the bioorganic phase that adheres to the surface of particles (Khalil *et al.*, 2024; Firdous *et al.*, 2025).

Antioxidant ability of nanoparticles

In this investigation, the silver nanoparticles were evaluated for their reactive oxygen species scavenging abilities at 20, 40, 60, and 80 µg/ml concentrations, resulting in inhibition percentages of 50.84%, 65.0%, 72.4% and 84.6%, respectively, against DPPH radicals. For comparison, ascorbic acid exhibited inhibition rates of 52.4%, 61.64%, 71.74% and 81.0 % at the corresponding concentrations. These results indicate that the antioxidant potential of AgNPs increases with concentration. At 80 µg/ml concentration, AgNPs demonstrated an inhibition of 84.64%, which was slightly higher than the 81.05% inhibition observed for the ascorbic acid, as illustrated in Fig. 6. This highlights the comparable antioxidant capacity of AgNPs, particularly at higher concentrations.

DISCUSSION

While silver can be toxic in higher concentrations, studies suggest that lower concentrations of AgNO₃ provide enhanced stability, biocompatibility, strength, and catalytic performance. Silver nanoparticles are recognized for their anticancer and antimicrobial properties, with the slow controlled release of silver ions offering a significant advantage over bulk silver metals. The convergence of traditional medicine and nanotechnology is fostering the emergence of cutting-edge bio-nano formulations (Hemlata *et al.*, 2020). Numerous studies have reported that nanoparticle peaks commonly fall within 400 to 600 nm range, and a sharp, single surface plasmon resonance peak around 400-500 nm is the indicator of stable AgNPs. In this study, UV spectroscopy showed a peak of *F. nubicola*-derived AgNPs at 420 nm. Biologically active compounds in the extract, such as proteins, flavonoids, phenols, carbohydrates, steroids and saponins, are known to act as capping agents, facilitating nanoparticle synthesis while also playing a crucial role in ion reduction and enhancing AgNP stability (Tailor *et al.*, 2020; Restrepo and Villa, 2021).

Our absorbance value was close to the absorbance of silver nanoparticles formed from *B. hainla* extract at 428 nm and *L. chinensis* with an absorbance of 422 nm (Tehri *et al.*, 2020; Budhathoki *et al.*, 2024). Several studies have reported the appearance of characteristic adsorption peaks for silver nanoparticles synthesized using plant extracts at wavelengths of 420, 422, 430, 434 and 435 nm (Flieger *et al.*, 2021). A 1 mM AgNO₃ concentration was selected based on the literature. No characteristic UV-Vis peak was observed at 0 hr, so the reaction was incubated in the dark overnight, and a color change was seen on next day, having a peak at 420 nm.

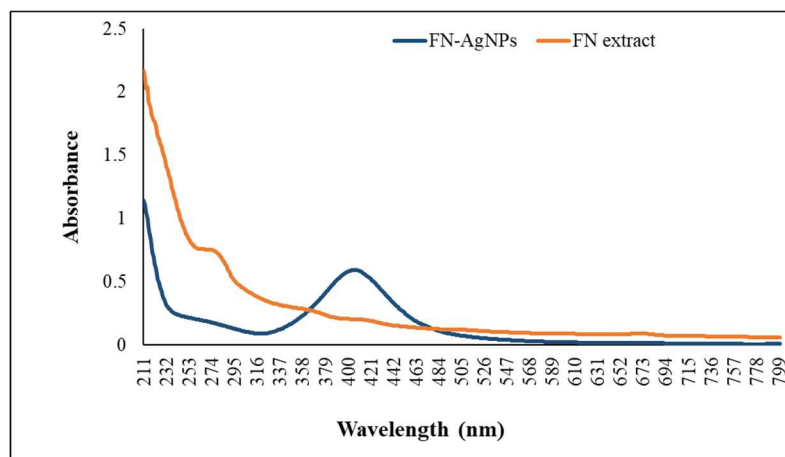


Fig. 1: UV-Vis absorption spectrum of biosynthesized AgNPs of *F.nubicola* plant extract

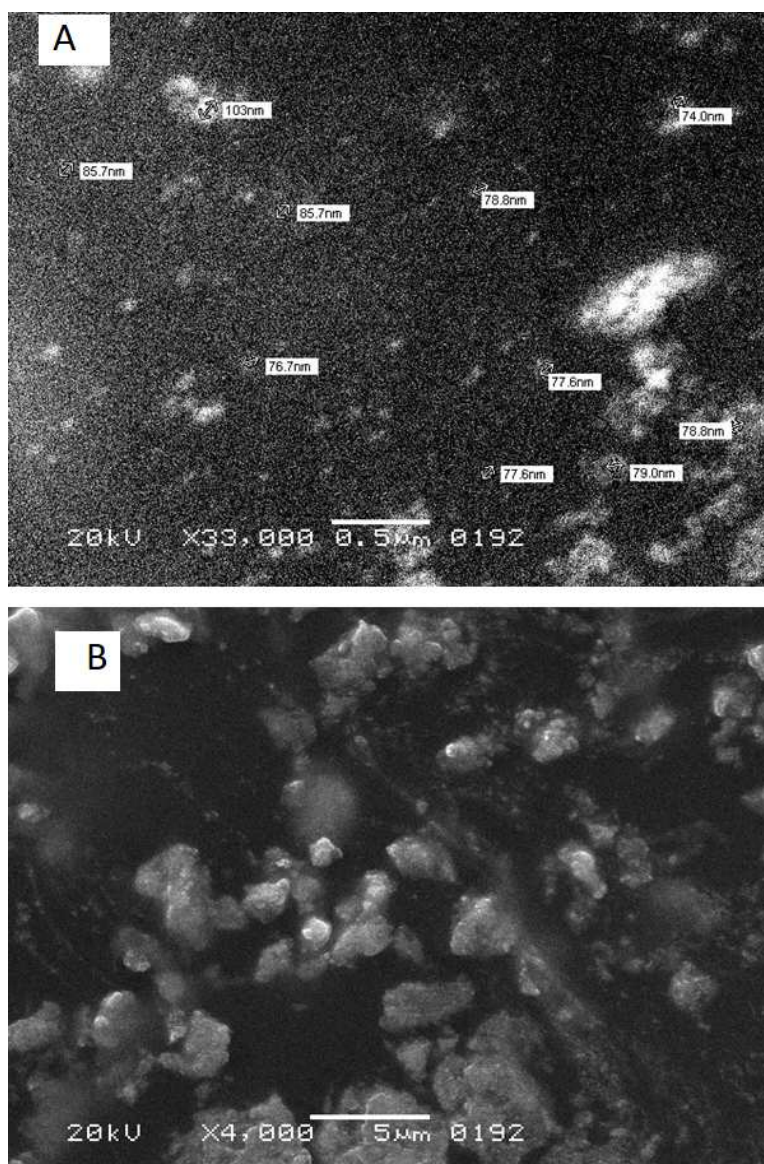


Fig. 2: SEM analysis of biosynthesized AgNPs of *F.nubicola* plant extract

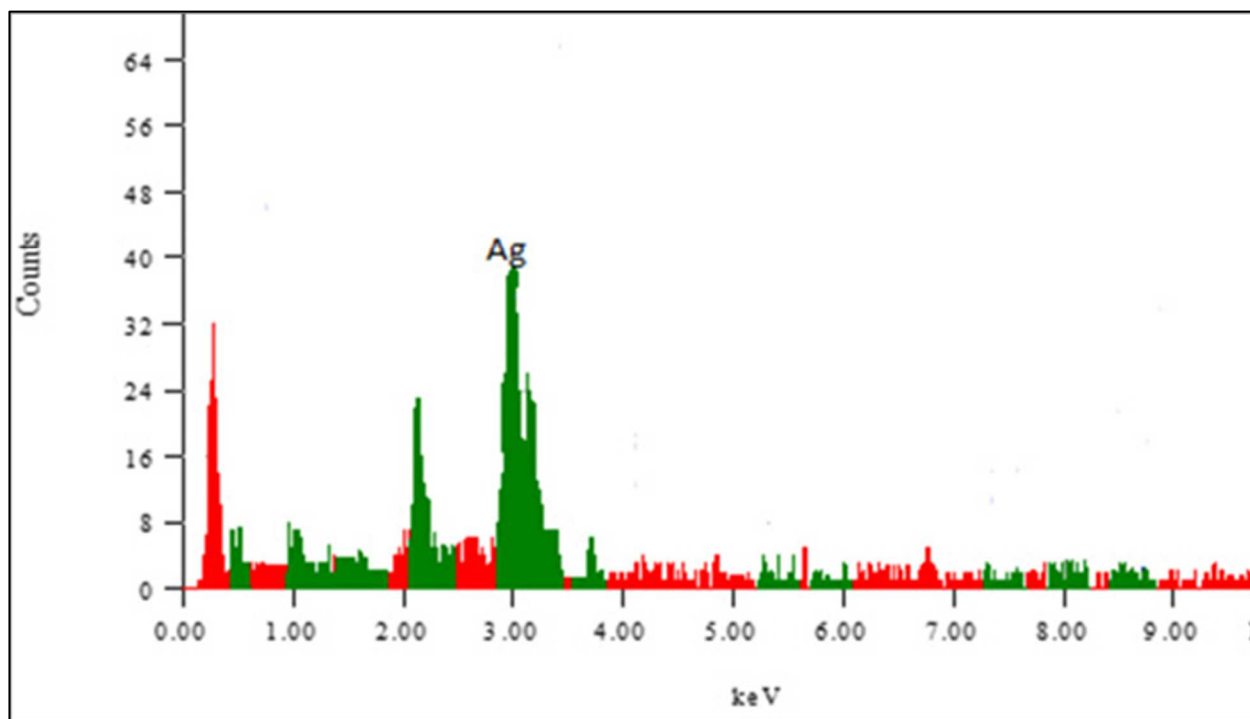


Fig. 3: EDX analysis of biosynthesized AgNPs of *F.nubicola* plant extract

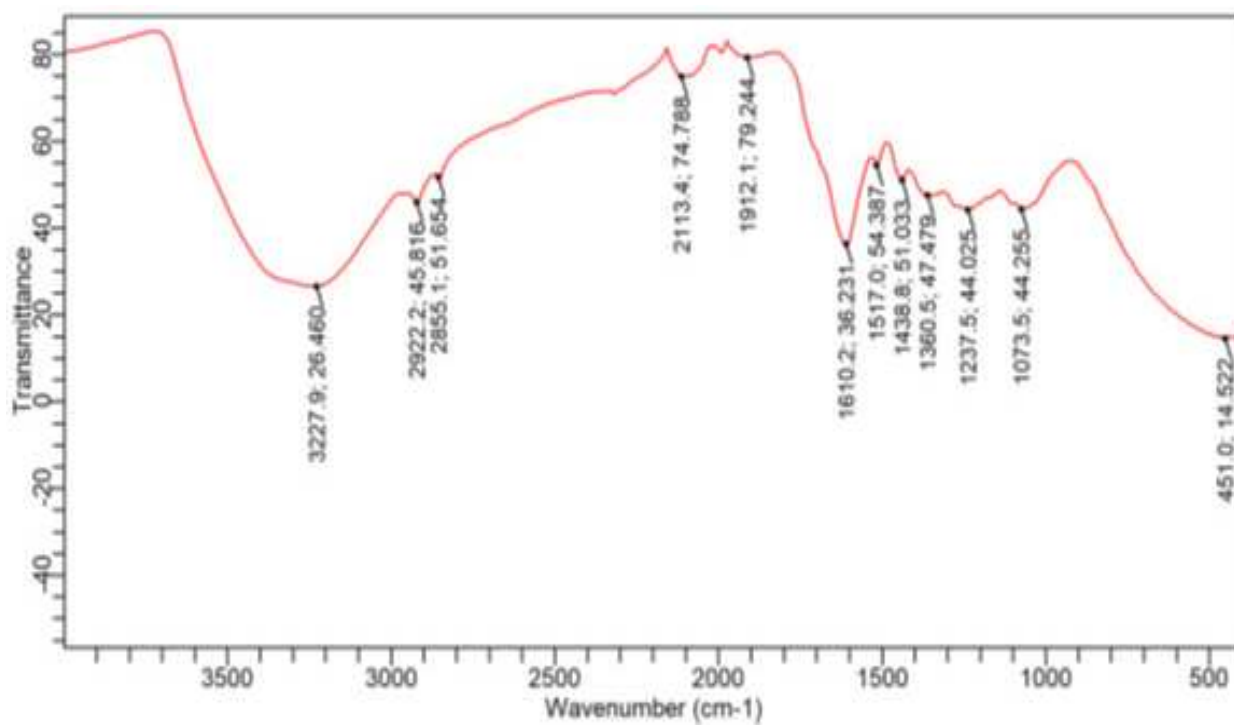
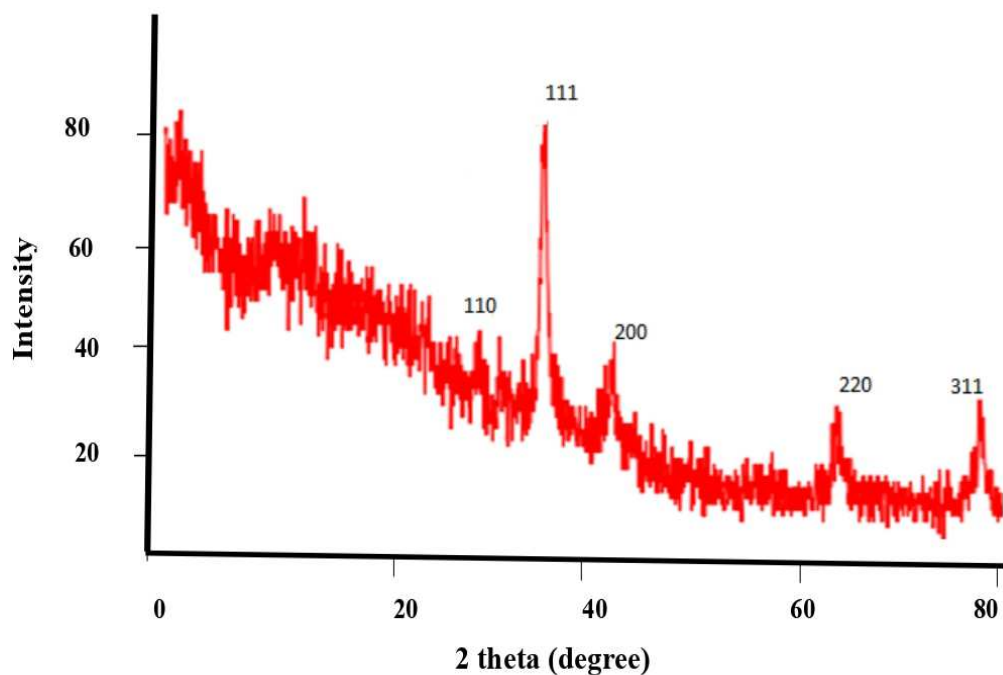
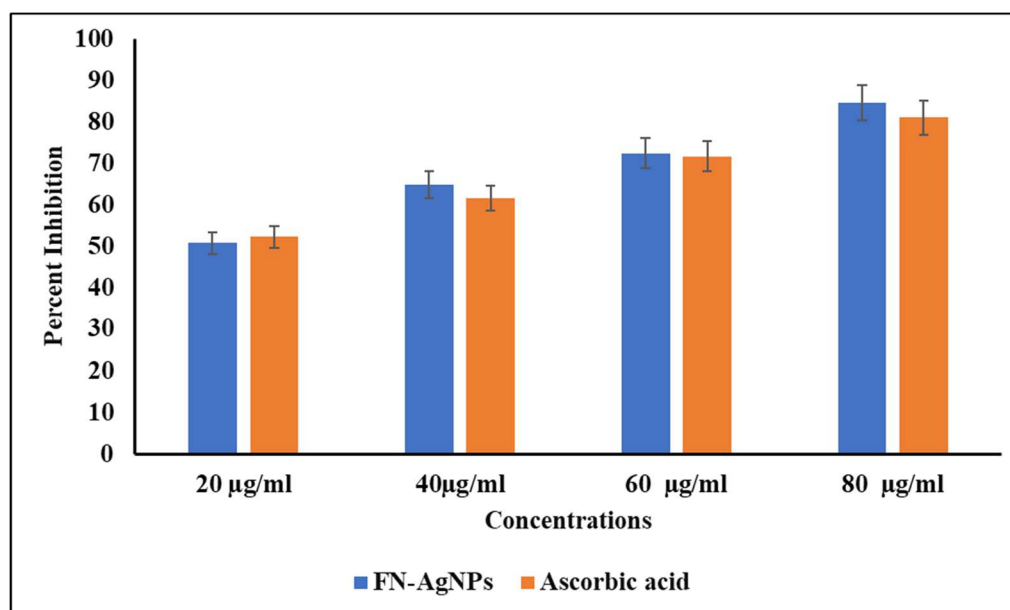


Fig. 4: FT-IR analysis of biosynthesized AgNPs of *F.nubicola* plant extract

Table 1: XRD results of biosynthesized AgNPs of *F. nubicola* plant extract

| 2 θ | θ | d-value | FWHM β degree | FWHM β radian | hkl | Size nm | Average size nm |
|------------|----------|---------|---------------------|---------------------|-----|---------|-----------------|
| 32.28 | 16.14 | 2.771 | 0.287 | 0.005009 | 110 | 30.07 | 69.5 |
| 38.22 | 19.11 | 2.629 | 0.08 | 0.001396 | 111 | 109.68 | |
| 44.4 | 22.20 | 2.039 | 0.114 | 0.00199 | 200 | 78.55 | |
| 64.46 | 32.23 | 1.44 | 0.263 | 0.00459 | 220 | 37.27 | |
| 77.26 | 38.63 | 1.234 | 0.115 | 0.002007 | 311 | 92.31 | |

**Fig. 5:** XRD analysis of AgNPs biosynthesized from *F. nubicola* plant extract**Fig. 6:** Percentage DPPH scavenging activity of biosynthesized AgNPs of *F. nubicola* plant extract and standard (Ascorbic acid) at different concentrations. Data obtained presented as mean \pm standard deviation ($n=3$)

The SEM examination of silver particles from *F. nubicola* revealed particles ranging 74-107 nm at 33,000 x, with dispersed morphology observed at 400 x and 1200x. Previously, SEM analysis of *Jacobaea maritima* exhibited the AgNPs varying from 28-52 nm at 37,000 magnifications (Althubiti *et al.*, 2023). The formation of larger nanoparticles may result from the clustering or aggregation of smaller particles. Variations in particle size can be attributed to agglomeration caused by polarity and electrostatic interactions among AgNPs. Similar findings were reported by (Firdous *et al.*, 2025), who observed silver nanoparticles with sizes ranging from 30 to 52 nm and an average size of 41.25 nm for *Carissa spinarum* due to agglomeration. Silver nanoparticles can indeed form in various shapes depending on the synthesis conditions of the agents. In the case of *F. nubicola* extract, somehow spherical nanoparticles were formed, likely due to the specific composition and properties of the extract. An increase in the concentration of extract typically leads to the development of larger nanoparticles, because it provides more reducing agents at the highest concentrated form, which in turn reduces more silver ions, by modifying the growth kinetics and promoting the formation of larger particles. However, this increased reduction can lead to the aggregation of silver ions, resulting in a larger particle size. Therefore, the larger nanoparticles can be associated with both higher availability of silver ions and the clustering effect during particle formation (Ahmad *et al.*, 2023). For instance, *Fragaria ananassa* (strawberry) seeds yielding nanoparticles in the 50–70 nm range and fruit pomace showing a size range of 10-20 nm, along with some irregular shapes, reflect the variability in the particle size and shape depending on the specific plant material and synthesis conditions (Alam, 2022; Ali *et al.*, 2022). Regarding *F. nubicola*, the strong peak at 3 keV observed by EDS is indeed a characteristic indication of the purity of metallic silver nanoparticles with a weight percentage of 29.04%, confirming the successful synthesis of AgNPs. The plant extracts can reduce metals such as gold, silver and platinum due to the presence of functional groups including hydroxyl, carboxyl, amino, and phenolic moieties. Phenolic acids, flavonoids and anthocyanins act as natural reducing and capping agents during nanoparticle synthesis (Meher *et al.*, 2024). In addition, the other signals detected in the EDS analysis indicate organic compounds, such as phytochemicals, absorbed on the surface of metallic nanoparticles. These organic substances of plant extract typically act as stabilizers for nanoparticles, which is a common observation in plant-mediated synthesis of nanoparticles (Choi *et al.*, 2019; Rautela and Rani, 2019). The functional groups present in the silver nanoparticles were analyzed using FTIR spectroscopy. The broad adsorption band at 3227.9 cm⁻¹ is attributed to the O-H stretching of phenolic compounds, indicating the role of polyphenols in reducing Ag⁺ ions. Peaks at 2922.23 cm⁻¹ and 2855.14 cm⁻¹ are associated with C-H stretching of aliphatic groups, while the band at 2113.40 cm⁻¹ indicates

C≡C stretching of alkynes. The peak at 1912 cm⁻¹ may correspond to cumulative double bonds (C=C=C) of allene groups. Additional signals between 1610 cm⁻¹ to 1360 cm⁻¹ suggest the presence of amide, nitro, ether, and alcohol groups, confirming the involvement of biomolecules such as proteins and polyphenols. These findings support the bio-reduction of Ag⁺ to Ag⁰ by phytochemicals present in *Fragaria nubicola* extract. Phenolic compounds act as reducing agents, while proteins contribute to nanoparticle stabilization through interactions involving amine (-NH₂) and carboxyl (-COOH) groups. Previous studies also highlight the involvement of carbonyl (C=O), hydroxyl (O-H), and amino (N-H) groups in green synthesis of AgNPs (Iravani *et al.*, 2014; Krithiga *et al.*, 2015; Flieger *et al.*, 2021). *Fragaria* species and other plant species such as *Ruta graveolens* are rich in flavonoids, anthocyanins, ellagitannins, hydroxybenzoic acid, hydroxyl cinnamic acid, and other bioactive molecules (Alam, 2022; Luo *et al.*, 2024), which enhance nanoparticles' stability through the surface (Alabraham *et al.*, 2025). Similar mechanisms have been reported in other plant-mediated AgNp synthesis. For example, flavonoids, catechins, and polyphenols in *Camellia sinensis*, *Typha angustifolia*, and *Eucalyptus globulus*, and amine and amide groups in *Solanum tuberosum* have been shown to play key roles in nanoparticle reduction and stabilization (Zeng *et al.*, 2020; Joudeh and Linke, 2022; Khan *et al.*, 2025). Likewise, in this study, hydroxyl and carbonyl functional groups of flavonoids and phenolics in *F.nubicola* extract could facilitate the reduction of Ag⁺ to Ag⁰ while acting as natural capping agents.

XRD analysis confirmed the crystalline nature by revealing the face-centered cubic (FCC) structure of the synthesized AgNPs. AgNPs with diverse morphologies, including spherical, nano bars, cubic, nanorods, triangular, and pyramidal structures, can be synthesized in a controlled manner by modulating the kinetics and thermodynamics of the reaction. This can be achieved through adjustments of factors such as solvent type, temperature and pH conditions during synthesis (Rodrigues *et al.*, 2024). A peak observed at 2 θ = 32.28 corresponds to the (110) plane, which is consistent with FCC geometry. Similarly, other peaks at 2 θ = 32, 44, 64 and 77 are consistent with 111, 200, 220, and 311, respectively. Five significant diffraction spikes were observed in the 110, 111, 200, 220 and 311 planes at 32.28°, 38.22°, 44.4°, 64.46° and 77.26° representing cubic crystalline structure of silver, respectively (Khan *et al.*, 2017; Asif *et al.*, 2022). Similar peaks were also reported in many plants derived silver nanoparticles including *Cinnamomum camphora*, *Senna siamea*, *Prunus dulcis*, and *Allium cepa* (Baran *et al.*, 2023). The average size of the synthesized silver nanoparticles in the present study was found to be 69.5 nm. However, nanoparticle size is highly dependent on the plant source and extraction conditions used during synthesis. For instance, AgNPs synthesized from the peel of *Eucalyptus edule* were

reported to be approximately 150 nm in size, whereas those obtained from the leaves of *Alternanthera dente* ranged between 50 and 100 nm. In contrast, the root extract of *Prangos ferulacea* yielded nanoparticles with sizes varying from 79 to 200 nm (Ahmed *et al.*, 2016; Mavaei *et al.*, 2020). A wide range of particle sizes has been documented in the literature of silver nanoparticles synthesized using various medicinal plants, including 10-80 nm for *Cassia angustifolia*, 160-260 nm for *Ficus krishnae*, 1-100 nm for *Nigella sativa*, 50-350 nm for *Aloe vera*, 20-70 nm for *Viburnum lanata*, and approximately 57 nm for *Moringa oleifera* (Khan *et al.*, 2025; Meher *et al.*, 2024). Similarly, gold nanoparticles (AuNPs) synthesized using medicinal plants have also been documented, such as 100-500 nm from *Annona muricata*, 154 nm from *Scutellaria barbata* and 20-200 nm from *Abies spectabilis*, all of which exhibited notable antioxidant and anticancer activities against bladder and pancreatic cancer cell lines (Khan *et al.*, 2025). Particle size plays a critical role in defining the biological performance of nanoparticles, particularly in biomedical applications. Previous studies have demonstrated that nanoparticles exhibit optimal cellular uptake and enhanced therapeutic efficacy (Khan *et al.*, 2025; Wu *et al.*, 2019). In biomedicine, AgNPs play a pivotal role in the development of advanced antimicrobial agents, novel drug delivery systems, and diagnostic approaches for various cancers. They have been engineered to enhance immune responses against infections through interactions at both extracellular and intracellular levels (Meher *et al.*, 2024). X-ray diffraction based on Bragg's law provides information on the crystalline structure and average particle size of NPs. In contrast, SEM coupled with EDS is employed to examine surface morphology, elemental composition and agglomeration. In the present study, SEM micrographs revealed that silver nanoparticles tended to agglomerate into small clusters of approximately 5-6 or more particles, which further merged to form larger aggregates due to their high surface energy. The particle size estimated from XRD was smaller than that observed in SEM, as XRD measures individual crystalline size, whereas SEM reflects the size of agglomerated particles, resulting in larger apparent dimensions (Joudeh and Linke, 2022; Long *et al.*, 2023).

Antioxidants are the chemical moieties that inhibit free radicals generated during various metabolic activities. Superoxide anion, singlet oxygen, and hydroxyl radicals are harmful reactive oxygen species produced during normal metabolic processes that damage proteins, lipids and DNA. Researchers have established a strong link between oxidative stress and the pathogenesis of numerous oxidative stress-related disorders such as cardiovascular complications, diabetes, and various cancers. Antioxidants play a crucial role by delaying and suppressing the initiation and propagation of oxidative chain reactions, thereby limiting the reactive oxygen species-induced cell injury. Remarkably, silver nanoparticles have shown a

narrow plasmon resonance, unique physicochemical features, and potent antioxidant, anticancer, and antimicrobial properties, which highlight their potential as promising therapeutic agents (Baran *et al.*, 2023; Khan *et al.*, 2025). Furthermore, the unique physicochemical properties of AgNPs render them a promising candidate for applications in wound dressings, bone implants, dental materials, and food quality enhancement (Nie *et al.*, 2023). DPPH assay is generally employed to find out the antioxidant potential of any sample, as it indicates successful antioxidant activity by changing color from violet to pale yellow. DPPH acts by capturing free electrons or hydrogen from species, and silver nanoparticles transfer electrons upon contact with DPPH, resulting in decolorization (Ali *et al.*, 2022). The silver nanoparticles derived from *F.nubicola* exhibited a higher percentage of inhibition as their concentration increased, indicating an enhanced antioxidant capacity. This improved efficacy can be attributed to bioactive hydroxyl and nitrogenous groups, which play a pivotal role in synthesis. These functional groups facilitate a reduction in ions but also contribute to stabilization, resulting in an enhanced ability to neutralize free radicals. These functional groups may enhance the reduction and stabilization process, leading to nanoparticles that are more effective in scavenging free radicals (Restrepo and Villa, 2021). Furthermore, these functional groups are responsible for preventing lipid oxidation and, by directly influencing free radical scavenging, they enhance the activity of antioxidant enzymes (Jalilian *et al.*, 2020). The enhanced antioxidant capacity of FN-AgNPs can be attributed to the presence of phytochemicals serving as capping agents, as previously reported for *Ixora* flower-mediated AgNPs by Patil and Raghavendra (2024) using the DPPH assay. Similarly, multifunctional nanoparticles synthesized from *Cassia fistula* extract have been reported to exhibit strong antioxidant activity, as confirmed by the DPPH assay, in which stable DPPH radical is reduced to its hydrogenated form through electron transfer (Mohamed *et al.*, 2024; Alabrahim *et al.*, 2025). Therefore, the antioxidant activity of NPs was primarily evaluated using the DPPH assay, as it is a widely accepted, rapid, and reliable method for assessing free radical scavenging efficiency compared with FRAP and ABTS. Based on the findings, the study concludes that nanoparticles can be successfully synthesized from readily available wild strawberries using a simple, cost-effective, and environmentally friendly method without the use of harmful chemicals or expensive equipment. Moreover, the bioactive metabolites highlight the potential of these NPs for applications in nanomedicine, particularly in managing arthritis and neurological disorders.

CONCLUSION

Silver nanoparticles were formed through a green chemistry approach by reducing a silver nitrate solution

using the ethanol extract of the whole *F. nubicola* plant. This biogenic synthesis is environmentally sustainable, efficient, and economically viable, offering a green alternative to conventional chemical methods due to numerous biohazards. The synthesized nanoparticles underwent comprehensive characterization through various techniques. SEM/EDX analysis verified their uniform morphology and elemental composition, while XRD confirmed their crystalline structure, indicating successful synthesis. Additionally, FTIR analysis highlighted the crucial role of secondary metabolites from *F. nubicola* in capping and stabilizing the nanoparticles, underscoring the dual function of plant extract as both a reducing and stabilizing agent in the synthesis process. Furthermore, the antioxidant potential of AgNPs was evaluated, showing significant free radical scavenging activity, which underscores their potential for therapeutic applications. Given their biocompatibility, stability, and notable antioxidant properties, AgNPs from *F. nubicola* hold promising prospects for use in various industrial sectors, particularly in medicine, for drug delivery, wound healing, and as antimicrobial agents.

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Author's contributions

R.R.: Conceptualization, Analysis, Methodology, Investigation, Writing – original draft preparation, Review. A.S., S.B., M. A., R.S.: Data curation. Y.Q.: Review. H. M. L.: Investigation, Data curation.

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