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# Green Synthesis of Silver Nanoparticles Using *Murraya Koenigii*: Characterization, Antioxidant, and Antibiofilm Potential

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## ABSTRACT

*This study investigates the green synthesis of silver nanoparticles (Ag-NPs) utilizing the leaf extract of Murraya koenigii as reducing and capping agent. The synthesized nanoparticles were characterized using various analytical techniques, including UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-ray Diffraction (XRD). The successful formation of AgNPs was confirmed by the appearance of a distinct surface plasmon resonance peak at 420 nm. Morphological analyses revealed that the nanoparticles predominantly spherical shape, with particle sizes range from 25 and 50 nm. Phytochemicals present in leaf extract played an important role in reducing silver ions and stabilizing nanoparticles. The biological activities of synthesized Ag-NPs were evaluated viz antioxidant and antibiofilm assays. Antioxidant potentials determined using hydrogen peroxide scavenging assay, while antibiofilm activity was assessed using tissue culture plate method against Staphylococcus aureus (MTCC 1678) and Escherichia coli (MTCC 737). The findings indicated that biosynthesized AgNPs exhibited considerable antioxidant and antibiofilm activities, suggesting their potential application as effective anti-microbial and bio medical agents.*

**Keywords:** *Murraya koenigii, Silver nanoparticles, Green synthesis, Antioxidant activity, Antibiofilm activity.*

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## Introduction

Green nanotechnology an emerging interdisciplinary field that has attracted considerable attention because it offers environmentally benign approaches for nanomaterials. Nanotechnology has emerged as a rapidly advancing field due to the distinctive physicochemical characteristics of nanoparticles, such as their large surface-area-to-volume ratio, excellent catalytic performance, enhanced thermal resistance, superior electrical properties, and greater mechanical durability when compared to bulk materials [1-2]. The elevated surface-area-to-volume ratio is especially significant in biological systems, as it promotes stronger interactions with microbial cells and various biomolecules. This enhanced interaction contributes to improved antimicrobial, antioxidant, and therapeutic activities, making nanoparticles highly valuable for biomedical applications [3]. Recent advances in nano-science accelerated the development of nanomaterials for biomedical, pharmaceutical, environmental, and agricultural applications [4].

The increasing prevalence of multidrug-resistant microorganisms created an urgent need for novel antimicrobial agents. Nanoparticles have emerged as promising alternatives due to its unique size-dependent physicochemical and biological properties [5]. Metal nanoparticles such as silver, gold, copper, platinum, and zinc oxide have been synthesized through physical, chemical, and biological routes; however, green synthesis methods are preferred because they are cost-effective, eco-friendly, and free from hazardous reducing agents [6,7]. Plant-mediated synthesis has gained particular importance as phytochemicals present in plant extracts can simultaneously act as reducing, capping, and stabilizing agents, resulting in the formation of stable nanoparticles with enhanced biological activities [8]. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have received significant attention because of their broad-spectrum antimicrobial, antioxidant, anti-inflammatory, anticancer, and antibiofilm properties [2,9]. AgNPs have found applications in medical devices, wound dressings, antimicrobial coatings, water purification systems, textiles, biosensors, drug delivery systems, and food packaging materials [10-11].

Silver is regarded as an oligodynamic metal exhibiting potent antimicrobial activity at low concentrations while maintaining relatively low toxicity toward mammalian cells [12]. The antimicrobial mechanism of AgNPs involves disruption of cell membranes, generation of reactive oxygen species, inhibition of essential enzymes, and interference with microbial DNA replication [13].

*Murraya koenigii* (L.) Spreng., commonly referred as curry leaf, is a medicinally important aromatic plant belonging to the Rutaceae family. Its leaves are widely utilized as a culinary spice in Indian food preparations and are recognized for a broad spectrum of therapeutic properties, including antioxidant, antimicrobial, antidiabetic, anti-inflammatory, hepatoprotective, and anticancer activities [14-15]. These biological effects are mainly attributed to the presence of various phytochemicals such as carbazole alkaloids, flavonoids, phenolic compounds, terpenoids, and essential oils [15-16]. The bioactive constituents facilitate the reduction of metal ions and contribute the stabilization of nanoparticles during green synthesis. Several studies have reported the effective biosynthesis of silver nanoparticles using *M. koenigii* leaf extract, resulting in the formation of stable nanoparticles with noteworthy antimicrobial properties [17-18]. More recent research has also demonstrated the enhanced antioxidant and antibacterial activities of *M. koenigii*-derived AgNPs, emphasizing their potential for diverse biomedical applications [19-20].

The advancement of green nanotechnology and plant-mediated nanoparticle synthesis [21]. Their investigations have demonstrated the successful biosynthesis of various metal oxide nanoparticles, including CuO, ZnO, CoO, FeO, and Ag nanoparticles, using medicinal and economically important plant extracts. Green synthesized CuO nanoparticles from *Angelonia angustifolia* exhibited promising antibacterial properties, highlighting the potential of phytochemical-assisted nanoparticle synthesis for biomedical applications [21,22]. Similarly, ZnO nanoparticles were reported to possess desirable structural characteristics and significant biological activities [23]. A comprehensive review on plant-mediated cobalt oxide nanoparticles further emphasized the growing importance of eco-friendly synthesis approaches and their applications in medicine, environmental remediation, and catalysis [24]. Dolichandrone-mediated mixed-phase iron oxide nanoparticles synthesized through green routes demonstrated effective antibacterial activity and structural stability [25]. And the biosynthesized silver nanoparticles have been successfully utilized as catalysts for the aqueous-phase synthesis of heterocyclic compounds, expanding the scope of green nanotechnology beyond antimicrobial applications [26]. These findings collectively support and exploration of plant-mediated synthesis of metallic nanoparticles and provide a strong foundation for the present investigation utilizing *Murraya koenigii* leaf extract for the synthesis of bioactive silver nanoparticles.

## Materials and Methods

### Selection and Collection of plant materials

Fresh leaves of *Murraya koenigii* (L.) Spreng. were collected and washed thoroughly under running tap water to eliminate adhering dust and impurities, followed by repeated rinsing with distilled water. The washed leaves were shade-dried at ambient temperature (25 ± 2 °C) until completely free from moisture. The dried material was then pulverized into a fine powder using a sterilized electric grinder.

To prepare the aqueous extract, 50 g of the powdered leaves was added to 150 mL of distilled water and heated at approximately 80 °C for 10 min under constant stirring. After heating, the mixture was allowed to cool to room temperature and subsequently filtered first through muslin cloth and then through Whatman No. 1 filter paper to obtain a clear extract. The filtrate exhibited a pale green color, suggesting the presence of water-soluble phytochemicals. The prepared extract was transferred into sterile containers, covered with aluminum foil to minimize contamination and light-induced degradation, and stored at 4 °C until further use in the synthesis of silver nanoparticles and related analyses. All glassware employed during the extraction procedure was thoroughly cleaned and sterilized before-hand to maintain experimental reliability and reproducibility (Fig. 1).

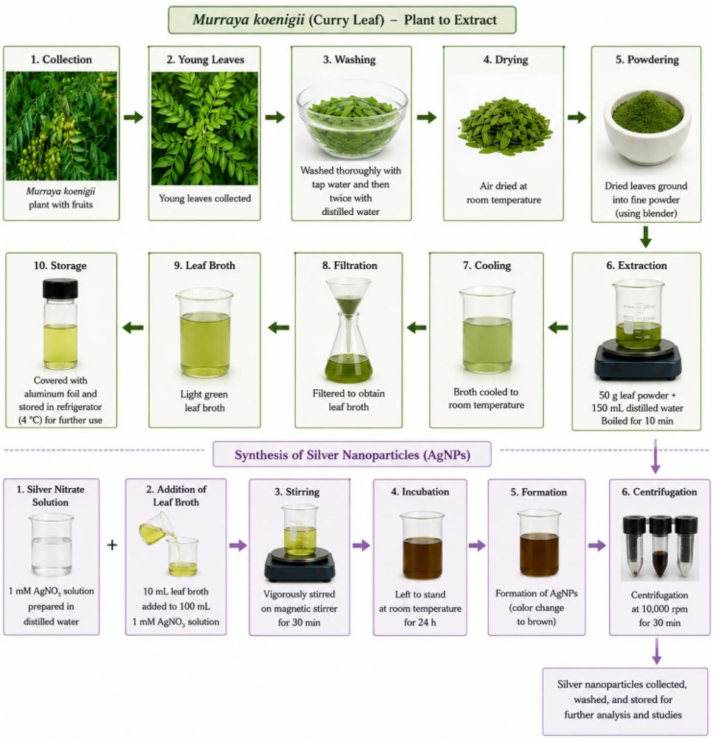


*Fig. 1: Synthesis of AgNPs from Murraya koenigii (L.) plants*

### Synthesis of Siver Nanoparticles (AgNPs) and Characterization

Silver nanoparticles (AgNPs) were bio-synthesized using aqueous leaf extract of *Murraya koenigii* through an environmentally friendly green synthesis method. 1 mM silver nitrate ($AgNO_3$) solution was prepared by dissolving the appropriate amount of analytical-grade $AgNO_3$ (Merck, Mumbai, India) in 250 mL of distilled water. For nanoparticle production, 10 mL of freshly prepared leaf extract was gradually introduced into 100 mL of the $AgNO_3$ solution under constant stirring at room temperature (25 ± 2 °C). The reaction mixture continuously agitated with magnetic stirrer for 30 minutes and promote homogeneous mixing and enhance the reduction of silver ions and bioactive compounds present in the extract. Following stirring, the mixture left un-disturbed at room temperature for 24 hours and to ensure complete reduction of $Ag^+$ ions and nanoparticle formation. Successful synthesis visually indicated by a noticeable color transition from light yellow to dark brown, resulting from the surface plasmon resonance phenomenon of silver nanoparticles. The synthesized AgNPs were subsequently separated by centrifugation at 10,000 rpm for 30 min.

The collected pellet was repeatedly washed with distilled water to eliminate excess reactants and impurities. The purified nanoparticles were then dried and preserved for subsequent characterization and biological activity studies [4].

### Characterization of Silver Nanoparticles

The synthesized AgNPs were characterized using a combination of spectroscopic and microscopic techniques to determine their physicochemical properties. UV–Visible spectroscopic analysis was carried out within the wavelength range of 250–800 nm to verify nanoparticle formation and detect the characteristic surface plasmon resonance peak. Fourier Transform Infrared (FTIR) spectroscopy was performed over the range of 400–4000 $cm^{-1}$ to identify functional groups involved in the reduction, capping, and stabilization of the nanoparticles. The surface morphology of the synthesized AgNPs was examined using Scanning Electron Microscopy (SEM), while Energy-Dispersive X-ray (EDX) analysis was employed to confirm the presence of silver and evaluate the elemental composition of the sample. Transmission Electron Microscopy (TEM) was used to investigate nanoparticle size, shape, and distribution patterns, providing detailed information regarding their structural characteristics.

### Antioxidant Activity of Silver Nanoparticles

The antioxidant activity of the synthesized silver nanoparticles (AgNPs) was evaluated using the hydrogen peroxide ($H_2O_2$) scavenging assay. Different concentrations of AgNPs (20, 40, 60, 80, 100, and 120 μg $mL^{-1}$) were prepared, along with a standard ascorbic acid solution for comparison. 2 mM hydrogen peroxide solution was freshly prepared in distilled water. Aliquots of AgNPs at different concentrations were mixed with the hydrogen peroxide solution and vortexed thoroughly to ensure proper mixing. The reaction mixtures then incubated at room temperature for 15 minutes. Following incubation, absorbance of each sample measured at 230 nm using a UV–Visible spectrophotometer. The hydrogen peroxide scavenging activity of the AgNPs calculated and compared with that of the standard ascorbic acid according to the method described. The assay was performed to assess the free radical scavenging potential of the synthesized silver nanoparticles [16].

### Anti-biofilm activity

The involving bacterial biofilm inhibition assay against MTCC 1678) and *E. coli* (MTCC 737), the present study put-forward the potential of AgNPs as an inhibitor for biofilm. And the involving 96 well plate overnight grown culture was diluted as 1:100 ratio in a fresh medium. Further AgNPs dose as 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, and 60 μg tested once added to 96 well titer plate and incubated at 37°C for 24 hours. Upon incubation medium removed and, wells were washed with the phosphate buffer saline. It was then added with 0.1% w/v crystal violet and incubated for 20 minutes. The crystal violet then removed by washing with phosphate buffer saline, attached cell quantification made by solubilizing remained crystal violet in absolute ethanol and final measurement made at 570 nm. Next reduction of the biofilm correlated with cells grown in the absence of AgNPs [16].

## Results and Discussion

### UV- visible spectroscopy of AgNPs

In the study *M. koenigii* reduced silver nanoparticles once synthesized was primarily tested for checking plasmon resonance of the AgNPs. UV-visible spectroscopy at possible peak range of 300-800 nm, recorded the typical peak at 420 nm which is characteristics features of the AgNPs as shown Fig. 1. This observation aligns with previous studies where the development of AgNPs results in a distinctive color shift and a surface plasmon resonance band, confirming their formation [3]. This characteristic absorption peak, typically observed within the 400-470 nm range, arises from the collective oscillation of conduction electrons in response to incident light, a phenomenon known as surface plasmon resonance [12].

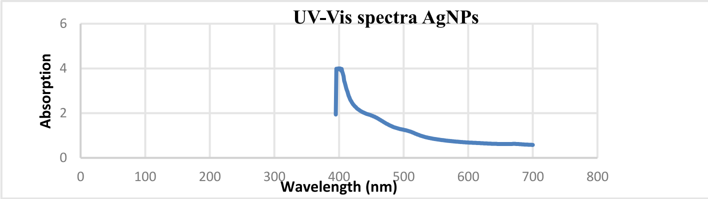


*Fig. 1: UV visible spectroscopy of the silver nanoparticles*

### FTIR and XRD of AgNPs

As per FTIR peaks recorded in the range of 500 to 4000 $cm^{-1}$ some typical peaks noted as 3455, 2883, 1632, and 1090 $cm^{-1}$ assigned to functional group as -OH, -CH, -C=C, and -NH respectively as shown in Fig.2. These distinct absorption bands collectively indicate the presence of biomolecules such as flavonoids, phenolic compounds, and proteins, which act as effective capping and reducing agents in the biogenic synthesis of silver nanoparticles . As per XRD analysis we recorded the typical spectra of AgNPs as shown in Fig. 3. And as per data analysis the average particle size found around 25-50 nm once calculated using Debye-Scherrer formula which indicates high surface area to volume ratio of nanocrystals. This crystalline nature is further corroborated, presence of Bragg reflections in the X-ray diffraction pattern, which align with established data for face-centered cubic silver. These diffraction patterns not only confirm the metallic silver composition but also provide critical information regarding crystallite size and lattice parameters, essential for understanding the material's properties and potential applications [14].

### SEM and TEM analysis of AgNPs

SEM analysis at various resolutions right form 2 μm to 100 nm, found that AgNPs remained in aggregated form and that may be because of the capping and stabilization by the plant biomolecules of *M. koenigii* as shown in Fig. 12 TEM data reported the defined globular shaped AgNPs with average size of 25-50nm. TEM analysis revealed the presence of clear lattice fringes, indicating the highly crystalline nature of the nanoparticles and confirming their structural integrity. The high-resolution TEM further elucidated these crystalline characteristics, showing a lattice spacing of approximately 0.234 nm, which corresponds to the plane of face-centered cubic silver [11].

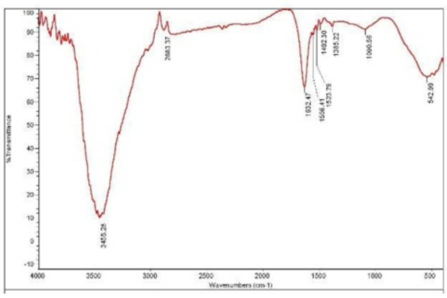

*Fig. 2: FTIR spectra of the AgNPs*

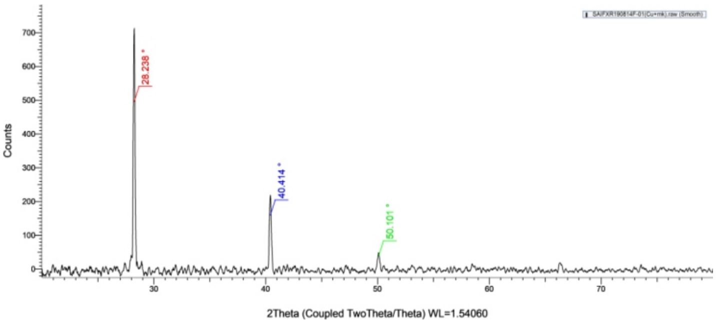


*Fig. 3: XRD spectra of the AgNPs*

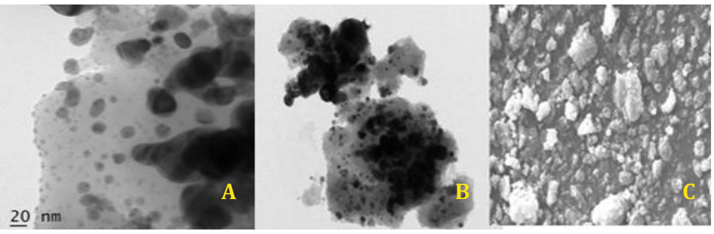


*Fig. 4: TEM (a), (b) and SEM images (c) of the AgNPs*

## Antioxidant activity of AgNPs

The ability to showcase antioxidant activity here screened with 20-2120 µg/ml dose of AgNPs along with standard ascorbic acid by Hydrogen peroxide assay. The recorded an excellent antioxidant activity of *M. koenigii* reduced AgNPs as concentration increases percentage of antioxidant increases. This result indicated that AgNPs potential as an equivalent antioxidant like that of ascorbic acid and needs to be tested further (Table 1). This enhanced antioxidant activity is often attributed to the increased surface area-to-volume ratio of the nanoparticles, which provides more active sites for radical quenching through electron transfer and valence transition [15]. The increasing AgNP concentration leads to improved radical scavenging, suggests a dose-dependent antioxidant capacity, aligning with findings from other studies [17].

*Table 1: Hydrogen Peroxide assay-based activity of the AgNPs along with positive control Ascorbic acid*

| Concentrations (µg/ml) | AgNPs | Std Ascorbic acid |
|---|---|---|
| | % inhibition | |
| **20** | 40.07 | 42.51 |
| **40** | 49.63 | 50.93 |
| **60** | 55.8 | 56.23 |
| **80** | 57.89 | 59.56 |
| **100** | 59.56 | 60.23 |
| **120** | 62.54 | 63.17 |

## Antibiofilm activity of AgNPs

As per crystal violet assay for antibiofilm inhibition assay by AgNPs against *E. coli* and *S. aureus*, we found that both pathogens are sensitive to get inhibited for their biofilm formation (fig.2). Increases with increase in concentration as per Table 2. This variation in efficacy underscores the need for species-specific optimization of AgNP concentrations for antibiofilm applications [18]. The precise mechanisms underlying this differential activity involve the disruption of bacterial cell membranes and interference with quorum sensing pathways, which are critical for biofilm development. Beyond direct membrane damage, AgNPs can also inhibit biofilm formation by interfering with bacterial adhesion and extracellular polymeric substance production [1].

**Figure 2.** Antibiofilm activity of green synthesized silver nanoparticles (AgNPs) against *E. coli* and *S. aureus* evaluated using the crystal violet staining method. A concentration-dependent reduction in biofilm formation was observed in both bacterial strains, confirming the inhibitory potential of AgNPs against bacterial biofilms.

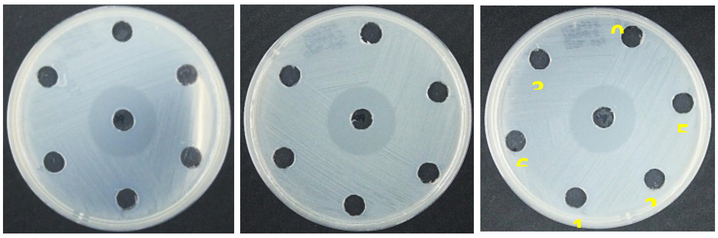

*Table 2: Antibiofilm activity recorded as per % inhibition based on dose response of AgNPs*

| Concentration µg/ml | *E. coli* (% inhibition) | *S. aureus* (% inhibition) |
|---|---|---|
| 10 | 22.67 | 56.16 |
| 20 | 25.02 | 58.72 |
| 30 | 25.49 | 55.81 |
| 40 | 29.71 | 53.91 |
| 50 | 71.06 | 65.91 |
| 60 | 78.43 | 72.55 |

## Discussion

The present investigation successfully achieved the green synthesis of silver nanoparticles (AgNPs) using *Murraya koenigii* leaf extract and demonstrated their notable antioxidant activity through hydrogen peroxide scavenging assays. The visual transformation of the reaction mixture from pale yellow to dark brown, together with UV–Visible spectroscopic confirmation, verified the effective reduction of $Ag^+$ ions and the formation of stable silver nanoparticles. Green synthesis approaches have become increasingly important because they provide an environmentally benign, economically feasible, and sustainable alternative to conventional chemical and physical nanoparticle synthesis methods. Similar observations have been reported in the synthesis of CuO nanoparticles using plant extracts, where naturally occurring phytochemicals functioned as reducing and capping agents, leading to nanoparticles with remarkable antioxidant and antibacterial activities [31]. The antioxidant performance of the synthesized AgNPs can be attributed to the combined influence of silver nanoparticles and the bioactive phytoconstituents derived from *M. koenigii*. Phytochemicals such as phenolic compounds, flavonoids, alkaloids, and terpenoids retained on the nanoparticle surface are known to enhance free radical scavenging activity and improve the biological functionality of nanoparticles. The findings have been documented for several medicinal plant-based nanoparticle systems, where enhanced antioxidant efficacy was associated with the presence of plant-derived secondary metabolites and their interaction with metallic nanoparticles [32]. A significant outcome of the present study is the demonstration that *M. koenigii*-mediated AgNPs can serve as multifunctional bioactive nanomaterials with potential applications in diverse sectors. Recent advancements in green nanotechnology have highlighted the importance of biologically synthesized nanoparticles in medicine, agriculture, and environmental management. For example, nanoparticles synthesized using *Azadirachta indica* extracts have shown protective effects against lead acetate-induced toxicity by reducing oxidative stress and cellular damage, thereby emphasizing the therapeutic value of phytogenic nanomaterials [33].

These findings further support the possibility of utilizing *M. koenigii*-derived AgNPs as effective antioxidant agents for biomedical and environmental applications. The relevance of nanoparticle-based technologies is also increasingly recognized in sustainable agriculture. Nano-enabled formulations, including nano-fertilizers and nano-biofertilizers, have emerged as innovative strategies for enhancing nutrient utilization efficiency, minimizing nutrient losses, and supporting climate-resilient agricultural practices [34,35], the development of plant-mediated nanoparticles offers an eco-friendly platform that may contribute to sustainable crop production and environmental protection. The antioxidant properties exhibited by the synthesized AgNPs in the present study further broaden their potential utility in agricultural systems subjected to oxidative stress conditions. Morphological and elemental characterization performed through SEM–EDX and TEM analyses revealed features consistent with previously reported plant-mediated metallic nanoparticles. The observed stability and structural integrity of the synthesized AgNPs may be attributed to the capping action of phytochemicals present in the leaf extract. Similar results have been reported for cobalt oxide nanoparticles synthesized using *Uraria picta*, where phytochemical-assisted synthesis enhanced nanoparticle stability and biological performance [36]. Numerous studies have likewise demonstrated that bioactive plant constituents play a critical role in nanoparticle formation, stabilization, and biological activity enhancement [37].

The broader significance of this study lies in its contribution to the growing field of sustainable nanobiotechnology. The use of *M. koenigii*, an easily available medicinal plant rich in bioactive compounds, provides a simple and eco-friendly route for the production of biologically active silver nanoparticles. The synthesized AgNPs exhibited substantial antioxidant potential, indicating their suitability for applications requiring oxidative stress mitigation, the study provides a foundation for future investigations on antimicrobial, anticancer, wound-healing, drug-delivery, and environmental remediation applications of phytogenic nanoparticles, the results indicate that *Murraya koenigii*-mediated silver nanoparticles possess considerable antioxidant potential and may serve as promising candidates for future pharmaceutical, agricultural, and environmental applications, the findings of the present study are in agreement with earlier reports describing the antioxidant and biological potential of medicinal plant-derived nanoparticles [38,39]. The successful synthesis and antioxidant efficacy of *M. koenigii*-mediated AgNPs highlight their promise as sustainable nanomaterials with potential applications in pharmaceutical, biomedical, agricultural, and environmental sectors. These results underscore the importance of integrating green chemistry principles with nanotechnology to develop safer and more effective functional materials for future applications.

## Conclusion

The study reported successfully synthesis of green silver nanoparticles having size 25-50 nm from *Muraya koinigii* leaf extract. Study also reported excellent antioxidant and antibiofilm activity against both gram-positive and gram-negative pathogens of these green nanoparticles. These results support the integration multipotential silver nanoparticles in nanotechnology for antimicrobial research, particularly for developing alternatives in response to antibiotic resistance.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests.

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