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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING FRESH AND SHADE-DRIED *MURRAYA KOENIGII* LEAF EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract
Green synthesis of silver nanoparticles (AgNPs) using plant extracts is a simple and eco-friendly approach. In the present study, AgNPs were synthesized using aqueous extracts of fresh and shade-dried *Murraya koenigii* leaves, and their antibacterial activity was evaluated against *Escherichia coli*. The effect of extract volume on AgNPs synthesis was investigated, and monitored by colour change and UV–Visible spectroscopy. The formation of AgNPs was indicated by an absorption band around 440–450 nm. Phytochemical screening showed a broader profile in the shade-dried leaf extract. However, AgNPs synthesized using the fresh leaf extract showed comparatively higher antibacterial activity against *E. coli*, with a zone of inhibition of 1.8 mm, compared with 1.3 mm for nanoparticles synthesized using the shade-dried extract. These preliminary findings indicate the potential of *M. koenigii* leaf extract for the green synthesis of AgNPs with antibacterial activity.

Keywords: *Murraya koenigii*, silver nanoparticles, green synthesis, phytochemical screening, antibacterial activity, *Escherichia coli*.

1. Introduction
Nanotechnology has emerged as one of the most rapidly advancing fields of science, with applications spanning medicine, agriculture, food technology, environmental remediation, and biotechnology. Among various nanomaterials, silver nanoparticles (AgNPs) have received particular attention because of their remarkable antimicrobial, antioxidant, catalytic, and biomedical properties (Nandasana et al., 2025).
Conventional methods for synthesizing silver nanoparticles generally involve chemical reducing agents and stabilizing compounds that may be toxic to humans and the environment. Consequently, green synthesis approaches employing biological materials have become increasingly popular because they are environmentally benign, cost-effective, and sustainable(Pram et al., 2024).
Plant-mediated synthesis utilizes naturally occurring phytochemicals such as phenolics, flavonoids, terpenoids, alkaloids, proteins, and sugars as reducing as well as stabilizing agents. This eliminates the requirement for hazardous chemicals while simplifying nanoparticle synthesis (Vinyagamoorthy et al., 2024).
Murraya koenigii (L.) Spreng., commonly known as the curry leaf plant, is a medicinal plant widely recognized for its rich phytochemical composition and diverse pharmacological activities. Leaves of *M. koenigii* contain numerous bioactive constituents capable of reducing silver ions into metallic nanoparticles. Despite increasing reports on plant-mediated nanoparticle synthesis, optimization of synthesis conditions remains necessary to obtain stable nanoparticles with desirable physicochemical properties.
Therefore, the present preliminary study aimed to optimize the green synthesis of silver nanoparticles using aqueous leaf extract of *Murraya koenigii* and monitor nanoparticle formation using UV–Visible spectroscopy.

2. Material and Methods

2.1 Sample Collection and processing
Fresh and healthy leaves of *Murraya koenigii* L. were collected from Navadra, Gujarat, India. Mature, undamaged, and disease-free leaves were carefully collected from healthy plants and transported to the laboratory in clean polyethylene bags. The leaves were thoroughly washed under running tap water, followed by

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repeated rinsing with double-distilled water to remove adhering dust and surface contaminants.

To investigate the effect of leaf drying on extract composition, silver nanoparticle biosynthesis, and biological activity, the collected *Murraya koenigii* leaves were divided into two portions. One portion was processed immediately for the preparation of the fresh leaf extract, while the second portion was shade-dried at room temperature (25–30 °C) for 12 days. The dried leaves were subsequently pulverized into a fine powder using an electric grinder and stored in airtight containers at 4 °C until further use.

2.2 Plant extract preparation

Aqueous extracts were prepared from the fresh and shade-dried leaves under identical extraction conditions. 5 g each of freshly collected leaves and shade-dried, finely powdered leaves were separately extracted with 100 mL of double-distilled water (5%, w/v) (Arumai Selvan et al., 2022; Nguyen et al., 2026). Both were incubated in a water bath at 70 °C for 1 h to facilitate the extraction of water-soluble bioactive phytoconstituents. Following extraction, the mixtures were filtered through Whatman No. 1 filter paper to remove insoluble plant residues. The resulting clear filtrates were designated as Fresh *M. koenigii* Leaf Extract (FMkLE) and Dried *M. koenigii* Leaf Extract (DMkLE), respectively, and stored at 4 °C until further analysis.

2.3 Qualitative Phytochemical Analysis

The aqueous extracts of fresh and shade-dried *Murraya koenigii* leaves, designated as Fresh *M. koenigii* Leaf Extract (FMkLE) and Dried *M. koenigii* Leaf Extract (DMkLE), respectively, were subjected to qualitative phytochemical screening to identify the major bioactive secondary metabolites involved in the biosynthesis and stabilization of AgNPs. Phytochemical assays were performed to detect the presence of alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenolic compounds, phytosterols, cardiac glycosides, proteins, and carbohydrates.

Alkaloids were detected using Mayer's test, in which 1 mL of each extract (FMkLE and DMkLE) was acidified with concentrated hydrochloric acid followed by the addition of a few drops of Mayer's reagent. The formation of a cream or white precipitate indicated the presence of alkaloids (Vinyagamoorthy et al., 2024).

Terpenoids were identified using the Salkowski test by mixing 0.5 mL of each extract with 2 mL of chloroform followed by the careful addition of concentrated sulfuric acid. The appearance of a reddish-brown ring at the interface confirmed the presence of terpenoids.

Steroids were detected using the Liebermann–Burchard test, where 0.5 mL of each extract was mixed with chloroform and concentrated sulfuric acid. The formation of a reddish-brown ring indicated a positive reaction.

The presence of tannins was determined using the Ferric chloride test, in which 1 mL of each extract was treated with 5% ferric chloride solution. The development of a dark blue or greenish-black colour confirmed the presence of tannins (Vinyagamoorthy et al., 2024).

Saponins were detected using the foam test. Approximately 0.5 mL of each extract was mixed with distilled water and vigorously shaken. The formation of a stable and persistent foam layer indicated the presence of saponins.

Flavonoids were identified by the alkaline reagent test, in which 0.5 mL of each extract was treated with 2 N sodium hydroxide solution. The appearance of a yellow colour indicated the presence of flavonoids (Vinyagamoorthy et al., 2024).

Phenolic compounds were detected using a modified Ferric chloride test, in which the extract was treated with glacial acetic acid and concentrated sulfuric acid. The formation of a bluish-green colour indicated the presence of phenolic compounds.

Phytosterols were determined using a modified Liebermann–Burchard reaction by dissolving the extract in chloroform followed by the addition of concentrated sulfuric acid. The appearance of a brown ring indicated the presence of phytosterols.

Cardiac glycosides were detected using the Keller–Kiliani test, where the extracts were treated with glacial acetic acid containing ferric chloride and carefully underlaid with concentrated sulfuric acid. The formation of a brown ring at the interface confirmed the presence of cardiac glycosides.

Proteins were detected using the Xanthoproteic test, in which concentrated nitric acid was added to each extract. The development of a

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yellow colour indicated the presence of proteins containing aromatic amino acids.

Carbohydrates were identified using Molisch's test by treating the extracts with alcoholic α-naphthol followed by concentrated sulfuric acid. The formation of a violet ring at the interface confirmed the presence of carbohydrates.

2.4 Optimization of Plant Extract Concentration

A small-scale optimization experiment was conducted to determine the ideal plant extract volume for the efficient biosynthesis of silver nanoparticles (AgNPs). FMkLE and DMkLE were evaluated at three different volumes—0.25 mL, 0.50 mL, and 0.75 mL per 5 mL of 1 mM silver nitrate ($AgNO_3$) solution. The reaction mixtures were incubated in the dark at room temperature (25 ± 2 °C) for 24 h to minimize the photoreduction of silver ions.

Nanoparticle formation was visually monitored for a colour transition from colourless/pale yellow to dark brown and spectroscopically validated by recording the Surface Plasmon Resonance (SPR) absorption band in the 400–500 nm wavelength range using a double-beam UV-Vis spectrophotometer (Bhardwaj et al., 2024).

2.5 Biosynthesis of Silver nanoparticles (AgNPs)

AgNPs were biosynthesized separately using the FMkLE and DMkLE as reducing and stabilizing agents. 5 mL of each extract was separately added to 100 mL of 1 mM aqueous silver nitrate ($AgNO_3$) solution, maintaining an extract-to-silver precursor ratio of 1:20 (v/v). The reaction mixtures were incubated at room temperature (25 ± 2 °C) under dark conditions to minimize photoreduction of silver ions (Gami et al., 2022; Pram et al., 2024, 2024; Vinyagamoorthy et al., 2024). The formation of AgNPs was initially indicated by a gradual colour change of the reaction mixtures from pale yellow to dark brown, suggesting the reduction of Ag^+ ions to metallic silver (Ag^0) nanoparticles (Ghosh et al., 2025; Shrivastava et al., 2024). The progress of nanoparticle formation was monitored periodically using UV–Visible spectroscopy by observing the development of the characteristic SPR absorption band. The AgNPs synthesized using FMkLE and DMkLE were designated as FMkAgNPs and DMkAgNPs, respectively. Following completion of the biosynthesis reaction, the nanoparticles were recovered by centrifugation at 10,000 rpm for 30 min (Vinyagamoorthy et al., 2024). The resulting pellets were washed with double-distilled water to remove unreacted silver ions and residual plant-derived phytochemicals. The purified FMkAgNPs and DMkAgNPs were subsequently stored in airtight containers until further characterization and biological evaluation.

2.6 Antibacterial Activity Assay

The antibacterial activity of the biosynthesized AgNPs was evaluated against *Escherichia coli* using the agar well diffusion method. 100 µL of actively growing bacterial culture was uniformly spread onto sterile Nutrient Agar plates, followed by the aseptic preparation of wells of approximately 6 mm diameter. The wells were separately loaded with 50 µL of each test preparation, including the biosynthesized nanoparticles AgNPs (FMkAgNPs and DMkAgNPs), their respective leaf extracts (FMkLE and DMkLE), 1 mM $AgNO_3$ and streptomycin used as the positive control. To comparatively assess the antibacterial efficacy of FMkAgNPs and DMkAgNPs, both preparations were evaluated at two test volumes (50 and 100 µL) under identical assay conditions. The inoculated plates were incubated at 37 °C for 24 h, and antibacterial activity was determined by measuring the diameter of the resulting zones of inhibition (ZOIs) in millimetres (mm).

3. Result and Discussion

3.1 Qualitative Phytochemical Analysis of FMkLE and DMkLE

Qualitative screening revealed differences in the phytochemical profiles of FMkLE and DMkLE (Table 1). Both extracts contained alkaloids, while DMkLE showed a broader profile with terpenoids, steroids, tannins, saponins, flavonoids, phenols, phytosterols, proteins, and carbohydrates. In comparison, FMkLE showed detectable terpenoids and carbohydrates in addition to alkaloids. Cardiac glycosides were not detected in either extract. Overall, DMkLE exhibited a broader range of detectable phytochemical constituents than FMkLE, suggesting that shade-drying influenced the phytochemical profile of the extract. However, the present qualitative screening indicates only the presence and

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relative reaction intensity of these phytochemical groups and does not provide quantitative information about their individual concentrations.

The intensity of colour development or precipitate formation was recorded qualitatively as absent (−), weak (+), moderate (++), strong (+++), or very strong (++++) and the results are presented in Table 1.

Table 1 :- Phytochemical analysis of prepared plant extract

No.	Test	Aqueous FMkLE	Aqueous DMkLE
1	Alkaloid	+++	+++
2	Terpenoids	+	+++
3	Steroids	-	+++
4	Tannins	-	+++
5	Saponins	-	+
6	Flavonoids	-	+
7	Phenols	-	++++
8	Phytosterol	-	+++
9	Cardiac glycosides	-	-
10	Protein	-	++
11	Carbohydrate	+	+++

3.2 Optimization of Plant Extract Concentration for AgNP Biosynthesis

The UV–Visible spectra showed distinct differences in AgNP formation with varying concentrations of FMkLE and DMkLE. For FMkLE, the 10 mL extract volume exhibited the highest absorbance (2.35) with an SPR maximum around 440–450 nm; however, the broader absorption profile at the higher extract volume suggested a possible tendency toward aggregation. Therefore, 5 mL FMkLE was selected as the optimum volume for subsequent AgNP synthesis. In the case of DMkLE, the highest absorbance (2.0) was observed with 5 mL extract, accompanied by a relatively distinct SPR band around 440–450 nm. Increasing the extract volume resulted in a broader and comparatively lower-intensity absorption profile. Hence, 5 mL of both FMkLE and DMkLE per 100 mL of 1 mM $AgNO_3$ was selected as the optimum condition for subsequent synthesis of FMkAgNPs and DMkAgNPs.

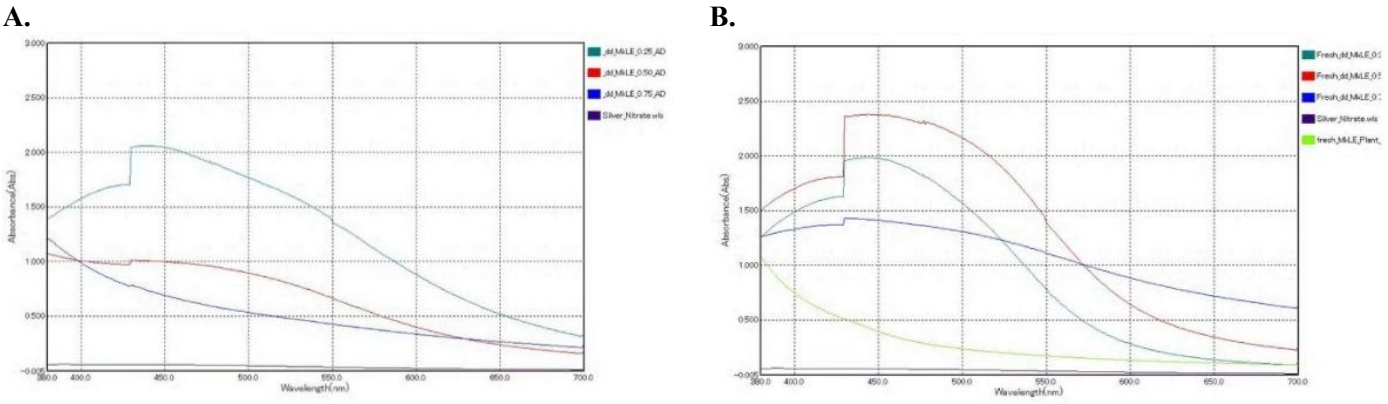


Fig.1 :- UV–Visible spectra showing the effect of different FMkLE concentrations on AgNP formation. A) Dried MkAgNPs B) Fresh MkAgNPs

3.3 Visual Confirmation of AgNP Biosynthesis

The biosynthesis of AgNPs was initially indicated by a visible change in the colour of

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the reaction mixtures following the addition of FMkLE and DMkLE to $AgNO_3$. The reaction mixture progressively changed from pale yellow to brown/dark brown, indicating the formation of silver nanoparticles. The colour development was more evident with increasing reaction time.



Fig. 2:- . Visual confirmation of AgNP biosynthesis using FMkLE and DMkLE before and after 24 h of incubation with $AgNO_3$.

3.4 UV–Visible Spectral Analysis

UV–Visible spectroscopic analysis demonstrated a progressive increase in absorbance during the biosynthesis of AgNPs using both FMkLE and DMkLE. An absorption band observed around 440–450 nm was attributed to the SPR of AgNPs. The highest absorbance was recorded after 24 h for both FMkAgNPs and DMkAgNPs, indicating progressive nanoparticle formation during the incubation period

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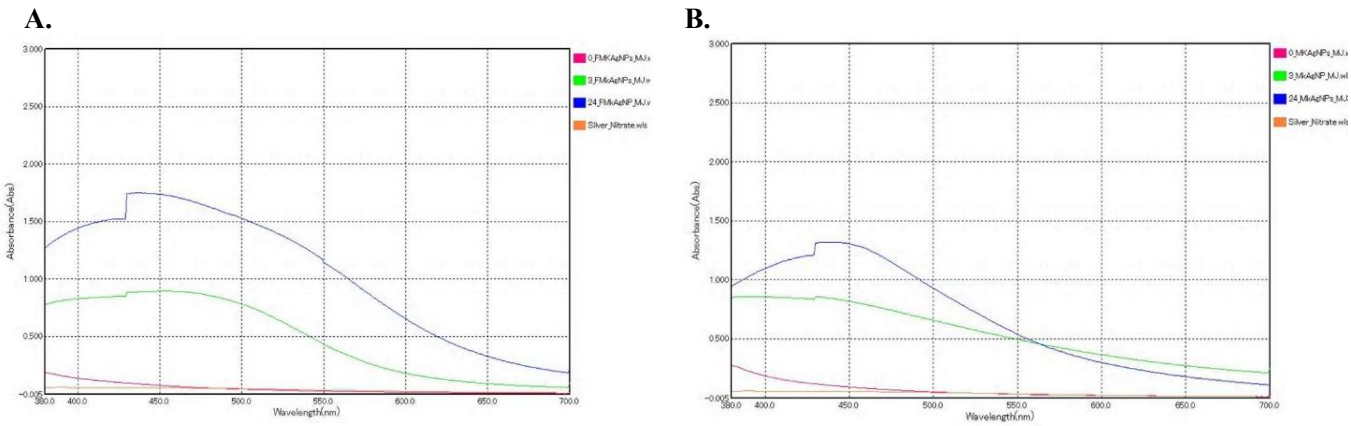


Fig. 3 :- Monitoring of A) FMkAgNPs and B) DMkAgNPs synthesis process through UV-Vis Spectrophotometer

3.5 Antibacterial activity assessment

The antibacterial activity of the synthesized AgNPs against *E. coli* is presented in Fig.4. FMkAgNPs exhibited a zone of inhibition of 1.8 mm, whereas DMkAgNPs showed a comparatively lower zone of inhibition of 1.3 mm. The corresponding FMkLE also exhibited antibacterial activity (1.7 mm), while DMkLE showed minimal inhibition (0.1 mm). $AgNO_3$ produced inhibition zones of 1.3 - 1.4 mm. Streptomycin exhibited the highest inhibition zones (3.4- 3.5 mm).

The comparative evaluation at different test volumes showed that FMkAgNPs produced inhibition zones of 1.2 and 1.5 mm at 50 and 100 µL, respectively. In contrast, DMkAgNPs showed 0.1 mm inhibition at 50 µL, which increased to 1.4 mm at 100 µL. Thus, FMkAgNPs demonstrated greater antibacterial activity than DMkAgNPs, particularly at the lower test volume. The observed difference may be associated with variations in the phytochemical composition of FMkLE and DMkLE resulting from leaf drying

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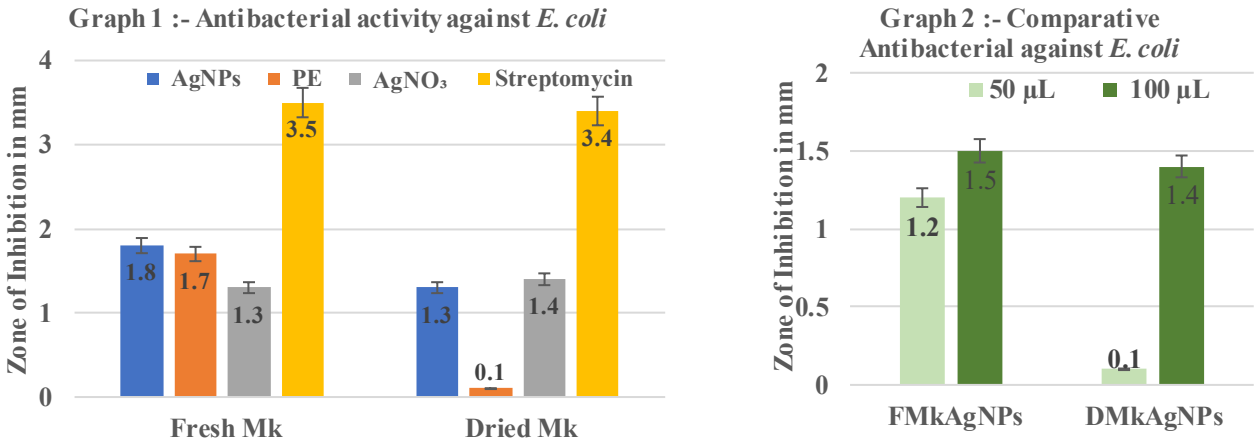


Fig.4 :- Antibacterial activity of FMkAgNPs, DMkAgNPs, FMkLE, DMkLE, $AgNO_3$, and streptomycin against *E. coli*.

4. Conclusion

The present study demonstrated the green synthesis of silver nanoparticles using aqueous extracts of fresh and shade-dried *Murraya koenigii* leaves. UV–Visible analysis confirmed AgNPs formation through the development of an SPR band around 440–450 nm. Phytochemical screening revealed a broader profile in DMkLE, whereas FMkAgNPs exhibited comparatively higher antibacterial activity against *Escherichia coli*. FMkAgNPs showed inhibition zones of 1.8 mm compared with 1.3 mm for DMkAgNPs, and retained activity at the lower test volume of 50 µL. Overall, the findings indicate that fresh *M. koenigii* leaves were more favorable for producing AgNPs with antibacterial activity against *E. coli* under the experimental conditions used, despite the broader phytochemical profile observed in the shade-dried extract. These findings support the potential of *M. koenigii* as a plant-based reducing and stabilizing source for the green synthesis of AgNPs.

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