

# Green Nanotechnology Approach for the Synthesis of *Musa paradisiaca*-Mediated Silver Nanoparticles and In-vitro Evaluation for Antidiabetic Efficacy

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## ABSTRACT

### Objective

This study was undertaken to develop silver nanoparticles utilizing *Musa paradisiaca* leaf extract through an eco-friendly green synthesis strategy and to investigate their potential antidiabetic efficacy.

### Methods

Silver nanoparticles were fabricated using an aqueous extract of *Musa paradisiaca* leaves, which served as both the reducing and capping agent. The synthesized nanoparticles were comprehensively characterized by UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), zeta potential analysis, X-ray diffraction (XRD), and transmission electron microscopy (TEM). Their antidiabetic potential was assessed through in vitro  $\alpha$ -glucosidase and  $\alpha$ -amylase enzyme inhibition assays.

### Results

Successful nanoparticle synthesis was evidenced by the appearance of a characteristic surface plasmon resonance band at 427 nm and the transformation of the reaction mixture to a dark-brown coloration. The prepared MPL-AgNPs exhibited a mean hydrodynamic diameter of  $83.4 \pm 2.76$  nm and a zeta potential of  $-23.8 \pm 3.47$  mV, reflecting satisfactory colloidal stability. FTIR spectra indicated the participation of bioactive phytochemicals in the reduction and stabilization processes, whereas XRD patterns confirmed the crystalline nature of the nanoparticles with a face-centered cubic silver lattice. TEM micrographs revealed predominantly spherical particles with uniform dispersion. Antidiabetic evaluation demonstrated superior enzyme inhibitory activity of MPL-AgNPs relative to the crude leaf extract. In the  $\alpha$ -glucosidase inhibition assay, MPL-AgNPs produced an  $IC_{50}$  value of 187.66  $\mu$ g/mL, markedly lower than that of the extract (390.87  $\mu$ g/mL), while acarbose exhibited an  $IC_{50}$  of 92.74  $\mu$ g/mL. Likewise, MPL-AgNPs showed enhanced  $\alpha$ -amylase inhibitory activity with an  $IC_{50}$  of 208.65  $\mu$ g/mL compared with 600.87  $\mu$ g/mL for the extract, whereas acarbose demonstrated an  $IC_{50}$  of 70.12  $\mu$ g/mL. The improved bioactivity of MPL-AgNPs may result from their reduced particle size, increased surface area, and favorable interactions between silver nanostructures and plant-derived phytochemicals.

### Conclusion

The synthesized *Musa paradisiaca*-derived silver nanoparticles exhibited notable inhibitory activity against key carbohydrate-hydrolysing enzymes, indicating their potential utility as a nanotechnology-based herbal intervention for diabetes management. Nevertheless, comprehensive in vivo investigations, mechanistic studies, and formulation optimization are required to validate their therapeutic applicability and safety profile.

**Keywords:** *Musa paradisiaca*; green nanotechnology; silver nanoparticles; enzyme inhibition; antidiabetic activity;  $\alpha$ -glucosidase;  $\alpha$ -amylase.

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Diabetes mellitus is a multifactorial metabolic disease characterized by sustained elevation of

## Introduction

blood glucose levels caused by inadequate insulin production, impaired insulin action, or a combination of both mechanisms. The disease has become a major healthcare challenge worldwide due to its escalating incidence and the development of severe complications, including cardiovascular disorders, diabetic nephropathy, neuropathy, retinopathy, and delayed tissue repair. The growing prevalence of diabetes, particularly in low- and middle-income countries, has been linked to urbanization, physical inactivity, obesity, and unhealthy dietary habits, thereby imposing a substantial socioeconomic burden on healthcare systems worldwide [1–3]. Although a variety of pharmacological agents are available for glycemic control, their prolonged use may result in undesirable effects, including hypoglycemic episodes, gastrointestinal discomfort, hepatic dysfunction, and poor treatment adherence. Consequently, the search for safer and more effective therapeutic alternatives remains an important focus of contemporary diabetes research [4,5].

Advances in nanotechnology have created new opportunities for the prevention and treatment of chronic metabolic disorders. Nanoparticles possess distinctive physicochemical attributes, including small particle dimensions, a large surface-to-volume ratio, enhanced bioavailability, and efficient cellular internalization, which can improve therapeutic performance [6,7]. Among the different metallic nanomaterials investigated for biomedical applications, silver nanoparticles (AgNPs) have attracted considerable interest owing to their diverse pharmacological activities. Previous studies have reported antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic properties of AgNPs [8–10]. In the context of diabetes management, AgNPs have been shown to influence glucose homeostasis, enhance insulin responsiveness, alleviate oxidative stress, and suppress the activity of key carbohydrate-digesting enzymes, including  $\alpha$ -amylase and  $\alpha$ -glucosidase [11–13]. Nevertheless, traditional approaches for nanoparticle production frequently require toxic reducing agents, elevated energy inputs, and complex processing conditions, raising concerns regarding environmental safety and biomedical suitability [14,15].

To overcome these limitations, green synthesis has emerged as a sustainable strategy for nanoparticle fabrication. This approach employs plant-derived biomolecules as natural reducing and capping agents, thereby minimizing the use of hazardous chemicals and reducing environmental impact [16,17]. Medicinal plant extracts contain a wide range of phytochemicals, such as flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, and saponins, which facilitate nanoparticle

formation and stabilization [18,19]. Furthermore, the presence of these bioactive constituents on the nanoparticle surface may contribute to enhanced biological efficacy through synergistic interactions with metallic nanostructures [20,21]. As a result, plant-mediated AgNPs often exhibit favorable biocompatibility, improved physicochemical stability, and enhanced therapeutic potential compared with conventionally synthesized counterparts [22].

*Musa paradisiaca* L. (Musaceae), commonly known as banana, is an economically important plant cultivated extensively throughout tropical and subtropical regions. Beyond its nutritional value, it has a long history of use in traditional medicine for the management of various ailments [23]. Different plant parts, including leaves, fruits, flowers, pseudostems, and peels, have been reported to possess diverse pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, wound-healing, and antidiabetic effects [24–27]. The therapeutic potential of *M. paradisiaca* has largely been attributed to its rich phytochemical profile, which includes polyphenols, flavonoids, tannins, and other bioactive metabolites capable of exerting beneficial biological effects. *Musa paradisiaca* leaves (MPL) are rich in polyphenols, flavonoids, dopamine, catecholamines, tannins, and other bioactive compounds that contribute to free radical scavenging and glucose-lowering effects [28,29]. Previous studies have reported that plant-derived phytochemicals from *Musa paradisiaca* can enhance insulin secretion, improve glucose utilization, and reduce oxidative stress associated with diabetic complications [30,31].

Although several medicinal plants have been explored for nanoparticle synthesis, limited studies have systematically investigated *Musa paradisiaca* leaf-mediated silver nanoparticles for antidiabetic applications [32]. The phytoconstituents present in *Musa paradisiaca* leaves may act synergistically with silver nanoparticles to enhance antioxidant and antihyperglycemic activity through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes, reduction of oxidative stress, and improvement in insulin signaling pathways [33,34]. Therefore, the present study aims to synthesize silver nanoparticles using *Musa paradisiaca* leaf extract through a green synthesis approach and evaluate their physicochemical characteristics and antidiabetic potential, with the objective of developing a safe, eco-friendly, and effective nanotherapeutic system for diabetes management.

## MATERIALS AND METHODS

### Materials

Silver nitrate ( $\text{AgNO}_3$ ) was purchased from Sigma-Aldrich Pvt. Ltd. (Mumbai, India). Brain Heart

Infusion (BHI) agar was supplied by HiMedia Laboratories Pvt. Ltd. (Mumbai, India).  $\alpha$ -Glucosidase derived from *Saccharomyces cerevisiae*,  $\alpha$ -amylase obtained from porcine pancreas, p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG), soluble starch, and the reference antidiabetic drug acarbose were sourced from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). All chemicals, solvents, and reagents employed throughout the study were of analytical-reagent grade and were used without further purification.

#### **Collection and Authentication of plant material**

Fresh leaves of *Musa paradisiaca* leaves were collected from Kamthana village and Sukhala Teertha, Bidar district (Karnataka), India, in November 2024. The plants were authenticated by Prof. (Dr.) Rajasamarsen Modi, Head, Department of Botany, Government First Grade College (GFGC), Bidar, Karnataka. Herbarium specimens were prepared, and voucher specimens (GFGC/BOT/HERBARIUM/2024-25) were deposited in the departmental herbarium. Identification was confirmed using Flora of Karnataka, Flora of Northeastern Karnataka, Flora of Gulbarga District, and Flora of Bidar District Karnataka.

#### **Preparation of aqueous MPL extract**

Fresh leaves of *Musa paradisiaca* were washed thoroughly with tap water and subsequently rinsed with double-distilled water to eliminate adhering dust and other impurities. A weighed quantity of 25 g of cleaned leaves was finely homogenized using a mechanical grinder and mixed with 250 mL of Milli-Q water in a suitable container. The suspension was maintained at 60 °C for 30 min under constant magnetic stirring to facilitate the extraction of bioactive constituents. Following extraction, the mixture was allowed to cool naturally to ambient temperature and then filtered successively through muslin cloth and Whatman filter paper to obtain a clear aqueous extract. The resulting filtrate was preserved at 4 °C until further use and served as both the reducing and capping agent during the biosynthesis of silver nanoparticles [33].

#### **Preliminary phytochemical estimation**

The aqueous leaf extract of *Musa paradisiaca* (MPL) was subjected to qualitative phytochemical investigation to identify the major groups of secondary metabolites present in the extract. Standard phytochemical assays were employed to screen for various bioactive constituents, including glycosides, alkaloids, saponins, carbohydrates, proteins, and tannins. The occurrence of these phytoconstituents was determined by observing specific colour reactions or precipitate formation according to well-established analytical procedures described in the literature [34,35].

#### **Preparation of AgNPs**

The synthesis parameters were selected based on preliminary optimization and literature precedence for plant-mediated silver nanoparticle synthesis. Different MPL extract-to- $\text{AgNO}_3$  ratios and pH conditions were initially screened to obtain rapid reduction, stable nanoparticle formation, and minimal aggregation. A 1:9 (v/v) MPL extract-to- $\text{AgNO}_3$  ratio provided sufficient phytochemicals for effective reduction while avoiding excessive capping, whereas a mildly alkaline pH (pH 8) favoured enhanced reduction kinetics and nanoparticle stability. These conditions consistently yielded well-dispersed and reproducible MPL-AgNPs and were therefore adopted for the final synthesis protocol.

Biosynthesis of silver nanoparticles was carried out using the aqueous leaf extract of *Musa paradisiaca* as a natural reducing and capping agent. Briefly, 10 mL of the plant extract was introduced gradually into 90 mL of a 1 mM aqueous silver nitrate ( $\text{AgNO}_3$ ) solution under constant stirring at ambient temperature. The reaction pH was adjusted to 8.0 using 1% (w/v) sodium hydroxide solution to promote efficient reduction of silver ions. The reaction mixture was then maintained in dark conditions for 24 h to ensure complete nanoparticle formation and to prevent photoinduced silver ion degradation. Successful synthesis of AgNPs was preliminarily evidenced by a distinct color transition of the reaction mixture from light green to reddish-brown, indicative of surface plasmon resonance associated with silver nanoparticles. Following completion of the reaction, the synthesized nanoparticles were separated by centrifugation at 10,000 rpm for 20 min at 4 °C. The collected pellet was repeatedly washed three times with Milli-Q water to eliminate unbound phytochemicals and residual reaction components. Finally, the purified nanoparticles were dried at 40 °C to obtain a fine powdered product, which was subsequently used for physicochemical characterization and biological evaluation [36,37].

### Visual and UV-Visible Spectrophotometric Analysis

The synthesis of silver nanoparticles was preliminarily assessed through visual inspection of the reaction mixture. The transformation of the solution colour from light green to dark brown served as an initial indication of nanoparticle formation due to the reduction of silver ions. To further verify this process, colorimetric observations were recorded at the beginning of the reaction (0 min) and after 24 h of incubation. The formation of MPL-mediated silver nanoparticles (MPL-AgNPs) and their characteristic surface plasmon resonance (SPR) behaviour were subsequently evaluated using a UV-Visible spectrophotometer (Shimadzu UV series). Absorption spectra were acquired within the wavelength range of 400–700 nm. The appearance of a prominent absorption band between 410 and 450 nm was considered characteristic of AgNPs formation and indicative of successful nanoparticle synthesis [37].

### Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted to investigate the functional groups responsible for the reduction of silver ions and stabilization of the synthesized nanoparticles. Spectra of the aqueous MPL extract, silver nitrate solution, and MPL-AgNPs were recorded using a Shimadzu Spirit-LX FTIR spectrometer over the spectral range of 4000–650  $\text{cm}^{-1}$  at ambient temperature. Dried samples were analyzed and the resulting spectra were compared to identify changes in band positions and intensities associated with nanoparticle formation. Variations in characteristic absorption peaks corresponding to hydroxyl (–OH), carbonyl (C=O), amine (–NH), and aromatic moieties were used to determine the participation of plant-derived biomolecules in nanoparticle synthesis. Such spectral modifications provide evidence for the involvement of phenolic compounds, flavonoids, proteins, and related phytochemicals in both the reduction of  $\text{Ag}^+$  ions and the stabilization of the resulting nanostructures [38,39].

### Particle Size Distribution and Polydispersity Index

The hydrodynamic diameter and polydispersity index (PDI) of MPL-AgNPs were determined by dynamic light scattering (DLS) using a Zetasizer instrument operated at 25 °C. Prior to analysis, nanoparticle suspensions were suitably diluted with Milli-Q water to minimize multiple-scattering effects and ensure accurate measurements. Each sample was analyzed in triplicate, and the results are presented as mean  $\pm$  standard deviation (SD) [40].

### Zeta Potential Measurement

The colloidal stability and surface charge characteristics of MPL-AgNPs were assessed through zeta potential analysis using a HORIBA Zetasizer (Japan) equipped with dedicated software. Measurements were carried out at 25 °C using a cell drive voltage of 150 mV. Three independent determinations were performed for each sample. Zeta potential values were calculated from electrophoretic mobility data while accounting for the dielectric constant and viscosity of the dispersing medium [41].

### Transmission Electron Microscopy (TEM)

The morphology, particle size, and dispersion characteristics of MPL-AgNPs were examined by transmission electron microscopy (TEM). A 5  $\mu\text{L}$  aliquot of the nanoparticle suspension was deposited onto a carbon-coated copper grid (300 mesh), and excess liquid was carefully removed using filter paper. The grid was allowed to dry at room temperature and subsequently stained with 1% uranyl acetate for 3–5 min before imaging. TEM micrographs were analyzed to assess particle shape, size distribution, and aggregation behaviour according to established procedures for biogenic silver nanoparticles [42].

### X-ray (XRD) Analysis

The crystalline properties of MPL-AgNPs were investigated using X-ray diffraction (XRD) analysis with  $\text{Cu-K}\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) operated at 40 kV and 30 mA. Freeze-dried nanoparticle powder was mounted on a glass sample holder, and diffraction patterns were recorded over a  $2\theta$  range of  $10^\circ$ – $80^\circ$  with a scanning speed of  $2^\circ \text{ min}^{-1}$  and a step interval of  $0.02^\circ$ . The diffraction peaks obtained were indexed by comparison with standard Joint Committee on Powder Diffraction Standards (JCPDS) data for metallic silver to verify the presence of a face-centered cubic (FCC) crystal structure [43].

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

where  $D$  is the crystallite size,  $K$  is the Scherrer constant (0.9),  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width at half maximum (FWHM), and  $\theta$  is the Bragg angle [60–61].

### In-vitro antidiabetic activity

#### In Vitro $\alpha$ -Amylase Inhibition Assay

The inhibitory effect of MPL extract and MPL-AgNPs on  $\alpha$ -amylase activity was assessed using a colorimetric method with minor modifications to previously reported procedures. Briefly, different concentrations of the test samples were prepared in distilled water and incubated with  $\alpha$ -amylase solution (1 U/mL) prepared in 0.02 M phosphate buffer (pH 6.9) containing 0.006 M sodium chloride. Following incubation at 37 °C for 10 min, a 1% soluble starch solution was added as the

substrate and the reaction mixture was further incubated for 15 min at the same temperature. The enzymatic reaction was terminated by the addition of dinitrosalicylic acid (DNSA) reagent, followed by heating in a boiling water bath for 5 min. After cooling, absorbance was recorded at 540 nm using a UV-Visible spectrophotometer. Acarbose was employed as the positive control [44].

$$\% \text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance of the test sample.

#### In Vitro $\alpha$ -Glucosidase Inhibition Assay

The  $\alpha$ -glucosidase inhibitory activity of MPL extract and MPL-AgNPs was evaluated using a standard spectrophotometric procedure. Various concentrations of the test samples were incubated with  $\alpha$ -glucosidase solution (1 U/mL) prepared in phosphate buffer (pH 6.8) at 37 °C for 10 min. Subsequently, p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) was added as the substrate and the reaction mixture was incubated for an additional 20 min. The reaction was terminated by adding 0.1 M sodium carbonate solution. The liberated p-nitrophenol was quantified by measuring absorbance at 405 nm using a UV-Visible spectrophotometer. Acarbose served as the reference inhibitor [45].

$$\% \text{Inhibition} = \frac{A_c - A_s}{A_c} \times 100$$

where  $A_c$  represents the absorbance of the control and  $A_s$  represents the absorbance of the test sample

#### Statistical Analysis

Experimental data are presented as mean  $\pm$  standard deviation (SD). Statistical evaluation was performed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc multiple-comparison test to determine differences among treatment groups. All analyses were conducted using GraphPad Prism software version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). A probability value ( $p < 0.05$ ) was considered indicative of statistical significance [46].

## RESULTS AND DISCUSSION

### Preparation and Characterization of MPL and Synthesized MPL-AgNPs

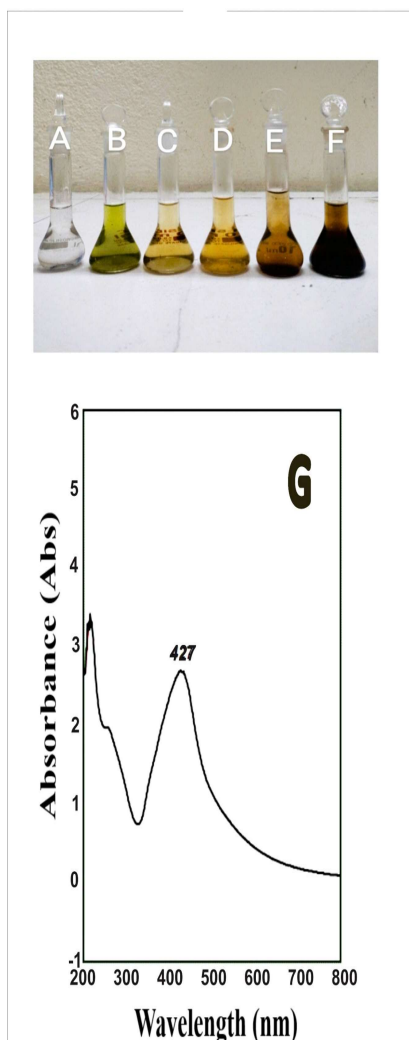
#### Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of the aqueous *Musa paradisiaca* leaf (MPL) extract revealed the presence of several important bioactive constituents, including phenolic compounds, flavonoids, tannins, glycosides, alkaloids, and saponins. These findings are in agreement with earlier reports demonstrating that *Musa paradisiaca* leaves are rich in polyphenolic and antioxidant phytochemicals possessing significant pharmacological potential.[47-49] Phenolic and flavonoid compounds are well

recognized for their strong reducing capacity and free radical scavenging activity, while tannins and glycosides contribute to metal ion chelation and stabilization of nanoparticle surfaces. The presence of these phytoconstituents suggests their potential involvement in the bio reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  and in the subsequent stabilization of biosynthesized silver nanoparticles during green synthesis. Moreover, the synergistic interaction between these bioactive compounds and silver nanoparticles may further enhance the biological and antidiabetic potential of MPS-AgNPs.

#### Visual Observation

The initial  $\text{AgNO}_3$  solution appeared colourless, while the MPL extract had a faint green colour at time zero. Upon addition of the MPL extract to  $\text{AgNO}_3$  solution along with incubation at room temperature for up to 24 h, the reaction mixture exhibited a progressive colour change from light green to dark brown (Fig. 1F). The visible colour transformation indicates reduction of  $\text{Ag}^+$  ions to metallic silver ( $\text{Ag}^0$ ), leading to AgNPs formation. Dark brown colour is widely recognized as a characteristic indicator of AgNPs formation due to SPR of the nanoparticles [50]. These results are in agreement with previous reports, such as those by [51-52] which attribute the brown coloration to the excitation of SPR—a phenomenon arising from collective oscillation of conduction electrons in response to light at specific wavelengths.



**Fig.1.A)** Visual analysis MPL extract Before and After addition of  $\text{AgNO}_3$  A)  $\text{AgNO}_3$  solution B) MPL extract C) Incubation after 1h D) Incubation after 6h E) Incubation after 12h and F) Incubation after 24h G) UV-Visible spectra of MPL-AgNPs after 24h time interval.

#### UV-Visible Spectroscopy

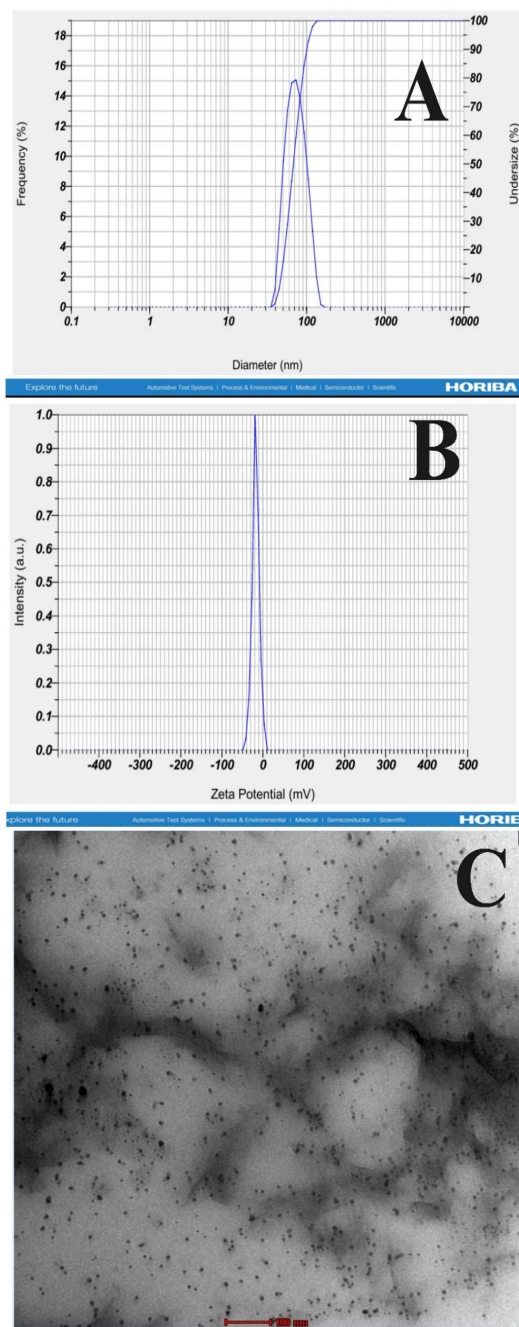
The biosynthesis of MPL-AgNPs has been further confirmed by UV-visible spectral analysis. The reaction mixture exhibited a surface plasmon resonance (SPR) peak at 427 nm (Fig. 1G), a typical signature of AgNPs [53]. The increase in absorption intensity with reaction time also supported the progressive reduction of  $\text{Ag}^+$  ions. The SPR peak noted in range of 410–455 nm has been generally ascribed to the presence of spherical AgNPs, the precise position and intensity of the SPR band depend on nanoparticle size, shape, and surrounding dielectric environment. Further emphasized that this range corresponds to the optical properties of spherical AgNPs. In this study, the absorbance peak at 427 nm falls within the reported SPR range, indicating successful

formation of predominantly spherical MPL-AgNPs. The  $\text{Ag}^+$  ions' quick reduction further suggests the efficiency of MPL extract in mediating nanoparticle synthesis.[54]

#### Particle size and Zeta Potential

Dynamic light scattering (DLS) measurements demonstrated that the *Musa paradisiaca* leaf extract-mediated silver nanoparticles (MPL-AgNPs) exhibited a mean hydrodynamic diameter of  $83.4 \pm 2.76$  nm. Zeta potential analysis further revealed a surface charge of  $-23.8 \pm 3.47$  mV. These findings confirm the successful fabrication of nanoscale silver particles with a relatively narrow size distribution, indicating effective stabilization by phytochemical constituents present in the plant extract [55]. The observed particle size falls within the nanometric range that is generally associated with enhanced biological performance, owing to increased surface area and greater accessibility to biological interfaces [56].

The zeta potential value of  $-23.8$  mV suggests satisfactory colloidal stability, as the negative surface charge generates electrostatic repulsive forces that help minimize particle aggregation during storage and dispersion [57]. This surface charge is likely imparted by the adsorption of phytochemicals, including phenolic compounds, flavonoids, and other biomolecules, which act as natural capping agents during the green synthesis process [58]. The combined effects of small particle size and stable surface characteristics may facilitate stronger interactions with biological macromolecules and improve cellular internalization, thereby enhancing the biological activity of MPL-AgNPs. Such physicochemical attributes may partially explain the superior antidiabetic efficacy observed for the nanoparticle formulation compared with the crude plant extract. Comparable results have been documented for other plant-derived silver nanoparticles, where favourable size distribution and surface properties contributed to improved biological and therapeutic performance [59].



**Fig.2.**MLP-AgNPs A) Particle size B) Zeta Potential; C) Surface Morphology at magnification 100 nm.

### Surface Morphology by TEM

Transmission electron microscopy (TEM) was employed to examine the morphology and dispersion characteristics of the synthesized *Musa paradisiaca*-mediated silver nanoparticles (MPL-AgNPs). TEM micrographs demonstrated that the nanoparticles were predominantly spherical and exhibited a relatively uniform size distribution. The particles were well separated from one another with

negligible signs of agglomeration, indicating efficient capping and stabilization by biomolecules present in the leaf extract.

The observed nanoscale dimensions and spherical morphology are desirable characteristics for biomedical applications, as they provide a larger effective surface area and facilitate enhanced interactions with biological systems [60]. The absence of significant aggregation further suggests that the phytochemical constituents of *M. paradisiaca* played an important role in maintaining nanoparticle stability during the biosynthesis process. These morphological features are in agreement with earlier studies on plant-mediated silver nanoparticles, where spherical, well-dispersed nanostructures were commonly reported as a result of successful green synthesis and effective phytochemical stabilization [61].

### Fourier-Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectroscopy was employed to identify the bioactive functional groups present in *Musa paradisiaca* leaf extract (MPL) and to elucidate their role in the biosynthesis and stabilization of silver nanoparticles (MPL-AgNPs). Comparative analysis of the FTIR spectra of the crude extract and synthesized nanoparticles revealed several characteristic absorption bands and notable spectral shifts, indicating the participation of plant-derived biomolecules during nanoparticle formation.

The FTIR spectrum of MPL displayed prominent absorption bands at 3848.35, 3742.65, and 3608.18  $\text{cm}^{-1}$ , which are attributable to free O–H stretching vibrations associated with alcohols and phenolic compounds. A broad absorption band centered at 3393.79  $\text{cm}^{-1}$  was assigned to hydrogen-bonded hydroxyl groups commonly present in polyphenols and flavonoids [62]. Peaks observed at 2982.57, 2883.55, and 2814.09  $\text{cm}^{-1}$  corresponded to aliphatic C–H stretching vibrations. The absorption signal at 2307.37  $\text{cm}^{-1}$  was attributed to C≡C or C≡N stretching modes, whereas the bands located at 1785.72 and 1699.05  $\text{cm}^{-1}$  were characteristic of carbonyl (C=O) groups present in esters, ketones, and carboxylic acid derivatives [63]. An additional band at 1514.60  $\text{cm}^{-1}$  was associated with aromatic C=C stretching vibrations, suggesting the presence of phenolic constituents. Peaks recorded at 984.60, 826.97, and 676.04  $\text{cm}^{-1}$  were assigned to C–O stretching, C–H bending, and aromatic ring deformation vibrations, respectively.

Following nanoparticle synthesis, substantial changes in the FTIR profile were observed, indicating interactions between silver ions and phytochemical constituents. In the spectrum of MPL-AgNPs, a broad absorption band appeared at 3280.12  $\text{cm}^{-1}$ , corresponding to O–H stretching vibrations of hydroxyl-containing compounds such as phenolics and alcohols. The shift of this peak

relative to the crude extract suggests the involvement of hydroxyl groups in the reduction of  $\text{Ag}^+$  ions to metallic silver [64]. Absorption bands at 3009.60, 2922.59, and 2856.19  $\text{cm}^{-1}$  were attributed to aliphatic C–H stretching vibrations. A distinct peak at 1615.68  $\text{cm}^{-1}$  was assigned to carbonyl or amide functional groups derived from proteins, polyphenols, or related biomolecules, indicating their contribution to nanoparticle capping and stabilization [65].

Additional bands observed at 1470.23, 1453.77, and 1406.96  $\text{cm}^{-1}$  corresponded to aromatic skeletal vibrations and C–H bending modes. Peaks at 1241.81, 1149.51, and 1038.31  $\text{cm}^{-1}$  were associated with C–O and C–N stretching vibrations characteristic of alcohols, ethers, and proteinaceous compounds. The absorption band at 717.12  $\text{cm}^{-1}$  was attributed to aromatic C–H out-of-plane bending vibrations. The observed shifts in peak positions together with variations in band intensity following nanoparticle formation provide strong evidence that phytochemicals present in the leaf extract actively participated in the reduction, capping, and stabilization processes. Bioactive constituents such as flavonoids, phenolic compounds, tannins, and proteins are therefore likely responsible for the successful green synthesis of MPL-AgNPs [66].

The FTIR findings are consistent with previous reports describing plant-mediated synthesis of silver nanoparticles, where naturally occurring phytochemicals served dual functions as reducing agents and stabilizing ligands, contributing to the formation of stable nanostructures with enhanced biological properties [67].

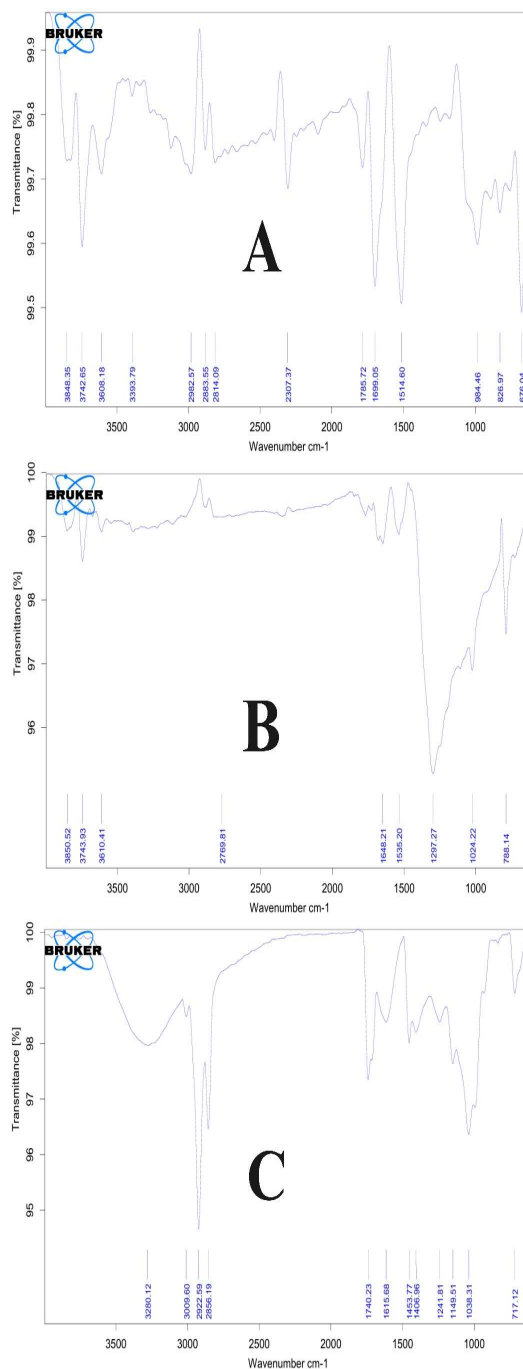
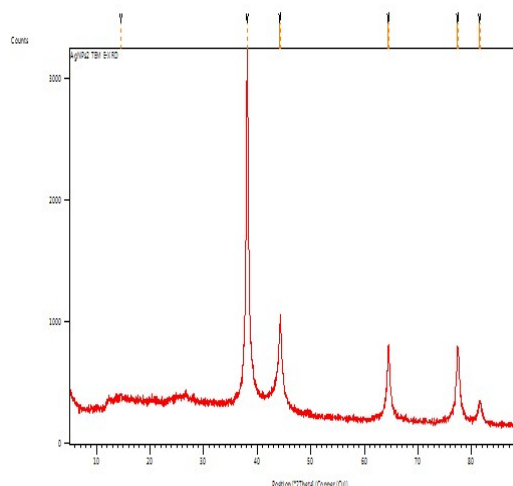


Fig.3.FTIR Spectra of A) MLP Extract B)  $\text{AgNO}_3$  C) MLP-AgNPs

#### XRD) Analysis

X-ray diffraction analysis was conducted to evaluate the crystalline characteristics of the *Musa paradisiaca*-mediated silver nanoparticles (MPL-AgNPs). The diffractogram exhibited distinct reflections at  $2\theta$  values of 38.13°, 44.23°, 64.46°, 77.39°, and 81.47°. These diffraction peaks were

indexed to the (111), (200), (220), (311), and (222) crystal planes, respectively, which are characteristic of the face-centered cubic (FCC) lattice structure of metallic silver [68]. The presence of these well-defined reflections confirms the successful formation of crystalline silver nanoparticles through the green synthesis process. The observed diffraction pattern is consistent with standard FCC silver and corroborates the effective reduction of silver ions into crystalline Ag<sup>0</sup> nanostructures by phytochemicals present in the *Musa paradisiaca* leaf extract. [68] Among the detected diffraction reflections, the peak located at  $2\theta = 38.13^\circ$  displayed the maximum intensity (1886 counts) and was assigned a relative intensity of 100%, indicating the preferential growth and dominant orientation of the (111) crystallographic plane in the synthesized silver nanoparticles. The presence of sharp, well-resolved, and highly intense diffraction peaks reflects the excellent crystalline nature of the nanoparticles and provides strong evidence for the efficient bio reduction of silver ions (Ag<sup>+</sup>) to metallic silver (Ag<sup>0</sup>) during the green synthesis process [69]. The predominance of the (111) plane is consistent with previous reports on plant-mediated silver nanoparticles, where this crystallographic orientation is frequently associated with enhanced structural stability and favourable physicochemical properties. Additional peaks at  $44.23^\circ$ ,  $64.46^\circ$ ,  $77.39^\circ$ , and  $81.47^\circ$  with corresponding intensities of 468, 399, 420, and 120 counts further supported the formation of crystalline AgNPs. Minor low-intensity peaks observed around  $14.56^\circ$  may be attributed to organic phytoconstituents from *Musa paradisiaca* leaf extract adsorbed on the nanoparticle surface, which act as reducing and stabilizing agents during green synthesis [70]. The obtained d-spacing values were also consistent with standard crystalline silver structures. Overall, the XRD findings confirmed the successful biosynthesis of highly crystalline and stable silver nanoparticles mediated by *Musa paradisiaca* leaf extract, which may contribute to their enhanced antidiabetic activity.



**Fig.4.XRD spectra of MLP-AgNPs**

### **In vitro Antidiabetic Activity**

#### **In Vitro $\alpha$ -Glucosidase Inhibition Assay**

*Musa paradisiaca* leaf extract (MPL) exhibits antidiabetic activity by inhibiting  $\alpha$ -glucosidase, a key intestinal enzyme responsible for the hydrolysis of oligosaccharides into absorbable glucose. Bioactive phytoconstituents such as flavonoids, phenolic acids, and tannins present in MPL interact with the enzyme's active site, reducing carbohydrate breakdown. This inhibition delays glucose absorption from the intestine, thereby lowering postprandial blood glucose levels. Consequently, MPL may help improve glycemic control and reduce glucose fluctuations associated with diabetes.[71]

The antidiabetic potential of *Musa paradisiaca* leaf extract (MPL) and its biosynthesized silver nanoparticles (MPL-AgNPs) was evaluated using an in vitro  $\alpha$ -glucosidase inhibition assay, with acarbose used as the standard reference drug. The inhibitory activity was expressed as IC<sub>50</sub> values, where lower IC<sub>50</sub> values indicate stronger enzyme inhibition. The IC<sub>50</sub> values obtained were 390.87  $\mu\text{g/mL}$  for MPL, 187.66  $\mu\text{g/mL}$  for MPL-AgNPs, and 92.74  $\mu\text{g/mL}$  for acarbose. The results clearly demonstrated that MPL-AgNPs exhibited significantly enhanced  $\alpha$ -glucosidase inhibitory activity compared to the crude plant extract.

The nearly two-fold reduction in IC<sub>50</sub> value following nanoparticle synthesis suggests that fabrication of AgNPs improved the biological efficacy of the phytoconstituents present in *Musa paradisiaca* leaves. This enhancement may be attributed to the nanoscale size, increased surface area, improved stability, and enhanced bioavailability of phytochemicals adsorbed onto the nanoparticle surface [71]. The inhibitory activity of MPL is likely associated with the presence of bioactive compounds such as phenolics, flavonoids, and tannins, which are known to inhibit

carbohydrate hydrolyzing enzymes and reduce glucose release from dietary carbohydrates [72]. Furthermore, the superior activity of MPL-AgNPs may result from synergistic interactions between silver nanoparticles and phytochemical capping agents, leading to stronger binding affinity toward the  $\alpha$ -glucosidase enzyme [73]. Green synthesized AgNPs are also reported to interfere with enzyme conformation and substrate accessibility, thereby enhancing inhibitory efficiency [74]. Similar findings have been reported for plant-mediated silver nanoparticles showing greater  $\alpha$ -glucosidase inhibitory activity than their corresponding crude extracts [75]. Although acarbose exhibited the strongest inhibition, MPL-AgNPs demonstrated considerable antidiabetic potential and may serve as a promising nanoherbal therapeutic approach for the management of postprandial hyperglycemia with potentially reduced adverse effects associated with synthetic drugs

#### **In Vitro $\alpha$ -Amylase Inhibition Assay**

MPL also demonstrates antidiabetic potential through inhibition of  $\alpha$ -amylase, the enzyme that catalyzes the initial digestion of dietary starch into smaller oligosaccharides and maltose. The polyphenolic compounds present in the extract bind to  $\alpha$ -amylase and interfere with its catalytic activity, slowing starch degradation. This reduction in carbohydrate digestion decreases the rate of glucose release and absorption in the gastrointestinal tract. As a result, MPL can effectively attenuate postprandial hyperglycemia and contribute to the management of type 2 diabetes mellitus.[76]

The in vitro  $\alpha$ -amylase inhibitory activity of *Musa paradisiaca* leaf extract (MPL) and its biosynthesized silver nanoparticles (MPL-AgNPs) was evaluated to investigate their potential in controlling postprandial hyperglycemia. Acarbose was used as the standard reference drug. The inhibitory activity was expressed as  $IC_{50}$  values, where lower values indicate stronger enzyme inhibition. The obtained  $IC_{50}$  values were 600.87  $\mu$ g/mL for MPL, 208.65  $\mu$ g/mL for MPL-AgNPs, and 70.12  $\mu$ g/mL for acarbose. The results demonstrated that MPL-AgNPs exhibited significantly enhanced  $\alpha$ -amylase inhibitory activity compared to the crude plant extract. The approximately three-fold reduction in  $IC_{50}$  value after nanoparticle synthesis indicates that nanoformulation substantially improved the biological efficacy of the phytoconstituents present in *Musa paradisiaca* leaves. This enhancement may be attributed to the nanoscale particle size, increased surface area, improved stability, and enhanced interaction of bioactive compounds with the enzyme active site [76]. The moderate inhibitory activity observed with MPL may be associated with phytochemicals such as flavonoids,

phenolics, tannins, and alkaloids, which are known to inhibit carbohydrate metabolizing enzymes and reduce starch hydrolysis into glucose [77].

Although acarbose showed the strongest inhibitory activity, MPL-AgNPs demonstrated considerable enzyme inhibition, suggesting synergistic effects between silver nanoparticles and phytochemical capping agents [78]. In addition, green synthesized AgNPs have been reported to alter enzyme conformation and interfere with substrate binding, thereby enhancing inhibitory efficiency [79]. Similar studies on plant-mediated silver nanoparticles have also reported improved  $\alpha$ -amylase inhibitory activity compared to crude plant extracts [80]. Overall, the findings indicate that MPL-AgNPs possess promising antidiabetic potential and may serve as an effective nanoherbal strategy for managing postprandial hyperglycemia.

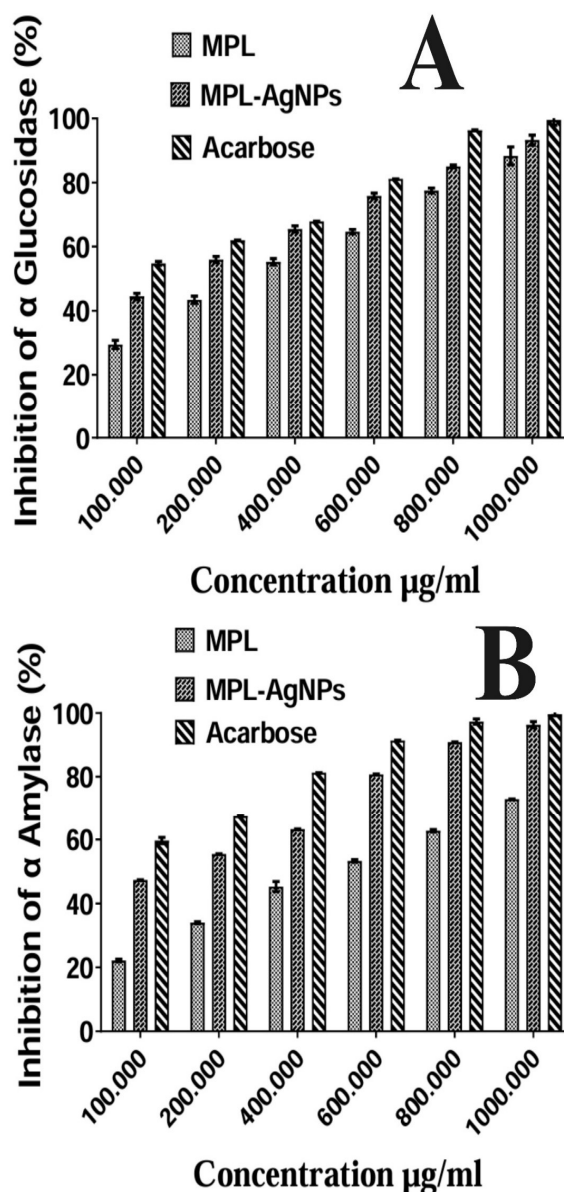


Fig.5: In vitro antidiabetic activity of MPL, MPL-AgNPs on A)  $\alpha$ -Glucosidase Inhibition Assay B)  $\alpha$ -Amylase Inhibition Assay

#### CONCLUSIONS

*Musa paradisiaca*-mediated silver nanoparticles (MPL-AgNPs) were successfully produced through a green and sustainable synthesis method. Physicochemical characterization confirmed the formation of stable, crystalline, and predominantly spherical nanoparticles with nanoscale dimensions. FTIR results indicated the active involvement of plant phytochemicals in nanoparticle formation and stabilization. In vitro antidiabetic evaluation revealed that MPL-AgNPs exhibited markedly stronger  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities than the crude leaf extract, suggesting enhanced bioactivity following nano formulation.

This improved efficacy is likely associated with the increased surface area and synergistic effects of silver nanoparticles and phytoconstituents. These findings highlight the potential of MPL-AgNPs as a nano herbal system for diabetes management, although further in vivo efficacy, safety, and formulation studies are required to support their therapeutic application.

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#### Conflict of interest

All authors declare that they have no conflict of interests.

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#### Authors Contribution

**Yogeshwari:** Writing – original draft, Investigation, Formal analysis. **Nayeem Khatib:** Writing – review & editing, Conceptualization, Methodology, Supervision, Project administration, **Shashtri Veerendra:** Writing – Review, editing, Conceptualization, Methodology. **Yogeshwari:** Writing – original draft, Visualization, Validation, Data curation.

**AI Use Statement-** "The author used Quill boat for grammar checking and improving sentence clarity. The author reviewed and edited the output and takes full responsibility for the final content."

#### Ethics approval and consent to participate

Applicable

#### Patient consent statement

Not Applicable

#### Permission to reproduce material from other sources

Not Applicable

#### Availability of data and material

Applicable on reasonable request

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