Green synthesis, optimization and characterization of copper oxide nanoparticles using *Iris kashmiriana* extract: Its anti-diabetic and anti-oxidant properties

Harish SL[a], Paarthipan Natarajan[a], Nisha B[b], Venkatesan Karthick[c*]

[a]Department of Radio-Diagnosis, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
[b]Department of Community Medicine, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
[c]Department of Pathology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai, 602 105, Tamil Nadu, India.

*** Corresponding author**
E-mail addresses: karthickv.smc@saveetha.com (V. Karthick)

Abstract

In the present study, copper oxide nanoparticles (CuO NPs) were synthesized using a green approach employing *Iris kashmiriana* extract as a reducing and stabilizing agent. The synthesized nanoparticles were subjected to comprehensive characterization. The FT-IR spectrum of CuONPs displayed a prominent peak at 1095 cm^{-1}, indicative of C–O stretching vibrations, along with C–N stretching vibrations observed at 915, 795, and 741 cm^{-1}, confirming the presence of phytoconstituents involved in nanoparticle stabilization. X-ray diffraction (XRD) analysis revealed distinct peaks at 2θ values of 32.40°, 39.16°, 47.89°, 54.08°, 57.18°, 62.11°, 66.41°, 68.41°, 71.43°, and 76.82°, corresponding to the (110), (111), (202), (020), (202), (113), (311), (113), (311), and (004) crystallographic planes, respectively, affirming the crystalline nature of the CuONPs. Morphological analysis indicated that the nanoparticles were spherical, well-dispersed, and free from significant aggregation, with sizes ranging from 45 to 90 nm. The anti-diabetic potential of the synthesized CuONPs was evaluated through *in vitro* inhibition assays of α-amylase and α-glucosidase enzymes. The CuONPs derived from *I. kashmiriana* extract exhibited a maximum inhibition of 12.65% against α-amylase and a significantly higher inhibition of 55.50% against α-glucosidase. These findings suggest a dominant inhibitory action on α-glucosidase, underscoring the therapeutic relevance of CuONPs in modulating postprandial hyperglycemia. In conclusion, this study demonstrates that phytofabricated CuONPs possess promising anti-diabetic and antioxidant properties, and highlight their potential application as future therapeutic agents in the management of diabetes.

Keywords
Iris kashmiriana
copper oxide nanoparticles
Eco-friendly approach
Antidiabetic activity
Antioxidant activity
SEM

1. Introduction

The chronic metabolic disease of diabetes is characterized by elevated levels of blood glucose (blood sugar), which can result in severe damage to the heart, blood vessels, eyes, kidneys, and nerves over time. About 830 million people live with diabetes worldwide, with the majority living in low- and middle-income countries. There are approximately 10% of people with type 1 diabetes and 90% of people with type 2 diabetes (Vaughan, 2024). The most common type of diabetes is type 2 diabetes, usually observed in adults, which occurs when the body becomes resistant to insulin or does not produce enough insulin (Karthick et al., 2025). Over the past three decades, the prevalence of type 2 diabetes has risen dramatically in countries of all income levels. It is estimated that more than half of diabetics do not receive treatment. It has been observed over the past few decades that both the number of people with diabetes as well as the number of people with untreated diabetes has been steadily increasing (WHO, 2024). Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus (Maritim et al., 2003). Reactive oxygen species (ROS)-induced alterations in metabolic and signalling pathways serve as a crucial link between type 2 diabetes mellitus and cancer. These reprogrammed pathways disrupt redox homeostasis, contribute to diabetic complications, and play distinct roles during the initiation and progression of cancer (Black, 2024).

The field of nanotechnology is gaining significant attention in the biomedical field due to its numerous applications. Emerging nanotechnology-based strategies, particularly those involving green-synthesized nanoparticles, are proving to be powerful tools for mitigating oxidative stress and managing chronic metabolic diseases such as diabetes (Balkrishna et al., 2021). The synthesis of nanostructured materials, particularly metallic nanoparticles, has garnered significant attention in recent years due to their unique physicochemical properties, which enable a wide range of applications across science and technology. However, a major challenge remains in developing efficient synthesis methods that yield nanoparticles with uniform size and shape while minimizing toxicity to human health and the environment (Kulkarni & Muddapur, 2014). It has been reported that metal oxide nanoparticles (NPs), such as Fe2 TiO2 NPs, ZrO2 O3 NPs, CuO NPs, MgO NPs, ZnO NPs, CeO2 NPs, are designed for the purpose of a variety of biological applications (Karthick et al., 2023; Nagaraj et al., 2023; Shanmugam et al., 2025). Additionally, these nanoparticles vary in terms of their morphologies, sizes, textures, as well as their intended uses. As one of the most widely used

metal oxide nanoparticles, CuO NPs have attracted a considerable amount of attention due to their wide range of applications in catalysis, magnetic phase shift, gas sensors, lithium ion batteries, optical, and electrical properties. In recent years, copper and copper oxide nanoparticles have gained significant attention due to their wide range of applications, prompting researchers worldwide to explore more efficient and environmentally friendly synthesis methods (Mohamed, 2020). Green synthesis is recognized as a safe and eco-friendly approach for nanoparticle production, utilizing plant-based extracts that serve simultaneously as solvents, stabilizing agents, and reducing agents (Altaf et al., 2025; Manigandan & Shanmugam, 2025).

Plant-mediated nanoparticles especially copper oxide nanoparticles (CuO NPs) combine the benefits of nanoscale properties with antioxidative phytochemical coatings, which enhance their free radical scavenging capacity. *Iris kashmiriana*, belonging to the family Iridaceae, is a perennial plant native to the Kashmir region of India, where it is frequently found in graveyards and cultivated in select areas of Pakistan (Chalotra et al., 2022). The plant holds significance due to its endemic presence in the region and its long-standing use in traditional medicine as an emetic, cathartic, diuretic, and expectorant (Hussain Mir, 2014). The present study aims to develop a green, eco-friendly method for the synthesis of CuO NPs using *I. kashmiriana* extract, optimize the synthesis parameters for enhanced nanoparticle stability and uniformity, and comprehensively characterize the physicochemical properties of the synthesized nanoparticles. Furthermore, the study evaluates the *in vitro* anti-diabetic and antioxidant potential of the CuO-NPs to explore their therapeutic relevance in managing oxidative stress and hyperglycemia.

2. Materials and methods

2.1. Materials

All chemicals utilized in this study were of analytical grade and procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich, and Merck Life Science Pvt. Ltd. (Mumbai, India). Aqueous suspensions were prepared using double-distilled, deionized water. All glassware was thoroughly cleaned and dried in a hot air oven prior to use.

2.2. Collection of plant material and preparation of leaf extract

The *I. kashmiriana* plant powder was purchased from a local herbal supplier. Ten grams of the powder were boiled in 100 mL of double-distilled water for 15 minutes. The mixture was cooled, filtered using Whatman No. 1 filter paper, and stored at 4°C for subsequent use.

2.3. Synthesis of copper oxide nanoparticles

Copper oxide nanoparticles (CuO NPs) were synthesized via the reduction of copper sulfate by phenolic compounds present in the plant extract. The reaction mixture was prepared by adding 1 mL of the plant extract to 100 mL of 1 mM $CuSO_4 \cdot 5H_2O$ solution in a 250 mL round-bottom flask. The mixture was subjected to reflux at 70 °C under continuous stirring for

2 hours. A distinct color change from green to dark brown indicated the formation of copper oxide nanoparticles. The green synthesis of CuO NPs using *I. kashmiriana* extract was optimized by varying key reaction parameters to achieve maximum efficiency, stability, and uniformity of nanoparticles. Initially, different volume ratios of plant extract to 1 mM copper sulfate solution (1:100, 1:90, 1:70, 1:50, and 1:30 mL) were tested. Among these, the 1:50 ratio was found to produce well-dispersed nanoparticles with optimal surface plasmon resonance, indicating efficient reduction of Cu^{2+} ions. To further optimize the synthesis, varying concentrations of copper sulfate (0.25, 0.5, 1.0, and 1.5 mM) were used with a fixed 10 mL volume of plant extract. The best nanoparticle yield and quality were observed at 1.0 mM. The influence of temperature was studied at 30, 40, 50, 60, and 70 °C, with 70 °C proving ideal for faster reaction kinetics and better particle morphology. The effect of pH was evaluated between 6.0 and 10.0; pH 7.5 was optimal, supporting effective phytochemical-mediated reduction under mildly alkaline conditions. Crucially, the reaction time was varied at 30, 60, 90, 120, and 150 minutes to assess the reduction efficiency and particle formation kinetics. Among these, 120 minutes was observed to be the most suitable duration, balancing complete reduction and preventing agglomeration. Two control setups one without plant extract and one without copper sulfate showed no nanoparticle formation, confirming the essential role of both components. These optimizations highlight the importance of precise control over reaction conditions to produce uniform, stable, and bioactive CuONPs suitable for further applications. The resulting brown precipitate was collected by centrifugation at room temperature, followed by multiple washes with absolute ethanol and distilled water to remove any unreacted constituents. The purified product was dried overnight at 80 °C and subsequently annealed in a muffle furnace at 400 °C for 2 hours to enhance crystallinity. The annealed powder was designated as the final CuONP sample for further analysis (Alhalili, 2022; Karthick et al., 2023).

2.4. Characterization of synthesized ZnO NPs

2.4.1. UV-Vis spectrophotometer analysis

UV–vis spectrophotometer (UV -1800, Shimadzu) was used to record the absorption spectrum and measure the optical property in the wavelength range 200–800 nm.

2.4.2. FTIR analysis

The purified pellets of synthesized CuO NPs were then dried and the powder was subjected to FTIR (Shimadzu 8400S, Japan) spectroscopy measurements in the diffuse reflectance mode at a resolution of 4000 to 400 cm^{-1} in KBr pellets.

2.4.3. XRD analysis

The crystal structure and phase purity of synthesized NPs were confirmed using X-ray diffractometer (XRD) with Cu-Kα (1.54) radiation (10/min). The average crystallite size of the synthesized CuONPs was calculated using the Debye–Scherrer equation, where D is the

crystallite size, K is the Scherrer constant (0.9), λ is the X-ray wavelength (1.5406 Å), β is the full width at half maximum (FWHM) in radians, and θ is the Bragg's angle.

2.4.4. SEM analysis

The surface morphology of the synthesized nanoparticles was analyzed using Scanning Electron Microscopy (SEM) (Model: FEI Quanta 200 FEG), which provided detailed imaging of particle shape and distribution.

2.5. In vitro antidiabetic activity

2.5.1. α -amylase inhibitory assay

Alpha-amylase inhibition was assessed by incubating 100 µL of test samples (25–200 µg/mL) with 1% starch in 20 mM phosphate buffer (pH 6.9, 6 mM NaCl) at 25°C for 10 min. Next, 100 µL of α-amylase (0.5 mg/mL) was added and incubated for another 10 min. The reaction was stopped with 200 µL of DNS reagent and heated at 100°C for 5 min. After cooling, 50 µL of the mixture was transferred to a 96-well plate and diluted with 200 µL of distilled water. Absorbance was measured at 540 nm (Thangaraj, 2016). Percent α-amylase activity was calculated as: % Activity = Absorbance of sample / Absorbance of control X 100.

2.5.2. α -glucosidase inhibitory assay

For the assay, 100 µL of sample (various concentrations) in 100 mM sodium phosphate buffer (pH 6.9) was mixed with 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) to initiate the reaction. After 5 min incubation at 37°C, 100 µL of buffer containing α-glucosidase (0.1 U/mL) was added. Following 30 min, absorbance was measured at 405 nm (Thangaraj, 2016). Percent α-glucosidase activity was calculated as: % Activity = Absorbance of sample / Absorbance of control X 100.

2.6. In vitro antioxidant activity

2.6.1. Radical scavenging activity using DPPH assay

In this assay, 100 µL of sample (25–200 µg/mL) was mixed with 100 µL of 0.1 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. Ascorbic acid was used as the standard, and a calibration curve was used to determine DPPH concentration. Ascorbic acid was used as standard (Amalraj et al., 2021). The free radical scavenging activity was expressed as IC_{50}, and % inhibition was calculated using: Scavenging activity (%) = [(Control OD–Sample OD)/Control OD] ×100].

2.6.2. ABTS radical cation decolourization assay

In this assay, 100 µL of sample (25–200 µg/mL) was mixed with 100 µL of 0.1 mM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. Ascorbic acid was used as the standard, and a calibration curve was used to determine DPPH concentration. Ascorbic acid was used as standard (Amalraj et al., 2021). The free radical scavenging activity was expressed as IC_{50}, and % inhibition was calculated using: Scavenging activity (%) = [(Control OD–Sample OD)/Control OD] ×100].

2.7. Statistical analysis

All experiments were conducted in triplicates, and the results are expressed as mean ± standard deviation (SD). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A p-value of less than 0.05 was considered statistically significant. Data analysis was carried out using GraphPad Prism software (version 8.0.2, GraphPad Software, Inc.) (Amalraj et al., 2021).

3. Results and Discussion

3.1. Optimization of green synthesized CuO NPs

The reaction mixture was periodically observed for visible color changes, indicating nanoparticle formation, and further analyzed using UV–visible spectrophotometry within the 200–800 nm range, as depicted in Figure 1. A distinct absorption peak was observed at 345 nm, which is characteristic of CuO NPs and indicates successful reduction of Cu^{2+} ions by the phytochemicals present in the *I. kashmiriana* extract. The sharp and well-defined peak at this wavelength suggests the formation of uniformly distributed nanoparticles with minimal aggregation. This optical behavior is consistent with the surface plasmon resonance typically associated with nanoscale CuO NPs. To achieve efficient and stable green synthesis of CuO NPs, various physicochemical parameters were systematically optimized, including the volume ratio of plant extract to precursor, precursor concentration, pH, reaction temperature, and reaction time. Each parameter was assessed individually while keeping other conditions constant.

The effect of varying the volume ratio between plant extract and 1 mM $CuSO_4 \cdot 5H_2O$ solution was evaluated at 1:100, 1:90, 1:70, 1:50, and 1:30 (v/v). Among these, the 1:50 ratio produced the most intense color change and maximum absorbance at 345 nm in UV–Vis spectroscopy, indicating efficient nanoparticle formation. Ratios with less extract (1:100, 1:90) showed slower or incomplete reduction, while higher extract volumes (1:30) resulted in broader peaks, likely due to excess biomolecules (Figure 2 a). Copper sulfate concentrations of 0.25, 0.5, 1.0, and 1.5 mM were tested. The optimum nanoparticle yield and size distribution were observed at 1.0 mM, balancing effective reduction and avoiding excessive agglomeration or particle growth. Higher concentrations tended to result in irregular shapes or larger aggregates (Figure 2 b). The pH of the reaction mixture was adjusted to 6.0, 7.0, 8.0, 9.0, and 10.0 to evaluate its influence on nanoparticle synthesis. The best results were obtained at pH 7.5–8.0,

where particle formation was rapid, and the absorption peak was sharp. At lower pH (acidic conditions), the reaction was sluggish, and at higher pH (>9), particle aggregation was observed (Figure 2 c). The effect of temperature on the reduction process was studied at 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C. The optimal temperature was found to be 70 °C, which promoted rapid reduction and formation of uniformly distributed, crystalline CuONPs. Lower temperatures showed delayed reaction kinetics, while excessively high temperatures (above 70 °C) were avoided to prevent degradation of phytochemicals (Figure 2 d). The reaction mixture was incubated for 30, 60, 90, 120, and 150 minutes. A gradual increase in absorbance was noted over time, with a sharp and stable SPR peak appearing at 90 minutes, indicating complete reduction and optimal nanoparticle stabilization. Prolonged incubation beyond 120 minutes did not significantly enhance synthesis and occasionally led to slight agglomeration (Figure 2 e).

Recently, Almeida et al. (2025), stable CuO-NPs were synthesized using nanofiltrated noni-leaf tea extract, which acted as a natural reducing and stabilizing agent; the reaction mixture was stirred vigorously and maintained at 90 °C for 5 hours to facilitate nanoparticle formation. In the study by Jabeen et al. (2024), CuO-NPs synthesized using Aloe vera extract under alkaline conditions (pH 10), at a temperature of 70–80 °C, and a reaction time of 4 hours exhibited high stability and uniformity, highlighting the critical role of optimized pH, temperature, and duration in efficient nanoparticle formation. According to the findings of Karimi et al. (2024) demonstrated that CuO-NPs synthesized using *Artemisia annua* extract under alkaline conditions at 70 °C for 24 hours exhibited high stability and antioxidant potential, indicating that prolonged reaction time and controlled temperature play a crucial role in enhancing nanoparticle quality. The results of the present study further confirm that optimized synthesis conditions are crucial for producing stable and uniform CuO nanoparticles through green routes. The observed optimal temperature of 70 °C promoted rapid reduction and crystallization, while the ideal pH range of 7.5–8.0 ensured efficient nanoparticle formation with minimal aggregation. The 1:50 extract-to-metal salt ratio and 1.0 mM $CuSO_4$ concentration yielded the most favorable outcomes, indicating the importance of balanced phytochemical availability and precursor concentration. Additionally, the reaction time of 90 minutes provided a sharp and stable SPR peak, suggesting complete reduction and stabilization. These findings are consistent with earlier reports, reinforcing that alkaline pH, moderate heating, and controlled incubation time are essential parameters across different plant-mediated syntheses. The current work also contributes new insight into the effect of varying extract volumes and precursor concentrations, factors often underexplored in prior studies.

3.2. Characterization of the green synthesized CuO NPs

3.2.1. FTIR analysis

The FTIR spectrum of the green-synthesized CuO NPs exhibited several characteristic absorption bands (Figure 3). Prominent peaks at 3775 cm^{-1} and 3637 cm^{-1} are attributed to O–H stretching vibrations from phenolic groups and adsorbed water. The distinct peak at 1414 cm^{-1} corresponds to C-C stretching vibrations of aromatic amines, indicating the presence of phytochemical capping agents. The band at 1086 cm^{-1} is assigned to C–O stretching, confirming the involvement of alcohol or ether functional groups from the plant extract.

Crucially, absorption peaks observed at 867 cm^{-1} and 574 cm^{-1} are indicative of Cu–O metal–oxygen stretching vibrations, confirming nanoparticle formation (Sankar et al., 2014). Together, these FTIR signatures demonstrate that phytochemicals from *I. kashmiriana* acted as both reducing and stabilizing agents during CuO NP formation, while the metal–oxygen bands confirm the successful synthesis of crystalline CuO NPs.

These findings are agreement with the results reported by Liang et al. (2025), who synthesized CuONPs using *Durio zibethinus* (durian husk) extract. Their FTIR analysis also identified characteristic O–H, C–O, and Cu–O bands, supporting the role of natural phytochemicals in both the reduction and stabilization processes. The similarity in FTIR profiles across different plant-based syntheses underscores the versatility of phytochemical functional groups particularly hydroxyls, amines, and carbonyls in facilitating green nanoparticle synthesis. These observations are supported by Jabeen et al. (2024), who reported similar FTIR spectral features in their biogenic synthesis of CuONPs using Aloe vera extract. Their spectrum revealed broad O–H stretching peaks, C=O and C–O functional groups, and strong Cu–O metal–oxygen vibrations, signifying the successful reduction and capping of Cu^{2+} ions by plant-derived biomolecules. The presence of hydroxyls, carbonyls, and phenolic groups played a crucial role in nanoparticle stabilization and morphology control in both studies. The presence of O–H stretching bands indicated phenolic compounds and moisture, while C–C and C–O stretching vibrations revealed the involvement of aromatic amines and alcohol/ether groups from the plant extract. Most importantly, the distinct peaks corresponding to Cu–O bonds provided clear evidence of nanoparticle formation. These results demonstrate that plant-derived phytochemicals play a crucial role as both reducing and capping agents, contributing to the structural integrity and stability of the synthesized CuO NPs.

3.2.2. XRD analysis

The crystalline structure of the green-synthesized CuO NPs was characterized using X-ray diffraction (XRD) analysis. The XRD pattern (Figure 4) revealed distinct diffraction peaks at 2θ values of 32.40°, 39.16°, 47.89°, 54.08°, 57.18°, 62.11°, 66.41°, 68.41°, 71.43°, and 76.82°, which correspond to the crystallographic planes (110), (111), (202), (020), (202), (113), (311), (113), (311), and (004), respectively and the average crystallite size was found to be approximately 28 nm. These diffraction peaks are in close agreement with the standard JCPDS data for monoclinic or crystalline CuO (Joint Committee on Powder Diffraction Standards card no. 05-0661), confirming the successful formation of phase-pure CuO NPs. The sharpness and intensity of the peaks further indicate a high degree of crystallinity in the synthesized particles. No additional peaks related to impurities or secondary phases were observed, suggesting the effectiveness of the *I. kashmiriana* leaf extract in promoting pure nanoparticle synthesis. The repetitive appearance of certain planes, such as (202) and (311), may be attributed to preferred orientation or overlapping reflections common in nanoscale materials. This crystalline nature plays a critical role in the functional properties of the CuONPs, particularly in their biological and catalytic applications.

Recently, Karimi et al. (2024) synthesized CuO NPs using *Artemisia annua* aqueous extract and confirmed their formation through XRD analysis. The XRD pattern revealed peaks corresponding to the monoclinic crystalline structure of CuO. The well-resolved and intense

diffraction peaks indicated a high degree of crystallinity. Using the Debye–Scherrer equation, the average crystallite size of the CuO NPs was calculated to be approximately 25 nm, confirming their nanoscale dimensions. Very recently, Kimta et al. (2025) reported the green synthesis of CuO NPs using a crude ethanolic extract and the n-butanol fraction of *Adiantum venustum*, the XRD analysis confirmed the crystalline nature of the nanoparticles, with average crystallite sizes of 14.65 nm and 18.73 nm, respectively.

3.2.3. SEM analysis

The surface morphology and particle size distribution of the green-synthesized CuO NPs were investigated using Scanning Electron Microscopy (SEM). As illustrated in Figure 5 a and b, the SEM micrographs revealed that the synthesized nanoparticles were predominantly spherical in shape, exhibiting a uniform surface texture and homogeneous distribution across the substrate. The particles were found to be well-separated, with minimal aggregation, suggesting effective capping and stabilization by the phytochemicals present in the *I. kashmiriana* extract. The particle size range was estimated to be between 45 to 90 nm, with an average diameter in the nanoscale regime. The relatively narrow size distribution and lack of significant clustering indicate that the biomolecules in the plant extract not only facilitated the reduction of Cu^{2+} ions but also acted as stabilizing agents, preventing uncontrolled growth and agglomeration of the nanoparticles. Furthermore, the spherical morphology observed under SEM is consistent with the successful nucleation and controlled growth mechanisms promoted under optimized synthesis conditions (pH, temperature, and precursor concentration). These findings corroborate the data obtained from UV–Vis and XRD analyses, reinforcing the formation of stable, crystalline, and nanoscale CuONPs with potential biomedical applications.

In a study by Nzilu et al. (2023), CuO NPs were synthesized using an aqueous extract of the whole *Parthenium hysterophorus* plant, and SEM analysis revealed that the particles were nearly spherical in shape with minimal signs of agglomeration. Relhan et al. (2024) reported that SEM images of CuO-NPs synthesized from *Allahabad Safeda* and *Hisar Safeda* extracts showed a polydisperse distribution with nanoparticles of varying diameters, exhibiting diverse shapes, irregular surfaces, and small structures with uneven edges. This indicates the potential of *I. kashmiriana* as a sustainable and efficient bio-reducing and stabilizing agent, offering a promising alternative for the eco-friendly production of metal oxide nanoparticles with applications in biomedical, environmental, and catalytic fields.

3.3. In vitro Antidiabetic activity

3.3.1. alpha amylase activity

The antidiabetic potential of the green-synthesized CuO NPs was assessed through an *in vitro* α-amylase inhibition assay conducted at various concentrations (25–200 µg/mL) Figure 6a. The results demonstrated a clear concentration-dependent inhibition pattern. At the lowest tested concentration (25 µg/mL), the CuO NPs exhibited an inhibition rate of 12.65%, which progressively increased with higher concentrations. At 50, 100, and 150 µg/mL, the inhibition rates were observed as 24.30%, 36.80%, and 47.45%, respectively. The maximum inhibitory

effect was recorded at 200 μg/mL, with a significant inhibition of 55.50% of α-amylase activity. These findings indicate that CuO NPs interfere effectively with the enzyme's catalytic action, possibly by binding to active or allosteric sites and thereby impeding starch hydrolysis. Statistical analysis showed that all treatment groups were significantly different from the control ($p < 0.001$). The half-maximal inhibitory concentration (IC_{50}) of the CuO NPs was determined from the dose-response curve and calculated to be approximately 142.9 μg/mL, confirming a potent antidiabetic effect at moderate nanoparticle concentrations. For comparison, the standard antidiabetic drug acarbose was used as a positive control, and showed the IC_{50} values for acarbose were 126.2 μg/mL. This enzyme inhibition profile highlights the potential of *I. kashmiriana*-mediated CuO NPs as a promising natural alternative for managing postprandial hyperglycemia.

In the study by Ameena et al. (2022), copper nanoparticles (CuNPs) were synthesized using a 3 mM copper acetate solution reduced by the aqueous leaf extract of *Cocculus hirsutus*, demonstrating an alpha-amylase inhibition activity of 64.5% ± 0.11%. Ramasubbu et al. (2023) reported that CuO NPs synthesized using *Sesbania grandiflora* (Hummingbird tree) leaf extract exhibited alpha-amylase inhibitory activity of up to 76.7%. This moderate yet consistent inhibitory profile, coupled with the eco-friendly synthesis approach, underscores the potential application of *I. kashmiriana*-mediated CuONPs in the development of alternative antidiabetic therapeutics. Furthermore, the use of a traditionally valued medicinal plant enhances the ethnopharmacological relevance of the formulation and opens new avenues for exploring its synergistic effects in managing postprandial hyperglycemia.

3.3.2. alpha glucosidase activity

The α-glucosidase inhibitory activity of the green-synthesized CuO NPs was investigated to evaluate their potential antidiabetic efficacy. As shown in Figure 6b, CuO NPs demonstrated a dose-dependent inhibitory effect on the enzyme activity. At a concentration of 25 μg/mL, the inhibition was modest at around 17.6%, while increasing the concentration to 50 μg/mL and 100 μg/mL led to more substantial inhibition levels of 27.4% and 36.9%, respectively. A further increase to 150 μg/mL showed a marked enhancement in inhibitory effect, reaching 45.1%, and the highest activity was recorded at 200 μg/mL, with an inhibition of 55.5%. These results highlight that CuO NPs effectively impede the activity of α-glucosidase in a concentration-dependent manner. Statistical analysis revealed highly significant differences ($p < 0.001$) between treated groups and control. The IC_{50} value, calculated from the dose-response curve, was approximately 122.07 μg/mL, indicating a potent inhibitory potential. For comparison, the standard antidiabetic drug acarbose was used as a positive control, and showed the IC_{50} values for acarbose were 102.7 μg/mL. This enzyme inhibition profile suggests that *I. kashmiriana*-derived CuONPs may serve as promising natural inhibitors of α-glucosidase, helping to modulate postprandial glucose spikes in diabetic individuals.

Ameena et al. (2022) reported the synthesis of CuNPs using an aqueous leaf extract of *Cocculus hirsutus* to reduce 3 mM copper acetate, which showed notable alpha-glucosidase inhibitory activity of 68.5% ± 0.11%. Ramasubbu et al. (2023) demonstrated that CuO NPs synthesized from *S. grandiflor* (Hummingbird tree) leaf extract effectively inhibited alpha-glucosidase activity, with a maximum inhibition of 72.1%. These findings highlight the

potential of *I. kashmiriana* as a sustainable and effective bioreductant and stabilizing agent in the green synthesis of CuO NPs with antidiabetic potential. The moderate yet consistent inhibition activity supports the use of this formulation in the development of plant-based nanotherapeutics aimed at controlling postprandial hyperglycemia, particularly as part of a multi-targeted strategy for managing type 2 diabetes.

3.4. In vitro antioxidant activity

3.4.1. DPPH assay

The antioxidant activity of the green-synthesized CuO NPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay Figure 7a. The nanoparticles were tested at various concentrations such as 25, 50, 100, 150, and 200 µg/mL to determine their dose-dependent radical scavenging potential. At the lowest concentration of 25 µg/mL, CuO NPs exhibited a scavenging activity of 18.89%, which increased progressively with higher concentrations. At 50 µg/mL, the inhibition rose to 31.43%, while 100 µg/mL showed a stronger effect at 39.34%. Further increases to 150 µg/mL and 200 µg/mL resulted in scavenging activities of 43.56% and 55.85%, respectively. The results revealed a concentration-dependent antioxidant response, indicating that the CuONPs possess free radical neutralizing ability, likely due to the presence of bioactive phytoconstituents from the *I. kashmiriana* extract acting as surface capping agents. The data were statistically significant ($p < 0.001$) at all tested concentrations compared to the control. Based on the inhibition curve, the IC_{50} value of the CuONPs in the DPPH assay was estimated to be approximately 116.5 µg/mL, whereas ascorbic acid showed an IC_{50} of 100.05 µg/mL, validating the superior efficacy of the standard, suggesting strong antioxidant capacity. These findings support the potential of *I. kashmiriana*-derived CuONPs as promising nanomaterials for oxidative stress-related therapeutic applications.

Ijaz et al. (2017) reported the green synthesis CuONPs using *Abutilon indicum* leaf extract, with the highest α-glucosidase inhibitory activity observed at 1000 µg/mL, corresponding to an IC_{50} value of 84 ± 0.32 µg/mL. CuONPs synthesized using *Magnolia champaca* floral extract exhibited a maximum DPPH radical scavenging activity of 76.30% at a concentration of 500 µg/mL, as reported by Santhoshkumar and Shanmugam (2020). These findings suggest that *I. kashmiriana*-derived CuONPs hold promise as a natural, eco-friendly antioxidant agent, with potential applications in pharmaceutical, nutraceutical, and cosmetic formulations aimed at mitigating oxidative stress. The study further adds value to the underexplored medicinal potential, supporting its use in green nanotechnology and bioactive material development.

3.4.2. ABTS assay

The antioxidant potential of green-synthesized CuO NPs was further assessed using the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay Figure 7b. CuONPs were tested at varying concentrations of 25, 50, 100, 150, and 200 µg/mL to evaluate their free radical neutralizing capacity. At 25 µg/mL, CuONPs exhibited a

scavenging activity of 17.91%, which gradually increased with concentration. At 50 μg/mL, the inhibition was 29.18%, followed by 36.72% at 100 μg/mL, and 41.18% at 150 μg/mL. The highest inhibition, 52.92%, was recorded at 200 μg/mL, indicating a strong antioxidant effect at elevated concentrations. This dose-dependent increase in ABTS radical scavenging activity reflects the effective electron-donating capacity of the CuONPs, likely facilitated by the presence of phenolic and flavonoid compounds from the *I. kashmiriana* extract acting as reducing and stabilizing agents during synthesis. All tested concentrations showed statistically significant inhibition ($p < 0.001$) compared to the control. The IC_{50} value derived from the inhibition curve was estimated to be approximately 118.5 μg/mL, whereas ascorbic acid showed an IC_{50} of 91.6 μg/mL, validating the superior efficacy of the standard, confirming a strong antioxidant potential. These findings support the role of *I. kashmiriana*-mediated CuONPs as promising candidates in free radical scavenging and oxidative stress mitigation strategies.

Santhoshkumar and Shanmugam (2020) reported that CuO NPs synthesized using *Magnolia champaca* floral extract exhibited a maximum ABTS radical scavenging activity of 88.53% at a concentration of 500 μg/mL. Vinothkanna et al. (2023) reported that CuO NPs synthesized using *Rubia cordifolia* bark extract exhibited ABTS radical scavenging activity of approximately 70.88% at a maximum concentration of 100 μg/mL. These outcomes support the potential of *I. kashmiriana* as a promising plant source for the eco-friendly production of bioactive nanoparticles. The demonstrated free radical scavenging activity enhances the scope of these CuONPs for applications in antioxidant therapies, functional formulations, and disease prevention strategies related to oxidative stress.

4. Conclusion

The present study successfully demonstrated the green synthesis of copper oxide nanoparticles (CuONPs) using *Iris kashmiriana* leaf extract as a natural reducing and stabilizing agent. The synthesis approach was simple, eco-friendly, and cost-effective, avoiding hazardous chemicals while yielding well-dispersed, spherical CuONPs with sizes ranging from 45 to 90 nm. Comprehensive characterization using FT-IR, UV–vis, SEM, and XRD confirmed the formation, morphology, crystallinity, and functional group interactions of the biosynthesized nanoparticles, with a crystallite size of approximately 22 nm. Optimization studies revealed that parameters such as temperature, pH, precursor concentration, plant extract volume, and reaction time significantly influenced nanoparticle yield and stability. Functionally, the CuONPs exhibited potent antioxidant activity, as demonstrated in DPPH and ABTS assays, and noteworthy antidiabetic potential, showing significant inhibition of both α-amylase and α-glucosidase enzymes. Although the activities were lower than the standard drugs (ascorbic acid and acarbose, respectively), the CuONPs still showed statistically significant effects in a concentration-dependent manner. These findings collectively highlight the dual therapeutic potential of *I. kashmiriana*-mediated CuONPs as natural antioxidant and antidiabetic agents, offering a promising platform for the development of safe, biocompatible nanomedicines. Their incorporation into therapeutic frameworks could offer a novel approach in managing oxidative stress-associated metabolic disorders such as type 2 diabetes mellitus.

Further *in vivo* studies and mechanistic investigations are recommended to validate their pharmacological efficacy and clinical applicability.

Conflict of Interest

Nil

Acknowledgements

This work is supported by the Department of Pathology, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Thandalam, Chennai-602 105, Tamil Nadu, India. The other authors would also like to thank their respective departments for providing facilities for the work.

Reference

Alhalili, Z. (2022). Green synthesis of copper oxide nanoparticles CuO NPs from *Eucalyptus Globoulus* leaf extract: Adsorption and design of experiments. *Arabian Journal of Chemistry*, *15*(5). https://doi.org/10.1016/j.arabjc.2022.103739

Altaf, I., Firdaus, N., Wani, I. A., Khan, S., & Owais, M. (2025). *Iris kashmiriana* Mediated Synthesis of Gold Nanoparticles: Characterization and Its Antibacterial Implications on Dental Appliances. *BioNanoScience*, *15*(1), 133. https://doi.org/10.1007/s12668-024-01693-6

Amalraj, S., Krupa, J., Sriramavaratharajan, V., Mariyammal, V., Murugan, R., & Ayyanar, M. (2021). Chemical characterization, antioxidant, antibacterial and enzyme inhibitory properties of *Canthium coromandelicum*, a valuable source for bioactive compounds. *Journal of Pharmaceutical and Biomedical Analysis*, *192*. https://doi.org/10.1016/j.jpba.2020.113620

Ameena, S., Rajesh, N., Anjum, S. M., Khadri, H., Riazunnisa, K., Mohammed, A., & Kari, Z. A. (2022). Antioxidant, Antibacterial, and Anti-diabetic Activity of Green Synthesized Copper Nanoparticles of *Cocculus hirsutus* (Menispermaceae). *Applied Biochemistry and Biotechnology*, *194*(10). https://doi.org/10.1007/s12010-022-03899-4

Balkrishna, A., Kumar, A., Arya, V., Rohela, A., Verma, R., Nepovimova, E., Krejcar, O., Kumar, D., Thakur, N., & Kuca, K. (2021). Phytoantioxidant Functionalized Nanoparticles: A Green Approach to Combat Nanoparticle-Induced Oxidative Stress. In *Oxidative Medicine and Cellular Longevity* (Vol. 2021). https://doi.org/10.1155/2021/3155962

Black, H. S. (2024). Oxidative Stress and ROS Link Diabetes and Cancer. In *Journal of Molecular Pathology* (Vol. 5, Issue 1). https://doi.org/10.3390/jmp5010007

Chalotra, R., Dhanawat, M., Maqbool, M., Lamba, N., Bibi, A., & Gupta, S. (2022). Phytochemistry and Pharmacology of *Iris kashmiriana*. *Pharmacognosy Research*, *14*(4). https://doi.org/10.5530/pres.14.4.52

da Silva Almeida, É., da Rocha, J. D. G., de Oliveira, D., & Hotza, D. (2025). Valorization of noni-leaf tea as a by-product of nanofiltration for the green synthesis of copper oxide nanoparticles with improved antimicrobial properties. *Materials Science and Engineering: B*, *317*, 118172. https://doi.org/10.1016/j.mseb.2025.118172

Hussain Mir, A. (2014). || ISSN 2277-4289 | www.gjrmi.com | International, Peer reviewed, Open access. In *Global Journal of Research on Medicinal Plants & Indigenous Medicine* || *GJRMI* || (Vol. 3, Issue 12). www.gjrmi.com

Ijaz, F., Shahid, S., Khan, S.A., Ahmad, W. and Zaman, S., 2017. Green synthesis of copper oxide nanoparticles using *Abutilon indicum* leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activitie. *Tropical Journal of Pharmaceutical Research,* 16(4), pp.743-753.

Jabeen, S., Siddiqui, V. U., Bala, S., Mishra, N., Mishra, A., Lawrence, R., Bansal, P., Khan, A. R., & Khan, T. (2024). Biogenic Synthesis of Copper Oxide Nanoparticles from *Aloe vera* : Antibacterial Activity, Molecular Docking, and Photocatalytic Dye Degradation. *ACS Omega*, *9*(28), 30190–30204. https://doi.org/10.1021/acsomega.3c10179

Santhoshkumar, J. & Shanmugam, V. (2020). Green Synthesis of Copper Oxide Nanoparticles from Magnolia Champaca Floral Extract and its Antioxidant & Toxicity Assay using Danio Rerio. *International Journal of Recent Technology and Engineering (IJRTE)*, *8*(5). https://doi.org/10.35940/ijrte.e6869.018520

Karimi, B., Mardani, M., Kaboutari, J., Javdani, M., Albadi, J., & Shirian, S. (2024). Green synthesis of copper oxide nanoparticles using *Artemisia annua* aqueous extract and its characterization, antioxidant, and burn wound healing activities. *Chemical Papers*, *78*(1). https://doi.org/10.1007/s11696-023-03069-8

Karthick, V., Zahir, A. A., Amalraj, S., Rahuman, A. A., Anbarasan, K., & Santhoshkumar, T. (2025). Sustained release of nano-encapsulated glimepiride drug with chitosan nanoparticles: A novel approach to control type 2 diabetes in streptozotocin-induced Wistar albino rats. *International Journal of Biological Macromolecules*, *287*. https://doi.org/10.1016/j.ijbiomac.2024.138496

Karthick, V., Zahir, A. A., Ayyanar, M., Amalraj, S., Anbarasan, K., Rahuman, A. A., Kadaikunnan, S., Kamaraj, C., Thiruvengadam, M., Gangapriya, P., & Tamizharasan, P. (2023). Optimization and characterization of eco-friendly formulated ZnO NPs in various parameters: assessment of its antidiabetic, antioxidant and antibacterial properties. *Biomass Conversion and Biorefinery*, 1–15. https://doi.org/10.1007/S13399-023-04363-X/METRICS

Kimta, N., Chauhan, A., Puri, S., Kumari, A., Sharma, R., Kumar, A., & Kapoor, D. (2025). Phytomediated Copper Oxide Nanoparticles Derived from the Fronds of Adiantum venustum D.Don: Evaluation of their Biomedical Potential. *Applied Biochemistry and Biotechnology*, *197*(1), 398–426. https://doi.org/10.1007/s12010-024-05031-0

Kulkarni, N., & Muddapur, U. (2014). Biosynthesis of metal nanoparticles: A review. In *Journal of Nanotechnology* (Vol. 2014). https://doi.org/10.1155/2014/510246

Liang, Y.-P., Chan, Y.-B., Aminuzzaman, M., Shahinuzzaman, M., Djearamane, S., Thiagarajah, K., Leong, S.-Y., Wong, L.-S., & Tey, L.-H. (2025). Green Synthesis and Characterization of Copper Oxide Nanoparticles from Durian (Durio zibethinus) Husk for Environmental Applications. *Catalysts*, *15*(3), 275. https://doi.org/10.3390/catal15030275

Manigandan, P., & Shanmugam, R. (2025). Green preparation of fluorescence carbon nanoparticles using honey and its biomedical applications- an in vitro study. *Journal of Drug Delivery Science and Technology*, *108*, 106919. https://doi.org/10.1016/j.jddst.2025.106919

Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. In *Journal of Biochemical and Molecular Toxicology* (Vol. 17, Issue 1). https://doi.org/10.1002/jbt.10058

Mohamed, E. A. (2020). Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. *Heliyon*, *6*(1). https://doi.org/10.1016/j.heliyon.2019.e03123

Nagaraj, K., Thangamuniyandi, P., Kamalesu, S., Dixitkumar, M., Saini, A. K., Sharma, S. K., Naman, J., Priyanshi, J., Uthra, C., Lokhandwala, S., Parekh, N. M., Radha, S., Sakthinathan, S., Chiu, T. W., & Karuppiah, C. (2023). Silver nanoparticles using Cassia Alata and its catalytic reduction activities of Rhodamine6G, Methyl orange and methylene blue dyes. *Inorganic Chemistry Communications*, *155*. https://doi.org/10.1016/j.inoche.2023.110985

Nzilu, D. M., Madivoli, E. S., Makhanu, D. S., Wanakai, S. I., Kiprono, G. K., & Kareru, P. G. (2023). Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-41119-z

Ramasubbu, K., Padmanabhan, S., Al-Ghanim, K. A., Nicoletti, M., Govindarajan, M., Sachivkina, N., & Rajeswari, V. D. (2023). Green Synthesis of Copper Oxide Nanoparticles Using *Sesbania grandiflora* Leaf Extract and Their Evaluation of Anti-Diabetic, Cytotoxic, Anti-Microbial, and Anti-Inflammatory Properties in an In-Vitro Approach. *Fermentation*, *9*(4). https://doi.org/10.3390/fermentation9040332

Relhan, A., Guleria, S., Bhasin, A., Mirza, A., & Zhou, J. L. (2024). Biosynthesized copper oxide nanoparticles by Psidium guajava plants with antibacterial, antidiabetic, antioxidant, and photocatalytic capacity. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-024-05544-y

Sankar, R., Manikandan, P., Malarvizhi, V., Fathima, T., Shivashangari, K. S., & Ravikumar, V. (2014). Green synthesis of colloidal copper oxide nanoparticles using Carica papaya and its application in photocatalytic dye degradation. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *121*. https://doi.org/10.1016/j.saa.2013.12.020

Shanmugam, R., Shree B, S., Anandan, J., Abalkhail, T., Alqahtani, A. M. S., & Roy, A. (2025). Green synthesis of iron oxide nanoparticles using Cissus rotundifolia and its antibacterial activity against wound pathogens. *Journal of the Indian Chemical Society*, *102*(3), 101599. https://doi.org/10.1016/j.jics.2025.101599

Thangaraj, P. (2016). Pharmacological assays of plant-based natural products. In *Progress in Drug Research* (Vol. 71).

Vaughan, N. (2024). Virtual Reality Meets Diabetes. In *Journal of Diabetes Science and Technology*. https://doi.org/10.1177/19322968231222022

Vinothkanna, A., Mathivanan, K., Ananth, S., Ma, Y., & Sekar, S. (2023). Biosynthesis of copper oxide nanoparticles using Rubia cordifolia bark extract: characterization, antibacterial, antioxidant, larvicidal and photocatalytic activities. *Environmental Science and Pollution Research*, *30*(15). https://doi.org/10.1007/s11356-022-18996-4

WHO. (2024). *Diabetes.* Https://Www.Who.Int/Health-Topics/Diabetes#tab=tab_1.

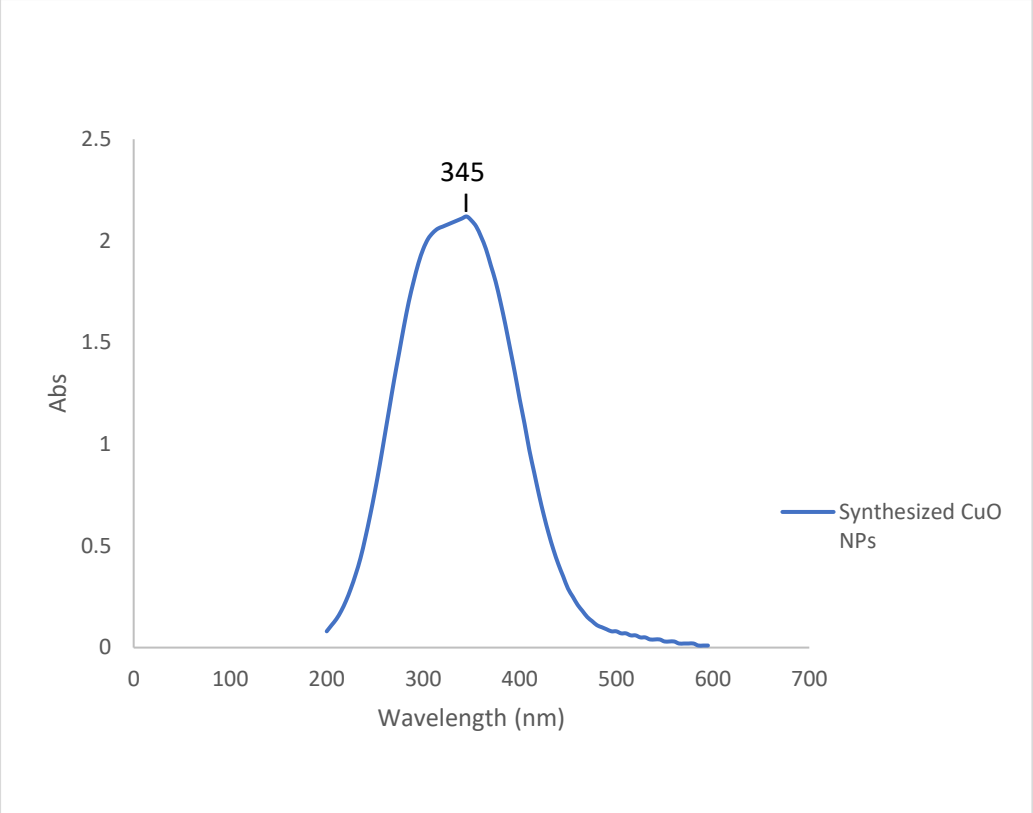

Figure 1: UV–Vis spectra of green synthesized CuO NPs using *Iris kashmiriana* extract.

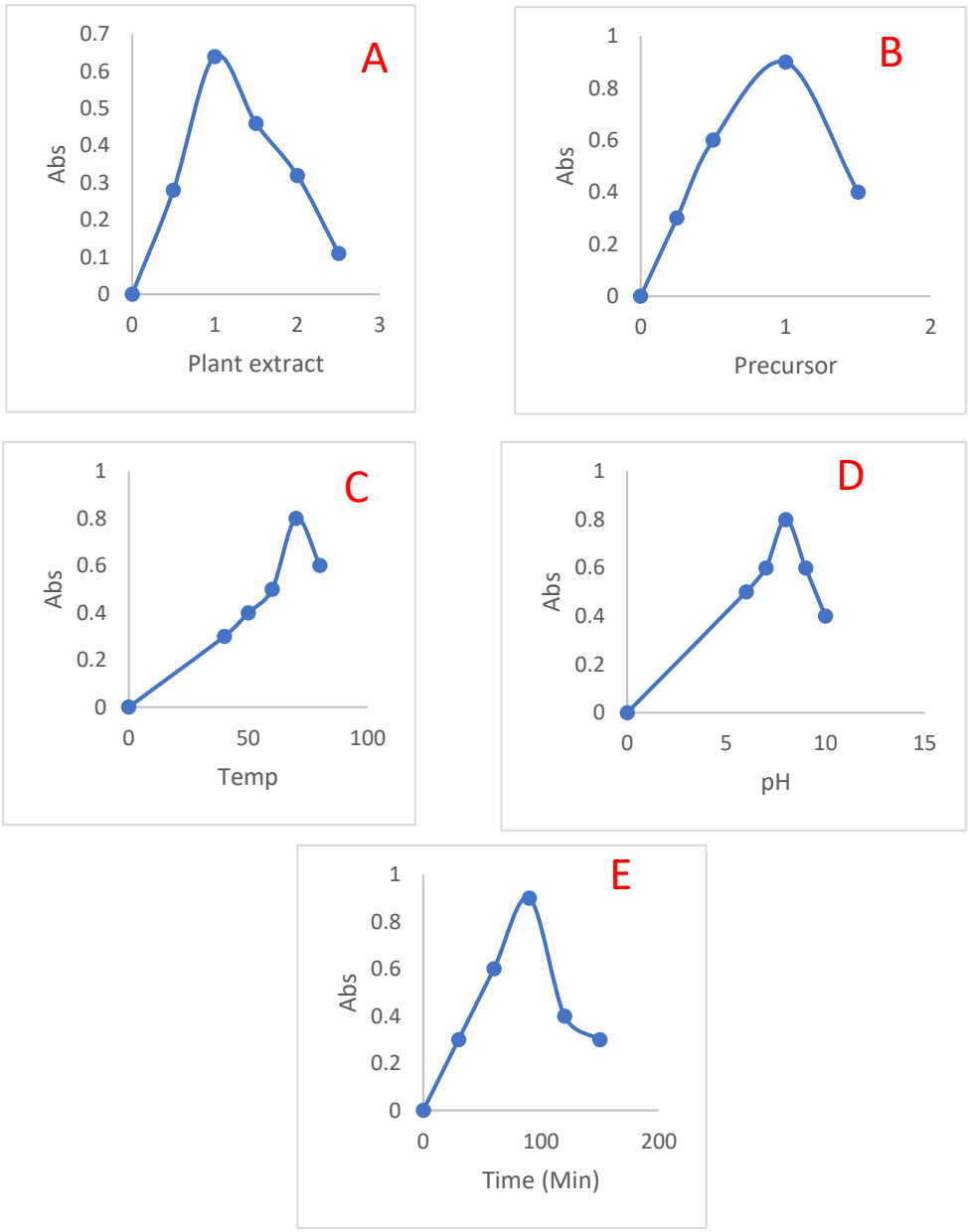

Figure 2: Optimizations of various parameters for the synthesis of CuO NPs. A. concentration of plant extract, B. precursor concentration, C. Temp, D. pH and E. reduction time

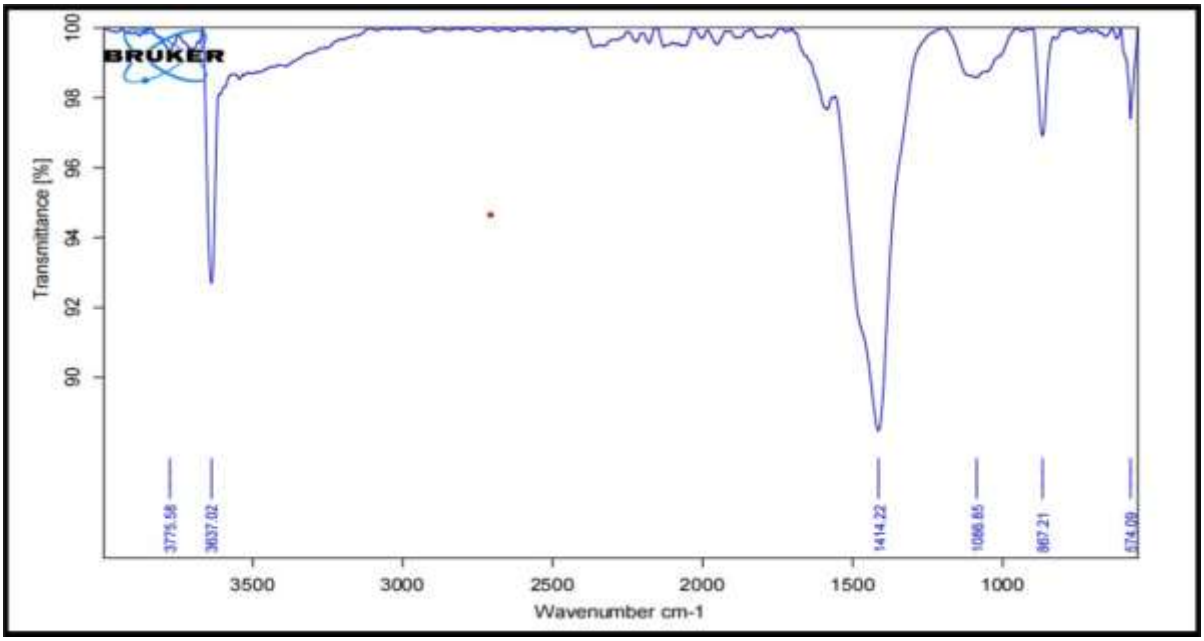

Figure 3: FT-IR spectrum of green synthesized CuO NPs

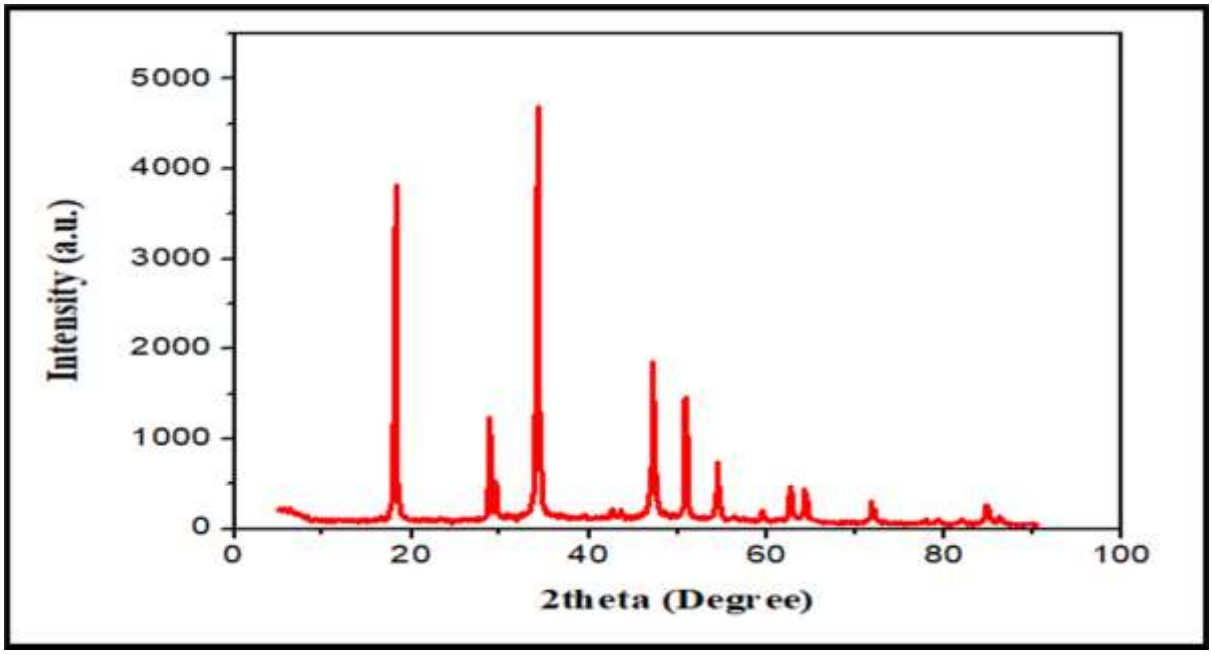

Figure 4: XRD spectrum of synthesized CuO NPs

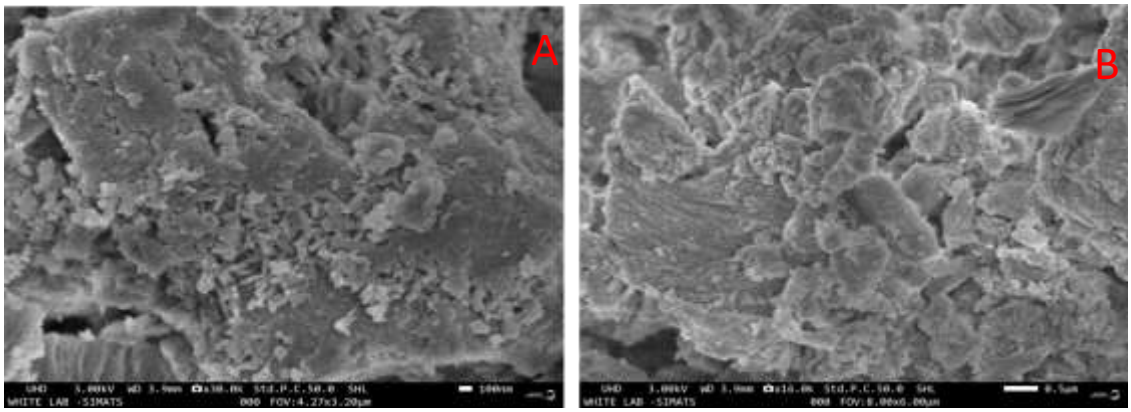

Figure 5: SEM Image of synthesized CuONPs at various magnifications, A. 100nm and B. 0.5 µm

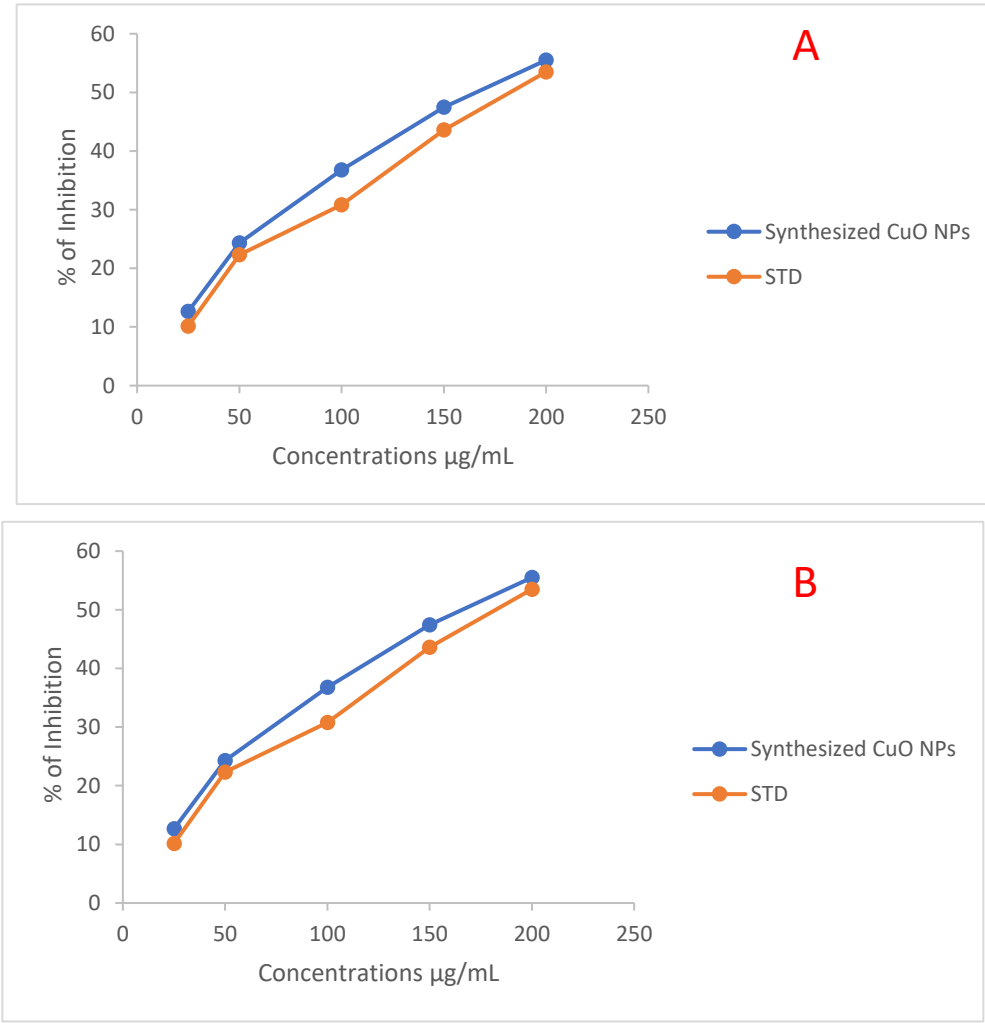

Figure 6: *In vitro* Antidiabetic Potential of synthesized CuONPs. A) Alpha Amylase activity and B) Alpha Glucosidase activity

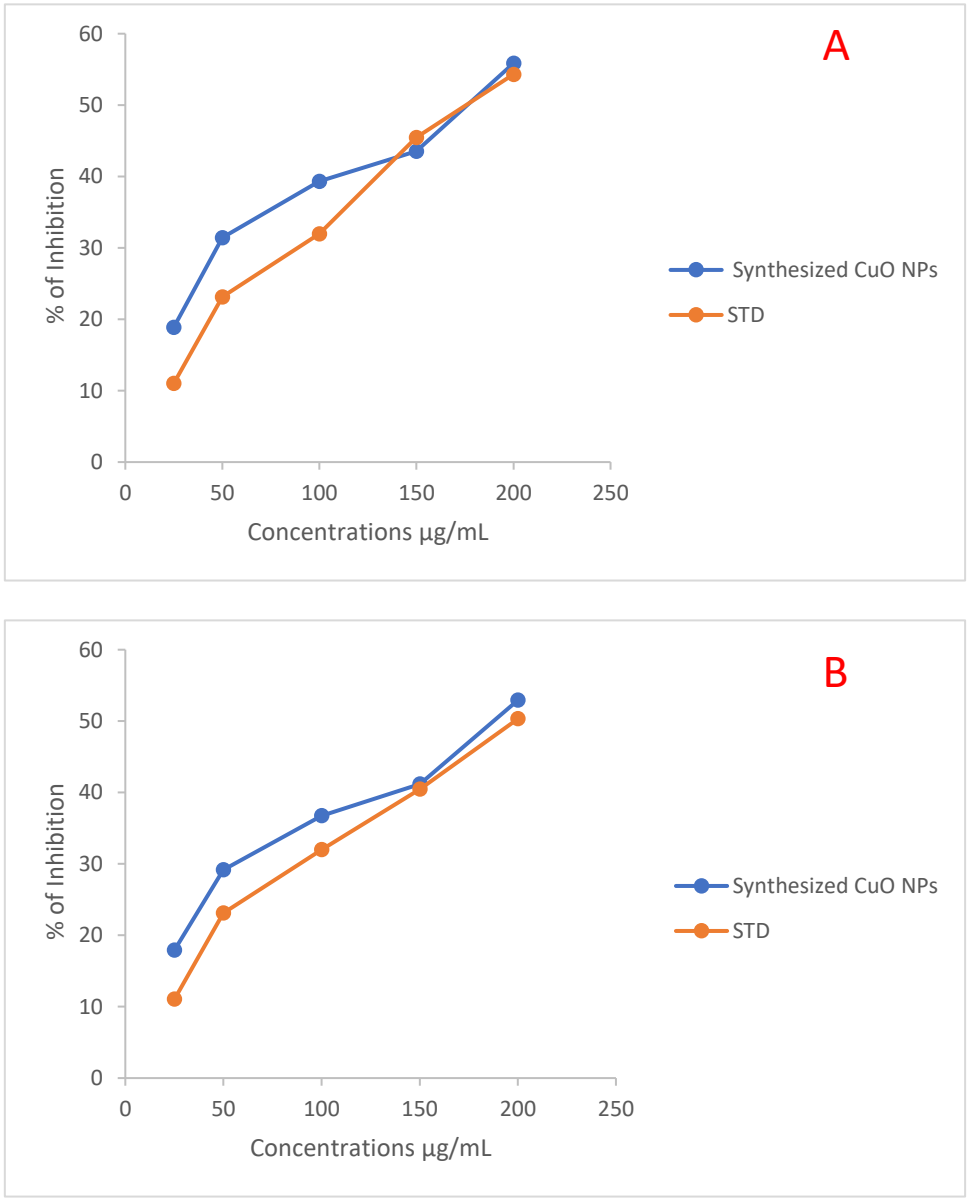

Figure 7: *In vitro* Anti-oxidant Potential of synthesized CuONPs. A) DPPH and B) ABTS