



GREEN SYNTHESIS AND CHARACTERIZATION OF COPPER OXIDE (CUO) NANOPARTICLES USING *ALOE VERA* LEAF EXTRACT: ANTIDIABETIC, ANTIOXIDANT, AND ANTIMICROBIAL APPLICATIONS

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ABSTRACT

The copper oxide (CuO) nanoparticles were bio synthesized using the aqueous extract of *Aloe vera* (*Aloe barbadensis* miller) leaf gel as a natural reducing and capping agent and aqueous ammonia for pH adjusting with copper sulfate pentahydrate (CuSO₄·5H₂O) as metal precursor. Synthesized nanoparticles were analyzed using Fourier-transform infrared (FTIR) spectroscopy, powder X-ray diffraction (XRD) and scanning electron microscopy (SEM). The characteristic Cu–O bond of CuO was confirmed by FTIR analysis, along with the presence of functional groups associated with the processes of bio reduction and capping performed by the phytoconstituents of *Aloe vera*. These were assigned as a single-phase monoclinic tenorite (CuO) structure, having an average crystallite size around 11–12 nm, as confirmed by XRD. SEM imaging showed dense collections of quasi-spherical to polygonal nanoparticles exhibiting sub-100 nm primary features, while EDX spectroscopy succeeded in confirming that only oxygen and copper (71.4 wt% Cu, 28.6 wt% O) elements existed in the product with no detectable element of impurity. The α -glucosidase inhibitory (antidiabetic) activity of the nanoparticles was also evaluated and the nanoparticles inhibited the enzyme with an IC₅₀ of 47.86 ± 0.12 μ g/mL while the standard acarbose had an activity of 551.1 ± 0.03 μ g/mL under the same assay conditions. The radical scavenging activity in a DPPH assay was weak with the same nanoparticles with IC₅₀ values exceeding the maximum dose tested (maximum 10.91% at 1000 μ g/mL), as opposed to their α -glucosidase inhibitory activity. The antibacterial screening through Agar well diffusion (Kirby Bauer) method exhibited organism dependent activity with moderate zone of inhibition in both case the *E. coli* and *B. subtilis* were active against most of the dosage used (In between 8.33 mm at 312.5 μ g to 13.67 mm at 5000 μ g and 11.67 mm at 5000 μ g, respectively), whereas for *S. aureus* and *P. aeruginosa* the activity observed was limited only with higher dosage tested (Between 16.33 mm at 5000 μ g and 11.33 mm at 5000 μ g at 5000 PPT and 10000 PPT respectively). All zones were much smaller than the appropriate ciprofloxacin positive control (24–31 mm for 3 μ g). Overall, the results suggest that *Aloe vera* mediated CuO nanoparticles could be a prospective, environmentally benign and low-cost nanomaterial with major biomedical advantage of α -glucosidase inhibition which has an additional concentration dependent antibacterial activity and comparatively low antimicrobial antioxidant activity.

Keywords: Green synthesis, CuO nanoparticles, α -glucosidase inhibition, Antidiabetic activity, DPPH.

INTRODUCTION

Copper oxide (CuO) nanoparticles are ideal for a variety of applications due to their desirable electrical, optical, catalytic and antimicrobial properties, such as sensors, catalysis, antibacterial coatings and photocatalytic water purification. Traditional chemical and physical synthetic methods require toxic reducing agents that require the input of high energy, and this is increasing the concerns for green

methods whereby plant extracts are used as environmentally friendly reducing and stabilizing agents. The bioactive phytoconstituents present in *Aloe vera* (*Aloe barbadensis* miller) leaf gel are highly abundant such as the polysaccharides (acemannan), flavonoids, phenolic acids and anthraquinones which can donate an electron to reduce the metal ions and form a capping layer on the resulting nanoparticles. The phytochemical profile makes *Aloe vera*

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a promising bio-reductant source using metal salts to produce nanoparticles like CuO in a cost-effective manner.

Because of increasing antimicrobial resistance and need for inexpensive and diverse nanomaterials, beyond their antimicrobial applications, metal oxide nanoparticles prepared by green route methods have been investigated for other biomedical applications, like enzyme-inhibitory (e.g., antidiabetic) and free-radical scavenging (antioxidant) agents. In the present study, copper sulfate pentahydrate, a metal precursor was selected to prepare CuO nanoparticles using aqueous extract of *Aloe vera* leaves, ammonia was used to adjust the pH of the reaction. Comprehensive physiochemical characterization of the synthesized nanoparticles was done by FTIR, XRD, SEM and EDX and their physiological evaluation was carried by antioxidant (DPPH) activity, In-vitro α -glucosidase inhibitory (antidiabetic) activity and antibacterial activity against four bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* which include both Gram negative as well as Gram positive bacteria were carried successfully to assess the biomedical potential of this green-synthesized nanomaterial.

MATERIALS AND METHODS

Materials

Fresh *Aloe vera* (*Aloe barbadensis* miller) leaves were used as the plant source. The metal precursor was copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, analytical grade) and the pH adjustment was carried out with aqueous ammonia solution. Throughout, distilled water was used as the solvent and Whatman No. 1 filter paper was used for filtering and collecting precipitates. The extraction and synthesis reaction was carried out using a magnetic stirrer, the separation of extract and precipitated nanoparticles was performed using a centrifuge, drying was performed in a hot air oven and calcination was performed in a muffle furnace. The bacterial strains (*Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 452), *Bacillus subtilis* (MTCC 1133) and *Pseudomonas aeruginosa* (MTCC 3541)) used in antibacterial screening were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

The extraction of *Aloe vera* leaf extract was performed as described below

The fresh leaves of *Aloe vera* were cut and washed with distilled water to get rid of dust and dirt on the leaves. The outer rind was removed and the mucilaginous gel extracted and divided into small pieces. This *Aloe vera* gel was processed into powder form and 15 g of *Aloe vera* powder was dissolved in 200 mL of distilled water. The bioactive compounds were extracted by heating the gel-water mixture on a magnetic stirrer at 40–50 °C for 2 h with continuous stirring at 500–1000 rpm after which it was filtered through filter paper. Residual solid debris was removed by centrifuging the filtrate at 2000 rpm for 15 min

and the clear supernatant was collected and utilized as plant extract for the preparation of nanoparticles.

The preparation of the precursor solution was the same as in Step

A solution of copper sulfate pentahydrate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, was prepared in 1000 mL of distilled water using the mass of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ required for the solution. A solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was prepared to a volume of 1000 mL of distilled water using the required mass of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The precursor solution was characterized with its blue colour before the reaction.

Reaction Mixture

Then 640 mL of precursor solution was mixed with 140 mL of the *Aloe vera* leaf extract in a beaker and stirred continuously on the magnetic stirrer at 50–60 °C for 3 h at 1000 rpm. The reaction mixture was stirred for 1 hour after which a drop of aqueous ammonia solution was added to it to bring the pH to a level that should facilitate the hydrolysis of copper ions to precipitate copper hydroxide intermediates. The reaction mixture changed color over time, as the nanoparticles began to form. The mixture was then centrifuged at 20,000 rpm when finished to get the precipitate and supernatant separated.

Collection and Calcination of CuO Nanoparticles

Whatman filter paper was used to collect precipitate and washed multiple times with distilled water to remove unreacted precursor ions, excess ammonia and residual plant biomolecules. The washed precipitate was dried at 80 °C in a hot air oven and further treated in a muffle furnace at 400 °C to eliminate the traces of organic impurities to produce the final crystalline CuO nanoparticles.

Characterization

Phytoconstituents that reduced, capped and complexed the nanoparticles were identified by Fourier-transform infrared (FTIR) (Shimadzu) spectroscopy in the range of 4000–400 cm^{-1} by observing the characteristic Cu–O vibration. Powder X-ray diffraction (XRD) was used to assess the phase purity and crystallite size in the 2θ range from 10 to 90°. Surface morphology was investigated using field-emission scanning electron microscopy (SEM) at an accelerating voltage of 10.0 kV and elemental composition was analysed with energy-dispersive X-ray (EDX) spectroscopy and mapping.

In-vitro α -Glucosidase Inhibitory (Antidiabetic) Assay

The α -Glucosidase inhibitory activity was determined as per the method described by Telagari & Hullatti (2015) using acarbose as a positive control. In a 96-well plate, the CuO–*Aloe vera* sample (20 μL , at concentrations of 0, 1, 10, 50, 100, 250, 500 and 1000 $\mu\text{g/mL}$) was pre-incubated with phosphate buffer (50 μL , 100 mM, pH 6.8) and α -glucosidase (10 μL , 1 U/mL, ex. yeast) at 37 °C for 15 min, followed by addition of p-NPG substrate (20 μL , 5 mM)

and further incubation. Reaction was terminated by adding 0.1 M Na₂CO₃ and absorbance of p-nitrophenol released was measured at 405 nm. The percent inhibition compared with the control was determined at each concentration, and the IC₅₀ was determined by GraphPad Prism 6. (Ref. Assay Performed by Aakaar Biotechnologies Pvt. Ltd., Lucknow) No. 25F62B.)

DPPH Radical Scavenging (Antioxidant) Assay

The free radical scavenging activity of the antioxidant was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging test method using ascorbic acid as the positive control. The CuO–*Aloe vera* solution at different concentration points (0, 1, 10, 50, 100, 250, 500 and 1000 µg/mL) was added to the 0.2 mL of 0.1 mM DPPH solution in methanol to the 96-well plate and incubated in dark for 30 min. Decolorization was measured at 517 nm using microplate reader (iMark, BioRad). Percent radical scavenging activity (%RSA) was determined as follows: %RSA = (Abs Control – Abs Sample) / Abs Control × 100 and IC₅₀ was calculated using GraphPad Prism 6. (Assay done by Aakaar biotechnology Pvt. Ltd., Lucknow; Ref. No. 25F62A.)

The antimicrobial activity was evaluated using the antibiotic activity assay (Kirby–Bauer Well Diffusion Method)

The antibacterial activity of CuO NPs–*Aloe vera* was assessed in four bacterial strains (*S. aureus* (MTCC 96), *E. coli* (MTCC 452), *B. subtilis* (MTCC 1133) and *P. aeruginosa* (MTCC 3541)) by the agar well diffusion (Kirby–Bauer) method. The respective bacterial inoculum was standardized to 0.5 McFarland (~1.5 × 10⁸ CFU/mL) and then 100 µL was uniformly inoculated onto Mueller–Hinton agar (MHA) plates. Inoculated agar was bored with uniform diameter and filled with test sample at the following working concentrations (Table 1) which correspond to the specific concentration required by the organism. In each assay, the solvent (vehicle) control was DMSO and the positive control was ciprofloxacin (3 µg/well). Plates were incubated for 24 hours at 37 °C and the diameter of clear zones around each well was measured. Each assay was done in 3 replicate plates (A, B, C) and the results are reported as mean ± SD (standard deviation).

RESULTS AND DISCUSSION

The FTIR spectrum of the CuO nanoparticles synthesized using *Aloe vera* leaf extract, recorded over the range 4000–400 cm^{–1}, is shown in Figure 1. The spectrum displays absorption bands corresponding to both the residual phytoconstituents of the *Aloe vera* extract and the inorganic Cu–O framework of the nanoparticles, summarized in Table 2.

Table 1. Assay-specific parameters for the antibacterial screening of CuO NPs–*Aloe vera* against each bacterial strain.

Organism	MTCC No.	Concentration range (µg/well)	Sample volume	Reference No.
<i>E. coli</i>	452	0–5000	50 µL	25F62F
<i>S. aureus</i>	96	0–5000	10 µL	25F62H
<i>B. subtilis</i>	1133	0–10000	50 µL	25F62G
<i>P. aeruginosa</i>	3541	0–5000	50 µL	25F62E

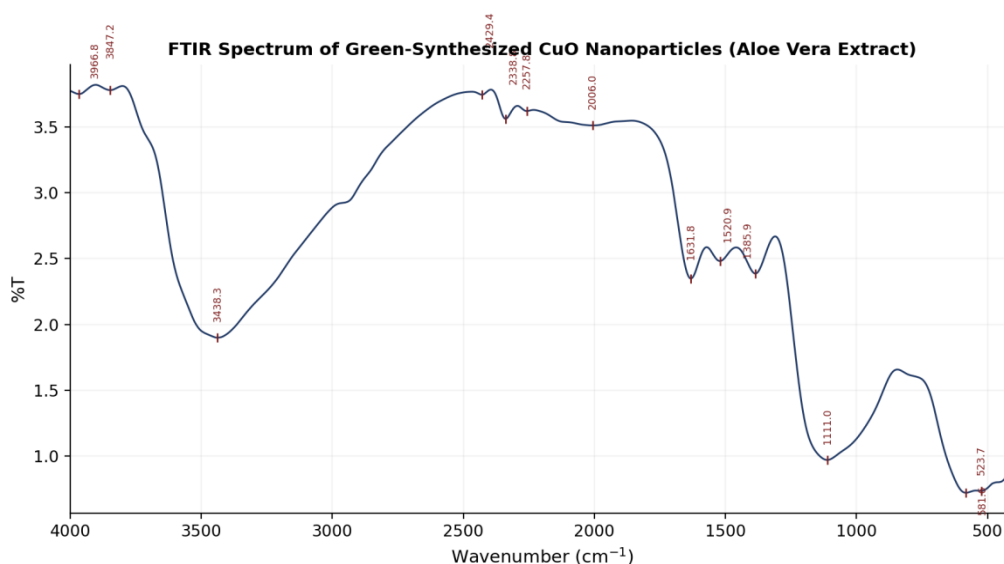


Figure 1. FTIR spectrum of green-synthesized CuO nanoparticles using *Aloe vera* leaf extract.

Table 2. FTIR peak positions and tentative band assignments for *Aloe vera*-mediated CuO nanoparticles.

Peak position (cm ⁻¹)	Tentative assignment
3438.3	O–H stretching (hydrogen-bonded hydroxyl/phenolic groups; adsorbed moisture)
2429.4 / 2338.8 / 2257.8	Weak bands; largely coincide with atmospheric CO ₂ interference / minor combination-overtone absorptions
2006.0	Weak combination/overtone band
1631.9	C=O stretching (asymmetric –COO ⁻ stretch / amide I); carbonyl of polyphenolic biomolecules
1520.9	N–H bending (amide II) / aromatic C=C skeletal stretching of flavonoid rings
1385.9	Symmetric –COO ⁻ stretching / phenolic O–H bending
1111.1	C–O–C / C–O stretching of polysaccharides (e.g., acemannan), consistent with <i>Aloe vera</i> polysaccharide content
581.6	Cu–O stretching vibration
523.7	Cu–O stretching vibration, characteristic of monoclinic CuO lattice

An O–H stretching vibration from hydrogen bonded hydroxyl groups and phenolic groups in the *Aloe vera* extract and the moisture that can be found on the sample's surface cause a broad band around 3438.3 cm⁻¹. The bands at 1631.9cm⁻¹ and 1520.9cm⁻¹ are consistent with carbonyl (C=O, asymmetric –COO⁻) and aromatic C=C stretching/amide stretching on the surface of the nanoparticles, respectively, thus supporting the idea of polyphenolic and flavonoid biomolecules, respectively, being on the surface of the nanoparticles, while the band at 1385.9 was assigned to the symmetric –COO⁻ stretching. This polysaccharide biomolecule is likely to be involved in the stabilization of nanoparticles together with the phenolic

components as polysaccharide bands appear at 1111.1 cm⁻¹ in association with the phenolic bands which represent C–O–C/C–O stretching of polysaccharides, like those found in acemannan, the main polysaccharide in *Aloe vera* gel. Reliably, the two bands at 581.6cm⁻¹ and 523.7cm⁻¹ are identified as Cu–O stretching vibrational modes of the CuO lattice, which are characteristic of the nutrient CuO formed by the lattice of biomolecules derived from *Aloe vera*. *Aloe vera* mediated CuO nanostructures powder XRD pattern is shown in figure 2 along with the reference CuO (Tenorite, COD entry 9015841) stick pattern recorded in 2θ range between 10–90°. All of these diffraction peaks can be distinguished and are summarized in Table 3.

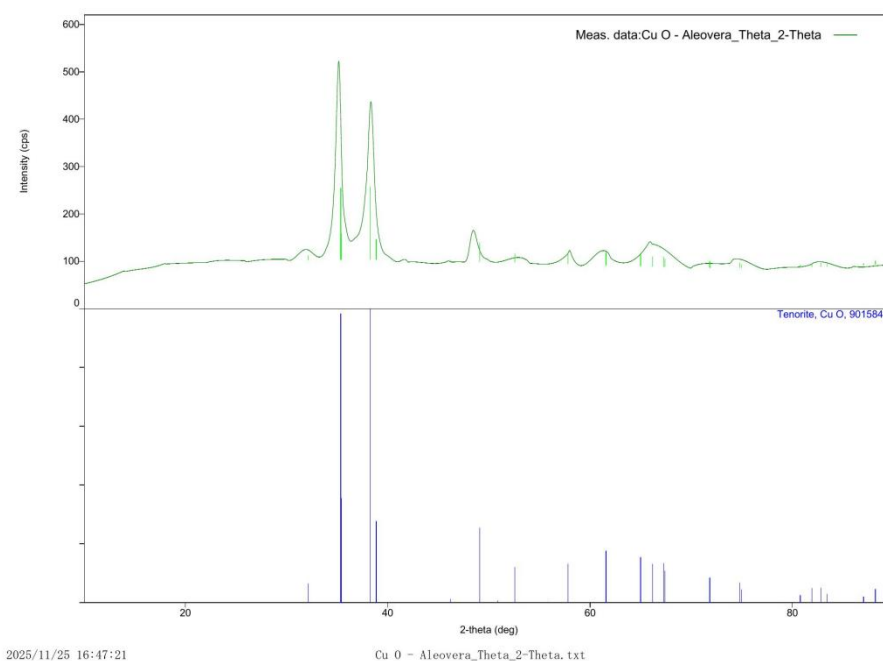
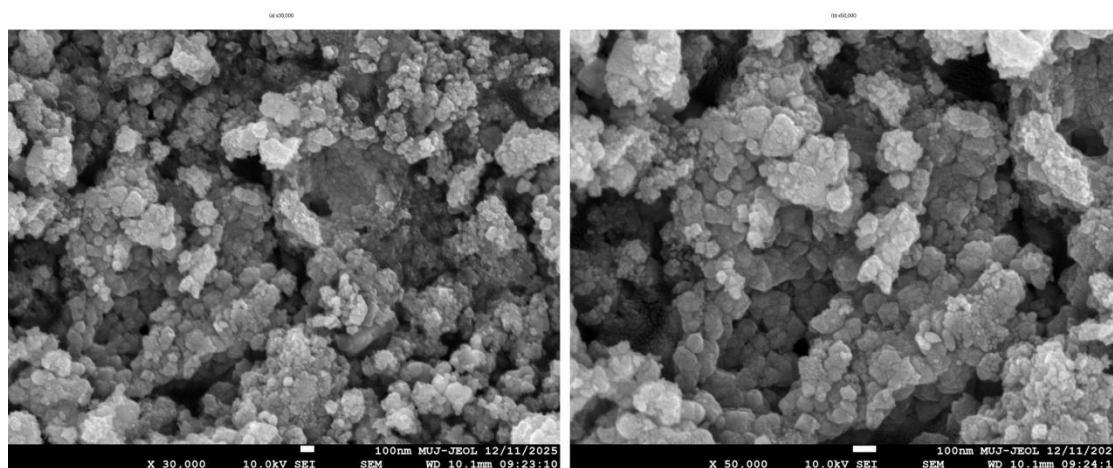
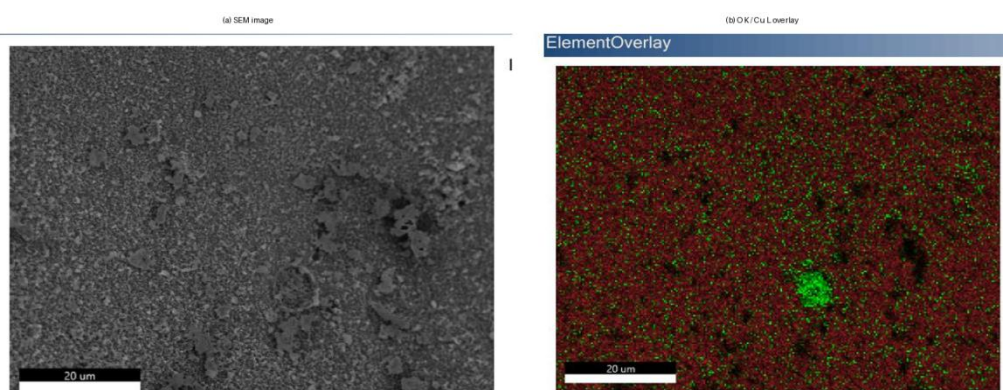
**Figure 2.** XRD pattern of green-synthesized CuO nanoparticles, matched against the tenorite (CuO) reference pattern (COD 9015841).

Table 3. XRD peak positions, d-spacings, FWHM, Scherrer crystallite sizes, and assigned (hkl) planes.

2 θ (deg)	d-spacing (Å)	FWHM (deg)	Int. I (cps·deg)	Crystallite size (nm)	Plane (hkl)
32.20	2.778	0.80	23	10.8	(110)
35.154	2.5507	0.590	260	14.7	(-111)
38.320	2.3470	0.79	280	11.2	(111)
48.421	1.8783	0.79	40.5	11.6	(-202)
53.04	1.7251	0.61	10.3	15.1	(020)
57.72	1.5959	0.67	17	14.1	(202)
61.21	1.5129	0.84	39	11.5	(-113)
66.16	1.411	2.98	92	3.3	(-311)
74.80	1.268	1.2	29	8.6	(004)

All nine reflections indexed closely to the monoclinic tenorite phase of CuO (space group C2/c), with the two most intense peaks at 35.154° and 38.320° corresponding to the (-111) and (111) planes the characteristic diagnostic doublet of CuO. No additional peaks attributable to unreacted copper precursor, copper hydroxide, or Cu₂O (cuprite) were observed, indicating that the calcined product is single-phase CuO. Crystallite sizes calculated for the individual reflections ranged from approximately 3.3 to 15.1 nm, with a mean of about 11.2 nm across all nine

peaks (approximately 12.2 nm excluding the anomalously broad 66.16° peak, likely arising from peak overlap or lower counting statistics). This places the synthesized nanoparticles well within the nanocrystalline regime, consistent with CuO nanoparticles reported using other plant leaf extracts. Surface morphology was examined by field-emission SEM at 10.0 kV. Representative micrographs at $\times 30,000$ and $\times 50,000$ magnification are shown in Figure 3.

**Figure 3.** SEM micrographs of *Aloe vera*-mediated CuO nanoparticles at (a) $\times 30,000$ and (b) $\times 50,000$ magnification (scale bar = 100 nm).**Figure 4.** (a) SEM image of the scanned area and (b) corresponding EDX elemental overlay map (green = O K, red = Cu L); scale bar = 20 μ m.

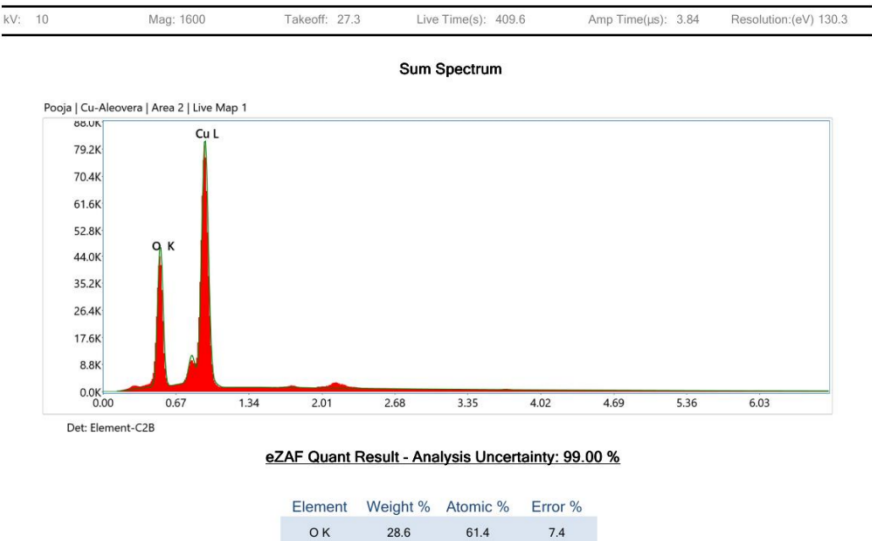


Figure 5. EDX sum spectrum and eZAF-quantified elemental composition of *Aloe vera*-mediated CuO nanoparticles.

Table 4. EDX-quantified elemental composition (eZAF quantification, analysis uncertainty 99.00%).

Element	Weight %	Atomic %	Error %
O K	28.6	61.4	7.4
Cu L	71.4	38.6	7.0

The micrographs reveal densely packed, quasi-spherical to polygonal nanoparticles extensively agglomerated into larger secondary clusters, with individual grain features approaching the sub-100 nm scale characteristic of biosynthesized metal oxide nanoparticles, where plant-derived biomolecules adsorb onto the particle surface during growth and promote clustering. The fine sub-grain texture is consistent with the nanocrystalline domain sizes (~11–12 nm) obtained independently from XRD line-broadening analysis (Section 3.2), indicating that the micron-scale features observed by SEM are agglomerates of much smaller primary CuO crystallites. EDX spectroscopy with elemental mapping confirmed the elemental composition. The SEM image and corresponding O K / Cu L elemental overlay map are shown in Figure 4, and the sum EDX spectrum with quantified composition is shown in Figure 5.

The EDX spectrum showed only oxygen (O K) and copper (Cu L) emission peaks, with no detectable peaks from sulfur, nitrogen, or other elements associated with the

precursor salt or plant extract, indicating effective removal of residual precursor ions and loosely bound organic matter during washing. Quantification gave 71.4 wt% (38.6 at%) copper and 28.6 wt% (61.4 at%) oxygen. The atomic O:Cu ratio of ~1.6:1 is somewhat higher than the ideal 1:1 stoichiometry of CuO, commonly attributed to the larger quantification error of the light O K line, combined with surface-adsorbed oxygen, hydroxylated surface layers, and residual oxygen-containing functional groups from the capping phytoconstituents identified by FTIR (Section 3.1). The elemental overlay map shows Cu L signal distributed fairly uniformly across the scanned area, consistent with a continuous CuO product, with one localized region of concentrated O K signal that may correspond to a residual plant-biomolecule-rich domain or a locally oxygen-enriched surface feature. CuO–*Aloe vera* exhibited concentration-dependent inhibition of α -glucosidase, rising from negligible activity at 1 μ g/mL to 76.44% inhibition at 1000 μ g/mL (Table 5, Figure 6), with an IC₅₀ of 47.86 $\pm$ 0.12 μ g/mL.

Table 5. Dose-dependent α -glucosidase inhibition by *Aloe vera*-mediated CuO nanoparticles.

Concentration (μ g/mL)	% Inhibition wrt control (mean)	SEM
0	0.00	$\pm$ 0.38
1	6.58	$\pm$ 1.51
10	20.00	$\pm$ 0.35
50	56.53	$\pm$ 1.04
100	70.32	$\pm$ 0.84
250	73.42	$\pm$ 0.48
500	74.79	$\pm$ 0.38
1000	76.44	$\pm$ 0.53

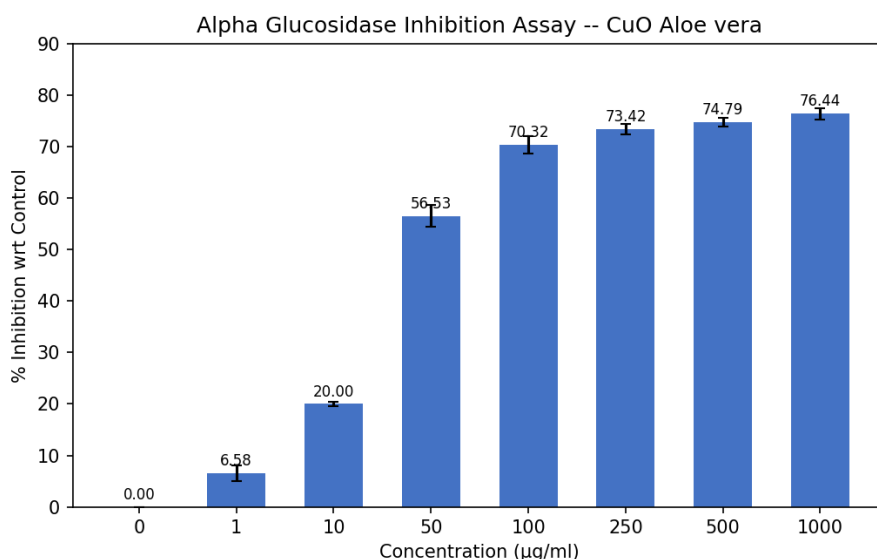


Figure 6. Dose-response curve of α -glucosidase inhibition by *Aloe vera*-mediated CuO nanoparticles.

Table 6. IC₅₀ comparison between *Aloe vera*-mediated CuO nanoparticles and the acarbose standard.

Sample	IC ₅₀ (µg/mL), Mean $\pm$ SEM
Acarbose (standard)	551.1 $\pm$ 0.03
CuO – <i>Aloe vera</i> (test sample)	47.86 $\pm$ 0.12

The IC₅₀ for CuO–*Aloe vera* is approximately 11.5-fold lower than that of acarbose obtained in the same assay, indicating that the biosynthesized nanoparticles are considerably more potent, on a mass basis, at inhibiting α -glucosidase in vitro than the standard antidiabetic drug. This activity is consistent with reports linking *Aloe vera* phytoconstituents and the CuO surfaces they cap to enzyme-inhibitory behaviour, and supports a potential

antidiabetic application for this green-synthesized nanomaterial, pending further mechanistic and in vivo validation. Unlike its pronounced α -glucosidase inhibitory activity, CuO–*Aloe vera* showed only weak, shallow dose-dependent DPPH radical scavenging (Table 7, Figure 7), reaching just 10.91% inhibition at the highest concentration tested (1000 µg/mL) far below the 50% threshold, so an IC₅₀ could not be calculated within the tested range.

Table 7. Dose-dependent DPPH radical scavenging by *Aloe vera*-mediated CuO nanoparticles.

Concentration (µg/mL)	% Inhibition wrt control (mean)	SEM
0	0.00	—
1	0.59	$\pm$ 0.15
10	2.50	$\pm$ 0.30
50	3.20	$\pm$ 0.30
100	5.66	$\pm$ 0.40
250	7.99	$\pm$ 0.70
500	9.20	$\pm$ 0.30
1000	10.91	$\pm$ 0.30

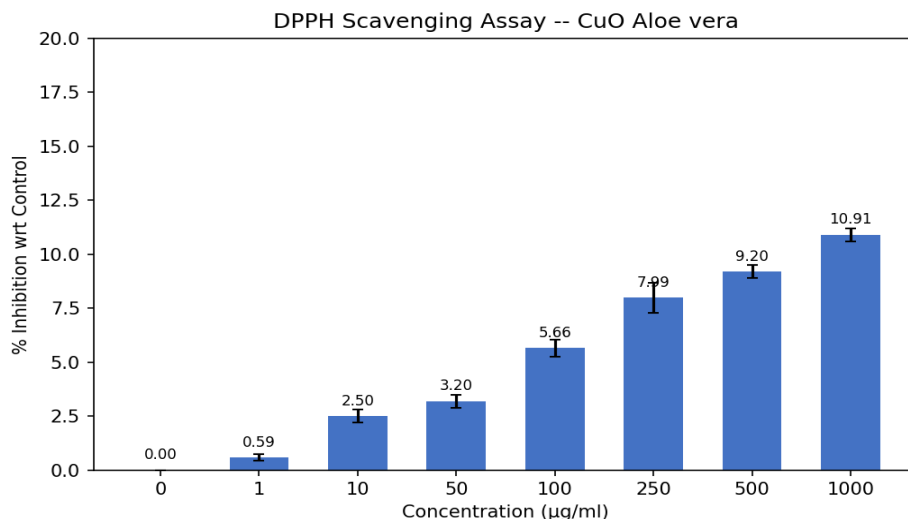


Figure 7. Dose-response curve of DPPH radical scavenging by *Aloe vera*-mediated CuO nanoparticles.

Table 8. IC₅₀ comparison between *Aloe vera*-mediated CuO nanoparticles and the ascorbic acid standard (DPPH assay).

Sample	IC ₅₀ (µg/mL), Mean ± SEM
Ascorbic acid (standard)	19.97 ± 0.02
CuO – <i>Aloe vera</i> (test sample)	Above maximum dose tested (>1000)

By comparison, the ascorbic acid standard reached an IC₅₀ of 19.97 ± 0.02 µg/mL. This contrast suggests that, in this preparation, the *Aloe vera*-derived phytoconstituents capping the CuO nanoparticles retain limited free radical-scavenging capacity, possibly because the calcination step used during synthesis (Section 2.5) removes or degrades much of the labile antioxidant phytochemistry (e.g., polyphenols) originally present in the extract, even though residual organic functional groups were still detectable by FTIR (Section 3.1). This indicates that the antidiabetic and antioxidant activities of this nanomaterial are not correlated and should be considered as separate, assay-specific

findings. The antibacterial potential of *Aloe vera*-mediated CuO nanoparticles was evaluated against four bacterial strains *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* using the Kirby–Bauer well diffusion method described in Section 2.9. Results for each organism are presented below. CuO–*Aloe vera* produced measurable, dose-dependent zones of inhibition against *E. coli*, rising from 8.33 mm at 312.5 µg to 13.67 mm at 5000 µg, and was judged effective at all tested amounts down to 312.5 µg (Table 9, Figure 8). No inhibition was observed at the DMSO vehicle-control well.

Table 9. Dose-dependent zone of inhibition against *E. coli* by *Aloe vera*-mediated CuO nanoparticles.

Amount (µg)	Zone of inhibition (mm), mean	SEM
0	0.00	—
312.5	8.33	±4.18
625	12.33	±0.33
1250	12.67	±0.33
2500	13.00	±0.58
5000	13.67	±0.33

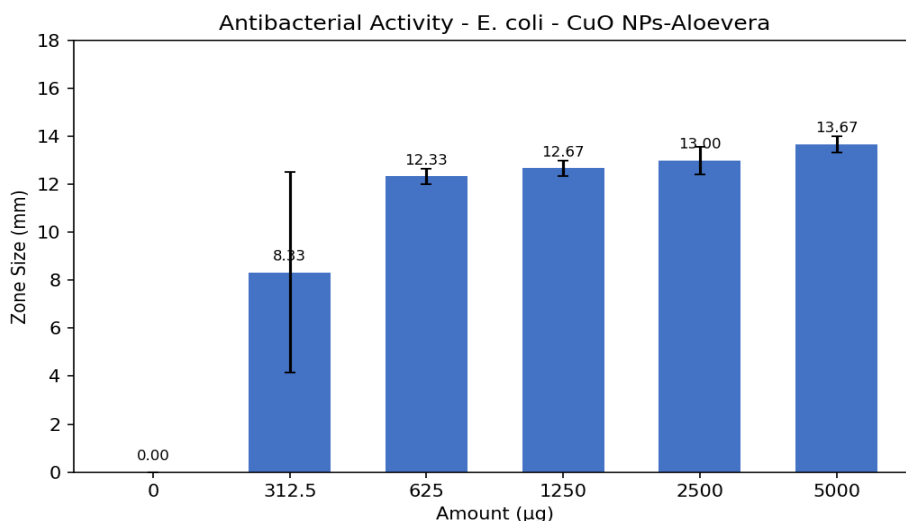


Figure 8. Dose-dependent zone of inhibition against *E. coli* by *Aloe vera*-mediated CuO nanoparticles.



Figure 9. Representative agar well diffusion plate: CuO NPs–*Aloe vera* against *S. aureus* (Ref. 25F62H, Plate C).

The zone produced by Ciprofloxacin (3 µg) which acted as a positive control, was 31 mm. CuO–*Aloe vera* zones were consequently, for the most part also smaller compared to the standard, implying mild antimicrobial efficacy against the Gram-negative microbe, with approximately 100 times as much CuO–*Aloe vera* needed to match even one half of the zone's size of the standard. Again, in parallel comparison, at the same effective loading (312.5 µg), CuO formed by Tulsi extract showed the largest zone of inhibition (12 mm) as compared to *Aloe vera* (8 mm) and Neem (4 mm) extracts suggesting that different capping plant extracts used in green synthesis definitely affect the antibacterial activity of CuO nanoparticles, which is probably attributed to the difference in sizes and surface phytochemical functionalization of the nanoparticles. A

mechanism of action of *E. coli* inhibitory effect of Gram-negative bacteria caused by the formation of ROS, release of Cu^{2+} ions affecting membrane transport and enzymatic function, and the direct disruption of the bacterial cell envelope have been proposed as the inhibitory mechanism of CuO NPs against Gram-negative bacteria. In the case of *S. aureus*, no zone of inhibition was observed at concentrations of up to 2500 µg/mL of CuO–*Aloe vera*, whereas a mean zone of inhibition of 16.33 ± 0.58 mm (individual replicates 16, 16 and 17 mm) was observed for the highest tested concentration of 5000 µg/mL (Table 10). Vehicle (DMSO alone) did not give any zone at any concentration indicating that the activity noted was because of the sample containing nanoparticles.

Table 10. Zone of inhibition (mm) produced by CuO NPs–*Aloe vera* against *S. aureus* across the tested dose range.

Amount (µg/mL)	Zone of inhibition (mm), mean $\pm$ SD	SEM
0	0.00 $\pm$ 0.00	0.00
312.5	0.00 $\pm$ 0.00	0.00
625	0.00 $\pm$ 0.00	0.00

1250	0.00 ± 0.00	0.00
2500	0.00 ± 0.00	0.00
5000	16.33 ± 0.58	0.33

The positive control, ciprofloxacin (3 µg), produced a substantially larger zone (24.0–25.33 mm across the plate set). This threshold-type response no activity until the highest dose contrasts with the more graded, dose-dependent response seen against *E. coli*, suggesting that *S. aureus*, a Gram-positive organism with a thicker peptidoglycan cell wall, may be comparatively less susceptible to nanoparticle/ion penetration at lower

loadings than the Gram-negative *E. coli*. CuO–*Aloe vera* inhibited the growth of *B. subtilis* only at the highest loading tested, 10000 µg/well, producing a mean zone of inhibition of 11.67 ± 0.58 mm (Table 11, Figure 10). No inhibition was observed at 625, 1250, 2500 or 5000 µg/well, nor at the DMSO vehicle-control well, across any of the three replicate plates.

Table 11. Zone of inhibition (mm) produced by CuO NPs–*Aloe vera* against *B. subtilis* across the tested dose range.

Amount (µg/well)	Zone of inhibition (mm), mean ± SD	SEM
PC (Ciprofloxacin, 3 µg)	25.00 ± 1.73	1.00
0 (DMSO vehicle)	0.00 ± 0.00	0.00
625	0.00 ± 0.00	0.00
1250	0.00 ± 0.00	0.00
2500	0.00 ± 0.00	0.00
5000	0.00 ± 0.00	0.00
10000	11.67 ± 0.58	0.33

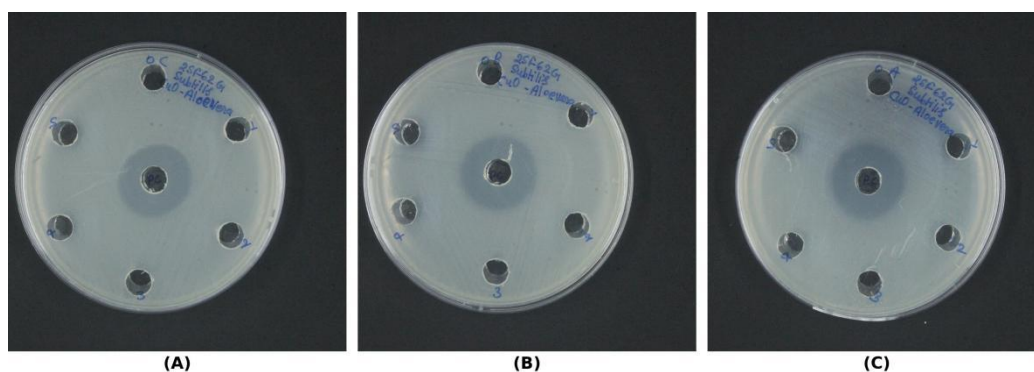


Figure 10. Representative well-diffusion plates of CuO NPs–*Aloe vera* against *B. subtilis* (replicates A–C).

This places CuO NPs–*Aloe vera* among only two formulations (the other being NiO NPs–Neem, 12 mm at 10000 µg) in the wider nine-sample screen (which also included Fe₂O₃ and NiO nanoparticles synthesized with Neem, Tulsi and *Aloe vera* extracts) to show any measurable antibacterial activity against *B. subtilis*; the remaining seven formulations showed no activity up to 10000 µg. Unlike the activity profile against *E. coli*, CuO–*Aloe vera* required more than 30-fold the effective concentration recorded against *E. coli* to show any effect on *B. subtilis*, and even then, produced only a modest zone well below the positive control (~25–26 mm at 3 µg). This threshold-like response, rather than a graded dose–

response, suggests *B. subtilis* is comparatively less susceptible to this nanoparticle preparation, potentially reflecting differences in cell-wall architecture and intrinsic resistance mechanisms. CuO–*Aloe vera* inhibited the growth of *P. aeruginosa* in a dose-dependent manner above a threshold loading (Table 12, Figure 11). No inhibition was observed at 0 (DMSO), 312.5, 625 or 1250 µg/well across any replicate. A zone first became apparent at 2500 µg/well, though only in two of the three replicate plates (Plate A: 0 mm, Plates B and C: 10 mm each; mean 6.67 ± 5.77 mm), and inhibition was consistent across all three replicates at 5000 µg/well (11, 11 and 12 mm; mean 11.33 ± 0.58 mm).

Table 12. Zone of inhibition (mm) produced by CuO NPs–*Aloe vera* against *P. aeruginosa* across the tested dose range.

Amount (µg/well)	Zone of inhibition (mm), mean ± SD	SEM
PC (Ciprofloxacin, 3 µg)	26.33 ± 1.53	0.88

0 (DMSO vehicle)	0.00 ± 0.00	0.00
312.5	0.00 ± 0.00	0.00
625	0.00 ± 0.00	0.00
1250	0.00 ± 0.00	0.00
2500	6.67 ± 5.77	3.33
5000	11.33 ± 0.58	0.33

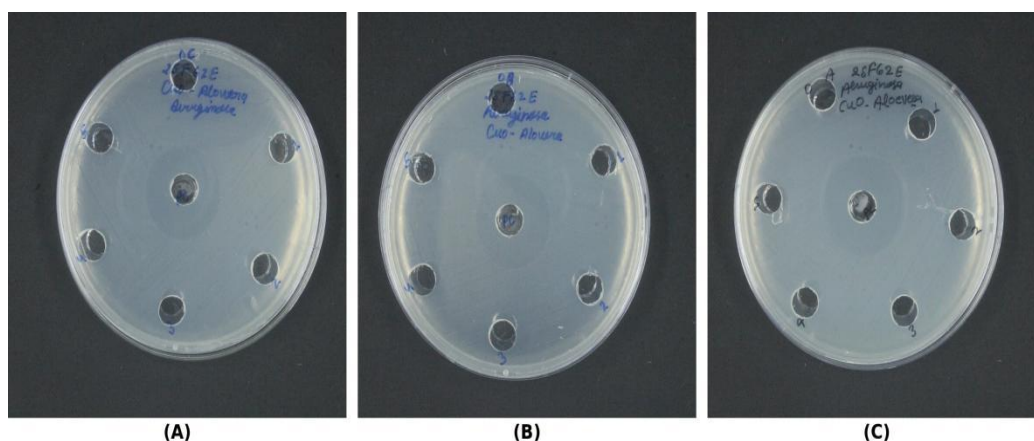


Figure 11. Representative well-diffusion plates of CuO NPs–*Aloe vera* against *P. aeruginosa* (replicates A–C).

One formulation (CuO NPs–*Aloe vera*) did exhibit any antibacterial activity against *P. aeruginosa* for the broader total of nine formulations, with NiO NPs–Neem as the other; the rest of the formulations showed no activity up to 5000 µg. Examining the *P. aeruginosa* data, the threshold response characteristic seen against *B. subtilis* appears not to be apparent against this organism, but rather a dose–response, particularly at the 2500 µg level (which is where a partial/variable response is shown, albeit with a large SD at this dose, possibly suggesting that exposure was near the minimum inhibitory threshold for *P. aeruginosa*). There was no difference between the zero and the zone dose, with both scores well below the dose of 3 µg in the positive control. The resistance of *P. aeruginosa* to a number of antimicrobial agents is probably more their intrinsic nature compared to that of other Gram-negative bacteria, because of its low outer-membrane permeability and low level "constitutive" expression of efflux pumps, as well as potential for forming biofilms that require increased loadings of CuO compared to *E. coli*.

The antibacterial activity of CuO NPs–*Aloe vera* in all four bacteria strains were summarized in Table 13. They

were highly active against *E. coli* (a quite resistant gram-negative) with activity as low as ~312.5 µg, moderately active against *P. aeruginosa* (intrinsically more resistant gram-negative) with activity as low as ~2500–5000 µg and only began to be active against *S. aureus* or *B. subtilis* with increasing loadings (activity at ~5000 µg or only at ~10000 µg, respectively). This result is in general agreement with other findings of the antibacterial mechanisms mediated by nanoparticles and Cu²⁺ being more effective against Gram-negative bacteria (less peptidoglycan layer, outer membrane more permeable) than Gram-positive bacteria, but somewhat counterintuitive because *P. aeruginosa* is more resistant in the Gram-negative group (than *E. coli*), probably due to the low count of *P. aeruginosa* in the sample and the presence of other resistance mechanisms related to efflux pumps and biofilm. In all cases the zone of inhibition produced by CuO NPs–*Aloe vera* is significantly smaller than that of the ciprofloxacin positive control (24–31 mm at 3 µg) suggesting that its antibacterial potency is modest compared with a clinical antibiotic and would require further optimization, such as dose, particle size, and surface functionalization before any practical microbial use could be considered

Table 13. Comparative summary of CuO NPs–*Aloe vera* antibacterial activity against four bacterial strains.

Organism	Gram type	Lowest effective conc.	Max. zone recorded (mm)	PC zone (mm)
<i>E. coli</i>	Negative	312.5 µg	13.67 (at 5000 µg)	31.0
<i>P. aeruginosa</i>	Negative	2500–5000 µg	11.33 (at 5000 µg)	26.3
<i>S. aureus</i>	Positive	5000 µg	16.33 (at 5000 µg)	24.0–25.3
<i>B. subtilis</i>	Positive	10000 µg	11.67 (at 10000 µg)	25.0

The results are combined and most clearly indicate the decreasing biomedical potential of this well characterized CuO–*Aloe vera* nanomaterial based on the three types of bioactivities published. The most promising target for the investigated material was the application as an α -glucosidase inhibitor, as the nanoparticles were found to be most effective as a single drug, on a mass basis, with more than a factor of 10 efficacy against acarbose, which is clinically available. Bacterial activity was found in this compound, but both organism-dependent and in absolute terms, it was low when compared to ciprofloxacin which makes use of the compound a secondary tool rather than first choice. The antioxidant (DPPH) activity was the lowest among the three which was likely explained by the retention of labile phenolic/flavonoid antioxidants during the 400 °C calcination treatment, even though the FTIR results showed other functional groups were still present on the surface of the nanoparticles but were not part of the labile antioxidants. These results together validate the need to investigate the antidiabetic activity of *Aloe vera* mediated CuO nanoparticles further at the mechanistic level and in vivo, in addition to its supplemental, concentration and organism dependent antidiabetic effect and antibacterial properties. The present study demonstrated the successful green synthesis of copper oxide nanoparticles (CuO-NPs) using aqueous *Aloe vera* (*Aloe barbadensis* Mill.) leaf gel extract as a natural reducing, stabilizing and capping agent. The use of plant extracts for nanoparticle synthesis represents an environmentally friendly alternative to conventional chemical approaches because phytochemicals can participate in metal-ion reduction as well as stabilization of the resulting nanoparticles (Gemachu *et al.*, 2024, Amin F *et al.*, 2021, Millavithanachchi SS *et al.*, 2025., Priya M 2023, Assefa ET *et al.*, 2024). Previous studies have also demonstrated the successful synthesis of CuO-NPs using different plant extracts, including *Azadirachta indica*, *Aerva javanica*, *Tithonia diversifolia* and *Morinda citrifolia*, supporting the suitability of plant-derived biomolecules for the biosynthesis of CuO nanomaterials.

In the present study, FTIR analysis confirmed the characteristic Cu–O vibration along with several functional groups associated with phytoconstituents of *Aloe vera*. These functional groups may have participated in the reduction, stabilization and capping of the synthesized nanoparticles. Similar FTIR observations have been reported in plant-mediated CuO-NP synthesis, where oxygen-containing functional groups of plant metabolites have been associated with nanoparticle formation and surface stabilization. The XRD analysis confirmed the formation of a single-phase monoclinic tenorite CuO structure with an average crystallite size of approximately 11–12 nm. This nanoscale crystallite size is comparable with previously reported plant-mediated CuO-NPs, in which crystallite dimensions varied according to plant source and synthesis conditions. In particular, studies reported monoclinic CuO nanoparticles synthesized using neem leaf extract, while study. demonstrated that precursor concentration, temperature and reaction time substantially influence the crystallinity and morphology of green-

synthesized CuO-NPs (Gemachu *et al.*, 2024, Amin F *et al.*, 2021, Millavithanachchi SS *et al.*, 2025). SEM analysis in the present study revealed dense aggregates of quasi-spherical to polygonal nanoparticles with sub-100 nm primary features. Such morphological variation and aggregation are commonly observed in biologically synthesized nanoparticles and may be associated with interparticle interactions and the presence of organic molecules originating from the plant extract. The polygonal and spherical morphology observed in the present investigation is also consistent with previous observations of green-synthesized CuO-NPs. EDX analysis confirmed the presence of copper and oxygen as the principal elements, accounting for approximately 71.4 wt% Cu and 28.6 wt% O, respectively, with no detectable major elemental impurities. This elemental profile further supports the successful formation and relative purity of CuO-NPs and agrees with previous reports in which EDX confirmed Cu and O as the predominant constituents of biosynthesized CuO nanoparticles.

A particularly important finding of the present investigation was the pronounced α -glucosidase inhibitory activity of the synthesized CuO-NPs. The nanoparticles exhibited an IC₅₀ value of 47.86 ± 0.12 μ g/mL, whereas the standard drug acarbose showed an IC₅₀ of 551.1 ± 0.03 μ g/mL under identical assay conditions. α -Glucosidase is an important carbohydrate-hydrolysing enzyme involved in the final stage of carbohydrate digestion, and its inhibition can delay glucose release and potentially reduce postprandial hyperglycaemia. Previous investigations have demonstrated significant α -glucosidase inhibitory activity in medicinal plant extracts and their phytochemical fractions, emphasizing the importance of plant-derived bioactive constituents in carbohydrate-metabolizing enzyme inhibition (Telagari *et al.*, 2015, Abubakar A *et al.*, 2020). The substantially lower IC₅₀ observed for the present CuO-NPs compared with acarbose indicates strong inhibitory activity under the experimental conditions employed. Nevertheless, this finding should be interpreted carefully because the observed activity may involve interactions between the nanoparticle surface, enzyme molecules and phytochemicals originating from *Aloe vera*. Surface-associated biomolecules may contribute to enzyme inhibition either independently or synergistically with the CuO nanostructure. Therefore, additional enzyme-kinetic and mechanistic investigations would be valuable to determine the precise mechanism underlying the observed α -glucosidase inhibition.

In contrast to the strong α -glucosidase inhibitory activity, the synthesized CuO-NPs exhibited comparatively weak antioxidant activity in the DPPH radical-scavenging assay. The maximum radical-scavenging activity observed was only approximately 10.91% at 1000 μ g/mL, and consequently the IC₅₀ exceeded the maximum concentration tested. This observation demonstrates that the biological activity of the synthesized CuO-NPs was assay-dependent and that strong α -glucosidase inhibition was not accompanied by comparable free-radical-scavenging capacity. Previous studies have shown that antioxidant

activity varies considerably according to plant species, extraction solvent, concentration and phytochemical composition (Abubakar A *et al.*, 2020., Imam MZ *et al.*, 2011). Christenson JC *et al.*, 2018. The weak DPPH activity observed in the present study may therefore be related to the relatively low availability of redox-active phytochemicals on the nanoparticle surface or to the intrinsic chemical properties of CuO-NPs. Thus, the present CuO-NPs appear to possess substantially greater α -glucosidase inhibitory potential than direct DPPH radical-scavenging activity. The antibacterial assay further demonstrated a concentration- and organism-dependent antimicrobial response. *Escherichia coli* and *Bacillus subtilis* exhibited measurable susceptibility at several of the tested concentrations. For *E. coli*, inhibition increased from approximately 8.33 mm at 312.5 $\mu\text{g/mL}$ to 13.67 mm at 5000 $\mu\text{g/mL}$, whereas *B. subtilis* exhibited an inhibition zone of approximately 11.67 mm at 5000 $\mu\text{g/mL}$. In contrast, *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed detectable inhibition predominantly at higher nanoparticle concentrations, with inhibition zones of approximately 16.33 mm at 5000 $\mu\text{g/mL}$ and 11.33 mm at 10,000 $\mu\text{g/mL}$, respectively. The concentration-dependent antibacterial activity observed in the present study is consistent with previous reports describing antimicrobial properties of green-synthesized CuO-NPs against both Gram-positive and Gram-negative bacteria.

The present findings also indicate that the antibacterial activity of the synthesized CuO-NPs was considerably lower than that of ciprofloxacin, which produced inhibition zones of approximately 24–31 mm at 3 μg . Therefore, although the nanoparticles demonstrated measurable antibacterial activity, their efficacy under the present agar-diffusion conditions was lower than that of the conventional antibiotic. Similar comparisons are important because antimicrobial susceptibility measurements can be affected by inoculum density, diffusion characteristics, concentration and experimental conditions. The agar well-diffusion/Kirby–Bauer approach used in the present study is a widely employed method for preliminary antimicrobial screening. Standardization of inoculum density using McFarland standards is also important for obtaining reproducible susceptibility results. Furthermore, laboratory diagnosis and interpretation of antimicrobial susceptibility should consider the organism, antimicrobial concentration and assay conditions. In the case of nanoparticles, agar diffusion may additionally be influenced by particle aggregation and restricted movement through the agar matrix; therefore, inhibition-zone measurements should preferably be complemented by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) studies. Overall, the present findings demonstrate that *Aloe vera*-mediated CuO-NPs possess a multifunctional biological profile, with particularly promising α -glucosidase inhibitory activity, moderate concentration-dependent antibacterial activity and comparatively weak DPPH radical-scavenging activity. However, the comparatively weak antioxidant activity indicates that the principal biological advantage of the

present nanoparticles may be their enzyme inhibitory and antimicrobial properties rather than their direct radical-scavenging capacity. Further studies involving MIC/MBC determination, enzyme-kinetic analysis, cytotoxicity evaluation, mechanistic investigations and in vivo validation are recommended before considering biomedical applications. Taken together, the findings suggest that *Aloe vera*-mediated CuO-NPs represent a promising, low-cost and environmentally benign nanomaterial with potential applications particularly in α -glucosidase inhibition and antimicrobial formulations.

CONCLUSION

Here, a well-known plant extract *Aloe vera* leaf extract prepared from aqueous medium was employed to reduce and cap the cuprate nanocrystals and copper sulphate pentahydrate and ammonia were utilized as metal salt and pH adjusting agent, respectively, in the successful preparation of CuO nanoparticles. FTIR analysis showed the presence of the characteristic Cu–O stretching vibrations of the CuO lattice along with the functional groups of polyphenols and polysaccharides from *Aloe vera* confirming that it acts as a bioreductant and as well as it acts as capping agent. To confirm the formation of a single phase, nanocrystalline CuO (tenorite) free from detectable impurity phases, XRD analysis was carried out and confirmed that. The average crystallite size of this novel formed CuO was about 11–12 nm. The elemental composition determined by EDX spectroscopy and the quasi-spherical to polygonal primary particles with sizes of less than 100 nm observed in SEM images confirmed that both the nanoparticles consist of pure CuO. The CuO nanoparticles synthesized in-vitro using *Aloe vera* extract were found to have significant potential as an antidiabetic agent with an IC₅₀ of $47.86 \pm 0.12 \mu\text{g/mL}$ which is much less than IC₅₀ of acarbose (standard drug) ($551.1 \pm 0.03 \mu\text{g/mL}$) with this support the further evaluation of this synthesized green nanomaterial is recommended as an antidiabetic agent.

However, DPPH radical-scavenging activities showed that the same sample exhibited low radical-scavenging activity. Modest, organism-dependent antibacterial activity was demonstrated using four bacterial strains in agar well diffusion assays, most pronounced and evident in the lowest concentrations against the Gram-negative *E. coli*, and substantially higher loadings needed to demonstrate an effect for the Gram-positive strains *S. aureus* and *B. subtilis*: The zones against all four were significantly less than the ciprofloxacin standard. In general, the findings show that the antimicrobial activity is a concentration and organism-dependent property that can be considered as a secondary effect of this green synthesized nanomaterial, the α -glucosidase inhibitory and antidiabetic activity of this nanomaterial points to its main biomedical strength, while antioxidant activity is a minor effect.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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