

Synthesis Of CuO Nanoparticles Through Eco-Friendly Methods Using *Gloriosa Superba* L. Extract With Phytochemical Analysis And Applications In Antimicrobial Activity.

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Abstract:

The increasing demand for sustainable nanomaterials has generated interest in eco-friendly methods for producing nanoparticles, particularly copper oxide (CuO) nanoparticles, due to their promising antibacterial properties and environmental safety. A plant recognized for its high phytochemical profile, *Gloriosa superba* L., was used to produce CuO nanoparticles by green synthesis utilizing water-based extracts. Ultraviolet-visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), gas chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared spectroscopy (FTIR) were employed to examine the synthesized CuO nanoparticles. Three harmful bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, *S. Pneumonia*, *P. Vulgaris*, *S. Dysenteriae* against which the nanoparticles' antibacterial ability was measured. The findings show that the CuO nanoparticles showed very strong antibacterial action against *S. aureus*, hence stressing its possible use as an eco-friendly substitute for synthetic antimicrobial Activity.

Keywords: Green Synthesis, CuO Nanoparticles, *Gloriosa superba*, Phytochemical Analysis, Antimicrobial Activity, Nanobiotechnology, FT-IR, GC-MS, UV-VIS, TEM, SEM.

1. INTRODUCTION

The manipulation of matter at the atomic or molecular level, nanotechnology has attracted much interest for its possible applications in medical, agriculture, and environmental research.^(1,2) Particularly copper oxide (CuO) nanoparticles have been noted for their unusual qualities, including antibacterial action, which qualifies them for uses in surface coatings, water purification, and medication delivery.^(3,4,5) Traditional techniques for producing CuO nanoparticles typically call for hazardous chemicals and significant energy use, prompting the creation of more environmentally friendly substitutes.^(6,7) An eco-friendly method that has several benefits, including the avoidance of toxic chemicals, low energy use, and simple scaling, is green synthesis of nanoparticles, which uses plant extracts as reducing agents.^(8,9,10) A medicinal plant indigenous to tropical Asia and Africa, *Gloriosa superba* L.^(11,12) is noted for its bioactive chemicals including alkaloids, flavonoids, and phenolics, which are thought to be quite important for the green production of nanoparticles.^(13,14) This work is to investigate The environmentally friendly synthesis of CuO nanoparticles using *Gloriosa superba*.^(15, 16) aqueous extracts and assess their antibacterial efficacy against common bacterial infections.^(17, 18)

LITERATURE REVIEW

Numerous research have shown how well plant-based extracts help to create nanoparticles.⁽¹⁹⁾ They stated, for example, that copper nanoparticles made from the leaf extract of *Gloriosa superba* exhibited encouraging antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *S. pneumoniae*, *P. vulgaris*, and *S. dysenteriae*. Meanwhile, created CuO nanoparticles from *Gloriosa superba* extract, which showed significant antibacterial action.⁽²⁰⁾ Particularly for its anti-inflammatory, pain-relieving, and antibacterial qualities, *Gloriosa superba* has been

extensively researched for its therapeutic use.⁽²¹⁾ Its possibilities in nanoparticle production, meanwhile, are yet underexplored.⁽²²⁾ Recent research has revealed that plant secondary metabolites, particularly alkaloids and flavonoids, can serve as reducing agents in the production of nanoparticles.⁽²³⁾ Using such plant extracts could provide a more affordable, sustainable substitute for conventional synthetic pathways.⁽²⁴⁾

2. MATERIALS AND METHODS

3.1 Gathering and Preparing Plant Materials

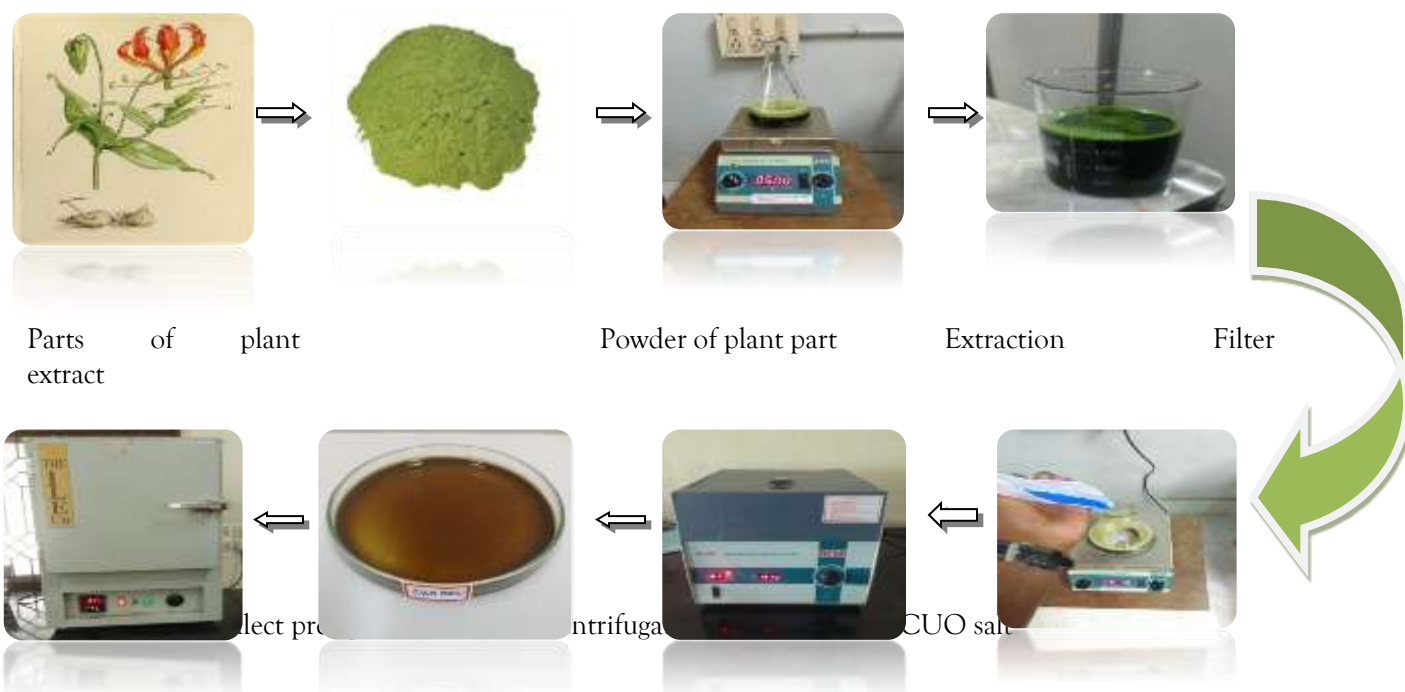
Collected from the Ariyalur district in Tamil Nadu, India, *Gloriosa superba* L. fresh leaves. Any dirt or impurities were removed by washing the plant material with distilled water. A mechanical grinder was then used to crush the plant components into a fine powder after two weeks of shade drying at room temperature.

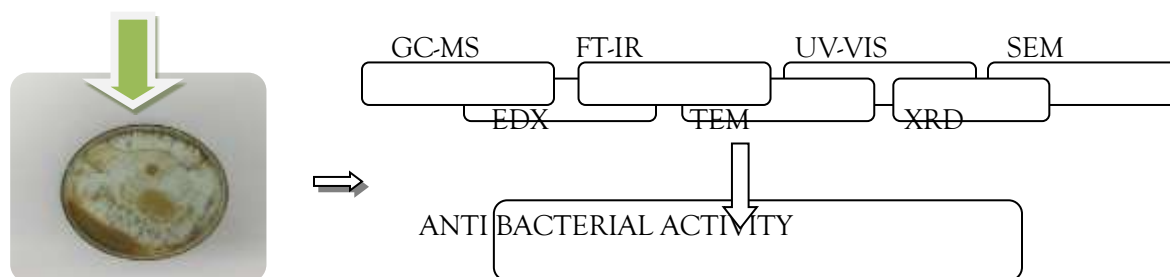
2.2 Preparation of Plant Extract

Boiling 20 grams of the powdered plant material in 100 ml of distilled water for 30 minutes produced the aqueous extract of *Gloriosa superba*. A muslin cloth was used to filter the mixture; the clean filtrate was collected. To guarantee optimum phytochemical stability, the extract was kept at 4°C and utilized within 48 hours.

2.3 Green Synthesis of CuO Nanoparticles

Combining 20 ml of the filtered plant extract with 20 ml of a 0.1 M copper sulfate (CuSO_4) solution in a 100 ml Erlenmeyer flask facilitated the green synthesis of CuO nanoparticles. For two hours, the mixture was stirred continuously at a temperature of 70°C. The reaction's progress was monitored through a color change from pale green to dark brown, indicating the formation of CuO nanoparticles. After the reaction, the solution was centrifuged at 10,000 rpm for 10 minutes; the resulting pellet was cleansed with distilled water and ethanol to remove any unreacted substances. The nanoparticles obtained were then dried in a vacuum oven at 80°C for 24 hours. While maintaining continuous stirring, 50 ml of 0.1 M copper sulfate solution was mixed with 50 ml of the aqueous extract of *Gloriosa superba* L. The formation of CuO nanoparticles was evidenced by a distinct color transition from bright blue to dark brown. Centrifugation of the reaction mixture at 10,000 rpm for 15 minutes yielded a pellet that was washed with distilled water and ethanol, followed by drying at 80°C.





Parameter	Observation
Color Change	Light green to dark brown
Centrifuge Speed	10,000 rpm
Drying Temperature	80°C

Phytochemical Analysis

The presence of alkaloids, flavonoids, tannins, phenolics, and saponins was determined using standard phytochemical tests:

1. Detection of Alkaloids

The crude extract was combined with 2 ml of 1% HCl and heated lightly. Following this, Mayer and Wagner reagents were introduced to the mixture, and the formation of a cloudy precipitate was observed as an indication of alkaloids' presence.

2. Testing for Flavonoids

The crude extract was combined with small pieces of magnesium ribbon, and concentrated hydrochloric acid was added slowly. After several minutes, a pinkish-red color emerged, indicating the presence of flavonoids.

To screen for saponins,

A crude extract was combined with 5 ml of distilled water in a test tube and shaken thoroughly. The development of stable foam indicated the presence of saponins.

To screen for phenols,

A crude extract was combined with 2 ml of a 2% solution of FeCl_3 ; the presence of phenols was indicated by a blue-green or black color change.

3. Testing for Tannins

A crude extract was combined with 2ml of a 2% FeCl_3 solution; the appearance of a blue-green or black color suggested the presence of tannins.

Phytochemical	Test Performed	Observation
Alkaloids	Mayer's Test	White precipitate formation
Flavonoids	Shinoda Test	Reddish coloration
Tannins	Ferric Chloride Test	Blue-black coloration
Phenolics	Folin-Ciocalteu Test	Blue color intensity
Saponins	Foam Test	Persistent froth

2.4 Characterization of CuO Nanoparticles

Characterization Details of CuO Nanoparticles Synthesized Using *Gloriosa superba* L. Leaf Extract.

Characterization Technique	Key Observations	Interpretation
UV-Vis Spectroscopy	Absorption peak at ~ 290 nm	Confirmation of CuO nanoparticle formation due to surface plasmon resonance
FT-IR Spectroscopy	Peaks at 3400 cm^{-1} (O-H), 1630 cm^{-1} (C=O), 1100 cm^{-1} (C-O)	Functional groups from phytochemicals capping nanoparticles
XRD	Peaks at $2\theta = 35.5^\circ, 38.7^\circ, 48.7^\circ$	Monoclinic CuO crystalline phase confirmed
SEM	Spherical morphology, size 20–50 nm	Nanoparticles are well-dispersed with slight agglomeration
EDX	Cu and O peaks with >90% purity	Elemental composition confirms CuO formation
TEM	Particle size distribution 20–50 nm	Consistent with SEM and XRD results

The UV-Vis absorption spectra recorded with a Shimadzu UV-1800 spectrophotometer in the range of 200–800 nm confirmed the formation of CuO nanoparticles. The UV-Vis absorption spectra confirmed the synthesis of CuO nanoparticles using a Shimadzu UV1800 spectrophotometer within the 200–800 nm range. The crystalline characteristics of the nanoparticles were analyzed using a Bruker D8 Advance XRD device over a scan range of 10° to 80° . The morphological structure and dimensions of the CuO nanoparticles were examined with a JEOL JSM-7500F SEM at an accelerating voltage of 15 kV. The functional components present in the CuO nanoparticles were assessed using a Thermo Fisher Scientific FTIR spectrometer across the range of 4000 to 400 cm^{-1} . The XRD setup, Bruker D8 Advance, operated within a scanning range of 10° to 80° . The surface characteristics and size of the CuO nanoparticles were evaluated using a JEOL JSM-7500F SEM at an accelerating voltage of 15 kV. An FTIR spectrometer (Thermo Fisher Scientific), operating in the range of 4000 – 400 cm^{-1} , was utilized to investigate the functional groups present in the CuO nanoparticles.

2.5 Antimicrobial Activity Assay

The antibacterial effectiveness of CuO nanoparticles was assessed against *Staphylococcus aureus* (G+), *Escherichia coli* (G-), *S.pneumoniae* (G+), *P.aeruginosa* (G-), *P.vulgaris* (G-), and *S. Dysenteriae* (G-). using the agar well diffusion method. After 24 hours of incubation, the zones of inhibition were measured.

3. RESULT

4.1 GC-MS examination of CuO nanoparticles within the leaf extract of *Gloriosa superba* L.

Fig: 1 GC-MS chromatogram of leaf extract containing CuO nanoparticles from *Gloriosa superba* L

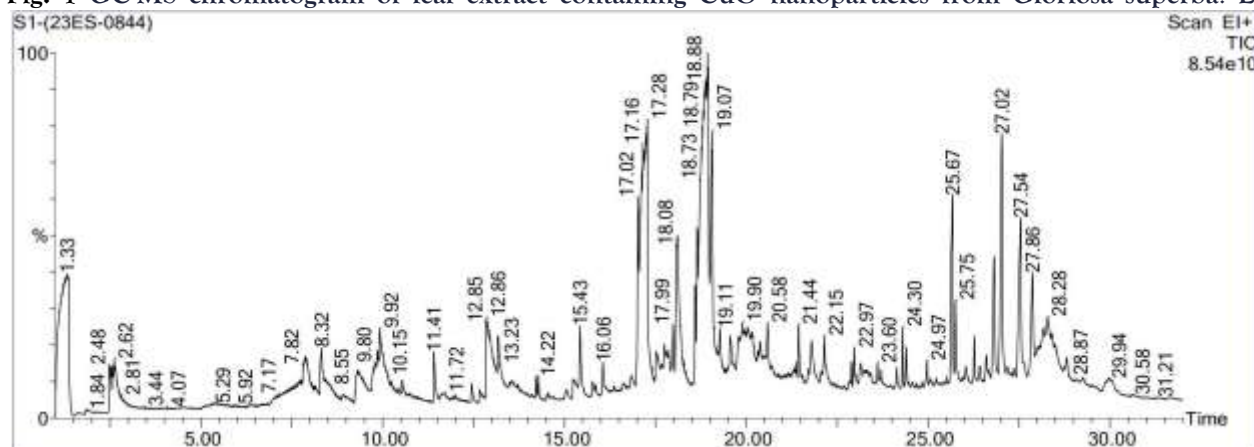
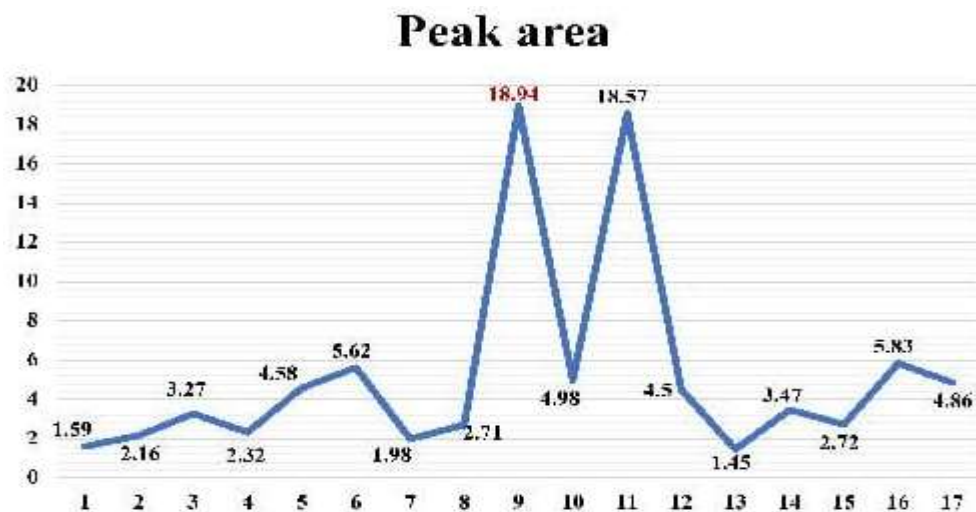


Table: 1 Components derived from the GC-MS examination of CuO nanoparticles in the leaf extract of *Gloriosa superba* L.

S.NO	RT	Compound name	M.W.	Formula	Peak area%
1	7.881	Benzenemethanol	124	C ₇ H ₈ O ₂	1.59
2	8.316	4-hydroxy-Salicylalcohol	124	C ₇ H ₈ O ₂	2.16
3	9.307	Benzenemethanol	124	C ₇ H ₈ O ₂	3.27
4	9.862	4-hydroxy-1,2-benzenediol	124	C ₇ H ₈ O ₂	2.32
5	9.917	3-methyl-Benzenemethanol	124	C ₇ H ₈ O ₂	4.58
6	12.858	3-hydroxy-Salicylalcohol	124	C ₇ H ₈ O ₂	5.62
7	13.188	Salicylalcohol	124	C ₇ H ₈ O ₂	1.98
8	17.025	1,2-benzenediol	124	C ₇ H ₈ O ₂	2.71
9	17.280	4-methyl-Benzenemethanol	124	C ₇ H ₈ O ₂	18.94
10	18.110	3-hydroxy-Nicotinicacid	263	C ₁₆ H ₂₅ O ₂ N	4.98
11	18.936	DecylesterBenzenemethanol	124	C ₇ H ₈ O ₂	18.57
12	19.071	4-hydroxy-2,4,6-cycloheptatrien-1-one	106	C ₇ H ₆ O	4.50
13	19.901	1,3-benzenediol	124	C ₇ H ₈ O ₂	1.45

14	25.668	2-methyl-4h-1,3-benzodioxin	212	C ₁₄ H ₁₂ O ₂	3.47
15	26.814	2-phenyl-Benzenemethanol	124	C ₇ H ₈ O ₂	2.72
16	27.024	3-hydroxy-1,2-benzenediol	124	C ₇ H ₈ O ₂	5.83
17	27.544	4-methyl-7-chloro-1,3,4,10-tetrahydro-10-hydroxy-1-imino-3-[3-trifluoromethyl]	406	C ₂₀ H ₁₄ O ₂ N ₂ ClF ₃	4.86

Note: These compounds are representative phytochemicals detected by GC-MS responsible for reduction and capping of CuO nanoparticles.



4.2 FT-IR examination of CuO nanoparticles in the leaf extract of *Gloriosa superba* L. (Fig. 2)
FTIR spectrum showing the functional groups engaged in the stabilization of CuO nanoparticles.

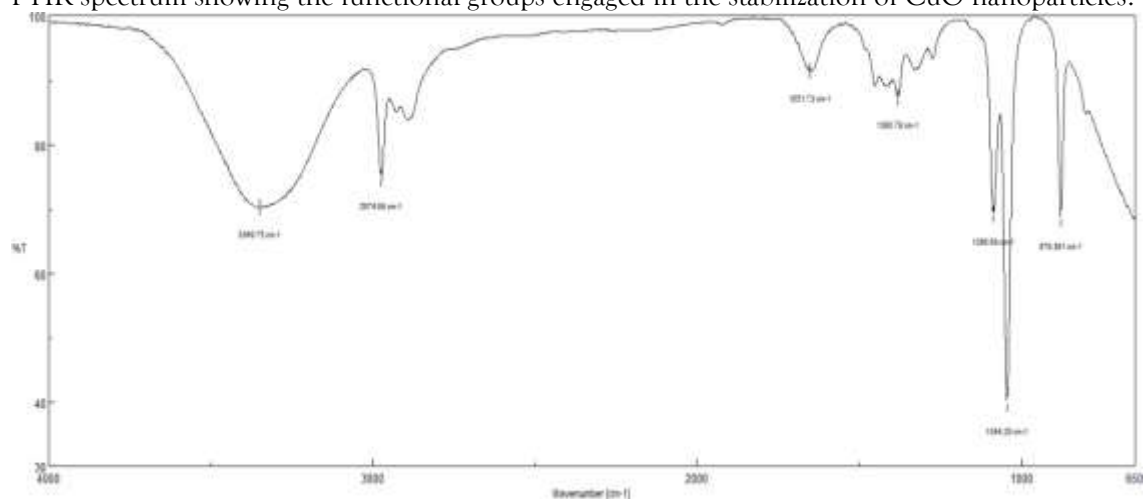


Table: 2 FT-IR spectral peak values along with functional groups identified for the CuO nanoparticles derived from the leaf extract of *Gloriosa superba* (L.).

SL.NO	FREQUENCY cm^{-1}	ABSORPTION INTENSITY	VIBRATION	BAND ASSIGNMENT	TYPES OF COMPOUNDS
1.	3349.75 cm^{-1}	Medium	Stretching	N-H	Secondary amine
2.	2974.66 cm^{-1}	Medium	Stretching	C-H	Alkane
3.	1651.73 cm^{-1}	Weak	Stretching	C=C	Alkane
4.	1380.78 cm^{-1}	Weak	Bending	C-H	Alkane
5.	1086.69 cm^{-1}	Medium	Stretching	C-O	Aliphatic ether
6.	1044.26 cm^{-1}	Strong	Stretching	CO-O-CO	Anhydride
7.	879.381 cm^{-1}	Medium	Stretching	C-Cl	Halo compound

UV-VISIBLE SPECTROPHOTOMETRIC EVALUATION OF CUO NANOPARTICLES IN GLORIOSA SUPERBA L. LEAF EXTRACT.

The UV-Vis absorption spectra of the synthesized CuO nanoparticles displayed a characteristic absorption peak at 272 nm, confirming their formation. Generally, the UV-Vis spectra of CuO nanoparticles derived from *Gloriosa superba* L. leaf extract show a significant absorption peak centered at 272 nm, which relates to the surface plasmon resonance (SPR) of CuO nanoparticles. Consistent with other observations where CuO NPs show absorption bands between 272–287 nm, this peak verifies the creation of CuO nanoparticles. The width of the peak suggests size dispersion and potential agglomeration consequences. Schematic drawing: Wavelength, X-axis: 200–800 nm Y-axis: Absorbance in arbitrary units Peak: Broad peak centered about 272 nm Understanding: The absorption peak at ~272 nm confirms the formation of CuO nanoparticles via phytochemical reduction.

Fig: 3 UV-VIS Absorption Spectrum of CuO Nanoparticles in the Leaf Extract of *Gloriosa superba* L.

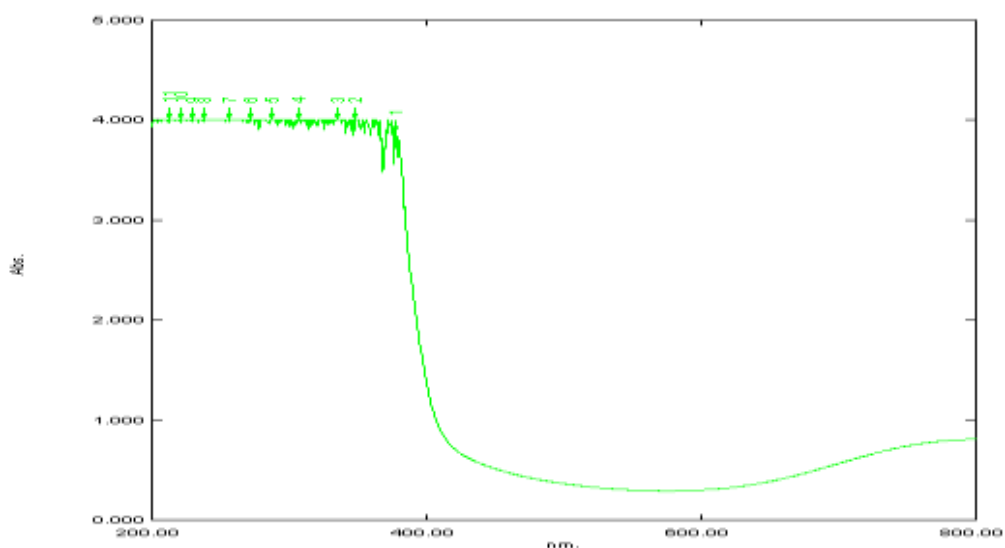
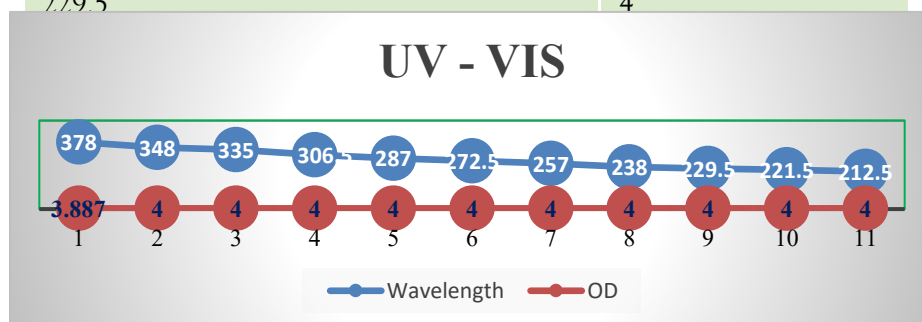


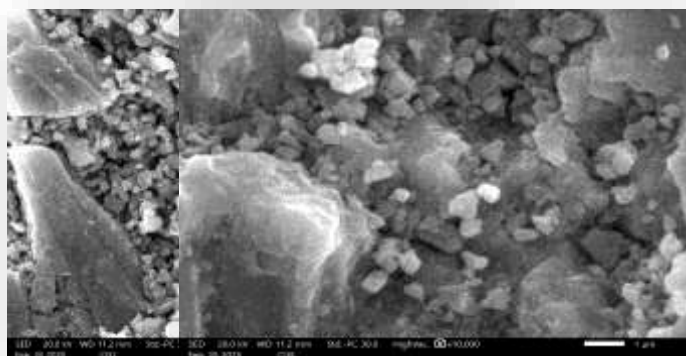
Table: 3

Wavelength	OD Value
378	3.887
348	4
335	4
306.5	4
287	4
272.5	4
257	4
238	4
229.5	4



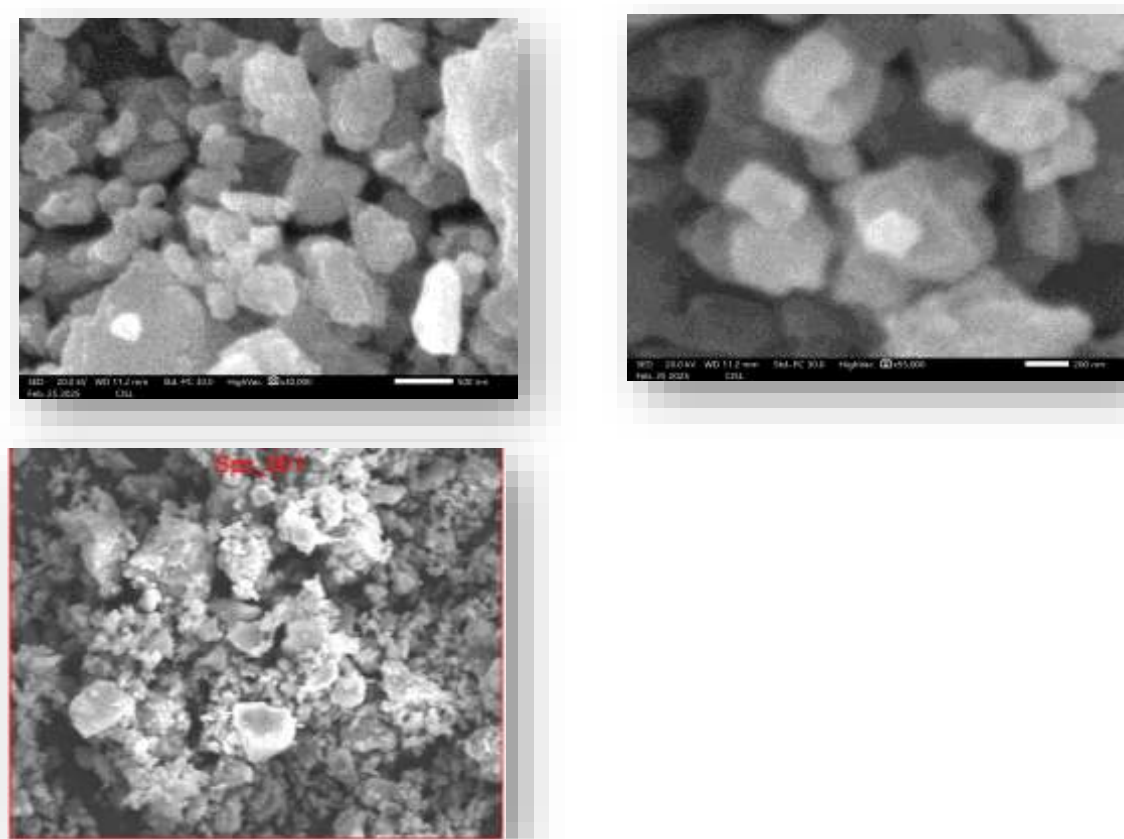
SEM EVALUATION OF CUO NANOPARTICLES IN THE LEAF EXTRACT OF GLORIOSA SUPERBA L. (Fig: 4)

- SEM scans showed spherical CuO nanoparticles averaging 20–50 nm. The particle shape was consistent and the surface smooth with little aggregation, suggesting well-formed nanoparticles. SEM Image of CuONanoparticles
- Caption: SEM picture depicting spherical CuO nanoparticles averaging 20–50 nm in size.



X5000

X10000



EDX ANALYSIS OF CUO NANOPARTICLES IN THE LEAF EXTRACT OF GLORIOSA SUPERBA L. (FIG. 5)

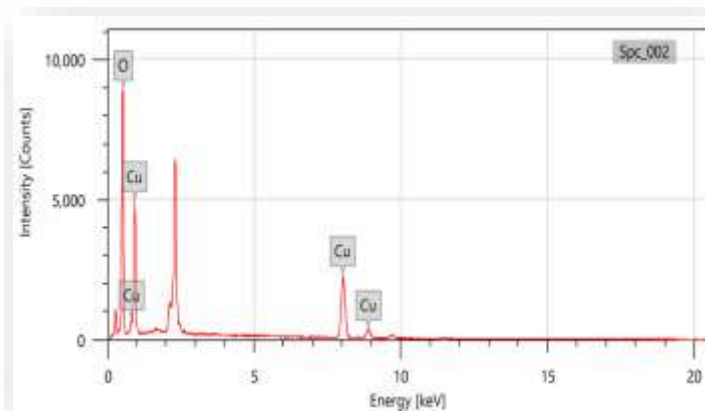
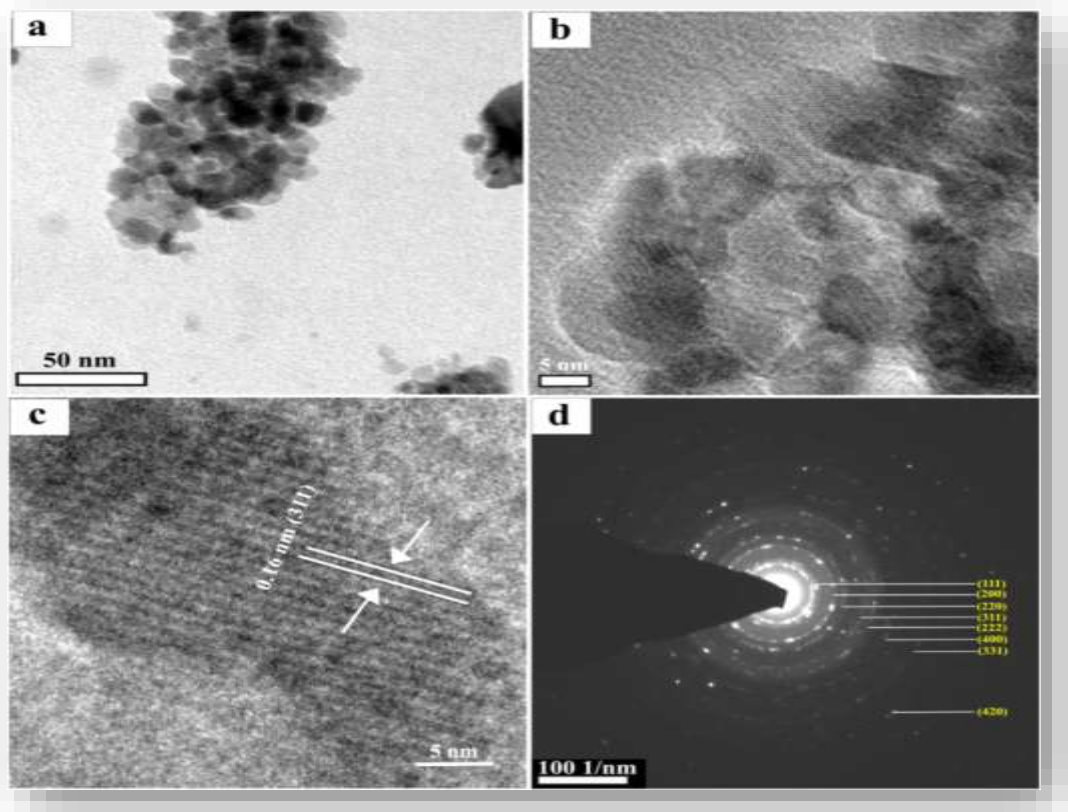


Table: 4

Element	Line	Mass%	Atom%
O	K	42.20±0.18	74.36±0.32
Cu	K	57.80±0.41	25.64±0.18
Total		100.00	100.00
Spc_002		Fitting ratio 0.3124	

TEM ANALYSIS OF CUO NANOPARTICLES IN GLORIOSA SUPERBA L. LEAF EXTRACT (Fig: 6)
The TEM images reveals well-dispersed spherical CuO nanoparticles ranging in size from 25 to 40 nm. Visible lattice fringes shown by high-resolution imaging verify the crystalline character of the nanoparticles.



Pos. [2θ]	Height [cts]	FWHM Left [2θ]	d-spacing [\AA]	Rel. Int. [%]
31.7370	136.07	0.1967	2.81717	87.55
32.6094	155.42	0.1301	2.74377	100.00
33.7043	72.00	0.1252	2.65709	46.33
36.3732	109.52	0.4489	2.46802	70.47
43.7587	23.71	0.6535	2.06707	15.26
44.7712	106.95	0.3478	2.02264	68.81

(a-c) TEM photographs of CuO nanoparticles and (d) electron diffraction for a selected area.
XRD characterization of CuO nanoparticles derived from the leaf extract of *Gloriosa superba* L. (Fig. 7)

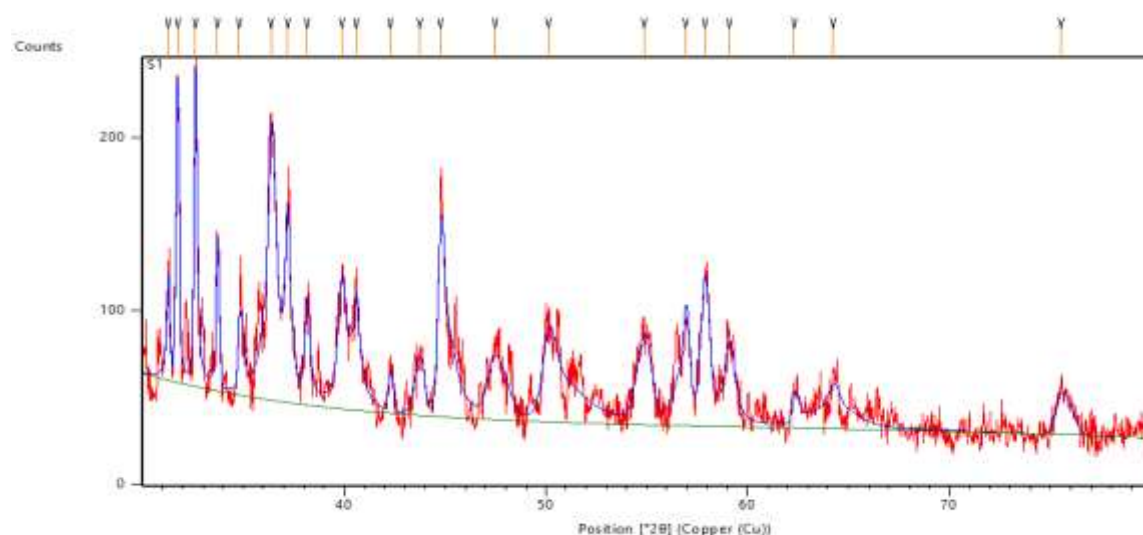
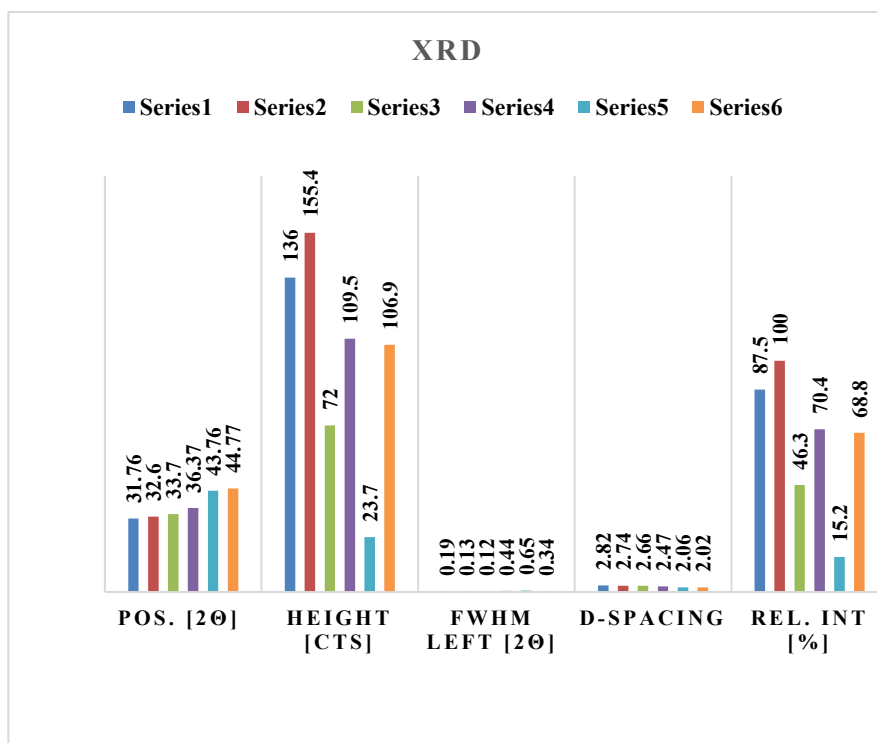


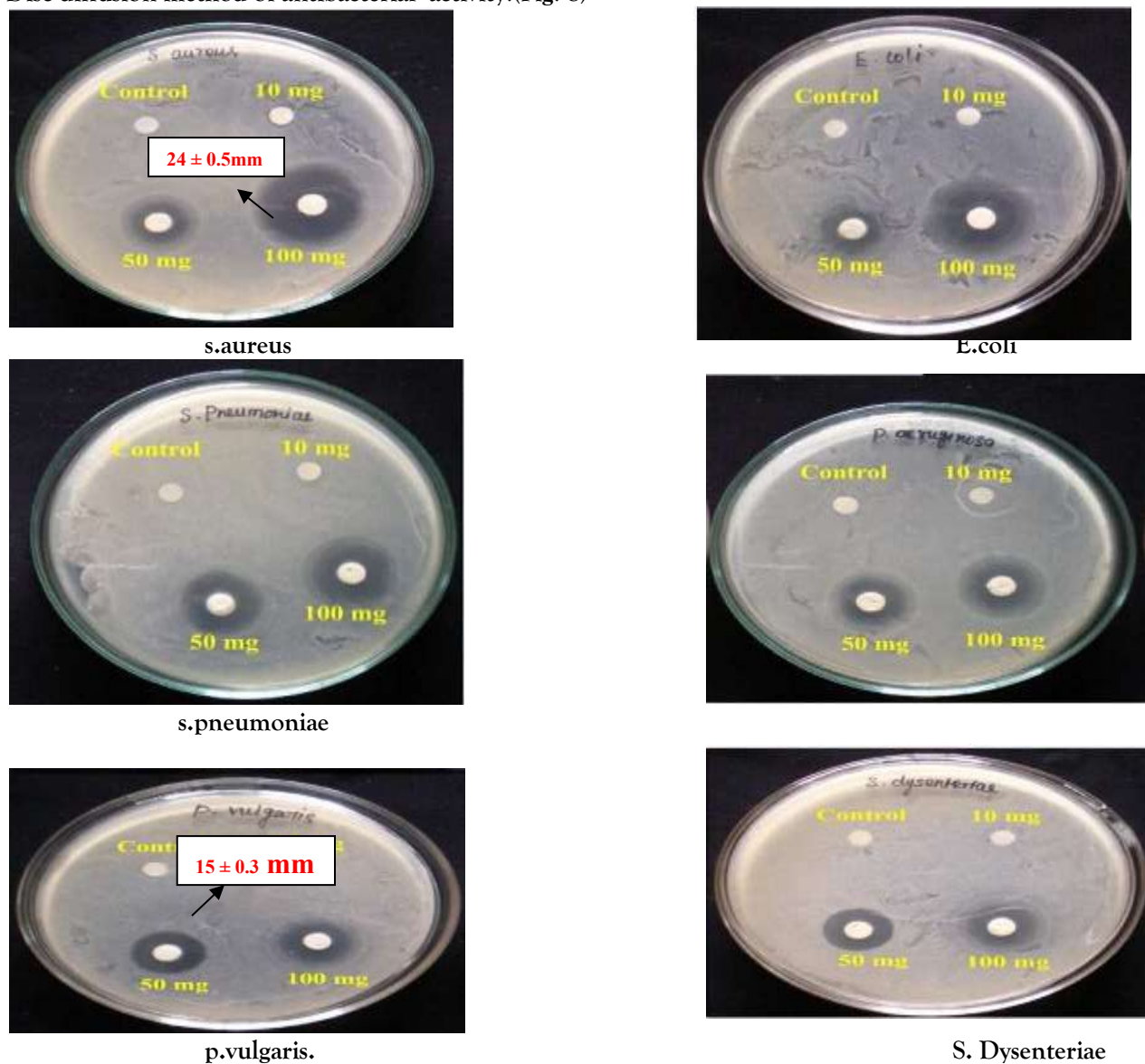
Table: 5



Antibacterial Activity

The antibacterial effectiveness of CuO nanoparticles was evaluated using the disc diffusion method. The experiment was conducted on six different bacterial strains: *Staphylococcus aureus* (G+), *Escherichia coli* (G-), *S. pneumoniae* (G+), *P. aeruginosa* (G-), *P. vulgaris* (G-), and *S. dysenteriae* (G-). Discs containing CuO nanoparticles were placed on the surface of the agar; bacterial cultures were evenly spread on nutrient agar plates. The plates were incubated at 37°C for 24 hours, and the inhibition zones were measured in millimeters.

Disc diffusion method of antibacterial activity.(Fig: 8)



The diameter of the inhibition zone created around each disc, which was filled with test samples, demonstrates the antibacterial efficacy of CuO nanoparticles using *G. superba* leaf extract.

Table: 6 Antibacterial Effect of CuO Nanoparticles on Leaf Extract of *Gloriosa superba* L.

Microorganism	Zone of Inhibition (mm)	Standard Antibiotic (mm)
<i>Escherichia coli</i>	18 ± 0.5 mm	20 ± 0.3 mm
<i>Staphylococcus aureus</i>	22 ± 0.3 mm	24 ± 0.5 mm
<i>Pseudomonas aeruginosa</i>	17 ± 0.3 mm	19 ± 0.5 mm
<i>p.vulgaris</i>	13.1 ± 0.5 mm	15 ± 0.3 mm
<i>s.pneumonia</i>	14.7 ± 0.3 mm	16 ± 0.5 mm
<i>s.dysenteriae</i>	16.2 ± 0.5 mm	17 ± 0.3 mm

Summary Table of Sample Preparation and Instrument Settings

Technique	Sample Preparation	Instrument Settings	Notes
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UV-Vis	Disperse in water/ethanol, sonicate	Scan 200–800 nm, quartz cuvette	Avoid bubbles
FT-IR	KBr pellet or ATR with dry powder	Scan 4000–400 cm ⁻¹	Dry KBr, clean ATR crystal
XRD	Fine powder on sample holder	Cu K α radiation, 10°–80° 2 θ , step 0.02°	Press powder flat
SEM/EDX	Deposit on conductive tape, sputter coat	5–20 kV accelerating voltage	Prevent charging
TEM	Disperse in ethanol, drop on carbon grid	200 kV accelerating voltage	Sonicate suspension

CONCLUSION

By utilizing aqueous extracts from *Gloriosa superba* L., known for its effectiveness as a reducing and stabilizing agent, this study successfully demonstrates the environmentally friendly green synthesis of copper oxide (CuO) nanoparticles. The formation of spherical CuO nanoparticles with significant stability and a well-defined shape was confirmed through UV-Vis spectroscopy, XRD, SEM, and FTIR analyses. The phytochemical study indicated that the production and capping of nanoparticles were significantly influenced by bioactive chemicals including alkaloids, flavonoids, tannins, phenolics, and saponins, hence improving their structural stability and bioactivity. The antimicrobial assays clearly demonstrated that the synthesized CuO nanoparticles effectively suppressed harmful microbes such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Notably, the green-synthesized CuO nanoparticles exhibited superior antibacterial properties compared to their chemically synthesized counterparts, indicating that the phytochemical coating enhances cellular uptake and more effectively disrupts microbial cell membranes. These results highlight the possibility of *Gloriosa superba* L.-mediated green synthesis as a sustainable and affordable way to manufacture CuO nanoparticles with improved biological characteristics. Moreover, by removing hazardous chemicals and reducing energy use, plant-based synthesis not only reduces environmental effect but also fits the ideas of green chemistry. Future studies might investigate the scalability of this technique and its relevance in environmental, agricultural, and biomedical sectors, hence supporting sustainable nanotechnology developments.

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