Green Synthesis of Cuo Nanoparticles Using Gloriosa Superba L. Extract by Phytochemical Analysis and Antimicrobial Applications

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Research Article

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Abstract

The growing need for sustainable nanomaterials has sparked interest in the green production of nanoparticles, especially copper oxide (CuO) nanoparticles, because of their encouraging antibacterial qualities and environmental friendliness. A plant recognized for its high phytochemical profile, Gloriosa superba L., was used to produce CuO nanoparticles by green synthesis utilizing water-based extracts. UV-Vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), Gas chromatography – mass spectrometry (GC-MS) and Fourier-transform infrared spectroscopy (FTIR) were used to analyze the produced 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 chemicals.

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 green production of CuO nanoparticles utilizing Gloriosa superba L.[15,16] aqueous extracts and evaluate their antibacterial effectiveness against prevalent bacterial infections.[17,18]

2. Literature Review

Numerous research have shown how well plant-based extracts help to create nanoparticles.[19] for example, documented the production of copper nanoparticles from *Gloriosa superba* leaf extract, which exhibited encouraging antibacterial activity against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa, S.Pneumonia, P.Vulgaris,, 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]

3. Materials and Methods

3.1 Plant Material Collection and Preparation

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.

3.2 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.

3.3 Green Synthesis of CuO Nanoparticles

Mixing 20 ml of the filtered plant extract with 20 ml of 0.1 M copper sulfate ($CuSO_4$) solution in a 100 ml Erlenmeyer flask produced the green synthesis of CuO nanoparticles. Over two hours, the mixture was constantly stirred at 70°C. Observing a color shift from pale green to dark brown, which indicated the creation of CuO nanoparticles, the reaction progress was tracked. The solution was centrifuged at 10,000 rpm for 10 minutes post-reaction; the pellet was washed with distilled water and ethanol to get rid of any unreacted contaminants. The resultant nanoparticles were vacuum oven dried at 80°C for 24 hours.Under continuous stirring, 50 ml of 0.1 M copper sulfate solution was combined with 50 ml of aqueous extract of Gloriosa superba L. The development of CuO nanoparticles was shown by a noticeable color shift from bright blue to dark brown. Centrifuging the reaction mixture at 10,000 rpm for 15 minutes produced a pellet that was washed with distilled water and ethanol and then dried at 80°C.

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

Phytochemical Analysis

Standard phytochemical tests were conducted to identify the presence of alkaloids, flavonoids, tannins, phenolics, and saponins:

1. ***Screening for Alkaloids***

Crude extract was mixed with 2ml of 1% HCL and heated gently.mayerand wagner reagents were then added to the mixture, and the turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2. ***Screening for Flavonoids***

Crude extract was mixed with a few fragments of magnesium ribbon, and concentrated HCL was added drop – wise. A pink scarlet color appeared after a few minutes, which indicated the presence of flavonoid.

3. ***Screening for Saponins***

Crude extract was mixed with 5 ml of distilled water in a test tube, and it was shaken vigorously. The formation of stable forms was taken as an indication of the presence of saponins.

4. ***Screening for phenols***Crude extract was mixed with 2ml of a2% solution of $Fecl_3$ a blue - green or black coloration indicated the presence of phenols .

5. ***Screening for Tannins***

Crude extract was mixed with 2ml ofa 2% solution of $Fecl_3$ a blue - green or black coloration indicated 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

3.4 **Characterization of CuO Nanoparticles**

Characterization Data 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 2θ = 35.5°, 38.7°, 48.7°	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

Recording the UV-Vis absorption spectra with a Shimadzu UV-1800 spectrophotometer in the range of 200–800 nm indicated the creation of CuO nanoparticles. An The UV-Vis absorption spectra indicated the formation of CuO nanoparticles usinga Shimadzu UV1800 spectrophotometer in the range of 200–800 nm. The crystalline nature of the nanoparticles was investigated throughout a scan range of 10° to 80° using a Bruker D8 Advance XRD device. The surface form and size of the CuO nanoparticles were studied using a JEOL JSM-7500F SEM at an accelerating voltage of 15 kV. The functional elements included in the CuO nanoparticles were investigated using a Thermo Fisher Scientific FTIR spectrometer ranging from 4000 to 400 cm\u207b\u00b9. XRD machine, Bruker D8 Advance, scanning range 10° to 80°. Using a JEOL JSM-7500F SEM at an accelerating voltage of 15 kV, the surface shape and size of the CuO nanoparticles were investigated. An FTIR spectrometer (Thermo Fisher Scientific) in the range of 4000–400 cm\u207b\u00b9 was used to examine the functional groups found in the CuO nanoparticles.

3.5 Antimicrobial Activity Assay

Using the agar well diffusion technique, the antibacterial effectiveness of the CuO nanoparticles was evaluated against *Staphylococcus aureus (G+) ,Escherichia coli (G-),Pseudomonas aeruginosa (G-), Aspergillus flavus, Aspergillus niger, Penicillium* . After 24 hours of incubation, zones of inhibition were assessed.

4. RESULT

4.1 GC-MS Analysis of cuo nanoparticles in Leaf extract of *Gloriosa superba L.*

Table: 1 Phyto-components obtained through GC–MS analysis of CUO nanoparticles in leaf extract of *Gloriosa superba* L.

S.NO	RT	Compound name	M.W.	Formula	Peak area%
1	7.881	Benzenemethanol	124	C7H8O2	1.59
2	8.316	4-hydroxy-Salicylalcohol	124	C7H8O2	2.16
3	9.307	Benzenemethanol	124	C7H8O2	3.27
4	9.862	4-hydroxy-1,2-benzenediol	124	C7H8O2	2.32
5	9.917	3-methyl-Benzenemethanol	124	C7H8O2	4.58
6	12.858	3-hydroxy-Salicylalcohol	124	C7H8O2	5.62
7	13.188	Salicylalcohol	124	C7H8O2	1.98
8	17.025	1,2-benzenediol	124	C7H8O2	2.71
9	17.280	4-methyl-Benzenemethanol	124	C7H8O2	18.94
10	18.110	3-hydroxy-Nicotinicacid	263	C16H25O2N	4.98
11	18.936	DecylesterBenzenemethanol	124	C7H8O2	18.57
12	19.071	4-hydroxy-2,4,6-cycloheptatrien-1-one	106	C7H6O	4.50
13	19.901	1,3-benzenediol	124	C7H8O2	1.45
14	25.668	2-methyl-4h-1,3-benzodioxin	212	C14H12O2	

					3.47
15	26.814	2-phenyl-Benzenemethanol	124	C7H8O2	2.72
16	27.024	3-hydroxy-1,2-benzenediol	124	C7H8O2	5.83
17	27.544	4-methyl-7-chloro-1,3,4,10-tetrahydro-10-hydroxy-1-imino-3-[3-trifluoromethyl]	406	C20H14O2N2ClF3	4.86

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

4.2 **FT-IR Analysis of cuo nanoparticles in 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 and functional groups obtained for the CUO nanoparticles in leaf extract of *Gloriosa superba* (L).

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

UV-VISIBLE SPECTROPHOTOMETER ANALYSIS OF CUO NANOPARTICLES IN LEAF EXTRACT OF *GLORIOSA SUPERBA* L.

The UV-Vis absorption spectra of the produced CuO nanoparticles revealed a typical absorption peak at 272nm, hence verifying their creation .Typically, the UV-Vis spectra of CuO nanoparticles produced from Gloriosa superba L. leaf extract reveals a large

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 CuOnanoparticles via phytochemic reduction.

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
221.5	4
212.5	4

SEM ANALYSIS OF CUO NANOPARTICLES IN 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.

EDX ANALYSIS OF CUO NANOPARTICLES IN LEAF EXTRACT OF *GLORIOSA SUPERBA* L. (Fig: 5)

TEM ANALYSIS OF CUO NANOPARTICLES IN LEAF EXTRACT OF *GLORIOSA SUPERBA* L. (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.

XRD analysis of cuo nanoparticles in leaf extract of *Gloriosa superba* L.(Fig: 7)

Table: 5

Antibacterial Activity

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	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

The disc diffusion technique was used to assess the antibacterial activity of CuO nanoparticles. Testing was done on three bacterial and fungal strains: *Staphylococcus aureus (G+) ,Escherichia coli (G-), Pseudomonas aeruginosa (G-), Aspergillus flavus , Aspergillus niger, Penicillium* . Discs loaded with CuO nanoparticles were set on the agar surface; the bacterial cultures were swabbed uniformly onto nutrient agar plates. The plates were kept at 37°C for 24 hours; the areas of inhibition were assessed in millimeters.

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

Size of the zone of inhibition formed around each disc, loaded with test samples, indicating the antibacterial activity CuO NPs using G. superba leaf extract.

Table: 6 Antimicrobial Activity of CuO Nanoparticles Against leaf extract of *Gloriosa superba L.*

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

Summary Table of Sample Preparation and Instrument Settings

Technique	Sample Preparation	Instrument Settings	Notes
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 Kα radiation, 10°–80° 2θ, 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

Using aqueous extracts of Gloriosa superba L., which functions as a good reducing and stabilizing agent, the current work effectively shows the eco-friendly green synthesis of copper oxide (CuO) nanoparticles. The creation of highly crystalline, spherical CuO nanoparticles with notable stability and well-defined shape was validated by UV-Vis spectroscopy, XRD, SEM, and FTIR. 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 tests unequivocally showed that the produced CuO nanoparticles significantly inhibited harmful microorganisms including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Especially, the green-synthesized CuO nanoparticles showed better antibacterial action than their chemically produced equivalents, suggesting that the phytochemical coating improves cellular absorption and more efficiently disturbs 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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Figures

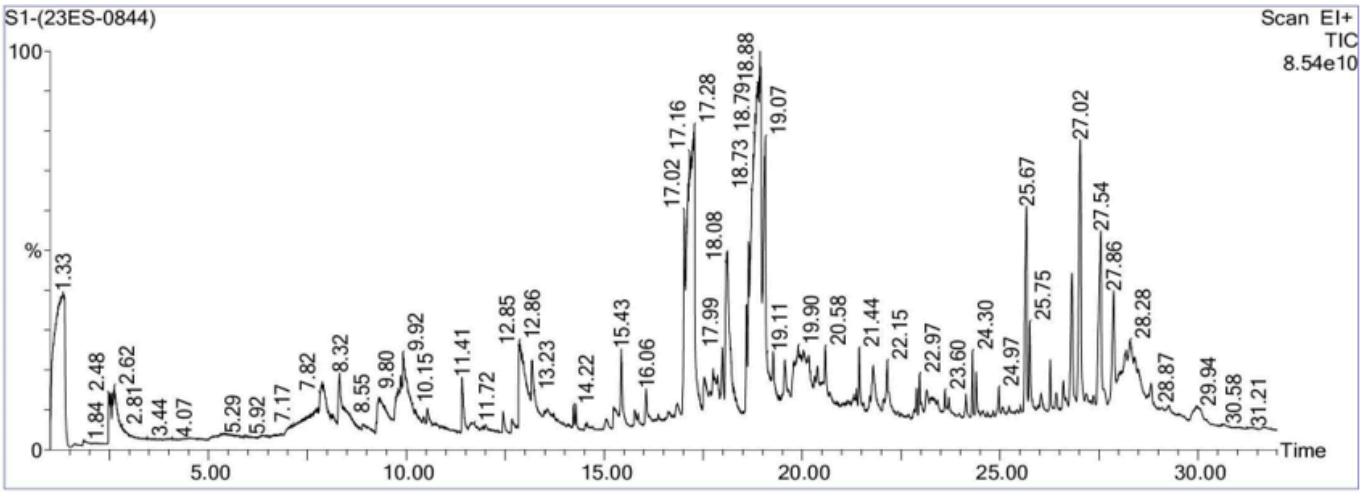

Figure 1

GC-MS chromatogram of leaf extract of cuo nanoparticles in *Gloriosa superba .L*

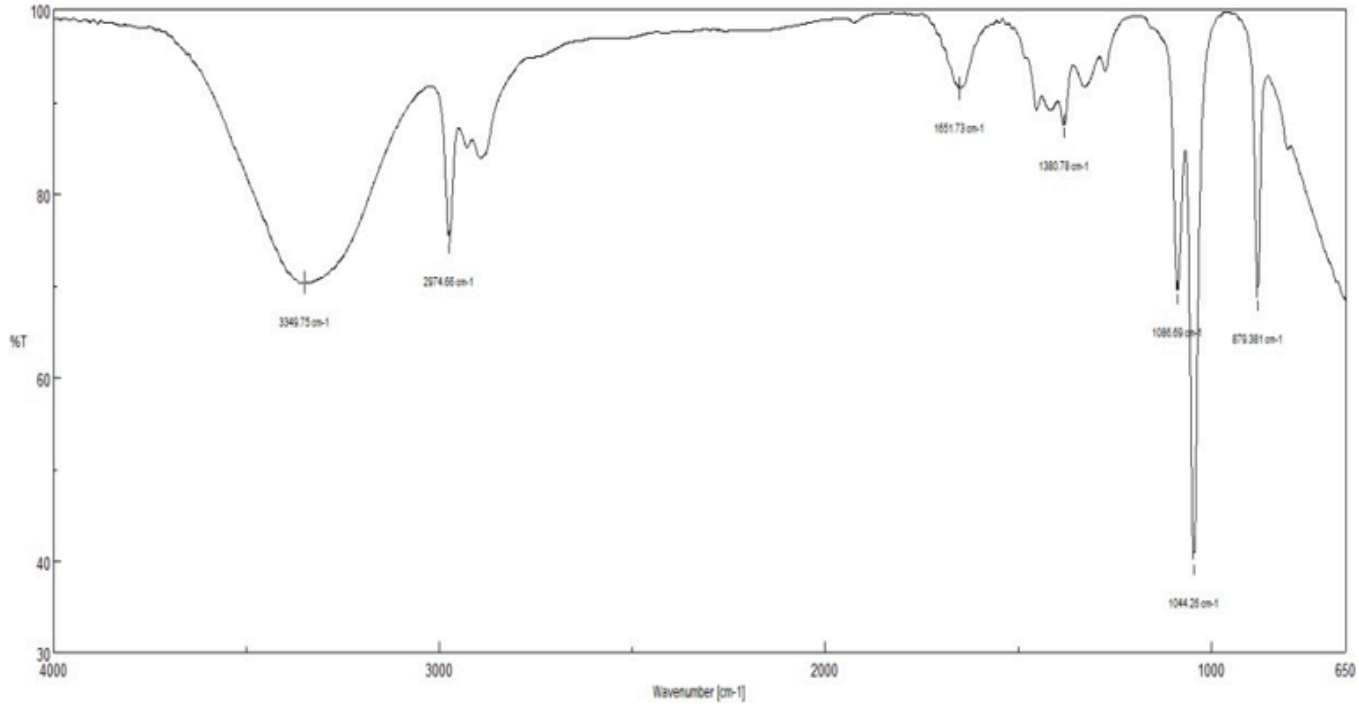

Figure 2

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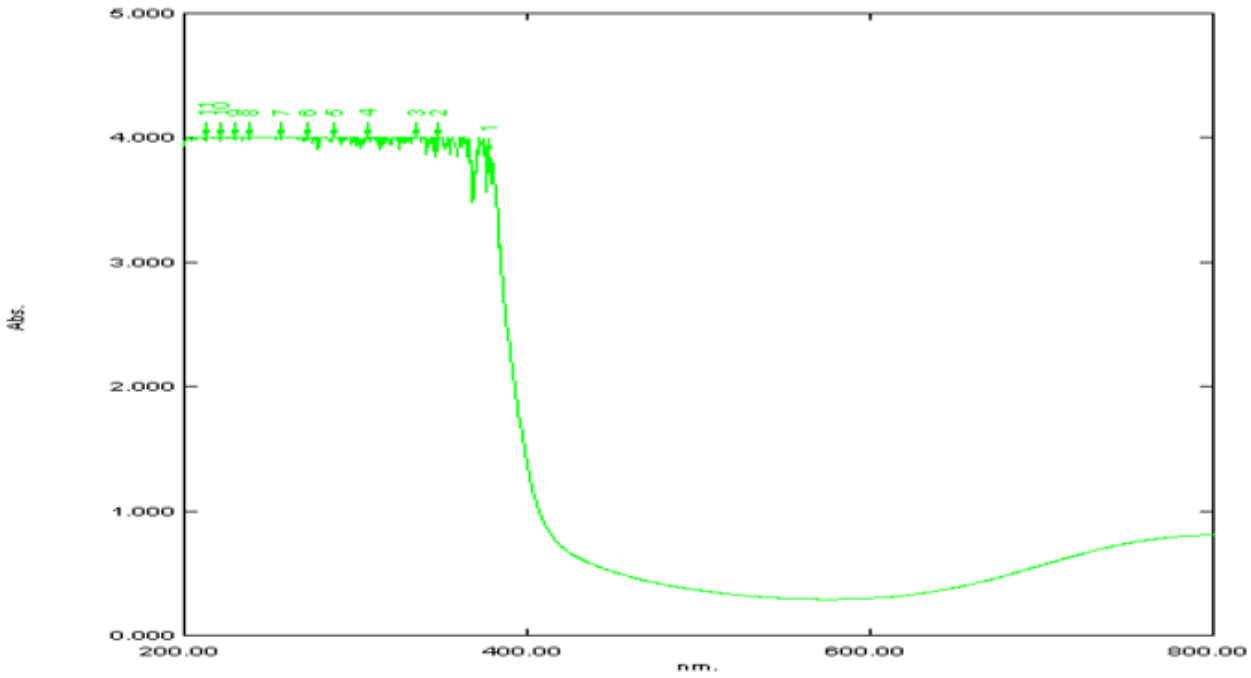

Figure 3

UV-VIS Absorption spectrum of CUO nanoparticles in leaf extract of *Gloriosa superba* L.

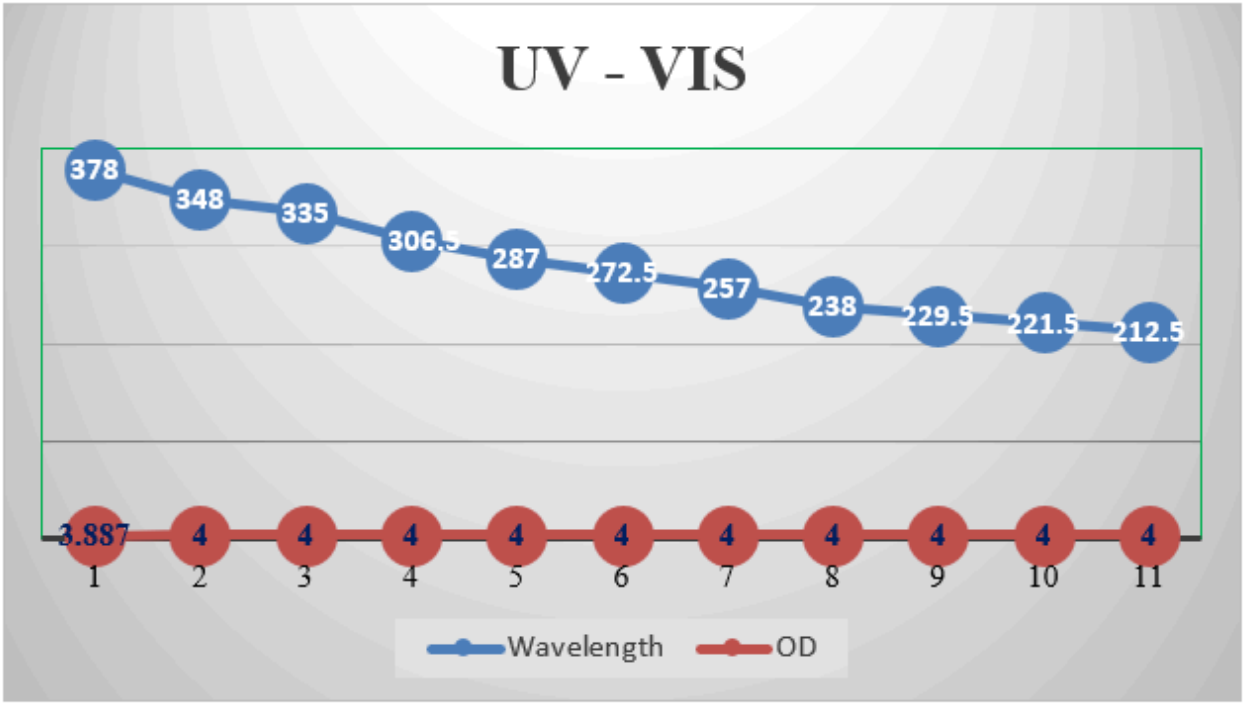

Figure 4

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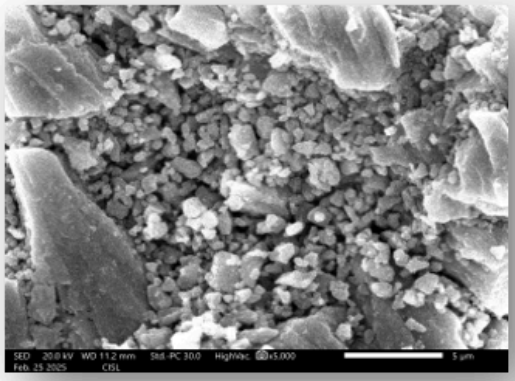

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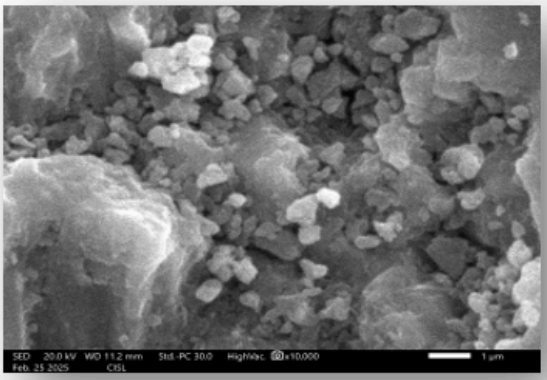

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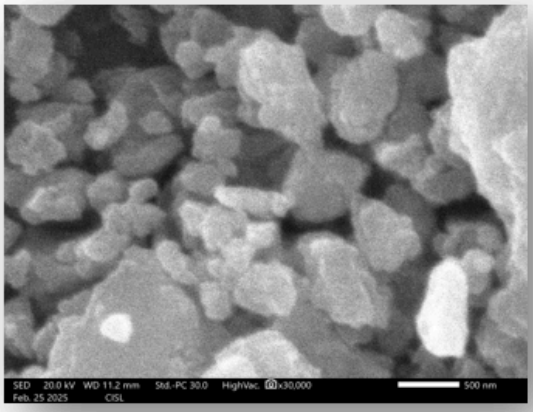
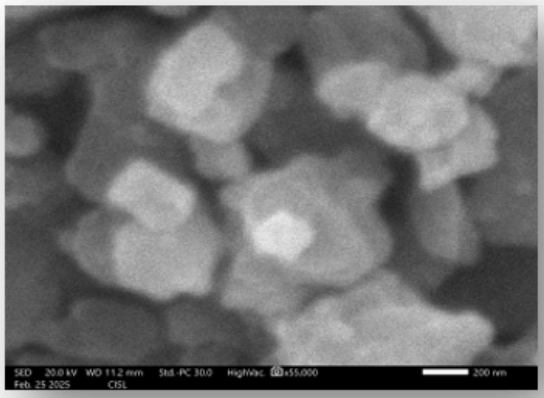


X30,000



X55,000

Figure 5

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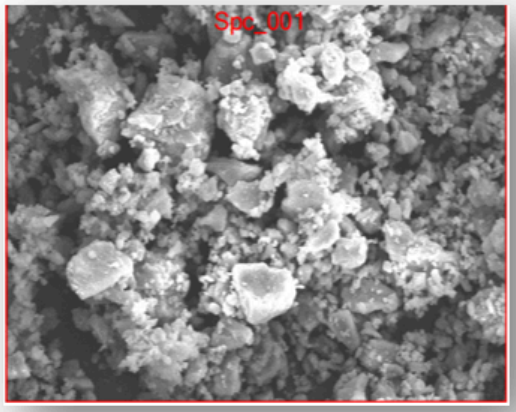

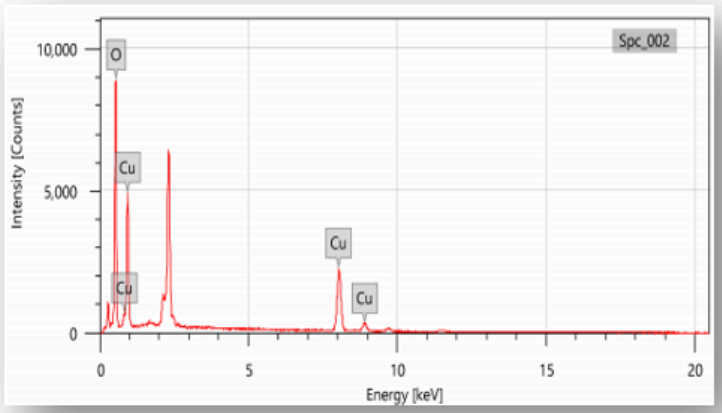

Element	Line	Mass%	Atom%
O	K	42.20±0.18	74.36±0.32
Cu	K	57.80±0.41	25.64±0.18
Total		100.00	100.00
Spc_002			Fitting ratio 0.3124

Figure 6

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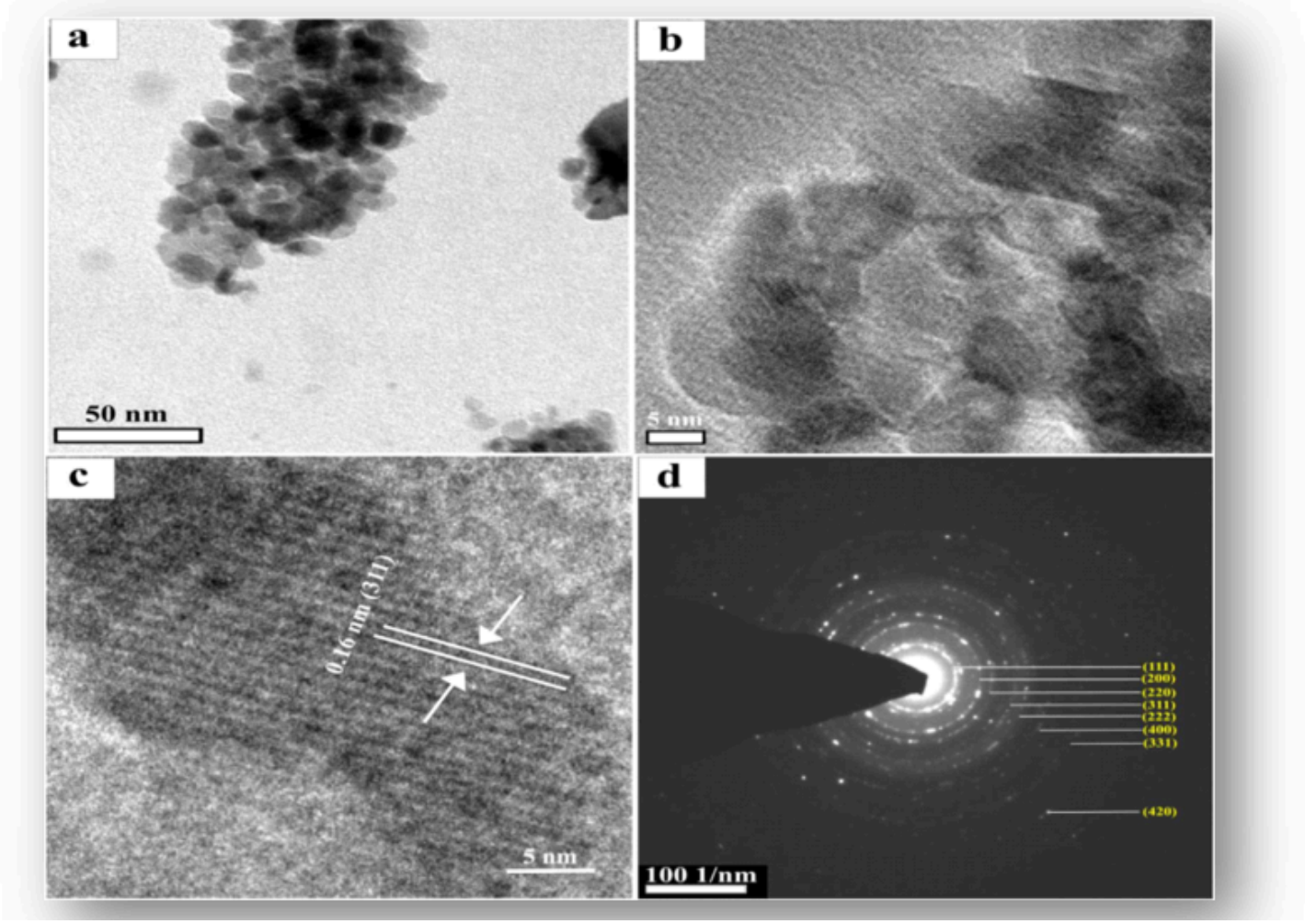
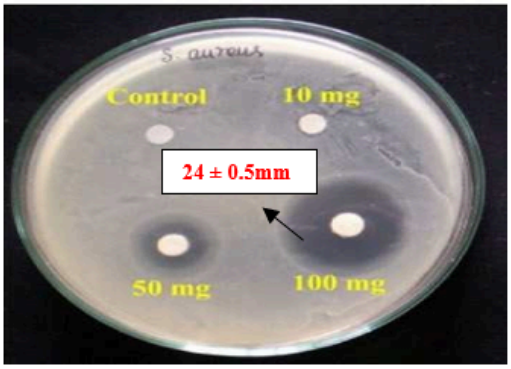
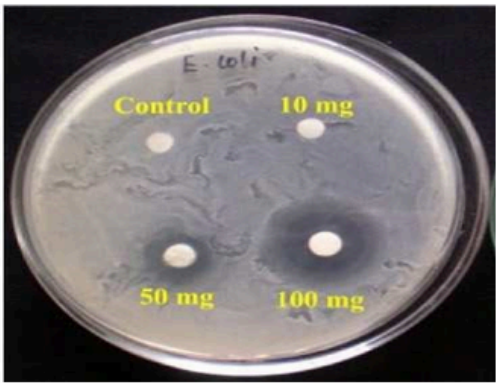


Figure 7

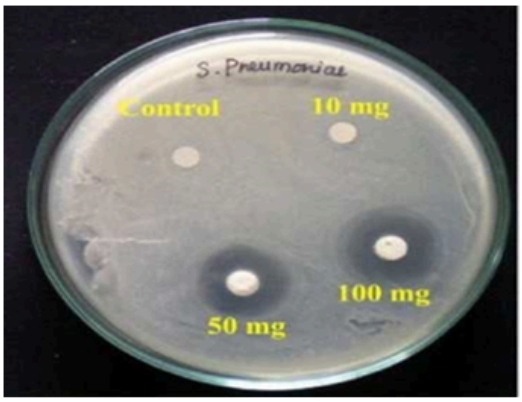
(a–c) TEM images of CUO NPs and (d) selected area electron diffraction.



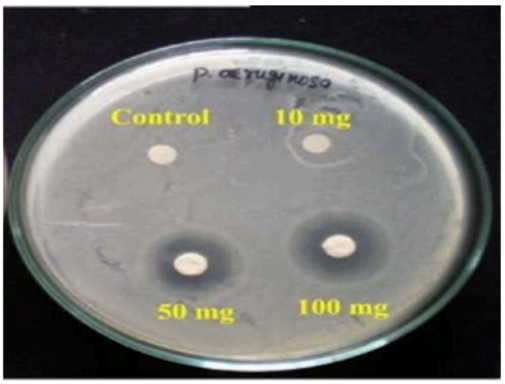
s.aureus



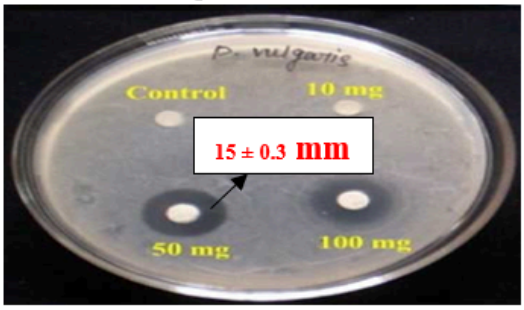
E.coli



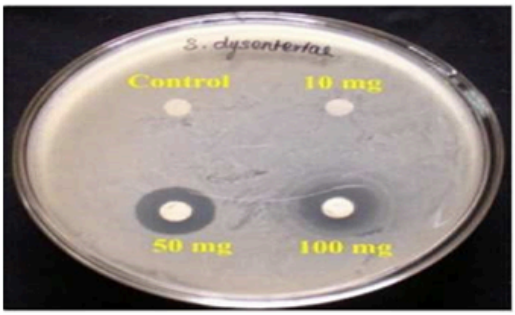
s.pneumoniae



p.aeruginosa



p.vulgaris.



S. Dysenteriae

Figure 8

Figure legend not provided with this version

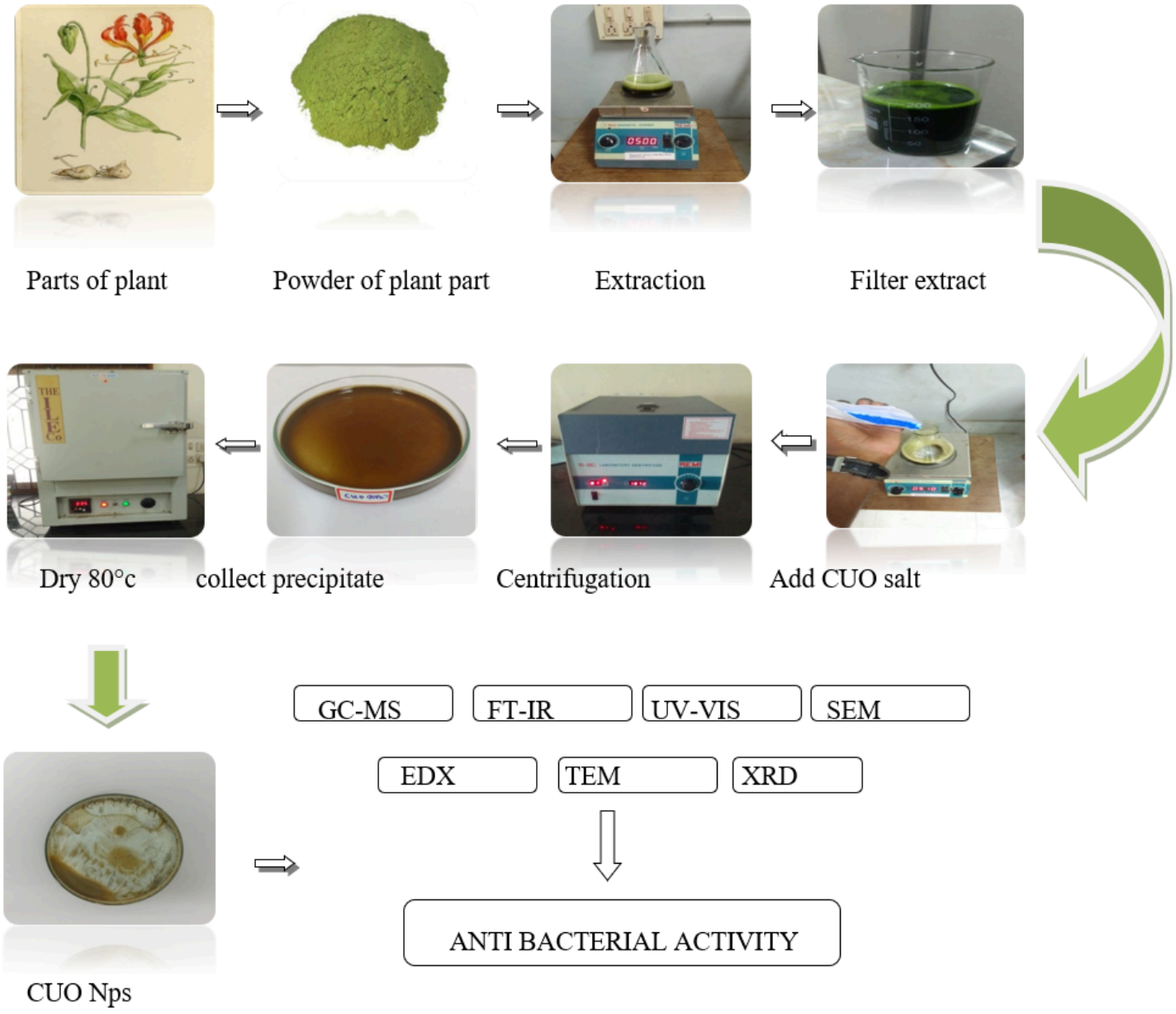


Figure 9

Unnumbered image in the Materials & Methods section.

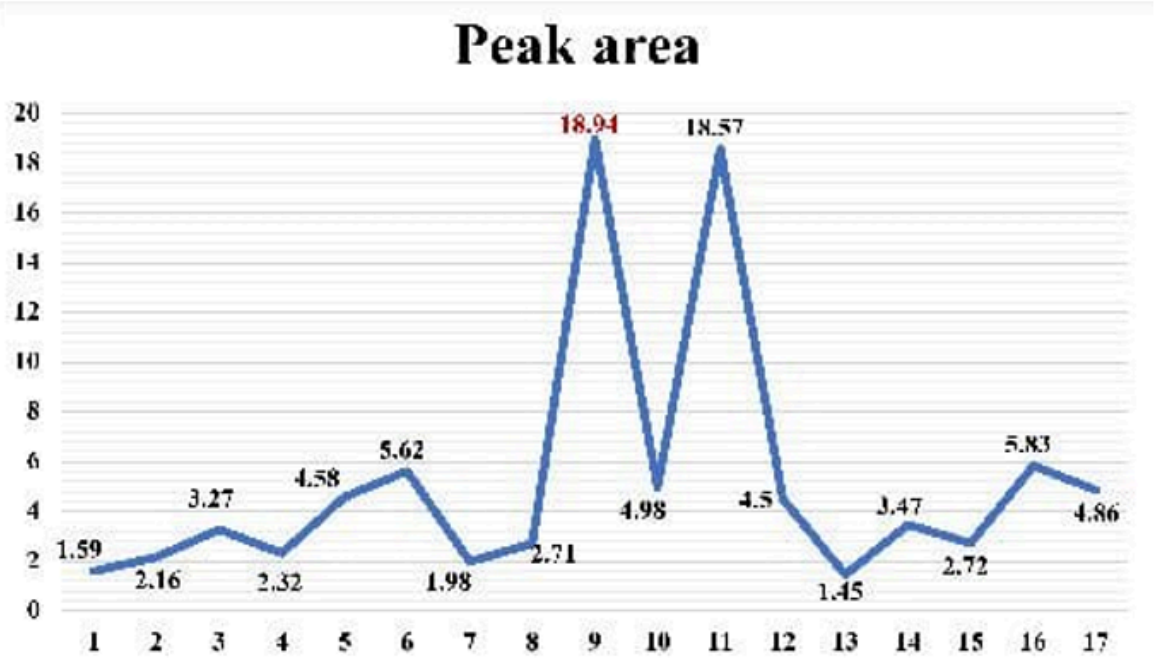


Figure 10

Unnumbered Image in the Result Section.

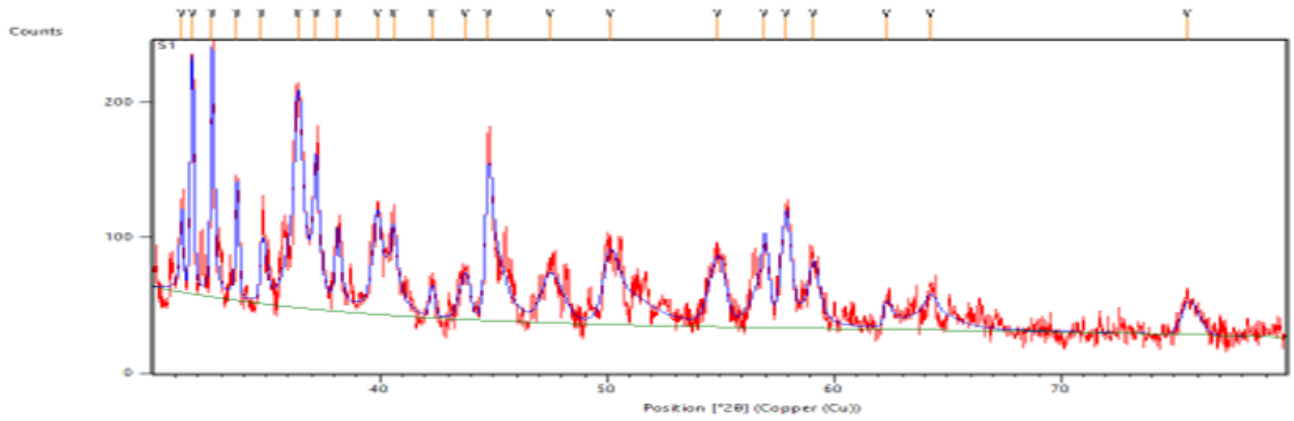


Figure 11

Unnumbered Image in the Result Section.

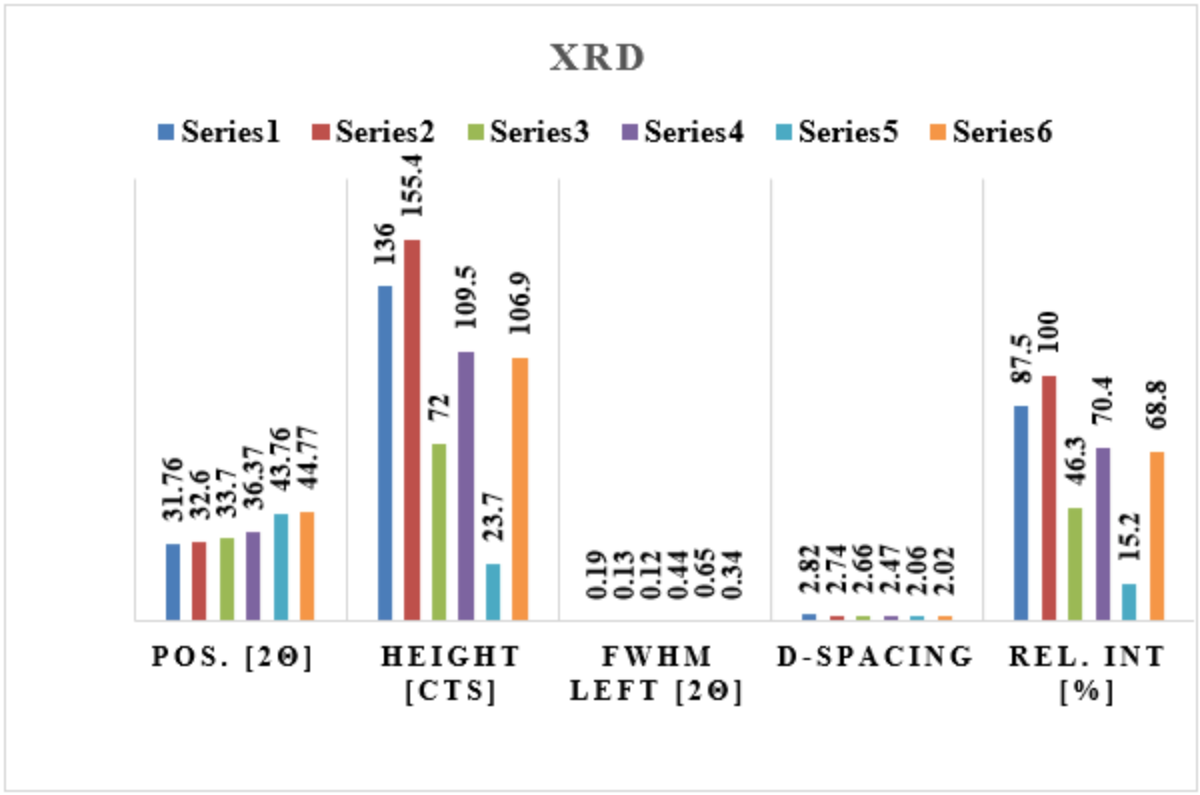


Figure 12

Unnumbered Image in the Result Section.