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Green Synthesis of Copper Oxide Nanoparticles using aqueous extract of *Sarcostemma acidum* and their Antibacterial properties

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Abstract
In the present study copper nanoparticles (CuNPs) were synthesized using *Sarcostemma acidum* stem extract and stabilizing agent under surfactant free conditions. The Synthesized copper oxide nanoparticles were characterized using UV-visible spectroscopy, FTIR spectroscopy, XRD techniques. Antibacterial activities were inversely proportional to the average nanoparticle sizes. According to UV-vis results the synthesized Cu NPs by this method are quite stable even after one month indicating the stablitiy of CuNPs.

Key-words Copper Nanoparticles, Green Synthesis, Stem Extract

Introduction
Nanotechnology and nanoscience is a multidisciplinary field to exploit the wide spectrum of the emerging area from fundamental sciences such as physics, chemistry electronics, and material science with novel techniques and produces unusual and unique properties of a nanomaterial at nano range [1]. Since copper nanoparticles (CuNPs) are less expensive than many metallic counterparts and exhibit unique optical, catalytic, electrical, and thermal conductivity characteristics, which are different from that of their bulk metals they have drawn a lot of attention.Due to their potential applications, there has been a special focus on the synthesis of CuNPs for the past few decades. CuNPs have been synthesized using chemical, biological, and physical techniques. Out of these three approaches, biological synthesis of CuNPs presents the greatest challenge to scientists because of severe oxidation issues [2].

Benefits of copper's superior antibacterial properties have long been recognized . The studies provide support for the utilizing of copper nanoparticles as a unique class of biocidal agents Numerous techniques, including metal vapor synthesis, the explosive wire method, vacuum vapor deposition, laser irradiation, and microemulsion, can be utilized to create this thin metal copper nanoparticle. [4-6]

Copper nanoparticles have prospective applications in optics, electronics, and medicine and in manufacturing of lubrications, nanofluids, conductive films, and antimicrobial agents. The preference of copper nanoparticles compared to silver is due to the lower cost of copper than silver, the physical and chemical stability, and ease of mixing with polymers. Smaller nanoparticles offer higher is their activity but may result in cluster formation causing a decrease in essential properties [7-9].

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Most of the current methods used for the synthesis of nanoparticles use either toxic chemicals and or complex processes, and uses a lot of energy. These substances can function as stabilizers to stop nanoparticle aggregation as well as reducing agents to convert different metal salts into zero-valent metal nanoparticles. The Hydrazine, N-N dimethylformamide and sodium borohydride are the popularly used reducing agents for various metal salts to their corresponding zero valent metallic nanoparticles or as stabilizing agents to prevent nanoparticles from agglomeration, which are highly toxic to living organisms and the environment [10-11].

This drives us to search for the clean, non-toxic and environmentally acceptable routes for the production of nanoparticles, synthesized the stable CuNPs by the treatment of aqueous solution of copper sulphate pentahydrate solution with sarcostemma acidum stem extract [12] with evaluation of antibacterial potential against E. coli which showed significant antibacterial activity. Amongst them, copper-based nanoparticles are of great interest due to their low cost and easy availability and because they possess properties similar to that of other metallic nanoparticles [13].It has been studied in sarcostemma acidum the antimicrobial properties and synthesis of nanoparticles.[14]

The aim of the present study was to develop a nontoxic and cost-effective biological method for the synthesis of CuNPs. It is a green approach for the synthesis of copper nanoparticles.

Material and Method

Plant collection

Fresh and healthy, Sarcostemma Acidum stem was collected from Rewa (M.P). It is a medically important plant.

Preparation of Plant Extract

To prepare the plant extract the fresh and healthy stem of sarcostemma acidum plant was collected from Rewa (M.P). The stem were cleaned with running tap water followed by distilled water the stem has to be dried naturally and then ground into powder with the help of mortar and pastel. The powder of S. Acidum stem was weighed (25g) were mixed with 100ml of double distilled water and boiled at 60^0C for 10 min. The boiled stem extract was cooled and filtered with whatmann no.1 paper to obtain an aqueous extract of stem. Thus the prepared extract was stored at 4^0C for further used for synthesis of copper Nps [13].

Preparation of Copper Nanoparticles

For the synthesis of copper nanoparticles an Erlenmeyer flask containing 100ml of 10 mM of ($CuSO_4.5H_2O$) was stirred for 1hour. 10 ml plant extract of sarcostemma acidum stem extract was added into 90ml of 10Mm of $CuSO_{4.}$ $5H_2O$ solution and kept in stirring for constant mixing under room temperature for 1 hour. A colour change of the solution was noted by visual examination this confirmed the synthesis of Cu Nps [15-16].

Characterization of synthesized Cu Nanoparticles

The characterization of synthesized nanoparticles was analyzed by different techniques such as, UV-Visible spectroscopy analysis.

Shape and size were analyzed using SEM & Crystalline structure of the synthesized nano copper analyzed using X-ray diffraction method (XRD).The Fourier transform infrared spectroscopy FTIR used to analyze phytochemicals responsible for nanoparticles synthesis [17-18].

Antibacterial Activity of Synthesized Nano Copper

The Antibacterial activity of synthesized nano copper was performed by the NAM disc diffusion method against Lactose fermenting bacteria and Non-lactose fermenting like E.Coli and Salmonella bacteria. Fresh overnight culture of each strain was swabbed uniformly into the individual plates containing sterilized [19].

The 4 mm diameter sterile discs were impregnated into the Cu Nps solution at different concentration 25, 50, 75 µl, respectively. The impregnated discs were placed onto the plates and incubated for 24h at 37^0C. Commercial Antibiotic discs Ampicillin, Tetracycline, Gentamycin were placed as control, after incubation, different levels of zonation formed around the discs were measured.

Results and Discussion

In the present work, we develop an ecofriendly, clean, non-toxic, facile chemically preparative method, for the generation of Cu NPs using the extract of stem of sarcostemma acidum, acting as reducing as well as stabilizing agent. Nanosized materials are having a great interest due to their

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unique optical properties. Nanoparticles were exhibit different array of colour while the synthesis process.

Copper (II) sulfate dissolved in water, dissociates into Cu^{2+} and SO_4. Cu^{2+} is reduced to Cu by reduction action of ascorbic acid, forming metallic copper nuclei, which initiate the growth stage .

Visual Observation

Copper sulphate converts into copper nanoparticles was primarily identified by the change of colour from light blue to pale yellow after adding the P.E and after maintaining the Ph of solution after hour the solution turns dark green colour solution

Fig 1: a) CuSO4 solution; b) CuSO4 and plant extract; c) CuO formed nanoparticles

After centrifugation of the solution the precipitation was observed indicates that the nanoparticles synthesis process was completed.[20]

UV-Visible absorption spectra

The UV-Vis absorption spectra of copper oxide Nps recorded at a different wavelength from 200-800nm shown in figure 2.

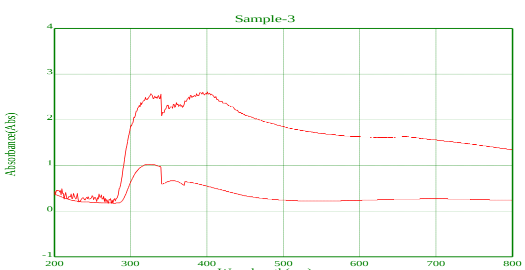

Fig 2: UV-Vis absorption spectra of copper nanoparticles using *S. acidum*

The Cu Nps are synthesized using $CuSO_4$&S.Acidum stem extract as a reducing agent displays an absorption peak at 375 nm. Broadened peak was observed in this UV-Vis spectrum confirmed that poly dispersed nanosized particles are synthesized [21].

FTIR spectrum of copper Nps

Substance specific vibrations of the molecules lead to the specific signals obtained by IR spectroscopy. FTIR spectra and functional group involved in CuNps synthesis illustrated peak in the range of 500-4000 cm^{-1} (Fig). Broad peaks obtained at 2359 corresponding to O=C=O stretching of strong bonds to 3571 corresponding to O-H groups, peaks from 3009 corresponds to N-H stretching, 1757 corresponds to C=O stretching [22-23].

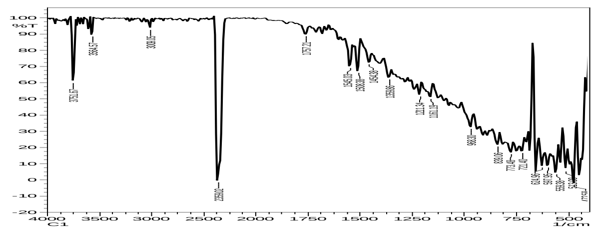

Fig 3: FTIR spectrum of copper nanoparticles synthesized using *S. acidum*

Table 1: Functional groups involved in CuNps synthesis analysed by FTIR

S.No	Absorption Peak	Functional groups
1	3751	O-H
2	3009	O=C=O
3	2359	C=O
4	1757	N-O
5	1545	C-H

X-ray diffraction (XRD)

XRD Spectra provides an insight about the crystallinity of Nps synthesized using stem extract nanoparticle . Represents XRD Spectra of Cu NPs.

The De Braggs reflection angles 35.06^0 and 60.1^0. These characteristic peaks represent that synthesized Cu nanoparticles are crystalline thus XRD used to nearly synthesized product is purely nano copper with high crystalline [24].

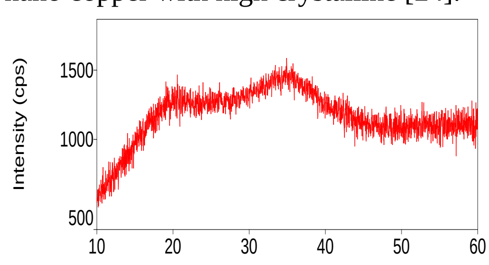

Fig 4: XRD spectrum of copper nanoparticles

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Table 2: Parameter calculation for average size calculation for nanoparticles

S. No.	2- theta	d -value	h k l
1	31.80	2.812	1 0 0
2	34.45	2.602	0 0 2
3	36.29	2.474	1 0 1
4	47.53	1.909	1 0 2
5	56.66	1.623	1 1 0
6	62.91	1.476	1 0 3
7	66.45	1.406	2 0 0

SEM Analysis

Scanning electron microscopy (SEM) of synthesized copper nanoparticles is illustrated in figure 5.

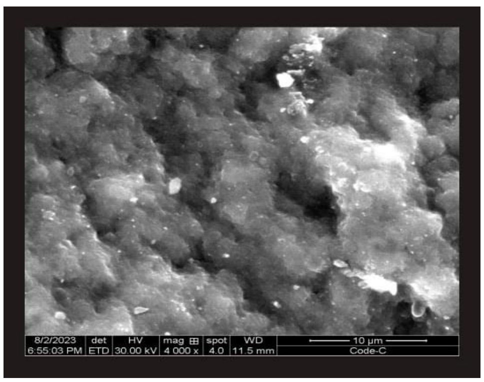

Fig 5: SEM of copper nanoparticles synthesized using *S. acidu*m

the surface morphology and size of the nano copper by this ecofriendly method showed the nearly nano dispersed of particle size the Cu nanoparticles have spherical shape with an average diameter of 11.5mm and some of them shows the undefined shape of nanoparticles [25].

TEM Analysis

Transmission electron microscope (TEM) study was performed to estimate the 2D structure of the nanoparticles. CuNPs synthesized were mostly seen in spherical shape and size varied from 10-30 nm when analyzed. [26]

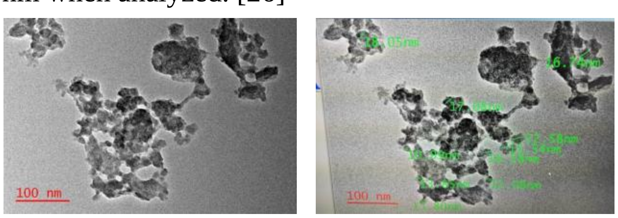

Fig 6: The Image of Cu nanoparticle synthesized using an aqueous extract of *S. acidium*

Antibacterial Activity of Cu Nanoparticles

From the above mentioned experiment can be found that the CuO

Nanoparticles are effecting in inhibiting a range of bacterial growth. The results of Antibacterial action of spherical shaped copper nanoparticles treated against the lactose fermenting bacteria E.coli the zone of inhibition around the 24mm in diameter. [27-30] The other observation was that the P.E also effecting in inhibiting arrange of bacterial growth in different concentrations of gram negative bacteria. For the L.F bacteria the zone of inhibition in 50µl is 40mm and in N.L.F bacteria the zone of inhibition in 75µl is 30mm.

Similarly some antibiotic like Tetracycline also shows the zone of inhibition for the L.F bacteria in the range of 40mm and for the N.L.F shows the 50mm zone of inhibition [31-32].

Table 3: Antibacterial activity of CuNps

Bacteria	40µl
L.F Bacteria	24mm
N.L.F Bacteria	No results

Table 4: Antibacterial activity of S.Acidum plant extract

Bacteria	50µl	75µl
L. F Bacteria	40mm	No results
N.L.F Bacteria	No results	30mm

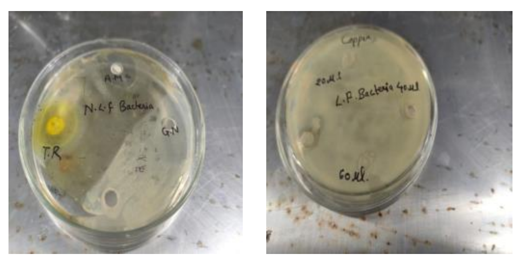

Fig 7: Bacterial culture showing the inhibition zone in E.coli and salmonella bacteria

Table 5: Antibiotic activity against pathogenic bacteria

Bacteria	Tetracycline
L.F Bacteria	40mm
N.L.F Bacteria	50mm

Conclusion

Studies have shown that the size, morphology, stability and properties are subject to experimental conditions. The present green method for the synthesis of Cu nanoparticles is simple, mild and environmental friendly .Green synthesis of copper nanoparticles was initially confirmed by the position of SPR band at 375 nm in uv-vis spectra. XRD spectrum shows crystalline nanostructured copper particles. SEM image shows spherical shape with an average particle size 11.5 mm. The particle size distribution of the synthesized Cu Nps is ranging between 10nm and 30nm. Cu Nps exhibited an excellent antibacterial activity against E.coli (lactose fermenting bacteria). So it can conclude that green synthesized Cu Nps using *Sarcostemma acidum* stem extract as environment friendly with low cost and antibacterial activity.

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