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GREEN SYNTHESIS AND CHARACTERIZATION OF COPPER NANOPARTICLES USING ALLAMANDA BLANCHETII FLOWER EXTRACT

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Abstract
The Allamanda Blanchetii flower extract is used for the biosynthesis of Copper nano particles and the successful formation of the resultant product was confirmed through several physico chemical techniques .The crystalline structure and crystallite size were investigated through an X-ray diffractometer (XRD). The antioxidant test is also carried out against 1, 1-diphenyl - 2- picryl hydrazyl free radicals and the antioxidant potential of CuNPs were found to be higher than ascorbicacid.

Keywords: Allamanda Blanchetii, Copper Nano particles, XRD, Anti oxidant acitivity, Biosynthesis

Introduction
Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials and devices. The technology promises scientific advancement in many sectors such as medicine, consumer products, energy, materials, and manufacturing. Nanoscience and nanotechnology represent an expanding research area, which involves structures, devices, and systems with novel properties and functions due to the arrangement of their atoms on the 1–100 nm scale. [i]The technology that utilizes it in practical applications such as devices etc. is called nano technology. [ii]Nano technology has already been embraced by industrial sectors, such as the information and communications sectors, but is also used in food technology, energy technology, aswell as in some medical products and medicines. Nano materials may also offer new opportunities for the reduction of environmental pollution. Nanotechnology has the potential to revolutionize ourlives. This is because it presents almost unlimited potential to make remarkable changes in virtually all fields ranging

from medicine, computer technology, construction, environmental remediation, food industry, to new energy sources.

Experimental

Materials

Copper acetate and distilled water (all at high laboratory standard) were used in this study, Allamanda Blanchetii flower extract was used as the reducing agent for the preparation of the copper acetate nano particles which was also used as the stabilizer for the newly synthesized nano-sized copper acetate colloids, Muffle furnace, hot air oven, Mortar and Pestle.

Synthesis of Allamanda Blanchetii flower extract

Aqueous extract of Allamanda Blanchetii was prepared using freshly amassed leaves (10g) which were collected from Tirunelveli, India. The surfaces of the leaves were cleaned with running tap water followed by distilled water and the leaves were subsequentlyboiledin100 ml distilled water at 120° C for 10 min. After 10 minutes the extract was filtered through Whatman No.1 filter paper and filter was collected.

Synthesis of the Copper Acetate Nanoparticles

0.1 M of copper acetate was prepared by using 100 ml of deionised water. To 50 ml of above solution, 50 ml of Allamanda Blanchetii was added and placed in the magnetic stirrer at 40^0C turned for 3 hours. The colour of the solution turned from dark green to pale green which indicated the formation of nanoparticles. The formed copper acetate nanoparticles were collected and stored in an airtight container.

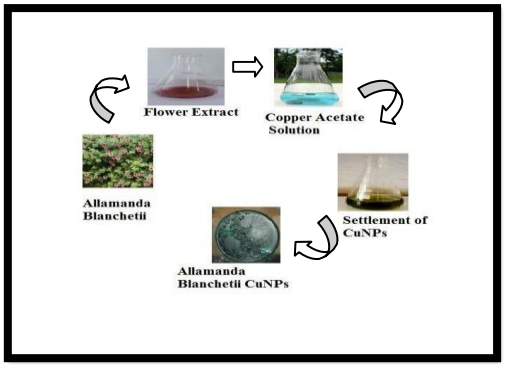

Figure1: A schematic representation of CuNPs from Allamanda Blanchetii flower extract

Results and discussion

UV Analysis

UV-visible absorption spectroscopy plays a very important role to investigate the optical properties of nanoparticles. Quantitative formation can be monitored and size of nanoparticles can be studied with the help of the UV technique. [iii] UV - Vis spectroscopy is also performed to confirm the formation, size, shape and stability of CuNPs. UV-visible absorption spectrum in the wavelength range 200–800 nm to confirm the formation and stability of Copper nanoparticles synthesized by green synthesis. [iv] UV analyses were used to identify the formation of CuNPs. The absorption peak is observed at 270 nm which preliminarily confirm the formation of CuNPs. [v] The following figure 2 shows the UV–Spectrum of CuNPs.

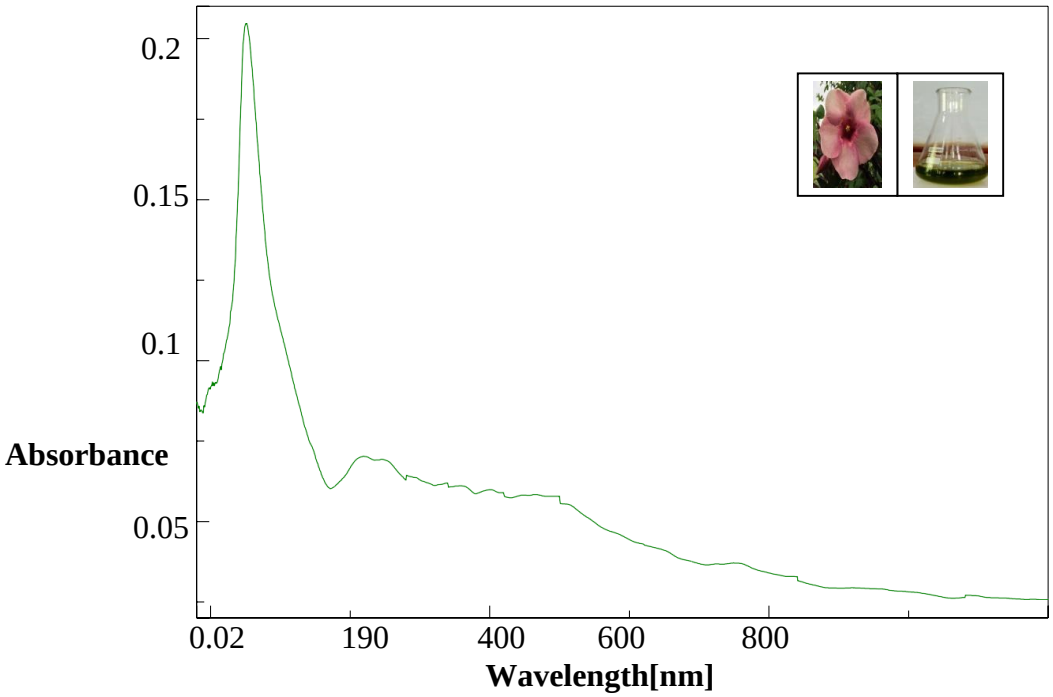

Figure 2: UV Spectrum of CuNPs

XRD pattern

XRD Spectra provides an insight about the crystalline of nano particle represents XRD Spectra of CuNPs synthesized using Allamanda Blanchetii. [vi] The size of the nanoparticles were calculated by using the Debye-Scherrer equation.

$$D=K \lambda / \beta \cos \theta$$

where, D-particle size in nm, x-ray wavelength, FWHM, Braggs angle of reflection.XRD spectra of CuNPs are given in figure3..XRD patterns of the CuNPs have broad peak indicate that they are crystalline. Average sizes of the synthesized nanoparticles were found to be 33nm.

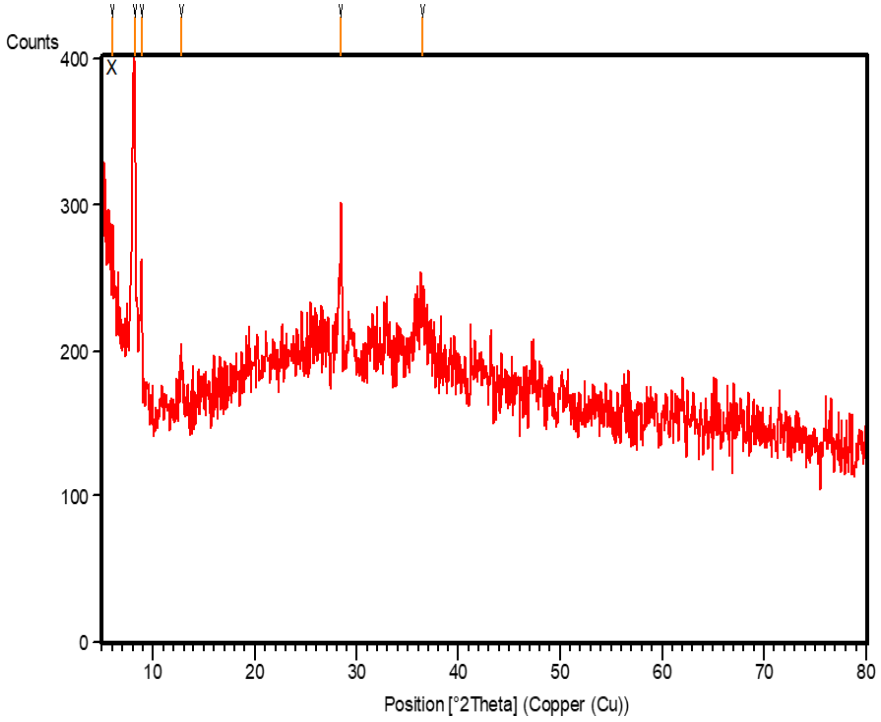

Figure 3: XRD pattern of CuNPs.

Antioxidant activity of copper nanoparticles

The percentage of Antioxidant Activity (AA %) of each substance was assessed by DPPH (1,1-diphenyl-2- picryl hydrazyl) free radical scavenging assay. Different concentrations of CuNPs were added to all the tubes except blank. Then 3mL of ethanol and 0.3mL of DPPH radical solution in ethanol was added. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Absorbance was read at 517 nm after 30 min of reaction. Same procedure was done for the standard BHT and its absorbance was also measured. [vii]The scavenging activity percentage (AA %) was calculated using the below formula.

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant studies. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517nm, which is induced by antioxidants.[viii]

$$\% \text{ Antioxidant Activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

Table1 : OD Value of Allamanda Blanchetii flower extract at 517 nm

S.No	Allamanda Blanchetii flower extract concentration (µg/ml)	OD Value at 517 nm(intriplicates)		
1	Control	0.684	0.646	0.619
2	500µg/ml	0.188	0.291	0.233
3	250µg/ml	0.297	0.308	0.334
4	100µg/ml	0.338	0.344	0.345
5	50µg/ml	0.355	0.357	0.364
6	10µg/ml	0.541	0.601	0.607
7	Ascorbicacid	0.08	0.11	0.12

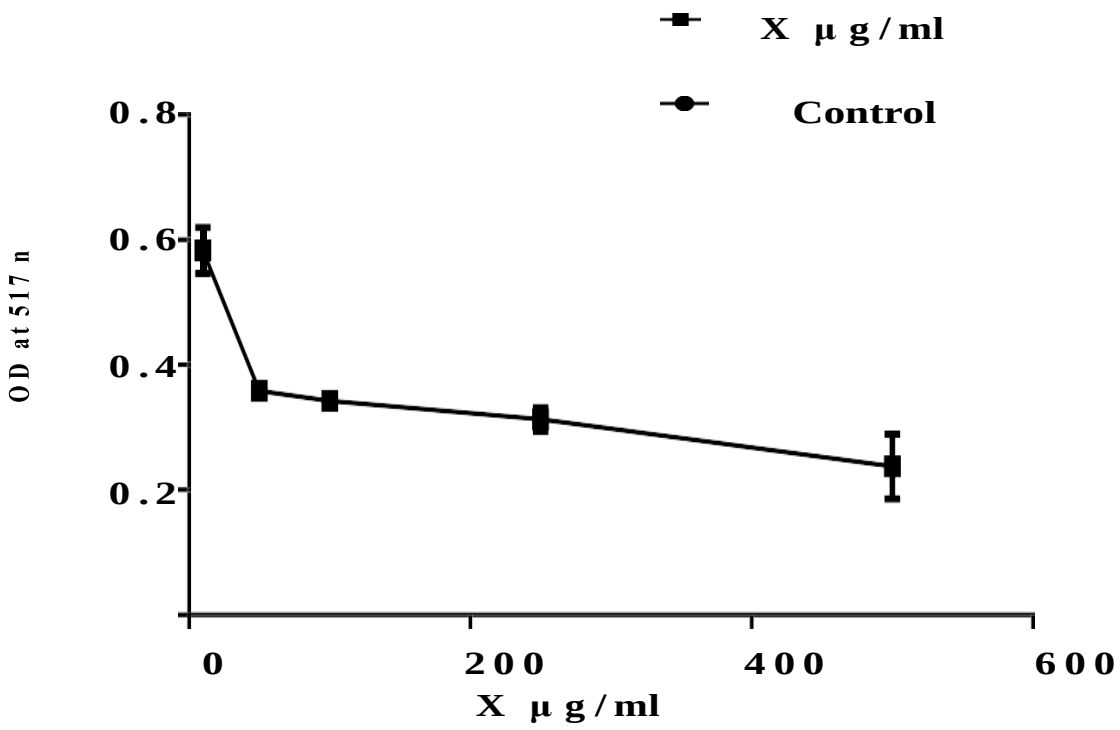

Figure 4: OD Value of A. Blanchetii flower extract at 517 nm

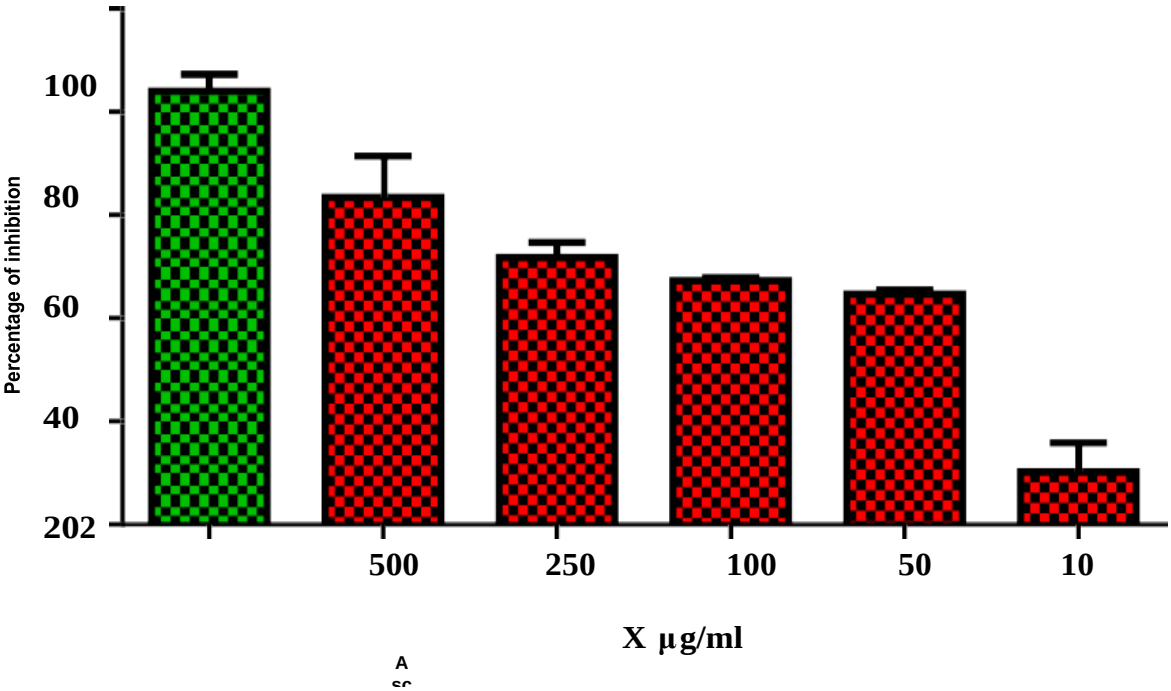

Figure 5: Percentage of DPPH radical scavenging activity of CuNPs

Table 2: Percentage of inhibition of CuNPs

S. No	Allamanda Blanchetii Flower extract concentration (µg/ml)	Percentage of inhibition(in triplicates)			Mean value (%)
1.	Ascorbic acid	87.67334	83.05085	81.51002	84.07807
2.	500 µg/ml	71.03236	55.16179	64.09861	63.43092
3.	250 µg/ml	54.23729	52.54237	48.53621	51.77196
4.	100 µg/ml	47.91988	46.99538	46.84129	47.25218
5.	50 µg/ml	45.30046	44.9923	43.91371	44.73549
6.	10 µg/ml	16.64099	7.395994	6.471495	10.16949

The % of DPPH radical scavenging activity of CuNPs is presented in Figure 5.The free radical in DPPH can be neutralized by the antioxidants present in CuNPs by transferring either their electron or hydrogen atoms to DPPH, thereby changing the colour from purple to yellow coloured diphenyl picryl hydrazine**.** [ix]Figure 6 shows the colour change occurred in DPPH assay with the standard BHT and the sample CuNPs. Table 3 shows that CuNPs demonstrated strong inhibitory activity of DPPH with an IC_{50} value of 45.65µg/ml

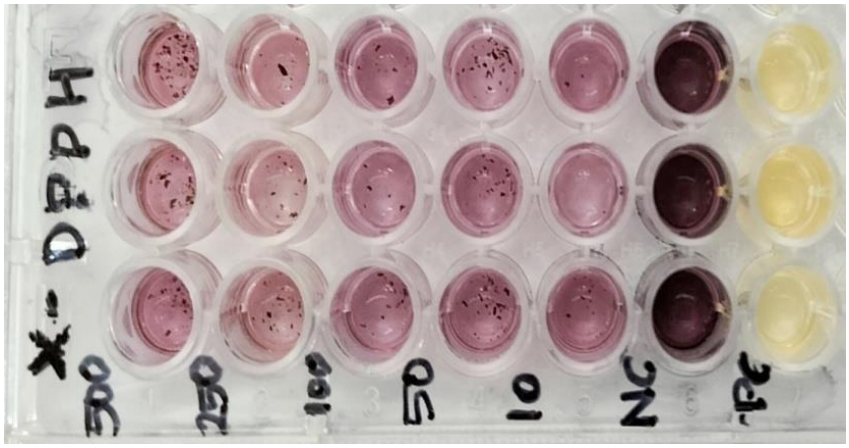

Figure 6: Colour change in standard BHT and CuNPs

Table 3: CuNPs inhibitory activity of DPPH with IC50 value

BEST FIT VALUES		
LogIC50		1.659
LlSlope		-1.331
IC50		45.65
Std.Error		
LogIC50		0.07520
HillSlope		0.2856
95% ConfidenceIntervals		
LogIC50		1.497 to 1.822
HillSlope		-1.948to-0.7146
IC50		31.40 to 66.35
GoodnessofFit		
DegreesofFreedom		13
Rsquare		0.8783
AbsoluteSumof Squares		2140
Sy.x		12.83
Numberofpoints		
Analyzed	3	15

In the DPPH assay, the antioxidant activity of the CuNPs synthesized from the Allamanda Blanchetii flower extract shows 63.4% of scavenging activity whereas the standard Ascorbic Acid shows 84.0% of scavenging activity. Hence, the synthesized Copper nanoparticles shows good antioxidant activity.

Conclusion

Copper nanoparticles have been synthesized with the help of Allamanda Blanchetii flower extract. It was the economically feasible route for synthesizing copper acetate nanoparticles and was confirmed through XRD analysis. The nanoparticles synthesized from Allamanda Blanchetii flower extract do not require any capping agentand remain stable for several weeks. It is simple to operate, cost-effective and reduces the use of toxic reducing agents.The Copper nanoparticles synthesized from the flower extract of Allamanda Blanchetii are evaluated for antioxidant activity by DPPH free radical scavenging assay. DPPH assay is used to study the antioxidant activity of the copper nanoparticles. It reveals that it is a potent antioxidant agent. Due to the better activity in free radical scavenging, it can be used in clinical therapeutic applications and future pharmaceutical applications.

It concludes that the synthesized Copper Acetate Nanoparticles are more efficient, compatible, and suitable for biological and environmental applications. This work can be extended for potential applications such as antimicrobial studies, bio-imaging, and drug delivery.

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