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Green Synthesis and Characterization of Copper Oxide Nanostructures from *Chromolaena odorata* Leaves Aqueous Extract for Potential Biomedical Applications: An Eco-friendly Approach

Samuel Jawahar B.^{1*} and Princess Rajendran A.²

¹Department of Zoology, St. Johns College, Palayamkottai, Tirunelveli, Tamil Nadu, India

²Department of Zoology, Rani Anna Government College for Women, Tirunelveli, Tamil Nadu, India

**Corresponding Author*

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Abstract: Nanoparticles (NPs) synthesis was applied in several applications using diverse methodologies, and tremendous development was achieved over past decades. One of the most exciting fields in today's century is the use of green chemistry concepts in nanotechnology (NT). The metal NPs intrinsic characteristics may be changed by their size and form and lead to possible uses. This study focuses on the critical function of phytochemicals (PCs) in techniques of green synthesis (GS) which have been mentioned so far in the manufacture of copper oxide (CuO) phytomaterial NPs. The major goal of the current study was the GS of CuO nanomaterials for ecofriendly applications utilizing aqueous extract (AQE) of *Chromolaena odorata*, capped and stabilized under ambient circumstances. The spherical CuONPs in the form of a 10 nm average have been manufactured effectively with the use of an AQE of *C. odorata*. The aqueous extract of leaves of *C. odorata* is a more cost-effective and environmentally friendly alternative to chemical production. CuONPs have been found to be a powerful antibacterial (ANB) and cytotoxic (CYT) agent in the ANB and CYT experiments. Our findings imply superior ANB activity for both Gram classes, with a maximal inhibition zone of 19, 22, 21 and 27 mm, in CuONPs. On the normal Vero line, the CuONPs demonstrated excellent non-toxic effects. The results have a favorable effect on the production of possible therapeutic molecules to address the ailment of concern.

Keywords: Phytogenic synthesis, Antibacterial activity, Well diffusion method, Vero cell line, *Chromolaena odorata*

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Introduction

Green NT is a mushrooming field of science, and our knowledge is generally unfunded and underexploited under the existing circumstances (Kulkarni *et al.*, 2006; Wu *et al.*, 2006; Namburu *et al.*, 2007; Karlsson *et al.*, 2008; Saif Hasan *et al.*, 2008). The important aspects in a GS method are

its eco-friendliness, economic prospects, safety, ease of operation and practicality that indicate a hopeful future for this area (Ren *et al.*, 2009). In the realm of green NT, CuONPs represent the usual and ideal target (Jadhav *et al.*, 2011). Due to the vast uses in catalysis, electronic sensors and

mainly antimicrobials (AMB) and anticancer (ANC) applications, it quickly increases in endless attention (Sun *et al.*, 2012; Siddiqui *et al.*, 2013). CuONPs are often manufactured through a number of very costly and sometimes dangerous physical and chemical processes including the utilization of dangerous and harmful substances, such as sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), hydrazine hydrate ($\text{H}_6\text{N}_2\text{O}$), sodium borohydride (NaBH_4), ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), and triethanolamine ($\text{C}_6\text{H}_{15}\text{NO}_3$). In view of the many magnificent CuONPs, the GS provides itself and demonstrates its power upwards (Ahamed *et al.*, 2014; Kayani *et al.*, 2015). Researches on biological syntheses involving microorganisms (MO's) such as fungus, bacteria and herbal extracts are published to date (Singh *et al.*, 2016). Whilst a variety of biological procedures are accessible, the utilization of plant extracts for the production of CuONPs is possibly favorable for simple, less bio-hazardous and complex cell culture maintenance operations over MO's or plant extracts (Alswat *et al.*, 2017). In addition, the use of leaves extract in CuONP synthesis is due to its eco-friendly, inexpensive and speedy approach towards green NT (Rehana *et al.*, 2017).

Due to its unique features, nano-crystalline noble metal has developed in recent years as an interesting application. CuO nanometal structures have attracted substantial interest among other noble metals as they show exceptional potential as a catalyst for extremely reactive, simple p-type metal-oxide semiconductors with monoclinic structure and a high surface volume (Ma *et al.*, 2020). In the synthesis of CuONPs, use of green chemical methods has been explored and the use of biological entities for NPs of the metals has been highlighted (Tao *et al.*, 2021). In this respect, this study focused on a combination of CuONPs and demonstrated their efficiency for stability of NPs with *Chloroxylon swietenia*, *A. albizia* and *C. odorata*, aqueous leaves extract at various time intervals and EDAX tests with best possible stability. Among the plant extracts tested *C.*

odorata, aqueous leaves extract alone produced CuONPs nanoparticles significantly. Hence the *C. odorata* aqueous extract based nanoparticles alone taken for further studies.

We describe an easy and environmentally favorable way to CuONPs synthesizing copper ion reduction using aqueous *C. odorata* extract which is bioactive. *C. odorata* belongs to Asteraceae family. Polyphenols are the principal component of *C. odorata* extract from aqueous leaves and it poses many biomedical applications such as anticonvulsant (Amazu *et al.*, 2013), anti-diabetic and anticataract (Onkaramurthy *et al.*, 2013), antioxidant (Uhegbu and Onwuegbuchulam, 2016), anti-diarrhoeal (Aba *et al.*, 2015), antifungal activity (Ngane *et al.*, 2006), Anti-inflammatory activity (Owoyele *et al.*, 2005), antiplasmodial activity (Ezenyi *et al.*, 2014), nematocidal agents (Thoden *et al.*, 2007), wound healing (Anyasor *et al.*, 2011) and anticancer activity (Lee *et al.*, 2008; Hung *et al.*, 2011; Prabhu and Ravi, 2012; Yajarla *et al.*, 2014; Adedapo *et al.*, 2016; Ahmad *et al.*, 2019; Yusuf *et al.*, 2021).

This study focuses on the critical function of phytochemicals (PCs) in techniques of green synthesis (GS) which have been mentioned so far in the manufacture of copper oxide (CuO) phytomaterial NPs. The major goal of the current study was the GS of CuO nanomaterials for ecofriendly applications utilizing aqueous extract (AQE) of *Chromolaena odorata* capped and stabilized under ambient circumstances.

Materials and Methods

The following materials and techniques for the green production of metal NPs have been adopted in this study. Prepared NPs were characterized using the following methods. In addition, the green synthesized CuONPs biological uses are explained in this section.

Plant used for the phytogenic synthesis of CuONPs:

In this study, the phytogenic production of CuONPs was based on diverse plant species including *C. swietenia*, *A. amara* and *C. odorata*

leaves. These are the most morphologically diversified, complex species of the biggest genera of the Rutaceae, Fabaceae and Asteraceae, respectively in Asia's forest and are an eternal green tree. These are used as an anti-pyretic, antioxidant, astringent, anti-diarrheal, anti-cancer, anti-dysenteric, anti-inflammatory and tumour therapy (Mancebo *et al.*, 2000; Choi and Hwang, 2005; Soto, *et al.*, 2011). In this study, plant leaves of *C. swietenia*, *A. amara* and *C. odorata* were obtained from Ponneri near Chennai, India. The samples are dried in shade for one week and pulverized to continue the procedure.

Chemicals and Reagents:

A metal precursor has been acquired from Hi-Media Laboratories, Pvt., Ltd., Mumbai, India for synthesizing CuONPs and other compounds such as Copper sulphate dehydrate ($\text{CuSO}_4 \cdot (\text{H}_2\text{O})_x$) analytical grade (AR) and Muller Hinton agar were obtained from Sigma-Aldrich Pvt. Ltd, India.

Preparation of plant extract:

25 g of *C. swietenia*, *A. amara* and *C. odorata* powders (separately) were taken in a conical flask with 500 ml distilled water (DW) and heated for 30 min with continuous stirring with magnetic stirrer. The extracts were filtered using Whatman No.1 filter paper, and all the extracts were exposed to rotary evaporators to remove solvents completely. For further examination, the extracts were kept at 4 C (Rajasekar *et al.*, 2020).

Phytosynthesis of CuONPs:

In order to prepare the CuONPs, 10 ml of the filtered extracts was mixed to 100 ml 1 mM $\text{Cu}(\text{OAc})_2$ solution and the mixture was heated at 80°C for 100 min. The formation of nanoparticles was confirmed by change in color to dark brown, an outcome of surface plasmon resonance excitation. The resulting sediments were rinsed thrice with deionized water, chloroform and ethanol successively and finally dried in air for 48 h (Rajasekar *et al.*, 2020).

Characterization of prepared CuONPs:

UV-Visible Spectroscopy:

Double beam spectrophotometer with a wavelength range between 300 and 700 nm has proved the creation of CuONPs. The stability of CuONPs was further examined by measuring the absorption at regular intervals between 300 nm and 700 nm (12 h, 24 h, 48 h, 5th day, 10th day, 20th day, and 30th day).

Scanning Electron Microscope (SEM) Analysis:

SEM analysis is a powerful method to analyze NPs crystalline morphology. The incoming electrons in SEM affect the sample's surface and are dispersed elastically by the atoms. While the incident electrons lose their fraction of the kinetic energy during elastic spreading, by activating more electrons or exciting the atoms in the sample. The Hitachi S-3400 SEM equipment produced SEM pictures of green manufactured NPs.

ANB activity by Well Diffusion Method:

Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and Gram-negative bacteria i.e. *Escherichia coli* and *Pseudomonas aeruginosa*, were studied for ANB activity in CuONPs well-diffusion test (Jahangirian *et al.*, 2013). The pathogens were acquired from The Bacterial Collection Centre, IBMS, Taramani, Chennai. On Muller-Hinton agar plates, aseptically, the newly cultivated bacterial suspensions (100 µl) were swabbed and wells were created using sterile cork borers. Positive control, Streptomycin (20 µg), plant extract (80 µg) and CuONPs (80 µg/ml) have been injected into the wells. During 24 h, plates were incubated at 37 C and there was an inhibition zone (ZOI, mm) around the wells.

Minimal Inhibitory Concentration (MIC) of CuONPs:

Gram-positive bacteria (*B.subtilis*, *S.aureus*) and gram-negative bacteria (*E. coli*, *P. aeruginosa*) were examined by the MIC of CuONPs in an agar well diffusion analysis (Boyanova *et al.*, 2005). In Muller-Hinton, aseptically the freshly cultivated

bacterial suspensions (100 µl) were swabbed and wells formed by the aid of a sterile cork borer. The wells were charged with various levels of biosynthesized CuONPs (25, 50, 75, 100 µg/ml). During 24 h, the plates were incubated at 37 C and there was an inhibition zone (ZOI, mm) around the wells.

CYT study using Vero cell line:

Cell Culture Maintenance:

Vero was obtained from the NCCS, Pune, India. DMEM was maintained with 10% (v/v) of inactivated FBS, 100 U/ ml penicillin, and 100 µg/ml streptomycin during the logarithmic growth phase. They were kept in an air humidified incubator at 37 C, with 5% CO₂ (Carmichael *et al.*, 1988).

CYT effect:

By means of MTT, the sample's CYT impact was examined against Vero cell line. The cells were planted in 96-wave microplates (1 x 10⁶ cells per well) and cultured in 5% CO₂ incubator at 37 C for 48 h, enabling a confluence of 70-80% to develop. Then the media was replenished and the cells treated with various levels of sample (12.5, 25, 50, 100, 125 µg/ml) for 24 h. Untreated (control) morphological alterations and treated cells were studied and photographed using the inverted digital microscope (40 x magnifications). The cells were then rinsed in saline with phosphate-buffer (PBS, pH-7.4) and to each well added 20 µl MTT solution (5 mg/ml in PBS). The plates then placed in the dark at 37 C for 2 h. Crystals from the formazan were dissolved in 100 µl DMSO. With a spectrometrical absorbance at 570 nm the cell viability percentage using the following formula has been computed:

$$\text{Cell Viability (\%)} = (\text{Absorbance of sample} / \text{Absorbance of Control}) \times 100$$

Graph was plotted using the cell viability (%) at Y-axis and concentration of the sample at X-axis.

Results and Discussion

Characterization methods have demonstrated the green production of CuONPs employing the AQE of

C. swietenia, *A. amara* and *C. odorata* leaves as reduction, capping and stabilization agents (Fig. 1). Double beam spectrophotometers with a wavelength range between 300 and 700 nm have proved the creation of CuONPs. The stability of CuONPs was further examined by measuring the absorption at regular intervals between 300 nm and 700 nm (12 h, 24 h, 48 h, 5th day, 10th day, 20th day, and 30th day).

The UV-Vis samples (Figs. 1 A, B, C, D) illustrate that it changed from light brownish to brick-red owing to surface resonant vibration (SPR) CuO NPs (SPR) excitement at 380-390 nm, which indicated CuONPs formation. The stability of CuONPs in *C. odorata* CuONPs has been discovered to be long-lasting for up to 30 days (Fig. 1) whereas other plants like *C. swietenia* CuONPs and *A. amara* CuONPs remain 30 min stable. Our observation on UV-Vis showed that *C. odorata* produced CuONPs based on AQE were very stable compared to the other two plant species. The phytosynthesized *C. odorata* CuONPs using this procedure are quite stable with no major uptake changes, even after 30 days (Fig. 1D), due to the presence in the leaves of *C. odorata* that surround the Cu⁺ ions, of a spectrum of PCs forming CuONPs. For the EDAX investigation, *C. odorata* CuONPs were taken to identify high-resolution sample pictures by scanning a focused electron beam over a surface and to detect secondary or back-scattered electron signal. An EDAX is also utilized to offer basic and quantitative identification data.

Morphological Features:

SEM evaluated the morphological characteristics and the size of CuONPs. It has been found that the spherical form is an average of 10 nm (Figs. 2A, B). The EDAX chart showed that elementary copper (Cu), oxygen (O₂) and carbon (C) were present and that copper was reduced to CuONPs (Fig. 2C). The SPR absorbance peak for CuONPs was in the UV vis. spectrophotometer, around 380-390, confirming that CuONPs were formed

Fig. 1: UV-Vis absorption spectra (A) *C. swietenia* CuO NPs, (B) *A. amara* CuO NPs, (C) *C. odorata* CuO NPs, and (D) Stability of *C. odorata* CuO NPs observed at different time intervals.

Fig. 2: (A and B) SEM image of *C. odorata* CuONPs; and (C) EDAX image of *C. odorata* CuONPs.

(Murugappan, and Sreeram, 2021). SPR usually depends on NPs dielectric and morphological characteristics (Rahdar *et al.*, 2021; Chandrasekar *et al.*, 2021; Hamami, and Javanbakht, 2021). Ganesan *et al.* (2020) explored the possible responsibility for synthesizing CuONPs of the PCs in the aqueous leaf extract of *C. odorata*. The SEM examination provided a 10 nm size distribution of CuONP, which was consistent with the absorption of the spectrophotometer UV-Vis. The presence of bio-molecules in the *C. odorata* aquatic extract, a stabilizer and reducing agent that regulates the formation of NPs, showed that the NPs were disseminated with minimal agglomerations. The size of the NPs might also be concluded crucially depending upon the features of the AQE.

The NPs may be synthesized by employing GS pathways that remove the usage of dangerous chemicals in different sizes and shapes. For further production of NPs in biomedical applications *C. odorata* AQE of different active reducing and stabilizing agents might be employed. Further investigation could be carried out to grasp fully the mechanism behind synthesized nanoparticles' ANB and CYT properties. In additional biomedical applications, these NPs can be employed when combined with polymers. The ultimate objective in this path is to increase the knowledge, the efficiency and the usefulness of these NPs and their biological activity for potential implementation. We present an easy and green technique to CuONPs synthesizing copper ions reduction utilizing AQE from physiologically active *C. odorata*. Polyphenols were the main components of *C. odorata* extract. Our work has shown that the *C. odorata* aqueous leaves extract may be employed as a stabilizer and as a reduction agent in the particle development process. The assessment of ANB and synthetic CuONPs' CYT using AQE has also been described.

Table 1 and Figure 3 illustrate anti-bacterial effectiveness of *C. odorata* mediated AQE CuONP. Different concentrations of aqueous *C. odorata* (80 µl), CUONP (80 µl) and Streptomycin (20 µl) were

used to treat the pathogenic bacterial strains as the positive control. The results demonstrated a greater activity in bacterial strains tested for CuONPs produced with *C. odorata* than that of AQE. In *S. aureus* the highest inhibitory zone than other bacterial species has been recorded. The positive control indicated that higher activity is possible for CuONPs because of Streptomycin's ANB nature. There is no clear understanding of the mechanism behind the ANB potential of CuONPs. Gordon *et al.* (2010) reported that the main bacterial Thiol respiratory enzyme interacts with CuONPs, which contribute to cell death. Some investigations have shown that the positive Cu⁺ ion interacts in the electrostatic contact and disrupts cell membrane on the negative bacterial cell walls.

The other proposed process is the development of irregular pits in the external membrane of the bacteria that cause permeability of the membrane (Khatami *et al.*, 2017; Awwad and Amer, 2020). The toxicity of CuONPs is likely indirectly affected by particulate features such as size, form, surface covering, reduction and stability agent. Because of their high volume to volume surface ratio, smaller NPs are more toxic and improve the penetration potential, which boosts overall ANB effects. This study showed that the ANB efficacy of CuONPs depends on the Gram class of bacteria, size and shape of the NPs. The stabilizing and reducing agents utilized also play a key role in controlling NPs, their size and structure.

Agar well diffusion test was used to assess its ANB activity. The medium has a 6 mm diameter and different concentrations of CuONP have been filled (25, 50, 75, 100 µl). As a positive check, streptomycin has been utilized. A clear region without growth of bacteria was specified for the diameter of the inhibition zone. Due to its outstanding inhibitory activity against pathogens in bacteria, the copper ions were used in many therapeutic preparations for the prevention of

Table 1: Bacterial growth inhibition of *C. odorata* AQE and its CuONPs

S. No.	Pathogen	Zone of inhibition (mm)		
		Streptomycin 20 μ l)	Aqueous extract 80 μ l	CuONPs 80 μ l
1	<i>B. subtilis</i>	23	No activity	13
2	<i>S. aureus</i>	29		26
3	<i>E. coli</i>	21		12
4	<i>P. aeruginosa</i>	23		15

Fig. 3: ANB activity of CuONPs synthesised using *C. odorata* AQE (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, (D) *P. aeruginosa*. Red coloured encircle indicates the zone of inhibition induced by CuONPs; yellow coloured encircle indicates the zone of inhibition induced by Streptomycin; and rose coloured encircle indicates the zone of inhibition induced by AQE.

Table 2: Minimal inhibitory concentration of *C. odorata* CuONPs

S. No.	Pathogen	Zone of inhibition in mm				
		Streptomycin 20 µl	CuONPs (µl)			
			25	50	75	100
1	<i>B. subtilis</i>	24	-	14	20	22
2	<i>S. aureus</i>	30	08	10	16	27
3	<i>E. coli</i>	22	-	10	15	21
4	<i>P. aeruginosa</i>	25	-	09	12	19

wound infections. Cupric interactions with the thiol groups of important respiratory enzymes in bacteria are linked to CuONPs significant ANB effects on bacterial cells. The study effectively evaluated the biosynthesized ANB activity of CuONPs against gram-positive (*B. subtilis*, *S. aureus*) and gram-negative (*K. pneumonia*, *E. coli*) pathogens. Our results clearly showed that the AQE leaves of *C. odorata* CuONPs had strong ANB activity, with a high inhibition zone of 19, 22, 21 and 27 mm for *P. aeruginosa*, *B. subtilis*, *E. coli* and *S. aureus*, between gram-positive and gram-negative bacteria (Table 2).

Figure 4 clearly shows that CuONPs showed a significant level of activity than Streptomycin antibiotic. CuONPs ANB activity is considerably enhanced with the increased concentrations and improved activity at a greater dose-dependent concentration of 100 µl. Synthesized CuONPs by leaves can minimize the problem of use of risky chemicals to reduce and stabilize *C. odorata* extracts, therefore making NPs biocompatible using an environmentally benign method. This work has therefore proved that CuONPs from *C. odorata* leaves AQE are a good ANB agent that may be exploited in pharmaceutical sectors.

CYT activities of CuONPs against Vero cell line:

MTT assays based on the measurement of Mitochondrial dehydrogenase (MDH) activity in live cells have determined the cytotoxic effect of CuONP on Vero cell line. Different levels of

CuONPs in the Vero cell line were exposed (12.5, 25, 50, 100 and 125 µg/ml). Following 24 h incubations, the inhibitor effect was detected. Results showed that considerable cell death occurred at greater doses. Figure 5 illustrates variations in the inhibition percentage of Vero cells treated with CuONPs. Vero cells were considerably suppressed when 12.5-125 µg/ml of material was treated with biosynthesized CuONPs. In the concentration 125 µg/ml the maximum cell growth inhibition was recorded at 28 per cent. The CuONPs were able to directly (dose dependent) lower the viability of the vero cell line. The vero cell development by 50% must be inhibited by a minimum of 100 µg/ml of CuONPs. The morphological alterations on cell line in different CuONPs concentrations are illustrated in Table 3 and Figure 5. Results showed that the increased cellular damage also caused cell death by the concentration of CuONPs.

Smaller CuONPs harm the main macromolecules such as proteins and DNA found in the cell through sulphide and phosphorous transmission. The harmful impact of CuONPs is presumably because CuONPs impede the correct functioning of cell protein, and hence lead to subsequent cell chemistry alterations. Based on the current investigations it might be defined as CuONPs breaking through the permeability of the external membrane resulting in the leaking of cellular components, the mechanism behind the CuONPs toxicity. CuONPs infiltrate the inner

Fig. 4: ANB activity of CuONPs synthesised using *C. odorata* AQE; (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, (D) *P. aeruginos*; C1, C2, C3 and C4 denotes 25, 50, 75 and 100 µl and S denotes Streptomycin.

Table 3: Percentage of viability on Vero cell line by CuONPs synthesized via *C. odorata* leaves AQE

Concentrations (µg/ml)	Absorbance		Average	Cell Viability (%)
	I	II		
Control	0.905	0.916	0.9105	100
12.5	0.922	0.91	0.916	100.6040637
25	0.879	0.866	0.8725	95.82646897
50	0.806	0.795	0.8005	87.91872597
100	0.411	0.424	0.4175	45.85392641
125	0.256	0.263	0.2595	28.50082372

Fig. 5: Percentage of viability on Vero cell line by CuONPs synthesized via *C. odorata* leaves AQE.

Fig. 6: Cytomorphological changes of biosynthesized CuONPs using *C. odorata* aqueous leaves extract in Vero cell line at different concentration (A) Control cells; (B) 12.5µg/ml; (C) 50µg/ml; and (D) 100µg/ml (E) 125 µg/ml.

membrane and make the respiratory chain inactivate, preventing breathing and cell development. At the same time, CuONPs could impact certain proteins and phosphate lipids and cause membrane collapses, which might finally lead to cell breakdown and death. The size, form and concentration of CuONPs relies mostly on the toxicity. When CuONPs have been biosynthesized, the chemical compositions in the aqueous leaves extract of *C. odorata* were influenced, leading to different bio-activities.

C. odorata aqueous leaves extracts were effectively synthesized with spherical, spherical CuONPs, average size 10 nm. *C. odorata* extract is a superior option to chemical production since the extract is cost-effective and environmentally acceptable. CuONPs were shown to be a powerful ANB and CYT agent in ANB and CYT. Our results indicated that CuONPs demonstrated higher ANB activity with a maximal inhibition zone of 19, 22, 21, and 27 mm in both Gram classes of bacteria. In the current work, the potential CYT of CuONPs against Vero cells is also explored. The *in vitro* CYT test was performed on the cell line of Vero. The results showed that the CuONPs are more cell-labile at all the different concentrations tested. The produced CuONPs are therefore not toxic towards the Vero cell line (Fig. 6). The use of *C. odorata* aqueous leaf extract for CuONP production is also an efficient alternative and is ideally designed to liberate CuONPs for therapeutic applications for harmful pollutants. The extract of *C. odorata* leaf is an agent which reduces and stabilizes the size and shape of the NPs. In targeted medication supply and clinically diagnosed use is made of green-synthesized CuONPs.

Conclusion

An easy and quick way to synthesizing CuONPs carried out with *C. odorata* aqueous leaves extract. The phytochemical resource is a plant-based resource for bio reducing and stabilizing CuONP production. Different characterization techniques such UV-vision spectroscopy and SEM-EDAX are

used to produce as-synthesized CuONPs. UV-Vis spectrum illustrates the CuONP reduction process; where the colour of the sample solution progressively shifts from light brownish to red brick owing to the CuONP excitation at 380-390 nm for the creation of CuONPs with the SPR vibration. The stability of CuONPs in *C. odorata* CuONPs lasts for up to 30 days and stays for 30 min in other plants such as *C. swietenia* CuONPs and *A. amara* CuONPs. This study on UV-Vis showed that *C. odorata* produced CuONPs based on AQE are very stable compared to the other two plant species. The phyto-synthesized CuONPs are highly stable even after 30 days, especially due to the presence of a spectrum of PCs on the leaves from *C. odorata* around Cu⁺ ions to produce CuONPs. CuONPs had no significant effect on absorption peaks. Spherical CuONPs were produced using *C. odorata* aqueous leaves extract, which had an average size of 10 nm. The extract of *C. odorata* is a superior option to chemical synthesis, given that it is economically viable and environmentally beneficial. CuONPs were shown to be a powerful ANB and CYT agent in ANB and CYT experiments. The AQE of *C. odorata* functions as a reduction and stabilizer that may provide regulated sizes and shape for NPs. In targeted medication delivery and clinical diagnosis the GS CuONPs are employed.

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