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Biogenic Synthesis of *Chromolanae Odorata*-mediated Copper Oxide Nanoparticles for Use as Antifungal and Photocatalytic Agent

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ABSTRACT

Plant-mediated nanoparticles (NPs) synthesis has been recently explored because of its numerous merits over chemical or physical methods. The present effort was to biogenically synthesize and characterize CuO NPs using aqueous extracts of *chromolanae odorata* for possible use as photocatalyst or an antimicrobial agent. The aqueous plant extracts as well as the CuO green NPs were characterized for their optical, structural, surface morphological, elemental and average particle sizes using Uv-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), x-ray diffraction spectroscopy, scanning electron microscope coupled with energy dispersive x-ray spectroscope (SEM-EDX) and dynamic light scattering (DLS) respectively. Results showed that both extracts and the green NPs absorbed in the Uv-regions at 300 nm and 250 nm respectively. FTIR indicated the presence important functional groups such as $C \equiv C$, amines, alcohols which are important in photocatalysis and or adsorption studies with Cu – O bond length at 801 cm^{-1}. XRD showed a crystalline phase of face-centered-cubic structure of monoclinic CuO NPs, with average crystallite size of 12.49±0.1 nm while SEM-EDX indicated large irregular shapes with large pore sized, having combination ratio, Cu:O of 1:2. In addition, DLS results showed a hydrodynamic particle size of 72.45 nm and polydispersity index (PDI) of 0.44; which were in variance with the crystallite size obtained from XRD and indicated possible agglomeration of the NPs. The important active sites, nano-particulate/crystallite size and hydrophobicity are indication that the NPs will be suitable both a multi-spectrum antimicrobial and photocatalytic agent.

Keywords: Nanoparticles, *Chromolanae-odorata*, Photocatalysis, CuO, Antimicrobial-agent.

1 Introduction

Research on green-nanoparticles synthesis has skyrocketed in the recent years. The importance attached to this novel approach sterns from its sustainability, economical-nature, feasibility, and eco-friendly nature of the products [1]. In contrast to the conventional synthetic techniques, which are considered disadvantageous because of the use of hazardous chemicals, biological risks, and high energy consumption; biogenic synthesis employs phytochemicals present in biomaterials such as plants-extracts and microorganisms as reducing, stabilizing and capping agent. These have made plant synthesis more eco-friendly, sustainable and less toxic and the most preferred [2].

Nanotechnology has transformed various scientific and allied fields by enhancing the design, fabrication and application of materials at the nanoscale, where unique physical and chemical properties emerge. It deals with the miniaturization and manipulation of matter into the nano-range dimensions, often between 1–100 nm in contrast with their bulk counterparts [2-4]. Materials in this category, otherwise called nanoparticles; are known to have higher surface-to-volume ratio and this has endeared them to scholars in various fields such as medical, chemical, food, electronic, and healthcare industries [3, 4].

As one of the most abundant elements in nature, in addition to its low cost and high conductivity (In comparison with silver or gold), Cu has drawn tremendous attention from nanotech-researchers [5-6].

Compared with other nanoparticles, metal oxide-based NPs have triggered overwhelming as a result of its versatility in terms of application in various fields such as medical, catalysis, and environmental remediation [4]. Due to their narrow to wide bandgap (1.35–3.5 eV), large surface area, minimal toxicity, accessibility, surface synthesis and enhanced oxygen adsorption capability; CuO nanoparticles are regarded as the most sought after antimicrobial agent as well as semiconductor photocatalysts under sunlight irradiation [7]. Green synthesized CuO nanoparticles have high potentials as both antibacterial and photocatalyst due to presence of secondary metabolites and phytochemicals such as phenols and flavonoids often found in either roots, stem and or leaves of the plant. The phytochemicals serve as reducing, capping and stabilizing agents presenting possible agglomeration and deformation of the NPs and enhancing adsorption [3, 6]. Their antifungal properties are particularly noteworthy; CuO nanoparticles have shown efficacy against a broad spectrum of fungal pathogens, which is crucial in combating fungal infections and mitigating the development of resistance [8].

Chromolaena odorata, otherwise called saem weed, Awolowo or Akintola weed in South west Nigeria; is a widely studied medicinal plant known for its extensive bioactivity. It is tropical herb, belonging to Asteraceae family, which has demonstrated a range of therapeutic properties such as antipyretic, anti-inflammatory, anti-ulcer, analgesic, anti-diarrheal, antihypertensive, diuretic, antimicrobial, and antipyretic due to its rich phytochemical profile [8]. The plant's extracts contain a variety of phytochemical compounds, such as flavonoids, terpenoids, quercetin, tannins, saponins, alkaloids and phenolic acids, together with two important amino acids, histidine and phenylalanine; both of which have strong reducing, capping and stabilizing capabilities [9 - 11]. Flavonoids that are found in the plant extracts has been noted to responsible for its high wound healing activity using free radical method [12Therefore, aqueous leave extracts of the c-odorata which often have pungent to aromatic odour possess high inhibitory properties against microbes and are also used to enhance hemostasis and blood coagulation [13]. These properties make *chromolaena odorata* an ideal candidate for the synthesis of metal nanoparticles with high economic prospects.

This study therefore explores the biogenic synthesis of CuO nanoparticles using *Chromolaena odorata* as a reducing stabilizing and capping agent and investigated its physicochemical properties as a potential antifungal and photocatalytic agents. It also leveraging the natural phytochemicals present in *chromolaena odorata*, this approach aims to enhance the efficacy and sustainability of CuO nanoparticles in various applications, paving the way for innovative solutions in both healthcare and environmental management.

2 Materials and Methods

2.1 Sample collection and Identification

The weed, *Chromolanae odorata* leave samples were collected from the Polytechnic environment, washed under running water so expunge dirt, dried in the laboratory under a limited sunlight for seven days [14]. Before drying, samples were first properly identified and confirmed at the Forestry Research Institute, Ibadan, Oyo State, Nigeria; and was assigned the voucher number FHI 109494 from their herbarium. It was thereafter washed under running water and It was later blend to powder (for faster extraction) using high capacity Binatone blender.

Other reagents used in this study were all analytical grade obtained from Sigma-Aldrich® hence, there was no further preparations.

2.2 Preparation of the C-odorata aqueous extracts and CuO GNPs

20 g of the C.odorata powder was measured into a 400 mL beaker, boiled for 20 minutes at 60^{o}C [10] and allowed to cool to room temperature, then; filtered using Whatman No.1 filter paper.

For the synthesis of the CuO green NPs, 50 mL of the *C-odorata* extracts was added to 200 mL of 0.1M CuSO4 (Sigma Aldrich Inc. USA) and stirred continuously for 2hr at 60oC, 300rpm. Formation of NPs was noticed with the gradual colour change of the bath from darkish-brown to dark colour. It was dried in oven for 24hr at 60oC, washed using distilled water and ethanol and calcined in a pyrolizing furnace at 400±1oC to remove impurities. The brownish-black CuO green NPs was stored in desiccator for further characterization [15].

2.3 Characterization of the prepared CuO GNPs

The fabricated green NPs were characterized for its optical, structural, surface morphological, elemental and particle size/hydrophobicity using Uv-visible spectrophotometer Jenway, 7315 Spectrophotometer (λ=200 - 700 nm), FTIR (Agilent technologies) with spectrum recorded using transmittance method in the 4000–650 cm^{-1} region at 30 sample scans, powder XRD (model X' Pert Pro – PAnalytic, Japan). Similarly, surface characteristics of green NPs was determined using scanning electron microscope coupled with energy dispersive x-ray spectroscopy (SEM-EDX) (model Phenom ProX, by phenomWorld Einhoven, The Netherlands) where sample was placed on a double adhesive on a sample stub, which was coated with sputter coater by quorum technologies model Q150R, with 5 nm of gold while average particle size and hydrophobicity was analyzed using dynamic light scattering equipment.

In addition, the XRD spectra was recorded at room temperature, using reflection-transmission spinner stage with 2θ settings, X-ray source; Cu Kα-radiation (λ=1.54Å to 0.154 nm) at scan rate of 4o/min in Bragg-Brentano geometry with programmable divergent slit optics and a 5mm width mask using Gonio scan. The 2θ starting position was 4o and terminates at 75^{o} degrees with a 2θ step of 0.026261 at 8.67 seconds per step, with a tube current of 40mA at a tension of 45VA. Average crystallites size of the biochar-doped ZnO NPs was estimated using Scherrer formula from the broadening of the diffraction peaks while instrumental resolution function was used to correct possible broadening effect of the instrument on the measured peaks so as to obtain accurate crystallite size. Full width height maximum (FWHM) was measured using peak detection method. The spectrum of the FTIR was recorded using transmittance method in the 4000–650 cm^{-1} region with 30 sample scans.

3 Results and Discussion

3.1 Uv-visible characterization

Bio-utilization of nanoparticles to a very large extent, depends on several factors such as charge present on surface, functional group attached, morphology, structure and size of a nanoparticle [16]. These properties absorb therefore determines whether the NPs will absorb either in the Uv, visible or near-infrared regions. This is as a result of surface plasmon resonance (SPR) phenomenon which explains the collective oscillation of surface electrons of nanoparticles. Dispersion of plasmonic nanoparticles produces one or more peaks that can be exploited to obtain relevant information on shape, size, and size distribution due to the SPR property of nanoparticles [17].

Figures 1a and b showed that the both the extracts and the NPs absorbed in the Uv-regions of the spectrum at 300 nm and 250 nm respectively. Absorption of the NPs at this wavelength is linked to surface plasmon absorption of ZnO nanoparticles while that of the extracts could be as a result of the chromophores in the phytochemicals [18]. SPR in metal nanoparticles is said to occur as a result of the collective oscillation of the free conduction band electrons which is excited by the incident electromagnetic radiation. The width and frequency of the SPR largely hinged on numerous factors such as shape, particle size, adjacent media and the dielectric constant of the metal [19]. This result agrees with the report of Kumar et al. [20]. The change in colour of the reaction mixture from darkish-brown to brownish-black was therefore due to this surface plasmon resonance phenomenon which indicated the formation of CuO green nanoparticles [20].

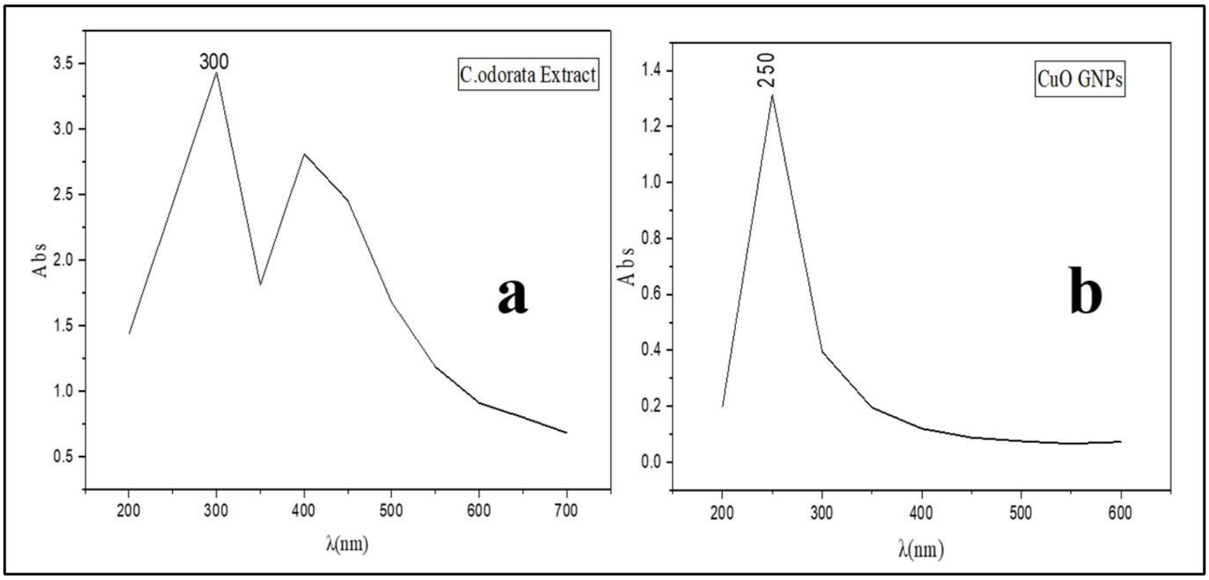

Figure 1: 1 a and b: UV-visible spectra of the C.odorata extracts and the CuO green NPs

3.2 FTIR Characterization

Functionalization of metal NPs has been reported to affect their bioactivity [16]. In order to properly understand the exact role played by the aqueous leave extract of the C-odorata as a reducing and capping, FTIR spectra of the green-fabricated CuO NPs (Fig. 2) was obtained in the wavenumber range of 400–4000 cm-1.

Peaks at 2116.1, 1640.6, 1422.8, 1297.1, 1062.4, 801.7 shows the presence of alkyne, primary amines, various aromatic alcohols, secondary alcohols and metal substituted species respectively. In addition, the low peak at 801 cm-1 is attributed to the Cu – O bond length or stretching, which indicates the successful synthesis of the NPs [6, 20]. The presence of –OH and $-NH_2$ in the sample is a clear testament that the extract provided the capping agent and that the NPs will be suitable as a photocatalyst (Khan *et al.*, 2023) [4].

From the result, the phytochemicals present in the extract were responsible for the reduction of the metal salt and stabilizing NPs against possible agglomeration [6, 23]. Indeed, these FTIR peaks are consistent with the crystalline biosynthesized CuO NPs pure phase characterized by XRD [15].

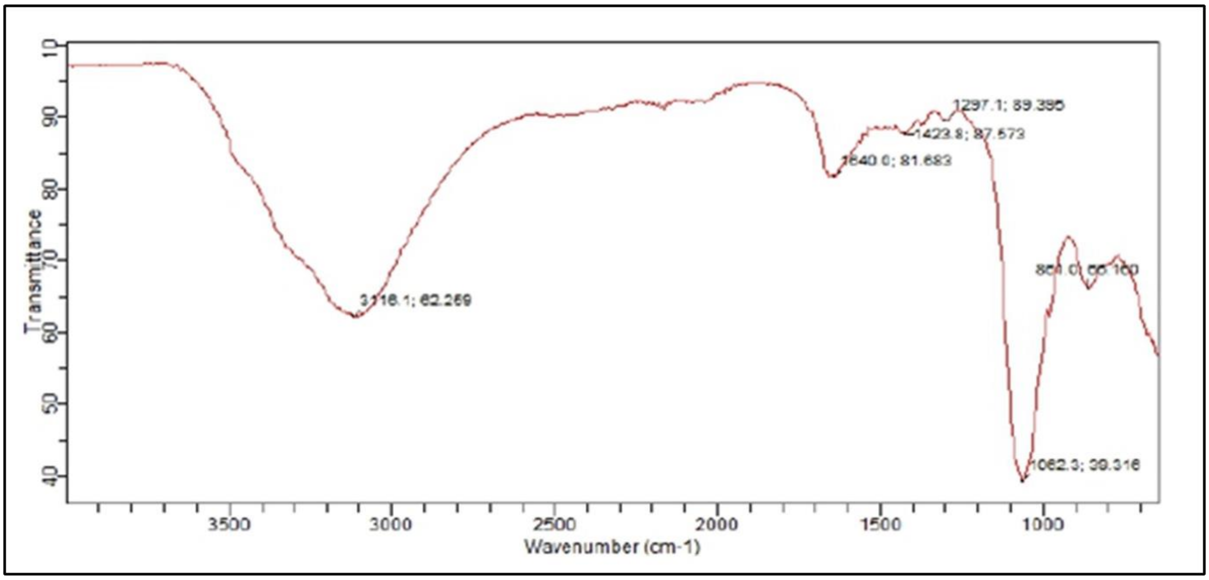

Figure 2: FTIR spectra of the synthesized CuO Green NPs

Table 1: FTIR analysis of the green synthesized ZnO NPs

Sample Name	Absorption cm-1	Intensity	Assignment	Class of compound
	2116.1	M	C≡C	Alkynes
	1640.6	S	N-H Bending	Primary amines
	1422.8	S	Ar-C-C stretching	Aromatics
	1297.1	m-s	Ar-OH (C-OH) Stretching	Aromatic alcohols
CuO GNPs	1062.4	m	C-O stretching	Secondary alcohols
	801.0	S	C-H bend	metal-disubstituted aromatic compound

3.3 XRD characterization

The X-ray diffraction pattern (X' Pert Pro – PAnalytic) with CuKα radiation (λ=1.54Å which is equivalent to 0.154 nm) was used to estimate the phase and crystalline quality of the CuO GNPs at 2θ range of 20° – 70°. Figure 3 showed amorphous-crystalline phases with diffraction prominent peaks at 36.4°, 39.3°, 42.6°, 44.3°, 47.4°, 49.4° , 51.9° and 57.4° corresponding to (002) (111), (-111), (-111), (-202), (202), (020), (113) which aligns with the face-centered-cubic structure of monoclinic CuO NPs according to JCPDS card no: 45-0937 [7, 19, 24-25]. Peaks observed at 42.6°, 44.3°, 47.4° could be ascribed to the presence of impurities, synonymous to Cu_2O [15].

The average crystallite size (D) was estimated by using the basic Scherrer equation [7, 15].

$$D = \frac{0.94\lambda}{\beta \text{Cos}\theta} \quad (1)$$

where λ is the X-ray wavelength (0.154056 nm), D is the crystallite size, β is the line broadening at half the maximum intensity (FWHM) and θ is the Bragg's diffraction angle.

The average particle size was estimated to be 12.49±0.1 nm for the CuO GNPs. Similar crystallite sizes has earlier been reported [18]. Although, this value is lesser than the particle size obtained from DLS, it shows there is less possibility of agglomeration of the NPs with time [26]. Moreover, it has been reported that the size and or shape of NPs controls both structural, chemical, physical, magnetic and other properties. This is because, as the size decreases, surface effects become more significant due to the rising fraction of surface atoms while decreasing the symmetry of the crystals as a result of incomplete atomic coordination [27]. The well-developed peaks at certain angles presenting sharp peak intensities are clear indication that the synthesized NPs are very crystalline.

Figure 4 showed that as the diffraction angle increases, the crystallite size increased until it reached maximum size at 15.01 nm the, continuously dropped. This could be as a result of the narrower peak width indicating more homogeneous crystal size distribution which also led to the XRD peak broadening pattern [28]. Increase in crystallite size could also be an indication of higher material properties such as thermal and or electrical conductivity [5].

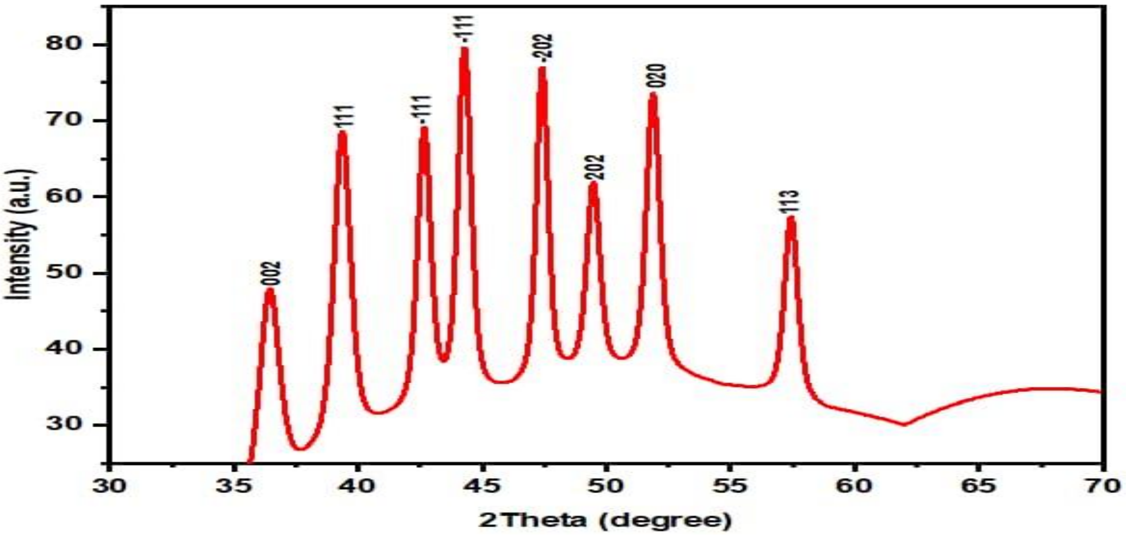

Figure 3: XRD of the synthesized CuO Green NPs

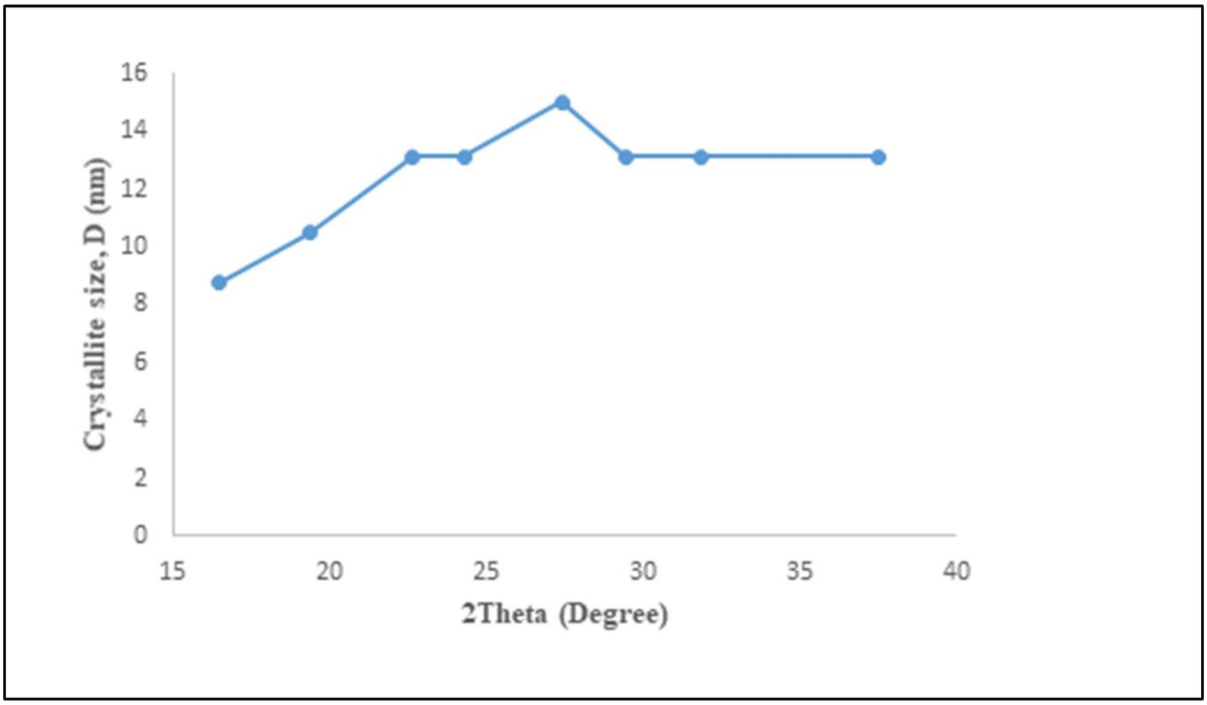

Figure 4: Variation of crystallite size of the CuO GNPs at increasing angle of diffraction

3.4 Surface morphology and elemental composition

Surface properties of NPs plays very important role in their applications because it analyzes their crystalline morphology. Figure 5a is a SEM micrograph of the green synthesized CuO NPs. The micrograph indicated mixed cloudy and large shapes, homogeneously distributed with relatively large pore sizes. As was manifested by the cloudy pattern of the micrograph, it indicated there could minimal or no agglomeration. This is a clear indication of the formation of nanoparticles [29]. Several factors may have influenced the morphology of the synthesized green nanoparticles during the synthesis process. These include the pH of a solution, the calcination temperature, precursor concentration, aging time, chelating agent, the kinetics of precursor analysis, and catalyst dosage [30].

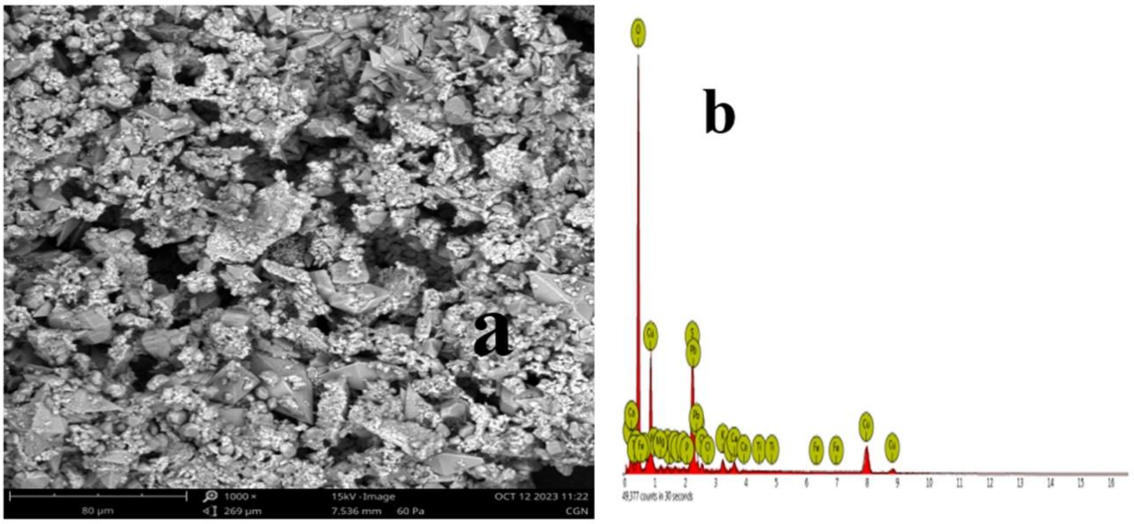

Figure 5: 5a and b: SEM micrograph and EDX elemental characterization of the synthesized CuO Green NPs

Similarly, figure 5b and table 2 shows the presence of important groups I – VII elements. The high concentration of oxygen could be as water used in reagent preparation as well as oxygen atoms accompanying the elements. The result also showed the elemental combination ratio of Cu:O as 1:2. This is typically due to the higher electronegativity and atomic radius of oxygen which enable it to displace Cu in its lattices. Also, the small amount chlorine in the sample could be as a result of potassium and or sodium present the sample which may have come from the plant extracts [31].

Table 2: EDX elemental characterization of the synthesized CuO Green NPs

Element Number	Element Symbol	Element Name	Weight Conc.
8	O	Oxygen	54.45
29	Cu	Copper	26.64
16	S	Sulfur	6.01
19	K	Potassium	3.30
82	Pb	Lead	3.01
20	Ca	Calcium	2.80
11	Na	Sodium	1.69
17	Cl	Chlorine	0.54
13	Al	Aluminium	0.46
12	Mg	Magnesium	0.46
14	Si	Silicon	0.34
15	P	Phosphorus	0.30
22	Ti	Titanium	0.00
26	Fe	Iron	0.00

3.5 Particle size distribution

The average hydrodynamic particle sizes of the green synthesized CuO NPs was determined using DLS which also showed their polydispersity index (PDI). Figure 6, and table 3 showed that the average hydrodynamic particle size and PDI of the of the NPs respectively were 72.45 nm and 0.44. The implication of HPI or particle size is that the synthesized nanoparticles were actually in nanoscale as it is between 1 – 100 nm. On the other hand, the PDI value indicated that the particles are not of equal sizes hence, there is the tendency of possible agglomeration [32]. Moreover, according to International Standard Organizations (ISOs); samples with PDIs less than 0.05 are considered to be monodispersed. Hence, the result obtained in this study showed that the particles are poly-dispersed [33].

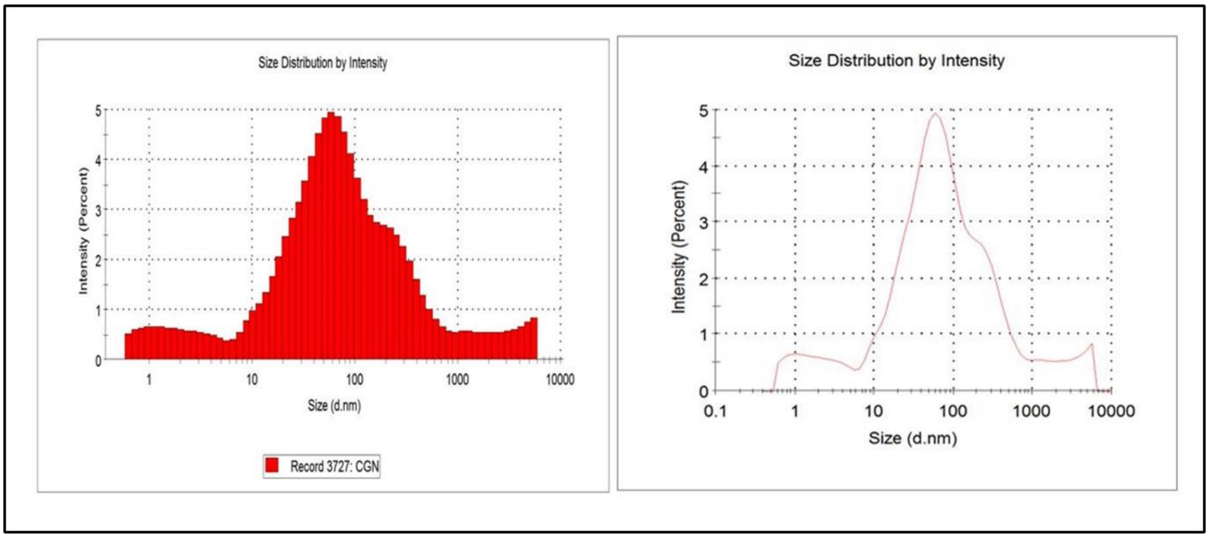

Figure 6: Particle size distribution of the synthesized CuO Green NPs

Table 3: Particle size distribution and polydispersity index of the synthesized CuO Green NPs

S/N	Average particle size (HDI) nm	Polydispersity index
1	72.45	0.44

4 Conclusion

The present study investigated the possibility of using a plant-mediated CuO nanoparticles synthesized using leave extracts of *chromolanae odorata* as a broad-spectrum antimicrobial agent as well as a photocatalyst for the removal obnoxious materials from the environment. Optical, structural, surface morphological, elemental and particle sized evaluation of the NPs indicated that it is a good candidate for the purposes envisioned. The study showed that *c.odorata's* potentials could be harnessed for sustainable economic and environmental purposes.

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