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Research Paper

Green synthesis Copper oxide nanoparticle using fresh leafs of Chenopodiumalbum and evaluating its in-vitro antibacterial activity

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ABSTRACT:

The *Chenopodium album* plant was collected and the fresh plant extract plays a key role in copper oxide nanoparticle synthesis. The reduction of Cu^{2+} ions to CuO NPs by aqueous leaf extract of *Chenopodium album* was visually observed by color variation in the reaction mixture. The UV-visible absorption peak arises from 300-380 nm denote the development of CuO NPs. In our study, the extreme absorption peak seemed at 365 nm directs the individual SPR band for CuO NPs with lesser particle size. In conclusion the copper oxide nanoparticle was synthesized using fresh leaf extract of *Chenopodium album*.

The synthesized nano particle was characterized and confirmed using UV-Visible, FT-IR, XRD, FE-SEM, EDX and SEM mapping analysis, the results showed that CuO Nps were synthesized properly. The *in-vitro* antibacterial assay depicts the effective antibacterial activity of CuO Nps. These conclude that the further study on CuO Nps helps for the drug development.

Keywords: Chenopodium album, CuO NPs, larvicidal activity, aqueous leaf extract, UV-Visible, FT-IR, XRD, FE-SEM, EDX

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Introduction

Bacterial infections constitute a significant cause of recurrent infections and death. Because of its cost-effectiveness and successful efficacy, antibiotics have become the favored therapeutic tool for bacterial infections. In reality, super bacteria, which are immune to almost all antibiotics, have evolved recently due to antibiotic misuse. Studies have shown that these bacteria carry a gene called NDM-1[1] which is super-resistance. Resistance mechanisms include expression of enzymes that modify or degrade antibiotics such as β-lactamases and aminoglycosides [2], modification of cell compounds such as vancomycin resistance cell wall and tetracycline resistance ribosomes [3], and expression of efflux pumps that provide simultaneous antibiotic resistance [4]. Most antibiotic resistance pathways are inaccessible to nanoparticles (NPs) because the mode of action of NPs is direct contact with the bacterial cell wall, without the need to enter the cell; this increases the possibility that NPs would be less vulnerable to fostering bacterial resistance than antibiotics. Hence the focus was on new and exciting NP-based materials with antibacterial activity [5].

. The bacteria are stuck together at this point, creating a barrier that can withstand antibiotics and provide a source for chronic systemic infections. Biofilms thus represent a serious threat to health [6,7]. In addition, Biofilm bacteria Biofilms in particular are less restrained by antibacterial agents than those of the respective planktonic bacteria [8].

Nanomaterials are products of at least one size (1–100 nm) in the spectrum of the nanometer scale or whose basic unit is beyond this spectrum in the three-dimensional space [9].

Overcoming the existing antibiotic resistance mechanisms

Most types of NPs can overcome at least one of the common mechanisms of resistance mentioned in the section "Antibacterial activity of NPs" (including disruption of bacterial membranes and impediment to biofilm formation) [10]. These effects are the result of the NPs bactericidal mode, which is based on their particular physicochemical properties [11]. Unlike typical antibiotics, the measurements of NPs are common, 100 nm. Their unusually small scale contributes to novel properties, such as improved cell activity due to a higher area-to-mass ratio and flexible and controllable application [6]. The processes by which NPs disrupt bacterial membranes are defined in detail in the section on "Antibacterial cycle of NPs;" alternatively, this section considers the interaction of NPs with cell barriers (including cell walls and membranes) and the synthesis of bacterial proteins. Because of its strongly conserved existence, the bacterial

cell membrane is hard to modify with only a few genetic changes, which also decreases the probability of bacterial drug resistance. Biofilm formation hindrance is an important mechanism in addition to disrupting bacterial membranes, as biofilms play an important role in the development of bacterial resistance [12]. The unique composition and structure of bacterial biofilms provide the embedded microorganisms with shelter or protection, helping them escape most antibiotics. Furthermore, bacterial biofilms are "a breeding place" for regular mutations of tolerance and the communication and modification between specific bacterial cells with such mutations.

Studies have shown that many NPs, including Au-based NPs [13], Ag-based NPs[14], Mg-based NPs[15], NO NPs[16,17], ZnO NPs[7], CuO NPs[18], Fe3O4 NPs[19], and YF NPs[20], can prevent or overcome the formation of biofilms. Greater biofilm prevention is achieved through a smaller size and a higher surface area-to-mass ratio, and the particle shape of NPs also has a remarkable effect on biofilm destruction (e.g., NPs with a rod-like shape are more effective than NPs with a spherical shape) [21].

Cupric Oxide (CuO)

CuO is one of copper's primary oxide and higher oxide. It is an anti-ferroelectric p-type semiconductor with a gap of about 1.4 eV in the narrow band. CuO has a unit cell and is monoclinical. It is highly insoluble and thermally stable oxide, and very suitable for applications in glass, optics and ceramic. CuO is used as a flux for CA metallurgy, as an optical polishing agent for glass, as a dye, and in galvanic electrodes. Since the starting material is low cost and easy accessibility, the preparation methods are inexpensive [22, 23], CuO has gained interest.

Objectives and Plan of Work

Objective:

- To synthesize copperoxide nanoparticle by using fresh leaf of *Chenopodium album*
- To characterize the synthesized copperoxide nanoparticle.
- To evaluate the *in-vitro* antibacterial activity using synthesized nanoparticle.

Plan of work

- Literature survey of synthesis of copperoxide nano particle using plant extracts.
- Selection of plant *Chenopodium album*
- Preparation of aqueous leaf extract

- Synthesis of copperoxide nanoparticle can be done using fresh leaf extract of selected plant.
- Characterization was determined using UV-Visible, FTIR, SEM, EDX, SEM mapping and XRD analysis.

Material and Methods

Chemicals

All materials were purchased from Nice and Loba chemicals. Solvents that were used during the reactions were of high purity and used without further purification.

Collection of plant materials

The plant material *Chenopodium album* was collected from the local places of Ariyalur area. Freshly collected whole plant was used for the synthesis of copper oxide nanoparticles.

Preparation of plant extract

The extract solution was equipped by using leaves of *Chenopodium album* plant. The leaves of fresh plant that had been rinsed with de ionized water and finely cut into small pieces. Then the plant material was boiled with 100 mL of distilled water at 100°C, filtered by using whatmann No. 1 filter paper and stored at 4°C for further experimentation.

Synthesis of Copper oxide nanoparticles

In the preparation of Copper Oxide nanoparticles, samples $Cu(NO_3)_2.3H_2O$ (0.1g) was first dissolved in enough quantity of de ionized water and mixed with 10mL of *Chenopodium album* plant extract solution under vigorous stirring with magnetic stirrer at 1000 rpm at room temperature for 3hr. Then 1ml of 10% NaOH solution was added to the reaction mixture to adjust the pH of the reaction mixture. The precipitated solid was filtered and dried. The crude product was maintained at 150°Cfor 12 hrs in oven. The obtained powder was calcined at 500 °C for 3 hrs and then crushed into fine powder by using pestle mortar.

Characterization studies

Copper oxide nano particles synthesized by using green chemistry technique were confirmed with the help of UV-Visible spectrophotometer (Shimadzu) and FT-IR spectrophotometer (Shimadzu) spectrum in the range 4000-400 cm^{-1}, Powder XRD, SEM and EDX examination.

Antibacterial activity

Antibacterial behavior of the aqueous leaf extract of *Chenopodium album* (**1)** CuO NPs (**2**) was checked against two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*

along with two gram positive bacteria *Staphylococcus aureus and Bacillius cereus*) that were preserved on the agar slants of the nutrient. The antimicrobial behavior was performed as defined by Institute of Clinical and Laboratory Standards [24]. Bacterial immunity to CuO NPs was tested using an assay to disperse the disks. Triplicates of the CuO NPs were used in sterile deionized water dilutions of (200, 100, 50, 25, and 12.5). Initially, the isolates were incubated at 4ºC for 15min, and then overnight at 37ºC. Good test outcomes were graded when an inhibition zone was found across the well after the incubation time then a digital vernier caliper was used to calculate the inhibition zone diameter [25].

Minimum inhibitory concentration (MIC) determination

The bacterial isolates, which were used to prepare 0.5 McFarland, were incubated at 37oC overnight. A minimum of 10ml tube nutrient broth medium was prepared and each sample was inoculated aseptically with 1ml of the respective bacterial suspension (approximately 108 CFU / mL). Five dilutions of aqueous leaf extract of *Chenopodium album* (**1)** CuO NPs (**2**) (200,100,50,25 and 12.5) were prepared in sterile deionized water and a negative control (without CuO NPs) was used. Tests for each isolate were performed in triplicates. The inoculated sets were overnight incubated to 37ºC. The apparent turbidity in each tube was examined during the incubation time. Of the measured strain the lowest concentration without turbidity is defined as the MIC. Tubes showed no turbidity on nutrient agar plates cultivated and incubated overnight at 37ºC.

Results and Discussion

The *Chenopodium album* plant was collected around Ariyalur and was identified using the *Flora of presidency of Madras* and the fresh plant extract plays a key role in copper oxide nanoparticle synthesis.

Optical Characterization

The reduction of Cu^{2+} ions to Cu^{0} NPs by aqueous leaf extract of *Chenopodium album* was visually observed by color variation in the reaction mixture. The gradual color change in solution from light green to sky blue. This indicates that the metal nitrates were reduces to form its respective nanoparticles.

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UV-Visible Spectroscopy

The UV-visible absorption peak arises from 300-380 nm denote the development of CuO NPs. In our study, the extreme absorption peak seemed at 365 nm directs the individual SPR band for CuO NPs with lesser particle size. **Figure 1** displays UV-vis spectra of CuO NPs synthesized by greener protocol.

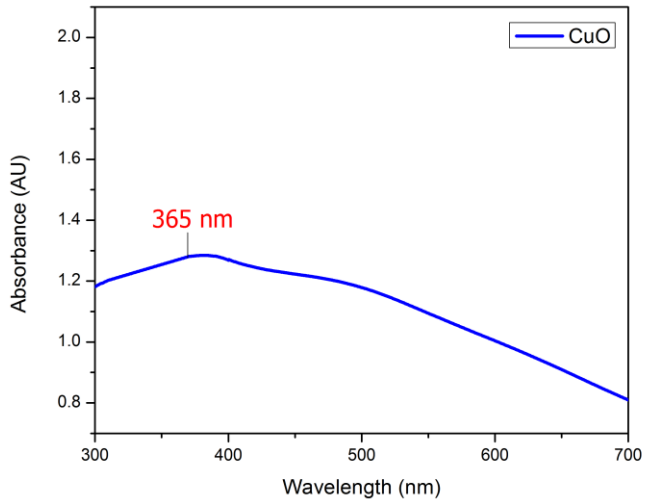

Figure 1 UV – Visible spectra of CuO nanoparticle.

FT-IR Analysis of metal oxide nanoparticles synthesized by using aqueous leaf extract of *Chenopodium album.*

The FT-IR spectrum noted in the ranges from 400-4000 cm^{-1}. A wide peak at 3428.71 cm^{-1} agrees to the O-H group which may be appeared as a result of the manifestation of Hydroxyl moiety131 [26]. The bands at 2800 - 3000 cm^{-1} signify the existence of C-H functional group of alkanes 130 [27]. The peaks (1626.66 cm^{-1}) showed the incidence of carbonyl moiety (C=O) which confirms the leaf extract having enzymes or proteins 129 [28]. The band at 516.34 cm^{-1} approves the existence of Cu-O vibrations 131 [29]. FT-IR analysis confirmed the presence of functional groups in the capping agent and also the formation of CuO NPs. FT-IR spectra of green synthesized CuO NPs was represented in **Figure 2**.

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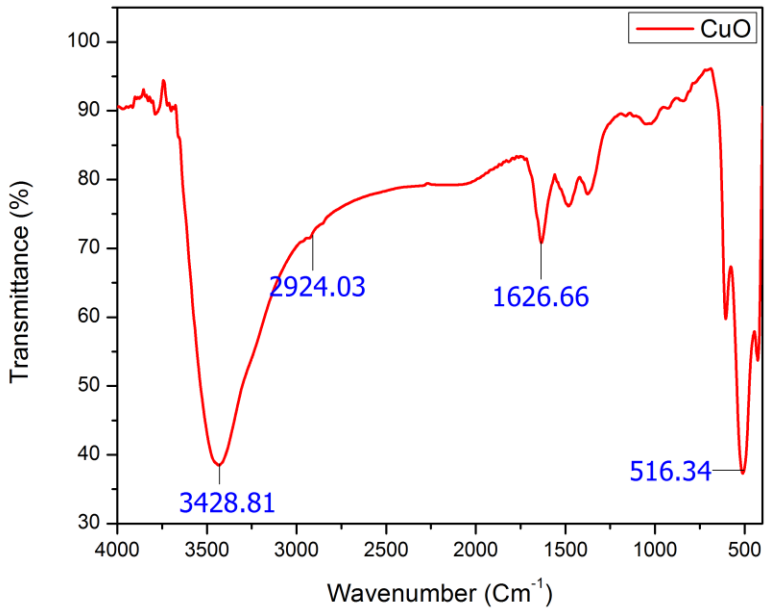

Figure 2. FT-IR spectra of CuO nanoparticle.

SEM and Mapping studies CuO nanoparticle

Scanning Electron Microscopy (SEM) investigation was performed to govern the size and morphology of the green synthesized CuO NPs by *Chenopodium album* (aqueous leaf extract). SEM image shown in Figure 3 confirmed that the obtained CuO NPs were flake like morphology. The green synthesized CuO NPs were dispersed as distinct particles and monodispersivity in nature. Phytochemicals in *Chenopodium album* aqueous leaf extract turn as a capping agent which prevents the aggregation of particles causes monodispersivity of CuO NPs. SEM mapping studies also conforms the synthesized nanoparticle was CuO. The blue dot corresponds to Copper atom and rose dots represents Oxygen atom. Figure 4 represents the SEM mapping studies of CuO nanoparticle.

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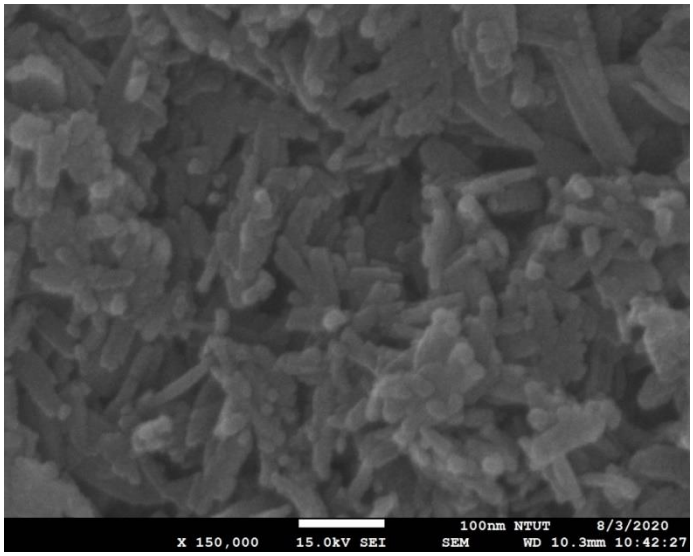

Figure 3.SEM image of CuO nanoparticle.

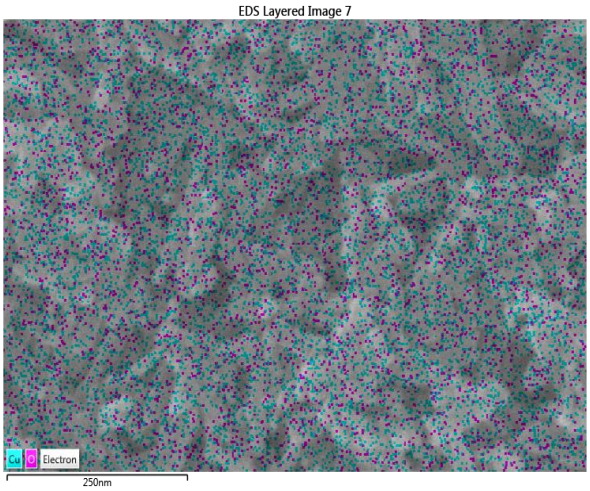

Figure 4.SEM image mapping of CuO nanoparticle.

EDX Analysis of CuO nanoparticle

The elemental composition of the synthesized CuO NPs was confirmed by EDX analysis. The manifestation of copper and oxygen peaks in the EDX spectra confirmed that the synthesized material was CuO NPs (Figure 5). The weight percentage of Copper and Oxygen atoms were 71.10 and 28.90 respectively. The further peaks extant in the spectra may be as a result of the existence of bioorganics or impurities in the solution. The elemental composition of CuO nanoparticle was represented in Table 2.

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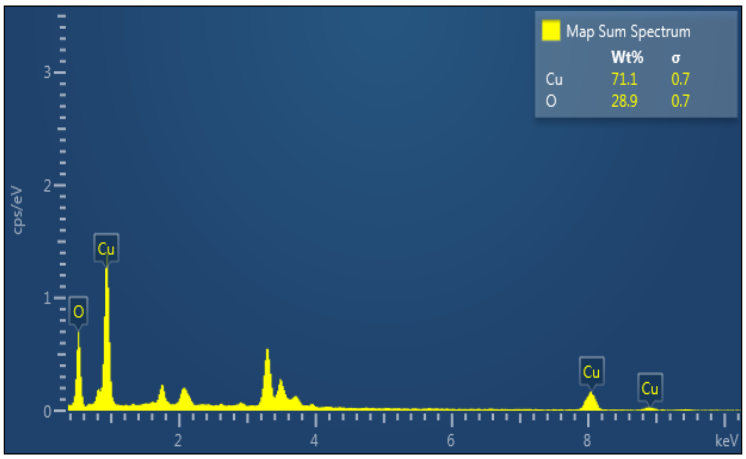

Figure 5. EDX spectra of CuO nanoparticle.

Table 2. Elemental composition of CuO nanoparticle

Element	Atomic Number	Weight %	σ
O	8	28.90	0.7
Cu	30	71.10	0.7
Total	-	100	-

XRD Analysis

The XRD pattern of aqueous leaf extract of *Chenopodium album* derived CuO NPs was represented in Figure 6. The diffraction peaks at 2θ = 32.4°, 35.5°, 38.7°, 46.1°, 48.8°, 53.6°, 58.4°, 61.6°, 66.2°, 68.0°, 72.4° and 75.2° were respectively indexed to (110), (111),(111), (112), (202), (020), (202), (113), (310), (220), (311) and (310) planes of monoclinic structure of CuO NPs. The obtained diffraction peaks were matched with of standard CuO NPs. All the diffraction peaks are in good agreement with the standard pattern for pure monoclinic phase of copperoxide nanoparticles (JCPDS No. 80-0076). There is no impurity peaks were observed. The intense peaks indicates the highly crystalline nature of the formed nanoparticles. From the observed main diffracted peak, the average crystalline size can be calculated using the Scherer equation,

$$D_{(hkl)} = \frac{k\lambda}{\beta\cos\theta}$$

Where, $D_{(hkl)}$ is the average crystalline size, k is shape constant (0.89), λ is the wavelength of the incident x-ray (Cukα source, λ = 0.15405 nm), β is the full width half maximum (FWHM), θ is

the incident angle of x-ray. The average crystallite size of the synthesized CuO nanoparticles was 12.45 nm.

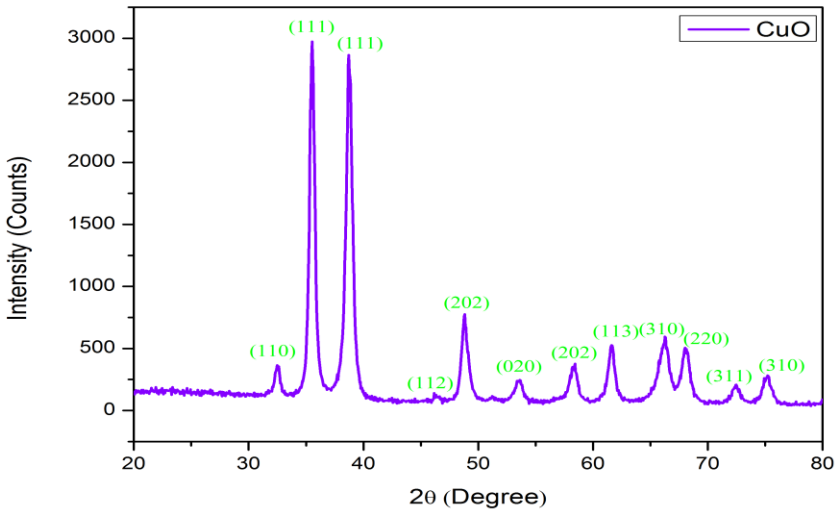

Figure 6. XRD spectra of CuO nanoparticle.

In vitro antibacterial activity

The in vitro antibacterial activity was screened against two Gram-negative bacteria namely, *E. coli*, *P. aeruginosa* and two Gram-positive bacteria namely, *S. aureus*, *B. cereus* using ciprofloxacin as standard drug. Minimum inhibitory concentration (MIC) values were determined using standard agar method. MIC values of the aqueous leaf extract of *Chenopodium album* (**1)** and synthesized CuO nanoparticle (**2)** were presented in Table 3. The synthesized CuO nanoparticle shows remarkable antibacterial activity than aqueous leaf extract of *Chenopodium album* (**1).** CuO nanoparticle**2** shows high antibacterial activity with the MIC value of 23.10 µg/mL than control **ciprofloxacin** with the MIC value of 25.00 µg/mL in *E. coli*. CuO nanoparticle **2** shows high antibacterial activity with the MIC value of 28.63 µg/mL than control **ciprofloxacin** with the MIC value of 50.00 µg/mL in *B. cereus*. CuO nanoparticle **2** displayed moderate activity in bacterial cultures *P. aeruginosa* and *S. aureus* with the MIC value of 36 and 28 µg/mL than standard **ciprofloxacin** Interestingly, The synthesized CuO nanoparticle (**2**) shows remarkable antibacterial activity than control **Ciprofloxacin** in both pathogens *E. coli* and *B. cereus* respectively.

Table 2. Antibacterial activity of aqueous leaf extract of *Chenopodium album* (**1)** and synthesized CuO nanoparticle (**2)**

Comp.No.	MIC µg/mL			
	E. coli	P. aeruginosa	S. aureus	*B. cereus*
1	30.56± 1.18	42± 0.64	34± 1.46	38.23 ± 2.68
2	23.10± 0.56	36± 1.28	28± 0.32	28.63± 1.34
Ciprofloxacin	25.00± 0.95	30± 0.0	20± 0.0	50.00± 1.75

[a] Value were the means of three replicates ± SD.

Conclusion

- In conclusion the copper oxide nanoparticle was synthesized using fresh leaf extract of *Chenopodium album*.
- The synthesized nano particle was characterized and confirmed using UV-Visible, FT-IR, XRD,FE-SEM, EDX and SEM mapping analysis, the results showed that CuO Nps were synthesized properly.
- The *in-vitro* antibacterial assay depicts the effective antibacterial activity of CuO Nps.
- These conclude that the further study on CuO Nps helps for the drug development.

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