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Original Article

First Report of green Synthesis of Copper Oxide Nanoparticles from Brassica Tournefortii Leaves Extract and Their Antibacterial Activity

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Abstract

In the present study, copper oxide nanoparticles (CuO NPs) were synthesized using a eco-friendly technique and evaluated their antibacterial activity. At the first time, Brassica tournefortii leaves extract mediated copper oxide-based nanoparticles which prepared by a bio-reduction method using an aqueous Brassica tournefortii leaves extract as both a reducing and stabilizing agent. Copper oxide nanoparticles were characterized by using ultraviolet-visible spectroscopy (UV-VIS), Fourier transform-infrared spectroscopy (FT-IR), x-ray diffraction (XRD) and scanning electron microscope (SEM) techniques. The synthesized CuO nanoparticles were also evaluated for their antibacterial activity against gram-positive (Staphylococcus aureus and Streptococcus spp) and gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains. UV-Vis and FT-IR results showed that the successful formation and stabilization of copper oxide nanoparticles due to biomolecules in Brassica tournefortii extract. XRD results indicated that the CuO nanoparticles were monoclinic of size 27.73 nm. SEM image showed that CuO nanoparticles had spherical morphology. Furthermore, Antibacterial activity showed that CuO nanoparticles have good antibacterial agent against both gram positive and gram-negative organisms. The antibacterial assay revealed that Pseudomonas aeruginosa a maximum zone of inhibition (44 mm) at 30 mg/ml concentration of CuO nanoparticles.

Keywords: Antibacterial activity, Bioreduction method, Brassica tournefortii leaves extract, CuO nanoparticles.

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Introduction

Antibacterial potency of metal oxide nanoparticles achieves abundant attention of the scientists. Metal oxide nanoparticles have an attracted interest due to broad effectiveness against pathogenic microorganisms [1]. The antibacterial activity of metal oxide nanoparticles is dependent on various parameters such as particle size, surface area, crystallinity, capping agent, morphology, concentration/ dosage, pH of the solution, and also the nature of the microorganisms [2].

Among the most promising of these novel metal oxide nanoparticles are cupric oxide nanoparticles, which have recently gained the attention due to its high stability, chemical reactivity, low cost, easiness of preparation and significant promising biological, catalytic and electric properties [3,4]. CuO nanoparticles have acquired much attention owing to their wide applications in various fields including lithium-ion electrode materials [5], sensors [6, 7] catalysis [8, 9] and especially in biomedical application [1]. CuO nanoparticles have been reported as exhibiting antibacterial potential agents against both pathogenic microorganisms [10-13]. Several physical and chemical methods have been reported for the synthesis of CuONPs [4]. However, these methods involve the hazardous chemicals, very expensive, and non-environmentally friendly chemicals. Recently, green synthesis of metal oxide nanoparticles is a field of current importance as it is simple, a non-toxic, cost-effective, eco-friendly, and easy operation procedure [14].

Various plants were used for synthesis of CuO nanoparticles using green synthesis. In this present investigation, Brassica tournefortii gouan (also known as Asian mustard, Sahara mustard) is an important medicinal plant which belongs to the Brassicaceae family. It has been reported as an anti-Alzheimer and anticancer [15]. Rahmani et al. reported that the Brassica tournefortii leaves richness by antioxidants like isothiocyanates and polyphenols, which the highest value of polyphenols was approximately about 33.2 mg GAE/g dr [16]. Where polyphenols can act as a reducing and capping agent for converting copper (II) ions to CuO nanoparticles and stabilizing. According to the literature, no studies have been reported before on the use of an aqueous extract of Brassica tournefortii leaves for the synthesis of CuO nanoparticles. Hence, the aim of this study is to synthesize CuO nanoparticles using Brassica tournefortii leaves extract and evaluate its antibacterial activity against both gram positive and negative bacterial strains.

Materials and Method

Fresh leaves of Brassica tournefortii plant were collected from Az-Zāwiyah, Libya.

Copper sulfate pentahydrate (CuSO4. 5H2O) and Sodium hydroxide (NaOH) were purchased from Sigma Aldrich.

Aqueous Brassica tournefortii Leaves Extract Preparation

The fresh Brassica tournefortii Gouan leaves were collected from Az-Zāwiyah, (March 2021). The leaves were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The (10 g) of leaves were cut into fine pieces and boiled with 100 ml of distilled water for 15 minutes. After the boiling process, the extract was filtered through Whatmann No.1 filter paper to obtain aqueous extract. The aqueous extract was stored at 4°C for future works [17].

Copper Oxide Nanoparticles Preparation

Copper oxide nanoparticles were synthesized by adding freshly prepared leaves extract to CuSO4 solution (0.1 M) in a 1:1 volume ratio. Then, the pH of the reaction mixture was adjusted to pH 9 by the addition of (0.1 M) NaOH. The mixture was stirred continuously at 50 °C for 30 min. the colour of the reaction mixture changed from blue to green, indicating the formation of CuO nanoparticles (see figure 1).

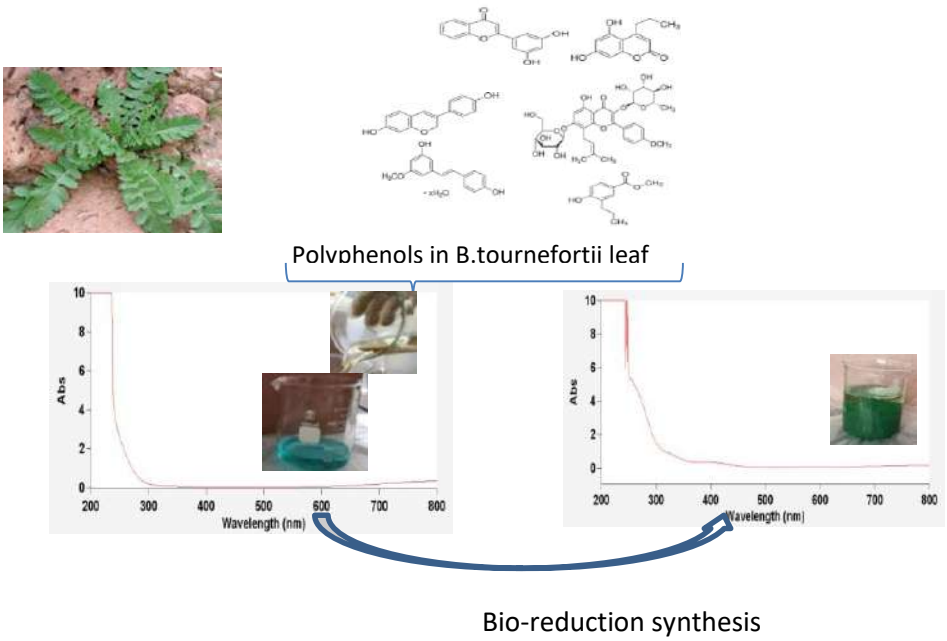

Figure 1. Green synthesis of synthesized CuONPs using *Brassica tourneforti*

Characterization techniques

UV–vis spectrophotometer (JASCO V 670) was used to monitor the green synthesis of CuONPs. The respective SPR peaks were recorded between 200 and 800 nm. The green colloidal solution was remove the unwanted organic matter. The resulting paste was dried in air for 48 h and calcinated in electronic oven at 600 °C for 2h. The obtained powdered CuONPs were analyzed using XRD (Shimadzu XRD-6100 diffractometer) with a Cu Kα radiation monochromatic filter in the range 35–80◦. Then, the size of CuONPs was calculated using Scherer equation. The morphology of the synthesized CuONPs were observed by scanning electron microscopy (SEM, LEO 1430VP), The FT-IR using IR Affinity-1s (Shimadzu) spectrometer, recorded in the wavenumber range of 500–4,000 cm-1 to find the functional groups present around the synthesized CuONPs.

Evaluation of Antibacterial Activity

Green synthesized CuO nanoparticles (CuONPs) using Brassica tournefortii leaf aqueous extract was studied for antibacterial activity using the agar diffusion technique. Both Gram-positive and Gram-negative strains including Staphylococcus
aureus and Streptococcus spp, Escherichia coli and Pseudomonas aeruginosa were tested. Chloramphenicol was used as a control antimicrobial agent. The pure cultures of bacteria were sub cultured on Mueller-Hinton Agar (MHA). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 5 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 20µl of the CuO NPs solution was poured onto each well on all plates. All petri dishes were kept at 37 °C for 24 h for incubation and diameter of zone of inhibition was calculated.

Results and discussion

Optical studies

Figure 2 illustrates the UV-VIS absorption spectra of synthesized CuO nanoparticles using Brassica tournefortii leaves extract. It shows the characteristic surface plasmon resonance (SPR) absorption with a maximum at 250–300 nm which is in close agreement with the earlier reports [18]. The formation of CuO nanoparticles was monitored by color changes. Polyphenols present in the Brassica tournefortii leaves extract playing a crucial role in the reduction process of Cu2+ ions. The blue shift reveals the information that polyphenolic constituents in the plant extract reduces the Cu2+ ions into Cu0, which Ester oxygen atom and phenolic hydroxyl group of phytochemicals form metal-phenolate complex when hydroxyl groups bind with metal by chelating effect. Then, oxidized to CuO spontaneously in oxygen because of higher oxidation potential of Cu0 [19, 20].

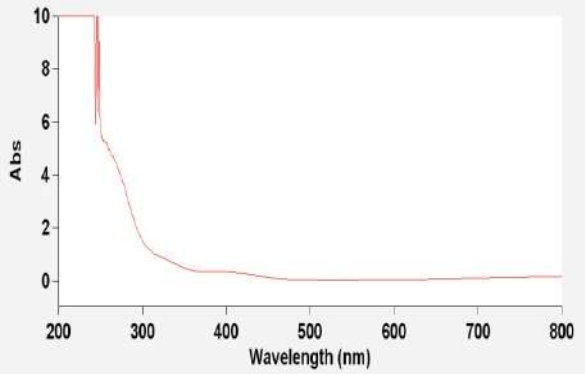

Figure 2. UV–Vis spectrum of synthesized CuO NPs using Brassica *tournefortii*

The band gap (Eg) value of the CuO nanoparticles has been studied. The optical band gap of CuO nanoparticles was measured by absorbance coefficient data as a function of wavelength using Tauc relation given as [21]:

$\alpha h\nu = A(h\nu - Eg)0.5$

Where α is the absorption coefficient, Eg is band gap energy, A is constant and hν is the photon energy. The plot of $(\alpha h\nu)2$ versus photon energy is a linear function of direct allowed transitions in CuO nanoparticles and is shown in figure 3. The direct band gap for CuO NPs found to be 4.16 eV, which is greater than (3.26 eV) for bulk CuO reported by shtewi et al. [22]. The increased band gap is attributed to the lowered particle size due to the quantum confinement effect [23].

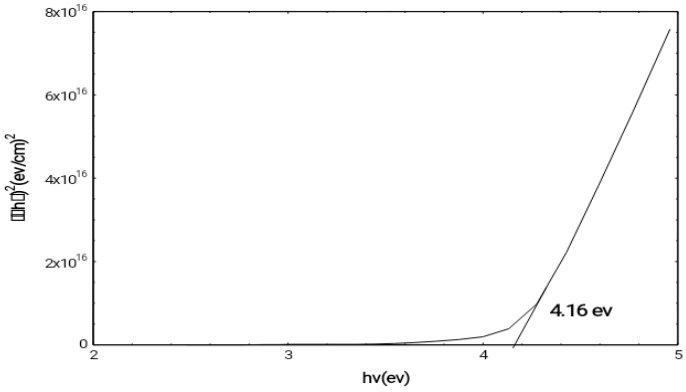

Figure 3. plot of band gap energy of CuO NPs using the *Brassica tournefortii* leaves extract.

Structural studies

FT-IR analysis was carried out to confirm the formation and stabilization of CuO nanoparticles using the Brassica tournefortii leaves extract. The FT-IR spectra of Brassica tournefortii leaves and CuO nanoparticles are shown in Figure 4. The main characteristic peaks of Brassica tournefortii at 3027, 2919, 2850, 1736, 1595, 1245, 1143, 1065 and 838 cm-1. The band around 3027 cm-1 represents

the C–H stretching of the aromatic ring. The bands at 2919 and 2850 cm-1 are attributed to asymmetric and symmetric C-H stretching mode of aliphatic hydrocarbon chains. The band near 1736 cm-1 shows presence of amide, ester and acids which are due to carbonyl group stretching vibrations and 1595 cm-1 represent –C=C– in the aromatic ring. The peaks at 1428 and 1245 cm−1 correspond to the C-N and O-H stretching vibrations of polyphenol. The intense peaks at 1143 and 1065 cm-1 depicts C–O stretching of phenolic compounds. A band at 838 cm-1 probably related to aromatic ring vibration [24]. The FT-IR spectrum of CuO nanoparticles has exhibited an absorption band at 3554 cm-1 mainly ascribed to −OH on the surface of the CuO nanoparticles [25-27]. The synthesis of CuO nanoparticles was confirmed by the appearance of the characteristic peaks of CuO NPs in specific regions, which can be attributed to the vibration bands of Cu-O at 515, 596 and 628 cm-1, further confirming the CuO nanoparticles formation

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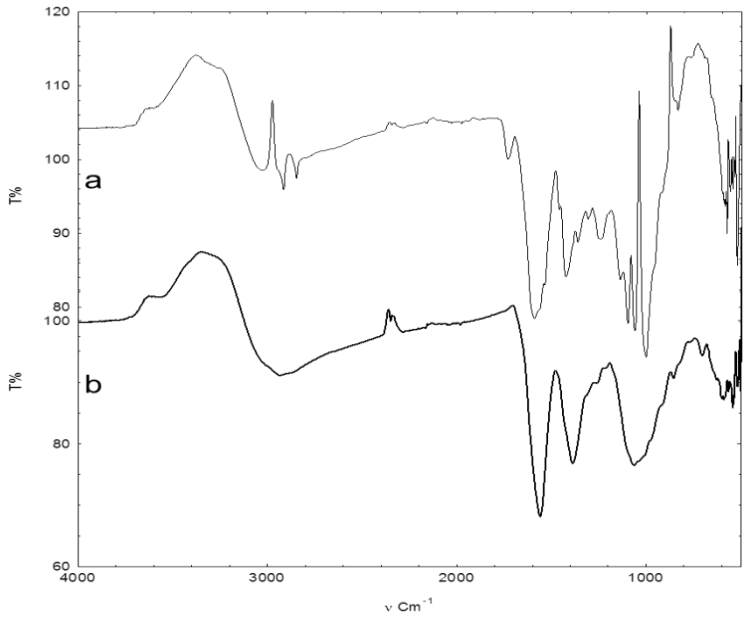

Figure 4**.** FT-IR spectrum of *Brassica tournefortii* (**a**), and synthesized CuO NPs (**b**).

The XRD analysis of copper oxide nanoparticles synthesized using Brassica tournefortii aqueous leaves extract is illustrated in figure 5. The typical XRD pattern of the prepared CuO nanoparticles is visible on both XRD patterns and the characteristic diffraction peaks at 2θ values of 33.26°, 36.26°, 39.45°, 49.48°, 58.94°, 62.24°, 66.95°, 68.76°, 73.02°,75.81° corresponds to the lattice planes (110), (111), (200), (202), (020), (202), (113), (311), (220) and (400) reflections respectively (JCPDS, file: 41-0254) which indicate the monoclinic

structure of tenorite phase. The sharp peaks indicated that the obtained nanoparticles contained high crystallinity nature. Similarly, our results agree with the reports recognized by other researchers [22, 28]. The average crystalline size of the prepared CuO nanoparticles was calculated by applying Scherer's equation,

$$D = 0.94 \lambda / \beta \cos\theta$$

Where D is the average crystallite size of the particle, λ is the wavelength of the electron beam (0.154 nm), β is the full width at half maximum (FWHM) of the peak and θ is the Bragg's angle of diffraction. The Crystalline size of green synthesized CuO nanoparticles was about 27.73nm.

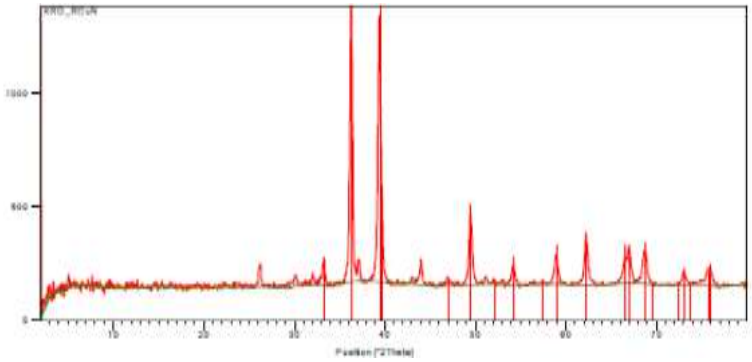

Figure 5. XRD patterns of synthesized CuO NPs using *Brassica tournefortii*

Surface morphology study

The morphology of synthesized CuO nanoparticles using Brassica tournefortii leaves extract was investigated by SEM image as shown in figure 6. SEM images of CuO nanoparticles which indicates that the morphology of CuO nanoparticles is spherical in shape. Clearly, small nuclear particles are self-aggregated and orient themselves to form larger spheres.

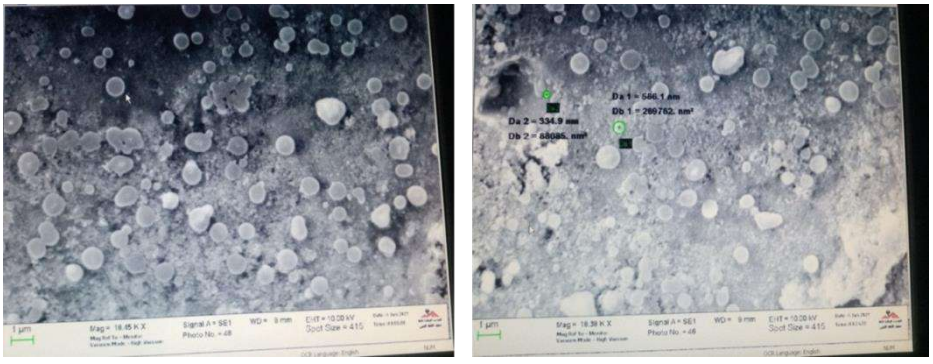

Figure 6. SEM images of synthesized CuO NPs using *Brassica tournefortii*

Antibacterial activity study

The synthesized CuO nanoparticles from Brassica tournefortii as a bactericidal agent were investigated. The results in table 1 and figure 7 supported the fact that CuO nanoparticles have promising antibacterial activity. Based on the inhibition zone obtained, it can be well assumed that the Gram-negative bacteria has greater antimicrobial activity than Gram-positive bacteria. The difference in activity against these two types of bacteria could be attributed to the structural and compositional differences of the cell membrane [10].

Table 1. Inhibition zone of synthesized CuO NPs.

Conc. of CuONPs	Inhibition zone			
	Gram-positive bacteria		Gram-negative bacteria	
	Streptococcus spp.	Staphylococcus aureus	E. coli	Pseudomonas aeruginosa
10 µg/ml	11	-	14	30
30 µg/ml	14	-	18	44
50 µg/ml	6	12	18	39

As it is observed, in table 1, the antibacterial activity experiment performed on Pseudomonas aeroginosa and E. coli confirmed that synthesized CuO nanoparticles have higher antibacterial effects and higher ZOI. Highest antibacterial activity was seen against Pseudomonas aeruginosa, which was the most sensitive to The 30 mg/ml concentration of CuO nanoparticles with a ZOI value of 44 mm. This kind of bacteria can cause various skin and soft tissue infections particularly when the skin or mucosal barriers have been breached. The antibacterial activity was moderate in Staphylococcus aureus and Staphylococcus spp. Our results are in agreement with previous studies [10, 29].

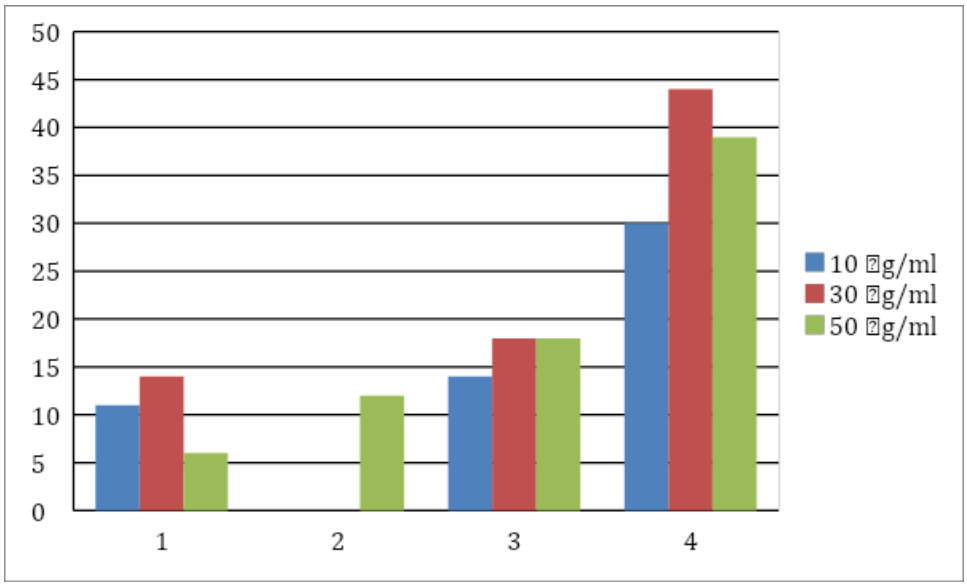

Figure 7. Antibacterial activity of synthesized CuO NPs

The smaller size of CuO nanoparticles (nm) compare to the pore size of bacterial cells (µm) allows the easy penetration of CuO nanoparticles into the cell membrane without any interference. The abundant functional groups such as amine and carboxylic groups on the surface of the cell membrane can attract the Copper cations towards the cell, causing damage to the cell membrane [1-3]. The destruction of the bacterial membranes by CuO nanoparticles could be via production of reactive oxygen species and radicals or by direct cell damage since superoxide and hydroxyl radicals are produced by CuO nanoparticles [3, 30].

Conclusion

The bio-reduction synthesis of CuO nanoparticles has been successfully synthesized for the first time using Brassica tournefortii aqueous leaves extract. The polyphenol content of the extract served as the reducing and capping agent revealed by FT-IR results. The XRD result and SEM results confirmed CuO nanoparticles have crystallite size 27.23 nm. The CuO nanoparticles showed their antibacterial properties on both gram positive and gram negative bacterial strains. The antibacterial activity experiment performed on Pseudomonas aeroginosa and E. coli confirmed that synthesized CuO nanoparticles have higher antibacterial effects and higher ZOI.

Abbreviations

CuONPs: Copper oxide nanoparticles
SPR: Surface plasmon resonance
FT-IR: Fourier transform infrared spectroscopy
UV-VIS: Ultraviolet–visible spectroscopy
XRD: X-ray diffraction analysis
SEM: Scanning electron microscopy
ZOI: zone of inhibition

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