



# Antioxidant and Catalytic Activity of Biosynthesized CuO Nanoparticles Using Extract of *Galeopsisida herba*

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## Abstract

The main objective of this study was synthesis of CuO nanoparticles using extract of *Galeopsisida herba* and were characterized by UV–Vis spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy (SEM), SEM with EDS profile and transmission electron microscopy (TEM) analysis. SEM images confirmed that synthesized nanoparticles were spherical in nature. EDS profile confirmed the signal characteristic of copper and oxygen. All the analyses revealed that synthesized CuO nanoparticles were  $10 \pm 5$  nm in size. The antioxidant behavior of synthesized CuO nanoparticles was evaluated by scavenging free radicals of 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH). The DPPH scavenging activity was monitored using UV–Vis spectrophotometer. The synthesized CuO nanoparticles presented very good catalytic activity in the reduction of malachite green.

**Keywords** CuO nanoparticles · Green synthesis · Antioxidant activity

## 1 Introduction

Nanotechnology concerns the arrangement of materials at the atomic stage to achieve nanoscale materials with unique physico-chemical and biological characteristics [1, 2]. Nanomaterials are part of a commercial revolution that has resulted in an explosion of hundreds of new products due to their diverse physico-chemical properties, enabling their usage in a wide range of innovative applications. In the last years, synthesis of metal oxide nanostructures with desired architecture has received significant attention due to their unique properties and applications [3–6]. In literature there are many physical and chemical methods synthesis of metal nanoparticles like physical vapor deposition, chemical vapor deposition, sol–gel method, microwave-assisted synthesis, ultrasonication method, electrochemical synthesis and chemical reduction of metallic ions. Moreover, these methods are usually expensive, potentially hazardous to the environment and living organisms. Recently researchers have tried to find biological methods for the synthesis of nanoparticles that

will be the alternative to chemical or physical methods [7]. The green synthesis techniques are generally synthetic routes that utilize relatively nontoxic solvents such as water, biological extracts, biological systems and microwave assisted synthesis [8]. In the biological methods the use of plants extract has advantages such as easily available, safe to handle and possess a broad viability of metabolites. Moreover, it is found that the extract of plants acts both as reducing and capping agents in the synthesizing process of the nanoparticles [9].

The obtained nanoparticles of metals or metal oxides are often combined with nanocomposites. The new combined material exhibits numerous new characteristics and properties that the single material does not have [10], and it has a wider spectrum of applications [11]. Given that the surface to volume ratio is large at the nanoscale, the addition of nanoparticles can influence the thermal, electrical, optical and dielectric properties of the polymer. For example, incorporation of  $Al_2O_3$  nanoparticles results in high conductivities of nanocomposites [12], and polymerization of  $TiO_2$  [13] or TiC nanoparticles produces electroactive polymers [14]. Good electrochemical response was observed for polymers grown into Montmorillonite-Cu [15] or Montmorillonite-Na [16].

This study presented the green synthesis of CuO nanoparticles using the extract of *Galeopsisida herba*. *G. herba*

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belongs to the Lamiaceae family. It is found in Europe and in the northern part of Asia. It is commonly found throughout Poland. In the past, it was used to treat tuberculosis, pneumonia, bronchitis, persistent and chronic cough and spleen oedema. *Galeopsis herba* contains irodooids, in particular galiridoside, harpagide, 8-0-acetylharpagide, antirrhinoside and 5-0-glucoside. It also contains saponins, flavonoids (among others the derivatives of scutellarein), about 6% of tannins and phenolic acids (such as vanillic acid, salicylic acid, p-coumaric acid, caffeic acid, cinnamic acid).

CuO nanoparticles have gained considerable attention in the past two decades due to their simplicity and the fact that they exhibit a range of potentially useful physical properties, depending strongly on their shape, size, and composition [17]. CuO nanoparticles have shown potential to replace noble metal catalysts for carbon monoxide oxidation and often found use as antimicrobial agents, semiconductors, heat transfer fluids in machine tools, and intrauterine contraceptive devices [18]. CuO is applied as an antimicrobial, anti-fouling, anti-biotic and anti-fungal agent when incorporated in coatings, plastics and textile [19]. The already known methods of synthesizing CuO nanoparticles include sol–gel, microwave irradiations, alkoxide based route, thermal decomposition of precursor one step solid-state reaction method, precipitation—pyrolysis. The biosynthesis of CuO nanoparticles by plants such as *Anthemis nobilis* [20], *Calotropis gigantea* [21], *Gloriosa superba* [22], *Cinnamomum camphora* [23], *Aloe vera* [17], *Carica papaya* [24] or *Emblica officinalis* [25] have been reported. An extensive literature survey revealed that there are no reports on the synthesis of CuO nanoparticles using the extract of *G. herba*. This work presents the synthesis of CuO nanoparticles using the extract of *Galeopsis herba*, as well as studies their antioxidant and catalytic activity in the degradation of malachite green.

## 2 Materials and Methods

### 2.1 Synthesis of CuO Nanoparticles

The synthesis of CuO nanoparticles was initiated by preparing the extract, which was made by mixing 4.5 g powdered *Galeopsis herba* with 300 ml double distilled water. The solution was stirred for 50 min at the temperature of 85 °C. The obtained solution was filtered through Whatman's No. 1 filter paper. The extract was mixed with Cu (NO<sub>3</sub>)<sub>2</sub> in the proportion: 90 [% weight]: 10 [% weight], and vigorously stirred for 4 h at 80 °C. Then, the solution was stored for 24 h in a dark place at the temperature of 25 °C.

### 2.2 Characterization of of CuO Nanoparticles

The synthesized of CuO nanoparticles using the extract of *Galeopsis herba* was monitored after precipitate formation using UV–visible spectrophotometer Cary E 500 at a wavelength range of 290–400 nm. The morphology of synthesized of CuO nanoparticles was examined scanning electron microscopy (SU3500), Hitachi with spectral imaging system Thermo Scientific NSS (EDS), the type of detector (BSE-3D), acceleration voltage (15.0 kV), working distance (11.6 mm), the pressure (in the case of a variable vacuum conditions)(40 Pa). CuO nanoparticles stretching frequencies were determined by FTIR measurement in a Perkin Elmer FTIR spectrometer using spectral range of 4000–400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>. The size and structure of synthesized CuO nanoparticles were characterized using a Transmission Electron Microscope JEOL JEM 1200 EXII, operating at 200 kV.

### 2.3 Antioxidant Activity Studies

This study determined the antioxidant activity of CuO nanoparticles synthesized using the *Galeopsis herba* extract by assessing their ability to neutralize the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The neutralization manifested itself by the reduction of the absorbance of the DPPH methanol solution during the reaction with the tested solution of CuO nanoparticles. Numerous dilutions were prepared, and they ranged from 0.01 to 5 µg/ml. The measurement of changes in the intensity of absorbance was made using the spectrophotometer Carry E 500. To the test tubes, which were protected from light, there were added 0.1 ml of the tested solution and 0.7 ml of the DPPH reagent at the concentration of 0.1 mM. The DPPH reagent was prepared 24 h earlier and was protected from light. The solutions were shaken in test tubes for 30 min. After that, the absorbance was checked at the wavelength  $\lambda = 515$  nm. Water (0.1 ml) and methanol (0.7 ml) were used as reference. The measurement of the absorbance of samples was preceded by the measurement of the absorbance of DPPH solution, which was made by checking the absorbance of the solution containing 0.1 ml of deionized water and 0.7 ml of DPPH. The ability to reduce free DPPH radicals was calculated based on the formula:

$$Aa = (A_o - A_i/A_o) \times 100\%$$

where Aa means antioxidant activity [%], A<sub>i</sub>—average absorbance of the tested solution, A<sub>o</sub>—average absorbance of the DPPH solution. The actual absorbance was taken as the absorbance difference of the control and the test sample and IC<sub>50</sub> value was determined. The actual absorbance was taken as the difference between the absorbance

of the control and the test sample, and IC50 value was determined.

## 2.4 Catalytic Activity Studies

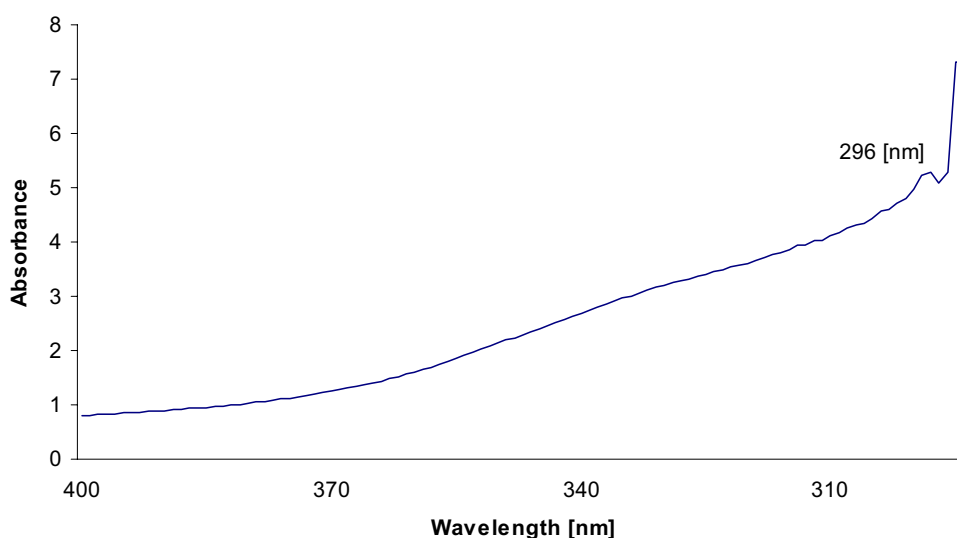
This study evaluated the catalytic activity of CuO nanoparticles synthesized using the extract of *G. herba*. The absorbance peaks were monitored using UV–Vis spectrophotometer Cary E 500. The absorbance was measured at room temperature, in the range of 400–800 nm. The sample no. 1 (MG1) was made by stirring 4 ml of malachite green ( $1 \times 10^{-4}$  M), 0.5 ml of *G. herba* water extract, 0.5 ml of prepared CuO nanoparticles and 3 ml of Milli Q water. During the next measurements, from MG2 to MG7, the amounts of the prepared solution of CuO nanoparticles were increased by 0.5 ml, and the amounts of Milli Q water were decreased by 0.5 ml.

## 3 Results and Discussion

### 3.1 UV–Visible Absorption

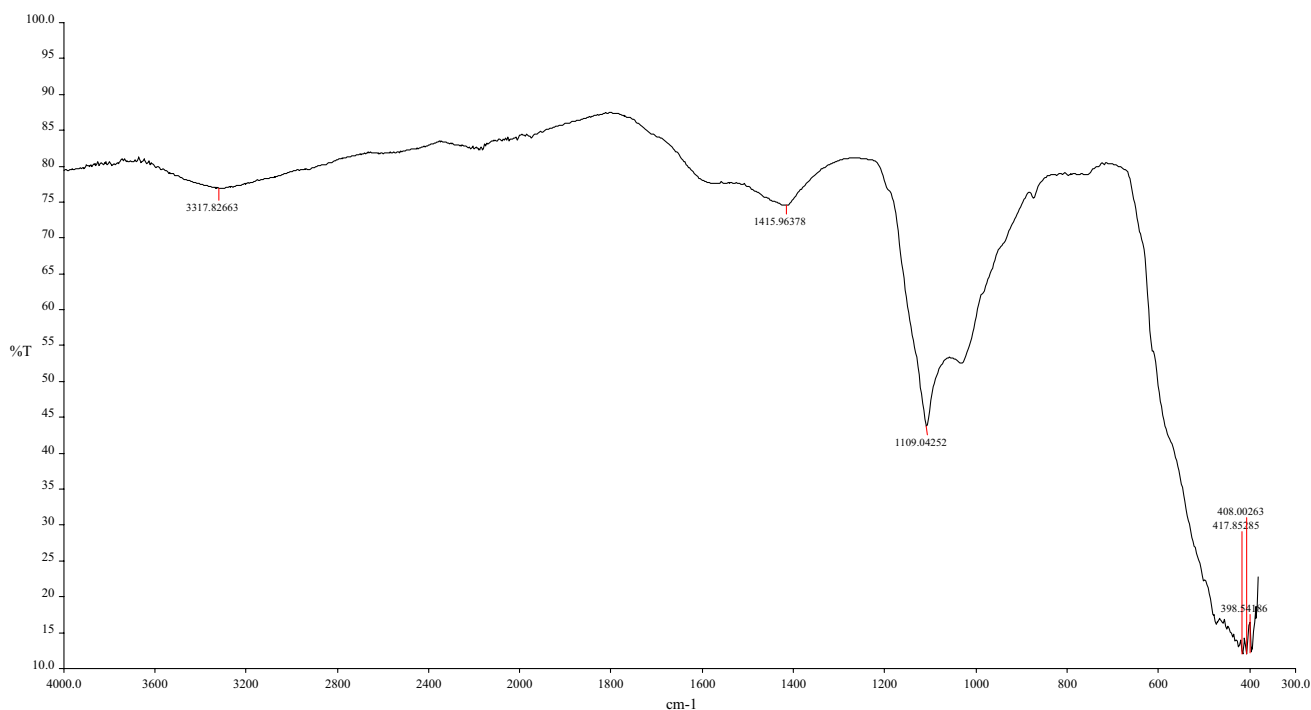
UV–Vis spectroscopy is an important technique to preview the morphology and stability of nanoparticles. In this work used range of 280–400 nm to identify CuO nanoparticles. Absorbance of the reaction mixture was monitored after 24 h stored the temperature of 25 °C in a dark place. Figure 1 presents UV–visible spectra of biosynthesized CuO nanoparticles using extract of *G. herba*. The maximum absorbance of synthesized CuO using extract of *G. herba* was around 296 nm due to the surface plasmon absorption of nanosized cupric oxide particles.

**Fig. 1** UV–visible spectra of CuO nanoparticles synthesized using extract of *G. herba*



### 3.2 FTIR Analysis

Fourier transform infrared spectroscopy (FTIR) has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules. Figure 2 presents the FTIR spectra of CuO nanoparticles synthesized using extract of *G. herba*. Clear and broad absorbance bands were observed at 3317, 1415, 1109, 417, 408, 398  $\text{cm}^{-1}$ . A broad peak at 3317  $\text{cm}^{-1}$  shows O–H stretching due to alcoholic group. The peak appears at 1410  $\text{cm}^{-1}$  correspond to C–O stretching of carboxylate ion bond to the CuO nanoparticles as bidentate ligand [26]. The spectrum showed bands at 417, 408  $\text{cm}^{-1}$ , 398  $\text{cm}^{-1}$  indicated the formation of metal–oxygen stretching of CuO nanostructure [27, 28]. According to Sharma et al. [21] the existence of prominent IR bands near 400–600  $\text{cm}^{-1}$  is conformed the formation of CuO nanoparticles using extract of *G. herba*. FTIR spectrum confirmed the presence of bioactive compounds in *G. herba*. The presence of flavonoids and phenolic acids in the extract of *G. herba* could probably be responsible for the reduction of metal ions and formation of CuO nanoparticles. The high antioxidant activity of flavonoids stems from the chemical structure of this group of compounds. The antioxidant activity of the compounds is a complex and multi-level process. Above all, it includes inhibition of enzymes that generate the reactive forms of oxygen, chelation of metal ions that contribute to the formation of free radicals, as well as reduction of the already produced active forms of oxygen. Thanks to that, flavonoids are natural antioxidants [29]. In the extract of *G. herba* *A. abrotanum* L., there were identified several phenolic acids, including vanillic acid, salicylic acid, p-coumaric acid, caffeic acid, cinnamic acid. Phenolic acids show antioxidant activity based on various action mechanisms. Phenolic acids, especially the derivatives of cinnamic acid, perform constitutive

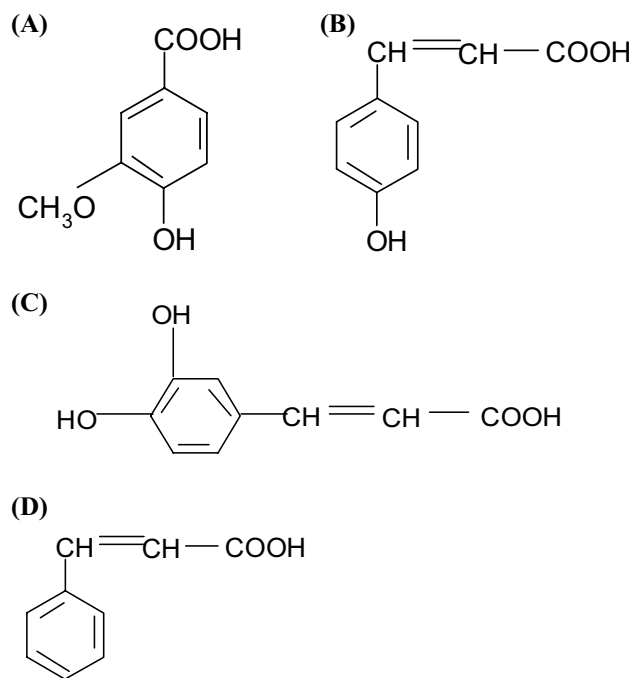


**Fig. 2** FTIR spectrum of CuO nanoparticles synthesized using extract of *G. herba*

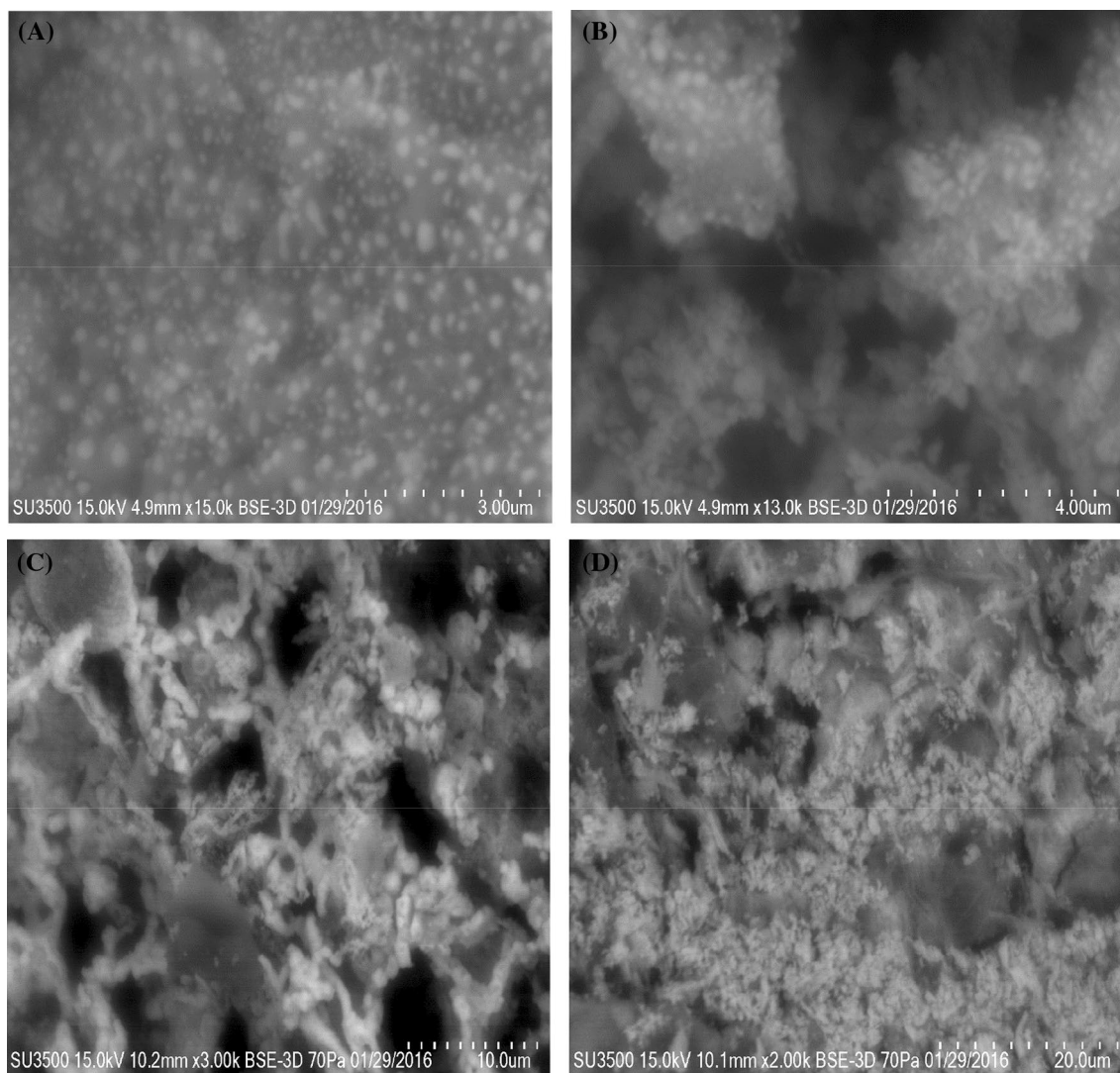
functions (they strengthen cell walls); they constitute part of proteins and polysaccharides, such as hemicelluloses, cell wall polysaccharides, in which phenolic acids (mainly ferulic acid and caffeic acid) are connected with the arabinoxylan chain. In numerous studies on the antioxidant properties of phenolic acids, scientists have proven that the properties depend on the chemical structure; namely, they are related to the number of hydroxy groups in a given particle and the level of their esterification. In compounds with one hydroxy group, the antioxidant activity is additionally increased by the presence of one or two methoxy groups in the ring. The introduction of a group with electron donors, an alkyl group or a methoxy group in the *ortho*-position increases the stability of the antioxidant properties of phenolic acids. The presence of such compounds undoubtedly contributes to the emergence and stabilization of CuO nanoparticles [30, 31]. Figure 3 shows the chemical structures of phenolic acids: vanillic acid, p-coumaric acid, caffeic acid and cinnamic acid. The presence of these compounds clearly contributes to the stabilization of received and CuO nanoparticles.

### 3.3 SEM Studies

The surface morphology and structure of CuO nanoparticles is investigated by using scanning electron microscopy analysis. Figure 4 present the SEM images of the CuO nanoparticles using extract of *G. herba*. The scale bar is (a) 3  $\mu\text{m}$ , (b)



**Fig. 3** The chemical structures of phenolic acids: vanillic acid (a), p-coumaric acid (b), caffeic acid (c) and cinnamic acid (d)



**Fig. 4** SEM images of the CuO nanoparticles using extract of *G. herba* where the scale bar is **a** 3  $\mu\text{m}$ , **b** 4  $\mu\text{m}$ , **c** 10  $\mu\text{m}$  and **d** 20  $\mu\text{m}$

4  $\mu\text{m}$ , (c) 10  $\mu\text{m}$  and (d) 20  $\mu\text{m}$ . The size of CuO nanoparticles was ranging from  $10 \pm 5$  nm.

### 3.4 SEM and EDS Profile

Figure 5 present the SEM images of CuO nanoparticles using extract of *G. herba* where (a) the scale bar is 10  $\mu\text{m}$  and (c) the scale bar is 25  $\mu\text{m}$  and EDS profiles (b, d). The EDS spectrum of CuO nanoparticles gives the elemental composition of CuO nanoparticles. Figure (b) and (d) present four peaks between 0.5 and 9 kV, which are identified as cooper and oxygen.

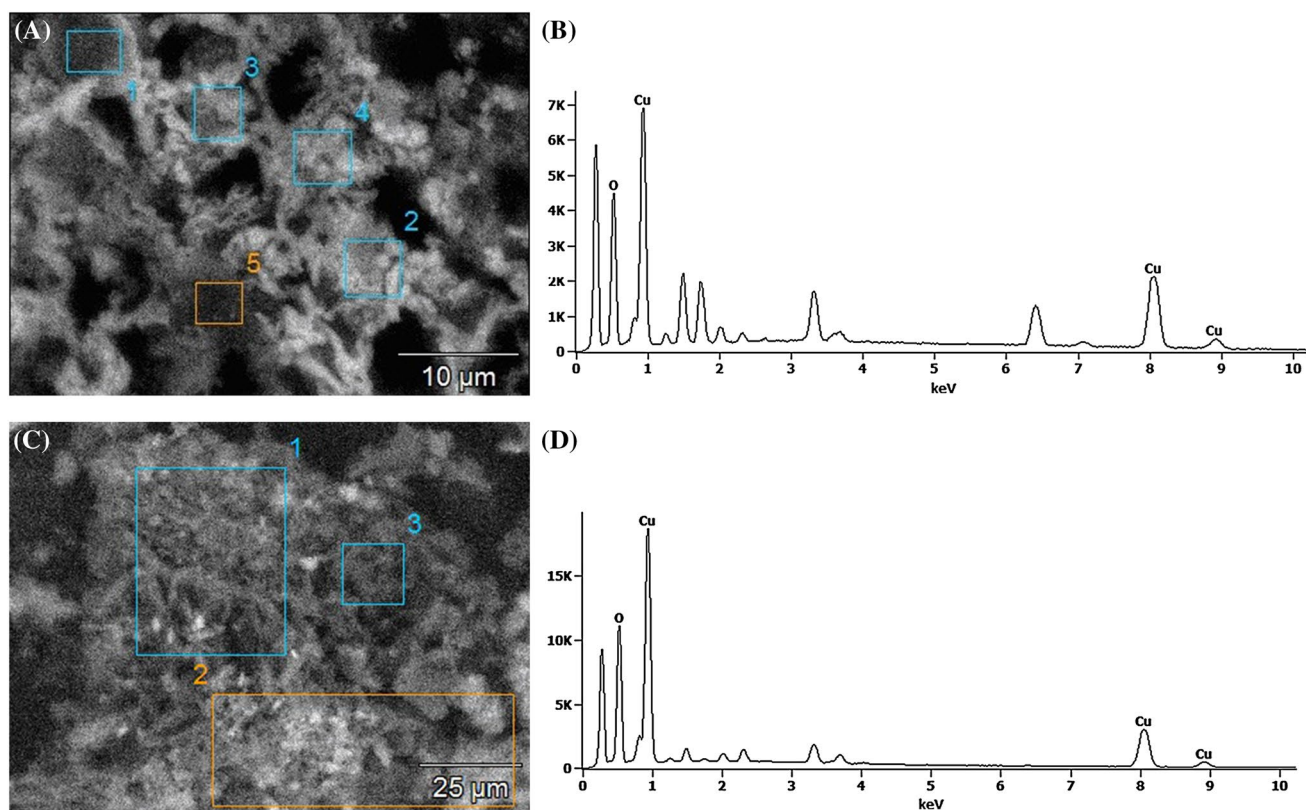
### 3.5 TEM Analysis

TEM images confirmed the connectivity between the spheres which observed in SEM pictures. Figure 6 shows

TEM images of the CuO nanoparticles synthesized using extract of *G. herba* where (a) the scale bar is 100  $\mu\text{m}$  and (b) the scale bar is 200  $\mu\text{m}$ . SEM and TEM analysis are used to determine the size and shape of nanoparticles. The SEM and TEM images reveal that particles are well dispersed, crystalline in nature [20]. The structure of prepared copper nanoparticles is spherical.

### 3.6 Antioxidant Activity of CuO Nanoparticles

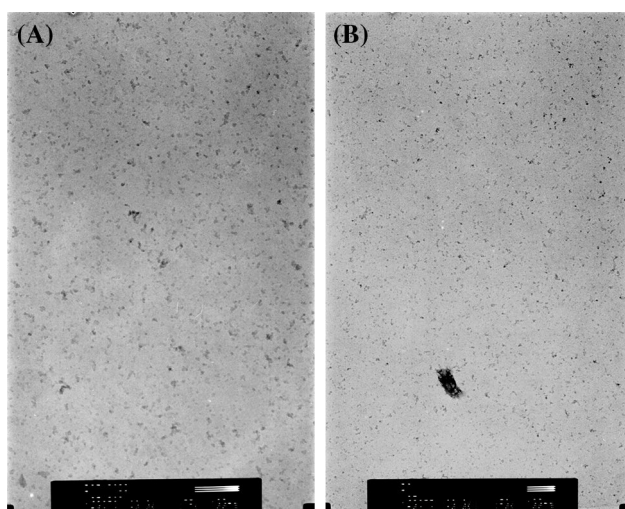
One of the most important basic studies in nano science and technology is the assessment of the antioxidant activity of nano materials [32, 33]. Antioxidants have a significant impact on the functioning of all bio-systems. In biological systems, free radicals are generated as a result of the interaction of biomolecules with molecular oxygen [34, 35]. The antioxidant activity of many kinds of both natural



**Fig. 5** SEM images of the CuO nanoparticles using extract of *G. herba* where **a** the scale bar is 10 µm and **c** the scale bar is 25 µm and EDS profiles (**b**, **d**)

and synthetic compounds has been examined by various researchers [36]. The DPPH scavenging assay is perceived as the most popular method of studying the antioxidant property of materials. This study determined the antioxidant activity of CuO nanoparticles synthesized using *G. herba*

extract, by means of a test that used the DPPH radical. In the DPPH method, antioxidants in the sample reduce the stable nitrogen radical DPPH, leading to the decrease in absorbance measured at the wavelength of 515 nm. Substances that can donate oxygen atom create the reduced form of DPPH, causing the solution to lose the violet color. In this work, the antioxidant properties of CuO nanoparticles synthesized using the extract of *G. herba* were determined by calculating IC<sub>50</sub> parameter, which indicates the ability to scavenge free radicals. A higher IC<sub>50</sub> parameter means that a given antioxidant is more reactive. For biosynthesized CuO nanoparticles, the value of the parameter was 4.12 µg/ml. The result indicated that the obtained CuO nanoparticles showed high antioxidant activity.



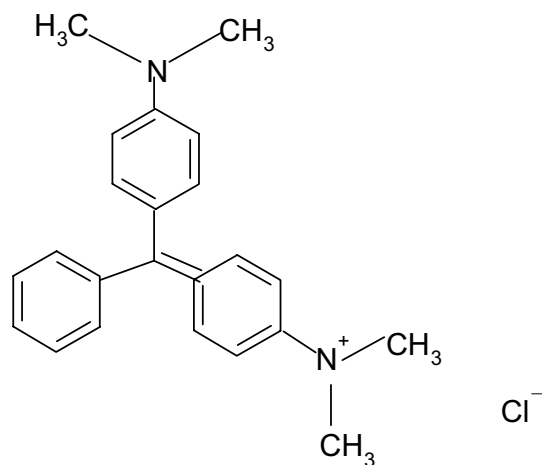
**Fig. 6** TEM images of the CuO nanoparticles synthesized using extract of *G. herba* where **a** the scale bar is 100 µm and **b** the scale bar is 200 µm

### 3.7 Catalytic Activity of CuO Nanoparticles

Malachite green is traditionally used as a dye for materials such as silk, leather and paper. It is highly toxic to mammalian cells, carcinogenic and can cause skin irritation. Therefore, removal of malachite green from effluent is essential to protect the environment. Conventional biological treatment used in order to remove dyes from wastewaters is generally ineffective as the dyes are resistant to microorganisms.

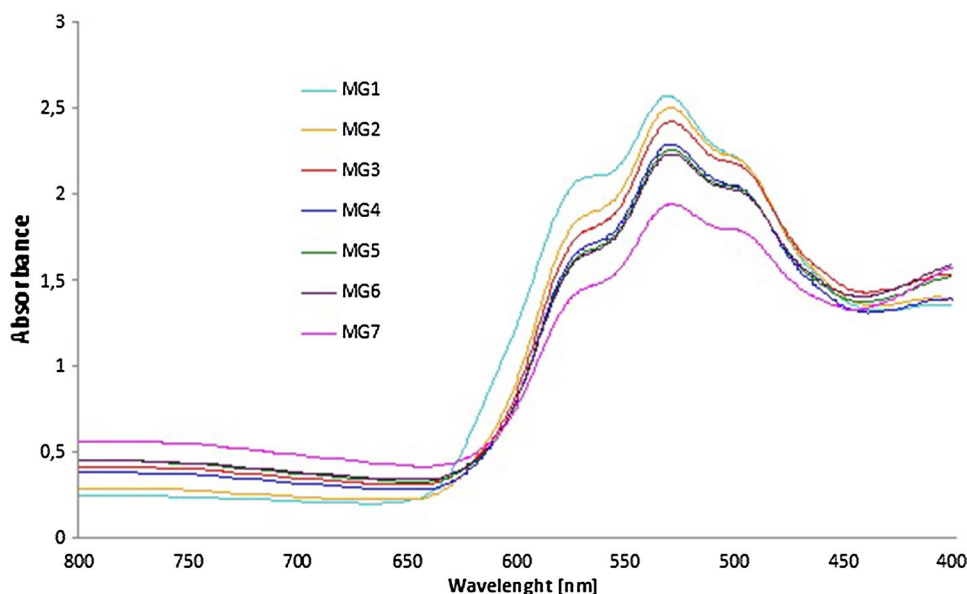
Moreover, the physico-chemical treatment methods are ineffective at higher effluent concentrations [37]. Therefore, the search for a simple method for the efficient degradation of dyes has gained greater significance. One of such solutions may be the application of metal oxide nanoparticles, which show enhanced catalytic activity in the degradation of organic dyes [38]. The maximum absorbance value of malachite green was recorded at 616 nm. This study investigated the catalytic degradation of synthetic dye malachite green by CuO nanoparticles synthesized using *G. herba* extract. Figure 7 presents the chemical structure of malachite green dye.

The absorbance was read after preparing the successive samples: from MG1 (4 ml of malachite green ( $1 \times 10^{-4}$  M), 0.5 ml of extract, 0.5 ml of prepared CuO nanoparticles and 3 ml of Milli Q water) to MG7, in which the amounts of the prepared solution of CuO nanoparticles were successively



**Fig. 7** Chemical structure of malachite green dye

**Fig. 8** UV–Vis spectra of malachite green reduction by CuO nanoparticles synthesized using extract of *G. herba*



increased by 0.5 ml, and the amounts of Milli Q water were successively decreased by 0.5 ml. Figure 8 shows UV–Vis spectra of malachite green reduction by CuO nanoparticles synthesized using extract of *G. herba*. There was observed a small decrease in absorbance related to the degradation of malachite green by the synthesized CuO nanoparticles. Therefore, the degradation of malachite green continued with the increase in the number of CuO nanoparticles.

## 4 Conclusion

In this study, the green synthesis of CuO nanoparticles using extract of *Galeopsidis herba* and studies their antioxidant and catalytic activity in degradation of malachite green was presented. The prepared CuO nanoparticles were characterized by UV–VIS, FTIR, SEM, SEM with EDS profile and (TEM) analysis. SEM and TEM studies supported the formation of CuO nanoparticles. EDS profile confirmed the signal characteristic of copper and oxygen. FTIR analysis confirmed the presence of active compounds responsible for reduction and stabilization of CuO nanoparticles. The size obtained CuO nanoparticles was about 10 nm. The synthesized CuONPs resulted in a slow degradation of malachite green, which was followed with increasing amounts of nanoparticles. For CuO nanoparticles, the value of the parameter for CuO nanoparticles was 4.12  $\mu\text{g}/\text{ml}$ . Also, CuO nanoparticles showed very good antioxidant activity.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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