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Antibacterial Activity of synthesized Copper Oxide Nanoparticles using *Malva sylvestris* Leaf Extract

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Abstract

The use of plant leaves extract in the biosynthesis of nanostructured materials can be eco-friendly, non-toxic and cost effective approach. In this paper, we report a facile and green synthesis of copper oxide nanoparticles using *Malva sylvestris* leaf extract. The biosynthesized copper oxide nanoparticles were characterized by Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and UV-vis spectroscopy. The particles are crystalline in nature with average size 14 nm. The morphology of the copper oxide nanoparticles can be controlled by tuning the amount of *Malva sylvestris* leaf extract and copper ions. The synthesized CuONPs are highly stable and have significant effect on both Gram-positive and Gram-negative bacteria.

Keywords: Antibacterial activity, Copper oxide nanoparticles, *Malva sylvestris* leaf extract.

Introduction

In recent years the interest in nanomaterials has increased dramatically due to their unique chemical and physical properties. As the experimental conditions used in the synthesis of these nanoparticles play an important role in determining the particle size. Various methods have been used for synthesis copper oxide nanoparticles CONPs including, hydrothermal approach (Teng et al., 2008; Volanti et al., 2008; Mohamed et al., 2014; Outokesh et al., 2011), sonochemical technique (Shui et al., 2013; Sufarifard and Morsali, 2012; Wongpisutpaisan et al., 2011), thermal oxidation route (Li et al., 2013; Chen et al., 2008), thermal decomposition (Salavati and Davar, 2009), reverse microemulsion method (Chu et al., 2013; Kumar et al., 2013,), precipitation method (Siddique and Karmakar, 2013; Phiwdang et al., 2013, Sahooli et al., 2013; Mustafa et al., 2013), solution combustion method (Umadevi and Christy, 2013), chemical method (Zhang et al., 2013; Harish et al., 2014). All employ toxic chemicals and energy intensive routes which make these choices eco-hazardous and preclude their applications in biology, medicine and clinical applications. Thus, developing environment friendly protocols is the need of hour in nanomaterial synthesis. Biosynthesis of copper oxide nanoparticles CuONPs using microorganisms such as bacteria, fungi, yeast have been reported in the literature (Singh et al., 2010; Rahman et al., 2009; Honary et al., 2012). Plants attracted few researchers to use their extracts in green synthesis of copper oxide nanoparticles such as *Carcia papaya* leaf extract (Sankar et al., 2014), *Aloe vera*leaf extract (Gunalan et al., 2012), and *Centella asiatica* leaves extract (Devi et al., 2014).

In this study, we report for the first time biological synthesis of copper oxide nanoparticles (CuONPs) using *Malva sylvestris* leaf extract. We investigated the effects of the reaction temperature, reaction time, plant leaf extract quantity on the particle size of copper oxide nanoparticles. We also evaluated antibacterial characteristics of the synthesized copper oxide nanoparticles.

Materials and Methods

Materials

Copper chloride dihydrate $CuCl_2.2H_2O$, and sodium hydroxide NaOH are analytical

grades purchased from Merck and used without purification. Deionized distilled water was used in all experimental work.

Preparation of *Malva sylvestris* Leaf Extract

Malva sylvestris leaves were collected in and around the campus of Royal Scientific Society, El Hassan Science City, Amman, Jordan. *Malva sylvesris* leaves were washed several times with distilled water to remove dust particles and then sun dried to remove the residual moisture. *Malva sylvestris* leaf extract was prepared by placing 5 g of dried fine cut in 500 ml glass beaker along with 400 ml of sterile distilled water. The mixture was then boiled for 5 minutes until the color of aqueous solution changed from watery to brown-yellow. Then the mixture was cooled to room temperature and filtered with Whatman No. 1 filter paper before centrifuging at 1200 rpm for 2 minutes to remove biomaterials. The extract was stored at room temperature in order to be used for further experiments.

Synthesis of Copper Oxide Nanoparticles

In a typical reaction mixture, 400 ml of aqueous 4mM copper chloride dehydrate $CuCl_2.2H_2O$ was treated with 10 ml aqueous leaf extract of *Malva sylvestris* and stirred magnetically at room temperature, until the light blue color changed to light green color. Then the mixture is heated at 80 ^oC for 2 minutes. Afterwards, the mixture was treated with 1M sodium hydroxide drop by drop. As soon as, the sodium hydroxide comes in contact copper ions spontaneous change the green mixture to brown precipitate, indicating the formation of water soluble monodispersed copper oxide nanoparticles. The concentrations of copper chloride solution and leaf extract were also varied from a 1 to 4 mM and 1% to 10% by volume, respectively. The brown precipitate was then taken out and washed repeatedly with deionized water followed by ethanol to remove the impurities for the final product. Then a brown powder was obtained after drying at 60 ^oC in vacuum oven over night. Fig. 1 shows the green synthesis route for copper oxide nanoparticles.

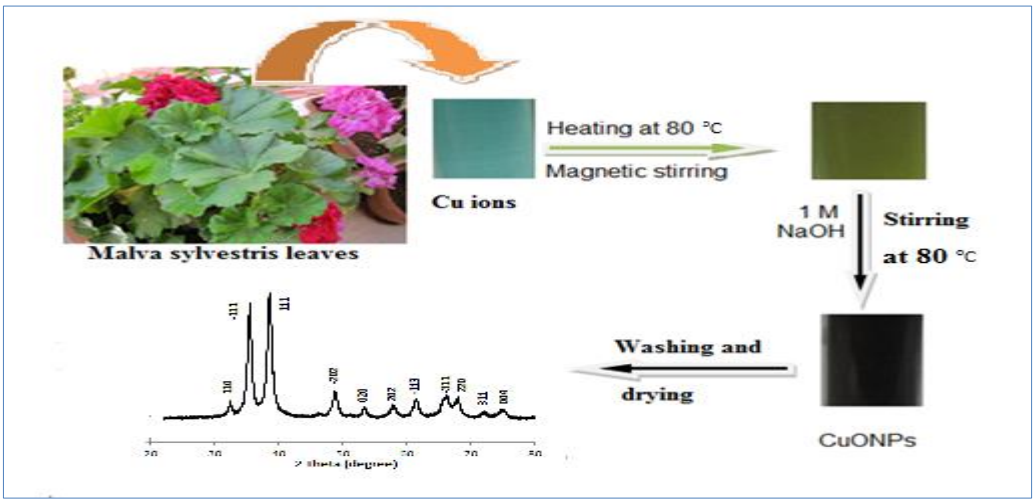

Fig. 1. Green synthesis route of copper oxide nanoparticles

Characterization of Copper Oxide Nanoparticles

Copper oxide nanoparticles synthesized by this green method were characterized by X-ray diffractometer, (XRD-6000, Shimadzu) equipped with Cu K α radiation source (λ =0.154056 nm) using Ni as filter at a setting of 30 kV/30mA. All XRD data were collected under the experimental conditions in the angular range $3^o \leq 2\theta \leq 80^o$. FT-IR spectra of plant leaf extract and synthesized copper oxide nanoparticles were obtained in the range 4000-400 cm^{-1} with IR-Prestige 21 spectrophotometer (Shimadzu) using KBr pellet method. UV-visible double beam spectrophotometer (UV-1601, Shimadzu) used for confirming the synthesized copper oxide nanoparticles. Scanning electron microscopy (SEM) images were taken using a field emission scanning electron microscopy (FEI Quanta 450 FEG) with 30 kV acceleration voltages.

Results and Discussion

X-ray diffraction Analysis

Fig. 2 shows the X-ray diffraction (XRD) pattern of the CuO powder synthesized from copper chloride dehydrate and sodium hydroxide in the presence of *Malva sylvestris* leaf extract at 80 ^oC. The XRD pattern revealed the orientation and crystalline nature of copper oxide nanoparticles. The peak position with 2θ values of 32.49o, 35.49^o, 38.96^o, 48.73^o, 53.45^o, 58.34^o, 61.53^o, 65.79^o, 66.25^o, 72.43^o and

75.03º are indexed as (110), (-111), (111), (-202), (020), (202), (-113), (-311), (220), (311), and (004) planes, which are in good agreement with those of powder CuO obtained from the International Center of Diffraction Data card (JCPDS-80-1916) confirming the formation of a crystalline monoclinic structure. No extra diffraction peaks of other phases are detected, indicating the phase purity of CuONPs. The average crystallite size of the synthesized copper oxide nanoparticles was calculated to be 14 nm using Debye-Scherrer equation (Klug and Alexander, 1954):

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

Where D is the crystallite size of copper oxide nanoparticles, λ represents wavelength of x-ray source 0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with value from 0.9 to 1 and θ is the Bragg angle.

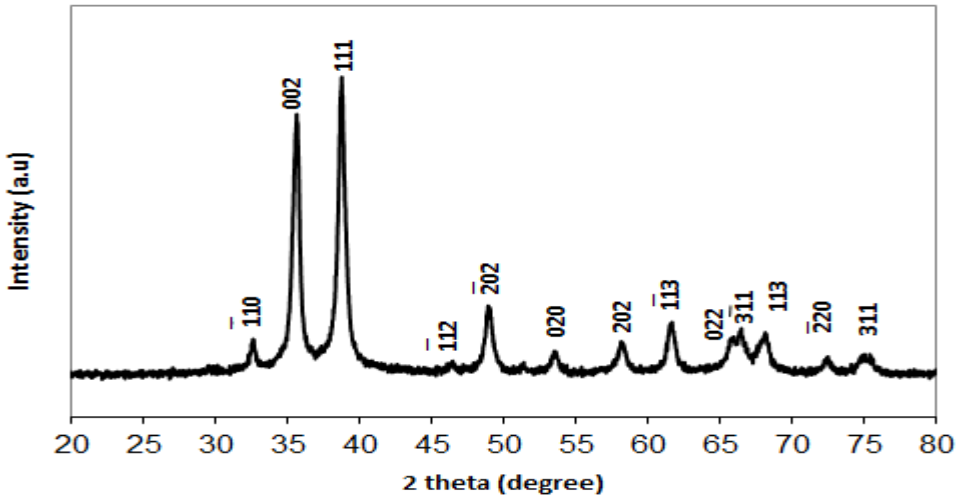

Fig. 2. XRD pattern of the synthesized copper oxide nanoparticles

Fourier transform infrared spectroscopy analysis

FT-IR analysis is used to identify and get an approximate ides of the possible biomolecules that are responsible for capping and stabilization of the CuONPs with the *Malva sylvestris* leaf extract.

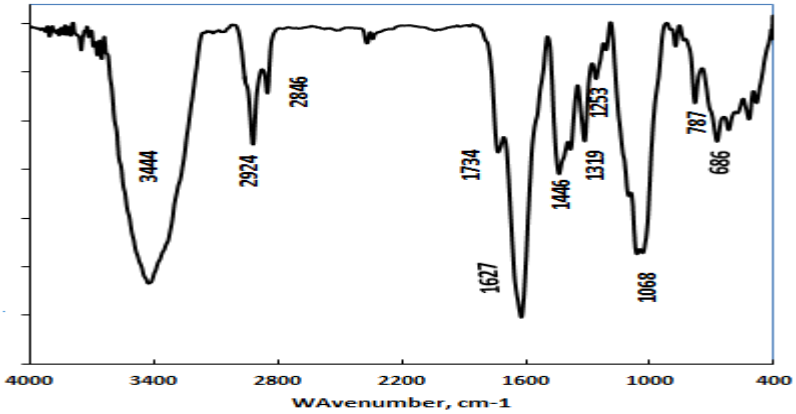

Fig. 3. FT-IR of *Malva sylvestris* leaf extract

(A strong peak at 3444 cm^{-1} can be attributed hydrogen bonded O-H groups of alcohols and phenols and also to the presence of amines N-H of amide, Fig. 3. This peak shifted to lower field at 3398 cm^{-1} in the synthesized CuONPs, Fig. 4. The bands at 2924 cm^{-1} and 2846 cm^{-1} are assigned to $–CH_2$ and C-H stretching mode in alkanes. The shoulder peak at 1734 cm^{-1} in *Malva sylvestris* leaf extract could be attributed to C=C stretching vibrations about C=O amide conjugated C=O of the proteins that are responsible for capping and stabilizing of CuONPs. The peaks observed in the range of 686-1446 cm^{-1} have been assigned to alcohols and phenolic groups, C-N stretching vibrations of aliphatic and aromatic amines. The major peak was observed to be 513 cm^{-1} should be a stretching of Cu-O. The bands at 1627 cm^{-1} of the leaf extract shifted to lower field at 1604 cm^{-1}, in the product.)

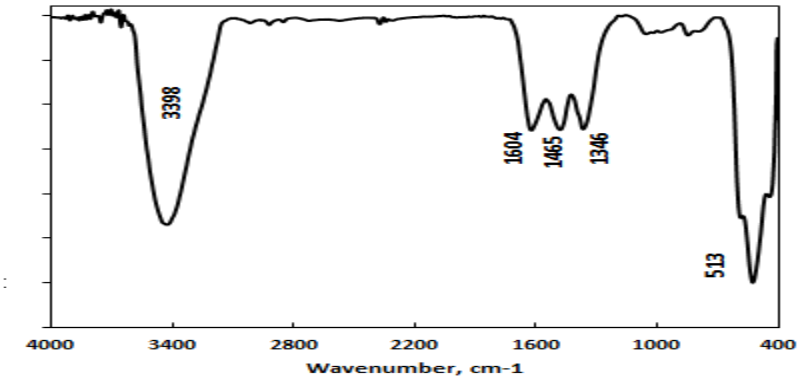

Fig. 4. FT-IR of synthesized CuONPs in *Malva sylvestris* leaf extract

(While the bands at 1446 cm^{-1} shifted to higher field at 1465 cm^{-1} and the peak at 1319 cm^{-1} shifted to 1346 cm^{-1}. These bands assigned to C-O stretching, C=O stretching and N-H bending. The shifting in these bands is clearly indicating that the coordination of carboxylic acids in the protein of *Malva sylvestris* leaf extract with CuONPs play a major role on dispersing, stabilizing and capping of CuONPS.)

Scanning electron microscopy analysis

Typical SEM micrograph for as prepared CuONPs is shown in Fig. 5. The SEM micrograph clearly showed rough agglomerations of nanostructural homogeneities with spherical morphologies of CuONPs. The SEM observation showed the presence of agglomerated nanospheres with an average diameter of 5–30 nm. This slight deviation of the particle size estimation compared to that calculated from XRD analysis can be attributed to the deviation of the spherical shape of the particles that is required for the Debye–Scherrer formula and the detection limit of the XRD diffractometer. Moreover, the observed strong agglomeration of the nanoparticles prepared by this method may be interpreted in terms of the increase in the catalytic activity of the surface of the nanoparticles. This may explain the pronounced antibacterial activity of the CuONPs prepared by the novel biosynthesis method using the *Malva sylvestris* leaf extract.

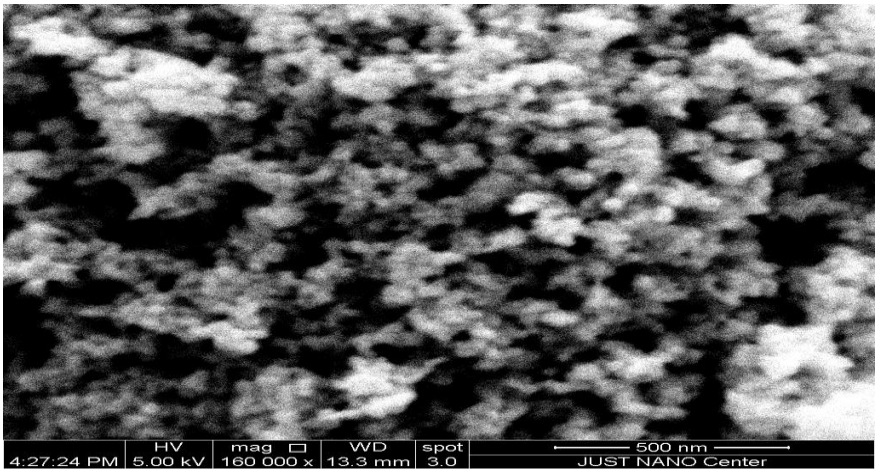

Fig. 5. SEM image of the synthesized CuO nanoparticles.

Antibacterial activity

The antibacterial activity of the synthesized copper oxide nanoparticles using *Malvas ylvestris* leaf extract was examined against both Gram-negative and Gram positive bacteria by using disc diffusion test. The radial diameter of the inhibition zone of *Shigella* and *Listeria* by CuONPS are 15 and 18 mm, respectively, **Fig. 6.**

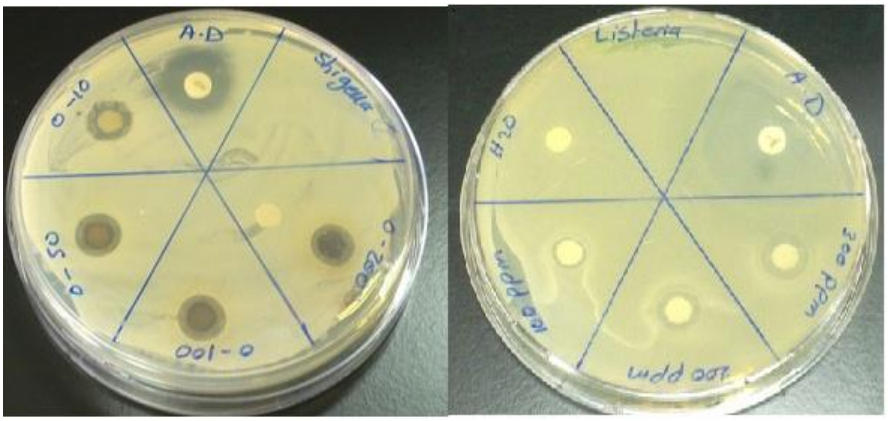

Fig. 6. Antibacterial assay: Zone of inhibition against *Shigella* and *Listeria* bacteria

Conclusion

In the present work, we first report an eco-friendly and simple method for the synthesis of copper oxide nanoparticles using *Malva sylvestris* leaf extract. Copper oxide nanoparticles (CuONPs) has been synthesized using copper chloride dehydrate and sodium hydroxide in the presence of *Malva sylvestris* leaf extract at 80 ^{o}C. The average size of the nanoparticles was found to be 14 nm which was calculated by Debye-Scherrer equation. FT-IR and XRD results corroborated the purity of the synthesized CuONPs. The synthesized CuONPs are highly stable and have significant effect on both Gram-positive and Gram-negative bacteria.

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