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Biosynthesis of copper oxide nanoparticles using *Ailanthus altissima* leaf extract and antibacterial activity

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An eco-friendly green route for synthesis copper oxide nanoparticles (CuONPs) by *Ailanthus altissima* leaf aqueous extract was reported. The synthesized copper oxide nanoparticles were characterized in terms morphology, crystalline nature, structural and antibacterial activity with UV-vis, SEM, TEM, FT-IR analysis tools. The synthesized copper oxide nanoparticles were well crystalline in nature with particle shape spherical and average particle size 20 nm. The antimicrobial activity of CuONPs was determined by disk diffusion method against some selected species of bacteria, demonstrated a significant inhibitory activity against *S. aureus* followed by *E. coli*. In view of promising activity, the CuONPs could possibly be employed as antibacterial agent.

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Capsule Summary: An eco-friendly green route for synthesis of CuONPs using *Ailanthus altissima* leaf aqueous extract and characterization was done by UV-vis spectroscopy, XRD, SEM and TEM techniques. CuONPs revealed promising antibacterial activity.

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INTRODUCTION

Copper oxide nanoparticles (CuONPs) with different morphological structures as antibacterial activity against bacteria was developed using various synthetic routes such as precipitation method (Phiwdang et al., 2013; Rahimi-Nasrabadi et al., 2013; Mayekar et al., 2014), sonochemical synthesis (Silva et al., 2019), thermal decomposition of copper salts (Shahsavani et al., 2016), electrochemical synthesis (Katwai et al., 2015), heat treatment approach (Baqer et al., 2018), nonionic water-in-oil microemulsions (Dodoo-Arhin et al., 2012), and sol-gel synthesis (Dörner et al., 2019). These routes for synthesis copper oxide nanoparticles have many disadvantages: Difficulty of scale up

the process, separation and purification of nanoparticles. Developing facile and green routes for synthesizing copper oxide nanoparticles are of importance and still a challenge for materials researchers. Plants extract such leaves, seeds, bark, flowers and, fruits have been suggested as valuable alternatives to chemical and physical methods for synthesis of copper oxide nanoparticles. Synthesis of copper oxide nanoparticles using plant extracts were reported in the open literature such as: *Ocimum basilicum* leaves extract (Altikatoglu et al., 2017), *Carica papaya* leaves extract (Sankar et al., 2014), leaf extract of *Calotropis goigantea* (Sharma et al., 2015), *Anthemis nobilis* flowers (Nasrollahzadeh et al., 2015), *Tinospora cordifolia* (Gowda et al., 2015), leaf extract of *Catha edulis* (Gebremedhn et al., 2019), *Gloriosa superba* L. extract (Naika et al., 2015),

Ocimum tenuiflorum (Sumitha et al., 2016), *Eichhornia crassipes* leaf extract (Hemalatha and Makeswari, 2017), *Murayya koenigii* aqueous leaf extract (Nordin et al., 2019), *Abutilon indicum* leaf extract (Ijaz et al., 2017), *Juglans regia* leaf extract (Aseman et al., 2019), *Punica granatum* leaf extract (Vidovix et al., 2019), *Aloe barbadensis* Miller leaf extract (Kumar et al., 2015), *Malva sylvestris* leaf extract (Awwad et al., 2015), *Ixora coccinea* leaf extract (Yedurkar et al., 2017), fruit extract of *Syzygium alternifolium* (Yugandhar et al., 2018), aqueous extract of *Pterospermum acerifolium* leaves (Saif et al., 2016), wheat seed extract (Bauzar et al., 2019), *Azadirachta indica* leaves (Dey et al., 2019), *Punica granatum* peels extract (Ghidan et al., 2016), banana peel extract (Aminuzzaman et al., 2017), *Cassia auriculata* leaf (Shi et al., 2017).

Besides, the aqueous extract of Oak fruit (Sorbiun et al., 2018), an aqueous black bean extract (Nagajyothi et al., 2017), *Camellia Sinensis* leaf extract (Jeronsia et al., 2019), *Ixora coccinea* plant leaves (Vishveshvar et al., 2018), *Desmodium gangeticum* aqueous root extract (Guin et al., 2015), leaves extract of *Leucaena leucocephala* L (Aher et al., 2017), *Albizia lebbek* leaf extract (Jayakumarai et al., 2015), *Acanthospermum hispidum* L. extract (Pansambai et al., 2017), aqueous extract of *Cordia myxa* L. Leaves (Thamer et al., 2018), *Ziziphus Mauritiana* L. extract (Ghotekar et al., 2017), *Enicostemma axillare* (Lam.) leaf extract (Mali et al., 2019), *Psidium guajava* leaf extract (Singh et al., 2019), *Tabernaemontana divaricate* leaf extract (Sivaraj et al., 2014), *Saraca indica* leaves (Prasad et al., 2017), Henna leaf powder (Fardood et al., 2018), *Azadirachta indica* leaf aqueous extract (Ansilin et al., 2016), *Ruellia tuberosa* leaf extract (Vasantharaj et al., 2019), *Artabotrys Hexapetalus* and *Bambusa Vulgaris* plant (Haseena et al., 2019), *Cordia sebestena* flower aqueous extract (Prakash et al., 2018), tea leaves (Khatami et al., 2019), *Phoenix dactylifera* L leaves extract (Berra et al., 2018), *Andean blackberry* (*Rubus glaucus* Benth.) fruit and leaf (Kumar et al., 2017), *Tecoma castanifolia* leaf extract (Sharmila et al., 2016), leaves of *Azadirachta indica*, *Pongamia pinnata*, *Lantana camara* and orange peel (Shiny et al., 2019) have also been applied for the preparation of bio-inspired NPs.

The present study described an eco-friendly and green synthesis route of CuONPs using an abundantly available *Ailanthus altissima* plants in Jordan. *Ailanthus altissima* leaves aqueous extract acted as the reducing and stabilizing agent for the potential application of the biosynthesized CuONPs as antimicrobial agent for positive-gram and negative-gram bacteria. The novelty of this work is that for the first time *Ailanthus altissima* leaf extract was used for the synthesis of CuONPs. The prepared CuONPs were characterized by advanced techniques and antimicrobial activity was also evaluated.

MATERIAL AND METHODS

Chemical and reagents

Copper (II) acetate $\geq 98\%$ $[\text{Cu}(\text{CH}_3\text{COO})_2]$ was obtained from Sigma-Aldrich, Germany. Fresh *Ailanthus altissima* leaves were collected from the trees planted in the gardens of the Royal Scientific Society, Amman, Jordan. *Ailanthus altissima* leaves were washed with distilled water to remove any dust particles and then left in our laboratory for 7 days to dry for the removal the moisture residue.

Aqueous extract preparation

A 5-20 g of the dried powder of leaves was boiled in 100 ml of de-ionized water for 10 min. The aqueous solution of *Ailanthus altissima* leaves was cooled at laboratory temperature (27°C) and filtered with Whatman No. 1 filter paper. The filtrate was kept in refrigerator for the experimental work.

Synthesis of copper oxide nanoparticles (CuONPs)

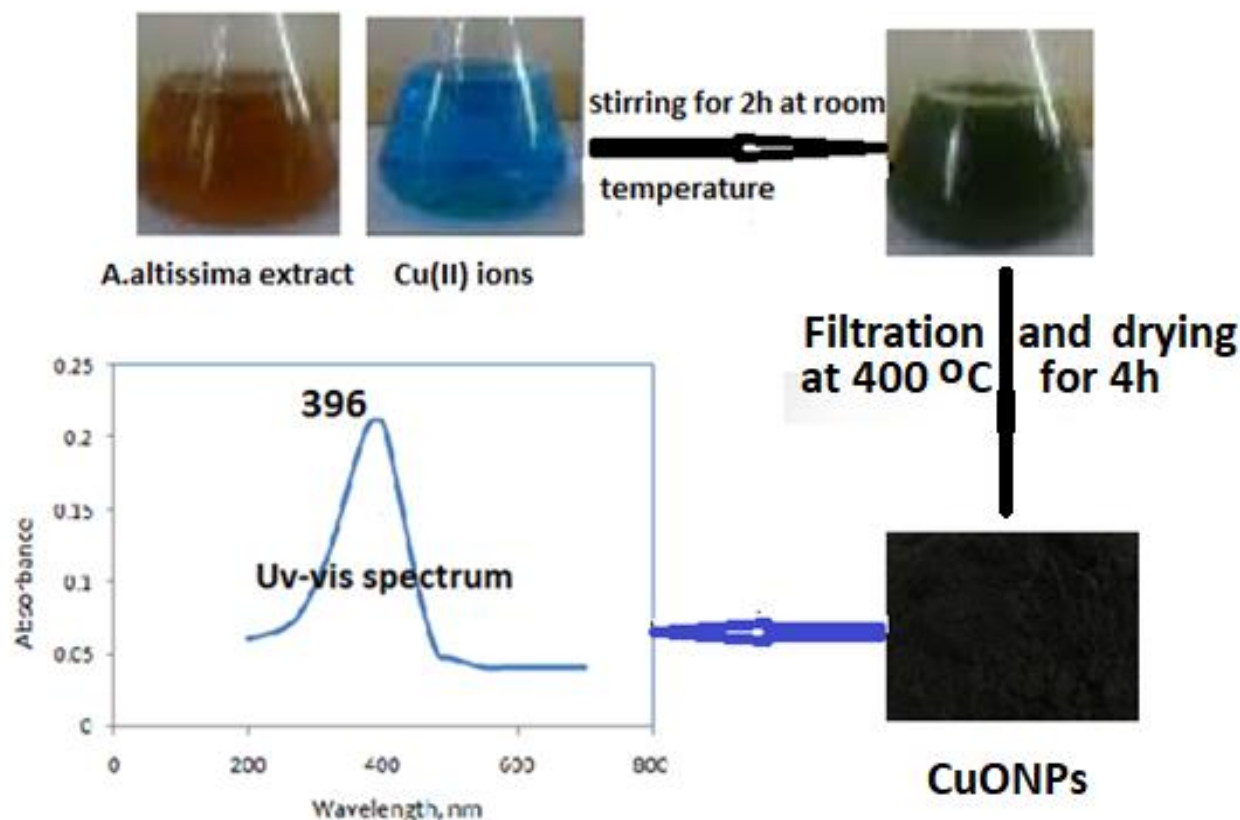
For the synthesis of CuONPs, 1-3g of copper acetate dissolved in 100ml de-ionized water in a glass beaker. Later, 10 ml of aqueous extract of *Ailanthus altissima* leaves extract was added drop wise and mixed homogeneously by magnetic stirrer for 10 min. The blue colour of copper acetate solution changes to brown deep colour due to the formation of CuOH NPs. Under continuous magnetic stirring for 4 h, the deep brown color of the mixture changed to brown-black. Afterwards, the brown-black mixture cooled to attain laboratory temperature and with centrifuging at 10,000 rpm for 10 min, the resulting paste was transferred into silicacrucible for heating in muffle furnace at 400 °C for 4h, subsequently black color nanopowder material achieved. Powder obtained was subjected to FT-IR, XRD, SEM and TEM analysis. The schematic presentation of synthesis is presented in Scheme 1.

Characterization

UV-vis spectrum of copper oxide nanoparticles was recorded, by taking 0.1 ml of the sample and diluting it with 2 ml de-ionized water, as a function of time of reaction using a Shimadzu 1601 spectrophotometer in the wave length region 200 to 700 nm operated at a resolution of 1 nm. Scanning electron microscopy (SEM) analysis of synthesized copper oxide nanoparticles was done using a Hitachi S-4500 SEM machine. Transmission electron microscopy was done using TEM CM 200, Philips. Powder X-ray diffraction (XRD) was performed using X-ray diffractometer, Shimadzu, XRD-6000 with $\text{CuK}\alpha$ radiation $\lambda = 1.5405 \text{ \AA}$ over a wide range of Bragg angles ($3^\circ \leq 2\theta \leq 80^\circ$). Fourier transform infrared (FT-IR) spectroscopic measurements were done using Shimadzu, IR-Prestige-21 spectrophotometer.

RESULTS AND DISCUSSION

UV-vis analysis



Scheme 1: UV-vis spectrum of CuONPs synthesized by using *Ailanthus altissima* leaf extract

The green approach for the formation of copper oxide nanoparticles using *Ailanthus altissima* leaf extract was studied. Formation of copper oxide nanoparticles were confirmed by UV-Vis spectrophotometer. Fig.1 showed the UV-vis absorption spectrum of copper oxide nanoparticles was recorded for the sample in the range of 200–700 nm. The spectrum showed that the absorbance peak at 396 nm corresponding to the characteristic band of copper oxide nanoparticles.

X-ray diffraction analysis

The XRD analysis of copper oxide nanoparticles synthesized using *Ailanthus altissima* leaf extract is illustrated in Fig. 2. The 2 θ peaks at 32.5°, 35.4°, 38.9°, 48.7°, 58.4°, 61.4°, 65.8°, 66.3°, 72.5° and 74.9° are attributed to the crystal planes of copper oxide at (110), (002), (111), (202), (020), (202), (113), (311), (113), (220), and (311), respectively.

Table 1: Antibacterial activity (zones of inhibition) of CuONPs on pathogenic bacteria strains

Conc. of CuONPs	<i>E. coli</i>	<i>S. aureus</i>
<i>Ailanthus altissima</i> extract	3	5
CuONPs – 20 μ g/ml	13	16
CuONPs – 40 μ g/ml	14	18
CuONPs – 60 μ g/ml	15	19
CuONPs – 80 μ g/ml	16	20
CuONPs -100 μ g/ml	18	20
CuONPs -120 μ g/ml	18	20
Chloromphenical drug	18	20

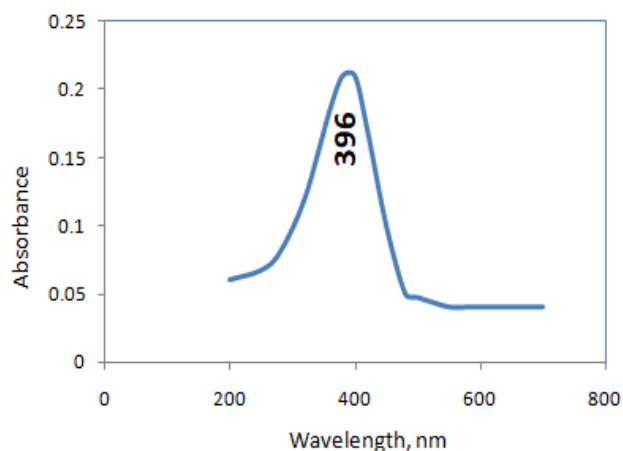


Fig. 1: UV-vis spectrum of CuONPs synthesized by using *Ailanthus altissima* leaf extract

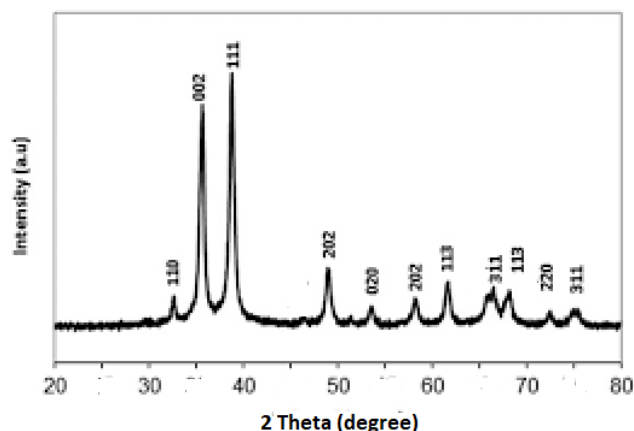


Fig. 2: XRD of synthesized copper oxide nanoparticles

The copper oxide nanoparticles (CuONPs) are well crystalline and the position and the relative intensity of the diffraction peaks match well with the standard phase CuONPs diffraction pattern of the International Center of Diffraction Data card (JCPDS-80-1916). The average particle sizes of the synthesized CuO nanoparticles were calculated using Debye-Scherrer formula (Eq. 1).

$$D = K\lambda / \beta \cos \theta \quad (1)$$

Where, D is the mean diameter of nanoparticles, β is the full width at half-maximum value of XRD diffraction lines, λ is the wavelength of X-ray radiation source 0.15405 nm, θ is the half diffraction angle–Bragg angle and K is the Scherrer constant with value from 0.9 to 1. The crystalline size of green synthesized copper oxide nanoparticles using *Ailanthus altissima* leaves extract calculated from Scherrer equation it was about 20 nm.

Scanning (SEM) and transmission (TEM) electron microscopy analysis

The morphology and size determination of green synthesized CuONPs using *Ailanthus altissima* aqueous extract was done by SEM and TEM analysis. Fig. 3 showed the green synthesized CuO NPs are spherical in shape with agglomeration of small particles appeared as cluster form. Different shapes and size of nanoparticles accelerate their performance in antibacterial activity studies attributable to large surface and higher reactive sites. TEM analysis also reveals the green synthesized CuONPs are in size range 5–20 nm. Moreover TEM pattern of green synthesized CuONPs confirmed the crystalline nature of nanoparticles.

FT-IR analysis of *Ailanthus altissima* leaf extract

The FT-IR analysis of *Ailanthus altissima* leaves aqueous extract and the synthesized copper oxide nanoparticles to identify the biomolecules responsible for the bioreduction copper salts to copper nanoparticles. The FT-IR spectrum of *Ailanthus altissima* leaves aqueous extract with the spectrum of synthesized copper nanoparticles showed that *Ailanthus altissima* spectrum have the peaks at 3444, 2920, 1735, 1616, 1543, 1450, 1327, 1215, 1041 cm^{-1} . Strong broad peak at 3444 cm^{-1} is due to the O-H groups of water, alcohols, or phenols and the presence of N-H amides stretching vibration. The band at 2920 cm^{-1} is assigned to $-\text{CH}_2$ and C-H stretching mode in alkenes. The strong peak at 1735 cm^{-1} in *Ailanthus altissima* leaves extract could be attributed to C=C stretching vibrations about C=O amide conjugated C=O of the proteins that are responsible for reducing, capping and stabilizing of CuONPs. The bands at 1616, 1543 and 1450 cm^{-1} can be allocated to the stretching vibration of C-OH bond from proteins (amide I) of the leaves extract. Peaks in the region 1327, 1215, and 1041 cm^{-1} may be attributed to the presence of the stretching vibrations of carboxylic acids and amino groups. The bands at 877, 774 and 602 cm^{-1} assigned for aromatic compounds residue in protein of *Ailanthus altissima* aqueous extract. The FT-IR analysis of synthesized copper oxide nanoparticles showed strong peaks at 3410 cm^{-1} , 2920, 1647 and 444 cm^{-1} . The band at 3410 cm^{-1} and 1647 represent C-O-H or C-N-H groups in the plant extract, which aid on the converting copper acetate to copper nanoparticles. While band at 2920 cm^{-1} represent $-\text{CH}$ in the protein structure of the extract. The band 444 cm^{-1} is due to the presence Cu-O nanoparticles.

Antibacterial activity

Green synthesized copper oxide nanoparticles (CuONPs) using *Ailanthus altissima* leaf aqueous extract were studied for antimicrobial activity against pathogenic bacteria by disc diffusion method. Chloromphenical was used as a control antimicrobial agent.

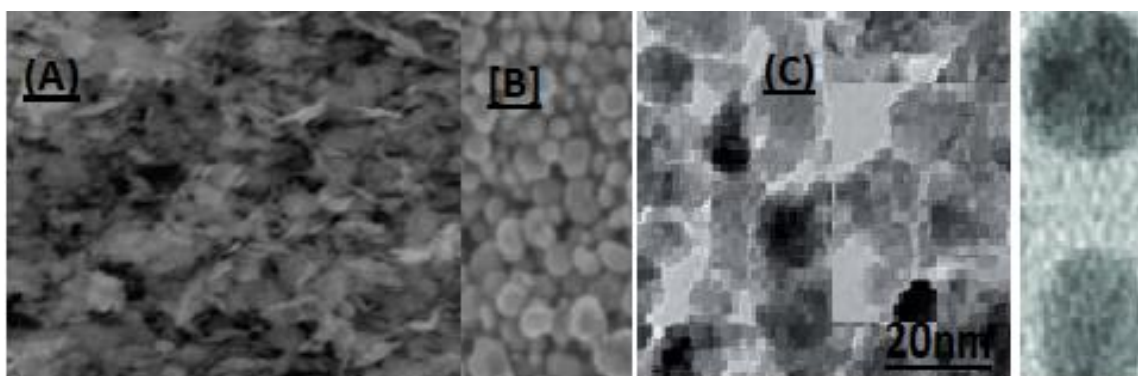


Fig. 3: SEM (A & B) and TEM (C) images of synthesized CuONPs

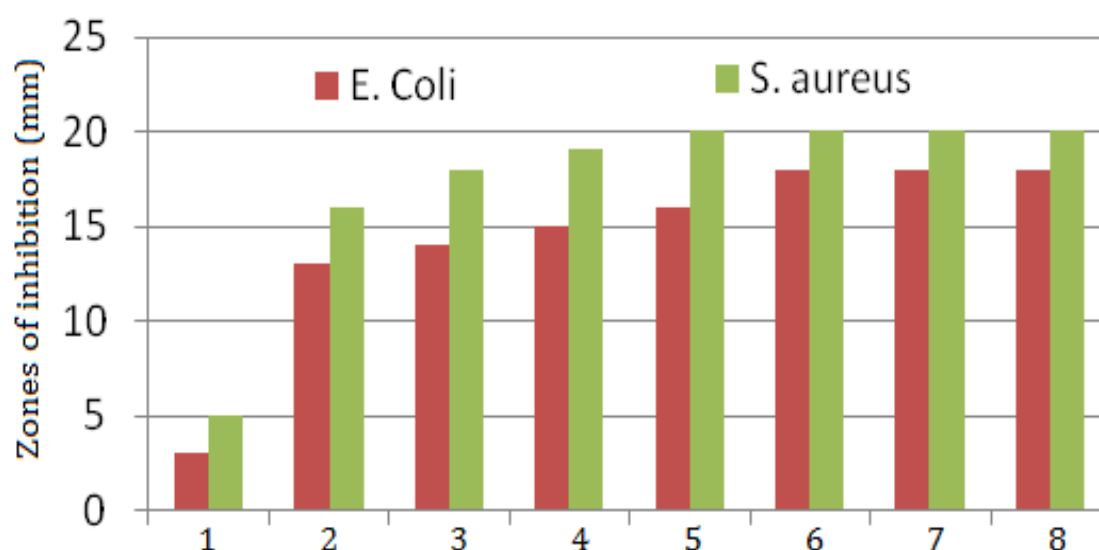


Fig. 4: Antibacterial activity of *Ailanthus altissima* leaf extract and synthesized copper oxide nanoparticles

[Where, 1 = *Ailanthus altissima* extract, 2 = CuONPs - 20µg/ml, 3 = CuONPs - 40µg/ml, 4 = CuONPs - 60µg/ml, 5 = CuONPs - 80µg/ml, 6 = CuONPs - 100µg/ml, 7 = CuONPs - 120µg/ml and 8 = chloromphenical drug]

The copper oxide showed inhibition zone against Gram negative *Escherichia coli* and Gram positive *Staphyococcus aureus* bacteria. Maximum zone of inhibition (MZI) are listed in Table 1 and plotted in Fig. 4. It was observed that an increase in CuONPs concentration increases the MZI of *E. coli* and *S. aureus* bacteria. Our results showed that the MZI for bacteria and average particles sizes of CuONPs are close to those reported previously (Remya et al., 2017; Berra et al., 2018; Igwe, O.U., Nwamezie, 2018; Awwad et al., 2015; Al Banna et al., 2020; Awwad et al., 2020ab).

CONCLUSIONS

The green chemistry route used in the present work for synthesis of copper oxide nanoparticles is simple, cost

effective and the resultant nanoparticles are highly stable and reproducible. There is no research work available in an open literature for the synthesis of copper oxide nanoparticle by using *Ailanthus altissima* leaves aqueous extract. The prepared copper oxide nanoparticles were spherical in shape and characterized using XRD, SEM and UV-Vis absorption techniques. The SEM image showed that most of the nanoparticles are spherical in shape. X-ray diffraction confirms the formation of crystalline monoclinic structure. Particle size of 20 nm was found, which indicates there is uniform size distribution of nanoparticles. Copper oxide nanoparticles prepared are expected to have more extensive applications such as efficient antibacterial agent. This process is an economical method for the preparation of nanocrystalline copper oxide with respect to energy, time

and simplicity and can be used for large scale synthesis of copper oxide nanoparticles.

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