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A novel route for the synthesis of copper oxide nanoparticles using *Bougainvillea* plant flowers extract and antifungal activity evaluation

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ABSTRACT

A green, cost-effective and eco-friendly method for the synthesis of copper oxide nanoparticles (CuO NPs) using *Bougainvillea* flower aqueous extract at room temperature was reported. The synthesized CuO NPs were characterized by UV–visible spectroscopy, fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and X-ray diffraction (XRD) tecniques. The synthesized particles were highly stable, spherical in shape with an average diameter of 12±4 nm. The CuO NPs were explored for their antifungal activity against *Aspergillus niger* and responses revealed that CuO NPs are highly efficient to inhibit the fungal growth and zone of inhibition were comparable with standard drug. The green route for the synthesis of CuO NPs is suggested in view of promising antifungal activity.

Capsule Summary: A green and fast-route for the synthesis of copper nanoparticles using aqueous solution of *Bougainvillea* flowers at room temperature was reported and NPs showed promising antimicrobial activity against panel of pathogens.

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INTRODUCTION

Synthesis of copper oxide nanoparticles (CuO NPs) can be performed using a number of routinely used chemical and physical methods such as chemical precipitation (Phiwdang et al., 2013; Luna et al., 2015; Rangel et al., 2020), sonochemical (Anandan et al., 2012; Silva et al., 2019), hydrothermal (Arun et al., 2015), thermal oxidation (Chen et al., 2008), sol-gel (Du et al., 2009), laser ablation in liquid (Khashan et al., 2016), chemical route [10], single-step wet synthesis (Naz et al., 2020), microwave irradiation (Wang et al., 2002), solid state thermal decomposition (Khalaji and Das; 2016) and simple chemical route (Mayekar et al., 2014). Using green method, stable copper oxide nanoparticles were synthesized by plant aqueous extract of *Bougainvillea* flowers both as reducing, stabilizing and capping agent. Plants and their related materials for production of nonmaterial are not only eco-friendly alternatives, but they are also cost-effective.

Copper oxide nanoparticles have been synthesized by using plants extract of leaves, fruits, roots, seeds, barks, stem, peels and flowers such as *Juglans regia* (Asemani and Anarjan; 2019), *Ailanthus Altissima* (Awwad and Amer., 2020), *Malva sylvestris* (Awwad et al., 2015), *Pneonix dactylifera* (Berra et al., 2018), *Abutilon indicum* (Ijaz et al., 2017), *Catha edulis* (Kelele et al., 2019), *Tabernaemontana divaricate* (Sivaraj et al., 2014), *Aloe vera* (Kumar et al., 2015), *Ocimum basilicum* (Altikatoglu et al., 2017), *Enicostemma axillare (Lam.)* (Mali et al., 2019), *Camellia Sinensis* (Jeronsia et al., 2019), *Gloriosa superba* L. (Naika et al., 2015), *Azadirachta indica* (Rafique et al., 2018), *Saraca indica* (Prasad et al., 2017), *Cassia auriculata* (Prasad et al., 2017), *Olea europaea* (Sulaiman et al., 2018), *Calotropis gigantean* (Sharma et al., 2015), *Bauhinia tomentosa* (Sharmila et al., 2018), *Lantana camara* (Chowdhury et al., 2020), *Aglaia elaeagnoidea* (Manjari wt al., 2017), *Punica granatum* (Ghidan et al., 2016), *Rheum palmatum* (Bordbar et al., 2017), *Desmodium gangeticum* (Guin et al., 2015), *Myristica fragrans* (Sasidharan et al., 2020), blackberry (*Rubus glaucus* Benth.) (Kumar et al., 2017), *Punica granatum* (Nazar et al., 2018), *Rumex crispus* seeds extract (Rostami-Vartooni; 2016), oak fruit hull (Sorbiun et al., 2018), *Caesalpinia bonducella* (Sukumar et al., 2020) and wheat root (Zhou et al., 2011).

In this research work for the first time, *Bougainvillea* flowers aqueous extract is used for synthesis of copper oxide nanoparticles in one step at room temperature. It has been reported that the size of CuO NPs can be controlled to 5-20 nm by the variation of the concentration of precursor and extracts. This study was designed to prepare of CuO NPs and to evaluate their antifungal activities. The synthesized CuO NPs were characterized by UV-visible, FT-IR, TEM and XRD techniques.

MATERIAL AND METHODS

Chemical and reagents

Copper acetate monohydrate $Cu(CH_3COO)_2.H_2O$ was obtained from Sigma-Aldrich, Germany with purity 99.9%. Purple flowers of *Bougainvillea* plant was collected from different gardens, Amman, Jordan. De-ionized water obtained from laboratories of the Royal Scientific Society, Amman, Jordan.

Preparation of aqueous extract

The fresh flowers of *Bougainvillea* were washed with distilled water to remove dust particles and left to dry in our laboratory at 27°C. Dried flowers were ground in a mortar and pestle. 5-10g of the fine powder of *Bougainvillea* flowers were mixed with 100ml of de-ionized water in a conical flask and heated for 10 min at 80°C. and then filtered by Whatman No. 2 filter paper to obtain clear purple solution of *Bougainvillea* flowers. It was then stored for further use in the refrigerator.

Green synthesis of copper oxide nannoparticles

Copper acetate monohydrate $[Cu(CH_3COO)_2{\cdot}H_2O]$ (5-10 g) was dissolved in 100 ml of de-ionized water and stirred magnetically at 27 °C for 2 min. Afterwards, *Bougainvillea* flowers aqueous extract was added drop wise under magnetic stirring as the flowers extract comes in contact with copper ions, the blue color of copper ions changed within few second to black-blue color, indicating the formation of copper oxide nanoparticles monodispersed in water. The black fine product was obtained and stored in an airtight glass container, which was used for characterization and antifungal activity evaluation (Fig. 1A).

Characterization

Copper oxide nanoparticles (CuO NPs) synthesized by this green method using flowers of *Bougainvillea* were characterized by X-ray diffractometer (XRD-6000, Shimadzu). FT-IR spectra of plant flower 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. Transmission electron microscopy (TEM) images were taken using Jeol/JEM 2100.

Antifungal activity evaluation

Antifungal activity of CuO NPs was monitored against *Aspergillus niger* (FCBP-0198). The Disc diffusion method was used for antifungal activity analysis. The Sabouraud dextrose agar media, pH 5.7 was autoclaved and poured in Petri plates under sterile conditions. Fungal lawns were prepared by inoculating spores on the surface of the media after that disc loaded with 5 µl of 5 mg/ml sample were placed on the surface of the Petri plate. The plates were incubated at 30 °C for 1 day and the zone of inhibition was measured, respectively.

RESULTS AND DISCUSSION

FT-IR analysis of flowers extract

Fourier transform infrared spectroscopy (FT-IR) is used to identify biomolecules in flower aqueous extract. FT-IR spectrum of aqueous solution flowers of Bougainvillea displays a number of absorption peaks reflecting its biomelcules nature. Broad peak at 3365 cm^{-1} attributed to hydrogen bonded –OH groups of alcohols, phenols and amines –N-H of amide. The band at 1643 cm^{-1} is characteristic of amide carbonyl group in amide I and II. The band at 602 cm^{-1} is assigned to aromatic groups. These functional groups are acting as reducing, dispersing, and stabilizing agents for CuO NPs during the synthesis process.

UV–Visible absorption spectra of CuONP

Green synthesis of CuO NPs using aqueous extract of *Bougainvillea* flowers, a change in the color from bluish to dark green within few seconds indicating the formation of CuO NPs. A strong absorption peak observed at around 274 nm was because of direct transition of electrons (Fig. 1B).

XRD analysis of copper oxide nanoparticles

Fig. 1A: Schematic presentation for the synthesis of CuO NPs using *Bougainvillea* aqueous extract

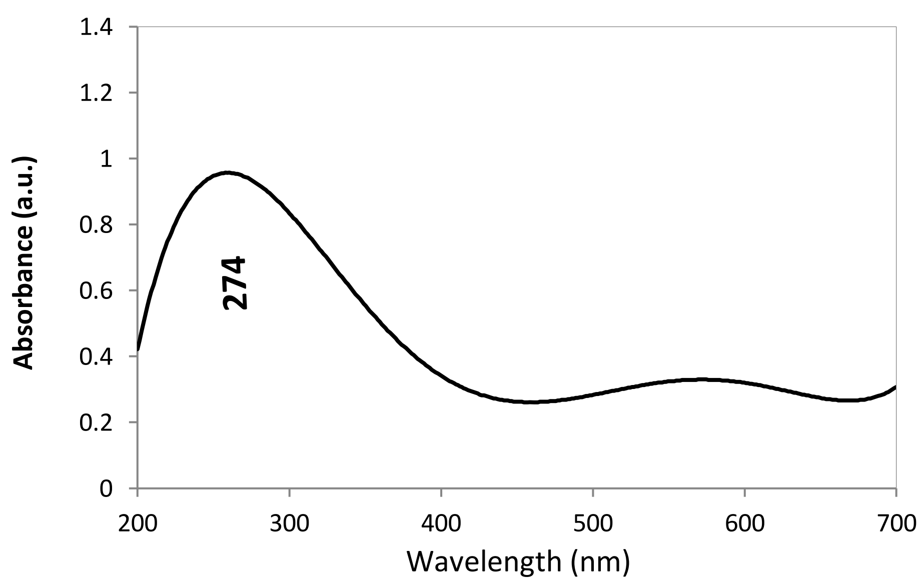

Fig. 1B: UV–Vis spectroscopic analysis of synthesized CuO NPs

The synthesized CuO NPs by flowers of *Bougainvillea* aqueous extract is illustrated in Fig. 2. The 2θ peaks at 32.4°, 35.2°, 38.7°, 48.6°, 58.4°, 61.3°, 65.6°, 66.3°, 72.4° and 74.8° are attributed to the crystal planes of copper oxide at (110), (002), (111), (202), (020), (202), (113), (311), (113), (220), and (311), respectively. The copper oxide nanoparticles (CuO NPs) are well crystalline and the position and the relative intensity of the diffraction peaks match well with the standard phase CuO NPs diffraction pattern of the International Center of Diffraction Data card (JCPDS-80-1916). The average particle sizes of the synthesized CuO NPs were calculated using Debye-Scherrer as shown in Eq. 1.

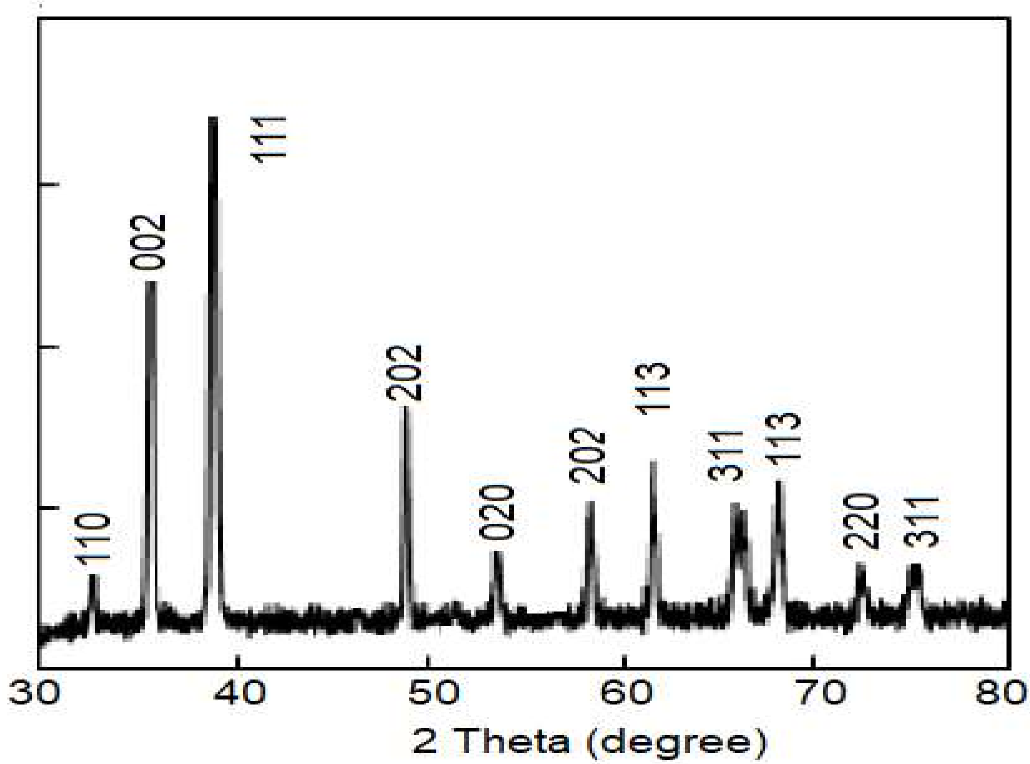

Fig. 2: XRD of CuO NPs synthesized using flowers aqueous extract of *Bougainvillea* (Y-axis = intensity counts, au)

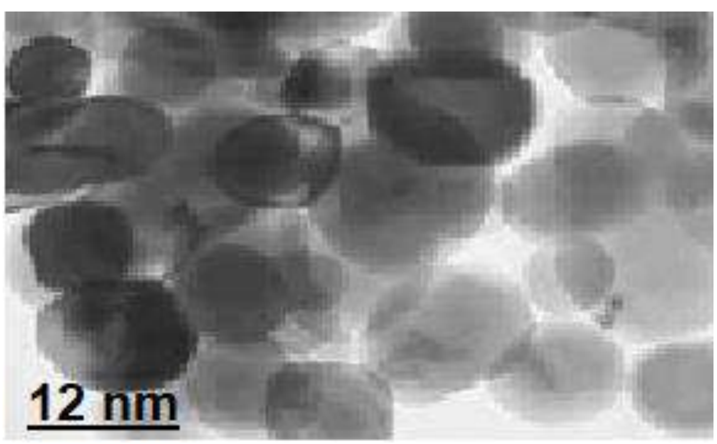

Fig. 3: TEM image of CuO NPs synthesized using flowers aqueous extract of *Bougainvillea*

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

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, 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 CuO NPs using aqueous extract of flowers of *Bougainvillea* was 18 nm.

Transmission electron microscopy (TEM)

The TEM of synthesized CuO NPs was used to study the morphology and particle sizes of micrograph, Fig. 3 clearly showed nanostructure homogeneities with spherical morphologies of CuO NPs. The TEM observation showed the nanospheres with an average diameter of 12 nm and 20 nm. This slight deviation of the particle size estimation compared to that calculated from XRD analysis can be

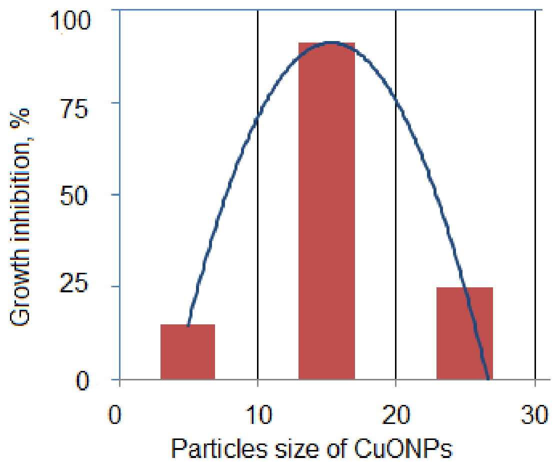

Fig. 4: Particles size distribution of CuO NPs and growth inhibition of *Aspergillus niger*

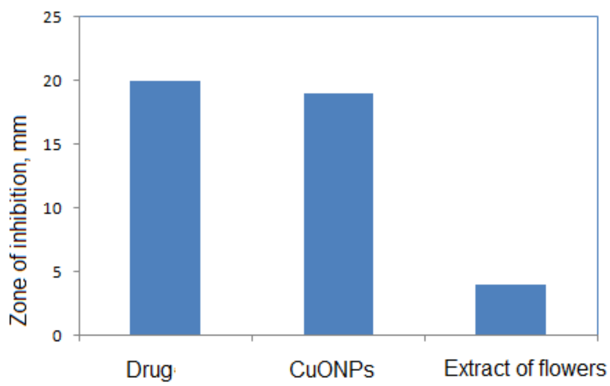

Fig. 5: Antifungal activity of CuO NPs against *Aspergillus niger* fungi, drug reference and *Bougainvillea* flowers aqueous extract

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. The magnified TEM image as shown confirms the uniform and regular spherical morphology.

A histogram of particle size distribution was drawn according to the CuO NPs size of 12 nm, Fig. 4. The average particle size was 12 nm with the standard deviation of 4 nm. The crystallite size of the synthesized CuO NPs was found to be 17.2 nm from XRD analysis, which is in an agreement with the result obtained from the TEM that shows a size distribution between 5 and 20 nm. A few very small spherical objects can be observed from the image which might be due to the residue of *Bougainvillea* flowers.

Antifungal activity of CuO NPs

Study of green synthesized CuO NPs inhibitory effect on *Aspergillus niger* growth was investigated under different concentrations of CuO NPs by a well diffusion assay on a Potato Dextrose Agar (PDA) plates. It was observed that CuO NPs have antifungal activities at different concentrations. The maximum inhibitory activity of 12±4 ppm, zone of inhibition was obtained and increased slightly at 80 and 100 ppm concentrations of CuO NPs. *Aspergillus niger* compared with positive drug control (antifungal drug fluconazole). From the results, it can be concluded that the activity of the CuO NPs showed antifungal activity comparable with standard antibiotic, Fluconazole drug. The results of antifungal evaluation and aqueous solution *Bougainvillea* flowers are shown in Fig. 5.

This suggests that 12 nm easily penetrate the cell membrane of fungus through their surfaces. We conclude from our results that particles of copper oxide nanoparticles with smaller size CuO NPs showed the highest growth inhibition activity. Antifungal activity of the aqueous extract of *Bougainvillea* used in green synthesis of copper oxide nanoparticles showed that the growth inhibition have 4-5 mm. *Bougainvillea* plant flowers extract found to be viable for the synthesis of CuO NPs with promising antifungal activity. Hence, this route can be adopted for the fabrication of CuO NPs for biomedical applications since green route is eco-benign and cost effective for the fabrication of NPs (Benassai et al., 2021; Bindu and Anila, 2021; Chen et al., 2021; Hu et al., 2021; Morales-Lozoya et al., 2021; Rambabu et al., 2021; Zhang et al., 2021).

CONCLUSIONS

The present study, reported a cost effective, eco-friendly and green approach method for synthesis of CuO NPs using aqueous extract of *Bougainvillea* flowers. The analytical tools like UV–vis spectroscopy and FT-IR studies revealed that the presence of phytochemicals like phenols and primary amines of proteins become helpful in reducing, capping and stabilizing of CuO NPs. Microscopic analysis with TEM instrument showed spherical shaped particles with a size range from 5–20 nm. These particles were mostly settled in non-agglomerated and poly-dispersed condition. The presence of higher concentration of phenols and proteins in *Bougainvillea* aqueous extract may be the reason behind for formation of narrow sized particles. Antifungal activity CuO NPs were determined against *Aspergillus niger* fungal strain and activity was compared with standard antifungal drug fluconazole. The results of the CuO NPs showed inhibition comparable with standard drug. This is the first report on green synthesis of CuO NPs using aqueous extract of *Bougainvillea* flowers, which could possibly be used for the fabrication of CuO NPs at nano-scale as an active antifungal agent.

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