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# Green synthesis of copper oxide nanoparticles using *Bougainvillea* leaves aqueous extract and antibacterial activity evaluation

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**A B S T R A C T**

The aim of present study is based on low cost, efficient, non-toxic and eco-friendly method for green synthesis of copper oxide nanoparticles (CuONPs) using *Bougainvillea* leaves aqueous extract. The green synthesized nanoparticles were subjected to characterization techniques UV–visible spectroscopy (UV–vis), X-ray diffraction (XRD), Fourier transform Infrared spectroscopy (FT-IR) and transmission electron microscope (TEM). The synthesized CuONPs were pure, predominantly spherical with sizes ranges from 8-20 nm. CuONPs showed excellent antimicrobial activity against various bacterial strains *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Moreover, *E. coli* and *E. faecalis* exhibited the highest sensitivity to CuONPs while *Staphylococcus aureus* was the least sensitive. Possible mechanisms of antimicrobial activity of CuONPs should be further investigated.



**Capsule Summary:** *Bougainvillea* leaves aqueous extract mediated green synthesis of copper oxide nanoparticles was investigated. The prepared CuONPs was of spherical shape with 8-20 nm size and active antibacterial agents.

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## INTRODUCTION

The CuONPs with different morphological structures was developed using various synthetic routes such as nonionic water-in-oil micro-emulsions (Dodoo-Arhin et al., 2012), precipitation method (Phiwdang et al., 2013; Ahamed et al. 2014; Mayekar et al., 2014), electrochemical method (Katwai et al., 2015), hydrothermal synthesis (Arun et al., 2015), solid state thermal decomposition (Shahsavani et al., 2016), sonochemical method (Silva et al., 2019), chemical and biogenic methods (Muthuvel et al., 2020), microwave-assisted method (Kannan et al., 2020), wet precipitation technique (Parekh et al., 2021), green and traditional chemical methods (Keabadile et al., 2020). These methods for synthesis of CuONPs 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 such leaves, seeds, bark, flowers and fruits have been suggested as valuable alternatives to chemical and physical methods for synthesis of CuONPs, i.e., *Polyalthia longifolia* (Nagore et al., 2021), *Ocimum basilicum* (Altikatoglu et al., 2017), *Catha edulis* (Andualem et al., 2020), *Juglans regia* (Asemani and Anarjan, 2019), *Ailanthus altissima* (Awwad and Amer, 2020), *Catharanthus roseus* (Begum et al., 2019), *Malva sylvestris* (Benhammada and Trache, 2021), *Adhatoda vasica* *nees* (Bhavyasree and Xavier, 2020), *Beta vulgaris L* (Chandrasekaran et al., 2020), *Lantana camara* (Chowdhury et al., 2020), *Psidium guajava* (Das and Goswami, 2019), *Chamomile* (Duman et al., 2016), *Catha edulis* (Gebremedhn et al., 2019), *Punica granatum* (Ghidan et al., 2016), *Ziziphus mauritiana L.* (Ghotekar et al., 2017), *Desmodium gangeticum* aqueous (Guin et al., 2015), *Abutilon indicum* (Ijaz et al., 2017), *Camellia Sinensis* (Jeronsia et al., 2019), *Cinnamomum malabatrum* (Krishna et al., 2020), *Andean blackberry* (Rubus glaucus Benth.) (Kumar et al. 2017) extracts have been employed for the green synthesis of NPs.

*Aloe vera* (Kumar et al., 2015), *Saraca indica* (Prasad, et al., 2017), *Anthemis nobilis* (Nasrollahazadeh, et al., 2015), *Papaya* (Phang et al., 2021), *Bougainvillea* (Shammout and Awwad, 2021), *Calotropis gigantean* (Sharma et al., 2015), *Tecoma castanifolia* (Sharmila et al., 2016), oak fruit hull (Sorbiun et al., 2018), *Cordia myxa L.* (Thamer et al., 2018), herbal tea (*Stachys Lavandulifolia*) (Veisi et al., 2021), *Ixiro coccinea* (Vishveshvar et al., 2018; Yedurkar et al., 2017), *Syzygium alternifolium* (Wt.) Walp (Yugandhar et al., 2018), *Punica granatum* (Vidovix et al., 2019), *Adiantum lunulatum* (Sarkar et al.,2020) also showed promising potential for the synthesis of NPs via green route. Moreover, *Cassia fistula and Melia azedarach* (Nasser et al., 2021). *Enicostemma axillare (Lam.)* (Mali et al., 2019), *Psidium guajava* (Singh et al., 2019), *Eletteria Cardamomum* (Venkatramanan et al., 2020), *Catharanathus* roseus (Dayana et al., 2021) and *Simaroubaglauca* (Deepthi et al., 2021) different plant parst showed promising efficiency for the synthesis of NPs.

Based on aforementioned facts, an eco-friendly and green route for the synthesis of copper oxide nanoparticles (CuONPs) using an abundantly available *Bougainvillea* leaves plants in Jordan is reported, which has not been reported previously. The CuONPs was characterized by various techniques (FT-IR, XRD, SEM and TEM) and antibacterial activity was also investigated against selected strains.

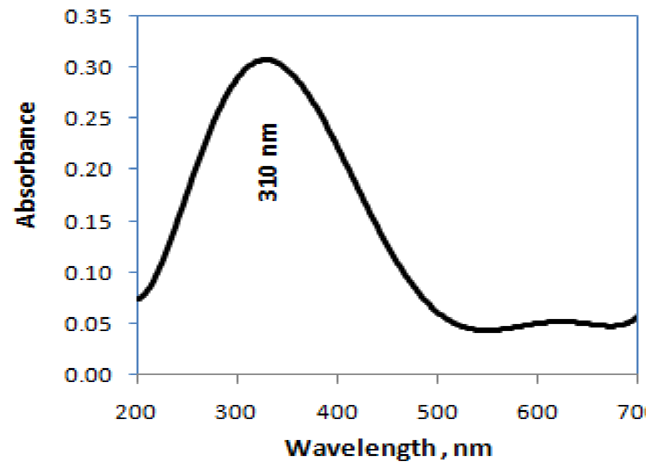



**Fig. 1:** UV-vis spectrum of CuONPs synthesized by green *Bougainvillea* leaves aqueous extract

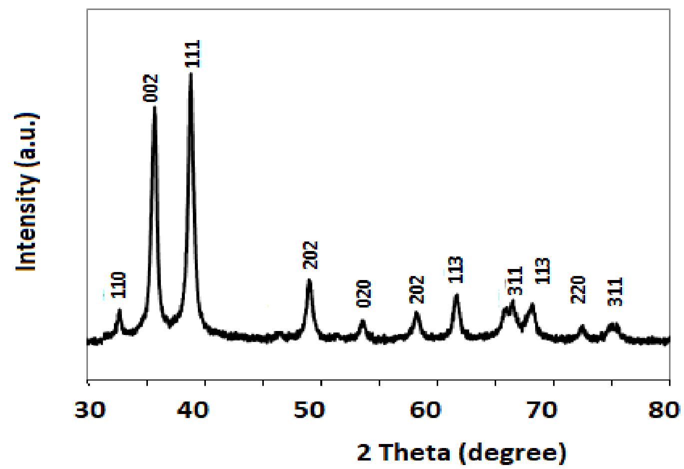



**Fig. 2:** XRD pattern of green synthesized CuONPs by *Bougainvillea* leaves aqueous extract

## MATERIAL AND METHODS

### Chemical, reagents and sample collection

Copper acetate $Cu(CH_3COO)_2$ was purchased from Sigma-Aldrich, Germany with purity ≥ 99.0%. *Bougainvillea* fresh leaves were collected from the plants at Al-Salt province, Jordan, which was washed with distilled water. Afterwards, leaves were dried at laboratory temperature (27$^{o}$C) for 7 days. Dried leaves were ground in a mortar and pestle to obtain powder leave particles ≤ 44 µm.

### Preparation of *Bougainvillea* leaves aqueous extract

5-10 g of the dried powder of leaves was boiled in 100 ml of de-ionized water for 10 min. The aqueous solution of *Bougainvillea* leaves was cooled at laboratory temperature and then filtered with Whatman No. 1 filter paper. The filtrate was kept in refrigerator for the experimental work.

### Synthesis of copper oxide nanoparticles (CuONPs)

A 5 g of copper acetate $Cu(CH_3COO)_2$ was dissolved in 100 ml de-ionized water in a glass beaker with stirring for 5 min. Afterwards, *Bougainvillea* leaves aqueous extract was added drop wise and mixed homogeneously by magnetic stirrer for 10 min. The blue color of copper acetate solution changes to green deep color due to the formation of CuOHNPs. Under continuous magnetic stirring for 4 h, the deep green 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 silica crucible for heating in furnace at 80 °C for 4 h, subsequently black color powder material achieved. Powder obtained was subjected to FT-IR, XRD, SEM and TEM analysis (Scheme 1).

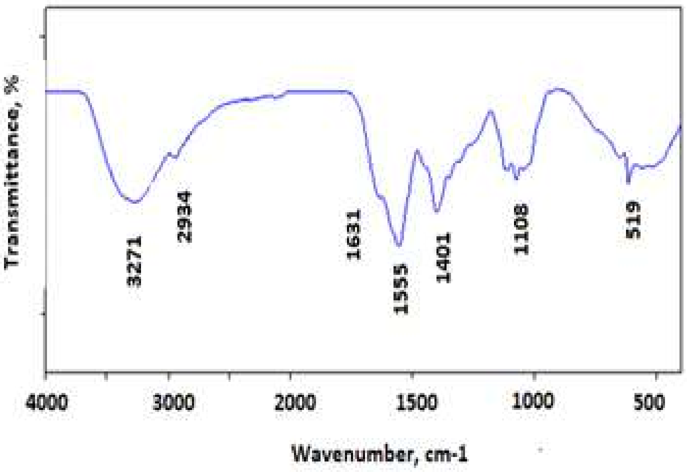



**Fig. 3:** FT-IR analysis of *Bougainvillea* aqueous leaf extract

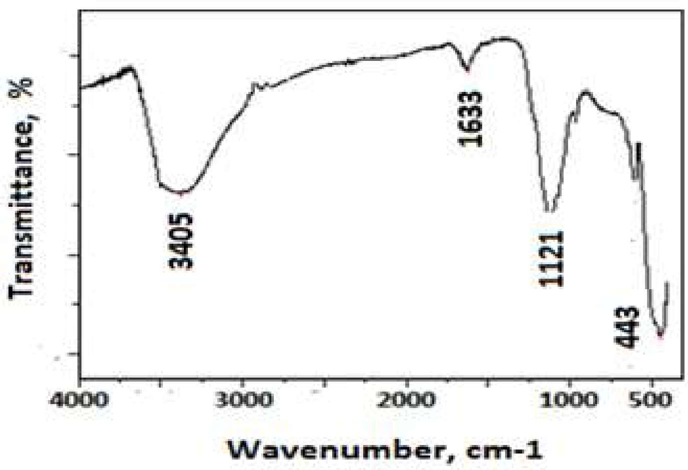



**Fig. 4.** FT-IR analysis of green synthesized CuONPs

## 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 Schimadzu 1601 spectrophotometer in the wave length region 200 to 700 nm operated at a resolution of 1 nm. Transmission electron microscopy was done using TEM CM 200, Philips. Powder X-ray diffraction (XRD) was performed using X-ray diffractometer, Shimadzu, XRD6000 with CuKα radiation λ = 1.5405 Å over a wide range of Bragg angles (3$^{o}$ ≤ 2θ ≤ 80$^{o}$). Fourier transform infrared (FTIR) spectroscopic measurements were done using Shimadzu, IR-Prestige-21 spectrophotometer.

## Antibacterial activities of CuONPs

The synthesized copper oxide nanoparticles (CuONPs) synthesized by *Bougainvillea* leaves aqueous extract effect on bacterial strains Gram negative bacteria *Escherichia coli* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and gram-positive bacterium *Staphylococcus aureus* (ATCC 25923). *E. coli*, *E. faecalis* and *S. aureus* were assayed by agar well diffusion and disc diffusion methods. All of the data from three independent replicate trials were subjected to analysis using statistical package. The data are reported as the mean±SD and significant differences between mean values were determined with one way analysis of variance. The minimum inhibitory concentration (MIC) was determined.

## RESULTS AND DISCUSSION

### UV-vis analysis

The green synthesis of copper oxide nanoparticles (CuONPs) using *Bougainvillea* leaves aqueous extract were studied. Formation of copper oxide nanoparticles were confirmed by UV-vis spectrophotometer (Fig. 1). UV–vis absorption spectrum of synthesized copper oxide nanoparticles was recorded for the sample in the range of 200–700 nm. The spectrum showed that the absorbance peak at 310 nm corresponding to the characteristic band of copper oxide nanoparticles.

### X-ray diffraction (XRD) analysis

The XRD analysis of green synthesized copper oxide nanoparticles (CuONPs) using *Bougainvillea* leaves aqueous extract as reducing and stabilizing agent (Fig. 2). The 2θ peaks at 32.5$^{o}$, 35.8$^{o}$, 38.4$^{o}$, 48.3$^{o}$, 58.3$^{o}$, 61.9$^{o}$, 64.9$^{o}$, 66.7$^{o}$, 72.7$^{o}$ and 75.4$^{o}$ 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 are well crystalline. 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).

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

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 *Bougainvillea* leaves aqueous extract calculated from Scherrer equation was in the range 8-20 nm.

### FT-IR analysis of extract and CuONPs

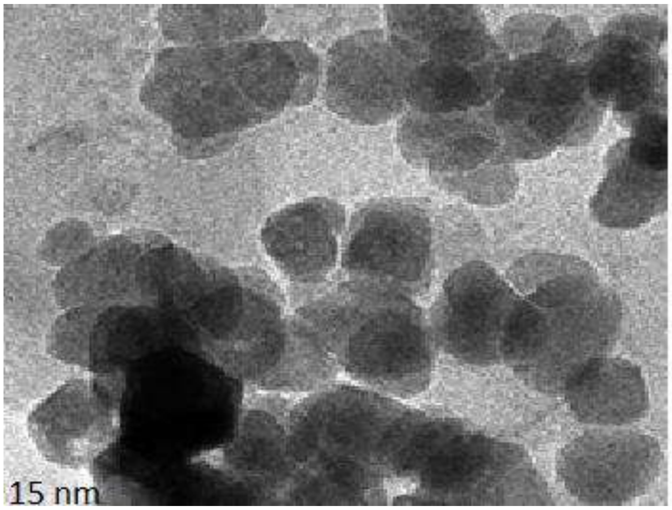



**Fig. 5:** TEM of green synthesize CuONPs by *Bougainvillea* leaves aqueous extract

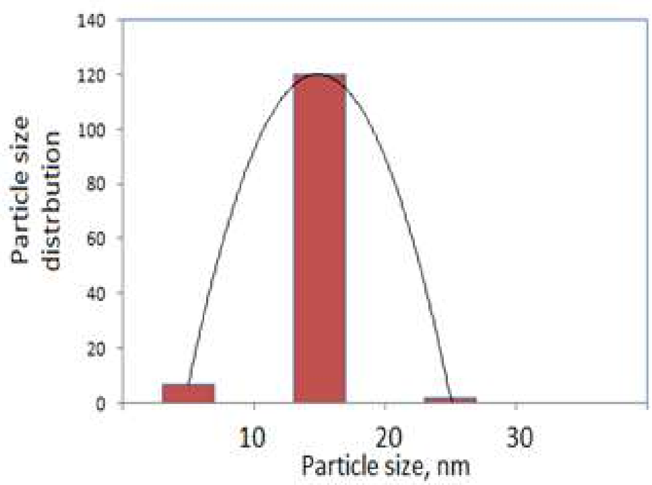



**Fig. 6:** Particle size distribution histogram of synthesized CuONPs

The FT-IR analysis of *Bougainvillea* leaves aqueous extract and the synthesized copper oxide nanoparticles to identify the biomolecules responsible for the bioreduction copper salts to copper oxide nanoparticles. The FT-IR spectrum of *Bougainvillea* leaves aqueous extract showed that *Bougainvillea* spectrum have the peaks at 3271, 2934, 1631, 1555, 1401, 1108 and 519 $cm^{-1}$ (Fig. 3). Strong broad peak at 3271 $cm^{-1}$ is due to the O-H groups of water, alcohols, or phenols and the presence of N-H amides stretching vibration. The band at 2934 $cm^{-1}$ is assigned to $-CH_2$ and C-H stretching mode in alkenes. The strong peak at 1631 $cm^{-1}$ in *Bougainvillea* leaves aqueous 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 1631, 1555, 1401, 1108 and 519 $cm^{-1}$ can be allocated to the stretching vibration of C-OH bond from proteins (amide I) of the leaves extract. Peak in the region 1601 $cm^{-1}$ may be attributed to the presence of the stretching vibrations of carboxylic acids and amino groups. The band at 519 $cm^{-1}$ assigned for aromatic compounds residue in protein of *Bougainvillea* leaves aqueous extract. The FT-IR analysis of synthesized copper oxide nanoparticles showed strong peaks at 3405 $cm^{-1}$, 1633, 1121 and 443 $cm^{-1}$. The bands at 3405 $cm^{-1}$ and 1633 $cm^{-1}$ represent C-O-H or C-N-H groups in the plant extract, which aid on the converting copper acetate to copper oxide nanoparticles. The band 443 $cm^{-1}$ is due to the presence CuO nanoparticles (Fig. 4).

**Transmission electron microscopy (TEM)**

The morphology and size determination of green synthesized CuONPs by *Bougainvillea* leaves aqueous extract was done by transmission electron microscopes (TEM). TEM of green synthesized CuONPs showed the nanoparticles are spherical in shape (Fig. 5). TEM analysis also reveals the green synthesized CuONPs are in size range 8–20 nm. Moreover. TEM pattern of green synthesized CuONPs confirmed the crystalline nature of copper oxide nanoparticles. The particle size distribution of the synthesized nanoparticles is ranging between 5 nm and 20 nm (Fig. 6). A narrow size distribution of the CuONPs was observed with average size 15 nm.

**Antibacterial activity**

Minimum inhibitory concentration (MIC) values were obtained for tested against *E. coli*, *E. faecalis* and *S. aureus*. The results are presented in Table 1 and Fig. 7, it can be seen that the copper oxide nanoparticles present the best antibacterial activity against the three strains tested in

**Table 1:** Antimicrobial activity (zone of inhibition) of synthesized CuONPs

| | CuONPs concentration | | | |
|---|---|---|---|---|
| Microorganisms | 20 µl | 40 µl | 60 µl | 80 µl |
| *E. coli* | 12 | 18 | 20 | 26 |
| *E. faecallis* | 10 | 17 | 18 | 22 |
| *S. aureus* | 8 | 11 | 16 | 18 |

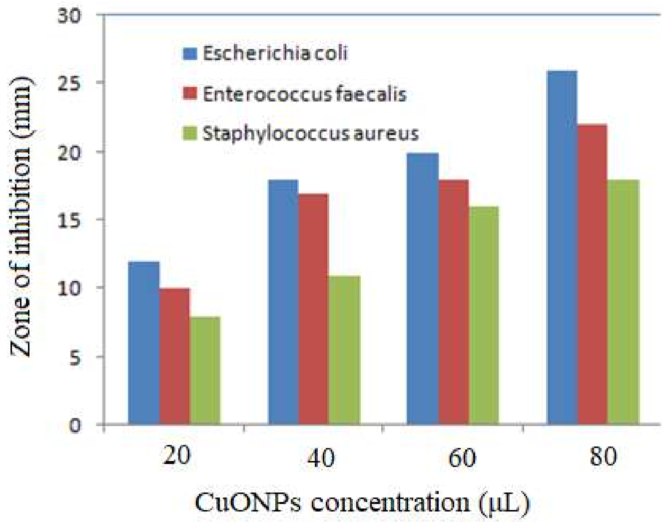
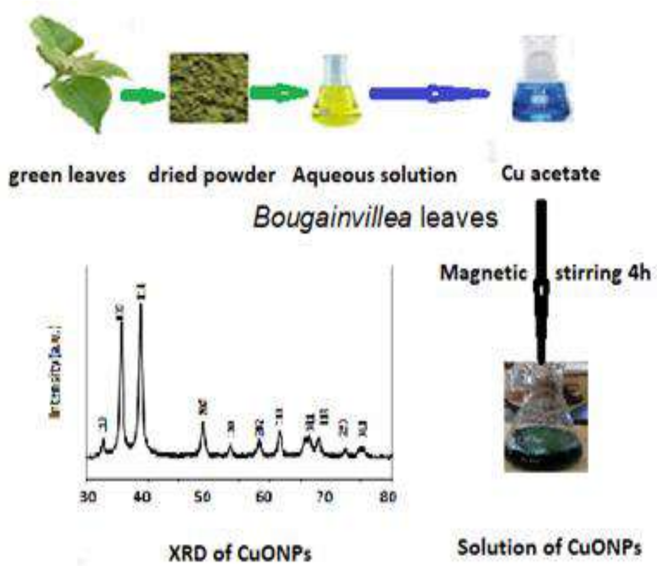



**Fig. 7:** Zone of inhibition (Well diffusion assay for CuONPs against microbial strains)




**Scheme 1:** Graphical presentation for the green synthesis of CuONPs

comparison with *Bougainvillea* leaves aqueous extract and the reference drug. The MIC of the copper oxide nanoparticles is higher when it is tested against *E. coli* and *E. faecalis* than when tested against *S. aureus*. These results refer to differences in the cell wall of each strain; the cell wall of Gram-negative strains (E. coli and *E. faecalis*) are wider than the cell wall of Gram-positive strains (*S. aureus*). This is probably of the toxicity of copper oxide ions on *E. coli* and *E. faecalis* include a rapid DNA degradation, followed by a reduction of bacterial respiration; it is also known that copper oxide ions inhibit certain cytochromes in the membrane. The *Bougainvillea* leaves aqueous extract was found to be highly efficient for the synthesis of CuONPs at nanoscale and the prepared CuONPs showed promising antimicrobial activity, which could have practical application to control bacterial infections. This study observed results reveal that the green synthesized CuONPs showed a significant effect as antibacterial studied compared with positive drug control. It could be explained by large surface area of CuONPs and partially its decomposition in wet medium to copper hydroxide ($Cu(OH)_3$) which gives better contact with microorganisms thus alter the microbial metabolism and penetrated inside the microorganisms. The equal volume (20 μl) of antibiotic: tetracycline (Das and Goswami, 2019). Hence, green synthesis is an efficient route to prepare CuONPs for biomedical application (Akintelu et al., 2020; Chandrasekar et al., 2021; Sathiyavimal et al., 2018).

## CONCLUSIONS

Green synthesis of copper oxide nanoparticles (CuONPs) is an eco-friendly, safer to environment and easy method. A fast, eco-friendly and convenient green method for the synthesis of CuONPs nanoparticles from copper acetate in aqueous extract of *Bougainvillea* leaves at ambient temperature. Spherical, polydispersity of CuONPs of particle sizes ranging from 8 to 20 nm with an average size of 15 nm are obtained. These results could be used in developing novel antibacterial agent, which may find potential applications in different fields with more safety since NPs could be prepared using green synthesis route and same route can be adopted for the fabrication of NPs based on different NPs metals.

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