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Green synthesis of copper nanoparticles by *Citrus limon* fruits extract, characterization and antibacterial activity

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A B S T R A C T

In view of advantages of green synthesis, a novel green route for the synthesis and stabilization of copper nanoparticles (CuNPs) using aqueous extract of *Citrus limon* fruits at room temperature was reported. The formation of CuNPs is monitored by recording the UV–vis absorption spectra for surface Plasmon resonance (SPR) peak (~579 nm). X-ray diffraction (XRD) pattern of the CuNPs agrees with the reported data for Cu metal and the crystallite average size is ~30 nm. Scanning and transmission electron microscopic (SEM and TEM) show uniform spherical particles obtained by this green method. The antimicrobial activity is found to be effective of CuNPs. Results revealed that the green synthesis is an efficient for the preparation of CuNPs as an active antimicrobial agent for practical applications.

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

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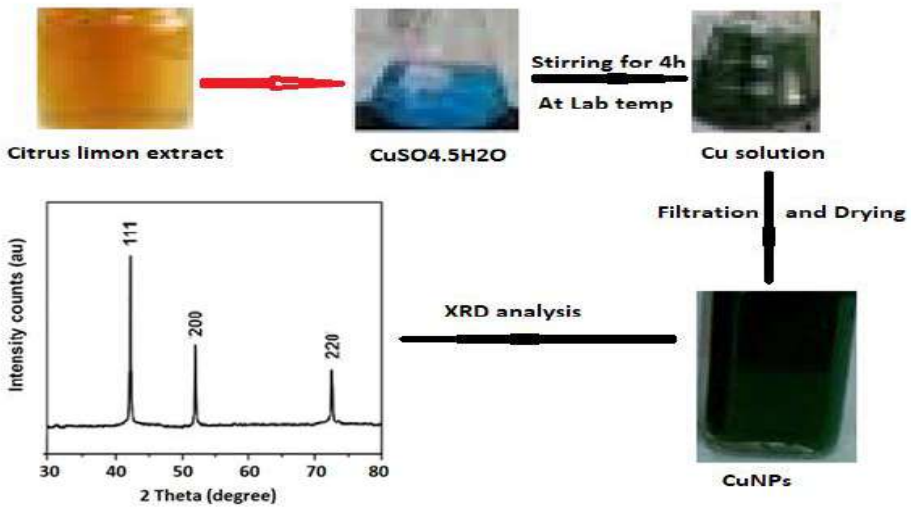
INTRODUCTION

The CuNPs have attracted considerable attention due to its optical, catalytic, mechanical and electrical properties. Moreover copper has the advantages in green-nano preparation. CuNPs have wide applications as super strong materials, sensors and antimicrobial activity against various bacterial and fungal strain. Many methods have been developed for preparation of copper nanoparticles such as electrochemical (Yang et al., 2003; Yu et al., 2008), electroreduction (Raga et al., 2008). Chemical reduction (Qiu-li et al., 2010; Granata et al., 2019), hydrothermal (Chen and Lee, 2010), thermal decomposition (Salavati-Niasari et al., 2008; Wei et al., 2010), Recently, researchers concentrated their scientific research on synthesis of CuNPs by green route using eco-friendly and non-toxic plant leaves, fruits, bark and flowers extracts, i.e., dried flower buds of *Syzygium aromaticum* (Subhankari and Nayak, 2013), *Ocimum sanctum* leaf (Kulkarni and Kulkarni, 2013), *Magnolia kobus* leaf (Lee et al., 2013), henna leaves (Cheirmadurai et al., 2014), *Nerium oleander* leaf (Gopinath et al., 2014), aqueous extract of the leaves of *Euphorbia esula L* (Nasrollahzadeh et al., 2014), *Vitis vinifera* leaf aqueous extract (Angrasan and Subbaiya 2014), *Gloriosa superba* L. extract (Raja et al., 2015), flower extract of *Aloe Vera* (Karimi and Mohsenzadeh, 2015), lemon extract as a reducer and curcumin as a stabilizer under certain conditions (Jayandran et al., 2015).



Scheme 1: A schematic presentation of CuNPs synthesis and characterization using *Citrus limon fruits* extract

Besides, *Gymnema sylvestre leaves extract* (Heera et al., 2015), *Magnolia kobus* leaf extract (Haleemkhan, et al., 2015), *Arevalanata* leaves extract (Hariprasad et al., 2016), *Aloe vera* extract (Pawlowska and Sadowsk, 2017), tea leaf aqueous extract (Mohindru and Garg, 2017), *Momordica charantia* fruit extracts (Ekezie et al., 2017), *Garcinia mangostana* leaf extract (Prabhu et al., 2017), *Eclipta prostrata* leaves extract (Chung et al., 2017), *Momordica charantia* fruit extracts (Ekezie et al., 2017), *Asparagus adscendens* Roxb. root and leaf extract (Thakur et al., 2018), mixture of Z*ingiber officinale,Tilia* extract (Hassanien et al., 2018), *Alchornea laxiflora* leaf extract (Olajire et al., 2018), *Quisqualis indica extract* (Mukhopadhyay et al., 2018), *Camelia sinensis* leaves extract (Ahmed et al., 2019), sodium citrate tribasic dihydrate, a solution of ascorbic acid and a solution of sodium hydroxide (Pariona et al., 2019), mixture of Z*ingiber officinale, Piper nigrum* and *Piper longum* extract (Shah et al., 2019), *Uncaria gambir* ROXB. leaf extract (Elisma et al., 2019), Hawthorn berries extract (Długosz et al., 2020), lemon extract as a reducer and curcumin as a stabilizer (Jayandran et al., 2020) have also been successfully be used for the synthesis of NPs. Previous reports revealed that the green synthesis offers various advantages and is also efficient for the fabrication of NPs for biological applications (Al Banna et al., 2020; Awwad and Amer, 2020; Awwad et al., 2020a; Awwad et al., 2020b; Igwe and Nwamezie, 2018; Izionworu et al., 2020; Remya et al., 2017).

In this research work, copper nanoparticles (CuNPs) were synthesized from copper sulfate pentahydrate and *Citrus limon* fruits aqueous extract at room temperature and characterized by advanced techniques. The synthesized CuNPs were tested for antimicrobial activity against Gram-positive and Gram-negative bacteria.

MATERIAL AND METHODS

Chemical and reagents

Copper sulfate pentahydrate ($CuSO_4.\ 5H_2O$, ≥ 98%) purchased from Sigma-Aldrich, Germany and used without further purifications. *Citrus limon* fruits were purchased from local market, Amman, Jordan. De-ionized and distilled water was obtained from our laboratory at Royal Scientific Society.

Preparation of *Citrus limon* fruits extract

Fresh samples of *Citrus limon* fruits were subjected to washing with distilled water and cutting to a small pieces and dropped into 100 ml de-ionized water in 250 ml glass beaker at 80 ^oC for 10 min. Afterwards, the extract was filtered on Whatman No. 41 filter paper to obtain *Citrus limon* fruits aqueous extract. The clear filtrate was kept in a stoppered glass flask in a refrigerator for the experimental work (Scheme 1).

Green synthesis of copper nanoparticles (CuNPs)

Citrus limon fruits aqueous extract (100 ml) was mixed with 4 g of copper sulfate pentahydrate under magnetic stirring at room temperature (27 ^oC) for 4 h. The blue color of copper sulfate pentahydrate changed to brown color within 10 minutes, indicating the formation of CuNPs due to reduction of copper ions from Cu(II) ions to Cu metal. The samples were then centrifuged at 3000 rpm for 10 min to get a clear supernatant at room temperature. The copper nanoparticles obtained were dried in an oven at 80-90 ^oC for 4 h for FT-IR, SEM and XRD analysis.

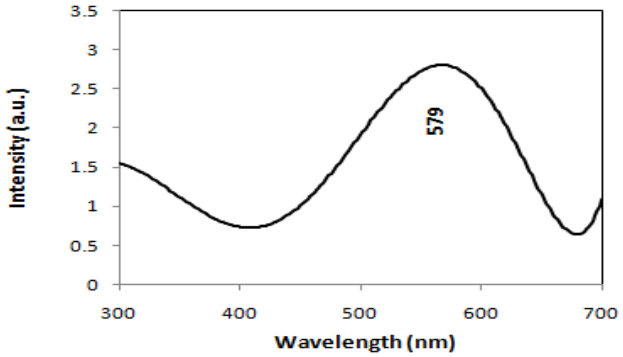


Fig. 1: UV-vis absorption spectra of copper nanoparticles using *Citrus limon* fruits extract

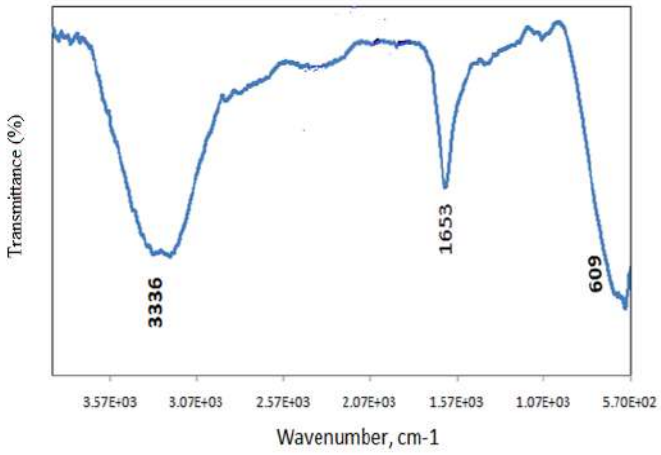


Fig. 2: FT-IR of *Citrus limon* fruits aqueous *extract*

Characterization of CuNPs

UV-Vis absorption spectrum was measured using Shimadzu UV-1601 spectrophotometer. Crystalline metallic copper nanoparticles were examined by X-ray diffractmeter (Shimadzu XRD-6000) equipped with Cu Kα radiation source using Ni as filter and at a setting of 30 kV/30 mA. All XRD data were collected under the same experimental conditions, in the angular range $20^o \leq 2\theta \leq 80^o$. FTIR spectra for *Citrus limon* fruits aqueous extract was obtained in the range 4000–400 cm^{-1} with IR-Prestige-21 Shimaduz FTIR spectrophotometer, using KBr pellet method. Scanning electron microscopy (SEM) analysis of copper nanoparticles analysis was done using Hitachi S-4500 SEM machine and transmission electron microscopy (TEM, Hitachi H7500).

Antibacterial activity evaluation

The effect of *Citrus limon* aqueous extract and the synthetized copper nanoparticles (CuNPs) on bacterial strains: *Escherichia coli* and *Staphylococcus aureus* were assayed by agar well diffusion method and disc diffusion method.

Statistical analysis

All of the data from three independent replicate trials were subjected to analysis using Statistical package: Statistics 8.0. The data are reported as the mean ± SD and significant differences between mean values were determined with one way analysis of variance.

RESULTS AND DISCUSSION

UV-Vis analysis

In the present study, the absorption spectra of copper nanoparticles synthesized using *Citrus limon* fruits extract, results revealed the conversion of copper ions to copper nanoparticles with almost 100% bioreduction of metal ions as evidenced by qualitative testing of supernatant after the purification of copper nanoparticles (Fig. 1). The difference in the rate of bioreduction observed may be assign to the differences in the activities of the amino acids present in *Citrus limon* fruits aqueous extract. The entire reaction mixture is turned to brown color, and exhibit an absorbance peak around 579 nm characteristic of CuNPs, due to its surface Plasmon resonance absorption band (Fig. 1). In the present investigation, the reaction mixtures showed a single SPR band revealing spherical shape of copper nanoparticles, which was further confirmed by TEM images.

FT-IR analysis

Fourier transform infrared spectroscopy (FT-IR) is used to identify and get an approximate identification of the possible biomolecules in the *Citrus limon* fruits extract. FT-IR spectrum (Fig. 2) obtained for *Citrus limon* fruits extract display a number of absorption peaks, reflecting its complex nature. Strong broad peak at 3336 cm^{-1} can be attributed hydrogen bonded O-H groups of alcohols, phenols and the N-H of amide. The band at 1653 cm^{-1} attributed to O-H stretching. The band at 609 cm^{-1} may be attributed to the presence of the stretching vibrations of carboxylic acids and amino groups. These functional groups are responsible on the reduction and stabilization of copper nanoparticles on the surfaces of the *Citrus limon* fruit.

X-ray diffraction analysis

XRD pattern of the synthesized CuNPs by green method using *Citrus limon* fruits extract (Fig. 3). The sharp peaks of the XRD pattern indicated the crystalline nature of CuNPs. The peaks at 43.3^o, 52.5^o and 73.8^o correspond to the Miller indices (111), (200) and (220), respectively (JCPDS card No: 89-2838). The green synthesized copper nanoparticles were found highly pure without impurities.

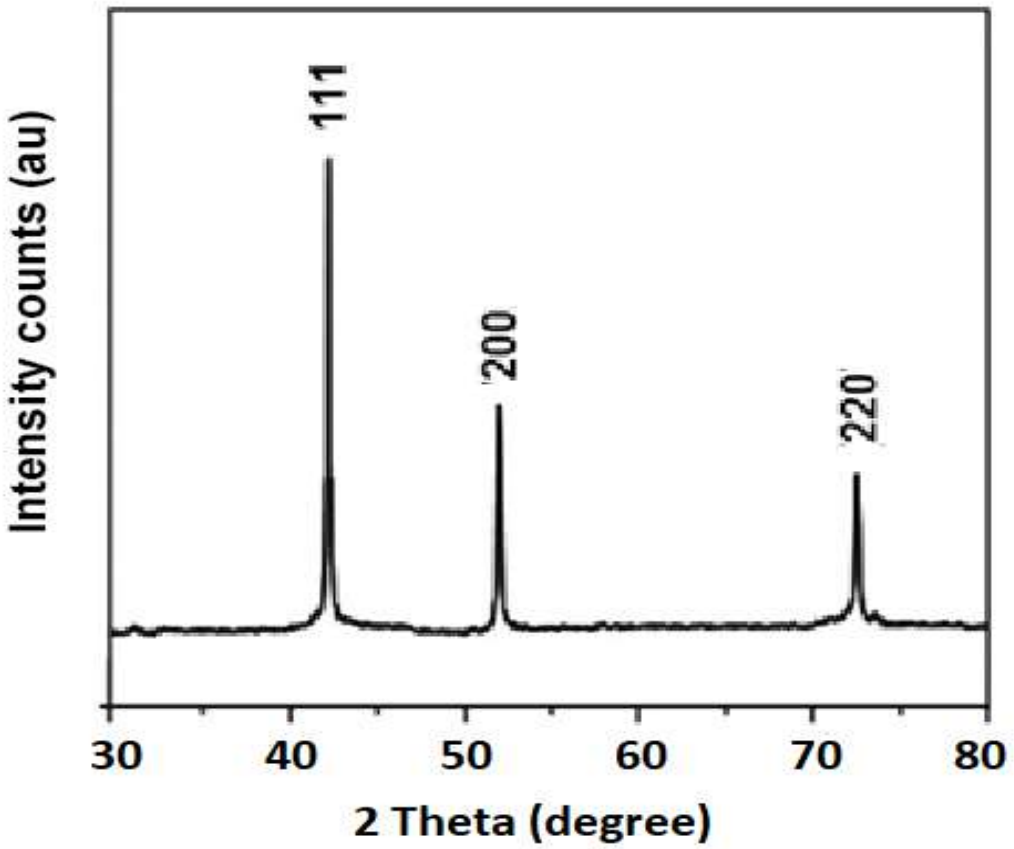


Fig. 3: X-ray diffraction (XRD) of green synthesized copper nanoparticles (CuNPs)

SEM and TEM analysis

Scanning electron microscopy (SEM) of the synthesized of copper nanoparticles is illustrated in Fig. 4. It was observed that the The CuNPs have spherical shape with an average diameter of 28 nm. Transmission electron microscopy (TEM) analysis (Fig. 5) revealed that CuNPs particles have the average particle size 18 nm. The particle size distribution of the synthesized copper nanoparicles is ranging between 5 nm and 28 nm (Fig. 6). A narrow size distribution of the copper nanoparticles (CuNPs) was observed with average size 28 nm.

Antibacterial activity

Minimum inhibitory concentration (MIC) values were obtained for tested against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 29213). The results are presented in Table 1, where it can be seen that the copper nanoparticles present the best antibacterial activity against the two strains tested in comparison with *Citrus limon* fruits aqueous extract and the reference drug. The MIC of the copper nanoparticles is higher when it is tested against *E. coli* than when tested against *S. aureus*. These results may refer to differences in the cell wall of each strain; the cell wall of Gram-negative strains (*E. coli)* is wider than the cell wall of Gram-positive strains (*S. aureus*). This is probably of the toxicity of copper ions on *E. coli* includes a rapid DNA degradation, followed by a reduction of bacterial respiration; it is also known that copper ions inhibit certain cytochromes in the membrane.

Table 1: Antimicrobial activity of CuNPs against selected microorganisms

Samples	Zone of inhibition (mm)	
	E. coli	*S. aureus*
Citrus limon fruits	4.5	2.2
Copper nanoparticles	25	20
Chloromphenical drug	20	20

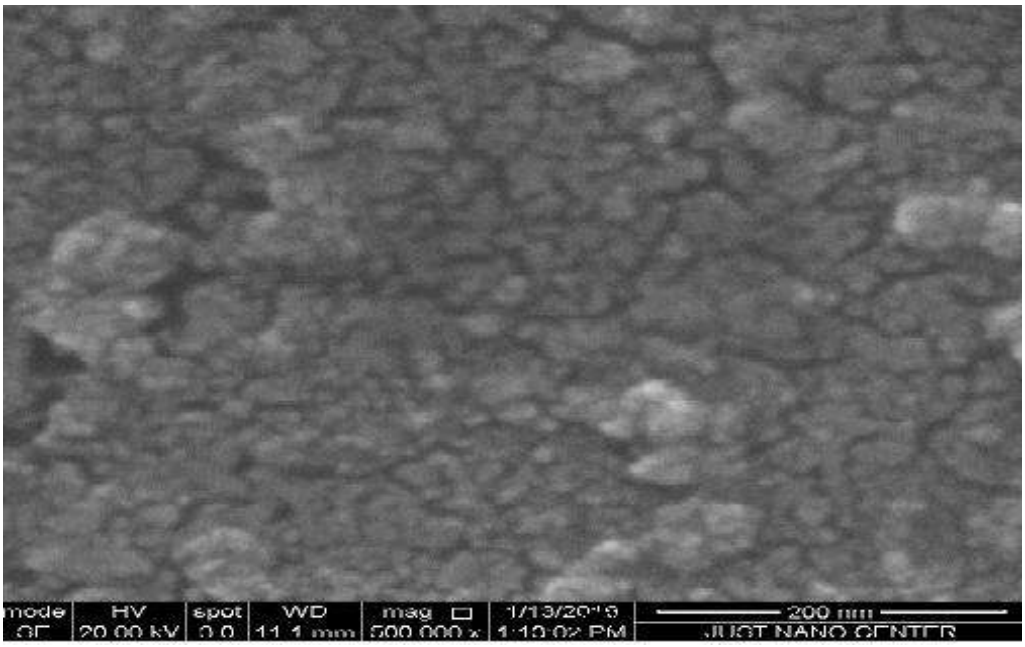


Fig. 4: SEM of the green synthesized copper nanoparticles

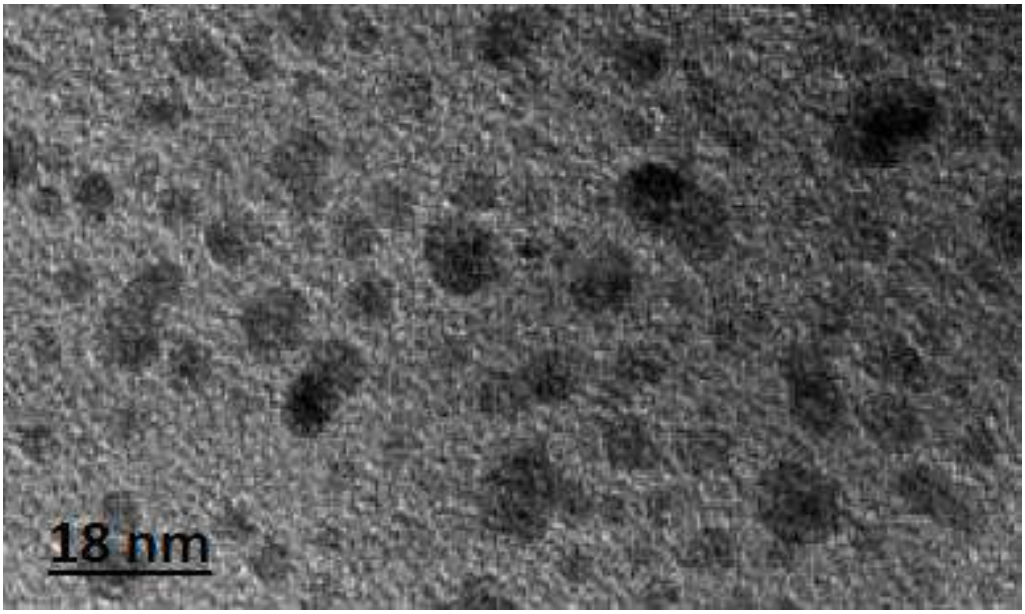


Fig. 5: TEM of of green synthesized CuNPs

Antibacterial activity showed the green synthesized copper nanoparticles displayed high antibacterial activity towards the two tested pathogenic strains *of E. coli* and *S. aureus* and *Citrus limon* fruits aqueous extract showed very low effect against microbial strains (Fig. 7). The *Citrus limon* fruits extract was found to be highly efficient for the synthesis of CuNPs at nanoscale and the prepared NPs showed promising antimicrobial activity, which could have practical application to control bacterial infections. In view of advantages offered by green synthesis route (Bandeira et al., 2020; Chandra et al., 2020; Hashemi et al., 2020; Hekmati et al., 2020; Jayarambabu et al., 2020; Pereira et al., 2020; Singh et al., 2020), it can be used for the preparation of NPs for different applications.

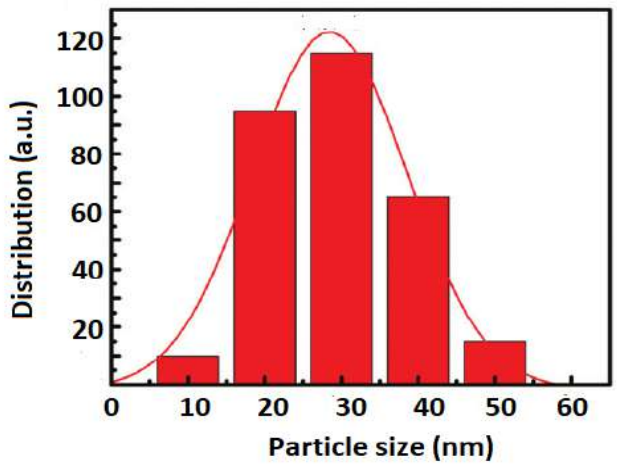


Fig. 6: Particle size distribution histogram of synthesized CuNPs

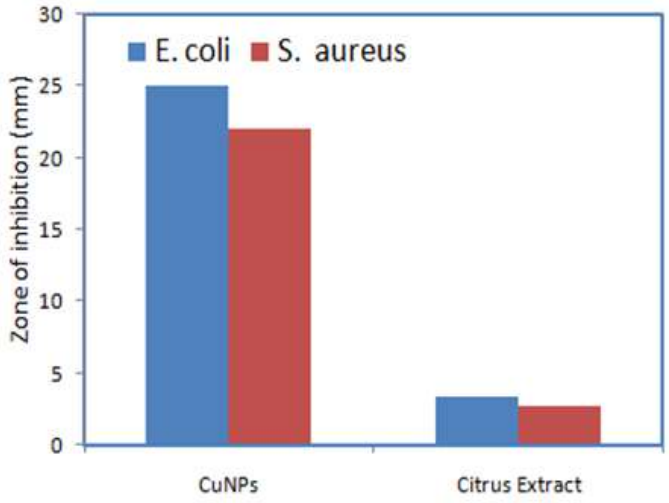


Fig. 7: Zones of inhibition (well diffusion assay for copper nanoparticles against microbial strains)

CONCLUSIONS

The green synthesis of copper nanoparticles performed using *Citrus limon* fruits extract without involving any toxic materials. The characterization of synthesized copper nanoparticles elucidated by microscopic and spectroscopic techniques which includes TEM, SEM, FTIR, XRD and UV-Visible confirm the formation of copper nanoparticles. CuNPs exhibited an excellent antibacterial activity against *E. coli* and *S. aureus* pathogenic bacteria. So it can concluded that green synthesized copper nanoparticles using *Citrus limon* fruits extract as environment-friendly with low-cost and excellent antibacterial activity.

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