



# PREPARATION, CHARACTERIZATION OF INORGANIC COPPER IODIDE NANOPARTICLES USING POMEGRANATE JUICE EXTRACT AND APPLICATIONS AGAINST *STAPHYLOCOCCUS AUREUS*

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## Abstract

In this study, copper iodide nanoparticles (CuI NPs) have been prepared by green method using pomegranate juice extract as a reductant and capping agents, because of the presence of anthocyanin molecules in its components. By using various analytical techniques CuI NPs were characterized, X-ray diffraction (XRD) analysis, Transmission Electron Microscopy (TEM), Fourier Transform Infra-Red Spectroscopy (FTIR) and UV-vis absorption Spectrophotometer. Scherer's formula has been utilized to calculate the size of the prepared samples. By using different amounts of pomegranate juice, the size of CuI was observed to be in the nano range and spherical shape. Also, the absorption spectrum showed shift to UV region. Furthermore, CuI NPs were showed antibacterial activity against *Staphylococcus aureus*.

**Key words:** Copper iodide (CuI), green synthesis, anthocyanin, antibacterial activity.

## Introduction

Nanotechnology is one of the most active areas in modern science (Veeranna *et al.*, 2013). It is a technology on the nanometer scale and deals with the atoms, molecules, or the macromolecules with the size of approximately 1-100 nm to create and use materials that have novel properties (Singh *et al.*, 2017; Gupta *et al.*, 2016). Copper iodide (CuI) is a binary metal halide semiconductor, which occurs in nature as a mineral called marshite and can also be synthesized by redox reactions of copper and iodine (Ezealigo *et al.*, 2017; Lin *et al.*, 2016). It is known as I-VII p-type semiconductor with unusual optical properties (Dhere *et al.*, 2010), since Cu and I are classified into groups one and seven in the periodic table, respectively. CuI NPs has attracted great attention due to its unusual properties, such as large band gap, unusually large temperature dependency, negative spin-orbit splitting, large ionicity, anomalous diamagnetism behavior, new high pressure phase, thus can be used in superionic conductor (Byranvand and Kharat, 2014), catalysis for the synthesis of organic compounds (Ziarati *et al.*, 2013), solid-state solar cells (Mohamed *et al.*, 2015; Zhao *et al.*, 2015), photonics, anti-microbial activity, drug delivery systems, etc. (Tavakoli *et al.*, 2013; Xu *et al.*, 2012). CuI NPs can be synthesis in a various chemical and physical methods, but these methods are high energy requirement, costly, toxic, difficult separation, high pressure and potentially hazardous (Sundrarajan and Gowri, 2011). However, the use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals and eco-friendly nature (Santhoshkumar *et al.*, 2017). The present study aim, a green, rapid, room temperature and cost efficient method for synthesizing CuI NPs by using pomegranate juice extract that is rich in anthocyanin content. This juice has been utilized as a reducing and capping agent. Generally, the extracts of plants or fruits act as stabilizers, capping and reducing agents to control crystal growth.

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## Materials and Methods

### Preparing pomegranate juice extract

Pomegranate juice (PJ) was obtained *via* pressing the arils in the blender. Then, it was filtered by a gauze. Subsequently, pomegranate juice was centrifuged at 10000 rpm for 15min. Finally, the red extract was stored in an air tight container in the fridge at 4°C until use.

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### Synthesizing copper (I) iodide nanoparticles

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (99.5%) and KI (99.5%) were purchased from Riedel De Hane and Labort Chemicka Companies, respectively and used without further purification. In a typical synthesis, a specific amount of pomegranate juice extract (10, 20 and 30 ml) has been added to the copper sulphate solution (0.07 M) under magnetic stirring. After that, potassium iodide solution (0.12 M) was added dropwise to this solution. The mixture was stirred for 30 min at room temperature. After the reaction was completed, the solutions which have been resulted were light green for sample 10 ml and dark brown for sample (20 and 30 ml) as shown in fig.1. The change in color was expected as indicator of the formation of CuI NPs (Vijayakumar and Rajagopal, 2016, BYRANVAND and KHARAT, 2014). The synthesized precipitant was separated by a centrifugation at 10000 rpm for 15min. afterwards, the precipitant was washed with ethanol and deionized for the purpose of removing any impurities for the ultimate product. Then, it was dried in air naturally and carefully collected. Finally, stored for further characterization purposes.

### Characterization of CuI NPs

The synthesized precipitates of CuI NPs were characterized using X-ray diffraction pattern (XRD) has been done on a Shimadzu XRD- 6000 diffractometer with the use of Cu K $\alpha$  irradiation (1.5405Å). The morphology, particle size were identified by transmitting electron microscope (TEM, Philips Model CM10). The functional groups on the surface of CuI NPs have been investigated by FTIR analyzing (“Shimadzu”), the spectra has been scanned in the range between 4000-400  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$ . The ultraviolet-visible (UV-vis)

spectra of CuI nanostructures that are suspended in de ionized water were measured using a Shimadzu UV-1800 spectrophotometer.

### Antibacterial activity of synthesized CuI NPs

CuI NPS were tested for antibacterial activity using two methods against virulent pathogenic microorganisms *Staphylococcus aureus* (Gram-positive) methicillin resistance (MRSA), supplemented from University of Baghdad /College of Science / Biotechnology department.

- Broth inoculation method: For detection CuI NPs ability to counteract the growth of selected pathogenic bacteria, Muller Hinton broth (Himedia/India) tubes are prepared and autoclave sterilization at 121°C for 15 minutes are done. Bacterial isolate is activated using Brain heart infusion broth (Himedia/India) and about 100  $\mu\text{l}$  activated bacterial culture is added to the individual tube as a control and another 100  $\mu\text{l}$  activated bacterial culture with 100  $\mu\text{g}/\text{ml}$  of CuI NPs (1mg/5ml) is added to individual tubes as a test. Then, the tubes were incubated at 37°C for (18, 20, 22 and 24 hrs.). Finally, the results are read using UV/vis spectrophotometer at 600 nm (Chatterjee *et al.*, 2011).

- Surface inoculation method and antimicrobial sensitivity test: This method is focused on the possible utilization of nanoparticles for improving the synergistic profiles of previously approved antibiotics for MRSA. Muller Hinton agar plates (Himedia/India ) are prepared and 100  $\mu\text{l}$  of activated bacterial culture is swabbed in a uniform in the separated plates with the use of sterile cotton swabs. Commercial antibiotic discs (Levofloxacin (LEV<sup>5</sup> $\mu\text{g}$ ), Norfloxacin (NOR<sup>10</sup> $\mu\text{g}$ ), Ceftriaxone (CRO<sup>30</sup> $\mu\text{g}$ ), Erythromycin (E<sup>10</sup> $\mu\text{g}$ ), Ciprofloxacin (CIP<sup>10</sup> $\mu\text{g}$ ), Trimethoprim (TMP<sup>10</sup> $\mu\text{g}$ ), Tetracycline (TE<sup>30</sup> $\mu\text{g}$ ) and Cefoxitin (FOX<sup>30</sup> $\mu\text{g}$ ) (Bioanalyse/Turkey) are placed as a control. Subsequently, 100  $\mu\text{l}$  activated culture previously treated with 100 $\mu\text{g}/\text{ml}$  of CuI NPs (1mg/5ml)solution is spread uniformly on Muller Hinton agar plates and antibiotic discs are placed as a test. Then, all plates are incubated at 37°C for 18 hrs. After incubation, various zonation levels are formed around the disc are measured.

## Results and Discussion

### X-ray diffraction

In order to understand the structural properties of CuI NPs prepared using a certain volumes (10, 20 and 30 ml) of pomegranate juice extract, X-ray

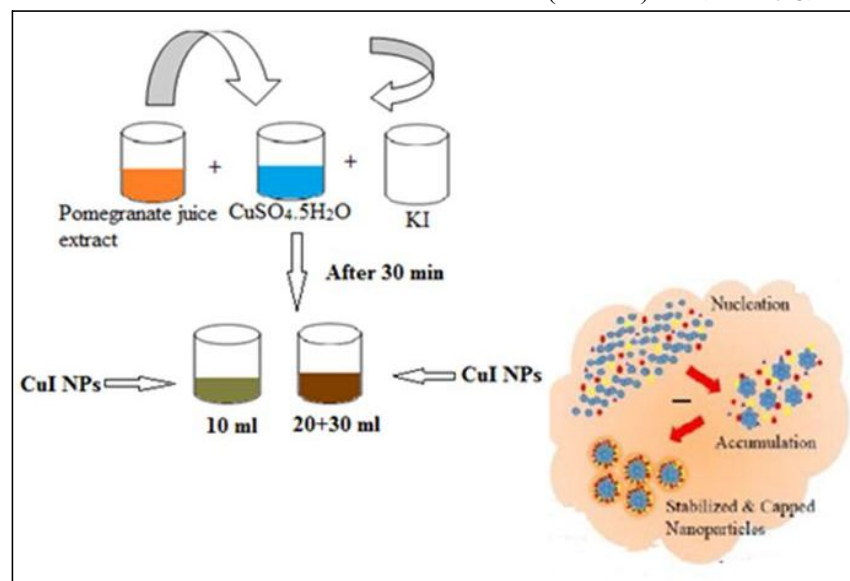


Fig. 1: Schematic representation of synthesis of CuI NPs.

diffraction analysis of CuINPs were carried out in the range of  $2\theta$  between  $20^\circ$ -  $80^\circ$ .

Fig. 2, shows agreement between XRD structure for all sample of CuI NPs and JCPDS standard card No. (006-0246) (McMurdie *et al.*, 1986), which have a polycrystalline structure and cubic phase. Also, no specific peaks due to any impurities were observed. The results have good agreements with Anushya and Revathy, Hammad *et al.*, and Ezealigo *et al* results (Ezealigo *et al.*, 2017; Vijayakumar and Rajagopal, 2016; Hammad R

Humud\*, 2017), respectively.

The pattern shows the detected (h k l) peaks are at  $2\theta$  values of  $25.5061^\circ$  plane (111),  $42.2457^\circ$  plane (220),  $49.9682^\circ$  plane (311),  $61.1639^\circ$  plane (400),  $67.3610^\circ$  plane (331) and  $77.2593^\circ$  plane (422) for 10 ml sample of pomegranate juice extract. While the increase of concentrations to (20 and 30 ml) were observed increase in the intensity of the crystallization peaks with appeared new peak are at  $2\theta$  value of  $29.5859^\circ$  plane (200) which disappeared in XRD pattern for sample 10 ml, this may

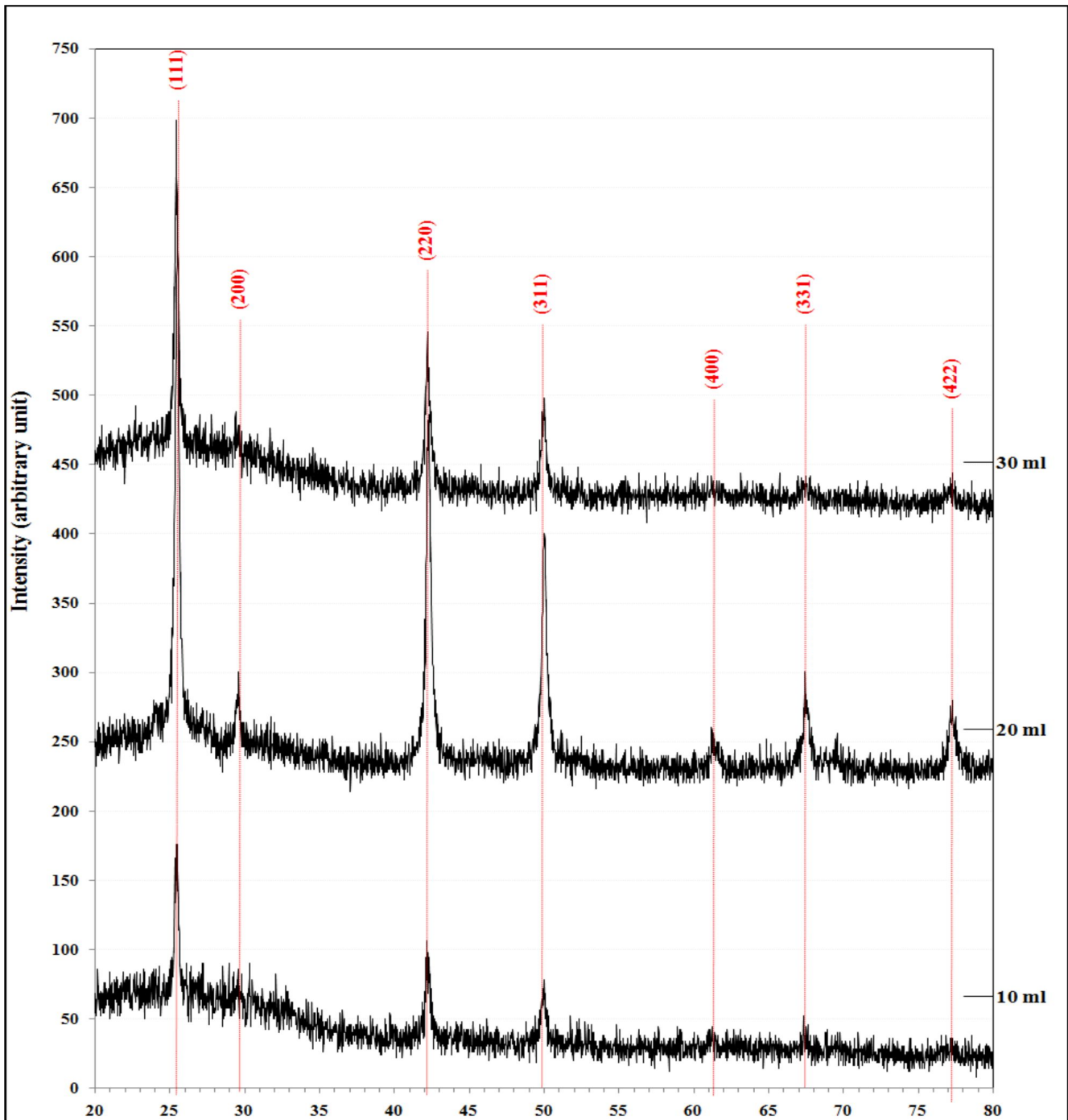


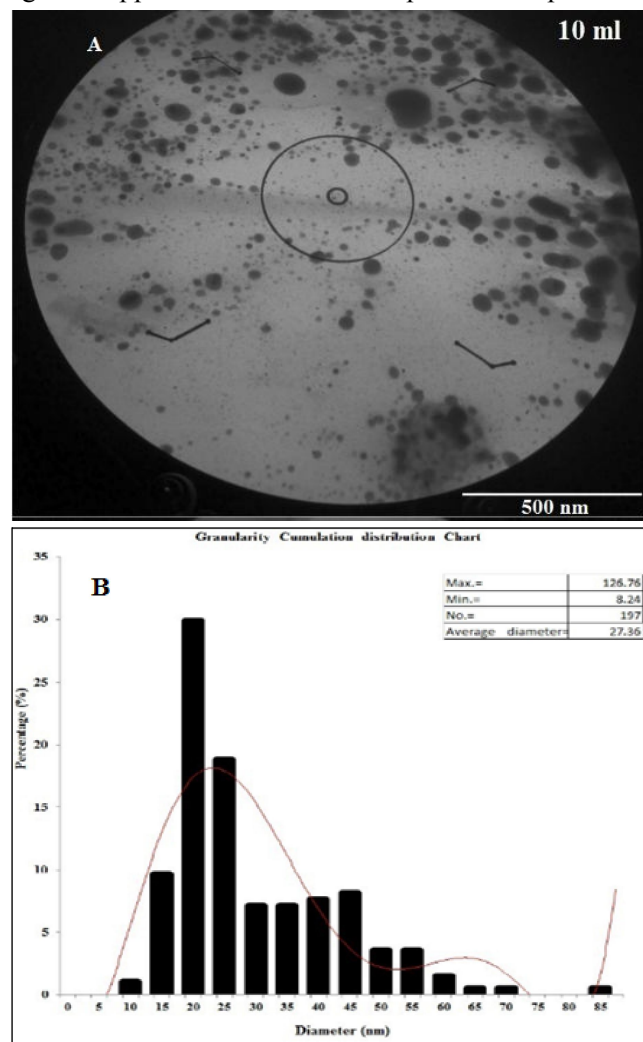
Fig. 2: Patterns of X-ray diffraction for CuI nanostructures synthesized by 10, 20 and 30 ml of pomegranate juice extract.

be due to the change of crystallites orientation at this peak toward the preferred peak (111) at this concentration of extract. However, the results showed that the intensity of the crystallization peaks had changed significantly with highest crystallization was observed for 20 ml of pomegranate juice extract.

The average crystallite size of the nanoparticles have been estimated using Scherer's formula [ $D = k\lambda/(\beta\cos(\theta))$ ] (Abass *et al.*, 2018), were found to be 35, 28 and 31 nm for sample (10, 20 and 30 ml) of pomegranate juice extract, respectively. The obtained sizes are smaller than the sizes reported by Mahdi and Ali (BYRANVAND and KHARAT, 2014).

### Transmission Electron Microscopy (TEM)

TEM was used for the characterization of the size, shape and morphology of synthesized CuI NPs. From the images, the bio-molecules that are acting as capping agents support the formation of spherical shaped with

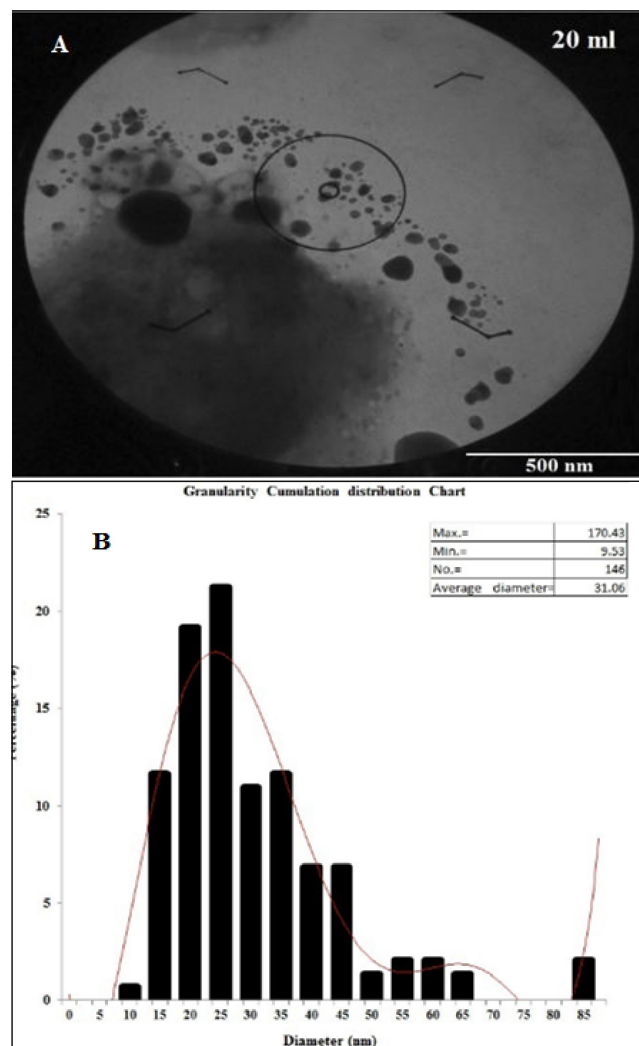


**Fig. 3:** (A) TEM image and (B) illustrates granularity accumulation distribution chart for CuI NPs synthesized by 10 ml of pomegranate juice extract.

**Table 1:** The optical density measurements at 600 nm for *S. aureus* in the different growth times in presence CuI NPs.

Times (hr)	Control (Broth + <i>S. aureus</i> )	Test (Broth + <i>S. aureus</i> + CuI NPs)
0	0	0
18	0.581	0.261
20	0.669	0.277
22	0.497	0.234
24	0.531	0.242

varying sizes which may prevent stabilizing functional groups of cuprous iodide nanoparticles from agglomeration as shown in figs. (3A, 4A and 5A). This result are good agreement with the research of Mohd *et al.*, (Johan *et al.*, 2012). Figs. (3B, 4B and 5B) illustrates the granularity accumulation distribution chart for CuI NPs. The average diameters were found to be (27.36, 31.06 and 28.41 nm) for sample 10, 20 and 30 ml, respectively.



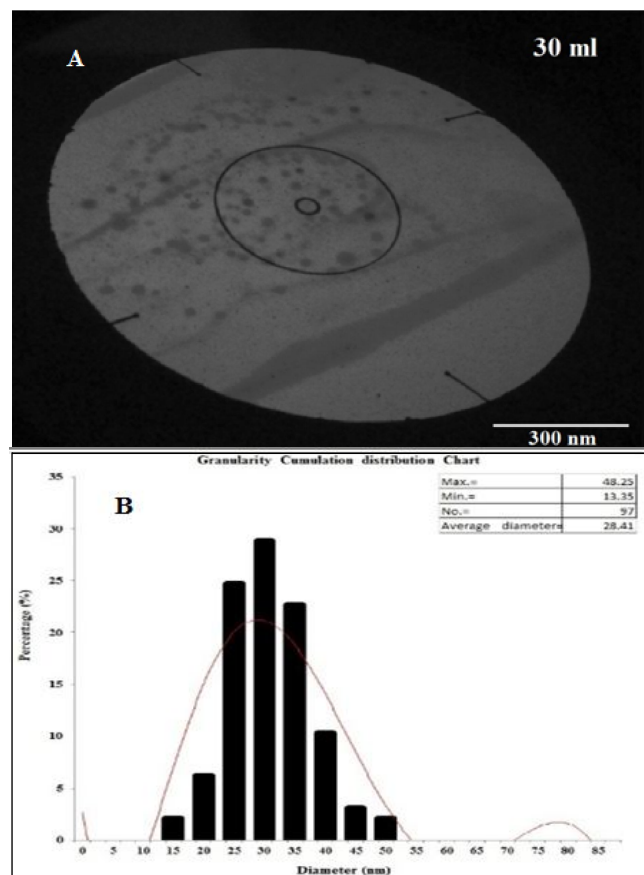
**Fig. 4:** (A) TEM image and (B) illustrates granularity accumulation distribution chart for CuI NPs synthesized 20 ml of pomegranate juice extract.

**Table 2:** Synergistic effect between some antibacterial agent and CuI NPs.

Antibiotic discs	Control (Bacteria plus antibiotic discs) Zone of inhibition (mm in diameter)	Bacteria treated with CuI nanoparticles in four time of incubation and antibiotic discs Zone of inhibition (mm in diameter)			
		Zone of inhibition (mm in diameter)			
		18 (hr)	20 (hr)	22 (hr)	24 (hr)
Levofloxacin	40	40	42	46	40
Norfloxacin	40	40	40	40	40
Ceftriaxone	30	32	31	32	30
Erythromycin	R	R	R	R	R
Ciprofloxacin	45	45	50	45	48
Trimethoprim	30	35	36	34	35
Tetracycline	25	28	29	29	29
Cefoxitin	R	R	R	R	R

### Fourier Transform Infra-Red spectroscopy (FTIR)

This measure has been performed for identifying the potential biomolecules that are responsible for the capping and reduction agent for the CuI NPs that have been synthesized by the extract of pomegranate juice. Fig. 6 shows FTIR spectrum for CuI NPs. The bands at (3442.68, 3424.60, 3442.68  $\text{cm}^{-1}$ ) are given to the O-H and N-H stretching vibration of phenols and proteins from the extract (Vadlapudi and Amanchy, 2017; Rao and Tang,



**Fig. 5:** (A) TEM image and (B) illustrates granularity accumulation distribution chart for CuI NPs synthesized by 30 ml of pomegranate juice extract.

2017). Other peaks at (2852.22, 2921.51, 3057.07, 2846.19, 2924.52 and 3020.92)  $\text{cm}^{-1}$  represent to C-H stretch of alkanes (Shivashankarappa and Sanjay, 2015). The peaks at 1641.17 $\text{cm}^{-1}$ , 1638.16 $\text{cm}^{-1}$  and 1644.18 $\text{cm}^{-1}$  assigned to C=O stretching mode in amine I group that is typically found in the protein (Loo *et al.*, 2012). Peaks at (1451.38, 1496.57, 1547.78, 1385.1, 1454.39, 1517.66, 1541.76, 1448.37 and 1532.72)  $\text{cm}^{-1}$  corresponding to N-O asymmetrical nitro compounds stretch (Vadlapudi and Amanchy, 2017). Peak at 1075.20 $\text{cm}^{-1}$  represent to alkoxy C-O, also the peak at

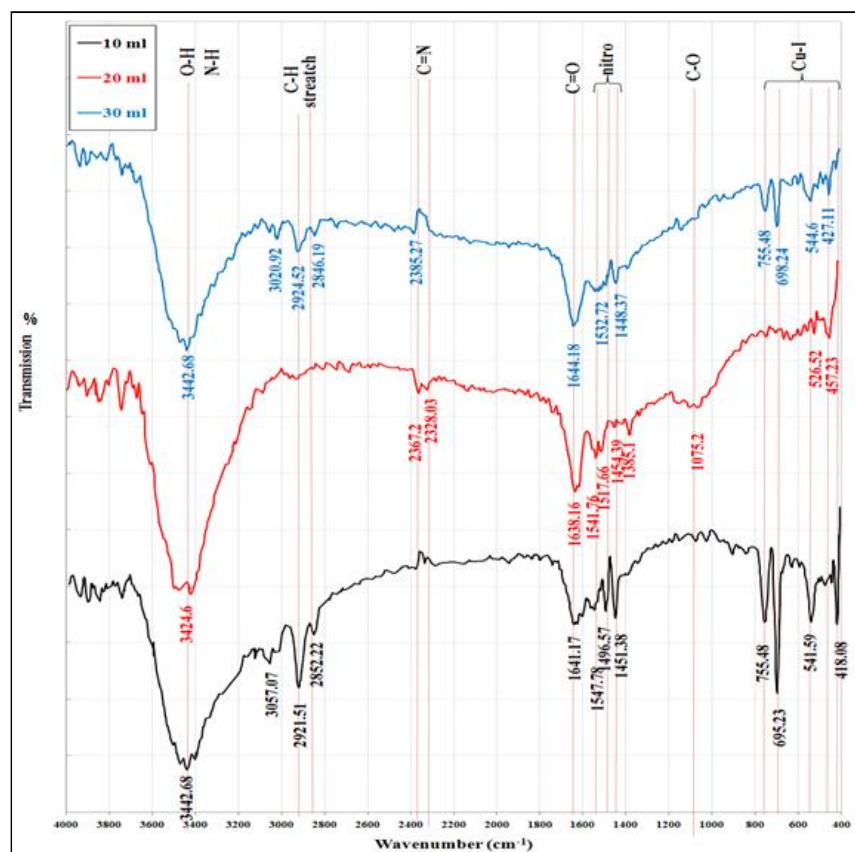
(2328.03, 2367.2 and 2385.27)  $\text{cm}^{-1}$  represent to C=N (Hammad R. Humud, 2017). The peaks at (418.08, 541.59, 695.23, 755.48, 457.23, 526.52, 427.11, 544.6 and 698.24) $\text{cm}^{-1}$  are assigned to Cu-I stretching vibrations. The same results are found in studies Hammad *et al.*, Hammad and Sawsan and Prakash (Prakash, 2011; Hammad R Humud\*, 2017; Hammad R. Humud, 2017), respectively. The dual influence of the plant extract as a reducing and capping agent and the existence of some functional groups has been proved by FTIR analyzing of CuI NPs. Capping agents play very important in stabilizing nanoparticles and capped CuINPs demonstrate a more efficient anti-bacterial activity in comparison with the uncapped CuINPs (Abdel-Mohsen *et al.*, 2013, Gnanadhas *et al.*, 2013).

### UV-visible analysis

The absorbance spectrum of CuI NPs was studied by dissolved powder in de ionized water fig. 7. An absorption onset about (226, 225 and 225) nm has been showed for three types of CuI nanostructures. There is a slight difference between the three concentrations, which is clarified by the shape and size of the particles. However, The decrease in particle size shifts the absorption edge from the visible to ultraviolet region of the electromagnetic spectrum as the band gap energy of the semiconductor increases (Yang *et al.*, 2016). It is a well known fact that, in case of nano-sized particles, the optical properties change when the particle size is changed, thus, the shift in absorption peak towards a lower wavelength can be assigned to a decrease in particle size and hence, an increase in the optical band gap as a result of the quantum confinement effect (Sharma *et al.*, 2006; Lin *et al.*, 2005).

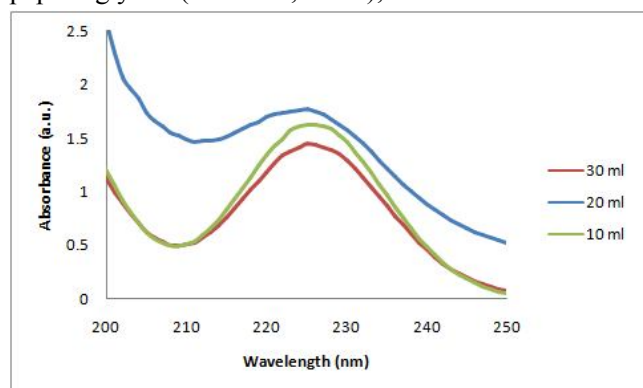
### Antibacterial activity of synthesized CuI NPs

- Broth inoculation method: The optical density for



**Fig. 6:** FT-IR spectrum of CuI NPs synthesized by different concentration of pomegranate juice extract.

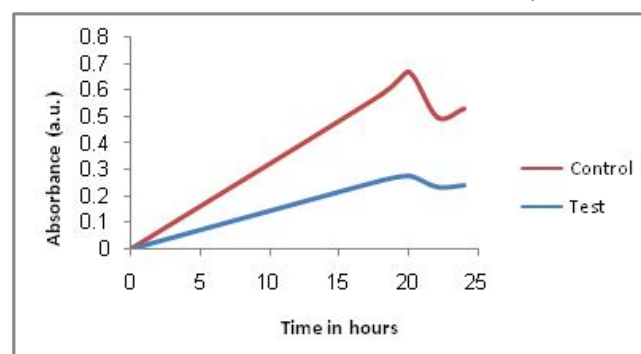
the inoculated bacterial suspension (control and the test) are shown in (Table 1, Fig. 8). Since types of bacteria are generally characterized by a cell wall, that lies outside the cell membrane and is composed mostly of a homogeneous peptidoglycan layer (which consists of amino acids and sugars). Thus, gram-positive bacteria have one cytoplasmic membrane with multilayer of peptidoglycan polymer (Fu *et al.*, 2005) and a thicker cell wall (20-80 nm). Whereas the wall of gram-negative bacteria is composed of two cell membranes, an outer membrane and a plasma membrane with a thin layer of peptidoglycan (Fu *et al.*, 2005), with a thickness of 7-



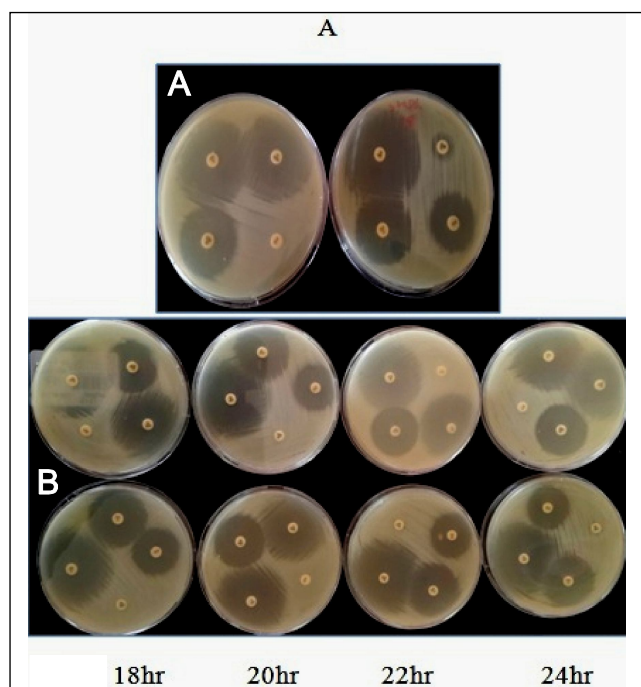
**Fig. 7:** Absorbance spectra of CuI NPs synthesized by 10, 20 and 30 ml of pomegranate juice extract.

8 nm. This study is therefore oriented towards gram-positive bacteria *S. aureus*. The results showed obviously decreasing in the test (MRSA) bacterial growth culture in compared with control, which was reflected the effect of CuI NPs in counteracting bacterial growth and this result is confirmed that reported by Alan and Cian (Hibbitts and O'Leary, 2018). This inhibition in the tested tubes is may be because of the disruption of the bacterial cell membrane and the formation of the "reactive oxygen species" (ROS) (Pramanik *et al.*, 2012). The disruption of the bacterial membrane happens when the charged ions of the nanoparticle bind to charged parts of the bacterial membrane, which results in pores in the membrane where contents of cytoplasm flow out of the cell, dissipating the H<sup>+</sup> gradient across the membrane that could cause cell death, this result documented by Knetsch and Koole and Lara *et al.*, (Knetsch and Koole, 2011; Lara *et al.*, 2010).

• Surface inoculation method and antimicrobial sensitivity test: In this study, the synergistic effects of CuI NPs with some antimicrobial agents were investigated against *S. aureus* (MRSA) (Fig. 9). The results in (Table 2) showed a positive increasing in the sensitivity of tested bacterial isolate (MRSA) against several tested antimicrobial agents. This results are agreed with Raghupathi and his workers (Raghupathi *et al.*, 2011), they mentioned about this elevation may be due to metallic ion-based NPs showed strong anti-microbial characteristics, because of the spherical shapes and size of nanoparticles ensures that a significantly large surface area of the particles is in contact with the bacterial effluent. Such a large contact surface is expected to enhance the extent of bacterial elimination (Pal *et al.*,



**Fig. 8:** Antibacterial activity of CuI NPs on *S. aureus*.



**Fig. 9:** Synergistic effect between some antibacterial agent and CuI NPs. (A) Control (B) Treated Bacteria.

2007). Thus, sulfur-containing proteins in the membrane or inside the cells and phosphorus-containing elements like DNA may be preferential sites for CuI NPs binding (Mlalila *et al.*, 2017). So, the mechanisms by which these NPs inhibited bacterial growth might be the release of ions ( $\text{Cu}^{+1}$ ) and production of ROS. As a matter of fact, capabilities of releasing ions by NPs were reported to be the one of the mechanisms by which different NPs showed antibacterial property (Sirelkhatim *et al.*, 2015). Therefore, we can suggest that, this type of NPs could in fact increase the possibility of drug sensitivity.

## Conclusions

In this paper, we report a green method for the synthesis of CuI NPs using pomegranate juice extract. This is a green, simple and efficient method to synthesize CuI NPs without using any capping or dispersing agent and any harmful reducing agents. The biosynthesized of CuI NPs were characterized by XRD, TEM, FTIR and UV-visible absorption spectroscopy. XRD pattern thus clearly illustrates that CuI NPs formed in this present synthesis are crystalline in nature and average particles size were (35, 28 and 31) nm for sample (10, 20 and 30) ml. The nanoparticles were spherical in shape with varying sizes ranging from 27.36 to 31.06 nm. CuI NPs exhibited good antibacterial activities against *S. aureus*.

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