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The Antibacterial Activity of the Biosynthesized Copper Oxide Nanoparticles by *Lantana camara* Flowers Extract Against Some Bacterial Isolated from Burns

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ABSTRACT

The study included 70 Surface swabs collected from patients in different ages of both genders suffering from burns infections. All the specimens were diagnosed by subculturing in differential and selective media, microscopically and by following the biochemical tests to confirm identifications. Antibiotic susceptibility test was done to all the bacterial isolates.The identification of the clinical isolates showed that the 16(22.9%) were belonged to *Staphylococcus aureus* and 6(8.6%) of *Staphylococcus epidermidis* , whereas 23(32.8%),20(28.6%) isolates were for *Pseudomonas aeruginosa* ,*Acinetobacter baumannii* respectively. Copper oxide nanoparticles were biosynthesized by *Lantana camara* flower extracts which acted as a reducing agent for copper nitrate salts, characterization of the metal oxide was done by using Ultra violate visible light Spectroscopy, Fourier transforms Infrared spectroscopy, X-ray Diffraction, Atomic Force Microscopy and Field Emission Scanning Electron Microscopy. The biological activity of Copper Oxide Nanoparticles was screened toward three clinical isolates that showed multiple drug resistance isolated from burns by following agar wells diffusion method which proved the ability of copper oxide nanoparticles to inhibit growth of the clinical bacterial isolates. The minimum inhibitory concentrations were determined toward the tested bacteria by using the microdilution method

KEYWORDS: Copper oxide nanoparticles; Burns infections; Antibacterial activity; Pathogenic bacteria; Antibiotics susceptibility; *Lantana camara*.

الخلاصة

تضمنت الدراسة جمع 70 مسحة من المرضى من مختلف الأعمار من كلا الجنسين يعانون من التهابات الحروق. تم تشخيص جميع المسحات باستخدام الاوساط الزرعية الانتقائية وتم فحص العزلات مجهريا حيث ظهر ان16(22.9%) و 6(8.6%) من العزلات السريرية تعود *Staphylococcus aureus* و *Staphylococcus epidermidis*, على الترتيب في حين كانت العزلات 20(28.6%) و 23 (32.8%), تعود الى *Acinetobacter baumannii* و *Pseudomonas aeruginosa*على التوالي . تم تحضير الجسيمات النانوية لأكسيد النحاس حيويًا بواسطة مستخلص زهرة *Lantana camara* التي عملت كعامل مختزل لأملاح نترات النحاس وتم توصيف أكسيد المعدن المحضر حيويا باستخدام جهاز المطياف الضوئي ، مطياف فوريه للأشعة تحت الحمراء ، جهاز حيود الأشعة السينية ، الفحص المجهري للقوة الذرية والمجهرالماسح الضوئي . تم فحص التأثير المضاد للبكتيريا لجزيئات أكسيد النحاس النانوية ضد ثلاث عزلات سريرية لها خاصية مقاومة لثلاث فئات أو أكثر من المضادات الحيوية باتباع طريقة الانتشار بالحفر التي أثبتت قدرة اوكسيد النحاس المحضر بالطريقة الحيوية على تثبيط نمو البكتيريا الممرضة ، وتم تحديد والتركيز المثبط الأدنى تجاه البكتيريا باستخدام طريقة التخافيف الدقيقة

INTRODUCTION

The most common forms of trauma are burns infections that cause serious thermal damages to skin which required serious attention immediately to minimize the rising rates of morbidity and mortality [1].These kinds of injuries are considered the main cause of high rates of mortality of children under the age of 14 where as in adults these injuries are causing mortality for males as twice as females in all ages groups .One of the serious complications that following burn injuries are burns infections, invasiveness of tissues and sepsis that followed by immediate colonization by skin normal flora such as *Staphylococcus aureues* , *Streptococcus pyogenes* and the patient internal normal flora that join the community on burned skin surfaces which become a rich environment in proteins containing avascular necrotic tissue (eschar) present a perfect environment for bacterial colonization and proliferation [2] . Skin surfaces are sterile at the time of thermal injury and later they become colonized with *Staphylococcus* spp. which

colonized deeper in skin glands of sweat hair follicles, these bacteria colonize the damaged skin surface during the first 48 hours and after 5 to 7 days. The damaged skin areas colonized with bacteria and fungi that come from the patient's normal flora or from the medical facility environment or are transferred by the direct contact with the hands of health care worker [3]. Unfortunately, these pathogens became a serious health problem as they had the ability to develop many resistance mechanisms against the most common used drugs in hospitals which made treatment of these infections more difficult mission. The resistance to antibiotics leads to an urgent need to find different ways, alternative drugs and materials and synthesized them in a safe low cost and ecofriendly approaches such as probiotics, medicinal plant and their extracts. Recently Nanoparticles and Nanotechnology is a famous and attractive field of researches since last century produced Nanoparticles (NPs) which include particulate with one dimension less than 100 nm. These NPs characterized by different techniques for the analysis of various physicochemical properties of NPs [4]. The biosynthesis technology is safe, low cost, and ecofriendly approach that have revealed that living systems are capable of utilizing their int rinsic organic chemical processes in the turning on inorganic ions of various metals into NPs [5]. Copper oxides are known to be a low cost and wide available metal oxides [6]. Copper elements is characterized by their high toxicity to bacteria but it is no toxic to animal cells. It is safe for human beings as they were widely used in food packaging and in treatment of water [7]. One of the most metal oxides is copper oxide which has a particular property. It is a semiconductor material with a significant optically, electrically, physically, and magnetic properties [8,9]. These metal oxide nanoparticles are stable and cheaper than other noble metals. They were applicable in many industrials' fields such as a colorant in ceramics including the preparation of slips and glazes, batteries, catalyst for chemical reactions, solar cell, chemical sensor, absorbent, thermal conductivity enhancer. Furthermore, they have an antifouling properties and wastewater treatment, they are biocontrol agent, drug delivery and have an anticancer activity and effective anti- microbial agent [10]. The green method can be carried out using algae, plant and their products and microorganisms including bacteria, yeast and fungi [11]. Many compounds have been found in plants extracts such as protein, alkaloids, flavonoids, reducing carbohydrates and phenols that can be as capping agent in the biosynthesis of nano-materials .these compounds reduce precursor metal salt to NPs detected by visible color change of salt solution[12].This study aimed to biosynthesize of CuO NPs by *Lantana camara* flowers extract and study their biological activity on drugs resistant bacterial strains and Isolation and identification of pathogenic bacteria from burns infections and determination of their susceptibility to antibiotics and determine the inhibitory effect at the minimum concentration (MIC) of the biosynthesized CuO NPs toward the tested bacteria by microdilution method

MATERIALS AND METHODS

Identification and laboratory diagnosis of bacterial isolates

Isolates were grown on MacConkey agar and Blood agar (Mast group, U.K) (incubated at 37°C for 24h) and microscopic examination was done by using Gram stain. Cell arrangement and shape were observed. Some of the isolated bacteria were subcultured on selective and differential media as follow: Eosin methylene blue agar, *S. aureus* and *S. epidermidis* were sub cultured on Mannitol salt agar (Himedia, India) Identification was done by biochemical tests (Indole, Methylred, Voges-Prouskauer, Citrate utilization), Oxidase test and Catalase test, when culture on (peptone water, urea agar base, Simmon Citrate Agar) Hi-media India [13]

Antibiotics sensitivity

Antimicrobial sensitivity test was done to bacterial species according to the CLSI (2021) instruction using Kirby- Bauer method, Different antimicrobial discs (Mast group, U.K), Table 1 were used with a maximum six discs were put at the of Mueller-Hinton agar media. The plates were wrapped with parafilm to prevent any possible contamination during incubation and incubated at 37°C for 24h. the inhibition zone of each antibiotic disc was noticed and measured.

Table 1. antibiotics discs and their potency used in the study

Antibiotic	Potency (µg/disc)	Antibiotic	Potency µg/disc
Penicillin	10	Tobramycin	10
Piperacillin	100	Ciprofloxacin	5
Ceftazidime	30	Levofloxacin	5
Cefoxitin	30	Imipenem	10
Cefotaxime	30	Meropenem	10
Amikacin	30	Vancomycin	30
Gentamycin	30	Trimthoprim sulfamethazol	1.25/23.75
Aztreonam	30		

Biosynthesis of copper oxide nanoparticles by *Lantana camara* flower extract

The preparation of copper oxide nanoparticles was done by dissolving 2 g of copper nitrate salts in 150 ml of deionized distilled water on magnetic stirrer using hot plate stirrer for 15 min at 80 C^o, then 50 ml of the aqueous flower extract was added to the solution with continuous stirrer for 1 h. After heterogeneity, 20 ml (3M) of sodium hydroxide (NaOH) was drop wise added to the solution to accelerate the precipitation process. The precipitation was washed with ethanol and deionized distilled water several times. Then dried in the oven at 100 for 3 hours. Finally, the powder was subject to heat-treatment at 600 ^OC for 4 hours to get a fine black powder[14]

Optimal Condition for Nanoparticle Synthesis

The concentration of *Lantana camara* flower extract was considered and subsequently added to a 5 ml solution of copper nitrate which were previously dissolved using 50 ml of deionized water. the concentrations of each solution were adjusted to pH 9.0 by using 0.1 N of "hydrochloric acid HCL and sodium hydroxide NaOH" [14].

Characterization of biosynthesized copper oxide nanoparticles

Several analysis techniques were used in the characterizations of Copper Oxide nanoparticles such as "Field Emission -Scanning Electron Microscopy (FE-SEM)" (Hitachi), Fourier Transforms Infrared Spectroscope (FTIR) "(Shimadzu, Japan), X-ray diffraction (XRD)(Bruker)", Ultra Violate- Visible Light Spectroscopy (UV-VIS) UV-1900i Shimadzu, and Atomic Force Microscopy (AFM) (AA-3000 Shimadzu)".

The Antibacterial activity of copper oxide nanoparticles

The bacterial suspension was prepared by taking a number of bacterial colonies by loop into brain heart infusion broth incubated for 18h at 37 °C. then suspension was compared with McFarland standard solution, which depends on the degree of turbidity of the bacterial suspension is close to 1.5 x 10^8 (CFU/ml).

Nanoparticle stock solution was prepared by dissolving a 2000 mg in 10 ml of deionized distilled water and stirrer the solution in an ultrasonic bath for 30 minutes to obtain the initial stock solution of the tested nanoparticles with final concentration 200 mg/ml that used then in the preparation of (12.5, 25, 50, 100) mg/ml concentration which then will be used in the next experiment.

Antibacterial activity *in vitro* of CuO NPs was analyzed by agar wells diffusion method according to Dadi [15]. The antimicrobial activity was tested against three multi- drugs resistance (MDR) strains include *A. baumannii*, *S. aureus*, *P. aeruginosa* which were isolated from burns infections. after culturing of the bacterial inoculum on Mueller-Hinton agar, five wells were made on the agar by sterilized cork borer. The five different concentrations of tested materials of 200 µl were inoculated to the wells using a micropipette, sixth well was considered as control by adding 100 µl of deionized distilled water, three repeated plates were kept in incubator for 24 h, at 37 °C**.**

Determination of Minimum Inhibition Concentrations (MIC)

The inhibitory effect at the minimum concentration was tested according to Duffy [16] with minor modification. The broth microdilution method using Muller- Hinton Broth (MHB) in 96 wells microplate. stock solution of nanoparticles was prepared by weighing powder of 20 mg and dissolved in 10 ml deionized water to get an initial stock of 2000 µg/ml, concentrations of the biosynthesized NPs were prepared (2000,1000, 500, 250, 125, 62.5, 31.25 and 15.63) µg/ml, the tested nanoparticles, bacterial suspension, and the sterile broth were always added to 96 wells microplate as following order: broth, NPs solution and finally the tested bacterial suspension. The first row (row A) was considered as a negative control

with 100µl MHB and 100 µl of the NP (NPs were added to the control to allow for any effect of light scattering due to the NPs). from Row B to G were contained 100µl of NP, 80 µl of MHB and 20 µl of ($1.5x10^5$) CFU/ml bacterial suspension. Row H was positive control contain only 180 µl of MHB and 20 µl of the bacterial suspension. Results were read at optical density OD 630 nm.

RESULTS AND DISCUSSION

Collection and Identification of clinical samples

Swabs have been collected from patients were admitted in Baquba Teaching Hospital, Diyala province, Iraq from September 2021 to the end of January 2022. The 70 swabs were taken from burn infections site of patients from both genders of different ages admitted in burn sections, The males sample number was 33 (47.1%), while the number of female 37 (52.9 %) **.**

After gram staining. *Staphylococcus* spp. showed gram positive cocci in grape-like clusters, *P. aeruginosa* was slender, short, plump, gram-negative bacilli with bipolar staining [13] *A. baumannii* appeared gram negative with some variations in shape from bacilli to cocco-bacilli. According to their growth phase, diplococcus and some had short chains [17] *Staphylococcus aureus* on blood agar were creamy colored, opaque with smooth edges. *S. epidermidis* in contrast to *S. aureus* have white colony with no pigment. they display γ- hemolysis whereas *S. aureus* colonies were surrounded by a zone of β-hemolysis [18]. *A. baumannii* colonies were small smooth non-hemolytic colonies [19]. *Pseudomonas aeruginosa* colonies were a blue-green color. The colonies are β-hemolytic [20]. Colonies of clinical isolates on MacConky agar showed variations in color and their ability to ferment sugars, *A. baumannii* colonies were pale pinkish with small colonies and have regular edges [21]. *P. aeruginosa* colonies were lactose non fermenting, with a brownish color [13]. Biochemical tests were done to confirm identification of clinical bacterial isolates. *Staphylococcus* spp. were identified depending on the cultural and the microscopical properties as well as biochemical tests. *S. aureus* and *S. epidermidis* were a positive for catalase, but they were negative for oxidase [13] as showed in Table 2.

In Table 3, All the isolates of *A. baumannii* were positive result for catalase test and citrate utilization but they showed negative results for oxidase, Indole, Methyl red, and Voges Proskauer and for Triple Sugar Iron (TSI), this pathogen did not have the ability to ferment sugars so the results are alkaline slant / no change for butt /H_2S negative / no gasses production, these bacteria showed negative urease test [22]. *P. aeruginosa* isolates were positive for oxidase and catalase, utilizing citrate as carbon source. These bacteria were negative for indole, methyl red and voges-proskauer, variable urease, TSI result showed alkaline slant and butt, H_2S positive, no gas production [23]. Final identification was done by VITEk-2 system that include 64 biochemical tests. the accuracy of diagnosing results up to 99%.

Table 2. Biochemical test used in identification of gram-positive bacteria

Bacteria	Biochemical test			
	Growth on mannitol salt agar	Catalase	oxidase	Gram staining
S. aureus	Yellow colonies	-VE	+VE	G+ve cocci
S. epidermidis	White colonies			

(+ve) positive, (-ve) negative

Table 3. Identification of gram-negative bacteria

Test type Bacterial Isolates	Catalase	Oxidase	Indole	Methyl red	Vegas Proskauer	Citrate Utilization	Urease	T S I
A. baumannii	+	-	-	-	-	+	V	K/K, H_2S-,G -
P. aeruginosa	+	+	-	-	-	+	V	K/K, H_2S+, G -

(-) Negative, (+) Positive, (V) Variable, (A) Acidic, (K) Alkaline, (G) gas

Isolation of pathogenic bacteria

The overall numbers of samples collected were 70 clinical samples and about 65 (92.8 %) were positive growth when sub-cultured on culture media whereas 5 (7.2%) of the samples did not show any growth n agar plates as showed in Table 4.

Table 4. Number of collected sample.

Bacterial growth	Number	% Percentage
Positive growth	65	92.8
No growth	5	7.2
Total number	70	100

The positive bacterial growth divided into *S. aureus*16 (22.9 %), *S. epidermidis* 6(8.6 %), *A. baumannii* 20 (28.6%), *Pseudomonas aeruginosa* 23(32.8%) as in Table 5.

Table 5. Numbers and distribution of clinical isolated bacteria from burns infections

Bacteria	Number	Percentage (%)
Staphylococcus aureus	16	22.9
Staphylococcus epidermidis	6	8.6
Acinetobacter baumannii	20	28.6
Pseudomonas aeruoginosa	23	32.8
No growth samples	5	7.1
Total number	70	100

Table 6 showed that 16 clinical isolates of *S.aureus* were tested to 10 antibiotics. *S. aureus* clinical isolates were resistance to Penicillin 14(87.5%), Cefoxitin16 (100%), Ciprofloxacin7 (43.8%), Levofloxacin8 (50%), Tobramycin10 (62.5%), Trimethoprim/sulfamethazole10 (62.5 %), Vancomycin1 (5%), Imipenem9 (56.2%), Meropenem12 (75 %), and Gentamicin9 (56.2%).

Table 6. Number and percentage of resistant and sensitive *Staphylococcus aureus*

Bacteria	*Staphylococcus aureus* N= 16 Percentage (%)			
Antibiotics	R	%	S	%
Penicillin	14	87.5	2	12.5
Cefoxitin	16	100	0	0
Gentamicin	9	56.2	7	43.8
Tobramycin	10	62.5	6	37.5
Ciprofloxacin	7	43.8	9	56.2
Levofloxacin	8	50	8	50
Trimthoprim sulfamethazole	10	62.5	6	37.5
Imipenem	9	56.2	7	43.8
Meropenem	12	75	4	25
Vancomycin	1	5	15	95

• R = Resistance, • S = Sensitive

Susceptibility test of the 20 clinical isolate of *Acinetobacter baumannii* c to 13 antibiotics was tested and Table 7 showed that the isolates were resistance to Pipracillin11 (55%), Ceftazidim14 (70%), Cefotaxime18 (90%), Amikacin11 (55%), Tobramycin9 (45%), Ciprofloxacin and Levofloxacin8 (40%), Imipenem16 (80%), Meropenem15 (75%), Trimethoprium/ sulfamethazole7 (35%), Doxycyclin10 (50%). All the 23 isolates of *P. aeruginosa* were tested for their susceptibility against 13 different antibiotics. the results showed highly resist to Pipracillin18 (78.3%), Cefotaxime18 (78.3) %, Ceftazidime23 (100) %, Amikacin16 (69.5%), Tobarmycin, Aztreonam, Imipenem and Trimthoprim Sulfamethazole 13 (56.5%), Ciprofloxacin14 (60.8) %, Levofloaxin11 (47.8%), Meropenem21 (91.3%), Doxycycline16 (69.5%) as in Table 7.

Table 7. susceptibility of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*

Bacteria	*Acinetobacter baumannii* N= 20percentage (%)				*Pseudomonas aeruginosa* N=23 percentage (%)			
Antibiotics	R	%	S	%	R	%	S	%
Piperacillin	11	55	9	45	18	78.3	5	21.7
Ceftazidime	14	70	6	30	23	100	0	0
Cefotaxime	18	90	2	10	18	78.3	5	21.7
Amikacin	11	55	9	45	16	69.5	7	30.5
Tobramycin	9	45	11	55	13	56.5	10	45.5
Aztreonam	-	-	-	-	13	56.5	10	45.5
Imipenem	16	80	4	20	13	56.5	10	45.5
Meropenem	15	75	5	25	21	91.3	2	8.7
Ciprofloxacin	8	40	12	60	14	60.8	9	39.2
Levofloxacin	8	40	12	60	11	47.8	12	52.2
Polymyxin B	14	70	6	30	8	37.7	15	65.3
Trimethoprim sulfamethazole	7	35	13	65	13	56.5	10	45.5
Doxycycline	10	50	10	50	16	69.5	7	30.5

• R = Resistance, • S = Sensitive

Biosynthesis of Copper Oxide by *Lantana camara* flower extract

The flowers of *Lantana camara* were chosen in this study to biosynthesis of copper oxide by using flower extract as a reducing agent (Figure 1).

Characterization of Copper Oxide nanoparticles

Ultraviolet – Visible Light spectroscope (UV-VIS)

The stability of the nanoparticles was diagnosed by using UV-vis spectrophotometer, as showed in Figure 2 The UV-vis absorption spectrum of CuONPs was recorded at 827nm this high absorbance wave length was higher than the wave length reported at 270 nm by Phang *et al*. [38] who prepared CuO NPs by adopting the green synthesis rout.

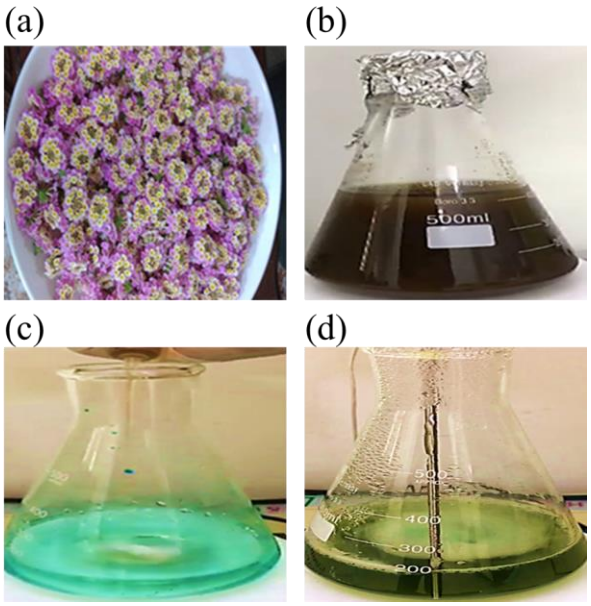

Figure 1. a) *Lantana camara* flowers, b) Flowers extract, c) Copper nitrate solution, d) The final colour changing indicates the biosynthesis of CuO nanoparticles by *Lantana camara* flower.

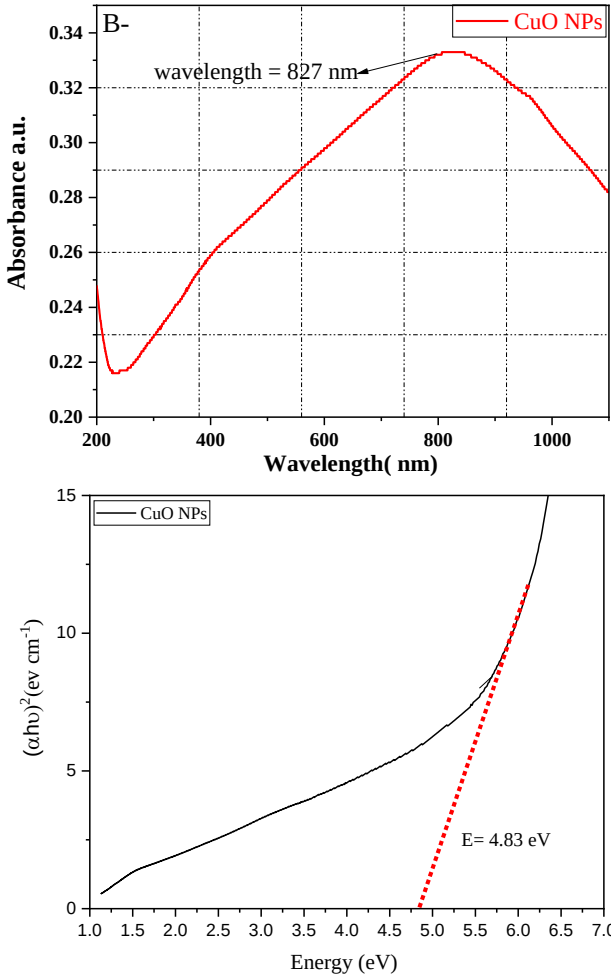

Figure 2. Ultra-violate visible light wavelength, absorbance and band gap of copper oxide nanoparticles.

X- Ray Diffraction (XRD) analysis

Diffraction of the X-ray of the biosynthesized CuO NPs using *L.camara* flower extracts was measured and the obtained peaks at 2θ = 31.8º , 34.4º, 36.3º ,47.5º, and 56.6ºcorrespond to the crystal structure of the obtained CuO NPs. The mentioned peaks are matched with the diffraction data standard of CuO NPs (JCPDS file no :01-080-1917) as in Figure 3. Additionally, those peaks correspond to the basal planes of (100), (002), (101), (102), and (110). The crystallite size of CuO NPs is calculated by Scherrer equation:

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

Where:

D: Average crystallite size (Diameter of the crystal)

β: Line broadening in radians (Full width at half maximum)

θ: Bragg angle is the angle obtained from 2Θ value corresponding to maximum intensity peak in XRD pattern.

λ: X-ray wavelength of the Cu-kα radiation.

K: Scherrer constant

the data of the strongest three peaks were used to estimate the crystallite size which was in 28.4 nm. CuO NPs as in Table 8. The recent study results matched with Thamer *et al*. [45] who reported that crystallite size was 27.2 nm of biosynthesized CuO NPs. the smallest crystallite size (less than 100 nm) refers to the nano-crystallinity nature of the biosynthesized nanoparticles [46].

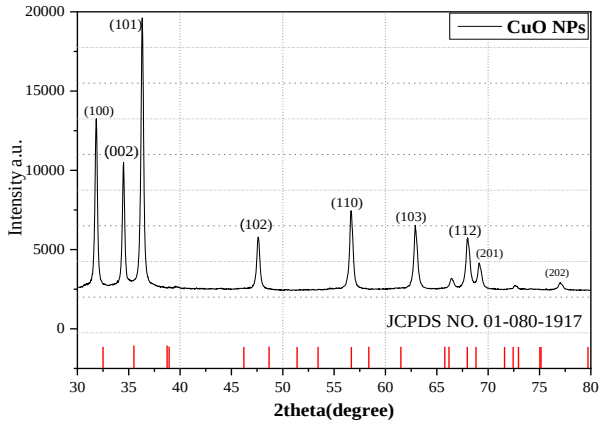

Figure 3. X-ray diffraction of copper oxide nanoparticles.

Table 8. Estimation of the crystallite size (CuO NPs)

2θ	FWHML	K	λ	D (nm) CuONPs	Average (nm)
31.83232	0.29401	0.94	0.15406	29.35	28.4
34.49211	0.29923			29.03	
36.31844	0.32564			26.82	

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Foureir Transform Infrared Spectroscopy (FTIR)

FT-IR spectra analysis was carried out to detect the probable biomolecules that are responsible for the formation of prepared nanoparticles as shown in Figure 4. The infrared spectra of *Lantana camara* flower extract show a broad absorption band at around 3412.06 cm^{-1}, corresponding to the hydroxyl groups (O-H) stretching vibrations, which are caused by the phenolic compounds. The C-H stretching vibration of the aromatic group is observed at 2927 cm^{-1} Moreover, the peak at 1269 cm^{-1} corresponds to the C=O stretching vibration, which might arise from the functional groups of ketones, aldehydes, and carboxylic acids. The peak at 1615.14 cm^{-1} corresponds to C=C stretch in aromatic rings

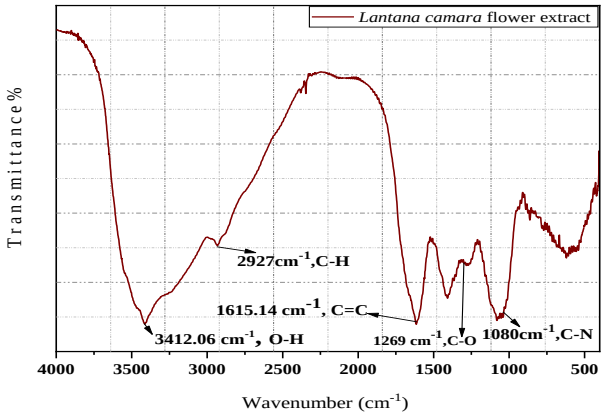

Figure 4. FTIR of Lantana camara flower extract.

In Figure 5, The FTIR spectral analysis of biosynthesized CuO NPs in the range 400–4000 cm^{-1}, The most significant absorption peaks were those observed at 2919.09 cm-1 N-H stretching, amines; suggested that the role of amines in the stabilization of CuO NPs, 1384.23cm^{-1} O-H Bending, phenols, 776.51cm^{-1} C-N stretching, amines and 531.27 cm^{-1} that correspond to the stretching vibration of the Cu-O which

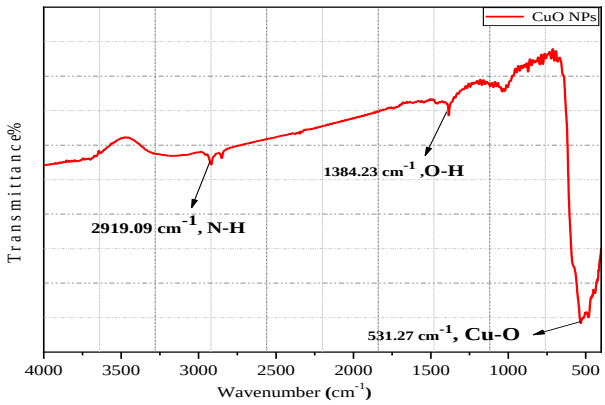

Figure 5. FTIR of the biosynthesized copper oxide nanoparticles.

Field Emission – Scanning Electron Microscope analysis

The FESEM image of CuO NPs sample showed the particle size were measured by Image J software as the following: (23.08, 26.17, 27.04, 28.30, 31.9, 34.48, 35.17, 36.34, 37.69, 40.81, 41.48, 42.43, 46.87, 50.67, 51.56, 62.06, 73.63, and 85.44) nm, Figure 6. The average of particles size was 43.06 nm which again confirmed the nanostructure nature of the metal oxide [8]

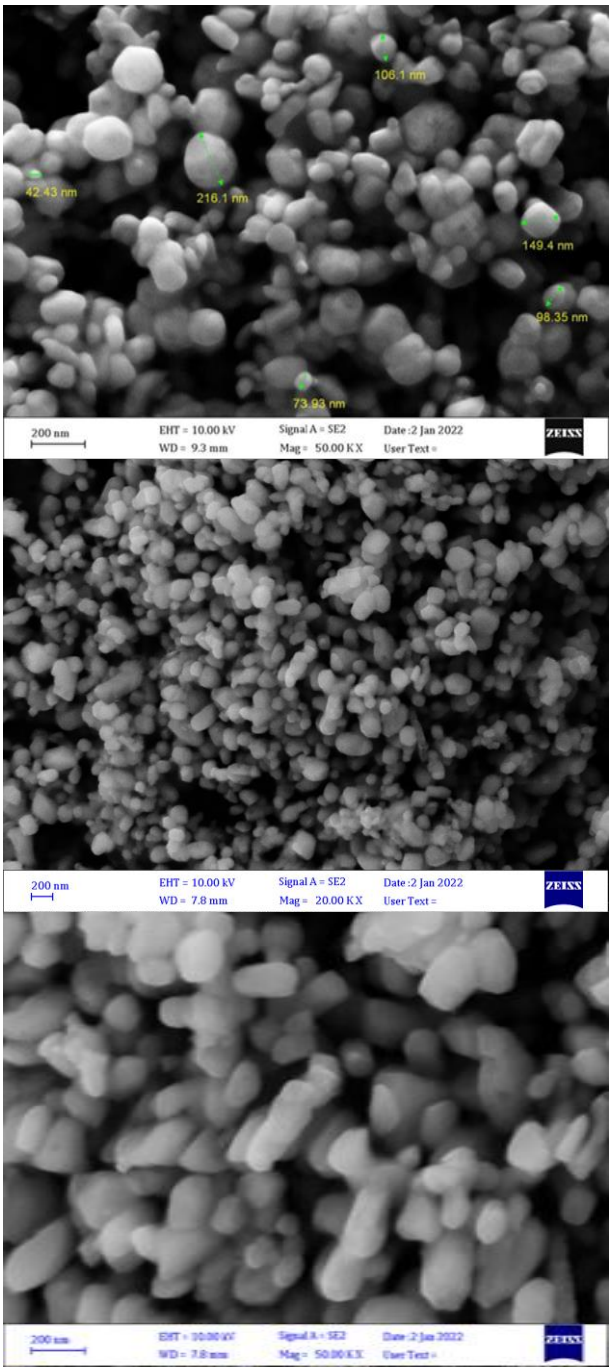

Figure 6. FE- SEM images of CuO nanoparticles.

Atomic Force Microscopy (AFM) analysis

In Table 9 and Figure 7, the Average diameters of CuO NPs biosynthesized by *L.camara* were 15.23 nm and the calculated sizes were ranged between (27.33 – 56.43)nm .These results demonstrated that the synthesized particles were ultrafine particles that has a diameter less than 100 nm proved that *L. camara* flower extract was efficient for synthesizing smaller NPs.

Table 9. Particle (grain) diameter size of CuO NPs.

Total particles number	100
Average diameter	15.23 nm
10% Diameter	7.21 nm
50% Diameter	15.17 nm
90% Diameter	23.2 nm

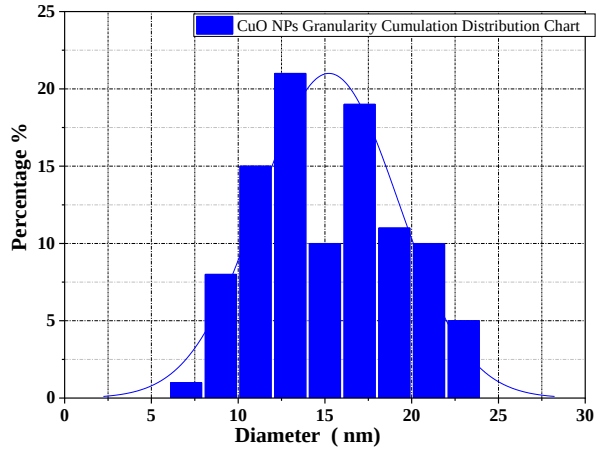

Figure 7. Particles (grain) size diameter distribution chart of CuO NPs.

Images of AFM 2D and 3D in Figure 8 also revealed that largest grain size was 6.47 nm, the histogram and roughness images also showed that the average roughness (RA) were 7.921 nm and the square root rate (RMS) 1.463 nm.

Antimicrobial activity of Copper Oxide nanoparticles

The antimicrobial activity of biosynthesized nanoparticles against pathogenic bacteria of the biosynthesized naonparticles were proved by testing them at the concentrations (12.5, 25, 50,100, 200) mg/ml The antimicrobial activity was evaluated by using agar well diffusion method against three kinds of pathogenic bacteria isolated from burns infections the bacterial isolates included *S.auerus, A.baumannii , P. aeruginosa* . as showed in Figure 9 and Table 10. The CuO NPs showed inhibitory effect and the widest inhibition zone was at 200 mg/ml of all the tested isolates (23 ,21, 21) mm respectively and the narrowest zone was at 12.5 mg/ml against the same isolates (15,14,14) mm respectively. These results corresponded to results reported by Taha *et al*. [51].

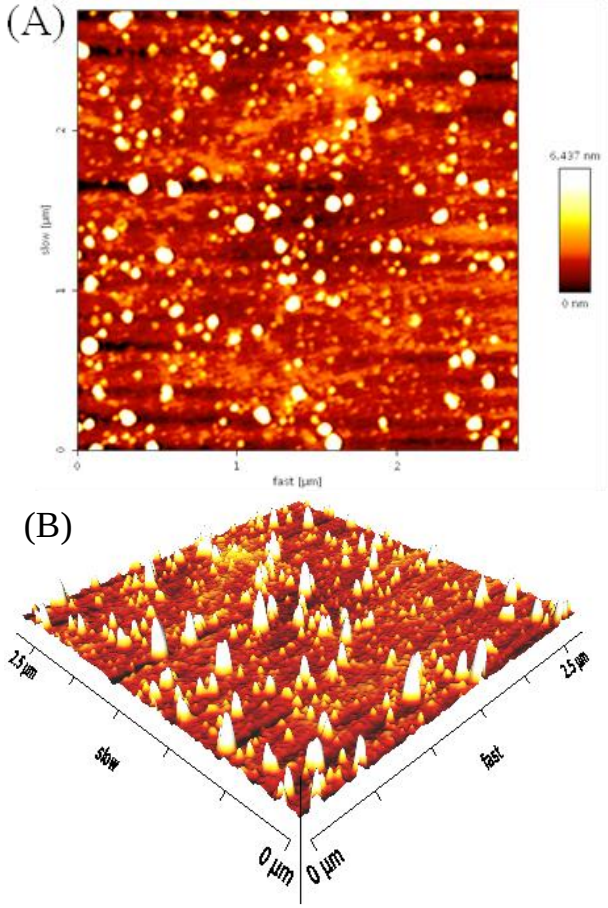

Figure 8. AFM images of CuO NPs, A) 2D, B) 3D.

Table 10. Antibacterial activity CuO NPs against bacterial isolates.

CuO NP conc.	Inhibition zone diameter (mm) Mean ± St. Error				
Bacteria	Conc. 12 mg/ml	Conc. 25 mg/ml	Conc. 50 mg/ml	Conc. 100 mg/ml	Conc. 200 mg/ml
S. aureus	15.00 ±0.00	16.00 ± 0.00	18.00 ±0.00	21.00 ±1.00	23.00 ±1.00
A.baumannii	14.00 ±2.00	15.00 ±1.00	18.00 ±2.00	20.00 ±2.00	21.00 ±3.00
P.aeruginosa	12.00 ±0.00	14.00 ±0.00	16.00 ±0.00	19.00 ±1.00	21.00 ±1.00

CuO NPs demonstrated a noticeable activity as inhibitory agent for bacterial growth of gram-positive *S. aureus* and gram-negative *A. baumannii* correspond to what was recorded by Hadler *et al*. [52].

A study was demonstrated successfully biosynthesized CuO NPs, showed a significant level of antibacterial activity against pathogenic *P. aeruginosa*.

The Minimum Inhibitory Concentration MIC of CuO nanoparticles was determined by microdilution method by using 96 wells sterile microplate the results were read by using an

automated absorbance (ELISA reader) at 630 nm to determine the MIC. A series of different concentrations from (2000,1000, 500, 250,125, 62.5, 31.25 and 15.63) µg/ml were performed. Table 11 the MIC of CuO NPs ranged from (125 to 1000) µg/ml.

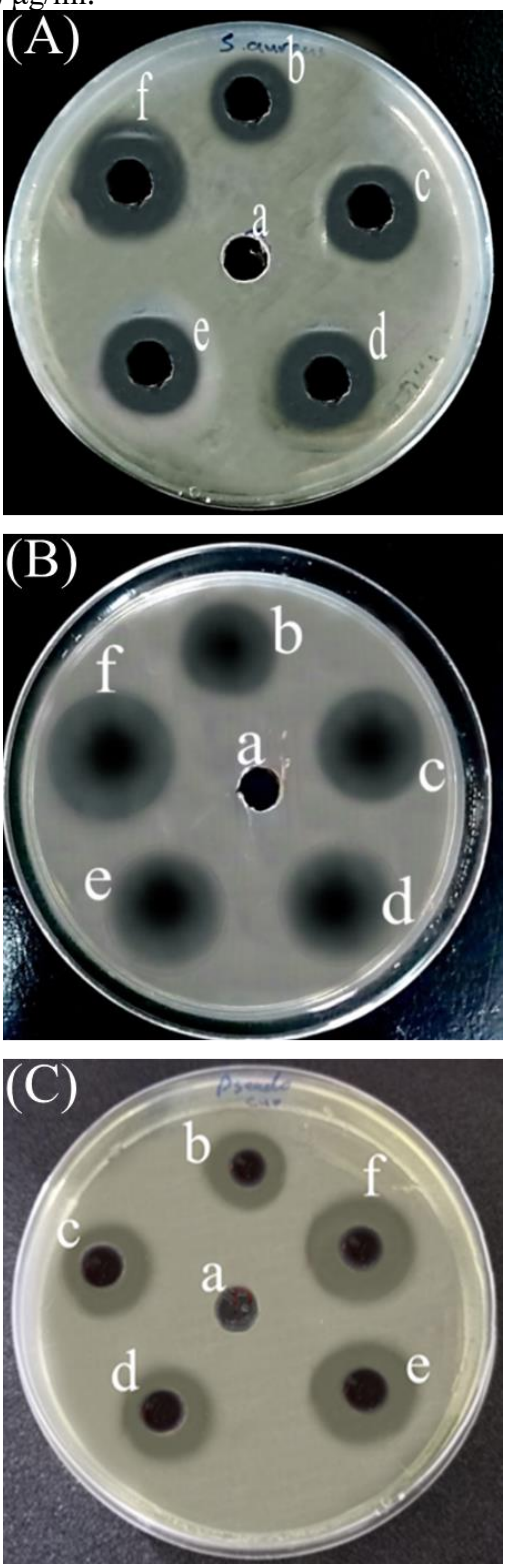

Figure 7. Antibacterial activity of CuO NPs, A) *S. aureus*, B) *A. baumannii*, C) *P. aeruginosa*. (a-0, b-12.5, c- 25, d-50, e- 100, f- 200) mg /ml.

Table 11. Minimum Inhibitory Concentration (MIC) of CuO NPs

Bacteria	MIC CuO NPs
S. aureus	125 µg/ml
A.baumannii	250 µg/ml
P.aeroginosa	1000 µg/ml

DISCUSSION

Bacterial Isolates Distribution

The results recorded that the highest rate of bacteria isolated from burns infections were belonged to *P. aeruginosa* followed by *A. baumannii* and *S. aureus*. The current results found that the numbers of Gram-negative bacteria isolated from burns were higher than that of Gram-positive bacteria, *S. aureus* and *S. epidermidis* were the most frequent gram-positive bacteria isolated from burn infections these results agreed with Nouri *et al.* [24]. The most repeated pathogenic bacteria isolated from burns was *P.aeruginosa* and *A. baumannii* can survive on dry surfaces and has the ability to tolerant nutrients limiting conditions , these pathogens are capable of colonization on medical instruments and hospital equipment 's which act as reservoirs. This pathogen can resist drought and remain alive for months. *Acinetobacter* spp. the most isolated pathogen found on inanimate objects and hands of medical workers comparing to *S. aureus* and *Pseudomonas* spp. [25].

Susceptibility of clinical bacterial isolated

S.aureus has many mechanisms of resistance that made them resistant to many groups of antibiotics such β-lactams ,Fluoroquinolones ,Folate pathways antagonist and Vancomycin [26]. These bacteria produce penicillinase which hydrolyze the penicillin β-lactam ring [27,28]. Tetracyclines resistances is mediated by ribosomal protection proteins and through the presence of efflux pumps. this pathogen is also resistant to trimethoprim-sulfamethazole which produces several enzymes that cause amino acid substitutions and made them resistant to the antibiotic combination. fluroquinolones resistance is either mediated through efflux pumps or through mutational amino acid substitutions in the fluoroquinolone binding site of topoisomerase IV and DNA gyrase [26]. the resistance of *S.aureus* to aminoglycosides is caused by a decrease in membrane permeability which

results in a decrease in drug intake [29]. Resistance of *S. aureus* to aminoglycosides is also mediated through enzymatic inactivation, specifically through enzymes that acetylate and phosphorylate aminoglycosides [30]. The lowest resistant percentage was toward Vancomycin 5%, this antibiotic has been considered the best drug for the treatment of severe *S.aureus* infections for long time [31]. Vancomycin resistance is mainly mediated by specific binding of the bacterial cell wall via peptidoglycan precursor small peptides, which are terminated with D-alanyl-D-alanine. this binding inhibits the elongation and cross-linking of bacterial cell wall peptidoglycans which cause repress to cell wall synthesis and leading to bacterial death [32, 33]. Resistance to aminoglycosides of *A.baumannii* is due the ability of this pathogen to produce almost all modifying enzymes act on aminoglycosides [34]. *A.baumannii* can develop a resistant mechanism against fluoroquinolones mediated through gene mutations in DNA gyrase and topoisomerase IV. In addition, this pathogen can reduce their affinity for fluoroquinolones, and through the producing qnr-type protection proteins which inhibit fluoroquinolones binding to topoisomerase IV and DNA gyrase. *A. baumannii* was known with the ability to produce efflux pumps and can decrease intracellular concentration of cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones [17] The results showed high resistant rate to Piperacillin, Ciprofloxacin, Imipenem. The results appeared that the percentage of bacterial resistant were moderate to Levofloxacin and Tobramycin. This pathogen can display resistance to many kinds of antibiotics groups including Aminoglycosides,quinolones and β-lactams [35].

Biosynthesis of CuO nanoparticles by *Lantana camara* flower extract

The biosynthesis process as showed in Figure 1. The reduction of copper nitrate into CuO NPs after adding the flower extracts was shown by change in colors of the salt solutions from light blue for CuO salts solutions to dark green solution which indicate the successful biosynthesis of the nanoparticles under optimized condition of pH and temperature. One of the most important advantages of nanoparticle biosynthesis is inexpensive, environmentally friendly, risk-free, simple to implement, and low in toxicity [36]. CuO NPS was obtained as afine black powder after drying in oven. The characteristics of NPs were studied using (AFM), (XRD), (UV-Vis), (FT-IR) and (FE-SEM) and the results of this study correspond to many studies which mentioned that plant extracts have a certain role in the biosynthesis process [37].

Characterization of copper oxide nanoparticles

UV-vis

Many studies reported that the extract types, pH, temperature and the synthesis method affect the surface plasmon resonance SPR of CuO NPs [39]. Researchers also reported that the high absorption peak resulted from the resonant oscillating of electrons at the conduction band induced by the incident electromagnetic radiation which is known as surface plasmon resonance[40] from the resulted high wave length there was need to estimate the band gap energy (Eg) of CuO NPs which estimated by Tauc's plot approach according to the equation $\alpha h\nu = A(h\nu - Eg)^{n/2}$[39] where (α) referred to the absorbance coefficient of nanoparticles, (*n*) known to be the electron transition nature between bands of valence and conduction such that n = 1 for the direct band gap, n = 4 for the indirect band gap[41], (A) is constant , (hν) defines as the incident photon energy .The construction of A plot of $(\alpha h\nu)^2$ versus hν and E_g and the extrapolation for the biosynthesized CuO NPs was estimated through extrapolation of the straight-line part to the x-axis. The E_g value of the CuO NPs was found to be 4.8 eV in this study. The feature of the plot indicated that it had a direct transition due to the linear absorption at the end of the plot as showed in Figure 2 [42]. The higher band gap energy of the CuO NPs than the reported value (1.9–2.1 eV) could be related to the quantum confinement effect that is the band gap increase with reduction in particle size [43]. These variations in surface plasmon resonance may be due to the spherical shape of CuO NPs [44,45].

FTIR

The FTIR of *Lantana camara* flower extract was done to compare with that of the prepared nanoparticles. Some intense bands were noted at various frequencies which specify the N–H, C=C, C–H, C=O, C-O and O-H skeletal vibrations, which designate the occurrence of various metabolites such as amines, phenolics, alkanes, aromatic ester, and aldehydes in the extract [47]. The strong peaks were due to C-O stretching

vibration in the carboxylic group and flavanones and the C-N stretching vibration peaks were due to the amine group. The strong aromatic C=C bending vibration and asymmetric and symmetric C-H stretching mode were due to the phenolic compounds, the broad O-H stretching vibration in alcohols. FTIR results confirmed the presence of proteins and phenolic compounds response that act as capping agent and the presence of these biologically active plant compounds might have been responsible for the reduction and capping of prepared NPs [48].

FESEM

The surface morphology was investigated by using the Field Emission-Scanning Electron Microscope analysis (FE-SEM), the images illustrate that some of the nanoparticles were well separated from each other, while most of them were in a lumpy form. This agglomeration is due to the electrostatic effects, this behavior is consistent with a similar behavior to nanoparticle agglomeration of another studies [49].

AFM

Surface morphology of copper oxide nanoparticles and the average grain size diameter were studied utilizing(AFM), This analysis gave clear results about the characterization of the biosynthesized NPs at the nanoscale, Nanoparticle size distributions, Direct visualization of the nanoparticles in 2D and 3D dimensions images and analysis of the size of nanoparticles as well as the size distributions are directly calculated from AFM images [50] the prepared nanoparticles antimicrobial activity is dependent on how Smaller the size of the nanoparticles which resulted in a higher surface area and that mean the NPs are more reactive, Smaller nanoparticles in size than cell membrane pores make them capable of crossing the cell wall and inhibiting the growth of bacteria [51].

ANTIBACTERIAL ACTIVITY

The ionic nature and surface charged of Cu and CuO NPs gave this metal and their oxides the ability to interact with different functional groups on the bacterial cell surfaces. CuO NPs can penetrate the bacterial membranes and this penetration is depend on the shape of the NPs. after penetration the nanoparticle will release ions within the cell, many mechanisms explain the action of CuO NPs as an antibacterial agent that include the deposition of NPs release of copper ions that found in nanostructure, the generation of reactive oxygen species on the surface of nanostructures or the direct interaction of particle surface with the biological target. The nanomaterial interacts through these mechanisms with bacterial cells and leads to Zones of inhibition around them [53]. The damages resulted from CuO NPs in the bacterial cell wall causes loss function of the cell wall which led to the inhibition of their growth and bacterial cell death [54].

The Minimum Inhibitory Concentration MIC

the antimicrobial lowest concentration that can inhibit the visible microorganism growth, after over- night incubation is called MIC. it is used to confirm resistance and consider as a tool to determine the activity of new antimicrobials *in vitro* and also to determine MICs break points [55]. The microbial sensitivity of metal oxide nanoparticles varies according to the microbial species and the concentrations of the metal oxides [15]. Our results recorded that *P. aeruginosa* were more resistant than the other isolates which inhibited at high concentrations of the biosynthesized CuO NPs, this can be explained as CuO NPs inhibitory action seem to be different on the tested bacteria depending on cellular walls that seem to impact the antimicrobial effect of tested NPs. High concertation of CuO NPs is required for for Gram negative bacteria, while for the grampositive species of *S. aureus* were killed at lower concentrations [56]. CuO nanoparticles cause inhibition to *P. aeruginosa* growth and *S. aureus* depending on the used dose and time [43]. the surface properties and size of nanoparticles is considered a significant factor as the smaller particles have large surface area a better antibacterial activity when compared with larger ones [57].

CONCLUSIONS

The possibility of using *Lantana camara* flower extracts in the biosynthesis of nanoparticles is a simple method, and economical cheap. The biosynthesized CuO nanoparticles showed antibacterial efficacy against bacterial strains isolated from human infections. The inhibitory effects of the nanoparticles showed variable activity toward pathogenic bacteria which depend on the bacterial type and concentration used. the potential of these nanoparticles can be used as a

new therapeutic drug synthesized by a very cost-effective, easily available plants and using this metal oxide in formulating many medicines such as anti-inflammatory or antibacterial cream**.**

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Author Contributions: All authors contributed equally in writing original draft preparation, all authors have read and agreed to the published version of the manuscript.

Informed Consent: All patients gave their written informed consents before inclusion.

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