

# Biosynthesis of Zinc Oxide nanoparticles by Lantana camara flowers extract characterization and their antibacterial potential against pathogenic bacteria isolated from wounds infections

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DOI: 10.47750/pnr.2022.13.S01.55

## Abstract

The recent study included biosynthesis of Zinc oxide nanoparticles by Lantana camara flower extract and investigate their biological potential toward pathogenic bacteria isolated from wounds infections. Characterization were made by U.V.-Visibl light Spectroskop,X-Ray Diffraction XRD ,Feild Emmission Scan Electron microscope FE-SEM , the results showed that the UV peaks were at 384.5 nm , XRD analysis estimated the crystallites sizes in 22.5 nm . FE-SEM analysis displayed average particles size were 45.15 nm . Clinical samples 100 were collected during October 2021 to the end of Febreuary 2022 from cared patients who have acute and chronic wounds infections, Isolation and identification by biochemical tests were done, susceptibilty test was investigated and antibacterial potential of ZnO nanoparticles was tested toward three clinical strains of *S. aureus* , *K. oxytoca* and *Proteus vulgaris*. that found to be resistance to at least three types of antibiotics in differernt antibiotics groups. The inhibition zones were observed at the concentration 200 mg/ml (25,22.5,21) mm. Sensitivity of the bacterial isolates to ZnO NPs was diagnosed by determination of Minimum inhibitory concentration by using microdilution method.

**Keywords:** Antibacterial, Antibiotics resistance, Biosynthesis, Burns infections, Lantana camara, , Wounds infections.

## INTRODUCTION

The skin form the first line defense that has many functions such as providing mechanic strength, regulation loss of water and salt , and provide protection to the body from different damages such as bacterial infections (Mihia et al.,2018) . Skin carry normal microflora that have their specific adaptation conditions to live on skin and their existence is determined by many factors that include age satge, nutrition state, geneder , echological and geographical factors (Byred et al.,2018). skin damage caused by many reasons for example trauma caused by cuts with sharp edges, abrasions , burns, surgery and diabetis ulcers (Mihia et al., 2018). Saprophytes microflora can cause contamination to wounds which caused wound infections and the most common bacterial species that caused infections of wounds include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Acinetobacter baumannii* , *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter spp.*, *Peptostreptococcus spp.* ( Halstead et al., 2015). Bacterial wounds infections result from either by direct contact with the pathogen through contaminated surgical equipment during surgery or airborne dispersal from a contaminated environment. Different microbial population are present at the beginning of a wound infection when pH is neutral after that as wounds become chronic pH increased and become alkaline wivch encourage anaerobic bacteria to be present (Hoque et al.,2017) . One of the most challenges that face the physcians and health care providers in treatment and management wounds infection is bacterial resistance to antibiotics which considered a health care challenge as pathogenic bacteria continuously develop resistance mechanisms to antibiotics which make the treatment of infections caused by them difficult to be treated (Stracy et al.,2022). As a result, there is an urgent need to find an alternative therapies such as probiotics, medicinal plants and their extracts and in the modern time the field of Nanotechnology which is one of the most active research field in modern material science. Nanoparticles exhibit new and improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology is also being utilized in medicine for diagnosis, therapeutic drug delivery and the development of treatments for many diseases and disorders (Sarvanthi et al., 2016) . Nanotechnology is produced materials

of various types at nanoscale level Nanoparticles (NPs) include particulate substances, which have one dimension less than 100 nm (Khan et al.,2019). Nanoparticle synthesis using microorganisms and plants by green synthesis technology is biologically safe, cost-effective and ecofriendly where the biological entities or their extracts are used for the green synthesis of metallic nanoparticles as they act as a reducing factors for the metallic oxides salts resulted in synthesis of nanoparticles ( Zhang et al.,2020).

ZnO nanoparticles have excellent applications including optical proertiy and electrical conductivity, UV-filtering, photochemical, photocatalytic, antiviral, antibacterial, antifungal and anticancer activities (Qi et al.,2017). This metal oxide is listed in the generally recognized as safe materials by the U.S. Food and Drug Administration, is biocompatible and non-toxic for human beings and animals ( Zhong et al .,2018).

## Materials and methods

### Collection of clinical samples

Clinical samples of 200 Surface swabs were collected from hospitalized patients in Baquba Teaching Hospital , Diyala province, Iraq during October 2021 to the end of february 2022. The surface swabs were taken from wound infections of patients from both genders of different ages.

### Prelaminry identification of bacterial samples

Surface swabs were subcultrued in Blood agar, Macckonkey agar and Mannitol salts agar ,and the prilaminry identification were done microscopically following by the biochemical tests (Procop et al .,2020). API 20E Diagnostic System depends on 20 tests and according to the manufacturer's instructions (Biomerieux) this system was used to identify the type of bacteria (genus and species) according to the system index. Confirmation of bacterial isolates identification was done by VITEK-2 system (Crowley et al.,2012).

### Antibiotics susceptibility test

Susceptibility test of the pathogenic isolates were tested according to the CLSI (2021) criteria using Kirby-Bauer method by transferring few bacterial coloneis to 2 ml of normal saline to make asuspension adjusted to McFarland turbidity (1.5×108) CFU/ ml .and then streaked on Muller-Hinton agar plates by using a sterile cotton swab. Different antimicrobial discs as showed in table (1) were used with a maximum six discs placed on the surface of inoculatd media after that the plates were wrapped with parafilm and incubated for 24 h at 37°C. the inhibition zone of each antibiotic disc was measured .

**Table (1)** antibiotics used in the study

Antibiotics	Concentration µg/disc	Antibiotics	Concentration µg/disc
Penicillin	10	Gentamicin	30
Pipracillin	100	Impenem	10
Ampicillin	10	Ciprofloxacin	5
Cefoxitin	30	Cefotaxime	30
Ceftazidime	30	Vancomycin	10
Amikacin	30	Azithromycin	15
Aztreonam	30	Trimethoprim- sulfamethoxazole	1.25/ 23.75
Doxycycline	30		

### Biosynthesis of ZnO nanoparticles by Lantana camara flower extract

#### Lantana camara flowers collection sources

Lantana camara flower were picked up from public gardens in Baquba city , Diyala province , Iraq during October 2021 washed carefully with distilled water to remove any dust or dirt and other contaminant, Cleaned flowers dried in shade at room temperature before grinding them by an electric grinder to get a fine powder which then were kept in sterile glass container till further analysis ( Thorat et al., 2021).

#### Preparation of nanoparticles

Zinc nitrate 2g are dissolved in 150 ml of deionized distilled water under magnetic stirrer for 15 min at 80 °C, then 50 ml of L.camara flower extract was added to the solution under stirrer for 2 h. After heterogeneity, 20 ml (3M) of sodium hydroxide (NaOH) was drop wise added to the above 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 °C for 3 h. the powder subjected to heat-treatment at 600 °C for 4 h to get a fine white powder. ( Chowdhury et al., 2020).

#### Optimal Condition for nanoparticle Synthesis

The concentration of Lantana camara flower extract was considered and subsequently added to a 5 ml solution of zinc nitrate which were previously dissolved using 50 ml of deionized water, the concentrations of each solution were optimized with pH 9.0 was taken into account, pH was controlled via the utilization of 0.1 N of NaOH. ( Chowdhury et al., 2020).

#### Characterization of ZnO nanoparticles

The UV-Vis spectrum analysis was achieved within the range of 200 to 1200 nm at a resolution of 0.86 nm using a UV-visible spectrophotometer ( UV-9001 Shimadzu ). The XRD analysis technique was used in this study to evaluate the physical properties, composition, crystal structure, and phase recognition of the biosynthesized ZnO NPs, FE-SEM technique was employed to investigate the morphological features of the prepared samples (A kintelu et al.2020).

#### The antibacterial potential of the biosynthesized ZnO nanoparticles

##### Preparation of bacterial inoculum

Bacterial suspension was prepared by taking a number of bacterial colonies by loop to tubes containing heart and brain infusion broth the tubes were incubated for 18 h at 37 °C, then the bacterial suspension was compared with McFarland standard solution, the degree of turbidity of the bacterial suspension is close to  $1.5 \times 10^8$  (CFU/ml).

##### Preparation of Zinc oxide nanoparticles stock solutions

Stock solution of ZnO NPs was prepared by dissolving 2000 mg in 10 ml of deionized distilled water in an ultrasonic bath for 30 min for powder dissolving completely. the initial stock solution was obtained with final concentration 200 mg/ml and was used to prepare four different concentrations (12.5,25,50,100) mg/ml that were used in the experiment.

##### Determination the antibacterial activity of Zinc Oxide nanoparticles

Antibacterial activity was analyzed by following the agar well diffusion method according to Dadi et al. (2019). Antibacterial activity was tested against three multiple drugs resistance (MDR) bacteria include *P.vulgaris*, *K. oxytoca*, *S.aureus*, which were isolated from wounds infections. Tested bacterium was cultured on Muller- Hinton agar by streaking method and the five wells 5 mm were made in the plate by sterilized cork borer. 200 µl of zinc oxide nanoparticles solution were added to wells using micropipette, the sixth well was taken as control add 200 µl of deionized water. Three repeats made for each bacterium and then incubated at 37 °C for 24 h.

##### Determination of Minimum Inhibition Concentration (MIC) of nanoparticles

The inhibitory effect at the minimum concentration was tested according to Duffy et al.(2018) with minor modification. The broth microdilution method using Mueller Hinton broth (MHB) in 96 well microplates. Stock solution of nanoparticles was prepared by powder of 20 mg dissolved in 10 ml of deionized water to get an initial stock solution of 2000 µg/ml. Eight concentrations of the selected NPs were prepared by 2-fold dilution in MHB. ( 2000,1000, 500, 250, 125, 62.5, 31.25 and 15.63) µg/ml, the addition to each 96 well plate was always in the order of broth, NP and then the tested bacteria. Row A was a negative control containing 100µl MHB and 100µl of the relevant NP.NPs were added to the control to allow for any effect of light scattering due to the NPs. Rows B to G contained 100µl of NP, 50µl of MHB and 50µl of ( $1.5 \times 10^7$ ) CFU/ml bacterial suspension. Row H was the positive control, containing 150µl of MHB and 50µl of the bacterial suspension. The MIC of each isolate was determined in duplicates on two plates. the results were read to an optical density OD 630 nm.

##### Statistical Analysis

Statistical analysis was done by using Social of Package Social Sciences SPSS version 24 ordinal variables were described in the form of number and percentage. As for the variables, they were described in the form of ( Mean± St.error) .the nanoparticles data and images were analysed by Image j programme and figures were replotted by Origin pro 2018.

## Results and discussion

### Collection, isolation, and Identification of pathogenic bacteria

The recent study included the collection of 100 surface swabs from patients with acute and chronic infection from

both sexes and different ages. All the swabs were subcultured in selective and differential media and after incubation the resulted growth and microscopical identification found that 93(93 %) showed positive bacterial growth divided into 46 (49.5%) of gram positive bacteria and 47 (50.5%) were gram negative bacteria and 7(7%) did not grow in culture media . confirmation of the clinical isolates was done by following the Biochemical tests as showed in table ( 2) and APIE20 was used to identify and differentiate members of Enterobacteriaceae family, The results showed complete agreement with the results of traditional biochemical assays. the results showed that the gram negative bacteria were 10(10.7%) of klebsiella pneumoniae , 8(8.7%) klebsiella oxytoca, 9(9.6%) proteus mirabilis , 9 (9.6%) proteus vulgaris and 11 (11.9%) Eschericia coli and for gram positive bacteria 34 (36.6%) belong to Staphy;ococuss aureus and 12 (12.9%) Staphylococuss epidermidis . these result matched with many studies recroded by reseachesrs found that the mentioned bacteria species were the most common and the most frequent pathogenic bacteria which colonise wounds infection and cause commplications that were hard to manage and difficult to treat( Shama et al .,2018

**Table (2)** Distribution of Numbers of bacterial species and their percentages

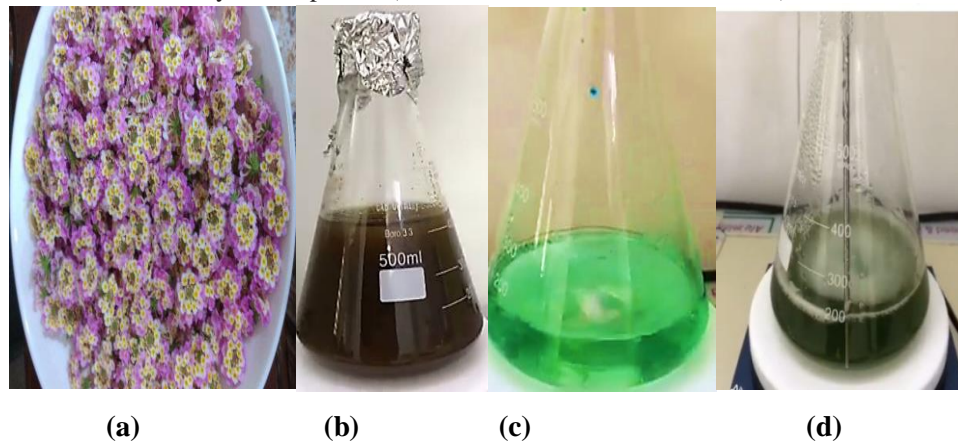
Bacterial isolates	No. of isolates	Pecentage (%)
S.aureus	34	36.6%
S. epidermidis	12	12.9%
K. oxytoca	8	8.7%
K. pneumonieae	10	10.7%
P. miribilis	9	9.6%
P. vulgaris	9	9.6%
E.coli	11	11.9%

#### Suseptibility patterns of the bacterial isolates

The resistance patterns against antibiotics was tested to three bacterial isolates which were the most frequent isolated from wound infections include Staphylococuss aureus , Proteus spp. and Klebsiella spp. The susceptibility test were diagnosed to 15 antibiotics which were Penicillin ,Piperacillin ,Ampicillin,Ceftazidime ,Cefotaxime, Gentamicin ,Trimethobrim sulfamethazole, Doxycycline ,Amikacin ,Impenenim, Ciprofloxacin, Vancomycin, Azithromycin , Aztreonam . It was noticed that the tested bacteria in the recent study exhibited an excellent performance in resistance to antibiotics, procedures, recent use of broad-spectrum antibiotics, exposure of patients to self-prescribed antibiotic because of the availability of these medications without prescription, and the horizontal transfer of antibiotic resistance genes by transport means. From each one of these bacterial isolates, one of MDR isolate, were selected to complete the current study.

#### Biosynthesis of Zinc Oxide nanoparticles by Lantana camara flower extract

The biosynthesis of ZnO NPs by Lantana camara flower extract as a reducing agent in the biosynthesis process as zinc nitrate were reduced to ZnO NPs after adding the flower extracts the success of the reaction can be observed in change in colors of the salt solutions from light green of ZnO salts solution to dark green solution that indicate the successful biosynthesis under optimized condition of pH and temperature as showed in figure (1) (Swamy et al.,2020 ) ZnO NPs were obtained as fine white powder after drying in oven , The results of this study correspond with many studies which mentioned that plant extracts have a certain role in the biosynthesis process( Mohammadi-Aloucheh et al., 2018).



**Figure (1)** Biosynthesis of zinc oxide nanoparticles by L. camara flower extract

a- Lantana camara Flowers

- b- Flowers extract
- c- Zinc nitrate solution (precursor)
- d- Color change after adding flower extract indicating successful synthesis of ZnO NPs

Characterization of zinc oxide nanoparticles

Ultraviolet – Visible light spectroscopy UV-VIS

The stability of the biosynthesized nanoparticles was diagnosed by using UV-VIS spectrophotometer, figure (2) showed the maximum absorbance peak of ZnO NPs that was observed at 384.5 nm which is similar to results reported by Swamy et al. (2021) under UV region indicates the formation of ZnO NPs.

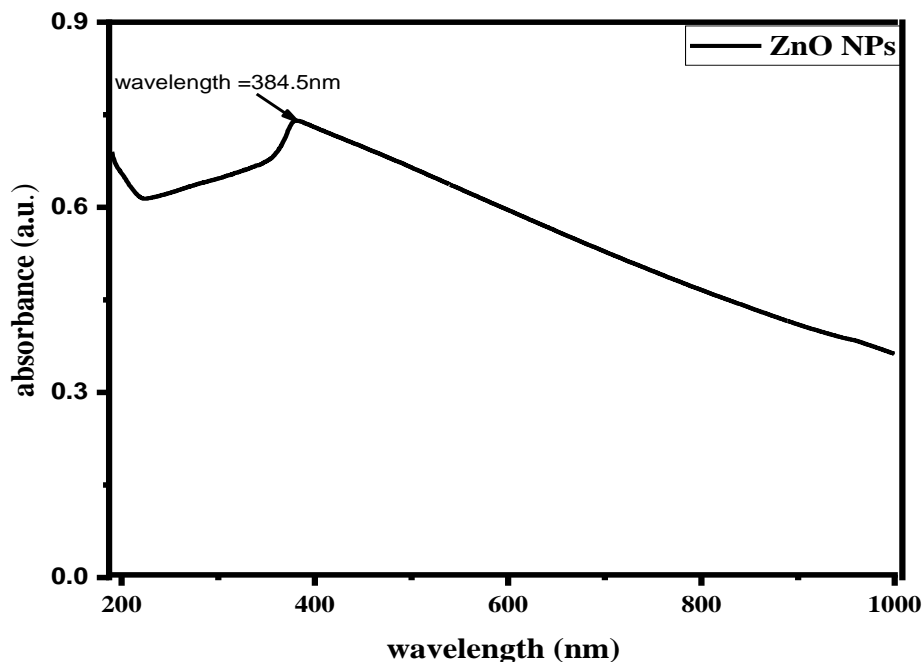
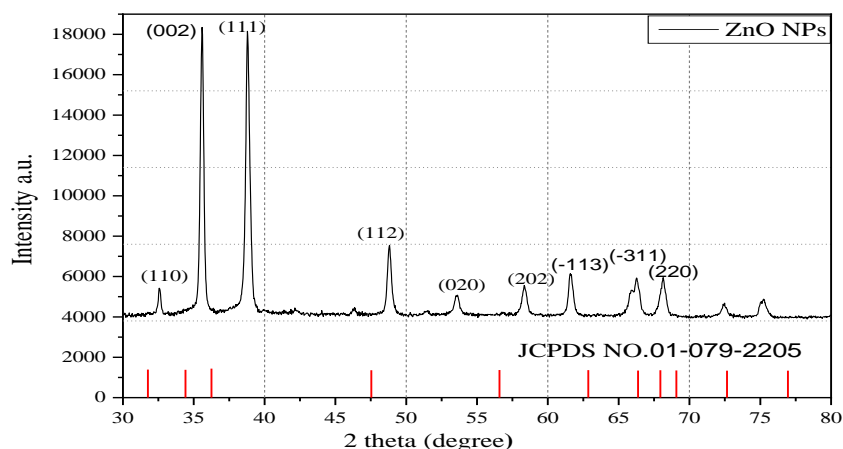


Figure (2) UV-VIS of the biosynthesized ZnO nanoparticle

X-Ray Diffraction (XRD) analysis

X-Ray diffractometer (XRD) of the biosynthesized ZnONPs using L.camara flower extract as a reducing agent is explained in Figure (3) and table (6). It could be perceived that the obtained peaks at  $2\theta = 32.5^\circ, 35.5^\circ, 38.7^\circ, 51.4^\circ,$  and  $68.1^\circ$  matched with the crystal structure of ZnO NPs, The mentioned peaks are matched with the diffraction data standard of ZnO NPs (JCPDS file no: 01-079220). those peaks correspond to the basal planes of (110), (002), (111), (112), and (220) which confirms the hexagonal phase without any impurities of biosynthesized ZnO NPs (Jayappa and Ramaiah Kumar,2020). the crystallite size of ZnO NPs was estimated according to the Scherrer equation and the measurements of the strongest three peaks were used to estimate the crystallite size of ZnO NPs in 22.52 nm (average) which confirm the nanostructure forms, The result of this study agreed with Swamy et al. (2020) who estimated crystallite size in 25 nm of ZnO NPs prepared by the same plant used in this study.



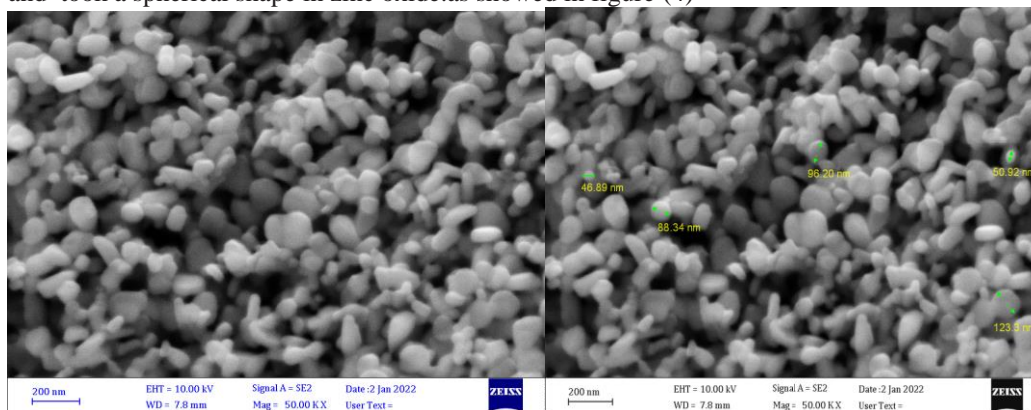
**Figure (3)** XRD of ZnO NPs

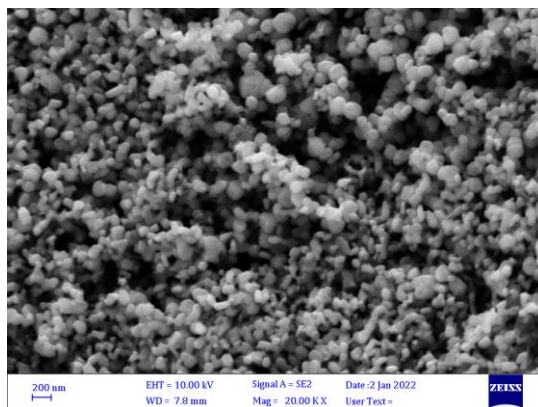
**Table (6)** The crystallite size of ZnO NPS

2 $\theta$	FWHML	K	$\Lambda$	Crystallite Size ZnONPs (nm)	Average crystallite size (nm)
35.58	0.29566	0.94	0.54016	27.75	22.52 nm
38.81	0.36821			22.49	
51.45	0.50000			17.34	

#### 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, FE-SEM images of ZnO NPs sample where the results showed the particle size were as following: ( 33.23, 35.49, 36.33, 37.35, 38.61, 40.44, 41.41, 42.52, 43.42, 44.36, 45.59, 47.47, 48.89, 49.59, 50.53, 51.62, 52.62, 53.62, 54.63 and 55.67 )nm, The average of the particles size was 45.15 nm. The surface morphology of biosynthesized nanoparticle shows that these nanoparticles which were under different magnification forces were irregular and took a spherical shape in zinc oxide, as showed in figure (4)





**Figure (3)** FE- SEM images of ZnO NPs.

#### Antibacterial activity of Zinc Oxide nanoparticles

The antibacterial activity of the biosynthesized nanoparticles have been proved with different concentrations (12.5, 25, 50, 100, 200) mg/ml. The results of the antibacterial activity of ZnONPs was dependent on the utilized concentrations, table (7). The narrowest zones of inhibition for the isolates *Proteus* spp., *Klebsiella* spp., *S. aureus* (12, 13, 18) mm respectively was recorded at the low concentration 12.5 mg/ml of ZnO NPs while the widest inhibition zone was recorded at the concentration 200 mg/ml for *S. aureus*, *Klebsiella* spp., *Proteus* spp. was (25, 22.5, 21) mm. Understanding the exact antibacterial mechanisms of ZnO NPs is crucial to elucidate da Silva et al. (2019) proposed that the small particle size and the high surface area can enhance antimicrobial activity of ZnO NPs. These nanoparticles have many mechanisms to explain their activity that makes the interpretation of the main mechanism responsible for antimicrobial activity more difficult. ZnO aqueous suspensions allow for chemical interactions between hydrogen peroxide and the proteins of the cell membrane. These proposed mechanisms are either by the production of reactive oxygen species (ROS), the loss of cellular integrity following contact between ZnO NPs and the cell wall, the release of Zn<sup>2+</sup> ions or by ZnO NPs internalization (da Silva et al., 2019).

**Table (7)** The antibacterial activity of ZnO NPs against bacterial growth

Tested material ZnO-NPs  Bacteria	Inhibition zone diameter (mm)				
	Conc. 12.5 mg/ml	Conc. 25 mg/ml	Conc. 50 mg/ml	Conc. 100 mg/ml	Conc. 200 mg/ml
<i>S. aureus</i>	18.00 ± 4.00	20.00 ± 4.00	21.00 ± 5.00	23.00 ± 5.00	25.00 ± 5.00
<i>Klebsiella</i> spp.	12.00 ± 0.00	14.00 ± 0.00	16.00 ± 0.00	18.00 ± 0.00	22.50 ± 0.70
<i>Proteus</i> spp.	13.00 ± 1.00	15.00 ± 1.00	17.00 ± 1.00	19.5 ± 1.50	21.00 ± 1.40

\* Mean ± St. Error

#### The Minimum Inhibitory Concentration of the biosynthesized ZnO nanoparticles

ZnO nanoparticles minimum inhibitory concentrations were determined by microdilution method. A series of different concentrations from (2000, 1000, 500, 250, 125, 62.5, 31.25 and 15.63) µg/ml were performed. In table (8) ZnO NPs from (125 to 500) µg/ml where *S. aureus* were more sensitive to ZnO NPs with MIC at the concentration (125) µg/ml where as *Klebsiella oxytoca* and *Proteus vulgaris* sensitivity were at the MIC concentration (250, 500) µg/ml respectively. The microbial sensitivity of metal oxide nanoparticles varies according to the microbial species and the concentrations of the metal oxides (Dadi et al., 2019).

**Table (8)** Minimum Inhibitory Concentration (MIC) of ZnO NPs

Bacteria	MIC of ZnONPs
<i>S. aureus</i>	125 µg/ml
<i>K. oxytoca</i>	250 µg/ml
<i>P. vulgaris</i>	500 µg/ml

## Conclusions

The results of this study confirmed that Lantana camara flower extracts can be used in the biosynthesis of nanoparticles successfully in a simple method, ecofriendly and economical cheap and These biosynthesized ZnO nanoparticles showed antibacterial efficacy against bacterial strains isolated from human infections classified as MDR that highlight the potential of these nanoparticles can be working as a new therapeutic drugs synthesized by a very cost-effective and easily available plants and using these metal oxide in formulating many medicines such as anti-inflammatory or antibacterial drugs .

## REFERENCES

1. Mihai, M. M., Preda, M., Lungu, I., Gestal, M. C., Popa, M. I., & Holban, A. M. (2018). Nanocoatings for chronic wound repair—modulation of microbial colonization and biofilm formation. *International journal of molecular sciences*, 19(4), 1179.
2. Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, 16(3), 143-155.
3. Halstead, F. D., Rauf, M., Moiemem, N. S., Bamford, A., Wearn, C. M., Fraise, A. P., ... & Webber, M. A. (2015). The antibacterial activity of acetic acid against biofilm-producing pathogens of relevance to burns patients. *PloS one*, 10(9), e0136190.
4. Hoque, J., Prakash, R. G., Paramanandham, K., Shome, B. R., & Haldar, J. (2017). Biocompatible injectable hydrogel with potent wound healing and antibacterial properties. *Molecular pharmaceutics*, 14(4), 1218-1230.
5. Stracy, M., Snitsler, O., Yelin, I., Amer, Y., Parizade, M., Katz, R., ... & Kishony, R. (2022). Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. *Science*, 375(6583), 889-894
6. Sravanthi, M., Muni Kumar, D., Usha, B., Ravichandra, M., Mahendra Rao, M., Hemalatha, K. P. J., & Ravichandra, M. (2016). Biological synthesis and characterization of copper oxide nanoparticles using *Antigonon leptopus* leaf extract and their antibacterial activity. *Int. J. Adv. Res.*, 4, 589-602.
7. Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry*, 12(7), 908-931.
8. Zhang D, Ma X-l, Gu Y, Huang H and Zhang G-w (2020) Green Synthesis of Metallic Nanoparticles and Their Potential Applications to Treat Cancer. *Front. Chem.* 8:799
9. Qi, K., Cheng, B., Yu, J., & Ho, W. (2017). Review on the improvement of the photocatalytic and antibacterial activities of ZnO. *Journal of Alloys and Compounds*, 727, 792-820.
10. Zhong, Q., Tian, J., Liu, T., Guo, Z., Ding, S., & Li, H. (2018). Preparation and antibacterial properties of carboxymethyl chitosan/ZnO nanocomposite microspheres with enhanced biocompatibility. *Materials Letters*, 212
11. Procop, G. W., Church, D. L., Hall, G. S., & Janda, W. M. (2020). *Koneman's color atlas and textbook of diagnostic microbiology*. Jones & Bartlett Publishers.)
12. Crowley, E., Bird, P., Fisher, K., Goetz, K., Boyle, M., Benzinger, Jr, M. J., ... & Johnson, R. L. (2012). Evaluation of the VITEK 2 gram positive (GP) microbial identification test card: collaborative study. *Journal of AOAC International*, 95(5), 1425-1432
13. Thorat, V. H., Tamboli, F. A., Jadhav, A. S., & Chavan, R. (2021). Phytochemical analysis and antimicrobial activity of *Lantana camara*. *Int J Pharm Chem Anal* 2021;8(4):171-173
14. Chowdhury, R., Khan, A., & Rashid, M. H. (2020). Green synthesis of CuO nanoparticles using *Lantana camara* flower extract and their potential catalytic activity towards the aza-Michael reaction. *RSC Advances*, 10(24), 14374-14385
15. Akintelu, S. A., Folorunso, A. S., Folorunso, F. A., & Oyebamiji, A. K. (2020). Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. *Heliyon*, 6(7), e04508
16. Dadi, R., Azouani, R., Traore, M., Mielcarek, C., & Kanaev, A. (2019). Antibacterial activity of ZnO and CuO nanoparticles against gram positive and gram negative strains. *Materials Science and Engineering: C*, 104, 109968.
17. Duffy, L. L., Osmond-McLeod, M. J., Judy, J., & King, T. (2018). Investigation into the antibacterial activity of silver, zinc oxide and copper oxide nanoparticles against poultry-relevant isolates of *Salmonella* and *Campylobacter*. *Food control*, 92, 293-300
18. Shama, M., Murugesan, K., & Vijayan, H. (2018). Isolation Identification and Antibiotic Sensitivity pattern of Pyogens from Pyogenic pathogens. *Biomedical & Pharmacology Journal*, 11(1), 463.
19. Luis, M., Pezzlo, M. T., Bittencourt, C. E., & Peterson, E. M. (2020). *Color atlas of medical bacteriology*. John Wiley & Sons.
20. Swamy M, M., & BS, S. (2021). Bio-mediated synthesis of ZnO nanoparticles using *Lantana Camara* flower extract: Its characterizations, photocatalytic, electrochemical and anti-inflammatory applications.
21. Mohammadi-Aloucheh, R., Habibi-Yangjeh, A., Bayrami, A., Latifi-Navid, S., & Asadi, A. (2018). Enhanced anti-bacterial activities of ZnO nanoparticles and ZnO/CuO nanocomposites synthesized using *Vaccinium arctostaphylos* L. fruit extract. *Artificial cells, nanomedicine, and biotechnology*, 46(sup1), 1200-1209
22. Jayappa, M. D., Ramaiah, C. K., Kumar, M. A. P., Suresh, D., Prabhu, A., Devasya, R. P., & Sheikh, S. (2020). Green synthesis of zinc oxide nanoparticles from the leaf, stem and in vitro grown callus of *Mussaenda frondosa* L.: characterization and their applications. *Applied nanoscience*, 10(8), 3057-3074.
23. da Silva, B. L., Abuçafy, M. P., Manaia, E. B., Junior, J. A. O., Chiari-Andréo, B. G., Pietro, R. C. R., & Chiavacci, L. A. (2019). Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. *International journal of nanomedicine*, 14, 9395
24. Yogesh Hole et al 2019 *J. Phys.: Conf. Ser.* 1362 012121