



## **Inhibitory Effect of the Biosynthesized ZnO Nanoparticles by *Musa acuminata* Fruits Peels against Pathogenic Bacteria Isolated from Diabetic Foot Ulcer**

Ussama Asaed Fazel\* and Abbas Yaseen Hasan

Department of Biology, College of Science, University of Diyala

\*[scibioms2103@uodiyala.edu.iq](mailto:scibioms2103@uodiyala.edu.iq)

Received: 28 February 2023

Accepted: 10 April 2023

DOI: <https://doi.org/10.24237/ASJ.02.02.738B>

### **Abstract**

Zinc Oxide nanoparticles was synthesized by green method with zinc nitrate as precursor and alcoholic extract of *Musa acuminata* fruits peels as a reducing agent, color changing from white to yellowish -white, which was an indication of the formation of ZnO NPs. Characterization were made by UV-Visible light spectroscopy (Uv-Vis), X-Ray Diffraction (XRD), Atomic force microscopy (AFM) and Field Emission Scan Electron Microscope (FE-SEM), the results showed the sharp UV peaks were at 374 nm, XRD analysis estimated the crystallites sizes in 25.9 nm, AFM was used to determine the average size and forme of the nanoparticles which was 40, 45, and 50 nm, FE-SEM analysis displayed average particles size were 75.6 nm. Clinical of 125 samples were collected from diabetic foot ulcer from patients admitted to Baquba Teaching Hospital during beginning of November 2021 to the end of January 2022, included isolation and diagnosis of 76 isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antibacterial activity test was done of ZnO NPs. which was tested toward these isolates. Different concentrations of ZnO NPs. (12.5, 25, 50, 100, 200) mg/ml were examined against pathogenic bacteria, the results showed the highest diameter of inhibition zone against *E.coli*, *K.pneumoniae*, *P.aeruginosa*, *S.aureus* and *S.epidermidis*



were (28, 27, 31, 29, 26) mm respectively at concentration 200 mg/ ml and the lowest zone at concentration 12.5 mg/ml to the same isolates were (22, 23, 24, 24, 21) mm respectively.

**Key words:** ZnO NPs, *Musa acuminata*, Pathogenic bacteria, diabetic foot ulcer, Antibacterial activity.

## التأثير التثبيطي للمادة النانوية ZnO المخلفة حيويًا من قشرة ثمار *Musa acuminata* ضد

### البكتريا المعزولة من قرحة القدم السكري

اسامة اسعد فاضل و عباس ياسين حسن

قسم علوم الحياة - كلية العلوم - جامعة ديالى

### الخلاصة

خلقت الجسيمات النانوية من أكسيد الزنك كعامل مختزل بالطريقة الخضراء للمستخلص الكحولي لقشور ثمار الموز *Musa acuminata*، ويعد تغير اللون من الابيض الى الابيض المصفر مؤشر على تكوين ZnO NPs. تم التوصيف بواسطة التحليل الطيفي للضوء فوق البنفسجي المرئي (Uv-Vis)، حيود الأشعة السينية (XRD)، مجهر القوة الذرية (AFM) والمجهر الإلكتروني الماسح (FE-SEM) Emmission Scan Electron Microscope

أظهرت النتائج أن قمم الأشعة فوق البنفسجية الحادة كانت عند 374 نانومتر، وحجوم البلورات في تحليل (XRD) هي 25.9 نانوميتر، وبلغ متوسط حجم الجسيمات النانوية 40,45,50 نانوميتر و 75.6 نانوميتر باستخدام AFM و FE-SEM على التوالي. جمعت 125 عينة سريرية من قرحة القدم السكرية لمرضى أدخلوا إلى مستشفى بعقوبة التعليمي خلال بداية تشرين الثاني 2021 الى نهاية كانون الثاني 2022، عزلت وشخصت منها 76 عزلة من بكتيريا *Escherichia coli*، *Staphylococcus epidermidis*، *S. aureus*، *Pseudomonas aeruginosa*، *Klebsiella pneumoniae* اجري الكشف عن فاعلية ZnO NPs تجاه العزلات البكتيرية المرضية وباستخدام التراكيز التالية من ZnO NPs (12.5، 25، 50، 100، 200) ملغم /مل، وأظهرت النتائج أعلى فطر لمنطقة التثبيط (26,29,31,27,28) واقل منطقة تثبيط (21,24,24,23,22) ملم ضد بكتيريا *E.coli*، *K.pneumoniae*، *P.aeruginosa*، *S. aureus*، و *S. epidermidis* على التوالي.

**الكلمات المفتاحية:** ZnO NPs، ثمار *Musa acuminata*، بكتريا المسببة للأمراض، قرحة القدم السكري، الفعالية المضادة للبكتريا.



## Introduction

A diabetic foot, which is characterized by foot ulcers connected to neuropathy and/or peripheral artery disease in the lower limb in a diabetic patient, is one of the most significant and crippling symptoms of diabetes [1]. Older persons are more likely to have the condition, which affects the diabetic population 4- 10% of the time [2]. Age and the length of diabetes both raise the risk of foot ulcers and limb amputation [3].

Finding the inhibitors of antibiotic resistance as a first step to creating a combination medication has grown in popularity as a result of the rising demand for an effective treatment against infectious diseases that are multidrug resistant. The use of nanotechnology, a method that makes it possible to introduce materials with nanoscale structures, was done to increase the therapeutic activity of the medicine and reduce its unpleasant side effects[4].

The use of medicinal plants, also known as medicinal herbs, in traditional medicine dates back to prehistoric times. These plants produce hundreds of chemical compounds that act as a form of defense against insects, fungi, diseases, and herbivorous mammals. Therefore, it has been used in pharmaceutical preparations to treat a wide range of diseases [5].

Nanomaterials synthesis is currently one of the most prominent research topics. They are small-sized particles ranging in size from (10-100nm). Nanoparticles are used in biomedical applications as they offer many advantages to larger particles including a higher surface-to-volume ratio and better magnetic properties [6].

A significant interest was received worldwide for the antibacterial activity of zinc oxide nanoparticles (ZnO NPs) [7]. This interest was due to their specific physicochemical properties including their small particle size, morphology, porosity, and their crystallinity, a feature that enhances their antimicrobial activity against pathogenic microorganisms [8].

### **Aim of the study**

1. Detection of bacterial isolates from diabetic foot ulcer patients in clinics and the



- Baquba Teaching Hospital, as well as identification of antibiotic-resistant bacteria.
2. Biosynthesis of zinc oxide from *Musa acuminata* fruit peel.
  3. Characterization of the nanomaterial by UV-vis spectroscopy, atomic force microscopy (AFM), scanning electron microscopy (SEM), and X-ray Diffraction (XRD) analysis.
  4. Determine the antibacterial activity of *Musa acuminata* extract against pathogenic bacterial isolates from diabetic foot ulcer patients.

## Material and methods

### Collection of the Samples

A total of 125 swabs were collected from diabetic foot ulcers from Baqubah Teaching Hospital during the period from the beginning of November 2021 to the end of January 2022. The specimens were cultured on a blood agar and macConkey agar. The isolates were purified on mannitol salt agar, EosinMethylene Blue, and Pseudomonas agar by streaking method. The agar plates were incubated for 24 h. at 37°C, then, biochemical and diagnostic tests were performed for the bacteria under the study of Amezquita Lopez *et al.* [9].

### Antibacterial susceptibility test

The susceptibility test of the pathogenic isolates was tested according to the CLSI (2021) criteria using the Kirby-Bauer method by transferring a few bacterial colonies to 2 ml of normal saline to make a suspension adjusted to McFarland turbidity ( $1.5 \times 10^8$ ) CFU / ml and then streaked on Muller Hinton agar plates by using a sterile cotton swab. Different antimicrobial discs as shown in table (1) were used with a maximum of discs placed on the surface of inoculated 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: The antibiotic discs used in this study**

| ABBREVIATION TERM FOR EACH ANTIBIOTIC | CONCENTRATION (MG /DISC) |
|---------------------------------------|--------------------------|
| Amikacin                              | 30                       |
| Amoxicillin                           | 25                       |



|                               |            |
|-------------------------------|------------|
| Aztreonam                     | 30         |
| Cefepime                      | 30         |
| Ceftriaxone                   | 250        |
| Chloramphenicol               | 30         |
| Ciprofloxacin                 | 5          |
| Clarithromycin                | 15         |
| Clindamycin                   | 2          |
| Gentamicin                    | 10         |
| Imipenem                      | 10         |
| Levofloxacin                  | 5          |
| Meropenem                     | 10         |
| Nitrofurantoin                | 300        |
| Ofloxacin                     | 5          |
| Piperacillin                  | 100        |
| Rifampicin                    | 5          |
| Streptomycin                  | 10         |
| Tetracycline                  | 30         |
| Trimethoprim                  | 5          |
| Trimethoprim-sulfamethoxazole | 1.25/23.75 |
| Vancomycin                    | 30         |

## Collection of *Musa acuminata* samples

*Musa acuminata* fruit used in this research was collected from the Baqubah bazaar in Baqubah municipality of Diyala province, the plant was classified by Prof. Dr. Khazal Dh. Wadi from the College of Science / Diyala University.

## Preparation of *Musa acuminata*

*Musa acuminata* fruit peels were washed thoroughly to remove impurities, then dried for 4 days at 35 °C in an oven, then crushed with an electric grinder to produce a fine powder, which was stored at 4 °C in a sterile and sealed glass vial [10].

## Biosynthesis of ZnO NPs from extract of *Musa acuminata*



In a typical preparation, 4 g of zinc nitrate was dissolved in 20 ml of ethanol under a magnetic stirrer for 15 min. Then 20 g of the plant extract added to the solution under the stirrer for 1 h. After homogenization, 3 ml of sodium hydroxide (NaOH) was added to the above solution. To obtain a white precipitation it was washed with ethanol and distilled water several times. Then dried the ZnO in the oven at 100 °C for 3 h. Next, the powder was processed at 450 °C for 4 h. [11].

## **Characterization of ZnO Nanoparticles**

The examination was carried out for both nanomaterials by a U.V. spectrum analysis, which was accomplished within 200 to 700 nm using a UV-visible spectrophotometer (UV-1800, Shimadzu(Japan)). The XRD analysis depends on the interaction between the monochromatic x- rays and the crystalline sample under test. The x-rays are created using a cathode tube of rays, and then the generated rays are filtered to generate monochromatic radiation directed and focused on the sample. The Scanning Electron Microscopy (SEM) analysis device scans the sample's surface through strongly focused electron radiation to obtain a microscopic image of the prepared nanoparticles while verifying the homogeneity of the examined. The Atomic Force Microscope (AFM) technique was introduced to investigate the surface morphology using AA- 3000, Shimadzu (Japan), with AFM communication mode. In particular, 5 drops of prodigiosin- ZnO NPs solution were dropped on a specific laboratory slide and kept for 30 min at 110 °C in an oven [12].

## **Antibacterial activity of ZnO NPs in vitro**

Muller – Hinton agar was prepared and poured into one petri dish. After solidifying, using a sterilized loop, all bacteria isolated from hospitals (gram-positive and gram-negative) were cultured in the dishes from the broth by streaking. After the culturing, five wells were made in the agar that did not touch the bottom of the dish to ensure proper distribution of the ZnO NPs and Ag NPs. 100µl of each dilution of ZnO NPs and Ag NPs was added to the well. Five different concentrations were prepared from the stock: (12,5 , 25 ,50 , 100 and 200) mg/ml. Each dish was sealed and left in an incubator at 37 °C overnight to be read the



next day. Each bacterium was cultured on Muller Hinton agar after comparison with McFarland tube ( $1.5 \times 10^8$ ) CFU/ml by streaking method, and the five wells 5mm were made in the plate by sterilized cork borer [13].

## Results and Discussion

### **Isolation of bacteria**

The study included collecting 125 samples from clinical sources (diabetic foot ulcers) of different ages for both sexes of patients in Baqubah Education Hospital in Diyala province during the period from the beginning of November 2021 to the end of January 2022, including taking direct swabs from patients. Samples were cultured on MacConkey agar and Blood agar by streaking method. The number of specimens according to their gender is shown in the table (2).

**Table 2:** The number of specimens according to their gender.

| GENDER | SPECIMENS NO. | PERCENTAGE(%) |
|--------|---------------|---------------|
| Male   | 60            | 48%           |
| Female | 65            | 52%           |
| Total  | 125           | 100%          |

### **Bacterial identification**

All bacterial isolates were diagnosed using selective and differential cultures media, microscopic examination, biochemical tests, and VITEC compact 2 system for confirmation. Table (3) results showed that the isolation percentage of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S.aureus*, and *S. epidermidis* from diabetic foot ulcers were 76, dividedas 9 (11.8%), 24 (31.6%), 12 (15.8%), 21(27.6%), and 10 (13.2%) respectively, these results agree with the study of Nour *et al.*[14].

**Table 3:** The number and percentage of bacteria isolated from diabetic foot ulcers



| ISOLATES                          | NO. OF DIABETIC FOOT ULCERS ISOLATES. (%) |
|-----------------------------------|---|
| <i>Escherichia coli</i>           | 9 (11.8%)                                 |
| <i>Klebsiella pneumoniae</i>      | 24 (31.6%)                                |
| <i>Pseudomonas aeruginosa</i>     | 12 (15.8%)                                |
| <i>Staphylococcus aureus</i>      | 21 (27.6%)                                |
| <i>Staphylococcus epidermidis</i> | 10 (13.2%)                                |
| Total                             | 76 (100%)                                 |

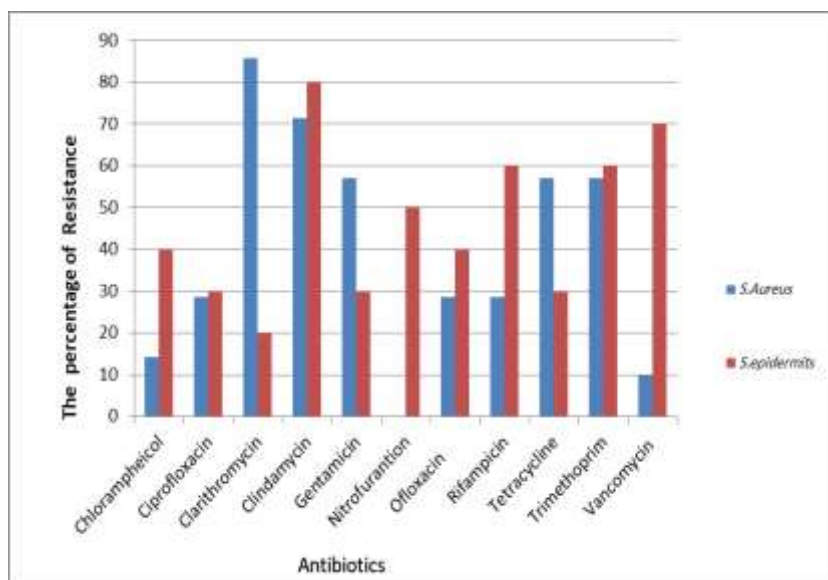
## Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed for 5 of the aforementioned pathogenic bacteria to identify the isolates of bacteria which they showed a multiple drug resistance (MDR). The results in the figures 1 showed that *S. aureus* was resistance to Chloramphenicol 3 (14.2%), Ciprofloxacin 6 (28.5%), Clarithromycin 18 (85.7%), Clindamycin 15 (71.4%), Gentamicin 12 (57.1%), Nitrofurantoin 0 (0%), Ofloxacin 6 (28.5%), Rifampicin 6 (28.5%), Tetracycline 12 (57%), Trimethoprim 12 (57%) and Vancomycin 2 (10%), Figure (1). While *Staphylococcus epidermidis*, The results indicated that from a total of 10 isolates revealed showed varied levels of resistance to Chloramphenicol 4 (40%), Ciprofloxacin 3 (30%), Clarithromycin 2 (20%), Clindamycin 8 (80%), Gentamicin 3 (30%), Nitrofurantoin 5 (50%), Ofloxacin 4 (40%), Rifampicin 6 (60%), Tetracycline 3 (30%), Trimethoprim 6 (60%) and Vancomycin 7 (70 %).

Figure 2 showed that Nine isolates of *E. coli* were resistance which include; Ofloxacin 0 (0%), Streptomycin 6 (66.6%), Gentamicin 6 (66.6%), Chloramphenicol 3 (33%), Ceftriaxone 6 (66.6%), Aztreonam 2 (22%), Piperacillin 3 (33.3%), Amoxicillin 8 (88.8%), Imipenem 2 (22.2%) and Tetracycline 8 (88.8%). While *K. pneumoniae*, the results of the analysis revealed 24 isolates showed variable resistance which include : Ofloxacin 3 (12%), Streptomycin 12 (50%), Gentamicin 15 (62%), Chloramphenicol 8 (33.3%), Ceftriaxone 20 (83.3%), Aztreonam 10 (41.6%) Piperacillin 5 (20.8%), Amoxicillin 22 (91.6%), Imipenem 8 (33.3%) and Tetracycline 22 (91.6%) Figure (2).



Figure 3 showed that a total of 12 of *P. aeruginosa* isolates were showed varied levels of resistance to the following antibiotics: Amikacin 0 (0%), Levofloxacin 2 (13%), Gentamicin 4 (27%), Ciprofloxacin 3 (20%), Nitrofurantoin 5 (33%), Aztreonam 3 (20%), Cefepime 11 (73%), Imipenem( 7%), Meropenem (13%) and Piperacillin (20%), Figure (3).

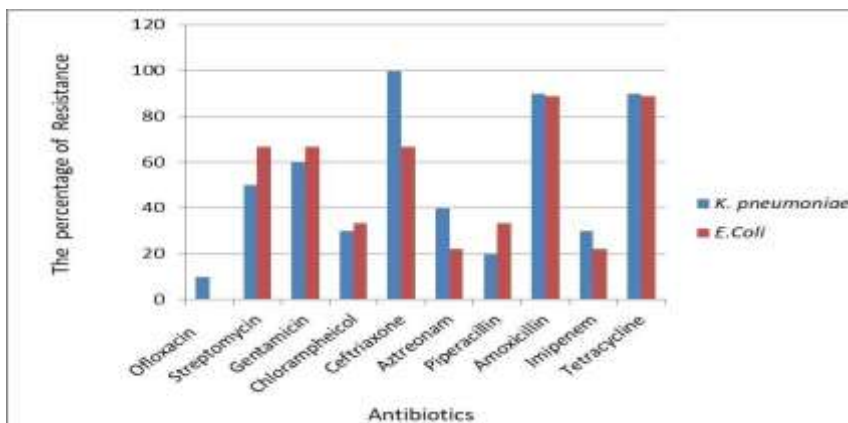


**Figure 1:** The resistance of antibiotics of *S. aureus* and *S. epidermidis*

The antimicrobial susceptibility of *S. aureus* isolates showed that 12 isolates (57.15%) and 15 (71.4%) were resistant to Gentamicin and Clindamycin respectively, these results resistance agreement with previous studies by Alsaadi [15] and Hasan[16] when reported the rates of resistance on the same antibiotics were 55.7% and 68% respectively.

The resistance of *S. epidermidis* isolates of the current study agreed with other studies such as Ayah [17], which reported that the resistance to ciprofloxacin was (33%) and to tetracycline (31%). According to the study recorded by Ali [18], which indicates that Rifampin resistance was 55%, these results agree with the present study. Shrestha *et al.*[19] revealed that

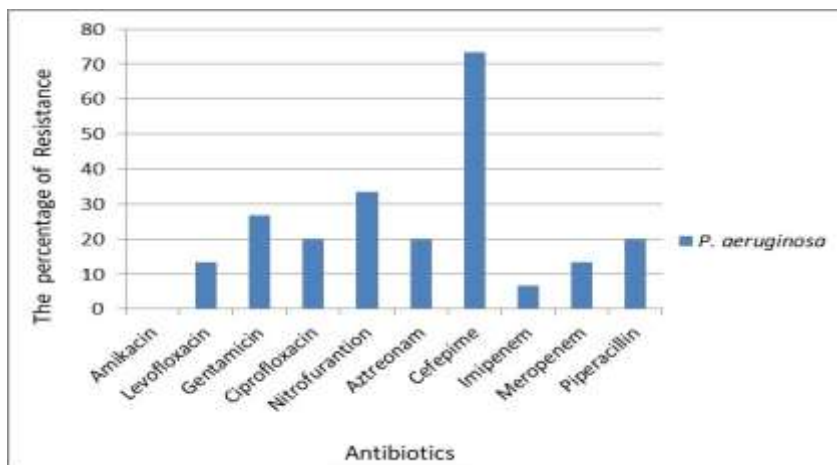
Nitrofurantoin resistance was 45% against *S. epidermidis*, this result agrees with the present study.



**Figure 2:** Antibiotic resistance of *E. coli* and *K. pneumoniae*

A study done by AL-Dolaymi [20] detected the resistance of *E. coli* to Streptomycin(70%), Gentamicin (60%), and Amoxicillin (90%), these results were close to present study.

The resistance percentage of *K.pneumoniae* isolates against Amoxicillin was great (91.6%), this result close to the result of study done by Al-Khafaji [21] who indicated the resistance rate of *K.pneumoniae* isolates against Amoxicillin was 89%.

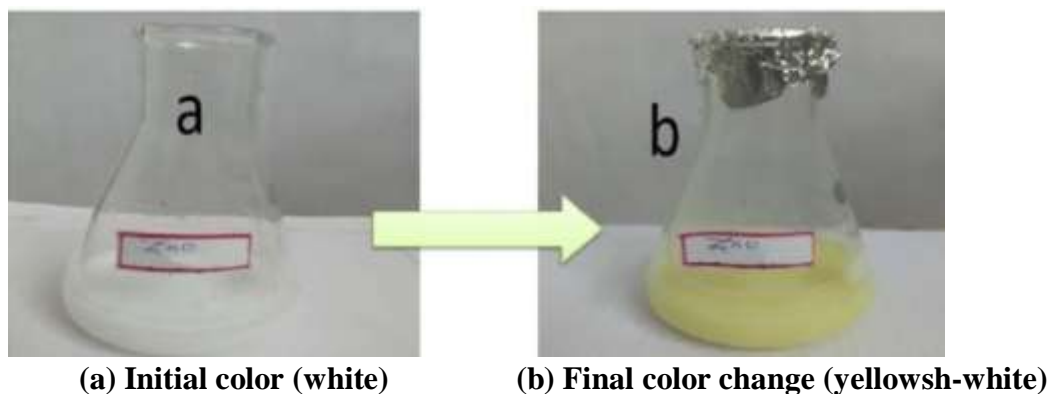


**Figure 3:** Antibiotic Resistance of *P. aeruginosa*

Al-saadi [15] showed the antibiotic resistance test for *P.aeruginosa* revealed that a high percentage of resistance was seen against Cefepime 73%, this result agrees with the present study but disagrees with the result of safferi *et al.* [22] which was 15%. Whereas a lesser percentage of antibiotic resistance was seen against Amikacin, Levofloxacin, and Meropenem (0%), and (13%), these results disagree with several studies such as sala *et al.* [23], and Namnq *et al.* [24] which revealed a high resistance against the same antibiotics.

### **Biosynthesis of ZnO NPs by *Musa acuminata* fruit peel extract**

Green method synthesis of nanoparticles increases the inhibition rate compared with conventional delivery and retains the antimicrobial efficacy for a longer time. The plant extract was confirmed by the appearance of yellowish-white color precipitates in the solution mixture, as shown in figure (4).



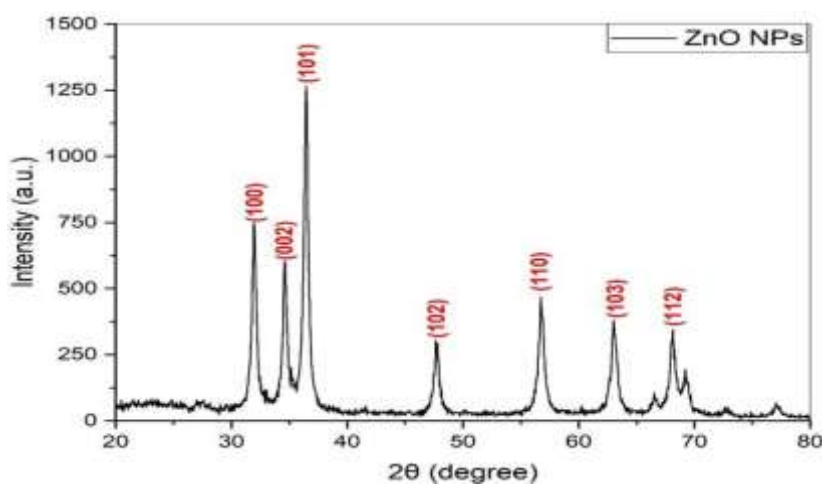
**Figure 4:** ZnO NPs synthesis by *Musa acuminata* fruit peel extract

### **Characterization of Zinc oxide Nanoparticles**

The characterization of ZnO NPs was carried out by using X-ray diffractometer (XRD) pattern analysis and UV-Visible Spectrophotometer where the nanomaterial has been characterized in the Physics department \Collage of Sciences \Diyala university. While Emission Scanning Electron Microscope (SEM) and Atomic Force Microscopy (AFM) It was characterized at Phi Nano-Science Center (PNSC) \Baghdad.

### **X-Ray Diffraction (XRD) analysis**

The biosynthesized ZnO nanoparticles were analyzed using X-ray diffraction devices to determine crystallinity and average particle size figures (5) show the XRD pattern of ZnO NPs prepared by green syntheses, the strongest peaks at angles  $31.9^\circ$ ,  $34.6^\circ$ ,  $36.4^\circ$ ,  $47.7^\circ$ ,  $56.7^\circ$ ,  $63^\circ$ ,  $68^\circ$  corresponding to planes (100), (002), (101), (102), (110), (103), (112) respectively. All these peaks agree with JCPDS Card No:(01-079-0205) and confirm the hexagonal phase of ZnO NPs. The crystallite size of ZnO NPs was calculated using Debye Scherrer's formula [25]. Where  $D$  is the crystal size,  $\lambda = 1.5406 \text{ \AA}$  is the X-ray wavelength,  $\beta$  is the Full-width half maximum (FWHM) of the peak in radians, and  $\theta$  is the Bragg angle. The mean crystallite size is about 25.9 nm and the small size of prepared nanoparticles gives us a special benefit for biological activity, especially for antimicrobial activity.

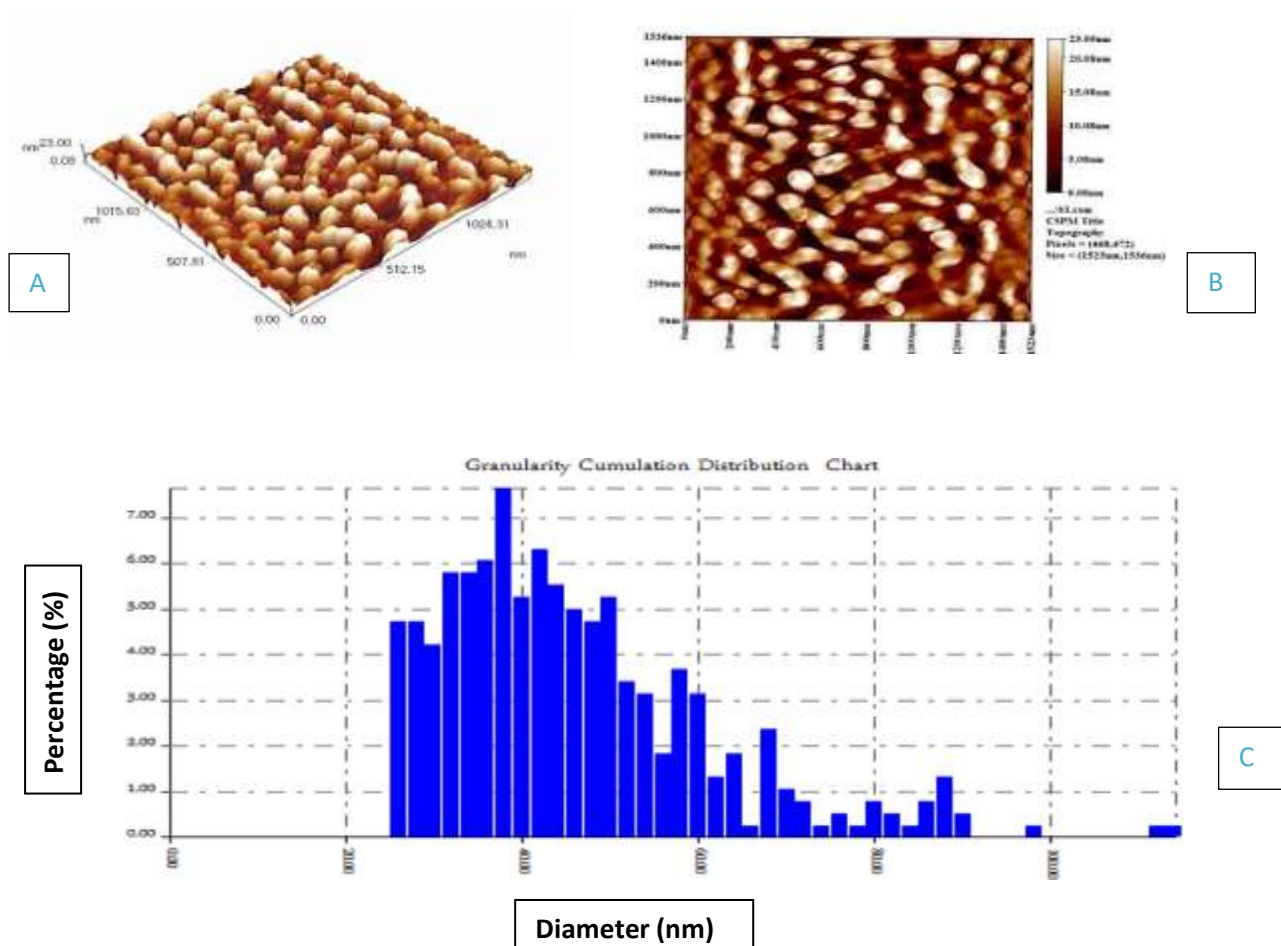


**Figure 5:** XRD pattern of ZnO NPs prepared by green synthesis

## Atomic Force Microscopy (AFM)

The surface roughness, topography, and morphology in this study were investigated using an Atomic Force Microscope (AFM) technique. This particular technique provides both two, and three-dimensional images of the desired nanoparticles at an atomic level [26] Figure (6). The average particle diameter of the synthesized ZnO nanoparticles is 40, 45, and 50 nm at 6.20, 5.50, and 5.15 respectively, it should be mentioned that the average size diameter of the scanned nanoparticles was estimated on the nano-scale, where the

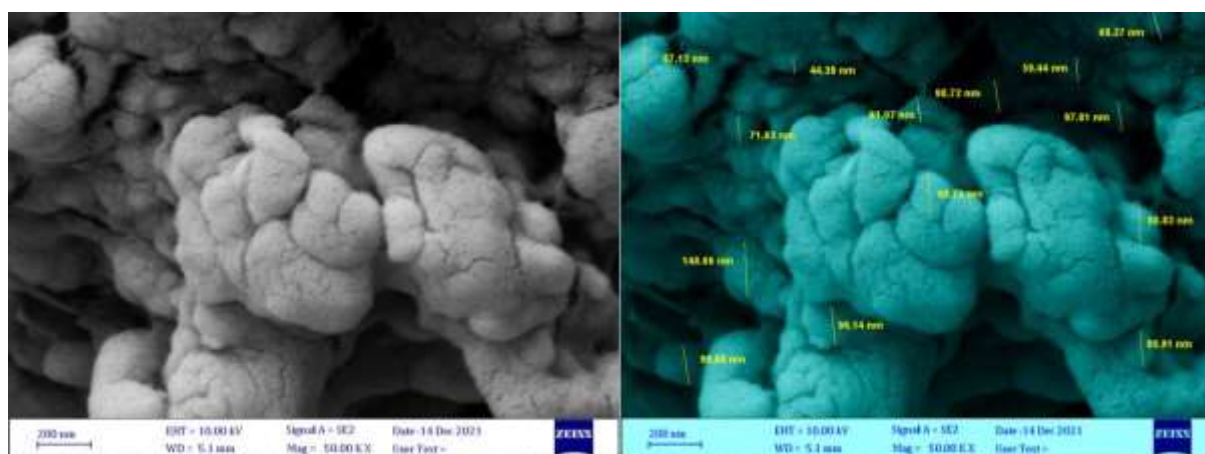
biosynthesized ZnO nanoparticles using *Musa acuminata* have investigated via the AFM technique. The average diameter was 45 nm.



## Scanning Electron Microscopy (SEM) analysis

The surface morphology obtained using the SEM technique was investigated, and the topographical analyses were presented based on the surface investigation. As illustrated in Figure(7) the image illustrates a low glomeration degree. Moreover, the prepared ZnO sample exhibited spherical particles and plate-like structures. The obtained structure was similar to

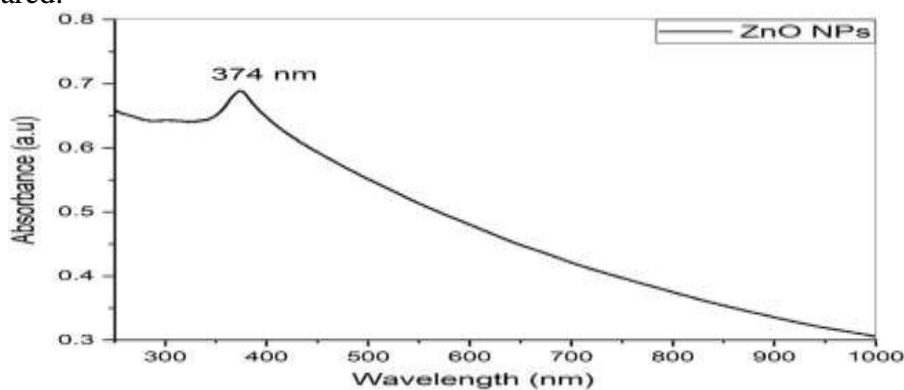
other researchers' outcomes by which plate-like morphology was also obtained with almost similar particle diameter and nanoparticle size around (28.2-55) nm. The results showed that the size of no NPS as follows: (87.13,44.38,68.27,59.44, 61.07,71.63,90.73,88.83, 96.14, 88.41) nm average size was 75.60.



**Figure 7:** SEM images of ZnO nanoparticles

## UV-Vis Spectroscopy

The optical properties of ZnO NPs have been studied by using UV-visible spectroscopy, as shown in Figure (8), the sharp peak of spectrum observed in the blue region at 374 nm, and this excitation wavelength gives us a strong indication of the behavior of ZnO NPs which has been prepared.



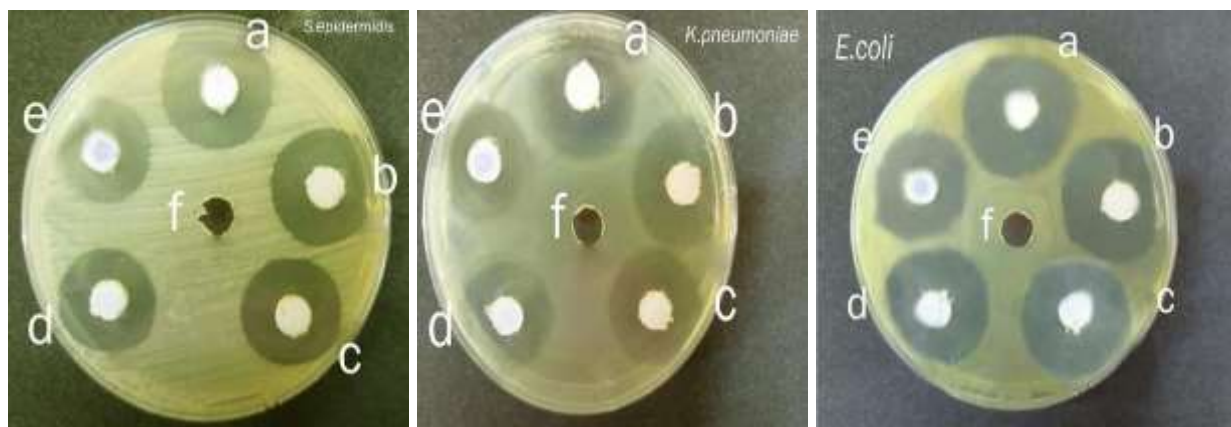
**Figure (8)** Uv-Visible spectroscopy of ZnO NPs prepared by green syntheses

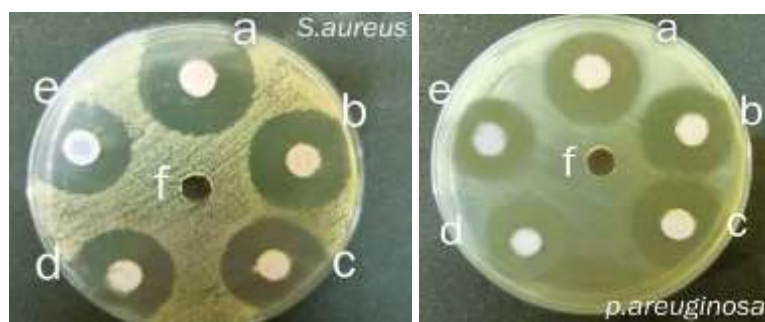
## Antibacterial activity of ZnO NPs against pathogenic bacteria

ZnO NPs show antibacterial activity against gram-positive and gram-negative bacteria (Multidrug-resistance) isolated from diabetic foot ulcers. Alcohol extract of *Musa acuminata* which using to biosynthesize ZnO NPs and the results are shown in table (3) and, figure (10). ZnO NPs showed the highest diameter of inhibition zone a concentration of 200 mg/ml of *E. coli*, *K. pneumoniae* *P. aeruginosa* *S. aureus*, and *S. epidermidis*, reaching (28, 27, 31, 29, 26) mm respectively, while the ZnO NPs recorded at concentration 12.5 mg/ml lowest areas of inhibition zone against the same isolates reaching (22, 23, 24, 24, 21) mm respectively.

**Table (3) Antibacterial activity of ZnO NPs on bacterial growth**

| ISOLATES              | AVERAGE OF INHIBITION ZONE DIAMETER(MM) |                          |                          |                           |                           |
|-----------------------|---|--------------------------|--------------------------|---------------------------|---------------------------|
|                       | concentration s 12.5 mg/ml              | concentration s 25 mg/ml | concentration s 50 mg/ml | concentration s 100 mg/ml | concentration s 200 mg/ml |
| <i>E. coli</i>        | 22                                      | 25                       | 26                       | 27                        | 28                        |
| <i>K. pneumoniae</i>  | 23                                      | 23                       | 24                       | 24                        | 27                        |
| <i>P. aeruginosa</i>  | 24                                      | 26                       | 31                       | 31                        | 31                        |
| <i>S. aureus</i>      | 24                                      | 27                       | 27                       | 28                        | 29                        |
| <i>S. epidermidis</i> | 21                                      | 24                       | 24                       | 25                        | 26                        |





**Figure 10:** antibacterial activity of ZnO NPs against *S. epidermidis*, *K. pneumoniae*, *E. coli*, *S. aureus*, and *P. aeruginosa* by agar well diffusion method. The letters (a, b, c, d, e) represent the concentrations (200, 100, 50, 25, 12.5)mg/ml respectively, and f (control).

The green synthetic method of ZnO NPs from *Musa acuminata* fruit peels is a recent approach that is the explanation of a cheap and eco-friendly methods [11]. Characterization of Zinc oxide Nanoparticles showed that crystallite size is about 25.9 nm when examined by XRD, this result was close to Ali *et al.* [27] who mentioned that the average size was 30-40 nm. The synthesized ZnO nanoparticle formation was confirmed by Atomic Force Microscopy (AFM) was 45 nm, this ratio was close to Elumalai and Velmurugan [28]. The surface morphology of the ZnO nanoparticle was investigated by Scanning Electron Microscopy (SEM) analysis with an average size was around (28.2-55) nm, this result agrees with Manjunatha *et al.*, [12]. The sharp peak of the spectrum was observed in the blue region at 374 nm when examined by UV-Vis Spectroscopy, this result agreed with Fakhari *et al.*, [29].

The ZnO NPs toxicity in bacteria is mainly due to the generation of reactive oxygen species (ROS), specifically, the ROS toxicity to the cell's wall is attributed to cellular constituent damage like proteins, lipids, and DNA. ROS generation is widely considered the major factor of antibacterial activity associated with ZnO photo-toxicity [30].

Nanotechnology and nanomaterials play an important role in damaging DNA, destroying the membrane, and killing cells through lipid peroxidation and oxidative stress. The nanoparticles, in general, interact with water molecules within the medium of the cell and also use the electrons capture pathway, which results in free radicals producing especially ROS [31].





## Conclusions

The present study revealed a simple, safe, cost-effective, and environmentally friendly method for producing ZnO NPs using an alcoholic extract of *Musa acuminata*. SEM, UV-vis, AFM, and XRD were used to investigate the obtaining efficiency of ZnO NPs. The nanostructures of ZnO NPs were verified by spectroscopy. These nanoparticles were also investigated for their ability to inhibit the growth of *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, and *S. epidermidis* in antibacterial tests. It was found that the nanoparticles had improved antibacterial qualities.

## References

1. M. H. Megallaa, A. A. Ismail, M. H. Zeitoun, M. S. Khalifa, Association of diabetic foot ulcers with chronic vascular diabetic complications in patients with type 2 diabetes, *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 13(2), 1287-1292(2019)
2. M. Edmonds, C. Manu, P. Vas, The current burden of diabetic foot disease, *Journal of clinical orthopaedics and trauma*, 17, 88-93(2021)
3. C. W. Hicks, E. Selvin, Epidemiology of peripheral neuropathy and lower extremity disease in diabetes, *Current diabetes reports*, 19, 1-8(2019)
4. H. Ibrahim, Nanotechnology and Its Applications to Medicine: an over view, *QJM: An International Journal of Medicine*, 11(1), (2020)
5. A. K. Shakya, Medicinal plants: Future source of new drugs, *International Journal of Herbal Medicine*, 4(4), 59-64(2016)
6. K. McNamara, S. A. Tofail, Nanoparticles in biomedical applications, *Advances in Physics: X*, 2(1), 54-88(2017)
7. A. Sirelkhatim, S. Mahmud, A. Seeni, N. H. M. Kaus, L. C. Ann, S. K. M. Bakhori, D. Mohamad, Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism, *Nano-Micro Letters*, 7(3), 219-242(2015)
8. S. E. Jin, H. E. Jin, Antimicrobial Activity of Zinc Oxide Nano/Microparticles and



- Their Combinations against Pathogenic Microorganisms for Biomedical Applications: From Physicochemical Characteristics to Pharmacological Aspects, *Nanomaterials (Basel)* 11(2), 263(2021)
9. B. A. Amezcuita-Lopez, M. Soto-Beltran, B. G. Lee, J. C. Yambao, B. Quiñones, Isolation, genotyping and antimicrobial resistance of Shiga toxin-producing *Escherichia coli.*, *Journal of Microbiology, Immunology and Infection*, 51(4), 425-434(2018)
  10. A. A. Mitiku, B. Yilma, A review on green synthesis and antibacterial activity of silver nanoparticles, *Int. J. Pharm. Sci. Rev. Res*, 46, 52-57(2018)
  11. Y. Sharma, K. Bala, Comparative study of different parts of fruits of *Musa* Sp. on the basis of their antioxidant activity, (2016)
  12. A. Pereira, M. Maraschin, Banana (*Musa* spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health, *Journal of ethnopharmacology*, 160, 149-163(2015)
  13. R. L. Manjunatha, K. V. Usharani, D. Naik, Synthesis and characterization of ZnO nanoparticles: A review, *Journal of Pharmacognosy and Phytochemistry*, 8(3), 1095-1101(2019)
  14. S. Shahid, S. A. Khan, W. Ahmad, U. Fatima, S. Knawal, Size dependent bacterial growth inhibition and antibacterial activity of Ag doped ZnO nanoparticles under different atmospheric conditions, *Indian Journal of Pharmaceutical Sciences*, 80(1), 173- 180(2018)
  15. S. Nour, M. Mukhtar, M. ElMakk, E. Mohamed, W. Elnour, M. Shamad, S. Ahmed, AEROBIC BACTERIAL PROFILE IN DIFFERENT GRAGES OF DIABETIC FOOT ULCERS OF SUDANESE PATIENTS: A CROSS SECTIONAL STUDY
  16. L. A. S. Alsaadi, Heavy Metals Tolerance and Antibiotics Susceptibility Profiles of *Staphylococcus aureus* Strains Isolated From Clinical Sources in Baquba city, *Diyala Journal for Pure Sciences*, 13(1), 130-144(2017)
  17. A. Y. Hasan, Antibiotic Susceptibility of Bacterial Isolates from Diabetes Patients



- withFoot Ulcers, Diyala Journal for Pure Science, 13(3- part 3), 164-175(2020)
18. N. F. Ayah, Comparative Study on Pathogenicity of Techoic Acid Extracted From Methicillin Resistance *Staphylococcus aureus* and *Staphylococcus epidermidis*, College of science/Baghdad university, (2019)
19. H. C. Ananda Murthy, B. Abebe, P. CH, K. Shantaveerayya, A review on green synthesis and applications of Cu and CuO nanoparticles, Material Science Research India, 15(3),279-295(2018)
20. A. M. Ali, Comparative study between *Staphylococcus epidermidis agr*<sup>+</sup> isolates and MRSA *agr*<sup>-</sup> isolates. M.Sc. Thesis, College of science, Baghdad University, (2018)
21. B. Shrestha, B. M. Pokhrel, T. M. Mohapatra, Antibiotic Susceptibility Pattern of Nosocomial Isolates of *Staphylococcus epidermidis* in a Tertiary Care Hospital, Nepal. J Nepal Med Assoc., 48(175),234-8(2020)
22. I. M. E. AL-Dolaymi, Detection of some toxin genes in Escherichia coli causing childrendiarrhea in Baquba city, Master thesis. College of Education for Pure Science / Diyala University, (2018)
23. M .H. M. Al-Khafaji, Biofilm formation by *Klebsiella pneumonia* contaminating external stabilizers and its resistance to antibiotics, Master Thesis, College of Science / Universityof Baghdad, (2019)
24. M. Saffari, S. Karami, F. Firoozeh, M. Sehat, Evaluation of biofilm-specific antimicrobial resistance genes in *Pseudomonas aeruginosa* isolates in Farabi Hospital, Journal of Medical Microbiology,66, 905–909(2017)
25. A. Sala, F. Ianni, I. Pelizzone, M. Bertocchi, D. Santospirito, F. Rogato, S. Flisi, The prevalence of *Pseudomonas aeruginosa* and multidrug resistant *Pseudomonas aeruginosa* in healthy captive ophidian, PeerJ, (2019)
26. A. Namuq, K. Ali, A. Al-Ani, Correlation between Biofilm Formation, Multi-Drug Resistance and AlgD Gene among *Pseudomonas aeruginosa* Clinical Isolates, Journal of University of Babylon for Pure & Applied Sciences, 27 (3), 1-8(2019)
27. M. A. Subhani, M. Irshad, A. Nazir, M. Hafeez, S. Ali, Synthesis and antibacterial



- potential of Loranthus pulverulentus conjugated silver nanoparticles, *Microscopy Research and Technique*, 85(11), 3530-3540(2022)
28. S. Chouhan, A. K. Bajpai, R. Bhatt, Analysis of topographical parameters and interfacial interaction of zinc oxide reinforced poly (vinyl alcohol-g-acrylonitrile) nanocomposite film surfaces using atomic force microscopy, *Nano-Structures & Nano-Objects*, 18, 100308(2019).
29. J. Ali, R. Irshad, B. Li, K. Tahir, A. Ahmad, M. Shakeel, Z. U. H. Khan, Synthesis and characterization of phytochemical fabricated zinc oxide nanoparticles with enhanced antibacterial and catalytic applications, *Journal of Photochemistry and Photobiology B: Biology*, 183, 349-356(2018)
30. K. Elumalai, S. Velmurugan, Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.), *Applied Surface Science*, 345, 329-336(2015)
31. S. Fakhari, M. Jamzad, H. Kabiri Fard, Green synthesis of zinc oxide nanoparticles: a comparison, *Green chemistry letters and reviews*, 12(1), 19-24(2019)
32. K. Gold, B. Slay, M. Knackstedt, A. K. Gaharwar, Antimicrobial activity of metal and metal-oxide based nanoparticles, *Advanced Therapeutics*, 1(3), 1700033(2018)
33. S. Li, X. Li, S. H. Ho, How to enhance carbon capture by evolution of microalgal photosynthesis, *Separation and Purification Technology*, 291, 120951(2022).