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Preparation of Ag and ZnO nanoparticles from (*Musa* x *paradisiaca* L.) fruit peels and studying its inhibitory effects against bacterial infection of diabetic foot ulcer

A Thesis
Submitted to College of Science - University of Diyala in Partial Fulfillment of the Requirements for the Degree of Master in Biology

By

Ussama Asaed Fazel

B. Sc. Department of Biology / College of Science
(2007)

Supervised by

Assist. Prof. Abbas Yaseen Hasan

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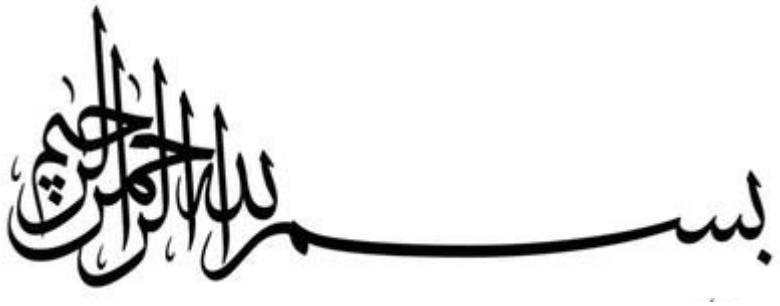

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Signature

Name: **Dr. Abbas Y. Hasan**

Assistant Professor

Date: / / 2023

In view of the available recommendations I forward this thesis for debate

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Signature:

Name: **Prof. Dr. Ibrahim Hadi Mohammad**

Head of department

Date: / / 2023

Scientific Amendment

I certify that the thesis entitled Inhibitory effect of **Preparation of Ag and ZnO nanoparticles from (*Musa* x *paradisiaca* L.) fruit peels and studying its inhibitory effects against bacterial infection of diabetic foot ulcer** presented by (Ussama Asaed Fazel) has been evaluated scientifically, therefore, it is suitable for debate by examining committee.

Signature:

Name: **Dr. Ahmed Jameel Sabr**

Title: Assistant Professor

Date: / / 2023

Signature:

Name: **D.Zahraa Jaafar Jameel**

Title: Assistant Professor

Date: / / 2023

Linguistic Amendment

I certify that the thesis entitled Inhibitory effect of **Preparation of Ag and ZnO nanoparticles from (*Musa* x *paradisiaca* L.) fruit peels and studying its inhibitory effects against bacterial infection of diabetic foot ulcer** presented by (Ussama Asaed Fazel) has been evaluated scientifically, therefore, it is suitable for debate by examining committee.

Signature:

Name: **Dr. Sanaa Nagem Abed Alhadidi**

Title: Lucturer

Date: / / 2023

Examination Committee Discussion

We certify that we have read this thesis entitled Inhibitory effect of **Preparation of Ag and ZnO nanoparticles from (*Musa* x *paradisiaca* L.) fruit peels and studying its inhibitory effects against bacterial infection of diabetic foot ulcer** we examined the student (Ussama Asaed Fazel) on its content and in what is related with it, and in our opinion it meets the standard of a thesis for the degree of master of science in Biology .

(Chairman)

Signature:
Name: **Khazal Dh. Wadi Al-Jibouri**
Title: Professor
Date: / / 2023

(Member)

Signature:
Name: **Dr. Tahseen Hussein Mubarak**
Title: Professor
Date: / / 2023

(Member/ supervisor)

Signature
Name: **D.Abbas Y. Hasan**
Title: Assistant Professor
Date: / / 2023

(Member)

Signature:
Name: **Dr. Fatima Ramadan Abdul**
Title: Assistant Professor
Date: / / 2023

Approved by the council of the Faculty of College of Science, University of Diyala

Signature:
Name: **Dr. Tahseen Hussein Mubarak**
Title: Professor
Date: / / 2023
(Dean of the College of Science)

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Summary

From November 2021 to January 2022, one hundred and twenty-five clinical specimens (diabetic foot ulcers) were collected from patients from Baquba Teaching Hospital. Samples were cultured on MacConkey and Blood agar media. The bacterial isolates were then initially diagnosed by using selective and differential media. Then biochemical tests were performed to confirm the diagnose of bacterial isolates and Identification of the isolates was confirmed using the VITEK-2 system. Based on the biochemical identification and the VITEK-2 the bacterial species were as following: *Klebsiella pneumoniae*, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis.* Which were first characterized by Carl Friedlander, Theodor Escherich, Carle Gessard, Alexander Ogston and Julius Rosenbach respectively.

Antimicrobial sensitivity was tested for all different bacterial isolates to 22 antimicrobials from each bacteria using disc diffusion method. The results showed that many bacterial isolates were multiple drugs resist (MDR). The following antibiotics were used: Amikacin, Amoxicillin, Aztreonam, Cefepime, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Clarithromycin, Clindamycin, Gentamicin, Imipenem, Levofloxacin, Meropenem, Nitrofuration, Ofloxacin, Piperacillin, Rifampicin, Streptomycin, Tetracycline, Trimethoprim, Trimethoprim-sulfamethoxazole andVancomycin.

Zink Oxid (ZnO) and Silver (Ag) Nanoparticles (NPs) were characterized by using Atomic Force Microscopy (AFM), which indicated that the average size diameter of ZnO NPs was 45 nm while Ag NPs was 76.1nm, and UV-Vis showed of ZnO NPs were at the wavelength of 374 nm while Ag NPs was 426 nm, and also the characterization of ZnO NPs by X-ray Diffraction (XRD) was found to be 25.9 nm while Ag NPs was 27 nm, Fourier transform infrared (FTIR) spectroscopy showed different functional groups of

biomolecules which were responsible for reduction and capping process, Scanning Electron microscopic (SEM) for ZnO NPs was 75.60 nm while Ag NPs was 71.69 nm.

Five concentrations (12.5, 25, 50 ,100 and 200) mg/mL of the *Musa* x *paradisiaca* extract were prepared to detect their inhibitory effect against multiple drugs resist (MDR). The results showed that the highest inhibition zone of isolates was at concentration 200 mg/ml and the lowest inhibition zone was at concentrate 12.5 mg/mL.

The antibacterial activity of Ag NPs against the selected multiple drugs resist (MDR) bacteria were determined by agar well diffusion method. It was observed that the growth of these bacteria was inhibited at 12.5 mg /mL. Ag NPs showed the highest diameter of inhibition zone at concentration 200 mg/ml against *S. aureus, S. epidermidis, P. aeruginosa, K. pneumonia* and *E. coli* reaching (20, 24, 15, 20, 18) mm respectively, while the ZnO NPs showed the highest diameter of inhibition zone at concentration 200 mg/ml against *S. aureus, S. epidermidis, P. aeruginosa, K. pneumonia and E. coli* reaching (29, 26, 31, 27, 28) mm respectively.

Finally, determination of MIC of Ag NPs was done by microdilution method. The MIC for Ag NPs against *S. aureus* was 8 µg/ml, *S. epidermidis*, *P. aeruginosa* and *K. pneumoniae* was 4 µg/ml, while *E. coli* was 2 µg/ml, the determination of MIC of Zno NPs was *S. aureus* 4 µg/ml, *S. epidermidis* and *E. coli* was 2 µg/ml, *P. aeruginosa* 12.5 µg/ml and *K. pneumoniae* was 8 µg/ml.

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List of Abbreviations

Abbreviation	Key
AFM	Atomic force microscope
Ag NPs	Ag NPs Silver nanoparticles
Bap	Biofilm related protein
CDC	Centers for Disease Control
CFU/ml	Colony Forming Unit per millimeter
CLSI	Clinical and Laboratory Standards Institute
D.D.W	Double Distal water
D.W	Distal water
DFU	diabetic foot ulcers
EMB	Eosin Methylene Blue
FTIR	Fourier transform infrared spectroscopy
HCl	Hydrochloric acid
IMViC	"I" is for indole test; "M" is for methyl red test; "V" is for Voges-Proskauer test, and "C" is for citrate test
JCPDS	Joint Committee on Powder Diffraction Standards
LPS	Lipopolysaccharide (Endotoxin)
MDR	Multiple drug resistant
MIC	Minimum inhibition concentration
MR	Methyl red
MRSA	Methicillin-resistant *Staphylococcus aureus*
nm	Nanometers
NPs	Nanoparticles
PDR	Pandrug-resistant bacteria
QS	Quorum sensing system
SCCmec	staphylococcus cassette chromosome
SEM	Scanning electron microscope
UTI	UTI Urinary tract infections
UV-Vis	Ultraviolet-visible spectrum
WHO	World Health Organization
XDR	Extensively drug-resistant
XRD	X-ray Diffraction
ZnO NPs	Zink oxid nanoparticles

Chapter one

Introduction

INTRODUCTION

Diabetic foot refers to a series of pathological abnormalities affecting the lower limbs that are typically caused by diabetic consequences such as peripheral neuropathy, vascular damage, and loss the sensation in the feet. High blood sugar and uncontrolled diabetes cause nerve damage in the feet and inadequate blood flow, resulting in numbness in the feet, and the patient does not feel if he has a wound or injury to the foot, increasing the risk of diabetic foot infection, if infections, ulcers, and serious consequences are not treated promptly, the diabetic foot may be amputated (Leone *et al*., 2012).

There are many mechanism resistance of bacteria to antibiotic, like enzyme inactivation, reduced cell permeability, target protection, target overproduction, changed target site/enzyme, and enhanced efflux due to efflux pump overexpression. Other more complicated phenotypes associated with antibiotic resistance in bacteria include biofilm development and quorum sensing (Davies and Davies, 2010).

The rising necessity for a good treatment against multidrug resistant infectious diseases has improved interest in discovering antibiotic resistance inhibitors as a first step toward developing a combination medication, nanotechnology which is a technique that enable the introduce of materials in nano- scale structure was applied in order to maximize the drug therapeutic activity and minimize its undesirable side effects (Ibrahim, 2020).

The medicinal plants were used have been discovered and have been used since prehistoric times in traditional medicine practices, medicinal and nutritious materials for work, and used in pharmaceuticals to treat many ailments, plants synthesis hundreds of chemical compounds for functions including defense against insects, fungi diseases and herbivorous mammals (Shakya, 2016).

Nanomaterials synthesis is currently one of the most prominent research topics. They are small sized particles ranging in size from (10 – 100)nm. 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 (McNamara and Tofail, 2017).

A significant interest was received worldwide for the antibacterial activity of zinc oxide nanoparticles (ZnO NPs) (Sirelkhatim *et al*., 2015). This interest was due to their specific physicochemical properties including their small particle size, morphology, porosity and their crystallinity, a feature that enhance their antimicrobial activity against pathogenic microorganisms (Jin and Jin, 2021).

Silver nanoparticles (Ag NPs) are among the most explored nanoparticles, because of their antimicrobial activity against a variety of commensal and pathogenic strains, silver nanoparticles are believed to be inhibitory against a variety of fungi , viruses, and bacterial strains (Mekawey and Helmy, 2017).

The aims of this study are:

1. Determine the antibacterial activity of *Musa* x *paradisiaca* extract against pathogenic bacterial isolates from diabetic foot ulcer patients.
2. Examination of the effectiveness of the nanomaterial and the plant extract on bacteria and the determination of Minimum Inhibition Concentration (MIC) of ZnO NPS and Ag NPS .
3. Identification of treatment-resistant bacteria by testing sensitivity to treatment.
4. Biosynthesis of zinc oxide and Ag nanoparticles from *Musa* x *paradisiaca* fruit peel.