



شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



**HANAA ALY**



شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم



# شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



**HANAA ALY**



شبكة المعلومات الجامعية  
التوثيق الإلكتروني والميكروفيلم

# جامعة عين شمس

## التوثيق الإلكتروني والميكروفيلم

### قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها  
على هذه الأقراص المدمجة قد أعدت دون أية تغيرات



### يجب أن

تحفظ هذه الأقراص المدمجة بعيداً عن الغبار



**HANAA ALY**



Ain Shams University  
Faculty of Science  
Microbiology  
Department



# **Biocontrol of some Pathogenic Microorganisms Using Milk Whey, Moringa plant and Nanoparticles.**

*A Thesis*

**Submitted for the Degree of Ph.D. of Science in Microbiology**

*By*

**Alshaymaa Abd el-Monaem Mohammed**

**B.Sc. (Microbiology), Botany Department, Faculty of Science,  
Zagazig University (2012)**

**M.Sc. In Microbiology, Microbiology Department, Faculty of  
Science, Zagazig University (2015)**

2021



Ain Shams University  
Faculty of Science  
Microbiology  
Department



# **Biocontrol of some Pathogenic Microorganisms Using Milk Whey, Moringa plant and Nanoparticles.**

*A Thesis*

**Submitted for the Degree of Ph.D. of Science in Microbiology**

*By*

**Alshaymaa Abd el-Monaem Mohammed**

B.Sc. (Microbiology), Botany Department, Faculty of Science,  
Zagazig University (2012)

M.Sc. In Microbiology, Microbiology Department, Faculty of  
Science, Zagazig University (2015)

*Under Supervision of*

**Prof. Dr.**

**Saadia Mohammed Hassanin  
Easa**

Prof. of Microbiology,  
Microbiology Department,  
Faculty of Science  
Ain Shams University

**Prof. Dr.**

**Mohammed Farok Ibrahim**

Prof. of Microbiology,  
Botany & Microbiology Department,  
Faculty of Science  
Zagazig University

**Prof. Dr. Seham Abdel-Shafi Awad-Alla**

Professor of Microbiology,  
Botany & Microbiology Department,  
Faculty of Science  
Zagazig University

2021

بسم الله الرحمن الرحيم  
(( وَمَا تَوْفِيقٍ إِلَّا بِاللهِ  
عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ ))  
صدق الله العظيم  
سورة هود الآية (٨٨)

# *Declaration*

*This thesis has not previously submitted for  
any other Universities*



# Acknowledgements

Praise and thanks to **ALLAH** Subhanah Wata'ala for blessing this work, until it has reached its end, as a part of his generous help, throughout my life.

Really I can hardly find the words to express my gratitude to **Prof. Dr. Saadia Hassanin Easa**, Professor of Microbiology, Faculty of science, Ain Shams University for here supervision, continuous help, encouragement throughout this work and great effort she has done in the meticulous revision of the whole work. It is a great honor to work under her guidance and supervision.

I am also, I would like to express my sincere gratitude to supervisor **Prof. Dr. Mohammed Farok Ghaly**, Professor of Microbiology, Faculty of Science, Zagazig University for his encouragement, valuable advice and continuous assistance during the work and revising of thesis.

Also, grateful to **Dr. Seham Abdel-Shafi Awad-Alla Hegazy**, Associate professor of Microbiology, Faculty of Science, Zagazig University for her continuous support of study and research, for her patience, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and help me with various problems that faced in my research.

Special and sincere thanks to **Dr. Ali Osman**, Associate Professor of Biochemistry, Faculty of Agriculture, Zagazig University, for his encouragement and helping me in my study. His guidance helped me in all the time of research.

Al-Shaymaa Abdel-Monaem

## **Abstract**

---

### **Abstract**

In this study the antibacterial activities of aqueous ethanolic extract of *Moringa olifera* collected from El- Shabanat village at Zagazig (Egypt) was examined. The organic solvent extract was tested against some pathogenic bacteria collected from different patients in Zagazig Hospital University (ZHU).The most sensitive bacteria for *Moringa olifera* after identification by PCR and 16SrRNA was *Staphylocooous pasteuri*.

The highest degree of antibacterial activity of whey and their fractions was shown by F2 fraction against *Staphylocooous pasteuri*. Transmission Electron Microscopy (TEM) of *Staphylocooous pasteuri* treated with aqueous ethanolic extracts of leaves and seeds of Moringa and whey fractions were manifested by signs of cellular deformation, partial and complete lysis of cell components. There is a great effect of *Moringa olifera* than different types of antibiotics against *Staphylococcus pasteuri* as the indicator organism. Phenolic, flavenoids compounds and antioxidant activity were quantitatively detected in leaves and seeds; the higher ratio of phenolic and flavenoid was detected in leaves. (584.7mg/g and 95mg/g respectively) and the higher ratio of antioxidant activity was detected in leaves (101.7mg/g) after 120 min..The ethanolic extract of leaves and seeds and also whey as raw material and their fractions were tested for their ability to formation of nanoparticles and their ability to inhibit isolated identified bacteria. Our results showed that the ability of nanoparticles of Moringa plant and whey to inhibit pathogenic bacteria decreases compared to raw plant and raw whey and their fractions.

## ***List of Abbreviation***

---

### **List of Abbreviation**

AgNPs	Silver Nano particles
AMPs	Antimicrobial peptides
APS	Ammonium per sulphate
AuNPs	Gold nano particles
BV	Biological value
BW	Buffalo whey
BWP	Buffalo whey protein
BWH	Buffalo whey hydrolzate
CMS	Compact mass spectrometer
CNS	Coagulase-negative staphylococcus
CNTs	Carbon nanotubes
CO <sub>2</sub>	Carbon dioxide
CPS	Coagulase-positive staphylococcus
CuNPs	Copper nano particles
DH	Degree of hydrolysis
DNase	Deoxyribonuclease
DPPH	Di Phenyl picryl hydrazene
EA	Ethyl alcohol
ESI	Electro spray ionization
F	Female
F1	Fraction 1
F2	Fraction 2
F3	Fraction 3
FT-IR	Fourier Transform Infra-Red
GMP	Glycol macro peptides
GSH	Glutathione
H	Hexane
<i>H. pylori</i>	<i>Helicobacter pylori</i>
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCMV	Human Cytomegalo virus
HIV	Human immunodeficiency virus
HPV	Human papilloma virus
IL-8	Inter leukin-8
IQE	Iso querçetin equivalents
IZD	Inhibition zones diameter
KBr	Potassium bromide
L	Lane marker
LDL	Low-density lipoprotein
LF	Lactoferrin

## ***List of Abbreviation***

---

M	Male
<i>M.pygmaea</i>	<i>Moringa pygmaea</i>
<i>M. arborea</i>	<i>Moringa arborea</i>
<i>M. borziana</i>	<i>Moringa borziana</i>
<i>M. corcanensis</i>	<i>Moringa corcanensis</i>
<i>M. drouhardii</i>	<i>Moringa drouhardii</i>
<i>M. hildebrandtii</i>	<i>Moringa hildebrandtii</i>
<i>M. longituba</i>	<i>Moringa longituba</i>
<i>M. oleifera</i>	<i>Moringa oleifera</i>
<i>M. ovalifolia</i>	<i>Moringa ovalifolia</i>
<i>M. peregrine</i>	<i>Moringa peregrine</i>
<i>M. rivae</i>	<i>Moringa rivae</i>
<i>M. ruspoliana</i>	<i>Moringa ruspoliana</i>
<i>M. stenopetala</i>	<i>Moringa stenopetala</i>
MIC	Minimum inhibitory concentration
MW	Molecular weight
MWCNTs	Multi-walled carbon nanotubes
Neg	Negative control
PC	Paper chromatography
PCR	Polymerase chain reaction
Pos	Positive control
S	Sample
SA	Serum albumin
SAgs	Super antigens
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC-F1	Size exclusion chromatography Fraction No. 1
SEC-F2	Size exclusion chromatography Fraction No. 2
SEC-F3	Size exclusion chromatography Fraction No.3
SEM	Scanning electron microscopy
SiNPs	Silicon nanoparticles
<i>Staph. pasteurii</i>	<i>Staphylococcus pasteurii</i>
SWCNTs	Single walled carbon nanotubes
TCA	Trichloro acetic acid
TEM	Transmission electron microscope
TEMED	Tetra Methyl Ethylen Diamine
TiO <sub>2</sub>	Titanium dioxide
TiO <sub>2</sub> NPs	Titanium dioxide nanoparticles
TSST-1	Toxic shock syndrome toxin
Urea-PAGE	Urea polyacrylamide gel electrophoresis
UTI	Urinary tract infection

## ***List of Abbreviation***

---

UV	Ultra violet
W	Water
WHO	World health organization
WP	Whey protein
ZHU	Zagazig hospital university
ZnO	Zinc oxide
ZnONPs	Zinc oxide nanoparticles
$\alpha$ -la	$\alpha$ -lactabumin
$\beta$ -lg	$\beta$ -lactoglobulin

## ***List of contents***

---

## **List of Contents**

<b><i>Subject</i></b>	<b><i>Page</i></b>
Abstract	I
List of abbreviations	II
List of contents	V
List of tables	VIII
List of figures	IX
Aim of the study.	XII
<b>I- Introduction</b>	1
<b>II- Review of Literature</b>	4
<b>III- Materials and Methods</b>	42
1. Collection of pathogenic bacteria	42
2. Media used for cultivation of tested bacteria	42
3. Biochemical tests	43
4. Molecular identification of selected isolate by using PCR	45
5. Methods of identification of tested microorganism by using PCR	47
6- Whey protein	50
6.1. Preparation of whey protein	50
6.2. Degree of hydrolysis	51
6.3. Production of milk protein hydrolysates and fractions	51
6.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	52
7.4. Urea polyacrylamide gel electrophoresis (Urea-PAGE)	56
8.4. Fourier Transform Infrared (FTIR) Spectroscopy	58
9.4. Antibacterial activity evaluation of buffalo whey protein and fractions	59
10.4. Determination of MIC values of whey protein	59
7. Plant Source	60
7.1. Preparation of moringa plant extract	60
7.2. Antibacterial activity evaluation of Moringa leaves and seeds ethanolic extract	60
7.3. Determination of MIC values of <i>M. oleifera</i> ethanolic extract	61
7.4. Quantitative inhibition of aqueous ethanolic <i>Moringa oleifera</i> extract	61
7.5. Antibacterial compounds isolation and purification	61
7.5.1. Chromatography	61
7.5.2. Fourier transform infrared (FTIR) spectroscopy	62

## ***List of contents***

---

7.5.3. UV spectroscopy (selection of $\lambda_{\max}$ )	62
7.5.4. Mass analysis	63
7.5.5. Determination of total phenolic compounds content	63
7.5.6 .Determination of total flavonoid compounds content	64
7.5.7. Antioxidant activity	65
8- Synthesis of nanoparticles from Whey and <i>M. oleifera</i>	66
8.1. Purification and concentration of AgNPs	66
8.2. Antibacterial activity of nanoparticles from Whey and <i>M. oleifera</i>	67
8.3. Scanning electron microscopy (SEM) for Nanoparticles	67
9. Transmission electron microscopy (TEM)	67
10.Chemicals and Reagents	69
11. Statistical analysis	69
<b>IV- RESULTS</b>	70
1. Isolation of pathogenic bacteria from patients	70
2. Screening of Moringa extracts and whey protein against bacterial isolates	75
3. Biochemical tests for selective bacteria	78
4. Molecular identification of the selected bacterium	79
5. Degree of hydrolysis of whey	82
6. Molecular masses of whey protein as obtained by SDS-PAGE	83
7. Molecular masses of whey protein as obtained by Urea Polyacrylamide gel electrophoresis (Urea-PAGE)	86
8. Fourier Transform Infrared (FT-IR) Spectroscopy	87
9. Antibacterial activity evaluation of buffalo whey protein and their fractions	90
10. Minimum Inhibitory Concentration (MIC) of buffalo whey protein	91
11. Evaluation of the antibacterial activities of the mixture of F2 whey protein and antibiotics	93
12. Transmission Electron Microscope (TEM) Image Analysis of F2	97
13. UV-vis spectroscopy	99
14. Scanning Electron Microscope analysis (SEM) for nanoparticles from F2	100
15. Inhibition of bacterial growth by nanoparticles from F2 whey protein	101
16. Antibacterial activity evaluation of moringa leaves and seeds	102
17. Minimum Inhibitory Concentration (MIC) of moringa	103

## ***List of contents***

---

leaves and seeds	
18. Quantitative inhibition of <i>Staphylococcus pasteurii</i> by aqueous ethanolic extract of leaves and seeds	105
19. Effect of antibiotic combination with moringa extracts	106
20. Detection and purification of antibacterial compounds by chromatography	110
21A. Fourier transforms infrared (FT-IR) spectroscopy of purified compounds from moringa leaves and seeds by paper chromatography	112
21 B. UV spectroscopy (selection of $\lambda_{\max}$ )	118
21 C. GC-Mass analysis	121
22. Transmission electron microscope (TEM) analysis of Staph. pasteurii treated with moringa leaves and seeds	130
23. Estimation of total Phenolic and flavenoids compounds in moringa extract	132
24. Estimation of antioxidant activity of moringa leaves and seeds ethanolic extract	136
25. UV-vis spectroscopy for nanoparticles from <i>M. olifera</i>	138
26. Scanning electron microscope analysis (SEM) for nanoparticles from moringa leaves	139
27. Inhibition of Staph.pasteuri growth by nanoparticles from moringa leaves nanoparticles	140
<b>V- Discussion</b>	141
<b>VI- English Summary</b>	155
<b>VII- Conclusion</b>	159
<b>VIII- Recommendations</b>	160
<b>IX- References</b>	161
<b>الملخص العربي</b>	