

**Faculty of Veterinary Medicine
Cairo University
Department of Microbiology**



Study on the antimicrobial activity of some plants

A Thesis Presented By

Doaa Mohamed Moawad El-Tahan

(B.V.Sc. Faculty of Vet. Medicine, Cairo University 2009)

**For The Degree of
Master in Veterinary Medical Science
(Microbiology)**

Under Supervision of

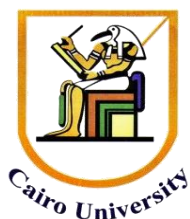
Prof. Dr. Heidy Mohamed Shawky Abo El-Yazeed

Professor of Microbiology
Faculty of Veterinary Medicine, Cairo University

**Dr.
Sherin Ibrahim Ibrahim**
Lecturer of pharmacology
Faculty of Veterinary Medicine
Cairo University

**Prof. Dr.
Ashraf Mohamed Barakat**
Head of Zoonoses Department
National Research Centre

2019



**Faculty of Veterinary Medicine
Cairo University
Department of Microbiology**



Supervision sheet

Study on the antimicrobial activity of some plants

A Thesis Presented By

Doaa Mohamed Moawad El-Tahan

(B.V.Sc. Faculty of Vet. Medicine, Cairo University 2009)

Under Supervision of

Prof. Dr. Heidy Mohamed Shawky Abo El-Yazeed

Professor of Microbiology
Faculty of Veterinary Medicine, Cairo University

Dr. Sherin Ibrahim Ibrahim

Lecturer of pharmacology
Faculty of Veterinary Medicine, Cairo University

Prof. Dr. Ashraf Mohamed Barakat

Head of Zoonoses Department
National Research Centre

2019



كلية الطب البيطرى
جامعة القاهرة
قسم الميكروبيولوجيا



لجنة الإشراف

دراسة عن النشاط المضاد للميكروبات لبعض النباتات

رسالة مقدمة من

ط.ب. دعاء محمد معوض الطحان

بكالوريوس العلوم الطبية البيطرية-جامعة القاهرة (2009)

تحت إشراف

أ.د. هايدى محمد شوقى أبو اليزيد

أستاذ الميكروبيولوجيا
كلية الطب البيطرى - جامعة القاهرة

د. شيرين إبراهيم إبراهيم

مدرس الأدوية
كلية الطب البيطرى
جامعة القاهرة

أ.د. أشرف محمد بركات

رئيس قسم الأمراض المشتركة
المركز القومى للبحوث

2019

Name: Doaa Mohamed Moawad El-Tahan **Birth date:** 17/1/1987

Nationality: Egyptian

Scientific degree: Master degree (Microbiology)

Title of Thesis: Study on the antimicrobial activity of some plants

Prof. Dr. Heidy Mohamed Shawky Abo El-Yazeed

Professor of Microbiology - Faculty of Veterinary Medicine - Cairo University

Dr. Sherin Ibrahim Ibrahim

Lecturer of pharmacology- Faculty of Veterinary Medicine - Cairo University

Prof. Dr. Ashraf Mohamed Barakat

Head of Zoonoses Department- National Research Centre

ABSTRACT

Many edible plants have been used since ancient time to control microbial infections. This study was designed for evaluating the antibacterial effect of the crude extracts of the tested herbal plants (*Citrillus colocynthis* fruits and seeds, *Cucurbita pepo* fruits and seeds, *Moringa oleifera* leaves) against some bacterial pathogens, namely methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella* Typhi, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Extraction method was applied for previously mentioned plants using methanol 70% then fractionation of these crude extracts was done by solubilization in water. The broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) of the studied extracts. Also the minimum bactericidal concentration (MBC) was determined by subculturing on antibiotic free nutrient rich agar plates. It was found that, MIC results of *Citrullus colocynthis* fruits and seeds extract revealed that the butanol and methanol extracts had the highest inhibition effect but hexane extract had the lowest one. Concerning MBC results of *Citrullus colocynthis* fruits and seeds extract it revealed that the methanol extract had the highest cidal effect but hexane and chloroform extracts had the lowest one. MIC results of *Cucurbita*

pepo fruits and seeds extract revealed that the butanol extract had the highest inhibition effect but hexane extract had the lowest one. Concerning MBC results of *Cucurbita pepo* fruits, it revealed that the methanol, hexane and butanol extracts had the highest cidal effect but chloroform extract had the lowest one. While MBC results of *Cucurbita pepo* seeds revealed that the chloroform extract had the highest cidal effect but hexane extract had the lowest one. MIC results of *Moringa oleifera* leaves revealed that the butanol extract had the highest inhibition effect specifically against *Pseudomonas aeruginosa* with MIC 160 µg/ml but hexane extract had the lowest one. Concerning MBC results, it revealed that the methanol and hexane extracts had the highest cidal effect while chloroform and butanol extracts had the lowest one. These results confirm the traditional claims and provide promising baseline information for the potential use of the tested extracts against bacterial infections.

Keywords:

Antibacterial, MRSA, *Salmonella* Typhi, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella pneumoniae*, Extract, MIC, MBC, *Moringa oleifera*, *Citrillus colocynthis*, *Cucurbita pepo*.

To

My beloved Mother,

My Father,

My husband Hegazy,

My mother in law

And my brothers

Acknowledgment

I am, and will always be indebted to Allah, the most Gracious and the most Merciful, the bounties of whom I can never reckon.

*It is a great pleasure to thank **Prof. Dr. Heidy Abo El-Yazeed**, Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, for her kind supervision, support, creative and comprehensive advice during the course of this study.*

*I would like to record my gratitude and my deep thanks to **Dr. Sherin Ibrahim**, lecturer of Pharmacology, Faculty of Veterinary Medicine, Cairo University, for her help and kind advice.*

*I wish to express my unlimited gratitude to **prof. Dr. Ashraf Barakat**, Professor of zoonoses, National Research Centre, for his useful help and assistance in this work,*

*I would like to express my gratitude to my track guide, **Dr. Mahmoud El-Hariri**, Assistant Professor of Microbiology, Faculty of Veterinary Medicine, Cairo University, whose expertise, understanding, and patience, added considerably to my graduate experience. His comments and kind help during the course of this work are greatly appreciated.*

*My gratitude extends, to **Prof. Dr. Abdel Mohsen Hammam**, Professor of Reproductive pharmacology, National Research Centre for his continuous help, support and advice.*

*I would like to express my special appreciation and thanks to **Prof. Dr. Ahmed El-Sherif**, Professor of Microbiology, Faculty of Pharmacy, Cairo University.*

*Finally, I would like to thank my dear friends **Heba Hussein** and **Nashwa Moatez** for their kind support and my colleagues **Dr. Ahmed Roshdy** and **Mr. Islam Hosny** for their endless help.*

LIST OF CONTENTS:

1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	5
2.1.Urgent need for new antimicrobial agents	5
2.2.Plants as a source of new antimicrobial compounds	7
2.3.Screening of natural products for drug discovery	12
2.4.Medicinal plants and antimicrobial activity	15
2.5.Examples of some plants used as antimicrobial agents:	16
2.5.1. <i>Citrullus colocynthis</i>	16
2.5.2. <i>Cucurbita pepo</i>	25
2.5.3. <i>Moringa oleifera</i>	34
3. MATERIAL AND METHODS.....	43
3.1.Materials:	43
3.2.Methods:	46
3.2.1. Reagent preparation	46
3.2.2. Collection and preparation of the selected plants	48
3.2.3. Preparation of plant extracts	48
3.2.4. Preparation of stock solution	49
3.2.5. Dilution of active principles	50
3.2.6. Preparation of the bacterial suspension	50
3.2.7. Determination of MIC for the plant extracts	51
3.2.8. Determination of MBC for the plant extracts	52
3.2.9. Statistical analysis	53
4.RESULTS.....	54
4.1.Plant material and extraction:	54
4.2.Determination of minimum inhibitory concentration (MIC) for the different plant extracts:	54
4.3.Determination of minimum bactericidal concentration (MBC) for the different plant extracts:	82
5.DISCUSSION.....	106
6.SUMMARY.....	118
7.REFERENCES.....	121

List of Figures:

Figure (1). Different parts of <i>C. colocynthis</i> fruits and seeds.....	17
Figure (2). Different parts of <i>Cucurbita pepo</i> fruits and seeds.....	26
Figure (3). <i>Moringa oleifera</i> leaves.....	35
Figure (4). Minimum inhibitory concentration (MIC) of methanol extract of different plants on the tested microorganisms.....	56
Figure (5). Minimum inhibitory concentration (MIC) of hexane extract of different plants on the tested microorganisms.....	58
Figure (6). Minimum inhibitory concentration (MIC) of chloroform extract of different plants on the tested microorganisms.....	60
Figure (7). Minimum inhibitory concentration (MIC) of butanol extract of different plants on the tested microorganism.....	62
Figure (8). Effect of different solvents on the inhibition of MRSA growth.....	64
Figure (9). Effect of plant extracts on the inhibition of MRSA growth.....	65
Figure (10). The combined effect of different solvents and plant extracts on the inhibition of MRSA growth.....	65
Figure (11). MIC test of <i>Citrullus colocynthis</i> seeds extract clarifying the inhibitory effect for MRSA.....	66
Figure (12). Effect of different solvents on the inhibition of <i>Salmonella</i> Typhi growth.....	68
Figure (13). Effect of plant extracts on the inhibition of <i>Salmonella</i> Typhi growth.....	68
Figure (14). The combined effect of different solvents and plant extracts on the inhibition of <i>Salmonella</i> Typhi growth.....	69

Figure (15). MIC test of <i>Citrullus colocynthis</i> seeds extract clarifying the inhibitory effect for <i>Salmonella</i> Typhi.....	69
Figure (16). Effect of different solvents on the inhibition of <i>Pseudomonas aeruginosa</i> growth.....	71
Figure (17). Effect of plant extracts on the inhibition of <i>Pseudomonas aeruginosa</i> growth.....	72
Figure (18). The combined effect of different solvents and plant extracts on the inhibition of <i>Pseudomonas aeruginosa</i> growth.....	72
Figure (19). MIC test of <i>Cucurbita pepo</i> seeds extract clarifying the inhibitory effect for <i>Pseudomonas aeruginosa</i>	73
Figure (20). MIC test of <i>Moringa</i> leaves extract clarifying the inhibitory effect for <i>Pseudomonas aeruginosa</i>	73
Figure (21). Effect of different solvents on the inhibition of <i>E.coli</i> growth.....	76
Figure (22). Effect of the plant extracts on the inhibition of <i>E.coli</i> growth.....	76
Figure (23). The combined effect of different solvents and plant extracts on the inhibition of <i>E.coli</i> growth.....	77
Figure (24). MIC test of <i>Cucurbita pepo</i> fruits extract clarifying the inhibitory effect for <i>E.coli</i>	77
Figure (25). MIC test of <i>Moringa</i> leaves extract clarifying the inhibitory effect for <i>E.coli</i>	78
Figure (26). Effect of different solvents on the inhibition of <i>Klebsiella pneumoniae</i> growth.....	80
Figure (27). Effect of plant extracts on the inhibition of <i>Klebsiella pneumoniae</i> growth.....	81

Figure (28). The combined effect of solvent and plant extract on the inhibition of <i>Klebsiella pneumoniae</i> growth.....	81
Figure (29). MIC test of <i>Cucurbita pepo</i> seeds extract clarifying the inhibitory effect for <i>Klebsiella pneumoniae</i>	82
Figure (30). Minimum bactericidal concentration (MBC) of the methanol extract of different plants on the tested microorganisms...	83
Figure (31). Minimum bactericidal concentration (MBC) for the hexane extract of different plants on the tested microorganisms.....	85
Figure (32). Minimum bactericidal concentration (MBC) of the chloroform extract of different plants on the tested microorganisms	86
Figure (33). Minimum bactericidal concentration (MBC) of the butanol extract of different plants on the tested microorganisms.....	88
Figure (34). The cidal effect of different solvents on MRSA growth.....	90
Figure (35). The cidal effect of plant extracts on MRSA growth.....	91
Figure (36). The combined cidal effect of different solvents and plant extracts on MRSA growth.....	91
Figure (37). The cidal effect of different solvents on <i>Salmonella</i> Typhi growth.....	93
Figure (38). The cidal effect of plant extracts on <i>Salmonella</i> Typhi growth.....	94
Figure (39). The combined cidal effect of different solvents and plant extracts on <i>Salmonella</i> Typhi growth.....	94
Figure (40). The cidal effect of different solvents on <i>Pseudomonas aeruginosa</i> growth.....	96

Figure (41). The cidal effect of plant extracts on <i>Pseudomonas aeruginosa</i> growth.....	98
Figure (42). The combined cidal effect of different solvents and plant extracts on <i>Pseudomonas aeruginosa</i> growth.....	98
Figure (43). The cidal effect of different solvents on <i>E.coli</i> growth.....	100
Figure (44).The cidal effect of plant extracts on <i>E.coli</i> growth....	101
Figure (45).The combined cidal effect of different solvents and plant extracts on <i>E.coli</i> growth.....	101
Figure (46). MBC test of different plant extracts clarifying the cidal effect for <i>E.coli</i>	102
Figure (47). The cidal effect of different solvents on <i>Klebsiella pneumoniae</i> growth.....	103
Figure (48).The cidal effect of plant extracts on <i>Klebsiella pneumoniae</i> growth.....	104
Figure (49). The combined cidal effect of different solvents and plant extracts on <i>Klebsiella pneumoniae</i> growth.....	104
Figure (50). MBC test of different plant extracts clarifying the cidal effect for <i>Klebsiella pneumoniae</i>	105

List of Tables

Table (1). The chemical composition of the dried <i>Cucurbita pepo</i> seeds.....	29
Table (2). The yield of different solvents used for extraction of each plant sample.....	54
Table (3). The minimum inhibitory concentration (MIC) of the methanol extract.....	55
Table (4). The minimum inhibitory concentration (MIC) of the hexane extract.....	57
Table (5). The minimum inhibitory concentration (MIC) of the chloroform extract.....	59
Table (6). The minimum inhibitory concentration (MIC) of the butanol extract.....	61
Table (7). ANOVA test explains the effect of different solvents and different plants on the inhibition of MRSA growth.....	63
Table (8). Pair wise comparison for the effect of different solvents on the inhibition of MRSA growth.....	64
Table (9). ANOVA test explains the effect of different solvents and different plants on the inhibition of <i>Salmonella</i> Typhi growth.....	67
Table (10). ANOVA test explains the effect of different solvents and different plants on the inhibition of <i>Pseudomonas aeruginosa</i> growth.....	71
Table (11). ANOVA test explains the effect of different solvents and different plants on the inhibition of <i>E.coli</i> growth.....	75
Table (12). Pair wise comparison for the effect of different solvents on the inhibition of <i>E.coli</i> growth.....	75

Table (13). ANOVA test explains the effect of different solvents and different plants on the inhibition of <i>Klebsiella pneumoniae</i> growth.	79
Table (14). Pair wise comparison for the effect of different solvents on the inhibition of <i>Klebsiella pneumoniae</i> growth.....	80
Table (15). The minimum bactericidal concentration (MBC) of the methanol extract.....	83
Table (16). The minimum bactericidal concentration (MBC) of the hexane extract.....	84
Table (17). The minimum bactericidal concentration (MBC) of the chloroform extract.....	86
Table (18). The Minimum bactericidal concentration (MBC) of the butanol extract.....	88
Table (19). ANOVA test explains the effect of different solvents and different plants on the cidal effect of MRSA growth.....	90
Table (20). ANOVA test explains the effect of different solvents and different plants on the cidal effect of <i>Salmonella</i> Typhi growth.....	93
Table (21). ANOVA test explains the effect of different solvents and different plants on the cidal effect of <i>Pseudomonas aeruginosa</i> growth.....	96
Table (22). Pair wise comparison for the cidal effect of plant extracts on <i>Pseudomonas aeruginosa</i>	97
Table (23). ANOVA test explains the effect of different solvents and different plants on the cidal effect of <i>E.coli</i> growth.....	100
Table (24). ANOVA test explains the effect of different solvents and different plants on the cidal effect of <i>Klebsiella pneumoniae</i> growth.....	103

List of Abbreviations

AIDS	Acquired Immune Deficiency Syndrome.
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
CFU	Colony Forming Unit.
DMSO	Dimethyl Sulfoxide.
HIV	Human Immunodeficiency Virus.
MBC	Minimum Bactericidal Concentration.
MDR	Multidrug Resistance.
MFC	Minimum Fungicidal Concentration.
MHA	Mueller-Hinton Agar.
MHB	Mueller-Hinton Broth.
MIC	Minimum Inhibitory Concentration.
mm	Millimeter.
mM	Millimolar.
M.O.	Microorganism
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
NOAEL	No Observed Adverse Effect Level.
SDS	Sodium Dodecyl Sulfate.
SM	Secondary Metabolites
WHO	World Health Organization.