



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرو فيلم



MONA MAGHRABY



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY



Ain Shams University
Faculty of Science
Microbiology
Department



Studies on the effect of oils extracted from medicinal plants on foodborne pathogens and their enzymatic activities

A Thesis

**Submitted for the degree of Doctor of Philosophy in
Science in Microbiology**

By

Sofia Mostafa Asim

**B.Sc. (Microbiology), Botany Department, Faculty of Science,
Tanta University (2009)**

**M.Sc. In Microbiology, Botany Department, Faculty of Science,
Zagazig University (2014)**

Under Supervision of

Prof. Dr.

Adel Ahmed El- Mehalawy

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

Prof. Dr.

Fawzy Gamal Khedre

Prof. of Botany
Botany and Microbiology Department,
Faculty of Science-Zagazig University

Dr.

Samar Samir Mohamed

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

Dr.

Ali Mohamed Saeed

Lecturer of Microbiology,
Microbiology Department,
Faculty of Science- Ain Shams University

2021



Ain Shams University
Faculty of Science
Microbiology
Department



Studies on the effect of oils extracted from medicinal plants on foodborne pathogens and their enzymatic activities

A Thesis

**Submitted for the degree of Doctor of Philosophy in
Science in Microbiology**

By

Sofia Mostafa Asim

**B.Sc. (Microbiology), Botany Department, Faculty of Science,
Tanta University (2009)**

**M.Sc. In Microbiology, Botany Department, Faculty of Science,
Zagazig University (2014)**

2021

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

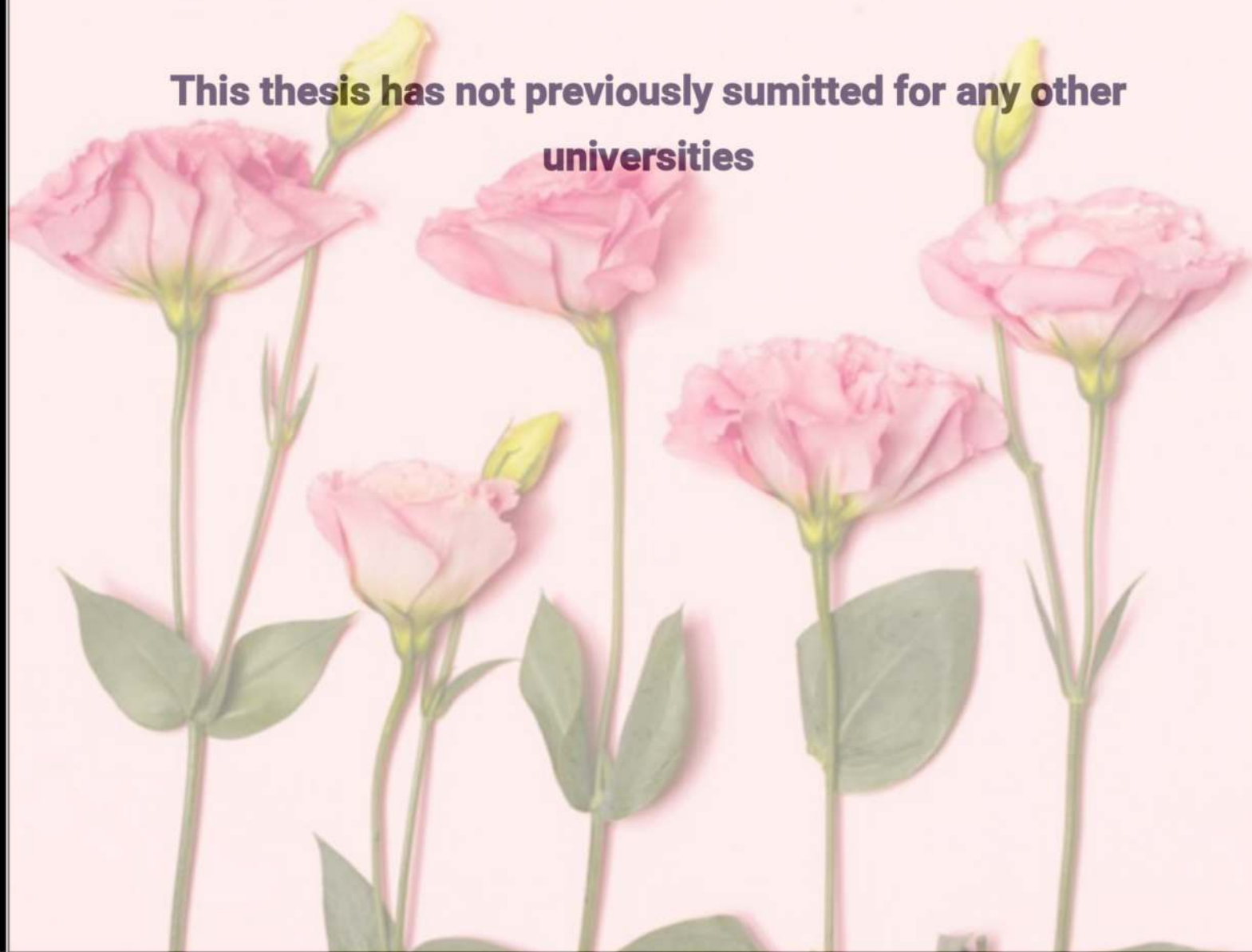
سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العليم

صدق الله العظيم

سورة البقرة الآية: ٣٢

Declaration

This thesis has not previously submitted for any other universities



ACKNOWLEDGMENT

Praise and thanks to Allah 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. Prof. Dr. Adel Ahmed El Mahalawy, Prof. of Fungi, Microbiology Department, Faculty of Science-Ain Shams University, for his supervision, continuous help, encouragement throughout this work and great effort he has done in the meticulous revision of the whole work. It's a great honor to work under his guidance and supervision.

Also, grateful to supervisor Prof. Dr. Fawzy Gamal Khedre, Prof. of Botany, Botany and Microbiology Department, Faculty of Science-Zagazig University, for his encouragement, valuable advice and continuous assistance during the work.

Also, I would like to express my sincere gratitude to Dr. Samar Samir Mohamed, Assistant Prof. of Microbiology, Microbiology Department, Faculty of Science-Ain Shams University, for her continuous support of study and research, for her patience motivation, enthusiasm and immense knowledge. Her guidance helped me with various problems that faced in my research. Special and sincere thanks to for her revising of thesis and her guidance that helped me in all the time of research.

Also, thanks to Dr. Ali Mohamed Saeed, Lecturer of Microbiology, Microbiology Department, Faculty of Science- Ain Shams University, for his encouragement and helping me in my study.

Sofia Mostafa Asim

Abstract

Abstract

Essential oils (EOs) are naturally occurring antimicrobials found in many plants that have been shown to be effective in a variety of applications by decreasing growth and survival of microorganisms. In addition, EOs exhibit antimicrobial properties that may make them suitable alternatives to antibiotics. These potential attributes and an increasing demand for natural food additive options have led to an interest in the use of EOs as potential alternative antimicrobials.

This study was to determine the efficacy of essential oils as antimicrobial against foodborne pathogens. Foodborne pathogens were isolated from different food sources as meat, chicken, fish, luncheon meat, bread, butter milk, liver of chicken shrimp, yogurt, pancake, tuna, mango juice, cocktail juice, cheese and tomato. The most common microorganisms were bacteria of (*E. coli*, *Pseudomonas sp.*, *Klebsella sp.* and *Bacillus sp.*) and fungi of (*Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus fumigatus*,). In this study, ten commercially available essential oils were used (*Tea tree oil*, *Lavender oil*, *Eucalyptus oil*, *Rosemary oil*, *Lemongras oil*, *Basil oil*, *Olbaum oil*, *Cumin oil*, *onion oil* and *Cress oil*) as antimicrobial against the isolated foodborne pathogens. The antimicrobial activity of the essential oils was assayed using the agar diffusion method.

The minimum inhibitory concentration (MIC) of the essential oils on the tested microorganisms was determined and the most effective essential oils as antibacterials were: *Lemongras* oil against *Pseudomonas sp.* with MIC of 0.031 µg/µl and *Eucalyptus* oil and *Tea tree* oil against *Bacillus sp.* with MIC of 0.125 µg/µl. The most effective essential oils as antifungals were: *Tea tree* oil against *Aspergillus flavus* with MIC of 0.125 µg/µl, *Tea tree oil* against *Aspergillus teries* with MIC of 0.062 µg/µl and *Lemongras* oil against *Aspergillus flavus* with MIC of 0.03 µg/µl.

It was found also that the selected essential oils had effect on the activity of (protease, lactase, lipase and amylase) enzymes of foodborne microorganisms. It was observed that enzyme activity for the microorganisms decreased with 80% after adding the essential oils to the growth media. GC mass was made to the most effective essential oil and the most effective components.

Keywords: Essential oils, Foodborne pathogens, *Lemongras oil*, *Basil oil*, *Olbaum oil*, protease, lactase, amylase, *Pseudomonas sp.*, *Bacillus sp*, *Aspergillus flavus*, *Aspergillus teries*, *Aspergillus fumegatus*.

List of Abbreviations

Symbols	List of Abbreviations
%	Percent
°C	Degree centigrade
µg	Microgram
µl	Microlitre
CFU	Colony forming units
g	gram
hrs	hours
TTO	Tea tree oil
Lav.	Lavender
Euc.	Eucalyptus
Ros.	Rosemary
Lmn.	lemongrass
Olb.	Olbaum
Cum.	Cumin
CAZ	Ceftazidime
AMB	amphotericin B
MIC	Minimum inhibitory concentration
MBC	Minimum Bactericidal Concentration
MFC	Minimum Fungicidal Concentration
TEM	Transmission electron microscopic
FL	Fibrillar layer
CW	Cell wall
PM	Plasma membrane
SP.	Space
V	Vacuole
TLC	Thin Layer Chromatography
GC-MS	Gas chromatography–mass spectrometry

List of Contents

Subject	Page
Abstract	I
List of abbreviations	IV
List of contents	V
List of tables	VII
List of figures	VIII
Aim of the study.	IX
1- Introduction	1
2- Review of Literature	6
3- Materials and Methods	31
3.1 Materials	32
3.2 Methods	38
3.2.1 Samples Collection:	38
3.2.2 Isolation of foodborne microorganisms	40
3.2.3 Coliform count (Most probable number)	41
3.2.4 Identification of foodborne microorganisms	42
3.2.4.1 Identification of foodborne bacteria	42
3.2.4.2 Identification of foodborne fungi	47
3.2.5 Antimicrobial activity of essential oils	48
3.2.6 Determination of MIC, MBC and MFC	49
3.2.7 Transmission electron microscopy	50
3.2.8 Fractionation of active compounds of lemongrass and tea tree oils by thin layer chromatography	51
3.2.9 GC-MS analysis	51
3.2.10 Effect of tea tree, lemongrass oils and active compounds on enzymatic activity of most sensitive pathogens	52
Statistical analysis	55
4- RESULTS	57
4.1. Isolation of foodborne microorganisms	58
4. 2. Identification of foodborne pathogens	61
4.2.1. Identification of foodborne bacteria	62
4.2.2 Identification of isolated foodborne fungi	63
4.3. Distribution and frequency of bacterial isolates among different food samples	66

List of contents

Subject	Page
4.4. Distribution and frequency of fungal isolates among different food samples	67
4.5. Antimicrobial activities of essential oils against foodborne bacterial and fungal pathogens	69
4.6. MIC, MBC and MFC values of tested essential oils against foodborne pathogens	73
4.7. Transmission electron microscope	76
4.7.1 Action mode of lemongrass oil as antibacterial mechanism through transmission electron microscopic (TEM) examination	82
4.7.2 Action mode of Tea tree oil as antifungal mechanism through transmission electron microscopic (TEM) examination	83
4.8. TLC and antimicrobial activity of fractions	84
4.9. GC–MASS analysis	86
4.10. Effect of essential oils and active compounds on enzymatic activity of <i>Pseudomonas aeruginosa</i> and <i>Aspergillus flavus</i>	88
5. Discussion	89
6. Conclusion	97
8. Summary	99
8. Summary (English)	100
7. References	106
8. الملخص العربي	-

List of Tables

No.	Title	Page
1	Food samples collected from different sources	39
2	Total bacterial, coliforms count and fungal count isolated from the collected food samples	58
3	Macromorphological characteristic of the food borne bacterial isolates.	61
4	Biochemical characteristics of foodborne bacterial isolates.	62
5	Micro and Macro morphological characters of isolated food borne fungi.	63
6	Distribution and frequency of bacterial isolates among different food sources.	67
7	Distribution and frequency of fungal isolates among different food sources	68
8	Effect of essential oils on bacterial growth	70
9	Effect of the tested essential oils on fungal isolates growth	73
10	MIC and MBC values of tested essential oils against bacterial foodborne pathogens	77
11	MIC and MFC values of the tested essential oils against the tested fungi	79
12	Antimicrobial activity of extracted fractions of lemongrass and tea tree oils against <i>Pseudomonas aeruginosa</i> and <i>Aspergillus flavus</i> , respectively.	85