



Antimicrobial and antitumor activities of some marine seaweed extracts

Thesis Submitted for Partial Fulfillment of the requirements for Master Degree in Microbiology

By Sohaila Ibrahim Ahmed Abotaleb

(B.Sc. Microbiology, 2013)

Supervisors

Prof. Adel Ahmed ELMehalawy

Professor of Microbiology,
Microbiology Department,
Faculty of science,
Ain Shams University

Prof. Nanis Gamal El din Alam

Professor of Microbiology, Microbiology Department, Faculty of Science, Tanta University

Dr. Ali Mohamed Ali Saeed

Lecturer of Microbiology, Microbiology Department, Faculty of science, Ain Shams University

Microbiology Department Faculty of Science Ain Shams University 2019

Acknowledgements

who ALLAH No words can ever express my sincere gratitude to guides, aids and enabled me to complete this work and blesses me in in my life everything and everywhere.

I wish to express my profound, sincere appreciation and supreme gratitude to my supervisor *Prof. Adel Ahmed ELMehalawy* Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for suggesting the topic of the present work, faithful guidance during practicing, his kind effort in constructing the data in the final form, unlimited help and guidance during all stages and critical reading and revising the manuscript.

I am deeply indebted to my supervisor *Prof. Nanis Gamal El din Alam* Professor of Microbiology, Microbiology Department, Faculty of Science, Tanta University who help, stimulating suggestions, her valuable advise during the study, revising the manuscript, encouragement and assistance during the practical work and writing of this thesis.

I am deeply indebted to my supervisor *Dr. Ali Mohamed Ali Saeed* Lecturer of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for his stimulating suggestions, valuable advise during the study, revising the manuscript, encouragement and help during the practical work and writing of this thesis.

I'm greatly appreciated to *Prof. Saly F. Gheda*, Assistant Professor of Phycology, Botany Department, Faculty of Science, Tanta University for her kind help for suggesting the point of research and for her valuable advice at the beginning of work.

Sohaila Abotaleb







Name: Sohaila Ibrahim Ahmed Abotaleb

Title: Antimicrobial and antitumor activities of some marine seaweed extracts

Supervisors

Prof. Adel Ahmed ELMehalaw

Professor of Microbiology, Microbiology Department, Faculty of science, Ain Shams University

Prof. Nanis Gamal El din Alam

Professor of Microbiology, Microbiology Department, Faculty of Science, Tanta University

Dr. Ali Mohamed Ali Saeed

Lecturer of Microbiology, Microbiology Department, Faculty of science, Ain Shams University

Examination committee

Prof. Ahmed Darwish Ahmed Al-Jamal

Professor of Phycology, Botany Department Faculty of Science, Al-Azhar University-boys

Prof. Heba Abdelmonem Elrefai

Professor of Natural and Microbial Products At the National Research Center

Prof. Adel Ahmed ELMehalaw

Professor of Microbiology, Microbiology Department, Faculty of science, Ain Shams University

Date of Discussion			Approval date	/	/
University Council approval	/	/			

Subject	
AIM OF THE WORK	
ABSTRACT	
1. INTRODUCTION	4
2. REVIEW OF LITERATURE	6
2.1. Seaweeds	6
2.2. Biological activities of seaweeds	11
2.2.1. Antibacterial activity	11
2.2.2. Antifungal activity	20
2.2.3. Anticancer and antitumoral activity of some seaweeds	24
2.3. Etiology (disease causatives)	
2.3.1. Bacteria causing UTI infection	
2.4. Antibiotics treatment and MDR bacteria	
2.5. Fungal pulmonary infections	
2.5.1. <i>Aspergillus</i> lives in the environment	
2.5.1.1. Aspergillus fumigatus	
2.5.1.2. Aspergillus flavus	
2.5.1.3. Aspergillus niger	
2.6. Fungal infections prevented, treated, and managed	
3. MATERIAL	
3.1. Sample collection	
3.1.1. Human bacterial and fungal species	

Subject	Page
3.1.2. Marine seaweeds	39
3.2. Preservation of seaweeds	40
3.2.1. Fresh methods	40
3.2.2. Dry methods	40
3.3. Identifiction of seaweeds	40
3.4. Media used for tested bacterial and fungal species.	40
3.4.1. Media used for bacterial species	40
3.4.2. Media used for fungal species	42
3.5. Solvent used for extraction	43
3.6. Reagents used	43
3.7. The antibiotics and antifungals used in sensitivity screening against bacterial and fungal species	43
3.7.1. Antifungals used in sensitivity screening against fungal species	43
3.7.2. The antibiotics used in sensitivity screening against bacterial species	44
3.8. Preservation of bacteria	44
3.9. Preservation of fungi	44
3.10. Preparation of algal extracts	44
3.11. Determination of antimicrobial activity of the seaweed extracts using disk diffusion method	45
3.12. Antibiotic susceptibility test of bacterial species	46
3.13. Antifungal susceptibility test of the fungal species	46

Subject	Page
3.14. Antioxidant activity	
3.14.1. Determination of total antioxidant activity	47
3.14.2. Antioxidant activity by phosphomolybdate antioxidant assay	48
3.15. Cytotoxicity assay	49
3.16. Determination of the minimum inhibitory concentration (MIC) using microtiter plates technique	51
3.17. Fractionation, purification and characterization of the antimicrobial crude extract of selected seaweeds	52
3.17.1. Colum chromatography	52
3.18. Determination of antimicrobial activity of the algal extracts fraction using disk diffusion method	
3.19. Determination of the chemical structure of antimicrobial compounds of selected seaweeds	
3.19.1. UV spectra of algal antimicrobial agent	
3.19.2. The infra-red spectra (IR) of the seaweed antimicrobial compound	
3.19.3. Gas chromatography mass spectrometry of the seaweed antimicrobial compound	
3.20. Statistical analysis	
4. RESULTS	
4.1. Identification of the seaweeds	
4.1.1. Chlorophyta (Green algae)	
4.1.2. Rhodophyta (Red algae)	
4.2. Antimicrobial activity	

Subject	Page
4.3. Antibiotic susceptibility of the pathogenic bacterial species.	66
4.4. Antifungal susceptibility of the pathogenic fungal species.	67
4.5. Determination of total antioxidant activity.	68
4.6. Cytotoxicity Assay	73
4.6.1. Cell line	74
4.7. The minimum inhibitory concentration (MIC).	77
4.8. Fractionation, purification and characterization of <i>U. lactuca</i> and <i>U. Fasciata</i> antibacterial crude extract.	78
4.8.1. <i>U. lactuca</i>	78
4.8.2. <i>U. fasciata</i>	80
4.9. Determination of the chemical structure of U. <i>lactuca</i> and U. fasciata active antimicrobial compound.	81
4.9.1. UV spectra analysis	81
4.9.2. Infra-red spectra (FTIR) spectra analysis.	83
4.9.3. Gas chromatography mass spectrometry GC-MS analysis.	84
4.9.3.1. <i>U. lactuca</i> extract	84
4.9.3.2. <i>U. fasciata</i> extract	86
5-DISCUSSION	88
SUMMARY	96
REFERENCES	100
ARABIC SUMMARY	

List of tables

Table .No	Title	Page
1	Human pathogenic bacteria	38
2	Human pathogenic fungi	38
3	Lists of antifungals used against fungal species	43
4	Lists of antibiotics used against bacterial species	44
5	Antimicrobial activity	65
6	The resistance of the tested bacteria against different authentic antibiotics	66
7	Antifungal susceptibility of clinical fungal isolates	67
8	Total antioxidant activity of the tested seaweed extract.	68
9	Antioxidant activity of tested seaweeds Phosphomolybdate antioxidant Assay	71
10	Cytotoxic activity of some compounds against human tumor cells	74
11	Minimum inhibitory concentration (MIC) of chloroform extract of <i>U. lactuca</i> and <i>U. fasciata</i> against the tested human pathogenic bacterial and fungal species	78
12	The antibacterial activities of different fractions obtained from the silica gel column chromatography against bacteria.	79
13	The antifungal activities of different fractions obtained from the silica gel column chromatography against fungi.	79
14	The fraction activities of different fractions obtained from the silica gel column chromatography against bacteria	80

List of tables

Table .No	Title	Page
15	The activities of different fractions obtained	81
16	GC-MS analysis of <i>U.lactuca</i> antimicrobial compound.	85
17	GC-MS analysis of <i>U.fasciata</i> antimicrobial compound.	87

List of figures

Figure No.	Title	Page
1	Map of Alexandria, Egypt (Abu Qir Bay area) after (Osman <i>et al.</i> , 2013)	39
2	Enteromorpha intestinalis (Linnaeus) Nees	59
3	Ulva lactuca (Linnaeus)	60
4	Ulva fasciata (Linnaeus)	61
5	Jania rubens (Linnaeus) J.V. Lamouroux	62
6	Gelidium spinosum (S.G. Gmelin) P.C. Silva	63
7	Determination of total antioxidant activity of U. lactuca chloroform extract	69
8	Determination of total antioxidant activity of U. Fasciata chloroform extract.	69
9	Determination of total antioxidant activityof E. intestinalis chloroformextract.	70
10	Determination of total antioxidant activity of G.spinosum chloroform extract.	70
11	Determination of total antioxidant activity of <i>J. rubens</i> chloroform extract.	71
12	Antioxidant activity of tested seaweeds Phosphomolybdate antioxidant Assay.	72
13	Cytotoxicity of <i>J.rubens</i> against the tested human tumor cell lines.	75
14	Cytotoxicity of <i>U.lactuca</i> aginst the tested human tumor cell lines.	75
15	Cytotoxicity of <i>U. fasciata</i> aginst the tested human tumor cell lines.	76
16	Cytotoxicity of <i>G. spinosum</i> aginst the tested human tumor cell lines.	76

List of figures

Figure No.	Title	Page
17	Cytotoxicity of DOX aginst the tested human tumor cell lines.	77
18	The UV spectrophotometer active fraction of <i>U.lactuca</i> and <i>U. Fasciata</i> crude extract resulted from column chromatography.	82
19	IR spectra of <i>U. lactuca</i> antimicrobial compound.	84
20	IR spectra of <i>U. fasciata</i> antimicrobial compound	84
21	The GC-MS chromatogram of the antimicrobial material of <i>U. lactuca</i> chloroform extract.	85
22	Chemical structure of <i>U.lactuca</i> antimicrobial compound.	86
23	The GC-MS chromatogram of the antimicrobial material of <i>U. fasciata</i> chloroform extract.	87
24	Chemical structure of <i>U. fasciata</i> antimicrobial compound	87

ABBREVIATIONS

ABPA	Allergic Broncho pulmonary Aspergillosis
CFU/ml	Colony forming unit/milliter
DMSO	dimethyl sulfoxide
E. coli	Escherichia coli
G	Gram
MS-GC	Gas chromatography mass spectrometry
GU	genitourinary
HIV	Human immunodeficiency virus
IR	red spectra The infra
Kb	Kilo base
MDR	Multi drug resistant bacteria
μg/ml	Microgram/milliter
μΙ	Microliter
MIC	Minimum Inhibitory Concentration
MRSA	Staphylococcus aureus Methicilin resistant
MS	Mass spectra
NB	Nutrient broth
P. aeruginosa	Pseudomonas aeruginosa
SD	Standard Deviation
SDA	Sabouraud's dextrose agar
U	Unit
UTI	Urinary tract infection
UV	Ultraviolet
WHO	World Health Organization
W/V	volume / Weight



The aim of this research work is to evaluate the algal extracts as potential natural antimicrobial substances, to study the effects of such extracts as antioxidant and anticancer.

ABSTRACT

The present study evaluated ethanol, methanol, ethyl acetate, hexane, chloroform and acetone extracts of five green and red seaweed species from Abu-Qir bay, Alexandria, Egypt for their antimicrobial, antioxidant activities and cytotoxicity against four cell lines. Chloroform extracts of *Ulva lactuca* and *Ulva fasciata* exhibited the highest inhibition zones against the tested pathogenic bacteria (Klebsiella pneumoniae and Proteus mirabilis) and fungi (Aspergillus flavus, Aspergillus fumingatus and Aspergillus niger) as measured by disc diffusion method. The extracts of *U*. lactuca and U. fasciata showed the highest antioxidant activity (IC₅₀ 6.32 ± 0.29 mg/ml and 6.61 ± 0.27 mg/ml, using DPPH respectively), (2, 2diphenyl-1picrylhydrazyl) scavenging method and total antioxidant capacity assay (2.13 and 1.51 mg ascorbic acid equivalent /gram dry weight, respectively).