



Antimicrobial activities of lectins of some leguminous seeds

Thesis submitted in partial fulfillment of the master degree of microbiology.

Microbiology Department Faculty of Science Ain Shams University

By

Mervat Mounir Soliman Mohammed

B.Sc. In Botany/ Chemistry (2006) Faculty of Science Ain-Shams University

Supervisors

Dr. Einas Hamed El-Shatoury

Dr. Magda Mahmoud Ibrahim EL-Araby

Associate Professor of Microbiology

Microbiology Department,

Faculty of Science,

Ain Shams University

Professor of Plant Physiology

Botany Department,

Faculty of Science,

Ain Shams University

Microbiology Department Faculty of Science Ain Shams University 2017





Antimicrobial activities of lectins of some leguminous seeds

Thesis submitted in partial fulfillment of the master degree of microbiology.

By

Mervat Mounir Soliman Mohammed

B.Sc. In Botany/ Chemistry (2006)
Faculty of Science
Ain-Shams University

Microbiology Department Faculty of Science Ain-Shams University 2017





Approval Sheet

Title: Antimicrobial activities of lectins of some leguminous

seeds

Degree: Master in Microbiology

Name of students: Mervat Mounir Soliman Mohammed

Supervisors:

Dr. Magda Mahmoud Ibrahim EL-Araby

Professor of Plant Physiology

Botany Department,

Faculty of Science,

Ain Shams University

Dr. Einas Hamed El-Shatoury

Associate Professor of Microbiology

Microbiology Department,

Faculty of Science,

Ain Shams University

Examination Committee

Dr. Mohie El- Deen Zohair El- Fouly

Professor of Microbiology

Atomic Energy Authority

Dr. Maimona Abd El-Aziz Kord

Professor of Plant Physiology

Faculty of Science, Cairo University

Dr. Magda Mahmoud Ibrahim EL-Araby

Professor of Plant Physiology

Botany Department,

Faculty of Science,

Ain Shams University

Dr. Einas Hamed El-Shatoury

Associate Professor of Microbiology

Microbiology Department,

Faculty of Science,

Ain Shams University

Postgraduates Affairs Approvals:

Date of discussion: Saturday 24/2/2018 Microbiology

Department

Approval Stamp: Approval Date:

Faculty Council Approval University Council Approval

Acknowledgments

First of all, I am deeply grateful to Almighty Allah, for his help, bless, and his guidance to finish this thesis. Peace and blessings are upon Muhammad (S A W) who brought peaceful in the world.

I owe my deepest gratitude to my supervisor **Dr. Einas Hamed El-Shatoury** for her continuous help with kindness, and encouragement. She helped me improve my skills in Microbiology. All respect and appreciation for her efforts and support.

I extend my special thanks to my supervisor **Dr. Magda Mahmoud Ibrahim EL-Araby,** for her support, patient, valuable supervision and for guiding me at every stage of this research. All words would not enough to express my appreciation for her.

I would like to thank my thesis committee **Dr. Mohie El- Deen Zohair El- Fouly** and **Dr. Dr. Maimona Abd El-Aziz Kord**

I would like to show my gratitude to **Dr. Seham Mohamed Ali, Dr. Khaled Zakaria El-Baghdady, and Dr. Sahar Tolba** for their kindness, and encouragement throughout my research.

I would like to thank **Dr. Nasser Eldin Ibrahim** for his support, helping, and cooperation.

I also owe grateful thanks to Dr. Nessma Maher, Dr. Ayat Salah, Dr. Azza Abd El-Azíz, Dr. Amal Mohamed Hosny, Dr. Reem Hassan Hadíd, and Dr. Haítham Mohamed Sabra.

Special thanks go to the heads and members of the **Microbiology Department, and Botany Department** Faculty of Science, Ain Shams University

I would like to take this opportunity to send warm thanks to **Unit of Central Laboratory** Ain Shams University, for moral support to complete this project.

For my family

This project would not have been possible without the support of all member of **my family** to whom I am greatly indebted.

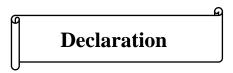
I am especially grateful to my parents, who supported me. I knew that you believed in me and wanted the best for me. Thank you to my mother for guiding me and for helping me throughout this journey.

I would like to thank my sisters, and my brother. I also dedicate my master thesis to my Daughters, and my

son.

Special thanks go to special person in my life my husband **Sayed Ahmed Mohamed** for supporting me throughout this journey.

Mervat Mounir Soliman Mohamed



This thesis is a presentation of original research work. Wherever this thesis has not been submitted previously for this or other degree in this university or any other university.

Mervat Mounir Soliman Mohamed

List of Figures		
Figure	Title	Page
(1)	Concentrations (mg/g) of fractionated lectins extracted from five leguminous seeds (Fava bean, lentil, Lima bean, Pea, and Soy bean). Lectin fractionation was done by precipitation with ammonium sulfate at 30, 70 and 90% saturations followed by extensive dialysis. Results are expressed as mean of three replicates with ± standard deviation.	50
(2)	Concentrations (mg/g) of fractionated lectins extracted from five <i>Phaseolus vulgaris</i> seeds (Bronco, Contender, 10YLHJ49, Diacole, and Shalatine). Lectin fractionation was carried out by precipitation with ammonium sulfate at 30, 70 and 90% saturations followed by extensive dialysis. Results are expressed as mean of three replicates with ± standard deviation	53

	1	
	Concentrations (mg/g) of 90% fraction of lectins extracted from (Fava bean,	
(3)	Lentil, and Pea) after purification by affinity chromatography.	55
(4-A)	SDS-PAGE profiles of fractionated partially purified Lectins extracted from five Leguminous seeds after Coomassie blue staining. Lanes 1,4,7,10 and 13 represent 90% saturation; Lanes 2,5,8,11 and 14 represent 70% saturation and Lanes 3,6,9,12 and 15 represent30% saturation. M=protein marker	57
(4-B)	SDS-PAGE profile after Coomassie blue staining of the fractionated lectins extracted from five <i>phaseolus vulgaris</i> seeds. Lanes 1,4,7,10 and 13 represent 30% saturation; Lanes 2,5,8,11 and 14 represent 70% saturation and Lanes 3,6,9,12 and 15 represent 90% saturation. M=protein marker	57
(5-A)	SDS-PAGE profiles after Coomassie blue staining of BLUeye protein marker and standard (PHA-P) lectin extracted from red kidney bean.	59

(5-B)	SDS-PAGE profile and its electropherogram of purified Lectins obtained by affinity chromatography (Fava bean, Lentil, and Pea) after Coomassie blue staining.	59
(6)	Hemagglutination activity of partially purified Lectins from Fava bean seeds (70and90% fractions) on blood group B.	61
(7)	Hemagglutination activity of partially purified Lectins from Shalatine seeds (70and90% fractions) on blood group B.	63
(8)	Antimicrobial activity measured as inhibition zones diameters (mm) of lectins extracted from five Leguminous seeds against tested microorganisms. The results are represented as mean of three replicates with ± standard deviation, and p-value≤0.05 &0.01 considered statistically significant (95&99 % confidence interval).	70

(9)	Antimicrobial activity (measured as inhibition zones diameters of lectins extracted from five cultivars of <i>Phaseolus</i> seeds against tested microorganisms. The results are represented as mean of three replicates with ± standard deviation, and p-value≤0.05 &0.01 considered statistically significant (95&99 % confidence interval).	73
(10)	shows the effect of 100µl of lectin (30,70, and 90% fractions) isolated from some tested seeds against Staphylococcus aureus Gram positive bacteria. C= control	74
(11)	shows the effect of 100µl of lectin (30,70, and 90% fractions) isolated from some tested seeds against <i>Pseudomonas aeruginosa</i> Gram - negative bacteria. C= control	75
(12)	shows the effect of 100µl of lectin (30,70, and 90% fractions) isolated from some tested seeds against <i>Candida albicans</i> . C= control	76

(13)	Effect of 100µl of lectin isolated from Shalatine seeds. A shows the inhibition zones with the Grampositive bacteria <i>Staphylococcus aureus</i> with lectin at 70% and 90% fractions. Scanning electron microscope images of	82
	Staphylococcus aureus are shown without B and with C 100µl lectin fraction 90%.	
(14)	Effect of 100µl lectin of Shalatine seeds. A shows the inhibition zones with the Gram -negative bacteria <i>Pseudomonas aeruginosa</i> with lectin at 30%, 70% and 90% fractions. Scanning electron microscope images of <i>Pseudomonas aeruginosa</i> are shown without B and with C 100µl lectin fraction 90%.	83
(15)	A shows the effect of 100µl of lectin (30,70, and 90% fractions) isolated from Fava bean seeds against <i>Staphylococcus aureus</i> Gram positive bacteria. Scanning electron microscope images B represent bacteria <i>Staphylococcus aureus</i> without treatment of lectins C, and D with treatment by 100µl (90% fraction) dialysed lectin and its purified form respectively.	86

(16)	A Shows the effect of 100µl lectin of Lentil seeds (30,70, and 90% fraction) against Gram-negative bacteria <i>Pseudomonas aeruginosa</i> . Scanning electron microscope images B represent bacteria <i>Pseudomonas aeruginosa</i> without treatment of lectins, C, and D with treatment by 100µl (90% fraction) dialysed lectin and its purified form respectively.	87
(17)	A Shows the effect of 100µl lectin of Pea seeds (30, 70, and 90% fraction) against tested fungi <i>Candida albicans</i> . Scanning electron microscope images B represent fungi <i>Candida albicans</i> without treatment of lectins, C, and D with treatment by 100µl (90% fraction) dialysed lectin and its purified form respectively	88

List of Tables		
Table	Title	Page
(1)	Concentrations (mg/g) of fractionated lectins extracted from five leguminous seeds (Fava bean, lentil, Lima bean, Pea, and Soy bean). Lectin fractionation was done by precipitation with ammonium sulfate at 30, 70, and 90% saturations followed by extensive dialysis. Results are expressed as mean of three replicates with ± standard deviation.	49
(2)	Concentrations (mg/g) of fractionated lectins extracted from five <i>Phaseolus vulgaris</i> seeds (Bronco, Contender, 10YLHJ49, Diacole, and Shalatine). Lectin fractionation was carried out by precipitation with ammonium sulfate at 30, 70, and 90% saturations followed by extensive dialysis. Results are expressed as mean of three replicates with ± standard deviation.	52
(3)	Concentrations (mg/g) of 90% fraction of lectins extracted from (Fava bean, lentil, and Pea) after purification by affinity chromatography.	54

(4)	Hemagglutination activity of lectins extracted from five cultivars of leguminous seeds on human blood groups (A, B, AB, and O). Highest activity (128) is shadowed.	60
(5)	Hemagglutination activity of lectins extracted from five cultivars of <i>Phaseolus</i> seeds on human blood groups (A, B, AB, and O). Highest activity (128) is shadowed.	62
(6)	Hemagglutination activity of lectins extracted from three leguminous seeds (after purification by affinity chromatography) on human blood groups (A, B, AB, and O).	64
(7)	Hemagglutination inhibition of Lectins (70% & 90% fractions) extracted from five leguminous seeds using different sugars.	65
(8)	Hemagglutination inhibition of Lectins (70 & 90% fractions) extracted from five cultivars of <i>Phaseolus</i> seeds using different sugars.	66
(9)	Hemagglutination inhibition of purified Lectins (90% fraction) extracted from Fava bean, Lentil, and Pea using different sugars	67

(10)	Antimicrobial activity measured as inhibition zones diameters (mm) of lectins extracted from five Leguminous seeds against tested microorganisms. The results are represented as mean of three replicates with ± standard deviation, and p-value≤0.05 &0.01 considered statistically significant (95 & 99 % confidence interval).	69
(11)	Antimicrobial activity (measured as inhibition zones diameters of lectins extracted from five cultivars of <i>Phaseolus</i> seeds against tested microorganisms. The results are represented as mean of three replicates with ± standard deviation, and p-value≤0.05 & 0.01 considered statistically significant (95 & 99 % confidence interval).	72
(12)	Antimicrobial activity of purified Lectins (90% fraction) extracted from three leguminous seeds against tested microorganisms.	77
(13)	Minimum inhibitory concentration (MIC) for antimicrobial activity using series of two-fold dilutions of lectins (90% fraction) of five leguminous seeds against the tested microorganisms. The MIC values with standards are also shown	78