



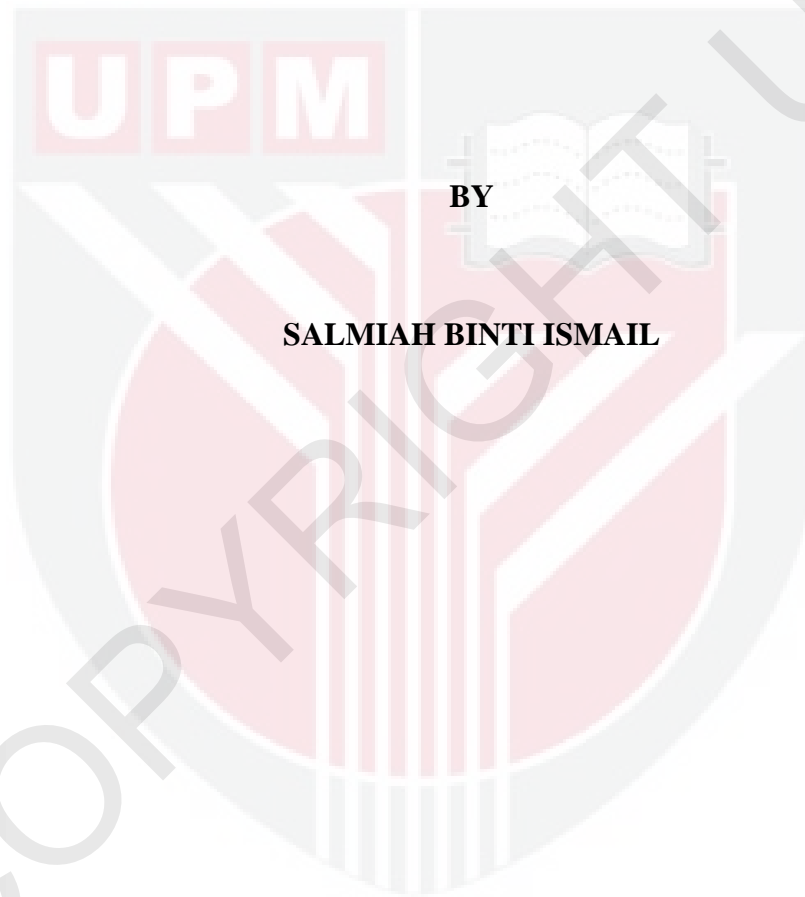
UNIVERSITI PUTRA MALAYSIA

**ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF *Allium Hirtifolium*
BOISS HYDROMETHANOLIC EXTRACT**

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FBSB 2011 42

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BOISS HYDROMETHANOLIC EXTRACT**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree Master of Science**

August 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

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By

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August 2011

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The emergence of multiple resistances exhibited by pathogenic microorganisms against commercial antimicrobial drugs, the undesirable side effects of certain antibiotics as well as the limited number of anticancer agents have led scientists to search for new antimicrobial substances from various sources such as medicinal plants. In fact, the usage of herbs or medicinal plants is still practiced until this modern time since these herbs are said to provide safe and effective treatments against many diseases. In line with discovery of plant-based drugs, the present study focused on studying medicinal properties of *Allium hirtifolium* known as Persian Shallot. This edible plant belongs to *Allium* genus, which has been used in folk medicine in many parts of Iran and Mediterranean region and it is believed to play an important role in the healthcare aspect.

In this current study, the effectiveness of hydromethanolic *Allium hirtifolium* Boiss bulbs was studied against six different pathogenic bacteria including *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Escherichia coli*, one fungus (*Candida albicans*), three different types of viruses Herpes Simplex Virus Type 1 (HSV-1), Herpes Simplex Virus Type 2 (HSV-2) and Respiratory Syncytial Virus (RSV), one type of normal human fibroblast cell line (MRC-5) and four different types of cancer cell lines consist of human breast cancer cells (MCF-7), human cervical cancer cells (HeLa), human liver cancer cells (HepG-2) and human colon cancer cells (HT-29) by *in vitro* system.

The result of disk diffusion assay showed that the extract at different concentrations (120, 60, 30, 15 mg/ml) was effective against all Grams positive bacteria as the zone of inhibition ranged from 14.25- 8.50 mm. Whereas, for Gram negative bacteria (*E. coli* and *E. coli* O157:H7), only higher concentrations (120, 60 and 30 mg/ml) were effective with the inhibition zones ranged from 12.50- 8.00 mm. The highest activity of the extract against *Salmonella typhimurium* was achieved only at the highest concentration (120 mg/ml). The significant result was obtained for the fungus *Candida albicans* with the zone of inhibition ranging from 24-17 mm compared to commercial antifungal drug (Amphotericin B) which was 14 mm in diameter. To confirm the antibacterial and antifungal properties of the extract, the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal/Fungicidal Concentration (MBC/MFC) were determined by Broth Dilution Assay. The starting concentration for this assay was 120 mg/ml and two fold serial dilutions were prepared from this concentration until the last concentration which

was 0.94 mg/ml. The MIC for all the tested pathogenic bacteria and fungus were ranged from 120.00- 3.75 mg/ml while MBC values were ranged from 120.00- 7.50 mg/ml indicating that shallot extract was effective. Moreover, morphological study using Scanning Electron Microscopy revealed that shallot extract altered the morphology of *Candida albicans* in comparison with Amphotericin B.

The MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) Assay was performed in order to determine the cytotoxicity of the extract on normal human cell line (MRC-5) as well as against cancer cells. This extract is shown to be less cytotoxic to the human normal fibroblast cells whereby 60% of the cells were viable at the concentration of 1 mg/ml even though at different time of incubations (24, 48 and 72 hours). A positive result was only obtained for MCF-7 and HeLa cancer cells with the IC₅₀ value 0.28 mg/ml and 0.11 mg/ml, respectively. At the concentration of 0.25 mg/ml, the anticancer property exhibited by the extract on MCF-7 and HeLa cancer cells was confirmed by Dual Assay whereby the induction of apoptosis through the activation of caspase-3 was observed at this concentration. In addition, cell cycle analysis showed that the treated cancer cells accumulated in the sub-G1 phase which is a hallmark of apoptotic cell death.

In terms of antiviral activity, the positive results were shown for HSV-1 and RSV through intracellular assay whereby the Selective Index values were 7.11 and 5.16, respectively. These findings suggest that the hydromethanolic extract of Persian Shallot might be useful in treatment of diseases caused by these viruses.

In general, the hydromethanolic extract of *Allium hirtifolium* Boiss has a strong potential to be used as an antibacterial, antifungal, antiviral as well as anticancer agents.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**ANTIMIKROBIAL DAN ANTIKANSER AKTIVITI BAGI EKSTRAK
HIDROMETANOLIK *Allium hirtifolium* Boiss**

Oleh

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Kewujudan masalah-masalah seperti mikroorganisma patogen yang rintang terhadap pelbagai jenis antibiotik atau antimikrob yang komersil, kesan sampingan terhadap pesakit yang menggunakan antibiotik serta jumlah agen antikanser yang terhad telah menyebabkan para saintis mencari bahan atau ubatan baru yang berasaskan pelbagai sumber, contohnya tumbuhan yang berpotensi dalam perubatan. Hakikatnya, penggunaan tumbuhan seperti pokok herba telah digunakan sejak dari zaman purbakala dan ianya masih diamalkan dalam era yang serba moden ini memandangkan tumbuhan ubatan atau herba ini dipercayai selamat untuk digunakan dan berkesan terhadap pelbagai jenis penyakit. Selari dengan penemuan serta penghasilan ubatan yang berasaskan daripada tumbuhan, maka penyelidikan ini dijalankan untuk mengkaji secara saintifik aspek biologi bagi tumbuhan *Allium hirtifolium* Boiss atau lebih dikenali sebagai “Persian Shallot”. Tumbuhan ini yang dikategorikan sebagai spesies bawang dan

dari genus *Allium* merupakan tumbuhan yang digunakan oleh kebanyakan masyarakat Iran atau mereka yang tinggal di kawasan Mediterranean sebagai bahan dalam masakan mahupun dalam perubatan tradisional. Ianya dipercayai memainkan peranan yang penting dalam aspek kesihatan.

Dalam kajian ini, keberkesanan ekstrak hidrometanolik *Allium hirtifolium* Boiss telah diuji terhadap enam jenis bakteria patogen yang berbeza iaitu *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *Staphylococcus aureus* (MSSA), *Salmonella typhimurium*, *Escherichia coli* O157:H7 dan *Escherichia coli*, satu jenis kulat iaitu *Candida albicans*, tiga jenis virus iaitu Herpes Simplex Virus Jenis 1 (HSV-1), Herpes Simplex Virus Jenis 2 (HSV-2) dan Respiratory Syncytial Virus (RSV) (HSV-1, HSV-2 dan RSV) satu jenis sel normal manusia (MRC-5) dan empat jenis sel kanser yang berlainan iaitu MCF-7, HeLa, HepG-2 dan HT-29. Kesemua kajian yang dijalankan adalah melalui sistem *in vitro*.

Keputusan dari Ujian Penyerapan Disk menunjukkan bahawa ekstrak ini pada kepekatan yang berbeza (120, 60, 30, 15 mg/ml) memberikan kesan positif terhadap semua bakteria Gram positif dengan diameter julat zon inhibisi dari 14.25- 8.50 mm. Bagi bakteria Gram negatif (*E. coli* O157:H7 dan *E. coli*) pula, hanya ekstrak pada kepekatan 120, 60 dan 30 mg/ml sahaja yang berkesan iaitu dengan zon inhibisi yang berjulat di antara 12.50- 8.00 mm. Manakala bagi *Salmonella typhimurium*, ekstrak hanya berkesan ke atas bakteria ini pada kepekatan 120 mg/ml sahaja. Keputusan yang signifikan hanya ditunjukkan oleh kulat *Candida albicans* yang dirawat oleh ekstrak di mana zon inhibisi adalah berjulat daripada 24- 17 mm dibandingkan dengan 14 mm oleh strain yang dirawat

oleh antikulat komersil iaitu Amfoterisin B. Bagi mengesahkan keputusan dari Ujian Penyerapan Disk, maka nilai MIC (Kepekatan Inhibitori Minimum) dan MBC/MFC (Kepekatan bakterisidal/ fungisidal Minimum) ditentukan melalui Ujian “Microdilution Broth”. Kepekatan bagi ekstrak yang digunakan bagi ujian ini bermula dari 120.00- 0.94 mg/ml dan pencairan ekstrak yang dilakukan adalah dalam gandaan 2 kali “two fold-serial dilution”. Nilai MIC bagi kesemua bakteria dan kulat adalah dalam julat 120.00- 3.75 mg/ml manakala nilai MBC pula adalah dalam julat antara 120.00- 7.50 mg/ml. Selain itu juga, kajian morfologi ke atas *Candida albicans* menggunakan “Scanning Electron Microscope” menunjukkan bahawa strain yang dirawat sama ada dengan menggunakan ekstrak atau agen antikulat komersil (Amfoterisin B) akan menyebabkan perubahan morfologi pada sel berbanding strain yang tidak dikenakan rawatan.

Ujian MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) juga telah dijalankan untuk menentukan kesan sitotoksik ekstrak ke atas sel normal manusia (MRC-5) dan ke atas sel kanser. Hasil daripada eksperimen menunjukkan bahawa ekstrak ini kurang sitotoksik terhadap sel normal dengan menunjukkan bahawa 60% sel adalah hidup pada kepekatan 1 mg/ml walaupun pada masa inkubasi yang berlainan iaitu 24, 48 dan 72 jam. Bagi sel kanser pula, keputusan yang positif hanya diperoleh bagi sel MCF-7 dan sel HeLa dengan nilai IC_{50} 0.28 mg/ml dan 0.11 mg/ml bagi setiap satu. Seterusnya, ujian konformasi “Dual Assay” telah dijalankan ke atas kedua-dua sel ini pada kepekatan 0.25 mg/ml dan keputusan menunjukkan bahawa berlakunya induksi apoptosis melalui pengaktifan kaspas-3. Selain itu juga, keputusan daripada analisis kitar sel juga menunjukkan bahawa berlakunya pengumpulan sel pada fasa Sub-G1 bagi sel

kanser yang dirawat dengan ekstrak yang mana merupakan petanda bahawa sel yang mati adalah disebabkan apoptosis.

Bagi aktiviti antiviral pula, keputusan yang positif diperoleh ke atas HSV-1 dan RSV melalui ujian intraselular di mana nilai Indeks Selektif adalah 7.11 dan 5.16 bagi setiap satu. Penemuan daripada kajian ini menunjukkan bahawa ekstrak hidrometanolik *Allium hirtifolium* Boiss berpotensi untuk merawat penyakit yang disebabkan oleh kedua-dua jenis virus ini.

Secara umumnya, ekstrak hidrometanolik *Allium hirtifolium* Boiss mempunyai potensi yang tinggi untuk digunakan sebagai agen antibakteria, antikulat, antivirus dan juga antikanser.

ACKNOWLEDGEMENTS

First of all, praise to Allah the Almighty as this research project would not have been possible without His will and favor.

I would like to express my gratitude and sincere appreciation to Dr. Fatemeh Jahanshiri, my supervisor for her encouragement and guidance throughout the study. I would also like to thank my supervisor committee members, Prof. Datin Paduka Dr. Khatijah Mohd. Yusoff and Dr. Zamberi Sekawi.

Sincere thanks to Farid Azizi Jalilian for teaching and assisting me throughout the project.

I also appreciate the friendship and assistance from all my labmates, Ema, Syazwan and especially my most helpful friend Syani.

Last but not least, special thanks to both my beloved parents and family members for words of support and endless love.



The thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science.
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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions

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Date: 02 August 2011

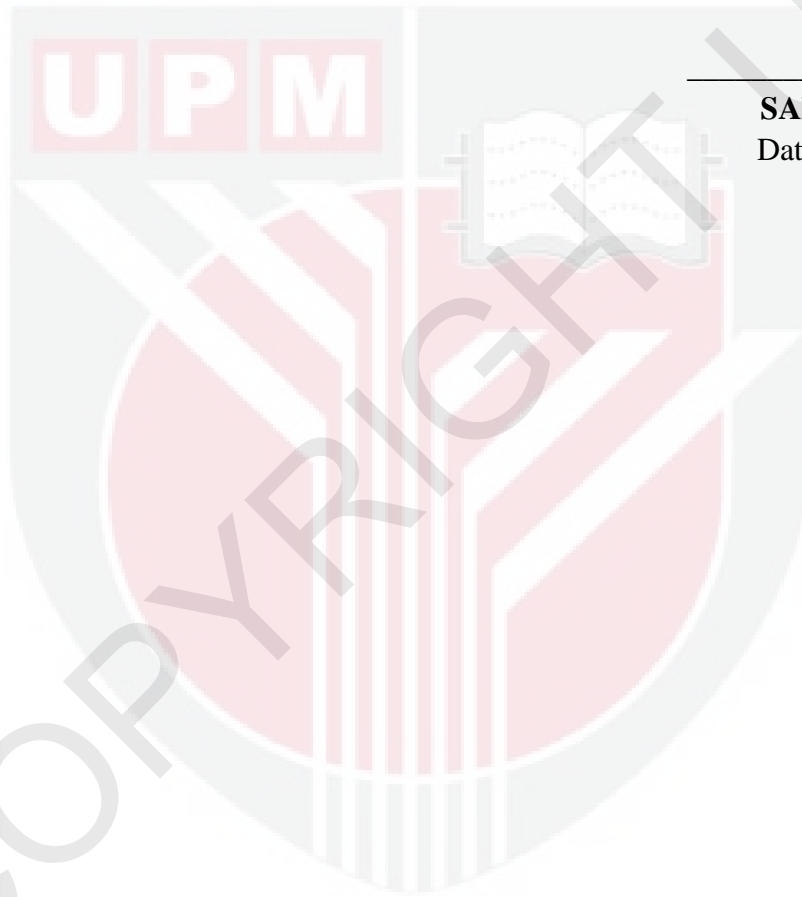


TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xi
DECLARATION	xiv
LIST OF TABLES	xviii
LIST OF FIGURES	xix
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1. INTRODUCTION	1
2. LITERITURE REVIEW	5
2.1 Plants and human life	5
2.1.1 History of medicinal plants	5
2.1.2 Medicinal plants-ancient remedies for modern times	7
2.2 Genus <i>Allium</i>	10
2.2.1 Therapeutic values of <i>Allium</i> plants	10
2.2.2 Chemical compounds in <i>Allium</i> plants	13
2.2.3 <i>Allium hirtifolium</i> Boiss	14
2.3 Drug resistance problem	17
2.4 Pathogenic bacteria and fungus	18
2.4.1 <i>Staphylococcus aureus</i>	18
2.4.2 <i>Salmonella typhimurium</i>	20
2.4.3 <i>Escherichia coli</i>	21
2.4.4 <i>Candida</i>	22
2.5 Pathogenic viruses	23
2.5.1 Herpes Simplex Viruses	23
2.5.2 Respiratory Syncytial Virus	27
2.6 Cancer	28
2.6.1 Colorectal cancer	29
2.6.2 Breast cancer	30
2.6.3 Cervical cancer	30
2.6.4 Hepatocellular carcinoma	31
3. MATERIALS AND METHODS	32

3.1	Plant material	32
3.2	Preparation of crude plant extract	32
3.3	Antibacterial and antifungal assay	33
	3.3.1 Microorganisms	33
	3.3.2 Disk diffusion assay	33
	3.3.3 Microdilution broth assay	34
	3.3.4 Scanning electron microscopy assay	35
	3.3.4.1 Preparation of samples	35
	3.3.4.2 Preparation of specimens	35
3.4	Cytotoxicity assay	36
	3.4.1 Cell line	36
	3.4.2 MTT assay	36
3.5	Anticancer assay	37
	3.5.1 Cell lines	37
	3.5.2 MTT Assay	38
	3.5.3 Dual assay	38
	3.5.4 Cell cycle analysis	39
3.6	Antiviral Assay	39
	3.6.1 Viruses and cell culture	39
	3.6.2 Cytotoxicity assay	40
	3.6.3 Titration of viruses infectivity	40
	3.6.4 Intracellular antiviral assay	41
	3.6.5 Extracellular antiviral assay	41
	3.6.6 Pre-treatment assay	42
	3.6.7 Data analysis	43
4.	RESULTS	44
	4.1 Antibacterial and antifungal assay	44
	4.1.1 Disk diffusion assay	44
	4.1.2 Microdilution broth assay	47
	4.1.3 Scanning electron microscopy assay	50
	4.2 Cytotoxicity and anticancer assay	53
	4.2.1 Principle of MTT assay	53
	4.2.2 Evaluation of MTT assay against normal and cancer cell lines treated with extract	54
	4.2.3 Morphological appearance of normal and cancer cell lines after exposure to non toxic concentration of the extract	57
	4.2.4 Evaluation of Dual assay for MCF-7 and Hela cancer cell lines	60
	4.2.5 Cell cycle analysis	62
	4.3 Antiviral property of the <i>Allium hirtifolium</i> Boiss hydromethanolic extrat	65
5.	DISCUSSION	70
	5.1 Plant extraction	71
	5.2 Antibacterial activity	71

5.3	Antifungal activity	74
5.4	Active compounds related to antibacterial and antifungal activities	75
5.5	Anticancer activity	77
5.6	Antiviral activity	82
5.6.1	Cytotoxicity evaluation (cells viability test) for Vero cells by MTT method	82
5.6.2	MNCC as starting concentration for antiviral assay	83
5.6.3	Intracellular antiviral assay	83
5.6.4	Extracellular antiviral assay	84
5.6.5	Prophylaxis assay	85
5.6.6	Comparison of different types of antiviral assay	85
5.6.7	Compounds related with anti-HSV-1 and anti-RSV	86
6.	CONCLUSION	88
	REFERENCES	90
	APPENDICES	113
	BIODATA OF STUDENT	123