UNIVERSITI PUTRA MALAYSIA

ANTI-VIRAL ACTIVITIES OF CENTELLA ASIATICA L., CURCUMA LONGA L. AND STROBILANTHES CRISPUS L. AGAINST PSEUDORABIES VIRUS IN ANIMAL CELL LINES

HANISA BINTI HOSNI

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By

HANISA BINTI HOSNI

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

July 2006

DECLARATION

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

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HANISA BINTI HOSNI

Date: 28 November 2006

To my husband, Mohd Azhar,

For encouraging and sustaining

To my children, Athirah and Azeem,

For making it all worthwhile

To my parents, Hosni and Patimah,

For making it possible

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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July 2006

Chairman : Professor Mohd Azmi Mohd Lila, PhD

Faculty : Veterinary Medicine

Herpes simplex virus (HSV) is a major opportunistic pathogen in immunosuppressed patients and a serious disease in high human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS) prevalence areas. The existence of resistant strains to the available drug is therefore an urgent need to identify new alternative agents for HSV. The aim of this study is to investigate anti-viral activities of the plants. Assays were developed to determine the characteristics of anti-viral activities, as anti-viral attachment, anti-prophylactic and virucidal. The pseudorabies virus (PrV) has been used as representative of HSV. Three medicinal plants have been used, which is *Centella asiatica* L. (*C. asiatica*), *Strobilanthes crispus* L. (*S. crispus*) and *Curcuma longa* L. (*C. longa*). The plants are reputed in traditional medicine for many treatments of diseases. Firstly, the potential cytotoxicity was evaluated for plant methanol extracts (ME) and aqueous extracts (AE) in cell line by using MTT assay. All three plant extracts were

found significantly ($P < 0.05$) non cytotoxic towards African Green Monkey Kidney (Vero) cells, Baby Hamster Kidney (BHK) cells and Rabbit Kidney (RK) cells. The plant ME was generally more cytotoxic than AE, showed lower (76 µg/ml) non-toxic limit concentration ($NTLC_{50}$) than plant AE (82 µg/ml). The least cytotoxic was the extract of *S. crispus* followed by *C. asiatica* and *C. longa*. The resistance of three different cell lines was also compared and it showed the BHK cells were the most toughest and resistant to the plant extracts. In anti-viral analysis, it was discovered that all plant extracts showed marked prophylactic activity, considerable anti-attachment and virucidal abilities up to 75% inhibition of cytopathic effect (CPE) formation. The *C. longa* extract was found as a potent anti-viral agent followed by *S. crispus* and *C. asiatica*. It exerted better prophylactic activity against PrV compared to the other two plants. Whereas *S. crispus* was found very effective as virucidal agent while *C. asiatica* as anti-viral attachment agent. Based on the results, it was also found that plant ME possessed better anti-viral activity than plant AE. These results also showed each of three plants possessed different anti-viral activities. The anti-viral activities were also varies in different cell lines tested. All three plants were analysed by liquid chromatography-electrospray ionisation-mass spectrometry (LC-ESI-MS) to identify plant compounds. The analysis revealed several compounds in *C. asiatica, S. crispus* and *C. longa* ME. This study discovered the promising anti-viral activities of *C. asiatica, S. crispus* and *C. longa* but not any identified plant compound against PrV *in vitro*. These results suggest that all three plant extracts have potent anti-viral agents against PrV as representative of HSV that can be exploited for development of an alternative medicine to prevent HSV infections.

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

ANTIVITI ANTI-VIRUS OLEH ***CENTELLA ASIATICA*** **L.,** ***CURCUMA LONGA*** **L. DAN** ***STROBILANTHES CRISPUS*** **L. TERHADAP VIRUS PSEUDORABIES DI DALAM BARISAN SEL**

Oleh

HANISA BINTI HOSNI

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Herpes simplex virus (HSV) adalah patogen oportunis utama kepada pesakit-pesakit yang sistem imunnya ditekan dan adalah satu penyakit serius di kawasan yang lazimnya mempunyai perebakan Virus Kurang Daya Tahan Penyakit (HIV) atau Sindrom Kurang Daya Tahan Penyakit (AIDS) yang tinggi. Kewujudan strain virus yang rintang kepada ubatan anti-virus menyebabkan keperluan mendesak untuk mencari agen alternatif bagi merawat HSV. Tujuan kajian ini adalah untuk mengkaji aktiviti anti-virus oleh tumbuhan. Kaedah-kaedah untuk menentukan sifat dan ciri aktiviti anti-virus pseudorabies (PrV) iaitu anti-perlekatan, anti-pre-rawatan dan membunuh virus telah dijalankan. PrV telah digunakan sebagai mewakili HSV. Tiga tumbuhan ubatan telah digunakan iaitu *Centella asiatica* L. (*C. asiatica*), *Strobilanthes crispus* L. (*S. crispus*) dan *Curcuma longa* L. (*C. longa*). Tumbuhan-tumbuhan ini mempunyai reputasi yang baik dalam merawat pelbagai penyakit di dalam perubatan tradisional. Pertama, potensi toksik ekstrak methanol (EM) and ekstrak akuas (EA) tumbuhan telah dianalisa ke atas

barisan sel dengan menggunakan kaedah MTT. Kesemua ekstrak tumbuhan dijumpai signifikan ($p < 0.05$) tidak menyebabkan toksik kepada sel Buah Pinggang Monyet Hijau (Vero), sel Buah Pinggang Anak Hamster (BHK) dan sel Buah Pinggang Arnab (RK). Secara amnya, EM tumbuhan lebih toksik ke atas sel berbanding AE tumbuhan, menunjukkan kepekatan tidak toksik pada 50% yang lebih rendah (76 µg/ml) daripada AE tumbuhan (85 µg/ml). Kerintangan ketiga-tiga barisan sel terhadap ekstrak tumbuhan juga dibandingkan dan menunjukkan sel BHK adalah paling kuat dan rintang kepada ekstrak tumbuhan. Kesemua ekstrak tumbuhan menunjukkan kebolehan yang jelas aktiviti pre-rawatan manakala anti-perlekatan dan kebolehan membunuh virus yang boleh dipertimbangkan sehingga 75% penekanan ke atas pembentukan Kesan Saitopatik (CPE). Ekstrak *C. longa* telah dijumpai sebagai agen anti-viral yang sangat berpotensi, diikuti ekstrak *S. crispus* dan *C. asiatica*. Ia menunjukkan aktiviti pre-rawatan yang lebih baik ke atas PrV berbanding dua tumbuhan tersebut. Manakala ekstrak *S. crispus* telah dijumpai menunjukkan kesan efektif sebagai agen membunuh virus dan ekstrak *C. asiatica* sebagai agen anti-perlekatan. Berdasarkan kepada keputusan, ia menunjukkan ME tumbuhan memberikan aktiviti anti-virus yang lebih baik berbanding AE tumbuhan. Keputusan juga menunjukkan bahawa setiap tumbuhan memberikan aktiviti anti-virus yang berbeza. Aktiviti anti-virus juga berbeza di dalam barisan sel yang berbeza. Ketiga-tiga tumbuhan telah dianalisa dengan menggunakan kromatografi cecair-ionisasi elektrospray-spektrometer jisim (LC-ESI-MS) untuk mengenalpasti bahan tumbuhan. Analisa tersebut mendedahkan bahan-bahan yang terkandung di dalam *C. asiatica, S. crispus dan C. longa* EM. Kajian ini menemui aktiviti anti-virus bagi *C. asiatica, S. crispus dan C. longa* tetapi bukan daripada mana-mana bahan tumbuhan yang

dikenalpasti terhadap PrV secara *in vitro.* Keputusan kajian juga mencadangkan bahawa ketiga-tiga tumbuhan mempunyai potensi sebagai agen anti-virus ke atas PrV sebagai mewakili HSV yang boleh dieksploitasikan bagi pembangunan ubatan alternatif bagi menghalang jangkitan HSV.

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I certify that an Examination Committee has met on 6th July 2006 to conduct the final examination of Hanisa Binti Hosni on her Master of Science thesis entitled Anti-viral Activities of *Centella Asiatica* L., *Strobilanthes Crispus* L. And *Curcuma Longa* L. Against Pseudorabies Virus in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 16 January 2007

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LIST OF ABBREVIATIONS

ACV	Acyclovir
ADV	Adenovirus
ADV-3	Adenovirus type 3
AE	Aqueous Extract
AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Type Cell Culture
ATV	Antibiotic Trypsin-Versine
BDMC	Bisdemethoxycurcumin
BHK	Baby Hamster Kidney cells
BSA	Bovine Serum Albumin
BVdU	E-5-(2-bromovinyl)-2'-deoxyuridine+
BVdU-TP	Brivudin-5'-triphosphate
CMV	Cytomegalovirus
CPE	Cytopathic effect
ddI	Dideoxyinosine
DAB	3,3'-diaminobenzidine
DLA	Dalton's Lymphoma Ascites
DMC	Demethoxycurcumin
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic Acid
EAC	Ehrlich Ascites Cells

EBV	Epstein Barr Virus
EMEM	Eagle's Minimal Essential Medium
ESI	Electrospray Ionisation
FCS	Fetal Calf Serum
GCV	Ganciclovir
GLM	General Linear Model
HBB	2-(hydroxybenzyl)-benzimidazole
HeLa	Human Epithelioid Cervical Carcinoma
HHV	Human Herpes Virus
HHV-1	Human Herpes Virus Type 1
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus Type-1
HPLC	High Performance Liquid Chromatography
HSV	Herpes Simplex Virus
HSV-1	Herpes Simplex Virus Type 1
HSV-2	Herpes Simplex Virus Type 2
HZV	Herpes Zoster Virus
IUdR	Iododeoxyuridine
LC-MS	Liquid Chromatography-Mass Spectrometry
LTR	Long Terminal Repeat
ME	Methanol Extract
MG	Maximum Growth; Cell treated with media only

MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide
MW	Molecular weight
NMR	Nuclear Magnetic Resonance
NTLC	Non-Toxic Limit Concentration
$NTLC_{50}$	Non-Toxic Limit Concentration at 50 percent
PAA	Polyacrylic acid
PBS	Phosphate Buffer Saline
PI	Post Infection
PrV	Pseudorabies Virus
PVAS	Polyvinylalcohol sulphate
PVS	Polyvinyl sulphonate
RK	Rabbit Kidney cells
RNA	Ribonucleic Acid
RRV	Ross River Virus
RSV	Respiratory Syncytial Virus
SAH	*S*- adenosylhomocysteine
SI	Selective Index
TE	Tris-EDTA
TECA	Titrated extract of *C. asiatica*
$TCID_{50}$	Tissue Culture Infective Dose at 50
TK	Thymidine kinase
TLC	Thin Layer Chromatography

TNF	Tumor Necrosis Factor
TSP	Thermospray
USA	United States of America
UV	Ultraviolet
VCV	Vanciclovir
VERO	African Green Monkey Kidney cells
VSV	Vesicular Stomatitis Virus
WHO	World Health Organization

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CHAPTER I

INTRODUCTION

Viral Infections

Viral infections are major health problem all over the world due to their morbidity and mortality. There are many types of transmissions of viral infections in man. They are respiratory or salivary spread, which is not readily controllable; faecal-oral spread that is controllable by public health measures and venereal spread, which is socially difficult to control. An example is HSV (Dawn and Robert, 1997).

The immune system appears as the controlling factor within the host that maintains beneficial microbes at harmless levels and prevents infections by dangerous agents. It is able to combat a variety of infections from birth on. Evolutionary pressure has forced most viruses to develop many strategies to subvert the immune system. Some of them are production of antigenic variants; adopted by influenza virus and HIV, avoiding recognition by lymphocytes; for example adenovirus, manipulate cytokine pathways to avoid destruction by the host immune response, the production of materials that interfere with the production of pro-inflammatory cytokines; for example vaccinia and cowpox viruses, and interfering with the alternate pathways of complement activation; for example HSV (Hans *et al*., 2000; Bruce, 2002).