IN VITRO ANTIVIRAL ACTIVITY OF *Polygonum minus* EXTRACTS AGAINST HERPES SIMPLEX VIRUS 1

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A dissertation submitted in partial fulfillment of the

requirements for award of the degree of

Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering

Universiti Teknologi Malaysia

FEBRUARY 2015

To my parents

ACKNOWLEDGEMENT

So it is done. First of all, I would like to extend my gratitude to my supervisor, Dr. Salehhuddin Hamdan, for sharing his expertise and critical advice throughout this project. He has been instrumental in supporting the project since day one. Thank you for giving me the opportunity to work with you.

To Ms. Sayang Baba who had taught me the rigors of research work, my sincerest appreciation. Her acute mentoring and perseverance in dealing with my folly were tremendous, bar none. A special acknowledgement to Dr. Saleha Shahar for her recommendations and willingness to discuss experimental protocols with me.

To past and present ATC lab members; Ms. Aqmar Bahaudin, Ms. Asita Elengoe and Ms. Norsyuhada Jaafar, thank you for the assistance and guidance during my lab work. Not forgetting friends and fellow class members who had certainly made the journey more awesome. I am grateful to have known such wonderful people in the short time studying here.

Finally, to my parents who supported me unconditionally all these years, thank you. There are no words to express my gratitude. I would not have made it if it weren't for them.

Cheers! :]

ABSTRACT

The use of medicinal plants as preventative and curative treatment may be integral when a complete treatment is not yet available. Hence, the purpose of this study was to conduct an evaluation on *Polygonum minus* extracts over its effect against Herpes Simplex Virus 1 (HSV-1) infection *in vitro*. Methanol, ethanol and aqueous extracts of *P.minus* were obtained through evaporation under reduced pressure via rotary evaporator. Cytotoxicity testing of *P.minus* extracts was conducted on Vero cells using MTT assay. DPPH and Folin-Ciocalteu assay were used to evaluate its radical scavenging activity and phenolic content respectively. To better understand its medicinal properties, an *in vitro* treatment was carried out by means of time-of-addition tests; simultaneous treatment, pre-treatment and post-treatment. Infection with HSV-1 was performed at MOI of 1 and inoculated with methanol and ethanol extracts at its maximum non-toxic dose concentration of 37.50 µg/ml. In simultaneous and pre-treatment, both extract appeared to exert inadequate effect against HSV-1 infected cells (where cell viability recorded well below 60%). However, in post-treatment test, only aqueous extract showed desirable effect where cell viability is maximally retained. This is despite the fact that aqueous extract displayed the lowest radical scavenging activity (IC_{50} = 146.58 µg/ml, with maximum inhibition at 8 mg/ml concentration), having the lowest phenolic content (61.68 ± 11.621 mg GAE/l at 8 mg/ml concentration), albeit a higher cytotoxicity (IC_{50} = 408.03 µg/ml) towards Vero cells compared to methanolic extract. Hence, preliminary finding suggests aqueous *P.minus* extract has curative potential towards HSV-1 infected cells despite its subpar antioxidant activity. Indeed, further studies are required to make clear the exact curative effect of *P.minus* towards HSV-1 infection before a more conclusive experimental findings be made.

ABSTRAK

Penggunaan tumbuhan ubatan sebagai rawatan pencegahan dan rawatan adalah penting apabila rawatan yang lengkap belum ada. Oleh itu, tujuan kajian ini dijalankan adalah untuk menilai kesan ekstrak *Polygonum minus* terhadap jangkitan virus Herpes Simplex 1 (HSV-1) *in vitro*. Ekstrak metanol, etanol dan akueus *P.minus* diperolehi melalui penyejatan di bawah tekanan. Ujian sitotoksiti ekstrak *P.minus* telah dijalankan ke atas sel-sel Vero menggunakan ujikaji MTT. Manakala, ujikaji DPPH dan Folin-Ciocalteu digunakan untuk menilai aktiviti-memerangkap-radikal dan kandugan fenolik. Bagi lebih memahami karakteristik perubatan *P.minus*, rawatan *in vitro* telah dijalankan melalui ujian berdasarkan masa-penambahan; rawatan serentak, pra-rawatan dan rawatan selepas. Jangkitan HSV-1 telah dilakukan pada MOI 1 dan didedahkan pada metanol dan etanol ekstrak pada kepekatan dos maksima tidak-toksik 37.50 µg/ml. Bagi ujian rawatan-serentak dan pra-rawatan, kedua-dua ekstrak menampakkan kesan perubatan yang tidak mencukupi terhadap sel dijangkiti HSV-1 (ini berikutan sel hidup dicatatkan di bawah 60%). Namun dalam ujian pasca rawatan, hanya ekstrak akueus menunjukkan kesan perubatan di mana sel-sel mampu hidup pada tahap maksima. Ini berikutan hakikat bahawa ekstrak akueus merekodkan aktiviti-memerangkap-radikal terendah (IC_{50} = 146.58 µg/ml, dengan perencatan maksimum pada kepekatan 8 mg/ml), mempunyai kandungan fenolik yang paling rendah (61.68 ± 11.621 mg GAE/l pada kepekatan 8 mg/ml), walaupun mencatatkan sitotoksiti yang lebih tinggi (IC_{50} = 408.03 µg/ml) pada sel-sel Vero berbanding dengan ekstrak metanol. Dapatan awal menunjukkan ekstrak akueus *P.minus* mempunyai potensi penyembuhan terhadap sel-sel dijangkiti HSV-1 sungguhpun merekodkan aktiviti antioksida yang rendah. Sememangnya kajian lebih lanjut adalah perlu bagi penjelasan terperinci berkenaan kesan penyembuhan *P.minus* terhadap HSV-1 jangkitan sebelum kesimpulan yang lebih muktamad boleh dibuat.

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LIST OF ABBREVIATIONS

SYMBOLS	DESCRIPTION
3D	three dimensional
ACV	acyclovir
ACV-TP	acyclovir-triphosphate
ANOVA	analysis of variance
CPE	cytopathic effect
CO_2	carbon dioxide gas
DENV2	Dengue virus type 2
dGTP	deoxyguanosine triphosphate
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FBS	Fetal Bovine Serum
F-C	Folin-Ciocalteu
F-D	Folin-Dennis
GAE	gallic acid equivalent
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus

HHV	Human herpesvirus
HSV-1	Herpes simplex virus 1
IC_{50}	half maximal inhibitory concentration
IM	Infection media
L15	Leibovitz 15 medium
MNTD	maximum non-toxic dose
MOI	multiplicity of infection
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
RSA	radical scavenging activity
SFE	supercritical fluid extraction
SFM	serum-free media
SPSS	Statistical Package for Social Science
$TCID_{50}$	50% Tissue culture infective dose
TPC	Total Phenolic Content
PBS	Phosphate Buffered Saline
pfu	plaque forming unit
P.minus	*Polygonum minus*
P/Strep	Penicillin-Streptomycin
qRT-PCR	quantitative Reverse Transcriptase-Polymerase Chain Reaction
µl	microliter
UAE	ultrasound assisted extraction
VSV	Vesicular Stomatitis Virus

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Herpes simplex virus 1 (HSV-1) is one of nine herpesviruses known to occur in man. The virus is the causative agent of oral herpes infection, of which no cure has been found yet. HSV-1 has the ability to establish latent infection in which virions are reactivated and propagated under certain circumstances (Griffiths, 2014). As a result, a person infected with HSV-1 may experience recurrent symptoms such as lesions near the oral or mucosal regions of body (Whitley and Roizman, 2001; Fatahzadeh *et al.*, 2007). Lifelong infection is thus notable in HSV infected person.

A synthetic drug known as acyclovir is commonly prescribed to control outbreaks of herpes infection (Tan *et al.*, 2013). In brief, acyclovir works by blocking viral DNA synthesis while maintaining cell DNA synthesis. When inside an infected cell, acyclovir is phosphorylated to its active form by the virus' thymidine kinase (Piret and Boivin, 2014). Meanwhile cells not infected with the virus are not subjected to the effects of the drug, attributing to its specificity. However, acyclovir only attacks virus that are active and not on virus in latency (Bdel-Haq and Asmar, 2001) thus making regular drug administration necessary to reduce outbreaks. In addition, there have been cases on the occurrence of drug-resistant HSV strain and is

a major concern especially in severely immunocompromised patients (Griffiths, 2014).

For many years, natural sources have been subjected to screening for antiviral properties. Screening studies are regularly conducted to identify potential medicinal values in natural sources. In this approach, cell biology techniques are employed to identify compounds that may inhibit virus replication. Viruses are inoculated in cell cultures and compounds are subsequently added at IC_{50} concentration. Evidence for viral inhibition may include cytopathic effect (CPE), cell viability and cell death, among others (Carter and Saunders, 2007). Following successful biological tests, crude extracts of natural source may then be subjected to compound fractionation for further evaluation (Balunas and Kinghorn, 2005). Screening compounds for antiviral properties is particularly useful when dealing with an undocumented natural source and is often the first step to drug discovery.

Polygonum minus is an herb plant of the Polygonaceae family. Due to its sweet, lemony and aromatic character, it is commonly used in traditional Southeast Asia's cooking as a flavor enhancer and interestingly, in folk medicine to treat digestive problems (Qader *et al.*, 2012a). In Malaysia, it is known as '*kesum*' in the Malay language (Burkill, 1996). The plant has been reported to exhibit potent antioxidant activity and antimicrobial properties (Faujan *et al.*, 2006; Uyub *et al.*, 2010). Previously, a screening study on 61 ethanolic extracts of medicinal plants in Malaysia found that *P.minus* was a potent antiviral agent against two types of viruses; HSV-1 and Vesicular Stomatitis Virus (VSV) (Ali *et al.*, 1996). Meanwhile, in this study two other solvent types were included; methanol and aqueous.

1.2 Problem Statement

P.minus has been documented to possess many medicinal properties. However, antiviral properties of methanolic and aqueous *P.minus* extracts against HSV-1 have not been extensively elucidated. This study was conducted to evaluate its potential as an antiviral agent against herpes simplex virus 1 *in vitro*.

1.3 Research Objectives

The objectives are:

1. To evaluate antioxidant properties of *P.minus* extracts based on DPPH radical scavenging activity.
2. To determine cytotoxicity of *P.minus* extracts on Vero cells based on the MTT assay.
3. To evaluate inhibitory effect of *P.minus* extracts against HSV-1 using crystal violet assay based on a time-of-addition study.

1.4 Scope of Research

The first part of the study involved with the preparation of plant extracts using rotary evaporation method followed by determining the antioxidant properties of plant extracts based on DPPH radical scavenging activity and Folin-Ciocalteu assay. Cytotoxicity of plant extracts toward Vero cells were tested to obtain its MNTD concentration. The second part was the quantification of virus stocks using end-point dilution assay. Finally, an *in vitro* antiviral treatment was carried out to

investigate extracts' efficacy as an antiviral agent. The antiviral treatment was done by incubating extracts at its MNTD concentration before virus inoculation (pre-treatment), after virus inoculation (post-treatment) and added together with virus (simultaneous-treatment). Cell viability was determined by crystal violet assay.

1.5 Significance of Study

The focus of this study is to evaluate the potential of a local herb in inhibiting viral infection *in vitro*. The study is hoped to help identify potential natural product-based treatment towards herpes infection and at large to spur a continuous interest on finding local plants that may exhibit medicinal properties against infectious diseases.

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