

THE PHOTOPROTECTIVE AND COLLAGEN STIMULATORY EFFECTS OF  
*LABISIA PUMILA VAR PUMILA* EXTRACT ON UVB  
IRRADIATED HUMAN SKIN FIBROBLAST (HSF1184) CELLS

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*To my beloved family*

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## ABSTRACT

This study mainly concentrates on the investigation of the photoprotective effect of *Labisia pumila* extract on the growth of Human Skin Fibroblasts cells (HSF1184) and collagen synthesis by the HSF1184 cells. In this study, the water extract of *Labisia pumila* and the methanolic extract of *Labisia pumila* showed growth stimulatory effect on HSF1184 cell line at the same concentration throughout the study, at  $1 \times 10^{-5}$   $\mu\text{g/ml}$ . At higher concentrations, both *Labisia pumila* plant extracts showed inhibitory effect on the growth of the cells, after 24 hours of treatment. After 48 hours of treatment, all concentrations of the water extract of *Labisia pumila* showed growth stimulatory effect on the HSF1184 cells in both non-ultraviolet B (UVB) irradiated and UVB irradiated cells cultured in media with fetal bovine serum (FBS) or without FBS. The growth stimulatory effect was more significant in HSF1184 cells culture media without FBS. These results demonstrate the time dependent effect of the water extract of *Labisia pumila* on the HSF1184 cells. Both plant extracts caused significant increase in the collagen production of the HSF1184 cells in both UVB and non UVB irradiated HSF1184 cells. At concentration of  $1 \times 10^{-5}$   $\mu\text{g/ml}$ , the water extract of *Labisia pumila* was able to increase the collagen synthesis in the HSF1184 UVB irradiated cells up to 70% whereas the methanolic extract of *Labisia pumila* has increased the collagen synthesis only by 20%, this concentration has proven to be the optimum concentration for both extracts of *Labisia pumila* throughout the course of this study and this result clearly indicate that the water extract of *Labisia pumila* is a better extract than the methanolic extract of *Labisia pumila*. In conclusion, the *Labisia pumila* plant extracts showed a significant photoprotective effect and has great potential to be developed and integrated into cosmeceutical product in the future.

## ABSTRAK

Kajian ini memberikan perhatian utama kepada kesan perlindungan cahaya Labisia pumila terhadap pertumbuhan sel fibroblast kulit manusia (HSF1184) dan penghasilan kolagen oleh sel HSF1184. Dalam kajian ini, Kedua-dua ekstrak akues Labisia pumila dan ekstrak methanol Labisia pumila menunjukkan kesan pertumbuhan pada sel HSF1184 pada kepekatan yang sama sepanjang kajian, iaitu pada  $1 \times 10^{-5} \mu\text{g} / \text{ml}$ . Pada kepekatan yang lebih tinggi, kedua-dua ekstrak tumbuhan Labisia pumila menunjukkan kesan yg menghalang pertumbuhan sel, setelah 24 jam rawatan. Selepas 48 jam rawatan, semua kepekatan yang digunakan menunjukkan kesan pertumbuhan pada sel HSF1184 yang dibiakkan dalam medium dengan serum tumbesaran ataupun dalam medium tanpa serum tumbesaran dalam kedua-dua keadaan sama ada terdedah kepada sinaran ultra-lembayung (UVB) ataupun tidak. Kesan pertumbuhan ini lebih ketara bagi sel HSF1184 yang dibiakkan di dalam medium tanpa serum tumbesaran. Ini menunjukkan kebergantungan kesan ekstrak akues dan ekstrak methanol Labisia pumila kepada tempoh pendedahan terhadap sel HSF1184. Kedua-dua ekstrak menyebabkan peningkatan ketara dalam pengeluaran kolagen sel HSF1184 dalam kedua-dua keadaan kajian. Pada kepekatan  $1 \times 10^{-5} \mu\text{g} / \text{ml}$ , ekstrak akues Labisia pumila mampu untuk meningkatkan penghasilan kolagen dalam sel HSF1184 yang terdedah kepada UVB sehingga 70% manakala ekstrak methanol Labisia pumila hanya menyebabkan kenaikan sebanyak 20%, ini juga menunjukkan kesan ekstrak akues Labisia pumila lebih efektif daripada kesan yang dihasilkan daripada ekstrak methanol Labisia pumila. Kesimpulannya, ekstrak tumbuhan Labisia pumila menunjukkan kesan perlindungan terhadap cahaya ultra-lembayung dan mempunyai potensi besar untuk dibangunkan dan diintegrasikan ke dalam produk kosmetik pada masa akan datang.

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## LIST OF ABBREVIATIONS

APC	-	Antigen Presenting Cells
AHA	-	Alpha-hydroxy acids
CO <sub>2</sub>	-	Carbon dioxide
CDKs	-	Cyclin Dependent Kinases
DPBS	-	Dulbecco Phosphate-buffered Saline
DMEM	-	Dulbecco Minimum Essential Medium
DMSO	-	Dimethyl Sulfoxide
DNA	-	Deoxyribonucleic Acid
EB	-	Ethidium Bromide
ECM	-	Extra Cellular Matrix
HSF	-	Human Skin Fibroblasts
FBS	-	Fetal Bovine Serum
FSH	-	Follicle Stimulating Hormone
GSG	-	Glycosaminoglycans
HRT	-	Hormone Replacement Therapy
IR	-	Infrared
KF	-	Kacip Fatimah
LH	-	Luteinizing Hormone
MHT	-	Menopausal Hormone Therapy
MMP	-	Matrix Metalloproteinases
mRNA	-	Messenger RNA
OA	-	Acridine Orange
PBS	-	Phosphate-Buffered Salines
PDT	-	Population Doubling Time
PICP	-	Procollagen C-End Terminal Pro Peptide
PINP	-	Procollagen N-End Terminal Pro Peptide
ROS	-	Reactive Oxygen Species
SCA	-	Sircol Collagen Assay
SR	-	Sirius red
TPC	-	Total Phenolic Content
TIMP	-	Tissue Inhibitor Metalloproteinase
rER	-	Rough Endoplasmic Reticulum
UV	-	Ultraviolet

## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Introduction**

Plant extract has been used since ancient time for cosmetic and pharmaceutical applications. Different parts of plant including leaves, fruits flowers, stem, barks, buds, and roots were used accordingly. Cosmetically, plant and plant extracts were used to provide moisturizing, whitening, tanning, colour cosmetics, sunscreen, free radical-scavenging, anti-oxidant, immunostimulant, washings, preservatives, and thickening effects (Blum et al. 2007). A lot of research has been done on different plant extract in order to get as much understanding as possible so that a detail chemical profile of the plant can be obtained. This fundamental procedure has been proven to be very important to the scientific community to determine the potentials and benefits of a plant extracts in cosmetic or pharmaceutical applications (Andrea et al. 2004).

Despite various use and benefits, the use of plants and plant materials is bounded by certain limitation: 1) the availability of the plant might be restricted through seasons, limited stocks, protection of the plant, problem in cultivation, and bad harvest . 2) the quality of the plants produced differ due to seasonal changes, methods in cultivation,



geography and clone types. These limitations should be overcome to realize the full potential of the plant extracts in health promotion (Blum et al. 2007). The advancement in technology has given us a solution to address these issues. For example, cell culture technology is used in cell based assay which is crucial in exploratory work in assessing bioactivities of plant extracts.

Cell culture technology is defined as a series of complex process by which cells are grown under controlled environment. The availability of this method developed in mid 1900s enables us to carry out a detail study on every possible aspect of cell properties, characteristics and metabolism that is affected by the addition of plant extracts in any cultured media or environment. In this particular study, human skin fibroblasts were being used as the subject for analysis as it is a well-established system for *in vitro* analysis of cell growth (Yamada et al. 2004), migration, and collagen metabolism (Nawrat et al. 2005). Human skin fibroblasts has been previously used to study skin aging (Hyun-Kyung Choi et al. 2009), wound healing (Saltzman, 2004), genetic disorder (Paradisi et al. 2005; Jones et al. 2004), evaluating cosmetic formulations toxicity (Losio et al. 1999; Gerhard et al 2009) and chemical cytotoxicity (Shrivastava et al. 2005).

The integration of cell culture technology and plant extract studies is expected to play a significant role in the advancement of the field of ethnopharmacology (Andrea et al. 2004). This field involves the study of cosmetics, cosmeceuticals and remedies to heal skin problems and diseases using traditionally used herbs and plant extracts (Aburjai et al 2003). Cell culture technology enables us to investigate the effect of plant extract on cell properties and characteristics. One of the most important properties and characteristics of cells are their ability to regenerate themselves as part of repair mechanism.

Cell regeneration or commonly known as cell growth is defined as the increase in cell population which is very important to maintain the integrity of a certain type of cells. Human skin for examples consists mainly of three major types of cells, namely keratinocytes, melanocytes and fibroblast (Kanitakis 2002). Human skin functions as an integument which simply means “covering” and acts more than an external body covering. Due to constant and direct exposure to the environment, the three components of the skin including important cell types experience stress conditions that can induce adaptive or degenerative pathways and influence ageing (Francois et al. 2005). One of the stress conditions that are very harmful to our skin is the chronic exposure to ultraviolet (UV) irradiation. UV irradiation is thought to be the real cause for skin damage that leads to premature ageing of the skin called photoaging (Kang et al. 2003; Jenkins, 2002).

Direct exposure to ultraviolet (UV) light especially UVB can interfere with normal molecular and biological functions of the fundamental components of human skin which is keratinocytes, melanocytes and fibroblasts cells (Xu et al. 2005; Rittie et al. 2002). These major types of cell are very important to maintain the physiological and histological functions of the skin (Chuong et al. 2002; Kanitakis 2002), therefore it is vital to protect the integrity of these cells. Constant exposure to UV light might also cause significant damage to skin cell directly that can lead to cell death. This might occur due to the increase in the number of reactive oxygen species (ROS) which is very damaging to the skin cells (Francois et al. 2005). These are the main reason why UV light exposure can speed up ageing process in human skin.

Skin regeneration is very important in this condition so that the skin can repair itself and function normally. However, in most situations full regeneration is not achieved as the skin cells have lost their ability to regenerate on their own capacity. Finding of chemical compounds or the use of plant extracts that can stimulate cell

growth after rigorous exposure to stressful environment might provide the solution for this problematic condition. The discovery of a natural product that address this issue might also become the alternative source of growth promoter that can be integrated into culture media to replace the expensive synthetic growth serum currently used in most culture media. In this study, an extract from a plant called *Labisia pumila var pumila* was used to investigate its effect on the growth of human fibroblast cells (HSF) and collagen synthesis stimulation when the plant extract is introduced to the cell in a tissue culture environment.

## 1.2 Research Background

*Labisia pumila* is one of the popular herbs in Malaysia and is popularly known as "Kacip Fatimah" domestically. The Malay people have been using Kacip Fatimah for many generations. It has been used extensively by the Malay women in childbirth due to its ability to induce and facilitate childbirth. Apart from that, Kacip Fatimah is also part of the traditional medication used as post-partum therapies (Burkill, 1935). There are three types of *Labisia pumila* that can be found in Malaysia namely, *Labisia pumila var. alata*, *Labisia pumila var. pumila* and *Labisia pumila var. lanceolata* (Stone, 1988). Differentiation of the three varieties of *Labisia pumila* is very important in terms of their chemical and physical characteristics as well as biological activity so that proper bioactive ingredients can be identified in order to produce a quality herbal product without jeopardising its safety and efficacy.

As mentioned in the earlier part of section 1.2, Kacip Fatimah has been the most favoured herbs among the indigenous people to treat menstrual irregularities and postpartum. Traditionally, certain parts of the plant are boiled and the water extract will be taken as a drink. Even though there are no scientific evidence on the efficacy of this traditional method of preparation, Kacip Fatimah has been continually used in traditional medicine. Recently a lot of interest has been shown in herbal medicine as it is said to be the natural way of healing. Due to the advances in technology, nowadays, people can get as much information as they want and be able to pick and choose, leading them to opt for herbal medicines as to their perception it is natural to the human body unlike the conventional drugs used in modern medicines. Therefore, a lot of studies have been carried out to determine the potential pharmacological application of herbal preparation. However, a lot of products that exploit the use of traditional herbs has been marketed commercially even without the proper knowledge of its mode of action. This is a very unhealthy practice but it shows the general acceptance of the public towards herbal-based products. Several studies of *Labisia pumila* (Jamal et al,1999, Ayida et al, 2007, Husniza, 2002) were carried out and the results of these studies clearly indicate the phytoestrogenic properties of *Labisia pumila*. One of the studies which used the ethanolic extract of the roots of *Labisia pumila* var. *alata* was tested on the human endometrial adenocarcinoma cells of the Ishikawa-Var I line, indicated that the plant extract has a weak but specific estrogenic effect on the cells by enhancing the secretion of alkaline phosphatase. Another recent studies conducted by the Institute of Medical Research (IMR) has showed that *Labisia pumila* plant extract was able to displace estradiol binding to antibodies raised against estradiol, making it similar to other estrogens such as estrone and estriol(Jamal et al,1999)

This study was carried out to evaluate the photoprotective effect of *Labisia pumila* on the growth of human skin fibroblast with and without exposure to UV irradiation and also to investigate the effect of the plant extract on the stimulation of collagen synthesis which is vital to skin health.

### **1.3 Problem Statement**

Premature skin ageing caused by ultraviolet irradiation from the sun has deleterious effects in human skin. These includes disruption in the growth of human skin cells (human skin fibroblast cells), and the impairment of collagen synthesis that play a very important role in maintaining the structural integrity of the human skin.

### **1.4 Hypothesis**

*Labisia pumila* (Kacip Fatimah) extracts promote the growth of human skin fibroblast cells (HSF1184) previously exposed to UVB irradiation and stimulate collagen synthesis in order to maintain the structural integrity of the human skin so that the effect of extrinsic ageing can be minimized.

### **1.5 Objectives of the study**

The objective of this study was to study the photo-protective effect of *Labisia pumila* extracts on UVB irradiated human skin fibroblast (HSF1184) cells. Its potential in promoting the growth of human skin fibroblast cells exposed to UVB irradiation and the stimulatory effect on collagen synthesis were investigated

## 1.6 Scopes of the study

This study consists of three main scopes.

1. To investigate the effect of *Labisia pumila* extracts on the growth of UVB irradiated human skin fibroblast (HSF1184) cells
2. To identify the difference between the effect of *Labisia pumila* extracts on normal HSF 1184 cells growth and UVB irradiated HSF 1184 cells
3. To investigate the potential of *Labisia pumila* extracts on the stimulation of collagen synthesis in HSF 1184 cells