Effects of *Labisia pumila* plant extract on the rate of growth of Human Skin Fibroblasts Cells (HSF 1184)

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Labisia pumila or commonly known as ‘Kacip Fatimah’ is traditionally used to facilitate and induce childbirth as well as post-partum medication amongst Malaysian women. This study concentrates on the effect of a standardized water extract of Labisia pumila on the rate of growth of Human Skin Fibroblasts Cells (HSF 1184). The effective concentration of the plant extract was determined by an efficacy test using spectrophotometry method involving MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). This step is very important as a non-suitable concentration of the Labisia pumila extract could be toxic to the cell culture environment and eventually lead to cell death. The rate of growth is defined as the growth of the cells monitored on daily basis. Data showed that the plant extract has caused significant growth promotion of the Human Skin Fibroblasts (HSF1184) cells.
CHAPTER 1: INTRODUCTION
**why** plant extract

- used since ancient time for cosmetic and pharmaceutical applications
- Different parts of plant including leaves, fruits, flowers, stem, barks, buds, and roots can be manipulated or use
- Cosmetically, plant and plant extracts are used due to their proven potential to provide moisturizing effect, whitening, sunscreen, antioxidant etc [1]
Labisia pumila...

- Popular herb in Malaysia, commonly known as ‘Kacip Fatimah’
- Traditionally used to induce and facilitate childbirth as well as post partum medication
- Interest has recently been shown in the herbal preparation to determine its mode of action and potential pharmacological application
- Two studies have shown that the plant exhibit oestrogenic properties [2]
• Recent study has showed that the plant extract has great potential in giving the photoprotective action to the human skin
Why choosing Human Skin Fibroblasts (HSF1184)

- Human skin fibroblasts cell line is used instead of primary cells because cell lines can continue growing through many subcultures
- The cells is available from ECACC United Kingdom
- Human skin fibroblasts cells are the major component of the skin dermis which are involved in the organization and production of ECM product
- Involves in maintaining the integrity of the human skin
- Widely used in cell culture environment
- They are a well established system for in vitro analysis of fibroblast growth, migration and collagen metabolism [3]
- been previously used to study skin aging [4], wound healing [5], genetic disorder [6], evaluating cosmetic formulations toxicity [7][8], and chemical cytotoxicity [9]
• commonly known as cell growth is defined as the increase in cell population
• very important to maintain the integrity of a certain type of cells
• constant and direct exposure to the environment can induce adaptive or degenerative pathways and influence ageing
• Skin regeneration is very important because skin can repair itself and function normally
Physiology of the skin..

• The skin is the largest organ of the body, weighing about four kilo-grams and covering about two square metres

• Skin is made up of several layers namely:
  – Stratum corneum
  – Epidermis
  – Dermis
  – Hypodermis

• Each layers of the human skin play a very important role in protecting the human body from the harmful environmental agents, particularly infective organisms (bacteria or viruses – ‘germs’), dirt, dust and sunlight
CROSS SECTION OF SKIN

- Dead cells – steadily shed from surface of skin
- Basal layer of keratinocytes containing evenly scattered melanocytes
- Stratum corneum
- Living epidermis
- Dermis
- Hypodermis
- Blood vessels
- Hair
- Sebaceous (oil) gland
- Hair root
- Nervous
- Fat

Cross-section of skin.
CHAPTER 2 : METHODS & MATERIALS
• Samples of dried, grounded *L. pumila* were extracted with a laboratory scale extractor in water at 100 °C for 4 h
• The extraction ratio between the dried, grounded raw material and water was 1:10 by mass
• the solid part was removed by filtration and the liquid part was directly spray dried
• The inlet temperature of the spray dryer was set at 180 °C and the outlet was set at 103 °C [4]
• Normal human dermal fibroblasts cells obtained from cell line HSF1184 were cultured in Dulbecco’s modified essential medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics
• All cells were maintained at 37ºC in a humidified atmosphere of 5% CO₂.
• normal Human skin Fibroblasts (HSF1184) cells from passage 8-10 were used.
MTT BIOASSAY

- laboratory test and standard colorimetric assays
- used to determine cytotoxicity of potential medicinal agents and other toxic materials, since those agents would result in cell toxicity and therefore metabolic dysfunction and therefore decreased performance in the assay
- Following treatment with L. pumila extract, culture medium was removed and MTT (0.33 g/L) solution was added for 90 min at 37 °C
- The supernatant was discarded and isopropanol was added to dissolve the formazan product
The intensity was measured colorimetrically at a wavelength of 570 nm by using ELISA reader.
• HSF 1184 cells were seeded at a density of $1 \times 10^5$ cells per well
• Human skin fibroblasts cells were cultured in fifteen 6-well plates
• All 6-well plates will represent the growth of the cells from day 0 until day 14
• The cells were treated with the *Labisia pumila* plant extract of $1 \times 10^4$ ug/ml concentration at day 0
Cultured media without plant extract (control)

Cultured media with the addition of plant extract
• Daily growth of the cells were monitored and cells population were counted using haemocytometer from day 0 until day 14
• Trypan blue exclusion test using a haemocytometer was used to do cell counting
• Trypan blue is excluded by live cells but accumulates in dead cells
• The number of stained (non-viable) cells and non-stained (viable) cells are counted under a light microscope
• The numbers of cells counted daily using haemocytometer were plotted in a graph
TRYPAN BLUE EXCLUSION TEST

Each small division is 0.05 mm.

Shaded area is one "cell."
• Statistical analyses are performed using Sigma Plot
• Values are expressed as mean ± SE with three independent experiments
• The results are represented as the means±S.E.M. All p values of less than 0.05 were considered statistically significant
CHAPTER 3: RESULT AND DISCUSSION
Effect of FBS on cell growth at different concentration of Labisia pumila

![Graph showing the effect of FBS on cell growth at different concentrations of Labisia pumila. The graph plots absorbance (% Control) against concentration (µg/ml). The x-axis represents the concentration levels from 1e-4 to 10000 µg/ml, while the y-axis shows absorbance ranging from 0.0 to 3.0. The graph compares the absorbance with FBS (black bars) and without FBS (gray bars) at each concentration.](image)
• that *Labisia pumila* has a very significant effect on the growth of HSF 1184 cell line

• This is true in both conditions, in the media with Fetal Bovine Serum (FBS) and in the media without Fetal Bovine Serum

• A medium alone without FBS will not provide a complete environment for the cell to survive

• Normally if a cell line is cultured in a medium without the existence of FBS, it will only take several days before the cells completely died

• This might suggest that *Labisia pumila* might be a possible substitute for a growth serum
• Daily growth of the cells were monitored and cells population were counted using haemocytometer from day 0 until day 14

• the effective concentration of the plant extract was determined and the concentration being chosen is $1 \times 10^{-4}$ ug/ml

• At this concentration, the growth stimulation of the cells was almost doubled and it did not show any toxic effect to the cells.
RATE OF GROWTH

Cells Density x10^7 (cells/ml) vs Day

- Control
- Labisia pumila
- Viability for control (%)
- Viability for Labisia (%)
• the addition of *Labisia pumila* extract has caused a significant increase in the growth of the cells
• Normally, the cells took about 14 days to be completely died, however after the addition of the plant extract; live cells were still available on day 14
• Even though the number of cells declined on the same day (day 7) for both conditions, this could happen due to the accumulation of waste products in the cell culture medium
• the cell number increases almost two-fold from day 1 to day 6 for cells treated with the plant extract
• this clearly indicates that *Labisia pumila* has a direct effect on the population doubling time (PDT) of the cells
• Results clearly show that *Labisia pumila* has significantly increased the growth and the rate of growth of HSF1184 cells

• Another studies should be done with constant replenishment of the cultured media to remove the accumulated waste products

• This could eliminate the effect of waste material on the stimulatory effect of *Labisia pumila*


THANK YOU