



THE EFFECTS OF WATER AND METHANOLIC EXTRACTS OF *LABISIA PUMILA* ON COLLAGEN SYNTHESIS IN HUMAN SKIN FIBROBLAST (HSF1184) CELLS

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ABSTRACT

Labisia pumila (Myrsinaceae) or commonly known as 'Kacip Fatimah' is traditionally used by Malaysian women to address reproductive health. In this study, we investigated the effects of standardized water extract and methanolic extract of *Labisia pumila* on the collagen synthesis in UVB-irradiated and non-irradiated Human Skin Fibroblast cells (HSF1184). 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 1×10^{-5} µg/ml, the standardized water extract of *Labisia pumila* promoted an increase of up to 70% in the collagen synthesis in for the HSF1184 UVB irradiated cells whereas in the methanolic extract at similar concentration the increase was only 20%. This concentration is shown to be the optimum concentration for both extracts of *Labisia pumila* throughout the course of the study. Beyond this concentration, the methanolic extracts indicated dose dependent inhibition effect. The result clearly indicates that the standardized water extract of *Labisia pumila* provides better effect than the methanolic extract.

1.0 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 are used for these applications. In Cosmetic, plant and plant extracts are used due to their proven potential to provide moisturizing, whitening, tanning, colour cosmetics, sunscreen, radical-scavenging, anti-oxidant, immune-stimulant, washings, preservatives, thickeners effect [1]. Every year, new researches are carried out on different plant extracts to get as much understanding as possible on their chemical profiles and bioactivities. This fundamental research was very important to the scientific community as we can explore and determine the potentials and benefits of

a plant extract for cosmetic or pharmaceutical applications [2]. In this particular study, human skin fibroblasts are being used as the subject for analysis as it is a well-established system for *in vitro* analysis of cell growth [3], migration, and collagen metabolism [4].

Human skin fibroblasts has been previously used to study skin aging [5], wound healing [6], genetic disorder [7], evaluating cosmetic formulations toxicity [8][9], and chemical cytotoxicity [10]. Even though, cell culture technology is not commonly used yet as a method to study the biological and chemical effect of plant extract on different types of cells available in cell culture, it has been proven to have very promising future. 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. This field involves the study of indigenous cosmetics, cosmeceuticals and remedies to address skin disorders using traditional herbs and plant extract [11]. Cell culture technology enables us to investigate the effect of plant extract on cells faster and at lower cost. 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 of three major types of cells, namely keratinocytes, melanocytes and fibroblast [12]. Due to constant and direct exposure to the environment, skin components including all three important cell types experience stress conditions that can induce adaptive or degenerative pathways and influence ageing [13]. Skin regeneration is very important in this condition so that the skin can repair itself and function normally. However, in most situations this condition 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. In this study, the standardize water extract and methanolic extract of *Labisia pumila* were used to investigate their effect on the growth of human fibroblast cells (HSF).

2.0 Materials and Methods

2.1 Preparation of *Labisia pumila* extract

The plant extract, *Labisia pumila var pumila*, used in this study was procured from Forest Research Institute Malaysia (FRIM). There are two types of *Labisia pumila* extracts used in this study, the water extract and the methanolic extract. To prepare the water extract of *Labisia pumila*; samples of dried, grounded *Labisia pumila var pumila* were extracted with a laboratory scale extractor in water at 100 °C for 4 hours. The extraction ratio between the dried, grounded raw material and water was 1:10 by mass. Following extraction, the solid residue was removed by filtration and the liquid part was directly spray dried (Production Minor GEA NIRO). The inlet temperature of the spray dryer was set at 180 °C and the outlet was at 103 °C. To prepare the methanolic extract of *Labisia pumila*; samples of dried, grounded *Labisia pumila var pumila* were extracted with a laboratory scale extractor in methanol at 100 °C for 4 hours. The extraction ratio between the dried, grounded raw material and water was 1:10 by mass. Following extraction, the solid part was removed by filtration

and the liquid part was directly spray dried (Production Minor GEA NIRO). The inlet temperature of the spray dryer was set at 180 °C and the outlet was at 103 °C. The samples were then stored at -20°C until further analysis. Both extracts were prepared from the same plants source. The source and the type of preparation for the plant extracts remain consistent at every stage of the study (Lee et al, 2011). 9 different concentrations of the water extract of *Labisia pumila* and methanolic extract of *Labisia pumila* were used in this study.

2.2 Cell culture

HSF 1184 cells (catalogue no. 90011883) (Human Skin Fibroblasts) (available from ECACC, United Kingdom) derived from normal Caucasian human skin cells are used for the purpose of this study. HSF1184 were cultured in Dulbecco's modified essential medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics. All cells were maintained at 37°C in a humidified atmosphere of 5% CO₂. For experiment normal Human skin Fibroblasts (HSF1184) cells from passage 8-10 were used.

2.3 Treatment with *Labisia pumila* plant extracts

Human Skin Fibroblast (HSF1184) cells were seeded at a density of 2×10^5 cells/well in 60 mm culture dishes and cultured in DMEM to 70% confluence. Cells were then starved in serum free DMEM for 24 hours and rinsed with phosphate-buffered saline (PBS). In this studies human skin fibroblasts cells were cultured in four 96-well plates. In each well plate, 60 wells were used for experimental purposes. 3 different concentrations of the standardized water extract and methanolic extract were used for this efficacy studies. 2 sets of 96-well plates were exposed to UVB irradiation whereas the remaining two sets of 96-well plates were not exposed to UVB irradiation. All cells were treated with their corresponding *Labisia pumila* extracts for 24 hours before cell viability assay procedure was carried out.

2.4 Ultraviolet (UVB) Irradiation

Human skin fibroblast cells (HSF1184) were seeded at a density of 2×10^5 cells/well in 60 mm culture dishes and cultured in DMEM to 70% confluence. Cells were then starved in serum free DMEM for 24 hours and rinsed with phosphate-buffered saline (PBS). Exposure to UVB irradiation was performed at 312 nm, 25 or 52 mJ/cm² by using a Philips F20T12/UV-B source (270–390 nm; containing 2.6% UVC, 43.6% UVB, 53.8% UVA), as measured with a SX-312 research radiometer (Uvitec, USA).

UVB-irradiated cells were cultured in DMEM with or without *Labisia pumila* extracts or ascorbic acid (Sigma, USA) for 48 hours [5].

2.5 MTT Bioassay

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay is a laboratory test and standard colorimetric assays (an assay which measures changes in colour) for measuring the activity of enzymes that reduce MTT to formazan, giving a purple colour. It can also be 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. The effect of the standardized water extract and methanolic extract of *Labisia pumila* on cell viability was assessed by using Thiazolyl Blue Tetrazolium Blue (MTT) assay. Following treatment with *Labisia pumila* extracts, culture medium was removed and MTT (0.33 g/L) solution was added for 180 minutes at 37 °C. The supernatant was discarded and isopropanol was added to dissolve the formazan product. The intensity was measured colorimetrically by using ELISA reader at a wavelength of 570 nm [5].

2.6 Sircol Collagen Assay

The Sircol Collagen Assay is a type of biological assay used in cell culture environment to evaluate the amount of acid and pepsin-soluble collagens and this procedure was based on a dye-binding method. The Sircol Collagen Assay is suitable test to be used to monitor and evaluate the amount of collagen produced in cell culture (*in-vitro*). This particular method is suitable in order to detect collagen, soluble in cold acid or pepsin, released into cell culture medium during cell growth and cell maintenance, and collagen, soluble in cold acid or pepsin, recovered from newly formed extracellular matrix that has been deposited onto cell culture treated plastic surfaces, (T-flasks and microwell plates). Cultured HSF1184 cells, standards and reagent blanks (100µl) were mixed with Sircol Dye reagent (1ml) in microcentrifuge tubes for 30 minutes. The collagen-dye complex formed precipitates out of solution. The complex was packed firmly to the bottom of the tubes by centrifugation at 12,000g for 10 minutes. Unbound dye was removed by inverting and draining tubes. Ice-cold acid-salt wash (750µl) was gently added to remove unbound dye from pellet surface and microcentrifuge tube. The tubes were centrifuged at 12,000rpm for 10 minutes and the wash drained off. Bound dye is released and dissolved via addition of alkali reagent (250µl or 1000µl depending on pellet size) and vortex mixing for 10 minutes. Released dye is measured spectrophotometrically at 555nm or colorimetrically with a blue-green filter using a multiwell plate reader [5].

2.7 Statistical Analysis

Statistical analyses were performed using Sigma Plot 10.0. Values were expressed as means ± SEM three independent experiments. Statistical significance of treatments was determined using the paired Student's *t* test. Once a *t* value is determined, a *p*-value can be found using a table of values from Student's *t*-distribution. If the calculated *p*-value is below the threshold chosen for statistical significance (usually the 0.10, the 0.05, or 0.01 level), the null hypothesis is rejected in favour of the alternative hypothesis.

3.0 Results and Discussion

Figure 1 shows the effect of standardized water extract of *Labisia pumila* on the collagen synthesis in HSF1184 cells after 48 hours of incubation. In this experiment only 3 concentrations were chosen as these 3 concentrations proved to be consistent in stimulating growth of the HSF1184 cells. In the previous study done by Mukrish et al. (2010), the growth of human skin fibroblast cells (HSF1184) cultured in DMEM was stimulated significantly at these 3 concentrations, 1×10^{-5} µg/ml, 1×10^{-4} µg/ml and 1×10^{-3} µg/ml. This result was also consistent with another study previously done Choi et al. (2010). According to the graph in Figure 1 all three concentration caused stimulation in collagen synthesis in the HSF1184 cells. The concentration that showed the most significant collagen stimulatory effect was at 1×10^{-5} µg/ml.

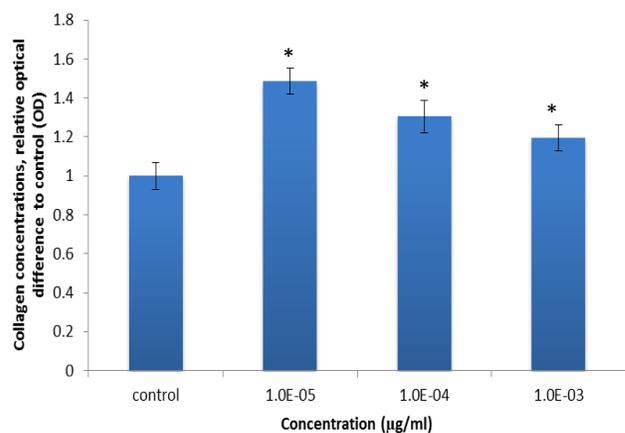


Fig. 1 The effect of standardized water extract of *Labisia pumila* on the collagen synthesis in HSF1184 cell line. (Control – HSF1184 cells without the addition of standardized water extract of *Labisia pumila*). Assays were performed in 3 replicates from 3 independent experiments. Values are means ± SEM (*, $p < 0.01$).

At this concentration the collagen synthesis increased up to 50% compared to the negative control (Control – HSF1184 cells without the addition of standardized water extract of *Labisia pumila*). The results were consistent with the previous

study done by Choi et al 2010 involving the same plant extract.

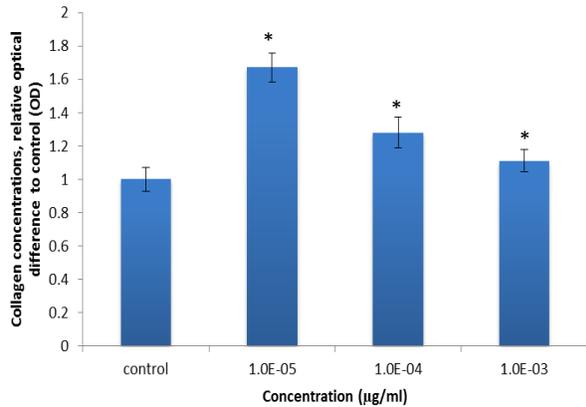


Fig. 2 The effect of standardized water extract of *Labisia pumila* on the collagen synthesis in UVB irradiated HSF1184 cell line. (Control – HSF1184 cells exposed to UVB irradiation but without the addition of standardized water extract of *Labisia pumila*). Assays were performed in 3 replicates from 3 independent experiments. Values are means \pm SEM (*, $p < 0.01$).

Figure 2 shows the effect of the standardized water extract of *Labisia pumila* on the collagen synthesis in UVB irradiated HSF1184 cells. In this experiment, the HSF1184 cells were cultured in media supplemented with FBS and were exposed to 80 mJ/cm² of UVB radiation.

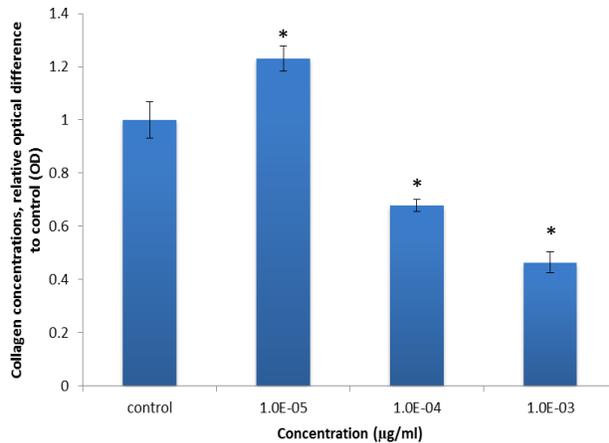


Fig. 3 The effect of methanolic extract of *Labisia pumila* on the collagen synthesis in HSF1184 cell line. (Control – HSF1184 cells without the addition of methanolic extract of *Labisia pumila*). Assays were performed in 3 replicates from 3 independent experiments. Values are means \pm SEM (*, $p < 0.01$).

A similar trend was observed in this experiment as in Figure 1, however at the same concentration of 1×10^{-5} µg/ml of the standardized water extract of *Labisia pumila*, the collagen production increased up to 60%, which was higher than the experiment without UVB irradiation. This observation has supported that the standardized water extract of *Labisia*

pumila was photoprotective against the damaging effect of the UVB irradiation as well as promote the collagen synthesis which is essential to avoid photoaging.

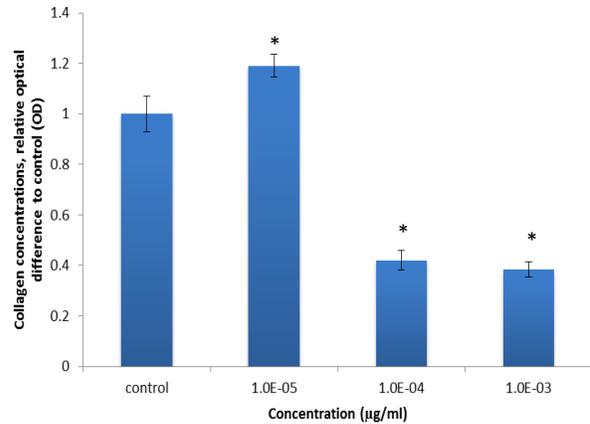


Fig. 4 The effect of methanolic extract of *Labisia pumila* on the collagen synthesis in UVB irradiated HSF1184 cell line. (Control – HSF1184 cells exposed to UVB irradiation but without the addition of methanolic extract of *Labisia pumila*). Assays were performed in 3 replicates from 3 independent experiments. Values are means \pm SEM (*, $p < 0.01$).

4.0 Recommendation

Further studies should be done focusing on the identification of the possible bioactive compounds or phytochemicals that are responsible for the photoprotective effect of the *Labisia pumila* plant extract. Apart from that, future studies should also try to identify the mechanism on how the *Labisia pumila* plant extract could cause growth stimulation and also increase collagen synthesis. The growth stimulatory effect on this study might occur due to the adaptive mechanism of the HSF1184 cells or it could occur due to the presents of some bioactive compounds in the plant that acted as the precursor to both growth and collagen synthesis pathways. Therefore, this study is important to get the confirmation on the actual mechanism on how the extract shows its photoprotective effects.

Culture media used in this study was supposed to imitate the blood circulatory system in the human body. In reality, our blood circulatory system has a mechanism to excrete metabolic waste products that are harmful to cell growth. This study was done without medium replenishment and this means, the metabolic waste products available in the media could cause cell death and eventually affect the result of the study. Therefore, further studies should be done with medium replenishment so that cell death does not happen due to the toxicity of the metabolic waste products.

5.0 Conclusion

The results of this study clearly indicate that both plant extracts caused significant increase in the collagen synthesis of the HSF1184 cells in both UVB non-irradiated and UVB irradiated cells. However, the effect of the standardized water extract of *Labisia pumila* was greater than the methanolic extract of *Labisia pumila*. At concentration of 1×10^{-5} $\mu\text{g/ml}$, the standardized water extract of *Labisia pumila* promoted an increase in 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%. Higher concentration of the methanolic extract of the plant showed inhibitory effect to the collagen synthesis, but this was not the case for the standardized water extract of *Labisia pumila* at both 1×10^{-4} $\mu\text{g/ml}$ and 1×10^{-3} $\mu\text{g/ml}$, as the collagen synthesis increased slightly in both concentrations. Therefore, the results of this study also prove that both *Labisia pumila* plant extracts showed photoprotective effect on human skin fibroblast cells (HSF1184) against UVB irradiation. This characteristic could be used to in cosmeceutical products.

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