# THE DERMATOXICOLOGICAL PROFILE OF *Labisia pumila* EXTRACT FOR COSMETIC APPLICATION

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# THE DERMATOXICOLOGICAL PROFILE OF *Labisia pumila* EXTRACT FOR COSMETIC APPLICATION

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*Dedicated to my beloved 'Jannah'…*

*Kanda..*
*Muhammad Syarzril Bin Muhammad Ghazi*

*Ayahanda..*
*Ali Bin Raub & Muhammad Ghazi Mahmod*

*Bonda..*
*Samidah Binti Asad & Sri Rahayu S. Sudarmaji*

*Adinda..*
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*&*

*Amir*

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# ABSTRACT

In recent years, there has been an increase in public concern on the safety and efficacy of cosmetic ingredients. Recently, the potential *Labisia pumila* (*L. pumila*) for cosmetic application was expanded as a skin moisturizer product. However, the general toxicity effect of *L. pumila* on skin is not well understood. Hence, the objective of this study was to carry out the safety assessment of *L. pumila* for cosmeceutical applications. This research encompassed cytotoxicity study, allergic dermatitis assessment, genotoxicity study, ocular irritancy and *in vivo* toxicological study. Based on the results, *L. pumila* was relatively non-cytotoxic up to 0.001 mg/mL. Penetration and absorption analyses revealed that overall recovery was in range of 100±15% and were considered acceptable with each of 500 mg/mL and 1000 mg/mL of extracts, respectively. The *L. pumila* extract did not cause mutagenic effects on *Salmonella thyphimurium* in the presence or absence of metabolic activation. Allergic contact dermatitis assessment through *in vitro* dermal irritation study showed the mean cell viability was found to be 91.5% with a standard deviation of ± 3.17%. However, sensitization analysis indicated that the extract is a sensitizer due to the average depletion of both peptides at 17.75%, more than 6.37% with low reactivity. The Bovine Corneal Opacity and Permeability (BCOP) examination indicated that non-ocular irritation occurred. After a single dose of dermal application in acute dermal toxicity study, estimation of lethal dose was 2000 mg/kg. The repeated dose for 28-days dermal toxicity study also indicated Non-Observed Adverse Effect Level (NOAEL) was noted up to 1000 mg/kg. From all of the analyses conducted, this pre-clinical safety assessment suggested that the *L. pumila* water extract is safe for present practices with the appropriate concentration for cosmetic formulation.

# ABSTRAK

Kesedaran masyarakat hari ini kian meningkat terhadap keselamatan serta keberkesanan bahan-bahan kosmetik. Kebelakangan ini, potensi *Labisia pumila* (*L. pumila*) diperluas dalam applikasi kosmetik seperti produk krim pelembab wajah. Walau bagaimanapun, kesan toksik kepada kulit secara amnya masih belum jelas. Oleh itu, kajian ini bertujuan menilai tahap keselamatan *L. pumila* bagi applikasi kosmetik. Kajian ini melibatkan ujian kesitotoksikan, penilaian alahan dermatitis, keradangan okular, ujian kegenotoksikan dan kajian toksikologi *in vivo*. Hasil kajian menunjukkan 0.001 mg/mL ekstrak adalah tidak sitotoksik secara relatif. Analisis ujian penembusan dan penyerapan mencatatkan nilai 100±15% kadar pemulihan bagi setiap 500 mg/mL dan 1000 mg/mL ekstrak. Ekstrak *L. pumila* tidak menyebabkan kesan mutagen kepada *Salmonella thyphimurium* dengan kehadiran atau ketidakhadiran pengaktifan metabolik. Penilaian alahan dermatitis melalui ujian kerengsaan kulit secara *in vitro* menunjukkan daya maju sel pada tahap 91.5% dengan sisihan piawai ± 3.17%. Walau bagaimanapun, analisis pemekaan mencatatkan purata nilai kedua-dua peptid 17.75% melebihi nilai piawaian sebanyak 6.37% dengan keaktifan yang rendah. Pemeriksaan Kelegapan dan Kebolehtelapan Kornea Bovin (BCOP) menunjukkan tiada alahan okular berlaku. Selepas applikasi dos tunggal ujian ketoksikan dermis akut, anggaran dos maut adalah 2000 mg/kg. Ujian applikasi berulang untuk 28 hari ujian ketoksikan dermis juga menunjukkan aras Kesan Buruk yang Tidak Diperhatikan (NOAEL) sehingga 1000 mg/kg. Daripada analisis yang dijalankan, penilaian keselamatan melalui kajian pre-klinikal ini menjangkakan bahawa ekstrak *L. pumila* adalah selamat untuk penggunaan buat masa ini dengan kepekatan yang sesuai untuk formulasi kosmetik.

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| | | |
|---|---|---|
| AMES | - | Bacteria Reverse Mutation Test |
| ANOVA | - | Analysis of Variant |
| AP-1 | - | Activated Protein 1 |
| BCOP | - | Bovine Corneal Oppacity and Permeability Assay |
| $CO_2$ | - | Carbon Dioxide |
| COLIPA | - | Cosmetics Europe-The Personal Care Association |
| DMEM | - | Dulbecco's Modified Eagle Medium |
| DMSO | - | Dimethylsulfoxide |
| DNA | - | Deoxyribonucleic Acid |
| ECACC | - | European Collection of Cell Cultures |
| ELISA | - | Enzyme Linked Immuno Sorbent Assay |
| FBS | - | Fetal Bovine Serum |
| FDA | - | Food and Drug Administration |
| GLP | - | Good Laboratory Practice |
| HaCaT | - | Human Culture Keratinocyte |
| IBD | - | Institute Bioproduct Development |
| IVIS | - | In vitro Irritancy Score |
| $LD_{50}$ | - | Median Lethal Doses |
| MEMT | - | Mammalian Erythrocyte Micronucleus Test |
| MMPs | - | Matrix Metaloprotienases |
| MTT | - | Yellow 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide |
| NOAEL | - | No Observe Adverse Effect Level |
| NF-kB | - | Nuclear Factor kappa-light-chain- activated B cells |
| OECD | - | Organization of Economic Cooperation and |

| | | |
|---|---|---|
| | | Development |
| PBS | - | Phosphate Buffer Solution |
| PCP | - | Personal Care Product |
| ROS | - | Reactive Oxygen Species |
| SE | - | Standard Error |
| SCCP | - | Scientific Committee of Cosmetic Product |
| SOD | - | Superoxide Dismutase |
| UN-GHS | - | United Nation Globally Harmonized System |
| UVA | - | Ultra-violet A |
| UVB | - | Ultra-violet B |
| UV | - | Ultra-violet |

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the Study

A large number of cosmetic consumers recently had shown progressive interest in the prevention of aging skin and maintaining their youthful appearance by using product from natural ingredients. Generally, the safety of the cosmetic is based on the safety of it active ingredient (Darr *et al*, 1996). This statement was in accordance with European cosmetic legislation and Scientific Committee of Consumer Product (SCCP), which prohibits final products containing hazardous ingredient that may affect human health (SCCP, 2006).

The growth of natural cosmetic industry nowadays is increasing tremendously with cosmetic consumers awareness. The term 'Natural Product' synonymously interpreted as 'safe' product due to its organic origin, even the fact that natural ingredient may have a greater variability of content compared to synthetically produced product. It must be noted that natural ingredients may also have a potential of batch to batch inconsistency and prone to contamination due to the harvesting process (Larry, 2001). It was also reported that many poisonous alkaloid came from the natural products and numerous products are lacking the

acceptable quality and safety standards. The issue was due to the product developer not following a scientifically bases to assess any product benefits and safety guidelines.

There is limited number of research journals in the area of evaluation in mode of action, efficacy, physiological and metabolic changes and also toxicity levels of the natural extract compared with amount of commercial products available in the market (Melissa *et al*, 2008; Ezumi *et al*: 2006). None of the toxicological profile was provided; a probability deleterious effect on human health was not evaluated. The safety assessment of natural ingredient is important in order to approach the general acceptance of basic systemic margin safety and attempt to propose any potential health risk due to application or exposure such as adverse dermal effect. The adverse dermal effect including dermatitis contact irritation, immediate or delay allergic reaction and also skin sensitization (Bakalli *et al.,* 2008) may develop upon exposure. With this respect, the safety issue of the natural ingredient must take into account (Eric *et al.,* 2011).

Kligman (1993) in his dermatologist guidelines, also indicates the safety of naturals ingredients including the potential of the active ingredient penetrating the various layers of the dermal skin and can delivered in sufficient concentration to the intended target consistently over time application. He also mentioned the important of specifying the bioactive compound mechanism of action in the target cells or skin. Such claims should be initiated together with any publication regarding the plant biological references, pre-review, pre-clinical trial or significant clinical trial to substantiate the efficacy claims.

The natural ingredients that were used in cosmetic product usually came from various plant extracts of wild herbal roots, leaves, or even bulbs. These natural ingredients were used more than a decade as traditional remedies among the local people either to treat internal diseases or topical application. One of the well-known

herbal among Malaysian women is *Labisia pumila* that is locally known as 'Kacip Fatimah'.

The *L. pumila* has been used traditionally to address for post-partum conditions (Zakaria and Muhammad, 1994; Ayida *et al*, 2008; Singh *et al*, 2009). It was also used widely for post-partum tonic, firm abdominal muscle, improve body strength, and regulate the menses (Shahrim, 2006, Yan *et al.,* 2012). A recent study also revealed that the extract benefited the skin by topical application. The *L. pumila* extract was also reported to promote cells regeneration and exhibits antiphoto-aging properties (Choi *et al*, 2010). Moreover, its also reported to inhibit microbial activity (Karimi *et al.,* 2011), anti-stress (Kaur *et al.,* 2010) and potentially used for polycystic Ovarian Syndrome (PCOS) treatment (Manneras *et al*, 2010).

Currently, the commercially prepared *L. pumila* extract is widely available in the market either in capsule, liquid or powder form as herbal supplement. The benefits of the extract also had been added into flavored drive such as pre-mixed tea/coffee as daily beverages. Besides that, its also found been added as active ingredient in cosmetic product such as daily moisturizer for skin improvement due to flavonoids, anti-oxidant properties present inside the extract. The antioxidant activity of the aqueous *L. pumila* extract has been reported to provide significant protection to human dermal fibroblasts, from cell damage caused by UV irradiation (Choi *et al.,* 2010). However, the safety issues of the extract itself on dermal application were not well reported.

Based on these considerations, the final research finding will give a better understanding of its effect on human skin when applied topically. Hence, this evaluation of the *L. pumila* extract towards hazard dermatological potential in cosmetic application will contribute to the cosmetic industry by giving the safety limit of the extract for cosmetic formulation.

## 1.2 Problem Statement

Consequently, according to previous studies it can be suggested that the *L. pumila* extract offers great potential for the prevention of skin aging and also benefited to improve the skin health based on its physiochemical and biological properties.

However, the assessment on dermatological effect on skin is not well reported. This evaluation is important for margin safety of cosmetic formulation especially under actual condition of use. The lack of information may develop dermal adverse effect that can cause various skin diseases such as contact dermatitis, severe irritation and chronic allergic reaction.

Therefore, this study provides a profile of the *L. pumila* extract to address safety issues for cosmetic formulation and may help in the development of new cosmetic product. It also gives the best correlation between risk and the benefit for developing a safe cosmetic product in the market.

## 1.3 Objective of The Study

The main objective of this research is to establish the dermatoxicological profile of *L. pumila* extract for cosmetic formulation.

## 1.4 Scopes of The Study

Following scopes of research were applied in order to fulfill the objectives:

**1.4.1** To determine cytotoxicity effect of *L. pumila* extract for pre-assessment toxicological studies using direct cells contact method.

**1.4.2** To investigate the penetration and absorption potential of *L. pumila* extract on Reconstructed Epidermis Human Skin (Epiderm $^{TM}$).

**1.4.3** To demonstrated the effect of *L. pumila* extract on genotoxicity and mutagenicity via AMES test on *Salmonella thypimurium*.

**1.4.4** To investigate the allergic contact dermatitis through sensitivity tests consisting of in vitro dermal irritation and sensitization test.

**1.4.5** To assess ocular opacity and permeability by BCOP analysis for eye irritation evaluation.

**1.4.6** To investigate NOEL and NOAEL of *L. pumila* on Sprague Dawley rats using acute dermal toxicity test and dermal repeated dose 28-days toxicity study.

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