UNIVERSITI PUTRA MALAYSIA

EVALUATION OF ANTIOXIDANT AND ANTI-PSORIATIC ACTIVITIES OF PURPLE SWEET POTATO (IPOMOEA BATATAS (L.) LAM) LEAVES AQUEOUS EXTRACT ON IMIQUIMOD-INDUCED PSORIASIS-LIKE DERMATITIS

NUR AFIQAH BINTI ARJUNAIDIE

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ABSTRACT

Evaluation of Antioxidant and Anti-psoriatic Activities of Purple Sweet Potato (*Ipomea batatas* (L.) Lam) Leaves Aqueous Extract on Imiquimod-induced Psoriasis-like Dermatitis

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Introduction: Psoriasis is a chronic autoimmune skin disease and is one of the most unpredictable and currently incurable and may greatly affect patients' quality of life. Conventional therapy using corticosteroids, Vitamin D analogs, calcineurin inhibitors and cytotoxic agents is associated with low success rate and their prolonged use may cause severe adverse effects. Thus, the discovery of more effective anti-psoriatic drugs with a minimal side effect is currently an active research area. In Malay traditional medicine, the leaf part of sweet potato (*Ipomoea batatas*) has been claimed as anti-psoriatic. However, scientific reports on its anti-psoriatic activity are very limited. **Objective:** The aim of this study is to evaluate the antioxidant and anti-psoriatic activities of purple sweet potato leaf aqueous extract (PSPLAE) on imiquimod (IMQ)-induced psoriasis-like dermatitis in Balb/C mice. **Methodology:** Several antioxidant-related phytochemical compositions in PSPLAE, namely total phenolic (TPC), total flavonoids (TFC) content, were investigated. In addition, the antioxidant activity of PSPLAE was tested using 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay. The PSPLAE was then subjected to Imiquimod, IMQ-induced psoriatic Balb/C mouse model to check the efficacy of PSPLAE against psoriasis-like dermatitis, where treatment was carried out for 15 days topically (PSPLAE cream at 5, 10 and 20%) and their Psoriasis Area Severity Index (PASI) was calculated. **Results:** From the phytochemical analysis, PSPLAE exhibited the corresponding values of 7.62 ± 1.91mg GAE/g dry extract (DE), and 2.74 ± 0.85 mg QE/g DE for TPC and TFC, respectively. In addition, the antioxidant activity of PSPLAE, which determined by the DPPH assay showed lower activity (EC_{50} = 244.8 ±13.6 µg/mL) when compared to the ascorbic acid standard (EC_{50} = 47.3 ±8.9 µg/mL). Interestingly, the antioxidant activity was found positively (r^2 = 0.59) correlated with the total phenolic content, TPC. As for the *in vivo* study, the effect of PSPLAE cream on the IMQ-induced psoriatic mouse model showed a dose-dependent response as evident through the PASI grading. **Discussion:** The results of the present investigation reveal the anti-psoriatic activity of the aqueous extract of *I. batatas* leaves against IMQ-induced psoriasis-like dermatitis in Balb/C mice. Based on the findings from this study and previous published reports, the mechanism of action of PSPLAE in ameliorating the psoriasis-like dermatitis in the experimental animals is closely related to the antioxidant activity of polyphenolic compounds present in the leaves part of *I. batatas*. **Conclusion:** This study suggests that sweet potato leaves are an inexpensive source of natural antioxidants with a possible anti-psoriatic activity.

Keywords: psoriasis, imiquimod, psoriasis area and severity index (PASI), DPPH radical scavenging assay, *Ipomoea batatas*

ABSTRAK

Penilaian Aktiviti Antioksidan dan Anti-psoriatik oleh Ekstrak Akues Daun Ubi Keledek Ungu (*Ipomoea batatas* (L.) Lam) Terhadap Dermatitis Seakan Psoriasis Aruhan Imiquimod

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Pengenalan: Psoriasis adalah penyakit kulit autoimun yang kronik dan merupakan salah satu penyakit yang paling tidak dapat diramalkan dan kini tidak dapat disembuhkan dan boleh mempengaruhi kualiti hidup pesakit. Terapi konvensional menggunakan kortikosteroid, analog Vitamin D, penghambat calcineurin dan agen sitotoksik dikaitkan dengan kadar kejayaan yang rendah dan penggunaannya yang berpanjangan boleh menyebabkan kesan buruk yang teruk. Oleh itu, penemuan ubat-ubatan anti-psoriatik yang lebih berkesan dengan kesan sampingan yang minimum kini menjadi kawasan penyelidikan yang aktif. Dalam perubatan tradisional Melayu, bahagian daun ubi keledek (*Ipomoea batatas*) telah dinyatakan sebagai anti-psoriatik. Walau bagaimanapun, laporan saintifik mengenai aktiviti anti-psoriatiknya sangat terhad. **Objektif**: Tujuan kajian ini adalah untuk menilai aktiviti antioksidan dan anti-psoriatik di dalam ekstrak akues daun ubi keledek ungu pada dermatitis psoriasis yang disebabkan oleh imiquimod pada tikus Balb/C. **Metodologi**: Beberapa komposisi fitokimia yang berkaitan dengan antioksidan dalam ekstrak akues daun ubi keledek ungu, iaitu jumlah fenolik dan jumlah flavonoid diselidiki. Sebagai tambahan, aktiviti antioksidan ekstrak akues daun ubi keledek ungu diuji dengan menggunakan ujian pemulih radikal bebas 2,2-diphenyl-1-picryhydrazyl (DPPH). Daun ubi keledek ungu kemudiannya diuji dengan Imiquimod, model tikus psoriatik Balb/C yang disebabkan oleh imiquimod untuk memeriksa keberkesanan ekstrak akues daun ubi keledek ungu terhadap dermatitis psoriasis, di mana rawatan dilakukan selama 15 hari secara topikal (krim PSPLAE pada kadar 5, 10 dan 20%) dan Indeks Keparahan Kawasan Psoriasis (IKKP) mereka dikira. **Keputusan**: Dari analisis fitokimia, ekstrak akues daun ubi keledek ungu menunjukkan nilai yang sesuai iaitu 7.62 ± 1.91mg GAE / g berat kering, dan 2.74 ± 0.85 mg QE / g berat kering untuk jumalah fenolik dan jumlah flavonoid, masing-masing. Di samping itu, aktiviti antioksidan di dalam ekstrak akues daun ubi keledek ungu, yang ditentukan oleh ujian DPPH menunjukkan aktiviti yang lebih rendah (EC50 = 244.8 ± 13.6 µg / mL) jika dibandingkan dengan piawai asid askorbik (EC50 = 47.3 ± 8.9 µg / mL). Menariknya, aktiviti antioksidan didapati positif (r2 = 0.59) berkorelasi dengan jumlah kandungan fenolik. Bagi kajian in vivo, kesan krim ekstrak akues daun ubi keledek ungu pada model haiwan tikus psoriatik yang diaruh oleh imiquimod menunjukkan tindak balas yang bergantung pada dos seperti yang ditunjukkan melalui penilaian IKKP. **Perbincangan**: Hasil penyiasatan ini menunjukkan, terdapat aktiviti anti-psoriatik oleh ekstrak akues daun *I. batatas* terhadap dermatitis psoriasis seperti imiquimod pada tikus Balb/C. Berdasarkan penemuan dari kajian ini dan laporan yang diterbitkan sebelumnya, mekanisme tindakan PSPLAE dalam memperbaiki dermatitis seperti psoriasis pada haiwan eksperimen berkait rapat dengan aktiviti antioksidan sebatian polifenolik yang terdapat

di bahagian daun *I. batatas.* **Kesimpulan**: Kajian ini menunjukkan bahawa daun ubi keledek adalah sumber antioksidan semula jadi yang mudah didapati dengan kemungkinan mempunyai aktiviti anti-psoriatik.

Kata kunci: psoriasis, imiquimod, Indeks Keparahan Kawasan Psoriasis (IKKP), ujian pemulih radikal bebas (DPPH), *Ipomoea batatas*

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AMP	Antimicrobial Peptide also recognized as host defence peptide
APC	Antigen Presenting Cells is a cell that bring the antigen to MHC.
BCG	Bacillus Calmette-Guerin vaccine used to fight tuberculosis
DPPH	2,2-diphenyl-1-picrylhydrazyl
IFN	Interferon is a protein
IFN-γ	Interferon – γ is a dimerized soluble cytokine
IL-1	Interleukin-1 is the prototypic pro-inflammatory cytokine
IL-6	Interleukin-6 play a role in pro-inflammatory cytokine
IL-17	Interleukin-17 is secreted by T-helper cell
IMQ	Imiquimod used to induce psoriasis
LL37	Known as Cathelicidin is an essential substance in immune system
PASI	Psoriasis Area and Severity Index to assess the intensity of psoriasis
PSPLAE	Purple Sweet Potato Leaf Aqueous Extract
pDC	plasmacytoid Dendritic Cells is an immune cell that produce type 1 IFN
ROS	Reactive Oxygen Species is a reactive chemical produced by oxygen gas.
TFC	Total Flavonoid Content
TLR-7/8	Toll-like Receptor – 7/8 is a protein that encoded by TLR7/8 gene
TLR-9	Toll-like Receptor – 9 is an essential receptor exhibited in immune system
TNF-α	Tumor Necrosis Factor – α is inflammatory cytokine released by macrophages
TPC	Total Phenolic Content
UV	Ultraviolet rays

CHAPTER 1

INTRODUCTION

1.1 Background

Psoriasis is a non-communicable and chronic inflammatory skin disease. Psoriasis is distinguished by white scales and strongly delineated erythematous plaques. Other than that, psoriasis also has been defined by Boehncke and Schön (2015) as genetic and immune-mediated disease that can be exhibited or occurred in both joint or skin or maybe either one only. This noncommunicable disease also greatly affects the quality of life of the patient. Psoriasis also has been linked with some comorbidities that need to be appropriately diagnosed and managed. The risk factor of psoriasis is categorized into two categories which are internal risk factor and external risk factor (Kamiya et al., 2019). Internal risk factors include obesity, diabetes mellitus, dyslipidemia, hypertension, and also mental stress. Besides, the external risk factors include mechanical stress, air pollution and sun exposure, drugs, vaccination, infection and lifestyle.

The diagnosis of psoriasis is usually done by observation of the erythematous scaly patches, plaques and papule presence which is often painful and pruritic. The classification of psoriasis can be categorized into plaque-type psoriasis, inverse psoriasis, erythrodermic psoriasis, localized pustular psoriasis, and guttate psoriasis (Weigle & McBane, 2013). The most common type is plaque-type psoriasis. It also can occur in non-dermatologic areas such as nail (psoriatic onychodystrophy) and psoriatic arthritis. Psoriasis is characterized by persistent inflammation, which results in uncontrolled keratinocyte growth and defective differentiation. According to Rendon and Schäkel (2019), the growth of keratinocytes was activated by the

inflammatory mediators through TNF-α, IL-17 and IFN-γ. Other than that, LL37-DNA complexes also trigger the activation of keratinocytes and lead to the increased secretion of type I IFNs. They also play an active role in the inflammatory cascade by secreting cytokines (IL-1, IL-6, and TNF-α), chemokines, and AMP. The experimental design in this study involves an induction of psoriasis-like dermatitis in Balb/C mice by topical cream, imiquimod (IMQ). IMQ is a medication that is used to cure genital warts and it acts by activating the Toll-like receptor-7/8 (TLR-7/8). This IMQ-induced psoriasis-like dermatitis model is used in this study to determine the therapeutic effect of purple sweet potato leaf aqueous extract (PSPLAE) on psoriasis disease.

Currently, there are a variety of therapy or medication that are available to cure or manage psoriasis. The treatment needed for psoriasis depends on its severity. The severity of the diseases is determined by the condition of the lesion, percentage of body area affected and how psoriasis affects the patient's quality of life (Mrowietz et al., 2011). Corticosteroids, vitamin D3 analogues and calcineurin are the examples of the conventional therapies and currently were used as a topical treatment for mild psoriasis (Na Takuathung et al., 2018). Menter et al. (2009) discussed that phototherapy or systemic medicines such as methotrexate, cyclosporine, and acitretin are frequently used to treat severe psoriasis. Other than that, biologic therapies for psoriasis therapies also have been developed and approved where it will play a role on the upregulated cytokine pathways. In several studies, antioxidant activity also has been proven to have a positive effect on the treatment of psoriasis. However, there are studies that report that the majority of these treatment have a well-documented list of side effects that appear to be the primary reason preventing patients from complying to long-term treatment of psoriasis, indicating a need for the development of a medicine with improved efficacy but fewer side effects.

Moreover, this study was proposed due to the unscientific findings of Malaysian patients that were not satisfied with the outcome of the conventional therapies but, after trying topical cream of purple sweet potato leaf, these patients found a significant remission of the treatment. Thus, this led to a new development of research to determine the antioxidant and anti-psoriatic activity of purple sweet potato leaf aqueous extract (PSPLAE).

1.2 Objectives

1.2.1 General Objective

The purpose of this study is to assess the antioxidant and anti-psoriatic activities of Ipomoea batatas leaves aqueous extract (PSPLAE) on imiquimod-induced psoriasis-like skin lesions in Balb/C mice.

1.2.2 Specific Objective

I. To measure the total phenolic content (TPC) of *I. batatas* leaves aqueous extract by using Folin-Ciocalteu assay and its total flavonoid content (TFC) by using aluminium chloride colorimetric assay.

II. To assess the antioxidant activity of PSPLAE using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.

III. To assess the relationship between TPC, TFC and antioxidant activity by using the Pearson's correlation coefficient, r.

IV. To measure the effects of topical application of PSPLAE on the imiquimod-induced psoriasis-like skin lesions using psoriasis area and severity index (PASI) score and cumulative score of psoriasis severity index.

1.3 Hypothesis

It is hypothesized that the relationship between TPC, TFC and antioxidant activity of purple sweet potato leaf aqueous extract (PSPLAE) is a positive correlation and that PSPLAE might exhibit anti-psoriatic activities on imiquimod-induced psoriasis-like skin lesions in Balb/C mice.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Psoriasis

Psoriasis is one of the most occurring inflammatory skin illness. The global incidence is evaluated to be around 2%, however it varies between countries. Asian and some African ethnicities have a lower incidence, while Caucasian and Scandinavian populations have up to 11% (Rendon & Schäkel, 2019). Psoriasis is characterized as a chronic inflammatory disease with a whitish scale and erythematous skin lesion. Kamiya et al. (2019) also described psoriasis as a chronic inflammatory skin illness that is distinguished by white scales and strongly delineated erythematous plaques. Other than that, Boehncke and Schön (2015) defined psoriasis as a genetic and immune-mediated disease that can be exhibited or occurred in both joint or skin or maybe either one only.

2.1.1 Risk Factor of Psoriasis

Psoriasis can happen at any age and the onset of the psoriasis can be affected by genetic background, environmental factors, and ethnicity. Based on Kamiya et al. (2019), they described that the risk factor for psoriasis can be categorized into two groups which are external and internal risk factors. The extrinsic factors include mechanical stress, air pollution and sun exposure, drugs, vaccination, infection and lifestyle. For instance, the oxidative stress from various air pollutants that include volatile organic compounds, heavy metals, ozone, oxides, particulate matter, polycyclic aromatic hydrocarbons, and UV will cause damage to the skin. In psoriasis, the air pollutants that usually affect the psoriasis pathogenesis is cadmium. Liaw et al. (2017) describe that higher blood cadmium were found in patients with serious psoriasis as compared to normal healthy population. This research reveals that

cadmium exposure in the environment may weaken immunity, and that microenvironmental disruption can predispose one to psoriasis exacerbation.

Another example of an extrinsic factor that triggers psoriasis is Bacillus Calmetter-Guerin (BCG) vaccination. According to Luca and Mihaescu (2013), BCG is a live attenuated strain of *Mycobacterium bovis* that is predominantly used for tuberculosis prevention. A case of erythrodermic pustular psoriasis which was induced by BCG immunotherapy in bladder cancer patients was also found (Wee et al., 2012). Unhealthy lifestyle such as smoking, and alcohol consumption are associated with the increasing cases of psoriasis. In the study done by Lee et al. (2017), a positive correlation between the duration of smoking and the psoriasis occurrence were found. There was also a trend that shows the risk of psoriasis will increase when the duration of smoking increases. For alcohol consumption, the researcher agreed that there were not enough studies or research to establish the relationship between psoriasis and consumption of alcohol. However, in a study done by Murzaku, Bronsnick and Rao (2014), the greater risk of psoriasis was observed in patients that consume alcohol when compared with the normal population.

The intrinsic factors include obesity, diabetes mellitus, dyslipidemia, hypertension, and mental stress (Kamiya et al., 2019). Obesity is a condition where there is an enlargement of white adipose tissue. Obesity was found to have a positive correlation with psoriasis. This is because the pathogenesis of psoriasis was contributed by the various mediators that are produced by adipose tissue and thus lead to a low-grade inflammatory state. TNF-α, IL-6, adiponectin and leptin were the pro-inflammatory adipokines that were secreted in adipose tissue. Leptin plays an important role as a hormone in adipose tissue. Leptin also functions as the metabolic status regulator, and it can affect the immune and inflammatory responses. According

to Zhu et al. (2013), psoriatic patients had a higher plasma or serum levels of leptin as compared with the healthy population. Another intrinsic factor that can trigger psoriasis is mental stress. Mental stress is a serious condition that occurs when the coping ability of a person or individual is exceeded by demands. Many physicians and psoriasis patients agreed that mental stress can trigger psoriasis and exacerbates or worsened the psoriasis. The exacerbation of psoriasis was induced by the itch-scratch-itch cycle and this condition was contributed by the daily increase of stress and worry. The researcher also concluded that more studies were needed to confirm the correlation between mental stress and psoriasis.

2.1.2 Clinical Classification of Psoriasis

Psoriasis usually affects the outer layer of skin or may be the joints. The clinical classification of psoriasis is usually done by observation of the erythematous scaly patches, plaques and papule presence which is often painful and pruritic. The classification of psoriasis can be categorized into plaque-type psoriasis, inverse psoriasis, erythrodermic psoriasis, localized pustular psoriasis, and guttate psoriasis. Plaque psoriasis, which is identified by precisely round or oval plaques that differ in size and frequently coalesce (Figure 1), affects approximately 90% of patients.

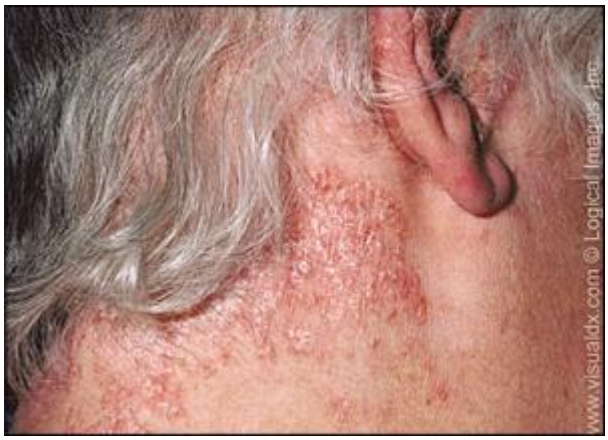


Figure 1. The area of the neck was affected by plaque-type psoriasis. (Source: Weigle & McBane, 2013)

It usually occurs on the extensor surfaces of the legs, arms, buttocks, trunk and scalp. Plaque psoriasis or psoriasis vulgaris are the most occurring type of psoriasis (Rendon & Schäkel, 2019). In Figure 2, it shows inverse psoriasis. Inverse psoriasis usually developed in skin folds such as axillary, inguinal, inframammary, intergluteal areas, and perineal. The development of inverse psoriasis is usually influenced by the infection, heat and trauma on the affected area.

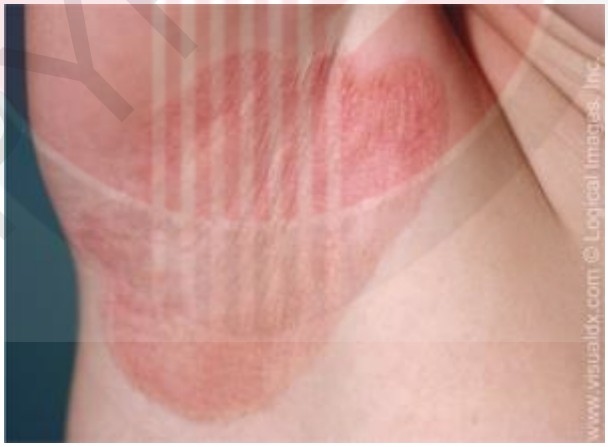


Figure 2. The axillary area was affected with inverse psoriasis. (Source: Weigle & McBane, 2013)

Another type of psoriasis is erythrodermic psoriasis. Erythrodermic psoriasis was distinguished as a widespread generalized erythema (Figure 3). It is usually associated with systemic symptoms. It can emerge gradually from long-term psoriasis or suddenly in patients with mild psoriasis (Menter et al., 2008). Pustular psoriasis (Figure 4) usually comprises pustules with no formation of plaque, and it usually forms on the palms and soles.

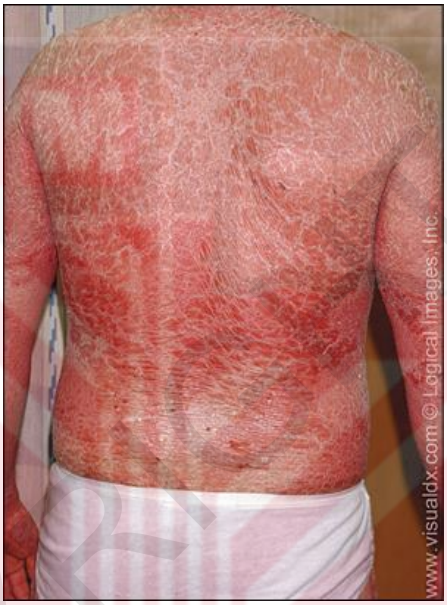


Figure 3. Erythrodermic psoriasis with confluent scaly plaques. (Source: Weigle & McBane, 2013)

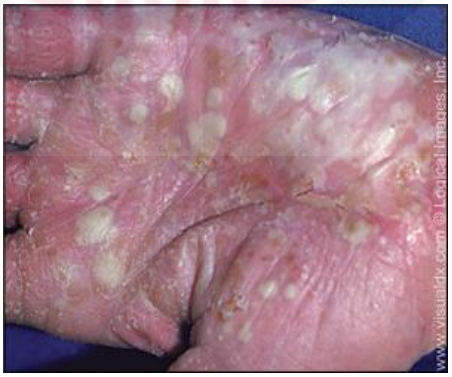


Figure 4. The hand was infected with localized pustular psoriasis on the hand. (Source: Weigle & McBane, 2013)

Guttate psoriasis frequently occurs in patients aged 30 years and below and only involves 2% of psoriasis cases. The lesions usually occur on the trunk. Guttate psoriasis is usually distinguished by the fine scaling on the pink papules where it is usually 1 to 10 mm in size (Figure 5).

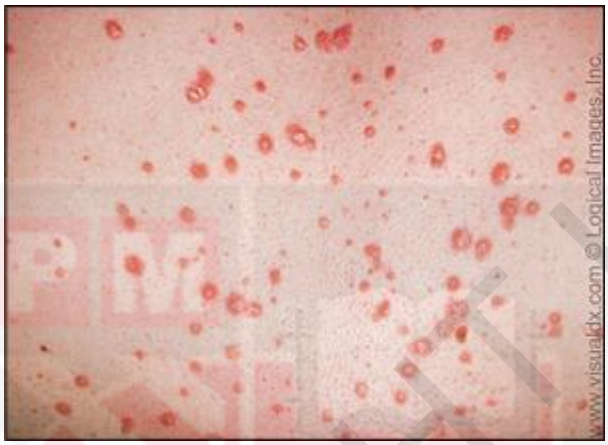


Figure 5. Pink erythematous papules in guttate psoriasis patients. (Source: Weigle & McBane, 2013)

It also can occur in non-dermatologic areas such as nails. Approximately 80 to 90% of patients have experienced nail disease or known as psoriatic onychodystrophy. It usually affects the fingernails more than toenails and it is known to be resistant to treatment (Menter et al., 2008). Psoriatic onychodystrophy is distinguished by the abnormal nail plate growth. This condition will cause subungual hyperkeratosis, onycholysis, and pitting on nails (Figure 6). Other than the nail, the condition that can occur is psoriatic arthritis. Psoriatic arthritis is a type of inflammatory arthritis that is seronegative and has a variety of clinical manifestations. It takes an average of 12 years following the development of skin lesions for it to manifest (Gottlieb et al., 2008).

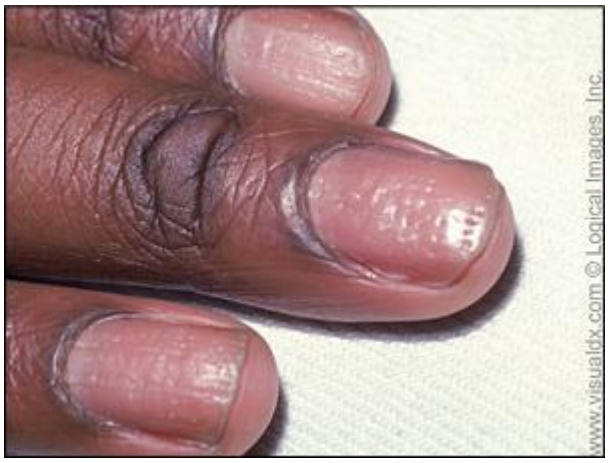


Figure 6. Nail pitting in psoriatic onychodystrophy patients. (Source: Weigle & McBane, 2013)

2.1.3 Immunopathophysiology of Psoriasis

Psoriasis is characterized by persistent inflammation, which results in uncontrolled keratinocyte growth and defective differentiation. One of the common features that present is neovascularization. Other than that, in the histology of psoriatic plaque, there will be presence of acanthosis or known as epidermal hyperplasia. The acanthosis (Figure 7) was overlain with the inflammatory infiltrates which consist of dermal dendritic cells, T cells, macrophage, and neutrophils.

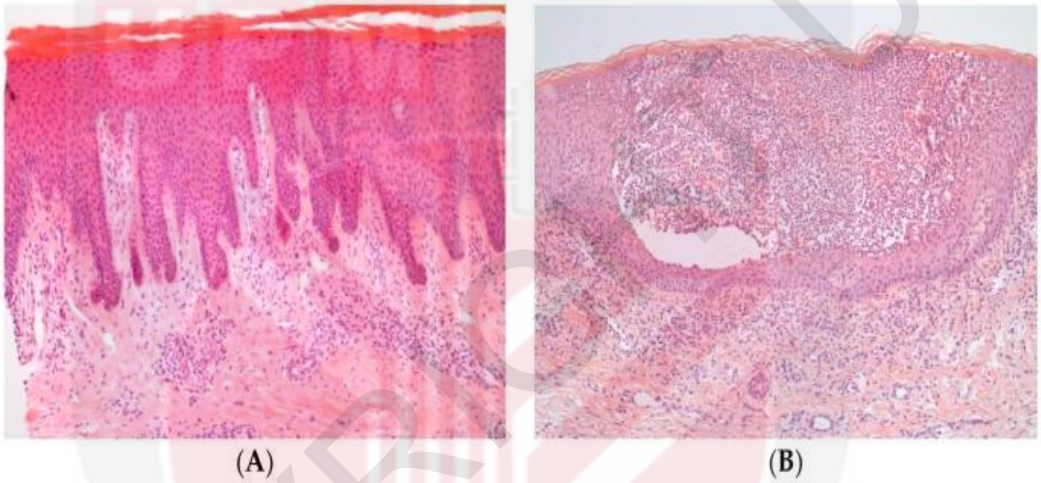


Figure 7. Psoriasis histopathology. (A) Psoriasis vulgaris or plaque-type psoriasis specifically shows acanthosis, parakeratosis, and dermal inflammatory infiltrates. (B) Acanthotic changes along with the epidermal predominantly neutrophilic infiltrates and lead to pustule formation in pustular psoriasis. (Source: Rendon & Schäkel, 2019)

According to Harden, Krueger, and Bowcock (2015), the development and maintenance of psoriasis inflammation is caused by the interruption of the innate and adaptive cutaneous immune system. In some cases, innate immune system activation triggered by endogenous threat signals and cytokines accompanied with autoinflammatory perpetuation, while in others, T cell-driven autoimmune reactions are observed. As a result, psoriasis has autoimmune-like characteristics on an autoinflammatory background, with both pathways intersecting and even triggering one another (Liang et al., 2017). The outermost surface of the skin, which is made up

of keratinocytes, is where the main findings of psoriasis were clinically found. Nevertheless, the growth of psoriatic plaque is not confined to inflammation in the epidermal layer only, but it is also affected by the interaction of several cell types (adaptive and innate immune cells, vasculature) in the dermal layer of the skin with the keratinocytes. An initiation phase that is usually activated by trauma or Koebner phenomenon, drugs or infection were conceptualized as the pathogenesis of psoriasis (Di Meglio, Villanova & Nestle, 2014). The maintenance phase of the plaque psoriasis is also indicated by the chronic clinical progression.

When injury occurs, keratinocytes secrete antimicrobial peptides (AMPs) and this secretion will be recognized by dendritic cells. AMPs also are found to be overexpressed in psoriatic skin. Dendritic cells, one of the antigen-presenting cells (APCs) known to play an essential role in the early phase of a disease. According to the study done by Morizane and Gallo (2012), LL37, β-defensins, and S100 proteins are the most studied psoriasis-associated AMPs. The initiation of toll-like receptor-9 (TLR-9) in plasmacytoid dendritic cells (pDCs) by the binding process of LL37 with DNA, is the initial point for the psoriatic plaque growth. LL37, also known as cathelicidin, was produced by damaged keratinocytes. The activation of pDCs is distinguished by the secretion of type I interferon (IFN) that include IFN-α and IFN-β. Type I IFN signaling enhances the phenotypic development of myeloid dendritic cells (mDCs) and has been linked to T-helper (Th) 1 and Th17 differentiation and function, as well as the production of IFN-γ and interleukin (IL)-17 respectively (Gregorio et al., 2010). Other than that, LL37 is also bound to RNA which will activate pDCs through TLR7. Through the TLR8, mDCs also will be activated by the LL37-RNA complexes (Morizane & Gallo, 2012). Tumor necrosis factor (TNF)-α, IL-23, and IL-12 are secreted by activated mDCs into draining lymph nodes, with the latter

two affecting the differentiation and proliferation of Th17 and Th1 cell subsets, respectively. Moreover, when LL37–RNA is activated, slan+ monocytes, which are significant pro-inflammatory cells seen in psoriasis skin lesions, produce a large amount of TNF-α, IL-12, and IL-23 **(**Hänsel et al., 2011).

The phase of psoriatic inflammation maintenance involves the stimulation of the adaptive immune response through the different T cell subsets (Nestle, Turka & Nickoloff, 1994). IL-17, IL-21 and IL-22 are Th17 cytokines which will trigger the activation of keratinocyte growth in the epidermis. In conclusion, the growth of keratinocytes was activated by the inflammatory mediators through TNF-α, IL-17 and IFN-γ. Other than that, LL37-DNA complexes also trigger the activation of keratinocytes and lead to the increased secretion of type I IFNs (Morizane et al., 2012). They also play an active role in the inflammatory cascade by secreting cytokines (IL-1, IL-6, and TNF-), chemokines, and AMP.

Intraepidermal penetration of activated polymorphonuclear leukocytes is a feature of early and active psoriatic lesions (Husna & Reddy, 2019). This causes an unrestricted production of reactive oxygen species (ROS), which causes pre-oxidative destruction to skin membranes, worsening the condition by escalating the seriousness of the lesions. The release of arachidonic acid mediators is increased when ROS activates phospholipase A2. By dilating capillaries in the dermis of the skin, stimulating keratinocyte cell development, and increasing leukocyte infiltration, prostaglandin generated by the cyclooxygenase pathway contributes to psoriasis **(**Amigó et al., 2007).

2.1.4 Management of Psoriasis

Psoriasis is a recurrent chronic illness that often involves long-term treatment. Basically, to determine the therapy that will be used to treat psoriasis usually depends on the severity of the disease, comorbidities, and accessibility to the healthcare. The severity of the diseases is usually divided into two groups which include mild or moderate to severe psoriasis and the intensity of diseases determined by the condition of the lesion, percentage of body area affected and how psoriasis affects the patient's quality of life (Mrowietz et al., 2011). Topical therapy in combination with glucocorticoids, vitamin D analogues, and phototherapy can be applied to manage mild to moderate psoriasis. Psoriasis that is moderate to severe generally necessitates systemic treatment.

Na Takuathung et al. (2018) also explains that corticosteroids, vitamin D3 analogues and calcineurin are the examples of the conventional therapies and currently were used as a topical treatment for mild psoriasis. Phototherapy or systemic medicines such as methotrexate, cyclosporine, and acitretin are frequently used to treat severe psoriasis (Menter et al., 2009). Biologic therapies for psoriasis therapies also have been developed and approved where it will play a role on the upregulated cytokine pathways. However, Augustin et al. (2011) reports that most of these treatments have a well-recognized list of side effects that appear to be the primary reason preventing patients from sticking to long-term psoriasis medication, indicating a need for the improvement of a medicine with improved efficacy but fewer side effects.

2.1.5 Imiquimod-induced Psoriasis Models

A psoriasis-like inflammation mouse model has been extensively used in the psoriasis study. This model depends on the effectiveness of the TLR7/8 agonist imiquimod (IMQ), which supports the TLR7/8 disease initiation concept. In addition, van der Fits et al. (2009) discussed that animals with IL-23 or IL-17R deficiency had no sensitivity to imiquimod, indicating that the IL-23/IL-17 axis is involved in skin inflammation and psoriasis-like pathogenesis. Imiquimod is a drug that has been applied to control and cure anogenital warts, superficial basal cell carcinomas and also actinic keratoses. It belongs to the class of medicines known as immune modulators. Other than that, imiquimod triggers innate and adaptive immune responses by binding to TLR7 and activating nuclear factor kappa-β, resulting in the release of cytokines and the entrance of plasmacytoid dendritic cells to the position of drug application, as well as the release of IFN-α and a Th1 response (Megyeri et al., 1995). Inflamed scaly skin lesions mimicking plaque type psoriasis were generated by daily administration of IMQ to the rear skin of mice. Increased epidermal growth, aberrant differentiation, epidermal neutrophil build-up in microabscesses, neoangiogenesis, and infiltrates of CD4(+) T cells, CD11c (+) dendritic cells, and plasmacytoid dendritic cells were found in these lesions (van der Fits et al., 2009). IMQ increased IL-23, IL-17A, and IL-17F expression in the epidermis, as well as splenic Th17 cells. As a conclusion, a dermatitis closely mimicking human psoriasis is swiftly induced by the innate TLR7/8 ligand IMQ, which is crucially reliant on the IL-23/IL-17 axis. This quick and easy example will enable more clarification of disease pathways and testing of innovative psoriasis therapy.

2.2 Purple Sweet Potato (*Ipomoea batatas* L.)

Purple sweet potato or also known as *Ipomoea batatas* L. come from the family of Convolvulaceae and it is known to be the world's six largest food crops (Scott, 1992). Purple sweet potatoes also are extensively grown in tropical and subtropical regions. The shape of the purple sweet potato leaves is an alternate heart-shaped, lobed leaves with medium-sized flowers. The root of the plant is commonly long and tapered. The scientific name of this plant is *Ipomoea batatas* L. and the common names for this plant are sweet potato, yam, nyamis (Africa), kumara (New Zealand) and camote (southwest United States) (Panda et al., 2012). The flower character of this plant is monoecious where both female and male flowers can be found in one plant.

Purple sweet potato is very popular as it is highly cultivated and consumed in many countries. It is consumed as food and as medicinal purposes in humans and animals. Based on the *Dietary Guidelines for Chinese Residents*, it is advisable to take about 250 to 400 gram per day of cereals and potatoes which equal to 50 to 100 g of potatoes. Moreover, the average daily anthocyanin intake per capita in the United States was around 12.5 mg/day, according to the daily dietary structure of American citizens (Wu et al., 2006). As a result, the purple sweet potato did not only contribute to the everyday consumption of cereals and potatoes in some people, but also helped to enhance the anthocyanins consumption to accomplish healthy life goals (Li et al., 2019).

2.2.1 Phytochemistry and Antioxidant Properties of *Ipomoea batatas* L.

A study done by Zhang et al. (2019) describes that purple sweet potato leaf extract expresses a broad range of activities that helps to improve health such as anti-oxidative, anti-cancer, anti-diabetic, anti-bacterial and anti-inflammation activity. These pharmacological activities have been reported to be strongly related to the bioactive compounds that appear in the leaf extract. In previous study, *Ipomoea batatas* has been reported to contains vitamins such as pantothenic acid (vitamin B5), pyridoxine (vitamin B6), thiamin (vitamin B1), niacin, and riboflavin (Islam, Yoshimoto & Yamakawa, 2003), polyphenols (anthocyanin) and phenolic (caffeic, monocaffeoylquinic, dicaffeoylquinic, and tricaffeoylquinic acids) (Choi et al., 2010), triterpenes (β-carotene and boehmeryl acetate) trace elements (iron, calcium and zinc), and proteins (Ishiguro et al., 2004). Interestingly, *Ipomoea batatas* leaves contain extra polyphenols than any other profit-oriented vegetable, including cabbage, spinach and lettuce (Islam, 2014). In a study conducted by Islam (2014), he mentioned that sweet potato leaves have at least 6 polyphenolic and 15 anthocyanins compounds in them. Furthermore, in the study done by Majid et al. (2019), it shows the existence of tannins, flavonoids, phenols, saponins, anthocyanin, and coumarins was confirmed by qualitative study of IPT-EA (Ethyl-acetate), IPA-EA, IPT-M (methanol), and IPA-M extracts, in tuber and aerial part, and there was no presence of terpenoids, which were lacking in IPA-M and triterpenoids in IPT-M.

Purple sweet potato leaves contain an attractive purple red colour due to the high content of anthocyanin, high content of phenol and high activity of antioxidants (Yoshinaga et al., 2010). If it is compared with orange-fleshed sweet potato, purple sweet potatoes have a higher content of anthocyanin (Xu et al., 2014). Other than that, purple sweet potatoes also contain the highest amount of anthocyanin if compared with

blackberries, blueberries, cranberries and also grapes (Enicole et al., 2010). Purple sweet potato anthocyanins (PSPAs) are a kind of anthocyanins by which their chemical structure is primarily made up of cyanidins and peonidins. It is usually in the shape of monoacylation and diacetylation. Due to its acylation form, PSPAs have an advantage where they have ultraviolet stability and high heat resistance which makes them useful as natural substances in food additives (Odake et al., 1994). Furthermore, the anthocyanin also contributes to the antioxidant and anti-inflammatory characteristic of the *Ipomoea batatas* L. especially when passing through the digestive tract. By consuming purple sweet potato, it may be able to reduce the risk of oxygen radicals and heavy metals posing a health danger. They can also help prevent atherosclerosis by being part of an anti-hypertensive diet (Mohanraj & Sivasankar, 2014).

Other than anthocyanin, the total phenolic and total flavonoid content also plays an essential role in sweet potato leaf. The total phenolic and flavonoid content was proven to have a contribution to the antioxidant activity of the sweet potato leaf. According to the study done by Hajihmahmoodi et al. (2008), the highest amount of total polyphenol was found in the olive pulp extract of Iranian olive cultivar. The olive pulp extract was one of the possible natural resources of antioxidant. Interestingly, in the study by Hue et al. (2012), the leave of sweet potato shows a higher level of total phenolic content compared to the olive pulp. This shows that the level of total phenolic content is high in sweet potato, and thus, it could serve as a possible resource of organic antioxidant. The role of phenolics is to scavenge free radicals in the human body and to aid in the maintenance of a healthy body by scavenging or eliminating reactive oxygen species (ROS). Hue et al. (2012) also added that the presence of flavonoids in green vegetables are contributing to their high activity of antioxidant. In addition to the toxicity of purple sweet potato, in the study done by Majid et al. (2019), they report

that there were no deaths occurred from the experimental groups where the rats were treated with the dose varying from 100 to 2000 mg/kg of rats. There were also no noticeable signs of toxicity detected. Majid et al. (2019) describes that these extracts were considered safe and reliable to explore their further pharmacological characteristic within the range of dose given.

Antioxidants are essential for preserving food quality and sustaining human health. Antioxidant means that it is against or opposes oxidation. Antioxidants can be used to treat a variety of inflammatory disorders, neurological ailments, and rheumatism (Ahmed & Iqbal, 2018). An antioxidant is a constituent that, at little concentrations matched to those of an oxidizable substrate, considerably slows or inhibits the oxidation of that substrate (Sehwag & Das, 2013). Antioxidants can aid in the treatment of psoriasis by preventing oxidative stress-related damage.

Purple sweet potato is known to have high antioxidant activity. In the study done by Teow et. al (2007), shows that purple sweet potatoes have the highest antioxidant activity than the other 19 different kinds of sweet potato which include white, light yellow, yellow, and orange. Another study done by Mohanraj and Sivasankar (2014) shows that purple-fleshed sweet potatoes have elevated total antioxidant activity in comparison with white-fleshed sweet potatoes. Purple sweet potatoes had 3.2 times more antioxidant activity than a variety of blueberry, according to one study. Sweet potatoes have a remarkable quantity of antioxidant activity in all of their parts. This is due to the content of anthocyanin, flavonoid and phenolic in purple sweet potatoes which can help to protect against oxidative stress-induced inflammation and cause oxidative stress markers to decrease.

2.3 Psoriasis Area and Severity Index (PASI) Scoring

Psoriasis Area and Severity Index or PASI scoring is chosen to be the gold standard method. PASI scoring was used to estimate the severity of psoriasis by measuring the area, erythema, scaliness and thickness of the psoriasis plaques (Fadzil et al., 2009). PASI scoring is also used to monitor the growth of psoriasis and the efficiency of psoriasis treatment. According to Rendon and Schäkel (2019), The PASI score has been widely utilized in clinical studies, particularly those involving biologic drug development. Psoriasis symptoms are assessed in terms of none to extremely severe, and the percentage of the body affected is calculated. Erythema, skin thickness, and desquamation or scaling were scored individually on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked.

CHAPTER 3

MATERIALS AND METHOD

3.1 Chemicals and Reagents

Folin-Ciocalteu reagent, DPPH solution, 2,4,6 tripyridyl s- triazine (TPTZ) solution, butylated hydroxytoluene (BHT), gallic acid, and other reagents were analytical grade, while the water used for the study was glass-distilled water. Imiquimod cream (Aldara) were purchased from 3M Pharmaceuticals and the hair removal cream, Veet was obtained from the local drugstore, Watson.

3.2 Collection and Preparation of *Ipomoea batatas* L.

The purple sweet potato leaves were acquired from the industrial sweet potato plantation in Sungai Pelek, Sepang, Selangor, Malaysia which is one of the largest, sweet potato farms in Selangor. The variety of sweet potato used in this study was the Anggun variety or purple sweet potato. The samples were validated and certified by the Institute of Bioscience, Universiti Putra Malaysia (Plant Voucher Number: MFI0188/20). Then, the leaves of the purple sweet potato (PSPL) were chilled at -80℃ and freeze-dried by using a freeze dryer, crushed into fine powder and kept at room temperature in a sealable bag prior to assessment.

3.3 Extraction of Aqueous *Ipomoea batatas* Leaf

The extraction for aqueous extract was prepared according to the technique explained by Mirna et al. (2014). Fifty grams of *Ipomoea batatas* dried leaf powder was put in a conical flask comprising 500 mL of distilled water, was thoroughly mixed and placed into the water bath for 24 hours at 80℃. Then, the PSPL aqueous extract was filtered by using Whatman's filter paper No. 1. Under the same condition, the residues were re-extracted and were repeated 4 times. Next, all of the filtrates were

combined and were dried by using an oven at 45℃. Then, the obtained extract was weighed, recorded, and kept in the -20℃ freezer prior to freeze drying. The volume of extract was measured; the percentage of extraction yield was estimated using the formula:

Yield (%) = (m(extract)) / (m (fine powder)) × 100Yield (%) = $\frac{m(extract)}{m\ (fine\ powder)}$ × 100,

where 'm (extract)' is the mass of the extract (g), 'm (fine powder)' is the mass of SPL powder.

3.4 Total Phenolic Content (TPC)

The total phenolic content or TPC was established using the Folin-Ciocalteau assay based on the approach by Lee et.al. (2014) with some alterations. Samples or a standard (20 µL) that have been diluted with distilled water (1mg/ml) were combined with 100 µL of diluted Folin-Ciocalteau reagent (1:10, v/v in distilled water) in a 96-well plate. After 5 minutes, 80 µL of 7.5% sodium carbonate (Na_2CO_3) were put in to each well. All reagents were mixed thoroughly with a vortexer before added into the well. The plate was cover up and held in the dark for 30 minutes on the bench. The absorbance was evaluated at 765 nm compared to a reagent blank. A standard calibration curve using gallic acid (7.8 – 500 µg/mL) was plotted, and all findings were done in triplicate and were conveyed as mg gallic acid equivalent (GAE)/g DE extract using the following formula:

$$\text{TPC for 1g of extract} = \frac{TPC\ per\ ml\ sample \times Dilution\ factor \times Total\ sample\ volume\ used}{Sample\ weight(1)}$$

3.5 Total Flavonoid Content (TFC)

The total flavonoid content or TFC was measured using an aluminium chloride colorimetric assay based on the approach explained by Belguith-Hadriche et al. (2013) with some adjustments. Samples or a standard (25 µL) that have been diluted with distilled water (1mg/ml) were mixed with 100 µL of distilled water (dH_2O) in a 96-well plate. Consequently, 7.5 µL of 5% $NaNO_2$ were add up to the mixture. After 5 min, 7.5 µL of 10% $AlCl_3.6H_2O$ were added. The mixture was left at room temperature for another 5 minutes. Then, 50 µL of 1M NaOH were add up. Instantly, 60 µL of dH2O were add up to the mixture, and the absorbance was evaluated at 510 nm using a microplate reader against a blank. All reagents were mixed thoroughly with a vortexer before added into the well. A standard calibration curve using quercetin (125 - 1000 µg/mL) was plotted, all results were done in triplicate and expressed as mg quercetin equivalent (QE)/g DE extract using the formula:

$$\text{TFC for 1g of extract} = \frac{TFC\ per\ ml\ sample \times Dilution\ factor \times Total\ sample\ volume\ used}{Sample\ weight (2)}$$

3.6 Antioxidant Assay: 2,2-diphenyl-1-picrylhydrazyl (DPPH)˙ Radical Scavenging Assay

The DPPH radical scavenging capacity was established according to the approach defined by Kong et al. (2012). Samples or a standard (50 µL) that have been diluted with methanol in 1mg/ml to prepare at various concentrations (3.9 – 250µg/mL) were mixed with 195 µL of DPPH solution (100 µM in methanol) in a 96-well plate and left in the dark room at room temperature for 30 minutes. All reagents were mixed thoroughly with a vortexer before added into the well. The absorbance of the reaction mixture was read at 515 nm. Butylated hydroxytoluene (BHT) was utilized

as standards. The scavenging capacity of the sample was evaluated using the following equation:

$$\text{Scavenging capacity (\%)} = \frac{A0-(A1-A2)}{A0} \times 100\%$$

where A0 is the absorbance of the control group (sample solution was substituted by extraction solvent); A1 is absorbance of sample or standard and A2 is the absorbance of blank sample (DPPH solution was substituted by methanol).

All results were done in triplicate and presented as EC_{50} which is the efficient concentration of samples and standards that scavenge 50% of DPPH radicals after specified exposure time.

3.7 Animal Ethics Application

All experiments were done by following the OECD guidelines (no. 442B, 2017) for the testing of chemicals. The procedure has been proposed and accepted by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia.

3.8 Anti-psoriatic Activity of Purple Sweet Potato Leaves Aqueous Extract (PSPLAE) on Imiquimod-induced Psoriasis-like Dermatitis in Balb/C mice

3.8.1 Experimental Design

Forty-two male Balb/C mice (weighing 20–30 g) at the age of 8 to 11 weeks were utilized for the research. These animals were freely permitted to get access to water and standard chow diet up to the end of the 15-day experimental phase. All the mice were held within a 12-hour light or dark cycle at a temperature of 23 to 25℃ and humidity of 55 to 60%.

These mice were distributed into 7 groups where each group comprises of seven mice (n=7). Firstly, all mice were bald on the back using hair removal cream,

Veet. After 24-hour, 62.5 mg of Imiquimod (translating 3.125 mg of active compound) was gently rubbed on the hairless back by using an applier brush for 15 following days. This step was repeated for all groups except for the normal control group and vehicle control group. The experimental groupings were as follows:

Group-I: Normal control group. This group was not treated and only tap water was rubbed on the shaved back until the end of the experiment, day 15.

Group-II: Negative (disease) control group. For 15 consecutive days, this group of mice was topically applied with Imiquimod 5% cream at a dose of 62.5 mg (translating 3.125 mg of active compound) on the shaved back.

Group-III: Vehicle control group. For 15 consecutive days, this group was topically treated with 62.5 mg cream base, the vehicle, on the shaved back.

Group-IV: Positive control group. The animals were induced with psoriasis which is the same as Group-III animals. On the 8th day, clobetasol propionate cream which acts as a positive control was put on topically once daily for the other 8 days.

Group-V: PSPLAE 5% cream group. This group was applied with psoriasis which is the same as Group-III animals. On the 8th day, 62.5 mg of PSPLAE 5% cream was topically induced for 8 days.

Group-VI: PSPLAE 10% cream group. This group was applied with psoriasis which is the same as Group-III animals. On the 8th day, 62.5 mg of PSPLAE 10% cream was topically induced for 8 days.

Group-VII: PSPLAE 20% cream group. This group was applied with psoriasis which is the same as Group-III animals. On the 8th day, 62.5 mg of PSPLAE 20% cream was topically induced for 8 days.

3.9 Scoring Severity of Imiquimod-induced Psoriasis-like Skin Lesions

The intensity of inflammation on the mice dorsal skin was observed and scored based on the clinical Psoriasis Area and Severity Index (PASI) Scoring severity of inflammation. Erythema, skin thickness, and desquamation or scaling were scored individually on a scale from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked. The total score (erythema score and scaling score and thickening score) served to signify the seriousness of psoriasis (scale 0–12) (Sun, Zhao & Hu, 2013).

3.10 Statistical Analysis

All research were conducted in triplicates. All the outcomes were shown as means ± standard error mean. For the phytochemical study, T-test was conducted by SPSS version 24. The relationship between the total phenolic, total flavonoid content and antioxidant activity were conducted using Pearson's Correlation Coefficient, r with a significant level of $p<0.05$. As for the anti-psoriatic study, two-way ANOVA along with Tukey's post hoc test was done to clarify the significance of differences between the groups. P values of less than 0.05 will be recognized as statistically significant.

CHAPTER 4

RESULTS

4.1 Extraction Yield of Purple *I. batatas* Leaf Aqueous Extract

Based on the study, the yield of purple sweet potato leaf aqueous extract (PSPLAE) is 17.2 ± 2.33 from three independent experiments.

4.2 Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Purple Sweet Potato Leaf Aqueous Extract (PSPLAE)

Total phenolic content (TPC) of the PSPLAE was calculated in terms of mg of gallic acid equivalents per gram dried extract (mg GAE/g DE) leaf samples based on the equation of the standard curve. The equation taken from the standard curve of gallic acid for the estimation of TPC is as follows: $y = 0.0038x - 0.0179$, $r^2 = 0.9997$. Data are conveyed as mean ± SEM from three separate analysis. The TPC of the aqueous extract of *I. batatas* leaf is presented in Table 1.

As for the total flavonoid content (TFC), it was determined in terms of mg of quercetin equivalents per gram dried extract (mg QE/g DE) leaf samples based on the equation of the standard curve. Data are conveyed as mean ± SEM from three separate study. The equation attained from the standard curve of quercetin for the calculation of TFC is as follows: $y = y = 0.0004x - 0.0278$, $r^2 = 0.9961$. Total flavonoid content of *I. batatas* leaf aqueous extract is presented in Table 1.

Table 1. Total phenolic (TPC) and total flavonoid (TFC) content of aqueous extract from purple sweet potato leaves.

Sample analysed	Total polyphenols (mg GAE/g DE)	Total flavonoids (mg QE/g DE)
Purple sweet potato leaf aqueous extract (PSPLAE)	7.62 ± 1.91	2.74 ± 0.85

4.3 Antioxidant Assay: 1,1-diphenyl-2-picrylhydrazyl (DPPH)˙ Radical Scavenging Assay

The activity of antioxidant in PSPLAE was estimated by 2,2-diphenyl-1-picrylhydrazyl, DPPH assay. The antioxidant activity of PSPLAE were compared with the antioxidant activity in standard, butylated hydroxytoluene (BHT) compounds by using DPPH radical scavenging assay. All results were done in triplicate and presented as EC_{50} which is the efficient concentration of samples EC_{50} is used to convey the quantity of effective concentration of extracts that enable to scavenge 50% of the free radicals. Moreover, the lowest EC_{50} value indicate the highest activity of antioxidant. Result from DPPH assay exhibit that PSPLAE showed lower antioxidant activity (EC_{50} = 82.00 ± 8.08 µg/mL) when compared to the standard, BHT (EC_{50} = 60.07 ± 4.18 µg/mL) (Table 2). Based on statistical analysis, PSPLAE was found to be not significantly different from standard, BHT. Thus, indicating that the antioxidant activity of PSPLAE is as active and effective as the standard antioxidant, BHT.

Table 2. Antioxidant activity of aqueous extract of I. batatas leaves and butylated hydroxytoluene, BHT in DPPH assay.

Sample analysed	EC_{50} in DPPH radical scavenging assay (µg/mL)
Purple sweet potato leaf aqueous extract (PSPLAE)	82.00 ± 8.08
Butylated hydroxytoluene (BHT)	60.07 ± 4.18

4.4 Correlation Between Total Phenolic Content (TPC), Total Flavonoid Content (TFC) with the Antioxidant Activity in Purple Sweet Potato Leaf Aqueous Extract (PSPLAE)

Results from the Pearson's coefficient correlation analysis show that both TPC and TFC in PSPLAE have significant positive correlation with the antioxidant activities in DPPH assay (Table 3). Moreover, the association between TFC and antioxidant activity showed a relatively higher correlation coefficient (r) value, indicating that flavonoids might contribute to higher antioxidant activity compared to phenolic content.

Table 3. Linear correlation coefficient (r^2) between TPC and TFC with antioxidant assay DPPH, of *Ipomoea batatas* leaf extract.

Components	Correlation coefficient (r^2) value
TPC vs DPPH	$r^2 = 0.590$, $p < 0.05$
TFC vs DPPH	$r^2 = 0.973$, $p < 0.05$

Note: Correlation is significant at $p<0.05$. TPC - total phenolic contents; TFC - total flavonoid contents; DPPH - 2,2-diphenyl-l-picrylhydrazyl.

4.5 Anti-psoriatic Activity of PSPLAE on Imiquimod-induced Psoriasis-like Dermatitis in Balb/C Mice

The Psoriasis Area and Severity Index (PASI) is a quantifiable rating scale that uses area coverage and plaque appearance to measure the severity of psoriatic injuries. Seven days after the application of imiquimod, indications of erythema, scaling and thickness were discovered on the dorsal skin of the Balb/C mice (Figure 8). Figure 8 indicates that the topical administrations of PSPLAE cream (both dosage of 5 and 10%) and the standard drug, Clobetasol propionate were capable to lessen the severity of psoriasis in imiquimod-induced mice. However, experimental animals in Group 7, which were treated with the highest dose of PSPLAE (10%) showed the most severe skin lesions. For group 1 (normal control group) and group 3 (vehicle control group), there were no observable psoriasis-like symptoms on the dorsal skin of Balb/C mice. While for group 2, which is the imiquimod-treated group, the imiquimod was applied for 15 consecutive days. Thus, the psoriasis-like symptoms were clearly observed (Figure 8) and the cumulative score of the PASI scoring were 5.67, where the result were conveyed as mean ± standard error of mean, SEM. Based on the statistical analysis, group 4 (positive control group), group 5 (IMQ + 5% PSPLAE), and group 6 (IMQ + 10% PSPLAE) were found significantly different from the group 2 (IMQ-treated group). However, experimental animals in Group 7, which were treated with the highest dose of PSPLAE (20%) showed the most severe skin lesions and were found not significantly different from Group 2, the IMQ-treated group.

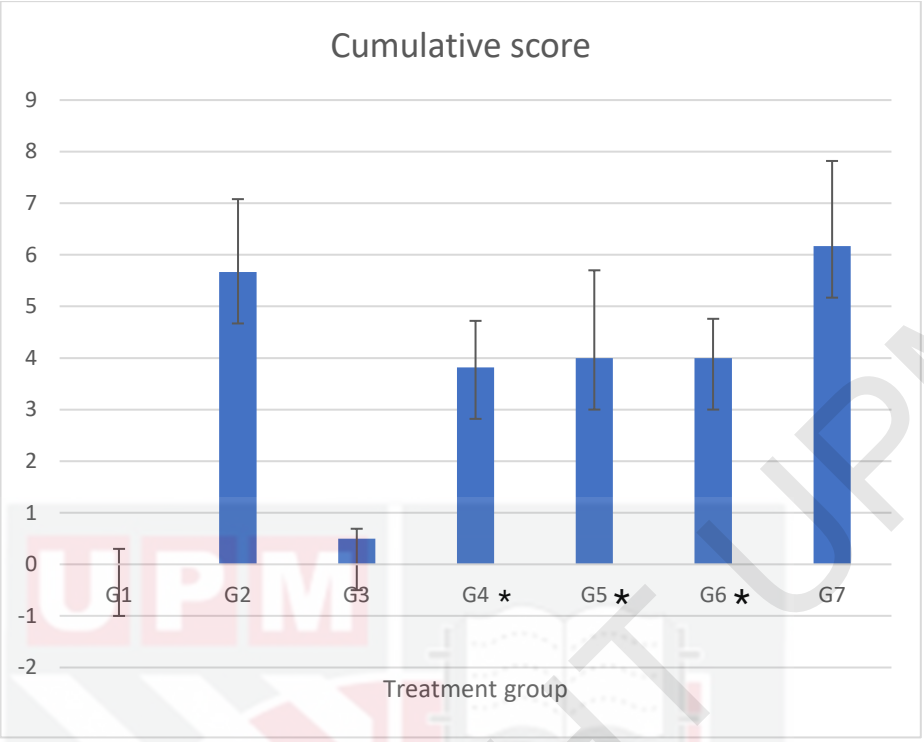
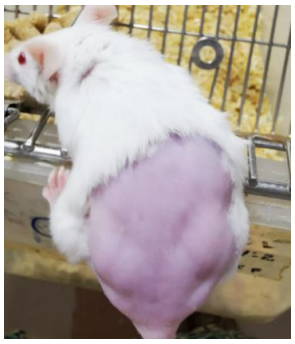
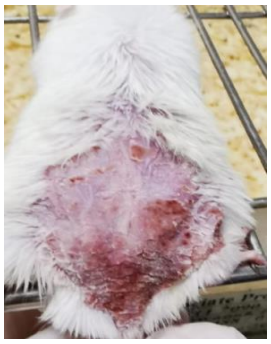


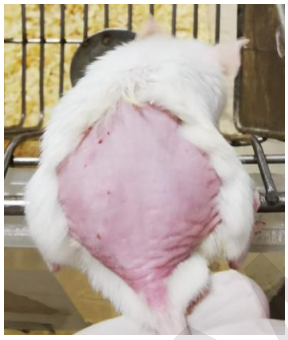
Figure 8. The cumulative score (erythema plus thickness plus scaling scores) (0-12) indicating the psoriasis severity index for different treatment groups at the end of the treatment (Day 15). The results represent mean ± standard error of mean, SEM. Values were considered significant at *P<0.05. G1, the normal group; G2, the negative control, IMQ-treated group, G3, the vehicle control cream base-treated group; G4, the positive control, IMQ + clobetasol propionate-treated group; G5, the IMQ + PSPLAE 5%-treated group; G6, the IMQ + PSPLAE 10%-treated group and G7, the IMQ + PSPLAE 20%-treated group.



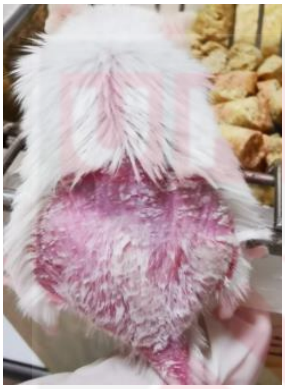
Group 1: Normal group



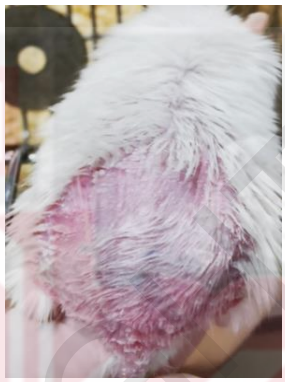
Group 2: Negative control group



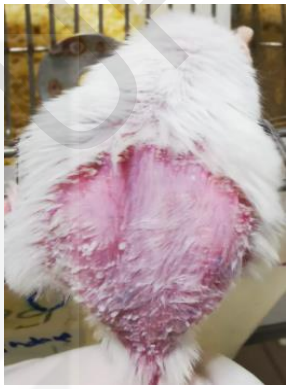
Group 3: Vehicle control group



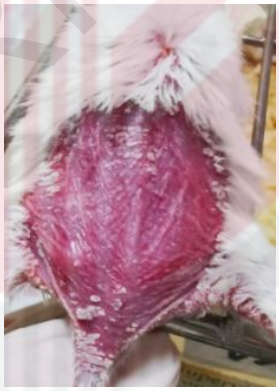
Group 4: Positive control group



Group 5: IMQ + 5% PSPLAE



Group 6: IMQ + 10% PSPLAE



Group 7: IMQ + 20% PSPLAE

Figure 9. IMQ-induced psoriasis-like dermatitis on the backs of Balb/C mice. The back skin of the three groups exhibited different grades of erythema, scales and infiltration. Photos were taken on day 15. IMQ, imiquimod; G1, the normal group; G2, the negative control, IMQ-treated group; G3, the vehicle control, cream base-treated group; G4, the positive control, IMQ + clobetasol propionate-treated group; G5, the IMQ + PSPLAE 5%-treated group; G6, the IMQ + PSPLAE 10%-treated group and G7, the IMQ + PSPLAE 20%-treated group.

CHAPTER 5

DISCUSSION

In a previous study, the leaves of *Ipomoea batatas* were discovered to have radical scavenging, anti-mutagenic, anti-cancer, and anti-bacterial properties (Islam, 2006). The presence of phenolics and flavonoid content in the leaves of *Ipomoea batatas* L. may contribute to their therapeutic characteristics. Based on the findings, the total phenolic content (TPC) was calculated in terms of mg of gallic acid equivalents per gram dried extract (mg GAE/g DE) leaf samples and were measured by using Folin-Ciocalteau assay. The Folin-Ciocalteau method was widely used to quantify total phenolics in the substrate, and it mainly employed gallic acids as a reference (Waterhouse, 2001). When phenolics are detected in the extracts, the colour of the Folin-Ciocalteau reagent changes from yellow to blue, which is caused by the chemical reduction of the mixture of tungsten and molybdenum oxides in the reagent. Due to the higher solubility of gallic acid in methanol than in water or other solvents (such as ethanol), methanol was employed to dilute the gallic acid standard in this study (Daneshfar et al., 2008). According to Islam (2006), total phenolics content in different types of *I. batatas* leaves ranged from 1.42 to 17.1 g/100g dry weight in previous research. In addition, geographical considerations, as well as varied growth practices, could explain the variation in total phenolic levels.

The total flavonoid content (TFC) was measured using an aluminium chloride colorimetric assay corresponding to the method explained by Belguith-Hadriche et al. (2013) with some adjustments. TFC value estimation has been done using this technique by researchers (Ghimeray et al., 2009). Flavonoids have a diverse variety of biological and pharmacological effects. These can act as reducing agents, singlet oxygen quenchers, metal chelators, ROS scavengers, and chain-breaking antioxidants

(Naseer et al., 2018). These flavonoids have inhibitory properties against the bacteria that cause plant diseases. Other than that, flavonoids' structure is thought to play a part in the extract's oxidative capabilities (Hue et al., 2012). Green leafy vegetables are known to have significant antioxidant activity, which is recognized in part to the existence of flavonoids in these plants. Thus, phenolic and flavonoid content is important due to its role of scavenge free radicals in the human body and to aid in the maintenance of a healthy body by scavenging or eliminating reactive oxygen species (ROS).

The antioxidant activity of PSPLAE were compared with the antioxidant activity in standard, butylated hydroxytoluene (BHT) compounds by using DPPH radical scavenging assay. BHT prevents unsaturated organic molecules from oxidizing. BHT is used in foods, cosmetics, and industrial fluid due to its function to inhibit oxidation and the production of free radical. EC_{50} is utilized to convey the level of effective concentration of extracts that enable to scavenge 50% of the free radicals. Moreover, the lowest EC_{50} value indicate the highest activity of antioxidant. From this study, the activity of antioxidant in purple sweet potato leaf was discovered to be not significantly different from the standard, BHT which indicates that the antioxidant activity of PSPLAE and BHT have the same strength and effectiveness. Moreover, there are also a positive association between the TPC and TFC with the antioxidant activity of the aqueous purple sweet potato leaf. This indicate that the antioxidant activity of PSPLAE were contributed by the total phenolic and total flavonoid content.

Due to the demand for a more safe and effective treatment of psoriasis, a less costly method, which is traditional medicine research have been generated. Purple sweet potato leaf has been used by ancient people to treat wound and other inflammatory diseases. But, due to the lack of scientific research and evidence, a new

study that involve purple sweet potato leaf has been done to see it anti-psoriatic effect on imiquimod-induced psoriasis-like dermatitis. Imiquimod or IMQ is a toll-like receptor-7/8 (TLR7/8) agonist that has been authorized to manage keratosis, external genital warts, and superficial basal cell carcinoma (Flutter & Nestle, 2013). The psoriasis-like dermatitis was observed on the hairless back of the mice after a few days of imiquimod application. The findings also shows that the Psoriasis Area and Severity Index (PASI) scoring was elevated in group 2, imiquimod-induced group (Figure 9). Other than that, with the application of 5% and 10% of PSPLAE cream, the cumulative score of PASI scoring shows a decrease in the skin thickening, erythema, and scaling. This indicate that the usage of PSPLAE up to 10% was capable to lessen the severity of the skin lesions in imiquimod-induced psoriasis-like skin lesion in Balb/C mice. However, the usage of 20% of PSPLAE which is the highest dose, shows no observable changes on the severity of the psoriasis-like skin lesion on mice. This indicates that the topical administration of 20% of PSPLAE cream was not able to reduce the imiquimod-induced skin inflammation in Balb/C mice. This is because, the concentration of the PSPLAE is too high and become toxic to the mice.

CHAPTER 6

CONCLUSION

In conclusion, the purple sweet potato leaf extract has shown the presence of total phenolic, total flavonoid, and the ability to scavenge free radicals. There is a definite correlation between antioxidant substances (TPC & TFC) and antioxidant activity (DPPH assay) in purple sweet potato leaf of aqueous extraction. The dosage of 5% and 10% of PSPLAE cream were competent to decrease the seriousness of psoriasis in IMQ-induced mice. Thus, the purple sweet potato leaf can be a good and affordable source of organic antioxidants and can be developed as an anti-psoriatic agent to reduce the symptoms of psoriasis. However, further research on the efficacy and safety of PSPLAE extracts as topical psoriasis treatment is required. In order to understand the mechanism of PSPLAE activity in IMQ-induced psoriasis-like skin lesion in Balb/C mice, further molecular research is required.

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APPENDICES

Data expression of cumulative score for PASI scoring

	Cumulative score	SEM
G1	0	0.13
G2	5.67	1.41
G3	0.5	0.19
G4	3.82	0.9
G5	4	1.7
G6	4	0.76
G7	6.17	1.65