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DEVELOPMENT AND CHARACTERIZATION OF COSMETIC CREAM FORMULATION INCORPORATING *Piper betle* LINN. EXTRACT

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

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ABSTRACT

Hyperpigmentation is a common and crucial aesthetic problem frequently encountered by women in which the darkened patches or spots appear on the skin. Therefore, cosmetic products containing skin lightening agents are used to inhibit the occurrence of the skin hyperpigmentation. The study aimed to obtain *Piper betle* aqueous extract containing hydroxychavicol (HC) from *Piper betle* leaves using subcritical water extraction method, to develop and optimize the formulation of cosmetic cream incorporating *Piper betle* crude extract and to characterize the optimized cream formulation incorporating *Piper betle* aqueous extract. Firstly, the *Piper betle* leaves were extracted using subcritical water extraction method to obtain *Piper betle* aqueous extract containing HC, a skin lightening agent. In order to determine the optimal extraction condition, extraction temperatures ranging from 40 to 180℃ and extraction time intervals of 10 to 90 min were tested. The results revealed that the highest amount of HC extracted was 7.53 ± 0.03%, w/w at a temperature of 100℃ with 60 min time interval with a total HC yield of 30.58 ± 0.12 mg/g *Piper betle* leaves. Next, the optimization of cream was conducted by incorporating stabilizers i.e. chelating agent of disodium ethylenediaminetetraacetic acid (EDTA) and complexing agents of hydroxypropyl-β-cyclodextrin (Hp-β-CD) and caffeine, which were added to stabilize HC in *Piper betle* extract and minimize degradation in the formulations. The preliminary stability study showed that F4 containing disodium EDTA minimized the HC degradation by 11.48 ± 1.13%. Following that, different cream formulations were subjected to a three-month stability study in different storage conditions at the temperatures of 40℃ and 30℃. Prior to stability study, three types of formulation were prepared and differentiated through the addition of aqueous extract of *Piper betle* (F1), aqueous extract of *Piper betle* with added disodium EDTA (F4), and dichloromethane (DCM) extract of *Piper betle* (F5). All formulations were characterized for physical analysis, pH determination, droplet size analysis, zeta potential determination, rheological analysis, microbial limit count assay, and HPLC assay to observe any HC degradation. After comparing all formulations, F4 was determined as the most stable formulation for yielding acceptable results within all parameters of cream characterization with the least degradation of HC of 26.32 ± 0.09% and 19.36 ± 0.30% at 40℃ and 30℃, respectively, within three months. As a result, an optimized and stable cosmetic cream incorporating *Piper betle* aqueous extract containing HC from *Piper betle* leaves was successfully developed.

خلاصة البحث

يعتبر فرط التصبغ مشكلة جمالية شائعة وعصيبة تعاني منها النساء في كثير من الأحيان حيث تظهر بقع داكنة أو علامات على الجلد. لذلك تستخدام مستحضرات التجميل المحتوية على مركبات مفتحة للجلد لمنع حدوث فرط تصبغ الجلد. هدفت الدراسة إلى الحصول على المستخلصات المائية لأوراق نبات التنبول (*Piper betle*) المحتوية على مركب هيدروكسيكافيكول (HC) باستخدام طريقة الاستخلاص المائية دون الحرجة لتطوير وتحسين صياغة كريم تجميلي محتوي على مستخلص نبات التنبول الخام وتوصيف تركيبة الكريم المحسنة مع إضافة المستخلصات المائية لنبات التنبول. تم أولا استخلاص أوراق نبتة التنبول باستخدام طريقة الاستخلاص المائية دون الحرجة للحصول على المستخلصات المائية لأوراق نبات التنبول المحتوية على مركب الHC والذي يعتبر عامل تفتيح للبشرة. من أجل تحديد حالة الاستخلاص المثلى تم اختبار درجات حرارة استخلاص تراوحت بين 40 إلى 180 ^{o}C وفترات زمنية من 10 إلى 90 دقيقة. أوضحت النتائج أن أعلى كمية من الHC المستخلص كانت 7.53±0.03٪ w/w عند درجة حرارة 100 ^{o}C بفترة زمنية دامت 60 دقيقة مع إنتاج إجمالي من الHC بلغ 30.58±0.12 مغ/غ من أوراق نبتة التنبول. بعد ذلك تم تحسين الكريم من خلال دمج مثبتات مثل العامل المستخلب حامض الإيثيلين ثنائي الأمين ثلاثي الأسيتات (EDTA) والعوامل المركّبة هيدروكسي بروبيل-β-دكسترين حلقي(Hp-β-CD) والكافيين، والتي أضيفت لتثبيت الHC في مستخلص نبات التنبول وتقليل التحلل في الصياغات. أظهرت دراسة الاستقرار الأولية أن F4 المحتوية على EDTA قللت من تدهور الHC بمقدار 11.48 ± 1.13٪. بعد ذلك تم وضع الصياغات المختلفة للكريم في دراسة استقرار لمدة ثلاثة أشهر في ظروف تخزين مختلفة في درجات حرارة بين 40 ^{o}C و 30 ^{o}C. قبل دراسة الاستقرار تم تحضير ثلاثة أنواع من الصيغ وتم التفريق بينهم من خلال إضافة المستخلص المائي لنبات التنبول (F1)، والمستخلص المائي لنبات التنبول مع إضافة EDTA (F4)، ومستخلص كلوريد الميثيلين (DCM) لنبات التنبول (F5). تم توصيف جميع الصيغ حسب التحليل الفيزيائي، والحموضة، وتحليل حجم القطيرات، وتحديد احتمال زيتا، والتحليل الريولوجي، واختبار كبية الميكروبات، والتحليل الكروماتوغرافي السائل عالي الأداء (HPLC) لمراقبة أي تحلل للHC. بعد مقارنة جميع الصيغ تم تحديد الصيغة F4 على أنها الأكثر استقرارًا للحصول على نتائج مقبولة في جميع المؤشرات توصيف الكريم بأقل تحلل للHC وذلك بمقدار 26.32±0.09٪ عند 40 ^{o}C و 19.36 ± 0.30٪ عند 30 ^{o}C في غضون ثلاثة شهور. ونتيجة لذلك تم بنجاح تطوير كريم تجميلي محسن ومستقر يحتوي على المستخلص المائي لنبتة التنبول بمركب الHC.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

ACS	American chemical society
ANOVA	Analysis of variance
ASE	Accelerated solvent extractor
ASEAN	Association of southeast asian nations
β-CD	β-cyclodextrin
ATR-FTIR	Attenuated total reflectance fourier transform infrared
CA	Cetrimide agar
DCM	Dichloromethane
DHI	5,6-Dihydroxyindole
DHICA	5,6 Dihydroxyindole-2-carboxylic acid
D(v, 10)	Diameter below which 10% of the sample exists
D(v, 50)	Diameter below which 50% of the sample exists
D(v, 90)	Diameter below which 90% of the sample exists
EDTA	Ethylenediaminetetraacetic acid
et al.	*(et alia)*: and others
F1	Formulation 1
F2	Formulation 2
F3	Formulation 3
F4	Formulation 4
F5	Formulation 5
HC	Hydroxychavicol
HLB	Hydrophile-lipophile balance
Hp-β-CD	Hydroxypropyl-β-cyclodextrin
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonization
i.e.	that is

IR	Infrared
L-DOPA	L-3,4-dihydroxyphenylalanine
LOD	Limit of detection
LOQ	Limit of quantification
MSA	Mannitol salt agar
n.d.	no date
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
PBS	Phosphate buffer solution
PDA	Photodiode array detector
psi	Pounds per square inch
RH	Relative humidity
RP-HPLC	Reversed-phase high performance liquid chromatography
RSD	Relative standard deviation
SD	Standard deviation
SDA	Sabouraud dextrose agar
SPSS	Statistical package for the social sciences
TAMC	Total aerobic microbial count
TSA	Tryptic soy agar
TSB	Tryptic soy broth
TYMC	Total yeasts and molds count
TRP1	Tyrosinase-related protein 1
TRP2	Tyrosinase-related protein 2
USP38/NF33	United States Pharmacopoeia 38/National Formulary 33
UTM	Universiti Teknologi Malaysia
UV	Ultraviolet
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

1.1.1 Cosmetic

Cosmetic is defined as articles intended to be rubbed, poured, sprinkled or sprayed on, introduced into, or applied to the human body parts for cleansing, beautifying, promoting attractiveness, or altering one's appearance. The examples of cosmetic products include skin moisturizers, perfumes, lipsticks, fingernail polishes, eye and facial make-up, cleansing shampoo, permanents waves, hair colours, deodorants and any substance intended to be used as a component of a cosmetic product (U.S. Food & Drug Administration, n.d.).

Besides enhancing the beauty of the skin, it is claimed that cosmetic products have a great potential in protecting the skin against harmful substances and reducing skin disorders such as hyperpigmentation, skin aging, wrinkles, and rough skin. Skin cleansers, toners, serums, and moisturizers are some examples of cosmetic products commonly used for minimizing these skin disorders. The benefits of cosmetics rely on the presence of natural or synthetic compounds incorporated in the formulation (Soni, 2015).

1.1.2 Skin

Skin is known as the largest and outermost organ of the human body which plays various crucial functions, for instance, as an agent for protection, sensory, absorption, excretion, thermoregulation, and metabolism (Darlenski, Kazandjieva & Tsankov,

2011). All of these functions are contributed by the complexity of the structure of the skin. There are three different layers that make up the skin structure, namely the viable epidermis, the vascularized dermis, and subcutaneous tissues as illustrated in Figure 1.1.

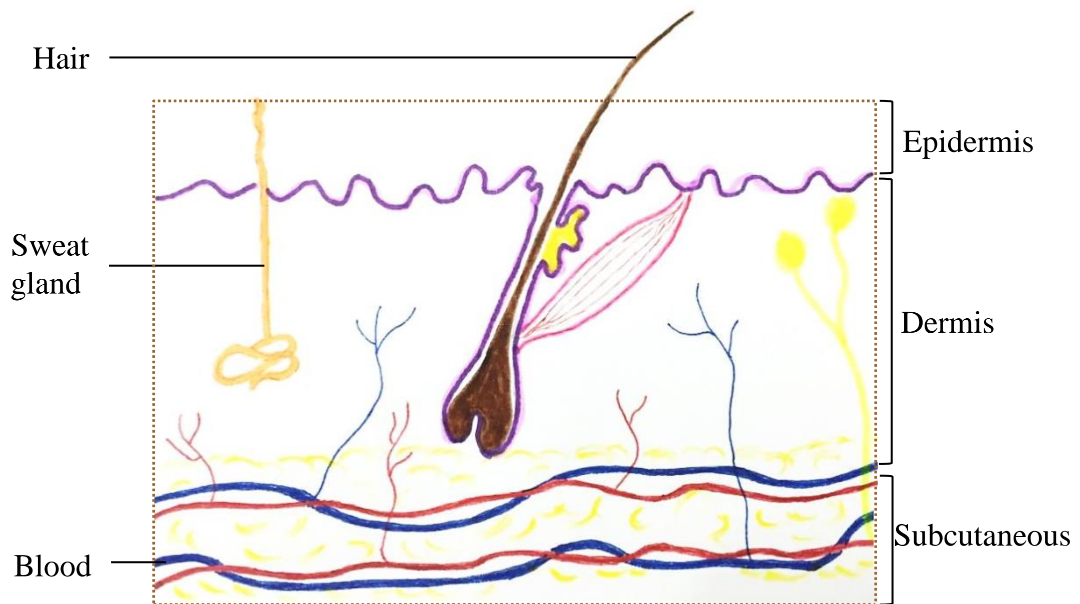

Figure 1.1 Skin structure (Adapted from McGrath, Eady & Pope, 2008).

The epidermis is primarily comprised of keratinocytes and melanocytes, and all components are arranged within four epidermal layers, namely stratum corneum, stratum granulosum, stratum spinosum and stratum germinativum as shown in Figure 1.2. As epidermis is the outermost skin layer, it is where skin barrier functions are carried out, especially within the superficial corneous layer i.e. stratum corneum (Baroni et al., 2012). The horny layer of stratum corneum is described as a simple two-compartment model of "brick-and-mortar", with 'brick' representing corneocytes residing in the intercellular lipid matrix containing fatty acids, ceramides, and cholesterol (Bouwstra et al., 2000). All of these components make up a complex and

intact skin barrier structure which is the rate-limiting unit for the permeability and penetration of exogenous substances or molecules such as destructive agents, allergens, bacteria, and viruses into the skin (Elias & Choi, 2005; Darlenski et al., 2009). Not only that, stratum corneum also prevents excessive loss of main and important components of human body system such as water, ions, and serum proteins. Even so, the permeability of skin barrier is characterized as flexible and dynamic in manner, and could be easily altered by several circumstances such as climate, physical factors, and skin and systemic diseases (Darlenski et al., 2009).

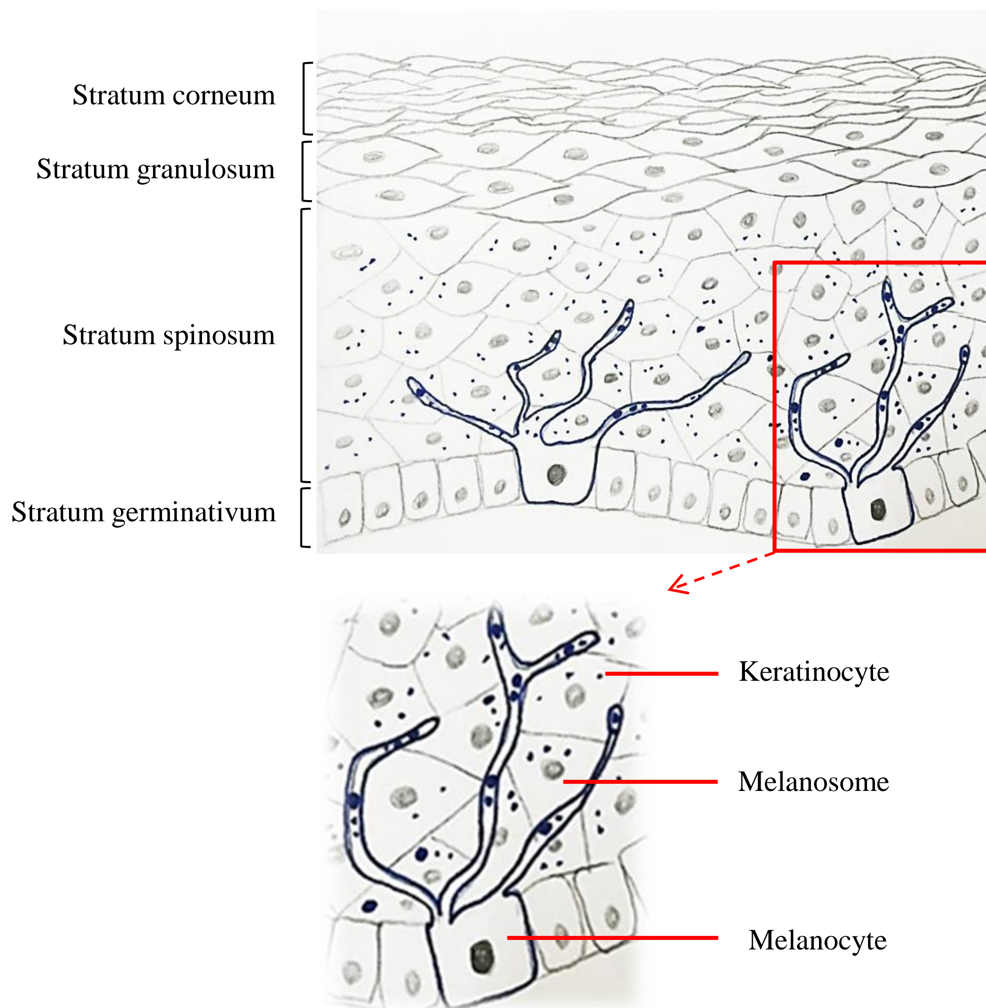

Figure 1.2 Layers of epidermis and an epidermal melanin unit (represented by a red box) (Adapted from Cichorek et al., 2013).

1.1.3 Hyperpigmentation

Hyperpigmentation is a skin disorder that is not uncommon. It may occur due to overproduction and accumulation of melanin pigment or due to increased amount of melanocytes expressing melanin synthesis within the skin layers (Leyden et al., 2011). Post inflammatory hyperpigmentation, solar lentigo, melasma, freckles, and age spots are examples of hyperpigmentation problems (Pillaiyar, Manickam & Jung, 2015). Melanin is a skin pigment that gives colour to the skin, hair, and eyes. As illustrated in Figure 1.2, there are epidermal melanin units (melanocytes, and keratinocytes which surround the former) arranged within the epidermis. The presence of melanin units is essential for melanin production. Other than deciding human skin colour, melanin plays vital protective roles in human skin against deleterious impacts of ultraviolet (UV) radiation, drugs, chemical substances, and other environmental factors (Maddodi, Jayanthy & Setaluri, 2012; Pillaiyar, Manickam & Jung, 2015).

The melanogenesis or melanin biosynthesis is a complex sequential pathway that occurs in the melanosomes i.e. membrane-bound organelles found in melanocytes, and is catalyzed by tyrosinase, a rate limiting enzyme (Narayanaswamy & Ismail, 2015). As shown in Figure 1.3, melanin biosynthesis is initiated by the catalytic hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosinase. Tyrosinase subsequently oxidizes L-DOPA to L-DOPAquinone. Following the formation of L-DOPAquinone, the pathway is divided into two processes of eumelanin (that is a dark brown-black insoluble polymer) synthesis and pheomelanin (that is a light red-yellow soluble polymer) synthesis (Gillbro & Olsson, 2011).

The production of eumelanin and pheomelanin can be distinguished by the presence of cysteine group; in the presence of cysteine, pheomelanin is produced. During pheomelanin synthetic pathway, L-DOPAquinone reacts with cysteine present

to form CysteinylDOPA, which is then oxidized and polymerized to pheomelanin. On the other hand, eumelanin is produced in the absence of cysteine. During the process, DOPAchrome is produced after the cyclization of L-DOPAquinone (Cichorek et al., 2013). Following the formation of DOPAchrome, the pathway is subdivided either to the spontaneous conversion into 5,6-Dihydroxyindole (DHI) or the enzymatic conversion into 5,6 Dihydroxyindole-2-carboxylic acid (DHICA) by Tyrosinase-related protein 2 (TRP2) (Gillbro & Olsson, 2011).

After enzymatic reaction by TRP2, Tyrosinase-related protein 1 (TRP1) further catalyzes the conversion of 5,6 Dihydroxyindole-2-carboxylic acid (DHICA) into Indole-5,6-quinone carboxylic acid. Meanwhile, after a spontaneous process of 5,6-Dihydroxyindole (DHI), it is converted into Indole-5,6-quinone. Finally, the polymerization of indole and quinone group takes place to produce eumelanin (Gillbro & Olsson, 2011). The variation in skin colour across various races is mainly determined by the size and amount of melanosomes, and the number, types, and distribution of melanin in suprabasal skin layer. Nevertheless, skin pigmentation can be triggered by various internal factors such as hormone regulation and inflammation, and also by external factors such as UV exposure and drugs (Leyden et al., 2011; Videira, Moura & Magina, 2013). Consequently, the production of melanin would increase and hyperpigmentation would occur.