THE EFFICACY OF 7% KAEMPFERIA PARVIFLORA CREAM FOR THE TREATMENT OF MELASMA

YEE YEE MON

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Author: Yee Yee Mon

Examination Committee:

Tanomkit Pawcsuntorn, M. D.	Chairperson
Paisal Rummaneethorn, M. D.	Member
Associate Professor Wongdyan Pandii, Dr. P. H.	Member

Advisors:

.....Advisor

(Paisal Rummaneethorn, M. D.)

Dean:

.....

(Karnt Wongsuphasawat, Ph. D.)

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Yee Yee Mon

Thesis Title	The Efficacy of 7% Kaempferia parviflora Cream for the Treatment of Melasma
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ABSTRACT

Background: Melasma is a common facial skin problem. Since people nowadays pursue the beauty of skin and its health, melasma has become a big concern for the quality of life and mental health of the affected persons. Many factors are liable for the occurrence of melasma including genetics, exposure to UV radiation, hormonal imbalance, pregnancy, and some medicines. The aim is to decrease melanocyte proliferation and elevate the breakdown of melanin in the treatment of melasma. There are many available and effective options for curing melasma. Some natural extracts from medicinal *herbs* are used for the treatment of melasma because of their proven improvement in melasma. *Kaempferia parviflora* is also widely known as "Thai ginseng". *Kaempferia parviflora* has been long used as herbal folk medicine. Based on safety and efficacy, *Kaempferia parviflora* has been chosen in Thailand as one of the top 5 medicinal products and has the target to produce more income to the country. *Kaempferia parviflora* extract can reduce oxidative stress and also has impressive anti-inflammatory and anti-tyrosinase benefits. We will focus on the anti-tyrosinase activity of 7% Kaempferia parviflora and study its potency in treating melasma as a facial cream.

Objective: To study the efficacy of 7% Kaempferia parviflora cream for the treatment of melasma.

Materials & Methods: This research is before and after comparison experimental study with 20 participants and the duration is 12 weeks period. The study population is healthy male and female between the age of 30-65 years old. They need to apply the 7% *Kaempferia parviflora* cream twice a day for 12 weeks. Follow-up appointments would be at 4th, 8th and 12th week. In this study we assess Melanin index

score, Modified MASI score, Doctor's evaluation and Patient satisfactory score to test the efficacy. Melanin Index assessment is done by Mexameter. To measure the improvement, photographs taken with VISIA® Complexion Analysis System will be evaluated by 3 dermatologists. The participants were assessed by physician and questionnaires to detect any adverse effects and participants' satisfaction.

Results: The outcome of modified MASI score and Melanin index were significantly improved (p value<0.001) in different visit. Thus, 7% Kaempferia parviflora cream can improve melasma. The result showed good improvement in 65% of the participants in Doctor's evaluation score. And 45 % showed very satisfied and 55% showed moderate satisfaction in Patient Satifactorion Score. is This study also achieved high participants' satisfactory score and doctors' evaluation score and no adverse effect was detected.

Conclusion: Based on the clinical study, it can be concluded that *Kaempferia parviflora* cream significantly reduce skin hyperpigmentation with great satisfaction. In summary, *Kaempferia parviflora* cream appears to be a safe and effective topical treatment for the treatment of melasma.

Keywords: Melasma, *Kaempferia parviflora*, Medicinal Product, Thai Ginseng

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INTRODUCTION

1.1 Background

Melasma is a common facial skin problem, characterized by irregular hyperpigmentation on both side of the face (Bagherani et al., 2015). Since people nowadays pursue the beauty of skin and its health, melasma has become a big concern for the quality of life and mental health of the affected persons (Tzouveka, 2014). It has a huge impact on the confident level during social communication (Adalatkhah et al., 2008).

"Melas" from melasma means dark coloration (Bandyopadhyay, 2009). Another name for melasma is "chloasma" indicating "mask of pregnancy" (Handel et al., 2014).

There are 3 types of facial melasma, based on their distribution, centrofacial, malar, and mandibular patterns (Mandry & Sánchez, 2000; Sanchez et al., 1981). Melasma is also classified as epidermal, dermal, or mixed type (Gilchrest et al., 1977).

Asian women over 30 years are more likely to get melasma (Newcomer, 1961). Many factors are liable in the occurrence of melasma including genetics, exposure to UV radiation, hormonal imbalance, pregnancy and some medicines such as phenytoin (Sarkar et al., 2014).

Wood's lamp can be helpful in determining the level of melanin predisposition and its nature (Sarkar et al., 2014). Curation of melasma is quite exigent for many of its aspect remain in the grey area (Shweta et al., 2014).

The aim is to decrease the melanocyte proliferation and elevate the breakdown of melanin in the treatment of melasma (Cestari et al.,2009).

There are many available and effective options in curing melasma. Some natural extract from medicinal herbs such as coffee berry and licorice are used for the treatment of melasma because of their proven improvement in melasma. They are affordable, consumer friendly (Cadiz-Gurrea et al., 2017).

Hydroquinone, being the standardized treatment, declines the tyrosinase enzyme and the production of RNA and DNA (Sarkar et al., 2014). Tranexamic acid, azelaic acid, ascorbic acid, arbutin are also popular alternatives in treating melasma. (Sarkar et al.,2014). There are furthermore options such as laser and light therapies and chemical peeling (Shankar et al., 2014).

Kaempferia parviflora is also widely known as “Thai ginseng”. The origin was in northeast of Thailand, and it can be found plentifully in Malaysia and Thailand (Wuttidharmmavej, 2002). *Kaempferia parviflora* has been long used as herbal folk medicine. Some studies have proved that Kaempferia has the following properties: allergy, asthma, mental stability, gastric ulcer and urinary tract infection (Elshamy et al., 2019).

Based on safety and efficacy, *Kaempferia parviflora* has been chosen in Thailand as one of the top 5 medicinal products and also has the target to produce more income to the country (Chuthaputti et al., 2013). *Kaempferia parviflora* extract can reduce oxidative stress and also has impressive anti-inflammatory and anti-tyrosinase benefits (Amic et al., 2007). We will focus on the anti-tyrosinase activity of *Kaempferia parviflora* and study its potency in treating melasma as a facial cream.

1.2 Research Question

Does facial cream made with 7% *Kaempferia parviflora* extract have an efficacy in the treatment of melasma?

1.3 Objectives

1.3.1 General Objective

To study the clinical efficacy of 7% *Kaempferia parviflora* extract cream in the field of melasma treatment.

1.3.2 Specific Objectives

1.3.2.1 Primary Outcomes

To discover the efficacy of 7% *Kaempferia parviflora* extract cream for melasma.

1.3.2.2 Secondary Outcomes

1. Uncovering the side effects of 7% *Kaempferia parviflora* cream in the treatment of melasma.

2. Assessment the patient's satisfactory score of 7% *Kaempferia parviflora* cream in treating melasma.

1.4 Hypothesis

1.4.1 Topical 7% *Kaempferia parviflora* cream has a good efficacy in the treatment of melasma.

1.4.2 Assessing the side effects of 7% *Kaempferia parviflora* cream in treating melasma.

1.5 Conceptual Framework

To achieve an attractive skin, pigmentation plays a major concern. A variety of treatment options are available to whiten the hyperpigmented lesions by reducing melanin levels. Tyrosinase is an oxidase and enzyme with copper and limited in rate in the process of melanogenesis. In melanogenesis, the 1st 2 steps were catalyzed by tyrosinase; (1) transformation of the hydroxyl action of tyrosine to Dopa (3, 4-dihydroxyphenylalaninine) and (2) the oxidation of Dopa to Dopaquinone .Therefore, this serum can inhibit melanogenesis. Since tyrosinase is the main character for synthesizing melanin, we will use anti tyrosinase activity of *Kaempferia parviflora* extract to get the skin lightening effect and decrease the production of melanin.

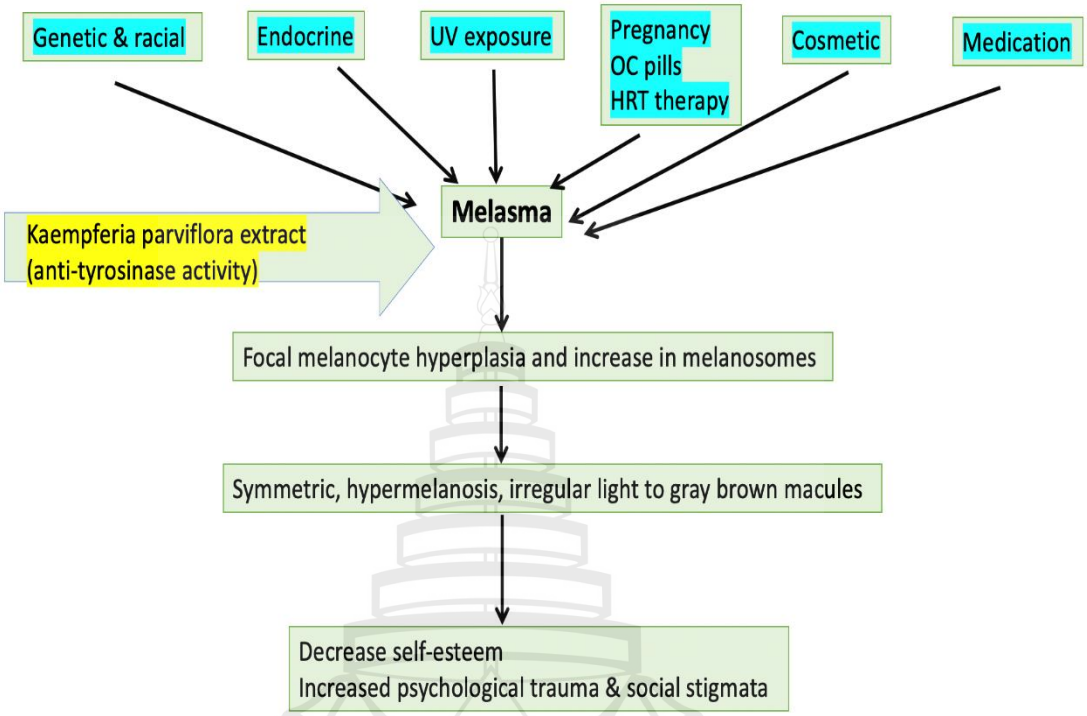


Figure 1.1 Conceptual framework

1.6 Scope of Study

The study group contains 20 subjects between the ages of 30 and 65 (Fitzpatrick skin types III-VI) with melasma and who met all inclusion and exclusion criteria. This is an experimental study which is conducted with 7% *Kaempferia parviflora* extract cream. The subjects are asked to apply the facial cream 2 times a day continuously for 12 weeks. Mild soap and sun creams are provided. The results are measured at 4th week, 8th week and 12th week by MASI score together with mexameter® MX18 measured and photographed by VISIA®. Moreover, volunteers' satisfactory scores and untoward reactions were assessed and noted by three dermatologists during the study period.

1.7 Definition

1.7.1 Melasma

An acquired skin disorder that presents with symmetric hyperpigmentation on sun exposed areas, mainly the face. The occurrence is higher in women and the precipitating factors contain family history, pregnancy, exposure to UV radiation, and stress (Kayla et al., 2020).

1.7.2 Tyrosinase

Tyrosinase is the enzyme responsible for the first steps in pathway of melanin synthesis. It is a binuclear type III copper containing protein (Hatcher et al., 2014).

1.7.3 Melanin

Melanin is a biopolymer essential for longevity of living beings (Solomon et al., 1996). Especially, melanin is accountable for protection against ultraviolet radiation, so it prevents impairment to DNA, proteins, and other key cellular elements (Murray et al., 2016).

1.7.4 Efficacy

Is defined as the ability to produce a desired or intended result.

1.7.5 Sunscreen

Is the cream applied to protect from sunlight.

1.7.6 Mexameter®

Is a device that measures by showing two skin components, melanocytes and red blood cells. It has high sensitivity to melanin and erythema providing the score from one to one thousand. Mean melanin index being the measure of mexameter® shows the result as one being equal to white and one thousand meaning dark. Even slight color changes can be tracked. Accuracy is above and below 4% (Okkar et al., 2020)

1.7.7 VISIA®

Is an equipment that visually measures skin appearance by producing a series of photographs through standard, UV, and cross-polarized light. VISIA® applies standardized flash to analyze spots, wrinkles, skin textures and pore size (Anne Goldsberry et al., 2014)

Figure 1.2 VISIA

1.7.8 Fitzpatrick Skin Type

Table 1.1 Fitzpatrick skin type scale

Skin	History	Examples
1	Always burns easily, never tans (sensitive)	Redhead, freckled, Celtic, Irish-Scots
2	Always burns easily, tans minimally (sensitive)	Fair-skinned, fair-haired, blue-eyed Caucasians
3	Burns moderately, tans gradually (to a light-brown) (normal)	Caucasians
4	Burns minimally, always tan well (to a moderate brown) (normal)	Mediterranean type Caucasians
5	Rarely burns, tans profusely (to a deak-brown) (insensitive)	Mid-Eastern. Some Latin America types
6	Never burns, deeply pigmented (insensitive)	Black skinned Negroids

1.7.9 Modified MASI Score

Used clinically to assess the severity of melasma (Handel et al., 2014). The area (A) and darkness level are two components mainly used.

For estimating the area (A), it must be scored out of a total of seven.

0 = absent, 1 = <10%, 2 = 10%-29%, 3 = 30%-49%, 4 = 50%-69%, 5 = 70%-89%, and 6 = 90%-100% (Amit et al., 2010).

To measure darkness (D), there are 4 grades.

0 =normal, absent of darkness, 1 = hyperpigmentation seen with naked eye, 2 = mild, 3 = marked, and 4 = severe darkness (Amit et al., 2010). Formulation for Modified MASI score Modified MASI score = 0.3A (f) D (f) + 0.3A (lm) D (lm) + 0.3A (rm) D (rm) + 0.1A (c) D (c)

Therefore, score was estimated at 0, 4th, 6th, 8th week. It is the area value associated with the sum of severity and uniformity of darkness for each of the 3 facial areas (Pandya et al., 2011).

1.7.10 *Kaempferia parviflora* Extract Cream

This is the cream to study its efficacy for the treatment of melasma. It is extracted from Black galingale (*Kaempferia parviflora*) and formulated with 7% of the extract with vehicles and basic ingredients of cream. It is manufactured by Skin Intimate Company from Thailand with registration number 12-1-6600015648.

CHAPTER 2

RELATED LITERATURE REVIEW

2.1 Melasma

Melasma is a common acquired hyperpigmentation disorder that affects up to 30% of child- bearing women in certain populations (Thierry et al., 2017) It is characterized by asymptomatic light to dark brown macule and patches with symmetrical distribution and irregular borders on sun exposed areas (Rajanala et al., 2019)

Melasma comes from “melas” which means dark color (Bandyopadhyay, 2009), also known as “melasma”, which means “mask of pregnancy” (Handel et al., 2014).

The effects on the quality of life of affected individuals are well documented and so new treatment strategies are needed (Thierry et al., 2018). It can also disrupt facial morphology as an important fact of self-confidence and dealing with others. Compared with men, most patients were women (Jiang et al., 2018).

2.1.1 History

Disease descriptions can be found in written medical writings and extended to reports by Hippocrates (470-360 BCE). Melasma usually becomes severe when exposed to UV rays, heat, coolness and redness of the skin (Handel et al., 2014).

First, there is a 20-year-old woman with hyperpigmented lesions on her upper lip. The nature of the lesions is made worse by exposure to ultraviolet light. In another study of 14 women with melasma on the face, 10 were pregnant and the rest had “melanosis of pregnancy” (Handel et al., 2014).

2.1.2 Epidemiology

Melasma is a common pigment abnormality that often prompts people to seek dermatological care. Its population prevalence varies by ethnic composition, skin phototype, and sun exposure. Race is one of the triggers of melasma (Ortonne et al. 2009).

Melasma and sex are the most common link, with women being 7 to 9 times more likely than men. In approximately 40-50% of female patients, the disease is triggered by pregnancy or the use of oral contraceptives. Melasma occurs in 8% to 34% of women taking COCs (combined hormonal oral contraceptives), which have also been reported after hormone replacement therapy (Ana et al., 2014).

Pregnancy is also strongly associated with melasma (50-70%). Hormonal factors such as estrogen and progesterone are included as risk factors. Research shows a significant reduction in prevalence after age 50, likely due to menopause and the reduction in melanocyte number and activity that occurs with aging (Videira et al., 2013).

2.1.3 Classification and Clinical Presentation

Melasma can be mainly classified into centrofacial, malar and mandibular types. Comparing these, the most common site is centrofacial area while mandibular is the least common area (Lynde et al., 2006).

Table 2.1 Frequency of facial features affected by melasma

Topographies	Percentage
Mandibular	18
Temporal	24
Glabella	25
Mentonian	29
Parotid	30
Nasal	40
Supralabial	51
Zygomatic	84

Source Handel et al. (2014)

Melasma can be subdivided into 4 types based on the primary pigment location: epidermal, dermal, mixed or indeterminate types (e.g. in a patient with very dark skin pigmentation). The contrast accentuated by examination with Wood's lamp is epidermal type and no accentuation is seen in dermal type (Cestari et al., 2009). According to its natural history , melasma can also be divided into transient type which

is within one year and persistent type which means more than one year duration (Bandyopadhyay, 2009).

2.1.4 Pathophysiology

The pathophysiology of melasma is still not clearly understood (Demirkan et al., 2017). Many risk factors like exposure to sunlight and hormonal imbalance have been found to have impact on the occurance of melasma (Kim et al., 2021).

Increased melanin production in melasma is well established. However, if the raised melanin is accompanied by an increase in the number of melanocytes or not is still debated (Grimes, 2005).

Exposure to UV radiation leads to increase in melanocyte stimulating hormone (MSH) receptors on melanocyte and accountable for the greater binding of hormones leading to upregulation in melanin synthesis (Rajanala et al., 2019).

Solar elastosis means accumulation of abnormal elastic tissue in dermis. It usually results from long term sunlight exposure or photoaging. Individuals affected with melasma have thicker and curler elastic fibers compared to normal skin in histology (Rajanala et al., 2019).

Melasma skin also has higher number of mast cells than unaffected skin (Videira et al., 2013). Histamine released from mast cells binds to H 2 receptor and activates the tyrosinase pathway inducing melanin synthesis (Yoshida et al., 2000). Mast cells also induce increase in vasculature by secreting proteins such as vascular endothelial growth factor (VEGF). But the effect of VEGF in melasma has not been clearly known (Chen et al., 2014).

Abnormality in basement membrane plays a role in melasma pathology. Damage from UV exposure activates matrix metalloproteinase 2 and 9 (MMP2 and MMP9) to degrade collagen type IV and VI in the basement membrane (Kwon et al., 2016). Cadherin 11 can then mediate interactions between melanocyte and fibroblasts and promote melanogenesis (Kim et al., 2016).

Chronic UV radiation causes inflammation in dermis and activates fibroblasts which produce stem cell factor (SCF). SCF may induce the melanogenesis in the epidermis (Lee et al., 2012).

Hormonal influence has been shown to play a role in production of melanin. It also explains the increased prevalence of melasma in post puberty, women using oral contraceptive pills and pregnant women (Chompootaweep et al., 1996).

2.1.5 Melanocytes

Melanocytes are melanin synthesizing dendritic cells. They saturated in theepidermis basal layer, the hair bulb, and hair follicles' outer sheath. That concerns with synthesize melanin pigment responsible for skin coloration (Yaar & Park, 2012).

Melanosomes are synthesized by epidermal melanin unit and then aretransferred adjacent keratinocytes bi-directionally throughout dendrites which are structures resembles the arms. A membrane which is unique organelle in that biosynthesis of melanin occurred as a group of cells cap covering the keratinocyte's nucleus is the melanosome (Bertrand et al., 2020).

2.1.6 Melanocyte Synthesis

Melanogenesis is the process in which production and distribution of melanin was taken responsible from the skin epidermal units. The main step in melanogenesis is the oxidation by the tyrosinase enzyme from tyrosine to dopaquinone. The main enzyme in regulation of the process melanogenesis which is a glycoprotein stayed within the melanosomes, is tyrosinase. Melanogenesis process starts while PAH (Phenylalanine hydroxylase) enzyme catalyze L-phenylalanine to L-tyrosine.

Subsequently key enzyme in regulation melanogenesis cooperates with TH-1 (Tyrosinase hydroxylase) to convert L-3,4 dihydroxyphenylalanine (DOPA) from L-tyrosine. DOPA is oxidized into DOPA quinone and it is converted to pheomelanin via pheomelanogenesis and eumelanin via eumelanogenesis. L-tyrosine can only be transported into the melanosomes by diffusion even if it is a building component for melanogenesis (Schallreuter & Wood, 1999).

Dopachrome is spontaneously changed to 5, 6-dihydroxyindole and is converted to 5, 6-dihydroxyindole-2carboxylic acid via enzymatic conversion by dopachrometautomerase (DCT), also known as tyrosinase-related protein-2 (TRP-2) in eumelanogenesis pathway. There are two tyrosinase-related proteins exist in melanosomes which called TRP1 and TRP-2. It has been promoted that TRP-1 raises the proportion of eumelanin to pheomelanin. Furthermore, they can augment the tyrosinase stability. Conversely, the function of TRP-1 and TRP-2 are not clear yet (Kanlayavattanakul & Lourith,

2018). Eumelanin formation can be led by the polymerization of quinines and phenols. The pheomelanin pathway varies from the eumelanin pathway by branching at the L-dopaquinone step and it is dependent on cysteine. Cysteine is actively transported through the melanosomal membrane. Cysteinyl-dopa can be formed by reaction of cysteine to L-dopaquinone. Subsequently, it is converted to alanine-hydroxyl dihydrobenzothazine, quinoleimine and polymerized to pheomelanin (Kanlayavattanakul & Lourith, 2018). Redox reaction is critical for the balance between the synthesis of pheomelanin and eumelanins. Reduction of glutathione (GSH) (low GSH for pheomelanin and high GSH for eumelanin) straightly determines this reaction. Therefore, melanogenesis is modified by the expression and functional activity of antioxidative enzymes (catalase, glutathione peroxidase, glutathione reductase and thioredoxinreductase) (Schallreuteret al., 1994). Melanin has a vital role in oxidative homeostasis in the skin (Gillbro & Olsson, 2011).

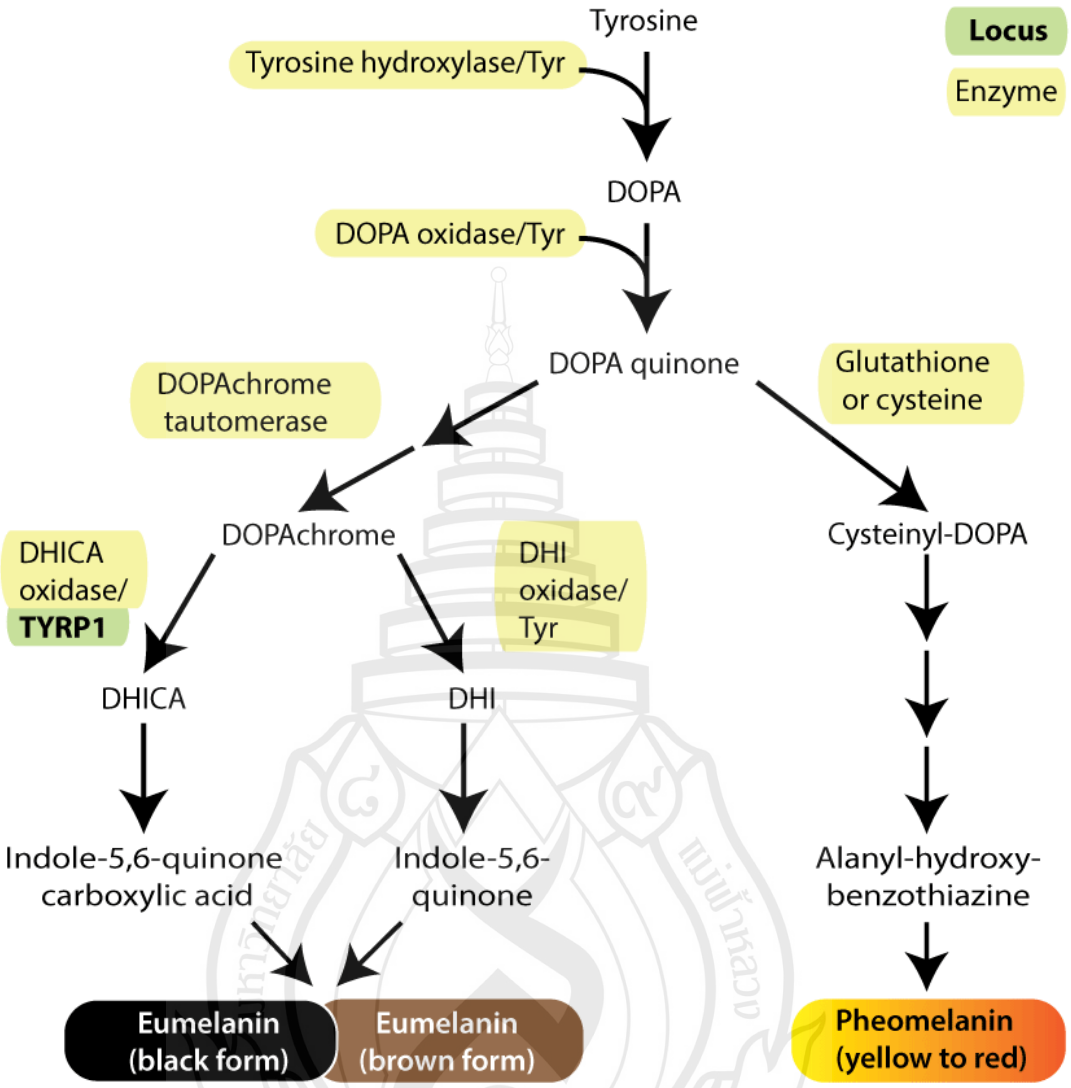


Figure 2.1 Melanin synthesis pathway

2.2 Approach to Melasma Treatment

Melasma's complex pathology and recurring nature make it difficult to treat therapeutically. The reduction in pigmentation within a minimal timeframe with little or no side effects is the main goal in the treatment of melasma (Gupta et al., 2006). Diligent protection from sunlight, hydroquinone and or steroid are the mainstream treatment. (Gupta et al., 2006).

2.2.1 Topical Treatment

Table 2.2 Topical treatment

Stage of melanin synthesis	Deposition	Active molecules
Before melanin synthesis	Tyrosinase transcription	Tretinoin, c-2 ceramide
	Tyrosinase glycosylation	$PaSSO_3Ca$
	Inhibition of plasmin	Tranexamic acid
During melanin synthesis	Tyrosinase inhibition	Hydroquinone, mequinol, azelaic acid, kojic acid, arbutin, deoxyarbutin, licorice extract, rucinol, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, *N*-acetyl glucosamide, resveratrol, ellagic acid, methyl gentisate, 4-hydroxyanisole
	Peroxidase inhibition	Phenolic compounds
	Reactive oxygen species scavengers	Ascorbic acid, ascorbic acid palmitate, thiotic acid, hydrocumarins
After melanin synthesis	Tyrosinase degradation	Linoleic acid, α-linoleic acid
	Inhibition of melanosome transfer	Niacinamide, serine protease inhibitors, retinoids, lecithins, neoglycoproteins, soybean trypsin inhibitor
	Skin turnover acceleration	Lactic acid, glycolic acid, linoleci acid, retinoic acid,
	Regulation of melanocyte environment	Corticosteroids, glabiridin
	Interaction with copper	Kojic acid, ascorbic acid
	Inhibition of melanosome maturation	Arbutin and deoxyarbutin
	Inhibition of protease activated receptor 2	Soybean trypsin inhibitor

2.2.1.1 Hydroquinone

The mechanism of action is to inhibit the melanogenesis. Hydroquinone has long been the conventional treatment. It can be used alone or with tretinoin. Kligman formula is a combination of 2% hydroquinone, 0.025% retinoic acid, and 1 % mometasone at (Nourmohammadi et al., 2019).

2.2.1.2 Azelaic acid

Azelaic acid, which normalizes keratinization, is a dicarboxylic acid that occurs naturally in grains and animal products (Graupe et al., 1996).

2.2.1.3 Tretinoin

Tretinoin inhibits tyrosinase and decreases melanin production. It has untowards effects such as inflammation, pruritus, scaling, dry skin, and photosensitivity, and it is also classified as pregnancy category C (Shankar et al., 2014).

2.2.1.4 Niacinamide

Nicotinamide can worsen tyrosinase and inhibit melanin transport (Navarrete-Solis et al., 2011). It is antioxidant property. Moreover, it inhibits inflammation and promotes barrier function (Bandyopadhyay, 2009).

2.2.1.5 Corticosteroids

Corticosteroids are anti-inflammatory because inflammation influences melanin production. It responds well to melasma but is not recommended on its own. In addition, long-term steroid use can lead to hypopigmentation (Bandyopadhyay, 2009).

2.2.1.6 Natural Remedies for Melasma

They are now popular for cosmetic ingredients due to their biological and physicochemical benefits. Also, their stability is comparable to synthetic products (Han et al., 2003). They are known for minimal adverse effects with long-term use (Bandyopadhyay, 2009).

1. Vitamin C

Ascorbic acid inhibits melanin, antioxidant and collagen synthesis properties. It’s effective for the photo damaging skin (Aboul-Einien et al., 2019).

2. Kojic acid

It has anti-tyrosinase and antioxidant properties and can be used with steroids to reduce inflammation and redness (Bandyopadhyay et al., 2009).

3. Soybeans

It stimulates collagen synthesis, UV protection, antioxidant and anti-inflammatory effects. In addition, it has a hydrating effect (Leyden et al., 2011).

4. Licorice

It has anti-tyrosinase activity in inhibiting melanin synthesis (Fu et al., 2005).

5. Mequinol

Mequinol is anti-tyrosinase, which can lead to inhibition of melanin production. It is beneficial in dealing with hypopigmentation without adverse effects (Draelos, 2006).

6. Arbutin

Arbutin blocks melanin synthesis and has been used with laser therapy (Han et al., 2011).

7. Glucosamine

Glucosamine reduces hyperpigmentation by reducing the melanin production (Espinal-Perez et al., 2004). Concomitant use with niacinamide is superior to monotherapy (Kimball et al., 2010).

8. Aloesin

It comes from aloe vera extract. Aloesin inhibits the production of melanin, so it can be used for hypermelanosis caused by ultraviolet radiation.

9. Mulberry Extract

Mulberry has the effect of inhibiting the activity of tyrosinase dopa oxidase, and also has the effect of scavenging superoxide (Halder et al., 2004).

10. Grape Seed Extract

Grape seed extract also has antioxidant properties and has been shown to lighten melasma when taken orally for six months (Yamakoshi et al., 2004).

11. Other external medicines

N-Acetyl-4-S-cysteinylphenol influences the inactivation of melanin. Features and side effects are less (Gupta et al., 2006).

12. Combination therapy

Combination therapies are more effective because they reinforce each other. Therefore, it also shortens the duration of the course and minimizes adverse effects. E.g., Krigman and Willis' formula. (Bagherani et al., 2015).

13. Oral and topical new therapies

Tranexamic acid inhibits the conversion of plasminogen to plasmin. It also has anti-inflammatory activity on arachidonic acid release and prostaglandin synthesis, which stimulates melanin production (Kim et al., 2015).

Melatonin is a potent antioxidant that inhibits receptors for alpha-melanocyte-stimulating hormone.

Glutathione is one of the most powerful endogenous antioxidants. It is a tripeptide of glutamic acid, cysteine , and glycine. It can inhibit tyrosinase (Sonthalia et al., 2016).

Methimazole, an oral antithyroid drug, causes depigmentation if used topically, and acts as a potent peroxidase inhibitor, inhibiting melanin synthesis (Kasraee et al., 2005, 2008).

14. Chemical peel

Chemical peels are adjunctive treatments for melasma because they increase epidermal remodeling and keratinocyte turnover (Gupta et al., 2006). Deep peeling is not recommended in melasma due to its side effects (Sarkar et al., 2012).

Suggested chemical peels are alpha and beta hydroxy acids such as TCA and salicylic acid with 10 to 30 percent salicylic acid, Jessner's solution, and 1% retinoic acid solution (Sarkar et al., 2010).

2.2.2 Treatment of Pregnant Women

Treatment of pregnant patients with melasma before delivery is not recommended. Furthermore, treatment during pregnancy is not easy due to hormonal instability (Lynde et al., 2006).

2.2.3 Device Therapy

Laser and light therapy are used as third-line treatments for melasma (Trivedi et al., 2017). Lasers are selectively targeted among various chromophores in the skin (Cui et al., 2018). Fractional laser therapy is effective in patients with persistent and drug-resistant variants (Rokhsar & Fitzpatrick, 2005).

2.3 *Kaempferia parviflora* Rhizome Extract

2.3.1 Introduction

Kaempferia parviflora also known as “Thai ginseng” (KP) is a medicinal plant, especially in Malaysia and Thailand. It is also pronounced Krachaidam in Thai. Also known as black ginger, it is popular as a health-promoting herb and is traditionally used as a folk remedy for urinary tract infections, fever, cough, asthma, and sexual dysfunction, especially in men (Saokaew et al., 2016). Additionally, it is well known for treating ulcers, osteoarthritis and improving blood flow (Akase et al., 2011).

Numerous studies have shown that it is good for allergies, redness, germs and ulcers, antidepressants, heart protection, anti-obesity and aphrodisiac. KP products are widely sold in the market as aphrodisiac boosters (Sudwan et al., 2007).

In addition, many other studies have shown that it has anti-fatigue, appetite-inducing, male sexual stimulation, anti-stomach pain, cardiac protection, anti-cancer, anti-peptic ulcer, anti-microbial, anti-allergic, anti-mutagenic, anti-cholinesterase activity, Inhibits intrinsic aging process, anti-osteoporosis, anti-obesity and improves physical endurance (Sripanidkulchai et al., 2019).

Kaempferia pariflora is one of the main products that increase the GDP of the country. However, clinical studies in humans are still limited (Chuthaputti, 2013).

2.3.2 Chemical Constituents of *Kaempferia parviflora*

The chemical profile of Kaempferia exhibits different types of secondary metabolites, such as terpenoids, especially isopentanols, diarylheptanes, flavonoids, and essential oils (Elshamy et al., 2019).

5,7-Dimethoxyflavones and 5,7,4-Trimethoxyflavones are the two main components. In addition, the flavonoids as constituents have antioxidant activity that protects neurons and enhances the cognitive abilities of the brain.

Methoxyflavonoids in Krachaidum have been reported to enhance sexual dysfunction. It also has anti-microbial action especially against Plasmodium falciparum. Moreover, it also has been showed that it has antifungal action against on Candida albican (Saokaew et al., 2016).

Methoxyflavones
3,5,7,3′,4′-pentamethoxyflavone (Compound-**1**)
3,5,7,4′-tetramethoxyflavone (Compound-**2**)
5,7,3′,4′-tetramethoxyflavone (Compound-**3**)
5,7,4′-trimethoxyflavone (Compound-**4**)
3,5,7-trimethoxyflavone (Compound-**5**)
5,7-dimethoxyflavone (Compound-**6**)
3,5-dihydroxy-7, 3′,4′-trimethoxyflavone (Compound-**7**)
5,3′-dihydroxy-3,7,4′-trimethoxyflavone (Compound-**8**)
5,4′-dihydroxy-7-methoxyflavone (Compound-**9**)
5-hydroxy-3,7,3′,4′-tetramethoxyflavone (Compound-**10**)
5-hydroxy-7, 3′,4′-trimethoxyflavone (Compound-**11**)
5-hydroxy-3,7,4′-trimethoxyflavone (Compound-**12**)
5-hydroxy-3,7-dimethoxyflavone (Compound-**13**)
5-hydroxy-7,4′-dimethoxyflavone (Compound-**14**)
5-hydroxy-7-methoxyflavone (Compound-**15**)
4′-hydroxy-5,7-dimethoxyflavone (Compound-**16**)

Figure 2.2 Structural identification of methoxyflavonoids isolated from S. chinensis

2.3.3 Preparation of the Extracts of the Rhizomes of S. chinensis

Kaempferia parviflora in front of the wall. Baker (rhizomes) are prepared as follows:

Step 1: Dry in a hot air oven

Step 2: Grinding

Step 3: Extraction with deionized water

Step 4: Low temperature evaporation in vacuum evaporator

Step 5: Dissolve in Butanediol Step 6: Standardize

Step 7: Black Ginger Extract

2.3.4 Biological Activity of Kaempferia Rhizome Extract

2.3.4.1 Anti-tyrosinase activity

In one study, the compounds 5-Hydroxy-7,3,4-trimethoxyflavone, 5,7,3,4-Tetramethoxyflavone, 5,3-dihydroxy-3,7,4-trimethoxyflavone and 5-hydroxy-3,7, 3,4-tetramethoxyflavone has anti-tyrosinase activity, can inhibit TRP 1 and 2 mRNA,

resulting in melanin production. We also identified several methoxyflavonoids with potent melanogenesis-inhibiting activity from methanolic extracts of K. parviflora rhizomes.

Some compounds are more effective than arbutin. In particular, 5,3-dihydroxy-3,7,4-trimethoxyflavone and 5-hydroxy-3,7,3,4-tetramethoxyflavone were 60 times more potent than arbutin (Ninomiya et al., 2015).

2.3.4.2 Antioxidant activity

Kaempferia parviflora contains a variety of flavonoids that play an important role as free radical scavengers.(Yenjai et al., 2004). Several studies have shown the potential of flavonoids to have antioxidant activity (Pietta, 2000).

2.3.4.3 Anti-aging activity

Skin aging such as intrinsic type is mainly caused by intracellular stress. The main reasons are cellular senescence and mitochondrial dysfunction (Lo´pez-Ot´ın et al., 2013). Mitochondrial homeostasis is associated with anti-aging (Gomes et al., 2013).

In one study, it slowed the intrinsic skin aging process by inhibiting cellular aging and mitochondrial dysfunction. Kaempferia parviflora prevents the formation of wrinkles and promotes the production of collagen and elastin (Park et al., 2017).

2.3.4.4 Anti-photoaging activity

UV irradiation mainly reduces collagen type I expression (Sardy, 2009). An abnormal increase in MMP production is one mechanism by which photoaging may occur. Therefore, the production of MMPs with reduced activity in natural materials may lead to protection against photodamage (Song et al., 2012).

One study showed that oral administration of K. parviflora inhibited UVB-induced expression of MMP-2, 3, 9 and 13. In addition, it protects against UVB-induced wrinkle formation, skin thickening, and collagen degradation, and promotes the expression of COL1A1, COL3A1, and COL7A1 in mice. Therefore, kaempferol is an effective natural anti-photoaging extract (Park et al., 2013).

2.3.4.5 Anti-inflammatory activity

Macrophage RAW264.7 cells can produce multiple inflammatory markers, such as PGE2, IL-1β, TNFα, and NO. One study showed that the ethanolic extract of Kaempferia has been used to inhibit lipopolysaccharide (LPS)-induced PGE2

expression in RAW264.7 cells. It has been reported that components such as 5-hydroxy-3,7,3,4 tetramethoxyflavone have effects on LPS-induced NO release and PGE2 production in RAW264.7 cells (Sae-wong et al., 2009).

Regarding toxicity, one study showed that 15% of the saffron was free of inflammation and swelling in the skin after 48 hours of use in rats. Histological examination showed no difference in the morphology of the skin of the test and control rats, indicating that the transdermal administration is safe. In addition, another study showed that LD50.

Kaempferia parviflora powder exceeds 13.33 grams per kilogram of body weight. At this dose, no abnormal histopathological changes were seen in various internal organs (Chivapat et al., 2010). A chronic toxicity study in rats at 5, 50 and 500 mg/kg/day for 6 months showed that it did not produce any signs of drug toxicity and did not show any signs of drug toxicity in various Any histopathological changes were induced in the study organ. The dose is 500 mg/kg, which is about 100 times higher than the dose used in humans (Sripanidkulchai et al., 2019).

Finally, the study focused on the treatment of melasma. While there are literature reviews on the oral use of Thai black ginger extract for other problems, it is less used for skin problems and topical melasma. In addition, there are few research papers on its anti-tyrosinase activity. Therefore, this study aimed to investigate the effectiveness and satisfaction of the extracts of *Kaempferia parviflora* in the treatment of melasma. The findings will support the diagnosis and treatment of melasma using *Kaempferia parviflora* as an alternative therapy for melasma.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Experimental study, before and after comparative.

3.2 Study Population

30-65 years old with Fitzpatrick skin type 3 to 5

3.3 Sample Size Determination

The subjects will be recruited according to inclusion and exclusion criteria. The subjects will be invited to the study by giving investigator voucher and advertising at MFU clinic and MFU school at Asoke.

The researcher selected sample sized calculation from a comparative study of the efficacy and safety of Nelumbo nucifera cream, which is a double blinded, randomized, placebo-controlled trial. The aim to study for facial whitening treatment.

Mexameter readings in groups using lotus cream after 12th week exhibited a significant reduction compared to the placebo group from 322.12 (± 48.75) at baseline to 274.23(±44.37), whose Mexameter readings deteriorated from 324.08 (± 47.26) at baseline to 313.43 (± 48.43) at 12th week.

Different change of total area in lotus cream=47.89 Different change of total area in standard cream base=10.65 Mean difference between two groups μd = 37.24

From the formula,

Let $\alpha = 0.05$ (two tail) $Z_{0.025} = 1.96$

$\beta = 0.1$ $Z_{0.100} = 1.28$

$n1 = 30$ $n2 = 30$

$$S_p^2 = \frac{[S_1^2(n_1-1)+ S_2^2(n_2-1)]}{[n_1+n_2-2]}$$

= 2157.0809

To calculate the sample size by two mean dependences, using formula

$$n = \frac{(z_{\alpha/2} + z_\beta)^2\sigma^2}{\mu_d^2}$$

Set

α = 0.05 (two tailed) $Z_{0.025}$ = 1.96

β = 0.10 (one tailed) $Z_{0.100}$ = 1.28

$$n = \frac{(z_{\alpha/2} + z_\beta)^2\sigma^2}{\mu_d^2}$$

$$= \frac{(1.96 + 1.28)^2 x\ 2157.0809}{(37.24)^2}$$

= 17

Where

n = sample size

S = σ = Variance

$S^2_p = \sigma^2$ =Pooled variance

The researcher expected 20% dropout rate. Thus, 20 subjects who wanted to join this study willingly were collected by researcher.

3.4 Selection Criteria

3.4.1 Inclusion Criteria

3.4.1.1 Healthy volunteers aged between 35 to 60 years with Fitzpatrick skin type 3 to 5

3.4.1.2 Volunteers with melasma on both faces were recruited

3.4.1.3 Volunteers who consent to apply 7% *Kaempferia parviflora* extract cream on both sides of the face

3.4.1.4 Volunteers who agree to restrict other kinds of melasma treatment during the study period except applying sunscreen.

3.4.1.5 Volunteers who is eager to take part in this study and can come for follow up once per month for 3 consecutive months.

3.4.1.6 Participants who are willing to sign the consent form.

3.4.2 Exclusion Criteria

3.4.2.1 Volunteers allergic to *Kaempferia parviflora* extract cream, soap and sun cream given

3.4.2.2 Pregnant women and breastfeeding women

3.4.2.3 Volunteers with skin impairments such as eczema or atopic dermatitis on the face.

3.4.2.4 Volunteers who has a history of keloid formation and delayed wound healing.

3.4.2.5 Volunteers taking medication that influences melanogenesis such as phenytoin, and birth control pills.

3.4.2.6 Volunteers using or have used hydroquinone within the last six months.

3.4.2.7 Volunteers using or have used tretinoin within the last three months

3.4.2.8 Volunteers who have lesions on the treatment area.

3.4.2.9 Volunteers who has undergone facial procedures such as chemical peeling, laser, and light therapies.

3.4.2.10 Volunteers with current usage of skin-lightening ointments or creams.

3.4.2.11 Volunteers whose career is exposed to sunlight for a long period.

3.4.3 Discontinuation Criteria

3.4.3.1 Volunteers who could not manage to apply the active cream and placebo as designated during the study period.

3.4.3.2 Volunteers with skin irritation after usage of active cream.

3.4.3.3 Volunteers with any reasons wanting to leave the study.

3.4.3.4 Volunteers who became pregnant.

3.4.3.5 Volunteers who are not good at cooperating and miss follow up in the study period.

3.4.3.6 Volunteers with other medical conditions or injury during the study

3.4.4 Early Termination Criteria

3.4.4.1 Individuals elect to leave the study or suffer from severe negative effects (for example: severe allergic reaction or pain).

3.4.4.2 The procedures and therapies cause the participants excessive discomfort or pain.

If 30% of volunteers develop severe negative effects such as excessive pain or severe irritation, the researcher will stop the study immediately.

3.5 Location

Mae Fah Luang University Hospital, Asoke, Bangkok, Thailand

3.6 Variables

The study's dependent variables were the modified MASI score, melanin index, physician and patient satisfaction scores, adverse reactions

3.7 Materials and Equipment Used in Research

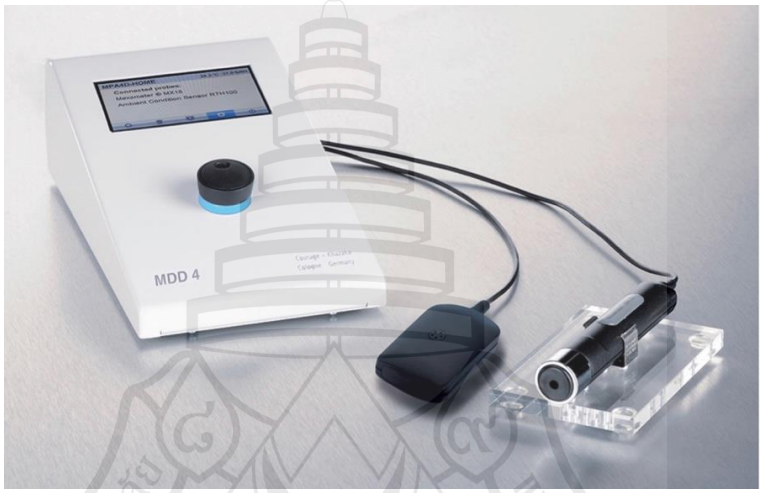
1. 7% *Kaempferia parviflora* extract cream - is the facial cream that includes 7% Kaempferia parviflora extract in a standard cream.
2. Sunscreen
3. Facial cleansing gel or soap with no active ingredients
4. VISIA®Complexion Analysis System (Canfield, Fairfield, NJ)
5. Mexameter ®MX 18 (Courage-Khazaka Electronic, Koln, Germany)
6. Patch test
7. Consent form

8. Docotor evaluation form

9. Patient satisfaction form

3.7.1 Mexameter

An instrument for measuring the intensity of melanin pigment which is beneficial to track and compare the changes better than the naked eye. The probe is handy and light weight for simple to use by giving constant pressure on the skin. the machine will access the reflected light and measure the value in 1 second.

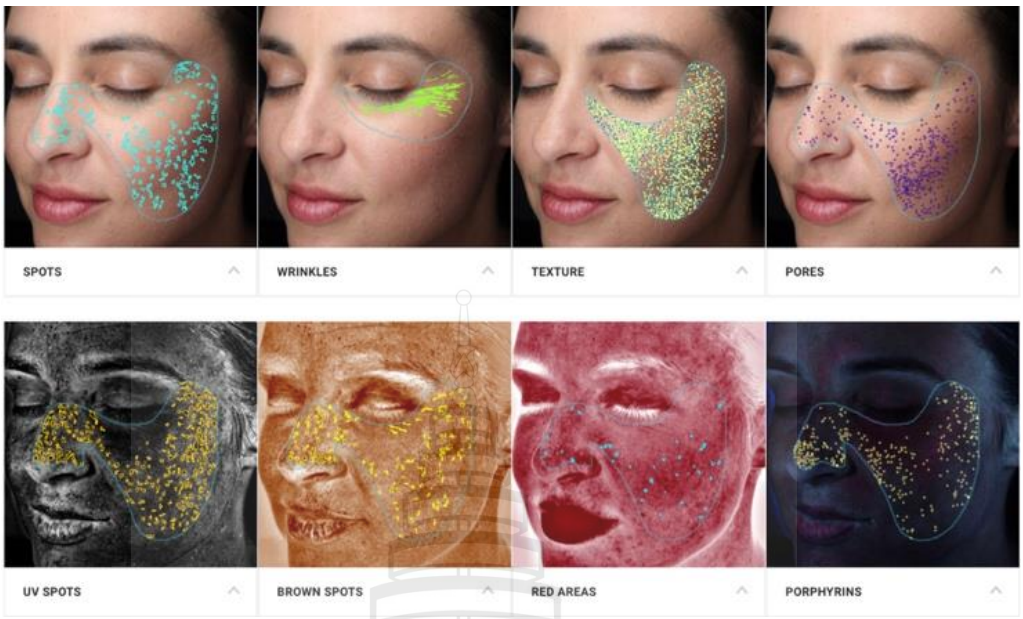


Source Courage+Khazaka electronic GmbH (n.d.)

Figure 3.1 Mexameter

3.7.2 VISIA Complexion Analysis

VISIA® Complexion Analysis System (Canfield, Fairfield, NJ) is high quality facial analysis with IntelliFlash®. It provides to visualize the skin appearance such as spots, wrinkles, texture, pores, UV spots, Brown spots, red areas and porphyrins.



Source Canfield Scientific (2025)

Figure 3.2 VISIA® complexion analysis system

3.7.3 Chemical Constituents of 7% *Kaempferia parviflora* Extract Cream

1. 7% *Kaempferia parviflora* Rhizome Extract
2. Aqua
3. Glycerin
4. Dimethicone
5. C 12-15 alkyl benzoate
6. Behenyl alcohol
7. Cetearyl Alcohol
8. Simmondsia
9. Chinensis seed oil
10. Glyceryl Stearate
11. Propanediol
12. Sodium polyacrylate
13. Ethylhexyleglycerin
14. PEG-40 Stearate
15. Ceteareth-20
16. Tocopheryl acetate

17. Ceterareth-20
18. Tocopheryl acetate
19. Disodium EDTA

3.7.4 Chemical Constituents of Suncream SPF 50, PA+++

1. Aqua
2. Ethylhexyl Methoxycinnamate
3. Diethylamino Hydroxybenzoyl Hexyl Benzoate
4. Bis-Ethylhexyloxyphenol Mehothoxyphenyl Triazine
5. Dibutyl Adipate
6. Nano Titanium dioxide
7. Dimethicone
8. Cyclodextrin
9. Tocopherol
10. Triethanolamine
11. Xanthan Gum
12. Lauryl Glucoside
13. BHT
14. Propylene Glycol
15. Oxybenzone

3.7.5 Chemical Constituents of Gentle Cleanser Soap

1. Aqua
2. Acetyl alcohol
3. Propylene glycol
4. Sodium Lauryl sulfate
5. Stearyl alcohol
6. Propylparaben
7. Butylparaben
8. Methylparaben

3.8 Quality Control

The extract cream will be collected with a container tube and one tube will contain 30g of the cream. This cream will be white in color and odourless and no silicone. The cream used in the research have approved Thailand FDA license.

3.8.1 Patch test

3.8.2 Consent form

3.8.3 Case record form

3.8.4 Patients' satisfaction record form

3.8.5 Adverse effects record form

3.9 Steps of Research

3.9.1 Treatment Procedures

3.9.1.1 According to the inclusion criteria, the researcher registered the volunteers for the study. The goal of this study, the benefits and feasible complications in the study period were explained clearly by the researcher. Subjects signed the informed consent forms to participate in the study.

3.9.1.2 Thorough history taking from the subjects.

3.9.1.3 Patch test is tested on the arm of each volunteer with waterproof patch containing 0.1 ml Kaempferis parviflora for 24 hours. Exercise, extreme activities, long duration sun exposure should be avoided. Re-examination is taken for any response for 48 to 96 hours. The results are noted as follows:

+? = suspected reaction (e.g., mild redness)

+ = weak positive reaction shows redness and thickened skin at contact site

++ = strong positive reaction shows red edematous skin with small, separated blisters

+++ = extreme positive reaction shows strong redness and swelling together with coalesced large blisters.

After removing the patch test, note the skin redness as follows:

NT= not tested

IR= irritant reaction

Volunteers who test positive in patch test with ++ score were excluded.

3.9.1.4 The photograph of volunteers with VISIA® complexion analysis system will be captured in 3 different positions (left, right and center) at 0, 4th, 8th and 12th week accordingly.

Volunteers' photos were kept as follow:

1. All volunteer information is kept completely private. The participant's results will only be used in scholarly research and presentations. Each participant's address and surname will be kept confidential in a folder which will be secured with a passcode that only researcher will have access to. All data about the volunteer's information will be delected from computer system in 1 year after publication.

2. Take only half a picture per page or only the area that needs evaluation

3. When taking a full face photo, use the black band to place the image on both eyes

4. Name the image file as a volunteer code rather than using a real name

3.9.1.5 Mean melanin index was assessed by the investigator using the Mexameter®, and the scores were calculated at weeks 0, 4, 8, and 12. Melanin index was measured on both sides of the face where there is marked hyperpigmentation.

3.9.2 Guidelines

3.9.2.1 Clean the face with gentle facial cleansing gel or soap by the following steps

1. Splash water on the face and use your finger to rub the facial cleanser provided.

2. Rinsed face thoroughly till there is no sensation of cleansers on face.

3. Dab the face with a dry, soft and clean facial tissue or towel.

3.9.2.2 Applied 0.5 g of active cream (one finger tip) on the face two times a day.

3.9.2.3 Suncream is applied after the extract cream, 20 minutes before exposing to sunlight.

3.9.2.4 Extract cream will need to be applied two times per day, in the morning and at night so 1 gram per day.

3.9.2.5 All volunteers have to follow the procedures strictly as stated in the consent form such as halt using other whitening agents or avoid sunlight exposure during 12 weeks of the study. Each participant will get a record form to record own adverse effects. If the subjects experience any serious side effects, they must halt using the cream, inform the researcher and follow up to hospital immediately

3.10 Follow Up

Participants were asked to follow up at weeks 4, 8, and 12 after the baseline visit to check and evaluate for improvement or Adverse Reactions.

3.11 Measurement and Collection of Data

3.11.1 Clinical Evaluation

In clinical, the modified MASI score is used to assess the severity level of melasma. The system uses mainly two parts: area and darkness. To estimate the area (A), it must be scored out of a total of seven. 0 means not involved, 1 means less than ten percent of the affected area, 2 means ten to twenty-nine percent of the area, 3 means thirty to forty-nine percent, and 3 means percentage between 30-39, 4 is 50 to 55 percent affected area, 5 is 70 to 89 percent affected area, 6 is 90 or more percent affected area.

To estimate darkness (D), the scale is zero to four. 0 is normal, 1 is naked eye hyperpigmentation, 2 is mild, 3 is moderate, and 4 is severely dark skinned. Therefore, a modified MASI score is measured at each visit.

3.11.2 Use VISIA® to take pictures at each visit to analyze the effectiveness of the Kaempferia parviflora extract cream.

3.11.3 The doctor evaluation score and Patient satisfactory scoring System is based on the Global Satisfaction Score and ranges as follows.

-1 = worse

0 = no change

1 = 1-25% fairness improvement

2 = >25-50% moderate improvement

3 = >50-75% good improvement

4 => 75-100% excellent improvement

3.11.4 Evaluate the satisfaction score of the patient from base line to 12th week of the study.

-1=Worse

0= No satisfaction, no change

1= Little satisfaction

2= Moderate satisfaction

3= Very satisfied

4= Extremely satisfied

3.11.5 For side effects and complication to be recorded, researchers asked the volunteers about the following untoward effect during the study period.

1. Pruritus score from 0 to 10.
2. Presence of erythema and if present, duration and severity
3. Other reaction like change in pigmentation, inflammation.

3.12 Data Analysis

3.12.1 Descriptive Analysis

3.12.1.1 The eligible volunteers for this study were determined according to the inclusion and exclusion criteria, and personal information was kept highly confidential.

3.12.1.2 Medical records and outcomes in this study were recorded Via Microsoft Excel 2019 and SPSS software.

3.12.1.3 Using descriptive statistical analysis, the demographic data were recorded.

3.12.1.4 Subjects' research profile data will be analyzed by using descriptive statistical analysis to provide descriptive information such as percentages, means, modes, medians, ranges and standard deviations.

3.12.1.5 Subjects' satisfaction will be analyzed by using descriptive statistical analysis.

3.12.1.6 Subjects'side effect will be analyzed by using descriptive statistical analysis.

3.12.1.7 Patient satisfaction scores were analyzed at week 12 employing the MeNemar test and descriptive statistics.

3.12.1.8 The researcher performed the following operations at a significance level of p-value < 0.05.

3.12.2 Inferential Data

The data will be statistically analyzed by paired t-test for normal distribution data. On the other hand, Wilcoxon signed-rank test will be used for analyze the nonparametric data. The significance levels of p-value < 0.05 is acceptable.

3.14.2.1 Comparing Global Aesthetics Improvement score before and after 4th, 8th, 16th weeks will be analyzed by using Repeated Measurement analysis of variance (ANOVA).

3.14.2.2 Comparing mexameter melanin index at before, and after 4th,8th and 16th weeks will be analyzed by using Repeated Measurement ANOVA.

3.13 Ethical Considerations

This study will strictly follow the Good Clinical Practice (GCP) guidelines that is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials which involves the participation of human subjects, provided by International Conference on Harmonization (ICH).

In GCP guidelines, following considerations are included;

1. Protection of human rights as an subject in clinical trial.
2. Assurance of the safety and efficacy of the newly developed compounds.
3. Standards on how clinical trials should be conducted.
4. Define the roles and responsibilities of clinical trial sponsors.
5. Clinical research investigators and monitors.

3.13.1 The volunteers are given a research information sheet by the researchers along with a description of the study's objectives, procedures, safety measures, symptoms, and any hazards

3.13.2 The participants must voluntarily sign a research information sheet from the researchers that contains information on the goals, methods, safety precautions, symptoms, and potential risks of the study. They can go at any time with no consequences

3.13.3 The subjects and the researcher will not have any conflict of interest. There will be no price for this research.

3.13.4 The 7% *Kaempferia parviflora* extract cream that the researcher is using in this investigation has been approved by the Thai FDA. Skin inflammation and erythema are possible side effects. Prior to the research, a complete medical history will be taken, and a patch test will be done to rule out allergies and hypersensitive reactions

3.13.5 The researcher will take responsibility for the subjects to the extent that it is practical. Participants are advised that in the unlikely event that this research causes them injury, the research team will provide them with free medical attention and make up any lost pay

3.13.6 All volunteer information is kept completely private. The participant's results will only be used in scholarly research and presentations. Each participant's address and surname name will be kept confidential in a folder which will be secured with a passcode that only researcher will have access to. All data about the volunteer's information will be deleted from the computer system in 1 year after publication.

CHAPTER 4

RESULTS

4.1 General Characteristics

Twenty healthy volunteers aged 30-65 years with Fitzpatrick skin type III to V who have melasma and compatible with all the inclusion criteria were registered. All twenty volunteers complete this study. The details of the demographic data were exhibited in Table 4.1.

Table 4.1 General characteristic

Demographic	**Participant n=20**	
	Number(n)	**Percentage**
Gender		
Male	5	25
Female	15	75
Age		
Mean+/-SD	45.1±8.1	
Min-Max	32-59	
Pregnancy or Lactation		
Yes	0	
No	20	100
Occupation		
Goverentment officer	0	0
Business owner	4	20
Housewife	7	35
Student	0	0
Employee	9	45
Others	0	0

Table 4.1 (continued)

Demographic	Participant n=20	
	Number(n)	**Percentage**
Underlying Disease		
Yes	0	0
No	20	100
Photosensitivity or Drug-induced hypersensitivity		
Yes	0	0
No	20	100
Personal medication		
Supplement	6	30
No	14	70
Treatment area		
Chemotherapy	0	0
Actvie inflammation or open wound at the treatment area	0	0
Malignant or premalignant lesion at treatment area	0	0
Food or Drug allergy		
Yes	1	5
No	19	95
Current facial product allergy		
Yes	0	0
No	20	100
History of previous treatment		
Yes	0	0
No	20	100
Average sun exposure		
Yes	20	100
No	0	0

Table 4.1 (continued)

Demographic	Participant n=20	
	Number(n)	**Percentage**
Sunlight exposure time (10 am to 4 pm)		
Mean+/-SD (min)	61.5±31.5	
Min-Max	30-120	
Fitzpatrick skin type		
Type III	14	70
Type IV	4	20
Type V	2	10

4.2 Clinical Evaluation

The demographic demographics of the participants in this study are shown in Table 4.1. There were total participants 20 individuals, with distribution of gender:5 males and 15 females. A total of 20 participants were initially enrolled in the study. Analyses were conducted with data from the 20 participants who completed the study. The average age of the participants was 45.1±8.1 years. The participants were 4 business owner (25%), 7 housewives (35%) while the remaining 9 were employees (45%). No participant has an underlying medical condition or photosensitivity or drug induced hypersensitivity, and none had received any treatment in the four weeks prior to the study. There was no report of current facial product sensitivity. One had food allergy while remaining 19 had no history of food or drug allergy. All 20 volunteers have sun exposure which is aggravating factor for melasma. In terms of skin type, 14 participants had Fitzpatrick skin type III (70%), 4 had Fitzpatrick skin type IV (20%) and 2 had Fitzpatrick skin type V (10%).

4.3 Modified Melasma Area and Severity Index (MASI)

Table 4.2 Statistical analysis of Modified MASI score that applied 7% *Kaempferia parviflora* cream.at baseline,4th ,8th and 12th week (n=20)

	Minimum	Maximum	Mean	Std. Deviation
Baseline	8.00	11.70	9.405	1.19
4th week	6.90	10.80	8.96	1.11
8th week	6.50	10.50	8.37	1.21
12th week	6.30	9.30	7.84	0.95

Note Repeated measure ANOVA, F = 52.459 (d.f=3), p<0.001

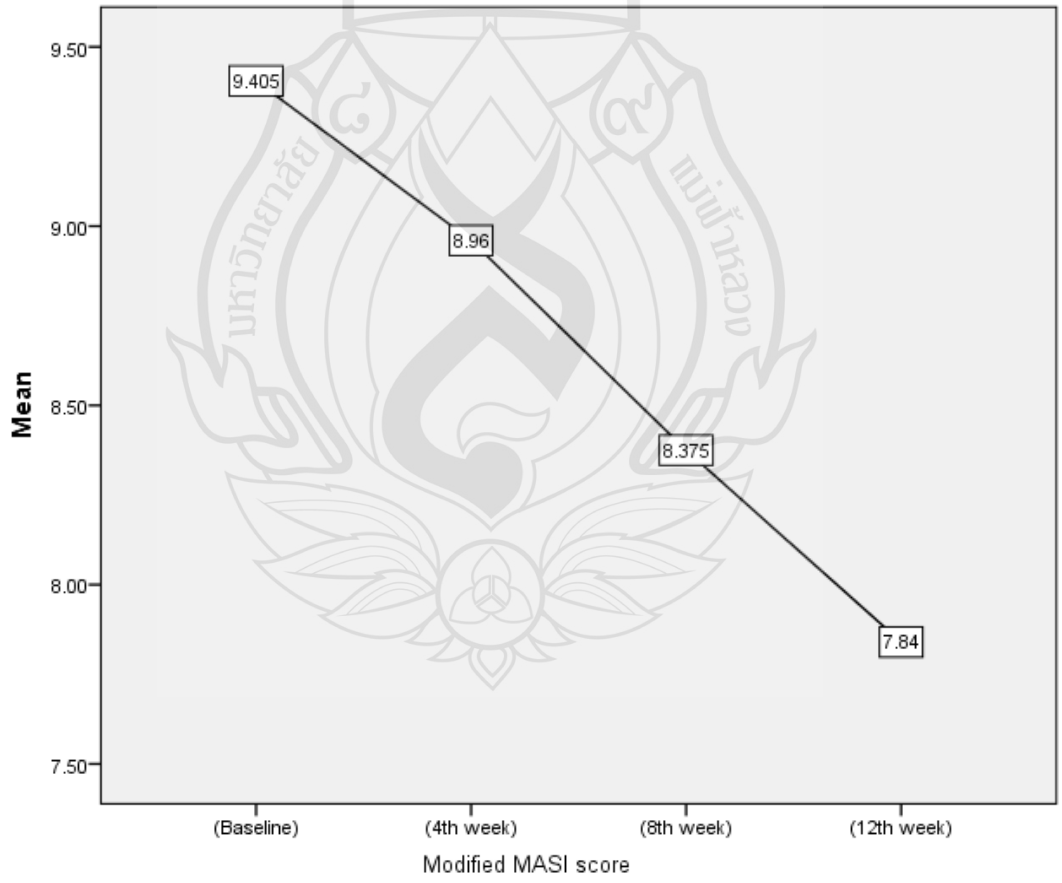


Figure 4.1 Line Graph showing Modified MASI score that applied 7% *Kaempferia parviflora* cream at baseline, 4th, 8th and 12th week

According to results from Table 4.2, and Figure 4.1, mean of modified MASI score in 7% *Kaempferia parviflora* extract cream before the treatment was 9.405±1.19 at the baseline. The results after applying extract from were at 4th week 8.96±1.11, 8th week 8.37±1.21, and 12th week 7.84±0.95. The mean of MASI score in 7% *Kaempferia parviflora* extract cream decreased statistically significant at the level of 0.05 ($p<0.001$). The results showed clinical difference and statistically significant.

Table 4.3 Multiple comparison of Modified MASI score that applied 7% *Kaempferia parviflora* cream.at baseline, 4th, 8th and 12th week

Duration	Pair comparison	Mean difference	p-value
Baseline	4th week	0.445	0.006*
	8th week	1.03	0.000*
	12th week	1.565	0.000*
4th week	Baseline	-0.445	0.006*
	8th week	0.585	0.001*
	12th week	1.12	0.000*
8th week	Baseline	-1.03	0.000*
	4th week	-0.585	0.001*
	12th week	0.535	0.004*
12th week	Baseline	-1.565	0.000*
	4th week	-1.12	0.000*
	8th week	-0.535	0.004*

Note Multiple comparison determines by the paired t test method

*The mean difference is significant at the 0.05 level.

Based on the data from above Table 4.3, modified MASI score on 12th week is significantly higher than baseline, week 4, week 8 with p value $p < 0.05$. Moreover, differences between each week is also statistically significant with p value of <0.05.

4.4 Melanin Index Score

Table 4.4 Statistical analysis of Melanin Index that applied 7% *Kaempferia parviflora* cream at baseline, 4th, 8th and 12th week (n=20)

Melanin Index	Median	IQR
Baseline	215.5	203.25-246.415
4th week	217.33	204-245.7475
8th week	207.3300	198.75-229.4975
12th week	205.5000	196.5825-224.83

Note Friedman test, Chi-Square=41.352 (d.f=3),p<0.001

According to Table 4.4, median of Melanin Index at baseline was 215.5 with Interquartile range (IQR) 203.25 – 246.3 and at 12 week median of Melanin Index was 205.5 with IQR 196.5 – 224.8 at the level of 0.05. The results showed there is improvement of melanin index from baseline to week 12 and it is statistically significant.

Table 4.5 Multiple comparison of Melanin Index that applied 7% *Kaempferia parviflora* cream at baseline, 4th, 8th and 12th week

Duration	Pair comparison	Mean difference	Z (Wilcoxon Signed Ranks Test)	p-value
Baseline	4th week	-0.866	-.374	0.709
	8th week	8.034	-3.846	0.000*
	12th week	12.517	-3.79	0.000*
4th week	Baseline	0.866	-.374	0.709
	8th week	8.9	-3.79	0.000*
	12th week	13.383	-3.823	0.000*
8th week	Baseline	-8.034	-3.846	0.000*
	4th week	-8.9	-3.79	0.000*
	12th week	4.483	-2.091	0.037
12th week	Baseline	-12.517	-3.79	0.000*
	4th week	-13.383	-3.823b	0.000*
	8th week	-4.483	-2.091b	0.037

Note Multiple comparison determines by the Wilcoxon Signed Ranks Test

*The mean difference is significant at the 0.05 level

Based on the data from above Table 4.5, mean difference on melanin index on 12th week and baseline is 12.5 which is significantly higher from the baseline.The mean difference on melanin index on 8th week and baseline showed 8.03 with p value $p < 0.05$. The results showed clinically difference and statistically significant. However mean difference on week 4 and baseline was -0.86 and p value 0.7 so statistically insignificant.

4.5 Dermatologist Evaluation Score

Table 4.6 Statistically Analysis of dermatologist evaluation score compared on 4th, 8th and 12th week

dermatologist evaluation score			
Improvement	**Week4**	**Week8**	**Week12**
-1 = worse	-	-	-
0 = no change	8(40%)	7(35%)	-
1 = 1-25% fairness improvement	9(45%)	8(40%)	6(30%)
2 = >25-50% moderate improvement	3(15%)	5(25%)	13(65%)
3 = >50-75% good improvement	-	-	1(5%)
4 => 75-100% excellent improvement	-	-	-

Note Friedman test -Chi-square =19.26,p=0.0001

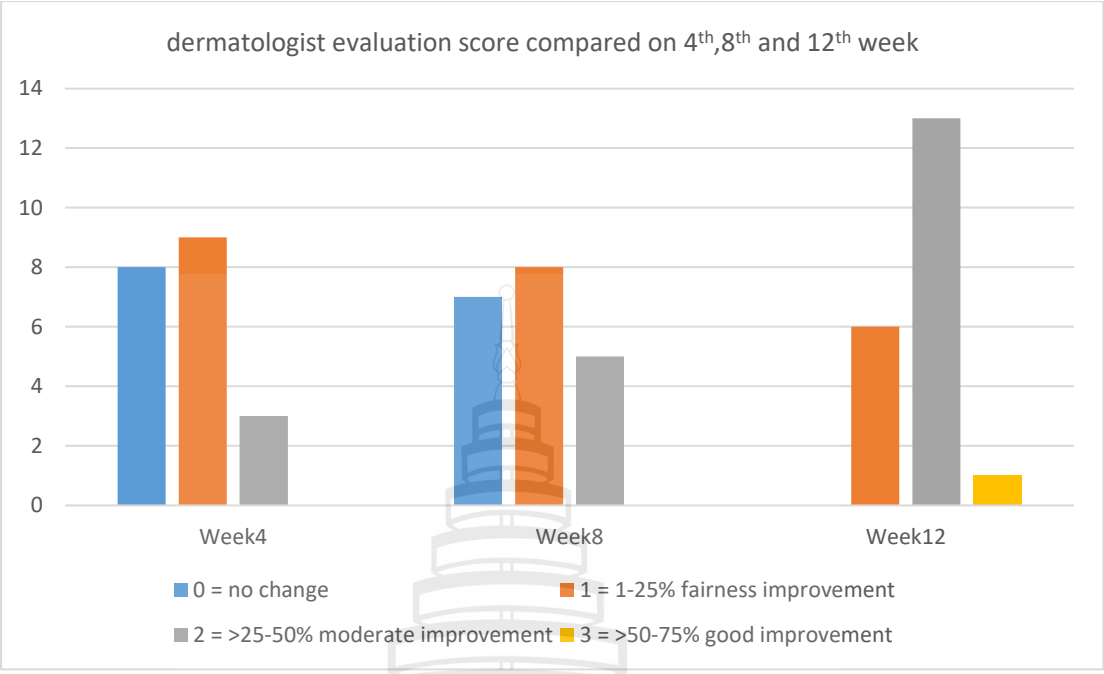


Figure 4.2 Presents the statistical analysis of evaluations by three dermatologists at the 4th, 8th, and 12th weeks for 7% *Kaempferia parviflora* cream

Table 4.6 and Figure 4.2 showed for the *Kaempferia parviflora* cream on week 4, 15% of the volunteers showed no changes according to dermatologists, 45% of subjects showed fair improvement and 15% showed moderate improvement. On Week 8, the result showed that 25% of subjects showed moderately improved, which can be compared to see that on week 12, 65% of subjects showed moderate improvement when evaluated by dermatologists. There were 35% of subjects who did not show any change until week 8 which is improved in week 12 where there is no subject evaluated as no change. On the end of the study week 12, 1 volunteer was scored as good improvement by dermatologists. Throughout the study period, no subject is tested as worsen and also no subject is tested as excellent improvement.

4.6 Patient Satisfaction Score

Table 4.7 Patient satisfaction score on 12th week

Patient Satisfaction score	Week12
-1=worse	-
0=No satisfaction, no change	-
1 = Little satisfaction	-
2= Moderate satisfaction	11(55%)
3= Very satisfied	9(45%)
4= Extremely satisfied	-

A statistical analysis for patient satisfaction for 7% *Kaempferia parviflora* extract cream revealed 45% reported "very satisfied", whereas 55% of patients experienced "moderate" satisfaction at Week 12.

4.7 Side Effects

Table 4.8 Frequencies of side effects and complication

	Kaempferia parviflora		
	4th week	**8th week**	**12th week**
Itching (scale 0 to 10)	0	0	0
Duration of erythema	-	-	-
Allergic contact dermatitis	-	-	-
Post-inflammatory hyperpigmentation	-	-	-
Post-inflammatory hypopigmentation	-	-	-

The assessment of adverse effects for Kaempferia parviflora revealed no occurrences of itching at any of the measured time points (4th week, 8th week, and 12th week). Additionally, there were no reports of duration of erythema, allergic contact dermatitis, post-inflammatory hyperpigmentation, or hypopigmentation throughout the study period.

CHAPTER 5

DISCUSSION, CONCLUSION AND SUGGESTION

5.1 Discussion

Melasma is primarily recognized as one of the most prevalent types of facial hyperpigmentation which significantly impacts life quality due to its aesthetic implication. The therapeutic goal is to reduce melanin synthesis, stop melanosome production, and increase the breakdown (Cestari et al., 2009).

There is an increasing trend towards utilizing plant-derived compounds in skincare, appreciated for their natural ability to lighten skin, and are noted for their safety and cost-effectiveness. In this study, we used *Kaempferia parviflora* cream, which is significant for its strong anti-tyrosinase activity. Furthermore, the extract has been found to possess anti-inflammatory, antioxidant, and UV protection, along with skin hydration. It also offers anti-aging, anti-photoaging, anti-acne, and anti-allergic activities

In this study, the researcher studied the efficacy of 7% *Kaempferia parviflora* cream for treating melasma in an experimental before and after comparison study design. There was a total of 20 participants with 5 males and 15 females. The demographic data revealed a diverse group with an average age of 45.1±8.1 years. None of the participants reported underlying medical conditions or photosensitivity, except one individual with a food allergy, and none had undergone any treatments in the four weeks preceding the study. All participants have exposure to sunlight which has significant aggravating factors for melasma. In terms of skin type, 14 participants had Fitzpatrick skin type III (70%), 4 had Fitzpatrick skin type IV (20%), and 2 had Fitzpatrick skin type V (10%).

The data were collected by VISIA scan, modified MASI score, and Mexameter to assess skin hyperpigmentation and melanin levels for participants treated with 7% Kaempferia parviflora. The results showed a statistically significant reduction in skin hyperpigmentation in melasma and also showed great satisfactory scores in doctors'

evaluation and patient's scores. The results from research showed that *Kaempferia parviflora* cream showed great efficacy in the treatment of melasma. The study results can be summarized as follows: the topical application of 7% *Kaempferia parviflora* cream did not result in any allergic reactions during the patch test, and no allergic cases were reported throughout the study. Based on this result, 7% *Kaempferia parviflora* cream might be safe for use for melasma treatment.

The mean of modified MASI score in 7% *Kaempferia parviflora* extract cream before the treatment was 9.405±1.19 at the baseline. The results after applying extract from were at 4th week 8.96±1.11, 8th week 8.37±1.21, and 12th week 7.84±0.95. The mean of MASI score in 7% *Kaempferia parviflora* extract cream decreased statistically significant at the level of 0.05 ($p < 0.001$). The results showed clinical difference and statistically significant. From pair t test, the result showed that modified MASI score on 12th week is significantly higher than baseline, week 4, week 8 with p value $p < 0.05$. Moreover, differences between each week is also statistically significant with p value of <0.05. This result suggested that the extract cream led to a great improvement in terms of modified MASI score for melasma treatment by 12th week when compared to modified MASI score from baseline and each week also showed significant improvement.

The present study's findings on 7% *Kaempferia parviflora* cream align closely with the results reported by a 5% *Kaempferia parviflora* extract in melasma patients over 12 weeks (Arjinajarn et al., 2016). Both studies demonstrated a progressive and statistically significant reduction in Modified MASI scores, with the current study showing a ~16.6% improvement compared to Arjinajarn et al.'s ~18% reduction. This suggests that increasing the concentration from 5% to 7% does not yield a substantially greater depigmenting effect, possibly indicating a plateau in efficacy beyond 5% KP. Both studies support *Kaempferia parviflora*'s role as an effective melanogenesis inhibitor, likely due to its anti-tyrosinase, antioxidant, and anti-inflammatory properties. The consistent findings across both studies reinforce the potential of *Kaempferia parviflora* as a safe and effective alternative to conventional melasma treatments, though further research is needed to determine the optimal concentration and long-term effects.

According to Friedman test for Melanin index, median of Melanin Index at baseline was 215.5 with Interquartile range (IQR) of 203.25 – 246.3, and in the 12th-week median of Melanin Index was 205.5 with IQR of 196.5 – 224.8 at the level of 0.05. The results showed there is improvement of melanin index from baseline to week 12, and it is statistically significant. Based on the data from Wilcoxon Signed Ranks Test, mean difference on melanin index on 12th week and baseline is 12.5 which is significantly higher from the baseline. The mean difference on melanin index on 8th week and baseline showed 8.03 with p value $p < 0.05$. The results showed clinically difference and statistically significant. From the data, we can conclude that Mean Melanin Index score decreased significantly by 12th week in comparison to baseline.

During the 12th-week follow-up visit, three dermatologists assessed the volunteers and rated 30% fair improvement, 65% moderate improvement and 5% good improvement. From the patients satisfactory score by week 12, it revealed 45% reported “very satisfied”, whereas 55%of patients experienced “moderate” satisfaction.

Based on this study and its results, 7% *Kaempferia parviflora* cream is a safe and effective topical treatment for melasma treatment. All participants tolerated the treatment well, with no reported side effects such as skin irritation, erythema, or hyperpigmentation throughout the study period. From the data, it can be concluded that 7% *Kaempferia parviflora* improved modified MASI score and reduces the melanin index, likely due to the antioxidant and skin lightening properties of the compounds present in the cream.

5.2 Suggestion for Future Research and Clinical Applications

5.2.1 Future studies can investigate the long-term effects of *Kaempferia parviflora* cream beyond 12 weeks.

5.2.2 Comparing *Kaempferia parviflora* cream with other anti-aging ingredients could clarify its relative effectiveness.

5.2.3 The potential of combining this cream with other treatments like injections and lasers should be explored more.

5.3 Conclusion

Based on the clinical study, it can be concluded that *Kaempferia parviflora* cream significantly reduce skin hyperpigmentation with great satisfaction. In summary, *Kaempferia parviflora* cream appears to be a safe and effective topical treatment for the treatment of melasma.

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APPENDIX A

CLINICAL EVALUATION FORM

Version....6.... Date...02/12/2024

CLINICAL EVALUATION

Table E1 Mean MASI scores of face applied with 7% *Kaempferia parviflora* cream on each visit

Number of subjects	Mean MASI Scores (N=20)			
	7% Kaempferia parviflora extract cream			
	Baseline	4th week	8th week	12th week
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				

Version....5.... Date...02/11/2024

Table E2 Mean melanin index scores of face applied with 7% *Kaempferia parviflora* cream on each visit

Number of subjects	Mean Melanin index (N=20)			
	7% *Kaempferia parviflora* cream			
	Baseline	4th week	8th week	12th week
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				

Version....5.... Date...02/11/2024

Table E3 Dermatologists evaluation scores for 7% *Kaempferia parviflora* cream on 4th, 8th and 12th week

No.	Dermatologists evaluation scores for 7% *Kaempferia parviflora* extract cream								
	0-4 week			0-8 week			0-12 week		
	Doc 1	Doc 2	Doc 3	Doc 1	Doc 2	Doc 3	Doc 1	Doc 2	Doc3
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									

Table E4 Patient satisfaction scores on 12th week compared on both sides applied with 7% *Kaempferia parviflora* cream and Standard cream

Number of patients (n)	Patient Satisfaction Scores
	7% *Kaempferia parviflora* cream
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	
20	
21	
22	

APPENDIX B

RESEARCH PROFILE

Version….6…… Date….02/12/2024

RESEARCH PROFILE (CONFIDENTIAL)

THE EFFICACY OF 7% *KAEMPFERIA PARVIFLORA* CREAM FOR THE TREATMENT OF MELASMA

STUDY ID:

_____ **Patient Record Form**

General Information

1. Date_ _/_ _/_ _
2. Participant No. ……
3. Gender a. Male b. Female
4. Pregnancy or lactation 1. Yes 2. No
5. Occupation
    1) Government officer
    2) business owner
    3) Housewife
    4) Student
    5) Employee
    6) Others
    7) Specify _
6. Underlying disease _
7. Photosensitivity or Drug Induced Hypersensitivity 1. Yes 2. No
8. Personal medication and supplement

Version…5…… Date….12/11/2024

1) Chemotherapy

2) Active inflammatory skin disease, open wound in the treatment area

3) History of malignant or premalignant lesions in the treatment area

13. History of food or drug allergy 1. Yes 2. No

If Yes, specify _

14. Current facial product allergy 1. Yes 2. No

If Yes, specify _

15. History of following treatment before this study? 1. Yes 2. No

If yes,

1. mesotherapy
2. laser
3. peeling
4. dermabrasion
5. derma roller

Average time exposure to the sunlight during 10 am to 4 pm

1. Yes 2. No

If Yes, Duration _ _ _ _ _ _ _ _ minutes

16. Fitzpatrick skin photo types (please circle) I II III IV V VI

APPENDIX C

DOCTOR RECORD FORM

Version....6.... Date...02.12.2024

Doctor Record Form

MASI score = ...

AREA

Area	**Forehead** **30%**	**malar** **60%**	**chin** **10%**
None=0			
0%-9%=1			
10%-29%=2			
30%-49%=3			
50%-69%=4			
70%-89%=5			
90%-100%=6			

MASI score = ...

DARKNESS

Darkness	**Forehead** **30%**	**malar** **60%**	**chin** **10%**
Absent=0			
Slight =1			
Mild =2			
Marked=3			
Maximum=4			

Melanin Index (MI) by Mexameter MX 18

Version....6.... Date...02.12.2024

Melanin Index

Baseline	**4th week**	**8th week**	**12th week**

CURRICULUM VITAE

NAME Yee Yee Mon

EDUCATIONAL BACKGROUND

2019 Bachelor of Medicine and Bachelor of Surgery,
University of Medicine, Mandalay, Myanmar.

WORK EXPERIENCE

2018-2019 Internship
Mandalay General Hospital,
Central Woman Hospital,
Children Hospital, Mandalay, Myanmar