# THE EFFICACY OF 10% ACACIA CONCINNA FRUIT EXTRACT FOR TREATMENT OF MELASMA

THAWEEPORN TREEPRAPHAKORN

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**THAWEEPORN TREEPRAPHAKORN**

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**Author:** Thaweeporn Treepraphakorn

**Examination Committee:**

| | |
|---|---|
| Assistant Professor Anon Paichitrojjana, M. D. | Chairperson |
| Tanomkit Pawcsuntorn, M. D. | Member |
| Associate Professor Wongdyan Pandii, Ph. D. | Member |

**Advisors:**

.....Advisor

(Tanomkit Pawcsuntorn, M. D.)

**Dean:**

.....

(Karnt Wongsuphasawat, Ph. D.)

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## ABSTRACT

Background: Melasma is a common hyperpigmentation skin disorders with prominent facial aesthetic disfigurement that can cause significant psychosocial distress and quality of life impairments. Sompoi (Acacia concinna) extract is known to have anti-tyrosinase effect and can inhibit melanin synthesis and thus providing a significant contribution in controlling the melasma. Clinically, the disease presents as a brown or grayish-brownish hyperpigmented patch and is more common among women, particularly those who are living in regions with very high levels of ultraviolet light. Although the exact reason for melasma is still a mystery, other things have been proposed as potential causes.

Objective: To study the efficacy of 10% Acacia concinna fruit extract for treatment of melasma.

Methods: 30 female volunteers with melasma, aged between 20 and 45 years old, who willingly wanted to get rid of their melasma were collected to participate in a split-face, double-blinded placebo-controlled trial for 8th weeks. 10% Acacia concinna extract and placebo were sprayed twice daily on designated sides of the face for 8 weeks. Various parameters such as melasma evaluation by dermatologists, MASI score and melanin index were assessed at baseline, 2nd, 4th, 6th and 8th weeks respectively.

Result: Statistically significant results of reduction in MASI score (p-value <0.001) and reduction in mean melanin index (p-value<0.001) were shown for Acacia concinna extract-treated side. No side effects were observed throughout the study.

Conclusion: This study statistically demonstrated that Acacia concinna extract reduced melanin production more than placebo when it is applied topically with no

harmful side effects. So, 10% Acacia concinna fruit extract could be safe, effective and can be used as an alternative for the treatment of melasma.

**Keywords:** Sompoi, Acacia Concinna, Melasma, MASI Score, Melanin Index

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# CHAPTER 1

# INTRODUCTION

## 1.1 Background

On sun-exposed skin, especially the forehead, cheeks, upper lip, and chin, symmetrical, localized, irregular, light-to-dark brown maculae are the hallmark of melasma, an acquired prominent hyper-melanosis (Werlinger et al., 2007). Melasma is the most frequent type of facial hyperpigmentation that causes cosmetic deformity. It has a psychological influence that leads to significant quality of life concerns (Tzouveka, 2014).

In the term “melasma”, the “melas” which meaning “black color” (Bandyopadhyay, 2009). “Chloasma,” refers to “pregnancy mask.” (Handel et al., 2014). The condition affects 50-70% of pregnant women and 38% of women taking oral contraception. Melasma can be caused by a variety of circumstances, the precise origin of which is uncertain (Tzouveka, 2014). Melasma treatment is quite difficult. Fear of post-inflammatory hyperpigmentation (PIH) following inflammation-inducing therapy is another issue (Abdallah, 2014). Melasma treatment can be complicated due to the depth of the pigment, exacerbating factors, likelihood of recurrence, and hormones (Gupta et al, 2006).

The specific cause has yet to be determined. Pregnancy, physiological factors like thyroid and female sex hormones, exposure to UV light, hereditary predispositions, and medications like phenytoin are all risk factors. It can also be diagnosed clinically by using a wood light to assess the depth of the melanin pigment (Sarkar et al, 2016). This disease's management is complicated because to an insufficient understanding of its pathophysiology, chronicity, and recurrence rates (Shweta et al, 2014).

The fundamental goal of the treatments is to either eradicate pigment that is already present in the epidermis and dermis or halt melanogenesis and melanin transfer from active melanocytes (Abdallah, 2014). Topical medicines that prevent melanogenesis, broad-spectrum sunscreen, and camouflage are among the first-line

treatments (Sheth & Pandya, 2011). Avoid the sun and use protective eyewear, helmets, caps, and clothing for those who must wear sunscreen every day (Cestari et al, 2009).

The gold standard of treatment for melasma is hydroquinone (HQ). HQ inhibits the tyrosinase enzyme, as well as RNA and deoxyribonucleic acid synthesis, resulting in melanocyte death. The second-line option is chemical peeling. Laser and light therapy are becoming more popular as treatment options to people who have failed previous treatments. Due to its unpredictability and pigmentation effects, laser therapy has a limited function in the therapeutic management of melasma (Shankar et al., 2014).

Herbal extracts are becoming increasingly popular in the beauty sector due to their efficacy and safety. Herbal extracts have been utilized in anti-aging cosmetic products since history. Carotenoids, flavonoids, and phenolics are among the phytochemical elements in their herbal extracts that have antioxidant and anti-tyrosinase effects (Cadiz-Gurrea et al., 2017).

Acacia concinna, also known as "Sompoi" or "Shikakai," is a member of the Fabaceae family and is found throughout Southern Asia. Acacia concinna fruit is a medicinal and vegetable plant in Thailand. Acacia concinna fruit has a lot of potent chemical ingredients; most of its parts, especially pods, barks, and leaves, contain numerous saponins. (Aung et al, 2020) Because of its anti-dermatophyte and antibacterial properties, it has been utilized in anti-dandruff shampoo. The antioxidant and anti-tyrosinase properties of the pod extracts have also been studied (Poomanee et al, 2015).

Strong anti-tyrosinase, anti-inflammatory, antioxidant, anti-cancer, anti-dermatophyte, antibacterial, and fungicidal properties are all present in Acacia concinna extract (Poomanee et al, 2015). Thus, to prevent the formation of melanin, we intend to investigate the anti-tyrosinase action. However, there are currently no reports of the extract in melasma studies. The key objective is to investigate the effectiveness of topical 10% Acacia concinna fruit extract in treating melasma.

## 1.2 Research Question

Does topical 10% Acacia concinna fruit extract have efficacy in melasma treatment?

## 1.3 Objectives

### 1.3.1 General Objective

To study the efficacy of 10% Acacia concinna fruit extract for treatment of melasma.

### 1.3.2 Specific Objectives

1.3.2.1 Primary Outcome

To explore the efficacy of 10% Acacia concinna fruit extract for treatment of melasma.

1.3.2.2 Secondary Outcomes

1. To observe the side effects of 10% Acacia concinna fruit extract in treating melasma.
2. To assess the participants' satisfaction between 10% Acacia concinna fruit extract and placebo.

## 1.4 Hypothesis

1.4.1 Topical 10% Acacia concinna fruit extract has a good efficacy for treatment of melasma. (reducing melanin and the face looks brighter)

1.4.2 Topical 10% Acacia concinna fruit extract is harmless to use and has minimal negative consequences.

## 1.5 Conceptual Framework

Many cosmetic and therapeutic sectors have prioritized the development of an aesthetically attractive skin pigmentary look. Several therapy techniques are being studied for their usefulness in lightening the skin and treating hyperpigmented lesions by lowering melanin concentration. Tyrosinase is the primary enzyme in melanogenesis. Because 10% Acacia concinna fruit extract possesses anti-tyrosinase activity, this fruit extract can inhibit melanin formation, making skin lighter, brighter, and relieving melasma.

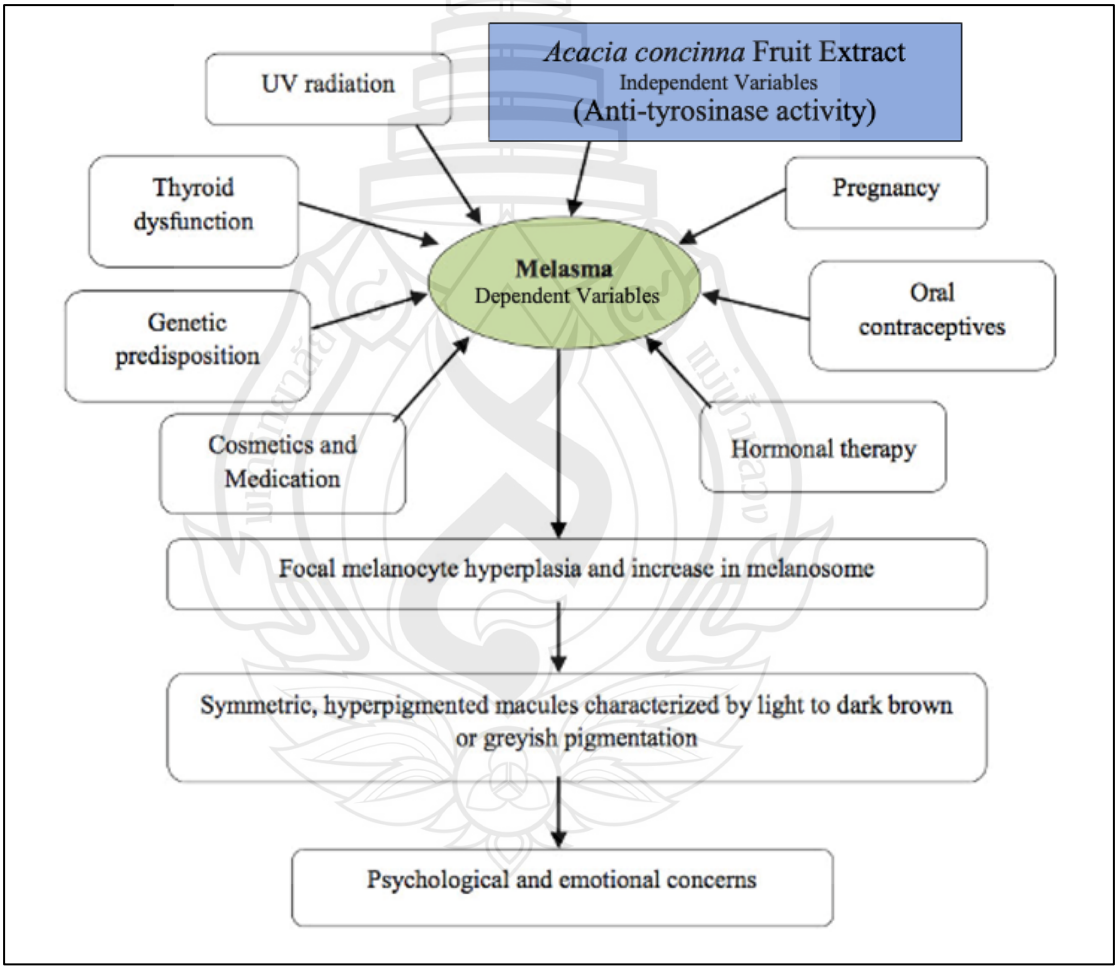


**Figure 1.1** Conceptual Framework

## 1.6 The Scope of Research

30 female volunteers with melasma, ages 20 to 45 (Fitzpatrick Skin Type III–VI), were inspected by a dermatologist; those who satisfied all inclusion requirements were enrolled. In a split face design, 10% Acacia concinna fruit extract and placebo were used in comparison. The color, consistency, and smell of 10% Acacia concinna extract & Placebo are comparable. For 8 weeks, volunteers sprayed and administered 10% Acacia concinna fruit extract & Placebo twice a day. The MASI score and the mexameter® MX18 measurement were used to measure melasma improvement at baseline, two, four, six, and eight weeks. Throughout the course of the study, three dermatologists monitored and assessed the volunteers' untoward effects and satisfactory results.

## 1.7 Definition

### 1.7.1 Melasma

Melasma is characterized by tanned or dark skin discoloration in the form of macules to patches. It is most common in premenopausal women.

### 1.7.2 Tyrosinase

Tyrosinase is the rate limiting enzyme in melanin synthesis.

### 1.7.3 Efficacy

The ability to achieve a desired or intended outcome is known as efficacy. (reducing melanin and the face looks brighter).

### 1.7.4 Placebo

Placebo is deionized water with no effect for reducing melanin and no effect for whitening.

### 1.7.5 Satisfaction

Satisfaction derived from the accomplishment of one's needs, wants, or aspirations.

### 1.7.6 Side Effect

Side effect means undesirable effect of a drug or medical treatment such as redness, rash, itching, swelling, blister, etc.

### 1.7.7 Mean Melanin Index

Mexameter parameters include the mean melanin index. It is very precise and provides a quantitative value on a board score scale of one to one thousand (One refers to white and one thousand refers to dark) for melanin to detect even the tiniest color shifts. Accuracy is ± 4 %.

### 1.7.8 Mexameter MX18

It is a piece of electronic equipment. It measures the reflectance of two skin components, melanocytes, and erythrocytes. The primary principle is absorption. Continuous measurements over a longer time period are optional. The highly sensitive measurement provides melanin and erythema readings on a broad scale (0-999), allowing even minor color changes to be detected. The precision may be easily verified at any moment. The apparatus is well-known throughout the world and is utilized in several scientific experiments. The Mexameter® MX 18 is offered as a standalone device, a device that can be connected to MPA systems, or as a wireless probe.

### 1.7.9 Modified MASI Score

Kimbrough-Green et al. submitted the "Modified MASI" in 1994. It can clinically assess the extent of melasma. It is the most popular tool to determine the extent of melasma. (Handel et al., 2014).

The mMASI system consists of two elements: the area (A) of active lesion and darkness (D). For the area (A) of active lesion, the investigator have to evaluate those affected with regard to normal skin and assign a numerical value depending on banding. A value of zero represents no involvement One represents under ten percent involved; Two represents 10-29% involved; Three indicates 30-49% involved; Four represents 50-69% impacted Five indicates 70-89% involved; and six represents a minimum of 90 percent involved.

Melasma’s darkness (D) is compared to normal skin and scored on a scale of from zero to four with the following values: Zero denotes normal skin tone; One

denotes hardly apparent hyperpigmentation; Two denotes mild hyperpigmentation; Three denotes moderate hyperpigmentation; and four represents severe pigmentation.

Modified MASI score = 0.3A (f) D (f) + 0.3A (lm) D (lm) + 0.3A (rm) D (rm) + 0.1A (c) D (c)

Given that we are conducting a half-face experiment, the formula would once more be altered to appear as what follows (Pandya et al. 2011).

2 x [(0.15) (A) (D) of forehead of one side + (0.3) (A) (D) of malar of one side + (0.05) (A) (D) of chin]

Accordingly, the Modified MASI score was calculated for each side of the participant's faces at baseline, the second, fourth, sixth, and eighth weeks. The MASI score is computed by summing the severity evaluations for darkening and uniformity for each of the three facial areas and multiplying by the area of involvement value (Pandya et al., 2011).

# CHAPTER 2

# REVIEW OF RELATED LITERATURE

## 2.1 Melasma

Melasma is derived from the word “melas,” which means “dark color” (Bandyopadhyay, 2009), and is also known as “chloasma,” which means “pregnancy mask.” (Handel et al., 2014)

Melasma is a type of hyperpigmentation that most typically affects the face. It is substantially more common in reproductive-age women. Genetic factors, ultraviolet (UV) radiation exposure, and hormonal impacts are the primary causal causes (Lee, 2015). It is distinguished by irregular light to dark brown spots on the face's forehead, cheeks, upper lip, and chin (Werlinger et al., 2007). Melasma can affect people of all races and ethnicities, however most patients are women and people with Fitzpatrick skin types IV through VI, which include Hispanic, Asian, and African American people (Rodrigues & Pandya, 2015). Melasma can also be found in women who use estrogen-progesterone oral contraceptives or hormone replacement treatment, as well as in men who use estrogen derivatives. Melasma, found as symmetrically, irregularly, hyperpigmentation especially on malar region (Mashiko et al., 2017).

Melasma is a common facial disease that causes pigmentation on the face. It influences mental health. It can also influence face morphology, which is vital for confidence and dealing with others. Female patients outnumber male patients (Jiang et al., 2018).

### 2.1.1 History

Disease descriptions can be found in medical writings dating back to Hippocrates' (470-360 BC) findings. Melasma typically worsens with UV exposure, hotness, coolness, and skin redness (Handel et al., 2014).

First, there was a 20-year-old woman with a hyperpigmented lesion on her upper lip. Because of the UV exposure, the nature of the lesion deteriorated. Another study

found that 10 of the fourteen women with melasma on their faces were pregnant, while the others had “melanosis of pregnancy” (Handel et al., 2014).

### 2.1.2 Epidemiology

The most important link is that between melasma and sex. Women have a 7th to 9th fold higher prevalence than men. And the age of onset is substantially related to the occurrence. It is most common between the ages of 30 and 65. Melasma development and family history have a substantial relative link (Passeron, 2013). Melasma is more common in those with darker complexion (Fitzpatrick skin types IV-VI), such as those of African, Asian, Latino, and Middle Eastern origin (Ortonne et al., 2009).

Melasma cannot be precisely diagnosed. Genetic predispositions, UV exposure, hormone imbalances, pregnancy, and medicines such as phenytoin can all contribute to it (Handel et al., 2014). Furthermore, ethnicity is one of the risk factors for melasma (Ortonne et al., 2009)

Melasma and sex is the most common link, with women being 7 to 9 times more likely than males. Pregnancy is very strongly linked to melasma (50-70%). Hormonal factors such as estrogen and progesterone are among the risk factors, particularly in women who use birth control tablets. Another important link between melasma and age is that it develops between the ages of 30 and 65 (Passeron, 2013).

### 2.1.3 Classification and Clinical Presentation

There are three major types such as centrofacial, malar and mandibular types. Among them, centrofacial type is the most common and mandibular type is the least common. Melasma is characterized based on the site of the lesion and the depth of involvement. Melasma can be classified into three clinical patterns based on the distribution of lesions: centro-facial (65%), malar (20%), and mandibular (15%). The centro-facial pattern affects the forehead, nose, cheeks, upper lip, and chin; the malar pattern affects the cheeks and nose; and the mandibular pattern affects the ramus of the jaw (Lynde et al., 2006). The melasma can also found rarely on upper part of the body and upper extremities (Handel et al., 2014).

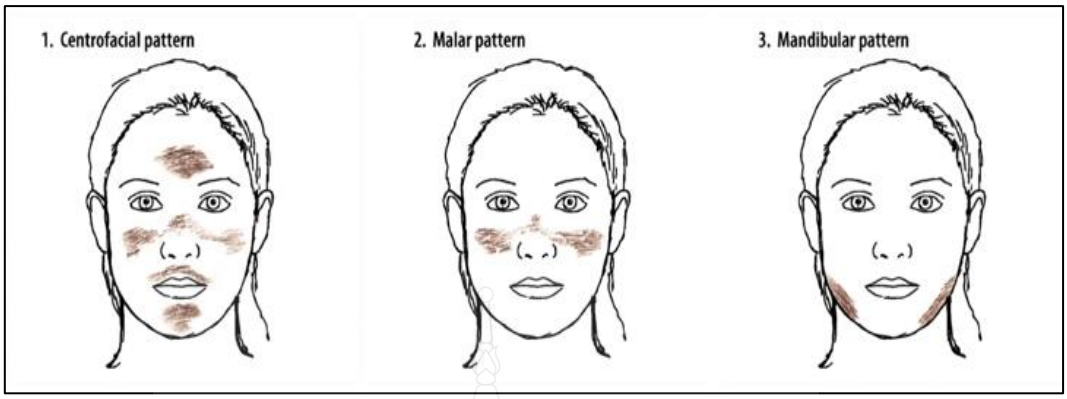


**Figure 2.1** Clinical facial features affected by melasma

### 2.1.4 Etiology and Risk Factors of Melasma

Melasma etiopathogenesis is complicated and unknown. Traditional affecting factors include genetic and hormonal factors, as well as UV radiation exposure. The UV component of sunlight is the primary activating and irritating factor, generating hyperplasia of localized melanocytes and an increase in melanosomes (Shankar et al., 2014). Other causes include cosmetic components, anti-seizure medications, phototoxic pharmaceuticals, and nutritional inadequacy, as well as endocrine problems such as ovarian or thyroid malfunction, hepatic dysfunction, and nutritional deficit. Idiopathic melasma accounts for up to one-third of cases in women and most cases in men (Handel et al., 2012).

Women account for 90% of instances (Demirkan et al., 2017). Melasma biology is poorly known. The main factors that might greatly boost tyrosinase activity include genetic predisposition, UV radiation, and endocrine hormones (Bagherani, 2015). UV exposure is the most important factor, owing to the increased quantity of melanosomes (Shankar et al., 2014).

Melasma patients exhibit higher levels of oxidative stress than the general population (Seckin et al., 2014). Other factors include some cosmetics, medicines, and ovarian and thyroid disorders (Demirkan et al., 2017).

### 2.1.5 Pathophysiology

The pathogenesis of melasma is complex (Demirkan et al., 2017). The factors such as sun exposure and estrogen effect on the melasma have been found in pathogenesis (Kim et al., 2012). Inflammatory processes have also been identified (Handel et al., 2014). Each etiologic component causes a distinct pigmentation illness, such as photoaging, post-inflammatory hyperpigmentation (PIH), or drug-induced pigmentation (oral contraceptives). PIH is one of the most common causes of melasma and lentigines-related hyperpigmentation (Nicolaidou et al., 2007).

UV irradiation, as previously stated, has a significant influence in the development of melasma (Tamega et al., 2013). Repeated suberythemal UV light exposure induces melanogenesis. Melasma is distinguished by excessive melanin deposition in the epidermis and dermis, which is indicative of particular hyperfunctional melanocytes (Grimes et al., 2005). UV irradiation stimulates melanogenesis by acting directly on melanocytes and indirectly on keratinocytes that release melanogenic substances (Gilchrest, 1996). UV photons have direct effects on DNA and melanocyte membranes, which cause melanogenesis (Eller et al.,1996).

UV irradiation causes melanocyte membranes to emit diacyl glycerol (DAG) and arachidonic acid (Carsberg et al., 1995). DAG is an endogenous component that activates protein kinase C (PKC), which is a key signal transduction route in melanogenesis (Park et al., 1993) (2). In reaction to UV exposure, keratinocytes generate NO, which has a melanogenic impact. Furthermore, cutaneous fibroblasts treated directly to UVA or UVB produce SCF, implying that cell-cell interactions between melanocytes and fibroblasts play a role in UV-induced melanogenesis (Shin et al., 2012).

The estrogen and progesterone receptors are also identified on melasma lesions. This fact has been updated for melasma pathogenesis (Pelletier & Ren, 2004). Melasma was reported as a side effect of birth control tablets containing levonorgestrel (Chompootaweep et al., 1996). Reports have suggested that progesterone in birth control pills has a preventive impact on melasma by reducing melanocyte formation without affecting tyrosinase enzymes and counteracting the effect of estrogen (Wiedemann et al., 2009).

The potential function of altered dermal vasculature in melasma patients and UV-irradiated persons has also been examined (Yano et al., 2005). VEGF has also been reported to cause an increase in the number and size of blood vessels, however the effect of VEGF in melasma has not been thoroughly described (Chen et al., 2014). Tranexamic acid can diminish pigmentation and vessel counts in melasma, which can modify blood vessel perspective (Na et al., 2013). Melasma has been shown to express stem cell factors (Kang et al., 2006). Melasma is caused by the traces of photodamaged skin, such as solar elastosis, vascular and barrier dysfunction (Passeron and Picardo, 2018).

### 2.1.6 Melanogenesis

Melanogenesis is the process through which melanin is produced primarily by melanocytes (Chang, 2012). Melanin is essential for preserving human skin from UV exposure and determining skin, hair, and eye color (Videira et al., 2013). The end products are classified as Eumelanin and pheomelanin (Chen et al., 2009).

The following is the pathway of the melanogenesis.

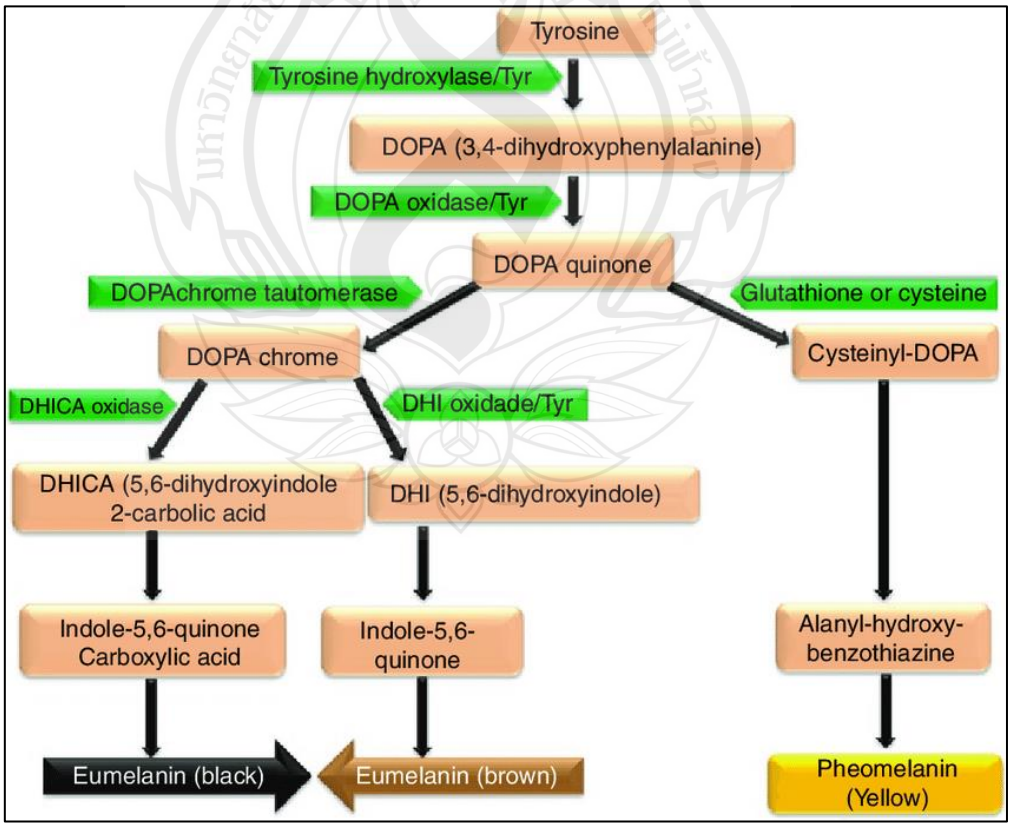


**Figure 2.2** Pathway of Melanogenesis

Eumelanin: Insoluble black-to-dark-brown pigment that exists in human black hair and the retina.

Pheomelanin: Alkali-soluble yellow-to-reddish-brown pigment that exists in red feathers and hair (Videira et al., 2013).

Both eumelanin and pheomelanin are formed from the same common precursor, dopaquinone, which is synthesized from tyrosine by the enzyme tyrosinase (Ito and Wakamatsu, 2008). Eumelanogenesis happens in three stages.

Copper is present in human melanosomes in quite high concentrations (Biesemeier et al., 2011). The conversion of DC is enhanced in the second step by various metal ions, particularly Cu2+ (Palumbo et al., 1987) or dopachrome tautomerase (Jackson et al.,1992).

The acidic pH environment of melanosomes is favorable for pheomelanogenesis over eumelanogenesis, resulting in a melanogenesis switch (Ito & Wakamatsu, 2008).

## 2.2 Treatment of Melasma

Melasma is difficult to cure due of its recurrence. The goal of melasma treatment is to diminish hyperpigmentation without creating hypopigmentation or discomfort on the surrounding skin. If melanin is prevalent in the epidermis, treatment is significantly easier; if it is present in the dermis, treatment is much more difficult (Gupta et al., 2006).

Photoprotective agents, antioxidant therapies, skin lighteners, exfoliants, and resurfacing procedures, as needed, should be included in the treatment. According to evidence-based studies, the first-line treatments are intensive photoprotection and topical lightening medications (Sarma et al., 2017).

### 2.2.1 Topical Treatment

2.2.1.1 Hydroquinone

Hydroquinone is one of the most commonly utilized melasma topical treatments. The mechanism of action is that it interferes with melanin formation by melanocytes by blocking the conversion of DOPA to melanin, resulting in a reduction in melanin pigment. It can be used as a stand-alone treatment or in conjunction with

other topical treatments. 4% is appropriate for monotherapy and 2% can be used for combination therapy. The action will respond clinically after 4-6 weeks. Side effects such as redness, itching, dryness, and mild irritating contact dermatitis are infrequent but not unheard of. Exogenous ochronosis, a bluish-gray staining, can result with prolonged usage of these medications (Shankar et al., 2014). It can be given alone or with tretinoin. Kligman's formula is the combination of three things including the hydroquione, tretinoin and mometasone with the concentration of 2%, 0.025% and 1% respectively. (Nourmohammadi et al.,2019). Exogenous ochronosis is one of the common adverse effect by using HQ (Shankar et al., 2014).

2.2.1.2 Azelaic acid

Azelaic acid has the action of normalizing the keratinization and is a dicarboxylic acid naturally found in cereals and animal products (Gupta et al., 2006). Azelaic acid is a dicarboxylic acid that occurs naturally in whole grains and animal products. Azelaic acid inhibits DNA synthesis and works on mitochondrial enzymes, resulting in a direct cytotoxic effect on melanocytes. It can also inhibit the generation of free radicals (Halder & Richards, 2004). Azelaic acid can be taken for a long time because it rarely causes side effects. However, mild discomfort may occur in sensitive skin or with pre-existing skin diseases such as eczema (Shankar et al., 2014).

2.2.1.3 Tretinoin

Tretinoin, commonly known as all trans retinoic acid, is a derivative. Tretinoin is a type of vitamin A derivative. The method of action involves blocking tyrosinase in melanin synthesis pathways, interfering with pigment transfer, and increasing the rate of cell turnover. Tretinoin, unlike other products, is quite irritating. The adverse effects are inflammation, itchiness, desquamation, dry skin and photosensitivity and also it is listed in pregnancy category C (Shankar et al., 2014).

2.2.1.4 Niacinamide

It serves as a precursor to nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (Zhu & Gao, 2008). Topical niacinamide offers antiaging qualities in addition to depigmenting capabilities since it reduces collagen oxidation and improves age-induced sallowness (Bissett et al., 2004). Niacinamide can degrade tyrosinase and hinder melanin transport (Navarrete-Solis et

al., 2011). It is anti-inflammatory and promotes barrier function (Bandyopadhyay, 2009).

2.2.1.5 Corticosteroids

The corticosteroid is claimed to have an inhibitory effect on prostaglandin and leukotriene synthesis, which affects the melanin formation pathway. With powerful or ultra potent steroids, there is a good therapeutic response. Because of the risks, monotherapy is not suggested. Prolonged usage of a powerful steroid can result in hypopigmentation, though it is reversible. Corticosteroids have anti-inflammatory properties because inflammation affects melanogenesis. It has a good response to melasma, however using it alone is not advised. Furthermore, long-term steroid use can result in hypopigmentation (Bandyopadhyay, 2009).

2.2.1.6 Naturally occurring remedies for melasma treatment

Due to their biological and physiochemical advantages, they are now widely employed as cosmetic components. Furthermore, their stability is comparable to that of synthetic compounds (Han et al., 2003). Furthermore, it is generally regarded for having minimal long-term negative effects (Bandyopadhyay, 2009).

1. Vitamin C

Ascorbic acid can diminish oxidized melanin and limit melanin production. It is an important chemical for skin health and useful in healing photodamaged skin since it contains antioxidant qualities and promotes collagen formation. Micro poring or laser treatment can be used to improve absorption because these techniques remove the epidermis's outermost layers. Ascorbic acid has the melanin inhibitory effect, antioxidant, and collagen synthesis properties. It’s effective for the photo damaging skin (Aboul-Einien, et al, 2019).

2. Kojic acid

Kojic acid has tyrosinase inhibitor and antioxidant properties (Deo et al., 2013). It is often used to treat hyperpigmented disorders such melasma (Shankar et al., 2014). Corticosteroids, which can minimize skin irritation, are the optimum combination (Bandyopadhyay, 2009). It is commonly used in Asia and Japan at concentrations ranging from 1% to 4%. Kojic acid creams can be applied topically twice a day (Halder and Richards, 2004). It has anti-tyrosinase and antioxidant action

and good to use with steroid to minimize the inflammation and redness (Bandyopadhyay et al., 2009).

3. Soybean

Other qualities of soybean include collagen stimulation, antioxidant, anti-inflammatory, UVB photodamage prevention, and moisturizing actions. Sun-induced pigmentation can be prevented by soybean extract (Paine et al., 2001). It can stimulate the synthesis of collagen, UV protective action, anti-oxidant and anti-inflammatory actions. Moreover, it also has hydration effects. (Leyden et al., 2011).

4. Licorice

Licorice extract can reduce melanogenesis by inhibiting tyrosinase activity via its main active component, glabridin (Fu et al., 2005). Liquiritin and isoliquirtin are two more popular licorice active compounds that function as melanin dispersers or removers of epidermal melanin (Toosi et al., 2013).

5. Mequinol

Mequinol can be used instead of HQ because it has fewer characteristics that cause skin irritation (Davis & Callender, 2010). Mequinol is a tyrosinase inhibitor, according to (Keeling et al., 2008). Mequinol has been shown to be beneficial in treating dyspigmentation, especially in darker skin types, with minimal side effects (Draelos, 2006).

6. Arbutin

Arbutin works by inhibiting the melanin formation pathway by binding tyrosinase without affecting tyrosinase messenger RNA transcription (Ebanks et al., 2009). The more the concentration, the better the outcome. Arbutin has been utilized as a component in a hydrogel mask with lasers or in combination with Ellagic acid to treat melasma (Ertam et al., 2008). Arbutin stops the melanin synthesis and has been used with the laser therapy. (Han et al., 2011).

7. Glucosamine

Glucosamine is a type of amino-monosaccharide present in all human tissues. N-acetyl glucosamine has been shown to lighten hypermelanosis by reducing melanin in melanocytes by preventing tyrosinase glycosylation (Iraji et al., 2009). Glucosamine lightens hyperpigmentation by reducing melanin synthesis (Espinal-Perez

et al., 2004). The combination with niacinamide is superior to monotherapy (Kimball et al., 2010).

8. Aloesin

Aloesin is a chemical found in aloe extracts. Aloesin can decrease melanin synthesis from mushroom and mouse sources. Aloesin could be utilized to treat UV-induced hypermelanosis. Melasma may benefit as well, as sun exposure has been linked to the development of melasma. It's made from aloe extracts. Because aloesin reduces melanin formation, it could be utilized to treat UV-induced hypermelanosis (Choi et al., 2002).

9. Mulberry extract

Mulberry inhibits dopa oxidase activity of tyrosinase and exhibits superoxide scavenging properties (Halder & Richards, 2004).

10. Grape seed extract

Grape seed extract contains antioxidants and taking it orally for six months exhibited a lightening effect on melasma (Yamakoshi et al., 2004).

2.2.1.7 Other topical agents

N-acetyl-4-S-cysteaminylphenol is also utilized as a topical treatment for melasma. It works particularly on melanocytes by inhibiting melanin activity. Under a light microscope, the skin treatment with N-acetyl-4-S-cysteaminylphenol resulted in a significant decrease in visible melanin in the epidermis. Furthermore, there are extremely little negative effects when utilizing this drug (Gupta et al., 2006).

2.2.1.8 Combination therapy

Combination therapies are effective more because they can boost each other. Therefore, it can also shorten the duration of the course and minimize the adverse effect. Eg. Kligman's and Willis' formula. (Bagherani et al., 2015). Tretinoin not only prevents HQ oxidation but also improves epidermal penetration, while the steroid minimizes irritation caused by the other two ingredients and lowers melanocyte activities, resulting in an early reaction in melasma. It has improved significantly because of the synergistic action of three topical medicines. Improvement is visible after 8 weeks with no major side effects (Shankar et al., 2014). According to certain evidence-based research, the triple combination of 4% HQ, 0.05% tretinoin, and 0.01% fluocinolone acetonide is the

most effective topical modality for melasma with a very minimal risk of skin atrophy (Torok, 2006).

2.2.1.9 New treatments of oral and topicals

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

Glutathione has the ability to inhibit of tyrosinase. Glutathione (GSH) is a highly effective endogenous antioxidant. Cells in the human body can make it. Mechanisms that can lighten the skin include tyrosinase inhibition and the ability to skew eumelanin to pheomelanin synthesis. The use of GSH intravenously has been linked to severe life-threatening events such as Stevens-Johnson syndrome and anaphylaxis (Sonthalia et al., 2016).

Methimazole is an oral anti-thyroid and if it is applied topically, it can lead to depigmentation and act as a potent peroxidase inhibitor that can inhibit melanin synthesis (Kasraee et al., 2005, 2008).

Cysteamine hydrochloride (ß-mercaptoethylanine hydrochloride) is a byproduct of the amino acid L-cysteine breakdown. Cysteamine is also a radioprotector, protecting cells from the mutagenic and other deadly consequences of ionizing radiation through direct hydroxy radical scavenging (Besouw et al., 2013).

2.2.1.10 Chemical peeling

Chemical peels can promote epidermal remodeling and keratinocyte turnover, making them an effective supplementary treatment option for melasma (Gupta et al., 2006). Deep peeling is not suggested in the treatment of melasma due to its negative effects (Sarkar et al., 2012).

Alpha and beta hydroxyl acids such as TCA and salicylic acid salicylic acid with a percentage of ten to thirty, Jessner's solution, and 1% tretinoin solution are advised chemical peels (Sarkar et al., 2010).

**2.2.2 Treatment for Pregnant Women**

It is not recommended to treat the melasma pregnant patients before childbirth. Moreover, it not easy to give treatment during pregnancy due to unstable hormones (Lynde et al., 2006).

### 2.2.3 Device Therapy

Laser and light therapy are a third-line treatment option for melasma. These techniques, like chemical peels, can speed up melanin removal but do not target melanin formation. They are at risk for PIH or a recurrence of melasma (Polder et al., 2011).

Lasers or other devices may aid to melasma treatment in the future by directly targeting pigment and facilitating the release of topical drugs, a technique known as laser-assisted drug delivery (LADD) (Haedersdal et al., 2016).

Microneedle (MN) technology, like LADD, generates micron-sized pores through the epidermis to enhance the delivery of therapeutic compounds into the epidermis (Donnelly et al., 2010).

## 2.3 Acacia Concinna Extract

**Figure 2.3** Acacia concinna or Sompoi

### 2.3.1 Introduction

Acacia concinna or Sompoi, a member of the Fabaceae family, is widely grown for medicinal uses in Southern and Southeast Asia. It has been identified as a component in holy water used to pay respect to old people in several important Thai

occasions, including the Songkran celebration. Because of its anti-dermatophyte and antibacterial properties, Acacia concinna pod has been utilized as an active ingredient in anti-dandruff shampoos, according to the Indian Ayurvedic pharmacopeia (Poomanee et al., 2015).

It heals leprosy and skin problems including oedema. According to folklore medicines Acacia concinna's analgesic, antibacterial, insect repellant, and wound healing properties are all well utilized. Its leaves are used to treat malaria, while decoctions of the pods are used to treat biliousness and as a purgative. Acacia concinna has traditionally been used as an oral rinse to treat halitosis, dental caries, mouth ulcers, and gum bleeding. When applied to problematic areas following a hot castor oil massage, it relieves leg, hip, and joint discomfort. This plant offers cleansing and anti-inflammatory qualities when used as a bath tincture or infusion, and it washes skin diseases such collected pus and exudates like skin rash (Biswal et al., 2019).

**Figure 2.4** Acacia concinna or Sompoi

Acacia concinna is used to treat pain, constipation, jaundice, dandruff, age spots, gum disorders, leprosy, and psoriasis, among other things. These plant remedies can be purchased commercially or made at home. The leaves, barks, and pods have all been utilized in herbal therapy to treat emetic, purgative, and expectorant symptoms. It has antioxidant, anti-coagulant, anti-platelet, anti-thrombotic, anti-dermatophytic, and immunological adjuvant effects (Biswal et al., 2019).

Saponin (20.8%) is the major chemical constituent and is responsible for its anti-dermatophyte and antibacterial capabilities. An antibacterial ointment containing Acacia concinna extract has been widely used to treat skin conditions. Several

dermatophytes, including Trichophyton rubrum, Trichophyton mentagrophyte, Microsporum nanum, and Epidermophyton floccosum, were killed by ethanolic, ethyl acetate, and hexane extracts of the Acacia concinna (Poomanee et al., 2015). The aqueous extracts of Acacia concinna has maximum activity against all types of microorganisms such as Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, followed by Pseudomonas aeruginosa and Staphylococcus aureus (Todkar et al, 2010).

Due to its high phenolic content, Acacia concinna extract via hydroethanolic maceration displayed the best antioxidant and antityrosinase actions of all extracts. Furthermore, the extract's safety profile on human peripheral blood mononuclear cells (PBMCs) was equivalent to that of ascorbic acid. As a result, Acacia concinna extract is thought to be a potential molecule for the topical treatment of microbiological infections, UV-induced skin diseases, and melasma (Poomanee et al., 2015).

Alkaloids, glycosides, reducing sugars, amino acids, phenolic compounds, saponins, carbohydrates, steroids, tannins, flavonoids, and starch were discovered to be the principal ingredients of the fruits (Aung et al., 2020). Acacia concinna, a green vegetable, can be a healthy alternative source of nutrients, including macronutrients and micronutrients. Its leaves have the highest dietary fiber macronutrient content and calcium micronutrient value. The leaves of Acacia concinna have a high antioxidant capacity as well as mildly cytotoxic effects on malignant cell lines such as human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma (Ganogpichayagrai & Suksaard, 2022).

### 2.3.2 Chemical Constituents of Acacia Concinna

Chemical profiles of Acacia concinna are 12-Hydroxyhydromethyl abietate, 5,7,8, 3´,4´-Pentamethoxy flavone, Esculentoside M, Fentanyl Nor, Ganderic acid G, Kaempferol-3-O-β-D-glucoside-7-O-α-L-arabinofuranoside, Luteolin-7-O[β-D-apiofuranosyl(1→6)]β-D-glucopyranoside, Nifedipine, Methyl-lucidenate P, 9-amino Minocycline, Ophiopogonanone A, Phosphatidyl ethanolamines, Pterosin Y, Rengyolester, Rengyoside C, Robinetin, Soyacerebroside I- 1, Sucrose, Schizonepetoside B, Portuloside A, Picrasinoside D, Ouabain, Methyl ganoderenate D, Ganoderic acid H, Daturametelin F, Ajugaside A and (Z)-(1S,5R)-β-Pinen-10-yl-β-vicianoside (Aung et al., 2020).

The polarity of the solvent used influences the phytochemical constituents of extracts. Hydrophilic chemicals are found in aqueous extracts, whereas hydrophobic and hydrophilic phytochemical constituents are found in ethanol extracts. All aqueous, benzene, chloroform, petroleum ether, butanol, and methanol extracts contain alkaloids, flavonoids, phytosterols, saponins, and phenolic chemicals. Except for petroleum ether and butanol, all extracts contain tannins, gums, and mucilage. Crystals of calcium oxalate and oil globules, as well as saponin-containing cells, characterize the powder. Saponins, which are foam-forming chemicals, are abundant in the bark. These pods contain saponin with trihydroxymonocarboxylic acid, which has acidic action as well as surfactant properties. Methanolic fraction extracts of Acacia concinna pods increase the activity of Th1 and Th2 helper T cells (Khanpara et al., 2012).

### 2.3.3 Preparation of Acacia Concinna Fruit Extract

Acacia concinna fruit extract is prepared as the following steps:

Step 1: Dried by hot air oven

Step 2: Grinding

Step 3: Extract with Deionized Water

Step 4: Evaporation by vacuum evaporator at low temperature

Step 5: Dissolved in Butylene Glycol

Step 6: Standardize

Step 7: Acacia concinna extract liquid

### 2.3.4 Biological Activities of Acacia Concinna Fruit Extract

2.3.4.1 Anti-tyrosinase activity

Tyrosinase is the rate-limiting enzyme in melanogenesis, which causes melasma and dark patches in skin cells. The Acacia concinna extracts were tested for their ability to inhibit the mushroom tyrosinase enzyme. IC50 values were shown and compared to a control, beta-arbutin. Among the extracts, HES demonstrated the strongest inhibition on hydroxylation of L-tyrosine (Poomanee et al., 2015).

2.3.4.2 Antioxidant activity

Acacia concinna contained a variety of flavonoids that play an important role as radical scavengers. Several studies showed that phenolic compounds and high level of flavonoids have the potential for antioxidant activity. Acacia concinna contains

the radical scavenging activity determined and confirmed by the DPPH- radical scavenging assay, ABTS- radical scavenging assay and Linoleic acid peroxidase assay (Poomanee et al., 2015). Another study evaluated antioxidant capacity using two different based assays, consisting of hydrogen atom transfer (HAT) and single-electron transfer. Acacia concinna extract showed good antioxidant capacity (Ganogpichayagrai & Suksaard, 2022).

2.3.4.3 Antimicrobial activity

Acacia concinna contains the phytochemicals such as terpenoids, saponins, tannin, alkaloids, and flavonoid. Therefore, it might be a factor of antibacterial activity of Acacia concinna and showed that maximum activity against Aspergillus niger followed by Penicillin sp. and Candida albican. Moreover, another study showed that has maximum activity against Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, followed by Pseudomonas aeruginosa and Staphylococcus aureus (Todkar et al, 2010). According to one study, Acacia concinna has considerable antidermatophytic activity against the dermatophytes Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton violaecum, Microsporum nanum, and Epidermophyton floccosum, with a MIC value of 62.5g/ml. Pitiriasis capitis can be controlled with Acacia concinna (Ediriweera et al., 2014).

2.3.4.4 Anticancer activity

The cytotoxicity of Acacia concinna leaves ethanolic extract against four malignant cell lines was determined using the REMA test for anticancer activities. Acacia concinna extract at 50 g/ml demonstrated minimal cytotoxicity against human hepatocarcinoma, breast cancer, lung cancer, and colon adenocarcinoma cell lines, with IC50 values ranging from 11.05% +/- 0.22% to 23.98% +/- 0.13% (Ganogpichayagrai & Suksaard, 2022). The phytocompounds may act synergistically with anticancer agents to conquer the resistance. The utilization of phyto-substances may also lead to the use of a low dosage of anticancer drugs. The cytotoxicity of the Acacia concinna was accessed on the MCF-7 cell line. The IC50 value calculated for Acacia concinna extract was 171.25 μg/ml (Shaikh et al., 2022). Three authentic saponins, kinmoonosides A-C (1-3), as well as a novel monoterpenoid (4), were identified from a methanolic extract of Acacia concinna fruits. 4-O-[(2E)- 6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl] was identified as the novel monoterpenoid

4.-D-quinovopyranose. Compounds 1-3 were significantly cytotoxic to human HT-1080 fibrosarcoma cells (Tezuka et al., 2000).

2.3.4.5 Anthelmintic activity

Farmers and traditional healers have long employed phytomedicine to treat parasite illness in animals. Natural chemicals derived from natural products have long played an important role in medication development. Despite their widespread ethnoveterinary use, scientific information on the antiparasitic efficacy of most plant products is weak. Acacia concinna is a widespread plant in India. Acacia concinna pods include kinmoonosides A C, triterpenoidal prosapogenols designated concinnosides A, B, C, D, and E, as well as four glycosides, acaciaside, julibroside A1, julibroside A3, albiziasaponin C, and its aglycone, acacic acid lactone. It serves as a purgative and relieves biliousness. Anthelmintic activity of Acacia concinna extract has been observed in vitro (Priya et al., 2013).

2.3.4.6 Anti-obesogenic activity

The anti-obesogenic chemicals in Acacia concinna pods were studied in this work. Saponins were isolated through chromatographic separation of the pod extract guided by pancreatic lipase inhibitory action. The saponins' chemical makeup was shown by decomposition analysis to be acacic acid, monoterpenes, and five types of sugars (glucose, xylose, rhamnose, quinovose, arabinose). The saponins' predicted structures from breakdown analysis were validated by LC-MS analysis, indicating that these saponins are a mixture of different monoterpene derivatives and sugar units. With an IC50 of 7.9 g/mL, these saponins substantially inhibited pancreatic lipase activity and reduced fat formation in 3T3-L1 adipocytes. Saponins also increased lipolysis in 3T3-L1 adipocytes at 3.1 or 6.3 g/mL via modulating the activity of the protein kinase A and extracellular signal-regulated kinase pathways, implying that this mechanism is partly responsible for the observed decrease in lipid content in adipocytes. The findings highlight Acacia concinna as a potential source of anti-obesogenic candidates for future obesity therapy and prevention (Zhouyue et al., 2021).

In summary, the study has been mainly focused on melasma treatment. And it was found that there have not been reports of the use of topical Acacia concinna fruit extract especially in human prior to the present study. Moreover, there were very few study papers for its anti-tyrosinase activity. Thus, this study is to explore the

effectiveness and satisfaction of Acacia concinna fruit extract in melasma treatment. The research outcomes will be used to support the diagnosis and treatment of the melasma by using Acacia concinna as an alternative treatment for melasma.

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Study Design

This study is a randomized controlled clinical trial that is comparative, double-blind (volunteers and physicians who performed the measurement), split face.

## 3.2 Study Population

The volunteers' range in age from twenty to forty-five years old and have melasma on their faces. The Fitzpatrick skin types range from 3 to 6.

## 3.3 Sample Size Calculation and Analysis

There is no report of the using the topical Acacia concinna fruit extract for treating melasma before. Therefore, the researcher has decided to choose the formula to show the result statistically significant of the study by using the similar report of the 5% Morinda citrifolia fruit extract topically in melasma treatment (Aung, 2021).

The mean melanin index of the 5% Morinda citrifolia fruit extract side showed improvement from 219.77+ 57.31 to 181.32+ 48.88 at the week 0 and week 8 respectively. On the placebo side, the mean melanin index showed increased from 223.70+ 65.31 to 228.14 + 67.06 at the baseline and week 8 respectively. The changes from week 0 of 5% Morinda citrifolia fruit extract side and placebo sides are 38.45 ($\mu$1) and – 4.44 ($\mu$2) respectively and the $\mu$d is 42.89. The number of sample size of group (n1, n2) are 16. The standard deviation of Morinda citrifolia fruit extract and placebo are 57.31 ($\sigma$1) and 48.88 ($\sigma$2) respectively.

From the formula, $\alpha = 0.05$ (two-tailed) $Z_{0.025} = 1.96$

$\beta = 0.10$ $Z_{0.10} = 1.28$

$S_p^2 = [(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2 ] / n_1 + n_2 - 2$

$S_p^2$ pooled $= [(16 – 1) (57.31)^2 + (16 – 1) (48.88)^2] / (16 + 16 – 2)$

$= 2836$

$n = (Z_{\alpha/2} + Z_{\beta})^2 \sigma_d^2 / \mu_d^2$

$= (1.96 + 1.28)^2 2836 / (42.89)^2$

$= 16.18$ or 17 subjects

Therefore, the sample size of this research is at least 16 people. In consideration for the risk of drop-out percentage 30% by the volunteer, the sample size will be 23 people. However, the researcher decided to do the research with the sample size to 30 participants in order to enhance the robustness of the findings.

## 3.4 Selection Criteria

### 3.4.1 Inclusion Criteria

3.4.1.1 The Women with melasma on both sides of their faces were recruited via a clinic and an internet platform.

3.4.1.2 Women between the ages of twenty and forty-five who have Fitzpatrick skin types 3 to 6.

3.4.1.3 Volunteers who have not received any melasma treatment in the last 60 days, other than sunscreen application.

3.4.1.4 Participants who are interested to engage in this study and can return for the follow-up appointment every 2 weeks (0, 2nd, 4th, 6th, and 8th weeks), for a total of five visits.

3.4.1.5 Volunteers who are willing to sign a consent form for information such as benefits, side effects, and photographing for public reporting.

### 3.4.2 Exclusion Criteria

3.4.2.1 Females that are pregnant at the current moment.

3.4.2.2 Female who is breastfeeding her child

3.4.2.3 Females who used birth control pills, hormones (phenytoin and spironolactone), or other drugs that can impair melanin formation.

3.4.2.4 Women who used hydroquinone six months previously

3.4.2.5 Women who used tretinoin three months ago

3.4.2.6 Women suffering from underlying conditions such as immunosuppression, blood disease, metabolic disease (Diabetes Mellitus), and photosensitivity.

3.4.2.7 Women who have a history of having difficulties healing wounds and getting atypical scars.

3.4.2.8 Women who used alcohol or drugs.

3.4.2.9 Women who have open wounds and are irritated on the treatment area.

3.4.2.10 Women with a history of cancer or a precancerous lesion in the treatment area.

3.4.2.11 Women who are currently undergoing treatment with a whitening agent.

3.4.2.12 Women who are exposed to sunlight for an extended period as a result of their job or leisure activities.

3.4.2.13 Women who are allergic to Acacia concinna fruit extract.

**3.4.3 Withdrawal Criteria**

3.4.3.1 Volunteer taking other melasma treatment not with Acacia concinna fruit extract

3.4.3.2 Volunteer who became pregnant.

3.4.3.3 Volunteers who experienced unfavorable effects or allergic reactions because of therapy, illness, or an accident

3.4.3.4 Volunteers that were willing to withdraw from this program for whatever reason

3.4.3.5 Volunteers who did not cooperate with the protocol or were unable to attend to a follow-up appointment for more than three visits.

## 3.5 Location

Mae Fah Luang University Hospital, Asoke, Bangkok, Thailand.

## 3.6 Variables

3.6.1 Independent variable of the study is using the placebo.

3.6.2 Dependent variables are modified MASI score, melanin index, doctor and patient satisfaction score, adverse effects.

## 3.7 Treatment

The 10% Acacia concinna fruit extract was treated on the half face of the subject and on another half with placebo.

## 3.8 Equipment

1. 10% Acacia concinna fruit extract (Color: Very Light brown, Odorless, Spray bottle, Volume 50ml., Produced by Health for life group Co., Ltd.)
2. Placebo (having same characteristic as 10% Acacia concinna fruit extract)
3. Mild Soap
4. Sunscreen
5. Moisturizing Cream
6. Patch test
7. Mexameter MX18 (Melanin measuring instrument)
8. Consent paper
9. Patient record form
10. Side effect record form
11. Letter of qualification
12. Satisfactory evaluation records for both doctors and volunteers

3.8.1 Placebo

**Table 3.1** Constituents of Placebo

| **Placebo** |
| --- |
| Deionized Water (99.99%) |
| Brown Iron Oxide (0.01%) |

3.8.2 "10% Acacia concinna fruit extract"

**Table 3.2** Constituents of 10% Acacia Concinna Fruit Extract

| **10% Acacia concinna fruit extract** |
| --- |
| 10% Acacia concinna fruit extract (10%) |
| Phenoxyethanol (1%) |
| Caprylhydroxamic acid (0.05%) |
| Deionized Water (88.95%) |

3.8.3 Mild Soap

**Table 3.3** Constituents of Mild Soap

| **Mild Soap Ingredients** |
| --- |
| Water |
| Cetyl Alcohol |
| Propylene Glycol |
| Sodium Lauryl Sulfate |
| Sodium Chloride |
| Stearyl Alcohol |
| Methylparaben |
| Propylparaben |
| Glyceryl Laurate |

3.8.4 Sunscreen SPF 50, PA+++

**Table 3.4** Constituents of Sunscreen

| **Sunscreen Ingredients** |
|---|
| Water |
| Alcohol |
| Ethylhexyl Methoxycinnamate |
| Ethylhexyl Triazone |
| Isopropyl Palmitate |
| Diethylamino Hydroxybenzoyl Hexyl Benzoate |
| Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine |
| Hydrogenated Polyisobutene |
| Sorbitan Distearate |
| Disodium EDTA |
| Phenoxyethanol |
| Sodium Hyaluronate |
| Agar |
| Sodium Hydroxide |

3.8.5 Moisturizing Cream

**Table 3.5** Constituents of Moisturizing Cream

| **Moisturizing Cream Ingredients** |
|---|
| Aqua |
| Glycerin |
| Dimethicone |
| C 12-15 alkyl benzoate |
| Behenyl alcohol |
| Cetearyl Alcohol |
| Simmondsia |
| Chinensis seed oil |
| Glyceryl Stearate |
| Propanediol |

**Table 3.5** (Continued)

| Moisturizing Cream Ingredients |
|---|
| Sodium polyacrylate |
| Ethylhexyleglycerin |
| PEG-40 Stearate |
| Ceteareth-20 |
| Tocopheryl acetate |
| Ceterareth-20 |
| Tocopheryl acetate |
| Disodium EDTA |

## 3.9 Process of Study

### 3.9.1 Procedure of Treatment

3.9.1.1 Volunteers were recruited based on inclusion and exclusion criteria. The researcher did research on the study's purpose, procedure, benefits, and drawbacks. The volunteers gave their permission for the study to continue.

3.9.1.2 Taking the history of the volunteers.

3.9.1.3 During the initial visit, volunteers' arms were treated with 10% Acacia concinna extract as part of a water-proof patch test, which was left on for a full day. During the patch test, avoid excessive perspiration and exposure to sunshine. At 48 and 96 hours, the results were read out. The International Contact Dermatitis Research Group approach was used to score any reactions that were seen. The scoring are as follows:

+? = doubtful reaction: mild redness only

+ = weak, positive reaction: red and slightly thickened skin

++ = strong positive reaction: red swollen skin with individual small blisters

+++ = extreme positive reaction: severe swelling and redness accompanied by the huge blister that have appeared or are spreading

After the patch is removed, the redness on the skin gets better.

3.9.1.4 The researcher takes photos to capture each volunteer for three different facial positions, such as left, right, and center, at weeks 0, 2nd, 4th, 6th, and 8th (a total of five times) on each visit.

3.9.1.5 The physicians employed the mexameter to analyze the mean melanin index and calculated scores at weeks 0, 2nd, 4th, 6th, and 8th. The physicians measured 2 centimeters below the lower eyelids at the mid pupillary line.

3.9.1.6 The physicians and participants will not know which water is put to which side of the face for randomization. The unrelated physician will generate block randomization by using a random sequence generator from the website and block randomization to disguise the sequence in opaque envelopes. The 30 numbers were then randomized from one to thousand, and these 30 randomized numbers were assigned to participants in the order of first come, first served. Odd numbers are represented by "1" and Even numbers by "2".

3.9.1.7 10% Acacia concinna fruit extract and placebo were labeled "A" and "B" respectively. All participants in this study, including the dermatologists, were blindfolded.

3.9.1.8 Because the trial is split face, each volunteer will be assigned to apply randomized to their right and left faces.

3.9.1.9 Two ways of the treatment were assigned.

1. (Right, Left) = Right face of the volunteer was to apply with the water A and Left face was to apply with the water B.

2. (Left, Right) = Left face of the volunteer was to apply with the water A and Right face was to apply with the water B.


**Figure 3.1** (Right, Left) of treatment as “1”


**Figure 3.2** (Left, right) of treatment as "2"

**3.9.2 Guidelines**

Water A and B were sprayed and applied two times a day. After the face was cleansed, the water was sprayed and applied about 0.5 ml (press spray 2 time) follow by applying moisturizing cream and sunscreen SPF 50, PA+++(Daytime)

## 3.10 Visit for the Follow Up

The volunteers were asked to attend for a follow-up visit every 2 weeks (0, 2nd, 4th, 6th, and 8th weeks), following the initial (week 0) appointment to examine, measure, and assess the adverse effect and improvement during the study period.

## 3.11 Measurement and Collection of Data

3.11.1 Clinical evaluation

In this investigation, a modified MASI score was applied. The MASI is commonly used in clinical settings to assess the severity of melasma. The approach primarily employs two components: area and darkness. To estimate the area (A), it must be scored seven times. 0 represents no involvement, 1 represents a percentage of less than ten percent, 2 represents a percentage of ten to twenty-nine percent, 3 represents a percentage of thirty to forty-nine percent, 3 represents a percentage of thirty to thirty-nine percent, 4 represents a percentage of fifty to fifty-five percent, 5 represents a percentage of seventy to eighty-nine percent, and 6 represents a percentage of more than 90 percent.

The darkness (D) is graded on a scale of zero to four. 0 represents normal, 1 represents hyperpigmentation with naked eyes, 2 represents mild, 3 represents moderate, and 4 represents severe skin darkness. As a result, Modified MASI scores were calculated for each visit.

As the study design is split face trial, the formula was as the following.

2x [(0.15) (A) (D) of forehead of one side + (0.3) (A) (D) of malar of one side + (0.05) (A) (D) of chin]

3.11.2 The effectiveness of the 10% Acacia concinna fruit extract and placebo were analyzed by taking photos at every visit.

3.11.3 The satisfaction and assessment scoring system by doctors is determined according to the Global Satisfaction Score as the following ranging. “-1” stands for worse, “0” stands for no improvement, “1” stands for fairly improvement (1-25%), “2” stands for moderate improvement (26-50%), “3” stands for good improvement (51-75%) and “4” stands for excellent improvement (76-100%).

3.11.4 For patients’ satisfactory scores were evaluated by using the grading scales from -1 to 4 in which “-1” stands for worse, “0” for no change, “1” for less satisfied, “2” for moderately satisfied, “3” for very satisfied and “4” for extremely satisfied.

3.11.5 To evaluate the adverse effects of 10% Acacia concinna fruit extract, the researcher requested the volunteers to follow up their doctors to see if volunteers have any side effect during the research period to record the scores for pruritus ranging from zero to ten, for duration of the redness and other symptoms such as inflammation, hypopigmentation, and hyperpigmentation features.

## 3.12 Data Analysis

3.12.1 Volunteers in this study were determined according to the inclusion and exclusion criteria and individual information was confidential.

3.12.2 The data analysis of the clinical evaluation and outcomes from this study was analyzed by using SPSS 18 software and Microsoft Excel.

3.12.3 The demographic information data was reported by descriptive statistical analysis.

3.12.4 The MASI score for each volunteers' face during treatment of the baseline, weeks 2nd, 4th, 6th, and 8th were evaluated clinically and then analyzed mean melanin index of the face for the whole 8 weeks by using Mexameter. MASI scores and mean melanin index results between 10% Acacia concinna fruit extract and placebo were analyzed through paired t test.

3.12.5 Comparison of the results between 10% Acacia concinna fruit extract and placebo at weeks 0, 2nd, 4th, 6th, and 8th by using repeated measure ANOVA test.

3.12.6 For data result of satisfaction by three dermatologists was described by using Descriptive statistics at weeks 0, 2, 4, 6 and 8 of the study periods.

3.12.7 At the 8th week of the research period, patient satisfactory score was analyzed, and adverse effects were noted. Data was reported by using Descriptive statistics.

3.12.8 The following was done by the researcher at significance levels of p-value<0.05.

# CHAPTER 4

# RESULT

## 4.1 Baseline Characteristics

The demographic information of thirty females aged 20 to 45 years old with Fitzpatrick skin type III-VI with melasma were enrolled from outpatient department (OPD), Mae Fah Luang University Hospital as well as internet advertisement. The diagnosis of melasma was based on the clinical features by physician and wood's lamp is being used to confirm and evaluate type of melasma.

**Table 4.1** Demographic Data

| **DEMOGRAPHIC** | **n=30** |
|---|---|
| Gender | |
| Female | 30 |
| Age (years) | |
| Mean +/- SD (years) | 29 ± 3.96 |
| min – max | 22 - 37 |
| Occupation, n (%) | |
| Housewife | 5 (16.67) |
| Employee | 25 (83.33) |
| Underlying disease, n (%) | |
| YES | 0 (0) |
| NO | 30 (100) |
| Photosensitivity, n (%) | 0 (0.0) |
| Personal medication, n (%) | |
| YES | 0 (0) |
| NO | 30 (100) |
| Food allergy, n (%) | 0 (0.0) |

**Table 4.1** (continued)

| DEMOGRAPHIC | n=30 |
|---|---|
| Treatment before, n (%) | |
| NO | 22(73.33) |
| Facial Whitening Agent | 3 (10) |
| Microdermabrasion | 3 (10) |
| Micro-needling | 1 (3.33) |
| Facial whitening treatment | 1(3.33) |
| Sunlight expose, n (%) | |
| YES | 30 (100) |
| NO | 0 (0) |
| Sunlight-expose duration (minute) | |
| Mean ± SD | 58.33 ± 17.19 |
| min - max | 30 - 120 |
| Fitzpatrick skin type, n (%) | |
| Type IV | 26 (86.67) |
| Type V | 4 (13.33) |
| Wood Lamp Histological Type, n (%) | |
| Epidermal type | 8 (26.67) |
| Mixed type (epidermal-dermal type) | 19 (63.33) |
| Dermal Type | 3 (10) |
| Clinical Pattern, n (%) | |
| Centro-facial Type | 18 (60) |
| Malar Type | 12 (40) |
| Mandibular Type | 0 (0) |

According to Table 4.1 that demonstrated the demographic data of the participants, mean age of the female subjects was 29±3.96 years, and there were 5 housewife (16.67%) and 25 employee (83.33%). There was no subject with underlying disease and take medicine regularly. Furthermore, eight subjects had history of melasma treatment before this study such as 3 subject who used microdermabrasion (10%) and 3 subject used facial whitening agent (10%), 1 subject had done microneedling (3.33%) and 1 subject used facial whitening treatment (3.33%). All of

these subjects used the respective treatments 1 or 2 years ago. All 30 subjects got exposed to sunlight with mean of duration was 58.33 ± 17.19 minutes. The twenty-six subjects had Fitzpatrick skin type 4 and the other four had Fitzpatrick skin type 5. The nineteen subjects had mixed type (63.33%), 8 subjects had epidermal type (26.67%) and the rest had dermal type (10%). Of all these volunteers, eighteen subjects had centro-facial type (60%) and 12 subjects had malar type (40%) and there is no mandibular type (0%). None of the volunteers had photosensitivity and food or drug allergy.

## 4.2 Melasma Area and Severity Index (MASI)

**Table 4.2** Statistical analysis of MASI for the 10% Acacia concinna and placebo side at baseline, follow-up on the 2nd, 4th, 6th, and 8th week (n=30)

| Follow-up | 10% Acacia concinna | Placebo | Mean difference | P-value† |
|---|---|---|---|---|
| | mean±SD | mean±SD | | |
| Baseline | 3.16±0.87 | 3.15±0.78 | 0.01 | 0.912 |
| 2nd week | 3.16±0.87 | 3.15±0.78 | 0.01 | 0.912 |
| 4th week | 3.08±0.87 | 3.16±0.77 | -0.08 | 0.343 |
| 6th week | 2.68±0.66 | 3.20±0.79 | -0.52 | <0.001* |
| 8th week | 2.44±0.60 | 3.22±0.79 | -0.78 | <0.001* |
| P-value‡ | <0.001* | 0.001* | | |

**Note** Data were analyzed with Repeated measure ANOVA‡ and paired t-test†

According to Table 4.2 which presents the results of statistical analyses, the mean MASI for the 10% Acacia concinna side at baseline, follow-up 2nd, 4th, 6th, and 8th week were 3.16±0.87, 3.16±0.87, 3.08±0.87, 2.68±0.66, and 2.44±0.60, respectively. The mean MASI at each visit decreased statistically significantly at the level of 0.05 (partial $\eta^2$ 0.542, $p<0.001$). In other words, 54.2% treatment effect of the 10% Acacia concinna on MASI.

On the other hand, the mean MASI for the placebo side at baseline, follow-up 2nd, 4th, 6th, and 8th week were 3.15±0.78, 3.15±0.78, 3.16±0.77, 3.20±0.79, and

3.22±0.79, respectively. The mean MASI at each visit increased statistically significantly at the level of 0.05 (partial $\eta^2$ 0.233, p=0.001). In other words, not using 10% Acacia concinna is associated with a 23.3% increase in MASI.

Comparing the MASI between the 10% Acacia concinna and the placebo side, the MASI in the 10% Acacia concinna side was significantly lower than in the placebo side at the follow-up 6th and 8th week, with significance at the 0.05 level (P<0.001).

**Table 4.3** Multiple comparison (Post-hoc) of MASI for the 10% Acacia concinna and placebo side (n=30)

| Pairwise | 10% Acacia concinna | | Placebo | |
|---|---|---|---|---|
| | **Mean difference** | **P-value** | **Mean difference** | **P-value** |
| Baseline - 2nd week | 0.00 | - | 0.00 | - |
| Baseline - 4th week | -0.08 | 0.816 | 0.01 | 1.000 |
| Baseline - 6th week | -0.48 | <0.001* | 0.05 | 0.107 |
| Baseline - 8th week | -0.72 | <0.001* | 0.07 | 0.016* |
| 2nd week - 4th week | -0.08 | 0.816 | 0.01 | 1.000 |
| 2nd week - 6th week | -0.48 | <0.001* | 0.05 | 0.107 |
| 2nd week - 8th week | -0.72 | <0.001* | 0.07 | 0.016* |
| 4th week - 6th week | -0.40 | 0.002* | 0.04 | 0.258 |
| 4th week - 8th week | -0.64 | <0.001* | 0.07 | 0.040* |
| 6th week - 8th week | -0.24 | 0.063 | 0.03 | 0.960 |

**Note** Data were analyzed with Bonferroni method

According to Table 4.3, which presents the results of multiple comparisons, the MASI for the 10% Acacia concinna side at the follow-up 6th, and 8th week was lower than at baseline. Additionally, the MASI for the 10% Acacia concinna side at the follow-up 6th and 8th week was lower than the 2nd week. Furthermore, the MASI for the 10% Acacia concinna side at the 6th and 8th week was also lower than the 4th week, which was statistically significant at the level of 0.05 (p<0.05). The decrease in the MASI for the 10% Acacia concinna side from baseline to the 8th week follow-up was 0.72.

For the placebo side, the MASI at the follow-up 8th week was higher than baseline, the follow-up 2nd, and 4th week, which was statistically significant at the level of 0.05 ($p<0.05$). The increase in the MASI from baseline to the 8th week follow-up was 0.07.

## 4.3 Mexameter

**Table 4.4** Statistical analysis of Mexameter for the 10% Acacia concinna and placebo side at baseline, follow-up on the 2nd, 4th, 6th, and 8th week (n=30)

| Follow-up | 10% Acacia concinna | Placebo | Mean difference | P-value† |
|---|---|---|---|---|
| | mean±SD | mean±SD | | |
| Baseline | 213.30±21.38 | 216.37±16.07 | -3.07 | 0.333 |
| 2nd week | 205.67±21.53 | 217.77±16.27 | -12.10 | <0.001* |
| 4th week | 196.17±21.21 | 219.23±15.58 | -23.07 | <0.001* |
| 6th week | 186.80±21.53 | 221.80±14.94 | -35.0 | <0.001* |
| 8th week | 178.67±20.94 | 224.57±17.07 | -45.9 | <0.001* |
| **P-value‡** | **<0.001*** | **<0.001*** | | |

**Note** Data were analyzed with Repeated measure ANOVA‡ and paired t-test†

*P<0.05 indicates statistical significance

According to Table 4.4 which presents the results of statistical analyses, the mean mexameter for the 10% Acacia concinna side at baseline, follow-up 2nd, 4th, 6th, and 8th week were 213.30±21.38, 205.67±21.53, 196.17±21.21, 186.80±21.53, and 178.67±20.94, respectively. The mean mexameter at each visit decreased statistically significantly at the level of 0.05 (partial $\eta^2$ 0.972, $p<0.001$). In other words, 97.2% treatment effect of the 10% Acacia concinna on mexameter.

On the other hand, the mean mexameter for the placebo side at baseline, follow-up 2nd, 4th, 6th, and 8th week were 216.37±16.07, 217.77±16.27, 219.23±15.58, 221.80±14.94, and 224.57±17.07, respectively. The mean mexameter at each visit increased statistically significantly at the level of 0.05 (partial $\eta^2$ 0.425, $p<0.001$). In

other words, not using 10% Acacia concinna is associated with a 42.5% increase in mexameter.

Comparing the mexameter between the 10% Acacia concinna and the placebo side, the mexameter in the 10% Acacia concinna side was significantly lower than in the placebo side at the follow-up 2nd, 4th, 6th and 8th week, with significance at the 0.05 level. (P<0.001)

**Table 4.5** Multiple comparison (Post-hoc) of mexameter for the 10% Acacia concinna and placebo side (n=30)

| Pairwise | 10% Acacia concinna | | Placebo | |
|---|---|---|---|---|
| | Mean difference | P-value | Mean difference | P-value |
| Baseline - 2nd week | -7.63 | <0.001* | 1.40 | <0.001* |
| Baseline - 4th week | -17.13 | <0.001* | 2.87 | <0.001* |
| Baseline - 6th week | -26.50 | <0.001* | 5.43 | <0.001* |
| Baseline - 8th week | -34.63 | <0.001* | 8.20 | <0.001* |
| 2nd week - 4th week | -9.50 | <0.001* | 1.47 | 0.010* |
| 2nd week - 6th week | -18.87 | <0.001* | 4.03 | 0.007* |
| 2nd week - 8th week | -27.00 | <0.001* | 6.80 | <0.001* |
| 4th week - 6th week | -9.37 | <0.001* | 2.57 | 0.180 |
| 4th week - 8th week | -17.50 | <0.001* | 5.33 | 0.005* |
| 6th week - 8th week | -8.13 | <0.001* | 2.77 | 0.054 |

**Note** Data were analyzed with Bonferroni method

According to Table 4.5, which presents the results of multiple comparisons, the mexameter for the 10% Acacia concinna side at the follow-up 2nd, 4th, 6th, and 8th week was lower than at baseline. Additionally, the mexameter at the follow-up 4th, 6th and 8th week was lower than the 2nd week. Furthermore, the mexameter at the follow-up 6th and 8th week was lower than the 4th week, and the follow-up 8th week also lower than the 6th week, which was statistically significant at the level of 0.05 (p<0.05). The decrease in the mexameter from baseline to the 8th week follow-up was 34.63.

For the placebo side, the mexameter at the follow-up 2nd, 4th, 6th, and 8th week was higher than baseline. Additionally, the mexameter at the follow-up 4th, 6th and 8th week was higher than the 2nd week. Furthermore, the mexameter at the follow-up 8th week was higher than the 4th week which was statistically significant at the level of 0.05 ($p<0.05$). The increase in the mexameter from baseline to the 8th week follow-up was 8.20.

## 4.4 Dermatologist's Satisfactory Score

**Table 4.6** Statistical analysis of satisfactory score by 3 dermatologists on follow-up 2nd, 4th, 6th, and 8th week (n=30)

| Follow-up | Scale | 10% Acacia concinna | Placebo | P-value† |
|---|---|---|---|---|
| | | n | n | |
| 2nd week | No changes | 30 | 30 | - |
| 4th week | No changes | - | 30 | <0.001* |
| | Improved 1-25% (fair) | 30 | - | |
| 6th week | No changes | - | 30 | <0.001* |
| | Improved 1-25% (fair) | 8 | - | |
| | Improved 26-50% (moderate) | 22 | - | |
| 8th week | No changes | - | 30 | <0.001* |
| | Improved 26-50% (moderate) | 30 | - | |

**Note** Data were analyzed with Chi-square test

According to Table 4.6, which presents the results of statistical analyses for the dermatologist's satisfactory scores, there was no change in the satisfaction levels for either the 10% Acacia concinna or placebo side during the 2nd week follow-up for all subjects. At the 4th week follow-up, all subjects in the 10% Acacia concinna side showed a 1-25% improvement (fair), while all subjects in the placebo side showed no

change. This difference was statistically significant at the level of 0.05 ($p<0.001$). By the 6th week follow-up, 26.7% of the subjects in the 10% Acacia concinna side showed a 1-25% improvement (fair) and 73.3% showed a 26-50% improvement (moderate), while all subjects in the placebo side showed no change. This difference was statistically significant at the level of 0.05 ($p<0.001$). At the 8th week follow-up, all subjects in the 10% Acacia concinna side showed a 26-50% improvement (moderate), while all subjects in the placebo side showed no change. This difference was statistically significant at the level of 0.05 ($p<0.001$).

## 4.5 Patient's Satisfactory Score

**Table 4.7** Statistical analysis of patient's satisfactory score on follow-up 8th week (n=30)

| Scale | 10% Acacia concinna | Placebo | P-value |
|---|---|---|---|
| | n (%) | n (%) | |
| No changes | - | 25 (83.3%) | |
| Less satisfied 1-25% | - | 5 (16.7%) | |
| Moderately satisfied 26-50% | 29 (96.7%) | - | <0.001* |
| Very satisfied 51-75% | 1 (3.3%) | - | |

**Note** Data were analyzed with Chi-square test

According to Table 4.7, which presents the results of statistical analyses for the patient's satisfactory scores, 96.7% of subjects in the 10% Acacia concinna side were mostly rated as moderately satisfied, and 3.3% as very satisfied. In contrast, in the placebo side, 83.3% were mostly rated as showing no change, and 16.7% as less satisfied. This difference was statistically significant at the 0.05 level ($p<0.001$)."

## 4.6 Side Effect Evaluation

**Table 4.8** Side effects evaluation of 10% Acacia concinna fruit extract and placebo by 30 volunteers for eight weeks (n=30)

| Side Effect Category | Total volunteers | Incidents Reported | Remarks |
|---|---|---|---|
| Redness | 30 | 0 | No side effects observed |
| Flaky | 30 | 0 | No side effects observed |
| Pain/inflammation | 30 | 0 | No side effects observed |
| Itchy | 30 | 0 | No side effects observed |
| Acne | 30 | 0 | No side effects observed |
| Black patch/ ochronosis | 30 | 0 | No side effects observed |

According to Table 4.8, which evaluates the records for the patient's side effect evaluation during the using of 10% Acacia concinna and placebo for 8 weeks were observed from total 30 participants. There is no report of having any side effects observed during the 8 weeks period.

# CHAPTER 5

# DISCUSSION & CONCLUSION

## 5.1 Discussion

Melasma refers to a condition marked by acquired diffuse hyper-melanosis, presenting as symmetrical, localized, irregular patches ranging from light to dark brown on sun-exposed areas of the skin, notably on the forehead, cheeks, upper lip, and chin (Werlinger et al., 2007). It represents the most common form of facial hyperpigmentation, often resulting in cosmetic concerns and psychological distress, significantly impacting individuals' quality of life (Tzouveka, 2014). The objective of treatment is to diminish melanin production, halt the production of melanosomes, and enhance their degradation (Cestari et al., 2009). Topical tyrosinase inhibitors have the capability to impede melanogenesis, resulting in depigmentation.

There is a growing interest in plant-derived active compounds. Whitening agents containing natural ingredients are becoming increasingly popular due to their safety and cost-effectiveness (Bandyopadhyay, 2009). In this research, we utilized a 10% solution of Acacia concinna fruit extract to assess its anti-tyrosinase properties. Acacia concinna fruit extract is known for its potent anti-tyrosinase, anti-inflammatory, and antioxidant properties, as well as its antimicrobial, anticancer, anthelmintic and anti-obesogenic effects (Poomanee et al., 2015; Priya et al., 2013).

The research was carried out with 30 women between 20-45 years of age with different occupations were involved in the study and chose according to the exclusion and inclusion criteria. We also recorded Fitzpatrick skin type and the duration of sun exposure of each day.

The main objective was to study the efficacy of 10% Acacia concinna fruit extract for melasma treatment. The study design is a randomized controlled clinical trial that is comparative, double-blind and split face design. 30 female volunteers between 20-45 years of age with melasma selected from different occupations were chose according to the exclusion and inclusion criteria and recruited in the study. Fitzpatrick

skin type and the duration of sun exposure of each day was documented. MASI score and mean melanin index of the melasma were studied for 8th weeks with total 5 hospital visits (baseline, 2nd week, 4th week, 6th week and 8th week) follow up. Volunteers were needed to apply spray water on each side of the face two times per day. Moreover, moisturizing cream and sunscreen were given to nourish the skin and protect from ultraviolet radiation.

From previous research, Aung (2021) used 5% Morinda citrifolia fruit extract cream on the treatment of melasma for 12 weeks period. The study on 5% Morinda citrifolia fruit extract cream demonstrated significant reductions in the Melasma Area and Severity Index (MASI) and mexameter scores over 12 weeks. The MASI score decreased from 7.09±1.50 at baseline to 5.67±1.07 at the 12th week, and the mexameter score dropped from 219.77±57.31 to 166.36±43.65, both with high statistical significance ($p<0.001$). In contrast, the placebo cream showed no significant changes in MASI or mexameter scores. Comparing these results to the 10% Acacia concinna extract study, both treatments significantly reduced MASI and Mexameter from 3.16±0.87 (baseline) to 2.44±0.6 (8th week) and from 213.30±21.38 (baseline) to 178.67±20.94 (8th week) respectively within 8 weeks research period, but Acacia concinna extract achieved these reductions in MASI and Mexameter within 8 weeks, indicating a faster onset of action in decreasing mexameter scores compared to Morinda citrifolia. Both Acacia concinna and Morinda citrifolia contain flavonoids and phenolic compounds, which are known for their antioxidant and anti-tyrosinase properties, contributing to their effectiveness in treating melasma. However, Acacia concinna's rapid results may make it a more attractive option for quicker improvement, whereas Morinda citrifolia shows consistent improvement over a longer duration. Therefore, Topical 10% Acacia concinna fruit extract has a good efficacy for treatment of melasma and is consistent with hypothesis.

Moreover, there is no side effect for each participant throughout the research period and is consistent with the hypothesis stated that topical 10% Acacia concinna fruit extract has minimal, and less side effect and it is safe to use.

In conclusion, 10% Acacia concinna extract has some anti-tyrosinase action, which can reduce the patient's production of melanin at a reasonable cost and provide additional advantages as a potent antioxidant. Both the mean melanin index (p-

value<0.001) and the MASI score (p-value<0.001) decreased statistically significantly for the side treated with Acacia concinna extract. During the duration of the research, no adverse effects were noted. For hyperpigmented lesions like melasma, this extract may be a helpful alternative therapeutic option.

## 5.2 Conclusion

With no negative side effects, this study statistically showed that when administered topically, Acacia concinna extract decreased melanin formation more than a placebo. Therefore, 10% fruit extract from Acacia concinna could represent a safe, efficient, and viable substitute for melasma treatment.

## 5.3 Limitation

The study has limitation as it is relatively short research period (8 weeks), for study the recurrence of melasma, posing challenges in interpreting the results and forecasting potential adverse effects.

## 5.4 Suggestion

5.4.1 The findings of this research suggest that 10% Acacia concinna extract could serve as a viable alternative treatment for melasma.

5.4.2 This research data could serve as a foundation for future studies exploring the treatment of melasma.

5.4.3 The cosmetic benefits of Acacia concinna extract warrants further investigation, particularly from a cosmetic perspective, to fully understand its potential advantages.

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# APPENDIX A

# INFORMED CONSENT FORM

**หนังสือแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัย สำหรับอาสาสมัคร**

**(Informed Consent)**

ชื่อโครงการวิจัย- การศึกษาประสิทธิภาพการรักษาฝ้าด้วยสารสกัดส้มป่อย 10%

ข้าพเจ้า นาง/นางสาว ..... ที่อยู่.....

.....

ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย ฉบับวันที่ .....

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย และสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่างๆ ที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

ชื่อ-นามสกุล ผู้เข้าร่วมในโครงการวิจัย .....

(.....) (ตัวบรรจง)

วัน..... เดือน..... พ.ศ.....

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สะดวกสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

ชื่อ-นามสกุล ผู้วิจัย .....

(.....) (ตัวบรรจง)

ชื่อ-นามสกุล พยาน .....

(.....) (ตัวบรรจง)

วัน..... เดือน..... พ.ศ.....

# APPENDIX B

# RESEARCH PROFILE (CONFIDENTIAL)

STUDY ID: 6552001022

**Patient Record Form**

**การศึกษาประสิทธิภาพการรักษาฝ้าด้วยสารสกัดส้มป่อย 10%**

Patient Record Form

General Information

1. Date_ _/_ _/2024
2. Patient No._ _ _ _
3. Date of birth _ _ _ _ _ _ _ _ _ _ _ _
4. Address _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
5. Gender a. Male b. Female
   5.1 Pregnancy or lactation 1. Yes 2. No
6. Occupation
   1) Government officer
   2) business owner
   3) Housewife
   4) Student
   5) Employee
   6) Others
   7) Specify _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
7. Underlying disease _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
8. Photosensitivity or Drug Induced Hypersensitivity 1. Yes 2. No
9. Personal medication and supplement
   1) Chemotherapy
   2) Active inflammatory skin disease, open wound in the treatment area
   3) History of malignant or premalignant lesions in the treatment area
10. History of food or drug allergy 1. Yes 2. No

If Yes, specify _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

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

If Yes, specify _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

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

If Yes, ablative and non-ablative laser

1) Intense pulse light
2) Microdermabrasion
3) Skin needling
4) Chemical peeling
5) Facial whitening treatment
6) Facial whitening agent

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

1. Yes 2. No

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

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

15. Wood's lamp histological type

1) Epidermal type
2) Mixed type(epidermal-dermal) type
3) Dermal type

16. Clinical pattern

1) Centro-facial type
2) Malar type
3) Mandibular type

## Side-effect Record Form

Side effects (+/-)

1. Redness ( )
2. Flaky ( )
3. Pain/ inflammation ( )
4. Itchy ( )
5. Acne ( )
6. Black patch/ ochronosis ( )

Please circle the scoring of severity


Score range from 0 to 3

0 = None

1 = Mild

2 = Moderate

3 = Severe

## Doctor Record Form

MASI score = ……

**AREA**

Area right side =……..

| **Area** | **Right Forehead 15%** | **Right malar 30%** | **Right chin 5%** |
|---|---|---|---|
| None=0 | | | |
| 0%-9%=1 | | | |
| 10%-29%=2 | | | |
| 30%-49%=3 | | | |
| 50%-69%=4 | | | |
| 70%-89%=5 | | | |
| 90%-100%=6 | | | |

MASI score = ……

Area left side= ……..

| **Area** | **Left Forehead 15%** | **Left malar 30%** | **Left chin 5%** |
|---|---|---|---|
| None=0 | | | |
| 0%-9%=1 | | | |
| 10%-29%=2 | | | |
| 30%-49%=3 | | | |
| 50%-69%=4 | | | |
| 70%-89%=5 | | | |
| 90%-100%=6 | | | |

**DARKNESS**

Darkness Right side =…….

| **Darkness** | **Right Forehead 15%** | **Right malar 30%** | **Right chin 5%** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Absent=0 | | | |
| Slight =1 | | | |
| Mild =2 | | | |
| Marked=3 | | | |
| Maximum=4 | | | |

Darkness Left side = ……

| **Darkness** | **Left Forehead 15%** | **Left malar 30%** | **Left chin 5%** |
|---|---|---|---|
| Absent=0 | | | |
| Slight =1 | | | |
| Mild =2 | | | |
| Marked=3 | | | |
| Maximum=4 | | | |

Melanin Index (MI) by Mexameter MX 18

Melanin Index right side

| **Baseline** | **2th week** | **4th week** | **6th week** | **8th week** |
|---|---|---|---|---|
| | | | | |

Melanin Index left side

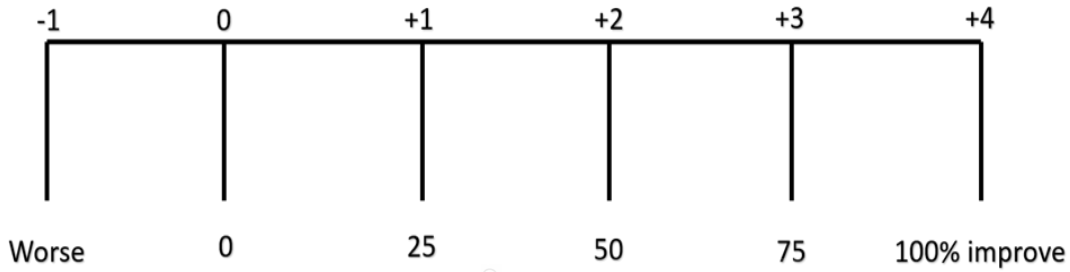
| **Baseline** | **2th week** | **4th week** | **6th week** | **8th week** |
|---|---|---|---|---|
| | | | | |

**Treatment of melasma Satisfaction questionnaires**

**การศึกษาประสิทธิภาพการรักษาฝ้าด้วยสารสกัดส้มป่อย 10%**

Global satisfactory score

Global satisfaction by dermatologist:(please draw the circle)




Score range from -1 to +4

-1 = Worse

0 = No changes

+1 = Improved -25% (fair)

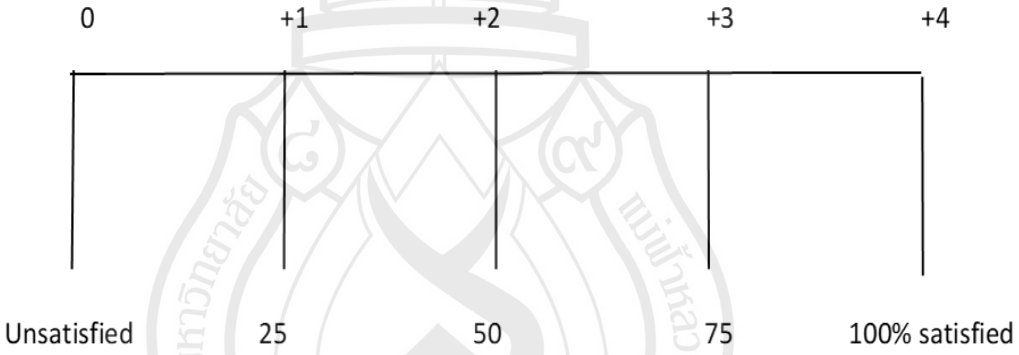
+2 = Improved 26-50% (moderate)

+3 = Improved 51-75% (good)

+4 = Improved 76-100% (excellent)

Satisfactory score

Satisfactory Evaluation by patients/volunteers (please draw the circle) on 8th week




Score range from 0 to +4

0 = No changes

+1 = less satisfied 1-25%

+2 = moderately satisfied 26-50%

+3 = very satisfied 51-75%

+4 = extremely satisfied 76-100%

# APPENDIX C

## STANDARDIZED PHOTOGRAPHS OF SUBJECTS


**Figure C1** Before and after 8 weeks of pictures of one of the volunteers who applied 10% Acacia concinna extract (right)

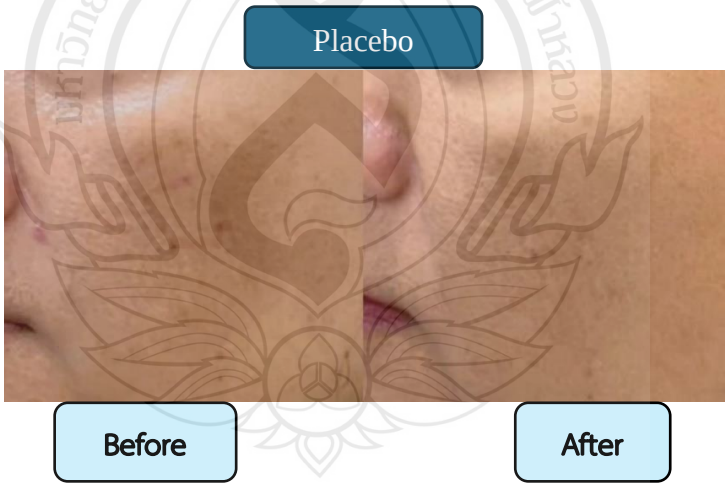



**Figure C2** Before and after 8 weeks of pictures of one of the volunteers who applied Placebo Spray (left)


**Figure C3** Before and after 8 weeks of pictures of one of the volunteers who applied 10% Acacia concinna extract (left)

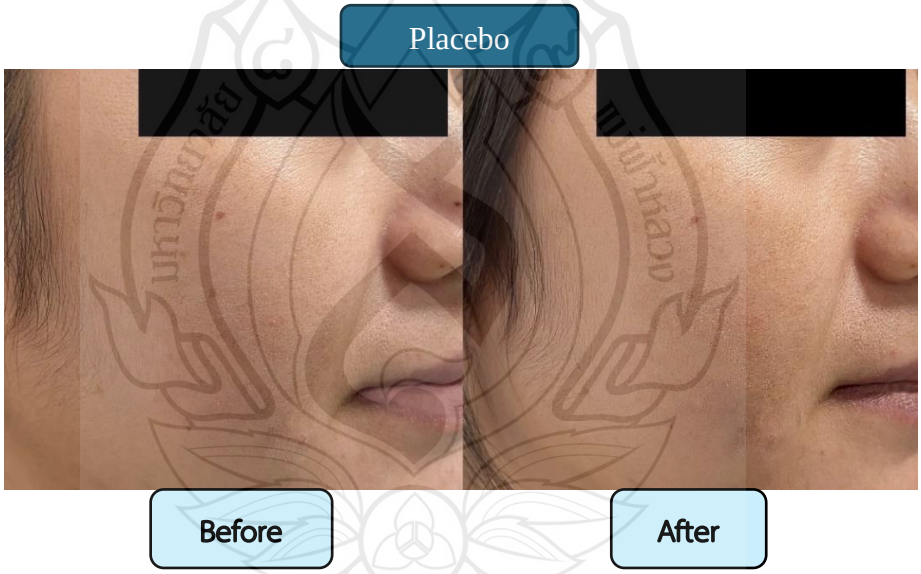



**Figure C4** Before and after 8 weeks of pictures of one of the volunteers who applied Placebo Spray (right)

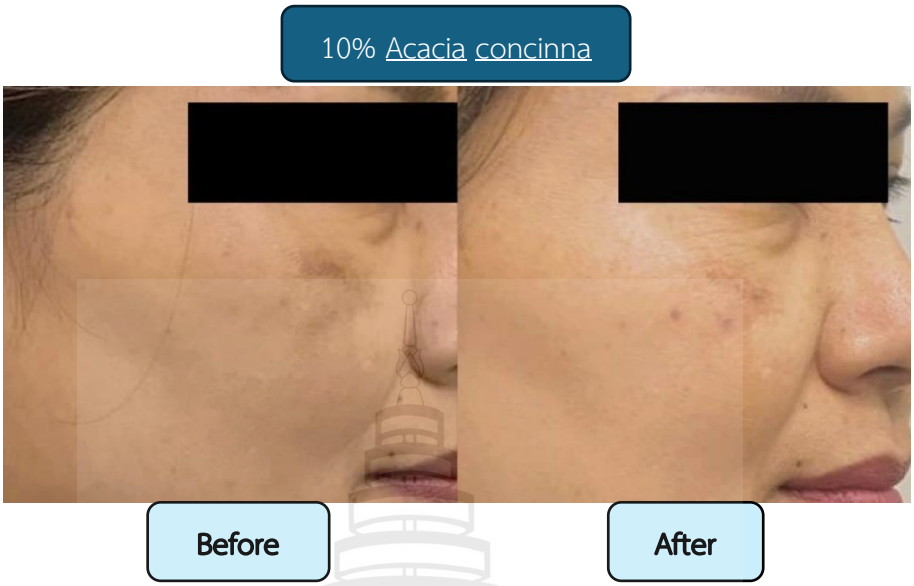



**Figure C5** Before and after 8 weeks of pictures of one of the volunteers who applied 10% Acacia concinna extract (right)

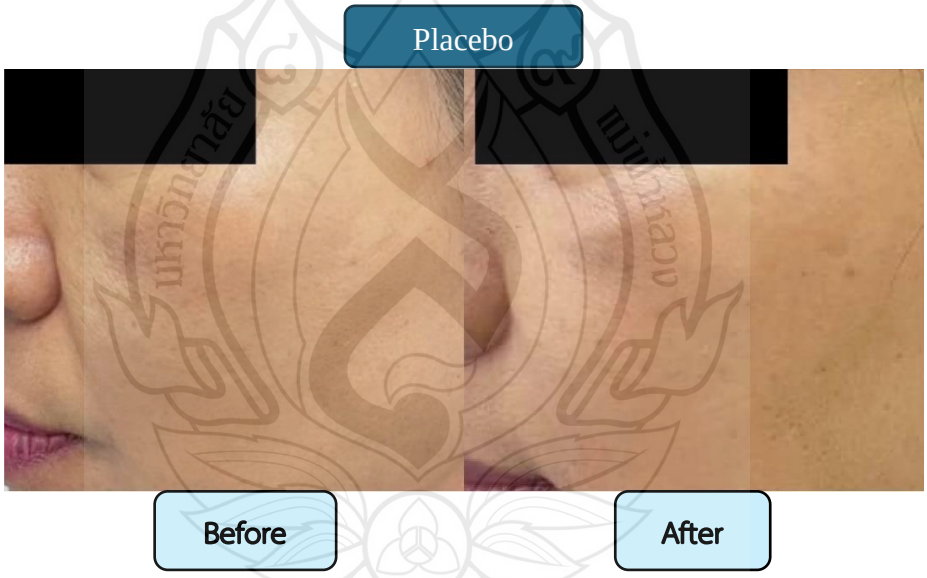



**Figure C6** Before and after 8 weeks of pictures of one of the volunteers who applied Placebo Spray (left)


**Figure C7** Before and after 8 weeks of pictures of one of the volunteers who applied 10% Acacia concinna extract (left)

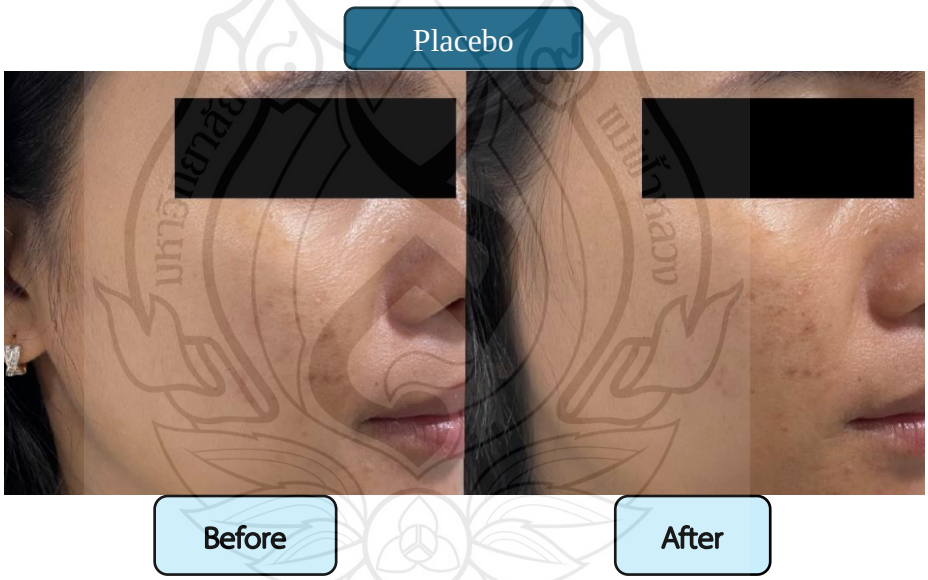



**Figure C8** Before and after 8 weeks of pictures of one of the volunteers who applied Placebo Spray (right)


**Figure C9** Before and after 8 weeks of pictures of one of the volunteers who applied 10% Acacia concinna extract (left)

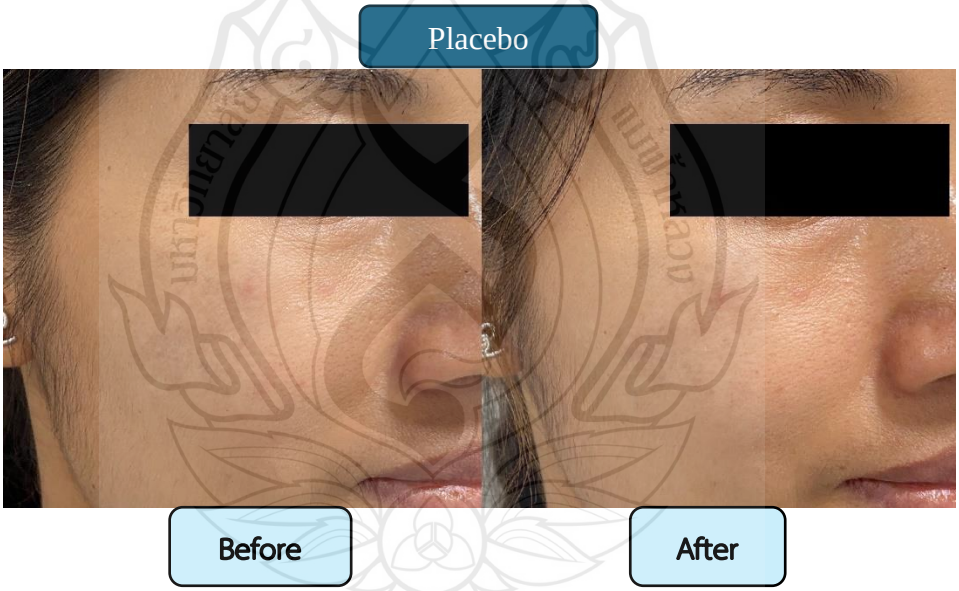



**Figure C10** Before and after 8 weeks of pictures of one of the volunteers who applied Placebo Spray (right)

# APPENDIX D

# MATERIAL


**Figure D1** Packaging of 10% Acacia concinna extract and placebo spray


**Figure D2** Packaging of 10% Acacia concinna extract and placebo spray

# APPENDIX E

# CLINICAL EVALUATION

**Table E1** Mean MASI scores of face applied with 10% Acacia concinna extract on each visit

| Number of subjects | Mean MASI Scores (N=30) | | | | |
|---|---|---|---|---|---|
| | 10% Acacia concinna extract | | | | |
| | **Baseline** | **2th week** | **4th week** | **6th week** | **8th week** |
| 1 | 4.6 | 4.6 | 4.55 | 4.55 | 3.35 |
| 2 | 3.9 | 3.9 | 3 | 3 | 2.3 |
| 3 | 2.8 | 2.8 | 2.8 | 2.2 | 2.05 |
| 4 | 3.9 | 3.9 | 3 | 3 | 2.6 |
| 5 | 2.2 | 2.2 | 2.2 | 1.6 | 1.6 |
| 6 | 2.8 | 2.8 | 2.8 | 2.45 | 2.45 |
| 7 | 3.95 | 3.95 | 3.95 | 3.8 | 2.6 |
| 8 | 2.9 | 2.9 | 2.9 | 2.8 | 2.75 |
| 9 | 2.6 | 2.6 | 2.6 | 2.5 | 2.2 |
| 10 | 4.85 | 4.85 | 4.85 | 3.05 | 2.75 |
| 11 | 2.3 | 2.3 | 2.3 | 2.2 | 1.55 |
| 12 | 2.4 | 2.4 | 1.8 | 1.8 | 1.6 |
| 13 | 3.5 | 3.5 | 3.5 | 2.6 | 2.6 |
| 14 | 2.6 | 2.6 | 2.6 | 2.5 | 2.5 |
| 15 | 2.6 | 2.6 | 2.6 | 2.15 | 1.55 |
| 16 | 2.3 | 2.3 | 2.3 | 2.15 | 2.05 |
| 17 | 2.3 | 2.3 | 2.3 | 1.85 | 1.75 |
| 18 | 3.25 | 3.25 | 3.25 | 3.2 | 3 |
| 19 | 2.9 | 2.9 | 2.9 | 2.6 | 2.6 |
| 20 | 1.9 | 1.9 | 1.9 | 1.75 | 1.15 |
| 21 | 3.7 | 3.7 | 3.7 | 2.8 | 2.8 |
| 22 | 2.6 | 2.6 | 2.6 | 2.3 | 2.3 |
| 23 | 2.9 | 2.9 | 2.9 | 2.6 | 2.6 |

**Table E1** (continued)

| Number of subjects | Mean MASI Scores (N=30) | | | | |
|---|---|---|---|---|---|
| | 10% Acacia concinna extract | | | | |
| | Baseline | 2th week | 4th week | 6th week | 8th week |
| 24 | 3.3 | 3.3 | 3.3 | 2.4 | 2.4 |
| 25 | 3.5 | 3.5 | 3.5 | 3.1 | 3.1 |
| 26 | 2.55 | 2.55 | 2.55 | 2.25 | 2.25 |
| 27 | 3.4 | 3.4 | 3.4 | 3.35 | 3.35 |
| 28 | 5.85 | 5.85 | 5.85 | 3.75 | 3.75 |
| 29 | 2.95 | 2.95 | 2.95 | 2.7 | 2.7 |
| 30 | 3.4 | 3.4 | 3.4 | 3.35 | 2.9 |

**Table E2** Mean MASI scores of face applied with Placebo on each visit

| Number of subjects | Mean MASI Scores (N=30) | | | | |
|---|---|---|---|---|---|
| | Placebo | | | | |
| | Baseline | 2th week | 4th week | 6th week | 8th week |
| 1 | 4.7 | 4.7 | 4.7 | 4.7 | 4.7 |
| 2 | 3.6 | 3.6 | 3.6 | 3.6 | 3.6 |
| 3 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 4 | 3.8 | 3.8 | 3.8 | 3.8 | 3.8 |
| 5 | 2.5 | 2.5 | 2.6 | 2.6 | 2.6 |
| 6 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| 7 | 4 | 4 | 4 | 4.3 | 4.3 |
| 8 | 2.8 | 2.8 | 2.8 | 2.9 | 2.9 |
| 9 | 2.3 | 2.3 | 2.3 | 2.3 | 2.3 |
| 10 | 4.6 | 4.6 | 4.6 | 4.6 | 4.6 |
| 11 | 2.5 | 2.5 | 2.5 | 2.6 | 2.6 |
| 12 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| 13 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 14 | 2.6 | 2.6 | 2.6 | 2.6 | 2.6 |
| 15 | 2.65 | 2.65 | 2.65 | 2.65 | 2.65 |
| 16 | 3.4 | 3.4 | 3.4 | 3.5 | 3.5 |
| 17 | 2.2 | 2.2 | 2.3 | 2.3 | 2.3 |
| 18 | 3.7 | 3.7 | 3.7 | 3.7 | 3.7 |

**Table E2** (continued)

| Number of subjects | Mean MASI Scores (N=30) | | | | |
|---|---|---|---|---|---|
| | Placebo | | | | |
| | **Baseline** | **2th week** | **4th week** | **6th week** | **8th week** |
| 19 | 2.6 | 2.6 | 2.6 | 2.9 | 2.9 |
| 20 | 2.2 | 2.2 | 2.2 | 2.2 | 2.5 |
| 21 | 4.1 | 4.1 | 4.1 | 4.4 | 4.4 |
| 22 | 2.6 | 2.6 | 2.6 | 2.6 | 2.6 |
| 23 | 2.9 | 2.9 | 2.9 | 2.9 | 2.9 |
| 24 | 2.4 | 2.4 | 2.4 | 2.4 | 2.4 |
| 25 | 2.9 | 2.9 | 2.9 | 2.9 | 2.9 |
| 26 | 2.55 | 2.55 | 2.55 | 2.55 | 2.55 |
| 27 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| 28 | 4.95 | 4.95 | 4.95 | 4.95 | 5.1 |
| 29 | 3.1 | 3.1 | 3.1 | 3.1 | 3.4 |
| 30 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |

**Table E3** Mean melanin index scores of face applied with 10% Acacia concinna extract on each visit

| Number of subjects | Mean Melanin index (N=30) | | | | |
|---|---|---|---|---|---|
| | 10% Acacia concinna extract | | | | |
| | **Baseline** | **2th week** | **4th week** | **6th week** | **8th week** |
| 1 | 232 | 226 | 217 | 211 | 197 |
| 2 | 221 | 217 | 203 | 198 | 184 |
| 3 | 175 | 164 | 156 | 142 | 137 |
| 4 | 213 | 208 | 202 | 188 | 179 |
| 5 | 196 | 191 | 182 | 171 | 163 |
| 6 | 185 | 181 | 169 | 155 | 147 |
| 7 | 229 | 218 | 210 | 196 | 189 |
| 8 | 232 | 228 | 216 | 198 | 193 |
| 9 | 187 | 182 | 174 | 168 | 157 |
| 10 | 230 | 216 | 203 | 192 | 187 |
| 11 | 172 | 165 | 158 | 149 | 138 |

**Table E3** (continued)

| Number of subjects | Mean Melanin index (N=30) | | | | |
|---|---|---|---|---|---|
| | 10% Acacia concinna extract | | | | |
| | Baseline | 2th week | 4th week | 6th week | 8th week |
| 12 | 215 | 211 | 198 | 190 | 181 |
| 13 | 212 | 201 | 193 | 181 | 176 |
| 14 | 201 | 195 | 188 | 180 | 169 |
| 15 | 205 | 198 | 186 | 180 | 172 |
| 16 | 192 | 181 | 173 | 166 | 159 |
| 17 | 210 | 203 | 192 | 185 | 180 |
| 18 | 202 | 193 | 181 | 175 | 170 |
| 19 | 220 | 211 | 203 | 197 | 188 |
| 20 | 208 | 201 | 189 | 181 | 272 |
| 21 | 217 | 210 | 201 | 193 | 184 |
| 22 | 201 | 194 | 184 | 179 | 170 |
| 23 | 214 | 202 | 194 | 188 | 181 |
| 24 | 225 | 214 | 206 | 194 | 188 |
| 25 | 224 | 212 | 202 | 192 | 187 |
| 26 | 240 | 234 | 220 | 208 | 202 |
| 27 | 223 | 215 | 210 | 203 | 189 |
| 28 | 283 | 277 | 268 | 262 | 250 |
| 29 | 219 | 212 | 205 | 193 | 188 |
| 30 | 216 | 210 | 202 | 189 | 183 |

**Table E4** Mean melanin index scores of face applied with placebo on each visit

| Number of subjects | Mean Melanin index (N=30) | | | | |
|---|---|---|---|---|---|
| | Placebo | | | | |
| | Baseline | 2th week | 4th week | 6th week | 8th week |
| 1 | 252 | 252 | 252 | 252 | 252 |
| 2 | 240 | 225 | 225 | 225 | 225 |
| 3 | 220 | 220 | 220 | 220 | 220 |
| 4 | 242 | 242 | 242 | 242 | 242 |
| 5 | 194 | 194 | 206 | 206 | 206 |
| 6 | 207 | 207 | 207 | 207 | 207 |
| 7 | 208 | 208 | 208 | 236 | 236 |
| 8 | 201 | 201 | 201 | 212 | 212 |
| 9 | 203 | 203 | 203 | 203 | 203 |
| 10 | 234 | 234 | 234 | 234 | 234 |
| 11 | 192 | 192 | 192 | 204 | 204 |
| 12 | 213 | 213 | 213 | 213 | 213 |
| 13 | 215 | 215 | 215 | 215 | 215 |
| 14 | 202 | 202 | 202 | 202 | 202 |
| 15 | 206 | 206 | 206 | 206 | 206 |
| 16 | 208 | 208 | 208 | 217 | 217 |
| 17 | 198 | 198 | 204 | 204 | 204 |
| 18 | 210 | 210 | 210 | 210 | 210 |
| 19 | 205 | 205 | 205 | 214 | 214 |
| 20 | 212 | 212 | 212 | 212 | 228 |
| 21 | 226 | 226 | 226 | 237 | 237 |
| 22 | 202 | 202 | 202 | 202 | 202 |
| 23 | 212 | 212 | 212 | 212 | 212 |
| 24 | 214 | 214 | 214 | 214 | 214 |
| 25 | 216 | 216 | 216 | 216 | 216 |
| 26 | 222 | 222 | 222 | 222 | 222 |
| 27 | 232 | 232 | 232 | 232 | 232 |
| 28 | 252 | 252 | 252 | 252 | 281 |
| 29 | 224 | 224 | 224 | 224 | 238 |
| 30 | 229 | 229 | 229 | 229 | 229 |

**Table E5** Dermatologists evaluation scores for **10% <u>Acacia</u> <u>concinna</u> extract** on $2^{th}$, $4^{th}$, $6^{th}$ and $8^{th}$ week

| No. | Dermatologists evaluation scores for 10% Acacia concinna extract | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-2 week | | | 0-4 week | | | 0-6 week | | | 0-8 week | | |
| | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 |
| 1 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 |
| 2 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 |
| 3 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 2 | 2 |
| 4 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 5 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 |
| 6 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 3 |
| 7 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 3 | 2 | 2 |
| 8 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 2 |
| 9 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 |
| 10 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 3 |
| 11 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 12 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 13 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 2 |
| 14 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 15 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 16 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 |
| 17 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 |
| 18 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 |
| 19 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 |
| 20 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 21 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 22 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 23 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 24 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 3 |
| 25 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 2 | 2 |
| 26 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 2 |
| 27 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 28 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 2 | 1 | 2 | 2 | 2 |
| 29 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 30 | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 2 |

**Table E6** Dermatologists evaluation scores for Placebo on 2th,4th,6th and 8th week

| No. | Dermatologists evaluation scores for Placebo | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0-2 week | | | 0-4 week | | | 0-6 week | | | 0-8 week | | |
| | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 | Doc 1 | Doc 2 | Doc 3 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table E7** Patient satisfaction scores on 8th week compared on both sides applied with 10% Acacia concinna extract and placebo

| Number of patients (n) | Patient Satisfaction Scores | |
|---|---|---|
| | **10% Acacia concinna extract** | **Placebo** |
| 1 | 2 | 0 |
| 2 | 2 | 0 |
| 3 | 2 | 1 |
| 4 | 2 | 0 |
| 5 | 2 | 0 |
| 6 | 2 | 0 |
| 7 | 2 | 0 |
| 8 | 2 | 1 |
| 9 | 2 | 0 |
| 10 | 2 | 0 |
| 11 | 2 | 0 |
| 12 | 2 | 0 |
| 13 | 2 | 1 |
| 14 | 2 | 0 |
| 15 | 2 | 0 |
| 16 | 2 | 0 |
| 17 | 2 | 0 |
| 18 | 2 | 0 |
| 19 | 2 | 0 |
| 20 | 2 | 0 |
| 21 | 2 | 0 |
| 22 | 2 | 0 |
| 23 | 3 | 1 |
| 24 | 2 | 0 |
| 25 | 2 | 1 |
| 26 | 2 | 0 |
| 27 | 2 | 0 |
| 28 | 2 | 0 |
| 29 | 2 | 0 |
| 30 | 2 | 0 |

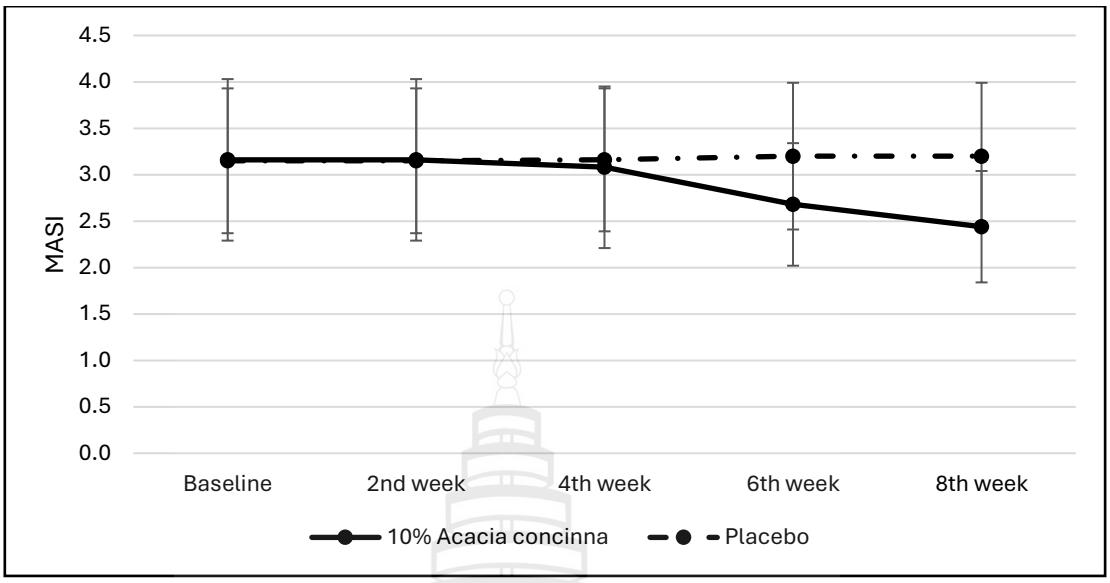



**Figure E1** Displays a line graph showing the Melasma Area and Severity Index (MASI) for the 10% Acacia concinna and placebo side from baseline to the follow-up 8th week

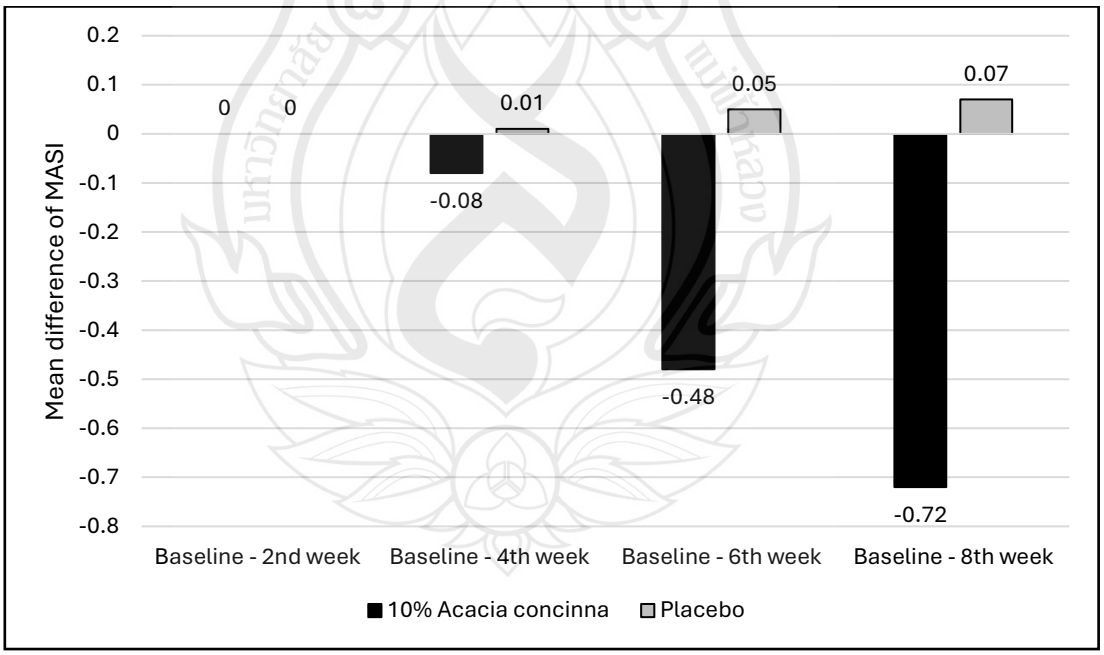



**Figure E2** Displays a bar graph showing the mean difference of MASI at the follow-up 2nd, 4th, 6th, and 8th week with baseline for the 10% Acacia concinna and placebo side

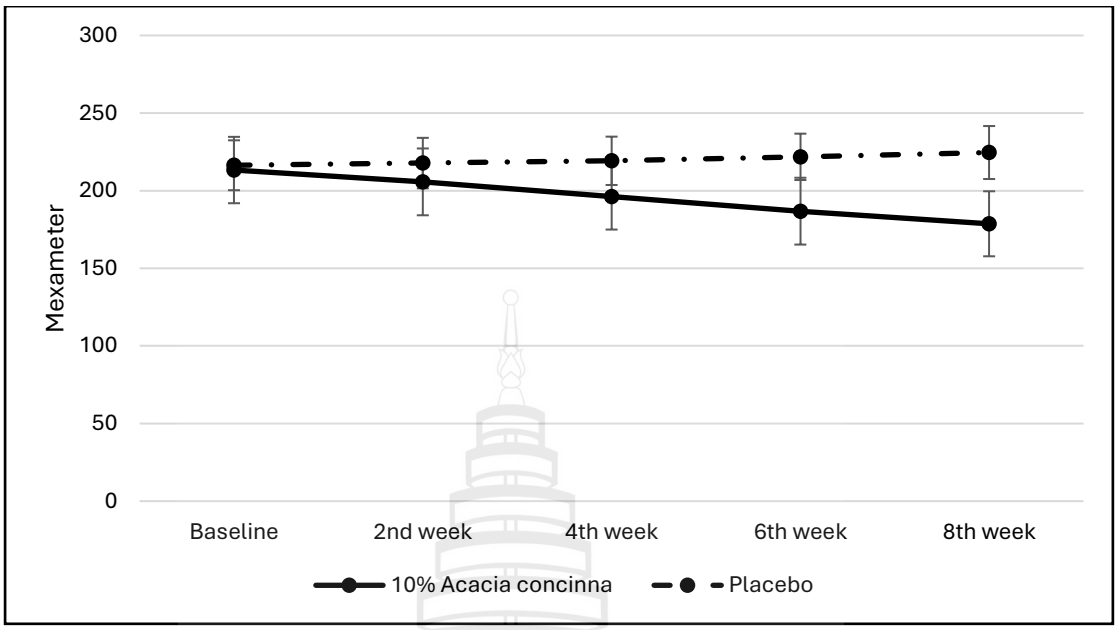



**Figure E3** Displays a line graph showing the Mexameter for 10% Acacia concinna and placebo side from baseline to the follow-up 8th week

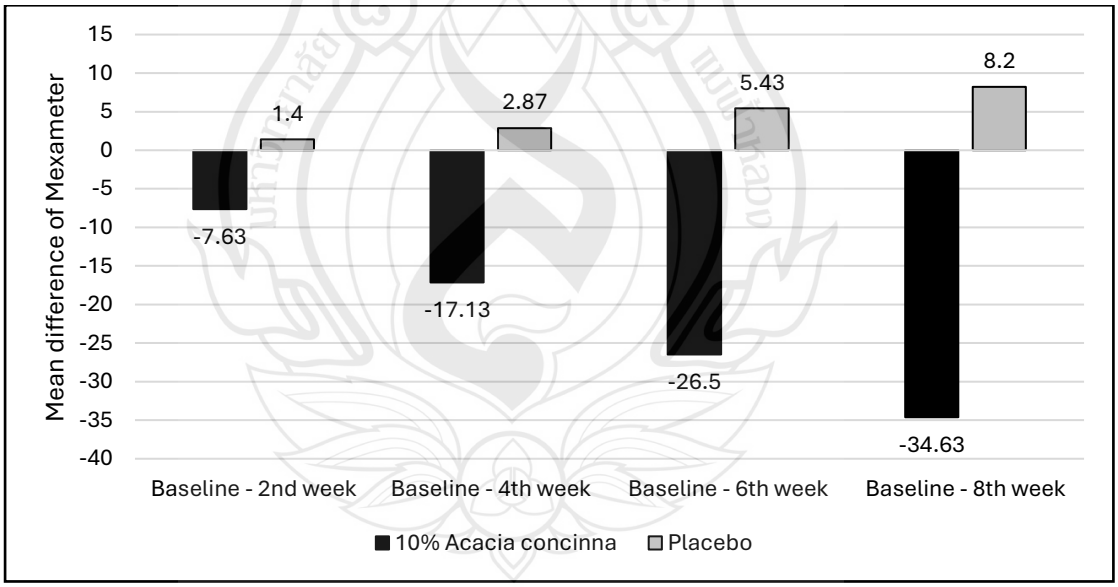



**Figure E4** Displays a bar graph showing the mean difference of mexameter at the follow-up 2nd, 4th, 6th, and 8th week compared with baseline for the 10% Acacia concinna and placebo side

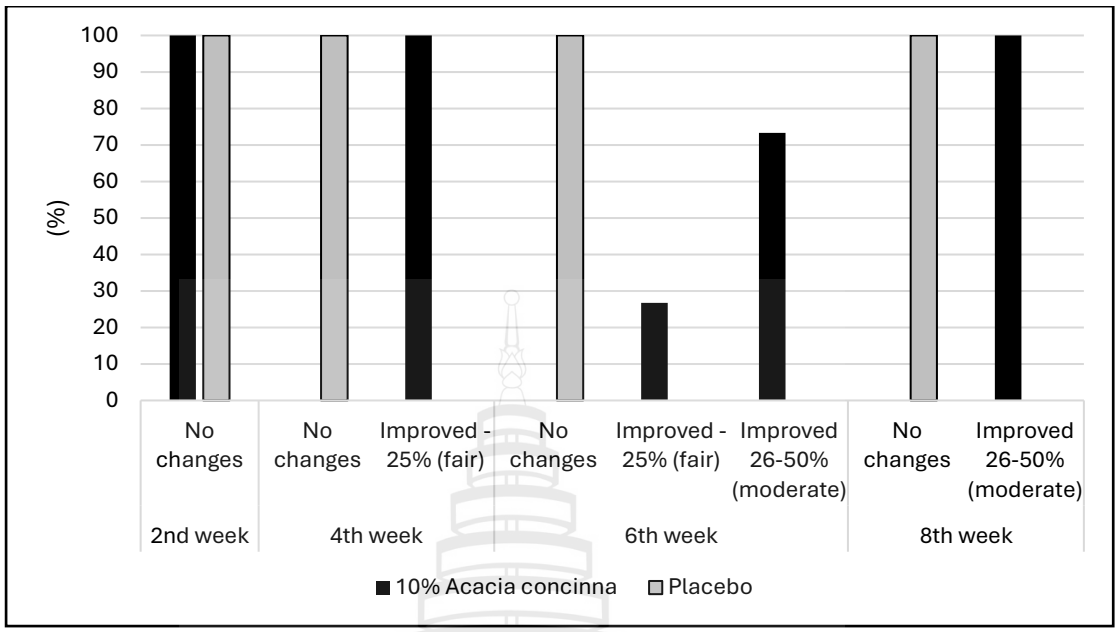



**Figure E5** Displays a bar graph showing the global satisfactory scale for 10% Acacia concinna and placebo side at the follow-up 2nd, 4th, 6th, and 8th week

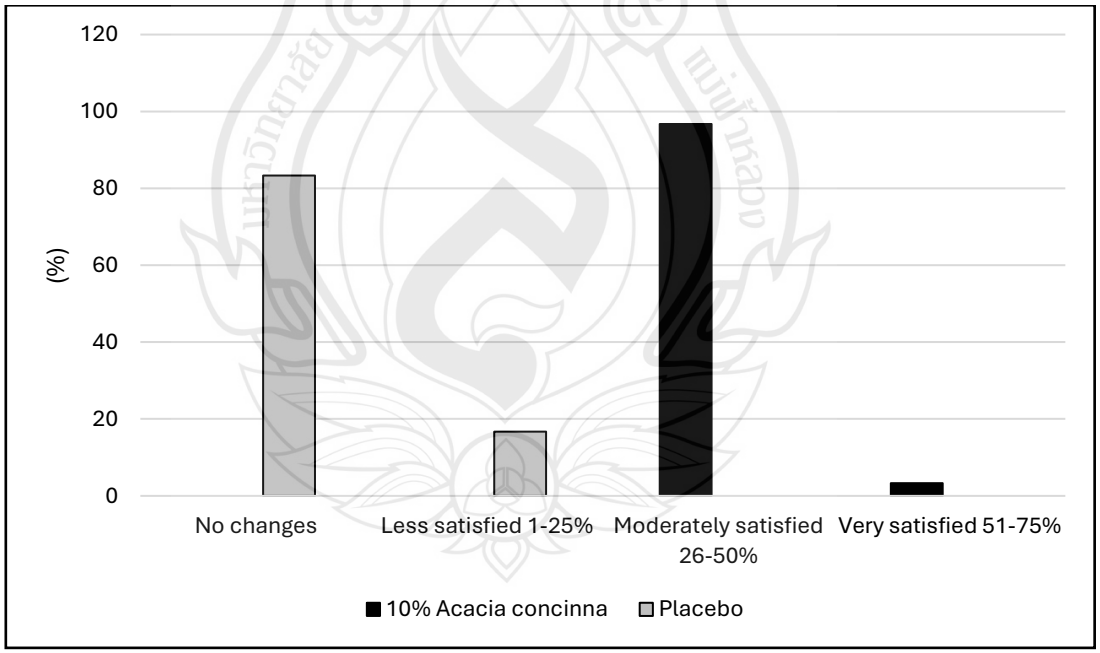



**Figure E6** Displays a bar graph showing the satisfactory scale for 10% Acacia concinna and placebo side at the follow-up 8th week

# CURRICULUM VITAE

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| **NAME** | Thaweeporn Treepraphakorn |
| **EDUCATIONAL BACKGROUND** | |
| 2007 - 2013 | Doctor of Medicine, Chiang Mai University |
| **WORK EXPERIENCE** | |
| September 2021 | Diploma American Board of Hair Restoration Surgery (ABHRS) |
| July 2015 - March 2021 | Lannawadee clinic |
| July 2015 - June 2016 | Nakhon Ping Chiang Mai Hospital, Thailand |
| October 2013 - June 2015 | Studied Fellowship Cosmetic Surgery, Korean College of Cosmetic Surgery (KCCS), South Korea |
| May 2013 - October 2013 | Lampang Regional Hospital, Thailand |
| **ACADEMIC CONFERENCE ATTENDED** | |
| April 2024 | Hand on Filler injection in Allergan Medical Institute (Mid Face) |
| January 2024 | Hand on Filler injection in Allergan Medical Institute (Lower Face) |
| June 2023 | ISHRS Europe 2023 Live Surgery Workshop, Manchester, United Kingdom |
| February 2023 | 2nd ATAP International Congress “The Aesthetics of a better future 2023” |
| November 2022 | Annual meeting “Pearls and Pitfalls” in Dermatology |
| October 2022 | Hand on Filler injection in Allergan Medical Institute |
| September 2022 | 12th Annual meeting of TAARM “How to live in the Covid-19 Era” |

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| May 2022 | Hand on Filler injection in Allergan Medical Institute (Upper Face) |
| March 2022 | World Congress of Aesthetic Surgery, Antiaging Medicine |
| October 2021 | 29th World Congress International Society of Hair Restoration Surgery, Lisbon, Portugal |
| May 2021 | ISHRS 2021 Advanced / Board Review Course |
| April 2021 | Basic Life Support, National CPR Foundation, USA |
| October 2020 | 28th World Congress International Society of Hair Restoration Surgery |
| February 2020 | Professional Aesthetic Surgery, Cosmetic & Anti-aging Leader |
| November 2019 | 27th World Congress International Society of Hair Restoration Surgery |
| May-August2019 | Recent Advances in Aesthetic Dermatology |
| February 2019 | Rhinoplasty LIVE Surgery Workshop, 35th Conference of KSKCS, Korea |
| December 2018 | 3rd American Board of Laser Surgery in AEC Symposium |
| November 2018 | 3rd ASEAN Meeting of Aesthetic Surgery and Medicine (AMAS) |
| September 2018 | 10th A4M Congress on Anti-Aging and Aesthetic Medicine |
| June 2018 | International Congress and Live Surgery of the Korean Society of Hair Restoration Surgery (KSHRS 2018), Korea |
| March 2018 | 43th Dermatological Society of Thailand annual scientific meeting |

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|---|---|
| November 2017 | 38th Annual meeting of the International Society for Dematologic and Aesthetic Surgery (ISDS) |
| August 2017 | Aesthetic Anti-aging Surgery Congress (A2S) |
| June 2017 | Hand on Cosmetic Surgery at Teerawat Hospital, Thailand |
| May 2017 | Attended International Congress and Live Surgery of the Korean Society of Hair Restoration Surgery (KSHRS 2017), Korea |
| October 2016 | Asian Fat Congress Live Surgery Workshop, Seoul, Korea |
| October 2016 | Ramathibodi Training Course in Cosmetic Dermatology |
| December 2015 | Blepharo-Rhinoplasty Workshop & Hand on Course, Hong Kong |