THE EFFICACY AND SAFETY OF TOPICAL HEMP SEED EXTRACT IN TREATMENT OF ACNE VULGARIS: A SPLIT-FACE, DOUBLE-BLINDED, RANDOMIZED, CONTROLLED TRIAL

BY

PURIDA JARUTHITI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (DERMATOLOGY) CHULABHORN INTERNATIONAL COLLEGE OF MEDICINE THAMMASAT UNIVERSITY ACADEMIC YEAR 2021

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Was approved as partial fulfillment of the requirements for
the degree of Master of Science (Dermatology)

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Thesis Title	THE EFFICACY AND SAFETY OF TOPICAL HEMP SEED EXTRACT IN TREATMENT OF ACNE VULGARIS: A SPLIT-FACE, DOUBLE-BLINDED, RANDOMIZED, CONTROLLED TRIAL
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ABSTRACT

A prevalent chronic inflammatory skin condition affecting teenagers and young adults is acne vulgaris. The disease may have a negative influence on wellbeing in both physical and psychological ways. Treatment of acne vulgaris is depends on disease severity. Currently, first-line medical therapy for acne vulgaris mostly come with side effects varies from irritation to teratogenicity.

Cannabidiol (CBD), one of the most well-known phytocannabinoid from *Cannabis Sativa* L. plant, is shown to have an ability to cure acne vulgaris by its antilipogenic, anti-proliferative and anti-inflammatory effects. Several studies have been proven that hemp seed and its extract contain CBD as a major composition of all phytocannabinoids. Recent studies about potential of CBD and hemp seed extract for treating acne vulgaris is still in vitro, but lack of clinical trial in human.

Therefore, this study compares the effectiveness of topical hemp seed extract to a placebo in the treatment of acne vulgaris. For a period of 12 weeks, topical treatments will be given twice daily to either the left or right side of their faces, at random. The result will be interpreted objectively by acne lesion count, erythema index, sebum level and subjectively by physichian's clinical assessment score and patient's satisfaction score.

The result revealed a statistically decreased of acne lesion count particularly in inflammatory acne lesions. Moreover, sebum level was also found significantly

reduction by decreased 44.79% from baseline. However, contrasting from previous research, we established that there was no significant improvement in erythema index. Adverse events found in hemp seed extract cream were mild, including skin dryness and itchiness. There was no serious adverse effects presented in both interventions. In conclusion, hemp seed extract cream is safe and effective alternative treatment option for mild to moderate acne vulgaris.

Keywords: Acne vulgaris, Cannabidiol, CBD, hemp seed, hemp seed extract, hempseed oil

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LIST OF ABBREVIATIONS

Symbols/Abbreviations	Terms
AEA	Anandamide
ALA	Aminolevulinic acid
BP	Benzoyl peroxide
CAMs	Complementary and alternative medications
CB	Cannabinoid
CBC	Cannabichromene
CBD	Cannabidiol
CBDV	Cannabidivarin
CBG	Cannabigerol
CBGV	Cannabigovarin
COCs	Combined oral conceptive
EAE	Encephalomyelitis
ECBs	Endocannabinoids
ECS	Endocannabinoids system
ENT	equilibrative nucleoside transporter
Etc.	(et cetera; Latin) and the other things
Et al.	(et alia; Latin) and others
FDA	Food and Drug Administration
GI	Glycemic index
GL	Glycemic load
IGF-1	Insulin like growth-factor 1
MAL	Methyl- aminolevulinic acid
n	sample size
NRIP	Nuclear receptor interacting protein
PASI	Psoriasis Area and Severity Index
PCBs	Phytocannabinoids
PEA	Palmitoyl ethanolamide
PPAR	Peroxisome proliferative activated receptor

Symbols/Abbreviations	Terms
SCs	Synthetic cannabinoids
THC	Tetrahydrocannabinol
THCV	Tetrahydrocannabivarin
TLR	Toll like receptor

CHAPTER 1
INTRODUCTION

1.1 Background and rational

Acne vulgaris is a common inflammatory disease of the pilosebaceous unit. It is found in about 85% of adolescents and can persist into adulthood. Not only cause physical destruction but acne can also cause psychosocial discomfort to patients due to chronic inflammation and scarring [1].

It is generally recognized that acne is a complicated multifactorial illness; psychological stress, changes in hormonal and nutritional status, and other factors can cause the development and aggravation of the symptoms [2]. The increase sebum production, follicular hyperkeratinization, Propionibacterium acne proliferation and inflammation are four main pathological factors of developing acne vulgaris [1].

The intensity and degree of inflammation are typically taken into account while treating acne clinically. In addition, skin type, acne severity, and previous acne scars may all be considered in making therapy recommendations for acne. Acne may be treated in a variety of ways, such as with appropriate skin care, benzoyl peroxide, topical and oral antibiotics, and topical and oral retinoids [3]. Unfortunately, most of the treatment modalities cannot treat acne by suppress all of the pathological factors and they also have some side effect ranges from irritation to teratogenicity.

Cannabis-based skincare products have been increasingly popular on the market recently. A broad spectrum of substances known as cannabinoids are structurally and biochemically identical to the main psychoactive component obtained from Cannabis sativa delta(9)-tetrahydrocannabinol (THC). Endocannabinoids, which are generated by humans naturally, phytocannabinoids, which are found in the cannabis plant, and synthetic cannabinoids, which are created in laboratories, are the three primary kinds of cannabinoids [4, 5].

Tetrahydrocannabinol (THC) and cannabidiol are two of the most widely available phytocannabinoids (CBD). Unlike THC, CBD has no psychoactive effects. According to recent researches, CBD may be helpful in treating a variety of skin

conditions, especially atopic dermatitis, persistent pruritus, and acne [6]. Furthermore, several studies indicated that CBD could also have ability for treating acne vulgaris due to its anti-lipogenic, antiproliferative, anti-inflammatory and antimicrobial properties [7, 8].

Hemp seed and its extract have been proven that mainly contain CBD than other phytocannabinoids [9, 10]. Additionally, a human clinical investigation of cannabis seed extract revealed a considerably lower sebum and erythema levels in cheek skin. The researchers believed that cannabis seeds extract might be valuable in management of acne vulgaris [8].

By far, there are many studies about potential of CBD and hempseed extract for treating acne vulgaris in vitro. However, there is little clinical evidence to back up their usage. Result from its property of CBD in hemp seed extract, we decided to find out how well and safely topical hempseed extract worked for treating acne vulgaris.

1.2 Objectives and hypothesis

1.2.1 Primary objective

To evaluate the efficacy of topical hemp seed extract in the treatment of acne vulgaris.

1.2.2 Secondary objectives

1.2.2.1 To evaluate the safety of topical hemp seed extract in the treatment of acne vulgaris.

1.2.2.2 To evaluate the efficacy of topical hemp seed extract in sebum reduction.

1.2.3 Hypothesis

Topical hemp seed extract might be more effective in the treatment of acne vulgaris compare to placebo.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Acne vulgaris

2.1.1 Epidemiology

Teenagers and young adults frequently suffer from the persistent, inflammatory skin condition acne vulgaris. The ailment is not life-threatening, but therefore can be both physiologic and psychologic disease burden.

Acne vulgaris affects 85% of adolescents, and may persist into adulthood. For females, the average age of acne beginning is 11, while for boys, it is 12. Due to the earlier development of the condition, younger people have recently been shown to have acne. Boys are tending to have prevalence of acne more than girls. Despite the fact that the frequency of acne declines with age after adolescence. Instead of a newly developed acne illness, adult acne often refers to chronic, persistent acne from adolescence [3]. However, the persistent nature of many acne instances may result in discomfort, mental distress, deformity, and even permanent skin scars [1].

2.1.2 Etiology and pathogenesis

The pathogenesis of acne is complex of multifactorial. Over the past decade, we believed that there are 4 main pathogenic factors of acne include, follicular hyperkeratinization, excessive sebum production, Propionibacterium acne (Presently known as Cutibacterium acnes) proliferation, and inflammation [11]. Follicular hyperkeratinization or follicular plugging results from abnormal differentiate of follicular epithelial cell. This process leads to the development of microcomedone which then progressively enlarge to form noninflammatory comedones [3]. Sebum is a nutrient source for *C. acnes*. The main component of sebum, triglycerides, is important in acne pathogenesis. Triglycerides are broken down into free fatty acid and promote *C. acnes* colonization and induction of inflammation. Lipoperoxides, another component in sebum, can increase sebum production through peroxisome proliferator-activated receptor (PPAR) pathway [12]. Androgen hormones especially testosterone leads to increase sebum production. Acne vulgaris is more prone to develop in

association with androgen-rich conditions such polycystic ovarian syndrome, congenital adrenal hyperplasia, and different endocrine malignancies [3]. When the immune system discovers C. acnes, the inflammatory process starts. Inflammatory lesions develop when chemotactic agents like neutrophils, lymphocytes, and macrophages are delivered [1].

2.1.3 Clinical manifestation

The face, back, chest, and shoulders are areas with highly developed sebaceous glands where acne vulgaris is most commonly observed. [13]. Clinical manifestation of acne is characterized by noninflammatory lesion or comedone and inflammatory lesion. Both types of acne lesions can also find together in one location. Whiteheads or close comedone is characterized by a slightly elevated whitish papule without follicular opening. Whereas blackhead or close comedone appears as a flat or slightly elevated skin with visible black central orifice due to follicular plugging. Consequently, inflammatory lesions range in size from tiny erythematous papules and pustules to substantial nodules [14]. Scar and post-inflammatory hyperpigmentation are consequence of acne. Although the pigmentary changes can be improved spontaneously over time but unfortunately, scar either hypertrophic or atrophic can be permanent [15].

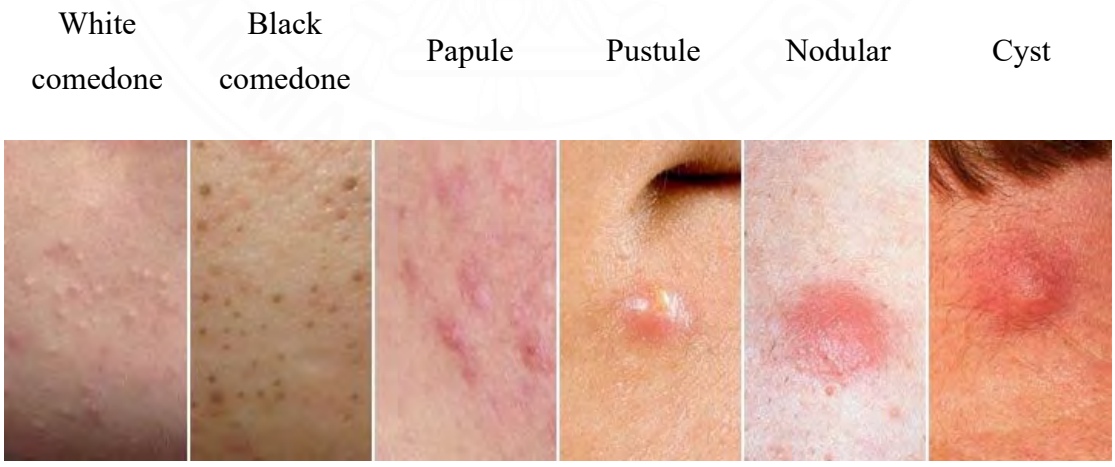

Figure 2.1 Clinical manifestation of acne [16]

2.1.4 Disease severity assessment

There are several ways to estimate the severity of acne vulgaris, ranging from straightforward techniques like counting the number of lesions to complex tools like polarized light photography, fluorescent photography, and photography [17]. Acne lesion counting and grading system are two commonly used methods to assess acne severity. Lesion counting has good accuracy, but also time consume. Acne severity may be graded accurately using the Global Acne Grading System (GAGS) and Investigator Global Assessment of acne (IGA).

2.1.4.1 Investigator Global Assessment of acne (IGA)

Investigator Global Assessment of Acne was approved by the American Food and Drug Administration (FDA) in 2018 as a technique for assessing the severity of face acne [18]. According on the morphology of acne on the face, the score ranges from 0 to 4. The grading scale typically defined as:

0 : Clear (no acne lesion)

1 : almost clear (few comedones and few papules)

2 : mild (some comedones, papules and pustules involved less than half of the face)

3 : moderate (more than half of the face is involved with comedones, papules and pustules or present of a single nodule)

4 : severe (entire face is involved with comedones, papules and pustules or present of nodulocystic acne)

2.1.4.2 Global Acne Grading System (GAGS)

The GAGS contemplate six areas on the face, including the chest and upper back, assigning a surface area factor (forehead = 2, right cheek = 2, left cheek = 2, nose = 1, chin = 1, chest and upper back = 3), as well as distribution and density of pilosebaceous units, to each place. Each of the six locations would be given a grade according to type of the most severe lesion (0 = No lesion, 1 = ≥ one comedone, 2 = ≥ one papule, 3 = ≥ one pustule, 4 = ≥ one nodule). The grade (0-4) multiplied by factor of each location gives the local score. The global score is then calculated by adding up the local scores. Thus, global score of 1-18 indicates mild acne vulgaris, score of 19-30 as moderate, score of 31-38 as severe and score more than 38 as very severe acne vulgaris [18, 19].

Location	Factor × Grade (0–4)* = Local score
I Forehead	2
II Right cheek	2
III Left cheek	2
IV Nose	1
V Chin	1
VI Chest and upper back	3

Global score = ____

0	None
1–18	Mild
19–30	Moderate
31–38	Severe
>39	Very severe

*0, No lesions; 1, ⩾ one comedone; 2, ⩾ one papule; 3, ⩾ one pustule; 4, ⩾ one nodule.

Figure 2.2 Global Acne Grading System (GAGS) [19]

2.1.4.3 Dermatology Life Quality Index (DLQI)

A 10-item questionnaire called the Dermatology Life Quality Index (DLQI) is frequently used to assess how well dermatology patients have thriving with their skin conditions. Each question is score from 0 to 3 and the sum of the item is the DLQI total score, which ranges from 0 (no impact of skin disease in quality of life) to 30 (maximum implications for quality of life) [18, 20]. The interpretation of DLQI implies from total score as:

0-1 : no effect on patient's life

2-5 : small effect on patient's life

6-10 : moderate effect on patient's life

11-20 : very large effect on patient's life

21-30 : extremely large effect on patient's life

2.1.4.4 Cardiff Acne Disability Index (CADI)

A 5-item questionnaire called the Cardiff Acne Disability Index (CADI) is used to determine how acne affects a person's quality of life. [19]. Each question is score from 0-3. The sum of the score could be range from 0-15. The worse the quality of life, the higher the score. (grade of impairment; 0 no impairment, 1-5 mild impairment, 6-10 moderate impairment, and 11-15 severe impairment) [18, 21].

2.1.5 Treatment

Treatment options for acne vulgaris, according to American Academy of Dermatology's 2017 treatment guidelines, depend on how severe the condition is [22].

Among mild acne vulgaris, first line treatment recommended mainly consist of topical treatments, include topical retinoid or benzoyl peroxide alone, or a combination of benzoyl peroxide and topical antibiotic (erythromycin or clindamycin); benzoyl peroxide and topical retinoid; benzoyl peroxide and both topical antibiotic and topical retinoid [22, 23].

A topical combination therapy, such as one that combines benzoyl peroxide with a topical antibiotic, topical retinoid, or both antibiotic and retinoid, is the first line of treatment for moderate acne vulgaris. Oral antibiotics also contribute to the management of moderate acne vulgaris. Either a combination of benzoyl peroxide, topical retinoid and oral antibiotic or a combination of benzoyl peroxide, topical retinoid, oral and topical antibiotics are also treatment regimen for moderate acne vulgaris [22, 23].

For severe acne vulgaris, oral isotretinoin is thought to be the best option. Moreover, a combination of oral antibiotics, benzoyl peroxide, and topical antibiotic; oral antibiotics, benzoyl peroxide, and topical retinoid; oral antibiotics, benzoyl peroxide, topical antibiotic and topical retinoid are also considered to be the first line treatment for severe acne vulgaris [22, 23].

According to the guideline for management of acne vulgaris, which can conclude that a combination therapy should be given to treat acne vulgaris due to It may aim at several acne etiology factors. As a result, combination therapy is superior to monotherapy in the treatment of acne vulgaris.

Table 2.1 First line treatment for acne vulgaris [23]

	Mild	Moderate	Severe
1st Line Treatment	Benzoyl Peroxide (BP) or Topical Retinoid -or- Topical Combination Therapy** BP + Antibiotic or Retinoid + BP or Retinoid + BP + Antibiotic	Topical Combination Therapy** BP + Antibiotic or Retinoid + BP or Retinoid + BP + Antibiotic -or- Oral Antibiotic + Topical Retinoid + BP -or- Oral Antibiotic + Topical Retinoid + BP + Topical Antibiotic	Oral Antibiotic + Topical Combination Therapy** BP + Antibiotic or Retinoid + BP or Retinoid + BP + Antibiotic -or- Oral Isotretinoin

2.1.5.1 Pharmacological treatment

(1) Retinoid

The US Food and Drug Administration (FDA) has authorized retinoid, a class of vitamin A derivatives, for the topical or oral treatment of acne vulgaris[24].

For mild to severe acne vulgaris, topical retinoids are the initial line of treatment. Due to their anti-comedogenic and anti-inflammatory properties, topical retinoids including adapalene, tretinoin, and tazarotene help reduce the severity of acne [25]. However, tazarotene is illegal for acne treatment in UK. The use of topical retinoids alone as monotherapy or in combination with other therapeutic modalities can result in more successful treatment. A topical retinoid alone is mainly recommended for comedonal acne [22]. According to property of it anti-comedogenic and anti-inflammatory effects, topical retinoids assist in reducing the numbers of comedonal and inflammatory acne lesions [8]. Moreover, retinoid can also help in atrophic scar improvement and reduce post acne skin hyperpigmentation [1, 24]. Each retinoid has slightly differences in activity, efficacy and tolerability due to binding to different set of retinoic acid receptor [23]. Adapalene is the best tolerated retinoid and least effective whereas tazarotene is the most poorly tolerated and most effective retinoid [23, 24]. Because of the side effects of irritation, dryness, and redness, the initial treatment should be start at low concentration [24]. In addition, teratogenicity is the worst side effect of retinoid. Tretinoin and adapalene are pregnancy category C, while tazarotene

is category X [23]. Retinoids should not be used by female patients who are of reproductive age.

The most effective treatment for acne vulgaris is isotretinoin, an oral retinoid. Recently, the only medication that effectively combats the four etiology of acne vulgaris is isotretinoin [1, 26]. This medication is approved by FDA and usually reserved for severe and refractory acne [23, 24]. The recommended dose of oral isotretinoin for the greatest results is 1 mg/kg per day, or a total of 150 mg/kg for the course of treatment [27]. As a result in side effects of cheilitis, dry skin, photosensitivity, hyperlipidemia and teratogenicity, the usage of isotretinoin should be aware [27]. According to its teratogenicity, oral isotretinoin is contraindicated in pregnancy and it would be safe if women of childbearing age have contraception during use of retinoid [24, 25, 27].

(2) Benzoyl peroxide

Benzoyl peroxide has comedolytic and antibacterial effects against acne vulgaris [1, 23]. For the treatment of mild to moderate acne vulgaris, benzoyl peroxide is equally effective when used alone or in conjunction with topical retinoids or antibiotics. Benzoyl peroxide's main negative effects include dryness, burning, erythema, stinging, and burning [1]. Due to its local irritation, low concentration, less frequency and less duration use of benzoyl peroxide should be initiate.

(3) Antibiotic

For mild to moderate acne vulgaris, topical antibiotics like clindamycin and erythromycin are most frequently utilized. They improve acne by direct effect against *C.acnes* and reduce inflammation [1, 27]. However, Due to the potential development of antibiotic resistance, topical antibiotics should not have been administered as monotherapy. In order to prevent bacterial resistance, it is therefore preferable to combine the use of topical antibiotics with benzoyl peroxide or topical retinoid [1, 22, 24, 27].

Only moderate to severe acne requires oral antibiotics. Doxycycline, erythromycin, tetracycline and minocycline are commonly used. The choice of antibiotic depends on patient’s preference, side effects, and cost. Antibiotics taken orally should not be administered alone, just like topical antibiotics [18,19]. Oral

antibiotics should be provided for the shortest amount of time feasible, along with topical benzoyl peroxide or retinoids, and should be evaluated after three to four months of treatment to limit the chance of building a resistance [22, 27, 28].

(4) Hormonal

(4.1) Oral contraceptive pills

Due to its ability to lower excessive sebum production, combined oral contraceptives (COCs), which include both estrogen and progestogen, are a therapy option for severe acne. COCs predominantly decreased both inflammation acne and comedonal acne [23, 24]. However, progesterone only contraceptive pills should be avoided because they can worsen acne [25, 27]. For the treatment of female acne vulgaris, oral contraceptives can be used alone or in conjunction with other therapies. The minimum of 12 months duration must be prescribed for treating acne by oral contraceptive, as the results are only apparent after 3-6 months of treatment [1]. Minor side effects of oral contraceptive include abnormal menstruation, weight gain, nausea and vomits are normally seen, whereas more severe side effects of venous thromboembolic events such as thrombophlebitis and pulmonary embolism can be rarely occurred [29].

(4.2) Spironolactone

The potassium-sparing diuretic or spironolactone, is an aldosterone receptor antagonist which can help decreased inflammatory acne lesions [1, 22-24]. Although it is presently not FDA-approved for the treatment of acne, it is often used as an off-label medication to treat hormonal acne in women [23, 24]. Spironolactone frequently causes diuresis, irregular menstruation, painful breasts, enlarged breasts, headaches, lethargy, and dizziness as side effects [23]. Serious side effect is hyperkalemia, but fortunately rare in healthy individuals, so the screening of electrolyte is not necessary in healthy person without abnormal hepatic, adrenal and renal function [23, 24].

2.1.5.2 Non pharmacological treatment

(1) Role of diet

The evidence for diet's influence in acne is growing. Diets with a high glycemic index and dairy intake might be correlated to acne [22, 24, 30]. As we know that there are 4 main pathological factors associated with acne vulgaris; follicular

hyperkeratinization, increase sebum production, Propionibacterium acne, and inflammation. Not only androgen that affect in increment of sebum production but also insulin-like growth factor-1 (IGF-1). Milk-derived amino acids stimulate hepatic IGF-1 production and increase insulin secretion [31]. IGF-1 plasma level is strongly correlated with acne severity [32].

A systematic review and meta-analysis published in 2018 [31] found studies on dairy consumption and acne vulgaris in children, adolescents, and young adults. They concluded by establishing that the odd ratio for acne vulgaris was increased by any dairy product, including milk, cheese, and yogurt.

Similar to a systematic review about diet and acne was conducted by Dall'Oligo et al in 2021 [33]. They reached the conclusion that foods with a high glycemic index (GI), a high glycemic load (GL), dairy products, fatty foods, and chocolate are all variables that contribute to acne whereas consuming fatty acids, fruit, and vegetables protects against acne.

The first randomized controlled trial in dietary glycemic load on the clinical assessment of acne was conducted by Smith et al in 2007 [34]. They conducted a 12-week study. 43 male acne patients were split into a low glycemic load group and a control group at random. At 12-week, they found that low glycemic load group had a higher decline in number of both total and inflammatory lesions than control group.

In 5 consecutive year, H.H.Kwon et al [35] conducted a clinical investigation on the use of a low glycemic load diet to cure acne vulgaris in 2012. This was a randomized, controlled experiment lasting 10 weeks period. A low glycemic load diet or a control group diet was randomly allocated to 32 individuals with mild to moderate acne vulgaris. According to the findings, the low glycemic load diet group experienced a considerable clinical improvement in the quantity of acne lesions, including those with and without inflammation. Additionally, histological analysis of skin samples from the reduced glycemic load group showed a reduction in sebaceous gland size, inflammation, and expression of interleukin-8 and sterol regulatory element-binding protein-1.

(2) Complementary and alternative medications (CAMs)

Another therapy option for acne is complementary and alternative medicine (CAM), which may be a more secure and efficient option. Various CAM therapies have been recognized for acne treatment in systematic review in 2006 [36].

(2.1) Tea tree oil

Skin care products for acne - prone skin frequently contain tea tree oil. Tea tree oil is an essential oil derived from the Melaleuca alternifolia plant, which is native to Australia. It contains 1,8-cineole and terpinen-4-ol. Terpinen-4-ol, a key ingredient, has anti-inflammatory and antibacterial properties that help treat acne [1, 28, 37, 38].

In order to examine the effectiveness and safety of 5 percent tea tree oil gel with 5 percent benzoyl peroxide in the treatment of acne vulgaris, Bassett et al [37] conducted a single blinded, randomized, controlled experiment with 124 participants. They concluded that the efficacy of 5 percent tea tree oil was equivalent to that of 5 percent benzoyl peroxide, which is a common therapy for mild to moderate acne vulgaris. However, tea tree oil required considerably more time responding than benzoyl peroxide. Enshaieh S. et al [39] found that a 5 percent tea tree oil gel was more effective in treating mild to moderate acne vulgaris than a placebo throughout the course of a 45-day, randomized, double-blinded, placebo-controlled research.

(2.2) Mangosteen fruit rind extract

Several studies suggested that alpha-mangostin from mangosteen fruit rind extract has an efficacy in acne treatment. Propionibacterium acnes and Staphylococcus epidermidis are both susceptible to the antimicrobial effects of alpha-mangostin [40-42].

In 2017, a randomized, double-blinded, split-face, 12-week study was conducted. The effectiveness of 0.5 percent topical mangosteen extract combined with 2.5 percent benzoyl peroxide in treating mild-to-moderate acne vulgaris was compared to 1 percent clindamycin combined with 2.5 percent benzoyl peroxide in 28 patients. After a 12-week treatment period, they discovered that mangosteen extract dramatically decreased both comedonal and inflammatory lesions (P 0.001)

with no negative side effects [41].

Another study about herbal extract in acne treatment has conducted in 2018 by Lubtikulthum et al. [42]. They compare the effectiveness of herbal extracts which composed of mangosteen, tea tree oil, and 4% niacinamide with 2.5% benzoyl peroxide in total 77 patients (39 patients in herbal extract group, 38 patients in benzoyl peroxide group). The result shows that herbal extracts was effective in decreased acne lesion count equal to benzoyl peroxide.

(3) Physical therapy

Apart from pharmacological treatment and nonpharmacological treatment, physical therapy can also be an adjunctive therapy for acne.

(3.1) Comedone extraction

Manual extraction of comedone can improve skin appearance. However, recurrence or residual scar can be occurred if the technique of comedone extraction is incorrect. Aseptic technique is required prior the procedure. Use of mechanical and exfoliative agents prior the procedure may help reducing the severity. Optimal pressure should applied directly on top of non-inflammatory acne lesions until all of the contents have been expelled [43, 44]. Furthermore, several authors have recommended that comedone extraction can be used together with isotretinoin to treat macrocomedone [43].

(3.2) Intralesional corticosteroid

Intralesional corticosteroid injections are rapidly impressive for segregate inflammatory acne lesions. Triamcinolone acetonide is the most frequently used corticosteroid. This procedure can lead to atrophic skin. Reduction in concentration of corticosteroid may decrease this adverse effect [1, 45].

(3.3) Optical treatment

Laser therapy, light sources, and photodynamic therapy are among the optical therapies for acne [1]. Blue light and red light are two most frequently used to treat acne. Blue light has the activity against P.acnes, whereas red light has anti-inflammatory effect [46]. As a result, by having synergistic effects, blue and red light combined may be more successful in treating inflammatory lesions.

A photosensitizing drug called photodynamic treatment (PDT) enhances the therapeutic effects of light or lasers. Famous photosensitizing

compounds include methyl-aminolevulinic acid (MAL) and aminolevulinic acid (ALA) [1, 46]. By eliminating C.acnes, heating the sebaceous gland, and reducing sebaceous gland activity, photodynamic treatment can improve acne. [47].

2.2 Hemp

2.2.1 Introduction

Hemp, also known as Cannabis sativa subsp. sativa, is a crop with numerous uses, including those for food, fiber, and medicine. Historically, hemp was believed to be first cultivate in China and then spread over Asia to Europe. Nowadays, only ruderal and industrial hemp plants exist [48, 49].

Hemp is commonly known as a versatile plant due to each component of this facility has a specific industrial application. The seed and its oil can be used as the food, and cosmetic. The stem is used for architecture, paper, textiles, and animal bedding (both cork and fiber). The remediation of heavy metals from the soil has led to a highly developed root system. Because the flowers' extract is high in cannabinoids, it can be used for both cosmetic and medicinal purposes. [49].

Due to its high nutritional content and useful properties, hemp seed, the fruit of the Cannabis sativa L. plant, is recently gaining attraction in the medical and cosmeceutical fields.

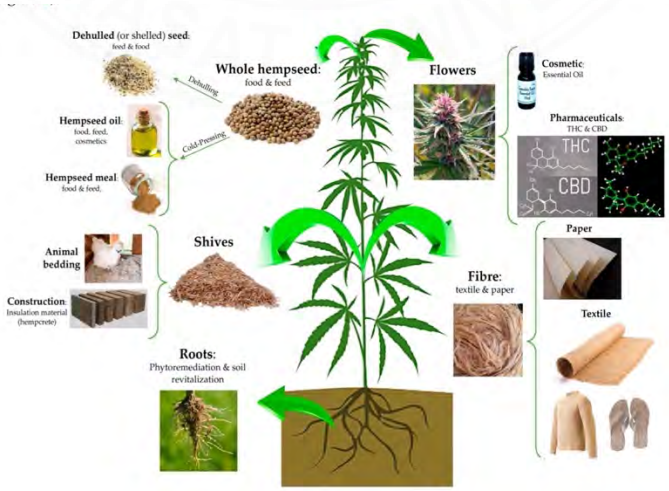

Figure 2.3 Hemp plant and its application [49]

2.2.2 Hemp seed composition

Hemp seed is becoming more well-liked because of its macronutrients and phytochemicals. Hemp seed composes of nutritional compounds such fatty acids (especially polyunsaturated fatty acid or PUFA), proteins, amino acids, carbohydrates, vitamins, and minerals. Moreover, Hemp seed is also abundant in bioactive peptides, tocopherols, carotenoids, phenolic compounds, and phytosterols, as well as antioxidants. [10, 49].

Cannabinoids, phenolic compounds, and other common chemicals are the primary secondary metabolites of cannabis. However, cannabinoids are the most important secondary metabolite of cannabis [50]. To identify a phytocannabinoids component in hemp seed and hempseed oil, several researchers conducted a study. Citti et al. [10] discovered THC, CBD, and other cannabinoids such as cannabichromene (CBC), cannabigerol (CBG), cannabinol (CBN), etc. by employing liquid chromatography techniques coupled with high-resolution mass spectrometry detection (HPLC-HRMS) in ten commercial hempseed oils from organic farm. Similar to Jang et al. [9], they were interested in acquiring a research employing gas chromatography-mass spectrometry (GC/MS) to determine the quantity of THC, CBD, and CBN in hemp seeds and hempseed oil. 77 hemp seed samples and 11 hempseed oil samples were used in this study. The samples were from a variety of countries, including Canada, South Korea, Australia, China, and others. They found that concentration of THC ranged from 0.2 to 19.73 µg/mL (mean 4.11 µg/mL), concentration of CBD ranged from 6.66 to 63.40 µg/mL (mean 31.26 µg/mL), and CBN concentration ranged from 0.11 to 2.31 µg/mL (mean 0.66 µg/mL). Furthermore, the concentration of THC was mostly ranged in 0.3 to 5 µg/mL, while the concentration of CBD was widely ranged in 20 to 30 µg/mL, and the concentration of CBD was commonly around of 0.5 to 1 µg/mL. According to previous studies, we can conclude that hemp seed extract predominantly contains CBD than other cannabinoids.

Even though hemp seed and hempseed oil contain THC. However, THC is highly lipophilic and has poor oral bioavailability. Oral administration of THC result in low absorption and low peak plasma concentration [51]. Furthermore, transdermal administration of THC was 10 times lower permeable than CBD due to its

less lipophilic [52]. Thus, hemp seed extract seems to be safe from THC psychogenic effect.

2.2.3 Hemp and health benefit

Hemp seed has been reported about numerous health benefit and potential therapies including improved cardiovascular health, improved diseases of central nervous system such as multiple sclerosis etc. [48]. As hemp seed contain macronutrients and phytocannabinoids, several research on hemp seed and hemp seed products as dietary supplements in humans have been published.

Schwab et al. [53] conducted a study to find the change in serum lipoprotein lipid concentrations and serum lipids by supplemented human food with hempseed oil and flaxseed oil. Total 14 participants were assigned to ingest each oil 30 ml daily for 4 weeks duration with 4 weeks washing in between. The result showed that in hempseed oil period, concentration of linoleic acid in both serum triglyceride and cholesteryl ester were significantly high compared with flaxseed oil period. Additionally, in comparison to flaxseed oil, hempseed oil had a smaller total-to-HDL ratio. These results could indicate a lower risk of coronary heart disease.

Hemp seed as a food supplement seems to have benefit in multiple sclerosis patients. In a research on individuals with multiple sclerosis, S. Rezapoue-Firouzi et al [54] investigated the effects of combining hemp seed and evening primrose oils with a hot nature diet. This study was a randomized, controlled trial that also was double blinded. Total of 65 multiple sclerosis patients were assigned into 3 groups which received different supplement. After a 6-month trial, they discovered that individuals with multiple sclerosis who also supplemented with evening primrose oil and hemp seed considerably improved their clinical scores and immunological measures.

2.2.4 Hemp and skin diseases

Clinical human study about hemp and skin disease is limited. There was a study indicated that hempseed oil improved atopic dermatitis. Callaway et al. conducted a study about dietary hempseed oil and atopic dermatitis in 2005. Twenty individuals with atopic dermatitis were split into 2 groups for this investigation. Each group was told to ingest 30 ml of either hempseed oil or olive oil per day. This trial lasted for eight weeks of crossover and four weeks of washout time (total 20 weeks).

The results demonstrated that oral hempseed oil significantly altered plasma fatty acid levels and alleviated atopic dermatitis clinical manifestations [55].

2.2.5 Hemp and acne vulgaris

Jin et al. conducted an in vitro research at how Propionibacterium acnes-induced inflammation and lipogenesis were affected by hemp seed hexane extract (HSHE), which has anti-microbial, anti-inflammatory, and anti-lipogenic properties. P. acnes was absolutely inhibited at 20 percent HSHE. For anti-inflammatory effect, the researchers found that HSHE can suppressed inflammatory enzymes iNOS and COX-2 and their products NO and PGE2 caused by P. acnes. Additionally, HSHE significantly lowered the inflammatory cytokines IL-1 and IL-8. Furthermore, HSHE inhibited intracellular lipid production in sebocytes by upregulating p-AMPK expression and downregulating p-mTOR, PPAR, SREBP1, and FAS expression. According to its anti-inflammatory, anti-lipogenesis, and anti-microbial actions, HSHE may be beneficial in combating acne vulgaris. [56].

The only clinical trial about hemp seed extract and its effects was conducted by Ali et al in 2015. They have performed a randomized, single-blinded comparative trial on a cream containing 3% cannabis seeds extract for erythema and sebum on human cheek skin. In this split face research, 11 healthy adults applied base cream on one side of their faces while applying base cream and 3 percent cannabis seeds extract on the other. After 12 weeks of twice-daily use, the side treated with cream containing 3 percent cannabis seeds extract produced significantly less cheek sebum and had significantly less erythema than the base-treated side. The researchers found that cannabis seeds extract cream was safe and there was no side effect during the study period. They came to the conclusion that a 3 percent cannabis seed extract could help with acne vulgaris. However, there were just a few participants in the research, and they were all free of acne vulgaris and in good health. [8].

According to previous studies, hemp seed extract seems to exert anti-inflammatory, anti-lipogenesis, and anti-microbial activity and might have potential in treating acne vulgaris.

2.3 Cannabidiol (CBD)

2.3.1 Introduction

The endocannabinoid system (ECS) is completed of many components include endocannabinoids which is signaling molecules, specific receptors, and enzymes. Standardize of the central nervous system and immune function of the body have been found as ECS function in the past decade. Up to recent year, several researchers have indicated that ECS also play a pivotal role in maintaining skin homeostasis and also barrier function.

The word "cannabinoids" is wide and refers to a huge class of pharmaceutical substances that interact with cannabinoid receptors. Endocannabinoids, phytocannabinoids, and synthetic cannabinoids are the three primary classes of cannabinoids [5, 57].

The substances that are naturally produced by the human body are known as endocannabinoids (ECBs). Two endocannabinoids that serve as prototypes are anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), whereas N-palmitoyl ethanolamide (PEA) is less known [57, 58]. Synthetic cannabinoids (SCs) are produced artificially in labs, whereas phytocannabinoids (PCBs) are cannabinoids that are only present in the Cannabis sativa L. plant. THC (delta-9-tetrahydrocannabinol) and CBD are the two phytocannabinoids that are most well-known (CBD). High levels of THC are present in marijuana, but high levels of CBD are present in hemp. Unlike THC, CBD has no psychoactive effects [5, 6].

Adams et al. isolated CBD for the first time in 1940 [59]. It is a significant non-psychoactive component of the cannabis sativa L. plant extract. Anxiolytic, antipsychotic, antiemetic, and anti-inflammatory characteristics are only a few of the many pharmacological effects of CBD [60].

Based on this knowledge, cannabis topical applications for skin disorders have gained interest.

2.3.2 Pharmacology

Raphael Mechoulam determined CBD's structure in 1963 [61] after Adams et al, originally isolated it in 1940 [59]. CBD is a significant non-psychoactive Its chemical name is 2-(1R-3-methyl-6R-(1-methylethenyl)-2cyclohexen-1-yl]-5-pentyl-1,3-benzenediol and the molecular formula is $C_{12}H_{30}O_2$. The molecular weight of CBD is 314.5 g/mol).

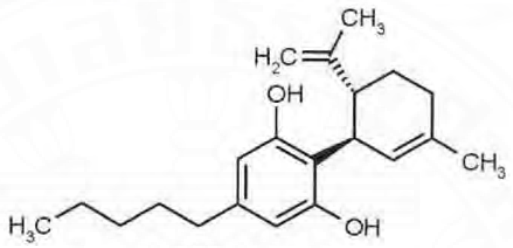

Figure 2.4 Structure of cannabidiol [62]

In the presence of stomach acid, there has been some proof that CBD can turn into THC. The possibility for this conversion has been shown in two in vitro investigations [63, 64] using simulated stomach fluid. They both concluded that without digestive enzymes, the production of delta-9-THC from CBD can be occurred in the acidic environment of the human digestive system. However, in a human in vivo investigation, a spontaneous transformation of CBD to THC has not been established. In a six-week clinical investigation in Huntington's disease patients who received 700 mg per day of CBD orally, Chonsroe et al. [65] found that the mean plasma concentration of CBD varied from 5.9 to 11.2 ng/mL, but no 9-delta-THC was found. Similar to a paper published by Gerhad, he also stated that a transition of oral CBD to THC seems not to appear in people after oral administration [66]. Thus, there was no evidence of a natural transformation of CBD to THC in people.

CBD can be taken orally, breathed, transdermally, or topically, among other possible delivery methods. The United States (US) Food and Drug Administration and the European Medicine Agency was first approved for oral drug formulation containing CBD include Epidiolex® and Sativex® indicated for adjunctive therapy of seizure associated with Lennox-Gastaut and Dravet syndrome. However,

administration CBD orally has lower bioavailability (6%) than administer through inhalation. Because of in gastrointestinal fluids, water solubility is extremely low, therefore first pass metabolism is crucial [67, 68]. Thus, other methods of CBD administration might be considered. Transdermal delivery of CBD provides numerous advantages compared to oral administration in terms of pharmacokinetics and efficacy [67, 69]. For limited symptoms, topical treatment may be helpful.

According to Lodzki et al [70], using an ethosomal carrier to administer CBD transdermally to mice resulted in a considerable buildup of the medication in the skin, and reaching a steady-state level at 24 hour and lasted at least 72 hours.

Propylene glycol is found to be best vehicle for topical CBD in terms of absorption rate and skin retention. Paraffin oil and lipophilic ointment also proven to be appropriate vehicles due to permeation performance [67].

A study developed by Giacoppo et al [71], 1% CBD cream was daily applied at both hind legs of mice for 28 days. The results indicated that the maximum plasma concentration of CBD (Cmax) was 8.3 2.1 ng/mL and that the steady-state plasma concentration of CBD (Css) was 6.1 1.9 ng/mL

Due to its high lipophilicity (Koctanol-water 6-7) and large volume of distribution (32 L/kg), CBD circulates quickly in the brain, adipose tissue, and other organs**.** Additionally, CBD is also highly bound to protein. Thus, the higher distribution might have occurred in patients with high adiposity [72].

CBD is metabolized mainly by CYP3A2, CYP3A4 and CYP2C8, CYP2C9, CYP2C19 enzymes, found extensively in the liver. CBD is mostly excreted via feces and lesser in the urine. The half-life is about 18-32 hours depending on route of administration [68, 72-74].

Considering drug interaction, there are some therapeutic agents that should be avoided to use concomitant with CBD, mostly because of their additive effect. As P450 isozymes, especially CYP2C and CYP3A, are strongly inhibited by CBD, those medications which are substrates for CYP3A4 are more likely to increase in plasma level when combined with CBD [72].

2.3.3 Therapeutic efficacy in dermatology

It is well recognized that the endocannabinoid system (ECS) is crucial to the modulation of skin function. Thus, it could be possible that topical cannabinoid might have an efficacy in treating various skin diseases especially inflammatory skin diseases. There are numerous studies about cannabinoid with skin diseases in vitro, but still limited data in vivo. Additionally, most clinical studies on the benefits of CBD and some other cannabinoids have used oral intake or intravenous injection rather than the less common topical or transdermal delivery methods [60]. Using ethosome as a carrier in mice, M. Lodzki et al [70] addressed effective transdermal administration of CBD in 2003.

In concordance with Giacoppo et al [71], an examination of 1% topical CBD with experimental autoimmune encephalomyelitis in mice was conducted. Myelin oligodendroglia glycoprotein peptide was used to induce encephalitis. 40 mice in total were randomly assigned to several experimental groups, including the native group, the experimental autoimmune encephalitis (EAE), the EAE+1 % CBD cream group, the EAE+vehicle cream group, the control group+1 % CBD cream, and the control group+vehicle cream. He found that Inflammatory cytokine expression is decreased by 1% CBD cream. Thus, CBD has a potential for anti-inflammatory effects.

Furthermore, the first pre-post observational study about topical CBD with atopic dermatitis was obtained by J. Maghfour et al in 2021. In this study, 14 patients with atopic dermatitis were assigned to apply 1% CBD-infused gel for 14 days. Questionnaire, Eczema Area and Severity Index (EASI), Visual Analogue Scale-Pruritus (VAS), and 5-D Pruritus Scales were used as an assessment tool. They found that there was significant reduction in EASI score ($P<0.05$), VAS-Pruritus ($P<0.05$), and 5-D Pruritus Scales ($P<0.05$). In conclusion, the researchers indicated that CBD might become an alternative therapy for atopic dermatitis due to its anti-inflammatory and anti-pruritic properties [75].

A study was performed by B. Palmieri et al. [76] to see if CBD-enriched ointment might affect severe chronic skin conditions and scarring. Twenty patients were entered to the experiment. 5 of them were suffering from psoriasis, 5 with atopic dermatitis, and 10 with scars. They were told to use the CBD ointment for three months, twice a day. The result showed significantly increased hydration, decreased

TEWL, and increased elasticity. Moreover, cutaneous blemishes and scar were significantly improved in patients with scar suffering and also improved PASI score in psoriasis patients ($p < 0.001$).

Cannabinoids have demonstrated potential in the treatment of psoriasis by its antiproliferative effect. Wilkinson et al, conducted research of the phytocannabinoid effect of inhibitory keratinocyte proliferation in vitro. He found that THC, CBD, cannabinol (CBN), and cannabigerol (CBG) all have the ability to inhibit keratinocyte proliferation through cannabinoid independent receptor activation. Moreover, of all tested phytocannabinoid, CBD has elicited the greatest overall activity [77].

According to three case studies [78], transdermal CBD improves speedier wound healing, less blisters, and analgesic alleviation in people with epidermolysis bullosa. The first case was a 6-month-old boy who had abundant intact blisters and aplasia cutis of the bilateral lower extremities at birth. He was diagnosed with recessive dystrophic epidermolysis bullosa. Apart from mupirocin, petroleum ointment, emu oil, and silicone-based dressing, his parents sprayed the CBD oil over his affected area 2 to 3 times daily. His parents then noticed that the blisters were significantly reduced, and morphine was no longer required since they started CBD spray. The second case was a 3-year-old girl with severe generalized epidermolysis bullosa simplex. She was first wound care with a mixture of petrolatum ointment with coconut oil, zinc oxide, and allantoin cream. Then her mother started to add emu oil and CBD oil to her blisters at least twice daily. She reported that the healing time of blisters was fastened and the number of the blisters were significantly reduced since she added the emu oil and CBD oil. Similar to the third case, a 10-year-old boy who was clinically diagnosed with localized EB, he noticed a significant reduction of blisters after he applied CBD oil and CBD cream. Moreover, after beginning to use topical CBD, he was able to stop using analgesic medications.

According to the potential of CBD in the field of dermatology, CBD has recently become popular ingredient among skincare products. CBD has shown to be potential for treating acne, eczematous disorder, prurigo, chronic pruritus, psoriasis, skin cancer, and atopic dermatitis, due to its anti-inflammatory, antipruritic, antiproliferative and antinociceptive properties [5, 6, 58].

2.3.4 Cannabidiol and acne vulgaris

As sebum over production and inflammation are two pathological factors of acne vulgaris. Any substances that have possibility to decrease sebum production and have anti-inflammatory effect could be useful for acne reduction. It is well established that the ECS acts a fundamental part in skin regulatory function, especially lipogenesis. Several studies suggested that CBD could have potential in acne treatment by acting through a pathway influencing the production of sebum, the growth of sebocytes, and inflammatory processes.

In cultured human sebocytes and skin cells culture, Olah et al. discovered in 2014 that CBD has the capacity to prevent the lipid formation of a variety of substances, particularly arachidonic acid (AA) and a mixture of linoleic acid and testosterone. This finding suggested that CBD has a universal lipostatic action. Furthermore, CBD reduced sebocyte proliferation by activating TRPV4 ion channels in a dose-dependent manner (1–10 M), but not altered cell viability. However, CBD induced sebocyte apoptosis and drove cytotoxicity at high concentrations of 50 M. Moreover, CBD exerted complex of antiinflammatory effects through A2a adenosine receptor-dependent upregulation of tribbles homolog 3 (TRIB3) and inhibition of the NF-κB signaling [7].

Apart from CBD, Olah et al. have studied more deeply about other phytocannabinoids in 2016. They discovered that modest doses of phytocannabinoids (10 M) may change the vitality of the sebocytes, but high doses (50 M) caused the sebocytes to undergo apoptosis. Cannabichromene (CBC) and tetrahydrocannabivarin (THCV) were superior in suppressed basal sebaceous lipid synthesis above cannabidivarin (CBDV). While lipid synthesis was elevated by cannabigerol (CBG) and cannabigerovarin (CBGV). Arachidonic-induced acne-like lipogenesis was considerably decreased by CBC, CBDV, and THCV [2].

To our knowledge, CBD might become a new treatment option for acne vulgaris, due to its property of antilipogenic and antiinflammation. According to several studies, hemp seed and hemp seed extract mainly contain CBD than other cannabinoids. We believed that hemp seed gel might have a potential to treat acne vulgaris. However, the available safety and effectiveness information is quite scarce.

In order to treat acne vulgaris, we aimed to investigate the effectiveness and safety of topical hemp seed cream.

2.3.5 Mechanism of action

While CB2 receptors are located in the peripheral nervous system, digestive system, and immune system, cannabinoid 1 receptors (CB1) are largely found in the central nervous system. CB1 and CB2 receptors are widely distributed in healthy skin and its appendages includes keratinocytes, hair follicles, sebaceous glands, fibroblasts, melanocytes, adipocytes, and nerves fibers. [6,7,10]. Endocannabinoids appears to bind to Transient Receptor Potential Vanilloid (TRPV) receptors in addition to cannabinoid receptors, which seem to be their primary targets. As they are implicated and maintenance of the epidermal barrier, cell differentiation, cell proliferation, immunological processes, and inflammatory responses, TRPV channels play the most significant function among non-CB receptors. TRPVs are primarily found in keratinocytes, hair follicles, mast cells, sebocytes, melanocytes, nerves fibers and other skin structures. Moreover, endocannabinoids engage PPAR (peroxisome proliferator-activated receptor) pathways. [57, 58]. Cannabinoids provide both agonist and antagonist responses on the ECS, which stimulate or inhibit cellular proliferation, sebum production, hair growth, and inflammation [4].

Normally, endocannabinoid enhances lipid production of sebocytes through activation of ERK1/2 MAPK pathway. AEA and 2-AG which are prototype of endocannabinoid activate the CB2 receptor agonist on sebocytes, resulting in activation of ERK1/2 MAPK and PPAR pathway. In contrast to endocannabinoid, CBD was discovered to be mediated by various molecular targets and independent of CB2. CBD inhibit lipid production by mediated Ca^{2+}influx via TRPV4, which then lead to the suppression of the prolipogenic ERK1/2 MAPK pathway and diminishes nuclear receptor interacting protein 1 (NRIP1). CBD's anti-inflammatory effects were controlled by suppressing the Toll-like receptors (TLR) 2 and 4, which then activated the A2A adenosine receptor, up-regulated tribbles homolog 3 (TRIB3), and therefore inhibited the proinflammatory p65 NF-κB pathway. Moreover, CBD also enhanced adenosine by inhibit equilibrative nucleoside transporter (ENT1) which subsequently promote A2A adenosine receptor activation. Hence, result in anti-inflammatory activity. [2, 7, 79].

In conclusion, CBD appears to have anti-inflammatory properties through the A2A adenosine receptor, hence decreasing the p65 NF-κB pathway, as well as exerting anti-acne triad effects by suppressing sebum excretion and sebocyte proliferation through TRPV4 activation. [57]. According to mechanism of action of CBD, CBD seems to be potential in acne therapy due to its anti-lipogenic, anti-proliferative, and anti-inflammatory effects.

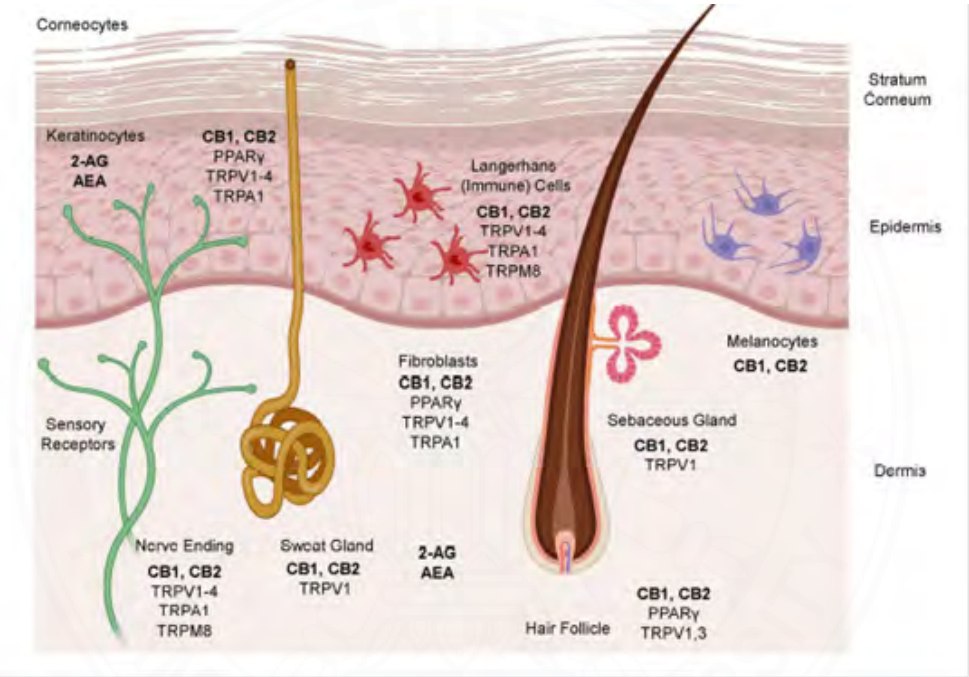

Figure 2.5 Endocannabinoid systems and various receptors among skin appendages [6]

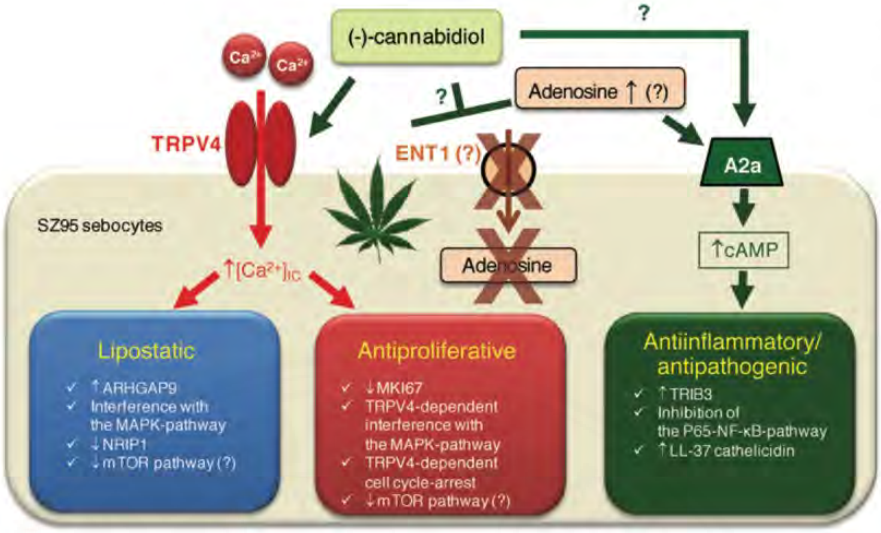

Figure 2.6 Mechanism of action of CBD [7]

2.3.6 Adverse effects

As aforementioned, the characteristic euphoric properties of THC are not present in CBD, and it also does not spontaneously transform in humans into THC. Several studies have demonstrated that delivering CBD through various methods is secure [60]. Oral CBD is found to be safe and also failed to produce significant adverse effects in healthy male subjects [80].

A total of 17 patients received intravenous fentanyl and CBD in a double-blind, placebo-controlled crossover study. Heart rate, blood pressure, respiratory rate and oxygen saturation were monitored during the study. CBD did not interfere with these vital sign. During the study, the negative effects of fentanyl were not made worse by CBD. No participant experienced any severe side effects, such as cardiovascular compromise or respiratory depression. Minor side effects such dizziness, itching, headache, abdominal discomfort, nausea/vomiting and diarrhea were reported. Thus, coadministration is safe, CBD may be used to treat opioid addiction [81].

However, there is still limited evaluation of topical CBD safety profile for being used as a therapeutic drug for dermatologic conditions. Using CBD as a topical ointment on patients with psoriasis, atopic dermatitis, and scarring had a

persistent administration impact, according to a research by B. Palmieri et al [76]. No negative effects were recorded, and the data indicated a favorable improvement. Similar to Ali et al [8], Cream containing 3 % cannabis seed extract has been determined to be safe for use on people's skin; no itching or allergic reactions were reported throughout the investigation.

Table 2.2 Treatment options and acne pathogenesis

Pathogenesis / Treatment	**Follicular hyperkeratinization**	**Increase sebum secretion**	***C. acnes***	**Inflammation**
Hemp seed extract	-	/	/	/
Benzoyl peroxide	/	-	/	-
Topical retinoid	/	-	-	/
Topical antibiotic	-	-	/	/
Oral contraceptive pills	-	/	-	/
Isotretinoin	/	/	/	/

CHAPTER 3
MATERIALS AND METHODS

3.1 Study sample

3.1.1 Target population

Patients with mild to moderate acne vulgaris, as determined by the Global Acne Grading System (GAGS), who presented to the Dermatology Out-Patient Department (OPD) unit at Benchakitti Park Hospital.

3.1.2 Sample size

By using N4study application combined with addition of 10% dropout rate, 20 patients need to be recruited in the study.

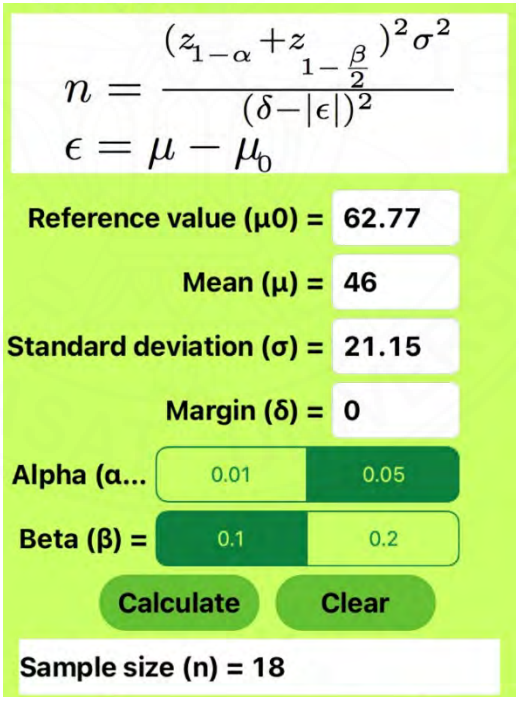

Figure 3.1 Calculation of Sample size by N4study application.

Sample size (n) = 18

Dropout rate 10% = 2

Total sample size = 20 subjects

3.1.3 Inclusion criteria

(1) Healthy males and females whose age more than 18-year-old.

(2) Mild to moderate acne vulgaris according to GAGS criteria.

Table 3.1 The Global Acne Grading System

The global acne grading system	
Location	Factor
Forehead	2
Right cheek	2
Left cheek	2
Nose	1
Chin	1
Chest and upper back	3

Grade (0 - 4)

Grade 0 : No lesion

Grade 1 : ≥ one comedones

Grade 2 : ≥ one papules

Grade 3 : ≥ one pustules

Grade 4 : ≥ one nodules

Local score = factor*grade

Global Acne Grading System (GAGS) = Sum of local score

Mild	=	1 - 18
Moderate	=	19 - 30
Severe	=	31 - 38
Very severe	>	39

3.1.4 Exclusion criteria

(1) Pregnancy or lactation.

(2) Patients who had other active skin lesions on face.

(3) Patients who had received oral retinoid within 6 months prior to the study.

(4) Patients who had used oral corticosteroids, oral contraceptives, or oral antibiotics within 1 prior to the study.

(5) Patients who experienced laser resurfacing or dermabrasion within 1 prior to the study.

(6) Patients who had received topical treatment for acne during the two weeks before to the trial, including corticosteroids, antibiotics, benzoyl peroxide, retinoid, and chemical peels.

(7) Patients who used either oral or topical therapy that effect skin facial oiliness eg. topical/oral retinoid, oral contraceptive pills, spironolactone.

(8) Patients who had been diagnosed with psychotic disorder.

3.1.5 Discontinuation criteria

(1) Patient declines to participate in this research.

(2) Any patient who has severe negative effects while participating in the trial.

(3) Patient with inadequate compliance and reliability.

3.2 Research Design

The study design is a clinical prospective, randomized, double blinded, split-face, controlled trial.

3.3 Material and Methods

3.3.1 Material

3.3.1.1 Hemp seed extract

Hemp seed cream in this study are compose of water, disodium EDTA, propylene glycol, carbomer 940, allantoin, IPP, mineral oil, tween 20, cetyl alcohol, lipomulse luxe, 1% hemp seed oil, triethanolamine (TEA), and phenoxyethanol.

3.3.1.2 Placebo

Composition of placebo are exactly the same as topical hemp seed cream, except for the experimental ingredient, 1% hemp seed extract.

3.3.2 Data collection

The data is collected in the paper document, text file, and imaging file in the computer in every visit.

3.3.3 Intervention

(1) The product of hemp seed extract and placebo will be repackaged in sterile container and randomly, using computer-generated randomization, assign to apply on patient's left or right side of the face.

(2) All patients are instructed to use two products twice daily, one on the right side of the face and the other on the left with different finger to avoid cross-contamination. The amount of each product is limit to 0.5 gm per application/half face.

(3) All patients are assigned to use the same cleanser (Acne aid™ Gentle cleanser; Stiefel Laboratories, Inc).

(4) All patients have to apply study products every day for 12 weeks.

(5) The topical gel container will be collected and checked at each visit to ensure compliance of the patients.

(6) Both objective and subjective measurement will be assessed at baseline and follow-up period at 2^{nd}, 4^{th}, 8^{th}, and 12^{th} weeks.

3.3.4 Outcome measurement

3.3.4.1 Objective outcome

(1) Acne lesion count

Number of both inflammatory acne lesions (papules, pustules, and nodules) and comedones on both sides of the patient's face will be counted from digital photographs by 2 blinded dermatologists at baseline, 2nd, 4th, 8th, and 12th weeks follow up.

(2) Digital photograph

Series of photograph will be taken by a standard digital camera (Full-Frame Mirrorless, Sony α7ii) at baseline and every follow-up visit. Five angles of the face will be taken at each visit, including straight, 45 and 90 degrees on the left, 45 and 90 degrees on the right side.

(3) Erythema index by Antera® 3D

Erythema index will be evaluated by Antera® 3D (Antera® 3D, Miravex Co., Ltd, Dublin, Ireland) on both cheeks at each visit to objectively assessed inflammatory erythema of hemoglobin index level.

(4) Sebum level by Cutometer dual MPA 580

Sebum level will be measured using Cutometer dual MPA 580 at both side of the cheeks at baseline, 2nd, 4th, 8th, and 12th weeks follow up. Subjects will be advised to clean their faces with cleanser and stayed in a temperature between 21-25 degree Celsius for 45 minutes prior to measurement.

3.3.4.2 Subjective outcome

(1) Patient's satisfaction score

Satisfaction score was graded by patients at each week of the follow-up. By using five-point scale which ranged from -2 to 2 (-2 = very dissatisfied, -1 = dissatisfied, 0 = neither satisfied nor dissatisfied, 1 = satisfied, and 2 = very satisfied).

(2) Physician's clinical assessment score

Score was graded by physician at each follow-up visit. By using five-point scale which ranges from -2 to 2 (-2 = very poor clinical outcome, -1 = poor clinical outcome, 0 = neither poor nor good clinical outcome, 1 = good clinical outcome, 2 = very good clinical outcome).

3.3.4.3 Adverse events

Any possible adverse events related to treatment including itching, burning, stinging, erythema, and scale will be observed and recorded as present or absent by physical investigator in every follow-up visit.

3.3.5 Data analysis

Software called SPSS (IBM SPSS statistic version 26) will be used to do the statistical analysis.

3.3.5.1 Summarization of data

Number and percentage were used to describe the category variables. Numerical variables were presented as mean, standard deviation, or median and range.

3.3.5.2 Statistical testing

Chi-square will be used for category variables in demographic data, while T-test will be used for numerical variables.

For clinical data, the comparison of mean acne lesion count, sebum level, and erythema index between two independent groups will be analyzed by paired t-test. As same as the comparison of mean acne lesion count, sebum level, and erythema index changed from pre and post treatment within each group will also be analyzed by paired t-test. All statistical comparisons will be based on significant level of $p < 0.05$.

3.4 Ethical consideration

The study protocol will be submitted to the Human Ethics Committee of Thammasat University.

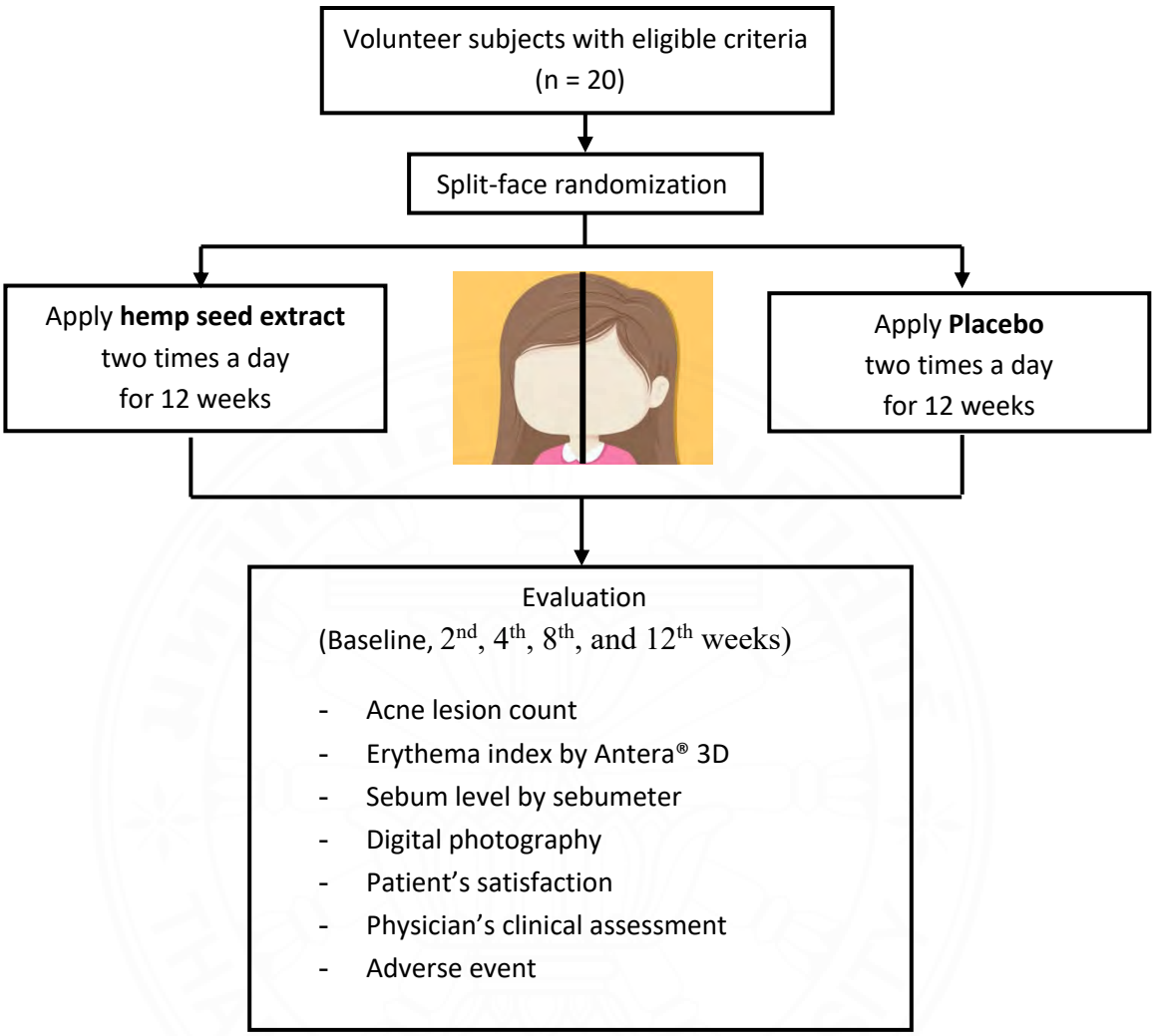

Figure 3.2 Study design

Table 3.2 Outcome measurement

Weeks / Measurements	Treatment and Evaluation				
	0	2	4	8	12
Digital photography	/	/	/	/	/
Acne lesion counts	/	/	/	/	/
Sebum level by Sebumeter	/	/	/	/	/
Erythema index by Antera® 3D	/	/	/	/	/
Patient's satisfaction		/	/	/	/
Physician's clinical assessment		/	/	/	/
Adverse event		/	/	/	/

3.5 Conceptual framework

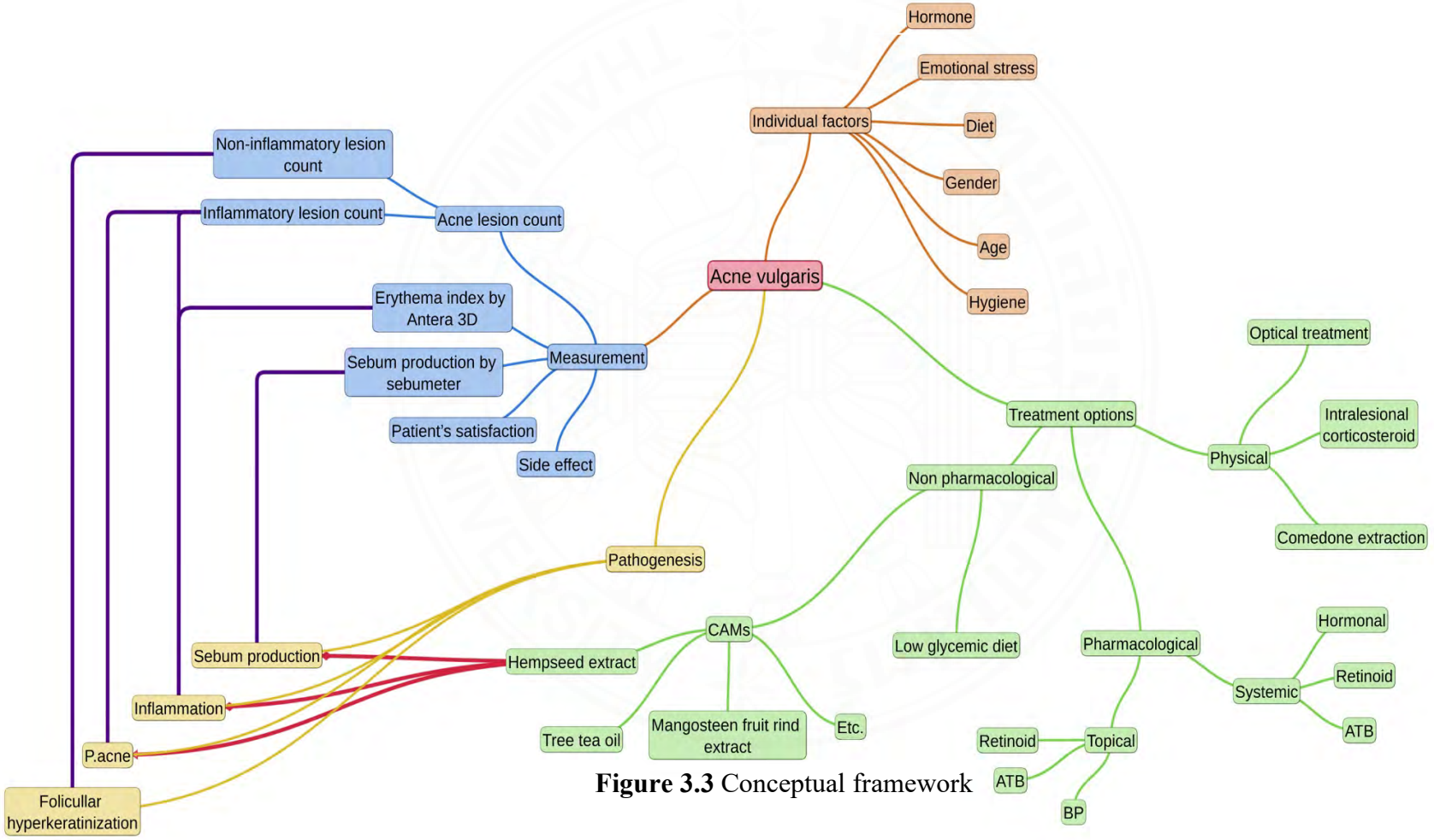

Figure 3.3 Conceptual framework

CHAPTER 4
FINDINGS AND RESULTS

4.1 Demographic data

From all of 20 subjects enrolled the study, 18 subjects had completed the study. There were 2 subjects who drop-out due to personal issue. The mean age was 25.30±4.73 years. Baseline characteristics are shown in Table 4.1. There was no significant difference in number of either inflammatory or non-inflammatory acne lesions between 2 sides of the patient's faces at baseline as shown in Table 4.2 and Table 4.3.

Table 4.1 Demographic data of the 20 subjects included in the analysis.

Demographic data	**Values (n = 20)**
Gender	
Male	9 (45%)
Female	11 (55%)
Age (years), mean ± SD	25.30 ±4.73
Acne severity (GAGS)	
Mild	16 (80%)
Moderate	4 (20%)

4.2 Efficacy evaluation

4.2.1 Acne lesion count

4.2.1.1 Inflammatory acne lesions

Within group analysis, mean number of inflammatory acne lesion was statistically significant decreased in hempseed extract cream-treated side, from the second week of the treatment continuously all the way through the study. The mean reduction of inflammatory acne lesions from baseline to week 12 was 12.27±5.52 to 7.05±3.43 (P<0.001) in hemp seed extract cream-treated side. Whereas in placebo-treated side, number of inflammatory acne lesions did not change during the study.

The mean inflammatory acne lesions count between these two different interventions showed statistically significant differences at week 4 (P = 0.03), week 8 (P < 0.001), and week 12 (P < 0.001) as shown in Table 4.2.

Table 4.2 Mean inflammatory acne lesions of each follow-up visit.

Time	Hemp seed extract		Placebo		P-value[b]
	Mean±SD	P-value[a]	Mean±SD	P-value[a]	
Week 0	12.27±5.52	1	11.32±5.08	1	0.056
Week 2	10.70±6.49	0.016*	11.27±5.32	0.888	0.501
Week 4	8.77 ±5.65	<0.001*	11.07±5.70	0.588	0.003*
Week 8	6.97±3.65	<0.001*	10.57±4.52	0.585	<0.001*
Week 12	7.05±3.43	<0.001*	12.02±7.57	0.341	<0.001*

Values are expressed as mean±SD. P-value is derived from paired t-test.
(P-value[a] < 0.05 comparison to baseline within group, P-value[b] < 0.05 comparison between hempseed extract and placebo)

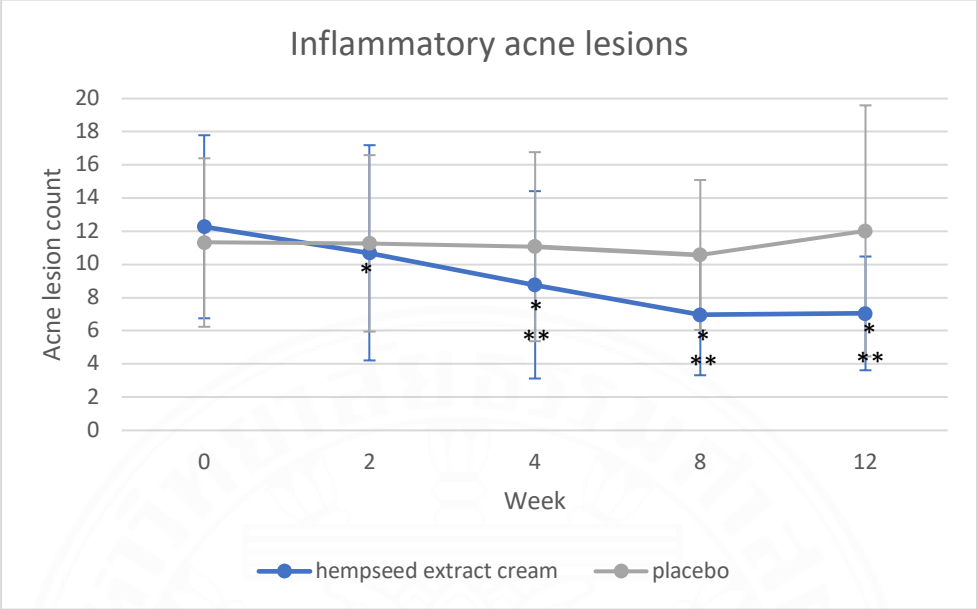

Figure 4.1 Chart of inflammatory acne lesion count.
* P-value < 0.05 comparison to baseline within group,
**P-value < 0.05 comparison between hempseed extract and placebo

4.2.1.2 Comedones

In terms of comedone, mean number of comedone lesion count in hemp seed extract treated side demonstrated reduction in number from 32.37±24.16 to 24.02±14.81 (baseline to week 12). Unfortunately, the decreased of comedone was no statistically significant differences between pre and post treatment of hemp seed extract cream.

Therefore, in placebo treated side, mean number of comedone showed minimally reduction from baseline to week 12. The mean reduction of non-inflammatory acne lesion from baseline to week 12 was 34.17±25.64 to 29.72±21.52. However, there was no statistically significant in the different of comedone reduction within group as shown in Table 4.3.

Even though, at week 8 and week 12 of the study, mean of non-inflammatory acne lesions showed statistically significant differences between hemp seed extract-treated side and placebo treated side with P-value < 0.05 as shown in the Table 4.3.

Table 4.3 Mean non-inflammatory acne lesions of each follow-up visit.

Time	Hemp seed extract		Placebo		P-value[b]
	Mean±SD	P-value[a]	Mean±SD	P-value[a]	
Week 0	32.37±24.16	1	34.17±25.64	1	0.584
Week 2	31.12±17.58	0.728	36.27±26.91	0.347	0.063
Week 4	28.85±21.40	0.271	32.90±26.02	0.647	0.150
Week 8	25.68±17.44	0.118	30.57±23.26	0.268	0.044*
Week 12	24.02±14.81	0.077	29.72±21.52	0.259	0.041*

Values are expressed as mean±SD. P-value is derived from paired t-test.
(P-value[a] < 0.05 comparison to baseline within group, P-value[b] < 0.05 comparison between hempseed extract and placebo)

In summary, compared to placebo, hemp seed extract cream significantly reduced the number of acne lesions, both inflammatory and non-inflammatory. Number of inflammatory acne lesions appeared to be significant difference to placebo since week 4 and continuously to week 12 which was the end of the study, while comedone began to show greater result at week 8 and week 12.

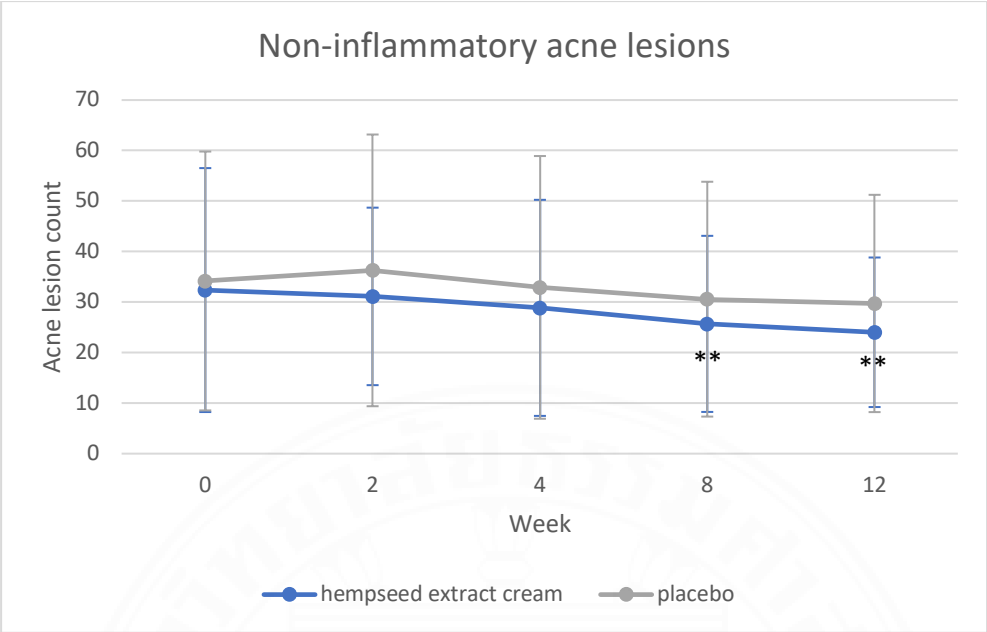

Figure 4.2 Chart of non-inflammatory acne lesion count.

**P-value < 0.05 comparison between hemp seed extract and placebo

4.2.2 Sebum level

The baseline sebum level between both groups was not statistically significant different (P-value = 0.059). In hemp seed extract-treated side, mean sebum level started to decreased after the second week of the study and became statistically significant difference at week 8 and 12 when compare to baseline (P-value = 0.006 and P-value = 0.004 respectively). Percentage reduction of sebum level within hemp seed extract group were 20.80%, 27.20%, 46.40%, and 44.90% at week 2, 4, 8, and week 12 when compare to baseline. Whereas in placebo-treated side, mean sebum level was slightly decreased after the second week of the treatment. However, the changed was statistically significant only at week 8 when compare to baseline. Within group of placebo, percent sebum reduction were less than 30% at every follow-up visit when compare to baseline. Even though at week 8 of the study, which was the greatest reduction outcome of this group, however percent reduction was only 29.50% when compare to pre-treatment.

The statistical analysis was evaluated to compare the sebum level between hemp seed extract-treated side and placebo-treated side. The results indicated that at week 8 and week 12 of the study, hemp seed extracted-treated side showed the

superior reduction of mean sebum level over placebo-treated side with statistically significant at P-value < 0.001 (see Table 4.4).

Table 4.4 Mean sebum level of each follow up visit.

Time	Hemp seed extract		Placebo		P-value[b]
	Mean±SD	P-value[a]	Mean±SD	P-value[a]	
Week 0	18.75±13.49	1	21.20±14.00	1	0.059
Week 2	14.85±8.06	0.135	16.30±9.96	0.070	0.280
Week 4	13.65±7.75	0.073	16.50±9.43	0.051	0.060
Week 8	10.05±6.08	0.006*	14.94±8.16	0.048*	<0.001*
Week 12	10.33±6.46	0.004*	16.94±10.84	0.152	<0.001*

Values are expressed as mean±SD. P-value is derived from paired t-test.
(P-value[a] < 0.05 comparison to baseline within group, P-value[b] < 0.05 comparison between hemp seed extract and placebo)

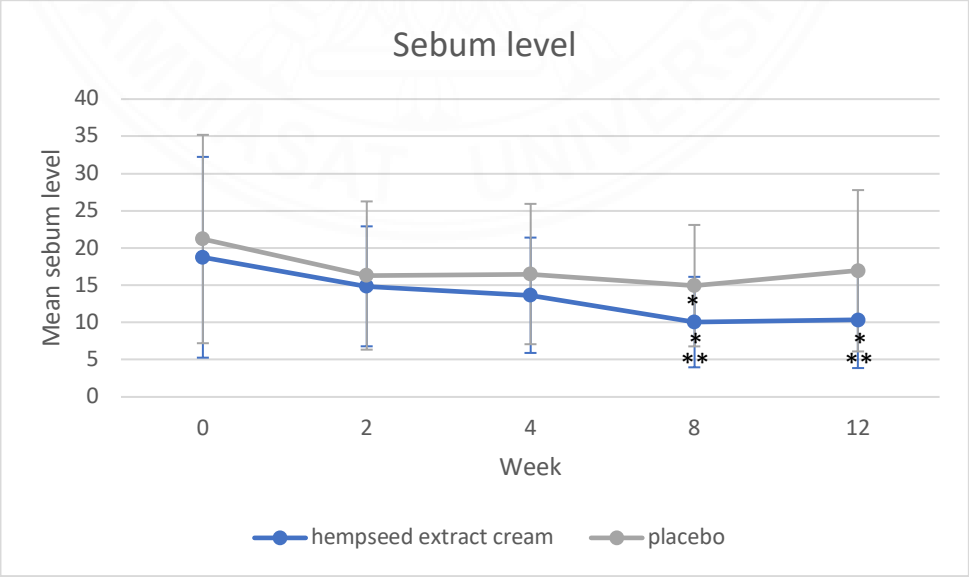

Figure 4.3 Chart of mean sebum level.
* P-value < 0.05 comparison to baseline within group,
**P-value < 0.05 comparison between hemp seed extract and placebo

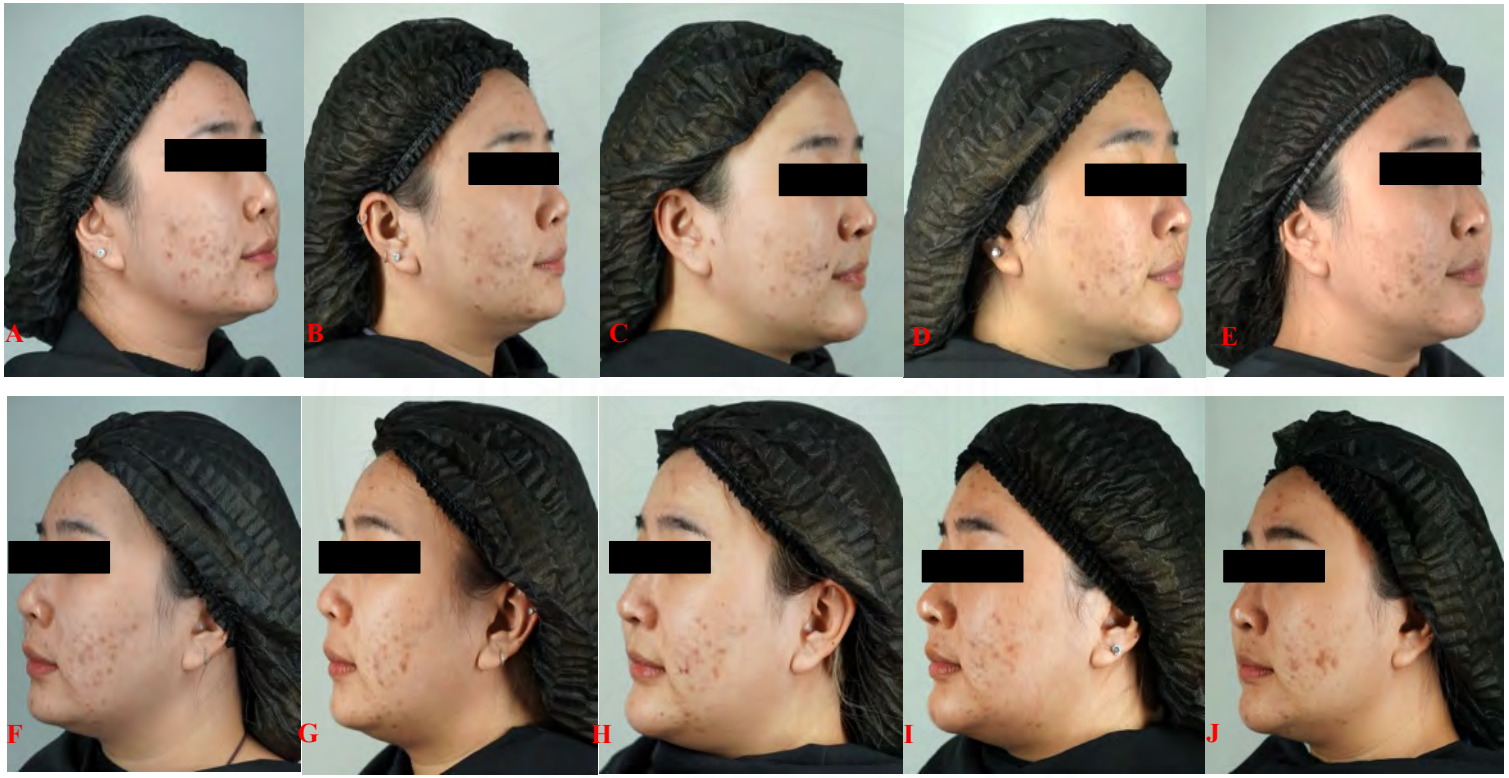

Figure 4.4

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.3)

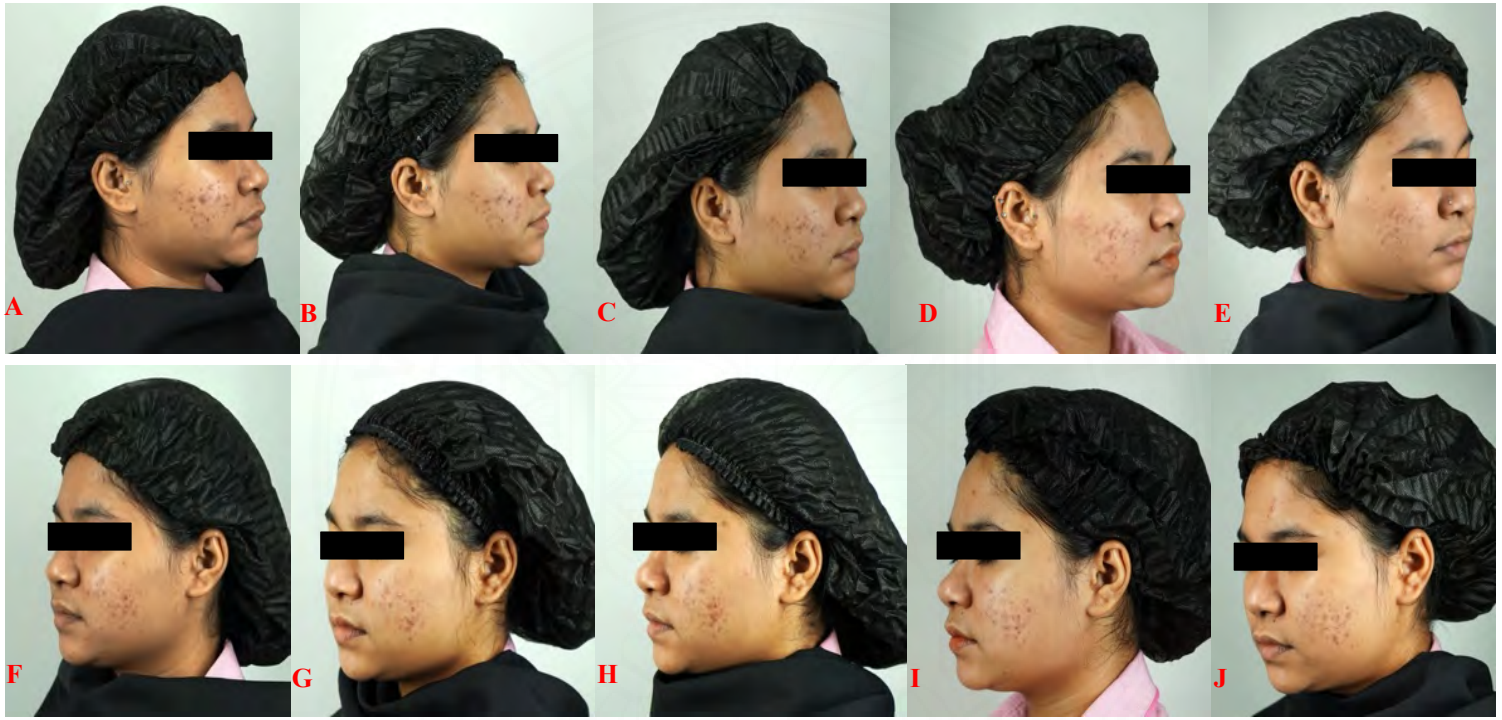

Figure 4.5 Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week 12. (Subject No.9)

4.2.3 Erythema index

Erythema index was measured by Antera 3D® with hemoglobin index level at every follow-up visit. Hemoglobin levels within the group of hemp seed extract-treated side did not significantly change from baseline. Similar to placebo-treated side, the hemoglobin index level by Antera 3D® showed no change of the hemoglobin index level along the study period and no statistically significant within group (see Table 4.5).

Therefore, when compared the mean different of hemoglobin index level between these two groups, the result indicated that there were statistically significant differences in mean hemoglobin index level at week 4 and week 12 of the study with P-value = 0.049 and 0.012 respectively as shown in Table 4.5.

Table 4.5 Mean hemoglobin index level by Antera 3D®of each follow up visit.

Time	Hemp seed extract		Placebo		P-value[b]
	Mean±SD	P-value[a]	Mean±SD	P-value[a]	
Week 0	1.41±0.18	1	1.40±0.19	1	0.496
Week 2	1.41±0.18	0.790	1.40±0.16	0.983	0.816
Week 4	1.41±0.15	0.854	1.44±0.13	0.076	0.049*
Week 8	1.40±0.17	0.512	1.44±0.16	0.307	0.073
Week 12	1.42±0.16	0.337	1.46±0.17	0.149	0.012*

Values are expressed as mean±SD. P-value is derived from paired t-test.
(P-value[a] < 0.05 comparison to baseline within group, P-value[b] < 0.05 comparison between hemp seed extract and placebo)

4.3 Adverse events

Adverse events were evaluated at every follow-up visit which was week 2, week 4, week 8, and week 12 of the study. Questionnaire evaluation on side effects including itchiness, scaling, burning, and erythema were given to the patients. No systemic side effects occurred during the study period in both interventions. In hemp seed extract-treated side, only minimal skin dryness and itchiness were reported during the study. Number of patient experienced scale around inflammatory papules were 1, 2, and 1 person at week 2, 4, and 12 respectively. Moreover, only one patient reported itchiness at week 12 of the study. The one who experienced the itchiness said that the itching sensation was just limited nearby inflammatory papules, not the whole area. Not only these two symptoms were reported in hemp seed extract-treated side, but itchiness and scaling were also reported in placebo-treated-side. Number of patients complained with scaling at week 2, 4, 8, and 12 were 2, 2, 1, and 2 persons respectively. Whereas itchiness was reported by 1 patient at week 2, 4, and 12.

According to the results, the adverse events of both hemp seed exact cream and placebo were comparable. Total of 4 subjects reported scaling in hemp seed exact-treated side, whereas 7 people noted scaling from placebo along the study period. For, itchiness, only 1 person in hemp seed extract group was claimed for itching sensation, while in placebo group 3 person informed that they also had itchiness. Data of patients experienced with sign of skin irritation in each follow-up visit are shown in Table 6.6.

Table 4.6 Frequency of patients experience with side effects at each follow-up visit.

Variables	Hemp seed extract-treated side n (%)	Placebo-treated side n (%)
Baseline		
-Itching	0 (0)	0 (0)
-Burning	0 (0)	0 (0)
-Erythema	0 (0)	0 (0)
-Scaling	0 (0)	0 (0)
Week 2		
-Itching	0 (0)	1 (5)
-Burning	0 (0)	0 (0)
-Erythema	0 (0)	0 (0)
-Scaling	1 (5)	2 (10)
Week 4		
-Itching	0 (0)	1 (5)
-Burning	0 (0)	0 (0)
-Erythema	0 (0)	0 (0)
-Scaling	2 (10)	2 (10)
Week 8		
-Itching	0 (0)	0 (0)
-Burning	0 (0)	0 (0)
-Erythema	0 (0)	0 (0)
-Scaling	0 (0)	1 (5.26)
Week 12		
-Itching	1 (5.56)	1 (5.56)
-Burning	0 (0)	0 (0)
-Erythema	0 (0)	0 (0)
-Scaling	1 (5.26)	2 (11.11)

4.4 Physician's clinical assessment score

Score was graded by physician at each week of follow-up. By using five-point scale which ranged from -2 to 2 (-2 = very poor clinical outcome, -1 = poor clinical outcome, 0 = neither poor nor good clinical outcome, 1 = good clinical outcome, 2 = very good clinical outcome).

According to physician's clinical assessment score, the score tends to increase during the study period in hemp seed extract-treated side. At second week of the study, percentage of patients who were given a score -1, 0, 1, and 2 were 15%, 30%, 50%, and 5% respectively. At the end of the study, majority of the patients were classified as good and very good clinical outcome by gotten a score 1 and 2 for 44.44% and 22.22% respectively. In placebo-treated side, at week 2 of the study percentage of patients who were given a score -1, 0, and 1 were 15%, 30%, and 55% respectively. At week 12, one third of the patients were classified very poor and poor clinical outcome. Percentage of patients who were given a score -2, -1, 0, 1, and 2 were 5.56%, 27.78%, 22.22%, 27.78, and 16.67% respectively.

To sum up, at the end of the study, physician's clinical assessment score in hemp seed extract group has shown more improvement of clinical outcome than placebo group. The percentage of patients who was assessed with good and very good clinical outcome was 66.66% in hemp seed extract group and 44.45% in placebo group.

Table 4.7 Physician's clinical assessment score at each follow-up visit.

Time	Hemp seed extract-treated side		Placebo-treated side	
	score	n (%)	score	n (%)
Week 2	-2 -1 0 1 2	0 (0) 3 (15) 6 (30) 10 (50) 1 (5)	-2 -1 0 1 2	0 (0) 3 (15) 6 (30) 11 (55) 0 (0)
Week 4	-2 -1 0 1 2	1 (5) 4 (20) 6 (30) 9 (45) 0 (0)	-2 -1 0 1 2	0 (0) 2 (10) 8 (40) 10 (5) 0 (0)
Week 8	-2 -1 0 1 2	0 (0) 2 (10.53) 5 (26.31) 8 (42.11) 4 (21.05)	-2 -1 0 1 2	0 (0) 3 (15.79) 6 (31.58) 6 (31.58) 4 (21.05)
Week 12	-2 -1 0 1 2	0 (0) 3 (16.67) 3 (16.67) 8 (44.44) 4 (22.22)	-2 -1 0 1 2	1 (5.56) 5 (27.78) 4 (22.22) 5 (27.78) 3 (16.67)

-2 = very poor clinical outcome, -1 = poor clinical outcome, 0 = neither poor nor good clinical outcome, 1 = good clinical outcome, 2 = very good clinical outcome

4.5 Patients' satisfaction score

Satisfaction score was graded by patients at each week of the follow-up. By using five-point scale which ranged from -2 to 2 (-2 = very dissatisfied, -1 = dissatisfied, 0 = neither satisfied nor dissatisfied, 1 = satisfied, and 2 = very satisfied). According to patients' satisfaction score, in hemp seed extract-treated side, the score tends to increase over time. At the second week of the study, percentage of patients given the score were 20%, 30%, 35%, and 15% for the score -1, 0, 1, and 2 respectively. At the end of the study, none of the participant reported dissatisfy. Majority of the patients (66.67%) were satisfied with hemp seed extract cream by given a score 1 and 2 for 38.89% and 27.28% respectively. Whereas in placebo-treated side, the score seemed to be stable over time. Therefore, at week 12, half of the patients were not satisfied by given a score -1 and 0 for 5.56% and 44.44% respectively. However, another half of the patients seemed to be satisfied by given a score 1 and 2 for 27.27% and 22.22% respectively.

In conclusion, at the end of the study, patients were more satisfied with hemp seed extract cream than placebo. The percentage of patients who satisfied (the one who given a score 1 or 2) with the product was 66.67% for hemp seed extract cream and 50% for placebo.

Table 4.8 Frequency of patients evaluated satisfaction score at each follow-up visit.

Time	Hemp seed extract-treated side		Placebo-treated side	
	score	n (%)	score	n (%)
Week 2	-2	0 (0)	-2	1 (5)
	-1	4 (20)	-1	4 (20)
	0	6 (30)	0	5 (25)
	1	7 (35)	1	6 (30)
	2	3 (15)	2	4 (20)
Week 4	-2	0 (0)	-2	0 (0)
	-1	7 (35)	-1	6 (30)
	0	3 (15)	0	5 (25)
	1	8 (40)	1	7 (35)
	2	2 (10)	2	2 (10)
Week 8	-2	0 (0)	-2	0 (0)
	-1	1 (5.26)	-1	0 (0)
	0	6 (31.58)	0	6 (31.58)
	1	8 (42.11)	1	10 (52.63)
	2	4 (21.05)	2	3 (15.79)
Week 12	-2	0 (0)	-2	0 (0)
	-1	0 (0)	-1	1 (5.56)
	0	6 (33.33)	0	8 (44.44)
	1	7 (38.89)	1	5 (27.78)
	2	5 (27.78)	2	4 (22.22)

-2 indicated very dissatisfied, -1 indicated dissatisfied, 0 indicated neither satisfied nor dissatisfied, 1 indicated satisfied, and 2 indicated very satisfied.

4.6 Discussion

Among young people, acne vulgaris is one of the most prevalent chronic inflammatory skin illnesses. Although it is not a life-threatening condition, but therefore can be both physiologic and psychologic disease burden. Acne vulgaris has four primary etiology, including follicular hyperkeratinization, increased sebum

production, C. acnes, and inflammation. According to various pathologic factors, the standard treatment recommends to use combination regimens. For the treatment of acne vulgaris, there are several choices, ranging from topical to systemic. Whether topical or systemic agents, most of the standard treatment have some side effects such as irritation from benzoyl peroxide, increase bacterial resistance from antibiotics, and teratogenic effect from isotretinoin [1, 3, 24].

Recently, hemp and cannabis-base skin care products are interested among the markets because it contains several phytocannabinoids. Of all phytocannabinoids, CBD has proven to be the major composition of phytocannabinoid in hempseed and its extract [9, 10].Several studies [7, 56, 71, 82] revealed that CBD and hemp have shown the ability in anti-lipogenic, anti-proliferative, and anti-inflammatory. CBD appears to have anti-inflammatory effects through the A2A adenosine receptor, which inhibits the p65 NF-κB pathway in addition to decreasing sebum production and sebocyte proliferation through TRPV4 activation [7]. By the mechanism of CBD from aforementioned, it may also use as an alternative treatment of acne vulgaris.

Our study revealed that hemp seed extract cream had ability to improved acne lesions in both inflammatory and non-inflammatory. Nonetheless, seemed to showed greater results in inflammatory lesions. These results were consistent with anti-inflammatory and anti-bacterial properties of CBD and hemp seed extract. There were several studies about anti-inflammatory properties of CBD in both vitro and vivo. A previous study from Giacoppo et al [71] was conducted in 2015. In this study, autoimmune encephalomyelitis mice were applied 1% topical CBD once daily for total 28 days. The researchers found that 1% CBD cream reduced the expression of inflammatory cytokines. Apart from animal studies, there were more human studies about anti-inflammatory properties of CBD. In 2019, Palmieri and a colleague [76] launched a research to explore upon how individuals with psoriasis, atopic dermatitis, and scars responded to CBD-enriched ointment. Both the clinical result and the objective metrics showed a considerable improvement in the outcome. Furthermore, another clinical study about CBD with atopic dermatitis was obtained by Maghfour et al [83] in 2021. They found that 1% CBD-infused gel enhanced both clinical severity of eczema and pruritic symptoms. For anti-bacterial effect of hempseed extract, Jin et al [56] detected that 0.6% of hempseed hexane extract reduced *P.acnes*-induced

inflammation in human keratinocyte cell line by down-regulation of pro-inflammatory cytokines, especially IL-1β and IL-8.

With the exception of standard treatment, complementary and alternative medicines are upcoming for an option for treating acne vulgaris. It has been demonstrated that herbal extracts such mangosteen fruit extract and tea tree oil could cure acne. A study of 0.5% topical mangosteen extract gel was done in 2018 [41]. In this split-face study, they compare the efficacy between 0.5% mangosteen extract plus 2.5% benzoyl peroxide and 1% clindamycin gel plus 2.5% benzoyl peroxide. The results after 12 weeks demonstrated that 0.5 % mangosteen extract was equivalent to 1 % clindamycin gel in terms of improvement of both inflammatory lesions and comedones. However, due to comedolytic and antibacterial effects against *P.acnes* of benzoyl peroxide, this may interfered outcome of the study. Another study about mangosteen [42] was also conducted in 2019. This study was a comparative study of herbal extracts, which composed of mangosteen, tea tree oil, and 4% niacinamide, vs 2.5% benzoyl peroxide in mild to moderate acne patients. They found that at the 12-week point both herbal extract and 2.5% benzoyl peroxide reduced inflammatory lesions for 40.54% and reduced comedones for 34.51% when compared to baseline. However, the outcomes were inconclusive when compare between the efficacy of herbal extracts and 2.5% benzoyl peroxide. The results of these two previous researches were similar to our study, which exhibited the greater improvement of inflammatory acne lesions than comedones. According to previous researches, mangosteen extract (contain alpha-mangostin) and tea tree oil seemed to be effective for acne treatment due to anti-inflammatory and anti-bacterial properties. However, our main ingredient, hemp seed extract function on more acne pathogenesis than those alternative treatments due to anti-lipogenic, anti-inflammation, and anti-microbial effects.

Moreover, we also evaluated erythema index by using Antera 3D® biometic camera, which gave the result of hemoglobin index level at every follow up visit. As a result of anti-inflammatory property of CBD, we expected that hemoglobin index level should be decreased, as well as the clinical improvement of acne lesions. Unfortunately, our results showed no significant decreased of hemoglobin index level within hemp seed extract treated-side. However, mean hemoglobin index level between hemp seed extract and placebo were significant difference at week 4 and week 12 of the study. Our

results of hemoglobin index were contrast to Ali's study in 2015 [82]. They revealed that 3% cannabis seeds extract cream showed significant reduction of erythema index in healthy male subjects. In details, these dissimilar results might be explained by two reasons. First, there were difference in subjects between Ali's and our study. Ali's subjects were all healthy males without any skin diseases, whereas our study consisted of acne patients. According to the different type of subjects, evaluation of erythema index between normal skin and post acne erythema might give the dissimilar results. Second, there were different tool assessment between these two studies. In our study, we used Antera 3D® biometic camera to measured hemoglobin level. This gave us an average hemoglobin level within the chosen area. Whereas Ali used mexameter from Courage and Khazaka Electronics GmbH, Cologne Germany. Probe of mexameter is small and be specific to the lesions. Further investigation should be done to get more accurate result of hemoglobin index.

As we all know that one of the key etiology of acne is increased sebum production. This excess sebum leads to the anaerobic, lipid rich environment which is suitable for *C.acnes* proliferation [84]. Topical retinoids and L-carnitine have been found to reduce sebum in both vitro, and vivo studies [85-87]. Nevertheless, skin irritation is commonly found among patients using topical retinoid. While topical L-carnitine alone is not wildly use in acne treatment. Systemic agents such as oral isotretinoin, spironolactone, and oral contraceptive pills are capable in sebocyte apoptosis and inhibiting sebocyte proliferation [85]. However, the use of these oral treatment regimens are limiting. In vitro study by Olah et al, CBD in hemp reduced sebum production by suppressed sebocyte proliferation via the activation of transient receptor potential vanilloid-4 (TRPV4) ion channels [7].

The anti-lipogenic activity of CBD in vitro is corresponded to a clinical study by Ali et al [82]. Apart from erythema index from aforementioned, Ali was also assessed human cheeks skin sebum level by using sebumeter (Courage and Khazaka, Germany). They revealed that 3% cannabis seeds extract was significantly reduced cheek sebum level both within group and when compared to cream base. Our results point in the same direction as Ali, we found that at the end of the study hemp seed extract cream reduced mean sebum level by 44.90% from pre-treatment. This theory might produce CBD above other alternative treatment because most topical treatment

options for acne vulgaris lacks of anti-lipogenic effect. However, some alternative treatment contains sebosuppressive effect. Nicotinamide, or niacinamide is a current alternative treatment option for acne vulgaris due to its anti-inflammatory and sebostatic effects [88-90]. A previous year, there was a comparison study of topical 2.5% benzoyl peroxide plus 5% niacinamide vs 2.5% benzoyl peroxide plus cream base in treatment of mild to moderate facial acne vulgaris. The results of this study indicated that niacinamide potentially reduced both inflammatory and non-inflammatory acne lesions. Moreover, this vitamin B3 was also showed decrease in sebum casual level at week 12 by 26% from baseline. However, the reductions were not statistically different from cream base [91]. When compare to our study, at week 12 hempseed extract cream exhibited greater result in sebum reduction by reduced 44.90% from baseline and also statistically significant when compared to placebo.

Apart from CBD, hemp seed oil also contains other functional ingredients such as linoleic acid, and alpha-linoleic acid which are polyunsaturated fatty acids (PUFA), tocopherols (alpha, beta, gamma, and delta), terpenes, and flavonoids [48, 49, 92]. As we all know that vitamin E or tocopherol has anti-oxidant and anti-inflammatory bioactivities [93]. Former studies [94-96] demonstrated that tocopherol or vitamin E improved clinical of atopic dermatitis both orally and topically. In addition to the fact that follicular keratinocyte proliferation is also regulated by linoleic acid. Low level of linoleic acid contributes to follicular hyperkeratinization, which result in comedone formation. Our study product, hemp seed extract cream consists of linoleic acid, CBD, and other several functional ingredients. Hence, linoleic acid in our hemp seed extract cream might diminish number of comedones. Moreover, there was a study indicated that these essential fatty acids in dietary hempseed oil improved clinical symptoms of atopic dermatitis [55]. According to aforementioned, CBD and other major constituents of hemp seed extract together might promote acne improvement.

Our study product, hemp seed extract cream, consists of 1% hemp seed oil which contain amount of CBD about 0.2%. Concentration of CBD and inflammatory skin diseases in former studies were varies from non-specified to 1%. There were studies about 1% topical CBD and anti-inflammatory property in animal and human in 2015 and 2021. An animal study by Giacoppo et al [71] indicated that daily applied 1% CBD cream for 28 days reduced the expression of inflammatory cytokines in

autoimmune encephalitis mice. Moreover, a clinical study by Maghfour et al [83] stated that 1% CBD-infused gel enhanced both clinical severity of eczema and pruritic symptoms. However, some study did not exhibit concentration of CBD such as a retrospective study by Palmieri and collogues [76]. They found that CBD-enriched ointment significantly improved clinical outcome and quality of life in inflammatory skin diseases such as psoriasis and atopic dermatitis. In addition, Ali et al [82] conducted a study of 3% cannabis seeds extract cream for human cheek skin sebum and erythema content. They indicated that their product of 3% cannabis seeds extract cream was significantly reduced in sebum production and hemoglobin index in healthy males. Unfortunately, they did not declare concentration of CBD in their cream. Nonetheless, our hemp seed extract cream with 0.2% CBD, showed great result in improvement of inflammatory acne lesions. Hence, this amount of CBD in our cream combined with another functional ingredients of hemp seed extract illustrated anti-inflammatory properties.

Considering the questionnaire evaluation on side effects including itchiness, scaling, burning, and erythema. Majority of patients reported no discomfort. However, scaling and itching sensation were found in minority of patients. These symptoms were commonly found in both interventions. This might caused by skin dryness due to we did not provide moisturizer for the participants, or other non-inactive ingredient in cream formulation. The former studies of topical CBD or hemp seed extract for dermatologic conditions were found to be safe with no irritant or allergic reaction in majority of people, however minimal skin irritation such as stinging sensation was found [76, 82, 83].

Hence, hemp seed extract cream could be the optional herbal medication in the treatment of acne vulgaris due to its effectiveness in majority of acne pathogenesis included inflammation, sebum production, and *C.acnes* proliferation with lessen side effects.

Nevertheless, the study's limitations were the limited the small sample size, loss of long term follow up period and mask wearing due to covid-19 situation.

CHAPTER 5
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In teens and young adults, acne vulgaris is a relatively prevalent chronic skin disorder. It negatively impacts patients' wellbeing in both physical and psychological dimensions. Although many risk factors and 4 main pathogenesis have been identified, the treatment is still challenging. Current standard treatments, whether topical or systemic agents have some side effects such as irritation from benzoyl peroxide, increase bacterial resistant from antibiotic, and teratogenicity, increase lipid profile from isotretinoin. Hemp seed extract which contains CBD as a major phytocannabinoid, was found to has an ability in anti-inflammatory, anti-lipogenic, and anti-proliferative properties. This study was inspired by a limitation of clinical human study of CBD or hemp seed extract in acne patients. Our research is the first prospective, split-face, randomized, double blinded study of hemp seed extract and acne vulgaris. The efficacy has been compared between hemp seed extract cream and placebo in total 12 weeks period of study. Our study revealed significant improvement in acne severity, particularly inflammatory acne lesions, which evaluated by acne lesion count. Moreover, sebum level was also found significantly reduction, which evaluated by sebumeter. Unfortunately, contrasting from previous research, we established that there was no significant improvement in erythema index. Adverse events found in hemp seed extract cream were mild, including skin dryness and itchiness. There was no serious adverse effects presented in both interventions. In conclusion, hemp seed extract cream might be safe and effective alternative treatment option for treatment of mild to moderate acne vulgaris.

5.2 Recommendations

5.2.1 Recommendation for clinical application

5.2.1.1 Our study recommends using hemp seed extract cream as an alternative treatment option for treatment of mild to moderate acne vulgaris.

5.2.2 Recommendation for future research

5.2.2.1 Further study with a larger population may provide the better precision and accuracy.

5.2.2.2 Evaluation of *C.acnes* such as number of porphyrin, which indirectly implied to the amount of *C.acnes* should be done to prove anti-microbial activity against *C.acnes*.

5.2.2.3 Evaluation of hemoglobin index by using different tools, such as mexameter should be done for more accuracy outcome of erythema index.

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APPENDICIES

APPENDIX A
CASE RECORD FORM

แบบฟอร์มบันทึกข้อมูล (Case record form)
Subject identification NO ☐☐
Part A: ประวัติผู้ป่วย
1.อายุ..... ปี 2.เพศ ☐ ชาย ☐ หญิง 3.วัน เดือน ปีเกิด (วันที่/เดือน/ปีพ.ศ.) ..... 4. โรคประจำตัว ☐ ไม่มี ☐ มี โปรดระบุ..... 5. ยาที่ใช้ประจำ ☐ ไม่มี ☐ มี โปรดระบุ.....
Part B: เกณฑ์แยกอาสาสมัครออกโดยแพทย์
1.อยู่ในระหว่างตั้งครรภ์ หรือให้นมบุตร ☐ ไม่ใช่ ☐ ใช่ 2.ได้รับยารักษา isotretinoin ภายใน 6 เดือนก่อนเข้าร่วมวิจัย ☐ ไม่มี ☐ มี 3.ได้รับยาปฏิชีวนะ, ยาเสตียรอยด์, ยาเม็ดคุมกำเนิด ชนิดรับประทานภายใน 1 เดือนก่อนเข้าร่วมวิจัย ☐ ไม่มี ☐ มี 4.ได้รับการทำเลเซอร์ที่ใบหน้าภายใน 1 เดือนก่อนเข้าร่วมวิจัย ☐ ไม่มี ☐ มี 5.ใช้ยาทารักษาสิว เช่น Benzoyl peroxide, antibiotic, retinoid, chemical peeling agent ภายใน 2 สัปดาห์ก่อนเข้าร่วมวิจัย ☐ ไม่มี ☐ มี 6.ประวัติแพ้ยาทาชนิด benzoyl peroxide หรือครีมที่มีส่วนผสมของ cannabidiol ☐ ไม่มี ☐ มี 7.ใช้ยากินหรือยาทาที่มีผลต่อความมันบนใบหน้า เช่น retinoid, OCP, spironolactone ☐ ไม่มี ☐ มี 8.มีอาการกำเริบของโรคผิวหนังอื่นๆบนใบหน้า ☐ ไม่มี ☐ มี 9.ได้รับการวินิจฉัยว่ามีโรคทางจิตเวช ☐ ไม่มี ☐ มี
Part C: เกณฑ์คัดเข้าโดยแพทย์
1.ผู้ป่วยมีอายุมากกว่า 18 ปี ☐ ไม่ใช่ ☐ ใช่ 2.ผู้ป่วยอ่านและยินยอมลงนามในแบบฟอร์ม inform consent ☐ ไม่ใช่ ☐ ใช่ 3.สิวความรุนแรงเล็กน้อยถึงปานกลาง (จาก Global Acne Grading System) ☐ ไม่ใช่ ☐ ใช่
ข้าพเจ้ายืนยันว่าข้อมูลด้านบนมีความถูกต้องและสมบูรณ์ ผู้เข้าร่วมการวิจัย..... วันที่..... แพทย์ผู้ตรวจสอบ..... วันที่.....

แบบฟอร์มบันทึกข้อมูล (Case record form)			
Global Acne Grading System			
Location	Factor	Grade	Local score (factor*grade)
Forehead	2		
Right cheek	2		
Left cheek	2		
Nose	1		
Chin	1		
Chest and upper back	3		
Global score			
GAGS ☐ None = 0 ☐ Mild = 1 – 18 ☐ Moderate = 19 – 30 ☐ Severe = 31-38 ☐ Very severe > 39			
* Grade 0 No lesion 1 ≥ one comedone 2 ≥ one papule 3 ≥ one pustule 4 ≥ one nodule			

วันที่	แบบฟอร์มบันทึกข้อมูล (Case Record Form)

Subject identification NO ☐☐ Week ☐☐

Part A: การตรวจร่างกายโดยแพทย์

1. Acne lesion counts

Dermatologist 1

Comedones		Inflammatory lesions	
Right side	Left side	Right side	Left side
		Papules: Pustules: Nodules: Cysts:	Papules: Pustules: Nodules: Cysts:

Dermatologist 2

Comedones		Inflammatory lesions	
Right side	Left side	Right side	Left side
		Papules: Pustules: Nodules: Cysts:	Papules: Pustules: Nodules: Cysts:

2. GAGS …….

3. Erythema index by Antera® 3D : Right side…….. Left side …….

4. Sebum level by sebumeter : Right side……... Left side …….

5. Physician's satisfaction (-2 to 2) : Right side…….. Left side ……..

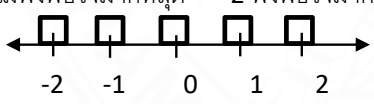
Part B: การประเมินความพึงพอใจและผลข้างเคียงโดยผู้เข้าร่วมวิจัย

ใบหน้าด้านขวา

1.ความพึงพอใจ

(ทำเครื่องหมาย x ในช่องตามความพึงพอใจ)

(-2ไม่พึงพอใจมากที่สุด 2 พึงพอใจมากที่สุด)



ผลข้างเคียง

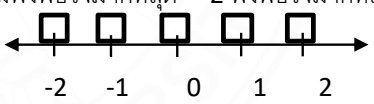
อาการคัน	☐ ไม่มี	☐ มี
อาการแสบร้อน	☐ ไม่มี	☐ มี
อาการแดง	☐ ไม่มี	☐ มี
อาการขุย	☐ ไม่มี	☐ มี

ใบหน้าด้านซ้าย

1.ความพึงพอใจ

(ทำเครื่องหมาย x ในช่องตามความพึงพอใจ)

(-2ไม่พึงพอใจมากที่สุด 2 พึงพอใจมากที่สุด)



ผลข้างเคียง

อาการคัน	☐ ไม่มี	☐ มี
อาการแสบร้อน	☐ ไม่มี	☐ มี
อาการแดง	☐ ไม่มี	☐ มี
อาการขุย	☐ ไม่มี	☐ มี

ข้าพเจ้ายืนยันว่าข้อมูลด้านบนมีความถูกต้องและสมบูรณ์

ผู้เข้าร่วมการวิจัย…… วันที่……..

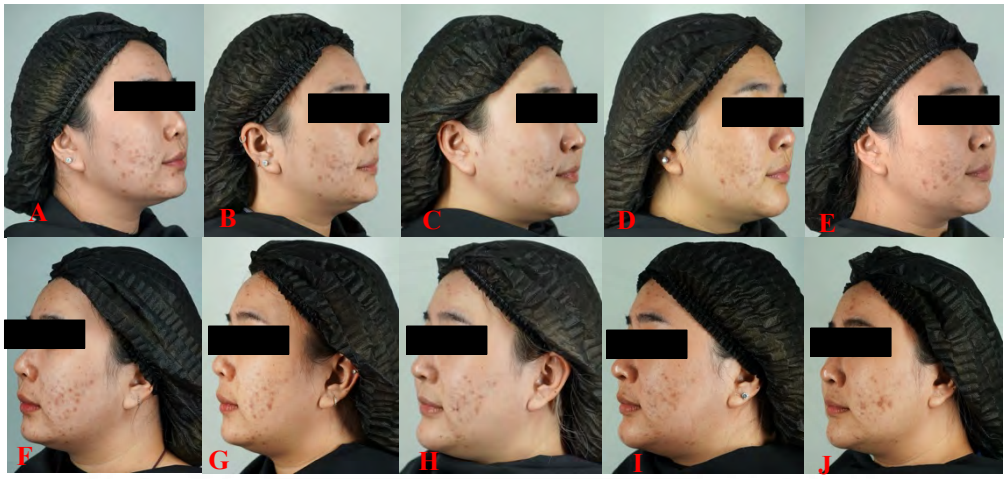
แพทย์ผู้ตรวจสอบ..... วันที่……..

APPENDIX B

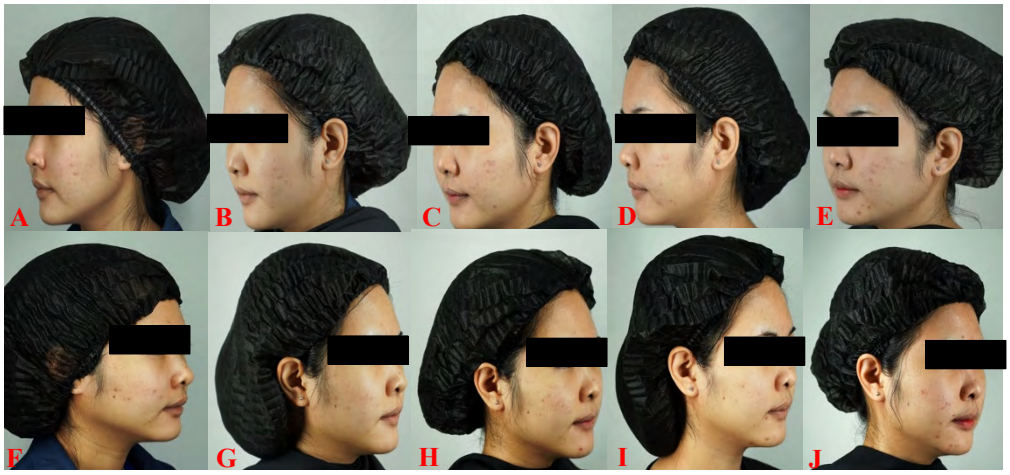
PICTURE OF PATIENTS

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.1)

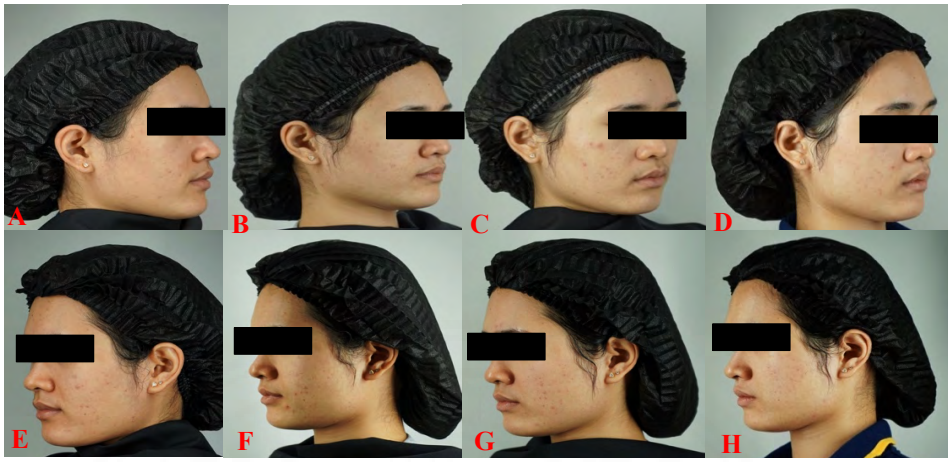
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.2)



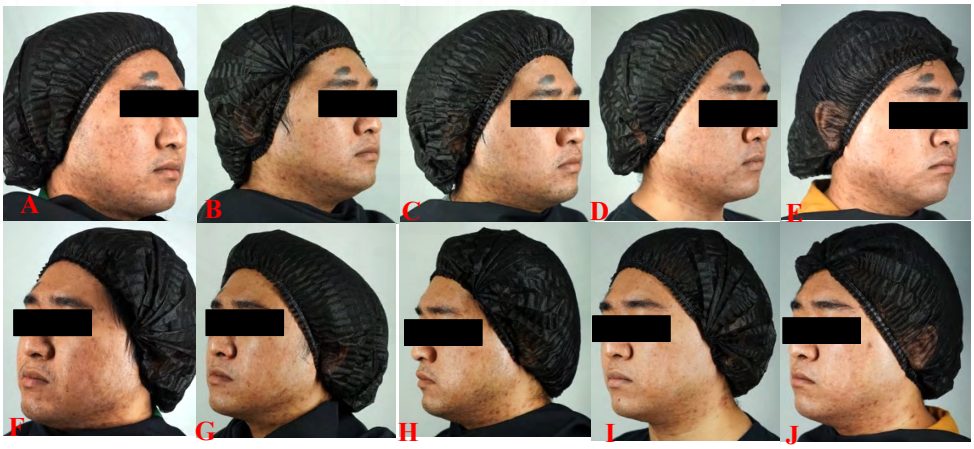
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.3)



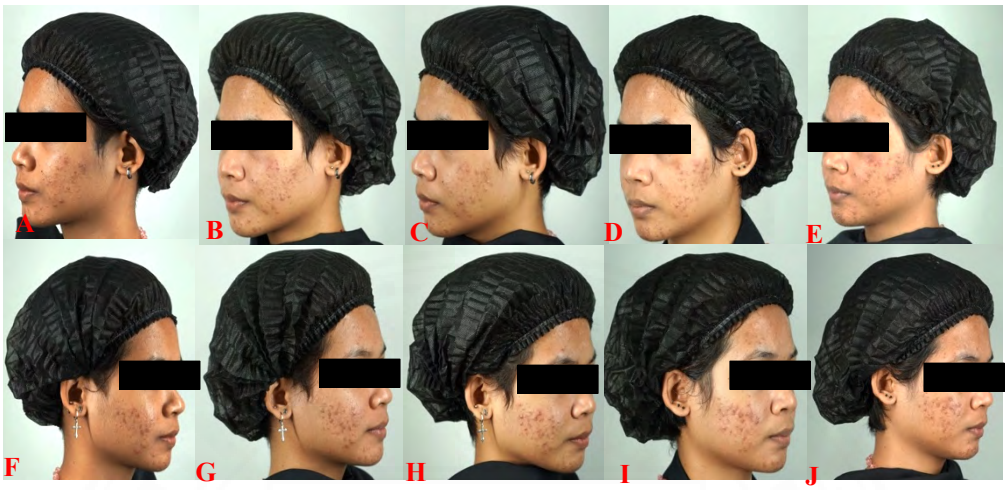
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.4)



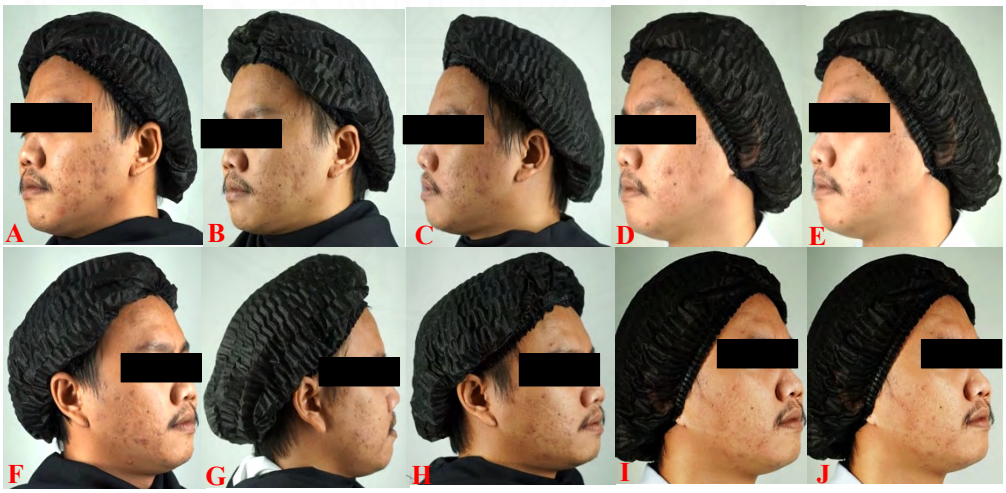
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-D) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (E-H) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.5)



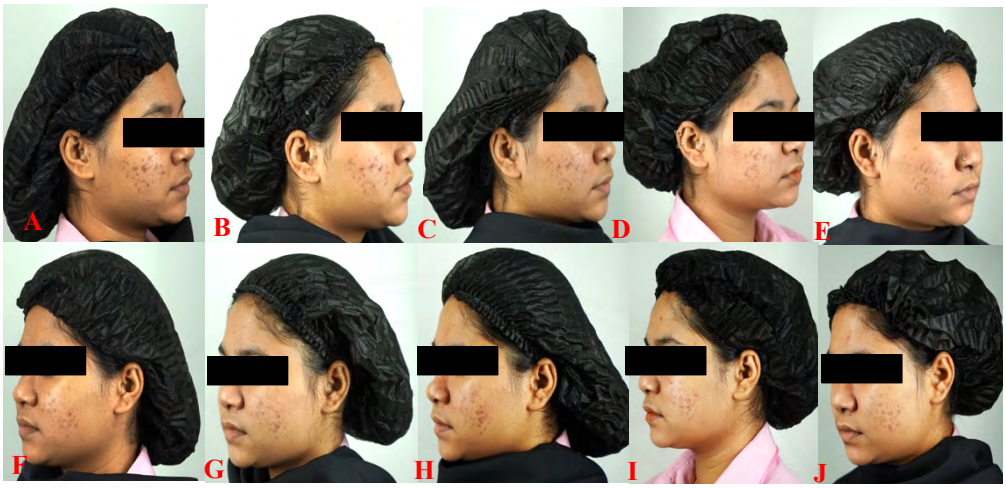
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.6)



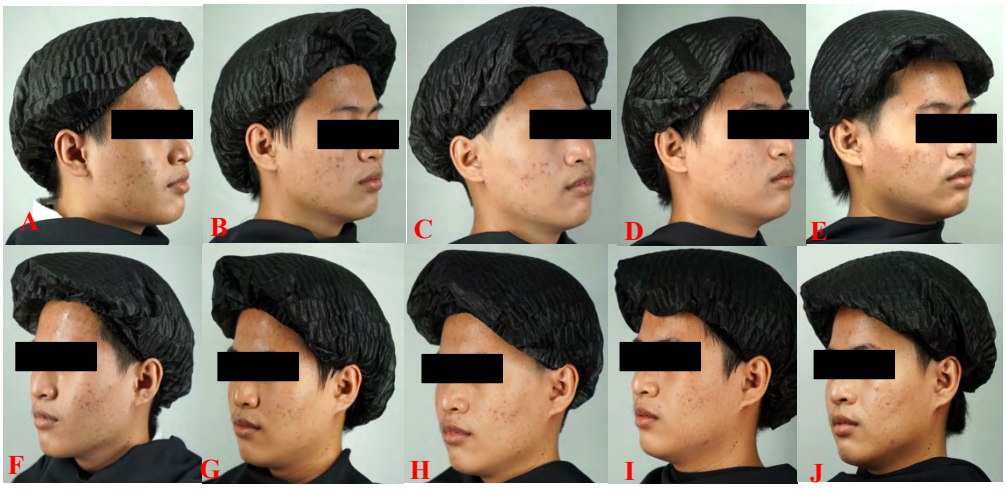
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.7)



Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.8)



Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.9)



Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.10)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.11)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.12)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.13)

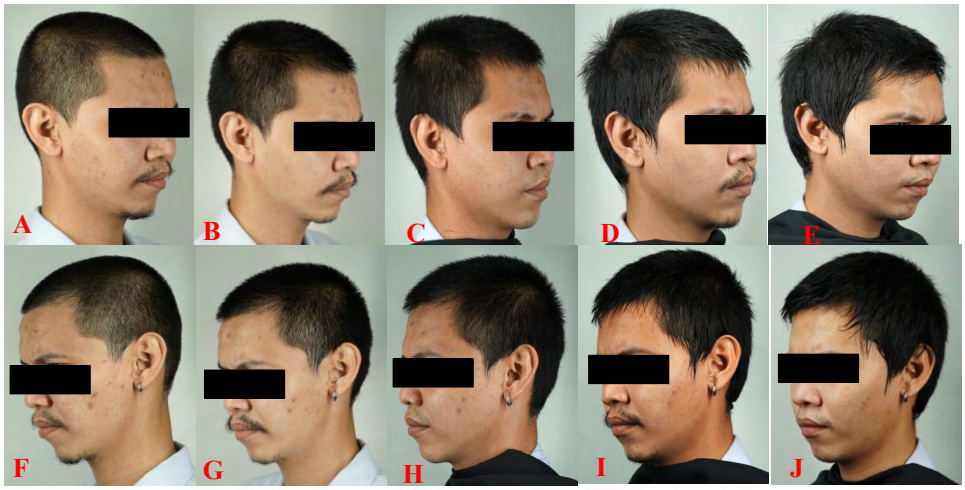
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-C) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (D-F) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.14)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.15)

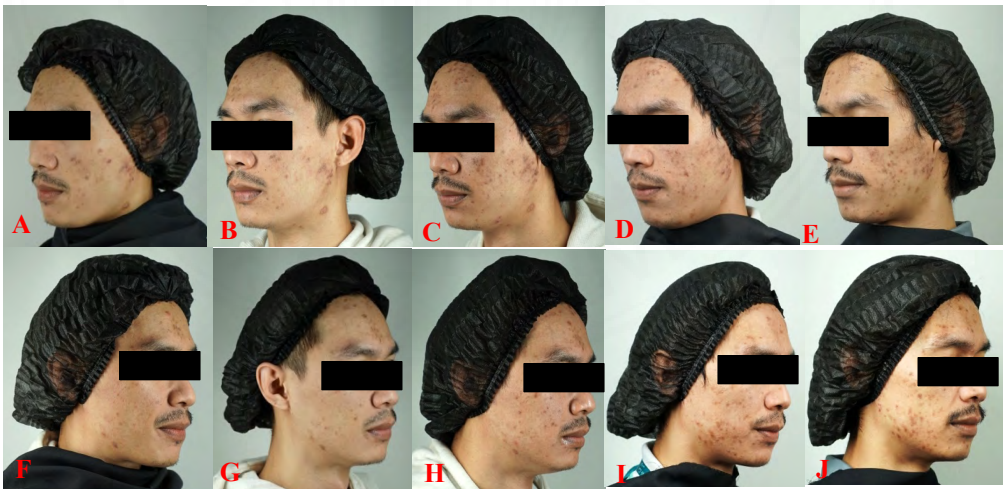
Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.16)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.17)

Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.18)



Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.19)



Evaluation of topical hempseed extract effect on acne patient using the split face study: (A-E) hempseed extract cream-treated side from baseline, week 2, week 4, week 8, to week 12. (F-J) placebo-treated side from baseline, week 2, week 4, week 8, to week12. (Subject No.20) *This subject was just recovery from covid-19 infection at week 12.

BIOGRAPHY

Name	Purida Jaruthiti
Date of Birth	May 10, 1994
Educational Attainment	Academic Year 2018: Doctor of Medicine, Rangsit University, Thailand
Work Experiences	2018 – 2020: General Practice Internship Queen Sirikit Naval Hospital