THE EFFICACY OF 0.05 % TOPICAL *ANDROGRAPHIS PANICULATA* GEL ON THE IMPROVEMENT OF MILD TO MODERATE INFLAMMATORY ACNE

THRATHIP PRASOPDAMKERNG

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IN
DERMATOLOGY

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Thesis Title	The Efficacy of the 0.05% Topical *Andrographis paniculata* Gel on the Improvement of Mild to Moderate Inflammatory Acne
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ABSTRACT

Background: Andrographolide is a major bioactive phytoconstituent found in various parts of *Andrographis paniculate* (AP). Andrographolide exhibits the remarkable antibacterial activity against *P. acnes,* which also has been involved in sebum production , and anti-inflammatory effects by inhibiting NF-kappa B binding to DNA, thus reducing the expression of proinflammatory proteins, which further induces keratinocyte hyperproliferation. This extract is found to inhibit the formation of oxygen-derived free radicals, and significantly increases the activities of antioxidant defense enzymes. Therefore, it can treat inflammatory acne vulgaris effectively by several mechanisms.

Objective: To evaluate the efficacy of 0.05% topical AP gel in improving acne and its adverse effects.

Methods: A randomized, double-blind, placebo-controlled, split-face study was performed on 12 mild to moderate acne patients. Patients applied 0.05% AP gel on one side of the face and placebo on another side twice daily for 8 weeks. Acne lesion counts and adverse effects were evaluated every 2 weeks.

Results: There were significant differences in total inflammatory acne counts between AP gel and placebo at the 4^{th} week ($p = 0.042$). No any adverse effect occurred.

Conclusion: Topical 0.05% AP gel can reduce inflammatory acne effectively. No any adverse effect occurred.

Keywords: Acne vulgaris, *Andrographis paniculata*, Anti-inflammatory effect, Topical gel, Andrographolide

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LIST OF ABBREVIATION

AG	Andrographolide
AHA	Alpha hydroxy acid
AP	*Andrographis paniculata*
BHA	Beta hydroxy acid
cAMP	Cyclic AMP
C. albicans	*Candida albicans*
CRH	corticotropin-releasing hormone
ECM	Extracellular matrix
EDTA	Ethylene diamine tetra-acetic acid
DNA	Deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
et al.	*et alii*
fMLP	N-Formylmethionyl-leucyl-phenylalanine
FTP	Finger tip unit
g	Gram
GASS	Global acne severity scales
GM-CSF	Granulocyte macrophage colony stimulating factor
G6PD	Glucose-6-phosphate dehydrogenase
IL	Interleukin
kg	Kilogram
LPs	Lipid peroxides
µg	Microgram

LIST OF ABBREVIATION (continued)

mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
MMPs	Matrix metalloproteinases
mol	Mole
MUFA	Monounsaturated fatty acids
Nd: YAG	Neodymium-doped yttrium aluminum garnet
OCP	Oral contraceptive pill
P. acnes	*Propionibacterium acnes*
PAF	Platelet-activating factor
PAR-2	Protease activated receptor-2
PDL	Pulse dye laser
PPARs	Peroxisome proliferator-activated receptors
PPD	Probing pocket depth
PIE	Post-inflammatory erythema
PIH	Post-inflammatory hyperpigmentation
PMNs	Polymorphonuclear cells
PPARs	Peroxisome proliferator-activated receptors
ROS	Reactive oxygen species
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
SREBPs	Sterol response-element-binding protein
TLR	Toll-like receptor
TNF	Tumor necrosis factor

LIST OF ABBREVIATION (continued)

VEGF	Vascular endothelial growth factor
W	Weight
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Acne is one of the most common skin diseases of the pilosebaceous follicles involving overproduction of sebum, hyperkeratinisation, inflammation and *Propionibacterium acnes* proliferation (Coenye et al., 2007; Kurokawa et al., 2009; Williams et al., 2012). Although acne does not cause a life-threatening condition, it produces long term scars and other diseases (e.g., anxiety and depression), social interactions, self-confidence, self-esteem and employment (Bowe et al., 2014; Bowe & Logan, 2011; Nguyen & Su, 2011). Acne vulgaris occurs at the area which contain high density of sebaceous gland (Kurokawa et al., 2009; Nguyen & Su, 2011). It commonly occurs in adolescents but is also observed in adults, and can occur at any age, such as neonatal acne and infantile acne. On an average 42.5% of men and 50.9% of women have got an acne (Bhate & Williams, 2013; Zaenglein et al., 2016).

Pilosebaceous units are the key important for the occurrence of acne and further induces hyperkeratinization (Sinha et al., 2014).

Propionibacterium acnes is known as one kind of bacteria of the skin. It can cause acne since long time ago. In addition, *P. acnes* is frequently found to form biofilms in the sebum in acne patients (Jahns et al., 2012). Moreover, *P. acnes* also has been involved in sebum production and lipogenesis by way of the corticotropin-releasing hormone (CRH)/CRH receptor pathway (Ebede et al., 2009). In USA, one research suggested that health- associated *P. acnes* strains exhibited less porphyrins acne-associated strains. In addition, porphyrins can generate reactive oxygen species lead to induction of inflammatory cascade (Johnson et al., 2016). One study showed that there was antibiotic resistance of *Cutibacterium acnes* ranged from 50.8% to 93.6% (Platsidaki & Dessinioti, 2018).

Cutibacterium acnes produces lipases, proteases, and hydrolases, inducing of inflammatory cascade by activation on TLR-2. This would incite the pro-inflammatory cytokines, such as IL-6 and IL-8 in keratinocytes, IL-8 and TNF-α in monocytes and IL-8 and IL-12 by macrophages, which will lead to keratinocyte hyperproliferation (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009).

Nowadays, There are several topical treatments of acne, including topical retinoid, which reduces keratinocyte hyperproliferation, inflammation and *Propionibacterium acnes* proliferation in acne vulgaris; benzoyl peroxide, which is a bactericidal agent, anti-inflammation and mild keratolytic agent; azelaic acid, which has keratolytic, bactericidal, and anti-inflammation. All of their common side effects are local irritation, including erythema, dryness, scaling, peeling, and itching. Bacterial resistance, high toxicity and side effects are also found in many forms of antibiotics (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014). Alternative treatment, such as *Andrographis paniculata*, may cure acne vulgaris. Many research have studied using plant derivative substances to cure acne vulgaris. (Sinha et al., 2014; Soleymani et al., 2020).

Andrographolide may be a nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB) inhibitor, which reduces pro-inflammatory cytokines. This will confirm the antibacterial efficacy of Andrographolide. Some research has showed that Andrographolide can moderately inhibit IL-8 and TNF-α on human keratinocyte HaCaT cells induced by heat-inactivated *P. acnes*. Andrographolide also decreased the DNA binding of NF-kappa B in whole cells and in nuclear extracts induced by PAF and fMLP. Thus, it acts as anti-inflammation by decreasing NF-kappa B binding to DNA, thus inhibiting the expression of proinflammatory cytokines, such as IL-8 and TNF-α (Beesetti et al., 2019; Rehan et al., 2021; Zhang et al., 2020). Andrographolide has also been showed to inhibit IL-2 production and T-cell proliferation in a mixed lymphocyte reaction (Jayakumar et al., 2013).

Therefore, the researcher intends to conduct the study on the efficacy of *Andrographis paniculate.*

1.2 Research Question

Does of the 0.05% topical *Andrographis paniculata* gel more effective in the improvement of mild to moderate acne vulgaris than placebo?

1.3 Objectives

1.3.1 To evaluate the efficacy of 0.05% topical *Andrographis paniculata* gel in improving facial acne vulgaris compared to a placebo gel.

1.3.2 To evaluate the side effects of 0.05% topical *Andrographis paniculata* gel.

1.3.3 To evaluate subjects' satisfaction after using 0.05% topical *Andrographis paniculata* gel as an acne treatment.

1.4 Research Hypothesis

0.05% topical *Andrographis paniculata* gel can improve inflammatory acne more effectively than placebo.

1.5 Variables of the Study

1.5.1 Independent Variable

0.05% topical *Andrographis paniculata* gel

1.5.2 Dependent Variables

1.5.2.1 Porphyrin counts

1.5.2.2 Acne lesion counts

1.5.2.3 Acne severity grading

1.5.2.4 Sebum level

1.5.2.5 Postinflammatory hyperpigmentation and postinflammatory erythema

1.5.2.6 Side effects

1.5.2.7 Patient satisfaction

1.6 Scope of Research

The volunteers with mild to moderate facial acne are enrolled into this study, ages between 18 to 45 years old, both females and males, will be randomly designated to receive the treatment of 0.05% topical *Andrographis paniculata* gel on one side of the face by using a computer-generated random sequence, while the contralateral side served as control. The volunteers will apply 0.05% topical *Andrographis paniculata* gel twice daily for eight weeks. The treatment efficacy, including acne lesion counts, acne severity grading, sebum level, porphyrin count, postinflammatory erythema and postinflammatory hyperpigmentation are measured at the first visit, the 2^{nd}, 4^{th}, 6^{th} and 8^{th} week. The side effects are reported at the 2^{nd}, 4^{th}, 6^{th} and 8^{th} week. The overall subject's satisfaction is reported at the 8^{th} week. The total duration of the study is about two months. The study was performed at Mae Fah Luang University Hospital, Bangkok.

1.7 Conceptual Framework

Propionibacterium acnes produces lipases, proteases, and hydrolases, and also activates on TLR-2, leading to incite the pro-inflammatory cytokines and inflammatory response in macrophages' surface, basal and infundibular keratinocytes. TLR-2 activation incites NF-kappa B triggering and then leads to pro-inflammatory cytokines occurrence, such as IL-1α, IL-6 and IL-8 by follicular keratinocytes, IL-1β, IL-8 and TNF-α by monocytes, IL-8 and IL-12in macrophages, including IL-17and granulocyte macrophage colony stimulating factor (GM-CSF), and then the inflammatory response that induces hyperkeratinization.

Moreover, *P. acnes* also has been involved in sebum production and lipogenesis, as it can stimulate the sebum production by way of the corticotropin-releasing hormone (CRH)/CRH receptor pathway (Ebede et al., 2009). *P. acnes* can also lead to the reactive oxygen species (ROS) formation, especially superoxide anions, by activation of the scavenger receptor CD36 on keratinocytes (Bhate & Williams, 2013; Tanghetti, 2013). In USA, one research suggested that health-

associated *P. acnes* strains exhibited less porphyrins acne-associated strains. In addition, porphyrins produce singlet oxygen from oxygen under ultraviolet exposure might exhibit squalene peroxide, a proinflammatory lipid.

AG acts as anti-inflammation by decreasing NF-kappa B binding to DNA, thus inhibiting the expression of proinflammatory cytokines, such as IL-8 and TNF-α, as figure 1.1. (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009). AG inhibited the oxygen-derived free radicals such as superoxide (32%), hydroxyl radicals (80%), lipid peroxidation (80%), and nitric oxide (42.8%) (Jayakumar et al., 2013; Mussard et al., 2019). The study in Malaysia showed that AP herbal gel at concentration 5.0% (w/w) exhibited the best antimicrobial activities for *P. acnes* and *S. epidermidis* (Kub et al., 2020). Some research has showed that Andrographolide can moderately inhibit IL-8 and TNF-α on human keratinocyte HaCaT cells induced by heat-inactivated *P. acnes* (Zhang et al., 2020). The detail of conceptual framework is as in figure1.1.

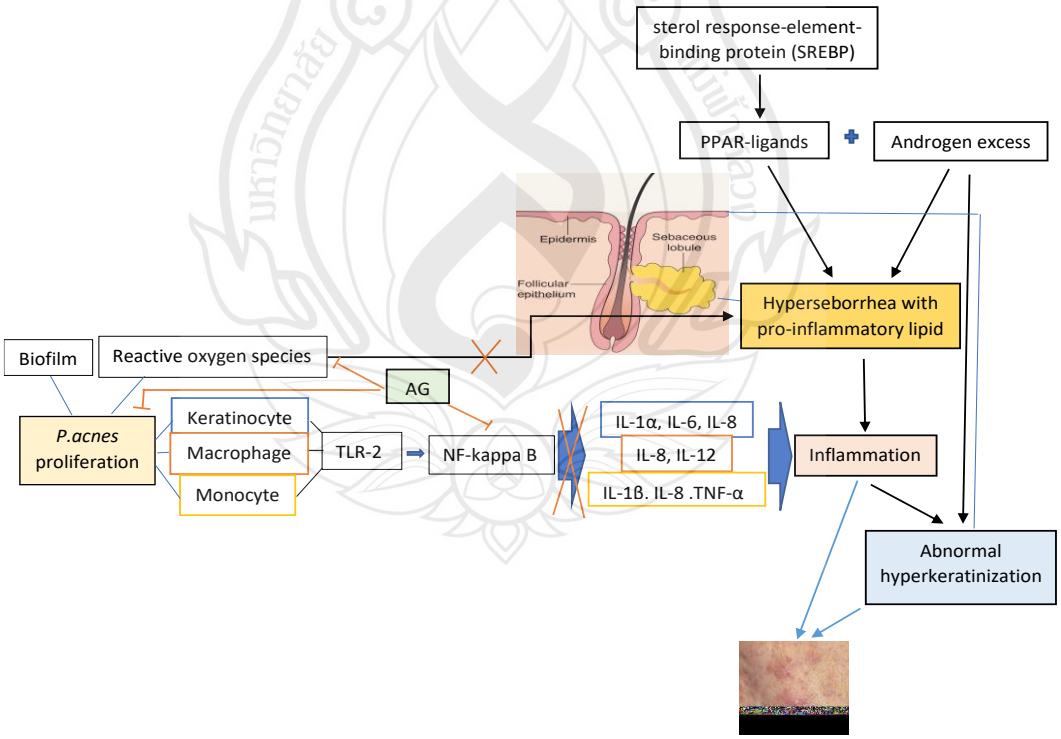


Figure 1.1 Conceptual framework of the mechanism of Andrographolide involved in acne vulgaris

1.8 Operational Definitions

1.8.1 Acne Vulgaris is a chronic inflammatory skin disorder. This disorder of the pilosebaceous unit is classified as non-inflammatory open/closed comedones and inflammatory included papules, pustules, or nodulocystic lesion (Sinha et al., 2014).

1.8.2 Efficacy of the improvement of inflammatory acne is measured from acne lesion counts, acne severity grading, sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts at week 2, 4, 6 and 8 compared to baseline.

1.8.3 Facial acne lesion counting is one of the evaluations of acne severity by counting all facial acne lesions (non-inflammatory and inflammatory acne) within each template segment. Hairline and jaw line defined boundaries of the face and the nose area are excluded, divided this into five facial segments (Lucky et al., 1996).

1.8.4 Porphyrin counts are used as a surrogate measure of *P. acnes* population. VISIA® is used to capture facial image with add-in software to analyze ultraviolet induced fluorescence spots.

1.7.5 Sebum level is evaluated with a photometric device (Sebumeter SM 815, Courage & Khazaka, Cologne, Germany). It is used to collect surface sebum by a special opaque plastic tape (surface area is 64 mm^2), the transparency of the tape after a slight pressure for 30 seconds on the skin is measured. The demonstrated values resuled from the sebum amount on the skin surface in the unit of micrograms per square centimeters (Dobrev, 2010)

1.8.6 Acne severity grading is assessed according to the Global Acne Severity Scale (GASS), which is divided into clear, almost clear, mild, moderate, severe, and very severe.

1.7.7 Side effects are evaluated at week 2, 4, 6 and 8 by history taking and physical examination.

1.7.8 Satisfaction evaluation is evaluated in term of reducing inflammatory acne to both 0.05% topical *Andrographis paniculata* gel and placebo by questionnaire at week 8, using Global Patient Satisfaction scales to evaluate satisfaction in term of

gel properties including colour, texture, viscosity, scent, absorption and result of reducing inflammatory acne.

1.8.9 *Andrographis paniculata*, commonly known as the “king of bitters,” is an herbaceous plant belonging to the Acanthaceae family,its major bioactive phytoconstituent is andrographolide ($C_{20}H_{30}O_5$) (Jayakumar et al., 2013).

1.8.10 *Andrographis paniculata* gel is composed of water, Andrographis paniculata, glycerin, sodium benzoate, triethanolamine, carbomer and disodium EDTA. It is mild green to yellow gel with slight turbidity and pH is approximately 6.

1.8.11 Placebo gel is a gel that contains the base ingredients of *Andrographis paniculata* gel, which are water, glycerin, sodium benzoate, triethanolamine, carbomer, and disodium EDTA. It has the same characteristic, such as colour, odour, and viscosity.

CHAPTER 2

LITERATURE REVIEW

2.1 Acne

Acne vulgaris is a chronic, inflammatory skin disorder of pilosebaceous units that commonly occurs in adolescents but is also observed in adults, and can occur at any age, such as neonatal acne and infantile acne, which is present in between 1 and 12 months. Globally around 85% of young adults aged 12–25 years old, approximately 8% of adults aged 25–34 years old, and 3% of adults aged 35–44 years old have got an acne vulgaris (Bhate & Williams, 2013; Zaenglein et al., 2016).

2.1.1 The Pathogenesis of Acne

The multifactorial pathogenesis of acne instigates at the pilosebaceous unit that consisted of multilobulated sebaceous glands, an epithelial lined follicular canal, and a hair. It is attributed to different notable factors, including:

2.1.1.1 Abnormal hyperkeratinization of the pilosebaceous unit with microcomedone formation.

Comedone contains mixture of keratin, sebum, bacteria (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009)

2.1.1.2 Sebaceous gland hyperplasia with seborrhoea, alteration in the quality of sebum lipids

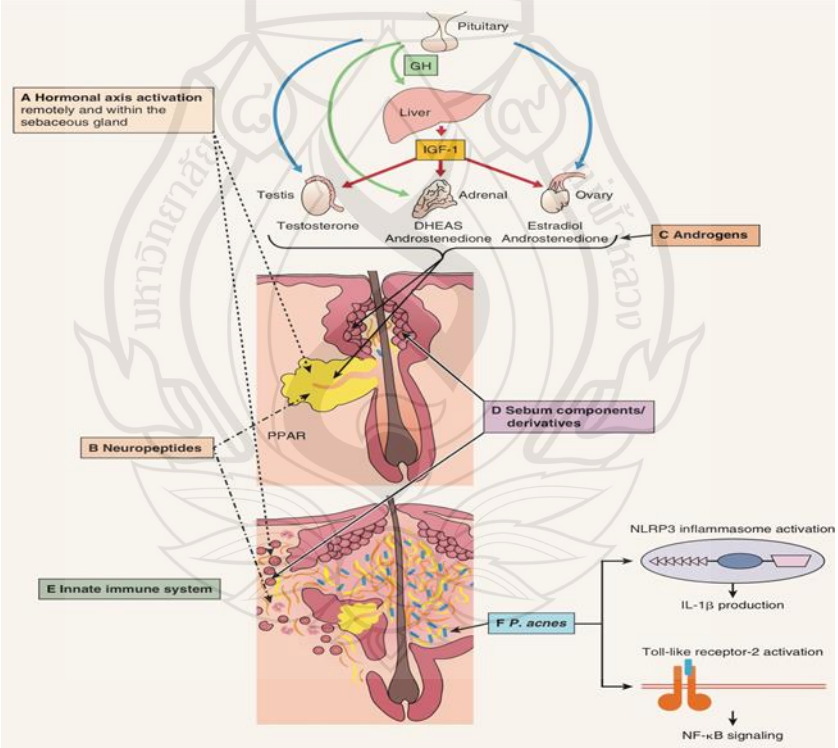
Pilosebaceous units or pilosebaceous follicles contain sebaceous glands, sensory end organs, arrector pili muscle, hair, and the hair follicle into the dermis. (Vasilache et al., 2020)

Excess sebum production occlude in the pilosebaceous unit. Monounsaturated fatty acids (MUFAs), lipid peroxides (LPs) are increased in sebaceous glands of patients with acne vulgaris, while linoleic acid is decreased. The

upregulation of the sterol response-element-binding protein (SREBP) pathway,a key regulator of lipogenesis, involves in differentiation and induction of lipogenesis. (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009; Sinha et al., 2014).

2.1.1.3 Inflammatory processes and immune response

Nuclear factor kappa B (NF-κ B), a transcription factor are showed for activation of pro-inflammatory cytokine genes, such as TNF-α, IL-1ß, IL-8 and IL-10. (Griffiths et al., 2016). Accumulation of lipid peroxides in comedones may induce inflammation leading to inflammatory acne. Lipid peroxides also activate peroxisome proliferator-activated receptors (PPARs), which are nuclear transcription factors that regulate inflammation and lipid metabolism. (Bhate & Williams, 2013; Tanghetti, 2013).



Source Bolognia et al. (2018)

Figure 2.1 Factors that play a role in the pathogenesis of acne vulgaris

2.1.1.4 *Propionibacterium acnes (P. acnes)* colonisation and proliferation

Propionibacterium acnes is an anaerobic Gram-positive bacterium. It also can produce lipasesand hyaluronidase, contributing to transmembrane receptors responding to microbial pathogens. TLR2, which recognizes the products produced by *P. acnes*, is on the surface of monocytes, macrophages and keratinocytes. Pro-inflammatory cytokines, IL-8, IL-12, IL-1α, IL-1β, and TNF-a are induced through the TLR2-dependent pathway. *P. acnes* can also lead to the reactive oxygen species (ROS) formation, especially superoxide anions, by activation of the scavenger receptor CD36 on keratinocytes. These may be other mechanisms of *P. acnes* that explain the involvement in inflammatory acne genesis. (Bhate & Williams, 2013; Tanghetti, 2013).

P. acnes also produces exoenzymes and chemotactically attracts neutrophils. Neutrophil ingests the intra-follicular *P. acnes* and elaborates hydrolases that weaken the wall. (Bhate & Williams, 2013; Dinulos, 2021).

Porphyrins, produced and secreted by *P. acnes*, are synthesized in all phylotype II strains, but not in IA strains. Porphyrin may act as a catalytic agent in squalene oxidation. Oxidised squalene from the bacteria may be another possible mechanism that involved in comedogenesis. *P. acnes* biofilm has also been proposed that it may act as a biological glue causing increased adhesiveness of corneocytes, result in obstruction and aggravating comedogenesis. *Propionibacterium acnes* produces porphyrins and shows red fluorescence under the Wood's lamp. This red fluorescence of comedones are caused by corproporphyrin III and protoporphyrin 9 from the *P. acnes* (Youn et al., 2009; Barnard et al., 2020).

The relationship between diet and acne is unclear as there is no randomized control trial study. However, a high level of glycemic diet, milk, chocolates or salt and high fat diet can be associated with severity of acne.

2.1.2 Types of Acne

Acne can be classified into non-inflammatory acne and inflammatory acne upon clinical appearance.

2.1.2.1 Non-inflammatory acne

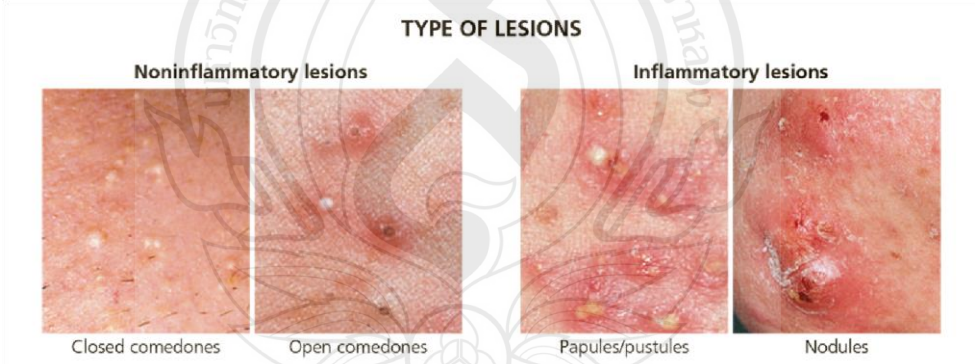
1. Closed comedones, which are known as whiteheads, are firm and white papules with a very small follicular orifice at the skin surface.

2. Open comedones, which are known as blackheads, are dilated follicular orifice filled with a keratin plug, which has a black colour due to the oxidation of tyrosine to melanin by tyrosinase (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

2.1.2.2 Inflammatory acne

Inflammatory acne is characterized by papules, pustules, nodules, and cysts.

1. Erythematous papules are 1 to 5 mm in diameter.
2. Pustules have the same size as erythematous papules, but they contain pus and/or microorganism such as *P. acnes*.
3. Nodules are markedly inflamed, and indurated.
4. Cysts are deeper than nodules, containing pus and serosanguinous fluid; may coalesce to form sinus tracts.



Source Dinulos (2021)

Figure 2.2 Clinical features show different types of acne lesion

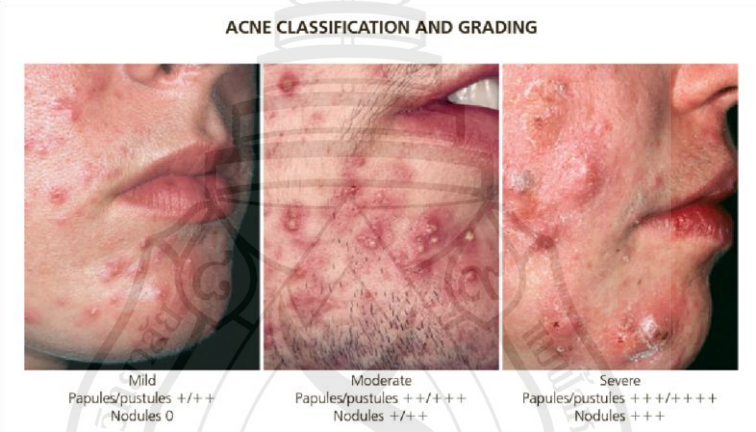
2.1.3 Grading Severity of Acne

According to Thai Clinical Practice Guidelines, the severity of acne can be divided into mild, moderate, and severe.

2.1.3.1 Mild acne mainly presents as comedones or not greater than ten of inflammatory acne.

2.1.3.2 Moderate acne is defined as more than ten small papules/pustules or less than five nodules.

2.1.3.3 Severe acne is defined as multiple inflammatory acne or chronic inflammation of acne and recurrent or presented sinus tract.



Source Dinulos (2021)

Figure 2.3 Grading severity of acne

Another scale used for evaluating the severity of acne is the Global Acne Severity Scale, as table 2.1.

Table 2.1 Global Acne Severity Scale

Grade	Value	Definition
Clear	0	Normal, clear skin with no evidence of acne vulgaris
Almost clear	1	Rare non-inflammatory lesions present, with rare non-inflamed papules (papules must be resolving and may be hyperpigmented, though not pink-red)
Mild	2	Some non-inflammatory lesions are present, with few inflammatory lesions (papules/pustules only, no nodulocystic lesions)
Moderate	3	Non-inflammatory lesions predominate, with multiple inflammatory lesions evident; several to many comedones and papules/pustules; there may or may not be one small nodulocystic lesions
Severe	4	Inflammatory lesions are more apparent, many comedones and papules/pustules, there may or may not a few nodulocystic lesions
Very severe	5	Highly inflammatory lesions predominate, variable number of comedones, many papules/pustules, and many nodulocystic lesions

Source Ak (2019)

2.1.4 The Pathogenesis of Acne Scar

Acne most often affects the face. Degree and duration of inflammation of acne can cause the scars. Postinflammatory erythema (PIE) and post-inflammatory hyperpigmentations (PIH) may develop in the healing process. These scars and post-inflammatory lesions can take many months to resolve, which can be emotionally and psychologically distressing. Moreover, if they persist, the appearance of scars may worsen with ageing or photodamage (Ak, 2019).

After producing cytokines/chemokines, the inflammatory process, follicular rupture, and perifollicular abscess formation occur. Then the wound healing process is initiated. The wound healing process can be divided into three phases: inflammation, granulation tissue formation or proliferation, and matrix remodeling or maturation.

2.1.4.1 Inflammation

The blood vessels contract causes vasoconstriction seen as blanching. After the blood flow has been stopped, inflammatory cytokines and chemokines are produced and caused vasodilatation and erythema. These inflammatory cytokines may stimulate melanogenesis. This step plays an essential role in the development of postinflammatory erythema and hyperpigmentation.

2.1.4.2 Granulation tissue formation (proliferation)

The goal of the proliferation stage is to reduce the lesion size. Angiogenesis, fibroplasia, and reepithelialization occur. Fibroblasts enter the wound site and form an extracellular matrix (ECM) by producing type III collagen and fibronectin. Reepithelialization is the process of the proliferation and migration of keratinocytes from the borders of the lesion.

2.1.4.3 Matrix remodelling (maturation)

This stage aims to get the appropriate tensile strength by reorganization, degradation, and resynthesis of the extracellular matrix (ECM). Fibroblasts and keratinocytes produce extracellular matrix (ECM), metalloproteinases (MMPs) and tissue inhibitors of MMPs. MMPs are extracellular matrix-degrading enzymes (Ak, 2019; Fabbrocini et al., 2010; Gonzalez et al., 2016).

2.1.5 Types of Acne Scar

Atrophic acne scars are caused by unusual production and degradation of collagen. Atrophic scars can be classified into three main types according to the depth and size of the scars: icepick, boxcar scars, and rolling.

2.1.5.1 Icepick scar

Icepick scars are narrow (less-than-2mm), deep, sharply demarcated edges that extend vertically to the deep dermis or subcutaneous tissue. The opening is wider than the infundibulum (V-shaped).

2.1.5.2 Boxcar scar

Boxcar scars are 1.5 to 4 mm in diameter, round to oval depressions with sharply demarcated vertical edges (U-shaped).

2.1.5.3 Rolling scar

Rolling scars are usually wider than 4 to 5 mm with the sloping edge, rolling appearance (M-shaped). Abnormal fibrous anchoring of the dermis to the subcutaneous tissue leads to superficial shadowing.

Hypertrophic and keloid scars are caused by too much connective tissue; collagen is produced during the healing process and decreases in collagenase activity. They commonly occur in the darker-skinned type.

Hypertrophic scars and keloids are commonly seen on the chest, back, shoulders and upper outer arm, especially in severe acne people. They have a smooth surface and are firm to palpation. They may be pruritic or painful; inhibit normal motion of adjacent tissues. The color can vary from pink–purple (early lesions) to skin- colored to hypo- or hyperpigmented. Both types of acne scar are less common and scarce. Hypertrophic scars are confined to wound margin in contrast to keloids (Ak, 2019; Bolognia et al., 2018; Fabbrocini et al., 2010).

2.1.6 Acne Treatment

Acne treatment aims to reduce inflammatory or non-inflammatory acne lesions. The selection of proper medication depends on its mechanism of action, type and severity of acne.

The patient should be given general information about the disease, causes, and factors. The patient should change lifestyles such as facial care, personal hygiene, avoidance of hyperglycemic diet and stress.

2.1.6.1 Topical Drugs

Topical drugs are most commonly prescribed, such as retinoids, benzoyl peroxide, and clindamycin. Although these drugs are highly effective in treating mild to moderate acne, they are irritating to some patients, resulting in poor compliance. It is supposed to choose the treatment that will be effective and well-tolerated.

1. Topical retinoids (vitamin A derivatives)

Topical retinoids reduce the abnormal mitosis of keratinocyte, hyper-keratinization and inflammation in acne vulgaris. Inflammation may occur while starting the treatment due to their effects of conversing closed comedones to open comedones. Continual using topical retinoids leads to thinning of the stratum

corneum, making the skin more irritant. Topical retinoids used for acne include tretinoin, adapalene, isotretinoin and tazarotene. Topical tretinoin has antiinflammatory activity,are reported to be less irritant, leading to be better tolerated than tretinoin. Adapalene does not cause sun sensitivity. 0.1 % Adapalene gel is as effective as 0.025% tretinoin gel for mild-tomoderate acne. The most common side effects are local irritation, including erythema, dryness, scaling, peeling, and itching. Fixed dose combination, including topical retinoids and benzoyl peroxide/topical antibiotics, can enhance the latter's efficacy due to the retinoid's effect of increasing penetration of the other drugs (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

2. Benzoyl Peroxide

Benzoyl peroxide is a bactericidal agent that can reduce the number of *P. acnes*; microbial resistance to benzoyl peroxide has not been reported. It also has mild comedolytic and anti-inflammatory activity, but it doesn't impact on sebum production. Benzoyl peroxide is a bleaching agent, so whitening of clothing and bedding can occur. It can also cause photosensitivity. There are many formulation and concentration available in the market, which are irritancy and bleaching. Water-based gels are less irritating, but alcohol-based gels are more effective. Allergic contact dermatitis from benzoyl peroxide is possible, but rare with other topical agents. Benzoyl peroxide is a Pregnancy Category C (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

3. Antibiotics

Topical antibiotics, such as clindamycin and erythromycin, should be prescribed in combination with topical retinoids or benzoyl peroxide. It is not supposed to prescribed topical antibiotics alone because of the significant increase of antibiotic-resistant *P. acnes* strains. Most formulations are alcohol-based, so the most common side effects are irritation, including xeroderma, erythema, burning, exfoliation, and pruritis. Clindamycin and erythromycin are Pregnancy Category B (Murphy & Le, 2018).

4. Azelaic Acid

Azelaic acid is a naturally occurring dicarboxylic acid that has comedolytic, antibacterial, and anti-inflammatory properties. It is helpful in the treatment of both non-inflammatory and inflammatory acne, as well as post-

inflammatory hyperpigmentation. It is applied twice daily. Azelaic acid does not cause antibiotic-resistant *P. acnes* strains. It does not cause sun sensitivity or significant local irritation. Azelaic acid is a Pregnancy Category B (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

5. Dapsone

5% and 7.5% of topical dapsone are used for non-inflammatory and inflammatory acne in a twice-daily dose. Topical dapsone is safe for use in patients with a G6PD deficiency. It should not be applied with concomitant benzoyl peroxide due to yellow-orange staining of skin and hair (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

2.1.6.2 Oral Drugs

Oral medications are prescribed in moderate to severe inflammatory Acne.

1. Isotretinoin

Isotretinoin (13-cis-retinoic acid), a synthetic vitamin A analogue, is an US FDA-approved medication for patients with severe, nodulocystic acne refractory to treatment, including conventional antibiotic therapies. It is also used to treat moderate acne that does not respond to other medications or relapse after discontinuing oral antibiotics. It is useful in sebum control in a patient with excessive oiliness. However, there are dose-dependent side effects such as xerosis, cheilitis, xerophthalmia, hearing loss, paronychias, inflammatory bowel disease, hair loss, elevated transaminases, elevated serum triglyceride and/or cholesterol levels, dryness of the oral and nasal mucosa. Significant but uncommon systemic effect is benign intracranial hypertension (especially when using with oral tetracyclines). After discontinuing isotretinoin, side effects are shortly reversible. Isotretinoin is a Pregnancy Category X (Dinulos, 2021; Griffiths et al., 2016; Kurokawa et al., 2009; Sinha et al., 2014).

2. Spironolactone

Spironolactone acts as a competitive androgen-receptor blocker and inhibitor of 5-α reductase. Because of antiandrogenic effects, it is used to treat acne and androgenic alopecia. Spironolactone has benefit in treating post-adolescent acne in women and men. Spironolactone can significantly reduce sebum production, leading to improve acne by competitively blocking dihydrotestosterone receptors in

the sebaceous glands. It can be used as combination therapy with antibiotics or oral contraceptives. Spironolactone is an alternative treatment with isotretinoin (Charny et al., 2017). Side effects are menstrual irregularity, headache, dizziness, breast pain, increased diuresis, occasional fluid retention and hyperkalemia. It can cause feminization of the male fetus, so it should be avoided in pregnancy (Sutaria et al., 2020). Spironolactone is a Pregnancy Category C (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

3. Oral Antibiotics

Oral antibiotics reduce the follicular colonization of *Propionibacterium acnes* and inhibit neutrophil chemotactic factors secreted during bacterial colonization. Oral antibiotics as treatment options for acne are tetracyclines, macrolides, trimethoprim, cephalosporins, and fluoroquinolones.

Tetracyclines, including doxycycline, tetracycline and minocycline, are the antibiotics of choice for nodulocystic and moderate-to-severe inflammatory acne (Zaenglein et al., 2016). Tetracyclines do not reduce sebum production, but they decrease the concentration of free fatty acids of sebum. As tetracycline should not be taken with food, antacids, and iron because of interfering with intestinal absorption. Moreover, tetracycline is contraindicated in children aged 8–12 years and pregnancy because of discolouration of the teeth and bones of the children and fetus (Muanda et al., 2017). Gastrointestinal upset is a common side effect of tetracyclin and doxycycline, which also has doxycycline-related phototoxicity. Doxycycline should be taken with food to reduce this side effect. Pseudotumor cerebri, which is increased intracranial pressure causing severe headache and papilledema, is a rare complication of tetracycline. Tetracyclines are classified as Pregnancy Category D.

Azithromycin, a dosage of 250 to 500 mg three times weekly, is often used in acne treatment.

Other antibiotics such as amoxicillin, trimethoprim/sulfamethoxazole, and cephalosporins are sometimes used. Amoxicillin is effective for gram-negative pustular acne. Cephalosporins are considered in case of antibiotic-resistant pustular acne. Trimethoprim/sulfamethoxazole is used when other antibiotics fail.

The treatment shows improvement typically after six weeks of antibiotic treatment. Oral antibiotic courses can be taken 3 to 6 months to reduce the

risk of antibiotic-resistant organisms. Pregnancy and breastfeeding patients should take erythromycin or amoxicillin instead of tetracyclines. (Dinulos, 2021; Griffiths et al., 2016; Kurokawa et al., 2009; Sinha et al., 2014).

4. Oral Contraceptive Pills (OCPs)

One of the signs of hyperandrogenic activity is acne. It can be found in polycystic ovary syndrome, Cushing's disease, anovulation, and androgen-secreting tumours. OCPs inhibit ovarian androgens by suppressing luteinizing hormone and adrenal production of androgens, reduce serum androgens and lower sebum production. Combined pills of estrogens and progestins with low androgenic activity are the most useful. They are practical and well-tolerated treatment in young adults with and without endocrine disorders. The contraindications to oral contraception are cerebrovascular disease or coronary artery disease, pregnancy, breastfeeding, active liver disease, migraine, and breast cancer (De Leo et al., 2016). Side effects include hypertension and thromboembolism. A significant reduction of TLR-2 expression leads to be particularly effective for inflammatory acne in the skin of adult females who used combined oral contraceptive (Dinulos, 2021; Griffiths et al., 2016; Kurokawa et al., 2009; Sinha et al., 2014).

2.1.6.3 Other therapies for acne

1. opical therapies such as topical nicotinamide, salicylic acid, sulfur,corticosteroids have some reports showing benefits.

2. Visible light – blue light can reduce inflammatory lesions compared to the control.

Other therapies include Light electrocautery and electrofulguration, intralesional injection of corticosteroid and chemical peels.

2.1.6.4 First-line and alternative treatment options

First-line treatment for mild comedonal/papulopustular acne vulgaris includes topical retinoids, adapalene and tretinoin-clindamycin (Nguyen & Su, 2011).

Second-line treatment includes topical dapsone, azelaic acid, salicylic acid. Additional options would be comedone extraction.

First-line treatment for moderate papulopustular acne includes topical retinoid together with oral antibiotic, with or without benzoyl peroxide; topical retinoid together with benzoyl peroxide, with or without topical antibiotic.

Second-line treatment includes alternative topical retinoids together with alternative oral antibiotic with or without benzoyl peroxide/azelaic acid. Additional treatments are comedone extraction and oral contraceptive.

First-line treatment for severe nodulocystic acne includes oral isotretinoin.

Second-line treatment includes topical retinoid together with oral antibiotic and benzoyl peroxide. Additional treatments are oral contraceptive intralesional injection of corticosteroid (Dinulos, 2021; Griffiths et al., 2016; Sinha et al., 2014).

2.2 *Andrographis paniculata*

Andrographis paniculata, commonly known as the “king of bitters,” is an herbaceous plant belonging to the Acanthaceae family. It is native to India and cultivated in China. Thailand, it is known as Fah Tha Lai Joan or Nam Laai Phangphon.

This herb consists of diterpenoid lactone compounds, diterpene glucoside compounds, flavonoid compounds, steroid, phenolic compounds and organic acids (Jayakumar et al., 2013).

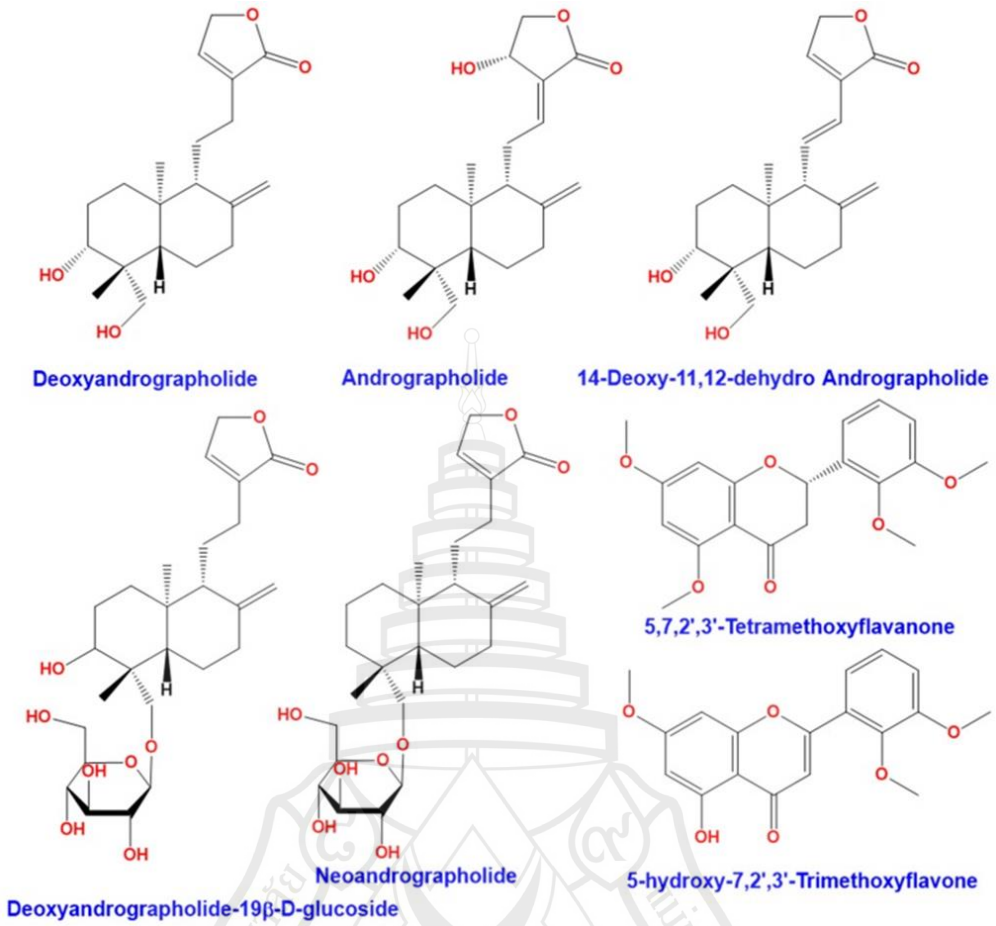


Figure 2.4 Compounds found in the leaves of *Andrographis paniculata*.

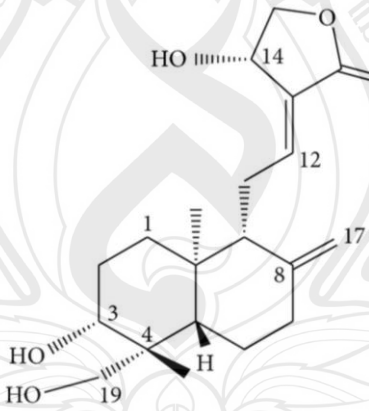
2.2.1 Chemical and Physical Properties

Andrographolide is a major bioactive phytoconstituent found in various parts of A. paniculate (Figure 2.6), but particularly in the leaves. Its molecular formula and weight are $C_{20}H_{30}O_5$ and 350.4 (C 68.54%, H 8.63%, and O 22.83%), respectively. It is not very soluble in water, it is soluble in acetone, chloroform, ether, and hot ethanol. Its melting point of andrographolide is 235.3°C (Jayakumar et al., 2013).

The natural oil mainly consisted of triglycerides of oleic and linoleic acids. When natural oil was added in the gel base and natural lipids, the crystal structure of the gel changed from cubic to reverse haxagonal form, which was found to have the most favorable sustained release properties (Jayakumar et al., 2013).

Source Jayakumar et al. (2013)

Figure 2.5 Morphology of *Andrographis paniculate*



Source Jayakumar et al. (2013)

Figure 2.6 Chemical structure of andrographolide

2.2.2 Pharmacology and Biochemistry

The pharmacological activities of *Andrographis paniculata*, andrographolide show antiallergic, antiplatelet aggregation, antihyperglycemic, antioxidant, antipyretic, antivenom, antigastric ulcer, analgesic, antiviral, antibacterial, antiparasitic, antifeedant, renoprotective, stimulation of smooth muscle, antifilarial activity, antifertility, antihypertensive, antidiarrheal, hypolipidemic and hepatoprotective (Jayakumar et al., 2013; Mussard et al., 2019).

Its bio-availability, however, decreased four-fold when a 10-times-higher dose was used. It is most likely and dose dependency metabolized (Panossian et al., 2000; Bera et al., 2014). The data of the drug absorption and serum drug levels after applying topical *Andrographis paniculata* is still unavailable.

2.2.3 Drug and Medication Information

2.2.3.1 Indications and uses

1. Thai FDA-approved indications:

1) Oral administration for an antipyretic remedy

2) Oral administration for the symptomatic relief of the common cold

3) Oral administration for the relief of sore throat and cough with thick sputum

4) Oral administration for the treatment of non-infectious diarrhea

5) Oral administration for the treatment of asymptomatic and mild symptomatic COVID-19 infection

2. Off-label uses of oral *Andrographis paniculata*:

1) Oral administration for the treatment of gastro-intestinal upsets (dyspepsia, loss of appetite, flatulence) and acute diarrhoea.

2) Oral administration for the treatment of upper respiratory tract inflammation, including influenza

3) Oral administration for the treatment of other diseases as follows: for inflammations including rheumatoid arthritis, familial Mediterranean fever, inflammatory bowel disease.

3. Over-the-counter products are now available as hard capsule/ tablet /pill in two form, including crude(total lactone calculated as Andrographolide not less than 6 % w/w and Andrographolide not less than 1 % w/w) and extract (Andrographolide not less than 4 % w/w).

2.2.3.2 Drug interactions

If *Andrographis paniculata* and antihypertensive drugs, such as captoprilenalapril, losartan, valsartan, diltiazem, amlodipine, hydrochlorothiazide and furosemide, are administered together; this might cause blood pressure to go too low.

If *Andrographis paniculata* and anticoagulant/antiplatelet drugs, such as aspirin, clopidogrel, diclofenac, ibuprofen, naproxen, dalteparin, enoxaparin, heparin and warfarin, are administered together; this might increase the chances of bruising and bleeding.

If *Andrographis paniculata* and immunosuppressants drugs, such asb azathioprine, basiliximab, cyclosporine, daclizumab, muromonab, mycophenolate, tacrolimus, sirolimus, prednisone and corticosteroids, are administered together; this might decrease the effectiveness of immunosuppressants drugs.

2.2.3.3 Adverse effects

The common adverse effects of *Andrographis paniculata* are minor such as rash, pruritus, paraesthesia, breath discomfort, cough, peeling skin, fatigue, nausea, gastric discomfort, anorexia, throat tightness, ocular hyperaemia, wheezing, urticaria, hypoxia, hyperhidrosis, angioedema, dizziness, headache and palpitation. Other severe adverse effects, which are rare, include anaphylactic shock , anaphylactic reaction, erythema multiforme and Stevens Johnson syndrome (Suwankesawong et al., 2014).

2.2.3.4 Contraindications

Contraindications of *Andrographis paniculata* include known allergy or hypersensitivity to products that contain *A.paniculata* or its constituents, pregnancy or lactation.

2.2.4 Drug Monitoring

The data of the drug monitoring of *Andrographis paniculata* is still unavailable.

2.2.5 Safety and Hazards

2.2.5.1 *Andrographis paniculata* is possibly unsafe when taken by mouth during pregnancy. There is a concern that it might cause miscarriages. Not enough is known about the safety of *Andrographis paniculata* during breast-feeding.

2.2.5.2 *Andrographis paniculata* might slow blood clotting. This might increase the risk of bleeding or bruising in people with bleeding disorders.

2.2.5.3 *Andrographis paniculata* might lower blood pressure. This has not been seen in humans. In theory, however, andrographis might lower pressure too much if taken by people who already have low blood pressure.

2.2.5.4 *Andrographis paniculata* might cause the immune system to become more active, and this could increase the symptoms of auto-immune diseases.

2.2.5.5 Animal research suggests that *Andrographis paniculata* might interfere with reproduction, but this hasn't been shown in people.

2.2.6 Toxicity

Hamasakwattanakul investigated the cytotoxicity of AP gel on human periodontal ligament fibroblast and on the migration of these cell. It was found that AP gel at concentration of 5.625 mg/ml resulted in almost 100% cell death (Jayakumar et al., 2013).

A chronic toxicity study on dogs showed no pathological changes after administering 15 times the clinical dosage of Andrographolide. In one randomized control trial study, participants were monitored for changes in liver and kidney function, blood counts and any other laboratory measures. No related toxic effects in rats was found. (Dey et al., 2013)

2.3 Related Literature Review of Topical *Andrographis paniculata*

There were several studies about the benefit of topical *Andrographis paniculata* in various concentrations and formulations. Topical *Andrographis paniculata* was also studied to prove its benefit versus other modalities. Atsawasuwan compared the clinical and microbiological effects between subgingival application of the AP gel and metronidazole gel as adjunct to scaling and root planing. They found that both treatments improved clinical and microbiological parameters. However, the AP gel tended to improve the disease condition faster than the metronidazole gel. Proportions of the black-pigmented anaerobes were also significantly reduced in the AP gel group. Boonchaipanichwatana compared the clinical and microbiological effects of minocycline ointment and AP gel. And they found that both AP gel and minocycline ointment had similar effects in the reduction of probing pocket depth (PPD). The AP gel group also demonstrated more consistent increased percentage of cocci and decreased in percentage of motile rods throughout 4 months (Jayakumar et al., 2013).

Moreover, one study in Thailand found that the gel formulation containing the herbal ball extract, which included *Andrographis paniculata*, Centella asiatica, Benchalokawichian remedy and the stem bark power of Hesperethusa crenulata (Thanaka),with the concentration of 5% w/w showed antioxidants and antimicrobial activity against *P. acnes* according to the DPPH assay and the agar well diffusion study. The study in India concluded that the formulated herbal anti-acne moisturizer,including *Azadiracta indica*, Aloe, *Andrographis paniculata*, Liquorice, was associated with significant reduction in microbial growth which causes acne by using disc diffusion method and also was found to produce mosturizing effect (Jantarat et al., 2018). The study in India showed that 1%, 2.5%, and 5% poly herbal cream ointment and gel, containing *Abutilon indicum, Aristolochia bracteolate and Andrographis paniculata* have potential activity against these microorganisms, including *Trichophyton rubrum*, *Microsporum canis*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Propionibacterium acnes* (Raja & Pandiyan, 2017).

The study in Malaysia showed that AP herbal gel at concentration 2.5% (w/w) showed the antimicrobial effect of *S. aureus* and *C. albicans*, while 5.0% (w/w) resulted the best antimicrobial activities for *P. acnes* and *S. epidermidis*. *Andrographis paniculata* gel can treat acne effectively (Kub et al., 2020). One study in Thailand found that there was significantly difference between 0.0256% *Andrographis paniculate* extract gel group and control group for satisfaction of efficacy of 0.0256% *Andrographis paniculate* extract gel in the treatment of acne vulgaris, including postinflammatory erythema, number of comedone and inflammatory acne, and severity of comedone and inflammatory acne for 4 weeks (Chusinkul, 2015).

One pilot study suggested that gel formulation containing glabridin, andrographolide, and apolactoferrin is safe and effective in improving the cosmetic appearance of epidermal melasma in adult Caucasian women (Cantelli et al., 2020). Andrographolide can cure hyperpigmentation disorders (Zhu et al., 2015). Yuh-Chiang Shen et al. reported that andrographolide can prevent production of oxygen radical. (Shen et al., 2002; Zhu et al., 2015)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Clinical trial study in subjects with mild to moderate acne vulgaris to evaluate the efficacy of 0.05% topical *Andrographis paniculate* in the improvement of inflammatory acne by a randomized, double-blind (volunteers and researcher), placebo-controlled, split-face study.

3.2 Study Population

Thai subjects, aged between eighteen to fourty-five years old with mild to moderate facial acne vulgaris.

3.3 Sample

Thai healthy females and males subjects with mild to moderate acne vulgaris at the face, age between 18 - 45 years old, follow up at Mae Fah Luang University hospital, Bangkok.

3.4 Sample Size Determination

Due to limiting of study in comparing efficacy of topical *Andrographis paniculata* for the improvement of facial acne vulgaris in split-face study, so the researcher selects similar research study, so the sample size comes from papules at 0 and 12th week in the efficacy of 0.025% Licochalcone A cream for moderate to

severe facial acne vulgaris treatment. The confident interval 95% with these followings:

α = 0.05 then $Z_{\alpha/2}$= 1.96

β = 0.2 (power = 80%)

Z_{β} = 0.84

μ_1 = 5.17 μ_2 = 9.00

S_1 = 4.16 S_2 = 5.19

n_1 = 15

n_2 = 15

The amount of sample who would attend in this study was calculated from

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

$$22.12 = \frac{4.16^2(15-1) + 5.19^2(15-1)}{15+15-2}$$

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 S_p^2}{(\mu_1 - \mu_2)^2}$$

$$n = \frac{(1.96+0.84)^2 22.12}{(5.17-9)^2}$$

$$n = 11.82$$

The estimation of drop-off rate was 20%, so 15 subjects (n = 15) were enrolled in this study.

3.5 Selection Criteria

3.5.1 Inclusion Criteria

3.5.1.1 Female or male, Thai healthy subjects aged group 18-45 years old.

3.5.1.2 Subject with facial acne with mild-to-moderate acne severity diagnosed by a doctor.

3.5.1.3 No history of allergy to *Andrographis paniculate.*

3.5.1.4 Sign the informed consent accepting publication of benefits, photographs, complications and risks associated

3.5.1.5 Subjects can come to follow-up every appointment to evaluate the result and follow the research guideline.

3.5.1.6 No lactation or pregnancy or intending to become pregnant.

3.5.1.7 No apply of any forms of medication, including retinoids, antibiotics, azelaic acid, hormonal treatment, benzoyl peroxide, AHA, and BHA within 1 month.

3.5.1.8 No history of laser therapy or any other facial treatment within 1 month.

3.5.2 Exclusion Criteria

3.5.2.1 Patch test positive for 0.05% *Andrographis paniculata*

3.5.2.2 History of facial active skin disorders

3.5.2.3 Uncontrolled chronic disorders, including central nervous system diseases, hypertension, immunocompromisation, diabetes mellitus, heart diseases, autoimmune diseases or kidney diseases.

3.5.2.4 History of sex hormone disorders; polycystic ovarian syndrome, Cushing's disease.

3.5.3 Discontinuation Criteria

3.5.3.1 Subjects want to leave the research.

3.5.3.2 Pregnancy, serious illness, or dying during participation in the study

3.5.3.3 Subjects have severe complications from the therapy.

3.5.3.4 Loss follow up.

3.6 Study Location

Mae Fah Luang University Hospital, Bangkok

3.7 Materials and Equipment

3.7.1 The information sheet

3.7.2 The informed consent

3.7.3 The patient record form

3.7.4 The record form of the research result and side effects of the treatment

3.7.5 The questionnaire for subjects' satisfaction and evaluation

3.7.6 The transparent plastic sheet

3.7.7 The permanent marker

3.7.8 The VISIA® Skin Analysis by Canfield Scientific, Inc.

3.7.9 Sebumeter® SM815 (Courage & Khazaka, Cologne, Germany)

Figure 3.1 The VISIA® Skin Analysis

3.7.10 0.05% *Andrographis paniculata* gel, composed of water, *Andrographis paniculata*, glycerin, sodium benzoate, triethanolamine, carbomer and disodium EDTA. It is clear gel in a white plastic squeeze tube in size of 15 ml. The pH is approximately 6. It is manufactured by Skinnex (Thailand) Co., Ltd.

3.7.11 Placebo gel, which is composed of water, glycerin, sodium benzoate, triethanolamine, carbomer, and disodium EDTA. It has the same characteristic as 0.05% *Andrographis paniculata* gel, such as colour, odour, and viscosity containing in a white plastic squeeze tube in size of 15 ml. It is manufactured by Skinnex (Thailand) Co., Ltd.

3.7.12 Facial cleansing agent: Acne Aid Gentle Cleanser

3.8 Study Procedures

3.8.1 Enrolled volunteers according to the exclusion and inclusion criteria.

3.8.2 Informed volunteers about the study's objectives, procedures, measurement, advantage, and possible side effects.

3.8.3 Subjects signed the consent form before the study has begun.

3.8.4 Subjects filled in the information sheet, including underlying disease, food/drug allergy, patient history and general information for the study.

3.8.5 Subjects were tested for allergic reaction to 0.05% *Andrographis paniculata* gel by patch test; using the gel on the inside upper arm in size of three by three centimeter square, then covering with water-proof hypoallergic plaster for 48 hours. And 48 hours later, removed the patch. After 30 minutes, the allergic reaction was observed for 30 minutes. If the allergic responses were positive, subjects would be informed immediately.

3.8.6 Subjects washed the face with a provided cleansing agent, Acne Aid Gentle Cleanser and used tissue paper to pat the face dry. Rested in the room temperature of 25ºC for 30 minutes. Other topical lotion must not be applied.

3.8.7 A physician, who did not involve in this study, taked a random of the control and intervention sides of the face by choosing one sequence from 0 to 999 that the website, http://random.org/sequences/, generates, and then repeatedly picked the following 15^{th} sequence to get the total of 15 sequences, which were assigned to 14 subjects, one by one. The odd number sequences were substituted by A, and the even number sequences were covered by B.

A represented applying 0.05% *Andrographis paniculata* gel at the left side of the face and applying a placebo at the right side of the face.

B represented applying 0.05% *Andrographis paniculata* gel at the right side of the face and applying a placebo at the left side of the face.

After randomization, the results were secretly kept until the data analysis had finished.

3.8.8 The same physician, who taked a random of the intervention and control sides, prepared the gel, of which label informed the applying side – right or left side and gave them to the researcher.

3.8.9 Marked the postinflammatory hyperpigmentation, postinflammatory erythema and acne lesion counts via a permanent marker on a transparent plastic sheet to locate the lesions at week 0 and use it as a follow-up tool.

3.8.10 Separately assessed acne grading severity and lesion counts into two groups of right or left sides of the face by two physicians, who were not involved in this study, before the treatment started (baseline), at week 2, 4, 6 and 8 after the study had begun.

3.8.11 Took three photos and multi-image analysis to assess postinflammatory hyperpigmentation and postinflammatory erythema before the treatment started (baseline) and every follow-up visit; 37 degrees left, front view and 37 degrees right photos by VISIA® skin analysis.

3.8.12 Porphyrin counts were measured by ultraviolet-induced fluorescence spots using VISIA® with add-in software by manual masking feature into two groups on left and right sides of each volunteer. Evaluation was done before the treatment (baseline), at week 2, 4, 6 and 8 after the study had begun.

3.8.13 Sebum levels of both cheeks were obtained after rest 30 minutes in room temperature, using Sebumeter® (SM815 Courage & Khazaka, Cologne, Germany). Evaluation was done three times and found average score into two groups on left and right sides of each volunteer. Evaluation was done before the treatment (baseline), at week 2, 4, 6 and 8 after the study had begun.

3.8.14 The researcher gave the gel to the subjects and informed instruction and possible side effects.

Applied 0.5 the finger tip unit (FTU) or 0.5 gram of the gel on the label side of the face. Washed the hands and applied it on the another side of the face. Applied two times per day, morning and evening bed for 8 weeks.

3.8.15 Adviced subjects to cease applying other acne treatments, including products such as creams, serums and masks. Also, subjects avoided applying cosmetics and sunlight, including sunscreen. Stored the gel in a dry and cool place.

When any side effects occurred, the subjects stopped applying the gel and contacted the researcher as soon as possible.

3.8.16 Subjects were evaluated side effect at week 2, 4, 6 and 8 and treatment satisfaction at week 8 after the study had begun.

3.8.17 The researcher collected the data, analyzed, discussed and concluded the study.

3.9 Outcome Evaluation and Data Collection

3.9.1 Acne Lesion Counts and Acne Grading

Assess numbers of lesion counts and acne grading by the physician who is not involved in this study before the first treatment, at week 2, 4, 6 and 8.

3.9.1.1 Acne lesion counts

Facial acne lesions are counted and classified by types of acne lesions, i.e., non-inflammatory lesions and inflammatory lesions, as table 3.1. Noninflammatory lesions include open and closed comedones. Inflammatory lesions have papules, pustules, nodules, and cysts. They are located on a transparent plastic sheet via permanent marker at week 0 are compared with the lesions on the subject's face at the 2nd week if the lesions at the 2nd week are new or old.

Table 3.1 Acne lesion counts form

	Right Side					**Left side**				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										

Table 3.1 (continued)

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										

3.9.1.2 Acne grading

Global Acne Severity Scale is used in table 3.1.

3.9.2 Sebum Level

Sebum level is collected by Sebumeter® in the units of microgram/cm2.

3.9.3 Photos Analysis

3.9.3.1 The postinflammatory hyperpigmentation, postinflammatory erythema lesions are located on a transparent plastic sheet via permanent marker at week 0 are compared with the lesions on the subject's face at the 2nd week if the lesions at the 2nd week are new or old. Subject's photos and multi-image analysis by VISIA® are collected every visit; at first visit, week 2, 4, 6 and 8.

3.9.3.2 Porphyrin counts are measured by ultraviolet- induced fluorescence spots using VISIA®. Two separate images of each volunteer's face are taken to analyze the treatment area. Each area is manually mapped by the investigator. The porphyrin counts represent in spots of feature counts by VISIA® at first visit, week 2, 4, 6 and 8.

3.9.4 Satisfaction Evaluation

Subjects are evaluated their overall satisfaction in term of serum properties, including colour, texture, viscosity, scent, absorption and result of reducing inflammatory acne at week 8 to both 0.05% *Andrographis paniculata* gel and placebo by questionnaire and using Global Patient Satisfaction scales as table 3.2.

Table 3.2 Global patient satisfaction scales

Scale	Satisfaction level
1	Very unsatisfied
2	Unsatisfied
3	Neutral
4	Satisfied
5	Very satisfied

3.9.5 Side Effects Evaluation

Side effects were evaluated at week 2, 4, 6 and 8 by history taking and physical examination.

3.10 Data Analyses

3.10.1 Descriptive Statistics

3.10.1.1 Analyze qualitative data, for example, gender, history of acne treatment, side effects and acne severity grading. The results are presented as numeric data and percentage.

3.10.1.2 Analyze quantitative data, for example, age getting acne, sebum level, PIE, PIH and porphyrin counts. The results are presented as mean ± standard deviation.

3.10.2 Inferential Statistics

Acne lesion counts are compared between placebo *and Andrographis paniculata* gel before and after the treatment at week 2, 4, 6 and 8 using Anova test, Mann-Whitney U Test, repeated measured ANOVA and Kruskal-Wallis Test for non-parametric data and level of confidence is set at p-value = 0.05.

3.11 Ethical Considerations

This study was conducted under Good Clinical Practice (GCP) guidelines. This standard provided public assurance of the safety, rights and well-being of the subjects.

Generally understanding, considerations were as following

3.11.1 Volunteers completely understood the objective, procedure, and possible side effects of the study.

3.11.2 Volunteers wanted to attend the study and sign the consent form before starting the study.

3.11.3 Volunteers did not pay any cost and were able to quit this study anytime without prohibits.

3.11.4 If any side effects occured, the researcher could help as much as possible.

3.11.5 All volunteers' information was confidential.The researcher substituted patient’s picture file names and identical names by the serial number. The photographs were taken in lateral views only and censored by eye censor bars.

3.11.6 All protocol of this study was conducted after receiving approval from the Mae Fah Luang University Ethics Committee on Human Research (approval number COA: 033/2022)

CHAPTER 4

RESULTS

The study was conducted to evaluate the efficacy of 0.05% topical *Andrographis paniculata* gel in treating facial acne vulgaris compared to a placebo gel, the side effects of 0.05% topical *Andrographis paniculata* gel, and patients' satisfaction after using 0.05% topical *Andrographis paniculata* gel as an acne treatment. The results were reported in 10 parts.

1. General characteristics of subjects
2. Comparisons of non-inflammatory acne counts between 0.05% topical *Andrographis paniculata* gel and placebo
3. Comparisons of non-inflammatory acne counts among time
4. Comparisons of inflammatory acne counts between the 0.05% topical *Andrographis paniculata* gel and placebo
5. Comparisons of inflammatory acne counts among time
6. Comparisons of mean percentage changes between 0.05% topical *Andrographis paniculata* gel and placebo
7. Comparisons of severity of acne
8. Side effects evaluation
9. Subjects' satisfaction
10. Physicians' evaluation and photos analysis

4.1 General Characteristics of Subjects

The study included 15 subjects that 12 subjects completed the study at Mae Fah Luang University Hospital, Bangkok, for 8 weeks from March to May 2022. Three volunteers discontinued due to COVID-19 situation concern since the 6th week of the study. The demographic data of all volunteers demonstrated in Table 4.1

Table 4.1 General characteristics

Characteristics	n
Age	
Mean ± SD	28.83 ± 7.58
Min	20
Max	42
Gender	
Male	3
Female	9
Occupation	
Private company employees	6
Nurses	3
Government employees	2

The mean age of the subjects was 28.83 ± 7.58 ranging from 20 to 42 years old. There were 3 males and 9 females participating in this study. Their occupation consisted of 6 private company employees, 3 nurses, 2 government employees and 1 student. There was no subject with underlying diseases or food and drug allergies.

General information of subjects on acne was shown in Table 4.2. The mean of age getting acne was 15.92 ± 2.39 which the minimum age was 12, and the maximum age was 20. Most of the subjects started to get acne at the age of 15 to 19. The subjects had acne for 4 to 32 years with an average of 11.92 years. Most of them had acne for 5 to 9 years. Ten subjects had oily skin as aggravating factor. Five subjects found that taking high-fat diet caused acne breakouts. Four female subjects reported that their breakouts coincided with menstruation. Cosmetics and skincare usage was another factor that caused acne in 4 subjects. Three subjects got acne breakouts if they took a chocolate. One subjects got acne breakouts if they took a cake. Twelve subjects had not had acne treatment before. The severity of acne was graded by Global Acne Severity Scale, referring to the more severe side of the face; 4 subjects were mild, and 8 subjects were moderate.

Table 4.2 General information of subjects on acne

Characteristics	n
Age getting acne	
Mean ± SD	15.92 ± 2.39
Min	12
Max	20
< 15 years old	3
15 – 19 years old	8
≥ 20 years old	1
Duration of acne	
Mean ± SD	11.92 ± 8.32
Min	4
Max	32
< 5 years	1
5 – 9 years	5
10 – 14 years	2
≥ 15 years	4
Influencing factors	
Oily skin	10
High-fat diet	5
Cosmetics and skincare usage	4
Menstrual cycle	4
Chocolate	3
Cake	1
Previous acne treatment	
None	12
Severity of acne	
Mild	4
Moderate	8

4.2 Comparisons of Non-Inflammatory Acne Counts between the *Andrographis paniculata* Gel and Placebo

The descriptive statistics of total non-inflammatory acne, closed comedone, and open comedone counts was shown in table 4.3. The data was a normal distribution that was tested by the Kolmogorov-Smirnov test ($p > 0.05$).

Table 4.3 Acne counts of total non-inflammatory acne, closed comedones, and open comedones (n = 12)

	Group					
	***Andrographis paniculata* gel**			**Placebo**		
	Min	**Max**	**Med, IQR**	**Min**	**Max**	**Med, IQR**
Total non-inflammatory acne						
Baseline	0	15	0.50 (0.00, 3.00)	0	7	2.50 (0.00, 5.00)
Week 2	0	8	0.00 (0.00, 3.75)	0	13	0.00 (0.00, 5.75)
Week 4	0	1	0.00 (0.00, 1.00)	0	2	0.00 (0.00, 0.75)
Week 6	0	0	0.00 (0.00, 0.00)	0	2	0.00 (0.00, 0.75)
Week 8	0	3	0.00 (0.00, 0.00)	0	4	0.00 (0.00, 0.00)
Closed comedones						
Baseline	0	10	0.50 (0.00, 3.00)	0	7	2.50 (0.00, 5.00)
Week 2	0	8	0.00 (0.00, 3.75)	0	13	0.00 (0.00, 5.75)
Week 4	0	1	0.00 (0.00, 1.00)	0	2	0.00 (0.00, 0.75)
Week 6	0	0	0.00 (0.00, 0.00)	0	2	0.00 (0.00, 0.00)
Week 8	0	3	0.00 (0.00, 0.00)	0	4	0.00 (0.00, 0.00)
Open comedones						
Baseline	0	5	0.00 (0.00, 0.00)	0	0	0.00 (0.00, 0.00)
Week 2	0	0	0.00 (0.00, 0.00)	0	0	0.00 (0.00, 0.00)
Week 4	0	0	0.00 (0.00, 0.00)	0	0	0.00 (0.00, 0.00)
Week 6	0	0	0.00 (0.00, 0.00)	0	1	0.00 (0.00, 0.00)
Week 8	0	0	0.00 (0.00, 0.00)	0	0	0.00 (0.00, 0.00)

The comparisons of total non-inflammatory acne, closed comedone, and open comedone counts between the *Andrographis paniculata* gel and placebo at baseline, week 2, 4, 6, and 8 were shown in table 4.4.

The data showed that between the *Andrographis paniculata* gel and placebo were no significant difference ($p > 0.05$).

Table 4.4 Non-inflammatory acne counts compared between the *Andrographis paniculata* gel and placebo (n = 12)

Acne Count			Z	p-value
	***Andrographis paniculata* gel**	**Placebo**		
	Mean ± SD	**Mean ± SD**		
Total non-inflammatory acne				
Baseline	2.33 ± 4.25	2.75 ± 2.86	-.882	0.378
Week 2	1.92 ± 2.94	3.08 ± 4.44	-.387	0.699
Week 4	0.42 ± 0.52	0.42 ± 0.79	-.487	0.626
Week 6	0.00 ± 0.00	0.33 ± 0.65	-1.809	0.070
Week 8	0.25 ± 0.87	0.58 ± 1.38	-.653	0.514
Closed comedones				
Baseline	1.92 ± 2.94	2.75 ± 2.87	-.882	0.378
Week 2	1.92 ± 2.94	3.08 ± 4.40	-.387	0.699
Week 4	0.42 ± 0.52	0.42 ± 0.79	-.487	0.626
Week 6	0.00 ± 0.00	0.25 ± 0.62	-1.445	0.149
Week 8	0.25 ± 0.87	0.58 ± 1.38	-.653	0.514
Open comedones				
Baseline	0.42 ± 1.44	0.00 ± 0.00	-1.000	0.317
Week 2	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
Week 4	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
Week 6	0.00 ± 0.00	0.08 ± 0.29	-1.000	0.317
Week 8	0.00 ± 0.00	0.00 ± 0.00	.000	1.000

Note p-value determined by Mann-Whitney U Test

4.3 Comparison of Non-Inflammatory Acne Counts Among Time

Table 4.5-4.10 showed total non-inflammatory acne, closed comedone, and open comedone counts compared between baseline and during treatment (within-group).

On *Andrographis paniculata* gel side, significant difference were observed in total non-inflammatory acne counts at baseline compared with week 6 (p = 0.005) and week 8 (p = 0.020), week 2 compared with week 6 (p = 0.016). On placebo side, significant difference were observed in total non-inflammatory acne counts at baseline compared with week 4 (p = 0.039), week 6 (p = 0.018) and week 8 (p = 0.024) as shown in table 4.5.

The *Andrographis paniculata* gel also had significant differences on closed comedones counts at baseline compared with week 6 (p = 0.005) and week 8 (p = 0.020), week 2 compared with week 6 (p = 0.016). The placebo also had significant differences on closed comedones counts at baseline compared with week 4 (p = 0.036), week 6 (p = 0.014) and week 8 (p = 0.021) as shown in table 4.7.

Table 4.5 Acne counts of total non-inflammatory acne among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean diff	p-value
Andrographis paniculata gel					
Baseline	2.33 ± 4.25	Week 2	1.92 ± 2.94	0.41	0.707
		Week 4	0.42 ± 0.52	1.91	0.342
		Week 6	0.00 ± 0.00	2.33	0.005
		Week 8	0.25 ± 0.87	2.08	0.020
Week 2	1.92 ± 2.94	Week 4	0.42 ± 0.52	1.50	0.566
		Week 6	0.00 ± 0.00	1.91	0.016
		Week 8	0.25 ± 0.87	1.66	0.051
Week 4	0.42 ± 0.52	Week 6	0.00 ± 0.00	0.41	0.066
		Week 8	0.25 ± 0.87	0.16	0.169
Week 6	0.00 ± 0.00	Week 8	0.25 ± 0.87	-0.25	0.643

Table 4.5 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean diff	p-value
Placebo					
Baseline	2.75 ± 2.86	Week 2	3.08 ± 4.40	-0.33	0.781
		Week 4	0.42 ± 0.79	2.33	0.039
		Week 6	0.33 ± 0.65	2.41	0.018
		Week 8	0.58 ± 1.38	2.16	0.024
Week 2	3.08 ± 4.40	Week 4	0.42 ± 0.79	2.66	0.180
		Week 6	0.33 ± 0.65	2.75	0.098
		Week 8	0.58 ± 1.38	2.50	0.106
Week 4	0.42 ± 0.79	Week 6	0.33 ± 0.65	0.08	0.596
		Week 8	0.58 ± 1.38	-1.67	0.807
Week 6	0.33 ± 0.65	Week 8	0.58 ± 1.38	-0.25	0.859

Note p-value determined by Kruskal-Wallis Test

Table 4.6 Acne counts of total non-inflammatory acne among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	2.33 ± 4.25	Week 2	1.92 ± 2.94	0.801
		Week 4	0.42 ± 0.52	0.152
		Week 6	0.00 ± 0.00	0.084
		Week 8	0.25 ± 0.87	0.136
Week 2	1.92 ± 2.94	Week 4	0.42 ± 0.52	0.105
		Week 6	0.00 ± 0.00	0.045
		Week 8	0.25 ± 0.87	0.069
Week 4	0.42 ± 0.52	Week 6	0.00 ± 0.00	0.067
		Week 8	0.25 ± 0.87	0.504
Week 6	0.00 ± 0.00	Week 8	0.25 ± 0.87	0.339

Table 4.6 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Placebo				
Baseline	2.75 ± 2.86	Week 2	3.08 ± 4.40	0.813
		Week 4	0.42 ± 0.79	0.019
		Week 6	0.33 ± 0.65	0.015
		Week 8	0.58 ± 1.38	0.035
Week 2	3.08 ± 4.40	Week 4	0.42 ± 0.79	0.055
		Week 6	0.33 ± 0.65	0.053
		Week 8	0.58 ± 1.38	0.059
Week 4	0.42 ± 0.79	Week 6	0.33 ± 0.65	0.723
		Week 8	0.58 ± 1.38	0.438
Week 6	0.33 ± 0.65	Week 8	0.58 ± 1.38	0.463

Note p-value determined by repeated measured ANOVA

Table 4.7 Acne counts of closed comedones among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	1.92 ± 2.94	Week 2	1.92 ± 2.94	0.00	0.707
		Week 4	0.42 ± 0.52	1.50	0.342
		Week 6	0.00 ± 0.00	1.92	0.005
		Week 8	0.25 ±0.87	1.66	0.020
Week 2	1.92 ± 2.94	Week 4	0.42 ± 0.52	1.50	0.566
		Week 6	0.00 ± 0.00	1.92	0.016
		Week 8	0.25 ±0.87	1.67	0.051

Table 4.7 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Week 4	0.42 ± 0.52	Week 6	0.00 ± 0.00	0.42	0.066
		Week 8	0.25 ±0.87	0.17	0.169
Week 6	0.00 ± 0.00	Week 8	0.25 ±0.87	-2.50	0.743
Placebo					
Baseline	2.75 ± 2.86	Week 2	3.08 ± 4.40	-0.33	0.519
		Week 4	0.42 ± 0.79	2.33	0.036
		Week 6	0.25 ± 0.62	2.50	0.014
		Week 8	0.58 ± 1.38	2.16	0.021
Week 2	3.08 ± 4.40	Week 4	0.42 ± 0.79	2.67	0.146
		Week 6	0.25 ± 0.62	2.83	0.071
		Week 8	0.58 ± 1.38	2.50	0.095
Week 4	0.42 ± 0.79	Week 6	0.25 ± 0.62	0.17	0.723
		Week 8	0.58 ± 1.38	-0.16	0.826
Week 6	0.25 ± 0.62	Week 8	0.58 ± 1.38	-0.33	0.893

Note p-value determined by Kruskal-Wallis Test

Table 4.8 Acne counts of closed comedones among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	1.92 ± 2.94	Week 2	1.92 ± 2.94	1.00
		Week 4	0.42 ± 0.52	0.108
		Week 6	0.00 ± 0.00	0.045
		Week 8	0.25 ±0.87	0.101
Week 2	1.92 ± 2.94	Week 4	0.42 ± 0.52	0.105
		Week 6	0.00 ± 0.00	0.045
		Week 8	0.25 ±0.87	0.069

Table 4.8 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Week 4	0.42 ± 0.52	Week 6	0.00 ± 0.00	0.057
		Week 8	0.25 ±0.87	0.504
Week 6	0.00 ± 0.00	Week 8	0.25 ±0.87	0.339
Placebo Baseline	2.75 ± 2.86	Week 2	3.08 ± 4.40	0.813
		Week 4	0.42 ± 0.79	0.019
		Week 6	0.25 ± 0.62	0.014
		Week 8	0.58 ± 1.38	0.035
Week 2	3.08 ± 4.40	Week 4	0.42 ± 0.79	0.055
		Week 6	0.25 ± 0.62	0.051
		Week 8	0.58 ± 1.38	0.053
Week 4	0.42 ± 0.79	Week 6	0.25 ± 0.62	0.438
		Week 8	0.58 ± 1.38	0.438
Week 6	0.25 ± 0.62	Week 8	0.58 ± 1.38	0.305

Note p-value determined by repeated measured ANOVA

Table 4.9 Acne counts of open comedones among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	0.42 ± 1.443	Week 2	0.00 ± 0.00	0.42	0.317
		Week 4	0.00 ± 0.00	0.42	0.317
		Week 6	0.00 ± 0.00	0.42	0.317
		Week 8	0.00 ± 0.00	0.42	0.317
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	0.00	1.000

Table 4.9 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Placebo					
Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	0.00	1.000
		Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.08 ± 0.29	0.00	0.317
		Week 8	0.00 ± 0.00	0.00	1.000
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.08 ± 0.29	-0.08	0.317
		Week 8	0.00 ± 0.00	0.00	1.000
Week 4	0.00 ± 0.00	Week 6	0.08 ± 0.29	-0.08	0.317
		Week 8	0.00 ± 0.00	0.00	1.000
Week 6	0.08 ± 0.29	Week 8	0.00 ± 0.00	0.08	0.317

Note p-value determined by Kruskal-Wallis Test

Table 4.10 Acne counts of open comedones among time (by repeated measured ANOVA)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	0.42 ± 1.443	Week 2	0.00 ± 0.00	0.339
		Week 4	0.00 ± 0.00	0.339
		Week 6	0.00 ± 0.00	0.339
		Week 8	0.00 ± 0.00	0.339
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	NA
		Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	NA
Placebo Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	NA
		Week 4	0.00 ± 0.00	NA
		Week 6	0.08 ± 0.29	0.339
		Week 8	0.00 ± 0.00	NA

Table 4.10 (continued)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	NA
		Week 6	0.08 ± 0.29	0.339
		Week 8	0.00 ± 0.00	NA
Week 4	0.00 ± 0.00	Week 6	0.08 ± 0.29	0.339
		Week 8	0.00 ± 0.00	NA
Week 6	0.08 ± 0.29	Week 8	0.00 ± 0.00	NA

Note p-value determined by repeated measured ANOVA

4.4 Comparisons of Inflammatory Acne Counts between the *Andrographis paniculata* Gel and Placebo

The descriptive statistics of inflammatory acne, papule, pustule, nodule, and cyst counts was shown in table 4.11. The data was not normally distributed that was tested by the Kolmogorov-Smirnov test ($p < 0.05$).

Table 4.11 Acne counts of total inflammatory acne, papules, pustules, nodules and cysts (n = 12)

	Group					
	Andrographis paniculata gel			**Placebo**		
	Min	**Max**	**Median, IQR**	**Min**	**Max**	**Median, IQR**
Total inflammatory acne						
Baseline	1	15	4.50 (3.25, 7.50)	0	10	3.50 (1.00, 7.00)
Week 2	0	12	2.00 (0.00, 7.00)	0	13	3.00 (1.00, 9.25)
Week 4	1	7	1.00 (1.00, 3.75)	0	8	3.00 (1.25, 4.50)
Week 6	0	7	1.50 (0.00, 4.75)	0	9	2.50 (1.00, 3.75)
Week 8	0	4	1.50 (0.00, 3.00)	0	11	1.00 (0.00, 3.75)
Papules						
Baseline	1	6	3.50 (2.25, 5.00)	0	10	1.50 (0.25, 4.00)
Week 2	0	11	2.00 (0.00, 6.25)	0	9	2.50 (1.00, 6.50)
Week 4	0	5	1.00 (1.00, 1.75)	0	7	3.00 (1.00, 3.75)

Table 4.11 (continued)

	Group					
	Andrographis paniculata gel			Placebo		
	Min	**Max**	**Median, IQR**	**Min**	**Max**	**Median, IQR**
Week 6	0	7	1.50 (0.00,4.75)	0	9	1.50 (1.00, 3.00)
Week 8	0	4	1.00 (0.00, 3.00)	0	11	1.00 (0.00, 3.00)
Pustules						
Baseline	0	12	0.50 (0.00, 3.75)	0	8	0.00 (0.00, 2.75)
Week 2	0	3	0.00 (0.00, 0.00)	0	8	0.00 (0.00, 2.00)
Week 4	0	4	0.00 (0.00, 1.00)	0	2	0.00 (0.00, 1.00)
Week 6	0	0	0.00 (0.00, 0.00)	0	3	0.00 (0.00, 0.00)
Week 8	0	1	0.00 (0.00, 0.00)	0	1	0.00 (0.00, 0.00)
Nodules						
Baseline	0	0	0	0	0	0
Week 2	0	0	0	0	0	0
Week 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0
Week 8	0	0	0	0	0	0
Cysts						
Baseline	0	0	0	0	0	0
Week 2	0	0	0	0	0	0
Week 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0
Week 8	0	0	0	0	0	0

The comparisons of total inflammatory acne, papule, pustule, and nodule counts between the *Andrographis paniculata* gel and placebo at baseline, week 2, 4, 6 and 8 were shown in table 4.12.

The data showed that compared with placebo, the *Andrographis paniculata* gel significantly decreased total inflammatory acne counts at week 4 ($p = 0.042$). The *Andrographis paniculata* gel also significantly decreased papule counts at week 4 ($p = 0.046$). The *Andrographis paniculata* gel also significantly decreased pustules counts at week 6 ($p = 0.049$). For nodules, the *Andrographis paniculata* gel and placebo were not statistically significant difference ($p > 0.05$).

Table 4.12 Acne counts of inflammatory acne compared between *Andrographis paniculata* gel and placebo

	Acne Count		**Z**	**p-value**
	***Andrographis paniculata* gel**	**Placebo**		
	Mean ± SD	**Mean ± SD**		
Total inflammatory acne				
Baseline	5.92 ± 4.01	4.17 ± 3.49	-.987	0.324
Week 2	3.42 ± 4.30	4.67 ± 4.46	-1.052	0.293
Week 4	2.17 ± 2.17	4.67 ± 2.25	-1.268	0.042
Week 6	2.33 ± 2.61	2.75 ± 2.63	-.558	0.577
Week 8	1.67 ± 1.67	2.17 ± 3.19	-.030	0.976
Papules				
Baseline	3.58 ± 1.73	2.67 ± 2.93	-1.431	0.152
Week 2	3.08 ± 3.75	3.42 ± 2.91	-.702	0.483
Week 4	1.50 ± 1.51	3.25 ± 2.05	-1.494	0.046
Week 6	2.33 ± 2.61	2.25 ± 2.42	-.206	0.836
Week 8	1.50 ± 1.57	2.00 ± 3.16	.000	1.000
Pustules				
Baseline	2.33 ± 3.63	1.50 ± 2.39	-.504	0.614
Week 2	0.33 ± 0.89	1.25 ± 2.38	-1.025	0.305
Week 4	0.67 ± 1.23	1.22 ± 0.67	-.173	0.863
Week 6	0.00 ± 0.00	1.20 ± 1.17	-1.446	0.049
Week 8	0.17 ± 0.39	0.17 ± 0.39	.000	1.000
Nodules				
Baseline	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
Week 2	0.00 ± 0.800	0.00 ± 0.00	.000	1.000
Week 4	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
Week 6	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
Week 8	0.00 ± 0.00	0.00 ± 0.00	.000	1.000

Note p-value determined by Mann-Whitney U Test

4.5 Comparisons of Inflammatory Acne Counts Among Time

Table 4.13-4.20 showed total inflammatory acne, papule, pustule and nodule counts compared between baseline and during treatment (within-group).

On the *Andrographis paniculata* gel side, significant differences were observed in total inflammatory acne counts at baseline compared with week 2 (p = 0.022), week 4 (p = 0.014), week 6 (p = 0.010) and week 8 (p = 0.002), as shown in table 4.13. The *Andrographis paniculata* gel also had significant differences on papule counts in week 2 compared with week 4 (p = 0.006) and week 8 (p = 0.008), as shown in table 4.15. The *Andrographis paniculata* gel also had significant differences on pustule counts in baseline compared with week 2 (p = 0.032), week 6 (p = 0.002) and week 8 (p = 0.025) as shown in table 4.17. There was not significant difference in nodule counts, as shown in table 4.19. However, on the placebo side, no significant difference was observed.

Table 4.13 Acne counts of total inflammatory acne among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Time	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	5.92 ± 4.01	Week 2	3.42 ± 4.30	2.50	0.022
		Week 4	2.17 ± 2.17	3.75	0.014
		Week 6	2.33 ± 2.61	3.58	0.010
		Week 8	1.67 ± 1.67	4.25	0.002
Week 2	3.42 ± 4.30	Week 4	2.17 ± 2.17	1.25	0.863
		Week 6	2.33 ± 2.61	1.08	0.771
		Week 8	1.67 ± 1.67	1.75	0.430

Table 4.13 (continued)

Time	Mean ± SD	Time	Mean ± SD	Mean difference	p-value
Week 4	2.17 ± 2.17	Week 6	2.33 ± 2.61	-1.66	0.905
		Week 8	1.67 ± 1.67	0.50	0.537
Week 6	2.33 ± 2.61	Week 8	1.67 ± 1.67	0.66	0.618
Placebo					
Baseline	4.17 ± 3.49	Week 2	4.67 ± 4.46	-0.50	0.816
		Week 4	4.67 ± 2.25	-0.50	0.619
		Week 6	2.75 ± 2.63	1.42	0.366
		Week 8	2.17 ± 3.19	2.00	0.127
Week 2	4.67 ± 4.46	Week 4	4.67 ± 2.25	0.00	0.681
		Week 6	2.75 ± 2.63	1.92	0.334
		Week 8	2.17 ± 3.19	2.50	0.095
Week 4	4.67 ± 2.25	Week 6	2.75 ± 2.63	1.92	0.534
		Week 8	2.17 ± 3.19	1.00	0.134
Week 6	2.75 ± 2.63	Week 8	2.17 ± 3.19	0.58	0.346

Note p-value determined by Kruskal-Wallis Test

Table 4.14 Acne counts of total inflammatory acne among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	5.92 ± 4.01	Week 2	3.42 ± 4.30	0.076
		Week 4	2.17 ± 2.17	0.001
		Week 6	2.33 ± 2.61	0.021
		Week 8	1.67 ± 1.67	0.006
Week 2	3.42 ± 4.30	Week 4	2.17 ± 2.17	0.269
		Week 6	2.33 ± 2.61	0.401
		Week 8	1.67 ± 1.67	0.226
Week 4	2.17 ± 2.17	Week 6	2.33 ± 2.61	0.809
		Week 8	1.67 ± 1.67	0.515
Week 6	2.33 ± 2.61	Week 8	1.67 ± 1.67	0.322
Placebo				
Baseline	4.17 ± 3.49	Week 2	4.67 ± 4.46	0.568
		Week 4	4.67 ± 2.25	0.293
		Week 6	2.75 ± 2.63	0.342
		Week 8	2.17 ± 3.19	0.156
Week 2	4.67 ± 4.46	Week 4	4.67 ± 2.25	0.207
		Week 6	2.75 ± 2.63	0.233
		Week 8	2.17 ± 3.19	0.187
Week 4	4.67 ± 2.25	Week 6	2.75 ± 2.63	0.636
		Week 8	2.17 ± 3.19	0.451
Week 6	2.75 ± 2.63	Week 8	2.17 ± 3.19	0.641

Note p-value determined by repeated measured ANOVA

Table 4.15 Acne counts of papules among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Time	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	3.58 ± 1.73	Week 2	3.08 ± 3.75	0.50	0.267
		Week 4	1.50 ± 1.51	2.08	0.006
		Week 6	2.33 ± 2.61	1.25	0.145
		Week 8	1.50 ± 1.57	2.08	0.008
Week 2	3.08 ± 3.75	Week 4	1.50 ± 1.51	1.58	0.766
		Week 6	2.33 ± 2.61	0.75	0.764
		Week 8	1.50 ± 1.57	1.58	0.468
Week 4	1.50 ± 1.51	Week 6	2.33 ± 2.61	-0.83	0.812
		Week 8	1.50 ± 1.57	0.00	0.740
Week 6	2.33 ± 2.61	Week 8	1.50 ± 1.57	0.83	0.547
Placebo					
Baseline	2.67 ± 2.93	Week 2	3.42 ± 2.91	-0.75	0.430
		Week 4	3.25 ± 2.05	-0.58	0.769
		Week 6	2.25± 2.42	0.42	0.746
		Week 8	2.20 ± 3.16	0.67	0.327
Week 2	3.42 ± 2.91	Week 4	3.25 ± 2.05	0.17	0.791
		Week 6	2.25± 2.42	1.17	0.330
		Week 8	2.20 ± 3.16	1.42	0.100
Week 4	3.25 ± 2.05	Week 6	2.25± 2.42	1.00	0.338
		Week 8	2.20 ± 3.16	0.75	0.162
Week 6	2.25± 2.42	Week 8	2.20 ± 3.16	0.25	0.403

Note p-value determined by Kruskal-Wallis Test

Table 4.16 Acne counts of papules among time (by repeated measured ANOVA)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	3.58 ± 1.73	Week 2	3.08 ± 3.75	0.684
		Week 4	1.50 ± 1.51	0.005
		Week 6	2.33 ± 2.61	0.183
		Week 8	1.50 ± 1.57	0.008
Week 2	3.08 ± 3.75	Week 4	1.50 ± 1.51	0.172
		Week 6	2.33 ± 2.61	0.488
		Week 8	1.50 ± 1.57	0.215
Week 4	1.50 ± 1.51	Week 6	2.33 ± 2.61	0.147
		Week 8	1.50 ± 1.57	1.000
Week 6	2.33 ± 2.61	Week 8	1.50 ± 1.57	0.241
Placebo				
Baseline	2.67 ± 2.93	Week 2	3.42 ± 2.91	0.463
		Week 4	3.25 ± 2.05	0.920
		Week 6	2.25± 2.42	0.730
		Week 8	2.20 ± 3.16	0.601
Week 2	3.42 ± 2.91	Week 4	3.25 ± 2.05	0.526
		Week 6	2.25± 2.42	0.346
		Week 8	2.20 ± 3.16	0.323
Week 4	3.25 ± 2.05	Week 6	2.25± 2.42	0.555
		Week 8	2.20 ± 3.16	0.547
Week 6	2.25± 2.42	Week 8	2.20 ± 3.16	0.834

Note p-value determined by repeated measured ANOVA

Table 4.17 Acne counts of pustules among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Time	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	2.33 ± 3.63	Week 2	0.33 ± 0.89	2.00	0.032
		Week 4	0.67 ± 1.23	1.66	0.218
		Week 6	0.00 ± 0.00	2.33	0.002
		Week 8	0.17 ± 0.39	2.16	0.025
Week 2	0.33 ± 0.89	Week 4	0.67 ± 1.23	-0.34	0.360
		Week 6	0.00 ± 0.00	0.33	0.360
		Week 8	0.17 ± 0.39	0.16	0.925
Week 4	0.67 ± 1.23	Week 6	0.00 ± 0.00	0.67	0.067
		Week 8	0.17 ± 0.39	0.50	0.312
Week 6	0.00 ± 0.00	Week 8	0.17 ± 0.39	-1.70	0.412
Placebo					
Baseline	1.50 ± 2.39	Week 2	1.25 ± 2.38	0.25	0.665
		Week 4	0.42 ± 0.67	1.08	0.318
		Week 6	1.20 ± 1.17	0.30	0.220
		Week 8	0.17 ± 0.39	1.33	0.098
Week 2	1.25 ± 2.38	Week 4	0.42 ± 0.67	0.83	0.629
		Week 6	1.20 ± 1.17	0.05	0.403
		Week 8	0.17 ± 0.39	1.08	0.224
Week 4	0.42 ± 0.67	Week 6	1.20 ± 1.17	-0.78	0.543
		Week 8	0.17 ± 0.39	0.25	0.320
Week 6	1.20 ± 1.17	Week 8	0.17 ± 0.39	1.03	0.859

Note p-value determined by Kruskal-Wallis Test

Table 4.18 Acne counts of pustules among time (by repeated measured ANOVA)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	2.33 ± 3.63	Week 2	0.33 ± 0.89	0.042
		Week 4	0.67 ± 1.23	0.051
		Week 6	0.00 ± 0.00	0.048
		Week 8	0.17 ± 0.39	0.057
Week 2	0.33 ± 0.89	Week 4	0.67 ± 1.23	0.104
		Week 6	0.00 ± 0.00	0.220
		Week 8	0.17 ± 0.39	0.586
Week 4	0.67 ± 1.23	Week 6	0.00 ± 0.00	0.087
		Week 8	0.17 ± 0.39	0.191
Week 6	0.00 ± 0.00	Week 8	0.17 ± 0.39	0.166
Placebo Baseline				
	1.50 ± 2.39	Week 2	1.25 ± 2.38	0.793
		Week 4	0.42 ± 0.67	0.097
		Week 6	1.20 ± 1.17	0.266
		Week 8	0.17 ± 0.39	0.075
Week 2	1.25 ± 2.38	Week 4	0.42 ± 0.67	0.175
		Week 6	1.20 ± 1.17	0.157
		Week 8	0.17 ± 0.39	0.162
Week 4	0.42 ± 0.67	Week 6	1.20 ± 1.17	0.795
		Week 8	0.17 ± 0.39	0.339
Week 6	1.20 ± 1.17	Week 8	0.17 ± 0.39	0.394

Note p-value determined by repeated measured ANOVA

Table 4.19 Acne counts of nodules among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Time	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	0.00	1.000
		Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	0.00	1.000
Placebo					
Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	0.00	1.000
		Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	0.00	1.000
		Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	0.00	1.000
		Week 8	0.00 ± 0.00	0.00	1.000
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	0.00	1.000

Note p-value determined by Kruskal-Wallis Test

Table 4.20 Acne counts of nodules among time (by repeated measured ANOVA)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	NA
		Week 4	0.00 ± 0.00	NA
		Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	NA
		Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	NA
Placebo				
Baseline	0.00 ± 0.00	Week 2	0.00 ± 0.00	NA
		Week 4	0.00 ± 0.00	NA
		Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 2	0.00 ± 0.00	Week 4	0.00 ± 0.00	NA
		Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 4	0.00 ± 0.00	Week 6	0.00 ± 0.00	NA
		Week 8	0.00 ± 0.00	NA
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	NA

Note p-value determined by repeated measured ANOVA

4.6 Comparisons of Mean Percentage Changes between the *Andrographis paniculata* Gel and Placebo

Mean percentage changes of total non-inflammatory acne and total inflammatory acne counts from baseline compared between the *Andrographis paniculata* gel and placebo were shown in table 4.21-4.24.

Table 4.21 Mean percentage changes of acne counts from the baseline to week 2, 4, 6, and 8 of total non-inflammatory acne (by Cochran's Q test)

	***Andrographis paniculata* gel**		**Placebo**	
	Without acne	**With acne**	**Without acne**	**With acne**
Baseline	6	6	5	7
Week 2	7	5	7	5
Week 4	7	5	9	3
Week 6	12	0	9	3
Week 8	11	1	10	2
Cochran's Q	11.680		7.619	
df	4		4	
Aysmp. Sig	.020		.107	

Note p-value determined by Cochran's Q test

Table 4.22 Mean percentage changes of acne counts from the baseline to week 2, 4, 6, and 8 of total non-inflammatory acne (by McNemar Test)

Time	Time	p-value
Andrographis paniculata gel		
Baseline	Week 2	1.000
	Week 4	1.000
	Week 6	.031
	Week 8	.125
Week 2	Week 4	1.000
	Week 6	.063
	Week 8	.125
Week 4	Week 6	.063
	Week 8	.125
Week 6	Week 8	1.000

Note p-value determined by McNemar Test

Table 4.23 Mean percentage changes of acne counts from the baseline to week 2, 4, 6, and 8 of total inflammatory acne (by Cochran's Q test)

	***Andrographis paniculata* gel**		**Placebo**	
	Without acne	With acne	Without acne	With acne
Baseline	0	12	2	10
Week 2	5	7	1	11
Week 4	2	10	1	11
Week 6	5	7	2	10
Week 8	5	7	5	7
Cochran's Q	9.636		6.353	
df	4		4	
Aysmp. Sig	.047		.174	

Note p-value determined by Cochran's Q test

Table 4.24 Mean percentage changes of acne counts from the baseline to week 2, 4, 6, and 8 of total inflammatory acne (by McNemar Test)

Time	**Time**	**p-value**
Andrographis paniculata gel		
Baseline	Week 2	.063
	Week 4	.500
	Week 6	.063
	Week 8	.063
Week 2	Week 4	.375
	Week 6	1.000
	Week 8	1.000
Week 4	Week 6	.453
	Week 8	1.000
Week 6	Week 8	1.000

Note p-value determined by McNemar Test

On the *Andrographis paniculata* gel side, significant difference was observed in total non-inflammatory acne counts at baseline compared with week 6 ($p = 0.031$). However, on the placebo side, no significant difference was observed.

4.7 Comparison of Severity of Acne

Severity of acne was separately graded by Global Acne Severity Scale in *Andrographis paniculata* gel side and the placebo side;

Clear was defined as normal, clear skin with no evidence of acne vulgaris and valued as 0.

Almost clear was defined as rare non-inflammatory lesions present, with rare non-inflamed papules (papules must be resolving and may be hyperpigmented, though not pink-red) and valued as 1.

Mild was defined as some non-inflammatory lesions are present, with few inflammatory lesions (papules/pustules only, no nodulocystic lesions) and valued as 2.

Moderate was defined as non-inflammatory lesions predominate, with multiple inflammatory lesions evident; several to many comedones and papules/pustules; there may or may not be one small nodulocystic lesions and valued as 3.

Severe was defined as inflammatory lesions are more apparent, many comedones and papules/pustules, there may or may not a few nodulocystic lesions and valued as 4.

Very severe was defined as highly inflammatory lesions predominate, variable number of comedones, many papules/pustules, and many nodulocystic lesions and valued as 5.

The frequency distribution of severity of acne was shown in table 4.25. Comparing between the *Andrographis paniculata* gel and placebo showed that at week 6 and 8, there were no significant difference, as shown in table 4.26.

Table 4.25 The severity of acne at baseline, 2nd, 4th, 6th and 8th week (n = 12)

	***Andrographis paniculata* gel**				**Placebo**			
	Mild	**Moderate**	**Severe**	**Very severe**	**Mild**	**Moderate**	**Severe**	**Very severe**
Baseline	6 (50.0%)	6 (50.0%)	0	0	1 (8.3%)	11 (91.7%)	0	0
Week 2	4 (33.3%)	8 (66.7%)	0	0	3 (25.0%)	9 (75.0%)	0	0
Week 4	6 (50.0%)	6 (50.0%)	0	0	5 (41.7%)	7 (58.3%)	0	0
Week 6	7 (58.3%)	5 (41.7%)	0	0	6 (50.0%)	6 (50.0%)	0	0
Week 8	7 (58.3%)	5 (41.7%)	0	0	8 (66.7%)	4 (33.3%)	0	0

Table 4.26 The severity of acne compared between *Andrographis paniculate* gel and placebo (n = 12)

	Severity of acne		**Z**	**p-value**
	***Andrographis paniculata* gel**	**Placebo**		
	Mean ± SD	**Mean ± SD**		
Baseline	3.33 ± 0.49	3.33 ± 0.78	-.135	0.893
Week 2	3.08 ± 0.79	3.17 ± 0.84	-.300	0.764
Week 4	2.83 ± 0.94	2.75 ± 0.75	-.062	0.950
Week 6	2.58 ± 0.67	2.67 ± 0.78	-.191	0.848
Week 8	2.50 ± 0.67	2.42 ± 0.67	-.371	0.710

Note p-value determined by Mann-Whitney U Test

Table 4.27 showed the severity of acne comparing between baseline and during the study. On the *Andrographis paniculata* gel side, significant differences were observed in severity of acne at baseline compared with week 4 (p = 0.043), week 6 (p = 0.008) and week 8 (p = 0.003). On the placebo side, significant differences were observed in severity of acne at baseline compared with week 6 (p = 0.042) and week 8 (p = 0.004), week 2 compared with week 8 (p = 0.019).

Table 4.27 The severity of acne among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	3.33 ± 0.49	Week 2	3.08 ± 0.79	0.25	0.289
		Week 4	2.83 ± 0.98	0.50	0.043
		Week 6	2.58 ± 0.67	0.75	0.008
		Week 8	2.50 ± 0.67	0.83	0.003
Week 2	3.08 ± 0.79	Week 4	2.83 ± 0.98	0.25	0.338
		Week 6	2.58 ± 0.67	0.50	0.113
		Week 8	2.50 ± 0.67	0.58	0.057
Week 4	2.83 ± 0.98	Week 6	2.58 ± 0.67	0.25	0.531
		Week 8	2.50 ± 0.67	0.33	0.344
Week 6	2.58 ± 0.67	Week 8	2.50 ± 0.67	0.08	0.749
Placebo					
Baseline	3.33 ± 0.78	Week 2	3.17 ± 0.84	0.16	0.598
		Week 4	2.75 ± 0.75	0.58	0.083
		Week 6	2.67 ± 0.78	0.66	0.042
		Week 8	2.42 ± 0.67	0.91	0.004
Week 2	3.17 ± 0.84	Week 4	2.75 ± 0.75	0.42	0.228
		Week 6	2.67 ± 0.78	0.50	0.132
		Week 8	2.42 ± 0.67	0.75	0.019
Week 4	2.75 ± 0.75	Week 6	2.67 ± 0.78	0.08	0.763
		Week 8	2.42 ± 0.67	0.33	0.258
Week 6	2.67 ± 0.78	Week 8	2.42 ± 0.67	0.25	0.407

Note p-value determined by Kruskal-Wallis Test

Table 4.28 The severity of acne among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	3.33 ± 0.49	Week 2	3.08 ± 0.79	0.275
		Week 4	2.83 ± 0.98	0.111
		Week 6	2.58 ± 0.67	0.005
		Week 8	2.50 ± 0.67	0.002
Week 2	3.08 ± 0.79	Week 4	2.83 ± 0.98	0.191
		Week 6	2.58 ± 0.67	0.057
		Week 8	2.50 ± 0.67	0.052
Week 4	2.83 ± 0.98	Week 6	2.58 ± 0.67	0.275
		Week 8	2.50 ± 0.67	0.166
Week 6	2.58 ± 0.67	Week 8	2.50 ± 0.67	0.586
Placebo Baseline	3.33 ± 0.78	Week 2	3.17 ± 0.84	0.438
		Week 4	2.75 ± 0.75	0.067
		Week 6	2.67 ± 0.78	0.025
		Week 8	2.42 ± 0.67	0.002
Week 2	3.17 ± 0.84	Week 4	2.75 ± 0.75	0.096
		Week 6	2.67 ± 0.78	0.056
		Week 8	2.42 ± 0.67	0.005
Week 4	2.75 ± 0.75	Week 6	2.67 ± 0.78	0.723
		Week 8	2.42 ± 0.67	0.104
Week 6	2.67 ± 0.78	Week 8	0.00 ± 0.00	0.275

Note p-value determined by repeated measured ANOVA

4.8 Comparisons of Sebum Level, Postinflammatory Hyperpigmentation (PIH), Postinflammatory Erythema (PIE) and Porphyrin Counts between the *Andrographis paniculata* Gel and Placebo

The descriptive statistics of sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts was shown in table 4.29. The data was not normally distributed that was tested by the Kolmogorov-Smirnov test ($p < 0.05$).

Table 4.29 Sebum level, PIH, PIE and porphyrin counts (n = 12)

	Group					
	***Andrographis paniculata* gel**			**Placebo**		
	Min	**Max**	**Med, IQR**	**Min**	**Max**	**Med, IQR**
Sebum						
Baseline	10	58	34.00 (24.00, 54.34)	13	64	29.50 (20.59, 47.17)
Week 2	4	69	19.17 (10.19, 43.92)	4	54	23.17 (9.92, 36.67)
Week 4	5	52	26.50 (12.33, 34.92)	4	56	23.34 (13.67, 35.17)
Week 6	6	41	17.34 (10.75, 25.60)	8	42	23.00 (13.00, 36.00)
Week 8	6	42	20.67 (12.50, 30.75)	9	37	17.50 (13.50, 35.67)
PIH						
Baseline	0	10	0.00 (0.00, 1.75)	0	10	0.00 (0.00, 3.00)
Week 2	0	12	0.00 (0.00, 6.25)	0	12	0.00 (0.00, 4.00)
Week 4	0	3	0.00 (0.00, 0.00)	0	3	0.00 (0.00, 0.00)
Week 6	0	14	0.00 (0.00, 0.00)	0	16	0.00 (0.00, 0.00)
PIE						
Baseline	0	36	5.50 (0.00, 13.25)	0	42	5.50 (0.75, 20.00)
Week 2	0	35	3.50 (1.25, 10.50)	0	54	3.00 (0.25, 12.25)
Week 4	0	48	3.00 (0.25, 15.00)	0	40	4.50 (0.00, 14.25)
Week 6	0	35	1.00 (0.25, 3.00)	0	43	2.50 (0.00, 7.75)
Week 8	0	32	0.00 (0.00, 7.25)	0	32	0.00 (0.00, 6.25)

Table 4.29 (continued)

	Group					
	***Andrographis paniculata* gel**			**Placebo**		
	Min	**Max**	**Med, IQR**	**Min**	**Max**	**Med, IQR**
Porphyrin counts						
Baseline	300	2100	739.00 (379.75, 645.00)	326	1911	884.00 (556.50,1608.00)
Week 2	281	2088	772.50 (545.00,1333.75)	320	2603	742.50 (540.75,1258.00)
Week 4	154	1432	742.50 (503.75,1048.50)	108	1782	742.50 (410.50,1100.75)
Week 6	150	1279	473.00 (242.00,930.25)	120	1458	479.00 (277.00, 934.00)
Week 8	40	1575	472.00 (326.25,968.25)	20	2352	488.00 (384.75, 964.50)

The comparisons of sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts between the *Andrographis paniculata* gel and placebo at baseline, week 2, 4, 6 and 8 were shown in table 4.30.

The data showed that for sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts, the *Andrographis paniculata* gel and placebo were not statistically significant difference ($p > 0.05$).

Table 4.30 Sebum level, PIH, PIE and porphyrin counts compared between *Andrographis paniculata* gel and placebo (n = 12)

Sebum level, PIH, PIE and porphyrin counts			**Z**	**p-value**
	***Andrographis paniculata* gel**	**Placebo**		
	Mean ± SD	**Mean ± SD**		
Sebum				
Baseline	36.42 ± 16.86	35.28 ± 17.94	-.289	0.773
Week 2	26.20 ± 20.17	23.72 ± 16.08	-.173	0.862
Week 4	25.03 ± 13.67	25.56 ± 14.94	-.058	0.954
Week 6	19.96 ± 11.32	23.58 ± 11.80	-.752	0.452
Week 8	21.67 ± 10.55	22.53 ± 11.52	-.145	0.885

Table 4.30 (continued)

Sebum level, PIH, PIE and porphyrin counts			Z	p-value
	Andrographis paniculata gel	Placebo		
	Mean ± SD	Mean ± SD		
PIH				
Baseline	1.75 ± 3.46	2.00 ± 3.30	-.231	0.690
Week 2	2.50 ± 4.42	2.58 ± 4.05	-.087	0.740
Week 4	0.25 ±0.86	0.25 ± 0.86	-.231	1.000
Week 6	1.33 ± 4.03	1.50 ± 4.60	-.522	0.965
Week 8	0.00 ± 0.00	0.00 ± 0.00	.000	1.000
PIE				
Baseline	8.42 ± 11.51	11.75 ± 14.14	.000	0.590
Week 2	7.25 ± 9.8	9.83 ± 16.08	-.203	0.887
Week 4	9.67 ±14.32	8.08 ± 11.86	-.058	0.932
Week 6	4.75 ± 9.91	8.00 ± 13.84	-.405	0.630
Week 8	4.42 ± 9.41	4.67 ± 9.78	-.227	0.977
Porphyrin counts				
Baseline	968.17 ± 644.01	999.08 ± 561.98	-.462	0.644
Week 2	953.92 ± 607.79	986.00 ± 673.72	-.116	0.908
Week 4	742.58 ± 356.72	762.50 ± 458.22	-.144	0.885
Week 6	570.08 ± 363.49	613.58 ± 425.06	-.347	0.729
Week 8	612.90 ± 460.17	721.50 ± 640.17	-.681	0.496

Note p-value determined by Mann-Whitney U Test

4.9 Comparisons of Sebum Level, Postinflammatory Hyperpigmentation (PIH), Postinflammatory Erythema (PIE) and Porphyrin Counts Among Time

Table 4.31-4.38 showed sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts compared between baseline and during treatment (within-group).

On the *Andrographis paniculata* gel side, significant differences were observed in sebum level at baseline compared with week 6 (p = 0.015) and week 8 (p = 0.024). On the placebo side, no significant difference was observed. The *Andrographis paniculata* gel also had significant differences on PIH at baseline compared with week 8 (p = 0.033), week 2 compared with week 8 (p = 0.033). The placebo also had significant differences on PIH counts at baseline compared with week 8 (p = 0.018), week 2 compared with week 4 (p = 0.040), week 2 compared with week 8 (p = 0.012).

However, there was not significant difference in postinflammatory erythema (PIE) and porphyrin counts as shown in table 4.35-4.38.

Table 4.31 Sebum level among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	36.42 ± 16.86	Week 2	26.20 ± 20.17	10.22	0.106
		Week 4	25.03 ±13.67	11.39	0.118
		Week 6	19.96 ± 11.33	16.46	0.015
		Week 8	21.67 ± 10.55	14.75	0.024
Week 2	26.20 ± 20.17	Week 4	25.03 ±13.67	1.17	0.817
		Week 6	19.96 ± 11.33	6.24	0.644
		Week 8	21.67 ± 10.55	4.53	0.862

Table 4.31 (continued)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Week 4	25.03 ±13.67	Week 6	19.96 ± 11.33	5.07	0.453
		Week 8	21.67 ± 10.55	3.36	0.525
Week 6	19.96 ± 11.33	Week 8	21.67 ± 10.55	-1.71	0.603
Placebo					
Baseline	35.28 ±17.93	Week 2	23.72 ± 16.08	11.56	0.088
		Week 4	25.56 ± 14.94	9.72	0.184
		Week 6	23.58 ± 11.80	11.70	0.118
		Week 8	22.53 ± 11.52	12.75	0.133
Week 2	23.72 ± 16.08	Week 4	25.56 ± 14.94	-1.83	0.773
		Week 6	23.58 ± 11.80	0.14	0.908
		Week 8	22.53 ± 11.52	1.19	0.954
Week 4	25.56 ± 14.94	Week 6	23.58 ± 11.80	1.97	0.862
		Week 8	22.53 ± 11.52	3.02	0.707
Week 6	23.58 ± 11.80	Week 8	22.53 ± 11.52	1.05	0.885

Note p-value determined by Kruskal-Wallis Test

Table 4.32 Sebum level among time (by repeated measured ANOVA)

Time Mean ± SD		Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	36.42 ± 16. 86	Week 2	26.20 ± 20.17	0.183
		Week 4	25.03 ±13.67	0.056
		Week 6	19.96 ± 11.33	0.015
		Week 8	21.67 ± 10.55	0.017
Week 2	26.20 ± 20.17	Week 4	25.03 ±13.67	0.878
		Week 6	19.96 ± 11.33	0.344
		Week 8	21.67 ± 10.55	0.491
Week 4	25.03 ±13.67	Week 6	19.96 ± 11.33	0.138
		Week 8	21.67 ± 10.55	0.403
Week 6	19.96 ± 11.33	Week 8	21.67 ± 10.55	0.485
Placebo Baseline	35.28 ±17.93	Week 2	23.72 ± 16.08	0.066
		Week 4	25.56 ± 14.94	0.068
		Week 6	23.58 ± 11.80	0.056
		Week 8	22.53 ± 11.52	0.051
Week 2	23.72 ± 16.08	Week 4	25.56 ± 14.94	0.689
		Week 6	23.58 ± 11.80	0.978
		Week 8	22.53 ± 11.52	0.782
Week 4	25.56 ± 14.94	Week 6	23.58 ± 11.80	0.519
		Week 8	22.53 ± 11.52	0.311
Week 6	23.58 ± 11.80	Week 8	22.53 ± 11.52	0.549

Note p-value determined by repeated measured ANOVA

Table 4.33 PIH among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean diff	p-value
Andrographis paniculata gel					
Baseline	1.75 ± 3.46	Week 2	2.50 ± 4.42	-0.750	0.891
		Week 4	0.25 ±0.86	1.500	0.143
		Week 6	19.96 ± 11.33	0.417	0.425
		Week 8	21.67 ± 10.55	1.750	0.033
Week 2	2.50 ± 4.42	Week 4	0.25 ±0.86	2.250	0.122
		Week 6	1.33 ± 4.03	1.167	0.404
		Week 8	0.00 ± 0.00	2.500	0.033
Week 4	0.25 ±0.86	Week 6	1.33 ± 4.03	-1.083	0.547
		Week 8	0.00 ± 0.00	0.250	0.317
Week 6	1.33 ± 4.03	Week 8	0.00 ± 0.00	1.333	0.149
Placebo	2.00 ± 3.30				
Baseline		Week 2	2.58 ± 4.05	-0.583	0.890
		Week 4	0.25 ± 0.86	1.750	0.056
		Week 6	1.50 ± 4.60	0.500	0.170
		Week 8	0.00 ± 0.00	2.000	0.018
Week 2	2.58 ± 4.05	Week 4	0.25 ± 0.86	2.333	0.040
		Week 6	1.50 ± 4.60	1.083	0.131
		Week 8	0.00 ± 0.00	2.583	0.012
Week 4	0.25 ± 0.86	Week 6	1.50 ± 4.60	-1.250	0.587
		Week 8	0.00 ± 0.00	0.250	0.650
Week 6	1.50 ± 4.60	Week 8	0.00 ± 0.00	1.500	0.319

Note p-value determined by Kruskal-Wallis Test

Table 4.34 PIH among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	1.75 ± 3.46	Week 2	2.50 ± 4.42	0.609
		Week 4	0.25 ±0.86	0.180
		Week 6	19.96 ± 11.33	0.806
		Week 8	21.67 ± 10.55	0.108
Week 2	2.50 ± 4.42	Week 4	0.25 ±0.86	0.070
		Week 6	19.96 ± 11.33	0.529
		Week 8	21.67 ± 10.55	0.076
Week 4	0.25 ±0.86	Week 6	19.96 ± 11.33	0.377
		Week 8	21.67 ± 10.55	0.339
Week 6	19.96 ± 11.33	Week 8	21.67 ± 10.55	0.276
Placebo Baseline	2.00 ± 3.30	Week 2	2.58 ± 4.05	0.717
		Week 4	0.25 ± 0.86	0.111
		Week 6	1.50 ± 4.60	0.785
		Week 8	0.00 ± 0.00	0.060
Week 2	2.58 ± 4.05	Week 4	0.25 ± 0.86	0.066
		Week 6	1.50 ± 4.60	0.573
		Week 8	0.00 ± 0.00	0.060
Week 4	0.25 ± 0.86	Week 6	1.50 ± 4.60	0.372
		Week 8	0.00 ± 0.00	0.339
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	0.283

Note p-value determined by repeated measured ANOVA

Table 4.35 PIE among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	8.42 ± 11.51	Week 2	7.25 ± 9.8	1.167	0.861
		Week 4	9.67 ±14.32	-1.250	0.906
		Week 6	4.75 ± 9.91	3.667	0.575
		Week 8	4.42 ± 9.41	4.000	0.257
Week 2	7.25 ± 9.8	Week 4	9.67 ±14.32	-2.417	0.977
		Week 6	4.75 ± 9.91	2.500	0.170
		Week 8	4.42 ± 9.41	2.833	0.059
Week 4	9.67 ±14.32	Week 6	4.75 ± 9.91	4.917	0.292
		Week 8	4.42 ± 9.41	5.250	0.083
Week 6	4.75 ± 9.91	Week 8	4.42 ± 9.41	0.333	0.201
Placebo					
Baseline	11.75 ± 14.14	Week 2	9.83 ± 16.08	1.917	0.540
		Week 4	8.08 ± 11.86	3.667	0.482
		Week 6	8.00 ± 13.84	3.750	0.349
		Week 8	4.67 ± 9.78	7.083	0.060
Week 2	9.83 ± 16.08	Week 4	8.08 ± 11.86	1.750	0.860
		Week 6	8.00 ± 13.84	1.833	0.682
		Week 8	4.67 ± 9.78	5.167	0.107
Week 4	8.08 ± 11.86	Week 6	8.00 ± 13.84	0.083	0.768
		Week 8	4.67 ± 9.78	3.417	0.165
Week 6	8.00 ± 13.84	Week 8	4.67 ± 9.78	3.333	0.184

Note p-value determined by Kruskal-Wallis Test

Table 4.36 PIE among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel				
Baseline	1.75 ± 3.46	Week 2	2.50 ± 4.42	0.609
		Week 4	0.25 ±0.86	0.180
		Week 6	19.96 ± 11.33	0.806
		Week 8	21.67 ± 10.55	0.108
Week 2	2.50 ± 4.42	Week 4	0.25 ±0.86	0.070
		Week 6	19.96 ± 11.33	0.529
		Week 8	21.67 ± 10.55	0.076
Week 4	0.25 ±0.86	Week 6	19.96 ± 11.33	0.377
		Week 8	21.67 ± 10.55	0.339
Week 6	19.96 ± 11.33	Week 8	21.67 ± 10.55	0.276
Placebo				
Baseline	2.00 ± 3.30	Week 2	2.58 ± 4.05	0.717
		Week 4	0.25 ± 0.86	0.111
		Week 6	1.50 ± 4.60	0.785
		Week 8	0.00 ± 0.00	0.060
Week 2	2.58 ± 4.05	Week 4	0.25 ± 0.86	0.058
		Week 6	1.50 ± 4.60	0.573
		Week 8	0.00 ± 0.00	0.055
Week 4	0.25 ± 0.86	Week 6	1.50 ± 4.60	0.372
		Week 8	0.00 ± 0.00	0.339
Week 6	0.00 ± 0.00	Week 8	0.00 ± 0.00	0.283

Note p-value determined by repeated measured ANOVA

Table 4.37 Porphyrin counts among time (by Kruskal-Wallis Test)

Time	Mean ± SD	Pair comparison	Mean ± SD	Mean difference	p-value
Andrographis paniculata gel					
Baseline	968.17 ± 644.01	Week 2	953.92 ± 607.79	14.25	0.954
		Week 4	742.58 ± 356.72	225.59	0.604
		Week 6	570.08 ± 363.49	398.09	0.106
		Week 8	612.90 ± 460.17	355.27	0.210
Week 2	953.92 ± 607.79	Week 4	742.58 ± 356.72	211.34	0.488
		Week 6	570.08 ± 363.49	383.84	0.064
		Week 8	612.90 ± 460.17	341.02	0.129
Week 4	742.58 ± 356.72	Week 6	570.08 ± 363.49	172.50	0.225
		Week 8	612.90 ± 460.17	129.68	0.235
Week 6	570.08 ± 363.49	Week 8	612.90 ± 460.17	-42.82	0.947
Placebo					
Baseline	999.08 ± 561.98	Week 2	986.00 ± 673.72	13.08	0.686
		Week 4	762.50 ± 458.22	236.58	0.299
		Week 6	613.58 ± 425.06	385.50	0.065
		Week 8	721.50 ± 640.17	277.58	0.210
Week 2	986.00 ± 673.72	Week 4	762.50 ± 458.22	233.50	0.419
		Week 6	613.58 ± 425.06	372.42	0.083
		Week 8	721.50 ± 640.17	264.50	0.129
Week 4	762.50 ± 458.22	Week 6	613.58 ± 425.06	148.92	0.326
		Week 8	721.50 ± 640.17	41.00	0.488
Week 6	613.58 ± 425.06	Week 8	721.50 ± 640.17	-107.92	0.692

Note p-value determined by Kruskal-Wallis Test

Table 4.38 Porphyrin counts among time (by repeated measured ANOVA)

Time	Mean ± SD	Pair comparison	Mean ± SD	p-value
Andrographis paniculata gel		Week 2	953.92 ± 607.79	0.939
Baseline	968.17 ± 644.01	Week 4	742.58 ± 356.72	0.232
		Week 6	570.08 ± 363.49	0.059
		Week 8	612.90 ± 460.17	0.255
Week 2	953.92 ± 607.79	Week 4	742.58 ± 356.72	0.061
		Week 6	570.08 ± 363.49	0.058
		Week 8	612.90 ± 460.17	0.060
Week 4	742.58 ± 356.72	Week 6	570.08 ± 363.49	0.052
		Week 8	612.90 ± 460.17	0.229
Week 6	570.08 ± 363.49	Week 8	612.90 ± 460.17	0.210
Placebo		Week 2	986.00 ± 673.72	0.944
Baseline	999.08 ± 561.98			
		Week 4	762.50 ± 458.22	0.483
		Week 6	613.58 ± 425.06	0.055
		Week 8	721.50 ± 640.17	0.488
Week 2	986.00 ± 673.72	Week 4	762.50 ± 458.22	0.057
		Week 6	613.58 ± 425.06	0.055
		Week 8	721.50 ± 640.17	0.056
Week 4	762.50 ± 458.22	Week 6	613.58 ± 425.06	0.115
		Week 8	721.50 ± 640.17	0.984
Week 6	613.58 ± 425.06	Week 8	721.50 ± 640.17	0.302

Note p-value determined by repeated measured ANOVA

4.10 Side Effects Evaluation

Side effects were evaluated every visit by history taking and physical examination. There was no one that got side effect, including erythema, dryness, scaling, peeling, and itching.

4.11 Subjects' Satisfaction

Subjects' satisfaction was evaluated by a questionnaire about subjects' feeling for the change of the number of acnes every visit. On week 8, subjects assessed subjects' satisfaction with the *Andrographis paniculata* gel property and anti-inflammatory effects on both the *Andrographis paniculata* gel and the placebo sides. For subjects' feeling for the change of the number of acnes shown in table 4.39, subjects felt that the number of acnes on the *Andrographis paniculata* gel side compared with placebo were no significantly difference.

Subjects graded at the 8th week for subjects' satisfaction with the *Andrographis paniculata* gel property using Global Patient Satisfaction scales, as shown in table 4.40.

Very unsatisfied was valued as 1.

Unsatisfied was valued as 2.

Neutral was valued as 3.

Satisfied was valued as 4.

Very satisfied was valued as 5.

Table 4.39 The changes of the number of acnes evaluated by subjects at 2nd, 4th, 6th and 8th week (n = 12)

	***Andrographis paniculata* gel**			**Placebo**			**p-value**
	Decreased	**Same**	**Increased**	**Decreased**	**Same**	**Increased**	
Week 2	9 (75.0%)	3 (25.0%)	0	9 (75.0%)	3 (25.0%)	0	1.000
Week 4	9 (75.0%)	3 (25.0%)	0	9 (75.0%)	3 (25.0%)	0	1.000
Week 6	12 (100.0%)	0	0	9 (75.0%)	3 (25.0%)	0	0.082
Week 8	11 (91.7%)	1 (8.3%)	0	11 (91.7%)	1 (8.3%)	0	1.000

Note p-value determined between decreased acne of *Andrographis paniculate* gel and the placebo by Fisher's exact test

Table 4.40 Subjects' satisfaction with the *Andrographis paniculate* gel property (n = 12)

Property	Level of satisfaction					Mean+SD
	Very satisfied	Satisfied	Neutral	Unsatisfied	Very Unsatisfied	
Color	6 (50.0%)	5 (41.7%)	1 (8.3%)	0	0	4.42 ± 0.67
Scent	5 (41.7%)	4 (33.3%)	3 (25.0%)	0	0	4.17 ± 0.84
Texture	9 (75.0%)	2 (16.7%)	1 (8.3%)	0	0	4.67 ± 0.65
Viscosity	4 (33.3%)	5 (41.7%)	3 (25.0%)	0	0	4.17 ± 0.84
Absorption	5 (41.67%)	5 (41.67%)	2 (16.7%)	0	0	4.25 ± 0.75
Overall satisfaction	2 (16.7%)	8 (66.7%)	2 (16.7%)	0	0	4.00 ± 0.60

The data in table 4.40 showed that 83.4% of the subjects were impressed with this gel formula.

For subjects' satisfaction with the anti-inflammatory effect, subjects graded at the 8th week by using Global Patient Satisfaction scales, the same scale as the *Andrographis paniculate* gel property. The results were shown in table 4.41 that subjects were impressed with the anti-inflammatory effect of the *Andrographis paniculate* gel not significantly greater than placebo.

Table 4.41 Subjects' satisfaction with the anti-inflammatory effect compared between the *Andrographis paniculate* gel and placebo (n = 12)

	***Andrographis paniculata* gel**	**Placebo**	**Z**	**p-value**
Mean ± SD	4.14 ± 0.690	3.80 ± 0.447	-.971	0.332

Note p-value determined by Mann-Whitney U Test

4.12 Physicians' Evaluation and Photos Analysis

The *Andrographis paniculata* gel side got inflammatory acne less than the placebo side from history taking and physical examinations. The physicians noted that the overall skin appearance looked better over time.

4.12.1 Anti-Inflammatory Effect

Figure 4.1 Red areas imaging from VISIA® Skin Analysis on the *Andrographis paniculata* gel side (above) and the placebo side (below) on baseline, 2nd, 4th, 6th and 8th week from left to right

According to VISIA® Skin Analysis, red areas represented several conditions, such as acne, inflammation, rosacea or spider veins. Acne spots and inflammation were round in shape and varied in size. Rosacea is usually larger and diffuse compared to acne. Spider veins typically were short, thin and interconnected in a dense network.

The physicians noted that both the *Andrographis paniculata* gel side and placebo side decreased the redness of the skin over time, corresponded with the imaging from VISIA® Skin Analysis. From figure 4.12, the redness on the right cheek (the row above, the *Andrographis paniculata* gel side) and left cheek (the row below, the placebo side) appeared lighter than baseline.

4.12.2 Anti-Melanogenic Effect

Figure 4.2 Brown spots imaging from VISIA® Skin Analysis on the *Andrographis paniculata* gel side (above) and the placebo side (below) on baseline, 2nd, 4th, 6th and 8th week from left to right

Brown Spots represents a surplus of melanin produced by melanocytes in the bottom layer of the epidermis. They can appear as freckles, areas of hyperpigmentation, lentigines, and melasma.

The physicians noted that both the *Andrographis paniculata* gel side and placebo side decreased the darkness of the skin, corresponded with the imaging from VISIA® Skin Analysis. From figure 4.13, the darkness on the left cheek (the row above, the *Andrographis paniculata* gel side) and right cheek (the row below, the placebo side) appeared lighter than baseline.

4.12.3 Antibacterial Effect

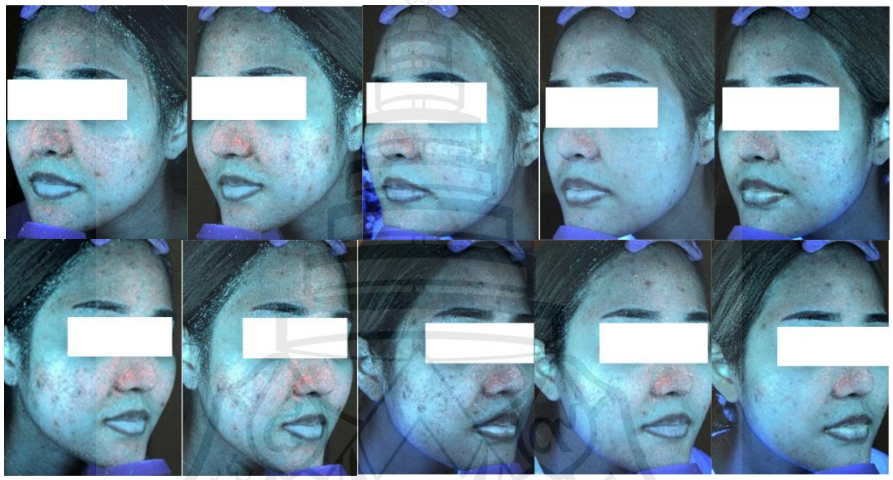


Figure 4.3 Porphyrin counts imaging from VISIA® Skin Analysis on the *Andrographis paniculata* gel side (above) and the placebo side (below) on baseline, 2nd, 4th, 6th and 8th week from left to right

According to VISIA® Skin Analysis, The VISIA Skin Analysis determines the skin's levels of the bacterial component known as porphyrins. Porphyrins represent natural bacteria that can live in pores and cause swelling. Porphyrins are an indication of clogged pores and the potential for acne to develop. These substances can also contribute to the appearance of other skin conditions. Porphyrins fluoresce in UV light and exhibit circular white spot characteristics.

The physicians noted that both the *Andrographis paniculata* gel side and placebo side decreased the porphyrin counts of the skin over time, corresponded with the imaging from VISIA® Skin Analysis. From figure 4.14, the porphyrin counts on

the left cheek (the row above, the *Andrographis paniculata* gel side) and right cheek (the row below, the placebo side) appeared less than baseline.

4.12.4 Post postinflammatory hyperpigmentation (PIH) and postinflammatory erythema (PIE) and acne counts

The *Andrographis paniculata* gel side seemed to get less acne counts compared with the placebo side, as shown in figure 4.15, 4.16 and 4.17. Though PIE and PIH faded away over time.

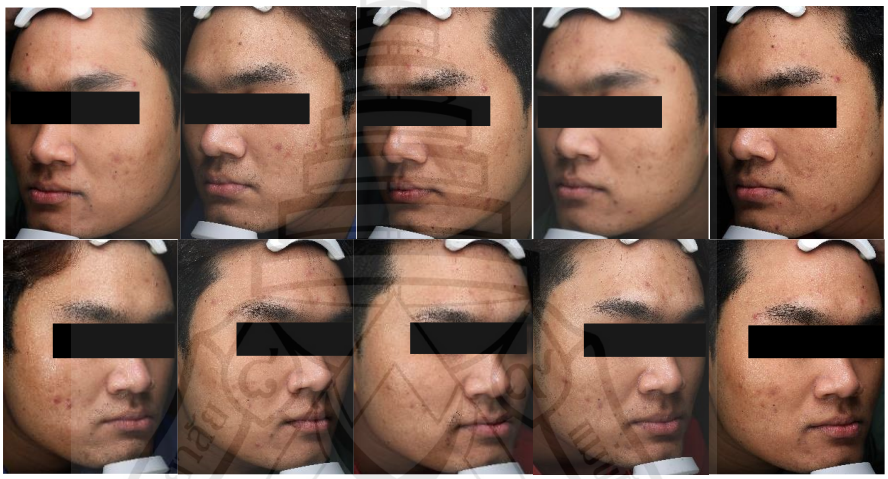


Figure 4.4 Subject's photos on the *Andrographis paniculata* gel side (above) and the placebo side (below) on the baseline, 2nd, 4th, 6th and 8th week from left to right

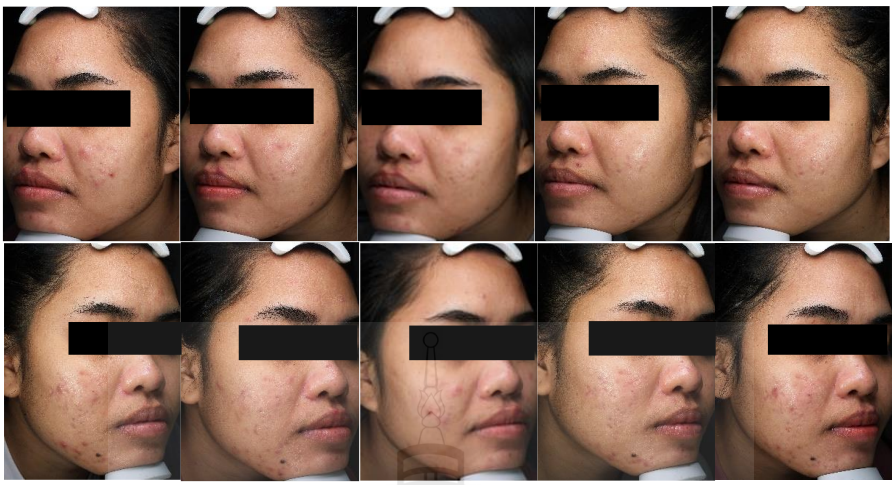


Figure 4.5 Subject's photos on the *Andrographis paniculata* gel side (above) and the placebo side (below) on the baseline, 2nd, 4th, 6th and 8th week from left to right

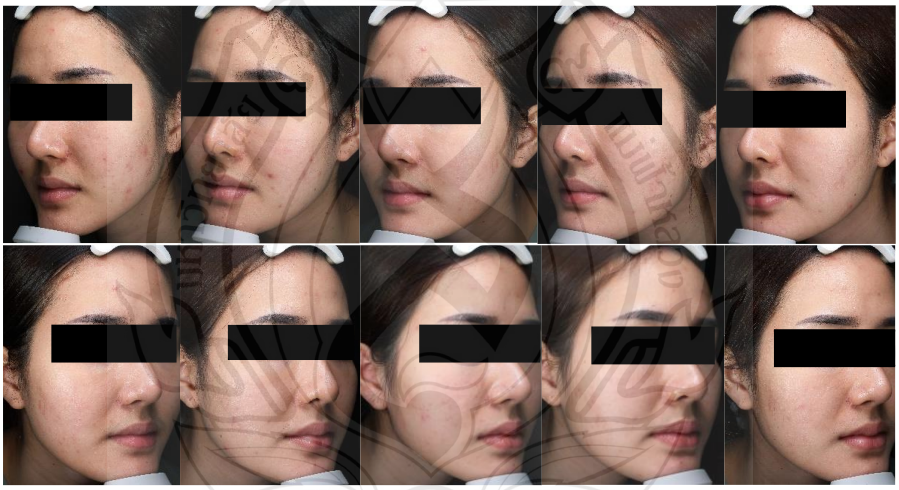


Figure 4.6 Subject's photos on the *Andrographis paniculata* gel side (above) and the placebo side (below) on the baseline, **2nd**, 4th, 6th and 8th week from left to right

CHAPTER 5

DISCUSSION AND CONCLUSION

The study aimed to evaluate the efficacy of 0.05% topical *Andrographis paniculata* gel in treating facial acne vulgaris compared to a placebo gel, the side effects, and satisfaction after using 0.05% topical *Andrographis paniculata* gel as an acne treatment by a randomized, double-blind, placebo-controlled, split-face study. Acne lesion counts, acne grading, sebum level, postinflammatory hyperpigmentation (PIH), postinflammatory erythema (PIE) and porphyrin counts were measured at the first visit, the 2nd, 4th, 6th, and 8th week. The side effects were reported at the 2nd, 4th, 6th, and 8th week. And the overall subject's satisfaction was reported at week 8th.

5.1 Discussion

5.1.1 General Characteristics

There were 12 subjects who completed the study. The mean age of the subjects was 28.83 ± 7.58 ranging from 20 to 42 years old, which was in adolescents and young adults. There were 3 males and 9 females participating in this study that was 25% and 75%, respectively. The number of male volunteers was more minor than females. Their occupation mostly was private company employees (50%). Others were nurses, government employees and student. There was no subject with medical conditions or food and drug allergies.

The mean age getting acne of the subjects was 15.92 ± 2.39, correlated with the peak incidence of acne vulgaris in adolescents. The minimum age that getting acne was 12, which was the onset of starting puberty. And the maximum age was 20 that was in young adults. Most of the subjects began to get acne when beginning puberty (15 – 19 years old). An average acne duration was 11.92 ± 8.32, of which most of them had acne for 10 to 14 years as acne was a chronic disease.

Oily skin is one of aggravating factors of 83.33 % of the subjects as an increase in sebum production was one of the pathogenesis of acne. Other factors increasing acne included coincidence with menstruation, cosmetics and skincare usage, a high-fat diet, chocolate and cake. These were well-known factors making breakouts (Coenye et al., 2007; Kurokawa et al., 2009; Williams et al., 2012). All subjects had not had acne treatment before.

5.1.2 Non-inflammatory Acne Counts

The total non-inflammatory acne counts, closed comedone counts and open comedone counts compared between the *Andrographis paniculata* gel and placebo in every visit were not statistically different ($p > 0.05$). Comparing acne counts at different times, the non-inflammatory acne counts, closed comedone counts on AP gel and placebo side significantly decreased since week 6 and week 4, respectively. This result show that non-inflammatory acne and closed comedone on both AP gel and placebo side would be faded over time without significant differences.

Developing comedone covers greasy plugs comprising mixture of keratin, sebum, bacteria (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009). Pilosebaceous units or pilosebaceous follicles contain sebaceous glands, sensory end organs, arrector pili muscle, hair, and the hair follicle into the dermis. Pilosebaceous units are the key important for the occurrence of acne and further induces hyperkeratinisation (Sinha et al., 2014).

Mean percentage changes were negative at week 4, 6, and 8 on both *Andrographis paniculata* gel and placebo without significance.

Means of total non-inflammatory acne counts, closed comedone counts on the placebo side at week 2 were increased from baseline, week 8 increased from week 4 and 6, on the AP side at week 6 increased from week 4 without significant differences.

Some subjects had to put on makeup for work. And some subjects liked to eat high-fat diet, chocolate or cake. The relationship between diet and acne is unclear as there is no good quality study. However, there are some study showing that worsening of acne vulgaris has been associated with high level of glycemic, use of milk, chocolates or salt (Ismail et al., 2012; Meixiong et al., 2022).

5.1.3 Inflammatory acne Counts

This study found that compared with placebo, topical 0.05% AP gel significantly reduced total inflammatory acne counts at week 4 of the study ($p = 0.042$), reduced papules as papule counts decreased significantly at week 4 ($p = 0.046$), and reduced pustules as pustule counts decreased significantly at week 6 ($p = 0.049$).

Comparing acne counts at different times, on AP gel side, total inflammatory acne and pustule counts significantly decreased since week 2, papule counts significantly decreased since week 4, confirming the anti-inflammatory effects of AP in terms of reducing inflammatory acne.

However, on placebo side, there was no significant decrease, confirming the anti-inflammatory effects of AP in terms of reducing inflammatory acne.

One of the pathogeneses of acne vulgaris is inflammation caused by the innate immune response to *P. acnes* colonies in the follicles (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009).

AG acts as anti-inflammation by decreasing NF-kappa B binding to DNA, thus inhibiting the expression of proinflammatory cytokines, such as IL-8 and TNF-α (Bhate & Williams, 2013; Das & Reynolds, 2014; Kurokawa et al., 2009). Some research has showed that Andrographolide can moderately inhibit IL-8 and TNF-α on human keratinocyte HaCaT cells induced by heat-inactivated *P. acnes*. (Zhang et al., 2020). AG inhibited the oxygen-derived free radicals such as superoxide (32%), hydroxyl radicals (80%), lipid peroxidation (80%), and nitric oxide (42.8%) (Jayakumar et al., 2013; Mussard et al., 2019). The study in Malaysia showed that AP herbal gel at concentration 5.0% (w/w) exhibited the best antimicrobial activities for *P. acnes* and *S. epidermidis* (Kub et al., 2020).

Currently, studies regarding efficacy of 0.05% topical *Andrographis paniculata* gel in treating facial acne vulgaris have not been conducted before.

Mean percentage changes of total inflammatory counts on both the *Andrographis paniculata* gel and placebo side were negative at week 4, 6, and 8. These changes were significant differences at week 4 ($p = 0.020$).

Means of total inflammatory counts and papule counts on the placebo side at week 2 and 4 were increased from baseline, on the AP side at week 6 increased from week 4 without significant differences. And means of pustule counts on placebo sides at week 6 were increased from week 4, on the AP side at week 4 increased from week 2 and week 8 increased from week 6 without significant differences.

Work from home policy was a confounding variable. Some subjects got breakouts after back to work in hot workplace environment then leading to oily skin during the working time (Vasilache et al., 2020). Another possible factor was coincidence with menstruation.

5.1.4 Severity of Acne

The grade of acne was not significantly different between the AP gel and at any time. Comparing acne counts at different times the severity of acne on the placebo side was significantly decreased sine week 6. While on the AP gel side, the severity of acne was significantly decreased sine week 4. This result show that severity of acne on both AP gel and placebo side would decrease over time without significant difference.

5.1.5 Sebum Level, Postinflammatory Hyperpigmentation (PIH), Postinflammatory Erythema (PIE) and Porphyrin Counts

The sebum level, PIH, PIE and porphyrin counts compared between the *Andrographis paniculata* gel and placebo in every visit were not statistically different ($p > 0.05$). Comparing acne counts at different times, the sebum level on AP gel side significantly decreased since week 6, and PIH on both AP gel and placebo side significantly decreased at week 8. This result show that PIH on both AP gel and placebo side would be faded over time without significant difference. On AP gel side, sebum level was decreased faster than placebo side, but not significant difference. However, PIE and porphyrin counts did not significantly decrease over time, it might be caused by low AP gel percentage.

Excess sebum production causes occlusion in the pilosebaceous unit and increases cell turnover in the follicular canal. Moreover, *P. acnes* also has been involved in sebum production and lipogenesis, as it can stimulate the sebaceous glands and sebum synthesis by way of the corticotropin-releasing hormone

(CRH)/CRH receptor pathway (Ebede et al., 2009). In USA, one research suggested that health- associated *P. acnes* strains exhibited less porphyrins acne-associated strains. In addition, porphyrins produce singlet oxygen from oxygen under ultraviolet exposure might exhibit squalene peroxide, a proinflammatory lipid.

The study in Malaysia showed that AP herbal gel at concentration 2.5% (w/w) showed the optimum antimicrobial effect of *S. aureus* and *C. albicans*, while 5.0% (w/w) exhibited the best antimicrobial activities for *P. acnes* and *S. epidermidis*. (Kub et al., 2020) Moreover, one study in Thailand found that the gel formulation containing the herbal ball extract, which included *Andrographis paniculate* with the concentration of 5% w/w showed antioxidants and antimicrobial activity against *P. acnes* according to the DPPH assay and the agar well diffusion study (Jantarat et al., 2018). AG can effectively suppress melanin content and Tyrosinase (TYR) activity in B16F10 melanoma cells, Human Epidermis Melanocyte (HEM) cells and UVB-induced brown guinea pig skin (Zhu et al., 2015).

Means of sebum level on the AP side at week 8 were increased from week 6, on the placebo side at week 4 were increased from week 2. Means of PIH on the AP side at week 6 were increased from week 4, on the placebo side at week 2 were increased from baseline, week 6 increased from week 4. Means of PIE on the AP side at week 4 were increased from baseline, week 4 increased from week 2. Means of porphyrin counts on both the AP side and placebo side at week 8 were increased from week 6 without significant differences.

Some subjects got breakouts after they went back to work in hot workplace environment and had to wear the mask then leading to oily skin during the working time (Vasilache et al., 2020).

5.1.6 Side Effects

As in the previous studies, There was no any irritation effects of *Andrographis paniculata* gel (Chusinkul, 2015). And this study, There was no one that got any side effects, including erythema, dryness, scaling, peeling, and itching. While, several standard topical treatments of acne got their common side effects are local irritation, including erythema, dryness, scaling, peeling, itching and bacterial resistance.

5.1.7 Subjects' Satisfaction

Subjects felt that the number of acnes on the *Andrographis paniculata* gel compared with placebo did not significantly different.

For the gel property, 83.4% of the subjects were impressed with this gel formula. Means of each of gel properties were more than 4, which implied that this was favorite formula.

Subjects were also satisfied with the *Andrographis paniculata* gel's anti-inflammatory effect. However, the *Andrographis paniculata* gel did not get the satisfaction score more than placebo significantly.

5.1.8 Physicians' Evaluation and Photos Analysis

In this study, the physicians also found that the *Andrographis paniculata* gel decreased the red areas of the skin, corresponded with the imaging from VISIA® Skin Analysis. However, the *Andrographis paniculata* gel did not decrease the brown spots of the skin, corresponded with the imaging from VISIA® Skin Analysis.

5.2 Conclusion

Topical 0.05% *Andrographis paniculata* gel can reduce inflammatory acne counts, especially papules and pustules, effectively at the 4th week and 6th week after the treatment in mild to moderate facial acne, confirming the anti-inflammatory effects. Moreover, it seemed to help sebum level decrease faster than placebo, and finally resulted in less oily skin, confirming the antioxidant effects, which inhibited proinflammatory lipid. However, AP gel did not seem to reduce PIE, PIH or porphyrin counts, it was probably due to low AP gel percentage. There was no any adverse effects, including erythema, dryness, scaling, peeling, itching and bacterial resistance. They were commonly found in several standard topical treatments of acne. Subjects were impressed with the *Andrographis paniculata* gel's anti-inflammatory effect and its formula. Hence, topical 0.05% *Andrographis paniculata* gel can be prescribed as an adjunctive treatment of mild to moderate facial inflammatory acne, Moreover, topical 0.05% *Andrographis paniculata* gel can be used for protection against oily skin, which is one of the influencing factors of acne.

5.3 Suggestions

5.3.1 In the future study, other higher percent concentration and other formula of *Andrographis paniculata* should be included for more significant result.

5.3.2 Moreover, the study should compare between *Andrographis paniculata* and placebo in treatment of melasma, psoriasis or the other inflammatory diseases in the future.

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APPENDICES

APPENDIX A

CERTIFICATE OF APPROVAL

The Mae Fah Luang University Ethics Committee on Human Research
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หนังสือรับรองด้านจริยธรรมการวิจัย

COA: 033/2022 **รหัสโครงการวิจัย:** EC 21243-20

ชื่อโครงการวิจัย : ประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง

ชื่อผู้วิจัยหลัก: แพทย์หญิงธราทิพย์ ประสพดำเกิง

สำนักวิชา: เวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ

ผู้สนับสนุนทุนวิจัย: ทุนส่วนตัว

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(6) ผู้วิจัย และผู้วิจัยร่วม	

- แพทย์หญิงธราทิพย์ ประสพดำเกิง

ขอรับรองว่าโครงการดังกล่าวข้างต้นได้ผ่านการพิจารณารับรองจากคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง ว่าสอดคล้องกับแนวทางจริยธรรมสากล ได้แก่ ปฏิญญาเฮลซิงกิ (Declaration of Helsinki) รายงานเบลมองต์ (Belmont Report) แนวทางจริยธรรมสากลสำหรับการวิจัยในมนุษย์ของสภาองค์การสากลด้านวิทยาศาสตร์การแพทย์ (CIOMS) และแนวทางการปฏิบัติการวิจัยที่ดี (ICH-GCP)

วันที่รับรองด้านจริยธรรมของโครงร่างการวิจัย: 23 กุมภาพันธ์ 2565
วันสิ้นสุดการรับรอง: 22 กุมภาพันธ์ 2566
ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: 1 ปี

ลงนาม .....

(รองศาสตราจารย์ พลตรีหญิง แพทย์หญิง แสงแข ชำนาญวนกิจ)
ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง

APPENDIX B

INFORMATION SHEET

เอกสารชี้แจงข้อมูลแก่อาสาสมัครที่เข้าร่วมในโครงการวิจัย

(Research Subject Information sheet)

ชื่อโครงการวิจัย: ประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง

ผู้วิจัยหลัก: แพทย์หญิง ธราทิพย์ ประสพดำเกิง
สำนักวิชาเวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ มหาวิทยาลัยแม่ฟ้าหลวง

ชื่อผู้วิจัยร่วม (ทุกคน): ไม่มี

ผู้สนับสนุนทุนวิจัย: มหาวิทยาลัยแม่ฟ้าหลวง

ท่านได้รับการเชิญชวนให้เข้าร่วมในโครงการวิจัยนี้ เนื่องจากท่านเป็นโรคสิวอักเสบเล็กน้อยถึงปานกลาง ก่อนที่ท่านจะตกลงใจเข้าร่วมหรือไม่ โปรดอ่านข้อความในเอกสารนี้อย่างถี่ถ้วน เพื่อให้ทราบถึงเหตุผลและรายละเอียดของโครงการวิจัยนี้ว่า หากเข้าร่วม ท่านจะต้องทำอะไรบ้าง รวมทั้งข้อดีและข้อเสียที่อาจจะเกิดขึ้นในระหว่างการวิจัย

หากท่านมีข้อสงสัยใด ๆ กรุณาซักถามจากทีมงานผู้วิจัย ซึ่งจะเป็นผู้ตอบคำถามจนกว่าท่านจะเข้าใจอย่างชัดแจ้ง ท่านสามารถปรึกษาญาติพี่น้อง เพื่อน หรือแพทย์ที่ท่านรู้จัก ก่อนตัดสินใจว่าจะเข้าร่วมหรือไม่เข้าร่วมในโครงการวิจัยนี้

การเข้าร่วมในโครงการวิจัย จะต้องเป็น**<u>ความสมัครใจ</u>**ของท่าน ไม่มีการบังคับหรือชักจูง ถ้าท่านตัดสินใจเข้าร่วม กรุณาลงนามในเอกสารแสดงเจตนายินยอมเข้าร่วมโครงการวิจัยนี้

เหตุผลความเป็นมา และวัตถุประสงค์ของโครงการวิจัย

สิวเป็นโรคทางผิวหนังที่พบได้บ่อย เกิดจากการอักเสบของหน่วยรูขุมขนและต่อมไขมัน มักเป็นบริเวณ ใบหน้า ลำคอ และลำตัว โดยสิวพบมากในวัยรุ่นและวัยผู้ใหญ่ตอนต้น แต่อย่างไรก็ตามสิวอาจเป็น ๆ หาย ๆ ทุกช่วงอายุ นอกจากนี้สิวทำให้เกิดผลทางกายภาพ เช่น รอยดำ รอยแดง แผลเป็น

มีผลต่อการเข้าสังคม รวมทั้งจิตใจ เช่นความเครียด ความวิตกกังวล ยารักษาสิวในรูปแบบทาเช่น ยาในกลุ่มวิตามินเอ และยาทาในกลุ่มละลายเคราตินส่วนมากมักมีผลข้างเคียงเช่น อาการคัน แสบผิวแดง แห้งและลอก รวมทั้งยาฆ่าเชื้อมักทำให้เกิดแบคทีเรียดื้อยาได้บ่อย

ดังนั้นจึงมีความจำเป็นที่จะหาแนวทางการรักษาใหม่ๆที่จะแก้ไขปัญหาที่พบได้บ่อยเหล่านี้ จากการทบทวนวรรณกรรมพบว่าสมุนไพรไทยอย่างฟ้าทะลายโจรมีคุณสมบัติในการต้านเชื้อแบคทีเรีย สารต้านอนุมูลอิสระ สารต้านการอักเสบ และสารลดรอยดำ รวมทั้งมีงานศึกษาวิจัยที่พบว่าในรูปแบบการทาที่ผิวนั้นไม่ก่อให้
เกิดการแพ้ ระคายเคือง หรืออาการข้างเคียงที่ร้ายแรงอื่น ๆ

งานวิจัยนี้จะศึกษาประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลางซึ่งได้แก่ การลดจำนวนและความรุนแรงของสิว ลดรอยแดงและรอยดำจากสิว ลดความมัน ลดเชื้อแบคทีเรียที่ทำให้เกิดสิว อาการข้างเคียงและความพึงพอใจ เพื่อหาทางเลือกใหม่ในการรักษาสิวให้มีประสิทธิภาพมากขึ้นและผลข้างเคียงที่ลดลง

<u>วัตถุประสงค์หลัก</u>

เพื่อศึกษาประสิทธิภาพประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอก ในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง

<u>วัตถุประสงค์รอง</u>

1. เพื่อศึกษาผลข้างเคียงของเจลฟ้าทะลายโจร0.05%ชนิดใช้ภายนอก
2. เพื่อศึกษาความพึงพอใจของอาสาสมัครหลังจากการใช้เจลฟ้าทะลายโจร0.05%ชนิดใช้ภายนอกเป็นยารักษาสิว

ท่านได้รับเชิญให้เข้าร่วมในโครงการวิจัยนี้เพราะคุณสมบัติที่เหมาะสมดังต่อไปนี้

1. เพศชายหรือเพศหญิง สุขภาพแข็งแรง อายุ18 ถึง 45 ปี
2. ได้รับการวินิจฉัยโดยแพทย์ว่าเป็นโรคสิวโดยมีระดับความรุนแรงน้อยถึงปานกลาง
3. อาสาสมัครยินยอมเข้าร่วมงานวิจัยด้วยความสมัครใจและลงลายลักษณ์อักษรในใบยินยอมเข้าร่วมโครงการ
4. อาสาสมัครปฏิบัติตามแนวทางการรักษาที่ระบุไว้และสามารถมาตรวจติดตามการรักษาตามนัดได้
5. ไม่ใช่หญิงตั้งครรภ์ให้นมบุตรหรือวางแผนที่จะมีบุตร
6. ไม่มีประวัติได้รับการรักษาโรคสิวทั้งในรูปแบบกินและทา ภายในระยะเวลา 1 เดือนก่อนเข้าร่วมการวิจัยเช่น ยาในกลุ่มวิตามินเอ, ยาทาในกลุ่มละลายเคราติน, ยาปฏิชีวนะ, ยาคุมกำเนิด, การใช้สารเคมีลอกชั้นหนังกำพร้า เป็นต้น

7. ไม่มีประวัติได้รับการรักษาด้วยแสงเลเซอร์หรือการรักษาบนใบหน้าชนิดใด ๆ ภายใน 1 เดือน

ท่านไม่สามารถเข้าร่วมในโครงการวิจัย หากท่านมีคุณสมบัติดังต่อไปนี้

1. แพ้เจลฟ้าทะลายโจร 0.05% โดยการทดสอบด้วยแพทช์เทสท์

2. มีประวัติหรือกำลังมีโรคผิวหนังบริเวณใบหน้า เช่น โรคผื่นภูมิแพ้ผิวหนังอักเสบ โรคผื่นผิวหนังอักเสบบริเวณผิวมัน เป็นต้น

3. ผู้ที่มีโรคประจำตัว เช่น โรคเบาหวาน ความดันโลหิตสูง โรคหัวใจ โรคมะเร็ง โรคไต โรคหลอดเลือดสมองอุดตัน ผู้ที่มีภาวะภูมิคุ้มกันบกพร่อง เป็นต้น

4. มีประวัติฮอร์โมนเพศผิดปกติ เช่น โรคถุงน้ำรังไข่หลายใบ โรคคุชชิ่ง เป็นต้น

สถานที่ทำการวิจัย และจำนวนผู้เข้าร่วมในโครงการวิจัย

โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร

ผู้เข้าร่วมโครงการ 15 คน

วิธีดำเนินการวิจัย

การวิจัยนั้นเป็นการศึกษาแบบสุ่มและแบ่งครึ่งหน้าเพื่อทดสอบประสิทธิผลของเจลฟ้าทะลายโจร0.05% โดยอาสาสมัครและแพทย์ผู้วิจัยจะไม่ทราบว่าได้รับการรักษาด้วยวิธีใดบนใบหน้าแต่ละข้าง โดยมีวิธีดำเนินงานวิจัยดังนี้

1. อาสาสมัครจะได้รับการซักประวัติทั่วไป บันทึก อายุเพศ ประวัติ เกี่ยวกับสิวและประวัติอื่น ๆ ส่วนข้อมูลส่วนบุคคลของอาสาสมัครจะมีการบันทึกเป็นเลขรหัสแทนตัวอาสาสมัคร โดยจะไม่สามารถบ่งชี้ตัวบุคคลของอาสาสมัครได้ ส่วนชื่อและนามสกุลของอาสาสมัครนั้น ผู้วิจัยจะเก็บรวบรวมข้อมูลส่วนนั้นแยกไว้เป็นความลับโดยผู้วิจัยเป็นผู้ที่เข้าถึงข้อมูลเท่านั้น

2. วินิจฉัยโรคสิวโดยการตรวจร่างกายโดยแพทย์ ด้วยการนับจำนวนสิวแต่ละชนิด เพื่อประเมินระดับความรุนแรงของโรคสิว

3. แพทย์ผู้ทำวิจัยให้ข้อมูลและขั้นตอนในการปฏิบัติ ทำความเข้าใจแก่อาสาสมัครถึงประโยชน์ที่จะได้รับ และผลข้างเคียงโดยละเอียด

4. อาสาสมัครผู้เข้าร่วมการวิจัยกรอกประวัติส่วนตัวในแบบสอบถาม และลงลายลักษณ์อักษรยินยอม เข้าร่วมการวิจัย

5. อาสาสมัครต้องเดินทางมาพบผู้วิจัยในวันพุธ เวลา 10.00น. ถึง 14.00น. ให้อาสาสมัครทดสอบอาการแพ้ของสารที่นำมาวิจัยด้วยการทำการทดสอบทางผิวหนังด้วยแพทช์เทสท์โดยทาเจล

ฟ้าทะลายโจร 0.05% ที่บริเวณแขนในส่วนท้องแขนข้างซ้ายของอาสาสมัคร บนพื้นที่ขนาด 3x3 ตารางเซนติเมตร โดยปล่อยทิ้งไว้ให้แห้งแล้ว จึงปิดทับบริเวณที่ทาด้วยพลาสเตอร์ใสปิดแผลกันน้ำ เป็นระยะเวลา 2 วัน หรือ 48 ชั่วโมง โดยไม่ล้างออกเมื่อครบเวลาที่กำหนดจึงประเมินการแพ้โดยการสังเกตลักษณะผิวหนังด้วยตาเปล่าที่เวลา 30 นาที หลังจากแกะพลาสเตอร์ออก หากมีอาการแพ้จะแจ้งให้อาสาสมัครทราบทันที

6. อาสาสมัครต้องเดินทางมาพบผู้วิจัยในวันศุกร์ เวลา 10.00 น. ถึง 14.00 น. อาสาสมัครผู้เข้าร่วมการวิจัยล้างหน้าให้สะอาดโดยใช้สบู่ล้างหน้าที่จัดเตรียมไว้ให้ ซับหน้าเบา ๆ ให้แห้งด้วยกระดาษทิชชู่และนั่งรอในสถานที่ที่จัดไว้ให้โดยมีอุณหภูมิ 25 องศาเป็นเวลา 30 นาที ก่อนไปวัดผล

7. แพทย์สองคนที่ไม่มีส่วนร่วมในการศึกษาวิจัยนี้ทำการสุ่มเลือกฝั่งที่ทาเจลฟ้าทะลายโจร 0.05% และฝั่งที่ทาเจลยาหลอกที่ด้านข้างของใบหน้า โดยเลือกหนึ่งลำดับจาก 0 ถึง 999 ที่เว็บไซต์สร้างขึ้น

8. แพทย์สองคนที่ไม่เกี่ยวข้องกับการศึกษาวิจัยนี้ใช้ปากกาเคมีทำเครื่องหมาย รอยดำ รอยแดงจากสิวและรอยโรคสิวลงบนแผ่นพลาสติกใส หลังจากพัก 30 นาทีในอุณหภูมิห้อง อาสาสมัครจะได้รับการวัดระดับความมันด้วยเครื่องวัด และหลังจากนั้นอาสาสมัครจะได้รับการวัดพอร์ไฟรินด้วยเครื่องวัด โดยแบ่งออกเป็นสองกลุ่มของใบหน้า ด้านซ้ายและด้านขวาของอาสาสมัคร ครั้งที่ 1 ก่อนเริ่มการวิจัย (การตรวจวัดพื้นฐาน) ครั้งที่ 2, 3, 4 และ 5 ในสัปดาห์ที่ 2, 4, 6 และ 8 หลังจากเริ่มการวิจัยแล้วตามลำดับ

9. ถ่ายภาพอาสาสมัครด้วยเครื่องถ่ายภาพ โดยถ่ายภาพทั้งหมด 3 ภาพ แบ่งเป็น ใบหน้าตรง 1 ภาพ ใบหน้าด้านซ้ายเอียง 37 องศา 1 ภาพ, ใบหน้าด้านขวาเอียง 37 องศา 1 ภาพ โดยจะถ่ายภาพก่อนเริ่มงานวิจัยและทุกครั้งที่มาติดตามการรักษา ภาพถ่ายจะถูกเก็บเป็นความลับ และจะนำแถบสีดำมาคาดบริเวณตา เพื่อไม่ให้สามารถระบุตัวตนได้ และใช้เพื่อการศึกษาวิจัย เท่านั้น ครั้งที่ 1 ก่อนเริ่มการวิจัย (การตรวจวัดพื้นฐาน) ครั้งที่ 2, 3, 4 และ 5 ในสัปดาห์ที่ 2, 4, 6 และ 8 หลังจากเริ่มการวิจัยแล้วตามลำดับ

10. ในงานวิจัยนี้อาสาสมัครทุกท่านจะได้รับยา 2 ขวด คือ ขวดหนึ่ง คือ เจลฟ้าทะลายโจร 0.05% และ อีกขวดหนึ่งเป็นเจลยาหลอกซึ่งยาหลอก คือ ยาที่มีลักษณะ รูป รส กลิ่นเหมือนกับยาที่ทำการทดลอง แต่ไม่มีผลใด ๆ ในการรักษาโรค ยาแต่ละขวดจะมีฉลากระบุไว้ว่าให้ทาที่ใบหน้าข้างใด ซึ่งผู้สุ่มจะทำการสุ่มเพื่อกำหนดว่าใบหน้าข้างใดจะได้รับยาใดและจะเปิดเผยผลการสุ่มหลังจากสิ้นสุดการวิเคราะห์ผล

11. แพทย์ผู้ทำวิจัยจ่ายยาที่ได้รับมาให้แก่อาสาสมัครผู้เข้าร่วมวิจัยพร้อมทั้งแนะนำวิธีการใช้ยา ผลข้างเคียงที่อาจเกิดขึ้น และข้อควรปฏิบัติต่าง ๆ ดังนี้ วิธีใช้ยาคือ ทาเจลขนาดเส้นผ่านศูนย์กลาง 5 มิลลิเมตรตามความยาวจากปลายนิ้วชี้ถึงข้อแรกของนิ้วชี้ ซึ่งเท่ากับ 0.5 กรัม แล้วใช้นิ้ว

ลูบยาทาให้ทั่วทั้งใบหน้าข้างที่ฉลากกำหนดไว้ห้ามทา สลับข้างกัน แล้วล้างยาที่เหลือบนนิ้วออกให้หมดด้วยสบู่ และทายาอีกข้างด้วยวิธีเดียวกัน ปล่อยทิ้งไว้จนยาแห้ง โดยห้ามล้างหน้า หรือล้างยาออก ทายาเช้า และก่อนนอนทุกวัน เป็นเวลาติดต่อกัน 8 สัปดาห์ โดยจะมีการตรวจติดตามในงานวิจัย ในสัปดาห์ที่ 2, 4, 6 และ 8

12. แนะนำให้อาสาสมัครหยุดใช้เครื่องสำอาง พร้อมทั้งแนะนำให้อาสาสมัครหลีกเลี่ยงการโดนแสงแดดและสามารถทาครีมกันแดดที่ท่านมีอยู่ได้ตลอดการวิจัย เก็บยาไว้ในที่แห้งและหลีกเลี่ยงแสงแดดโดยตรง ไม่ใช้ครีมหรือผลิตภัณฑ์อื่น ๆ เช่น ครีมลอก หรือผลัดผิว และครีมละลายสิว หากเกิดอาการระคายเคืองจากการใช้ยา ให้หยุดทายานั้นๆทันที และติดต่อเข้าพบแพทย์ผู้ทำวิจัย

13. อาสาสมัครผู้เข้าร่วมการวิจัยจะต้องช่วยประเมินความพึงพอใจต่อการรักษา โดยใช้แบบสอบถามเพื่อประเมินความพึงพอใจ

14. อาสาสมัครผู้เข้าร่วมการวิจัยจะได้รับการสอบถามเกี่ยวกับอาการข้างเคียงที่เกิดขึ้น กรณีที่อาสาสมัครผู้เข้าร่วมการวิจัยมีอาการไม่พึงประสงค์หรือสิวมีความรุนแรงมากขึ้น สามารถมาพบผู้วิจัยได้ก่อนการนัดติดตามและมีสิทธิออกจากโครงการวิจัยได้ก่อนกำหนด ได้เสมอเมื่อใดก็ได้ทันทีที่ต้องการ หากเกิดผลข้างเคียง ต่อการรักษาที่รุนแรง หรือสิวมีความรุนแรงมากขึ้น จะได้รับการดูแลรักษาผลข้างเคียงและสิวที่มีความรุนแรงมากขึ้นที่เกี่ยวข้องกับงานวิจัยโดยตรงจนหายเป็นปกติอีกด้วย

ความรับผิดชอบของผู้เข้าร่วมในโครงการวิจัย

ข้อปฏิบัติสำหรับผู้เข้าร่วมในโครงการวิจัย ได้แก่

1. การให้ความร่วมมือในการทายาทั้ง 2 ชนิดที่รอยโรคสิวในแต่ละด้านของใบหน้า เช้าและก่อนนอน

2. การงดใช้ยาทาภายนอกชนิดอื่นๆ ยารับประทานกลุ่มยาปฏิชีวนะ ยาคุมกำเนิด การรักษาด้วยแสงเลเซอร์หรือการรักษาบนใบหน้าชนิดใดๆ ภายในระยะเวลาที่ทำการศึกษาวิจัย

3. สังเกตอาการผลข้างเคียงจากการใช้ยา เช่น อาการแสบ แดง คัน หากมีอาการเกิดขึ้นให้แจ้งผู้วิจัยโดยเร็ว

4. ท่านจะต้องเข้ามารับการรักษาและตรวจติดตาม ตามที่แพทย์นัดทุกครั้ง

5. หลังให้การรักษาแล้ว แพทย์จะให้ใบประเมินความพึงพอใจการรักษากับท่าน ท่านกรุณากรอกใบประเมินตามความเป็น จริงเพื่อแพทย์ผู้ทำการวิจัยจะได้นำไปใช้ในการวิเคราะห์ข้อมูลต่อไป

ความไม่สุขสบาย หรือความเสี่ยงที่อาจเกิดขึ้น และการดูแลรักษา

ผู้วิจัยจะดำเนินการทุกอย่างเพื่อที่จะทำให้อาสาสมัครมีความปลอดภัยมากที่สุด และมีความเสี่ยงน้อยที่สุด โดยความเสี่ยงทั้งหมดที่อาจเกิดขึ้นกับอาสาสมัคร ได้แก่ การแพ้และระคายเคือง เช่น อาการคัน แสบ แดงและลอก ความไม่สบายใจ และการเปิดเผยความลับ โดยระหว่างการเข้าร่วมโครงการหากมีข้อสงสัย อาสาสมัครสามารถติดต่อสอบถามและแจ้งผู้วิจัยได้ตลอดเวลา หากมีอาการข้างเคียงจากการทายาเช่น ผิวหนังแสบ คัน แดง มีรอยดำ ตุ่มน้ำ การเกิดความเสียหายจากการวิจัย เช่น กรณีผลการทดสอบปรากฏว่า Placebo และสารที่ใช้ทดลอง ให้ผลแตกต่างกันอย่างมีนัยสำคัญ อาการไม่ดีขึ้น หรือ มีสิวที่มีความรุนแรงระดับปานกลางถึงรุนแรง และอาสาสมัครจะได้รับการรักษาโดยแพทย์ผู้เชี่ยวชาญด้วยวิธีการรักษาตามมาตรฐานตำแหน่งใบหน้าด้านที่มีอาการข้างเคียง เกิดความเสียหาย อาการไม่ดีขึ้น หรือ มีสิวที่มีความรุนแรงระดับปานกลางถึงรุนแรง จนกว่าอาการจะดีขึ้น โดยไม่เสียค่าใช้จ่าย ผู้วิจัยจะจ่ายค่าชดเชยและค่ารักษาพยาบาลแก่อาสาสมัครตามความเสียหายที่เกิดขึ้น ด้วยจำนวนเงินขั้นต่ำ 300 บาทต่อคน

ประโยชน์ที่คาดว่าจะได้รับจากโครงการวิจัย

1. เป็นวิธีใหม่ทางการแพทย์ซึ่งใช้เป็นทางเลือกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง โดยที่ยังไม่สามารถรับรองได้ว่า จำนวนหรือความรุนแรงของโรคสิวจะลดลงอย่างแน่นอน
2. การสร้างองค์ความรู้ใหม่ ๆ ในเรื่องประสิทธิภาพและผลข้างเคียงของสมุนไพรอย่างฟ้าทะลายโจรที่ปลูกได้ในประเทศไทย ทำให้สมุนไพรที่ปลูกได้ในประเทศไทยมีมูลค่าเพิ่มมากขึ้น
3. เพื่อใช้เป็นข้อมูลพื้นฐานในการทำวิจัยในเรื่องประโยชน์จากการใช้สารสกัดจากฟ้าทะลายโจรความเข้มข้นร้อยละ 0.05 ในการรักษาโรคผิวหนัง สำหรับผู้วิจัยคนอื่นๆต่อไปในอนาคต

ทางเลือกอื่น หากไม่เข้าร่วมในโครงการวิจัย

ท่านไม่จำเป็นต้องเข้าร่วมในโครงการวิจัยนี้ เนื่องจากมีแนวทางการรักษาอื่น หรือโรงพยาบาลอื่น สำหรับรักษาโรคของท่านได้ ดังนั้น ท่านควรปรึกษากับแพทย์ของท่าน ก่อนตัดสินใจเข้าร่วมในโครงการวิจัย

ค่าใช้จ่ายที่ผู้เข้าร่วมในโครงการวิจัยจะต้องรับผิดชอบ

ท่านไม่ต้องรับผิดชอบค่าใช้จ่ายใด ๆ ในการวิจัยนี้ เช่น ค่ายา เนื่องจากได้รับสนับสนุนจากผู้วิจัยหรือผู้สนับสนุนทุนวิจัย

ค่าตอบแทนที่จะได้รับ เมื่อเข้าร่วมในโครงการวิจัย

ค่าตอบแทนที่จะได้รับเมื่อเข้าร่วมในโครงการวิจัย เป็นค่าตอบแทนสำหรับค่าเดินทางมาเพื่อประเมิน การรักษาในการเข้าร่วมการวิจัยแต่ละครั้งเป็นจำนวนเงิน ครั้งละ 200 บาท รวมทั้งหมด 5 ครั้ง ครั้งที่ 1 ก่อนเริ่มการวิจัย (การตรวจวัดพื้นฐาน) ครั้งที่ 2, 3, 4 และ 5 ในสัปดาห์ที่ 2, 4, 6 และ 8 หลังจากเริ่มการวิจัยแล้วตามลำดับ โดยจะไม่รวมถึงวันที่มาทำการทดสอบอาการแพ้

การรักษาความลับของข้อมูล

การเก็บข้อมูลจะมีการป้องกันการเปิดเผยความลับและไม่เก็บข้อมูลที่ระบุตัวบุคคล ปกปิดความลับของอาสาสมัคร รักษาความลับของข้อมูลการวิจัยโดยการถ่ายภาพสิวเฉพาะบริเวณใบหน้าและปิดตาอาสาสมัครด้วยการคาดสีดำทึบที่บริเวณตาทั้งสองข้างของอาสาสมัครเพื่อปกป้องความเป็นส่วนตัวของอาสาสมัคร จะไม่ถ่ายภาพในบริเวณที่ไม่เกี่ยวข้องกับการรักษาและมีการรักษาความลับในการเก็บไฟล์ภาพโดยระบุชื่อภาพเป็นรหัสของอาสาสมัคร ไม่มีการระบุตัวตนของอาสาสมัครเช่น ชื่อหรือนามสกุล โดยผู้ที่สามารถเข้าถึงภาพถ่ายจะเป็นเพียงผู้วิจัยเท่านั้น และจะไม่ถูกนำไปใช้ในงานวิจัยอื่น ๆ โดยเมื่อเสร็จสิ้นโครงการวิจัยและเผยแพร่งานวิจัยแล้วจะทำลายข้อมูลส่วนตัวทั้งหมดดังกล่าว

การถอนตัวหรือการสิ้นสุดการเข้าร่วมในโครงการวิจัย

หากท่านไม่สมัครใจจะเข้าร่วมการศึกษาแล้ว ท่านสามารถถอนตัวได้ตลอดเวลา การขอถอนตัวออกจากโครงการวิจัยจะไม่มีผลต่อการดูแลรักษาโรคหรือสิทธิต่าง ๆ ที่ท่านพึงได้รับ

ผู้วิจัยอาจถอนท่านออกจากการเข้าร่วมการวิจัย เพื่อเหตุผลด้านความปลอดภัยของท่าน เช่น มีอาการข้างเคียงที่อาจเกิดอันตรายต่อท่าน หรือท่านไม่สามารถปฏิบัติตามคำแนะนำของผู้วิจัย เป็นต้น

การติดต่อ หากมีคำถามเกี่ยวกับการวิจัย

หากมีคำถามที่เกี่ยวข้องกับการวิจัยหรือเกิดอันตรายใด ๆ ท่านสามารถติดต่อผู้วิจัยได้ตลอด 24 ชั่วโมง โดยติดต่อแพทย์หญิง ธราทิพย์ ประสพดำเกิง เบอร์โทรศัพท์ : 0926895335

หากท่านได้รับการปฏิบัติที่ไม่เป็นธรรมในโครงการวิจัยนี้ ท่านสามารถติดต่อได้ที่ห้องปฏิบัติการคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง อาคารบริการวิชาการ (AS) ชั้น 4 มหาวิทยาลัยแม่ฟ้าหลวง โทรศัพท์ 053-917-170 ถึง 71 โทรสาร 053-917-170 หรืออีเมล rec.human@mfu.ac.th

หากมีข้อมูลใหม่ที่เกี่ยวข้องกับโครงการวิจัย (ในกรณีที่เป็นการวิจัยเกี่ยวข้องกับการใช้ยา)

หากมีข้อมูลใหม่ที่เกี่ยวข้องกับโครงการวิจัย ผู้วิจัยจะต้องแจ้งให้ท่านทราบ เพื่อการตัดสินใจว่า จะยังคงเข้าร่วมหรือถอนตัวจากโครงการวิจัยนี้

APPENDIX C

INFORMED CONSENT

หนังสือแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัย สำหรับอาสาสมัคร

(Informed Consent)

ชื่อโครงการวิจัย ประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง

ข้าพเจ้า นาย/นาง/นางสาว.....

ที่อยู่.....

ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย ฉบับวันที่ .....

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย และสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้อง จะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

..... ลงนามผู้เข้าร่วมในโครงการวิจัย

(.....) ชื่อ-สกุล ผู้เข้าร่วมในโครงการวิจัย (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้นประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

..... ลงนามผู้วิจัย

(แพทย์หญิง ธราทิพย์ ประสพดำเกิง) ชื่อ-สกุล ผู้วิจัย (ตัวบรรจง)

..... ลงนามพยาน

(.....) ชื่อ-สกุล พยาน (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

APPENDIX D

PATIENT RECORD FORM

แบบบันทึกและประเมินโดยอาสาสมัคร

เรื่อง การศึกษาประสิทธิผลของเจลฟ้าทะลายโจร 0.05% ชนิดใช้ภายนอกในการรักษาสิวอักเสบเล็กน้อยถึงปานกลาง

เลขที่บันทึกข้อมูล.....

ข้อมูลทั่วไปของผู้ป่วย(**Patient demographic information**)

วัน เดือน ปี ที่เก็บข้อมูล.....

1. อายุ.....ปี เบอร์โทรศัพท์.....

2. เพศ ( ) ชาย ( ) หญิง

3. อาชีพ

( ) ข้าราชการ ( ) พนักงานบริษัท ( ) แม่บ้าน ( ) นักศึกษา

( ) กิจการส่วนตัว ( ) อาชีพอิสระ ( ) อื่นๆ โปรดระบุ.....

4. โรคประจำตัวอื่น ๆ.....

5. ยา วิตามิน อาหารเสริมที่รับประทานประจำ.....

6. ประวัติแพ้ยา.....

สำหรับเพศหญิง

วันแรกของการมีประจำเดือนครั้งสุดท้ายวันที่.....

ประวัติการคุมกำเนิด ( ) ไม่ได้คุมกำเนิด

( ) คุมกำเนิด โดยวิธี โปรดระบุ.....

ท่านกำลังตั้งครรภ์ใช่หรือไม่ ( ) ใช่ ( ) ไม่ใช่

ท่านกำลังให้นมบุตรใช่หรือไม่ ( ) ใช่ ( ) ไม่ใช่

ท่านกำลังวางแผนมีบุตรใช่หรือไม่ ( ) ใช่ ( ) ไม่ใช่

ประวัติเกี่ยวกับโรคสิว

ท่านเป็นสิวที่ใบหน้ามานาน.....ปี.....เดือน

เริ่มเป็นสิวเมื่ออายุ .....ปี

<u>ปัจจัยกระตุ้นที่ทำให้เกิดสิว</u>

( ) ผิวหน้ามัน ( ) การมีประจำเดือนผิดปกติ ( ) การใช้เครื่องสำอาง

( ) อาหารที่ทานมีไขมันสูง ( ) อาหารบางชนิด โปรดระบุ.....

( ) อื่น ๆ โปรดระบุ.....

ประวัติการรักษาสิว

1. ในช่วง 1 เดือนที่ผ่านได้มาท่านใช้ยาทาหรือยากินรักษาสิวหรือไม่

( ) ไม่ใช่ ( ) ใช่ (โปรดระบุ).....

2. ในช่วง 1 เดือนที่ผ่านมาท่านได้ รักษาสิวด้วยเลเซอร์หรือแสงรักษาสิวชนิดอื่นหรือไม่

( ) ไม่ใช่ ( ) ใช่ (โปรดระบุ).....

3. ในช่วง 1 เดือนที่ผ่านมาท่านได้ รักษาสิวด้วยวิธีอื่นๆหรือไม่

( ) ไม่ใช่ ( ) ใช่ (โปรดระบุ).....

APPENDIX E

DOCTOR RECORD FORM

แบบบันทึกและประเมินโดยแพทย์

เลขที่บันทึกข้อมูล……..

Week 0 Date……..

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										
Postinflammatory hyperpigmentation										
Postinflammatory erythema										
Sebum level										

Acne grading……

2nd Week Date……..

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										
Postinflammatory hyperpigmentation										
Postinflammatory erythema										
Sebum level										

Acne grading……

Side effects on the right side……..……..……...……..

Side effects on the left side …….....……

4th Week Date……..

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										
Postinflammatory hyperpigmentation										
Postinflammatory erythema										
Sebum level										

Acne grading……

Side effects on the right side …….....……..

Side effects on the left side …….....……

6th Week Date……..

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										
Postinflammatory hyperpigmentation										
Postinflammatory erythema										
Sebum level										

Acne grading……

Side effects on the right side ……..……..

Side effects on the left side …….....……

8th Week Date……..

	Right Side					Left side				
	Forehead	**Cheek**	**Nose**	**Chin**	**Others**	**Forehead**	**Cheek**	**Nose**	**Chin**	**Others**
Closed comedones										
Open comedones										
Total noninflammatory lesions										
Papules										
Pustules										
Nodules										
Cysts										
Total inflammatory lesions										
Postinflammatory hyperpigmentation										
Postinflammatory erythema										
Sebum level										

Acne grading……

Side effects on the right side ……..……..

Side effects on the left side …….....……

APPENDIX F

PATIENT'S SATISFACTION QUESTIONNAIRES

แบบประเมินความพึงพอใจ

เลขที่บันทึกข้อมูล.....

คำชี้แจง : ขอความกรุณาอาสาสมัครทุกท่านให้คะแนนความพึงพอใจของการรักษา คณะผู้วิจัยมีความสนใจการประเมินของท่านเกี่ยวกับผลการรักษา กรุณาใส่เครื่องหมายถูกต้องที่ใกล้เคียงความเป็นจริงมากที่สุด

	ใบหน้าข้างซ้าย			**ใบหน้าข้างขวา**		
จำนวนสิว	ลดลง	เท่าเดิม	มากขึ้น	ลดลง	เท่าเดิม	มากขึ้น
สัปดาห์ที่ 2						
สัปดาห์ที่ 4						
สัปดาห์ที่ 6						
สัปดาห์ที่ 8						

หลังจบการวิจัย โดยรวมแล้ว ท่านมีความพึงพอใจต่อผลการรักษาสิวระดับใด

ความพึงพอใจในด้าน	**ระดับความพึงพอใจ**				
	มากที่สุด	มาก	ปานกลาง	น้อย	น้อยที่สุด
สี					
กลิ่น					
เนื้อผลิตภัณฑ์					
ความหนืด					
การดูดซึม					
ผลในการลดสิวอักเสบ					
ใบหน้าข้างซ้าย					
ใบหน้าข้างขวา					
ความพึงพอใจโดยรวม					

Global Patient Satisfaction scales

Scale	Satisfaction level
1	Very unsatisfied
2	Unsatisfied
3	Neutral
4	Satisfied
5	Very satisfied

APPENDIX G

PACKAGING AND LABELING

Figure G1 The *Andrographis paniculata* gel and placebo were containing in a white plastic squeeze tube in size of 15 ml

Figure G2 The labels on the white plastic squeeze tubes informed the applying side – left or right side