THE EFFECTIVENESS OF 2% ANDROGRAPHIS PANICULATA EXTRACT CREAM FOR PERIORBITAL WRINKLE REDUCTION AND PERIORBITAL HYPERPIGMENTATION REDUCTION

NANN PHOO PHOO MON

MASTER OF SCIENCE

IN

DERMATOLOGY

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE

MAE FAH LUANG UNIVERSITY

2024

THE EFFECTIVENESS OF 2% ANDROGRAPHIS PANICULATA EXTRACT CREAM FOR PERIORBITAL WRINKLE REDUCTION AND PERIORBITAL HYPERPIGMENTATION REDUCTION

NANN PHOO PHOO MON

THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
DERMATOLOGY

SCHOOL OF ANTI-AGING AND REGENERATIVE MEDICINE
MAE FAH LUANG UNIVERSITY
2024

THESIS APPROVAL

MAE FAH LUANG UNIVERSITY

FOR

MASTER OF SCIENCE IN DERMATOLOGY

Thesis Title: The Effectiveness of 2% Andrographis Paniculata Extract Cream for Periorbital Wrinkle Reduction and Periorbital Hyperpigmentation Reduction

Author: Nann Phoo Phoo Mon

Examination Committee:

Professor Thamthiwat Nararatwanchai, Ph. D.	Chairperson
Sirintip Chaichalotornkul, Ph. D.	Member
Assistant Professor Tawee Saiwichai, Ph. D.	Member
Associate Professor Wongdyan Pandii, Dr. P. H.	Member

Advisors:

S. Chaichalotornkul ..... Advisor

(Sirintip Chaichalotornkul, Ph. D.)

T. Saiwichai .....Co-Advisor

(Assistant Professor Tawee Saiwichai, Ph. D.)

Dean:

Karnt W. .....

(Karnt Wongsuphasawat, Ph. D.)

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my deepest appreciation to my main advisor, Ajarn Sirintip, for granting me the opportunity to pursue this research and for her unwavering support and guidance throughout this journey. Her insights and encouragement were pivotal to the successful completion of this thesis. I would also like to express my sincere gratitude to my co-supervisor, Dr. Tawee Saiwichai, for his expertise and continuous guidance during this process. His valuable contributions have greatly supported my academic progress.

In addition, I am truly thankful to Asst. Prof. Lt. JG. Dr. Thep Chalermchai and Assoc. Prof. Dr. Wongdyan Pandii for their thoughtful advice and assistance, particularly in statistical analysis. Their input has been very helpful in shaping this research, and I deeply appreciate their support.

I would also like to acknowledge my friends, whose advice, support, and encouragement have been a constant source of strength throughout this journey. A special thanks to the volunteers who participated in this research. Their commitment and dedication to following the research protocols made this project possible, and I am sincerely grateful for their contributions.

Lastly, I am deeply grateful to my parents for their endless love and unwavering support throughout this process. Their emotional and mental encouragement has been my greatest source of strength, and without their belief in me, this accomplishment would not have been possible.

Nann Phoo Phoo Mon

Thesis Title	The Effectiveness of 2% Andrographis Paniculata Extract Cream for Periorbital Wrinkle Reduction and Periorbital Hyperpigmentation Reduction
Author	Nann Phoo Phoo Mon
Degree	Master of Science (Dermatology)
Advisor	Sirintip Chaichalotornkul, Ph. D.
Co-Advisor	Assistant Professor Tawee Saiwichai, Ph. D.

ABSTRACT

Background: The appearance of youth and vitality is highly valued in modern society, influencing social interactions and self-esteem. Facial wrinkles, particularly in the periorbital region, are one of the most noticeable signs of aging, driven by cumulative changes in skin structure and physiology. The aging process, accelerated by oxidative stress and sun exposure, leads to the degradation of skin components such as collagen and elastin. Herbal extracts, rich in antioxidants, have emerged as promising solutions for combating the visible effects of aging. Andrographis paniculata, a medicinal herb with potent antioxidant and anti-inflammatory properties, has been traditionally used for a variety of health conditions. Its active compound, andrographolide, has shown potential in preventing oxidative damage and reducing the effects of skin aging.

Objective: To study the effect of 2% *Andrographis paniculata* extract on improving wrinkles and periorbital hyperpigmentation.

Study Design: This study employed a double-blinded, randomized, controlled, and split-face clinical trial to assess the efficacy of Andrographis paniculata (AP) extract on periorbital wrinkles over a 12-week period. The study enrolled 19 healthy male and female volunteers aged between 25 and 50 years, all of whom expressed a desire for treatment of their periorbital wrinkles. Prior to participation, all volunteers provided informed consent, and they were instructed to follow the study's protocols consistently for the duration of the trial. Skin evaluations were performed at four

intervals: baseline, 4 weeks, 8 weeks, and 12 weeks, using the VISIA, Cutometer, and Mexameter devices to monitor changes in wrinkles, skin elasticity, and pigmentation.

Result: The results demonstrated a significant reduction in periorbital wrinkles and periorbital hyperpigmentation in the treatment group compared to the control group. The treatment group exhibited a notable decrease in wrinkle, as well as melanin score, indicating the extract's effectiveness in combating oxidative stress and improving hyperpigmentation. No adverse effects were reported during the study.

Conclusion: The study concluded that *Andrographis paniculata* extract is an effective natural solution for reducing periorbital wrinkles, improving skin elasticity, and minimizing hyperpigmentation. Its potent antioxidant properties played a key role in protecting the skin from oxidative stress and promoting the production of collagen and elastin, which are essential for maintaining skin elasticity.

Keywords: *Andrographis paniculata* Extract, Skin Elasticity, Hyperpigmentation, Antioxidant, Oxidative Stress, Collagen, Elastin

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

A youthful appearance is linked with high self-esteem and improved social interactions. It has been proved that an attractive appearance positively impacts on social functioning (Gupta & Gilchrest, 2005). It has been demonstrated that human face is not only the reflection of inner self, nature, and personality, but also the signs and symptoms of aging and overall health. Common signs of aging face include wrinkles, receding hairline, frown lines, nasolabial folds, excessive eyelid skin and undereye hollowness. In today's world, due to the influence of beauty standards, people want to look younger and more attractive than they already are (Panda & Chowdhary, 2021).

As periorbital area is where it gets the most visual attention, it's one of the most important points for evaluation of aging (Bae et al., 2017; Samson et al., 2010). The periorbital area, the first place we start noticing where skin aging, are composed of the crow's feet and infraorbital region, which is not easily be treated. This is the part of the face most people are most worried about. (Kaczvinsky et al., 2009) The human skin is also the essential organ of emotional expression, in addition to being serve as a shield against environmental factors and threats (Kligman & Koblenzer, 1997).

Visible folds or creases in the skin are known as wrinkles. Wrinkles smaller than 1 mm width and depth are being identified as fine wrinkles. Wrinkles, bigger 1 mm or more in width and depth, are what we called coarse wrinkles. (Manríquez et al., 2014) and these are the hallmark of facial aging. (Prystowsky & Franck, 2000) It has been stated that wrinkles are caused by changes in dermal cells. It has also been proved that older skin has decreased collagen, elastin, and hyaluronic acid levels in the skin. (Baumann, 2005) Photo-ageing is accelerated in areas frequently exposed to sunlight (ultraviolet radiation), which in turn leads to dryness, dyspigmentation, wrinkling, and elasticity in the skin (Binic et al., 2013).

Skin aging, a part of normal “aging mosaic,” is also happening in other organs with time. Although we cannot see or detect the aging process and signs of internal organs visually, the skin is the organ that shows the noticeable changes, mostly on the skin areas, which frequently expose to the sunlight (Zouboulis et al., 2019). Clinical signs of the aging skin include thinning of the skin layers, a decrease in levels of elastin and collagen fibers, skin lipids, which promote the formation of wrinkles (Farage et al., 2009). Long term exposure to UV lights accelerates the aging, by a process known as the photoaging (Binic et al., 2013).

Intrinsic aging and extrinsic aging are two kinds of aging. Intrinsic aging is responsible for human genetics changes, which is completely inevitable (Baumann, 2007). The intrinsic aging, of what we call, ‘the biologic clock’, damages the skin in the same way as it does to the various internal organs, with the slow and irreversible tissue breakdown (El-Domyati et al., 2002). So many factors such as the ionizing radiation and environmental stresses (physical and psychological), drinking and lifestyle habits, nutrition, environmental pollution, and UV radiation are associated with extrinsic aging (Mccullough. 2006).

Premature skin aging can be caused by long term exposure to UV radiation (Fisher et al., 1997). By frequent exposure to UVB irradiation, an enzyme called, matrix metalloproteinase-1(MMP-1) is produced by skin cells called fibroblasts and keratinocytes (Pandel et al., 2013). UVB induced TNF-α release from endothelial cells and keratinocytes promote formation elastases and collagenases, which breaks down skin cells resulting in skin aging (Bashir et al., 2009). The gravity impact, muscles action, loss of superficial and deep facial fat volume, and bony skeleton support also the impacts on aging. These factors contribute to the facial skin loosening, changes in shape and characteristics (Ganceviciene et al., 2012). For the time being, we can’t prevent or reverse the aging process of the skin caused by intrinsic skin aging. But we can control or decrease the factors that cause extrinsic aging (Puizina-Ivić, 2008).

Wrinkles are inevitable and cannot be treated completely. But there are so many ways we can adjust the appearance of fine lines and wrinkles. We can use agents like topical antioxidants, procedures like lasers, chemical peels, photo rejuvenation, fat and volume restoration, skin augmentation, as well as systemic agents with antioxidants and hormones (Zouboulis et al., 2019).

The chemical peels which are done superficially can be safe if it is used in proper ways, but skin reactions like itchiness, redness, changes in skin sensitivity and pigmentation can still happen. Some type of chemical peels could even activate herpes viral infection, and scar formation (Akbar, 2011). In one study, deep peels are no longer trendy among Indian people nowadays because they can lead to milia, secondary skin infection, and scarring (Nikalji et al., 2012). Some of the complications of Botox injections and dermal fillers are asymmetry, infections, and some injection site complications such as ecchymosis, erythema, edema, pain, allergy and hypersensitivity reactions (Kassir et al., 2020). Complications of IPL therapy have been proved to have burning, blisters, crusts, infection, oedema, discomfort. (Fodor & Bota, 2020).

As topical treatment is the safest way in treating wrinkles, people have been paying more attention to cosmetic products, which are formulated with the natural products such as herbal extracts, with anti-inflammatory, antioxidant, UV-protective, and other antiaging effects (Naeimifar et al., 2020). Herbal extracts, which are enriched in antioxidants, have been used widely to reduce wrinkles and aging skin (Mukherjee, et al. 2011).

Andrographis paniculata is a herb which has been used in Asian countries for the treatment of common colds and respiratory tract infections (Burgos et al., 2020). *Andrographis paniculata* belongs to the family Acanthaceae (Chandrasekaran et al., 2010). It's called the "king of bitters", mainly used in China, India, and other Southeast Asian countries.

Andrographolide is the active ingredient and has so many physiological benefits, such as anti-inflammatory, antibacterial, antitumor, antimalarial, and hepatoprotective (You et al., 2015). The aerial parts of the plant are most used mostly. In some cases, the whole plant or roots are also used for some purposes (Akbar, 2011). Andrographolide can prevent free-radical formation by inhibition of specific ROS-producing enzymes. It can also enhance antioxidants by activating Nrf2 signaling (Mussard et al., 2019). It has been proved in previous studies that diterpenoids and flavonoids are the major chemical compounds which are responsible for the pharmacological effects of *Andrographis paniculata* (Hanh et al., 2020).

Reactive oxygen species (ROS) can lead to oxidative stress that can lead to damages lipids, proteins in the skin which contributes to skin aging (Gu et al., 2020). It

was observed that andrographolide has this significant antioxidative function by scavenging the stable free radicals (Krithika et al., 2013). Andrographolide inhibited production of reactive oxygen species by reducing NADPH oxidase (NOX) and increasing the action of erythroid 2-related factor 2 (Nrf2) expression (Liang et al., 2018). Andrographolide sodium bisulfate has also demonstrated a protective effect against UVB-induced skin aging in mice and may offer benefits for cosmetic applications. Therefore, it has been demonstrated that it could be beneficial for cosmetic purposes (Zhan et al., 2016). In terms of acnes, AP extract gel is also proved to be effective at 2.5 % against S. aureus and C. albicans, and 5% against P. acnes and S. epidermis (Katta, 2007). It also has been proved that APE activates the epidermal stem cells proliferation. It also appeared to improve skin hydration, elasticity, wrinkling and sagging in this clinical study (You et al., 2015).

Source *Andrographis paniculata* (2024)

Figure 1.1 *Andrographis paniculata*

1.2 Research Question

Does the extract of 2% *Andrographis paniculata* improve the wrinkles and hyperpigmentation around periorbital area?

1.3 Objectives

1.3.1 General Objective

To study the effectiveness of 2% *Andrographis paniculata* extract on improving wrinkles and periorbital hyperpigmentation.

1.3.2 Specific Objectives

1.3.2.1 Primary Objective

To compare the percentage of improvement of periorbital wrinkle reduction by 2% *Andrographis paniculata* extract eye cream with baseline to each visit and week-12.

To compare the percentage of improvement of periorbital hyperpigmentation by 2% *Andrographis paniculata* extract eye cream with baseline to each visit and week-12.

1.3.2.2 Secondary Objective

To study the effectiveness of 2% *Andrographis paniculata* extract on the improvement of skin elasticity.

To observe the side effects of 2% *Andrographis paniculata* extract.

To observe the participant's satisfaction of the volunteers after using 2% *Andrographis paniculata* extract.

1.4 Research Hypothesis

2% *Andrographis paniculata* extract eye cream has shown to have better efficacy and positive evaluation in the reduction of wrinkles around the periorbital area than the placebo eye cream.

2% *Andrographis paniculata* extract eye cream has shown to have better efficacy and positive evaluation in the reduction of hyperpigmentation around the periorbital area than the placebo eye cream.

1.5 Benefits

1.5.1 To apply 2% *Andrographis paniculata* as an alternative method of treatment for treating periorbital wrinkles and periorbital hyperpigmentation.

1.5.2 To apply 2% *Andrographis paniculata* as an alternative method of treatment for treating periorbital hyperpigmentation.

1.5.3 To get the better understanding of *Andrographis paniculata*.

1.5.4 To assist the future studies of *Andrographsc paniculata*.

1.6 Conceptual Framework

Generation of mitochondrial ROS is caused by chronological or intrinsic aging via the oxidative metabolism. Ultra-violet radiation (UVR) also increases ROS production which means the oxidative stress which can cause DNA damage, which leads to the inflammatory response and reduced production of antioxidants and induces matrix metalloproteinases (MMPs). MMPs cause the degradation of collagen and elastic fibers in the skin, which lead to the wrinkles. *Andrographis paniculata* acts as the antioxidant to reduce wrinkles by suppressing the reactive oxygen species formation.

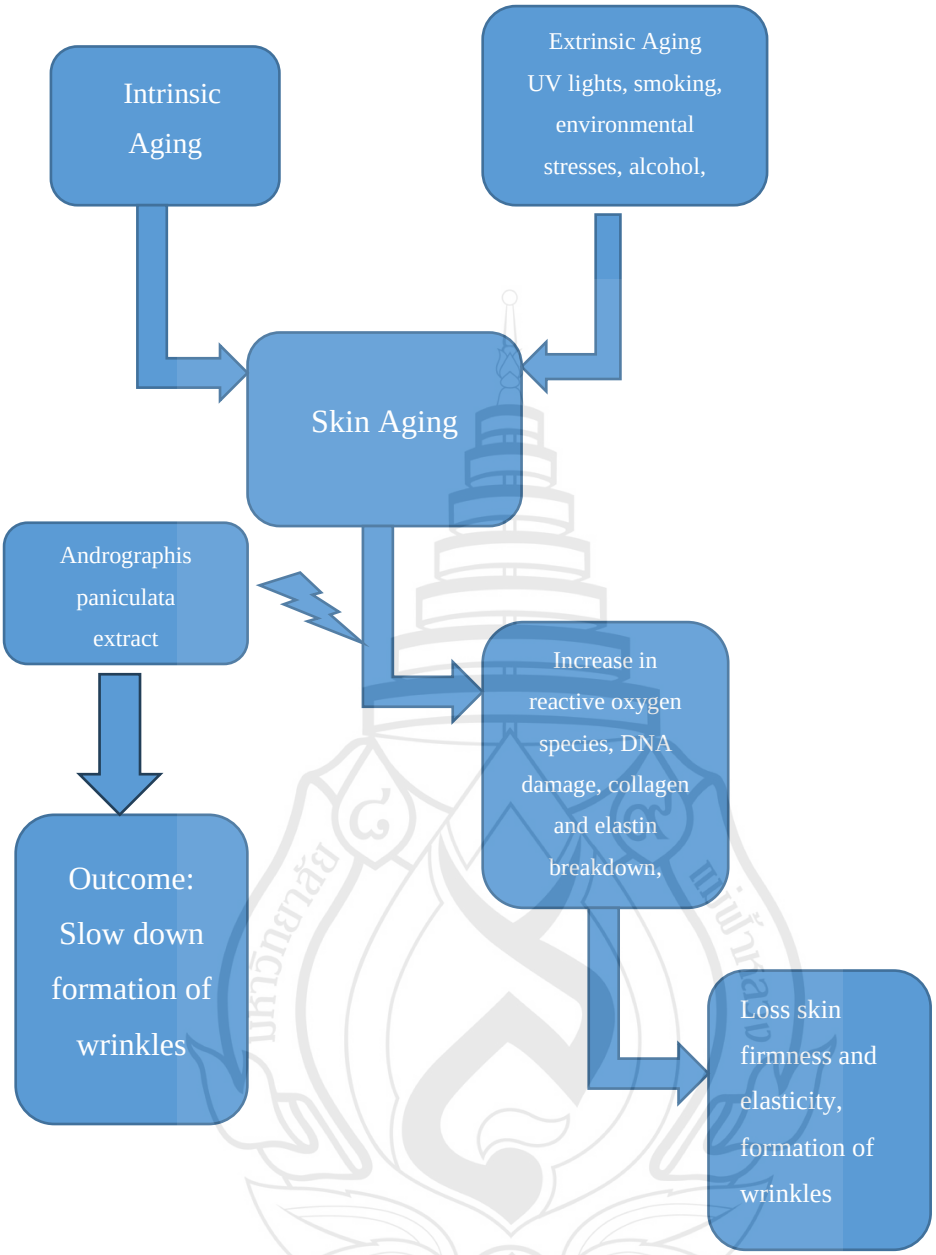


Figure 1.2 Conceptual Framework

1.7 The Scope of Research

This study will be focused on the effect of 2% *Andrographis paniculata* extract on improving periorbital wrinkles and periorbital hyperpigmentation. The researcher will choose healthy male and female between 25 to 50 years, who want to be treated periorbital wrinkles. They must apply the *Andrographis paniculata* extract on

periorbital area. The research will take 12 weeks and will evaluate the baseline before the treatment and every 4 weeks, at 4th, 8th and 12th weeks respectively by the VISIA® Complexion Analysis System for Wrinkle Score, Cutometer® and Mexameter®.

1.8 Operational Definitions

1.8.1 APE: *Andrographis paniculata* extract (APE 2% is proved to be effective in improving skin elasticity)

1.8.2 Antioxidants: a compound for preventing oxidation, which is a chemical process that generates free radicals

1.8.3 Wrinkles: a normal part of skin aging, which are most frequently found on sun-exposed skin, like face, neck, hands, and forearms.

1.8.4 MMPs: Matrix metalloproteinases are enzymes capable of breaking down various types of extracellular matrix proteins.

1.8.5 ROS: Reactive oxygen species which are generated during mitochondrial oxidative metabolism

1.8.6 Periorbital Hyperpigmentation: Periorbital hyperpigmentation refers to the presence of bilateral, uniform darkened macules and patches that predominantly affect the lower eyelids. In some cases, it may extend to the upper eyelids, eyebrows, malar regions, temples, and the lateral root of the nose.

1.8.7 Skin elasticity: The skin laxity is related to loss of dermal collagen fibers. Histological features of loose skin indicate atrophy of the dermis

1.8.8 Collagen: Collagen is the primary structural protein present in the skin and connective tissues.

1.8.9 Elastin: Elastin is an essential structural protein found in the connective tissue of elastic structures.

1.8.10 UV Radiation: Ultraviolet (UV) radiation is from the wavelength range of 100 to 400 nm, which is a higher frequency and shorter wavelength than visible light.

1.8.11 Nuclear factor erythroid 2-related 2 (Nrf2): a transcription factor which is important in the activating antioxidant enzymes

1.8.12 Effectiveness is a quantitative measure of the clinical trial to indicate

whether the intervention made in the trial show desired effect

1.8.13 Side effects: any unwanted effects of using the AP cream and placebo

1.8.14 Satisfaction: The degree to which the volunteers' expectation of a product or service are met or exceeded

1.8.15 Efficacy: the ability of a treatment, intervention, or product to produce a desired effect under ideal or controlled conditions

CHAPTER 2

LITERATURE REVIEW

The researcher focused on investigating the efficacy of *Andrographis paniculata* extract on the improvement of periorbital wrinkles and periorbital hyperpigmentation. So, this chapter will include literature review on basic anatomy and function of the skin, skin aging, wrinkles, wrinkle treatments, and *Andrographis paniculata.*

2.1 Skin Structure and Function

Skin is the largest organ of the body and divided into three parts: the epidermis, dermis and hypodermis (Yousef et al., 2021). It has also been demonstrated to function as the barrier which protects from the external stressors. Moreover, the skin allows the sensory transmission, plays a role in maintaining homeostasis and are involved in the thermoregulation of the body (Kolarsick et al., 2011).

2.1.1 Epidermis

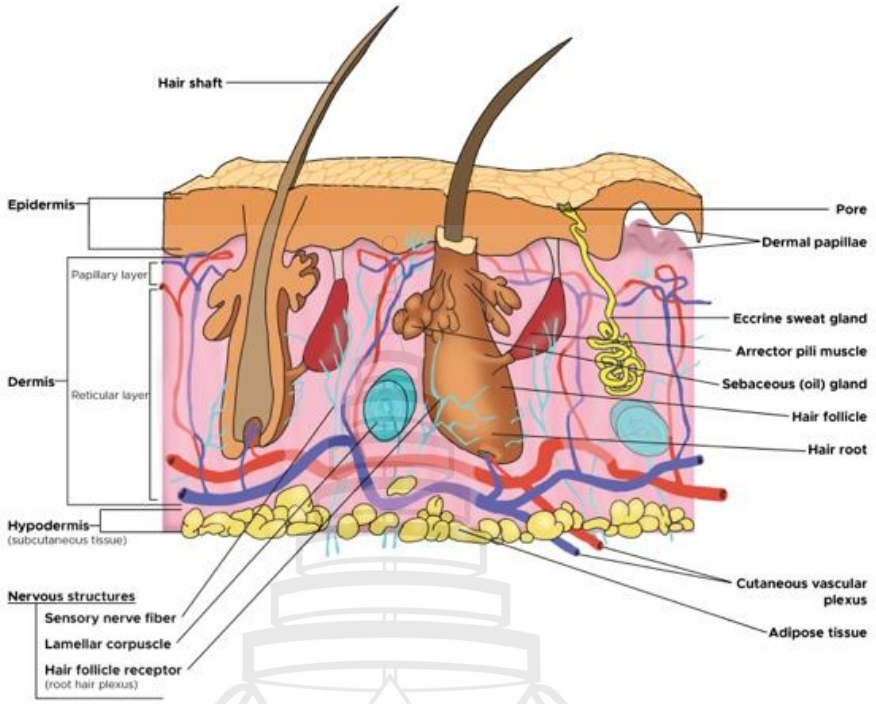
Skin cells such as keratinocytes, melanocytes, dendritic cells, and sensory cells make up this layer of the skin.

2.1.2 Dermis

Dermis is composed of two layers of connective tissues The hair follicles, sweat glands, and collagen fibers are located in this layer (Yousef et al., 2021).

2.1.3 Hypodermis

It’s a layer where subcutaneous adipose tissue, neurons, vessels and skin appendages are located (Yousef et al., 2021).



Source Yousef et al. (2021)

Figure 2.1 Layers of the skin, hair follicles and sweat glands

2.2 Wrinkles

Wrinkles are folds of the skin, commonly found on the face and the hand areas, with increasing in numbers as we age (Audonneau et al., 1999). Wrinkles appear along the pattern of polyhedral mesh structures on the skin's surface, which can be appeared as prominent skin furrows (Akazaki et al., 2002). When we age, we suffer from the deterioration of connective tissue in the skin, which causes elastin and collagen fibers degradation leaving fine lines and wrinkles (Al-Atif, 2022). It has been shown in one study that frequently exposing to UVB radiation at sub erythemal doses decreases the skin elasticity which can lead to the wrinkle formation (Imokawa, 2009). We have not only photodamage by UV rays, but also intrinsic aging, hormonal status, and intercurrent diseases as the risk factors for wrinkles (Manríquez et al., 2014).

2.2.1 Predisposing Factors

Wrinkles can be caused by two processes of aging. These are intrinsic aging and intrinsic aging. (Rowe & Guyuron, 2010) Intrinsic aging is also known as the innate aging, and it has impacts on the skin the way it does to the internal organs. Not like the extrinsic aging, even the areas which are protected from the sun, are also affected in intrinsic aging. (Uitto, 1997) Fine wrinkles, atrophy of the dermis, subcutaneous adipose tissue reduction are characteristics of the innate aging even in the protected areas of the skin. The histological findings from these areas of tissue from the same person are frequently fragmented fibers, with reduction in number and diameter of the elastic fibers. (Uitto et al., 1989) In terms of the extrinsic aging, it has the interaction with environmental factors like UV rays, air pollution, climate factors, nutrition, and other factors. (Krutmann et al., 2021)

2.2.2 Etiology and Pathogenesis of Wrinkles

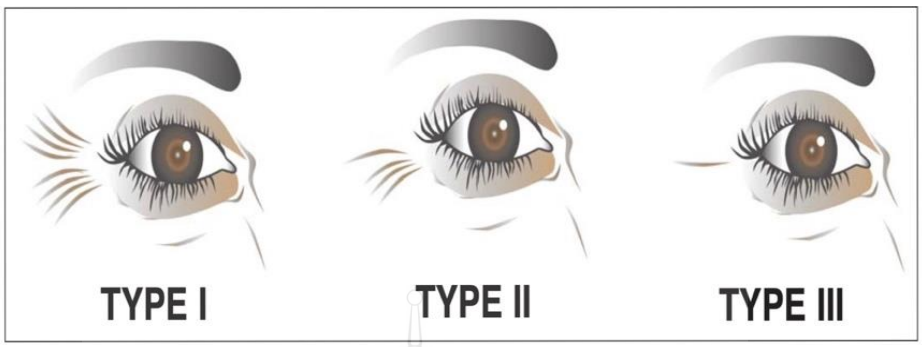
Wrinkles are formed by matrix metalloproteinases (MMP-1, MMP-9 and MMP-12), which are induced by external factors such as UV rays. (Fisher et al., 1996) Wrinkle formation is also associated with many factors including volume loss of the skin and its adipose tissues. Additionally, it has been noted that muscle contractions and their alteration through surgery or injections significantly influence the extent to which wrinkles appear. (Pessa et al., 2014)

2.2.3 Classification of Wrinkles

Type I: Wrinkles located laterally to the outer corner of the eye, extending from the brow down to the zygomatic arch.

Type II: Wrinkles lateral to the outer corner of the eye, extending from the eye's outer corner to the zygomatic arch, with no wrinkles present in the upper lateral area.

Type III: Wrinkles present only along the line of the outer corner of the eye.



Source Tamura and Odo (2011)

Figure 2.2 Primary classification of wrinkles at periorbital area

Table 2.1 Fitzpatrick's wrinkle classification (perioral and periorbital facial areas)

Class	Score	Wrinkling	Degree of Elastosis
I	1-3	Fine wrinkles	Mild (fine textural changes with subtly accentuated skin lines)
II	4-6	Fine to moderate depth wrinkles, moderate number of line	Moderate (distinct popular elastosis, individual papules with yellow translucency, dyschromia)
III	7-9	Fine to deep wrinkles, numerous line, with or without redundant skin	Severe (multipapular and confluent elastosis, thickened yellow and pallid cutis rhomboidalis)

2.2.4 Treatment of Wrinkles

It has been stated that these methods are used to treat the skin aging: cosmetic skin care, topical applications, systemic agents, preventing risk factors and invasive methods. (Ganceviciene et al., 2012)

2.2.4.1 Topical agents

Stratum corneum functions as the skin barrier to protect the skin from environmental stressors and prevent the water loss from the skin making it smooth and soft. Daily use of skin care with moisturizing agents prevent skin dryness but the effect is only temporary. (Tabata et al., 2000) Common topical agents that are used for the skin aging are retinoids, alpha-hydroxy acid, Niacinamide, Vitamin C, hyaluronic acid,

peptides and other antioxidants. (Imhof & Leuthard, 2021) But if we want to prevent the wrinkles, we have to stop the degradation of collagen, elastin and HA, the three primary constituents of the skin, which are proved to decline as we grow old. (Baumann, 2007)

2.2.4.2 Invasive procedures

Some of the minimally invasive procedures are mesotherapy, chemical peelings and micro needling. Chemical peels and micro needling procedures are supposed to create injury to the skin so that it can stimulates growth and exfoliation to regenerate the skin. (Lee et al., 2016)

Chemical peeling is the effective treatment that has been used for fine wrinkles and to remove some superficial lesions. (Moy et al., 1993) The classification and side effects depend on its depth of penetration to the skin layers. There may be some immediate and delayed complications after the procedures such as burning, itching sensation, blistering up to the emergency ones like anaphylactic reactions, inadvertent ocular mucosal splashes, and cardiac arrhythmia, depending on the ingredients that are used. (Samargandy & Raggio, 2022)

People also use mesotherapy as skin rejuvenation but that has not been proved in studies yet so far. (El-Domyati et al., 2012) (Amin et al., 2006) Micro needling, minimally invasive procedure, is one of the effective treatments for wrinkles, skin rejuvenation and acne. Dyspigmentation is something to be worried about using the procedure in darker skin phototypes (Fitzpatrick IV, V, VI), but it's a rare complication without the UV exposure on the treatment areas. (Alster & Graham, 2018)

After IPL treatment, skin biopsy shows that type 1 and type 3 collagens, the amount of fibroblasts are increased, elastin and collagen fibers were more neatly arranged. (Babilas et al., 2010) Its mechanism is causing thermal injury to the dermis and activates the fibroblast synthesis, which in turn produces the new collagen and extracellular matrix materials. It has been proved that wrinkles can be effectively reduced by IPL treatment but we should be aware of complications like mild pain and erythema. (Li et al., 2008) It has been stated that novel non ablative laser Nd:YAG system has statistically no significance in treating wrinkles and side effects were hyperpigmentation and scarring. (Menaker et al., 1999)

2.2.4.3 Systemic therapies

Some of the hormone level declines with age and it occurs free radicals concentration in the body. (Hertoghe, 2006) Hormone replacement therapy (HRT) has been proved to increase skin thickness, promote wound healing with regarding to wrinkle treatment. (Warren & Halpert, 2004) But we still have to keep in mind that risks factors of HRT such as increasing risk in breast cancer and cardiovascular diseases. (Brower, 2003)

2.2.4.4 Injection of botulinum toxins and fillers

Botox injections are more effective in treating dynamic wrinkles, which are caused by hyperactivity of the muscles, while the static wrinkles are better treated by dermal fillers. (Dastoor et al., 2007) Botox injection relieves skin wrinkles by inhibiting muscle contraction by blocking the release of acetylcholine from the neuromuscular junction. (Boulle et al. 2010) The complications of Botox such as ptosis, asymmetry and injection site complications should still be looked out for.(Kassir et al., 2020) Dermal fillers are one of the treatment options for fine lines and wrinkles. (Ballin et al., 2015) However, we still shojld be careful with the complications such as swelling or bruising, product sensitivity in patients with hypersensitivity to collagen, hyaluronic acid and poly-L-lactic acid, inappropriate placement and infection. (Cohen, 2008)

2.3 Periorbital Hyperpigmentation

Periorbital hyperpigmentation is one of the common cosmetic issues. The periorbital hyperpigmentation can be seen as bilateral, evenly distributed dark patches around the eyes. It can be found in both upper and lower eyelids. (Agrawal, 2018) Additionally, it’s more common in people of color compared to Caucasians. (Roberts, 2014).

2.3.1 Causes of Periorbital Hyperpigmentation

It’s still difficult to acknowledge what exactly causes the periorbital hyperpigmentation. (Ranu et al., 2011) The cause of periorbital hyperpigmentation can be so many factors such as post inflammatory hyperpigmentation (PIH), melanin accumulation, contact dermatitis, anemia, genetics, stress, edema of the periorbital

region, vascular structures being superficial. Moreover, loosening of the skin, deficiency in some nutrients, and even underlying diseases can cause periorbital hyperpigmentation. (Mendiratta et al., 2019)

2.3.2 Treatments of Periorbital Hyperpigmentation

There are so many treatment options for periorbital hyperpigmentation such as topical agents, chemical peels, lasers, PRP, autologous fat transplantation, fillers, surgery/blepharoplasty and carboxy therapy. (Sarkar et al., 2016)

2.3.3 Topical Agents

Among the treatment options for periorbital hyperpigmentation, topical agents have been stated that it is the most used type of treatment clinically. Topical depigmenting agents are mostly made from the naturally active ingredients like lactic acid, beta-carotene, azelaic acid, alpha-arbutin, and niacinamide etc. The aim of using topical agents is to control the melanin and tyrosinase biosynthesis pathway which is related to the complex pathophysiology of periorbital hyperpigmentation. (Sawant & Khan, 2020)

2.3.4 Chemical Peels, Lasers, Fillers, and Surgery

Chemical peels with the combination treatment of trichloroacetic TCA 3.75% and lactic acid 15% has been proved to show significant outcomes. (Vavouli et al., 2013) In carboxy therapy, carbon Dioxide is injected subcutaneously to induce increasing oxygen and nutrients to the skin, thereby improving the appearance of the injected skin. But it’s important to note that it has some contraindications like acute untreated cardiovascular diseases, connective tissue diseases, skin infections and uncontrolled diabetes. (Zenke,r 2012)

It has been proved that dermal melanocytosis can be treated by Q-switch Ruby laser. (Watanabe et al.,2006) For the vascular type of periorbital hyperpigmentation, due to hypervascularity, along with the thin, translucent lower eyelid skin, Long-pulsed 1,064-nm Nd:YAG-laser treatment has been proved to be effective and safe. But care must be taken to protect the eyes from laser radiation. (Ma et al., 2012)

Tear trough deformity is one of the causes of periorbital hyperpigmentation and this condition can be corrected by using with hyaluronic acid fillers. Special care should be taken to avoid overcorrection, too superficial injections, and choice of an inappropriate filler to avoid unwanted side effects like swelling, bruising, pain,

erythema, and asymmetry. (Sharad, 2012) Autologous fat transplantation is also one of the effective treatment options for the vascular type of periorbital hyperpigmentation, which occurs due to the thin layer of the lower eyelids which covers the orbicularis oculi muscle. (Roh et al., 2009) Nano grafting method is proved to be significance in treating the vascular type of dark circles without visible lumps fat, contour irregularities, or fat necrosis, except minimal erythema and post-operative edema. (Oh et al., 2014)

Platelet-rich plasma is an autologous preparation consisting of a concentrated amount of human platelets in a small volume of plasma. It acts as a stimulant for fibroblast proliferation and collagen release. It has been considered that PRP might have been effective in the treatment of periorbital dark circles by relating to color homogeneity. (Mehryan et al., 2014) Blepharoplasty may also be a useful treatment for removing dark circles which are associated with shadows that are caused by fat deposits. It's important to choose a skillful surgeon to avoid an overly aggressive removal of fat and skin which can worsen the dark circles. (Gendler, 2005)

2.4 *Andrographis paniculata*

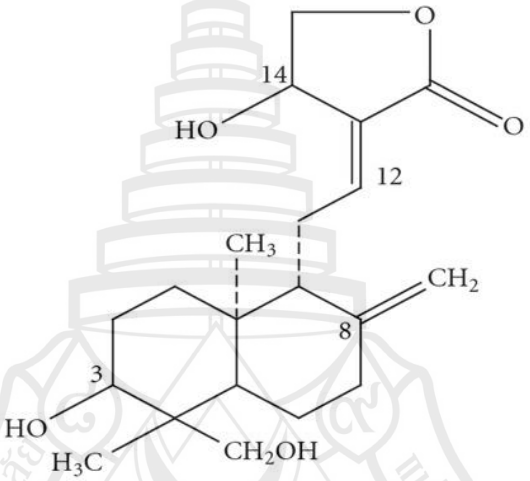
2.4.1 General Information

Andrographis paniculata, also known as the king of bitters, belong to the family *Acanthaceae*. It has been reported that for centuries, people in Asian countries have utilized it to treat gastrointestinal disorders, infections such as upper respiratory tract infections, herpes, and other infectious diseases. (Mishra et al., 2007) It also has been stated that *Andrographis paniculata* have anti-pyretic, anti-inflammatory and anti-malarial properties. The plant also has the protective activity to the liver disorders. (Jain et al., 2000) A*ndrographis paniculata* is commonly found in India, Pakistan and Srilanka, favorable for hot and shade places. The plant is wide usely in many in many Ayurvedic formulations. (Katta, 2007)

2.4.2 Chemical Constituents of *Andrographis panniculata*

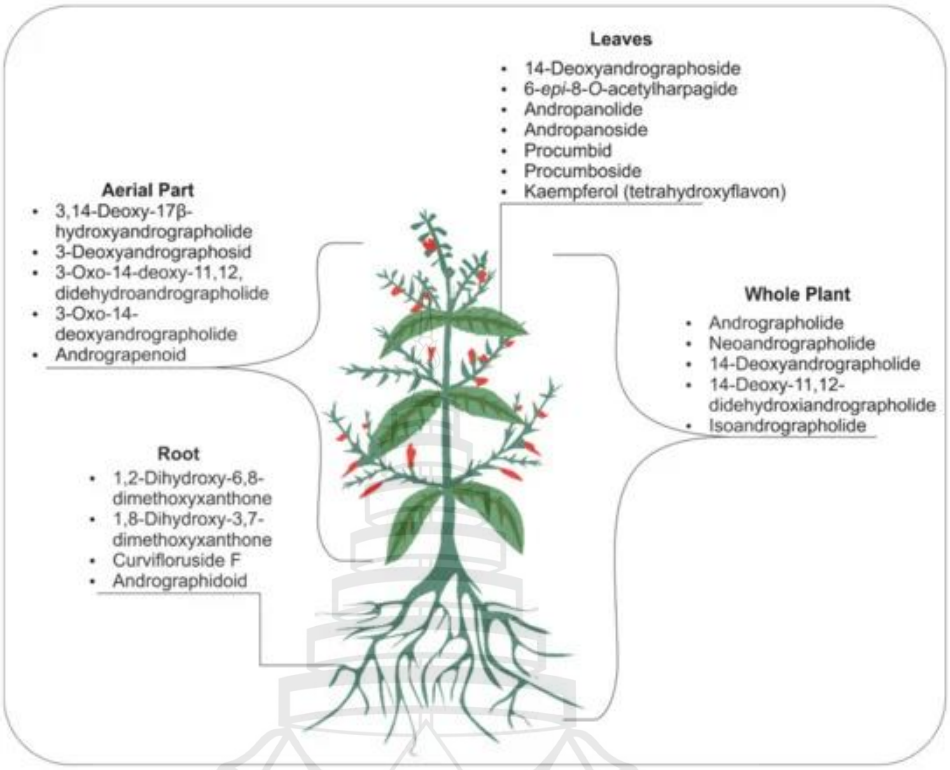
Andrographis paniculate is a well-known medicinal plant and has so many chemical constituents like flavonoids, andrographolide diterpenoids, and polyphenols.

(Hanh et al., 2020) The primary bioactive compound found in Andrographis paniculata is Andrographolide, an ent-labdane diterpenoid lactone. (Brahmachari, 2017). Its chemical structure is defined as (3-[2-[decahydro-6-hydroxy-5-(hydroxymethyl) -5,8-dimethyl-2-methylene-1-naphthalenyl] ethylidene] dihydro-4-hydroxy-2(3H)-furanone). (Varma et al., 2011) Andrographolide is mainly extracted from the leaves of the plant, and it has potent immunomodulatory and anti-angiogenic activities in tumorous tissues. (Varma et al., 2011)



Source Varma et al. (2011)

Figure 2.3 Structure of principle of phytochemical compound of andrographolide

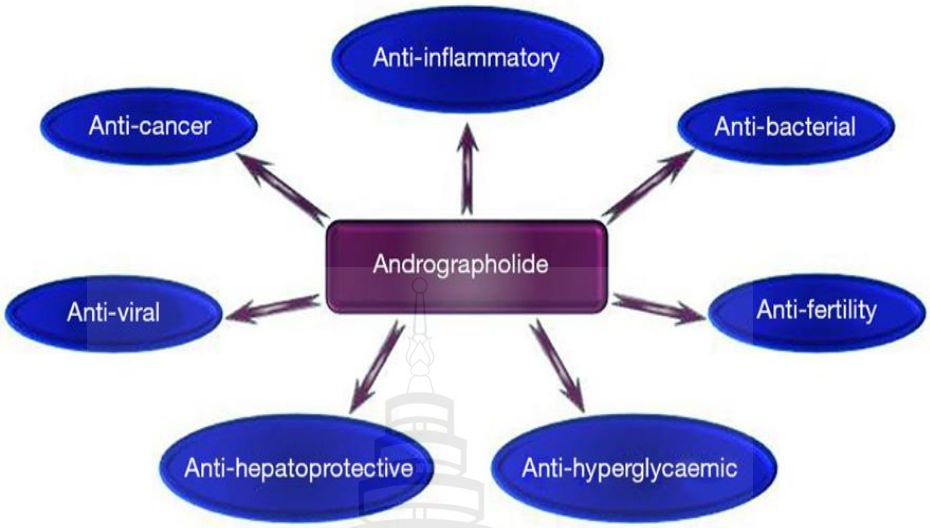


Source Adiguna et al. (2021)

Figure 2.4 The compounds in Andrographis paniculata. It is divided into 4 parts: whole plant, aerial plant, leaves, and roots. The whole plant includes all components of the plant. The aerial part includes stems, twigs, leaves, and flowers

2.4.3 Biological Properties of *Andrographis paniculata*

Biological properties of *Andrographis panniculata* is summarized in figure 2.6. (Vetvicka & Vannucci, 2021) It has been proved that APE has antiviral activity (Kim et al., 2018) The immunomodulatory exhibit by promoting cytotoxic T cells, natural killer (NK) cells, phagocytosis, and antibody-dependent cell-mediated cytotoxicity (ADCC). These. Properties, therefore, prevent virus replication and virus-induced pathogenesis. (Gupta et al., 2017) Some derivatives of *Andrographis panniculata* are potential anti-cancer treatment. (Jada et al., 2008) The aqueous extract of *Andrographis panniculata* tested in the rats with ethanol induced liver toxicity is found to have protective effect. (Uthirapathy, 2012)



Source Vetvicka and Vannucci (2021)

Figure 2.5 Major biological properties of Andrographolides

2.4.4 Role of Andrographis paniculata in Dermatology

Andrographolide is shown to have anti-inflammatory effect by suppressing inflammatory mediator pathways and reducing oxidative stress. (Guo et al., 2012) Reactive oxygen species from environmental aging risk factors such as UV rays, smoking and pollutions, as well as the intrinsic aging, is the major factor to skin inflammation. (Pillai et al., 2005) It has been stated that the matrix metalloproteinases (MMP-1, MMP-3)are induced by UV radiation, resulting in degradation of collagen and elastin levels, which leads to wrinkles and skin laxity. (Kahari & Saarialho-Kere, 1997) Andrographolide sodium bisulphate (ASB), which is a water soluble form of andrographolide, has been stated to suppress the induction of MMP enzymes. (Zhan et al., 2016)

2.4.5 Wrinkles and *Andrographis paniculata*

It has been stated that APE promotes proliferation of human epidermal stem cell (EpSCs). Increased expression of integrin β1 and production of VEGF in APE treated stem cells promote Type 1 collagen production in normal human fibroblasts (NHFs). It has been stated that there was a significance improvement in skin wrinkles and APE has anti-aging properties. (You et al., 2015)

2.4.6 Periorbital Hyperpigmentation and *Andrographis paniculate*

Andrographis paniculata leaf extract at a concentration of 25 μg/ml has been shown to be a safe and effective skin lightening agent by inhibiting melanin production through the suppression of TYR, MITF, and related proteins. It is considered a safe and reliable anti-melanogenic agent for treating hyperpigmentation. (Adam et al., 2022)

2.4.7 Safety and Toxicity

The systemic side effects of *Andrographis paniculata* are associated with gastrointestinal, skin, and subcutaneous disorders. But the serious side effect such as anaphylactic reaction is rare. It's contraindicated in pregnant women, lactating women and in those with history of allergy to the plant with Acanthae family. (Worakunphanich et al., 2021) In some studies, *Andrographis paniculate* is considered to be safe. (Thompson-Coon & Ernst 2004; Hu et al., 2017; Cáceres et al., 1999) Semi-solid forms of *Andrographis paniculate* has no reported side effects. (Oh et al., 2014)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

The researcher used a double-blinded, randomized, controlled and split face clinical study of AP extract on the periorbital winkles and periorbital hyperpigmentation for 12 weeks with those who want their peri orbital wrinkles and periorbital hyperpigmentation to be treated.

3.2 Study Population

3.2.1 Population

In this study, the researcher will enroll healthy male and female volunteers aged between 25years to 50 years, who would like to seek treatment for their wrinkles and periorbital hyperpigmentation.

3.2.2 Sample

All volunteers must have wrinkles age between who must not be treated by topical anti wrinkles treatment and periorbital hyperpigmentation for 6 weeks before the study. This study was conducted at Mae Fah Luang University, Thailand**.** All participants were required to fit the inclusion criteria and exclude from the exclusion criteria. Follow-up visits took place at Mae Fah Luang University Hospital, Bangkok, Thailand.

3.2.3 Sample Size Determination

The researcher used the study of the efficacy of Ziziphus jujuba extract for periorbital reduction by Shune (2021), was used to calculate the sample size determination.

The results of skin parameters for Ziziphus jujuba extract group showed a significant drop from 0.382±0.174 at baseline to 0.52±0.174 at 12th week. Those skin parameters were measured by using cutometer.

Different change from baseline of Ziziphus jujuba extract, μ= 0.382-0.52 = - 0.138

$\sigma_{before} = S_{1=} 0.174$ $\sigma_{after} = S_2 = 0.174$

Where

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

$$S_p^2 = \frac{0.174^2\,(14 - 1) + 0.174^2\,(14 - 1)]}{[14 + 14 - 2]}$$

$= 0.030276$

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

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

Set

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

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

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

$$= \frac{(1.96 + 1.28)^2 0.030276}{(-0.138)^2}$$

$= 16.69$

≈ 17

Where

n = sample size

$S = \sigma$ = Variance

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

The expected dropout rate is 20%. Thus, 20 volunteers (n=20) should be recruited.

3.2.4 Selection Criteria

3.2.4.1 Inclusion criteria

1. Healthy individuals
2. Male or female
3. Age between 25years to 50 years old
4. Fitzpatrick skin type from III to V
5. Female participants must not be pregnant, or breast feeding and must discontinue taking contraceptive pills at least 3 months prior to the participation
6. Participants must agree to not use any other skin care products during study
7. Subjects who agree to use *Andrographis paniculata* extract eye cream on their faces
8. Subjects must not take any facial skin procedure such as laser, peeling, mesotherapy, roller and derma abrasion.
9. All participants must be able to follow the research guidelines and attend the follow-up visits.

3.2.4.2 Exclusion Criteria

1. Subjects with a history of allergic reactions from *Andrographis paniculata.*
2. Subjects who are received by topical anti-wrinkle treatments for 6 weeks before the study
3. Subjects who have skin disorders such as dermatitis and skin infections
4. Pregnant or breastfeeding female subjects.
5. Subjects with drinking, smoking and drug abuse.
6. Subjects who have abnormal wound healing or scarring conditions like keloids
7. Subjects who work in direct sunlight or who has a preference for tanning
8. Participants who have received fillers with semi-permanent or non-permanent type at periorbital area and radiofrequency rejuvenation treatment in 12 months prior to the study

9. Participants who had received botulinum toxin injection at periorbital area and ablative or non-ablative laser 6 months prior to study

10. Participants currently using any topical or systemic treatments that could interfere with the study's assessment

3.2.4.3 Discontinuation Criteria

1. Participants who would like to discontinue the program due to any reasons

2. Participants who get serious side effects and adverse complications

3. Failure to attend the follow-up visits

4. Participants who cannot follow with the instructions and guidelines of the study

5. Participants get pregnant during the trial

6. Participants who suffered illness, diseases and medical conditions during the trial

3.3 Location of the Study

Mae Fah Luang University Hospital, Bangkok, Thailand.

3.4 Variables of the Study

3.4.1 Independent Variables

2% Andrographis paniculata extract cream

Placebo cream

Smoking

Alcohol

Hormones

UV light

3.4.2 Dependent variables

Wrinkles

Hyperpigmentation

Patient satisfaction score

Adverse effects

DNA damage

Collagen Breakdown

3.5 Research Instrument

3.5.1 Patient record form

3.5.2 Participant information sheet

3.5.3 Informed consent form

3.5.4 Clinical evaluation record form

3.5.5 2% *Andrographis panniculata* extract eyecream

3.5.6 Standard cream base

3.5.7 Patch Test

3.5.8 Questionnaires and Satisfaction Assessment Form

3.5.9 Adverse Effect Record Form

3.6 Equipment

3.6.1 The VISIA® Complexion

Source Surface Imaging Solutions Limited (n.d.)

Figure 3.1 The VISIA® skin complexion analysis system

The VISIA® a device used for scanning the facial skin that detects data by means of a multi-spectral imaging and analysis. It shows different aspects of skin complexion and appearance, including spots like wrinkles, pores, skin textures, UV spots, red areas, brown spots and porphyrins (even the presence of bacteria), all under a controlled and consistent environment. The wrinkle score by VISIA® scan are the larger, the better.

3.6.2 Cutometer® MPA 580

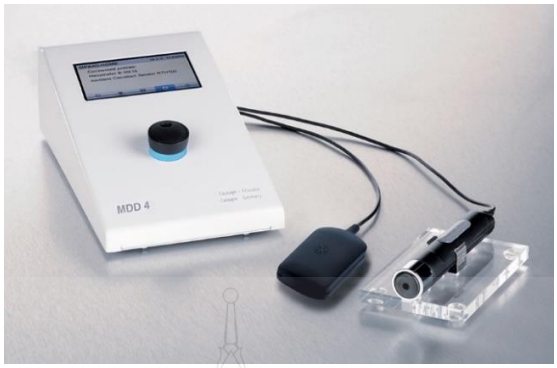
Source Courage + Khazaka electronic GmbH (n.d.)

Figure 3.2 Cutometer® MPA 580

The Cutometer is a device that we can use to measure the upper skin layer elasticity by applying negative pressure through a suction method. This measurement technique provides data on the skin's elastic and mechanical properties, allowing for an objective evaluation of skin aging.

3.6.3 Mexameter®MX18

Mexameter®MX18 is a spectrometer which is used to measure two major chromophores: hemoglobin, and melanin, based on light reflection and absorption. There are different wavelengths of light are emitted from the probe, 568nm, 660nm and 870nm. The emitted light is reflected by the skin and detected by a receiver in the probe. Results are generated within one second as index numbers ranging from 0 to 999. For accurate readings, the probe head must be pressed consistently against the skin.



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

Figure 3.3 Mexameter MX 18

3.6.4 Extract Cream which Consists of 4% *Andrographis paniculata* Extract Cream in the Standard Cream Base

1. DI WATER	Solvent
2. Sodium Chloride	Thickener agent
3. Glycerin	Humectant
4. *Andrographis Paniculata* Leaf Extract	Skin conditioning
5. Cyclopentasiloxane, PEG-10 Dimethicone, Disteardimonium Hectorite	Emulsifier/Thickener
6. Dimethicone	Emollient
7. Caprylic/Capric Tryglyceride	Emollient
8. Hydroxyacetophenone, Caprylyl Glycol, Dipropylene Glycol, Dipotassium Glycyrrhizinate	Preservative

3.6.5 Standard Cream Base which may have a Little Anti-wrinkle Effect

1. DI WATER	Solvent
2. Sodium Chloride	Thickener agent
3. Glycerin	Humectant
4. Cyclopentasiloxane, PEG-10 Dimethicone, Disteardimonium Hectorite	Emulsifier/Thickener
5. Dimethicone	Emollient
6. Caprylic/Capric Tryglyceride	Emollient
7. Hydroxyacetophenone, Caprylyl Glycol, Dipropylene Glycol, Dipotassium Glycyrrhizinate	Preservative

3.7 Study Procedures

3.7.1 Recruitment of Volunteers

The researcher recruited the volunteers with periorbital wrinkles and periorbital hyperpigmentation based on the inclusion and exclusion criteria.

3.7.2 Explanation about Research

Each volunteer was fully explained of the aim and benefits of research, the steps of the procedure, and potential side effects of the treatment by the researcher.

3.7.3 Informed Consent Taking

Before participating, each of the volunteers was asked to sign the informed written consent form by the researcher.

3.7.4 History Taking

Volunteers were asked to disclose the general information such as age, sex, address, phone numbers, jobs, and medical history that are related to wrinkles and periorbital hyperpigmentation.

3.7.5 Patch Test

Before the participant was given the AP creams and placebo creams, a patch test was done before application to these creams around eyes.

Volunteers were instructed to apply 2% *Andrographis paniculata* extract and placebo cream on the arms of the subjects and then left for 48 hours. Participants were instructed to avoid activities that cause excessive sweating and exposure to intense sunlight during the test period, and they were examined at 48 and then 96 hours, respectively.

According to the system from the International Contact Dermatitis Research Group:

+ = mild redness

+ = redness and slight skin thickening

++ = red, swollen skin with small blisters

+++ = extreme positive reaction, characterized by severe redness and large blisters

score ++ or higher were excluded by the researcher.

3.7.6 Photo Taking of Faces of the Volunteers

The researcher used VISIA® Complexion Analysis System to take photographs of each participant at baseline, 4th, 8th, and 12th weeks. The following conditions were maintained during the photo taking process: the room lights is switched off, and images are captured at 12-megapixel resolution with automatic white balance correction and autofocus. Facial positions were right 45°, left 45°, and center 0°. Multispectral imaging was used, with standard daylight fluorescent lighting, UV lighting and cross-polarized flash, and.

3.7.7 Randomization

3.7.7.1 *Andrographis paniculata* extract cream and a placebo cream base, with the same consistency, color, and odor, were placed in identical packages labeled as cream "A" and cream "B." Both the researcher and participants were blinded to the contents.

3.7.7.2 The physician, who was not related to this the study, planned which side of the face will be treated with cream A and cream B by “Block Randomization” and kept the results in opaque envelopes.

3.7.7.3 Total of 20 volunteers joined, resulting in total 38 faces. Each block contained two subjects, with faces are RIGHT, which means right face and LEFT, that means left face. There two groups of Right (cream A applied to right, cream B applied to left side) and left (cream A applied to left, cream B applied to right side).

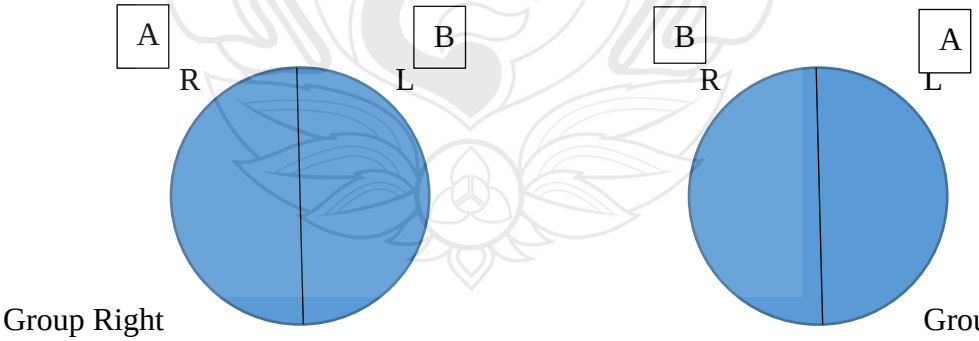


Figure 3.4 Serum A and B application on the face sides in group RIGHT and LEFT

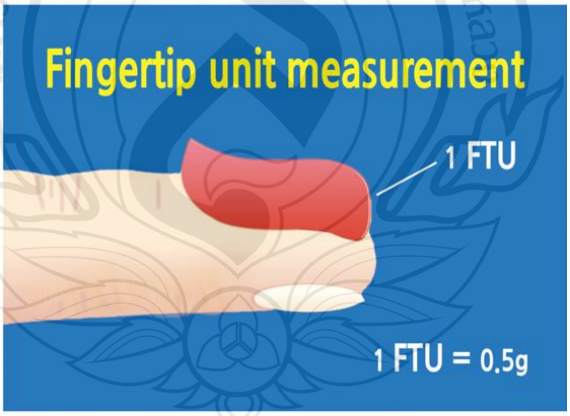
3.7.7.4 Random Sequence Generator from was used (https://www.random.org).

Figure 3.5 Random sequence generator

3.7.7.5 Arabic numbers from 1 to 1000 were randomized then each of these 20 numbers was assigned to 20 volunteers, by means of first-come, first-served order. The randomization is done by the physician who was not related to this study.

Fingertip unit

The amount of ointment which is produced from the nozzle of 5mm in size of a standard tube, align from the distal crease of the interchanges to the finger end. It weighs around 0.5 g and this can be considered as a useful method for the estimation of the prescription needed. (Finlay, 2012)



Source Yun et al. (2017)

Figure 3.6 Fingertip unit measurement

3.7.9 Eye Cream Application and Instructions to Follow

1. After washing face gently with mild soap, dry with towel or tissues.

2. After face washing, apply extract cream and standard base cream on the periorbital areas of the face in the morning and in the evening according to the instructions.

3. The participant were instructed to apply the cream with 1 fingertip unit.

4. Throughout the research, all subjects acted in accordance with the guidelines precisely as mentioned in the consent.

5. A side effect record form was given to record if there was any.

6. If any side effects occurred, the participants are informed to stop using the cream, notify the researcher as soon as possible.

3.8 Data Collection

3.8.1 Brief history taking about personal data

3.8.2 Skin elasticity score by using Cutometer MPA 580 at 4 time points: 0^{th}, 4^{th}, 8^{th}, 12^{th} weeks

3.8.3 Melanin index by Mexameter MX 18 at 4 time points: 0^{th}, 4^{th}, 8^{th} 12^{th} weeks

3.8.4 Wrinkle scores by VISIA at 4 time points: 0^{th}, 4^{th}, 8^{th} 12^{th} weeks

3.8.5 Participant's treatment satisfactory scores were collected by using the grading scales, score 1 to 4.

Score 1 = No satisfaction

Score 2 = Little satisfaction

Score 3 = Average satisfactions

Score 4 = Most satisfaction

3.9 Statistical Analysis

3.9.1 Volunteers were carefully selected by inclusion and exclusion criteria. Additionally, personal data of each volunteer is kept highly confidential in data analysis.

3.9.2 At Mae Fah Luang University Dermatology Clinic, the medical records of the volunteers in this study were collected.

3.9.3 Analysis of demographic data of the volunteers were made using descriptive statistics, including means, medians, modes, standard deviations and ranges.

3.9.4 Skin elasticity, measured by Cutometer scores, was analyzed using the Paired t-test and Repeated Measures ANOVA.

3.9.5 A post hoc test (Bonferroni method) was applied to determine significant differences between specific time points at the 0th, 4th, 8th, and 12th weeks.

3.9.6 Analysis of wrinkles score of each participant was done by 3 dermatologists, at each 4 time points: baseline, 4^{th}, 8^{th}, 12^{th} week by using descriptive statistics.

3.9.7 The researcher used level of significant at p-value <0.05.

3.10 Ethical Consideration

3.10.1 The study was approved by the ethic committee at Fah Luang University.

3.10.2 All research activities adhered to Good Clinical Practice (GCP) guidelines throughout the study.

3.10.3 Written informed consent was obtained from all participants after providing detailed research information.

3.10.4 Participant identities and data were securely stored to ensure confidentiality.

3.10.5 Participation was voluntary, with the right to withdraw at any time without consequences.

3.10. 6 Participants were informed of the study's objectives, and results will be shared appropriately.

CHAPTER 4

RESULTS

4.1 General Demographic of the Participants

The demographic demographics of the participants in this study are shown in Table 4.1. There were total participants 20 individuals, with distribution of gender: 9 males and 10 females. A total of 20 participants were initially enrolled in the study. However, one participant dropped out before completion, resulting in a final sample size of 19. Analyses were conducted with data from the 19 participants who completed the study. The average age of the participants was 26.95±2.12 years. The participants were students (14), while the remaining (5) were government officers. Only one participant reportedly has an underlying medical condition, and none had received any treatment in the four weeks prior to the study. The median duration of daily sunlight exposure among participants was 30 minutes, with an interquartile range (IQR) of 32.5 to 19.5 minutes, and exposure times ranging from 15 to 60 minutes. In terms of skin type, many participants had combination skin (11 individuals), with the rest having oily skin (8 individuals).

Table 4.1 General characteristics

Demographic data	**n = 19**
Gender	
Male	9
Female	10
Age (years)	
Mean±SD	26.95±2.12
Occupation	
Student	14
Government officer	5
Underlying disease	1
Treatment 4 weeks before	0

Table 4.1 (continued)

Demographic data	n = 19
Duration of sunlight exposure (minute/day)	
Median (IQR)	30 mins (32.5 – 19.5) minutes
Min - Max	15 - 60 minutes
Skin type	
Combination	11
Oily	8

4.2 Clinical Evaluation

4.2.1 Score for Skin Elasticity in Treatment Cream and Placebo Group

Table 4.2 Statistical analysis of skin elasticity at crow's feet and under eye areas comparing the sides of Andrographis paniculata extract and placebo group at baseline and after treatment at 4th, 8th, and 12th week of study (n=19)

	***Andrographis paniculata* extract**	**Placebo**	**Paired differences±SE**	**p-value[(a)]**
	Mean±SD	**Mean±SD**		
Crow's feet				
Baseline	78.21±10.04	80.12±11.67	-1.91±3.53	0.592
4th week	83.94±10.90	79.93±16.34	84.01±4.51	0.380
8th week	87.76±10.55	81.01±12.86	6.75±3.82	0.086
12th week	92.77±9.44	80.31±17.39	12.46±4.54	0.010
P-value[(b)]	**<0.001***	**0.996**		
Under eye				
Baseline	67.20±10.27	72.81±10.04	-5.61±3.29	0.097
4th week	78.25±12.01	73.45±13.02	4.80±4.06	0.045
8th week	87.04±10.96	73.01±13.09	14.03±3.92	0.001
12th week	96.11±14.34	74.79±13.67	21.32±4.55	<0.001
P-value[(b)]	**<0.001***	**0.966**		

Note Data were analyzed with Paired t-test and Repeated measure ANOVA

p-value (a): compares the treatment effect of *Andrographis paniculata* cream vs.

placebo.

p-value[(b)] : significant change within a group across time points.

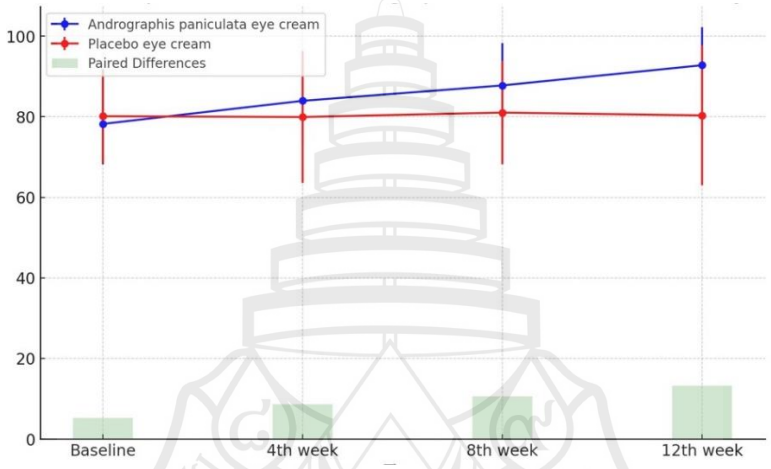
Table 4.2 shows that the mean Cutometer scores for crow's feet on the *Andrographis paniculata* eye cream side were 78.21±10.04 at baseline, 83.94±10.90at the 4th week, 87.76±10.55 at the 8th week, and 92.77±9.44 at the 12th week. The increase in these scores across all time points was statistically significant, with p-values of <0.001*. On the placebo side, the mean scores were 80.12±11.67 at baseline, 79.93±16.34 at the 4th week, 81.01±12.861at the 8th week, and 80.31±17.39 at the 12th week, with p value of 0.996 (p > 0.05). When comparing the scores between the two sides, the *Andrographis paniculata* side demonstrated higher Cutometer scores at the 12th weeks, with p-values of 0.010 (<0.05).

Regarding the under-eye area, the mean Cutometer scores for the *Andrographis paniculata* were 67.20±10.27 at baseline, 78.25±12.01at the 4th week, 87.04±10.96 at the 8th week, and 96.11±14.34 at the 12th week. These scores increased is statistically significantly over time, with p-values of less than 0.001.The placebo eye cream side had mean scores of 72.81±10.04 at baseline, 73.45±13.02 at the 4th week, 73.01±13.09 at the 8th week, and 74.79±13.67 at the 12th week. However, the differences in mean scores between the time points for the placebo side were minor and not statistically significant, with p value of 0.966 (>0.05). When comparing the two sides, significant differences in cutometer scores were found at the 4th, 8th, and 12th weeks, with p-values of 0.045, 0.001, and less than 0.001, respectively.

Table 4.5 Mean Changes of skin elasticity by cutometer of *Andrographis paniculata* extract eye cream over 12 weeks

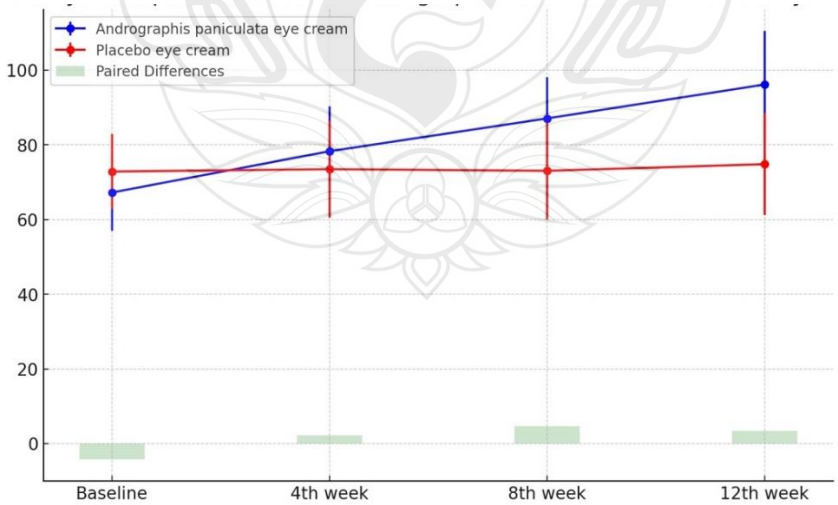
Variables	**Mean Changes from Baseline**		**p-value**	
Skin Elasticity by Cutometer	**Under Eye (Mean ± SD)**	**Crow Feet (Mean ± SD)**	**Under Eye**	**Crow Feet**
Baseline – week4	11.05±10.79	5.73±10.89		0.034
Baseline– week 8	19.98±11.97	9.55±13.01		0.005
Baseline – week12	28.91±16.99	14.57±10.20		<0.001
Week4 – week 8	8.93±8.19	3.82±8.23	<0.001	0.058
Week 4 – week12	17.86±12.61	8.83±9.86		0.001
Week8 – week12	8.93±9.94	5.01±9.77	0.001	0.038

Based on the data from above table, the score of skin elasticity by cutometer on 12th week is significantly higher than baseline, week 4, week 8 with p value of 0.001 (<0.05) at under eye regions. Moreover, differences between baseline and each week is also statistically significant with p value of <0.001. In terms of crow's feet, differences between baseline to each week are higher p-value of 0.035, 0.005 and <0.001. However, data from week 12 is significantly higher than only from week 4 and week 8 with p value of 0.001 and 0.038 (<0.05).



X axis = time point, Y axis = skin elasticity score

Figure 4.1 Comparison of Andrographis paniculata eye cream and placebo over time (Crow's Feet)



X axis = time point, Y axis = skin elasticity score

Figure 4.2 Comparison of *Andrographis paniculata* eye cream and placebo over time (Under Eye)

4.2.2 Score for Melanin Index in *Andrographis paniculata* Eye Cream and Placebo Eye Cream

Table 4.3 Melanin index for the crow's feet and under eye comparing between *Andrographis paniculata* extract and placebo group at baseline and after the 4th, 8th, and 12th week of the study (n=19)

	***Andrographis paniculata* eye cream side**	**Placebo eye cream side**	**Paired**	**p-value[(b)]**
	Mean±SD	**Mean±SD**		
Crow's feet				
Baseline	245.86±65.94	236.42±62.63	1.63±6.94	0.780
4th week	245.86±65.94	236.42±62.63	1.63±6.94	0.780
8th week	235.20±63.00	247.37±67.66	10.37±20.19	0.614
12th week	222.34±61.00	231.74±52.11	19.47±13.61	0.170
P-value[(b)]	**0.004**	**0.140**		
Under eye				
Baseline	214.04±52.58	201.47±52.38	0.440±5.13	0.864
4th week	202.89±53.50	210.58±48.47	7.70±4.30	0.727
8th week	196.40±52.10	218.26±50.23	12.10±4.15	0.923
12th week	190.20±53.00	210.21±48.62	16.80±4.20	0.005
P-value[(b)]	**0.229**	**0.210**		

Note Data were analyzed with Paired t-test and Repeated measure ANOVA

p-value [(a)]: compares the treatment effect of *Andrographis paniculata* cream vs. placebo.

p-value [(b)]: significant change within a group across time points.

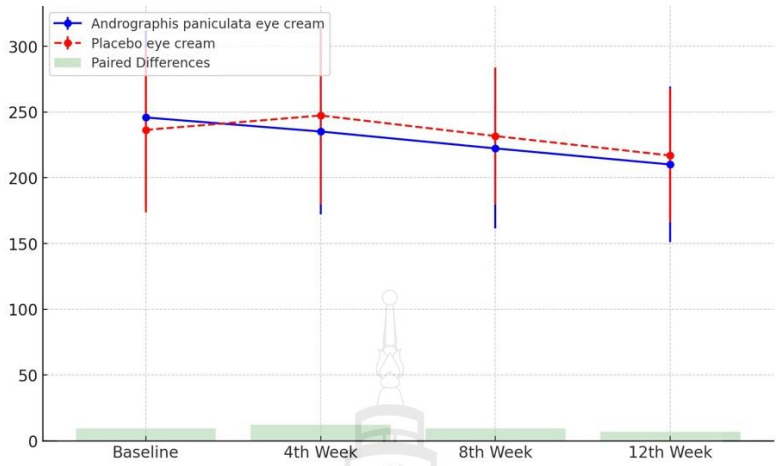
The mean scores for Crow's feet on the *Andrographis paniculata* eye cream side were 245.86±65.94 at baseline, 235.20±63.00 at the 4th week, 222.34±61.00 at the 8th week, and 210.12±59.00 at the 12th week. The results show a statistically significant reduction of melanin index in Crow's feet with time, with p-values of 0.004 (<0.05). In comparison, the placebo eye cream side had mean scores of 236.42±62.63 at baseline, 247.37±67.66 at the 4th week, 231.74±52.11 at the 8th week, and 216.95±50.85 at the 12th week, showing no significant improvement (P-value = 0.140). However, the paired differences between the two sides, the *Andrographis paniculata* cream showed not significantly statistically.

Regarding the under-eye area, the mean scores for the *Andrographis paniculata* side were 214.04±52.58 at baseline, 202.89±53.50 at the 4th week, 196.40±52.10 at the 8th week, and 190.20±53.00 at the 12th week. These results showed a decrease in melanin index, but with p-values of 0.229 (>0.05). Therefore, it can be determnined that the melanin index scores were decreased but it is not statistically significant. On the placebo eye cream side, it exhibited mean scores of 201.47±52.38 at baseline, 210.58±48.47 at the 4th week, 218.26±50.23 at the 8th week, and 210.21±48.62 at the 12th week, with no statistically significant improvement with p-value of 0.210 (>0.05). However, when comparing the two sides, the *Andrographis paniculata* extract eye cream showed significantly greater improvements in the under-eye area at 12th week, with p-value of 0.005(<0.05).

Table 4.6 Mean Changes of melanin index by mexameter of *Andrographis paniculata* extract eye cream over 12 weeks

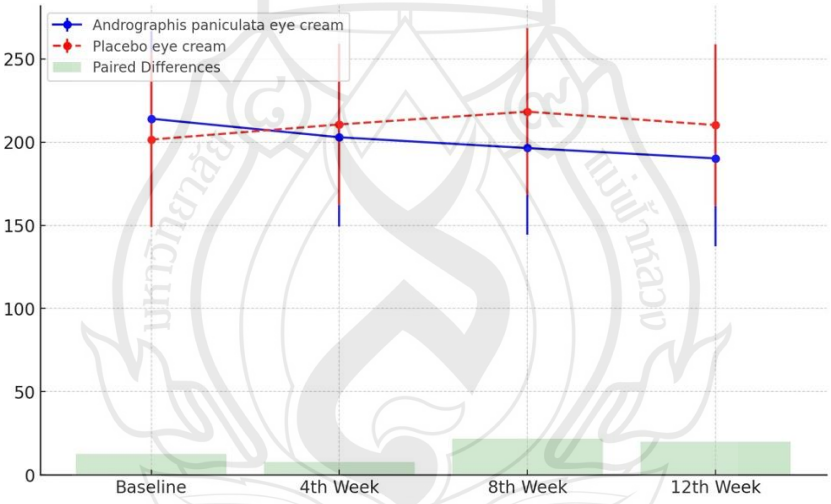
Variables	**Mean Changes from Baseline**		**p-value**	
Melanin Index by Mexameter	**Under Eye (Mean ± SD)**	**Crow Feet (Mean ± SD)**	**Under Eye**	**Crow Feet**
Baseline – week4	-8.32±73.08	-27.63±82.79	0.626	0.163
Baseline– week 8	7.16±104.72	-52.37±54.84	0.769	0.001
Baseline – week12	-43.05±73.30	-28.21±69.80	0.020	0.095
Week4 – week 8	15.47±75.79	-24.74±71.64	0.385	0.150
Week 4 – week12	-34.74±59.31	-0.58±72.63	0.020	0.973
Week8 – week12	-50.21±81.72	24.16±76.92	0.015	0.188

According to the above table, mean changes of melanin index by mexameter from week 8 is significantly lower than week baseline with p-value of 0.001 at crow feet region. At undereye area, mean changes from week 12 is significantly lower than baseline and week 4 with p value of 0.020 (<0.05).



X axis = time point, Y axis = melanin index score

Figure 4.3 Comparison of *Andrographis paniculata* Eye Cream and Placebo Over Time (Crow's Feet)



X axis = time point, Y axis = melanin index score

Figure 4.4 Comparison of *Andrographis paniculata* Eye Cream and Placebo Over Time (Under eye)

Table 4.4 Wrinkle score comparing between *Andrographis paniculata* extract and placebo eye cream on baseline, follow- up 4th, 8th, and 12th week (n=19)

	***Andrographis paniculata* extract**	**Placebo**	**Paired differences±SE**	**p-value[(a)]**
	Mean±SD	**Mean±SD**		
Baseline	49.63±2.24	56.16±3.01	-5.68±6.90	0.432
4th week	51.63±2.48	54.95±4.42	-2.76±4.50	0.019
8th week	54.68±3.42	54.05±7.65	3.88±4.32	0.935
12th week	57.89±4.89	51.74±8.86	9.67±3.72	0.010
P-value[(b)]	**0.002**	**0.196**		

Note Data were analyzed with Wilcoxon Signed Ranks test

p-value [(a)]: compares the treatment effect of *Andrographis paniculata* cream vs. placebo.

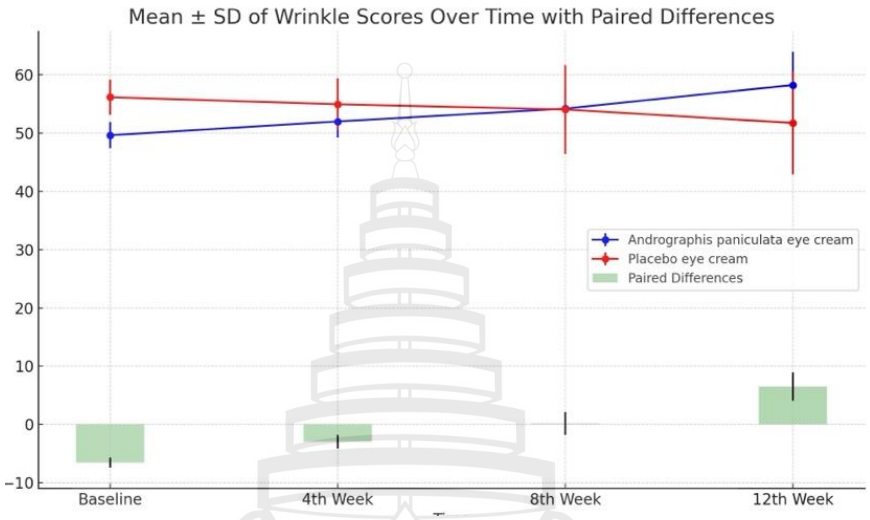
p-value [(b)]: significant change within a group across time points.

According to the analysis of Visia scores, the results showed significant improvements for the *Andrographis paniculata* eye cream compared to the placebo eye cream side, at 4th week and 12th week. At baseline, the AP extract cream side had the score of 49.63±2.24, 51.63±2.48, 54.68±3.42, 57.89±4.89 with p value of 0.002 (<0.05), which is statistically significant. At the placebo eye cream side, the results did not show any improvement with p value of 0.196(>0.05).

Table 4.7 Mean Changes of wrinkle score by VISIA scan of *Andrographis paniculata* extract eye cream over 12 weeks

Variables / **Wrinkle Scores by Visia Scan**	**Mean Changes from Baseline**	**p-value**
Baseline – week4	2.37±2.06	<0.001*
Baseline– week 8	4.58±3.32	<0.001*
Baseline – week12	8.63±4.88	<0.001*
Week4 – week 8	2.21±2.68	0.002
Week 4 – week12	6.26±4.12	<0.001*
Week 8 – week12	4.05±3.44	<0.001*

According to the analysis from the above table, mean changes were statistically significant over baseline to 4th, 8th, 12th week respectively with p value of less than 0.001. Moreover, baseline to week 4, week 8 and week 12 also showed statistically significant with p value of <0.001.



X axis = time point, Y axis = Wrinkle score

Figure 4.5 Comparison of Andrographis paniculata eye cream and placebo over time

4.3 Patients' Satisfaction Score

Table 4.8 Statistical analysis of patients' satisfaction score compare between Andrographis paniculata eye cream side and placebo eye cream side

	***Andrographis Paniculata* Extract, n(%)**	**Placebo group n(%)**	**p-value**
No satisfaction (0)	0 (0.0)	8 (42.1%)	
Little satisfaction (1)	0 (0.0)	6 (31.6%)	
Average satisfaction (2)	3 (15.8%)	5 (26.3%)	<0.001
More satisfaction (3)	10 (52.6%)	0 (0.0)	
Most satisfaction (4)	6 (31.6%)	0 (0.0)	

Note Data were analyzed with McNemar test

A statistical analysis comparing patient satisfaction between the *Andrographis paniculata* extract eye cream and the placebo eye cream revealed 31.6% reported "Most satisfaction" with the *Andrographis paniculata* eye cream, whereas 26.3% of patients experienced "Average satisfaction," respectively, with placebo eye cream, resulting in a statistically significant difference with a p-value of less than 0.001. Satisfaction scores were collected using a 5-point Likert scale, where 0 represents "no satisfaction" and 4 represents "most satisfaction."

4.4 Dermatologists' Evaluation Score

Table 4.9 Frequencies of dermatologists' evaluation score

	***Andrographis paniculata* extract**			**Placebo**		
	4th week	**8th week**	**12th week**	**4th week**	**8th week**	**12th week**
Worse (-1)	-	-	-	11	6	5
No change (0)	-	-	-	5	5	8
Fair improvement (1)	11	5	3	3	8	6
Moderate improvement (2)	8	10	10	-	-	-
Good improvement (3)	-	4	6	-	-	-
Excellent improvement (4)	-	-	-	-	-	-

Table 4.10 Statistical analysis of evaluation by 3 dermatologists on follow-up 4th, 8th, and 12th week

	***Andrographis paniculata* extract**	**Placebo**	**P-value(a)**
	Median (IQR)	**Median (IQR)**	
4th week	0.7(0.7-1.3)	-0.7 (-0.7-0.7)	0.7(0.7-1.3)
8th week	1.3(1.3-2)	0.7(0-0.7)	1.3(1.3-2)

Table 4.10 (continued)

	***Andrographis paniculata* extract**	**Placebo**	**P-value[a]**
	Median (IQR)	**Median (IQR)**	
12th week	1.3(1.3-2)	0.7(0-0.7)	1.3(1.3-2)
P-value[b]	**17.55(2), p<0.0001**	**7(2), p=0.03**	

Note Data were analyzed with Wilcoxon Signed ranks test (a), and Friedman test (b)

Table 4.9 presents the statistical analysis of evaluations by three dermatologists at the 4th, 8th, and 12th weeks for both the *Andrographis paniculata* extract eye cream and the standard eye cream. For the *Andrographis paniculata* eye cream, the median evaluations were 0.7 (IQR: 0.7-1.3) at the 4th week, 1.3 (IQR: 1.3-2) at the 8th week, and 1.3 (IQR: 1.3-2) at the 12th week. In contrast, the standard eye cream side had median evaluations of -0.7 (IQR: -0.7-0.7) at the 4th week, 0.7 (IQR: 0-0.7) at the 8th week, and 0.7 (IQR: 0-0.7) at the 12th week. The statistical significance of the differences between the two treatments was evaluated using the Mann-Whitney U test and the Friedman test, with p-values of less than 0.0001 and 0.03, respectively, indicating a significant difference in the evaluations over time.

4.5 Side Effects

Table 4.11 Frequencies of side effects and complication

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

The assessment of adverse effects for both the *Andrographis paniculata* eye cream and placebo eye cream treatment revealed no occurrences of itching at any of the measured time points (4th week, 8th week, and 12th week) for either treatment. Additionally, there were no reports of duration of erythema, allergic contact dermatitis, post-inflammatory hyperpigmentation, or post-inflammatory hypopigmentation for either the *Andrographis paniculata* eye cream side or the placebo side throughout the study period.

CHAPTER 5

DISCUSSION, CONCLUSION, SUGGESTION

5.1 Discussion

In this study, the researcher studied the efficacy of 2% *Andrographis paniculata* eye cream in reducing periorbital wrinkles and periorbital hyperpigmentation. The participants were 52.63% females and 47.37% males, with an average age of 26.95±2.12 years. The participants were students (73.68%), while the rest were government officers (26.32%). Only one participant reported an underlying medical condition, and none had undergone any treatments in the four weeks preceding the study. Most subjects had combination skin (57.89%), while the remaining participants had oily skin (42.11%).

The data were collected by VISIA scan, Cutometer, and Mexameter to assess skin elasticity, and melanin levels, and wrinkle scores comparing the side treated with *Andrographis paniculata* eye cream to the side treated with a placebo eye cream. The results showed a statistically significant reduction in periorbital wrinkles and periorbital hyperpigmentation on the *Andrographis paniculata*-treated side compared to the standard cream side. Additionally, the *Andrographis paniculata* extract eye cream showed greater efficacy and provided more satisfactory results than the standard cream.

The study results can be summarized as follows:

Firstly, the topical application of *Andrographis paniculata* eye cream did not result in any allergic reactions during the patch test, and no allergic cases were reported throughout the study. Based on this, it can be concluded that *Andrographis paniculata* eye cream is safe for use around the eye area.

The mean Cutometer score at the crow's feet area on the *Andrographis paniculata* eye cream side showed a statistically significant increase at 12th week with p value of 0.010(<0.05) at crow's feet area. Similarly, the mean Cutometer score at the undereye area on the *Andrographis paniculata* -treated side showed a statistically significant increase at 4^{th} week (0.045), 8^{th} week (0.001) and 12^{th} week (<0.001),

compared to the placebo treated side. Moreover, there was statistically significant change improvement across all time points at crow's feet and under eyes regions on AP cream treated side with p value (<0.001) However, there was no statistically significant change in Cutometer scores at the crow's feet and undereye areas for the placebo eye cream. These findings indicate that *Andrographis paniculata* eye cream enhances skin elasticity around the eye area more effectively than the standard eye cream.

The *Andrographis paniculata* eye cream was also found to reduce the melanin index, as measured by Mexameter, at the crow's feet area from baseline to the 4th week, 8th week, and 12th week with p value of 0.004(<0.05). In contrast, the Mexameter scores at the crow's feet on the placebo side did not show a statistically significant difference across visits at crow's feet area, with p value of 0.140. The Mexameter score on AP cream treated side at the under-eye area showed a statistically significant reduction at the 12th week, with a significance level of 0.005 ($p<0.05$), compared to the placebo treated side. These results suggest that the *Andrographis paniculata* eye cream led to a greater decrease in melanin index around the under-eye area by the 12th week compared to the standard eye cream at under eye area.

Based on the wrinkle scores by Visia scan, the side treated with *Andrographis paniculata* eye cream showed a statistically significant improvement between baseline and the 4^{th}week, 8^{th} week and 12^{th} week with p value of 0.002 ($p<0.05$). In contrast, the VISIA scores for the side treated with the placebo eye cream did not show a statistically significant difference with p value of 0.196(>0.05). This indicates that *Andrographis paniculata* eye cream is more effective at reducing skin wrinkles and hyperpigmentation compared to the placebo eye cream. Regarding patients' satisfaction, *Andrographis paniculata* eye cream treated side had statistically greater result than placebo eye cream treated side at follow up 12^{th} week.

During the 12th-week follow-up visit, three dermatologists assessed the volunteers and rated 3 of them as having shown good improvement, 10 as having moderate improvement, and 6 as having good improvement on the side treated with *Andrographis paniculata* extract cream. In comparison, the majority (6 volunteers) were rated as having fair improvement on the side treated with the placebo eye cream.

The above data demonstrated a significant increase in skin elasticity on the side treated with *Andrographis paniculata* eye cream. This effect may be due to its ability

to counteract the action of matrix metalloproteinases (MMP-1, MMP-3), which are known to be triggered by UV radiation, leading to the breakdown of collagen and elastin, and leading to wrinkles and skin laxity (Kahari & Saarialho-Kere, 1997). Andrographolide sodium bisulphate (ASB), a water-soluble form of andrographolide, has been proved to inhibit the activation of MMP enzymes (Zhan et al., 2016). *Andrographis paniculata* extract can also stimulate collagen production and this results in a noticeable improvement in skin wrinkles. (You et al., 2015). Therefore, *Andrographis paniculata* eye cream enhances skin elasticity and can be an effective treatment for wrinkles around the eyes.

Andrographis paniculata leaf extract has been shown to significantly reduce the melanin index, making it a potent and safe natural ingredient for skin lightening. It effectively inhibits melanin production by suppressing TYR, MITF, and related proteins. Additionally, the extract is considered a reliable and safe option for managing hyperpigmentation, offering a natural solution for skin lightening (Adam et al., 2022). This anti-melanogenic property of AP cream can be the cause in reduction of melanin index and good for periorbital hyperpigmentation.

Based on this study and its results, *Andrographis paniculata* extract eye cream is a safe and effective topical treatment for wrinkles and hyperpigmentation around the eyes. All participants tolerated the treatment well, with no reported side effects such as skin irritation, erythema, or hyperpigmentation throughout the study period. From the data, it can be concluded that *Andrographis paniculata* extract eye cream enhances skin elasticity and reduces the melanin index, likely due to the antioxidant and skin lightening properties of the compounds present in the cream.

5.2 Suggestion for Future Research and Clinical Applications

5.2.1 Future studies can investigate the long-term effects of *Andrographis paniculata* extract eye cream beyond 12 weeks.

5.2.2 Extending the duration of the study would be beneficial. Comparing *Andrographis paniculata* with other anti-aging ingredients could clarify its relative effectiveness.

5.2.3 The potential of combining this eye cream with other treatments like fillers or laser therapy should be explored.

5.3 Conclusion

Based on the clinical study, it can be concluded that *Andrographis paniculata* eye cream significantly enhances skin elasticity and reduces hyperpigmentation in participants without causing harmful side effects. In summary, *Andrographis paniculata* eye cream appears to be a safe and effective topical treatment for periorbital wrinkles and periorbital hyperpigmentation.

REFERENCES

Adiguna, S. B. P., Panggabean, J. A., Atikana, A., Untari, F., Izzati, F., Bayu, A., . . . Putra, M. Y. (2021). Antiviral activities of andrographolide and its derivatives: mechanism of action and delivery system. *Pharmaceuticals*, *14*(11), 1102. https://doi.org/10.3390/ph14111102

Agrawal, S. (2018). Periorbital hyperpigmentation: Overcoming the challenges in the management. *Nepal Journal of Dermatology, Venereology & Leprology*, *16*(1), 2-11. https://doi.org/10.3126/njdvl.v16i1.19411

Akazaki, S., Nakagawa, H., Kazama, H., Osanai, O., Kawai, M., Takema, Y., & Imokawa, G. (2002). Age-related changes in skin wrinkles assessed by a novel three-dimensional morphometric analysis. *British Journal of Dermatology*, *147*(4), 689-695. https://doi.org/10.1046/j.1365-2133.2002.04874.x

Akbar, S. (2011). Andrographis paniculata: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*, *16*(1), 66-77.

Al-Atif, H. (2022). Collagen supplements for aging and wrinkles: a paradigm shift in the fields of dermatology and cosmetics. *Dermatology practical & conceptual*, *12*(1). https://doi.org/10.5826/dpc.1201a18

Alster, T. S., & Graham, P. M. (2018). Microneedling: a review and practical guide. *Dermatologic Surgery*, *44*(3), 397-404. https://doi.org/10.1097/DSS.0000000000001248

Amin, S. P., Phelps, R. G., & Goldberg, D. J. (2006). Mesotherapy for facial skin rejuvenation: a clinical, histologic, and electron microscopic evaluation. *Dermatologic surgery*, *32*(12), 1467-1472. https://doi.org/10.1111/j.1524-4725.2006.32353.x

Andrographis paniculata. (5 November 2024). *Wikipedia.* https://en.wikipedia.org/wiki/Andrographis_paniculata

Babilas, P., Schreml, S., Szeimies, R. M., & Landthaler, M. (2010). Intense pulsed light (IPL): a review. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, *42*(2), 93-104. https://doi.org/10.1002/lsm.20877

Bae, J. S., Kim, J. M., Kim, J. Y., Choi, C. H., Kim, J. Y., Moon, W. K., . . . Seo, J. S. (2017). Topical application of palmitoyl-RGD reduces human facial wrinkle formation in Korean women. *Archives of Dermatological Research*, *309*, 665-671. https://doi.org/10.1007/s00403-017-1763-y

Ballin, A. C., Brandt, F. S., & Cazzaniga, A. (2015). Dermal fillers: an update. *American journal of clinical dermatology*, *16*, 271-283. https://doi.org/10.1007/s40257-015-0135-7

Bashir, M. M., Sharma, M. R., & Werth, V. P. (2009). UVB and proinflammatory cytokines synergistically activate TNF-α production in keratinocytes through enhanced gene transcription. *Journal of Investigative Dermatology*, *129*(4), 994-1001. https://doi.org/10.1038/jid.2008.332

Baumann, L. (2005). A dermatologist's opinion on hormone therapy and skin aging. *Fertility and Sterility*, *84*(2), 289-290. https://doi.org/10.1016/j.fertnstert.2005.03.032

Baumann, L. (2007). Skin ageing and its treatment. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *211*(2), 241-251. https://doi.org/10.1002/path.2098

Binic, I., Lazarevic, V., Ljubenovic, M., Mojsa, J., & Sokolovic, D. (2013). Skin ageing: natural weapons and strategies. *Evidence-Based Complementary and Alternative Medicine*, *2013*(1), 827248. https://doi.org/10.1155/2013/827248

Brahmachari, G. (2016). Andrographolide: A molecule of antidiabetic promise. *Discovery and Development of Antidiabetic Agents from Natural Products; Brahmachari, G., Ed*, 1-27.

Branchet, M. C., Boisnic, S., Frances, C., & Robert, A. M. (1990). Skin thickness changes in normal aging skin. *Gerontology*, *36*(1), 28-35. https://doi.org/10.1159/000213172

Brower, V. (2003). A second chance for hormone replacement therapy? Hormone replacement therapy for older women has been vilified after two major studies showed that it increased their susceptibility to various diseases. But scientists maintain that, used differently, it can still help to protect against neurological and cardiovascular diseases. *EMBO reports*, *4*(12), 1112-1115. https://doi.org/10.1038/sj.embor.7400043

Burgos, R. A., Alarcón, P., Quiroga, J., Manosalva, C., & Hancke, J. (2020). Andrographolide, an anti-inflammatory multitarget drug: all roads lead to cellular metabolism. *Molecules*, *26*(1), 5. https://doi.org/10.3390/molecules26010005

Caceres, D. D., Hancke, J. L., Burgos, R. A., Sandberg, F., & Wikman, G. K. (1999). Use of visual analogue scale measurements (VAS) to asses the effectiveness of standardized Andrographis paniculata extract SHA-10 in reducing the symptoms of common cold. A randomized double blind-placebo study. *Phytomedicine*, *6*(4), 217-223. https://doi.org/10.1016/S0944-7113(99)80012-9

Chandrasekaran, C. V., Gupta, A., & Agarwal, A. (2010). Effect of an extract of Andrographis paniculata leaves on inflammatory and allergic mediators in vitro. *Journal of ethnopharmacology*, *129*(2), 203-207. https://doi.org/10.1016/j.jep.2010.03.007

Cohen, J. L. (2008). Understanding, avoiding, and managing dermal filler complications. *Dermatologic Surgery*, *34*, S92-S99. https://doi.org/10.1111/j.1524-4725.2008.34249.x

Contet-Audonneau, J. L., Jeanmaire, C., & Pauly, G. (1999). A histological study of human wrinkle structures: comparison between sun-exposed areas of the face, with or without wrinkles, and sun-protected areas. *British Journal of Dermatology*, *140*(6), 1038-1047. https://doi.org/10.1046/j.1365-2133.1999.02901.x

Coon, J. T., & Ernst, E. (2004). Andrographis paniculata in the treatment of upper respiratory tract infections: a systematic review of safety and efficacy. *Planta medica*, *70*(04), 293-298. https://doi.org/10.1055/s-2004-818938

Courage + Khazaka electronic GmbH. (n.d.). *Scientific product*. https://www.courage-khazaka.com

Dastoor, S. F., Misch, C. E., & Wang, H. L. (2007). Botulinum toxin (Botox) to enhance facial macroesthetics: a literature review. *Journal of Oral Implantology*, *33*(3), 164-171. https://doi.org/10.1563/0-835.1

De Boulle, K., Fagien, S., Sommer, B., & Glogau, R. (2010). Treating glabellar lines with botulinum toxin type A-hemagglutinin complex: a review of the science, the clinical data, and patient satisfaction. *Clinical Interventions in Aging*, 101-118. https://doi.org/10.2147/CIA.S9338

El-Domyati, M., Attia, S., Saleh, F., Brown, D., Birk, D. E., Gasparro, F., ... & Uitto, J. (2002). Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Experimental dermatology*, *11*(5), 398-405. https://doi.org/10.1034/j.1600-0625.2002.110502.x

El-Domyati, M., El-Ammawi, T. S., Moawad, O., El-Fakahany, H., Medhat, W., Mahoney, M. G., & Uitto, J. (2012). Efficacy of mesotherapy in facial rejuvenation: a histological and immunohistochemical evaluation. *International journal of dermatology*, *51*(8), 913-919. https://doi.org/10.1111/j.1365-4632.2011.05184.x

Farage, M. A., Miller, K. W., Berardesca, E., & Maibach, H. I. (2009). Clinical implications of aging skin: cutaneous disorders in the elderly. *American journal of clinical dermatology*, *10*, 73-86. https://doi.org/10.2165/00128071-200910020-00001

FFinlay, A. Y. (2012). Defining the fingertip unit. *BMJ: British Medical Journal (Online)*, *344*.https://doi.org/10.1136/bmj.e3061

Fisher, G. J., Datta, S. C., Talwar, H. S., Wang, Z. Q., Varani, J., Kang, S., & Voorhees, J. J. (1996). Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature*, *379*(6563), 335-339. https://doi.org/10.1038/379335a0

Fisher, G. J., Wang, Z., Datta, S. C., Varani, J., Kang, S., & Voorhees, J. J. (1997). Pathophysiology of premature skin aging induced by ultraviolet light. *New England Journal of Medicine*, *337*(20), 1419-1429. https://doi.org/10.1056/NEJM199711133372003

Fodor, L., & Bota, O. (2020). Complications After IPL Treatment. *Aesthetic Applications of Intense Pulsed Light*, 195-216.

Ganceviciene, R., Liakou, A. I., Theodoridis, A., Makrantonaki, E., & Zouboulis, C. C. (2012). Skin anti-aging strategies. *Dermato-endocrinology*, *4*(3), 308-319. https://doi.org/10.4161/derm.22804

Gendler, E. C. (2005). Treatment of periorbital hyperpigmentation. *Aesthetic Surgery Journal*, *25*(6), 618-624. https://doi.org/10.1016/j.asj.2005.09.018

Gu, Y., Han, J., Jiang, C., & Zhang, Y. (2020). Biomarkers, oxidative stress and autophagy in skin aging. *Ageing research reviews*, *59*, 101036. https://doi.org/10.1016/j.arr.2020.101036

Guo, W., Liu, W., Chen, G., Hong, S., Qian, C., Xie, N., ... & Xu, Q. (2012). Water-soluble andrographolide sulfonate exerts anti-sepsis action in mice through down-regulating p38 MAPK, STAT3 and NF-κB pathways. *International immunopharmacology*, *14*(4), 613-619. https://doi.org/10.1016/j.intimp.2012.09.002

Gupta, M. A., & Gilchrest, B. A. (2005). Psychosocial aspects of aging skin. *Dermatologic Clinics*, *23*(4), 643-648. https://doi.org/10.1016/j.det.2005.05.012

Gupta, S., Mishra, K. P., & Ganju, L. (2017). Broad-spectrum antiviral properties of andrographolide. *Archives of virology*, *162*(3), 611-623. https://doi.org/10.1007/s00705-016-3166-3

Hanh, T. T. H., My, N. T. T., Cham, P. T., Quang, T. H., Cuong, N. X., Huong, T. T., . . . Van Minh, C. (2020). Diterpenoids and Flavonoids from Andrographis paniculata. *Chemical and Pharmaceutical Bulletin*, *68*(1), 96-99. https://doi.org/ 10.1248/cpb.c19-00662

Hertoghe, T. (2005). The “Multiple Hormone Deficienc” Theory of Aging: Is Human Senescence Caused Mainly by Multiple Hormone Deficiencies?. *Annals of the New York Academy of Sciences*, *1057*(1), 448-465. https://doi.org/10.1111/j.1749-6632.2005.tb06150.x

Hossain, M. S., Urbi, Z., Sule, A., & Rahman, K. H. (2014). Andrographis paniculata (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. *The Scientific World Journal*, *2014*(1), 274905. https://doi.org/10.1155/2014/274905

Hu, X. Y., Wu, R. H., Logue, M., Blondel, C., Lai, L. Y. W., Stuart, B., . . . Lewith, G. (2017). Andrographis paniculata (Chuān Xīn Lián) for symptomatic relief of acute respiratory tract infections in adults and children: A systematic review and meta-analysis. *PloS one*, *12*(8), e0181780. https://doi.org/10.1371/journal.pone.0181780

Imhof, L., & Leuthard, D. (2021). Topical over-the-counter antiaging agents: an update and systematic review. *Dermatology*, *237*(2), 217-229. https://doi.org/10.1159/000509296

Imokawa, G. (2009, August). Mechanism of UVB-induced wrinkling of the skin: paracrine cytokine linkage between keratinocytes and fibroblasts leading to the stimulation of elastase. In *Journal of Investigative Dermatology Symposium Proceedings* (Vol. 14, No. 1, pp. 36-43). Elsevier. https://doi.org/10.1038/jidsymp.2009.11

Jada, S. R., Matthews, C., Saad, M. S., Hamzah, A. S., Lajis, N. H., Stevens, M. F. G., & Stanslas, J. (2008). Benzylidene derivatives of andrographolide inhibit growth of breast and colon cancer cells in vitro by inducing G1 arrest and apoptosis. *British journal of Pharmacology*, *155*(5), 641-654. https://doi.org/10.1038/bjp.2008.368

Jain, D. C., Gupta, M. M., Saxena, S., & Kumar, S. (2000). LC analysis of hepatoprotective diterpenoids from Andrographis paniculata. *Journal of pharmaceutical and biomedical analysis*, *22*(4), 705-709. https://doi.org/10.1016/S0731-7085(99)00297-6

Kaczvinsky, J. R., Griffiths, C. E., Schnicker, M. S., & Li, J. (2009). Efficacy of anti-aging products for periorbital wrinkles as measured by 3-D imaging. *Journal of cosmetic dermatology*, *8*(3), 228-233. https://doi.org/10.1111/j.1473-2165.2009.00444.x

Kähäri, V. M., & Saarialho-Kere, U. (1997). Matrix metalloproteinases in skin. *Experimental dermatology*, *6*(5), 199-213. https://doi.org/10.1111/j.1600-0625.1997.tb00164.x

Kassir, M., Gupta, M., Galadari, H., Kroumpouzos, G., Katsambas, A., Lotti, T., . . . Goldust, M. (2020). Complications of botulinum toxin and fillers: A narrative review. *Journal of cosmetic dermatology*, *19*(3), 570-573. https://doi.org/10.1111/jocd.13266

Kim, A. Y., Shim, H. J., Shin, H. M., Lee, Y. J., Nam, H., Kim, S. Y., & Youn, H. S. (2018). Andrographolide suppresses TRIF-dependent signaling of toll-like receptors by targeting TBK1. *International Immunopharmacology*, *57*, 172-180. https://doi.org/10.1016/j.intimp.2018.02.019

Kligman, A. M., & Koblenzer, C. (1997). Demographics and psychological implications for the aging population. *Dermatologic clinics*, *15*(4), 549-553. https://doi.org/10.1016/S0733-8635(05)70464-2

Kolarsick, P. A., Kolarsick, M. A., & Goodwin, C. (2011). Anatomy and physiology of the skin. *Journal of the Dermatology Nurses' Association*, *3*(4), 203-213. https://doi.org/10.1097/JDN.0b013e3182274a98

Krithika, R., Verma, R. J., & Shrivastav, P. S. (2013). Antioxidative and cytoprotective effects of andrographolide against CCl4-induced hepatotoxicity in HepG2 cells. *Human & experimental toxicology*, *32*(5), 530-543. https://doi.org/10.1177/0960327112459530

Krutmann, J., Schikowski, T., Morita, A., & Berneburg, M. (2021). Environmentally-induced (extrinsic) skin aging: Exposomal factors and underlying mechanisms. *Journal of Investigative Dermatology*, *141*(4), 1096-1103. https://doi.org/10.1016/j.jid.2020.12.011

Lee, J. C., Daniels, M. A., & Roth, M. Z. (2016). Mesotherapy, microneedling, and chemical peels. *Clinics in plastic surgery*, *43*(3), 583-595. https://doi.org/10.1016/j.cps.2016.03.004

Li, Y. H., Wu, Y., Chen, J. Z., Gao, X. H., Liu, M., Shu, C. M., . . . Chen, H. D. (2008). Application of a new intense pulsed light device in the treatment of photoaging skin in Asian patients. *Dermatologic surgery*, *34*(11), 1459-1464. https://doi.org/10.1111/j.1524-4725.2008.34309.x

Liang, E., Liu, X., Du, Z., Yang, R., & Zhao, Y. (2018). Andrographolide Ameliorates Diabetic Cardiomyopathy in Mice by Blockage of Oxidative Damage and NF-κB-Mediated Inflammation. *Oxidative Medicine and Cellular Longevity*, *2018*(1), 9086747. https://doi.org/10.1155/2018/9086747

Lobo, R. A., Kelsey, J., & Marcus, R. (Eds.). (2000). *Menopause: biology and pathobiology*. Academic press.

Ma, G., Lin, X. X., Hu, X. J., Jin, Y. B., & Chen, H. (2012). Treatment of venous infraorbital dark circles using a long-pulsed 1,064-nm neodymium-doped yttrium aluminum garnet laser. *Dermatologic surgery*, *38*(8), 1277-1282. https://doi.org/10.1111/j.1524-4725.2012.02457.x

Manríquez, J. J., Cataldo, K., Vera-Kellet, C., & Harz-Fresno, I. (2014). Wrinkles. *BMJ clinical evidence*, *2014*.

McCullough, J. L., & Kelly, K. M. (2006). Prevention and treatment of skin aging. *Annals of the New York Academy of Sciences*, *1067*(1), 323-331. https://doi.org/10.1196/annals.1354.044

Mehryan, P., Zartab, H., Rajabi, A., Pazhoohi, N., & Firooz, A. (2014). Assessment of efficacy of platelet-rich plasma (PRP) on infraorbital dark circles and crow's feet wrinkles. *Journal of cosmetic dermatology*, *13*(1), 72-78. https://doi.org/10.1111/jocd.12072

Menaker, G. M., Wrone, D. A., Williams, R. M., & Moy, R. L. (1999). Treatment of facial rhytids with a nonablative laser: a clinical and histologic study. *Dermatologic surgery*, *25*(6), 440-444. https://doi.org/10.1046/j.1524-4725.1999.08158.x

Mendiratta, V., Rana, S., Jassi, R., & Chander, R. (2019). Study of causative factors and clinical patterns of periorbital pigmentation. *Indian Dermatology Online Journal*, *10*(3), 293-295. https://doi.org/10.4103/idoj.IDOJ_158_18

Mishra, S. K., Sangwan, N. S., & Sangwan, R. S. (2007). Phcog rev.: Plant review Andrographis paniculata (Kalmegh): A review. *Pharmacognosy Reviews*, *1*(2), 283-298.

MOY, L. S., MURAD, H., & MOY, R. L. (1993). Glycolic acid peels for the treatment of wrinkles and photoaging. *Dermatologic Surgery*, *19*(3), 243-246. https://doi.org/10.1111/j.1524-4725.1993.tb00343.x

Mukherjee, P. K., Maity, N., Nema, N. K., & Sarkar, B. K. (2011). Bioactive compounds from natural resources against skin aging. *Phytomedicine*, *19*(1), 64-73. https://doi.org/10.1016/j.phymed.2011.10.003

Mussard, E., Cesaro, A., Lespessailles, E., Legrain, B., Berteina-Raboin, S., & Toumi, H. (2019). Andrographolide, a natural antioxidant: An update. *Antioxidants*, *8*(12), 571. https://doi.org/10.3390/antiox8120571

Naeimifar, A., Ahmad Nasrollahi, S., Samadi, A., Talari, R., Sajad Ale-nabi, S., Massoud Hossini, A., & Firooz, A. (2020). Preparation and evaluation of anti-wrinkle cream containing saffron extract and avocado oil. *Journal of cosmetic dermatology*, *19*(9), 2366-2373. https://doi.org/10.1111/jocd.13284

Nikalji, N., Godse, K., Sakhiya, J., Patil, S., & Nadkarni, N. (2012). Complications of medium depth and deep chemical peels. *Journal of cutaneous and aesthetic surgery*, *5*(4), 254-260. https://doi.org/10.4103/0974-2077.104913

Oh, D. S., Kim, D. H., Roh, T. S., Yun, I. S., & Kim, Y. S. (2014). Correction of Dark Coloration of the Lower Eyelid Skin with Nanofat Grafting. *Archives of Aesthetic Plastic Surgery*, *20*(2), 92-96. https://doi.org/10.14730/aaps.2014.20.2.92

Panda, A. K., & Chowdhary, A. (2021). Non-surgical modalities of facial rejuvenation and aesthetics. *Oral and maxillofacial surgery for the clinician*, 661-689.

Pandel, R., Poljšak, B., Godic, A., & Dahmane, R. (2013). Skin photoaging and the role of antioxidants in its prevention. *International Scholarly Research Notices*, *2013*(1), 930164. https://doi.org/10.1155/2013/930164

Pessa, J. E., Nguyen, H., John, G. B., & Scherer, P. E. (2014). The anatomical basis for wrinkles. *Aesthetic surgery journal*, *34*(2), 227-234. https://doi.org/10.1177/1090820X13517896

Pillai, S., Oresajo, C., & Hayward, J. (2005). Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation–a review. *International journal of cosmetic science*, *27*(1), 17-34. https://doi.org/10.1111/j.1467-2494.2004.00241.x

Puizina-Ivic, N. J. A. D. A. (2008). Skin aging. *Acta Dermatovenerologica Alpina Panonica Et Adriatica*, *17*(2), 47.

Ramli, F., Hamid, M. A., & Bohari, S. P. M. (2023). Melanogenesis inhibition effect of ethanolic Andrographis paniculata leaf extract via suppression of tyrosinase and MITF expression. *Journal of Applied Pharmaceutical Science*, *13*(1), 128-138. https://doi.org/10.7324/JAPS.2023.130107

Ranu, H., Thng, S., Goh, B. K., Burger, A., & Goh, C. L. (2011). Periorbital hyperpigmentation in Asians: an epidemiologic study and a proposed classification. *Dermatologic surgery*, *37*(9), 1297-1303. https://doi.org/10.1111/j.1524-4725.2011.02065.x

Roberts, W. E. (2014). Periorbital hyperpigmentation: review of etiology, medical evaluation, and aesthetic treatment. *Journal of drugs in dermatology: JDD*, *13*(4), 472-482.

Roh, M. R., Kim, T. K., & Chung, K. Y. (2009). Treatment of infraorbital dark circles by autologous fat transplantation: a pilot study. *British Journal of Dermatology*, *160*(5), 1022-1025. https://doi.org/10.1111/j.1365-2133.2009.09066.x

Rowe, D. J., & Guyuron, B. (2010). Environmental and genetic factors in facial aging in twins. *Textbook of Aging Skin*, 441-446.

Samargandy, S., & Raggio, B. S. (2019). *Skin Resurfacing Chemical Peels.* StatPearls Publishing.

Samson, N., Fink, B., Matts, P. J., Dawes, N. C., & Weitz, S. (2010). Visible changes of female facial skin surface topography in relation to age and attractiveness perception. *Journal of Cosmetic Dermatology*, *9*(2), 79-88. https://doi.org/10.1111/j.1473-2165.2010.00489.x

Sarkar, R., Ranjan, R., Garg, S., Garg, V. K., Sonthalia, S., & Bansal, S. (2016). Periorbital hyperpigmentation: a comprehensive review. *The Journal of clinical and aesthetic dermatology*, *9*(1), 49.

Sawant, O., & Khan, T. (2020). Management of periorbital hyperpigmentation: An overview of nature-based agents and alternative approaches. *Dermatologic Therapy*, *33*(4), e13717. https://doi.org/10.1111/dth.13717

Sharad, J. (2012). Dermal fillers for the treatment of tear trough deformity: a review of anatomy, treatment techniques, and their outcomes. *Journal of cutaneous and aesthetic surgery*, *5*(4), 229-238. https://doi.org/10.4103/0974-2077.104910

Sheeja, K., Shihab, P. K., & Kuttan, G. (2006). Antioxidant and anti-inflammatory activities of the plant Andrographis paniculata Nees. *Immunopharmacology and immunotoxicology*, *28*(1), 129-140. https://doi.org/10.1080/08923970600626007

Surface Imaging Solutions Limited. (n.d.). *The VISIA® Skin Complexion Analysis System.* https://www.surfaceimaging.co.uk/visia-4

Tabata, N., O'Goshi, K., Zhen, Y. X., Kligman, A. M., & Tagami, H. (2000). Biophysical assessment of persistent effects of moisturizers after their daily applications: evaluation of corneotherapy. *Dermatology*, *200*(4), 308-313. https://doi.org/10.1159/000018393

Tamura, B. M., & Odo, M. Y. (2011). Classification of periorbital wrinkles and treatment with Botulinum Toxin Type A. *Surg Cosmet Dermatol*, *3*(2), 129-34.

Uitto, J. (1997). Understanding premature skin aging. *New England Journal of Medicine*, *337*(20), 1463-1465. https://doi.org/10.1056/NEJM199711133372011

Uitto, J., Fazio, M. J., & Olsen, D. R. (1989). Molecular mechanisms of cutaneous aging: age-associated connective tissue alterations in the dermis. *Journal of the American Academy of Dermatology*, *21*(3), 614-622. https://doi.org/10.1016/S0190-9622(89)70228-0

Vance, M. L. (2003). Can growth hormone prevent aging?. *New England Journal of Medicine*, *348*(9), 779-780. https://doi.org/10.1056/NEJMp020186

Varma, A., Padh, H., & Shrivastava, N. (2011). Andrographolide: a new plant-derived antineoplastic entity on horizon. *Evidence-Based Complementary and Alternative Medicine*, *2011*(1), 815390. https://doi.org/10.1093/ecam/nep135

Vavouli, C., Katsambas, A., Gregoriou, S., Teodor, A., Salavastru, C., Alexandru, A., & Kontochristopoulos, G. (2013). Chemical peeling with trichloroacetic acid and lactic acid for infraorbital dark circles. *Journal of cosmetic dermatology*, *12*(3), 204-209. https://doi.org/10.1111/jocd.12044

Vetriselvan, S., & Subasini, U. (2012). Hepatoprotective activity of Andrographis paniculata. *International journal of Research in Pharmaceutical and Nano Sciences*, *1*(2), 307-316.

Vetvicka, V., & Vannucci, L. (2021). Biological properties of andrographolide, an active ingredient of Andrographis Paniculata: A narrative review. *Annals of Translational Medicine*, *9*(14). https://doi.org/10.21037/atm-20-7830

Vijaykumar, K., Murthy, P. B., Kannababu, S., Syamasundar, B., & Subbaraju, G. V. (2007). Estimation of Adrographolide in Andrographis paniculata herb, extracts and dosage forms. *International Journal of Applied Science and Engineering*, *5*(1), 27-39.

Warren, M. P., & Halpert, S. (2004). Hormone replacement therapy: controversies, pros and cons. *Best Practice & Research Clinical Endocrinology & Metabolism*, *18*(3), 317-332. https://doi.org/10.1016/j.beem.2004.02.005

Watanabe, S., Nakai, K., & Ohnishi, T. (2006). Condition Known as "Dark Rings Under the Eyes" in the Japanese Population is a Kind of Dermal Melanocytosis Which can be Successfully Treatedby Q-Switched Ruby Laser. *Dermatologic surgery*, *32*(6), 785-789. https://doi.org/10.1111/j.1524-4725.2006.32161.x

Worakunphanich, W., Thavorncharoensap, M., Youngkong, S., Thadanipon, K., & Thakkinstian, A. (2021). Safety of Andrographis paniculata: A systematic review and meta-analysis. *Pharmacoepidemiology and Drug Safety*, *30*(6), 727-739. https://doi.org/10.1002/pds.5190

You, J., Roh, K. B., Li, Z., Liu, G., Tang, J., Shin, S., . . . Jung, E. (2015). The antiaging properties of Andrographis paniculata by activation epidermal cell stemness. *Molecules*, *20*(9), 17557-17569. https://doi.org/10.3390/molecules200917557

Yousef, H., Alhajj, M., & Sharma, S. (2017). *Anatomy, skin (integument), epidermis*. StatPearls Publishing

Yun, Y., Ko, Y., Ahn, J. H., Jang, B. H., Kim, K., Ko, S. G., & Choi, I. (2017). Topical application of Jaungo in atopic dermatitis patients: study protocol for a randomized, controlled trial. *Trials*, *18*, 1-8. https://doi.org/10.1186/s13063-017-1920-9

Zenker, S. (2012). Carboxytherapy: Carbon dioxide injections in aesthetic medicine. *Prime Europe*, *2*(1), 42-50.

Zhan, J. Y. X., Wang, X. F., Liu, Y. H., Zhang, Z. B., Wang, L., Chen, J. N., ... & Lai, X. P. (2016). Andrographolide sodium bisulfate prevents UV-induced skin photoaging through inhibiting oxidative stress and inflammation. *Mediators of inflammation*, *2016*(1), 3271451. https://doi.org/10.1155/2016/3271451

Zouboulis, C. C., Ganceviciene, R., Liakou, A. I., Theodoridis, A., Elewa, R., & Makrantonaki, E. (2019). Aesthetic aspects of skin aging, prevention, and local treatment. *Clinics in dermatology*, *37*(4), 365-372. https://doi.org/10.1016/j.clindermatol.2019.04.002

APPENDIX A

DOCUMENTARY PROFF OF ETHICAL CLEARANCE

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

หนังสือรับรองด้านจริยธรรมการวิจัย

COA: 85/2024 รหัสโครงการวิจัย: EC 23148-20

ชื่อโครงการวิจัย : การศึกษาประสิทธิภาพของครีมสกัดจากฟ้าทะลายโจร 2% ต่อการลดเลือนริ้วรอยและรอยคล้ำรอบดวงตา

ชื่อผู้วิจัยหลัก: พญ. แนน พู พู มน

สำนักวิชา: เวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ

ผู้สนับสนุนการวิจัย: ทุนส่วนตัว

การรับรอง :

(1) โครงการวิจัย	ฉบับที่ 3 วันที่ 11 มีนาคม 2567
(2) เอกสารข้อมูลและขอความยินยอมเข้าร่วมการวิจัย	ฉบับที่ 3 วันที่ 11 มีนาคม 2567
(3) เอกสาโฆษณา	ฉบับที่ 3 วันที่ 11 มีนาคม 2567
(4) แบบสอบถาม และ แบบบันทึกข้อมูล	ฉบับที่ 3 วันที่ 11 มีนาคม 2567
(5) ผู้วิจัย และผู้วิจัยร่วม	
- พญ. แนน พู พู มน	- ดร. พญ.สิรินทิพย์ ชัยชโลทรกุล

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

วันที่รับรองด้านจริยธรรมของโครงการวิจัย: 17 เมษายน 2567

วันสิ้นสุดการรับรอง: 16 เมษายน 2568

ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: 1 ปี

ลงนาม .....

(รองศาสตราจารย์ พลตรีหญิง แพทย์หญิง แสงแข ชำนาญวนกิจ)

ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

CERTIFICATE OF APPROVAL

COA: 85/2024 **Protocol No: EC 23148-20**

Title: Preparation of chitosomes loaded with *Eleutherine palmifolia* extract for use as cosmetic active ingredient

Principal investigator: Nann Phoo Phoo Mon, M.D.

School: Anti-Aging and Regenerative Medicine

Funding support: Personal funding

Approval:

1)	Research protocol	Version 3 Date March 11, 2024
2)	Information sheet and informed consent documents	Version 3 Date March 11, 2024
3)	Advertisement	Version 3 Date March 11, 2024
4)	Questionnaire form and case record form	Version 3 Date March 11, 2024
5)	Principal investigator and Co-investigators	
	- Nann Phoo Phoo Mon, M.D.	- Sirintip Chaichalotornkul, M.D.

The aforementioned documents have been reviewed and approved by the Mae Fah Luang University Ethics Committee on Human Research in compliance with international guidelines such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice (ICH - GCP)

Date of Approval: April 17, 2024
Date of Expiration: April 16, 2025
Frequency of Continuing Review: 1 year

Somphae
.....
(Assoc. Prof., Maj. Gen. Sangkae Chamnanvanakij, M.D.)
Chairperson of the Mae Fah Luang Ethics Committee on Human Research

APPENDIX B

INROMED CONSENT FORM

ข้าพเจ้า _____ ตัดสินใจเข้าร่วมการวิจัยเรื่อง การศึกษาประสิทธิภาพของครีมสารสกัดจากฟ้าทะลายโจร 2% (Andrographis paniculata Extract Cream) ต่อการลดเลือนริ้วรอยรอบดวงตา ซึ่งข้าพเจ้าได้รับข้อมูลและคำอธิบายเกี่ยวกับการวิจัยนี้แล้ว และได้มีโอกาสซักถามและได้รับคำตอบเป็นที่พอใจแล้ว ข้าพเจ้ามีเวลาเพียงพอในการอ่านและทำความเข้าใจข้อมูลในเอกสารให้ข้อมูลสำหรับผู้เข้าร่วมการวิจัยอย่างถี่ถ้วน และได้รับเวลาเพียงพอในการตัดสินใจว่าจะเข้าร่วมการวิจัยนี้

ข้าพเจ้ารับทราบว่า ข้าพเจ้าสามารถปฏิเสธการเข้าร่วมการวิจัยนี้ได้โดยอิสระ และระหว่างการเข้าร่วมการวิจัย ข้าพเจ้ายังสามารถถอนตัวออกจากการวิจัยได้ทุกเมื่อ โดยไม่ส่งผลกระทบต่อการดูแลรักษา หรือสิทธิที่ข้าพเจ้าพึงมี

โดยการลงนามนี้ ข้าพเจ้าไม่ได้สละสิทธิใด ๆ ที่ข้าพเจ้าพึงมีตามกฎหมาย และหลังจากลงนามแล้ว ข้าพเจ้าจะได้รับเอกสารข้อมูลและขอความยินยอมไว้จำนวน 1 ชุด

ลายมือชื่อผู้เข้าร่วมการวิจัย _____ วัน-เดือน-ปี _____

(_____)

..... (กรณีที่ผู้เข้าร่วมการวิจัยอ่านหนังสือไม่ออกแต่ฟังเข้าใจ) .....
ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในเอกสารข้อมูลและขอความยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนามหรือพิมพ์ลายนิ้วมือของข้าพเจ้าในหนังสือนี้ด้วยความสมัครใจ
ลงนาม/พิมพ์ลายนิ้วมือผู้เข้าร่วมการวิจัย _____ วัน-เดือน-ปี _____
(_____)

ลายมือชื่อผู้ขอความยินยอม _____ วัน-เดือน-ปี _____

(_____)

คำรับรองของพยานผู้ไม่มีส่วนได้เสียกับการวิจัย (กรณีที่ผู้เข้าร่วมการวิจัยอ่านหนังสือไม่ออกแต่ฟังเข้าใจ)

ข้าพเจ้าได้อยู่ร่วมในกระบวนการขอความยินยอมและยืนยันว่า ผู้ขอความยินยอมได้อ่าน/อธิบายเอกสารข้อมูลให้แก่ _____ ซึ่งผู้มีชื่อข้างต้นมีโอกาสซักถามข้อสงสัยต่าง ๆ และได้ให้ความยินยอมเข้าร่วมการวิจัยโดยอิสระ หลังจากรับทราบข้อมูลที่มีอยู่ตรงตามที่ปรากฏในเอกสารนี้แล้ว

ลายมือชื่อพยาน _____ วัน-เดือน-ปี _____

(_____)

APPENDIX C

RESEARCH PROFILE (CONFIDENTIAL)

Questionaries: Patient Record Form

THE EFFICACY OF 2% ANDROGRAPHIS PANICULATA EXTRACT FOR PERIORBITAL WRINKLE REDUCTION

Patient Record Form

General Information

1. Date_ _/_ _/_ _
2. Hospital No._ _ _ _
3.DOB _ _ _ (in year)
4. Name _ _ _ _ _ _ _ _ _ _ _
5. Tel _ _ _ _ _ _ _ _ _ _
6. E-mail _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
7.Address _
8.Gender ■ Male ■ Female
   8.1 Pregnancy or lactation ■ 1. Yes ■ 2.No
9. Occupation
   - Government officer
   - Business owner
   - Housewife
   - Student
   - Employee
   - Others Specify _
10. Underlying disease _
11.Photosensitivity or Drug Induced Hypersensitivity 1. Yes 2. No
12.Personal medication and supplement

12.1Chemotherapy

12.2Active inflammatory skin disease, open wound in the treatment area

12. History of malignant or premalignant lesions in the treatment area

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

if yes, specify _

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

If yes, specify _

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

If yes

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

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

APPENDIX D

STANDARDIZED PHOTOGRAPHS OF SUBJECTS

2% Andrographis paniculata extract cream	
	
BEFORE	AFTER

Figure D1 Photos of one of the volunteers at baseline and 12 weeks after using 2% Andrographis paniculata cream (Right)

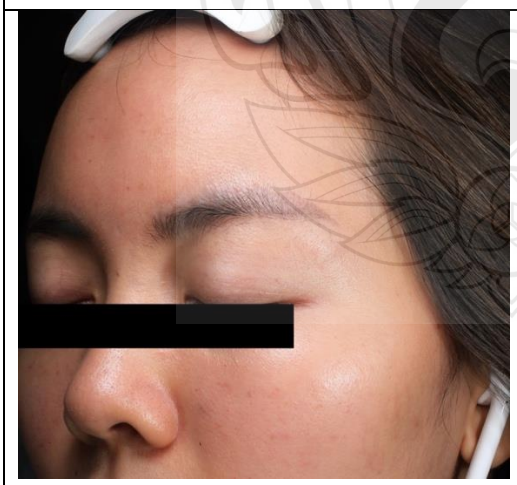
Placebo cream	
	
BEFORE	AFTER

Figure D2 Photos of one of the volunteers at baseline and 12 weeks after using placebo cream (Left)

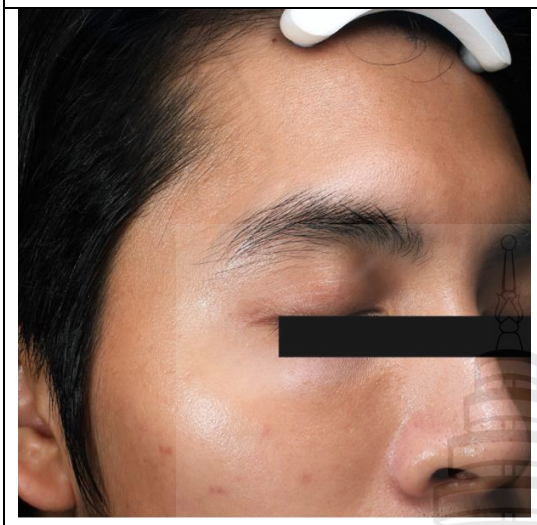
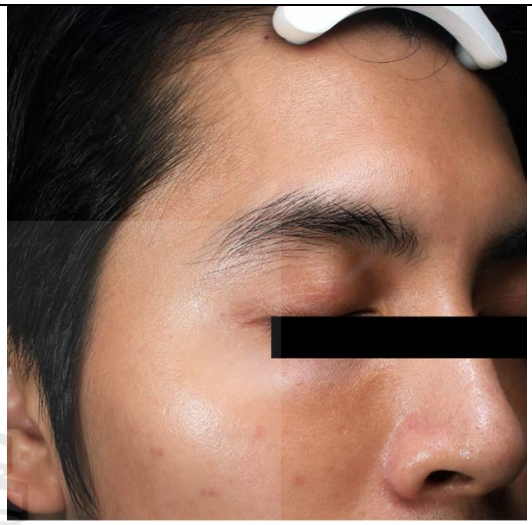
2% Andrographis paniculata extract cream	
	
BEFORE	AFTER

Figure D3 Photos of one of the volunteers at baseline and 12 weeks after using 2% Andrographis paniculata cream (Right)

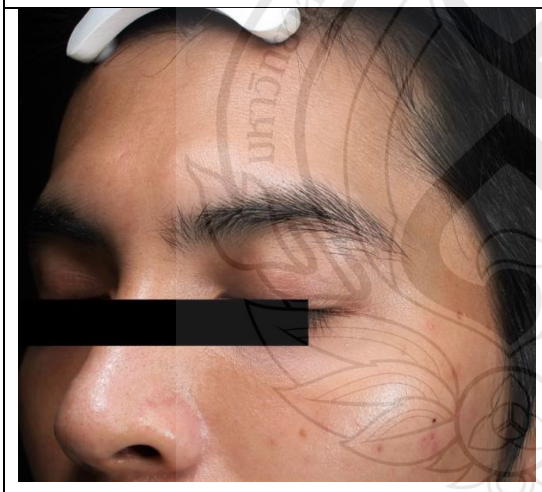
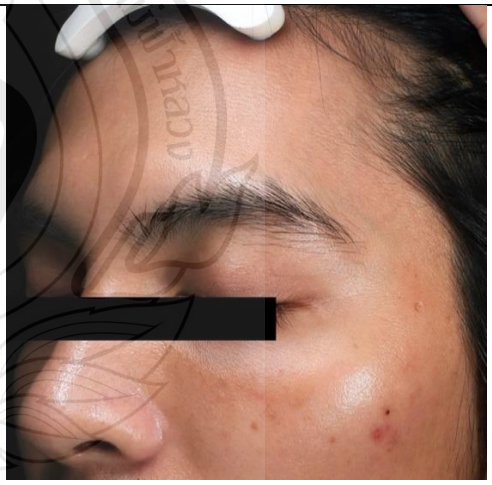
Placebo cream	
	
BEFORE	AFTER

Figure D4 Photos of one of the volunteers at baseline and 12 weeks after using placebo cream (Left)

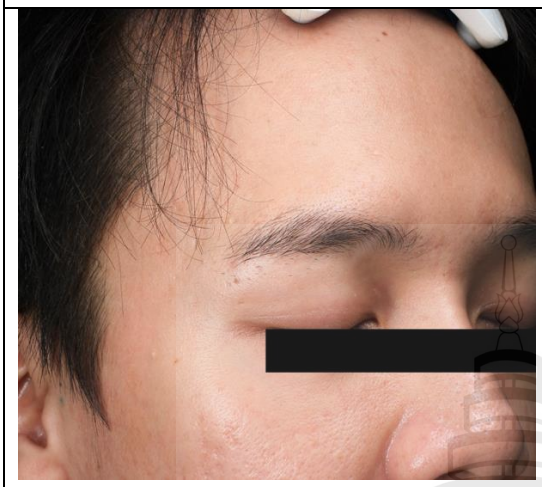
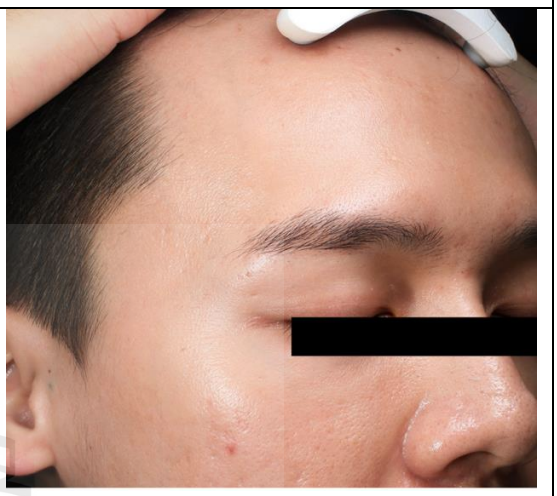
2% Andrographis paniculata extract cream	
	
BEFORE	AFTER

Figure D5 Photos of one of the volunteers at baseline and 12 weeks after using 2% Andrographis paniculata cream (Right)

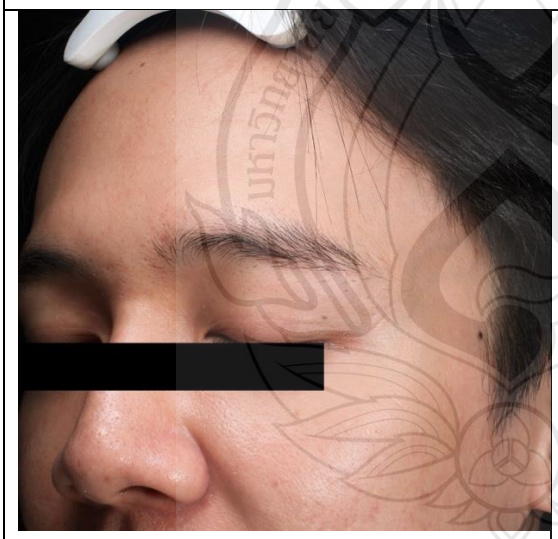
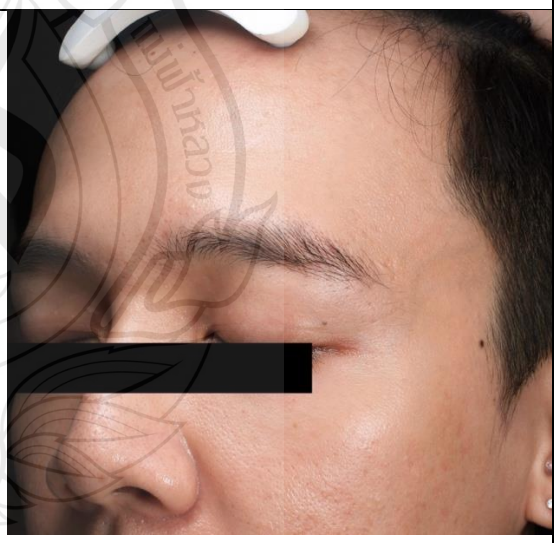
Placebo cream	
	
BEFORE	AFTER

Figure D6 Photos of one of the volunteers at baseline and 12 weeks after using placebo cream (Left)

APPENDIX E

MATERIAL

Figure E1 Packaging of 2% Andrographis paniculata extract cream and placebo cream

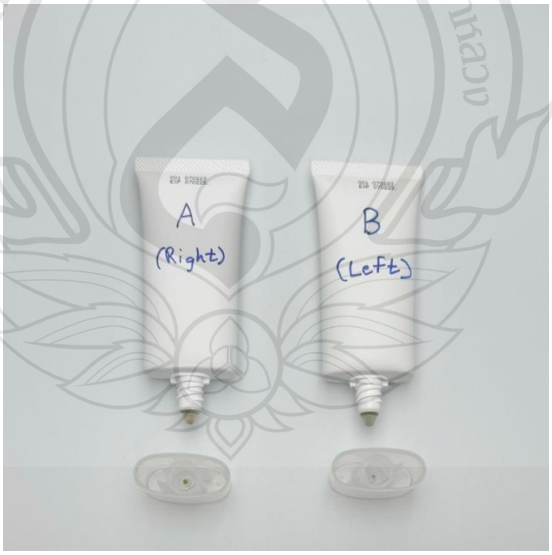


Figure E2 Photo of 2% Andrographis paniculata extract cream and placebo cream

APPENDIX F

RESEARCH RECORD FORM

RESEARCH RECORD: RESEARCHER'S PART CASE RECORD FORM, CRF

Volunteer number.....

1. Cutometer MP 580

Cutometer	No.	Right	Left
Week 0	1		
	2		
	3		
	Average		
Week 4	1		
	2		
	3		
	Average		
Week 8	11		
	2		
	3		
	Average		
Week 12	1		
	2		
	3		
	Average		

2. VISIA® complexion analysis system

Visia	No.	Right	Left
Week 0	1		
	2		
	3		
	Average		
Week 4	1		
	2		
	3		
	Average		
Week 8	11		
	2		
	3		
	Average		
Week 12	1		
	2		
	3		
	Average		

3. Mexameter

Mexameter	No.	Right	Left
Week 0	1		
	2		
	3		
	Average		
Week 4	1		
	2		
	3		
	Average		
Week 8	11		
	2		
	3		
	Average		
Week 12	1		
	2		
	3		
	Average		

4. Rao-Goldman 5-Points Visual Score evaluated by 3 Dermatologists

Dermatologist 1	Week 0	Week 12
Left		
Right		
Dermatologist 2	Week 0	Week 12
Left		
Right		
Dermatologist 3	Week 0	Week 12
Left		
Right		

APPENDIX G

CLINICAL EVALUATION

Table G1 Periorbital skin elasticity measurement by Cutometer® on 2% Andrographis paniculata eye cream treated side

ID No.	2% Andrographis paniculata extract cream							
	Week 0		Week 4		Week 8		Week 12	
	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye
161201	77.9	70.84	62.9	77.23	62.91	84.23	90.98	88.51
161202	77.03	72.28	78.33	76.83	79.03	81.74	88.72	83.83
161203	69.14	64.57	82.55	66.05	104.48	78.17	91.5	79.04
161204	75.43	62.44	62.76	72.51	85.96	89.2	91.16	103.41
161205	63.07	74.57	75.51	76.83	75.3	80.09	76.95	87.59
161206	80.87	77.2	85.93	102.61	90.47	106.82	78.62	118.59
161207	65.8	73.04	81.46	86.83	77.98	92.55	88.56	109.85
161208	55.45	65.8	80.52	75.7	93.46	80.71	94	69.69
161209	82.15	49.16	85.23	55.22	87.49	65.5	107.39	80.77
1612010	82.43	62.14	87.66	95.18	88.02	86.58	85.08	113.14
1612011	93.23	69.83	97.61	94.35	99.37	96.95	98.46	103.2
1612012	81.7	64.5	86.33	69.73	87.85	77.01	93.12	82.58
1612013	88.51	69.37	89.57	76.82	99.4	95.75	109.98	99.61
1612014	79.01	62.53	98.2	64.03	92.28	78.29	97.85	112.71
1612015	87.99	72.38	109.16	71.46	105.7	73.37	112.95	79.01
1612016	71.03	72	82.36	75.64	85.67	96.1	86.67	99.79
1612017	76.39	85.64	78.46	88.1	86.64	104.23	86.7	109.66
1612018	93.65	70.35	83.3	91.32	76.86	93.29	91.67	96.81
1612019	85.12	38.08	86.97	70.3	88.51	95.81	92.3	108.28

Table G2 Periorbital skin elasticity measurement by Cutometer® on placebo eye cream treated side

ID No.	Placebo Cream							
	Week 0		Week 4		Week 8		Week 12	
	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye
161201	82.61	72.5	59.07	50.33	80.11	46.56	75.5	90.64
161202	84.05	71.63	108.66	75.79	81.63	80.66	75.71	74.41
161203	76.34	63.74	77.89	79.04	77.05	101.28	66.93	78.63
161204	74.2	70.03	74.34	50.12	85.09	63.74	95.29	40.16
161205	86.34	57.67	68.68	68.75	59.97	62.87	59.48	56.54
161206	88.96	75.47	104.44	69.36	92.71	82.84	90.46	62.11
161207	84.8	60.4	88.66	77.44	88.44	66.4	101.72	63.54
161208	77.56	50.05	77.53	76.07	76.59	86.77	61.57	82.43
161209	60.93	76.75	57.07	68.34	61.38	72.33	72.65	102.76
1612010	73.91	77.03	97.02	57.28	82.47	73.03	105.02	68.89
1612011	81.59	87.84	96.19	57.21	82.83	55.06	55.08	69.46
1612012	76.27	76.3	78.58	87.48	62.88	66.22	74.46	81.09
1612013	103.13	83.12	78.65	74.68	91.64	81.43	91.49	82.4
1612014	74.3	73.61	65.88	101.9	74.17	85.22	104.59	88.27
1612015	84.14	82.6	73.3	74.08	99.26	89.72	62.89	79.32
1612016	83.77	65.63	47.48	78.76	91.99	63.35	62.67	70.28
1612017	97.4	70.99	79.94	83.29	100.12	77.8	101.54	71.34
1612018	82.12	88.26	93.16	80.13	59.17	64.92	68.69	78.89
1612019	49.85	79.72	92.13	85.49	91.7	71.04	100.16	79.85

Table G3 Periorbital hyperpigmentation measurement by Mexameter® on 2% Andrographis paniculata eye cream treated side

ID No.	2% Andrographis paniculata extract cream							
	Week 0		Week 4		Week 8		Week 12	
	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye
161201	244	170	367	269	237	258	301	117
161202	254	183	172	118	191	200	204	100
161203	330	102	159	171	194	304	234	187
161204	207	186	308	203	236	288	251	211
161205	282	174	285	205	134	183	186	188
161206	233	222	275	179	136	247	223	81
161207	232	232	275	236	179	230	211	185
161208	318	313	234	146	209	268	216	121
161209	300	264	179	195	241	146	165	226
1612010	300	184	240	209	312	232	212	210
1612011	332	167	193	230	275	252	240	140
1612012	247	240	297	241	213	105	296	163
1612013	291	145	226	143	221	135	267	134
1612014	225	310	183	121	161	90	337	187
1612015	267	276	215	271	221	182	165	241
1612016	237	189	261	221	205	234	262	138
1612017	252	124	200	163	242	275	221	217
1612018	285	285	183	286	172	200	339	162
1612019	192	208	251	209	254	281	162	148

Table G4 Periorbital hyperpigmentation measurement by Mexameter® on placebo eye cream treated side

ID No.	Placebo							
	Week 0		Week 4		Week 8		Week 12	
	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye	Crow's Feet	Undereye
161201	188.87	198.54	249.67	207.71	141.88	197.19	147.92	216.48
161202	213.42	184.17	313.49	231.02	144.88	240.48	247.28	201.74
161203	230.89	152.85	192.38	211.44	119.17	250.74	132.27	232.01
161204	122.2	266.66	251.31	145.73	143.28	281.24	279.7	189.98
161205	303	302.86	254.73	234.75	330.01	206.07	124.63	170.46
161206	263.09	343.33	254.1	170.64	322.23	262.5	213.58	249.3
161207	162.14	312.54	248.76	162.73	167.7	227.67	180.47	270.51
161208	173.7	129.43	185.16	289.62	175.11	191.65	285.35	272.51
161209	226.5	160.38	240.79	147.25	133.29	183.96	218.57	209.96
1612010	151.91	238.15	206.4	157.17	204.22	289.47	234.62	208.9
1612011	169.5	276.44	134.93	247.47	261.86	197.09	290.24	159.18
1612012	321.61	252.91	205.36	231.41	234.21	197.86	203.32	112.09
1612013	339	104.15	247.52	205.59	106.84	240.26	246.65	233.56
1612014	200.73	180.62	248.39	165.05	278.55	267.18	215.61	271.59
1612015	304.58	318.98	218.77	274.33	170.07	182.86	249.48	231.62
1612016	273.84	152.05	217.18	180.57	157.82	205.98	230.01	219.57
1612017	175.58	229.65	180.38	239.71	238.8	218.87	215.21	166.74
1612018	214.74	290.27	242.19	186.24	176.82	102.2	218.69	135
1612019	393.32	174.66	266.88	165.67	307.54	277.73	244.12	215.55

Table G5 Periorbital wrinkle measurement by Visioscan® on 2% Andrographis paniculata eye cream treated side

No.	ID No.	2% Andrographis paniculata extract cream			
		Week 0	**Week 4**	**Week 8**	**Week 12**
1	161201	50	51	53	54
2	161202	48	48	50	52
3	161203	52	53	55	56
4	161204	47	48	48	52
5	161205	51	52	52	56
6	161206	53	52	53	56
7	161207	49	51	52	55
8	161208	46	54	53	55
9	161209	52	55	56	70
10	1612010	50	52	51	56
11	1612011	51	53	57	60
12	1612012	49	51	54	59
13	1612013	48	50	51	54
14	1612014	52	58	60	68
15	1612015	50	54	59	58
16	1612016	47	49	60	64
17	1612017	53	57	60	70
18	1612018	46	49	53	55
19	1612019	49	51	53	57

Table G6 Periorbital wrinkle measurement by Visioscan® on placebo eye cream treated side

ID No.	2% Andrographis paniculata extract cream			
	Week 0	**Week 4**	**Week 8**	**Week 12**
161201	55	53	51	51
161202	60	59	60	62
161203	52	51	50	51
161204	58	59	57	56
161205	56	54	53	55
161206	54	53	54	52
161207	59	57	60	56
161208	57	56	57	55
161209	53	52	54	52
1612010	58	57	61	57
1612011	55	53	55	52
1612012	56	51	30	21
1612013	52	42	40	39
1612014	64	61	62	60
1612015	54	55	53	52
1612016	58	60	57	56
1612017	56	58	57	49
1612018	53	54	58	51
1612019	57	59	58	56

Table G7 Dermatologists' evaluation score for 2% Andrographis paniculata eye cream treatment at 4^{th}, 8^{th}, 12^{th} week

Volunteer No.	Doc 1	Doc 2	Doc 3
	4^{th} week		
161201	1	0	1
161202	0	1	1
161203	1	0	1
161204	2	2	0
161205	2	1	1
161206	2	0	2
161207	1	2	1
161208	0	2	2
161209	1	1	2
1612010	2	0	2
1612011	1	1	0
1612012	0	1	1
1612013	2	0	0
1612014	2	2	0
1612015	3	0	1
1612016	0	2	0
1612017	0	1	1
1612018	1	0	1
1612019	1	1	0
	8^{th} week		
161201	1	1	0
161202	3	2	1
161203	3	0	3
161204	3	3	0
161205	2	2	2
161206	1	2	1

Table G7 (continued)

Volunteer No.	Doc 1	Doc 2	Doc 3
161207	2	0	2
161208	2	1	1
161209	0	2	2
1612010	1	1	2
1612011	2	2	0
1612012	1	1	2
1612013	2	0	2
1612014	2	1	1
1612015	1	2	1
1612016	1	3	2
1612017	0	3	3
1612018	1	0	1
1612019	0	1	1
	12th week		
161201	1	1	0
161202	3	2	1
161203	0	3	3
161204	3	0	3
161205	2	1	3
161206	2	2	0
161207	2	1	1
161208	3	3	0
161209	2	0	2
1612010	0	2	2
1612011	2	2	2
1612012	3	1	2
1612013	2	2	0
1612014	1	1	2

Table G7 (continued)

Volunteer No.	Doc 1	Doc 2	Doc 3
1612015	3	0	3
1612016	0	2	2
1612017	1	2	1
1612018	2	0	2
1612019	0	1	1

Table G8 Dermatologists' evaluation score for placebo eye cream treatment at 4th, 8th, 12th week

Volunteer No.	Doc 1	Doc 2	Doc 3
	4th week		
PP01	0	-1	-1
PP02	1	-2	-1
PP03	0	-2	0
PP04	1	0	-1
PP05	0	1	1
PP06	-2	1	-1
PP07	0	1	-1
PP08	1	0	1
PP09	1	1	0
PP10	2	0	0
PP11	1	0	1
PP12	-1	1	0
PP13	-1	-1	0
PP14	0	-1	-1
PP15	0	1	1
PP16	-2	1	-1
PP17	0	-1	-1

Table G8 (continued)

Volunteer No.	Doc 1	Doc 2	Doc 3
PP18	-1	-1	0
PP19	-1	0	-1
8th week			
PP01	-1	0	-1
PP02	1	1	0
PP03	1	0	1
PP04	2	-1	1
PP05	-1	2	1
PP06	1	0	1
PP07	1	0	-1
PP08	1	1	0
PP09	1	2	-1
PP10	0	-1	1
PP11	0	1	1
PP12	1	0	1
PP13	1	1	0
PP14	0	1	1
PP15	-1	1	0
PP16	2	-1	1
PP17	1	0	1
PP18	-1	-1	0
PP19	-1	0	-1
12th week			
PP01	-1	-1	0
PP02	1	1	0
PP03	0	1	1
PP04	1	0	1
PP05	0	-1	1

Table G8 (continued)

Volunteer No.	Doc 1	Doc 2	Doc 3
PP06	0	1	1
PP07	1	1	0
PP08	2	-1	1
PP09	-1	2	1
PP10	0	1	1
PP11	1	0	-1
PP12	1	1	0
PP13	-1	1	2
PP14	0	1	1
PP15	-1	1	0
PP16	1	0	1
PP17	1	-1	2
PP18	-1	0	-1
PP19	0	-1	-1

Table G9 Patients' satisfaction score for 2% Andrographis paniculata extract cream

Volunteer No.	Satisfaction level
PP01	2
PP02	4
PP03	4
PP04	3
PP05	4
PP06	3
PP07	4
PP08	3
PP09	3
PP10	4

Table G9 (continued)

Volunteer No.	Satisfaction level
PP11	3
PP12	3
PP13	4
PP14	3
PP15	2
PP16	3
PP17	3
PP18	2
PP19	2

Table G10 Patients' satisfaction score for placebo eye cream

Volunteer No.	Satisfaction level
PP01	0
PP02	2
PP03	2
PP04	1
PP05	2
PP06	1
PP07	2
PP08	1
PP09	1
PP10	1
PP11	1
PP12	1
PP13	1
PP14	0
PP15	0

Table G10 (continued)

Volunteer No.	Satisfaction level
PP16	0
PP17	0
PP18	0
PP19	0

CURRICULUM VITAE

NAME Nann Phoo Phoo Mon

EDUCATIONAL BACKGROUND

2019	Bachelor of Medicine and Bachelor of Surgery, University of Medicine, Mandalay, Myanmar
2015	Bachelor of Medicine and Bachelor of Surgery, University of Medicine, Magway, Myanmar

WORK EXPERIENCE

2018-2019	Internship Mandalay General Hospital, 300 Bedded Children Hospital, 300 Bedded Mandalay Orthopedic Hospital, Central Woman Hospital, Mandalay, Myanmar.
2019-2020	Medical Officer Lynn Thit Sar Hospital, Kalaw, Myanmar Medical Officer Grand Mandalay Hospital, Mandalay, Myanmar