EFFICACY OF 3% EUTERPE OLERACEA EXTRACT CREAM FOR REDUCTION OF PERIORBITAL WRINKLES

HSU MON ZAW

MASTER OF SCIENCE
IN
DERMATOLOGY

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

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Author	Hsu Mon Zaw
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ABSTRACT

Background: Periorbital wrinkle occurs because of extrinsic and intrinsic aging. Asides from aging, genetics, smoking, drinking and even certain sleeping positions can contribute to wrinkle development. Free radical damage is proven to be one of the main reasons for aging and wrinkles. Several anti-aging and anti-wrinkle treatments are being studied upon these days but the efficacy of *Euterpe oleracea* has not been established yet. *Euterpe oleracea* or the acai palm is known to possess the highest amount of antioxidant capacity among fruits and vegetables. Some of the various constituents found are anthocyanin, orientin, scoparin, isovitexin and resveratrol. Among them, anthocyanins are known as free radical scavengers against harmful oxidants such as reactive oxygen and nitrogen species. They are also found to be photoprotective.

Objective: To study the efficacy of topical 3% *Euterpe oleracea* extract cream in decreasing periorbital wrinkles

Methods and materials: 20 subjects of age between 30 to 50 years were recruited for this clinical experimental study. Both male and female participated and the study lasted for 12 weeks. Volunteers were instructed to apply 3% *Euterpe oleracea* extract cream twice daily to periorbital area during the 12-week period. Skin

parameter measurements such as skin elasticity, hydration and wrinkle score assessment by dermatologists were taken at baseline, 4^{th}, 8^{th} and 12^{th} week respectively.

Result: The mean of cutometer score (skin elasticity) increased from the start to week 12 and it was statistically significant. The mean corneometer score (skin hydration) also improved significantly from baseline to week 12. A rise in wrinkle evaluation score was also recorded from week 4 to week 12 with a significance. There was no adverse effect in all subjects throughout the study with high patients' satisfactory score.

Conclusion: The study demonstrated that Acai palm extract decreased periorbital wrinkles when it is applied topically with no adverse effects. It could be an alternative anti-wrinkle treatment.

Keywords: *Euterpe oleracea*, Acai, Wrinkle, Elasticity, Hydration

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CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

People have always had tendencies to reduce the aging process of their skin. This is a phenomenon that is deeply rooted in the history of human civilization. It is widely known that Cleopatra, the queen of Egypt and a symbol of beauty, would often take two baths a day in donkey milk as it contains alpha-hydroxy acids for its role in the anti-aging process. There are many more topical treatments that were popularized throughout our history such as apple cider vinegar, honey and turmeric to more manmade treatments such as botulinum toxin, filler, thread lift and laser treatments.

The treatments mentioned above among with a plethora of others tackle similar aging issues like wrinkles, dark spots, dull skin and laxity. Wrinkles are skin folds or creases caused by a combination of factors. The first one is aging which can be classified into intrinsic and extrinsic aging. Intrinsic aging is a chronological process that is mainly influenced by genetical and hormonal factors whereas extrinsic aging is primarily caused by ultraviolet [UV] irradiation called Photoaging. UV lights degrade the collagen and elastin fibers by free radicals oxidation and expression of matrix metalloproteinases. As these layers are destroyed, the skin becomes weaker and wrinkles appear. Smoking also plays a role in the wrinkle formation by accelerating the aging process. Alcohol consumption promotes dehydration and dry skin are more prone to developing wrinkles. Other causes include repeated facial muscle expression, exposure to pollutants, toxins and diet.

Even though having wrinkles is not a disease, it imposes a discomfort for many people. Wrinkle formation cannot be stopped but there are various modalities targeted at reducing its appearance, varying from skincare application, botulinum toxin and filler injections, microdermabrasion, laser therapies to cosmetic surgeries.

Among them,botanical extracts are increasingly used for anti-aging purposes. They are also being used as an alternative to conventional treatments owing to their fewer side effects. Various research have shown their anti-microbial, anti-inflammatory, anti-proliferating, photo protective and wound healing properties (Reuter et al., 2010). The emergence of botanical extract as an anti-aging ingredient since they are rich in antioxidants. Besides, they are cost effective and easily available.

The acai fruit, coming from a specific palm tree called *Euterpe oleracea*, may prove to be one of the most promising botanical extracts constituting powerful antioxidants. Largely cultivated in the Brazilian Amazon, acai tree extracts have a growing demand around the globe exactly for their anti-inflammatory, antinociceptive, wound healing and cholesterol lowering properties (Sadowska-Krępa et al., 2014; Favacho et al., 2011; Oliveira de Souza et al., 2010; Kang & Kim, 2018). In addition to its use for anti-aging and acne treatment purposes, different parts of the acai palm trees are commonly used as alternative treatments for other medical problems in Brazil and Peru. A few examples include diarrhea being treated by the oil of the fruit, skin ulcers being washed with the fruit grind and jaundice, malaria, fever relief, diabetes and hair loss being treated by the infusion of the root. Even though there has always been a historical interest in acai berries regarding anti-aging, the data and enough research on it has been lacking.

When we dive into further details, its properties become more apparent. It is demonstrated that the fruit pulp consumption causes an increase in plasma antioxidant capacity (de Oliveira et al., 2019). Additionally, it is also proven that the dietary supplement of the fruit modulates ROS production and improves the gene expression of antioxidant enzymes in rats' liver under oxidative stress (Guerra et al., 2011). In a recent study, acai berry water extract was shown to promote wound healing. It also increases type I collagen, VEGF, and fibronectin productions whilst MMP-1 and IL-1β expression are inhibited. MMP-1 or matrix metalloprotenase 1 is a collagenase. Inhibition of MMP-1 results in reduction of collagenase and accelerates wound healing. IL-1β is responsible for T cell activation which in turn promotes inflammatory cells like lymphocytes and monocytes. Inhibition of IL-1β can be attributed to anti-inflammatory effects in the wound healing process (Kang & Kim, 2018).

According to previous studies, some of the constituents found in acai fruits are claimed to be polyphenols including anthocyanins, flavonoids, gallic acid and ferulic acids, lignans and lipids (Schauss et al., 2006). The constituents mentioned above are able to actively scavenge free radicals and/or chelate the metal iron, leading them to act as effective antioxidants (Guerra et al., 2011). There is good evidence that confirm the extracts can counteract the oxidative stress and aging processes in *Caenorhabditis elegans* [14]. Moreover, Petruk et al pretreated BALB/3T3 fibroblasts with acai extracts before inducing oxidative stress by UV-A radiation (100 J/cm^2). It was found that acai extract inhibits ROS (reactive oxygen species), intracellular glutathione depletion (GSH form) and lipid per oxidation thus protecting the fibroblasts against the negative effects of UV-A induced oxidative stress. The most active compounds responsible for the effects were found to be cyanidin and malvidin (Petruk et al., 2017). Based on these health-promoting benefits, the acai extract has been gaining interest in anti-aging cosmetic products.

E. oleracea, or the acai berry, has shown to have potential in preventing or retarding age-related diseases like cardiovascular diseases, cancer and aging skin. The fruit is consisted of such compounds as polyphenols and flavonoids that exert strong antioxidant actions. In the matter of choosing anti-aging products, those formulated from plants containing antioxidant, anti-inflammatory and free-radical-scavenging functions are preferred. The extract would be useful in reducing wrinkles because it has the properties of inhibiting the aging process. Evidence presents the acai berry water extract can increase type 1 collagen production and the dietary supplement can reduce oxidative stress. More importantly for this research, the extract has the quality of lowering MMP-1 expression.

The main reason of this study is to conduct that the antioxidant action of acai palm extract cream would be useful in treatment of wrinkles on face. Thus, the actual purpose is to study the efficacy and satisfaction of *E. oleracea* extract cream in wrinkles reduction.

1.2 Research Question

Can *Euterpe oleracea* extract cream be used to reduce periorbital wrinkles?

1.3 Objective

1.3.1 General Objective

To study the efficacy of topical 3% *Euterpe oleracea* extract cream in decreasing periorbital wrinkles

1.3.2 Specific Objective

1.3.2.1 Primary Objective

To compare the mean change of periorbital wrinkle reduction by topical 3% *Euterpe oleracea* extract cream from baseline to 12^{th} week visit, assessed by using Corneometer, Cutometer and VISIA® Complexion Analysis System

1.3.2.2 Secondary Objective

1. To observe safety and adverse effects of topical 3% *Euterpe oleracea* extract cream

2. To evaluate the participants' satisfaction score of topical 3% *Euterpe oleracea* extract cream

1.4 Hypothesis

1.4.1 Topical 3% *Euterpe oleracea* extract cream has good efficacy in periorbital wrinkle reduction.

1.4.2 Topical 3% *Euterpe oleracea* extract cream can be used safely, measured by assessment of adverse effects (pruritus, erythema, and others such as allergic contact dermatitis, post inflammatory hypopigmentation and hyperpigmentation).

1.4.3 Topical 3% *Euterpe oleracea* extract cream has high patients' satisfactory score for reduction of periorbital wrinkles.

1.5 Benefits

1.5.1 To use as an alternative method for decreasing the periorbital wrinkles

1.5.2 To use as a reference data for further research

1.6 Conceptual Framework

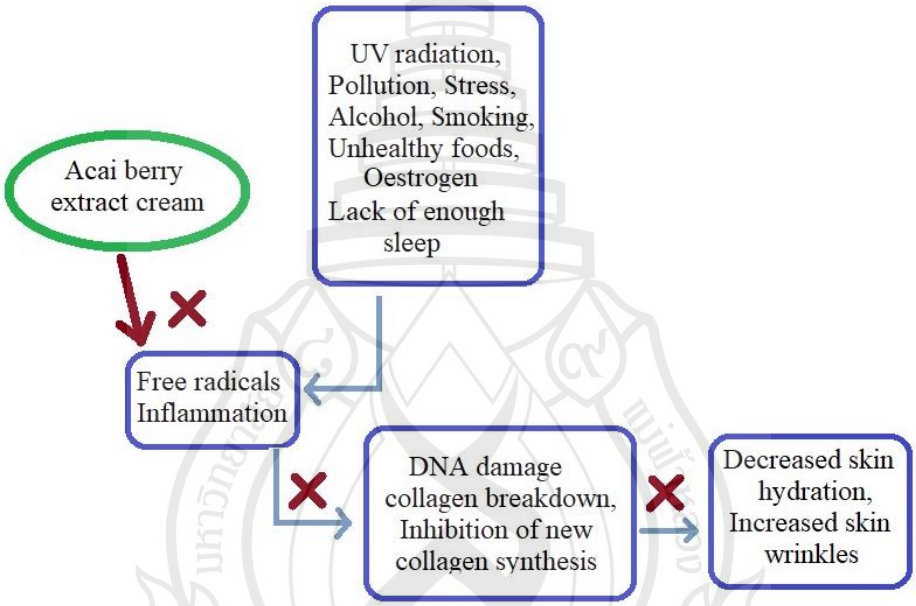


Figure 1.1 Conceptual framework

1.7 Scope of Research

This study is to evaluate the efficacy and satisfaction of *E. oleracea* extract cream on periorbital wrinkles. A total of 20 subjects ranging from age 30 to 50 of both genders with mild to moderate periorbital wrinkles were selected according to the inclusion and exclusion criteria. The topical cream of 3% acai palm extract was instructed to be applied on both sides of the face around the eyes for 12 weeks. Cutometer and Corneometer were used to measure skin moisture and elasticity.

The VISIA® complexion analysis system was also used to get report scores and wrinkle scores for all subjects. All parameters were tested at baseline, 4^{th}, 8^{th}, and 12^{th} weeks. Adverse effects were observed and satisfactory scores were recorded.

1.8 Definitions

1.8.1 Wrinkle

Folds, ridges or creases in skin are known as wrinkles. As well as intrinsic, activities including smoking and alcohol consumption leading to extrinsic aging can cause wrinkles in cheeks, crow's feet and perioral areas. VISIA Complexion Analysis System (Canfield, Fairfield, NJ) is a useful quantifier of wrinkles. Using multi-spectral imaging and analyzing the areas that affect the skin's wrinkles, appearance, UV spots, brown spots, pores, texture and porphyrins under identical environment, it scans the skin and produces important visual information.

1.8.2 Periorbital Wrinkle

Is simply defined as wrinkles around the eyes. The wrinkles of the periorbital region can be classified into (Tamura & Odo, 2011):

1.8.2.1 Type I – Wrinkles lateral to the external canthus of the eye, extending from the brow to the zygomatic arch

1.8.2.2 Type II – Wrinkles lateral to the external canthus of the eye, extending from the line of the external canthus of the eye to the zygomatic arch (absence of wrinkles in the superior lateral region)

1.8.2.3 Type III – Presence of wrinkles in the line of the external canthus only

Source Tamura and Odo (2011)

Figure 1.2 Primary classification of the wrinkles located in the periorbital area

These three types of wrinkle can occur together with:

1. A – Absence of lower eyelid wrinkles
2. B – Presence of lower eyelid wrinkles, according to the following sub classification:
    1) B1 – Lateral wrinkles
    2) B2 – Medial wrinkles
    3) B3 – Wrinkles in the internal canthus

Figure 1.3 Classification of wrinkle of lower eyelid

1.8.2.4 The skin's ability to stretch and then return to its original state is called Skin Elasticity. The elasticity of the skin is measurable using the Cutometer MPA 580. It deforms the skin mechanically based on the suction method that will allow the elasticity to be numerated by the device.

1.8.2.5 A healthy, smooth and renewed complexion can be achieved by hydration or increasing the amount of water in skin cells. Corneometer measures the skin hydration of the epidermis, the stratum corneum, by way of capacitance measurement of a dielectric medium that reacts to changes of capacity that are induced by the high dielectricity-constant of water.

1.8.2.6 An FTU, or a finger-tip-unit, is approximately the amount of an ointment or cream squeezed out from a tube with a 5 mm diameter opening stretching from the tip of the index finger to the distal skin-crease. More accurately, 1 FTU is equivalent to 0.5 g. An application of a gram of the cream will roughly cover a skin area of 100 cm2 (Long & Finlay, 1991)

Source Long and Finlay (1991)

Figure 1.4 One finer-tip unit

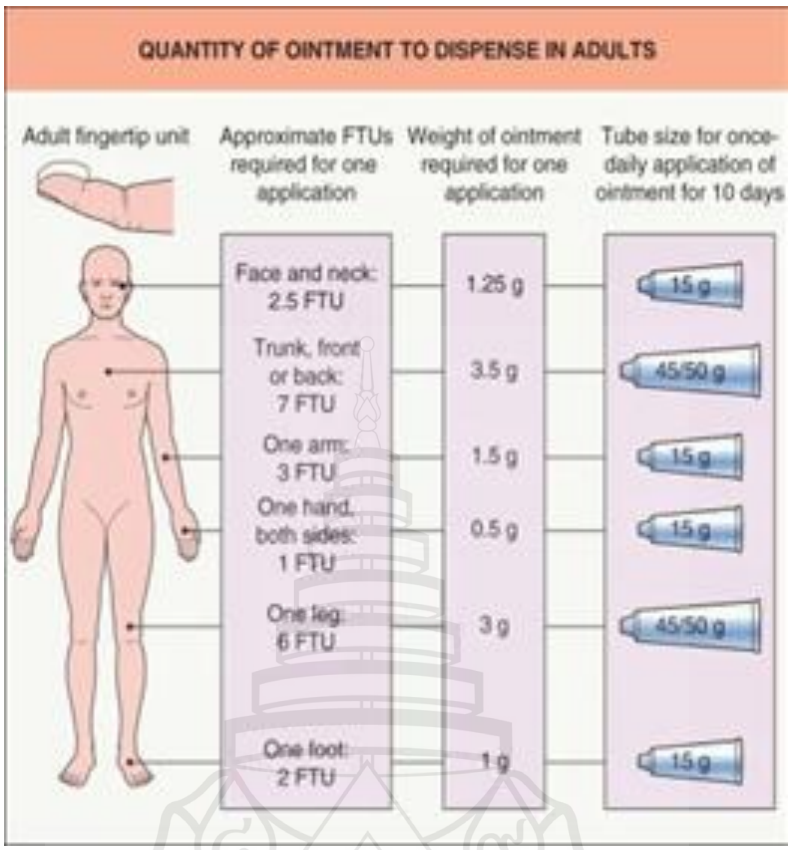


Figure 1.5 Quantity of ointment to dispense in adults

1.8.3 Efficacy

Efficacy means the ability to produce the desired or intended result after the study. In this study, the desired result is reduction of periorbital wrinkles. Hence, good efficacy refers to a decrease in the periorbital wrinkles after use of the topical 3% Euterpe oleracea extract cream.

1.8.4 Safety

Patient's safety is the prevention of errors and adverse effects to patients associated with the research procedure. Adverse effects such as allergy, contact dermatitis, irritation were closely monitored to participants during the study duration of 12 weeks.

CHAPTER 2

REVIEW OF RELATED LITERATURE

2.1 Pathophysiology

The skin is the largest organ in the human body and comprises of 3 layers: epidermis, dermis and hypodermis. The epidermis contains keratinocytes with keratin protein, melanocytes or pigment producing cells, dendritic cells and nerve endings. Being the outmost layer of the skin, it is in direct contact with the external environment. The second layer underneath is dermis and it contains fibroblasts, hair follicles, sebaceous, sweat glands, collagen and elastic fibers that account for skin elasticity. The bottom layer is called the hypodermis, or the subcutaneous tissue and it constitutes of fat cells, vessels and nerve fibers.

Being the largest organ in the human body, the skin performs a plethora of functions crucial to the human body such as protection from environment and UV radiation, thermoregulation, pressure, touch and temperature sensations, prevention of water loss and electrolyte imbalance, cell regeneration and repair among other things.

2.2 Skin Changes During Aging

Inevitably, the skin goes through the effects of aging like any other organ in the body. The phenomenon of aging can be caused by multiple factors. Thin and dry skin, wrinkles, roughness and loss of elasticity are common symptoms of aging. The process of aging impacts all three layers of the skin [16,17].

Specifically, there are epidermal thinning, flattening of dermo-epidermal junction, loss of rete ridges, reduction of lipid content and subcutaneous fat and less elasticity all leading fragility and increased risk of injury.

2.2.1 Epidermal Changes

The proliferation rate of cells declines with age which results in thinning of the epidermis by about 6.4% per decade. Such signs of aging tend to appear earlier in women compared to men due to a drop in estrogen level in midlife. Exposed areas of the skin like face, neck, upper chest and extensor surface of the forearm can appear especially thin (Farage et al., 2013). The wound healing capacity decreases as the cell turnover rate decreases by age (Khavkin & Ellis, 2011). Uneven pigmentation and reduced melanin production are also common symptoms as functional melanocytes can deteriorate up to 20% per decade. Keratinocytes becomes shorter and fatter in contrast to corneocytes which become bigger. There is also deterioration in functional melanocyte numbers up to 20% per decade leading to reduced melanin production and uneven skin pigmentation in aged skin. In aged epidermis, there is also a reduction of the number and the responsiveness of Langerhans cells. This results in diminished antigen presenting capacity, which contributes to weakened cutaneous immunity in the elderly.

Loss of dermal papillae results in flattening of the dermo epidermal junction. This, in turn, results in less surface area for nutrients and oxygen supply to avascular epidermis from vascular dermis which explains the elderly being more susceptible to wrinkles and blisters.

2.2.2 Dermal Changes

The number of fibroblasts and mast cells drop as part of aging. Loss of vessels and cells, decreases in hyaluronic acid, glycosaminoglycans and ground substances in the dermis are the driving factors of said aging.

A decrease in fibroblast collagen production causes an increase in collagen-degarding MMP production, fragmented collagen bundles and a decrease in overall collagen level (mainly I and III). There is also modifications to the organized architectures of the elastic microfibrils that contain elastin and fibrillin (glycoprotein). The loss of fibrillin accounts of permanent wrinkles and deposits of poorly organized elastin cause deep wrinkles or solar elastosis (Bonté et al., 2019). These changes make the aged skin more rigid, fragile, lax and cause an increase in risk of tear-type injuries.

2.2.3 Changes in Hypodermis

The subcutaneous adipose tissue erodes with age despite the proportion of body fat increasing until approximately the age of seventy. Aging changes fat distribution as face hands and feet experience decreases as increases are felt around thighs, waists and abdomen.

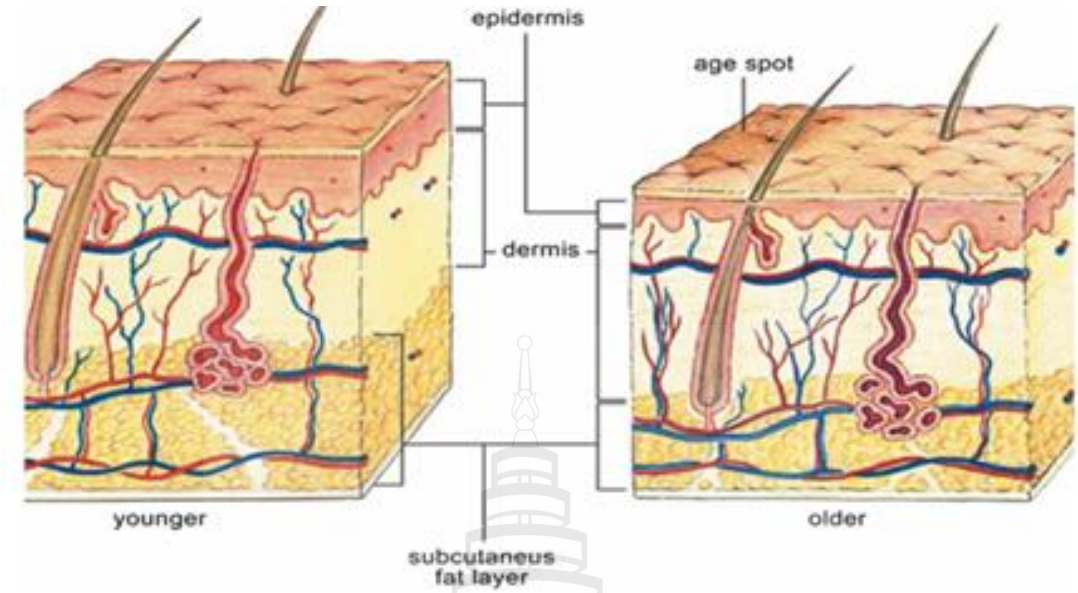
2.2.4 Lipid and Water Content

The skin experiences a reduction of natural water and fat emulsion as water content decreases in the stratum corneum. Global lipid content of the aged skin is reduced as much as 65%. The water binding capacity is reduced by the changes in amino acid composition as it reduces the cutaneous natural moisturizing factor. Due to the reduction of water content, baseline TEWL decreases with age as well.

2.2.5 Changes in Epidermal Appendages

Although there is no change in the number of sebaceous glands, there is a decline in sebum production. A fall in size and output of both eccrine and apocrine glands is found in elderly skin.

Greying and thinning of hair are common indicators of aging. Nearly half of the population has at least 50% of gray scalp hair by the age of sixty. Caused by a combination of reduced ability to handle oxidative stress and loss of melancolytes in the hair bulb, everyone has experienced some amount of greying. Nail growth decreases from about the age of twenty-five. Nails become more rough in the old age and develop beaded ridging due to a reduction in lipophilic sterols and free fatty acids.



Source Farage et al. (2013)

Figure 2.1 Changes in younger and elderly skin

Aged skin is prone to developing numerous dermatological conditions ranging from benign lesions like seborrheic keratosis and solar lentigines, inflammatory dermatoses like xerosis and contact dermatitis, vascular disorders such a pressure ulcers and stasis dermatitis, viral infections like herpes zoster or shingles, cutaneous expression of autoimmune disorders to more critical disorders of malignant skin cancers.

2.3 Causes of Skin Aging

Due to its positioning, the skin experiences intrinsic (chronological) aging that generally genetic and hormonal influence account for. Environmental factors cause extrinsic aging which also lead to reduced structural integrity and loss of physiological function (Tobin, 2017)

2.3.1 Intrinsic Aging

Intrinsic or chronological aging is a natural process that occurs over time and it is under the influence of several factors: genetic, metabolic, hormonal, etc (Bonté et al., 2019). The proliferation rate of basal cells decreases as the skin ages, and this is known as Cellular Senescence. This is visible in melanocytes, keratinocytes and fibroblast. Epidermal and dermal atrophy follows with loss of collagen and elastin fibers (Farage et al., 2013). Signs of intrinsic aging are not visible until much older even though the changes normally begin in around the age of 25 (Masnec & Situm, 2010). These signs include fine wrinkle, saggy, thin, dry and transparent skin, unwanted hair, grey hair, hair loss, loss of subcutaneous fat or inability to sweat sufficiently (Kammeyer & Luiten, 2015).

2.3.2 Extrinsic Aging

Our skin can be prematurely aged when external or extrinsic factors are involved. Solar radiation, cigarette smoke, air pollution, repeated facial expressions, gravity and sleeping positions are a few examples (Masnec & Situm, 2010).

2.3.2.1 Sun Exposure

The primary factor for extrinsic aging is exposure to UV radiation or Photoaging, account for 80% of facial aging (Zhang & Duan, 2018). Photoaging is different for each individual and is dependent on factors such as sun exposure, skin pigment and skin type. People spending more time outdoors, living in sunnier regions and have lighter skin experience photoaging more. People with skin types I and II experience a greater degree of photoaging compared to those of type III or higher (Tobin, 2017).

Sallow, deeply wrinkled, with numerous telangiectasis, atrophic, dryness, loss of elasticity, laxity, pigmentation disorders and roughly textured skin is a clear sign of a photodamaged skin. Thickening of the epidermis, disorganization and cytologic atypia of the keratinocytes, uneven distribution of melanocytes in basal layer with significant decrease of Langerhans cells and abnormal deposition of elastic tissue in the papillary dermis (Bonté et al., 2019). Solar elastosis is a vivid characteristic of photoaged skin with abnormal deposition of elastic tissue deep in the dermis (Zhang & Duan, 2018). Development of benign and maglinant neoplasms on

photoaged skin are common as a variety of premalignant skin tumors like acrochordons, keratoses, actinic keratoses and sebaceous gland hyperplasia are frequently found on extrinsically aged skin (Masnec & Situm, 2010).

2.3.2.2 Cigarette Smoking

The relation between smoking and wrinkling was noted as early as 1856 and cigarettes cause biochemical changes in the body that accelerate aging (Kammeyer & Luiten, 2015). A typical 'smoker's face' is described as having facial wrinkles stemming from the corner of the eyes light gray pigment or a red hue. This sign is not limited to smokers. However, smoking men and women can display wrinkled facial skin prematurely. Wrinkling gradually increases with pack years of smoking. When coupled with excessive sun exposure, cigarette smoking has multiplied effect on wrinkling. The risk of developing wrinkles for individuals with excessive sun exposure and heavy smoking is 11.4 times higher than that of normal age-controlled population (Landau, 2007). The increased formation of wrinkles in smokers seems to be both of molecular origin, through the activation of MMPs and the increase in elastosis, and of behavioral origin, due to repeated pursing of the lips and contraction of the facial muscles. Young children exposed to cigarette smoke are more likely to develop atopic eczema than non-exposed children (Bonté et al., 2019).

2.3.2.3 Air Pollution

It was shown in 2010 that exposure to atmospheric pollution significantly correlated with signs of extrinsic aging to leading dark spots and wrinkles. Air pollution both outdoors and indoors through pollutants such as O3, CO2, CO, SO2, NO2 and volatile organic compound are all causes. Many of these pollutants promote formations of dark spots on the skin, the oxidation of surface lipids and the formation wrinkles through activation of the aryl hydrocarbon receptor. Volatile organic compounds inhibit the proteasome, promote apoptosis, alter mitochondrial membrane potential, induce a decrease in Lon protease, stimulate the production of free radicals and induce lipid peroxidation (Bonté et al., 2019).

Exposure to increased ground levels of ozone can be associated with wrinkle formation in the face. Depleting antioxidants from the stratum corneum, regular contact with ozone increases lipid peroxidation and protein oxidation in mouse skin.

2.3.2.4 Repeated Facial Expressions

Fine lines and wrinkles are also caused by repetitive facial expressions. The face is particularly impacted as it suffers from the effects of repeated muscular contractions relating to facial expressions (Bonté et al., 2019). A groove is formed every time a facial muscle is used and this is how we see lines formed with each facial expression. The skin springs back to the original state as it ages and loses its elasticity, becoming permanent resulting in wrinkles and fine lines.

2.3.2.5 Gravity and Sleeping Positons

The effects of gravity become more apparent as skin declines in elasticity. Gravity causes the tip of the nose to droop, the ears to elongate, the eyelids to fall, the jowls to form, and the upper lip to disappear while the lower lip becomes more pronounced. Resting your face in the same way every night for years eventually also leads to wrinkles. Sleep wrinkles become etched on the surface of the skin and no longer disappear.

2.4 Mechanism of Skin Aging

The molecular basis of aging can be defined by a number of mechanisms which are oxidative stress, DNA damage, telomere shortening, microRNA (miRNA) regulation, genetic mutation, advanced glycation end product accumulation, and inflammaging or chronic inflammation.

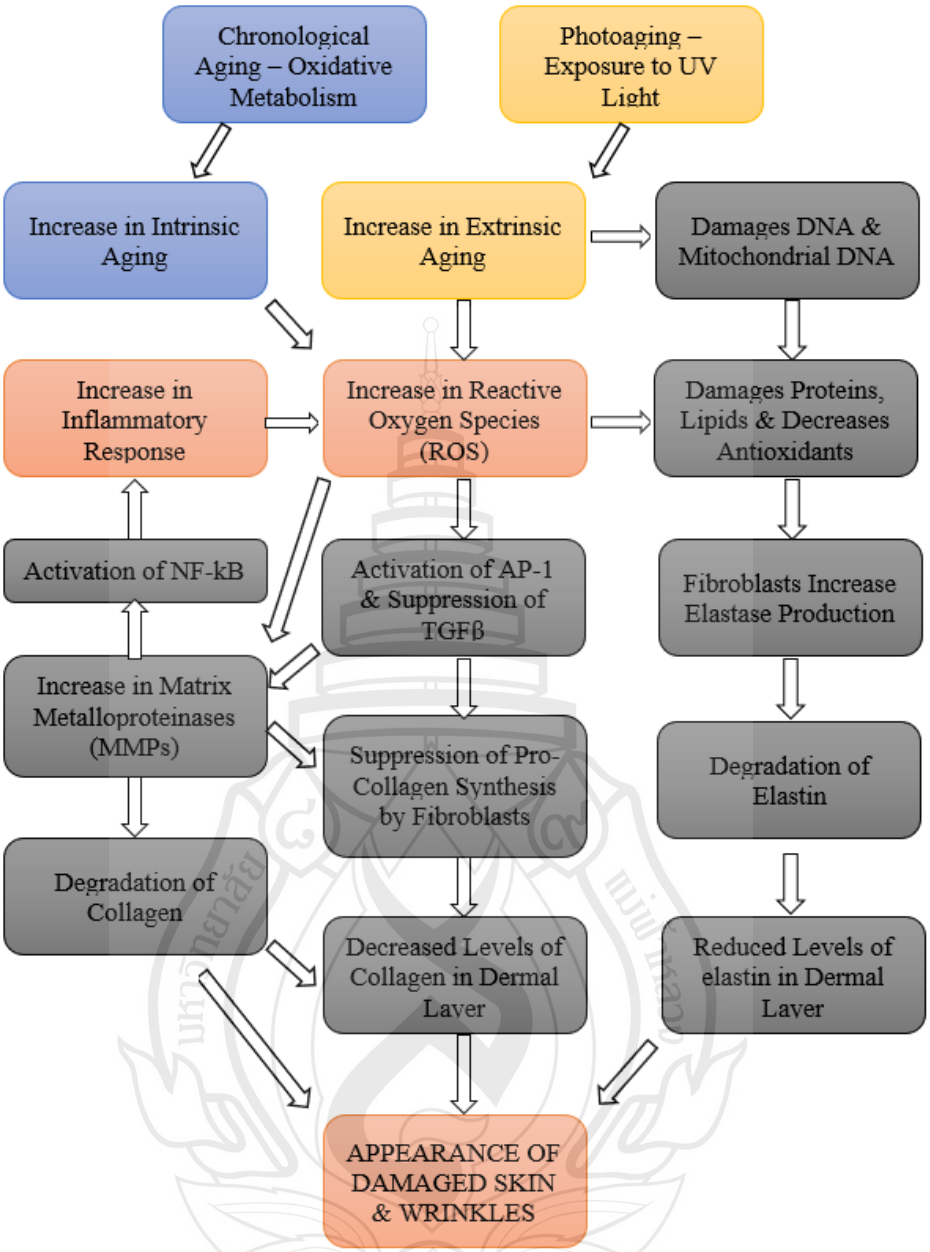


Figure 2.2 Mechanism of aging

2.5 Wrinkles and Aging

Wrinkles are due to repeated activity of facial muscles over a long period of time (hyperdynamic lines) or redundant skin due to depletion of collagen and elasticity. The wrinkles especially in the periorbital region start to form as elastin start to break down in the early 20s. Between the late 30s and mid-40s, the nasolabial fold starts to deepen, the cheek starts to sag (jowling), crow's feet (lateral orbital commissure line) starts to appear and dynamic wrinkles become visible in forehead and glabellar areas. At the age of 50s, platysmal bands of muscles, redundant skin around the neck, and marionette lines starts to appear. In the 60s and 70s, the face significantly loses more fat that support shape and volume, especially malar fat pad which gives youth and definition. All of the wrinkles and worry lines become more prominent. Generally, it is only the aging wrinkles that really bother people. There are two basic approaches to the amelioration of these signs of aging: prevention and treatment (Tun, 2018).

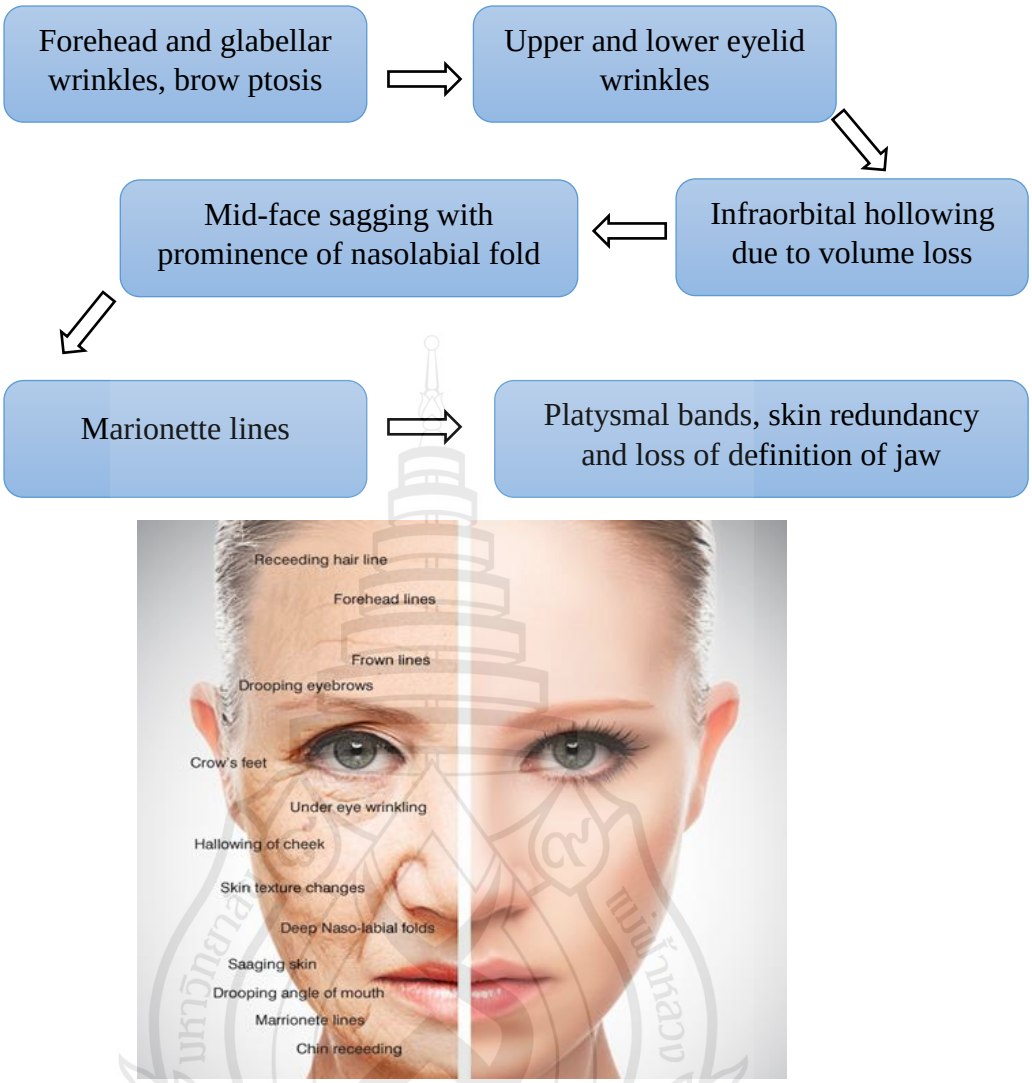


Figure 2.2 Signs of aging

2.6 Treatment Options for Wrinkle Reduction

Several therapies are available to fight against wrinkles. Topical wrinkle treatments are, in general, much more effective for fine lines. Deeper creases may require more invasive techniques, such as injection of fillers, botulinum toxin, or plastic surgery.

2.6.1 Topical Medication

2.6.1.1 Topical retinoid, vitamin A derivatives, are effective treatments for wrinkles as they can induce the biosynthesis of collagen, reduce the expression of MMP 1, promote keratinocytes proliferation, strengthen the protective function of the epidermis, and restrain transepidermal water loss (Zasada & Budzisz, 2019). They also reduce other signs of photoaging like loss of elasticity and pigmentation.

2.6.1.2 Application of topical antioxidants like vitamin C, vitamin E, coenzyme Q10, alpha lipoic acid can also help remove wrinkles by fighting free radical formation, rebuilding collagen and moisturizing the skin (Kyi, 2019).

2.6.2 Invasive Procedures

2.6.2.1 Chemical peels are methods to cause a chemical ablation of defined skin layers to induce an even and tight skin. Chemical peels are classified into three categories. Superficial peels [α-β-, lipo-hydroxy acids (HA), trichloroacetic acid (TCA) 10–30%] exfoliate epidermal layers without going beyond the basal layer; medium-depth peels (TCA above 30 to 50%) reach the upper reticular dermis; deep peels (TCA > 50%, phenol) penetrate the lower reticular dermis. An increase in collagen fiber content, water and GAG in the dermis has been reported after chemical peel treatment. Improvement in wrinkles after chemical peeling can be attributed to increase of elastic fibers, as well of a dense rearrangement of collagen fibers.

2.6.2.2 Laser, light source and radiofrequency which is wounding (ablative) laser resurfacing. When the dermis is heated, the epidermis is destroyed by a laser beam. From this process, it stimulates the development of newly collagen fibers. Moreover, when the wound heals, new skin will be smoother and tighter than the old one. While the ablative laser takes several months to heal, non-ablative laser pulses, light sources and radiofrequency devices do not damage to the epidermis. But the dermis is heated and triggering new collagen and elastin formation. The patients may feel firmer and appears refreshed skin after several treatments. However, patients may have burn, redness, post inflammatory pigmentation.

2.6.2.3 Botox (Botulinum Toxin Type A). Botulinum toxin blocks the chemical signals and causes the muscles to relax. Generally, it is injected in small multiple doses into the targeted muscles, giving a less wrinkled and smoother looking

appearance. Botox is effective for lines between the eyes (frown lines), on the forehead and crow's feet. As the treatments normally last about three to four months, the injections are repeated. Patient may have side effects of bruise and ptosis.

2.6.2.4 Face lift, a rhytidectomy, a type of cosmetic surgery. It aimed to giving a more youthful or flawless appearance. Rhytidectomy usually removes the excess facial skin and fat, with or without tightening of the underlying tissues. But, healing times may be long and the patient need to hope swelling and bruising for a few weeks after surgery.

2.6.2.5 Dermabrasion which is a surgical procedure and abrasion of the upper layers of skin with sandpaper or some other mechanical. Dermabrasion is for the removal of scars, fine wrinkles, tattoos and nevi (moles). But, the results are not usually immediate and may be taken to several months. There will be some scabbing, redness and swelling, which will be surprisingly gone after two weeks but the pinkness, may take several months to fade.

2.6.2.6 Microdermabrasion (MDA) is a mechanical exfoliation. It is superficial skin resurfacing utilizing a refined abrasive element such as diamond-tipped pad or aerosolized crystals to gently remove the outermost layer of skin. This controlled wounding stimulates cell renewal with regeneration of a healthier epidermis. It helps to give a fresher to the skin, smoother appearance, diminishing the wrinkles, lines, coarse skin, enlarged pores, hyperpigmentation, acne, scars and photoaging. Patients may have a little redness in the treated skin. Repeated treatments are needed because of the temporary results.

2.6.2.7 Filler injections

These include hyaluronic acid or fat. Generally, they are injected into the deeper layers of face, increasing volume and flattening the wrinkles and folds. Unfortunately, patient may have swelling, bruising and redness in the treated skin for a short period. Moreover, good results depend on several factors, where the wrinkles are located, how deep they are, and skillfulness of the doctor. However, there is no known current treatment that can stop the aging (Aye, 2018)

2.7 Factors Affecting Wrinkle Formation

Factors promoting wrinkle formation include the following:

2.7.1 UV radiation

2.7.2 Smoking

2.7.3 Facial expressions

2.7.4 Sleeping positions

2.8 Prevention of Wrinkles

The main preventive measures we can take are to minimize sun exposure and not smoke. These measures can, at best, delay wrinkles.

2.9 Free Radicals and Antioxidants

2.9.1 Free Radicals

Free radicals are chemical species capable of an independent existence that contain one or more unpaired electrons. They are consistently formed as byproducts of aerobic metabolism in the human body and are highly reactive. During the reaction of free radicals with a new compound, more free radicals are generated, and this chain reaction leads to thousands of events. Approximately 1%–4% of oxygen taken up in the body is converted as free radicals. The most damaging effect of oxygen is due to the formation of oxygen free radicals. The oxidative metabolism continuously produces oxygen-centered free radicals and other reactive oxygen species (ROS), including superoxides (O2•–), hydroxyl radicals (OH•), hydrogen peroxides (H2O2,) lipid peroxides (LOO•), etc.

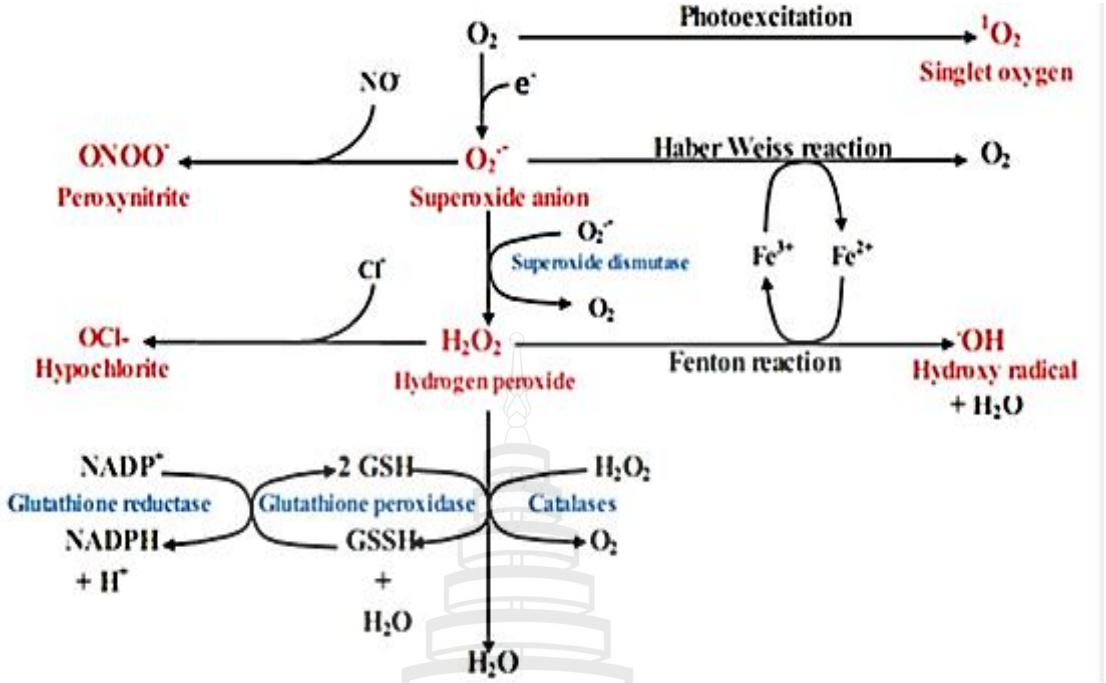
2.9.1.1 Sources

Free radicals are either derived from endogenous or exogenous sources. Endogenous sources comprise the normal metabolism, enzymes and transport molecules (e.g., xanthine oxidase, cyclooxygenase, etc.), auto-oxidation reactions of biological molecules (e.g., flavins, catecholamines, hemoglobin, etc., as well as metal

ions, Cu, Fe, Cd, As, Hg, Cr) are common sources. Exogenous sources consist of air pollutants, medications (especially anticancer agents), ionizing radiations, chemical pollutants (e.g., pesticides), viruses, bacteria, parasites, and smoking (both active and passive). Excessive alcohol and stress conditions may also cause the formation of free radicals.

2.9.1.2 Mechanism of Action

Free radicals are beneficial to cellular function up to a threshold. When in excess, they produce their deleterious response by peroxidizing cell-membrane lipids, causing an alteration in membranous structural integrity and or changes in the microenvironment of membrane proteins, causing severe oxidative stress. At the same time, DNA damage, such as DNA breaks or crosslinking, may also be observed. Free radicals disrupt the equilibrium of biological systems by damaging their major constituent molecules, which lead to cell death .Oxidative stress has been associated with many chronic and degenerative diseases including vascular diseases, diabetes, cancer, and overall aging (Schauss et al., 2006). ROS induces gene expression pathways resulting in increased degradation of collagen and accumulation of elastin. ROS not only directly destroys interstitial collagen, but also inactivates tissue inhibitors of matrix- metalloproteases and induces the synthesis and activation of matrix degrading metalloproteases. This is important as the MMPs' over expression can cause an increase in collagen degradation and diminish the collagen synthesis, resulting in periorbital wrinkles.

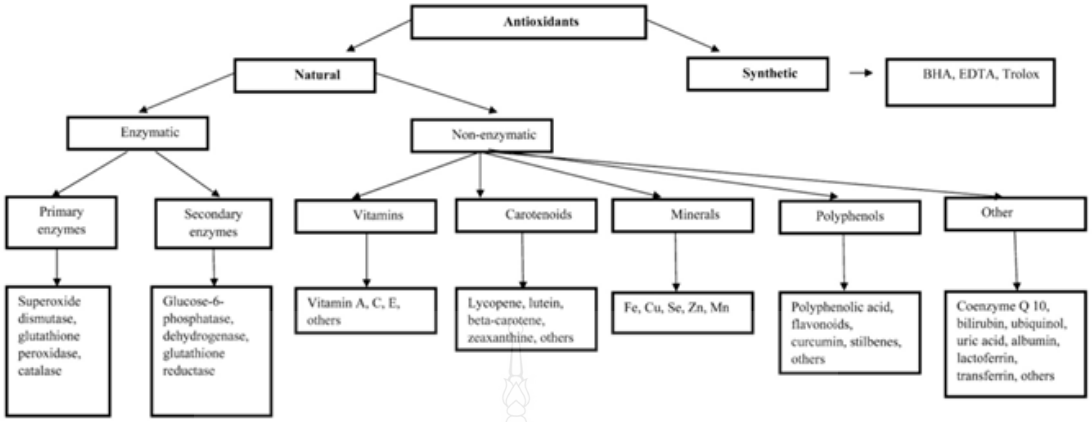


Source Miracle (2017)

Figure 2.4 Generation of ROS

2.9.2 Antioxidants

Antioxidants are molecules that can neutralize free radicals by donating or receiving an electron. They are natural scavengers of free radicals that cause cellular damage and other age-related disorders including wrinkling of the skin. Antioxidants' anti-aging benefit is due to their anti-inflammatory effect, delay or prevention of cancer, diabetes, and so on.



Source Sotler (2019)

Figure 2.5 Classification of antioxidants

2.10 *Euterpe oleracea (Acai Palm) Extract*

2.10.1 General Information

The scientific name for acai palm is Euterpe *oleracea* which belongs to the Arecaceae family and it is commonly known as Acai, Huasaí, Cabbage palm or palm tree, Tucum, Manaka palm, Pina palm, Naidí palm or Morroke. This fast-growing palm tree is native to northern South America, where it is appreciated for its nutritious fruit. It grows in northern Brazil, Colombia, Venezuela, Ecuador, French Guyana, Surinam, Guyana, Trinidad and Venezuela, and in the Magdalena Medio and the Pacific region in Colombia. Brazil is one of the main producing countries and the biggest exporter of palm heart.

This monoecious palm tree with multiple stems, sometimes slightly inclined, can reach 10-20 m high and 7-18 cm in diameter, with roots that often appear on the surface. It has 4 to 8 well developed stems, and the pinnate leaves are 2 to 3 m long. They are arched, hanging leaves, with the bases forming a green or somewhat brown capital, and they are found in groups between 8 and 14 per trunk. They have abundant, linear and hanging leaflets. Spike inflorescences with flowers purple in color and 2 mm in diameter emerge from the lower part of the capital and are covered

with whitish-brown hairs. The globose fruit is dark purple or purplish black in color and 1-2 cm in diameter. Each fruit has a hard brown seed, 6 mm in diameter.

The fruits have been traditionally used by natives of the Amazon to increase vitality and promote healthy living. In Europe and America, the fruit has started to be marketed as a food supplement in the form of energy bars, juice, sweets or even as the whole fruit, for its energy and nutrient value. Recently, acai palm has been classified as a "superfruit" (like goji and acerola), as it acts as a general tonic for the body, providing strength and energy to anyone who takes it. Moreover, it provides fiber, it improves digestion, cholesterol levels, sleep, skin, and heart health.

The fruit also has medicinal properties against fever, malaria, jaundice, diabetes, bleeding, muscle aches, and liver and kidney diseases. Moreover, it is said that it has a very powerful antioxidant activity, being 33 times higher than that of grapes (powerful antioxidants), and that it leads to a longer life.

Figure 2.6 The tree of acai

2.10.2 Chemical Constituents of *Euterpe oleracea* Extract

It has a varied chemical composition, which includes an important amount of polyphenols such as anthocyanins (cyanidin-3-glucoside, cyanidin-3-rutinoside), and proanthocyanins and other flavonoids (orientin, scoparin, isovitexin). It also contains

tannins, carotenoids, lignans, vitamins (A, C, E and group B vitamins), resveratrol, and aspartic, glutamic, myristic and arachidonic acid. Other compounds found in the fruits include p-hydroxy-benzoic acid, gallic acid, ellagic acid, protocatechuic acid, p-coumaric acid, ferulic acid, and vanillic acid. It is worth noting that the fruit is particularly rich in fatty acids, the unsaturated fraction being the most abundant, and specifically monounsaturated fatty acids (60%), especially oleic acids. Besides, minerals and sterols have been identified (Schauss et al., 2006; Pacheco-Palencia et al., 2008).

Benefits of Different Chemical Structures Identified in *Euterpe oleracea* Anthocyanins

The Acai fruit gets its deep purple color from a group of polyphenols called Anthocyanins. There compounds are known as free radical scavengers against harmful oxidants such as reactive oxygen and nitrogen species (ROS and RNS) (Mattioli et al., 2020). They prevent cells from UVA-induced oxidative damage and UVB-induced inflammation (Petruk et al., 2017). The most important benefits of their antioxidant properties are cardioprotective and neuroprotective benefits (Bendokas et al., 2019). Other significant benefits include flow-, ediated vasodilation, anti-imflammatory, anti-proliferative and photoprotective capacities (Oliveira et al., 2020).

1. Orientin, Isovitexin

They are a category of flavonoids called flavones and mainly exert anti-inflammatory and antioxidant actions through influencing cytokines (Yu et al., 2022). Moreover, these flavones reportedly have anti-cancer, anti-tumor, hepatoprotective activities among many others (Xiao et al., 2015).

2. Resveratrol

Resveratrol is a phenolic compound that exihibit pleiotropic effects in human subjects. Many studies demonstrate it has high antioxidant potential. Anti-inflammatory, anti-carcinogenic, cardioprotective, vasorelaxantm phytoestrogenic, neuprotective and other bioactive effects have also been reported (Salehi et al., 2018).

2.10.3 Biological Action of *Euterpe oleracea*

2.10.3.1 Antioxidant Activity

1. Oxygen Radical Absorption Capacity with fluorescence (ORACFL), including lipophilic assays (LORAC), hydrophilic assays (HORAC)

The original ORACFL assay was designed to measure the antioxidant capacity of foods toward peroxyl radicals, and it can be conducted to measure both hydrophilic and lipophilic antioxidants. The results of the ORAC assays show that acai palm has a much higher antioxidant capacity than any other fruit or vegetable studied to date, since its results are the highest in the LORAC and HORAC test, expressed as Trolox Equivalents (TE). The values obtained in the LORAC test analyzing fruit ranged from 0.05 μmol TE/g (Golden apples without skin) to 5.52 μmol TE/g (avocados). In vegetables, values ranged from 0.09 μmol TE/g (beetroot and beans) to 6.36 μmol TE/g (peas). Based on this reference, acai palm has a much higher value of 29.60 μmol TE/g. The same applies to the HORAC test, where the values obtained in fruit ranged from 1.23 μmol TE/g (melon) to 92.56 μmol TE/g (blueberry). In vegetables, values ranged from 0.87 μmol TE/g (cucumber skin) to 145.39 μmol TE/g (red beans). Acai palm obtained a much higher result, with a value of 996.9 μmol TE/g. (Schauss et al., 2006; Wu et al., 2004).

2. Superoxide radical scavenging ability

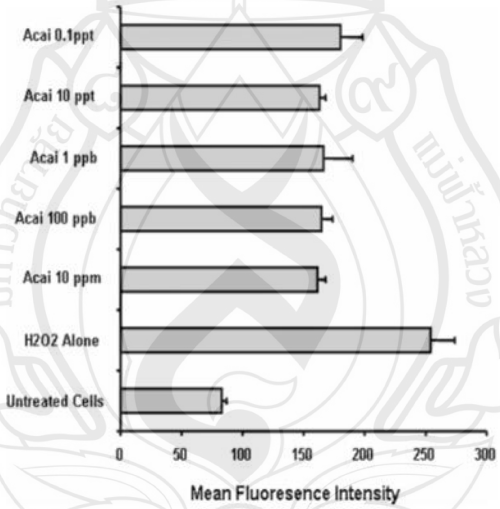
The result of acai fruit extract's ability to scavenge superoxide radicals (1614 units/g) was also much higher than that of fruits and vegetables that had been tested in earlier studies. This is a very important parameter to preserve the body's overall antioxidant capacity. Superoxide (O2-) is believed to be the cause of other ROS formations such as hydrogen peroxide, peroxynitrite, and hydroxyl radicals. Therefore, O2- scavenging capacity in the human body is the first line of defense against oxidative stress. It has been reported that over expression of superoxide dismutase and catalase in transgenic flies extended life-span by as much as one-third, perhaps, due to decreased oxidative stress reflected by lower protein carbonyl contents (Schauss et al., 2006). Different studies performed on healthy women, which consumed 200 g/day of açai pulp for 4 weeks, revealed that some antioxidant enzymes, i.e. superoxide dismutase, catalase and glutathione, were increased (Petruk et al., 2017).

3. Hydroxyl radical averting capacity (HORAC)

HORAC was performed to measure antioxidant capacity of acai toward hydroxyl radical. It is similar to that of grapes but lower than that of dark colored berries.

4. Inhibition of the formation of reactive oxygen species (ROS) on human neutrophils.

The study of its capacity to scavenge ROS yielded very positive results, as acai fruit extract clearly inhibited their formation. It is worth mentioning that, as this was a study conducted on human cells, it is even more relevant, as it is close to a more realistic situation on the skin. In addition, the extract was effective at very low concentrations (0.1 parts parts per trillion), significantly reducing the harmful effect of hydrogen peroxide.



Source Schauss et al. (2006)

Figure 2.7 Freeze-dried acai palm reduced the H2O2-induced formation of reactive oxygen species (ROS) in freshly purified human neutrophils

5. Increases antioxidant enzymes

Healthy women, which consumed 200 g/day of açai pulp for 4 weeks, revealed that some antioxidant enzymes, i.e. superoxide dismutase, catalase and glutathione, were increased.

2.10.3.2 Anti-inflammatory action

In in vitro research, freeze-dried acai fruit pulp and skin powder displayed selective inhibitory activity against COX-1 and COX-2 enzymes (Schauss et al., 2006). The extract also reduces the levels of IL- 1β. IL- 1β is a pro-inflammatory cytokine that causes T cells activation to produce inflammatory cells. Inhibition of IL-1β results in an anti-inflammatory manner (Kang & Kim, 2018). Moreover, the polyphenols present in the fruits can effectively downregulate the expression of another pro-inflammatory transcription factor, NF-KB. NF-kB has been considered signaling a pro-inflammatory pathway, targeting pro-inflammatory cytokines such as interleukins and tumor necrosis factor a (TNF-α). Açai polyphenols downregulated the gene expression of NF-KB-target genes, TNF-α, MCP-1, IL-6, IL-8, IL-1β, and INF-β. TNF-α protein levels were also downregulated by açai polyphenols (Martino et al., 2016).

2.10.3.3 Anti-collagenase Action

Açaí berry water extract can activate mRNA expression of levels of type I collagen, VEGF, and fibronectin while downregulating the expression of MMP-1. MMP-1 is a collagenase that breaks down collagen. Histologically, higher concentrations of collagen with regular alignments were also found in the excisional wound of rats after treatment with the extract (Kang & Kim, 2018). This effect can be useful in treating the signs of aging as aging of the skin is associated with a decrease in collagen level and an increase in MMPs. The potential of acai palm extract in wound healing was also described in human dermal fibroblasts. The accelerated wound closure was associated with the presence of trace elements like iron and zinc, and high value of fatty acids in the extract (Matta et al., 2020).

2.10.3.4 Photoprotective Action

Acai extract has been shown to protect immortalized fibroblasts against UV-A induced oxidative stress. The high number of anthocyanins are responsible for this action (Petruk et al., 2017).

2.10.3.5 Cholesterol Lowering and Cardioprotective Effect

When rats were fed a hypercholesterolemic diet supplemented with 2% açaí pulp, there was a significant decrease in serum total cholesterol, low-density lipoprotein cholesterol, and increased high-density lipoprotein cholesterol and cholesterol excretion in feces. The hypocholesterolemic effect was observed through an increase in the expression of ATP-binding cassette, subfamily G transporters, and LDL-R (low density lipoprotein receptor) genes. Increased hepatic LDL-R expression results in an improved clearance of plasma LDL-C, which has been strongly associated with a decreased risk of the development of cardiovascular disease in humans (de Souza et al., 2012). A study where women consumed acai pulp for 4 weeks was conducted and interestingly, it was shown to trigger an increase in apolipoprotein A-I concentration and the transfer of cholesterol esters to HDL. This suggests an improvement in the metabolism of this lipoprotein, and it is well known that HDL is protective against atherosclerosis (Pala et al., 2018).

2.10.4 Preparation of *Euterpe oleracea* Extract

Grinding of fresh fruits including seeds

Water extraction and concentration

Addition of preservatives

Homogenization

Filtration

Final product – *Euterpe oleracea* extract in liquid form

2.10.5 Safety

A lack of toxic effects was noted with oral intake of acai palm at acute doses up to 2,000 mg/kg of body weight in laboratory animals (similar to a human consumption of 140 g at one time). According to secondary sources, Brazilians commonly drink up to a liter (34 ounces [oz]) of acai juice daily. Anecdotal reports suggest that the roots of the acai palm tree are used medicinally, prepared as a decoction of which 1–2 cups are taken daily. Additional suggested doses from secondary sources include 1 oz of powder mixed with 10–12 oz of water once or twice daily or freeze-dried acai palm in capsules or tablets at 1–2 g daily. In healthy, overweight subjects, a specific acai palm product used for 1 month reportedly had a

lack of adverse effects (Ulbricht et al., 2012). However, there is no data available for topical application of *Euterpe oleracea* extract on human skin. For this reason, this study was conducted to elucidate the efficacy of 3% *Euterpe oleracea* extract cream as a treatment of periorbital wrinkles.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

Experimental study

3.2 Study Population

20 healthy male and female volunteers aged between 30 and 50 with prominent periorbital wrinkles were enrolled in the study. Written consent was obtained before participation.

3.3 Sample Size Determination

To calculate the sample size, used the reference data from similar article “The efficacy of 3% *Emblica officinalis* extract cream for periorbital wrinkle reduction” by (Aye, 2018)

According to this study, the results of skin parameters for 3% *Emblica officinalis* extract group revealed a significant drop from 0.83±0.091 at baseline to 0.92±0.06 at 12th week. For the standard base serum, the results showed slight decline from 0.83±0.09 at baseline to 0.87±0.07 at 12th week. Those skin parameters were measured by using cutometer.

Different change from baseline of 3% *Emblica officinalis*, μ1= 0.83-0.92 = -0.09

Different change from baseline of standard cream, μ2 = 0.83-0.87 = -0.04

Mean difference between two groups, μd = (-0.09) – (-0.04) = -0.05

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) $Z_{0.100} = 1.28$

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

$$S_p^2 = \frac{0.06^2\,(20 - 1) + 0.07^2\,(20 - 1)]}{[20 + 20 - 2]}$$

$= 0.00425$

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

$$= \frac{(1.96 + 1.28)^2 0.00425}{(-0.05)^2}$$

$= 17.84$

≈ 18

Where

n = sample size

S = σ = Variance

S^2_p = σ^2 = Pooled variance

The expected dropout rate is 10%. Thus, 20 volunteers (n=20) should be recruited.

3.4 Inclusion Criteria

3.4.1 Female and male volunteers of age between 30 and 50 years

3.4.2 Volunteers have accepted to use 3% Euterpe oleracea extract cream on both sides of the face

3.4.3 Accept to restrict no other eye cream and retinol around the eye during the study. Volunteers can continue using other routine skincare steps including sunscreen.

3.4.4 Accept to have no facial treatment like laser and radiofrequency treatment during study

3.4.5 Accept to have no facial botox and filler injection before study

3.4.6 All volunteers agree to be involved in the treatment and have follow ups for 3 consecutive months.

3.5 Exclusion Criteria

3.5.1 Volunteers who were allergic to 3% Euterpe oleracea extract cream

3.5.2 Underlying active skin disease that rule out any sort of application

3.5.3 Pregnancy or breastfeeding

3.5.4 Subjects with alcohol, smoking or drug abuse within last one month

3.5.5 History of abnormal scarring or poor wound healing

3.5.6 Volunteers who had facial tightening treatments such as botox, filler, laser, radiofrequency, HIFU 6 months before the study

3.5.7 Medical illness such as coagulopathy, poorly controlled diabetic mellitus, photosensitivity and taking immunosuppressant

3.5.8 History of strong UV exposure in some career or leisure

3.6 Discontinuation Criteria

3.6.1 Cannot apply 3% Euterpe oleracea extract cream continuously

3.6.2 Severe allergy or side effect or skin irritation

3.6.3 Subjects who got pregnant

3.6.4 Other medical condition such as illness or accident

3.6.5 Subject who wanted to leave the program with any reasons

3.6.6 Subject who non-cooperated the protocol or lost follow up for more than three visits

3.7 Variables of the Study

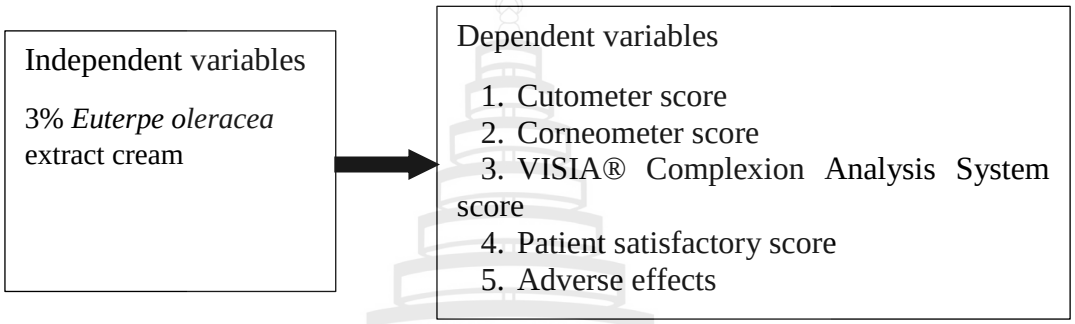


Figure 3.1 Variables of the study

3.8 Equipment

3.8.1 Cutometer MPA 580 for Measurement of Skin Elasticity

The Cutometer® does all the basic measurements regarding cosmetic applications. It is used for efficacy testing which is important for all kinds of cosmetic products especially anti-aging products, anti-cellulite products or firmness enhancing and formulation. It uses negative pressure to measure the elasticity of the upper skin layer. Depending on the suction method, the machine collects important information about the skin like its mechanical and elastic properties, allowing to numerically understand the skin aging.

Figure 3.2 Cutometer MPA 580

3.8.2 Corneometer

To measure the hydration of the epidermis, the outer layer of stratum corneum, we will use the Corneometer. Based on capacitance measurement of a dielectric medium, the recordings are collected within a set timeframe.

3.8.3 The VISIA complexion Analysis System for Wrinkle Score

VISIA® Complexion Analysis System (Canfield, Fairfield, NJ) detects the areas of the facial skin's appearance and health; wrinkles, pores, texture, spots, brown spots, UV spots, red areas and porphyrins (evidence of bacteria under the identical environment). It uses multi-spectral imaging and analysis system.

Figure 3.3 VISIA® Complexion Analysis System

3.8.4 3% *Euterpe oleracea* Extract Cream

The cream for the research was ordered from Skinnex co., ltd. The color is white and it has a characteristic smell. Each packaging contains 15grams of the cream.

3.9 Step of Research

3.9.1 The inclusion and exclusion criteria were followed when enrolling volunteers.

3.9.2 The goal of the research, process, benefits and possible side effects were all thoroughly explained to the volunteer by the researcher.

3.9.3 The volunteers had to sign an informed consent form for participation in the research.

Then apply 0.5 g of *Euterpe oleracea* extract cream around the periorbital area on each side in the morning and at night.

3.9.4 History taking was done. General information such as age, sex, occupation, address, contact number and previous medical history related to wrinkles were collected.

3.9.5 Patch test will be performed prior to applying *E. oleracea* cream. The volunteer will be asked to apply 1 finger-tip-unit of the cream on their arm under waterproof conditions. This means the volunteer will be asked to stay indoors if possible, avoiding the outdoors, sunlight and sweating excessively. The researcher will be in contact with the volunteer for the next 3 days to see if there are any reactions. All reactions recorded will be in accordion with the international contact dermatitis research group system as follows:

- = negative

+? = any doubtful reaction for example only mild redness

+ = weak positive reaction; appears redness and the skin become slightly thickened

++ = strong positive reaction; appears red swollen skin with individual small water blister

+++ = extreme positive reaction; appears intense redness and swelling with coalesced spreading reaction or large blisters.

NT = not tested

IR = irritant reaction

Volunteer with positive patch test scoring ++ or higher were excluded.

3.9.6 Taking photographs of volunteers' faces

The photographs of each volunteer were taken at every appointment (baseline, 4^{th}, 8^{th}, 12^{th} weeks) using the VISIA complexion analysis system. The eye area of the participants will be covered with black lines in the photographs for privacy. Favorable condition for the procedure is to turn the light off and capture with 12 megapixel resolution, automatic white balance correction, automatic focus, facial position: Right 37, left 37, Center 0, multi-spectral imaging (cross polarized flash and ultraviolet lighting, standard daylight fluorescent lighting).

3.9.7 Measurement of skin elasticity and hydration

Hydration and skin elasticity were measured by Cutometer MPA 580 and Corneometer at 0, 4^{th}, 8^{th} and 12^{th} week at two landmarks. These landmarks were at 1.5 cm lateral to lateral canthus (A) and 1.5 cm from the lower eyelid at the mid papillary line (B). The data was recorded and analyzed for each visit.

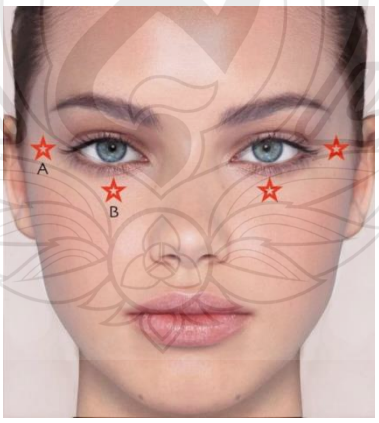


Figure 3.4 Landmarks to measure skin moisturization and elasticity

3.9.8 Manual for volunteers

Wash the face using mild soaps and pat the face with dry and clean tissues or towels. After washing the face, squeeze 1 finger-tip unit of topical 3% *Euterpe oleracea* extract cream through tube (1 finger-tip unit = 0.5 grams) and apply to left side around the periorbital region using index finger. Carefully place small dots over eyelid and under the eye, starting in the innermost corner and moving outward. Be sure to pat the product on the skin and NOT drag it or rub it. Go over the same steps to apply the cream in right periorbital area. Wait 2 minutes for the cream to fully absorb. Other routine facial skincare steps and sunscreen can be continued as usual. Aforementioned steps are to be followed twice in the morning and at night, meaning the applied amount is 2 gram per day.

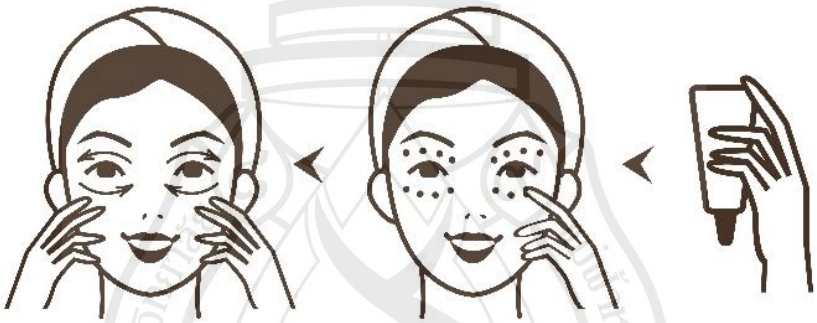


Figure 3.5 How to apply eye cream

Restrictions

Explain the participant thoroughly not to apply any other eye cream or retinol around the periorbital area during the study. If any side effect or reaction occurs, participants should stop using the cream and inform to researcher.

3.9.9 Follow-up

Duration of the study is 12 weeks and follow-up visits will be on 4^{th}, 8^{th} and 12^{th} weeks.

3.10 Data Collection and Analysis

3.10.1 High quality photos for assessment of periorbital wrinkles were recorded by VISIA® complexion analysis system under favorable conditions. Skin elasticity and moisture were measured by Cutometer MPA 580 and Corneometer. The records were taken at baseline and every follow-up.

3.10.2 Dermatologists' evaluation and Patient Assessments

3 different dermatologists evaluated the outcome for periorbital wrinkles from photos taken with VISIA® using grading scales as follows:

-1 = worse
0 = no changes
1 = 1-25% fair improvement
2 = >25-50% moderate improvement
3 = >50-75% good improvement
4 = >75-100% excellent improvement

3.10.3 The skin moisturization and elasticity score from Cutometer MPA 580 and Corneometer will be recorded at 0^{th}, 4^{th}, 8^{th} and 12^{th} weeks.

3.10.4 Treatment satisfactory score

The following grading scare was used by the volunteers to rate their satisfaction with the research:

Score 0 = No satisfaction
Score 1 = Little satisfaction
Score 2 = Average satisfaction
Score 3 = More satisfaction
Score 4 = Most satisfaction

3.10.5 Recording the adverse effects

The volunteer was asked by the researcher about the following adverse effects to see if they were experiencing any:

1. Pruritus score ranging from 0 to 10
2. Durations (days) of erythema
3. Others, such as allergic contact dermatitis, post inflammatory hyperpigmentation and hypopigmentation

3.11 Statistical Analyses

The participants' personal information was highly confidential in data analysis and the inclusion and exclusion criteria were used to determine their eligibility for this research. Mae Fah Luang University Dermatology Clinic in Bangkok, Thailand was used to conduct the research.

3.11.1 General demographic data of participants were recorded by using descriptive statistical analysis such as means, medians, modes, percentages, ranges, and standard deviations.

3.11.2 The mean changes in skin elasticity by Cutometer score, skin hydration by Corneometer score from baseline to 4th, 8th and 12th weeks were analyzed through Repeated measure ANOVA test. Post hoc test (Bonferroni method) was applied to identify which time point was different to other one on 0th, 4th, 8th and 12th weeks respectively. Wrinkle score analysis by 3 dermatologists was assessed by Friedman test on 4th, 8th and 12th week respectively and the data were analyzed between each visit with Post hoc test.

3.11.3 Adverse effects were recorded throughout the study and calculated with descriptive statistics

3.11.4 Patients' satisfactory score was analyzed on 12th week by descriptive statistics.

3.11.5 The researcher did the following at significant levels of p-value <0.05.

CHAPTER 4

RESULTS

4.1 Basic Demographic Data

General characteristics of twenty volunteers were recorded and expressed with descriptive statistics as shown in table 4.1.

Table 4.1 Characteristics of the volunteers (n = 20)

Characteristic	**n**
Gender, n	
Female	16
Male	4
Age group (Years)	
30-40	17
40-50	3
Mean ± SD	35.50±5.35
Min – Max	30 – 50
Occupation, n	
Housewife	5
Student	5
Employee	9
Shop Owner	1
Underlying Disease, n	
YES	0
NO	20

Table 4.1 (continued)

Characteristic	**n**
Personal Medication, n	
YES	0
NO	20
History of Drug Allergy, n	
YES	0
History of Drug Allergy, n	
YES	0
NO	20
Photosensitivity or Drug Induced Photosensitivity, n	
YES	0
NO	20
Average Time Exposure to Sunlight from 10 AM to 4 PM, n	
YES	15
NO	5
Treatment Before Study, n	
YES	0
NO	20
Skin Type, n	
Dry	5
Oily	4
Combination	11

According to the tables above, 80% of the subjects were female and 20% were male. The mean age of the volunteers was 35.50 ± 5.35 years and there were 5 housewives, 5 students, 9 employees and 1 shop owner. All participants were healthy with no underlying disease or drug allergy. None of them were taking any kind of medication. Majority of them had combination skin type (55%), 25% had dry skin and the rest 20% had oily skin.

4.2 Results of Cutometer Score for Skin Elasticity

Table 4.2 Statistical analysis of cutometer score at crow's feet

	Mean ± SD	**p-value**
Baseline	0.7103 ± 0.0845	< 0.001
Week 4	0.7481 ± 0.0922	
Week 8	0.8197 ± 0.0743	
Week 12	0.8630 ± 0.0898	

Note P-value determine by Repeated measure ANOVA test

Baseline (0.7103) Week 4 (0.7481) Week 8 (0.8197) Week 12 (0.8630)

Note *The unjoining line denotes that the pairs are significantly different

Figure 4.1 The comparison of mean of cutometer score at crow's feet at baseline, week 4, week 8 and week 12 by line joining variables

The mean cutometer score at crow's feet were 0.7103 ± 0.0845, 0.7481 ± 0.0922, 0.8197 ± 0.0743, and 0.8630 ± 0.0898 at baseline, 4^{th}, 8^{th} and 12^{th} weeks respectively. There was progressive increase with each visit and table 4.2 shows statistical significance ($p < 0.001$).

Figure 4.1 shows a multiple comparison result. The mean cutometer score at crow's feet at baseline is not significantly different from that at week 4. However, the baseline score differs significantly from week 8 and week 12. Similarly, week 4 cutometer score is statistically significant from week 8 and week 12. Moreover, the two mean cutometer scores at week 8 and week 12 have been shown to be statistically different.

Table 4.3 Statistical analysis of cutometer score at under eye area on baseline, follow-up 4th, 8th, and 12th week

	Mean ± SD	**p-value**
Baseline	0.6920 ± 0.1006	<0.001
Week 4	0.7363 ± 0.7827	
Week 8	0.7936 ± 0.0840	
Week 12	0.8505 ± 0.1042	

Note P-value determine by Repeated measure ANOVA

Baseline (0.692) 4th week (0.7363) 8th week (0.7936) 12th week (0.8505)

Note *The unjoining line denotes that the pairs are significantly different

Figure 4.2 The comparison of mean of undereye cutometer score at week 0, week 4, week 8 and week 12 by line joining variables

According to statistical analysis of undereye cutometer score from table 4.3, mean cutometer scores were 0.6920 ± 0.1006 at baseline, 0.7363 ± 0.7827 at 4th week visit, 0.7936 ± 0.0840 at 8th week and 0.8505 ± 0.1042 at 12th week. The data indicate that mean cutometer scores was significantly increased at level of 0.05 ($p < 0.001$). Therefore, mean cutometer score at undereye area were significantly increased on 4th, 8th and 12th weeks compared to baseline.

As seen in multiple comparison analysis of cutometer score at undereye area, mean different changes were seen in different time points. The mean change in cutometer score at the undereye area is statistically different in each week.

4.3 Results of Corneometer Score for Skin Hydration

Table 4.4 Statistical analysis of corneometer score at crow's feet at baseline, 4th, 8th, and 12th week

	Mean ± SD	p-value
Baseline	64.20 ± 12.05	< 0.001
Week 4	73.84 ± 12.17	
Week 8	79.42 ± 9.32	
Week 12	84.72 ± 12.01	

Note P-value determine by Repeated measure ANOVA test

Baseline (64.20) Week 4 (73.84) Week 8 (79.42) Week 12 (84.72)

Note *The unjoining line denotes that the pairs are significantly different

Figure 4.3 The comparison of mean of corneometer score at crow's feet at baseline, week 4, week 8 and week 12 by line joining variables

According to the statistical analysis of corneometer score at crow's feet from table 4.4, mean corneometer scores were 64.20 ± 12.05 at baseline, 73.84 ± 12.17 at 4th week visit, 79.42 ± 9.32 at 8th week and 84.72 ± 12.01 at 12th week. The mean corneometer scores were significant at the level of 0.05 ($p < 0.001$). Therefore, mean corneometer scores at crow's feet were significantly increased on 4th, 8th and 12th weeks compared to baseline.

Figure 4.3 describes multiple comparison results of corneometer score at crow's feet. All of the changes were statistically significant ($p < 0.05$) except from week 8 to week 12 ($p = 0.08$).

Table 4.5 Statistical analysis of corneometer score at undereye area at baseline, 4th, 8th, and 12th week

	Mean ± SD	**p-value**
Baseline	64.04 ± 13.11	< 0.001
Week 4	72.90 ± 10.77	
Week 8	78.06 ± 9.72	
Week 12	82.60 ± 11.44	

Note P-value determine by Repeated measure ANOVA

Baseline (64.04) 4th week (72.9) 8th week (78.06) 12th week (82.6)

Note *The unjoining line denotes that the pairs are significantly different

Figure 4.4 The comparison of mean of corneometer score at undereye area at baseline, 4th week, 8th week and 12th week by line joining variables

The mean corneometer scores at undereye area were 64.04 ± 13.11, 72.90 ± 10.77, 78.06 ± 9.72, and 82.60 ± 11.44 at baseline, 4th, 8th and 12th weeks which means there was progression with each visit. Table 4.5 shows this data is statistically significant at the level of 0.05 ($p < 0.001$). The multiple comparison analysis is shown below.

According to the comparison results from figure 4.4, it is found that corneometer score at undereye area was statistically significant at the level of 0.05 ($p < 0.05$) for each follow-up. Therefore, undereye corneometer score at 12th week was higher than those at baseline, week 4 and week 8.

4.4 Results of Wrinkle Evaluation by Dermatologists

Photographs taken by VISIA Complexion Analysis System were evaluated by 3 different dermatologists with the following grading scale:

-1 = worse

0 = no changes

1 = 1-25% fair improvement

2 = >25-50% moderate improvement

3 = >50-75% good improvement

4 = >75-100% excellent improvement

Table 4.6 Dermatologists' evaluation score of 3% acai palm extract cream

Grading scale	3% acai palm extract eye cream		
	Week 4	**Week 8**	**Week 12**
4 = excellent improvement	0	0	0
3 = good improvement	0	3	4
2 = moderate improvement	5	7	12
1 = fair improvement	15	10	6
0 = no changes	0	0	0
-1 = worse	0	0	0

Table 4.7 Statistical analysis of wrinkle evaluation score by dermatologists

Week	**Mean ± SD**
Week 4	1.30 ± 0.57
Week 8	1.85 ± 0.49
Week 12	2.30 ± 0.66
p-value	**< 0.001**

Note Data were analyzed by Friedman test.

$P < 0.05$ is significant.

Week 4 (1.30) Week 8 (1.85) Week 12 (2.30)

Note *The unjoining line denotes that the pairs are significantly different.

Figure 4.5 The comparison of mean of dermatologists' wrinkle evaluation score at week 4, week 8 and week 12 by line joining variables

The table 4.6 displays evaluation scores by three dermatologists. 15 of the participants showed fair improvement while there were 5 who showed moderate improvement at week 4. There was an overall progress amongst the 20 participants as 10 of them showed fair, 7 showed moderate and 3 showed good improvements. There was more progress at the end of the study at week 12 where 6 participants showed fair, 12 showed moderate and 4 showed good improvements.

The mean wrinkle evaluation score issued by the three dermatologists progressively increased with every visit with the $p < 0.001$, meaning it was statistically significant as shown in table 4.7. The mean scores were 1.3, 1.85 and 2.3 at 4^{th}, 8^{th} and 12 weeks respectively.

The mean wrinkle evaluation scores of week 4 to 8 were statistically significant ($p = 0.018$) according to the multiple comparison analysis (figure 4.5). The mean score of week 4 was also statistically significant from that of week 12 ($p < 0.001$) apart from the mean change from week 8 to 12 ($p = 0.058$).

4.5 Patients' Satisfaction Score

Table 4.8 Patients satisfaction score of 3% *Euterpe oleracea* extract eye cream on 12^{th} week

Satisfactory score	n
0 - no satisfaction	0
1 - little satisfaction	0
2 - average satisfaction	2
3 - more satisfaction	15
4 - most satisfaction	3

Above table 4.8 demonstrated patients' satisfaction score at 12^{th} week. The data suggested that most of the volunteers rated more satisfaction while 4 rated most satisfaction and 2 average satisfaction.

4.6 Side Effect

All 20 participants were closely monitored during patch test and the course of research. However, no side effects were recorded for any of the participants in the duration of this research.

CHAPTER 5

DISCUSSION, CONCLUSION, RECOMMENDATION

5.1 Discussion

Wrinkles can be caused by natural aging or due to external factors like UV exposure, smoking among many others. Oxidative stress produces ROS in excess of free radicals which can lead to increased MMP and collagen degradation. It also inhibits new collagen synthesis resulting in reduced skin elasticity and hydration, hence, wrinkles.

Due to their powerful antioxidant properties from anthocyanins and flavones such as orientin and isovitexin, *Euterpe oleracea* is known as superfood. Acai berries are also rich in other flavonoids and polyphenols.

This was the study of 3% *Euterpe oleracea* extract eye cream for reduction of periorbital wrinkles. Exactly 20 volunteers made up of both genders were recruited for the study duration of 3 months. All the subjects completed the study with monthly follow-ups. The mean age was 35.5 ± 5.35 years with minimum of 30 years and maximum of 50 years. They were all healthy with no underlying diseases. None of the subjects had photosensitivity, drug allergy, personal medication or treatment before the study. 11 of the participants had the combination skin type, 5 had dry skin and 4 had oily skin.

Cutometer was used to measure skin elasticity. Mean cutometer score at crow's feet increased significantly from baseline to consecutive follow-ups (table 4.2). Mean change in cutometer score at crow's feet progressively raised in each visit. Mean changes between the visits were all significant but for that from baseline to week 4. It indicates that elasticity at 12^{th} week was higher than that at baseline. As for undereye

elasticity, a progressive increase in mean cutometer score was observed in all follow-ups as well (table 4.3). Between follow-up phases, mean change in cutometer score was seen in all comparisons. Hence skin elasticity at undereye area was also greater in week 12 compared to baseline.

Measurement of skin hydration was done by using Corneometer. Mean corneometer scores at crow's feet were higher in 4^{th} week, 8^{th} week and 12^{th} week than in baseline. For comparison, mean changes from week 0 to week 4, week 0 to week 8, week 0 to week 12, week 4 to 8 and week 4 to 12 were shown to be statistically significant. However, the mean change from week 8 to 12 was not statistically significant. The results indicate improvement in skin hydration in crow's feet at week 12 when compared to baseline. For undereye, mean corneometer score went up in all visits. After comparison was done between the follow-ups, the mean change was statistically significant in all comparison groups. Hydration of the undereye also increased in week 12 compared to baseline.

Photographs taken by VISIA complexion analysis system were evaluated by 3 dermatologists and the scores were analyzed by Friedman test. The mean of evaluation score increased in each visit (table 4.7). The mean changes from week 4 to 8 and week 4 to 12 were statistically significant whereas that from week 8 to 12 was not. At week 12, wrinkle score was higher than that at week 0. Therefore, overall improvement is seen in all skin parameters after 12 weeks of using 3% *Euterpe oleracea* extract cream.

Additionally, volunteers were asked to rate their satisfaction from 0 to 4 after the study, with 0 being NO satisfaction and 4 being MOST satisfaction. From 20, 2 (10%) gave score 2 ratings, 15 (75%) gave score 3 ratings, 3 (15%) gave the study score 4. The data suggested that 3% acai palm extract cream has high patients' satisfactory score. Regarding adverse effects, itchiness, erythema, or any serious side effects were not observed with any participants during the patch test. Moreover, there were no occurrences of any side effects over 12 weeks of this study. Thus, 3% *Euterpe oleracea* extract cream is safe for topical application.

In this study, both skin hydration and elasticity were improved after regular use of 3% acai palm extract eye cream for 3 months. This might be due to the anti-collagenase action of acai palm extract. The proposed effect is associated with an increase in expression of type I collagen, VEGF, and fibronectin as well as downregulation of the expression of MMP-1 (Kang & Kim, 2018). Previous studies showed that it can scavenge superoxide and hydroxyl radicals. Amazingly, acai palm was found to have the highest antioxidant capacity than any other fruits or vegetables (Peixoto et al., 2016). The extract not only exhibited an inhibition of new ROS formation, but also increased some antioxidant enzymes like SOD, catalase, and glutathione when healthy women consumed acai pulp for 4 weeks (Peixoto et al., 2016).

The anti-inflammatory property of acai extract is largely related to its ability to downregulate the expression of pro-inflammatory transcription factor, NF-KB (de Souza et al., 2012). It is well understood that NF-KB is involved in the pathogenesis of aging by releasing several proinflammatory cytokines and increasing oxidative stress. By downregulation of NF-KB, the acai extract can inhibit the process of inflammation implicated in the formation of wrinkles. Owing to these informations, 3% acai berry extract cream showed its anti-wrinkle effects in line with the hypothesis.

5.2 Recommendations

This research may be used as a database for further research about skin rejuvenation and smoothness.

This study may be used as an alternative antiaging treatment for wrinkle reduction and the collected data may be used in comparison with other topical antiaging treatments. The collected data may also assist the usage of acai palm extract for skin diseases and other cosmetic purposes.

5.3 Conclusion

After 12 weeks of use of 3% Euterpe oleracea extract eye cream, the results demonstrated the gradual increase in the means of cutometer and cornrometer scores in each visit. This implies that the acai palm extract improves skin elasticity and hydration. More importantly, it did not give rise to any kind of adverse effects and the treatment satisfactory score received from the volunteers is also high. Lastly, the topical 3% Euterpe oleracea extract cream as a treatment for periorbital wrinkles is considered a safe and promising anti-wrinkle intervention.

5.4 Limitations

It is important to note that while the participants were strongly recommended to avoid outdoors, sun exposure and to get enough sleep while the study is going on, the researcher cannot control the lifestyles of the participants.

While there were improvements in skin elasticity and hydration, better improvements compared to baseline numbers could potentially be observed had the study been longer. Moreover, this study was done using a concentration of 3% of Euterpe oleracea extract in the cream and the researcher believes the effects of a cream with moderately higher acai palm concentration against wrinkles should be studied upon.

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APPENDICES

APPENDIX A

ETHICAL APPROVAL DOCUMENT

CERTIFICATE OF APPROVAL

COA: 073/2022

Protocol No: EC 22035-20

Title:	Efficacy of 3% Acai berry extract cream for reduction of periorbital wrinkles
Principal investigator:	Hsu Mon Zaw, M.D.
School:	Anti-Aging and Regenerative Medicine
Funding support:	Personal Funds

Approval:

(1) Research protocol	Version 2 date March 21, 2022
(2) Information sheet for research project participants	Version 2 date March 25, 2022
(3) Informed consent form	Version 2 date March 10, 2022
(4) Case Record Form and Assessment Form	Version 2 date March 21, 2022
(5) Questionnaire	Version 2 date March 21, 2022
(6) Research participant recruitment information	Version 2 date March 21, 2022

(7) Principal investigator and Co-investigators

- Hsu Mon Zaw, 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 18, 2022
Date of Expiration:	April 17, 2023
Frequency of Continuing Review:	1 year

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(Assoc. Prof., Maj. Gen. Sangkae Chamnanvanakij, M.D.)

Chairperson of the Mae Fah Luang Ethics Committee on Human Research

APPENDIX B

INFORMATION SHEET

1. Title

Efficacy of 3% *Euterpe oleracea* extract cream for reduction of periorbital wrinkles

2. Primary objective

To compare the mean change of periorbital wrinkle reduction by topical 3% *Euterpe oleracea* extract cream from baseline to 12^{th} week visit, assessed by using Corneometer, Cutometer and VISIA® Complexion Analysis System

3. Secondary objective

- To observe safety and adverse effects of topical 3% *Euterpe oleracea* extract cream
- To evaluate the participants' satisfaction score of topical 3% *Euterpe oleracea* extract cream

4. Background

People have always had tendencies to reduce the aging process of their skin. This is a phenomenon that is deeply rooted in the history of human civilization. It is widely known that Cleopatra, the queen of Egypt and a symbol of beauty, would often take two baths a day in donkey milk as it contains alpha-hydroxy acids for its role in the anti-aging process. There are many more topical treatments that were popularized throughout our history such as apple cider vinegar, honey and turmeric to more manmade treatments such as botulinum toxin, filler, thread lift and laser treatments.

The treatments mentioned above among with a plethora of others tackle similar aging issues like wrinkles, dark spots, dull skin and laxity. Wrinkles are skin

folds or creases caused by a combination of factors. The first one is aging which can be classified into intrinsic and extrinsic aging. Intrinsic aging is a chronological process that is mainly influenced by genetical and hormonal factors whereas extrinsic aging is primarily caused by ultraviolet [UV] irradiation called Photoaging. UV lights degrade the collagen and elastin fibers by free radicals oxidation and expression of matrix metalloproteinases. As these layers are destroyed, the skin becomes weaker and wrinkles appear. Smoking also plays a role in the wrinkle formation by accelerating the aging process. Alcohol consumption promotes dehydration and dry skin are more prone to developing wrinkles. Other causes include repeated facial muscle expression, exposure to pollutants, toxins and diet.

Even though having wrinkles is not a disease, it imposes a discomfort for many people. Wrinkle formation cannot be stopped but there are various modalities targeted at reducing its appearance, varying from skincare application, botulinum toxin and filler injections, microdermabrasion, laser therapies to cosmetic surgeries.

Among them, botanical extracts are increasingly used for anti-aging purposes. They are also being used as an alternative to conventional treatments owing to their fewer side effects. Various research have shown their anti-microbial, anti-inflammatory, anti-proliferating, photo protective and wound healing properties. The emergence of botanical extract as an anti-aging ingredient since they are rich in antioxidants. Besides, they are cost effective and easily available.

The acai fruit, coming from a specific palm tree called *Euterpe oleracea*, may prove to be one of the most promising botanical extracts constituting powerful antioxidants. Largely cultivated in the Brazilian Amazon, acai tree extracts have a growing demand around the globe exactly for their anti-inflammatory, antinociceptive, wound healing and cholesterol lowering properties. In addition to its use for anti-aging and acne treatment purposes, different parts of the acai palm trees are commonly used as alternative treatments for other medical problems in Brazil and Peru. A few examples include diarrhea being treated by the oil of the fruit, skin ulcers being washed with the fruit grind and jaundice, malaria, fever relief, diabetes and hair loss being treated by the infusion of the root. Even though there has always been a historical interest in acai berries regarding anti-aging, the data and enough research on it has been lacking.

When we dive into further details, its properties become more apparent. It is demonstrated that the fruit pulp consumption causes an increase in plasma antioxidant capacity. Additionally, it is also proven that the dietary supplement of the fruit modulates ROS production and improves the gene expression of antioxidant enzymes in rats' liver under oxidative stress. In a recent study, acai berry water extract was shown to promote wound healing. It also increases type I collagen, VEGF, and fibronectin productions whilst MMP-1 and IL-1β expression are inhibited. MMP-1 or matrix metalloprotenase 1 is a collagenase. Inhibition of MMP-1 results in reduction of collagenase and accelerates wound healing. IL-1β is responsible for T cell activation which in turn promotes inflammatory cells like lymphocytes and monocytes. Inhibition of IL-1β can be attributed to anti-inflammatory effects in the wound healing process.

According to previous studies, some of the constituents found in acai fruits are claimed to be polyphenols including anthocyanins, flavonoids, gallic acid and ferulic acids, lignans and lipids. The constituents mentioned above are able to actively scavenge free radicals and/or chelate the metal iron, leading them to act as effective antioxidants. There is good evidence that confirm the extracts can counteract the oxidative stress and aging processes in *Caenorhabditis elegans*. Moreover, Petruk et al pretreated BALB/3T3 fibroblasts with acai extracts before inducing oxidative stress by UV-A radiation (100 J/cm^2). It was found that acai extract inhibits ROS (reactive oxygen species), intracellular glutathione depletion (GSH form) and lipid per oxidation thus protecting the fibroblasts against the negative effects of UV-A induced oxidative stress. The most active compounds responsible for the effects were found to be cyanidin and malvidin. Based on these health-promoting benefits, the acai extract has been gaining interest in anti-aging cosmetic products.

E. oleracea, or the acai berry, has shown to have potential in preventing or retarding age-related diseases like cardiovascular diseases, cancer and aging skin. The fruit is consisted of such compounds as polyphenols and flavonoids that exert strong antioxidant actions. In the matter of choosing anti-aging products, those formulated from plants containing antioxidant, anti-inflammatory and free-radical-scavenging functions are preferred. The extract would be useful in reducing wrinkles because it has the properties of inhibiting the aging process. Evidence presents the acai berry

water extract can increase type 1 collagen production and the dietary supplement can reduce oxidative stress. More importantly for this research, the extract has the quality of lowering MMP-1 expression.

The main reason of this study is to conduct that the antioxidant action of acai palm extract cream would be useful in treatment of wrinkles on face. Thus, the actual purpose is to study the efficacy and satisfaction of *E. oleracea* extract cream in wrinkles reduction.

5. Study Location

Mae Fah Luang University Hospital, Asoke, Bangkok

6. Procedure

6.1 Patients were enrolled in the study according to inclusion and exclusion criteria.

6.2 The researcher intensively explained the aim of research, process during the research, benefits and possible side effects of the treatment.

6.3 The volunteers had to sign an informed consent form for participation in the research.

6.4 History taking was done. General information such as age, sex, occupation, address, contact number and previous medical history related to wrinkles were collected.

6.5 Patch test was performed before applying *Euterpe oleracea* cream

Apply 3% Euterpe oleracea extract cream on volunteers' arms under waterproof patch test and wait for 24 hours.

The volunteer should avoid excessive sweating conditions, heavy sunlight during the test and then examined for any responses until 48 and 96 hours. Any reaction reacted was scored according to the international contact dermatitis research group system as follow;

- = negative

+? = any doubtful reaction for example only mild redness

+ = weak positive reaction; appears redness and the skin become slightly thickened

++ = strong positive reaction; appears red swollen skin with individual small water blister

+++ = extreme positive reaction; appears intense redness and swelling with coalesced spreading reaction or large blisters.

NT = not tested

IR = irritant reaction

Volunteer with positive patch test scoring ++ or higher were excluded.

6.6 Taking photographs of volunteers' faces

The researcher took the photographs of each volunteer by using VISIA complexion analysis system at baseline, 4th, 8th and 12th week respectively. Favorable condition for the procedure is to turn the light off and capture with 12 megapixel resolution, automatic white balance correction, automatic focus, facial position: Right 37, left 37, Center 0, multi-spectral imaging (cross polarized flash and ultraviolet lighting, standard daylight fluorescent lighting).

6.7 Measurement of skin elasticity and hydration

Skin elasticity and hydration were measured by Cutometer MPA 580 and Corneometer at 0, 4th, 8th and 12th week at two landmarks. These landmarks were at 1.5 cm lateral to lateral canthus (A) and 1.5 cm from the lower eyelid at the mid papillary line (B). The data was recorded and analyzed for each visit.

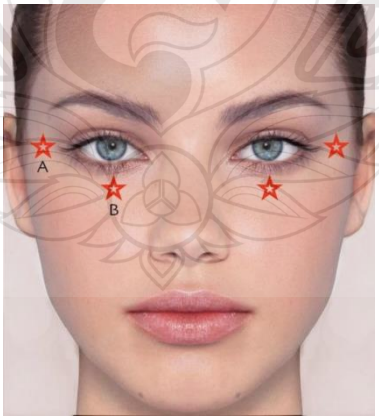

Figure B1 Landmarks to measure skin moisturization and elasticity

6.8 Manual for Volunteers

Instructions to follow

Topical 3% *Euterpe oleracea* cream will be used twice daily by the following steps:

1. Wash the face using mild soap and pat the face with dry and clean tissues or towels.

2. After washing the face, squeeze 1 finger-tip unit of topical 3% *Euterpe oleracea* extract cream through tube (1 finger-tip unit = 0.5 gram) and apply to left side around the periorbital region using index finger.

3. Gently tap small dots over eyelid and under the eye, starting in the innermost corner and moving outward. Pat the product into the skin. Avoid dragging or rubbing.

4. Go over the same steps to apply the cream in right periorbital area. Wait 2 minutes for the cream to fully absorb. Other routine facial skincare steps and sunscreen can be continued as usual.

5. All these steps are instructed to be repeated for two times in the morning and at night so that the cream amount will be 2 grams.

Figure B2 How to apply eye cream

Restrictions

Explain the participant thoroughly not to apply any other eye cream or retinol around the periorbital area during the study. If any side effect or reaction occurs, participants should stop using the cream and inform to researcher.

APPENDIX C

INFORMED CONSENT

หนังสือแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัย สำหรับอาสาสมัคร

ชื่อโครงการวิจัย Efficacy of 3% Euterpe Oleracea Extract Cream for Reduction of Periorbital Wrinkles
ประสิทธิภาพของครีม สารสกัดจาก อาซาอีปาล์ม 3% เพื่อลดเลือนริ้วรอยรอบดวงตา

ข้าพเจ้า นาย/นาง/นางสาว.....
ที่อยู่.....
ได้อ่านรายละเอียดจากเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัยวิจัย ฉบับวันที่ .....

ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย และสำเนาเอกสารแสดงเจตนายินยอมเข้าร่วมในโครงการวิจัยที่ข้าพเจ้าได้ลงนามและลงวันที่ ทั้งนี้ก่อนที่จะลงนาม ข้าพเจ้าได้รับการอธิบายโดยละเอียดจากผู้วิจัยถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้น ประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น

ข้าพเจ้ามีเวลาและโอกาสเพียงพอในการซักถามข้อสงสัย โดยผู้วิจัยได้ตอบคำถามต่าง ๆ ด้วยความเต็มใจไม่ปิดบังซ่อนเร้นจนข้าพเจ้าเข้าใจเป็นอย่างดีแล้ว

ข้าพเจ้ารับทราบจากผู้วิจัยว่า หากเกิดอันตรายใด ๆ จากการวิจัย ข้าพเจ้าจะได้รับการรักษาพยาบาล ตามที่ระบุในเอกสารชี้แจงข้อมูลแก่อาสาสมัครผู้เข้าร่วมในโครงการวิจัย

ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ การถอนตัวนี้ไม่มีผลต่อการรักษาพยาบาลและสิทธิอื่น ๆ ที่ข้าพเจ้าจะพึงได้รับต่อไป

ผู้วิจัยรับรองว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ การรายงานหรือสรุปผลการวิจัยจะไม่ระบุชื่อนามสกุลของข้าพเจ้า การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้องจะกระทำด้วยเหตุผลทางวิชาการเท่านั้น

ข้าพเจ้าได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินดีเข้าร่วมในการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารแสดงความยินยอมนี้

..... ลงนามผู้เข้าร่วมในโครงการวิจัย

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

วันที่ .....เดือน.....พ.ศ.....

ข้าพเจ้าได้อธิบายโดยละเอียดถึงวัตถุประสงค์ วิธีการวิจัย ความไม่สุขสบายหรือความเสี่ยงที่อาจเกิดขึ้นประโยชน์ที่คาดว่าจะได้รับจากการวิจัย และทางเลือกอื่น ให้ผู้เข้าร่วมในโครงการวิจัยได้ทราบและมีความเข้าใจดีแล้ว พร้อมทั้งลงนามในเอกสารแสดงเจตนายินยอมด้วยความสมัครใจ

..... ลงนามผู้วิจัย

(.....) ชื่อ-สกุล ผู้วิจัย (ตัวบรรจง)

..... ลงนามพยาน

(.....) ชื่อ-สกุล พยาน (ตัวบรรจง)

วันที่ .....เดือน.....พ.ศ.....

APPENDIX D

RESEARCH PROFILE (CONFIDENTIAL)

ID

General Information (Only official)

1. Date
2. Age
3. Sex ❑Male ❑Female
4. Pregnancy ❑Yes ❑No
5. Occupation
6. Underlying disease:
7. Personal medication and supplement:
    a. Chemo-radiotherapy
    b. Active inflammatory skin disease, open wound in the treatment area
    c. History of malignant of premalignant lesions in the treatment area
8. History of drug allergy
9. Current use of facial products
10. History of following treatment within 6 months before the study
    ❑ Yes .....Identify
        -Ablative and/or Non-ablative Laser Treatment
        -Microdermabrasion
        -Intense Pulse Light therapy
        -Botox
        -Radiofrequency
        -HIFU
    ❑ No
11. Photosensitivity or drug induced photosensitivity
12. Average time exposure to the sunlight during 10 am – 4pm.....min

APPENDIX E

PHYSICIAN RECORD FORM RESEARCH RECORD: (RESEARCHERS' PART)

EFFICACY OF 3% EUTERPE OLERACEA EXTRACT CREAM FOR REDUCTION OF PERIORBITAL WRINKLES

Volunteer number ……....

Tasks at the first visit

Date……..

Please use the "✓" symbol.

Checklist for first visit		
Process	**Yes**	**No**
Physical examination for fitness		
The volunteer has completed research profile		
All the profile data is fit to selection criteria		
Assure that the volunteer can understand information and how to use the treatment cream.		
The volunteer has written the informed consent form. The researcher asked the volunteer for any curiosity.		
The researcher answered the volunteer's questions.		
Photography of the face was performed using Cutometer, Corneometer was performed		

CASE RECORD FORM, CRF

Volunteer Number

1. Cutometer MPA 580

Cutometer	Right	Left
Week 0		
Week 4		
Week 8		
Week 12		

2. Corneometer

Corneometer	Right	Left
Week 0		
Week 4		
Week 8		
Week 12		

3. Facial Assessment Wrinkle Score by Dermatologist

Side	Baseline	4th week	8th week	12th week
Left				
Right				

-1 = worse

0 = no changes

1 = 1-25% fair improvement

2 = >25-50% moderate improvement

3 = >50-75% good improvement

APPENDIX F

SUBJECT'S SATISFATION QUESTIONNAIRE

Volunteer Number

1. Record of Adverse Effects

Please check the following signs and symptoms after each of the treatment by using the "✓" symbol.

Symptoms	Yes	No	Duration	Remark
Pruritus				
Erythema				
Other				

2. Treatment Satisfaction Score

Satisfactory evaluation by volunteers

Please draw the circle on 12th week.

0	1	2	3	4

Score 0 = no satisfaction

Score 1 = little satisfaction

Score 2 = average satisfaction

Score 3 = more satisfaction

Score 4 = most satisfaction

3. Will you undergo the same treatment in the future?

❑ Yes ❑ No

แบบสอบถามความพึงพอใจของอาสาสมัคร

รหัสอาสาสมัคร .....

1. อาการข้างเคียงที่ไม่พึงประสงค์

โปรดใส่เครื่องหมายถูก (✓) ลงในช่องว่างที่มีอาการไม่พึงประสงค์หลังการรักษาในแต่ละครั้ง

อาการ	ใช่	ไม่ใช่	ระยะเวลา	หมายเหตุ
อาการคัน				
แดง				
อื่นๆ				

2. คะแนนความพึงพอใจ

การประเมินความพึงพอใจ โดยอาสาสมัคร (โปรดทำเครื่องหมายวงกลม ○)

0	1	2	3	4
0	หมายถึง	ไม่แตกต่างจากเดิม		
1	หมายถึง	พึงพอใจระดับน้อย		
2	หมายถึง	พึงพอใจระดับปานกลาง		
3	หมายถึง	พึงพอใจระดับมาก		
4	หมายถึง	พึงพอใจที่สุด		

3. หากเลือกได้ ท่านยินดีที่จะรับการรักษาแบบเดียวกันนี้ในอนาคตอีกไหม

☐ รับ ☐ ไม่รับ

APPENDIX G

MATERIAL

Figure G1 Packaging of 3% Euterpe oleracea extract cream

STANDARDIZED PHOTOGRAPHS OF SUBJECTS

Figure G2 Comparison of the face of subject A before and after 12 week results

Figure G3 Comparison of the face of subject B before and after 12 week results

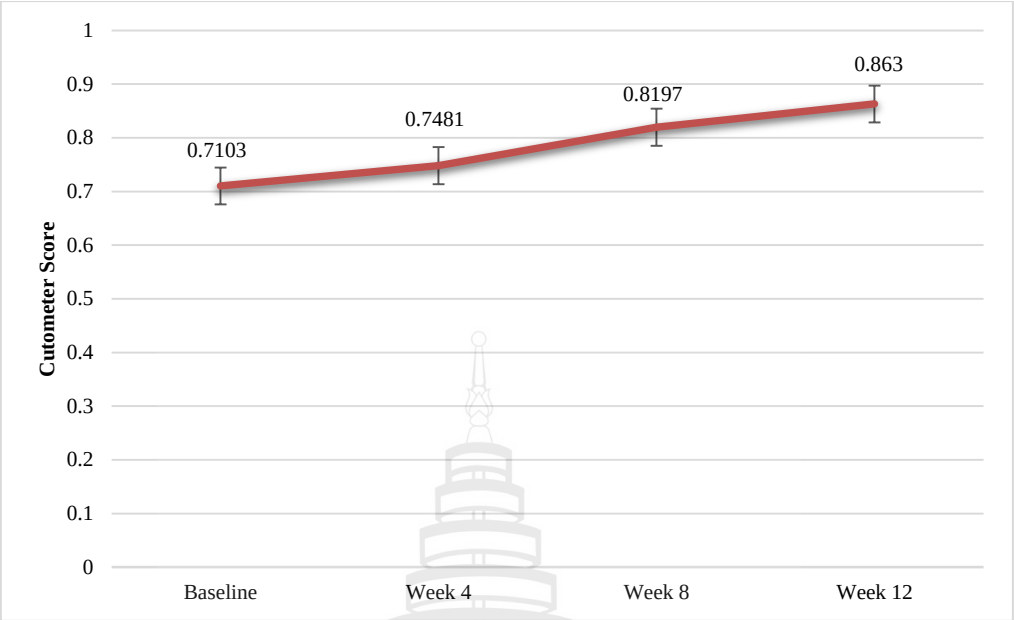

Figure G4 Line graph showing Mean cutometer score at crow's feet in each visit

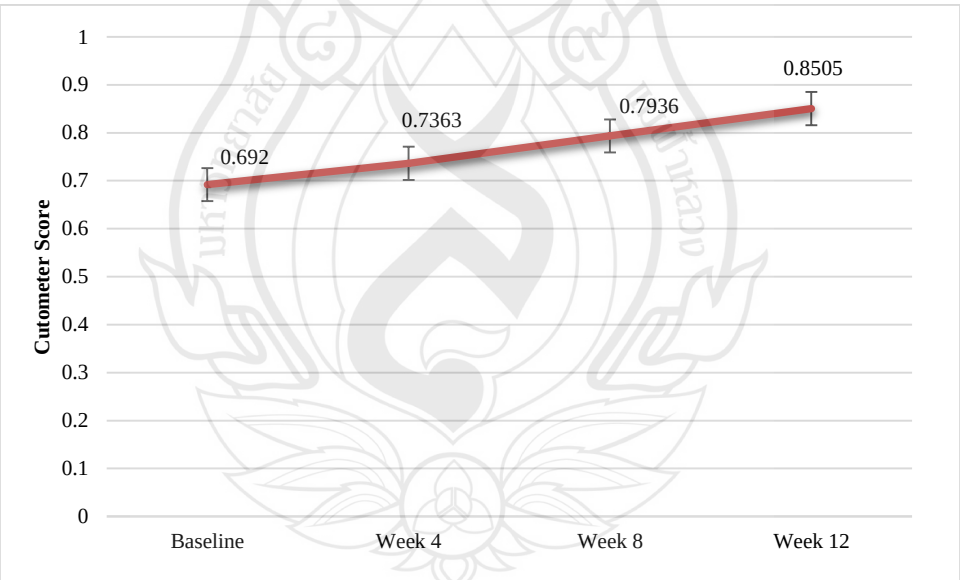

Figure G5 Line graph showing Mean cutometer score at under eye area in each visit

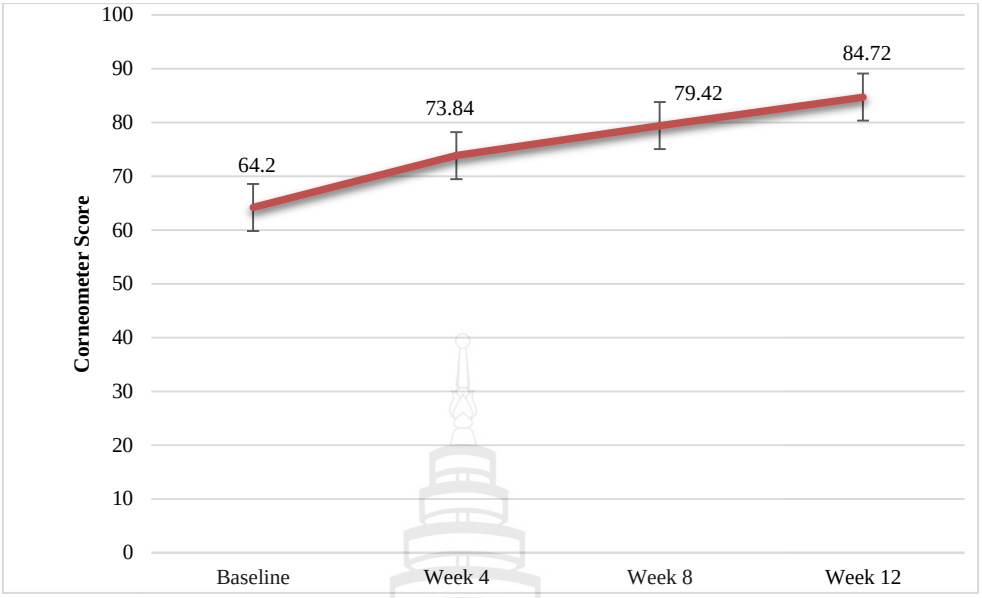

Figure G6 Line graph showing mean corneometer score at crow's feet area in each visit

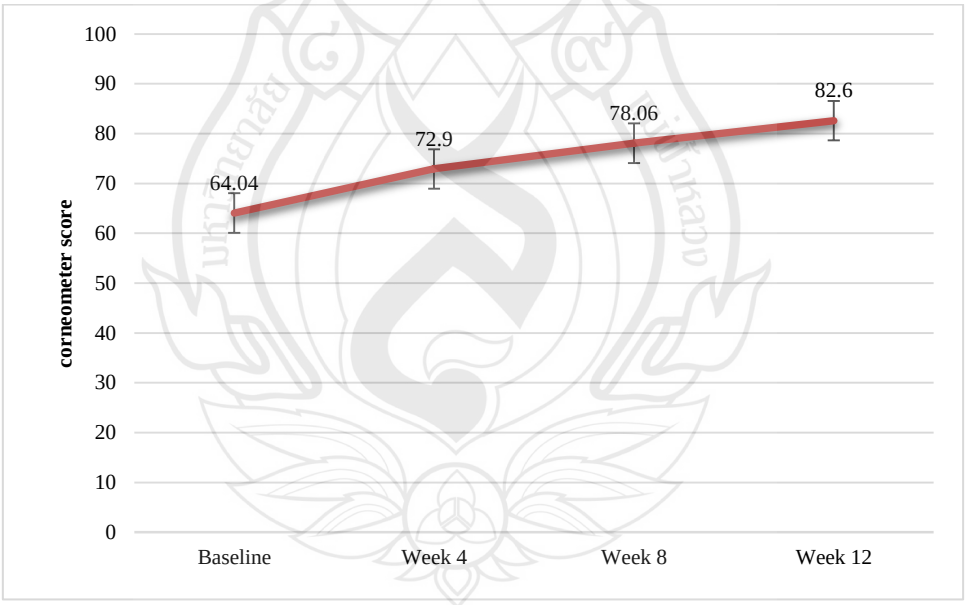

Figure G7 Line graph showing Mean cornemeter score at undereye area in each visit

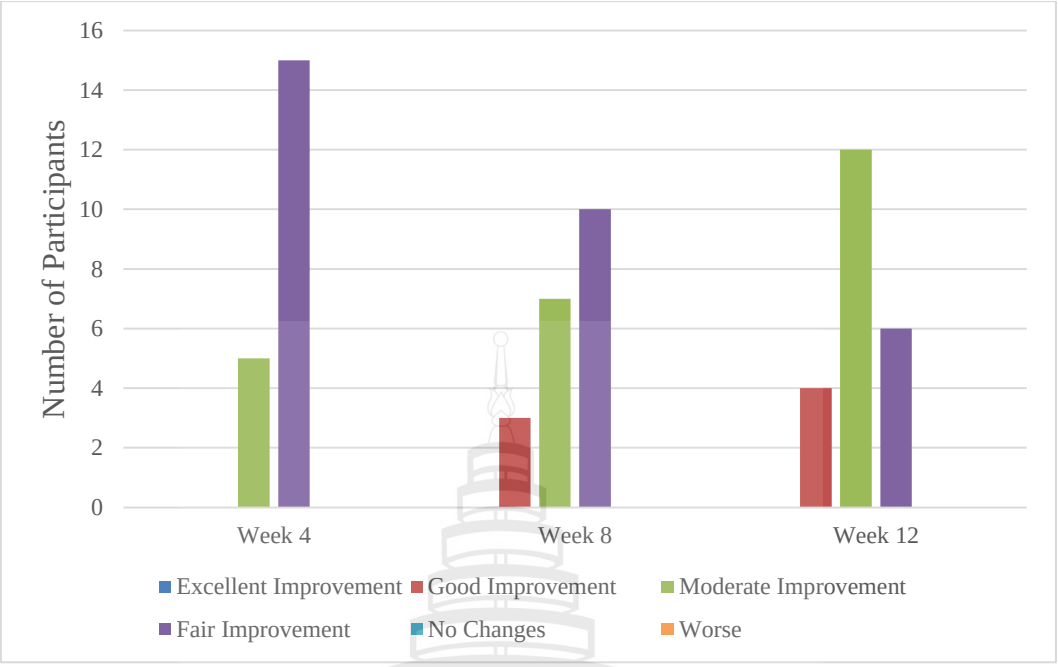

Figure G8 Column chart showing wrinkle evaluation score by 3 dermatologists at 4th week, 8th week and 12th week

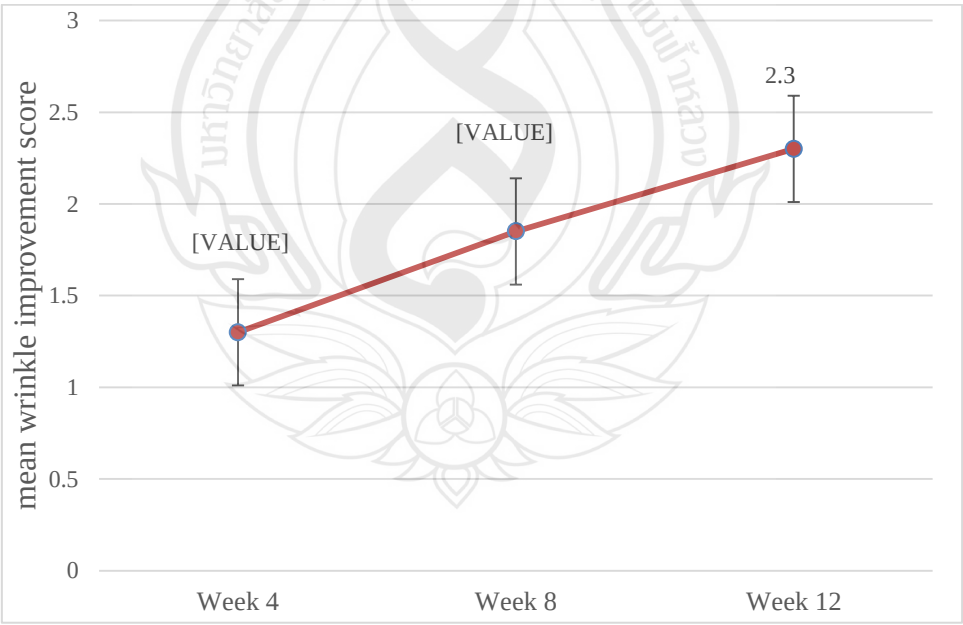

Figure G9 Linear graph showing mean wrinkle evaluation score by 3 dermatologists at 4th week, 8th week and 12th week

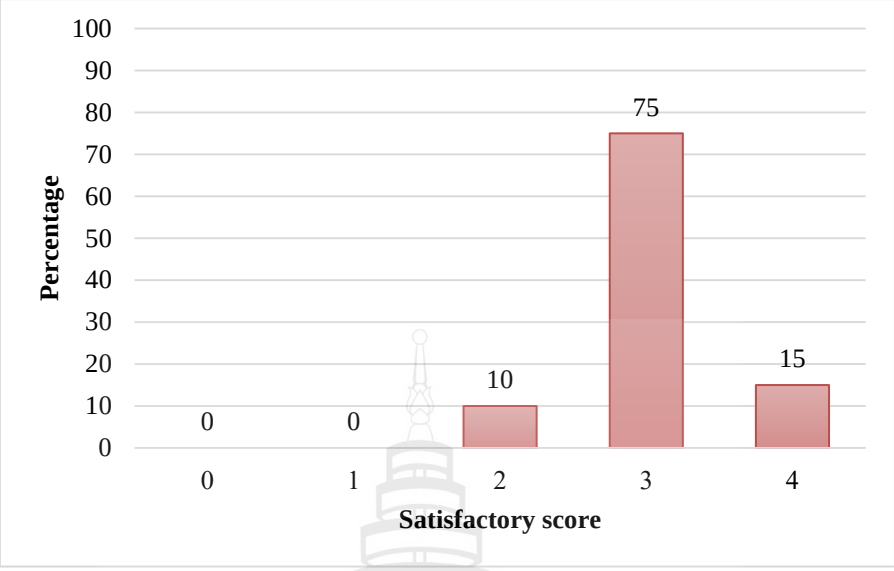

Figure G10 Bar graph showing frequencies of patient satisfaction score of 3% acai palm eye cream on 12th week