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The Efficacy of Topical 3% Morinda Citrifolia in the Treatment of Periorbital Wrinkles

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Abstract

Background: Wrinkles, or rhytids, are a fold, ridge, cease, in the skin. Skin wrinkle is naturally formed with the passage of time as a result of aging processes, such as glycation, loss of body mass, and sleeping position. Apart from these intrinsic factors attribute to wrinkle formation, extrinsic factors, including UV radiation, air pollution, and stress, can also cause premature skin aging. In cosmetic industry, recent research studies have found bioactive compounds that possess antioxidant property, which can scavenger free radical from skin cells, prevent trans-epidermal water loss, and contribute to protect skin from wrinkles, leading to glowing and healthy younger skin. Different parts of medicinal plants have been discovered to be antioxidant sources contain variety of bioactive compounds. *Morinda citrifolia* is a medicinal plant that has an extensive historical use as traditional medicine in Thailand owing to its antioxidant activity and proven health benefits. Due to this reason, it has led to further study on the efficacy of *Morinda citrifolia* in reducing the appearances of lateral canthal fine lines and wrinkles (crow's feet).

Objectives: To study the efficacy of *Morinda citrifolia* extract cream in periorbital wrinkle reduction.

Material and Methods: An 8-week clinical trial of a *Morinda citrifolia* (noni) extract eye cream regimen was conducted in 16 healthy Thai women, aged 50 between 60 years, at Mae Fah Luang hospital. Prior to enrollment of subjects, the participants' wrinkles were assessed by a physician using Rao-Goldman's 5-point

visual scale, by correlating the grade of the wrinkle in the reference photographs with the wrinkle in a participant's face. A score of 2 to 4 is assigned based on the visibility and depth of the wrinkles. The participants whose score between2 – 4 and meet all inclusion and non-exclusion criteria were included in the experiment. *Morinda citrifolia* extract eye cream and placebo cream (standard cream base with similar consistency, colour, smell) were randomly applied to the periorbital region, used block randomization, in a split face design (right and left), twice daily for 8 weeks and follow-up every 4 weeks. The periorbital wrinkle depth was measured by the Visioscan® VC98, and skin elasticity and firmness were measured by the Cutometer® MPA 580 at week 0 (baseline), week 4, and week 8 (end of study) respectively. Volunteer's side effects were assessed by questionnaires and physician observation. At the eighth week, participant's satisfaction was evaluated by questionnaires.

Results: Sixteen participants completed the 8-week clinical trial study. All tested results were statistically significant at the level of 0.05 (Paired t-test). The mean Cutometer scores have significantly increased on lateral canthus ($p<0.0001$) and lower eyelid ($p=0.005$ and $p<0.0001$). The mean Visioscan results have significantly reduced at week 8 with p-value of lateral canthus at $P<0.001$ and lower eyelid at $P<0.0001$. The participant satisfaction scores showed higher scores on the treated side with *Morinda citrifolia* extract eye cream compared to placebo. No side effects were noted throughout the study from applying both eye creams.

Conclusion: *Morinda citrifolia* extract eye cream was proven to be safe and effective for the periorbital wrinkle reduction by improving the appearance of lateral canthal fine lines and wrinkles (crow's feet) and increasing skin elasticity. The abstract, which summarizes the major contents of the GRP, must feature three main parts. The objectives and scope of the study; the methodology (including the type of population, sampling method and instrument(s) used); and the results. (Followed by a 100-300 word or 10-15 pages and 3-5 keywords)

Keywords: Morinda citrifolia, Anti-wrinkle, Wrinkle Reduction

Introduction

Human skin, like all other organs, undergoes aging. Skin aging is a complex biological process influenced by combination of intrinsic and extrinsic

factors(Ganceviciene, Liakou, Theodoridis, Makrantonaki, & Zouboulis, 2012).As skin changes with age, wrinkles are emerged as a natural part of aging and became more visible over time. Wrinkles is defined as a ridge, fold, or cease. Wrinkle formation is a prominent sign of skin aging that is characterized by reduced skin elasticity and degeneration of the extracellular matrix (ECM). ECM in the dermis is produced by fibroblasts and is composed of a mesh of fibrous proteins, such as, collagen and elastic fibers, and glycosaminoglycans that influence the outer of skin(Egbert et al., 2014).Skin wrinkle are typically formed as a result of aging process, such as glycation, habitual sleeping position, loss of body mass, or temporarily, as a result of an immersion in water(Danby, 2010).Apart from these intrinsic factors attribute to wrinkle formation, extrinsic factors, including ultraviolet radiation, air pollution, and stress, can also lead to premature aging skin. Major sign of intrinsic skin aging includes thin, atrophic, finely wrinkled and dry skin. In contrast to intrinsic skin aging, photoaged skin is premature aging skin caused by exposure to the ultraviolet prolonged radiation and is characterized by a thickened epidermis, mottled discoloration, deep wrinkles, laxity, dullness and roughness(Ganceviciene et al., 2012).

As skin physiology and skin aging are very complex processes, there has been an ongoing research effort in discovering more and more substances with beneficial effects on reduction of fine lines and skin wrinkles. It has been found that a wide variety of cosmeceuticals and formulas can facilitate the skin to repair wrinkles, fight against skin aging, and accelerate the synthesis of collagen, leading to a younger-looking face and healthy skin. A rational approach to anti-wrinkle skincare would focus on the application of skin cosmetics that provide moisture and board spectrum sun protection with a sun protection factor (SPF) of 15 or higher to minimize the UV rays penetration into skin cells. The most promising topical treatments incorporate antioxidants, hormone estrogen, vitamins and minerals that scavenge free radicals from skin cells known to contribute to physiological aging by permanently damaging cell structure and function. There are numerous anti-wrinkle synthetic skincare formulations containing active ingredients such as alpha hydroxyl acids (AHAs), hyaluronic acid, cohesive polydensified matrix (CPM) that protect skin from wrinkles and restore the skin damage(Mukherjee, Maity, Nema, & Sarkar,

2011). While the use of anti-wrinkle skincare products that contain synthetic ingredients is effective in reducing facial wrinkle, there are several potential adverse reactions, such as eczema, contact dermatitis, phototoxic and photo-allergic reactions (Pimple & Badole, 2014). Due to this reason, natural bioactive compounds have played a significant role in developing safer cosmetic ingredients that effectively improves the appearance of lateral canthal fine lines and wrinkles.

In recent years, bioactive compounds derived from natural resources have been successfully utilized in various applications of the skin care. Bioactive compounds are primarily added to the formulations due to different related properties such as anti-oxidant, anti-inflammatory, antiseptic and antimicrobial effects. In contrast to synthetic compounds, bioactive compounds from natural resources does not possess any adverse side effects on the human body instead of providing the body with nutrients and other useful minerals(Lee, Huang, & Chen, 2017). Studies have shown plant-derived polyphenolic substances such as alloin, catechin, epicatechin, curcumin, myricetin, and quercetin, are beneficial as anti-aging ingredients. Many herbal polyphenolic substances have found to be effective in reducing the rate and intensity of wrinkle formation(Pimple & Badole, 2014).*Morinda citrifolia* is a tropical tree, prevalently found in Thailand, contains various useful polyphenolic compounds that have anti-aging as well as anti-wrinkle effects.

Literature Review

Normal Skin Architecture

Skin is the body's largest organ system that covers the human body. It comprises of seven layers of ectodermal tissue that resides the underlying muscles, bones, ligaments, tendon, and organs. The surface tissue of the skin acts as a barrier, defending internal structures and surrounding fluids against dangerous microorganisms and toxins (OpenStaxCollege, 2013).Each layer plays crucial roles in the mechanical properties of the skin. The functions of skin include protection from harmful substances, temperature regulation, and tactile perception. Glands within the skin layers secrete sweat to prevent the body from overheating. Tiny hairs on skin provides insulation to the skin from cold temperatures. The skin also contains melanin which traps ultraviolet light, protecting the skin tissues from deleterious effects. Each

of these functions is essential to human survival. Skin is made up of three layers: the epidermis, the dermis, and the hypodermis(Sandford, Chen, Hunter, Hillebrand, & Jones, 2013).

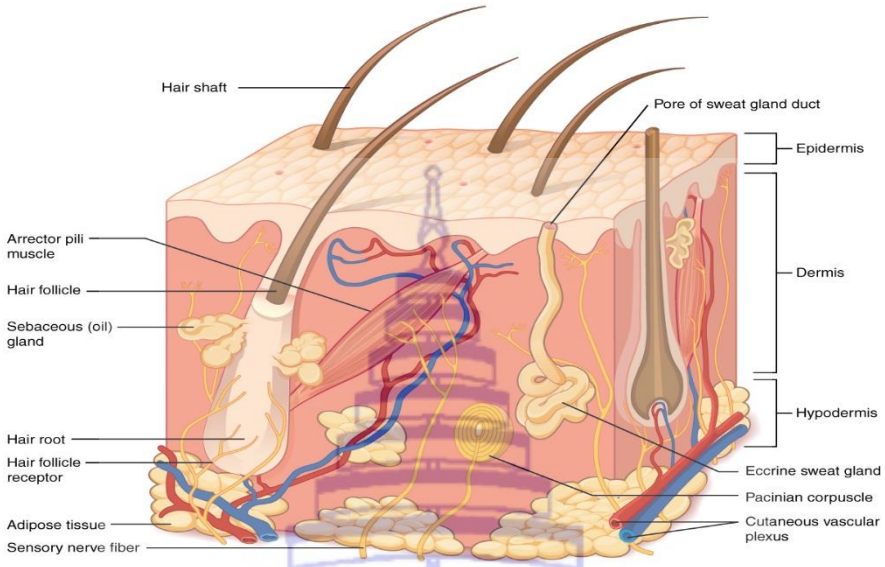


Figure 1 Three layers of human skin structure

Epidermis

The epidermis is the outermost layer of skin that is the keratinised stratified squamous epithelium undergoing continuous renewal. The epidermis layer guardsthe body against deleterious external factors, such as pressure, radiation, microbial penetration, and dehydration (Pavelka & Roth, 2010). The epidermis is avascular, which means no blood vessels is found withinthis layer. The epidermis is made of four or five layers of epithelial cells, which varied according to its location in the body. From deep to superficial, it forms distinctive epidermal layers: stratum basales or stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum(Khavkin & Ellis, 2011).Generally, thin skin covered most of the body parts, except for the palms of the hands and the soles of the feet. The fifth layer of the epidermal is called the stratum lucidum, which resides between the stratum corneum and the stratum granulosum (Kaufmann et al., 2018).Additionally, the cells in the epidermis are predominantly keratinocytes (Khavkin & Ellis, 2011).

Dermis

The dermis is the layer that lie beneath the epidermis. The dermis refers to the "core" of the integumentary system (derma means "skin"), as distinct from the epidermis (epi means "upon" or "over") and hypodermis (hypo means "below"). This layer consists of blood, vessels, nerves, and hair follicles, and sweat glands. The epidermis is the layer that has few or no blood vessel. Cells in this layer obtain oxygen and nutrients from capillaries in the dermis. There are two layers of connective tissue in the dermis that consisted of a network of elastin and collagenous fibers, synthesized by fibroblasts (Kaufmann et al., 2018).

Hypodermis

Hypodermis is a layer situated under the dermis. This layer is also called as the subcutaneous layer or superficial fascia. It joins the skin to the fascia (fibrous tissue) surrounding the muscles. Although this layer is not a part of the skin, the border between the hypodermis and dermis can be difficult to distinguish. The hypodermis contains of vascular, connective tissue, and dense adipose tissue, which severs as a fat storage providing support and insulation for theskin. Fascia is a dense connective tissue wrapping that surrounds skeletal muscles and groups of muscles (Kaufmann et al., 2018).

Cutaneous Aging

The prominent signs of cutaneous aging are periorbital wrinkles and fine lines. Cutaneous aging is an inevitable biological process that entails of two factors: intrinsic factor and extrinsic. Intrinsic structural changes happen as a part of the natural ageing process and are genetically determined. By definition, through this process aging is formed inevitably. In contrast, extrinsic ageing is caused by external factors that are introduced to the human body, such as cigarette smoking, overconsumption of alcohol, poor diet, and long-term exposure to UV radiation. Exposed to the elements falls under the voluntary realm and is not inevitable, representing premature skin ageing(Uitto, 1997).

Intrinsic Aging

Intrinsic or chronologic aging is predominantly influenced by genetic factors and is an unavoidable process in the skin caused by a passage of time. Age related skin changes are characterized by fine wrinkles, thin and transparent skin, a decline of

subcutaneous fat, skin laxity, and dehydration. Histologically, the epidermis layer becomes thinner with flattening of the dermal-epidermal junction(Montagna & Carlisle, 1979). The alteration of the dermal-epidermal interface leads to skin fragility and poor nutrient transfer between dermis and epidermis. Epidermal cell turnover declines, which results in delay wound healing and reduction in the desquamation rate. The dermis becomes atrophic with a smaller number of fibroblasts and reduced in subdermal adipose tissue. In aging, the solubility and thickness of collagen fibers decreases while the amount of type III collagen to type I collagen increases with increasing aging (Lovell et al., 1987)

Extrinsic Aging

Extrinsic aging is a result of the combination lifestyle and environmental factors. Unlike intrinsic aging, extrinsic ageing is largely preventable. Factors from exogenous sources, including smoking, poor diet and UV radiation exposure. These factors contribute to extrinsic, premature, cutaneous ageing (Baumann, 2007). Studies found that about 80% of facial ageing is caused by sun exposure, in which the phenomenon is known as photoaging(Uitto, 1997). The observable signs of photoaged skin include dehydration, rhytids, mottled pigment, loss of elasticity, and discolored spots. Photoaged skin or photodamaged skin is formed by massive accumulation of aberrant of elastin fiber below the dermal-epidermal junction, which is known as elastosis. Epidermal atrophy and disintegration of collagen and elastic fibers are related to photoaged skin(Baumann, 2007). From all of these external factors, UV exposure is considered to be the most harmful to the skin.

Wrinkle Formation

Skin aging occurs due to the intrinsic and extrinsic ageing factors. Wrinkle is one of the most prominent signs of skin aging. Wrinkles are caused by the decline of moisture retention property of epidermis and alternation of dermal structure due to loss of dermal collagen and the elastic fibers in the cutis(Masuda, Murata, & Uwaya, 2017).As time passes, the skin experiences cumulative low intensity of chronic inflammation that has frequently been referred to as “smoldering inflammation” or “inflammaging”(Franceschi & Campisi, 2014). Such chronic inflammatory response could build up with time and gradually causes the damage to the skin's structural components including collagen and elastin, thereby contributing to wrinkle formation.

As a matter of fact, it is difficult to differentiate the long-term aging events that are due to genetic factors from those that are caused by the accumulation of low level chronic inflammation resulting from oxidative stress, poor dietary habits, smoking, and chronic alcohol consumption (Rinnerthaler, Bischof, Streubel, Trost, & Richter, 2015; Weihermann, Lorencini, Brohem, & de Carvalho, 2017).Although intrinsic skin aging is a natural and inevitable process, it can be slow down by avoiding exposure to UV radiation from the sun. An accumulation of exposure to UV radiation damages the normal skin structure leading to early aging signs, which is also known as photoaging. The photoaging is the result of both UVA and UVB radiation exposure. UV radiation activates the of matrix metalloproteinases (MMPs), including MMP-1, MMP-3, and MMP-9. MMPs are accountable for the decline of the extracellular matrix (ECM) proteins such as fibronectin, proteoglycans, and collagen and elastic fibers.The matrix metalloprotein (MMP), collagen and elastic fibers are essential for supporting of muscles, tendons and joints. After skin exposed to UV radiation, it activated the expression of human leukocyte elastase (HLE). The decline of collagen is triggered by the activation of HLE. HLE slices the triple helix structure of type I collagen and breaks down elastin in the skin. Not only does exposure of skin to UV radiation trigger the release of enzymes that degrade collagen, but UVR reduces mRNA levels for both collagen I and III (J. Kim, Kim, Yun, & Hwang, 2017). Furthermore, the high levels of reactive oxygen species (ROS) are generated when the skin is overexposed to UV radiations, in which it induces the activation of elastase and hyaluronidase resulting in skin aging. Elastase and hyaluronidase are proteolytic enzymes in the dermis that are accountable for the breakdown of elastin and hyaluronic. Consequently, the decline of these essential tissues and/or oxidative damage of DNA causes a sagging skin and eventually leads to a formation of wrinkles(Garg, Khurana, & Garg, 2017).

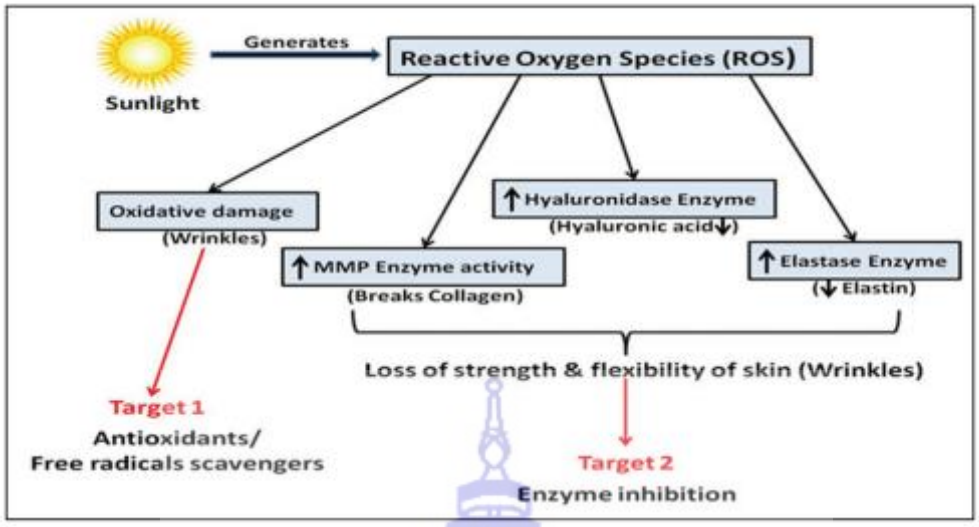


Figure 2 Schematic representation of molecular mechanisms of skin aging

Anti-Wrinkle Agents

In recent times, there has been an increase interest in minimizing the effects of aging. Most of anti-wrinkle cosmetics main functions include increasing moisture retention, accelerating keratinocyte turnover, and promoting collagen and elastin production through stimulated proliferation of skin fibroblasts. Although these anti-wrinkle cosmetics may help improve the appearance of wrinkles, they are only effective for shallow wrinkles and fine lines. Recently, there has been growing in popularity of the utilization of retinol, also known as retinoids, as a cosmetic ingredient due to its property in stimulating of collagen synthesis by fibroblasts in the cutis and improving skin tension. Retinol or vitamin A also reportedly effective in reducing pigment spots caused by melanin excreted by the epidermis, even though it has no inhibitory effects on the expression of tyrosinase and melanogenesis. However, retinoids also cause skin irritation and tend to have negative side effects such as dermatitis. Therefore, safer ingredients and more rational are continuously needed (Yaar& Gilchrest, 2007).

Morinda citrifolia

Figure 3 *Morinda citrifolia* (Noni)

General Information

Morinda citrifolia, also called as Noni, belongs to the genus of *Morinda*, species of *M.citrifolia*. It is a shrub or small tree of 3-10 m high, with a plenty of leaves (5-17 cm length, 10-40 cm width). The tree is cultivated throughout the tropical islands that include South Pacific, part of East Asia, Central America, Indian subcontinent, and in the Caribbean. The fruit and leaves of *Morinda citrifolia*, have a long-existing past of being used as food and for health promotion (Morton, 1992) . In traditional Japanese, Korean and Chinese medicine, *Morinda citrifolia*is selected as herb with biological properties(Potterat & Hamburger, 2007). *Morinda citrifolia,* has a wide range of therapeutic activities such as anticancer, analgesic, anti-inflammatory, anti-oxidant, immune-enhancing effects, and bacterial ,viral, and fungal infection prevention (Saminathan et al., 2014).

Phytochemical constituents of *Morinda citrifolia*

Morinda citrifolia consists of over 160 phytochemical compounds. The major micronutrients found in *Morinda citrifolia* are including alkaloid and phenolic compounds, organic acids, proteins minerals and vitamins. Among all phenolic compounds, the major compounds are anthraquinones damnacanthal,

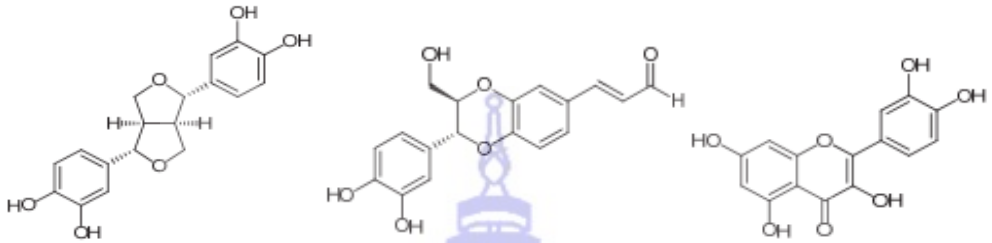
nordamnacanthal, morindone, rubiadin-1-methyl ether, alizarin, rubiadin, aucubin, asperuloside and scopoletin(Wang & Su, 2001). Caproic and caprylic acids are the organic acids that is mainly found in *Morinda citrifolia*(Dittmar, 1993). The alkaloid that is found is xeronine(Heinicke, 1985).Nevertheless, the complete phytochemical composition of *Morinda citrifolia* fruit has not been reported yet The chemical compositionis varied differently according to the parts of the plants. *Morinda citrifolia* fruit contains about 90% of water and the dry matter contents such as dietary fibers, soluble solids and proteins(Chunhieng, 2003). The fruit part is rich in protein content and amino acids, which are glutamic acid, aspartic acid and isoleucine(Chunhieng, 2003).

Inhibitory effect of *Morinda citrifolia* on wrinkle formation

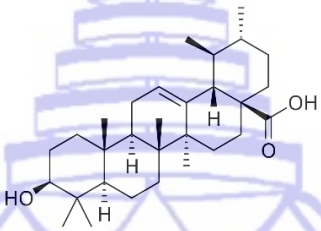
Ursolic acid and3, 3'-bisdemethylpinoresinol

In a recent study, Masuda et al. (2009) revealed that about 50% ethanolic extract of noni seeds displayed significant inhibitory effects ofelastase, tyrosinase, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. The seed extract was found to be more potent than noni leaf and fruit pulp extracts. The active compounds that suppress effect of tyrosinase and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities were identified through bioassay guided fractionationfollowed by chromatography. The result led to the isolation of 3, 3' bisdemethylpinoresinol, americanin A, and quercetin. Americanin A and quercetin exhibited SOD-like activity. In addition, ursolic acid was found to be a major active constituent responsible for a potent inhibitory effect of elastase secretion. Chronic UV radiation exposure causes a degradation of collagen and elastic fibers in the dermis, which leads wrinkles formation. During photoaging, neutrophil play a vital role, as they penetrate into the skin and triggers the release of active enzymes;namely, human leukocyte elastase (HLE) and Matrix metalloproteinases [collagenase (MMP-1) andgelatinase (MMP-9)] (Rijken, Kiekens, &Bruijnzeel, 2005). Among these enzymes, HLE slices the triple helix structure of type I collagen, diminishes the amount of elastic fiber in the skin, and activates the expression of MMP-1. In addition,tyrosinase is a key enzyme in the melanogenesis pathway. Abnormality in the melanin synthesis causes skin pigmentation disorders, such as melasma, freckles and aging spots (Matsuda, Murata, Itoh, Masuda, & Naruto, 2011).More recent

evidence, Masuda et al. (2012)reveals that the active compounds that possess an inhibitory effect of MMP-1 secretion was 3, 3'-bisdemethylpinoresinol, which possesses anti-melanogenesisproperty. Thus, the results from these studies suggest that,noni seed could be a suitable ingredient in cosmetics for prevention of skin wrinkles.



3, 3'-bisdemethylpinoresinol americana A quercetin



ursolic acid

Figure 4 3, 3'-bisdemethylpinoresinol, americana A, quercetin, ursolic acid

Iridoids, Scopoletin, and Acubin

Other phytochemicals in noni fruit also contribute to the protection of the extracellular matrix are Scopoletin and Iridoids. Scopoletin displays an inhibitory effect on collagenase and elastase activities in vitro (Bissonnette, Tremblay, Turmel, Pirotte, & Reboud-Ravaux, 2009; Oshima, Narukawa, Takeda, & Kiuchi, 2013). Likewise, Iridoids inhibits nuclear factor-kβ (NF-kβ), which is one of the mechanisms by which they exert their biological effects (West et al., 2016) . NF-kβ activates collagenases, such as MMP-1(Vincenti & Brinckerhoff, 2002). Furthermore, Ho et al.(2005) suggests thata direct inhibition of MMP-1 has been reported for acubin, which is structurally similar to the iridoids found in noni fruit. NF-kβ activation decreases type 1 collagen synthesis by downregulating expression of the alpha-1(I) collagen gene (Gao & Brigstock, 2005) . Thus, the inhibition of NF-kβ enhances type 1 collagen synthesis as well as prevents its degradation. Asperulosidic acid, the

second most abundant iridoid in noni fruit, as well as 6-O-(β-D-glucopyranosyl)-1-O-octanoyl-β-D-glucopyranose, a saccharide fatty acid ester found in noni fruit, shows an inhibitory effect on AP-1 activity in mouse epidermal cells (Liu et al., 2001) . AP-1 is a transcription factor that activates collagenase expression. Hence, such, inhibition of AP-1 represents another ECM protective mechanism.

Collagen-stimulating effect of *Morinda citrifolia*

In vitro study, the active constituents were identified in noni fruit extract. A compound exhibiting a type I collagen stimulatory effect was isolated and identified as 1, 4-dihydroxy-2-methoxy-7-methylanthraquinone through the technique of nuclear magnetic resonance, infrared spectroscopy, and mass spectrometry. This compound demonstrated a significant improvement in the production of glycosaminoglycans and procollagen type 1 C-terminal peptide. At the same time, it also suppressed the expression of the collagenase matrix metalloproteinase-1 dose-dependently in human dermal fibroblasts. Due to this reason, anthraquinone derived from Noni extract is a potential candidate as a new anti-wrinkle agent for its stimulatory effect of extracellular matrix components (S. Kim, Jo, Jeong, Choi, & Hwang, 2005).

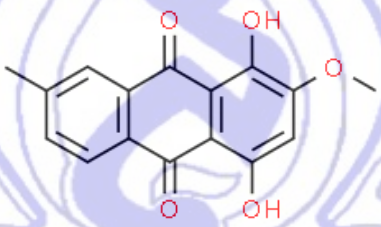


Figure 5 1,4-dihydroxy-2-methoxy-7-methylanthraquinone

Anti-wrinkle effect of *Morinda citrifolia*

In vivo study, the application of topical formulations with noni fruit juice improved the viscoelastic properties of the skin of adult participants. Daily application of a noni-based skin care regiment improved average skin firmness by up to 42.4% in 49 women, ages 38 to 55 years(West, Deng, Palu, & Jensen, 2009). In another clinical trial, a cream containing an ethanol extract of noni was applied to the face of 22 adult

women in the morning and evening for four weeks(S. H. Kim & Jang, 2016) . Daily application of a cream containing an ethanol extract of noni shows a significant improvement on the appearance of lateral canthal fine lines and wrinkles. When an ethanol extract of noni was evaluated in vitro, it decreased MMP-1 activity in human epidermal cells. Skin firmness and elasticity are directly related to dermal collagen and elastin networks (Bischoff, Arruda, & Grosh, 2000). Consequently, improved elasticity of the skin after using the topical noni-based treatments indicates that the active procollagen substances in noni fruit may be absorbed through the skin.

Research Methodology

Study Design

Comparative, double-blind, randomized, controlled and split face clinical trial

Subjects

Healthy female subjects, aged between 50 and 60 years old will be selected. They also have to give their written consent before participating in the study.

Study Location

Mae Fah Luang University Hospital, Bangkok, Thailand

Sample

1. Sample Size Determination

Total of subjects of female participants who participate in the study

Calculate the sample size by two mean independence.

$$n = \frac{\left(Z\alpha/_2 + Z_\beta\right)^2 \left(\sigma^2\right)}{d^2}$$

n =Sample size

$Z\alpha/2$ = the statistics under the curve while the level of statistical significance$\alpha/2$=0.05 is 1.96.

Z_β= The statistics under the curve while the authority on the test 80%, β=0.2 is0.842

σ^2 = pool variance

d^2 derives from the previous study on the wrinkle reduction measured by Visioscan®.The experiment compared between the placebo group and thetreatment group. The result showed that the placebo group had skin wrinkle (SEw) value of 72.84 whereas the treatment group had the skin wrinkle (SEw) value of 71.14 (Rasul & Naveed, 2012).

$$n = \frac{(1.96+0.84)^2\,(2.01)^2}{(72.84-71.14)^2}$$

$n = \approx$ 11.3, calculate drop out 40% =4.5≈5

Total sample size 11+5= 16 subjects

In this study, the sample size was 16 subjects and the researcher comparedbetweenactive formulation cream and placebo cream.

Inclusion Criteria

Female subjects of age between 50 and 60 years old. Participants whose score between2 – 4 in Rao-Goldman 5-point scale were included in the experiment. Participants accepted to use placebo cream (standard cream base) and *Morinda citrifolia* extract cream on each side of the face. Participants who are healthy and are willing to participate in the study. Participants who have not received facial Botox injection treatment within 6 months prior to study enrollment. Participants who have not received facial filler injection treatment within 6 months prior to study enrollment. Participants who do not take of any dietary supplement containing vitamins and minerals, such as vitamin E and vitamin C. Participants who have not taken retinoic acid supplement within 12 months of entry into the study. Participants who have not used an anti-wrinkle cream on the area of experiment within 6 weeks of entry into the study.

Exclusion Criteria

Participants who have atopic dermatitis on the area of experiment

Withdrawal and Discontinuation Criteria

Participants who exhibit allergic signs after using *Morinda citrifolia* extract eye cream or placebo cream (standard base cream). Participants cannot use active formulation cream continuously Pregnancy and breastfeeding. Other medical conditions

Equipment

1. Visioscan® VC98for Measurement of Skin Topography

The Visioscan® VC98 (by Courage and Kazaka) is a special UV light video camera with high resolution to study the skin surface directly camera with high resolution. The measuring principle is based on a graphic depiction of the living skin under special illumination and evaluation of this image according to four clinical parameters : Skin roughness (*SEr*), skin smoothness (*SEsm)*, skin scaliness (*SEsc*), skin wrinkles (*SEw*).. The parameters correspond to the state of the skin.

Figure 6 Visioscan® VC 98

2. Cutometer MPA 580 for Measurement of Skin Elasticity

The Cutometer® MPA 580 (by Courage and Khazaka) is a device used to measures elasticity of skin. The measuring principle is based on the suction method, which allows information on elasticity of skin and mechanical properties of skin surface to be collected and enables to objectively quantify skin aging.

Figure 7 Cutometer® MPA 580

3. Morinda citrifolia Extract Eye Cream is an Eye Cream that Contains Morinda citrifolia Extract in Standard Cream Base

Chemical compound of Morinda citrifolia extract eye cream *Morinda citrifolia* extract eye cream ingredient

1) *Morinda citrifolia* extract3%
2) Water
3) Methylparaben
4) Triethamolamine
5) Cetyl Alcohol
6) Stearic Acid
7) Glyceryl Stearate SE
8) Propylparaben
9) Isopropyl Myristate
10) FDA license number 10-1-6200004186

Chemical Compound of Placebo Cream (Standard Cream BaseComposition)

1) Water
2) Methylparaben
3) Triethamolamine
4) Cetyl Alcohol

5) Stearic Acid
6) Glyceryl Stearate SE
7) Propylparaben
8) Isopropyl Myristate

Step of Research

1. Photograph the face before the procedure

2. Patch test will do before apply creams.

Apply *Morinda citrifolia* extract eye cream on participants' arms under water proof patch test and wait for 24 hours. The participant needed to avoid excessive sweating conditions, heavy sunlight during the test and then examined for any responses until 48 and 96 hours. Any reaction that has been appeared was recorded according to the international contact dermatitis research group system as follow:

+? = 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 withcoalesced spreading reaction or large blisters

The red skin improved after the patch is removed.

NT = not tested

IR = irritant reaction

Volunteer with positive patch test from score ++ were excluded.

3. Skin elasticity is measured by the Cutometer® MPA 580 and periorbital wrinkle is measured by the Visioscan® VC98

Then apply2.5 g of Morinda citrifolia extract eye cream in one side and 2.5 g of placebo cream (standard cream base) in another side in the morning and at night.

However, neither the researcher nor the participant knows which side is *Morinda citrifolia* extract eye cream or placebo cream.

4. Randomization

3% *Morinda citrifolia* extract eye cream and placebo cream (standard cream base with similar consistency, color and smell) will be enclosed in two identical packages. Both packages will be labeled with "A" and "B" respectively. The participants and the physicians, who have to evaluate the results, are blinded.

The physician generated the randomization sequence which were randomly determined which side of the participants' face to be treated with cream A and cream B by using "Block randomization" and conceals the sequences in opaque envelopes.

This study was a split-face study and 16 participants were selected. Thus, there were thirty-two faces. Each block contains two sides of a participant's face which were RIGHT (right face) and LEFT (left face).

The treatment protocols were as follow:

1. (Right, Left) = the right face was applied cream A and the left face was applied cream B.

2. (Left, Right) = the left face was applied cream A and the right face was applied cream B.

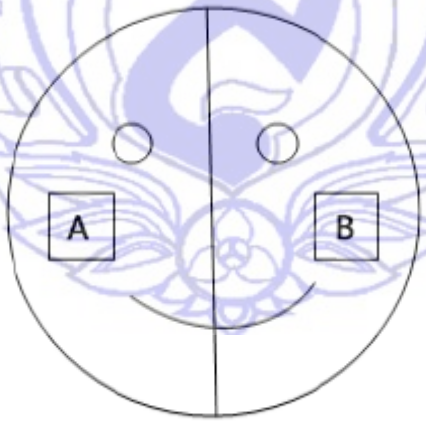


Figure 8 Way to apply cream A and B as "1"

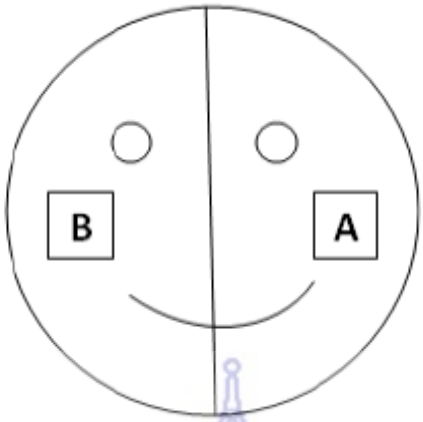


Figure 9 Way to apply cream A and B as “2”

5. The physician used the website http://www.random.org/sequences/It is the Random Sequence Generator.

Figure 10 Random Sequence Generator from Website

6. Randomized 16 Arabic numbers from 1 to 1000. 16 of these randomized numbers are referred to 16 participants according to first-come, first-served order.

7. Even number is “1” and odd number is “2”.

Instructions for eye cream application:

Use mild soaps two times per day as the following steps.

1. Splash the face with water and wash with mild soaps and then rub it to the face gently by using the fingers.

2. Rinse the face with water until there was no cleanser remained on face.

3. Pat the face with tissues or towels which are dry and clean.

Cream A and cream B will be used twiceper day by the following steps.

1. After washing the face, use the right index finger to press out the eye cream from the bottle labeled “Right side”. The amount of eye cream pressed out from the bottle was measured from the tip of a volunteer’s index finger to the first crease in the finger (1 fingertip unit (FTU) = 0.5 grams). The small amount of cream was then gently applied on the periorbital region, consisting of the eyelids and surrounding areas, using the right index finger, as shown in figure 3.7. All these steps took place twice daily, which were in the morning and at night. Therefore, the amount of cream applied was totaled to 2.5gram. In addition, it is be noted that the participants should use their own personal sunscreen and face wash.

2. Repeat the same steps for the left side. Use the left index finger to apply the cream from the bottle labeled “Left side” on the periorbital region, consisting of the eyelids and surrounding areas.

Figure 10 How to apply eye cream

Results

General Characteristics of subjects

Sixteen females completed this study at Mae Fah Luang hospital, Bangkok, for total 8 weeks from May 2020 to July 2020. The participants' demographic data demonstrates in table 4.1. The mean age of subjects was 54.24 ± 3.87 years ranging from 50-60 years old. There were 8 employees, 5 housekeepers, 2 business owner and 1 other, respectively. All subjects tested negative for allergic reaction through patch testing (16 subjects). Among all subjects, there were 1 subject with hypothyroid and 1subejct with high cholesterols.

Table 1 Demographics data

	Demographic	**n=16**
Age	Mean ± SD (years)	54.24 ± 3.87
	Min	50
	Max	60
Occupation, n		
	Employee/Government employee	8
	Business Owner	2
	Housekeeper	5
	Other	1
Allergy testing, n		
	Yes	1
	No	15
Congenital disease, n		
	Yes	2
	No	14
Eye wrinkle treatment, n		
	Eye cream	6
	No	10

Result of Cutometer for Skin Elasticity

Skin elasticity was compared between *Morinda citrifolia* side placebo group side at the lateral canthus and the lower eyelid on baseline, follow- up at week 4 and week8.

Table 2 Statistical analysis of Cutometer score compared between *Morinda citrifolia* side and placebo group side at the lateral canthus and the lower eyelid on baseline,follow- up at week 4 and week 8.

Skin	***Morinda Citrifolia* Extract Eye Cream**		**Placebo**		
Elasticity	Mean	S.D.	Mean	S.D.	P-value
Lateral canthus					
Week 0	0.633^{a}	0.123	0.624^{a}	0.170	0.201
Week 4	0.737^{b}	0.109	0.541^{b}	0.219	0.146
Week 8	0.832^{c}	0.809	0.700^{d}	0.149	0.0001
p-value		**<0.0001**		**0.006**	
Lower eyelid					
Week 0	0.632^{w}	0.113	0.638^{w}	0.205	0.493
Week 4	0.703^{x}	0.128	0.522^{x}	0.228	0.424
Week 8	0.827^{y}	0.094	0.707^{z}	0.092	0.007
p-value		**<0.0001**		**0.003**	

Note Significant differences between *Morinda citrifolia* and placebo groups are indicated with different superscript.

The lateral canthus mean score results applied *Morinda citrifolia* extract eye cream on week 0 was 0.633±0.123, week 4 was 0.737±0.109 and week 8 was 0.832 ±0.809. They increased on each visit at the level of 0.05 ($p<0.001$). As for the placebo mean score results, they also changed significantly on each visit according to the statistics analysis ($p=0.006$). Comparing Cutometer scores at lateral canthus between *Morinda citrifolia* and placebo side, there were statistically significant differences at week 8 ($p=0.0001$). The mean score results of lateral canthus applied with *Morinda citrifolia* extract eye cream was 0.832±0.809, which was significantly higher than the placebo side, 0.700 ± 0.149.

For lower eyelid, the mean score results applied *Morinda citrifolia* extract eye

cream on week 0 was 0.632±0.113, week 4 was 0.703±0.128 and week 8 was 0.827±0. 094.The differences were statistically significant. They increased on each visit at the level of 0.05 ($p<0.0001$). As for the placebo, the mean scores also changed significantly according to the statistics analysis ($p=0.003$). Comparing Cutometer scores at lower eyelid between *Morinda citrifolia* and placebo side, the differences were statistically significant at week 8 ($p=0.007$). The mean score results of lateral canthus applied with *Morinda citrifolia* extract eye cream was 0.827±0.094, which was significantly higher than the placebo side, 0.707 ± 0.092.

Result of Visioscan for Skin Wrinkle (Se_w)

Skin wrinkle was compared between *Morinda citrifolia* side placebo group side at the lateral canthus and the lower eyelid on baseline, follow- up at week 4 and week 8.

Table 3 Statistical analysis of Visioscan score compared between *Morinda citrifolia* side placebo group side at the lateral canthus and the lower eyelid on baseline, follow-up at week 4 and week 8.

Skin Wrinkle	***Morinda citrifolia* Extract Eye Cream**		**Placebo**		
	Mean	**S.D.**	**Mean**	**S.D.**	**P-value**
Lateral canthus					
Week 0	45.821^{a}	8.906	45.188^{a}	7.976	0.749
Week 4	38.198^{b}	5.151	39.218^{b}	5.100	0.329
Week 8	35.815^{c}	4.699	39.229^{b}	5.344	0.002
p-value	**<0.001**		**0.002**		
Lower eyelid					
Week 0	49.894^{w}	6.944	46.352^{w}	8.814	0.124
Week 4	39.946^{x}	6.127	40.313^{x}	6.050	0.781
Week 8	36.069^{y}	4.732	38.944^{z}	5.588	0.013
p-value	**<0.001**		**0.005**		

Note Significant differences between *Morinda citrifolia* and placebo groups are indicated with different superscript.

The lateral canthus mean score results applied *Morinda citrifolia* extract eye cream on week 0 was 45.821±8.906, week 4 was 38.198±5.151 and week 8 was

35.815±4.699. They decreased on each visit at the level of 0.05 ($p<0.001$). As for the placebo mean score results, they also changed significantly on each visit according to the statistics analysis ($p=0.002$). Comparing Visioscan scores at lateral canthus between *Morinda citrifolia* and placebo side, the differences were statistically significant at week 8 ($p=0.002$). The mean score results of lateral canthus applied with *Morinda citrifolia* extract eye cream was 35.815 ±4.699, which was significantly lower than the placebo side, 39.229 ± 5.344.

For lower eyelid, the mean score results applied *Morinda citrifolia* extract eye cream on week 0 was 49.894±6.944, week 4 was 39.946±6.127 and week 8 was 36.069±4.732. They decreased on each visit at the level of 0.05 ($p<0.0001$). As for the placebo, the mean scores also changed significantly according to the statistics analysis ($p=0.005$). Comparing Visioscan scores at lateral canthus between *Morinda citrifolia* and placebo side, the differences were statistically significant at week 8 ($p=0.013$). The mean score results of lower eyelid applied with *Morinda citrifolia* extract eye cream was 36.069 ±4.732, which was significantly lower than the placebo side, 38.944 ± 5.588.

Patient Satisfaction Score

The patient satisfaction scores of the *Morinda citrifolia* side and the placebo side were assessed after the end of the study (8weeks). For the *Morinda citrifolia* side, the results revealed that 10 participants rated high satisfaction. 5 participants rated substantial, and 1 participant rated moderate satisfaction. On the other hand, for the placebo side, there were only 4 participants rated high satisfaction. 3 participants rated substantial satisfaction, and 9 participants rated moderate satisfaction, respectively.

Table 4 Participant satisfaction scores

Satisfaction score	*Morinda citrifolia* Extract	Placebo

	Eye Cream	
	n	**n**
High satisfaction	10	4
Substantial satisfaction (75% improvement)	5	3
Moderate satisfaction (50% improvement)	1	9
Slight satisfaction (25% improvement)		
Not satisfaction (Unchanged)		
Total	**16**	**16**

1. Wrinkles decrease score

For the *Morinda citrifolia* side, 5 participants rated complete abolition of sighs and aging symptoms. 9 participants rated substantial improvement, and 2 participants rated moderate improvement. By contrast, for the placebo side, there was only 4 participants rated substantial improvement. 5 participants rated moderate and 7 participants rated slight improvement score, respectively.

Table 5 Participant satisfaction scores on wrinkles decrease

Wrinkles decrease	***Morinda citrifolia* Extract** Eye Cream	**Placebo**
	n	n
Complete abolition of sighs and aging symptoms	5	
Substantial improvement (75% improvement)	9	4
Moderate improvement (50% improvement)	2	5
Slight improvement (25% improvement)		7
Unchanged		
Total	**16**	**16**

2. Skin elasticity score

For the *Morinda citrifolia* side, 8 participants rated complete improvement. 6

participants rated substantial and 2 participants rated moderate improvement. On the other hand, for the placebo side, there was only 4 participants rated substantial improvement. 6 participants rated moderate and 6 participants rated slight improvement, respectively.

Table 6 Participant satisfaction scores on skin elasticity improvement

Skin elasticity	***Morinda citrifolia* Extract Eye Cream**	**Placebo**
	n	**n**
Complete abolition of sighs and aging symptoms	8	
Substantial improvement (75% improvement)	6	4
Moderate improvement (50% improvement)	2	6
Slight improvement (25% improvement)		6
Unchanged		
Total	**16**	**16**

3. Rao-Glodman 5-point visual scoring scale

Rao-Goldman 5-point visual scoring scale revealed a significant difference compared at week0, week4, and week8 in every group.

For the Morinda citrifolia extract eye cream group, the score was significant difference in both lateral canthus ($P<0.0001$) and lower eyelid ($P<0.0001$) while the placebo group also showed the same result, lateral canthus ($P=0.013$) and lower eyelid ($P<0.0001$).

Table 7 Statistical analysis of Rao-Goldman 5-point wrinkle score measurement at crow's feet and undereye applied with *Morinda citrifolia* extract eye cream and

Placebo on week 0, 4, and 8.

Rao-Glodman 5-point visual scoring scale	*Morinda citrifolia* Extract Eye Cream		Placebo	
	Mean	S.D.	Mean	S.D.
Lateral canthus				
Week 0	2.960^{a}	0.539	2.960^{a}	0.611
Week 4	2.360^{b}	0.490	2.600^{b}	0.500
Week 8	2.360^{b}	0.490	2.640^{b}	0.569
p-value	**<0.001**		**0.013**	
Lower eyelid				
Week 0	3.000^{x}	0.500	3.000^{x}	0.500
Week 4	2.520^{y}	0.510	2.720^{y}	0.458
Week 8	2.400^{y}	0.500	2.520^{y}	0.510
p-value	**<0.001**		**<0.001**	

Note Significant differences between *Morinda citrifolia* and placebo groups are indicated with different superscript.

Discussion and Suggestion

Wrinkles and *Morinda citrifolia*

Wrinkles, or rhytids, are defined as a visible fold, ridge, cease, in the skin. Wrinkles may differ in size and visible texture. Wrinkles with smaller than 1 mm in size are categorized as fine wrinkles and those with the size larger than 1 mm as coarse wrinkles. There are numerous findings underlying the formation of wrinkles. Wrinkles are caused by two major factors; namely, intrinsic factors (e.g., ageing, hormones, and diseases) and extrinsic factors (e.g., exposure to UV radiation and cigarette smoke). These factors result in thinning, fragile, sagging, and wrinkled skin. The severity of photodamage can be varied depending on individual's skin type, which include skin complexion, and tanning ability (Manríquez, Majerson Gringberg, & Nicklas Diaz, 2008).

Morinda citrifolia consists of several beneficial phytochemicals that possess free radical scavenging activity, inhibitory effect on wrinkle formation, and collagen-stimulating property. Americanin A and quercetin were the phytochemical compounds in *Morinda citrifolia* found to exhibit SOD-like activity, which is antioxidant that prevents and protects cellular components from being damaged by

reactive oxygen species (ROS). Moreover, Ursolic acid was found to be a major active constituent responsible for a potent inhibitory effect of elastase secretion, an enzyme that is responsible for the degradation of elastin along with collagen fibers during the UV radiation exposure. An overexposure to UV radiation is the major cause for a premature photodamaged skin, in which the phenomenon is also known as photoaging. UV radiation increases the expression of matrix metalloproteinases (MMPs), which include MMP-1, MMP-3, and MMP-9. MMPs are accountable for the breakdown of the extracellular matrix (ECM) proteins such as collagen, fibronectin, elastin, and proteoglycans. The matrix metalloprotein (MMP), collagen and elastic fibers are the major protein components that help supporting of muscles, tendons and joints. After skin exposed to UV radiation, the expression of human leukocyte elastase (HLE) is activated, in which HLE promotes the degradation of collagen. Among these enzymes, HLE slices the triple helix structure of type I collagen, breaks down elastin in the skin, and activate MMP-1. In the same way, an excessive exposure to UV radiation also increases the melanin production in the skin. Tyrosinase is a key enzyme that initiates skin pigmentation. Melanin is a skin pigment which is synthesized in melanosomes and is transferred to keratinocytes, in which the physiological process is called as melanogenesis. (Lin, Chiang, Lin, & Wen, 2008).3, 3'-bisdemethylpinoresinol is an active compound that have the inhibitory effect on MMP-1 secretion and tyrosinase activity. In addition, 1,4-dihydroxy-2-methoxy-7-methylanthraquinoneisan active single compound having a type I collagen-stimulating effect. 1,4-dihydroxy-2-methoxy-7-methylanthraquinoneis also found to have stimulated the synthesis of glycosaminoglycans and procollagen type 1 C-terminal peptide and also reduced the expression of MMP-1.

The efficacy of a *Morinda citrifolia* based skin care regimen has demonstrated the study of topical formulations with noni fruit juice improved the viscoelastic properties of the skin of adult participants. Daily application of a noni-based skin care regiment improved average skin firmness by up to 42.4% in 49 women, ages 38 to 55 years(West et al., 2009). Also, in another clinical trial, a cream containing an ethanol extract of noni was applied to the face of 22 adult women in the morning and evening for four weeks(S. H. Kim & Jang, 2016). Daily application of a cream containing an ethanol extract of noni showed a significant improvement on the appearance of lateral

canthal fine lines and wrinkles. Furthermore, in vitro study, an ethanol extract of Noni was evaluated and found that Noni has an inhibition effect on MMP-1 activity in human epidermal cells.

Skin Elasticity

Skin is the human body's largest organ which comprises of the epidermis, dermis and hypodermis layer. The skin tissue is heterogeneous, anisotropic and a non-linear viscoelastic material in nature (Ross, Kaye, & Pawlina, 2003). For this reason, the skin displays both viscous and elastic features during deformation (Silver, Freeman, & DeVore, 2001). The viscoelastic properties of the skin largely instigate from its dermis layer that is primarily consisted of extracellular matrix and fibroblasts. The dermis extracellular matrix is comprised of collagen fibers (type I and type III), elastin and proteoglycans (Ross et al., 2003). The mechanical properties of the skin are primarily determined by the orientation of collagen and elastin fibers. During the aging process, the dermis undergoes numerous changes. Collagen becomes fragmented and unevenly distributed due to increased activity of matrix metalloproteinases and decreased transforming growth factor-β signaling triggered by reactive oxygen species (ROS). The decrease in the amount of collagen deters the interaction between fibroblasts and the ECM. As a result, it leads to the decline of fibroblast function and further reduces in the amount of dermal collagen. Apart from the reduction of collagen, other ECM components, including elastic fibers, glycosaminglycans (GAGs), and proteoglycans (PGs), are declined in aged skin. However, there components accumulate abnormally in photoaged skin. A decrease in the levels of functional dermal components results in the clinical aging signs, such as wrinkles and reduced elasticity (Shin et al., 2019). Collagen is the major functional protein responsible for the structure, elasticity, and firmness of the skin. *Morinda citrifolia* constitutes of the active procollagen substances and may be absorbed through the skin, which increase skin elasticity, and improve the skin appearance(West et al., 2009).

As referred to the aforementioned concept, this research study results have shown a significant improvement of the skin elasticity scores from week 0 to 8.

Safety Assessment

There are many aspects related to the safety assessment of a bio-compound ingredient in order for it to be qualified as a safe skin care product. According West et al. (2009), the safe and efficacy of Noni skin care product were evaluated in 49 women, ages 38 to 55 years. The subjects applied three different formulations of Noni skin care product, including Noni juice, Noni seed oil and Noni leaf juice to the face and neck. Result shows a significant improvement in wrinkles and skin elasticity with no evidence of skin irritation. In addition, there were no signs of facial irritation were observed in any subjects during the clinical trial. From this research study, there were no side effect symptoms reported by the participants throughout 8 weeks of using both *Morinda citrifolia* extract eye cream and placebo. The result showed no redness, dryness, burn, sting, or itch signs. Overall, *Morinda citrifolia* extract eye cream was proven to be safe, nonirritating, and effective in reducing wrinkles.

Conclusion

The study has shown a significant reduction in wrinkle depth and improved in skin elasticity in periocular area over the period of 8- week clinical trial. The result showed significant changes as early as week 4 on the skin elasticity and wrinkle depth. In terms of patient satisfaction scores, Rao-Goldman 5-point wrinkle scores have statistically and significantly improved as well. There was neither side effect nor participant's dissatisfaction complaint during this clinical trial. From these results, we conclude that *Morinda citrifolia* extract eye cream is a safe and effective topical skincare product in reducing moderate to deep periorbital wrinkle.

Recommendations

1. This study provides insight for further research on skin rejuvenation, anti-aging modalities, alternative topical anti-wrinkle ingredient, and skin elasticity.

2. Morinda citrifolia could potentially be used as an alternative topical treatment option for improving signs of facial wrinkles.

3. The efficacy of Morinda citrifolia should be tested and compared with other topical ingredients.

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