THE EFFECTIVENESS OF 4% WHITE RADISH ROOT EXTRACT CREAM FOR FACIAL WHITENING IN THAIS

ARPORN KOOSUWAN

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

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Arporn Koosuwan

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Author	Arporn Koosuwan
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ABSTRACT

Background: Pale skin creates a vaguely supernatural aura. It can make an appearance look elegant, pretty and seductive. Southeast Asia and also Thai women want to look white like Japanese and Korean. The previous study showed that Bangkok women commonly used whitening cream for maintaining facial skin by purchased more than thousand baths every month. Synthetic products have been popular for several years in making the skin lighter. However, using these products provides serious drawbacks on the skin. The safer products are more expensive. Because of suitable for long term application, mild side effects and reasonable price, increasing attention has been paid to herbal plants for developing into modern medicine and cosmetic products. Previous study demonstrated that white radish root extract inhibits tyrosinase enzyme, the key enzyme in melanogenesis and also has antioxidant effect. White radish is cheap and grows easily in Thailand. The purpose of this research is to study the effectiveness of white radish root extract for facial whitening.

Objectives: To study the effectiveness of 4% white radish root extract cream for facial whitening in Thais.

Material and Methods: Thirty Thai volunteers, who matched with the inclusion criteria, were enrolled. 4% White radish root extract cream and standard cream base

(similar consistency, color and smell) were randomly applied, used block randomization, in a split face design (right and left sides), twice daily for 12 weeks. Furthermore, volunteers were received mild soap and broad spectrum sunscreen. Skin whitening was evaluated at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks by using mean melanin index measured by mexameter MX18. Volunteers' side effect was assessed by questionnaires and physician observation. Photographs from VISIA® Complexion Analysis System at 0^{th} (before treatment) and at 4^{th}, 8^{th}, $12^{th}$and 16^{th} (4 weeks after treated) weeks were compared and scored evaluation scales by 3 dermatologists. Volunteer satisfaction was evaluated at 12^{th} week by questionnaires.

Results: Twenty-eight volunteers completed the study. Mean melanin index of the sides that applied 4% White radish root extract cream and standard cream base were statistically significant reduction from the baseline. Paired difference between both sides had statistically significant with $p < 0.001$ from 4^{th} week of application without area dependent. The dermatologist evaluation and volunteer satisfaction rated for radish side as moderately satisfied and mildly satisfied for standard cream base, correspondingly. The side effect of 4% White radish root extract cream was very low.

Conclusion: The results of the study clearly demonstrated that white radish root extract was able to reduce melanin production in human volunteers with significant lightening effect and also less side effects. White radish root extract have a very promising potential for use as a safe, effective and economical whitening agent. Nevertheless, the highest concentration with lowest side effects, the duration that white radish root extract cream will reach its maximum lightening effect and more prolonged usage complication should be find out.

Keywords: White radish/Skin whitening agent/*Raphanus sativus* Linn./
Melanin reduction

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CHAPTER 1

INTRODUCTION

1.1 Background

Pale skin creates a vaguely supernatural aura. It can make an appearance look elegant, pretty and seductive. Many countries consider that pale skin is beautiful [1]. Southeast Asia and also Thai women want to look white like Japanese and Korean [2]. The previous study showed that women in Bangkok commonly used whitening cream for maintaining facial skin and purchased more than thousand baths every month [3].

Human skin color is the result of natural selection, comprised of genetic and extrinsic factors such as ultraviolet radiation and hormones. Ultraviolet irradiation and hormones stimulate melanin synthesis. The key enzyme in melanin synthesis is tyrosinase [4].

Skin whitening is the practice of using chemical substances or traditional herbal formulations, in an attempt to lighten skin tone or provide an even skin complexion by reduction the concentration of the melanin. Many of these actives have the tyrosinase-inhibiting effect leading to reduced total melanin production. The example of the tyrosinase inhibitors are hydroquinone, kojic acid, arbutin and different kinds of vegetal or herbal extracts. There are also molecules known to have an effect on the transfer of melanin from melanocytes to keratinocytes, leading to an overall lighter skin color such as nicotinic acid and soybean. Substances that increase the desquamation of the skin are also commonly used to remove excessive melanin content within the skin, for instance retinoic acid [5].

Synthetic products have been popular for several years in making the skin lighter and fairer. However, using these products provides serious drawbacks on the skin. Prolonged using of these treatment medications to the skin may provide adverse reactions and drug dependence. Individuals who are using synthetic products fail to provide natural

defenses of their skin from health problems, making them to utilize medications to counteract these side effects.

Over the past few years, natural cosmetic is a growing industry manufacturing. Increasing attention has been paid to herbal plants for developing into modern medicine and cosmetic products. Because of suitable for long term application and mild side effects, skin whitening containing natural ingredients from plants has become very popular [6]. Various tropical vegetables have been known for their antityrosinase activity which could be potentially prepared for skin whitening such as paper mulberry extract, emblicanin extract, white radish root extract…etc.

Radish (*Raphanus sativus* Linn., family Cruciferae) is grown mainly for its edible. The root can be eaten raw, cooked or preserved in salt. In East Asia, the crushed root is used to treat rheumatic pain, burns or bruises. Its squeezed juice used to treat cough and diarrhea. Traditionally, Thai women apply slices of fresh white radish root on their face for the treatment of melasma [6]. The previous research illustrated that Thai radish root extract has significant antityrosinase activity, the key enzyme in melanogenesis and also antioxidant activities [6, 7].

Therefore, the antityrosinase property of white radish root extract was investigated in vitro. The study in vivo has never been established. The objective of this study is to investigate the effectiveness of white radish root extract cream for facial whitening in Thais. If this hypothesis is proven with positive result, white radish root extract cream will be an alternative in whitening skin that cheaper, more effective and less side effects.

1.2 Objective

1.2.1 To study the clinical effectiveness of 4% White radish root extract cream for facial whitening in Thais.

1.2.2 To observe the side effect of 4% White radish root extract cream for facial whitening in Thais.

1.2.3 To evaluate the satisfaction of 4% White radish root extract cream for facial whitening in Thais.

1.3 Research hypothesis

4% White radish root extract cream is more effective than standard cream base for facial whitening in Thais

1.4 Conceptual framework

Skin whitening is the practice of using chemical substances or traditional herbal formulations, in an attempt to lighten skin tone or provide an even skin complexion by reduction the concentration of melanin. Tyrosinase is the key enzyme in melanin synthesis. White radish root extract has antityrosinase activity, so this substance can block melanin synthesis leading to skin whitening.

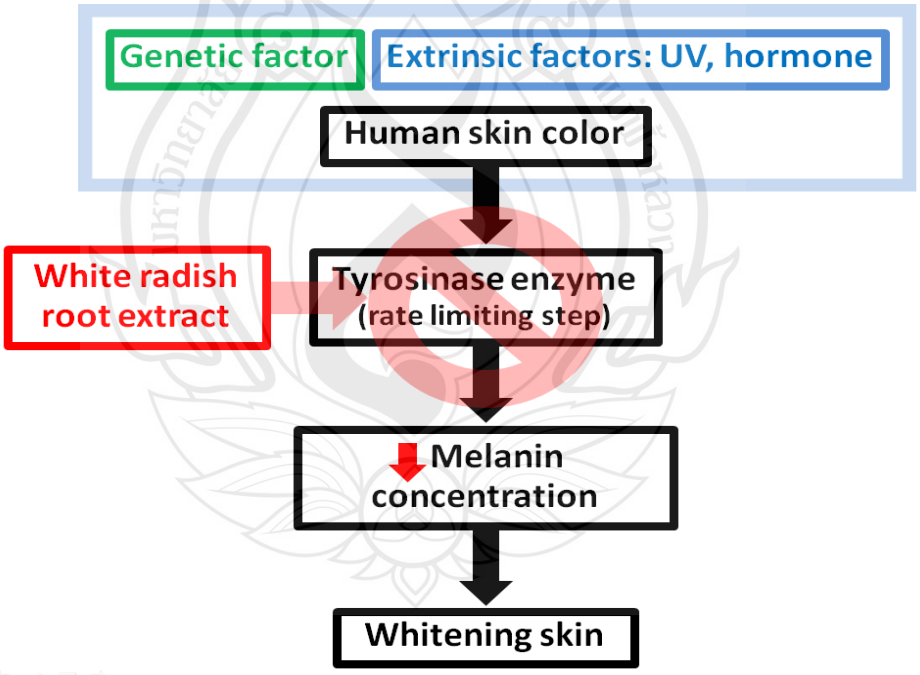


Figure 1.1 Conceptual framework

1.5 The scope of the research

Thirty Thai volunteers with Fitzpatrick Skin type 3-6 diagnosed by dermatologist, both males and females, ages 20-60 years, and matched with all inclusion criteria, were enrolled. 4% White radish root extract cream and standard cream base (similar consistency, color and smell) were randomly applied, used block randomization, in a split face design (right and left sides) twice daily for 12 weeks. Furthermore, volunteers were received mild soap and sunscreen. Skin whitening was evaluated at 4^{th}, 8^{th}, 12^{th} and 16^{th} (4 weeks after treatment) weeks by using mean melanin index measured by mexameter MX18. Volunteers' side effects were assessed by questionnaires and physician observation. Photographs from VISIA® Complexion Analysis System before treatment and at 4^{th}, 8^{th}, 12^{th} and 16^{th} (4 weeks after treatment) weeks were compared and scored evaluation scales by 3 dermatologists. Volunteers' satisfaction was evaluated at 12^{th} week by questionnaires. Moreover, dermatologists who measured the mexameter MX18 and evaluated the result of the treatment and volunteers were blinded.

1.6 Limitation of the study

Due to the small sample size, this may have problem with study interpretation.

1.7 Definition

1.7.1 Tyrosinase enzyme is the rate limiting step enzyme in melanogenesis.

1.7.2 IC50 (The half maximal inhibitory concentration) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance inhibitor is needed to inhibit a given biological process by half.

1.7.3 Patch test is a way of identifying whether a substance that comes in contact with the skin is causing inflammation of the skin (contact dermatitis). Any reaction seen is scored according to the International Contact Dermatitis Research Group system, as follows: +? = doubtful reaction: mild redness only, + = weak, positive reaction: red and

slightly thickened skin, ++ = strong positive reaction: red, swollen skin with individual small water blisters, +++ = extreme positive reaction: intense redness and swelling with coalesced large blisters or spreading reaction, IR = irritant reaction, Red skin improves once patch is removed, NT = not tested.

1.7.4 Standard cream base refers to the facial cream without whitening effect.

Standard cream base Ingredient
Glycerine
Cetearyl alcohol
Stearic acid ceteth-20
Glyceryl monosterate
Carbomer
Chlophenesin
Disodium EDTA
BHT
FDA license number: 10-1-5618288

Figure 1.2 Chemical compound of standard cream base

1.7.5 4%White radish root extract cream refers to the facial cream that contains 4% of white radish root extract in standard cream base.

4%White radish cream Ingredient
White radish root extract
Glycerine
Cetearyl alcohol
Stearic acid ceteth-20
Glyceryl monosterate
Carbomer
Chlophenesin
Disodium EDTA
BHT
FDA license number: 10-1-5618172

Figure 1.3 Chemical compound of 4%White radish root extract cream

1.7.6 Mild soap refers to the gentle facial cleaning gel.

Mild soap Ingredient
Water
Butylene glycol
Cocamidopropyl betane
Coconut diethanolamide
DMDM hydantoin
Methylparaben
PEG-120 Methyl glucose dioleate
Sodium methyl cocoyl taurate
Sodium PCA
FDA license number: 10-1-5618960

Figure 1.4 Chemical compound of mild soap

1.7.7 Sunscreen SPF60, PA+++ refers to the broad spectrum sunscreen that has SPF 60, PA+++.

Sunscreen SPF 60+++ Ingredient
Titanium dioxide
Oxybenzone
Zinc oxide
Octocrylene
Octyl methoxycinnamate
Behenyl alcohol
Propylene glycol
Propylparaben
Stearyl alcohol
Carbomer
Cetyl alcohol
Glycerol monostearate
Hydrogenated polydecene
Thai FDA number: 10-1-5201309

Figure 1.5 Chemical compound of sunscreen

1.7.8 UV irradiation means ultraviolet irradiation.

1.7.9 VISIA® Complexion Analysis System (Canfield, Fairfield, NJ) is an equipment for scanning the skin and captures key visual information, using multi-spectral imaging and analysis, of eight areas that affect the skin's health and appearance: pigmentation, pore size, porphyrins (evidence of bacteria), UV spots, photo damage, texture and wrinkles under the same environment.

1.7.10 The mexameter® MX 18 is an equipment to measure the 2 components, mainly responsible for the color of the skin: melanin and hemoglobin (erythema) by reflectance. The measurement is based on absorption and reflection by emit 3 specific light wavelengths. A receiver measures the light reflected by the skin. As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated. MX = 500 log INFRARED – REFLCTION + LOG 5 (Accuracy: ± 5%) The melanin is measured by specific wavelengths chosen to correspond to different absorption rates by the pigments. A spring in the probe head ensures constant pressure on the skin enabling exact, reproducible measurements.

1.7.11 Mean Melanin Index is a parameter from mexameter that is highly sensitive measurement gives values on a broad scale from 1-1000 (1 = white, 1000 = dark) for melanin so that even smallest changes in color become traceable. Accuracy is ± 4%.

1.7.12 Global satisfaction is a scale of the treatment satisfactory questionnaire for medication. Score ranges from -1 to +4; -1 = worse, 0 = not improved, +1 = fairly improvement (1-25%), +2 = moderate improvement (26-50%), +3 = good improvement and +4 = excellent improvement (76-100%)

1.7.13 Fitzpatrick skin phototype is a numerical classification schema for the color of skin. It was developed in 1975 by Thomas B. Fitzpatrick, a Harvard dermatologist, as a way to classify the response of different types of skin to ultraviolet radiation.

Skin Phototype	Typical features	Tanning ability
I	Pale, white skin, blue/harzel eyes, blond/red hair	Always burns, does not tan
II	Fair skin, blue eyes	Burns easily, tans poorly
III	Darker white skin	Tans after initial burn
IV	Light brown skin	Burns minimally, tans easily
V	Brown skin	Rarely burns, tans darkly easily
VI	Dark brown or black skin	Never burns, always tans darkly

Figure 1.6 Fitzpatrick skin phototype scale

CHAPTER 2

REVIEW LITERATURES

2.1 Human skin color

Human skin color is the result of natural selection. Skin pigmentation in humans evolved to the constitutive skin color and facultative skin color [4].

2.1.1 Constitutive Skin Color, Genetics

The difference in skin color between lightly and darkly pigmented individuals is due to the activity of melanocytes (quantity and relative amounts of eumelanin and pheomelanin), not the number (quantity) of melanocytes in their skin [8]. In dark skin, melanocytes produce melanin more than light skin. Melanosome in dark skin type is usually larger, darker in black brown color, more scattering and slower degradation [9].

2.1.2 Facultative Skin Color, Tanning

This process is under hormonal control, including the MSH and ACTH peptides that are produced from the precursor proopiomelanocortin, and ultraviolet irradiation.

There was an attempt to classify the response of different types of skin to UV irradiation by Fitzpatrick skin phototype;

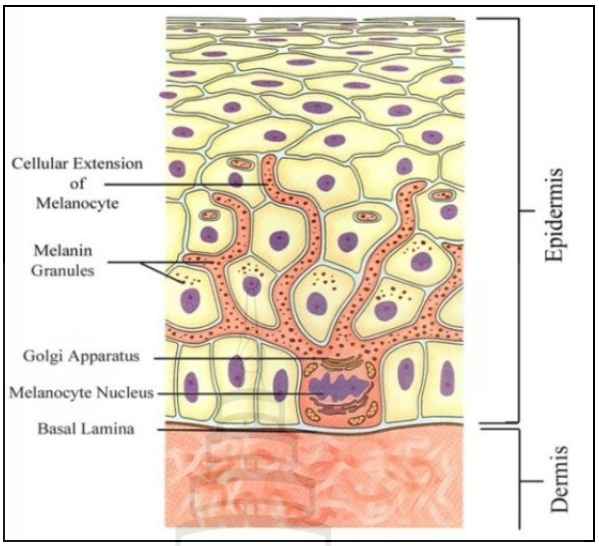
Skin Phototype	Typical features	Tanning ability
I	Pale, white skin, blue/harzel eyes, blond/red hair	Always burns, does not tan
II	Fair skin, blue eyes	Burns easily, tans poorly
III	Darker white skin	Tans after initial burn
IV	Light brown skin	Burns minimally, tans easily
V	Brown skin	Rarely burns, tans darkly easily
VI	Dark brown or black skin	Never burns, always tans darkly

Figure 2.1 Fitzpatrick skin phototype scale

2.2 Melanocyte

Melanocytes originate from the dorsal portions of the closing neural tube. After migration and differentiation, melanocytes are situated on the stratum basale of the epidermis, uvea, inner ears, meninges, bones and heart. The main function of melanocytes is producing melanin, the pigment primarily responsible for skin color [10].

In the skin, melanocytes are located in the stratum basale. Typically, melanocytes are 7 μm in length, between 1000 and 2000 cells per square millimeter (5-10%). One melanocyte is surrounded by approximately 36 keratinocytes. Together, they form so-called epidermal melanin unit. Once synthesized, melanin is contained in a special organelle called a melanosome, vesicle which package the chemical inside plasma membrane, and moved along arm-like structures called dendrites to reach the keratinocytes. The melanosome are organized as a cap protecting the nucleus of the ketatinocytes [10].



Source [11]

Figure 2.2 Melanocyte

2.3 Melanogenesis

Melanogenesis is enhanced by the activation of the key enzyme, tyrosinase. Tyrosinase is a glycoprotein located in the membrane of the melanosome. Melanogenesis takes place in the melanosomes and begins when Phenylalanine hydroxylase-PAH enzyme catalyzes L-phenylalanine to L-tyrosine. Then tyrosinase enzyme, a major rate-limiting enzyme of whole processes, cooperates with Tyrosinase hydroxylase 1-TH-1 to change L-tyrosine to L-3,4-dihydroxyphenyl-alanine (DOPA). DOPA is oxidized to DOPAquinone, converted to eumelanonin via eumelanogenesis and pheomelanin via pheomelanogenesis [12]. Even though L-tyrosine is the building stone for melanin, it can only be transported into the melanosome by facilitated diffusion. [13]

In the eumelanogenesis, dopachrome is either spontaneously converted to 5,6-dihydroxyindole or is enzymatically converted to 5,6-dihydroxyindole-2-carboxylic acid via enzymatic conversion by dopachrome tautomerase (DCT), also referred to as tyrosine-related protein-2 (TRP-2). There are two tyrosinase-related proteins, TRP-1 and TRP-2, reside in the melanosomes. It has been suggested that TRP-1 increases the ratio of

eumelanin to pheomelanin. In addition, they have been illustrated to increase tyrosinase stability. However, the role of TRP-1 and TRP-2 have not been yet clarified [5].

Finally, the polymerization of indoles and quinones leads to eumelanin formation. The pheomelanin pathway branches from the eumelanin pathway at the L-dopaquinone step and is dependent on the presence of cysteine which is actively transported through the melanosomal membrane. Cysteine reacts with L-dopaquinone to form cysteinyl-dopa. The latter is then converted to quinoleimine, alanine-hydroxyl dihydrobenzothazine and polymerizes to pheomelanin [5].

Redox reaction is crucial for the balance between the production of eumelanins and pheomelanins which is directly determined by reduced glutathione (GSH) (high GSH for eumelanin and low for pheomelanin). Therefore, the expression and functional activity of antioxidative enzymes such as catalase, glutathione peroxidase, glutathione reductase and thioredoxin reductase likely modify the melanogenesis [14].

Also, melanin itself has an important role in oxidative homoeostasis in the skin [5]. Eumelanin is a dark brown-black insoluble polymer with an ability to both scavenge and quench both oxygen- and carbon-derived free radicals. Pheomelanin is a light red-yellow sulfur-containing soluble polymer without an ability to eradicate free radical and maybe free radical itself when stimulated from UV irradiation.

2.4 Melanosome transportation

After completed synthesis, melanosome, containing melanin, is moved along arm-like structures called dendrites, so as to reach the keratinocytes. One melanocyte can produce melanin to 36 keratinocytes. Together, they form so-called epidermal melanin unit. This transportation is controlled by protease-activated receptor 2 (PAR-2). PAR-2 is a G-protein-coupled receptor which is stimulated by serine protease cleavage. Therefore, melanosome transportation is accelerated [10].

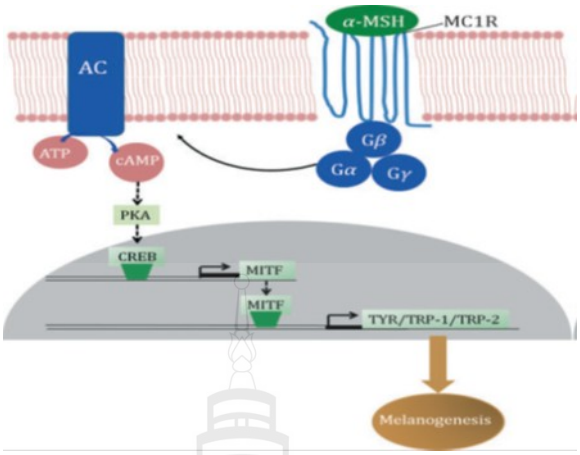
2.5 Melanogenic regulatory proteins

Melanogenic regulatory proteins are the gene encoding the basic helix–loop–helix leucine zipper microphthalmia-associated transcription factor gene (MITF). MITF appears to be the key of transcription factors and signaling pathways that control the survival, proliferation and differentiation of melanoblasts and melanocytes [15], tyrosinase, TRP-1 and TRP-2 [16] and also for Rab27A, a protein important for melanosome transport [17].

2.6 Tanning factors

2.6.1 Stimulate melanogenesis

There are many paracrine that stimulate the melanogenesis such as proopiomelanocortin (POMC)-derived peptides (a-MSH, b-MSH, ACTH). POMC expression in keratinocytes is stimulated by UV irradiation. a-MSH binds to and activates the Gs-protein-coupled receptor melanocortin receptor 1 (MC1R). The Gs family of G proteins (including Ga, Gb and Gc) transmits signals from MC1R to adenylyl cyclase (AC) which, in turn, catalyses the conversion of cytoplasmic ATP to cyclic adenosine 3', 5'-monophosphate (cAMP). Increased levels of cAMP act as a second messenger to activate protein kinase A (PKA), which, upon activation, translocates to the nucleus where it phosphorylates the cAMP-responsive element-binding protein (CREB) family of transcription factors. Phosphorylated CREBs then induce the expression of genes containing CRE (cAMP-responsive elements) consensus sequences in their promoters, such as the transcription factor MITF. The transcription factor MITF binds to the promoter of the pigmentary genes tyrosinase TRP-1 and TRP-2 (DCT) [5].



Source [19]

Figure 2.3 The melanocortin signaling pathway

Recently there was a study demonstrated that a-MSH can increase melanin synthesis by a mechanism independent of MC1R by binding to 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (6BH4), a competitive inhibitor of tyrosinase, and release the inhibitory effect on tyrosinase activity [18].

Even though the POMC peptides have important role on human skin color, there are other paracrine factors such as endothelin-1, stem cell factor, prostaglandins and catecholamines to mentionsome [20].

2.6.2 Stimulate melanosome transportation

This transportation is controlled by protease-activated receptor 2(PAR-2). PAR-2 is a G-protein-coupled receptor which is stimulated by serine protease cleavage. Therefore, melanosome transportation is accelerated. Substances with serine protease inhibiting activities will decrease melanosome transportation. Furthermore, there was the study showed that β amyloid precursor protein or sAPP can stimulate melanosome transportation by increasing movement of dendrites and growth factor (KGF/FGF7) increases the facilitated diffusion via phagocytosis [21].

2.7 Ultraviolet irradiation and skin color

Ultraviolet irradiation (UV irradiation) from sunlight stimulates epidermal melanin units via increase in size and number of melanosome. By the way, UV irradiation does not affect the number of melanocytes. Previous studies demonstrated that the amount of tyrosinase enzyme directly relates to the number of melanin and skin color. The comparative study showed that sun-approached skin has higher amount of DOPA than unexposed skin. There are also significantly change in hormones, cytokines and growth factors after sun exposure. Caused by UV irradiation, it accelerated the oxidation of tyrosine to DOPA and subsequently decreased sulhydryl substance in epidermis. Thus, the natural inhibitor of tyrosinase enzyme is minimized. Aside of that, increase in skin temperature also stimulate melanin production [22].

There are three types of UV: Ultraviolet A-UVA, ultraviolet B-UVB and ultraviolet C-UVC. UVA and UVB can pass through the atmosphere and touch the earth surface. In contrast, UVC is mostly reflected by ozone. UV-A causes immediately skin darkening. It occurs in 2-3 minutes after exposure to prolonged radiation for about 6-8 hours. The process is the oxidation of existing melanin. Both UV-A and UV-B have the same effect of delayed tanning, occurring in 2-3 days after radiation exposure and exists for 10-14 days. In this stage, tyrosinase function, numbers of melanin and melanosome transportation are increased. UV stimulates the formation of UV photon and free radicals. UV photon and free radicals break DNA into fragments such as thymidine nucleotides. Damaging process enhance DNA transcription by stimulate P53 gene, POMC, MSH production cause skin darkening [23].

2.8 Skin whitening

2.8.1 Tyrosinase inhibitors

The most common target for skin-whitening activities is tyrosinase inhibition. There are some of the most commonly used ones are reviewed.

2.8.1.1 Hydroquinone: Hydroquinone (1,4-dihydroxybenzene) has been the conventional standard for the treatment of hyperpigmentation for more than 40 years,

found in berries, wheat, beer, tea and coffee. The action is binding to histidines at the active site of the tyrosinase enzyme resulting in reduction in skin color. In addition, hydroquinone induced generation of reactive oxygen species, and leads to the oxidative damage of membrane lipids and proteins such as tyrosinase. Hydroquinone is also thought to inhibit pigmentation by depleting glutathione, reducing DNA and RNA synthesis with concomitant melanosome degradation and melanocyte damage. Nevertheless, the golden days of hydroquinone come to an end because it can lead to permanent damage of melanocytes leading to irreversible loss of inherited skin color [24]. Following long-term use, hydroquinone induces exogenous ochronosis and drug resistance [25].

2.8.1.2 Arbutin and Alpha-arbutin: Arbutin is another commonly used skin-lightening. It is a derivative of hydroquinone (hydroquinone-O-b-D-glucopyranoside) that is found in cranberries, blueberries, wheat and pears. Arbutin inhibits melanogenesis by competitively and reversibly binding tyrosinase without influencing the mRNA transcription of tyrosinase so arbutin has cytotoxicity less than hydroquinone [26]. The synthetically produced derivative of arbutin (deoxyarbutin) has been shown to be effective and safer skin-lightening agent. The comparative study of the effect of hydroquinone, arbutin and deoxyarbutin illustrated that all three compounds had similar inhibitory effects on tyrosinase activity but the protein expression of tyrosinase was neither affected by arbutin nor hydroquinone, whereas an effect on the protein level was seen by deoxyarbutin. Also, less cytotoxicity was seen in deoxyarbutin compared to the two other quinines. [27]

2.8.1.3 Kojic acid: Kojic acid (5-hydroxy-2-hydroxymethyl-4H-pyran-4-one) is a naturally occurring hydrophilic fungal metabolite obtained from species of Acetobacter, Aspergillus and Penicillium [28]. The activity of kojic acid is believed to arise from chelating copper atoms in the active site of tyrosinase as well as suppressing the tautomerization of dopachrome to 5,6-dihydroxyindole-2-carboxylic acid [29]. Although kojic acid is a popular treatment for melasma, it can cause contact dermatitis, sensitization and erythema [30].

2.8.1.4 Flavonoids: Flavonoids can be found in leaves, bark and flowers. The main action may be the ROS-scavenging properties and the ability to chelate metals at the active site of metalloenzymes. A number of flavonoids are frequently used in skin-lightening preparations such as aloesin, hydroxystilbene derivates and licorice extracts [31].

1. Aloesin has been proven to competitively inhibit tyrosinase and also been shown to inhibit TH and DOPA oxidase activities [32].

2. Resveratrol is found in red wine and has been shown to reduce not only tyrosinase activity [31] but also MITF expression in B16 mouse melanoma cells [33].

3. Licorice the main ingredient of the hydrophobic fraction of licorice extract is glabiridin. This ingredient has been shown to inhibit tyrosinase activity in B16 murine melanoma cells [34].

2.8.2 Melanosome transfer inhibitor

2.8.2.1 Nicotinamide: Niacinamide is a biologically active form of niacin (vitamin B3), found in yeast and root vegetables. It is an important precursor of NADH (nicotinamide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate). These co-enzymes are found in all living cells. The effect of niacinamide on hyperpigmentation is believed to occur through inhibition of melanosomal transfer [35]. Previous research showed that niacinamide has effect on inhibition of melanosome transfer in the coculture model and reduced cutaneous pigmentation by 35–68% [36].

2.8.2.2 Soymilk and soybean extracts: Soymilk and soybean extracts are natural skin-lightening remedies that are suggested to inhibit PAR-2 activation (PAR-2, the transmembrane G-protein-coupled receptor protease-activated receptor 2) [37].

2.8.2.3 Lectins: Lectins and their glycoconjugates have been shown to interrupt melanosome transfer by 15–44% [38]. The exact mechanism by which lectins are acting on melanosomal transfer remains to be elucidated.

2.8.3 Melanocyte cytotoxic agents

Azelaic acid: Azelaic acid (1,7-heptanedicarboxyilic acid) is a saturated dicarboxylic acid found naturally in wheat, rye, and barley. It is a natural substance that is produced from a yeast strain named Pityrosporum ovale [39] which is used to treat acne, rosacea, skin pigmentation, freckles, nevi and senile lentigines [40] by inhibit thioredoxin reductase, the synthesis of deoxyribonucleotides, the substrate for DNA synthesis in the S-phase of the cell cycle [41].

2.8.4 Antioxidants

The idea of using antioxidants for skin-lightening activities lies in the hypothesis that the oxidative effect of UV irradiation leads to activation of melanogenesis via many mechanisms. Antioxidants can also reduce the direct photooxidation of pre-existing melanin. Common antioxidants used in skin-lightening formulations are vitamin E, vitamin C and vitamin B [42].

2.8.5 Acceleration of epidermal turnover and desquamation

Chemical exfoliative skin agents will remove the uppermost layer of keratinocytes containing melanin such as salicylic acid, linoleic acid, retinoic acid and alpha-hydroxyacids [43]. Except for their activity on acceleration of epidermal turnover several of these acids have also been shown to have effect on tyrosinase. For example, unsaturated fatty acids such as linoleic acid show effect on tyrosinase activity, while retinoic acids have an inhibitory effect on tyrosinase transcription [44]. Also, hydroxyacids is complementing its action on desquamation with direct inhibition of tyrosinase without influencing mRNA or protein expression [45].

2.8.6 Conjugates to improve the stability and effect of skin-lightening agents

Many skin whitening agents are instable during storage. Therefore, several attempts have successfully been made to synthesize conjugates to improve their properties. For example,

2.8.6.1 Kojic acid–amino acid amides leading to superior effect with 90% increased antityrosinase activity [46].

2.8.6.2 Magnesium ascorbyl phosphate is improved stability of ascorbic acid (vitamin C), leading to reduced skin pigmentation [22].

2.8.6.3 3-aminopropyl dihydrogen phosphate (3-APPA) is a molecule that has been conjugated with both ascorbic acid and kojic acid that have proven the stability and lightening effect [47].

2.8.6.4 Broad spectrum sunscreens protect skin from ultraviolet radiation. Previous study demonstrated that using of broad spectrum sunscreen improved melasma [48].

2.9 Natural skin whitening agents

Over the past few years, natural cosmetic is a growing industry manufacturing. Increasing attention has been paid to herbal plants for developing into modern medicine and cosmetic products. Because of suitable for long term application and mild side effects, skin whitening containing natural ingredients from plants has become very popular [6]. Various tropical vegetables have been known for their anti-tyrosinase activity which could be potentially prepared for skin whitening such as paper mulberry extract, emblicanin extract, white radish root extract. Paper mulberry extract from the bark and the root contains high amount of Kazinol-F, which acts as a tyrosinase enzyme inhibitor [49]. Emblicanin extract, the dried fruit extract of emblic can be produced skin whitening. The main chemical compounds with interfere tyrosinase-related proteins, tyrosinase and peroxidase activities are Tannins emblicanin A and B [50].

2.10 White radish root extract

Source [51]

Figure 2.4 Radish

2.10.1 General information

The plant family of Cruciferae contains many important vegetables of economic importance. *Raphanus sativus* L. are round to cylindrical with a color ranging from white to red. A longer root form, ideal for cooking, grows up to 15 cm long, while the smaller, rounder form is typically eaten raw in salads. The flesh initially tastes sweet, but becomes bitter if the vegetable is left in the ground for too long. Leaves are arranged in a rosette, with sizes ranging from 10–15 cm in small cultivars, to up to 45 cm in large cultivars. They have a lyrate shape, meaning they are divided pinnately with an enlarged terminal lobe and smaller lateral lobes. The white flowers are borne on a racemose inflorescence [51].

2.10.2 Chemical constituents

There are many chemical constituents that found in root of radish, for example: Phenolic compound: Cyadinin, Kaempferol, Lutolin, Myricetin, Pelargonidin and Quercetin [6, 52], Flavonoid[6], L-ascorbic acid [6], Organic acid: Hydrocinnamic acid,

p-Hydroxybenzoic, Salicylic acid and vanillic acid [52], Coumarins: Aesculetin and Scopoletin [52]

2.10.3 Biological activities of white radish root extract

2.10.3.1 Anti-inflammatory activity [54]

2.10.3.2 Antioxidative activity: One study reported that the white radish root from methanolic extract exhibited hydroxyl radical scavenging potency 1.8-fold higher than that of L-ascorbic acid. It is suggested that flavonoids, together with sinapinic acid esters, may significantly contribute to the antioxidant activity of radish root [55].

2.10.3.3 Antiwrinkle activity [56]

2.10.3.4 Antityrosinase activities

2.10.4 Antityrosinase activity

White radish root extract has many beneficial properties, especially for cosmetic and dermatological applications. Traditionally, Thai women have used slices of fresh white radish roots for the treatment of melasma. There are some systematic studies about antityrosinase evaluation.

Table 2.1 Previous studies of *Raphanus sativus* (radish)

Author, Year	Type	Method	Outcome
Martha et al., 2004	Review article	-	Radish root contain phenolic compound and antioxidant properties
Pirodamornchai et al.,2005	Laboratory	Test of antityrosinase activity of nine vegetables using mushroom tyrosinase inhibition method compared to standard kojic acid	*Raphanus sativus* Linn is an active tyrosinase inhibitor (> 60%) compared to standard kojic acid.
Kamkaen et al., 2007	Laboratory	Test of antityrosinase activity of 16 vegetables extracted with four solvents: hexane, ethyl acetate, methanolic and 50% propylene glycol using mushroom tyrosinase inhibition method compared to standard kojic acid	*Raphanus sativus* Linn in 50% propylene glycol is an active tyrosinase inhibitor (88%) compared to standard kojic acid in 50% propylene glycol.

Table 2.1 (continued)

Author, Year	Type	Method	Outcome
Jakmatakul et al.,2009	Laboratory	Test of antityrosinase, antioxidant and cytotoxic activity of Thai radish root extracted by freeze dried and methanolic method	In freeze dried and methanolic extract of radish root found the contents of total phenolic, total flavonoids and L-ascorbic acid with potency of tyrosinase inhibition with IC50=3.09 and 9.62, and also the scavenging effects on DPPH radical, superoxide anion radical and singlet oxygen that reflexed the antioxidant activities. LDH leakage from fibroblast cells indicated that both extracts exhibited only mild cytotoxicity.

In year 2006, one study investigated antityrosinase activity of nine vegetables from nine families. The result illustrated that extract of five plants including *Raphanus sativus* Linn. (Brassicaceae) has tyrosinase inhibiting effect for skin-whitening preparations [7].

In year 2007, one study aimed to test anti-tyrosinase activity in 16 tropical vegetables from 10 families using mushroom tyrosinase inhibition method. Each plant was extracted with four solvents including hexane, ethyl acetate, methanol and 50% propylene glycol. The results showed that *Raphanus sativus* in 50% propylene glycol, exerted a considerable level of *in vitro* mushroom tyrosinase inhibition (88%) compared to positive controls of kojic acid in the same solvent-propylene glycol (82%) [57].

Table 2 Effect of sixteen vegetable extracts with four different organic solvents on mushroom tyrosinase inhibition

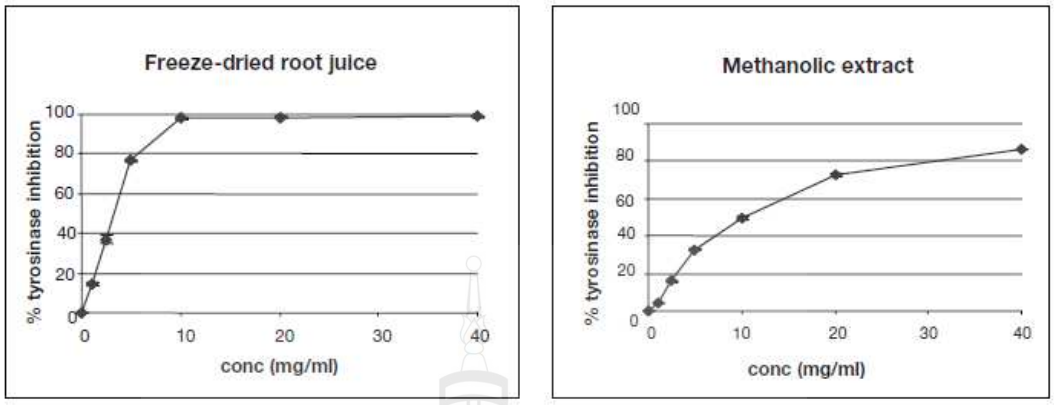
Scientific name	Inhibition of mushroom tyrosinase (%)			
	Hexane	Ethyl acetate	Methanol	50% Propylene glycol
Cymbopogon citratus	**46.57**	14.24	7.57	10.51
Piper longum	12.24	160.26	-89.73	-44.91
Raphanus sativus	38.43	**68.73**	53.51	**88.50**
Aloe vera	15.07	159.76	10.00	0.44
Sesbania grandiflora	-3.65	10.64	28.03	13.06
Ocimum basilicum	4.19	149.88	-371.34	-0.98
Momordica charantia	2.67	169.74	**78.98**	68.17
Hibiscus esculentus	25.52	-56.26	18.47	20.65
Boesenbergia pandurata	21.44	-437.50	6.80	7.82
Psophocarpus tetragonolobus	4.92	-489.29	-157.77	-13.95
Lycopersicon esculentum	4.27	-193.75	2.91	-1.70
Coriandrum sativum	13.56	-412.50	-24.76	9.32
Cucumis sativus	3.47	33.13	-21.60	-18.78
Ocimum sanctum	14.98	156.81	-98.30	-3.01
Mentha cordifolia	10.20	208.43	-218.20	36.27
Daucus sativus	16.18	39.84	2.18	-7.86
Kojic acid (reference)	**87.65**	**96.12**	**65.24**	**82.02**

Note: final concentration of vegetable extracts were 5 mg per well for hexane, ethyl acetate, and methanol extracts, and 10 mg per well for 50% propylene glycol extracts.

Source [57]

Figure 2.5 Sixteen tropical vegetables selected for screening of anti-tyrosinase inhibition

In year 2009, Jakmatakul et al. [6] were evaluated for antityrosinase and antioxidant activities of freeze-dried juice and methanolic extract from the root of Thai radish *(Raphanus sativus* L.). The freeze-dried juice showed higher potency of tyrosinase inhibition (IC50 = 3.09 mg/ml) than the methanolic extract (IC50 =9.62 mg/ml).



Source [6]

Figure 2.6 The relationship between percent of tyrosinase inhibition and the concentration of freeze-dried root juice and methanolic extract

2.10.5 Side effect

In the radish, the allyl isothiocyanate released enzymatically from sinigrin, a thioglycoside, was identified as a possible substance that can produce contact dermatitis [58].

2.10.6 Toxicology testing

A study from Japan using radish with methanolic extract tested in vitro with nitric oxide (NO) generation inhibitory activities in a murine macrophage cell line, RAW 264.7, stimulated with both lipopolysaccharide (LPS, 100 ng/ml) and interferon-gamma (IFN-gamma, 100 U/ml), showed strongly inhibited NO generation at a concentration of 200 mcg/ml by 70% or more with significant cell viability (>50%). While decrease in the concentration to 40 mcg/ml, it had any cell toxicity [59].

2.10.7 Genotoxicity

There was a research, used edible part of fresh white radish extracted by hexane, chloroform, methanol and freeze dried extract. The solvents were isolated by dry evaporation method and dissolved in dimethylsulfoxide. All of these extracts were tested with *Salmonella* (Mammalian microsome mutagenicity test). The result found

that there were no mutation in *S. typhimurium* TA98 and TA100 either in condition with or without S9 [60]. Apart of this, crude juice from white radish of 200 mcl was brought to test with *S. typhimurium* TA98 and TA100 in culture agar and had low genotoxicity [61].

2.10.8 Teratogenicity

Radish extract at the concentration of 175 mg/kg was given to the pregnant rats for 10 days to evaluate the teratogenicity. The result displayed that embryonic implantation was inhibited. However there were no teratogenic effects in pregnant mothers or fetuses [62].

In this study, the researcher used 4% of white radish root extract cream by methanol which equal to 40 mcg/mL. Refer to all previous studies, data indicated that the safety of it and no teratogenicity for certain. Crude juice solution in compare to freeze dried extract of 200 mcL has genotoxicity. Extract with other substances such as propylene glycol may increase potency of white radish but there is still no evidence enough to conclude the safety of them.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research design

Double-blind, randomized, controlled, split-face clinical trial

3.2 Population and sample

3.2.1 Study population

Thai volunteers, both male and female, ages 20-60 years old with Fitzpatrick skin type 3-6, diagnosed by dermatologist, wanted to whiten their facial skin at Mae Fah Luang University Hospital, Bangkok, who matched with all inclusion criteria.

3.2.2 Sample

Thai volunteers, both male and female, ages 20-60 years old with Fitzpatrick skin type 3-6, diagnosed by dermatologist, wanted to whiten their facial skin at Mae Fah Luang University Hospital, Bangkok, who matched with all inclusion criteria.

3.2.3 Sample size determination

The study of antityrosinase property of white radish root extract in vivo has never been established, so the researcher chose the most similar study, which is a comparative study of the safety and efficacy of 75% mulberry (*Morus alba*) extract oil versus placebo as a topical treatment for melasma: a randomized, placebo-controlled trial [63]

At 8th week, mexameter readings for the mulberry group showed a significant drop from 355.56 (± 59.51) at baseline to 312.52 (± 57.03) compared to the placebo group, whose mexameter readings deteriorated from 368.24 (± 46.62) at baseline to 372.12 (± 44.47)

From the formula [64]

α = 0.05 (two-tail) $Z_{0.025}$ = 1.96

β = 0.10 $Z_{0.100}$ = 1.28

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2(\sigma_1^2 + \sigma_2^2)}{\mu_d^2} \quad [62]$$

$$= \frac{(1.96+1.28)^2(57.03^2+44.47^2)}{(312.52-372.12)^2}$$

= 19

Note

n = Sample size per group

σ = Standard deviation, $\sigma 1$= 57.03 and$\sigma 2$= 44.47

μ_d = Mean difference

= 312.52-372.12

A drop-out rate of 20% was expected, so 23 volunteers (n=23) should be enrolled. The researcher collected 25-30 volunteers.

3.2.4 Selection criteria

3.2.4.1 Inclusion criteria

1. Healthy Thai volunteers, both male and female, ages 20-60 years old with Fitzpatrick skin type 3-6, diagnosed by dermatologist who can follow up at Mae Fah Luang University Hospital, Bangkok.
2. All subjects were required to sign an informed consent form of benefits, risks and possible complications of the treatment and publication of photographs.

3.2.4.2 Exclusion criteria

1. Ablative and non-ablative laser
2. Intense pulse light
3. Microdermabrasion

4. Skin needling
5. Chemical peeling
6. Facial whitening treatment
7. Facial whitening agent
8. Allergy to chemical compounds in 4% white radish root extract cream, standard cream base, mild soap and sunscreen SPF60, PA+++
9. Used of hormones or any medications that interfered the melanogenesis, for example: anticonvulsant
10. Medical illnesses such as poorly controlled diabetic mellitus, hypertension, cardiovascular disease, renal diseases, liver diseases and immunosuppressive agents
11. Photosensitivity or drug induced photosensitivity
12. Chemo-radiotherapy
13. Unable to avoid heavy sunlight
14. Active inflammatory skin disease, open wound in the treatment area
15. History of malignant or premalignant lesions in the treatment area
16. Pregnancy and lactation

3.2.4.3 Discontinuation criteria

1. Using facial whitening agents unless 4%white radish root extract cream
2. Developed severe side effect or allergic to cream
3. Pregnancy
4. Noncooperation to the protocol or lost to follow up
5. Patients want to leave the study

3.3 Variable of the study

Independent variable: Using 4% white radish root extract cream

Dependent variable: Decreased mean melanin index

3.4 Equipment

3.4.1 4% White radish root extract cream

3.4.2 Standard cream base

3.4.3 Mild soap

3.4.4 Sunscreen SPF 60, PA+++

3.4.5 Finn chamber for patch test

3.4.6 VISIA® Complexion Analysis System (Canfield, Fairfield, NJ)

3.4.7 Mexameter MX18® (Courage-Khazaka Electronic, Koln, Germany)

3.4.8 Patient profile record

3.4.9 Informed consent

3.4.10 Qualification letter

3.4.11 Side effect record form

3.4.12 Treatment satisfactory questionnaire for 3 independent dermatologists and volunteers

3.5 Study procedure

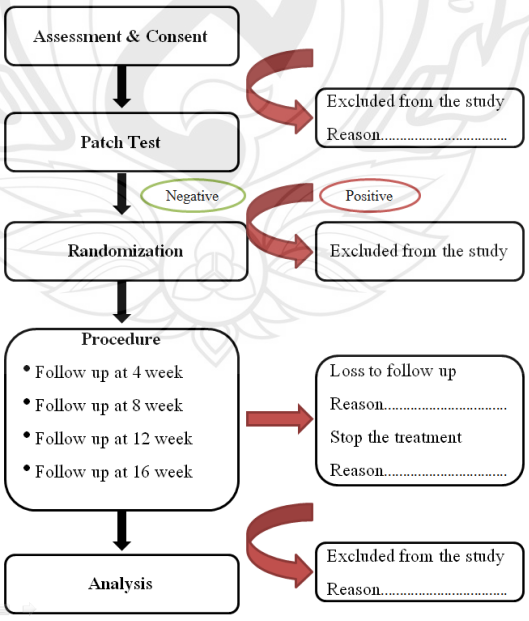


Figure 3.1 Study protocol

3.5.1 Treatment process

3.5.1.1 Patients were selected to enroll in the study in agreement with the selection criteria. The researcher intensively explained the purpose of the research, process during the study, benefits and possible complications of the treatment. The patients signed an informed consent form for participation in the study.

3.5.1.2 History taking

3.5.1.3 Performed the patch test by applied 4% white radish root extract cream on volunteers' back under the Finn chamber then left in place for 48 hours. During the test, volunteers should avoid excessive sweating condition or heavy sunlight and stop taking oral steroid at least 2 weeks before patch test were done, examined for any response at 48 and 96 hours. Any reaction seen is scored according to the International Contact Dermatitis Research Group system, as follows: +? = doubtful reaction: mild redness only. + = weak, positive reaction: red and slightly thickened skin. ++ = strong positive reaction: red, swollen skin with individual small water blisters. +++ = extreme positive reaction: intense redness and swelling with coalesced large blisters or spreading reaction.IR = irritant reaction. Red skin improves once patch is removed.NT = not tested. Volunteers with positive patch test since score ++ would be excluded.

3.5.1.4 The researcher took photographs of each volunteer using VISIA® Complexion Analysis System at before treatment, 4^{th}, 8^{th}, 12^{th} and 16^{th} (4 week after the last treatment) weeks, respectively. Of which the following were required: turn off the room light, 12 megapixel resolution, automatic focus, automated white balance correction, facial positions: left 37°, center 0°, right 37°, Multi-spectral Imaging (standard daylight fluorescent lighting, cross Polarized flash, and ultraviolet lighting).

3.5.1.5 The dermatologist measured the melanin index of each volunteer using mexameter MX18 then calculated into mean melanin index before treatment, at 4^{th} , 8^{th} , 12^{th} and 16^{th} (4 week after the last treatment) weeks, respectively. The landmarks were 1 centimeter above eyebrow at mid papillary line and 2 centimeter below lower lids at mid papillary line.

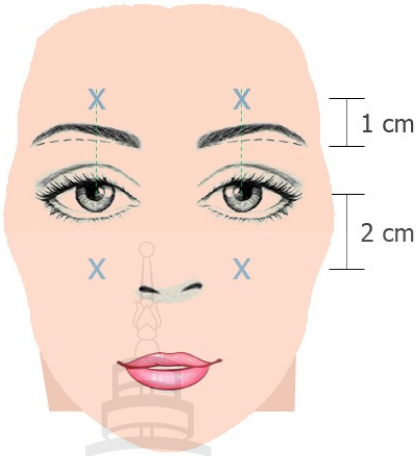


Figure 3.2 Landmark for measurement by mexameter MX18

3.5.1.6 Randomization

1. 4% White radish root extract cream and standard cream base (similar consistency, smell and color) were enclosed in the identical packages then labeled with "A" and "B". The volunteers and dermatologists, who evaluated the result, were blinded.

2. The physician, unrelated with the research, generated randomization sequence which randomly determined which side of the volunteers' face to be treated with cream A and which side with cream B by using "Block randomization" and conceals the sequence in opaque envelopes.

3. This study was split-face. Thirty volunteers were recruited, so there were 60 faces. Each block contained two members which were "Right" (Right face) and "Left" (Left face)

4. There were two ways for treatment;

1) (Right, Left) = Right face applied cream A, Left face applied cream B.

2) (Left, Right) = Left face applied cream A, Right face applied cream B

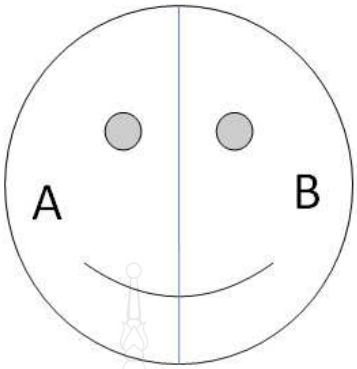


Figure 3.3 How to apply cream A and B as "1"

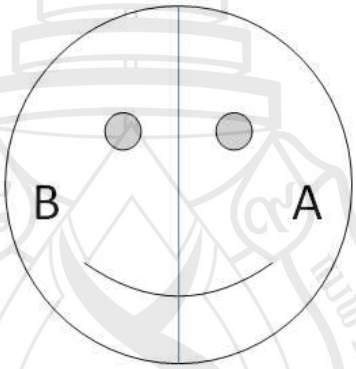


Figure 3.4 How to apply cream A and B as "2"

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

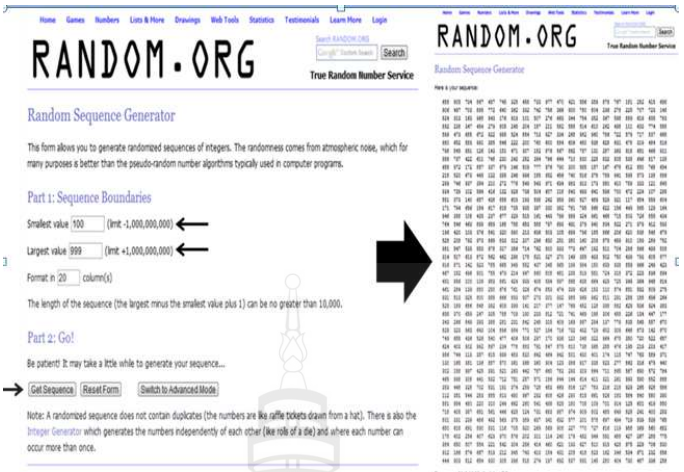


Figure 3.5 Random Sequence Generator from website

6. Randomized thirty Arabic numbers from minimal 1 to maximal 1000. Thirty of these randomized numbers were referred to thirty of volunteers according to first-come, first-served order.

7. Even number = “1”, Odd number = “2”

3.5.1.7 In this study, the researcher used 4% of white radish root extract cream by methanol which equal to 40 mcg/mL. Refer to all previous studies, data indicated that the safety of it and no teratogenicity for certain. Crude juice solution in compare to freeze dried extract of 200 mcL has genotoxicity. Extract with other substances such as propylene glycol may increase potency of white radish but there is still no evidence enough to conclude the safety of them. Direction for use as follow;

1. Mild soap used twice a day as following step:

1) Splashed the face with water.

2) Put a small amount of mild soap on the palm then rubbed the face gently with the fingers.

3) Rinsed the face with water. Repeat this until you remove all cleanser.

4) Patted the face dry with facial tissue or towels.

2. Cream A and B used twice a day as following step:

1) After washed the face

2) Use the measuring spoon (1 measuring spoon = 0.5 grams) scooped the cream, lightly. Run a spatula across the top to level the surface and scrape any excess back into the pot. Put a small amount of cream that labeled “right side” on right index and applied it on right side of the face. All of these steps were repeated for 3 times. The amount of cream that applied for half face was totaled at 1.5 gram

3) Repeated the same step as 2) on left index and applied it on left side of the face.

3. Sunscreen used once a day or 30 minutes before exposed to the sunlight as following step:

1) After applied cream A and B, poured sunscreen along right index length 1 fingertip then applied it on right side of the face.

2) Repeated the same step as 1) on left index and applied it on left side of the face.

During 16 weeks of the study, all volunteers had better strictly follow the treatment process as mentioned in the inform consent; Avoided using other whitening agents or exposing to heavy sunlight. Each volunteer was given a side effect record sheet to record the side effect. If volunteers experienced any severe side effect, they must stop using the cream, notify the researcher and come to follow up as fast as possible.

3.6 Follow up

3.6.1 Evaluated melanin concentration using mean melanin index measured by mexameter MX18 at 4^{th}, 8^{th}, 12^{th} and 16^{th} (4 weeks after treated) weeks by dermatologist.

3.6.2 Evaluated volunteers’ side effect by questionnaires and researcher observation at 4^{th}, 8^{th} and 12^{th} weeks.

3.6.3 Evaluation of the treatment between 4% White radish root extract and standard cream base via photographs from VISIA® Complexion Analysis System at 4^{th}, 8^{th} and 12^{th} weeks after treated. Evaluation of treatment by 3 independent dermatologists used global satisfaction scale. Score ranges from -1 to +4; -1 = worse, 0 = not improved, +1 =

fairly improvement (1-25%), +2 = moderate improvement (26-50%), +3 = good improvement, +4 = excellent improvement (76-100%)

3.6.4 Volunteers' satisfaction was evaluated at 12th week by questionnaires using global satisfaction scale.

3.7 Data analysis

3.7.1 Volunteers' research profile data: made descriptive statistical analysis to provide descriptive information, such as percentages, means, modes, medians, ranges, standard deviations.

3.7.2 Compared face that applied 4% white radish root extract cream or standard cream base before treatment with after treatment at 4th, 8th, 12th and 16th weeks using mean melanin index by mexameter MX18

3.7.2.1 In case there is normal distribution of data, we could use paired t-test statistics.

3.7.2.2 In case data is not along with normal distribution of data, Wilcoxon Match Pair sign rank test would be used.

3.7.3 Compared paired different of mean melanin index of forehead, cheek and total area that applied 4%White radish root extract with standard cream base after 4th, 8th, 12th and 16th weeks use paired t-test or Wilcoxon Match Pair sign rank test.

3.7.4 Evaluation of the treatment by 3 independent dermatologists.

3.7.4.1 In case there is normal distribution of data, we could use paired t-test statistics.

3.7.4.2 In case data is not along with normal distribution of data, Wilcoxon Match Pair sign rank test would be used.

3.7.5 Evaluation of patient satisfaction at 12th week between both sides using descriptive statistical analysis, the researcher did the following at significance levels of p-value < 0.05.

3.7.6 Complication using descriptive statistical analysis, the researcher did the following at significance levels of p-value < 0.05.

3.8 Ethical consideration

This study was strictly followed Good Clinical Practice (GCP) guidelines, an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects, provided by International Conference on Harmonization (ICH).

Good Clinical Practice guidelines include protection of human rights as a subject in clinical trial, assurance of the safety and efficacy of the newly developed compounds, standards on how clinical trials should be conducted; define the roles and responsibilities of clinical trial sponsors, clinical research investigators, and monitors. For general understanding, considerations were as followed.

3.8.1 Volunteers completely understand objective, methodology and possible side effect of the research

3.8.2 Volunteers are willing to sign informed consent before entering into the study. They can leave anytime without disadvantage.

3.8.3 This research is free of charge. There is no interest between the researcher and subjects.

3.8.4 4% White radish root extract cream is approved by Thai FDA. However, contact dermatitis or allergy to white radish root extract can be developed, thus all volunteers have to do patch test before enrolled.

3.8.5 In any case problem occurs; the researcher will help and pay responsibility with subjects as much as possible.

3.8.6 This study is spitted face design. After using 4% white radish root extract cream, if the hypothesis is right, half of their face will be brighter. The researcher advises volunteers to use cosmetics to conceal the difference during the study and the researcher will give 4% white radish root extract cream to all volunteers without expenses after finished study.

3.8.7 All volunteers' information is confidential.

3.9 Obstacles and strategy

Sunlight may affect the skin color. The researcher solved this problem by intensively explained the protocol and advised all volunteers to strictly following the direction.

CHAPTER 4

RESULTS

4.1 General characteristics

Thirty Thai volunteers, who wanted to whiten their facial skin at Mae Fah Luang University Hospital, Bangkok for 16 weeks from November 2013 to February 2014, were enrolled. Two volunteers were discontinued from the study due to loss to follow up. So volunteers were totaled at 28. Details of the demographic data were shown in Table 4.1.

Table 4.1 Demographic data

Characteristics		Data
Age		
	Mean (SD)	33.14 (11.86)
	Min-Max	19-60
Sex		
	Male	8
	Female	20
Occupation		
	Officer	22
	Housewife	2
	Student	4
Previous treatment		
Treatment		
	Cosmetic products	28
	Facial treatment	24
	Laser	16
Time exposure to the sunlight 10am - 4pm		
	Mean (SD)	18.57 min (14.25)
	Min-Max	5-60 min
Fitzpatrick skin type		
	skin type 3	8
	skin type 4	18
	skin type 5	2

Table 4.1 (continue)

Characteristics		Data
Underlying disease		
	Yes	2
	No	26
Drugs or supplements		
	Drugs	2
	Supplements	8
	No	18
Aggravating factors of melanin production		
	Sun expose	28

4.2 Clinical evaluation

There were 8 men and 20 women. The mean age of volunteers was 33.14±11.86 years, range from 19-60 years. There were 22 officers, two housewife and four students. All of the volunteers had history of using facial cosmetic products, 24 and 16 people used facial treatment and laser, correspondingly. The mean time exposure to the sunlight between 10am-4pm of volunteers was 18.57± 14.25 minutes, range from 5-60 minutes. Most of the volunteers had Fitzpatrick skin type 4, which were 18 people. Eight and two volunteers had Fitzpatrick skin type 3 and 5, respectively. Two volunteers had mild hypertensive disorder, the others had none. Eighteen volunteers took any medicine while two and eight volunteers were took antihypertensive drugs and supplements, individually. All of volunteers had aggravating factors of melanin production, which was sun exposure.

4.3 Parameter of the treatment

Table 4.2 Mean melanin index of face that applied 4% White radish root extract cream

Mean melanin index of the face that applied 4% White radish root extract cream										
	Before treatment		After treatment for 4 week		After treatment for 8 week		After treatment for 12 week		After stop treatment for 4 week	
	forehead	cheek	forehead	cheek	forehead	cheek	forehead	cheek	forehead	cheek
1	220	214	220	211	207	211	187	195	189	205
2	227	210	210	212	207	204	182	190	187	196
3	255	287	242	280	220	270	221	232	220	237
4	249	286	237	282	221	265	214	253	215	266
5	255	337	243	295	221	285	220	280	222	288
6	252	334	243	294	222	280	222	281	226	282
7	342	360	293	353	285	318	263	272	265	271
8	342	358	283	356	276	316	258	271	255	270
9	292	300	275	277	245	262	231	241	240	240
10	281	292	259	272	240	252	222	235	223	235
11	175	253	157	230	143	210	141	200	145	211
12	171	243	157	231	137	211	130	198	134	203
13	200	278	184	257	181	233	164	220	167	222
14	199	271	183	251	181	232	164	221	163	222
15	189	302	176	271	142	265	141	255	141	276
16	181	295	168	265	145	260	144	254	140	264
17	197	254	187	240	159	220	155	211	156	220
18	207	249	185	241	153	197	147	187	150	199
19	297	350	299	338	278	321	267	311	266	312
20	293	354	290	330	278	320	263	301	265	301
21	256	330	221	325	195	301	190	290	199	296
22	246	324	210	324	215	310	213	286	220	287
23	175	170	120	140	114	137	111	132	117	133
24	166	167	136	143	112	141	109	131	119	134
25	228	221	195	200	180	172	170	165	176	167
26	234	214	215	201	181	173	167	161	169	161
27	243	284	218	259	211	256	200	245	201	244
28	230	278	216	247	217	248	203	255	205	266

Table 4.3 Mean Melanin Index of the face that applied standard cream base

Mean melanin index of the face that applied standard cream base										
	Before treatment		After treatment for 4 week		After treatment for 8 week		After treatment for 12 week		After stop treatment for 4 week	
	forehead	cheek	forehead	cheek	forehead	cheek	forehead	cheek	forehead	cheek
1	215	200	210	210	204	199	200	205	202	204
2	209	198	208	200	198	202	199	207	200	210
3	266	274	262	264	255	260	265	261	260	259
4	262	264	258	264	254	261	260	266	259	259
5	260	348	257	336	250	333	255	343	260	345
6	254	336	255	322	251	320	249	323	251	325
7	358	360	342	367	325	345	344	344	350	345
8	358	354	345	362	320	342	345	341	355	344
9	294	300	288	311	275	301	288	294	290	300
10	286	298	277	303	261	311	285	299	291	305
11	198	246	177	238	189	239	190	244	195	245
12	197	245	174	232	195	241	196	234	197	241
13	191	281	185	272	196	275	181	276	187	270
14	194	286	183	270	202	272	187	278	178	279
15	192	395	191	380	201	378	199	389	200	390
16	191	297	188	287	200	288	190	278	201	285
17	221	241	200	245	182	243	210	244	220	267
18	219	237	199	225	178	235	219	213	201	233
19	300	375	290	370	286	363	290	365	299	366
20	300	367	295	371	301	369	295	354	298	356
21	253	321	245	313	220	322	245	330	250	333
22	250	319	244	310	221	320	246	329	254	333
23	160	179	150	161	136	159	158	177	163	187
24	165	175	147	159	127	141	163	168	165	167
25	261	215	231	199	220	210	257	211	260	222
26	255	206	235	203	221	200	254	201	253	202
27	267	272	257	265	254	281	257	263	260	270
28	265	272	240	264	245	278	255	266	256	270

4.4 The Comparison of mean melanin index of the face that applied standard cream base and 4% White radish root extract cream

Table 4.4 Comparison of mean melanin index of the forehead that applied standard cream base before and after treatment

	Standard cream base		
Forehead	**Mean±SD**	**Paired Differences**	**p-value**
Before treatment	244.32±51.23	11.00±8.33	<0.001
After treatment for 4 week	233.32±51.92		
Before treatment	244.32±51.23	16.93±16.24	<0.001
After treatment for 8 week	227.39±48.70		
Before treatment	244.32±51.23	5.68±5.07	<0.001
After treatment for 12 week	238.64±49.52		
Before treatment	244.32±51.23	3.07±6.37	0.017
After stop treatment for 4 week	241.25±50.99		

Note. Used Paired T test

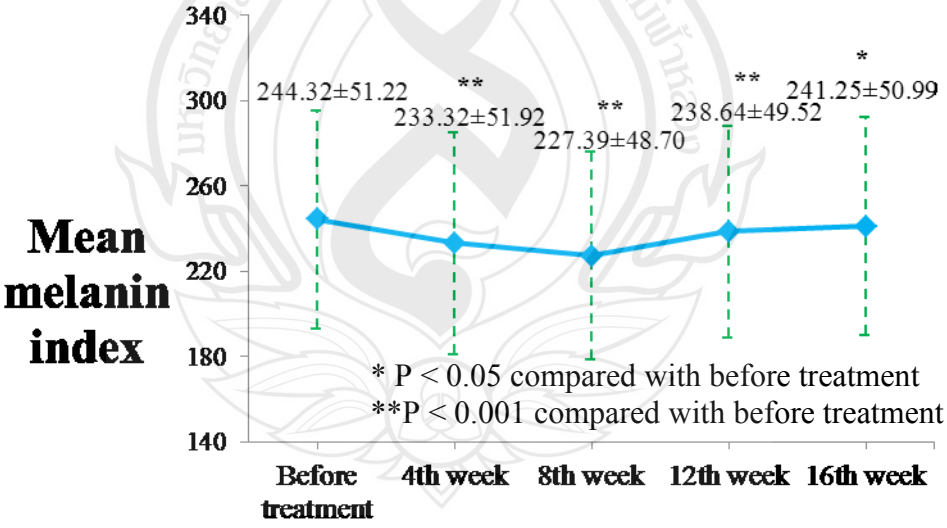


Figure 4.1 Linear graph shows mean melanin index of the forehead that applied standard cream base at 4th, 8th, 12th and 16th weeks

Table 4.4 and Figure 4.1 present the reduction of mean melanin index of the forehead after applied standard cream base with statistical significance ($p<0.001$) at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks. The reduction of mean melanin index at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks were 11.00±8.33, 16.93±16.24, 5.68±5.07 and 3.07±6.37, respectively. The researcher did the following at significance levels of p-value < 0.05.

Table 4.5 Comparison of mean melanin index of the cheek that applied standard cream base before and after treatment

	Standard cream base		
Cheek	**Mean±SD**	**Paired Differences**	**p-value**
Before treatment	280.75±62.04	5.64±8.84	0.002
After treatment for 4 week	275.11±62.04		
Before treatment	280.75±62.04	6.18±10.01	0.003
After treatment for 8 week	274.57±62.73		
Before treatment	280.75±62.04	5.64±8.39	0.001
After treatment for 12 week	275.11±60.36		
Before treatment	280.75±62.04	1.75±9.85	0.355
After stop treatment for 4 week	279.00±59.11		

Note. Used Paired T test

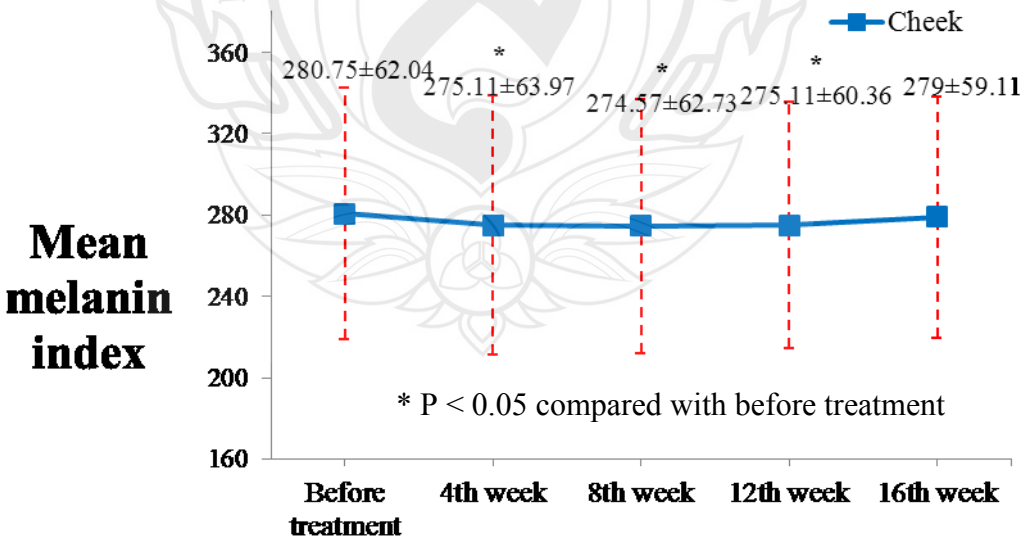


Figure 4.2 Linear graph shows mean melanin index of the cheek that applied standard cream base at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks

Table 4.5 and Figure 4.2 show the reduction of mean melanin index of the cheek after applied standard cream base with statistical significance at 4th(p=0.002), 8th(p=0.003), 12th(p=0.001) and 16th(p=0.355) weeks. The reduction of mean melanin index at 4th, 8th, 12th and 16th weeks were 5.64±8.84, 6.18±10.01, 5.64±8.39 and 1.75±9.85, correspondingly. The researcher did the following at significance levels of p-value < 0.05.

Table 4.6 Comparison of mean melanin index of the forehead that applied 4% White radish root extract cream before and after treatment

4%White radish root extract cream			
Forehead	**Mean±SD**	**Paired Differences**	**p-value**
Before treatment	234.68±48.97	19.61±16.38	<0.001
After treatment for 4 week	215.07±47.77		
Before treatment	234.68±48.97	35.89±17.67	<0.001
After treatment for 8 week	198.79±49.21		
Before treatment	234.68±48.97	45.43±16.94	<0.001
After treatment for 12 week	189.25±45.91		
Before treatment	234.68±48.97	42.71±16.73	<0.001
After stop treatment for 4 week	191.96±45.13		

Note. Used Paired T test

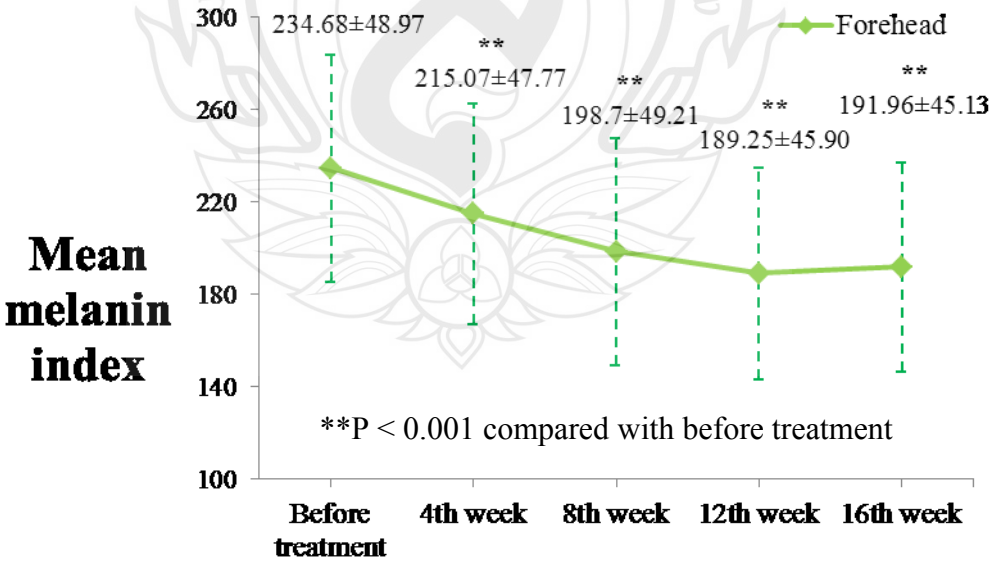


Figure 4.3 Linear graph shows mean melanin index of the forehead that applied standard cream base at 4th, 8th, 12th and 16th weeks

Table 4.6 and Figure 4.3 display the reduction of mean melanin index of the forehead after applied 4% White radish root extract cream with statistical significance ($p<0.001$) at week 4^{th}, 8^{th}, 12^{th} and 16^{th}. The reduction of mean melanin index at week 4^{th}, 8^{th}, 12^{th} and 16^{th} were 19.61±16.38, 35.89±17.67, 45.43±16.94 and 42.71±16.73, individually. The researcher did the following at significance levels of p-value < 0.05.

Table 4.7 Comparison of mean melanin index of the cheek that applied 4% White radish root extract cream before and after treatment

4%White radish root extract cream			
Cheek	**Mean±SD**	**Paired Differences**	**p-value**
Before treatment	279.11±54.63	17.50±12.02	<0.001
After treatment for 4 week	261.61±55.64		
Before treatment	279.11±54.63	33.75±12.93	<0.001
After treatment for 8 week	245.36±52.89		
Before treatment	279.11±54.63	47.93±16.15	<0.001
After treatment for 12 week	231.18±49.11		
Before treatment	279.11±54.63	43.11±18.92	<0.001
After stop treatment for 4 week	236.00±49.20		

Note. Used Paired T test

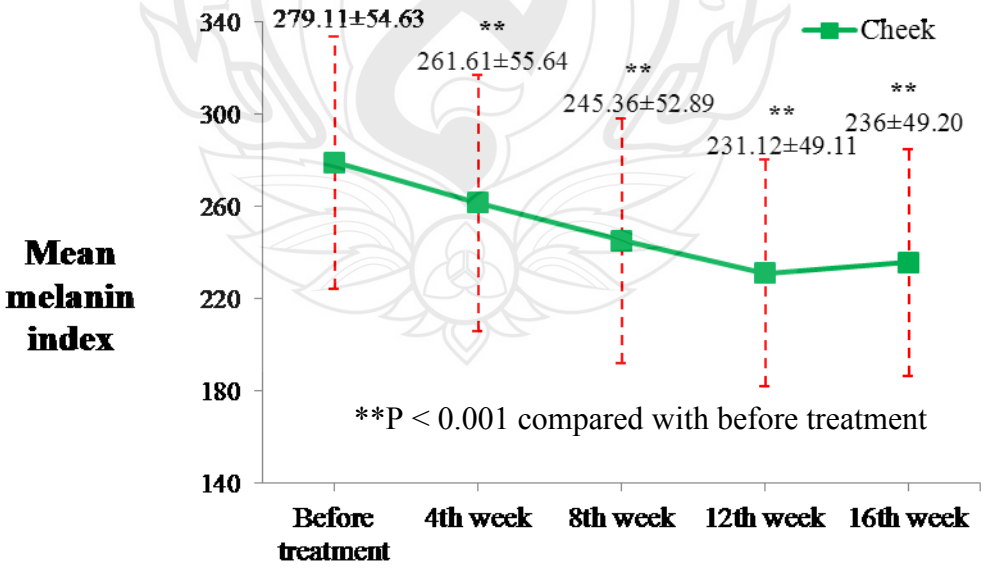


Figure 4.4 Linear graph shows mean melanin index of the cheek that applied 4% White radish root extract cream at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks

Table 4.7 and Figure 4.4 exhibit the reduction of mean melanin index of the cheek after applied 4% White radish root extract cream with statistical significance ($p<0.001$) at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks. The reduction of mean melanin index at 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks were 17.50±12.02, 33.75±12.93, 47.93±16.15 and 43.11±18.92, individually. The researcher did the following at significance levels of p-value < 0.05.

Table 4.8 Statistical analysis compared paired difference of mean melanin index of forehead, cheek and total area that applied 4% White radish root extract with standard cream base in each period

Comparison between Paired Difference of Mean Melanin Index	**Paired Difference**	**S.D.**	**p-value**
Differences dependent on topical agents			
Forehead (4%White radish root extract - standard cream base)			
Before treatment – 4^{th} week	8.61	15.99	0.008
Before treatment – 8^{th} week	18.96	15.47	< 0.001
Before treatment – 12^{th} week	39.75	18.01	< 0.001
Before treatment – 16^{th} week	39.64	18.50	< 0.001
Cheek (4%White radish root extract - standard cream base)			
Before treatment – 4^{th} week	11.86	11.22	< 0.001
Before treatment – 8^{th} week	27.57	14.65	< 0.001
Before treatment – 12^{th} week	42.29	13.62	< 0.001
Before treatment – 16^{th} week	41.36	17.62	< 0.001
Total area (4%White radish root extract - standard cream base)			
Before treatment – 4^{th} week	10.23	13.77	< 0.001
Before treatment – 8^{th} week	23.27	15.55	< 0.001
Before treatment – 12^{th} week	41.02	15.87	< 0.001
Before treatment – 16^{th} week	40.50	17.92	< 0.001

Table 4.8 (continued)

Comparison between Paired Difference of Mean Melanin Index	Paired Difference	S.D.	p-value
Differences dependent on facial areas			
4%White radish root extract (Forehead – Cheek)			
Before treatment – 4th week	2.11	21.03	0.600
Before treatment – 8th week	2.14	16.35	0.494
Before treatment – 12th week	2.50	13.86	0.350
Before treatment – 16th week	0.39	14.31	0.886
Standard cream base (Forehead – Cheek)			
Before treatment – 4th week	5.36	11.30	0.027
Before treatment – 8th week	10.75	19.54	0.007
Before treatment – 12th week	0.04	10.59	0.350
Before treatment – 16th week	1.32	11.20	0.886

Note. p-value compared between 2 groups with Wilcoxon Signs Ranks test or paired t-test

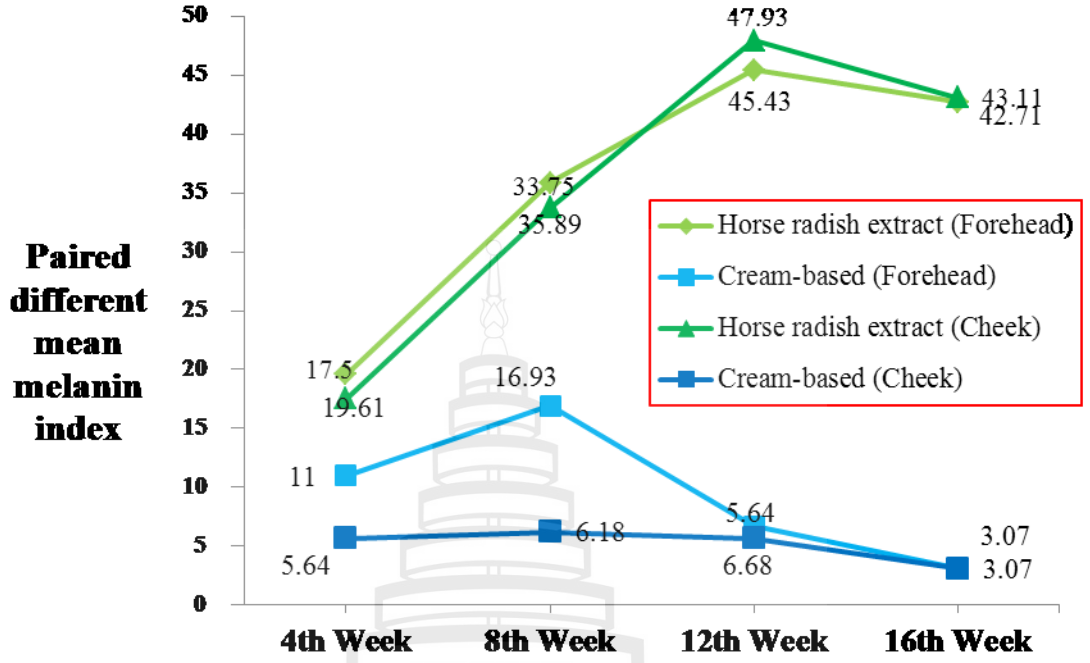


Figure 4.5 Linear graph shows comparison of paired difference of mean melanin index of forehead and cheek that applied 4% White radish root extract with standard cream base in each period

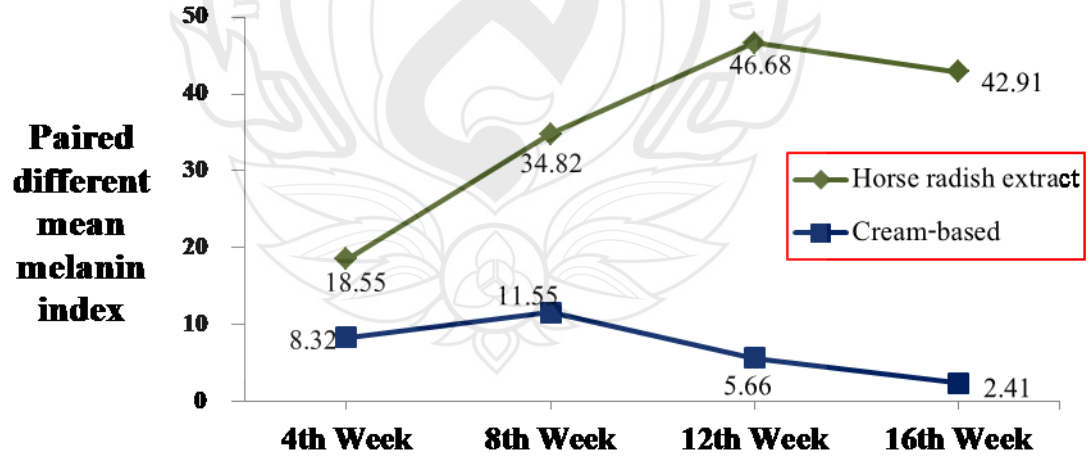


Figure 4.6 Linear graph shows comparison of paired difference of mean melanin index of total area that applied 4% White radish root extract with standard cream base in each period

Table 4.8 Figure 4.5 and Figure 4.6 exhibit paired difference of mean melanin index of forehead, cheek and total area between face that applied 4% White radish root extract and standard cream base in each period. After 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks, paired difference of forehead were 8.61±15.99, 18.96±15.47, 39.75±18.01 and 39.64±18.50 with statistical significance at $p=0.008$(4^{th} week) and $p<0.001$(8^{th}, 12^{th} and 16^{th} weeks). After 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks, paired difference of cheek were 11.86±11.22, 27.57±14.65, 42.29±13.62 and 41.36±17.62, respectively with statistical significance at $p<0.001$. After 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks paired difference of total area were 10.23±13.77, 23.27±15.55, 41.02±15.87 and 40.50±17.92, correspondingly with statistical significance at $p<0.001$. The researcher did the following at significance levels of p-value < 0.05. When compared the paired difference on 4%White radish root extract cream side between forehead and cheek, paired difference of mean melanin index were 2.11±21.03, 2.14±16.35, 2.50±13.86 and 0.39±14.31 after 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks with statistical insignificance at $p=0.600$, $p=0.494$,$p=0.350$ and $p=0.886$, respectively. For standard cream base side between forehead and cheek, paired difference of mean melanin index were 5.36±11.30, 10.75±19.54, 0.04±10.59 and 1.32±11.20 after 4^{th}, 8^{th}, 12^{th} and 16^{th} weeks with statistical significant at $p=0.027$ and $p=0.007$ and insignificance at $p=0.350$ and $p=0.886$, correspondingly. The researcher did the following at significance levels of p-value < 0.05.

4.5 Dermatologist evaluation and patient satisfaction

The Evaluation of the treatment between 4% White radish root extract and standard cream base via photographs from VISIA® Complexion Analysis System at 4^{th}, 8^{th} and 12^{th} weeks after treated. Scored satisfaction scales by 3 independent dermatologists using global satisfaction score ranges from -1 to +4;-1 = worse**,** 0 = not improved**,** +1 = fairly improvement (1-25%)**,** +2 = moderate improvement (26-50%)**,** +3 = good improvement**,** +4 = excellent improvement (76-100%)

Table 4.9 Dermatologist evaluation scores of face that applied standard cream base at 4, 8 and 12 week

	Dermatologist satisfaction score of the face that applied standard cream base								
	0-4 week			0-8 week			0-12 week		
	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3
1	0	1	0	0	1	0	1	0	1
2	0	0	1	0	0	1	0	1	1
3	0	0	0	0	0	0	0	0	1
4	0	0	0	0	0	0	1	0	0
5	1	1	0	1	1	1	1	1	1
6	0	0	0	0	1	0	1	1	1
7	0	0	0	1	0	1	0	0	0
8	0	0	1	0	0	1	0	0	0
9	0	0	1	0	1	1	1	0	1
10	0	0	0	0	1	1	0	1	1
11	0	0	0	0	0	1	0	0	1
12	0	0	0	0	0	1	1	1	0
13	1	1	0	1	1	1	1	1	1
14	0	1	0	0	1	0	1	1	1
15	0	0	0	0	1	0	0	0	0
16	0	0	0	1	0	0	0	0	0
17	0	0	0	1	0	1	1	0	1
18	0	0	0	1	0	1	0	1	1
19	0	0	0	0	0	1	0	0	1
20	1	0	0	1	0	0	1	0	0
21	0	0	0	1	0	1	1	1	1
22	0	0	0	1	0	1	1	1	1
23	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0
25	1	0	0	2	1	1	2	1	1
26	0	0	0	0	0	0	0	0	0
27	0	0	0	1	0	1	1	0	1
28	0	0	0	0	1	1	0	1	1

Table 4.10 Dermatologist evaluation scores of face that applied 4% White radish root extract cream at 4^{th}, 8^{th}, 12^{th} weeks

	Dermatologist satisfaction score of the face that applied 4%White radish root extract								
	0-4 week			0-8 week			0-12 week		
	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3
1	1	1	2	1	2	2	2	2	3
2	1	2	1	2	2	2	2	3	3
3	0	1	0	0	1	1	1	2	2
4	0	1	0	2	1	1	2	3	3
5	1	1	1	2	2	2	3	3	3
6	0	1	0	2	1	1	3	2	2

Table 4.10 (continued)

	Dermatologist satisfaction score of the face that applied 4%White radish root extract								
	0-4 week			0-8 week			0-12 week		
	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3	Dr1	Dr2	Dr3
7	1	2	1	2	3	2	3	3	2
8	0	1	0	2	1	1	2	2	3
9	2	1	1	2	1	1	2	2	1
10	1	1	2	1	2	2	2	2	3
11	2	1	1	2	3	3	2	3	3
12	0	1	1	1	2	2	1	2	2
13	0	1	1	2	3	2	2	3	2
14	2	1	1	2	2	2	3	3	3
15	0	1	1	2	3	2	3	2	2
16	2	1	1	2	2	2	3	3	2
17	2	1	1	2	3	2	2	2	3
18	0	1	0	2	1	1	2	2	1
19	0	1	1	2	3	2	2	2	3
20	1	1	2	2	2	2	3	3	2
21	1	2	1	1	2	1	1	2	2
22	2	1	1	2	3	2	2	3	2
23	0	1	1	2	2	2	3	3	3
24	2	1	1	2	3	2	3	2	2
25	0	1	1	2	2	2	3	3	2
26	0	1	1	2	3	2	2	2	3
27	1	1	1	2	1	1	2	2	1
28	1	0	1	2	1	1	2	2	1

Table 4.11 Statistically analysis of dermatologist evaluation scores compares between groups of 4% White radish root extract cream and standard cream base at 4^{th}, 8^{th}, 12^{th} weeks

	Dermatologist evaluation					
	4^{th} Week		8^{th} Week		12^{th} Week	
	Radish	Base	Radish	Base	Radish	Base
Extremely improved (75%)	-	-	1 (3.57)	-	9 (32.14)	-
Moderately improved (50%)	-	-	18 (64.29)	-	19 (67.86)	-
Mildly improved (25%)	22 (78.57)	2 (7.14)	9 (32.14)	12 (42.86)	-	15 (53.57)

Table 4.11 (continued)

	Dermatologist evaluation					
	4th Week		8th Week		12th Week	
	Radish	**Base**	**Radish**	**Base**	**Radish**	**Base**
No change (0%)	6 (21.42)	26 (92.86)	-	16 (57.14)	-	13 (46.43)
Mean	19.64	1.79	42.86	10.71	58.04	13.39
S.D	10.45	6.56	13.36	12.60	11.89	12.70
Median	25	0	50	0	50	25
(Max-Min)	(0-25)	(0-25)	(25-75)	(0-25)	(50-75)	(0-25)
p-value	< 0.001		< 0.001		< 0.001	

Note. Values were represented as number (percent).

As shown in Table 4.11, after applied 4% White radish root extract cream, at 4thweek dermatologists rated 22 volunteers (78.57%) as mildly improve and six volunteers (21.42%) as not improved. The mean satisfaction of dermatologists was 19.64±10.45 and median was 25. At 8thweek, dermatologists rated one volunteers (3.57%) as extremely improved, 18 volunteers (64.29%) as moderately improved and nine volunteers (32.14%) as mildly improved. The mean satisfaction of dermatologists was 42.86±13.36 and median was 50. At 12th week, dermatologists rated nine volunteers (32.14%) as extremely improved and 19 volunteers (67.86%) as moderately improved. The mean satisfaction of dermatologists was 58.04±11.89 and median was 50. For the face that applied standard cream base, at 4th week dermatologists rated two volunteers (7.14%) as mildly improve and 26 volunteers (92.86%) as not improved. The mean satisfaction of dermatologists was 1.79±6.56 and median was 0. At 8th week, dermatologists rated 12 volunteers (42.86%) as mildly improved and 16 volunteers (57.14%) as not improved. The mean and median satisfaction of dermatologists was 10.71±12.60 and 0, respectively. At 12th week, dermatologists rated 15 volunteers (53.57%) as mildly improved and 13 volunteers (46.43%) as not improved. The mean satisfaction of dermatologists was 13.39±12.70 and median was 25.

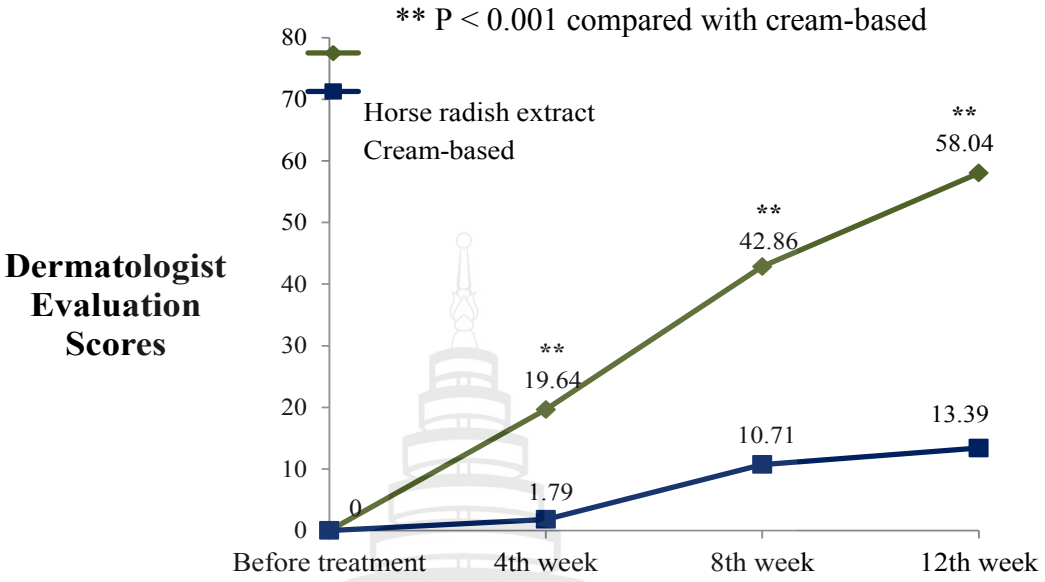


Figure 4.7 Linear graph compared difference of mean changes of dermatologist satisfaction scores between 2 groups

Table 4.11 and Figure 4.7 present the difference of mean changes of dermatologist evaluation scores between 2 groups with statistical significance ($p<0.001$) at 4^{th}, 8^{th} and 12^{th} weeks. The researcher did the following at significance levels of p-value < 0.05.

Table 4.12 Patient-satisfaction-score statistical analysis after treatment for 12^{th} weeks

	Patient satisfaction on 12^{th} week		
	Radish extract	**Cream based**	**p-value**
3 = Very satisfaction	9 (32.14)	-	< 0.001
2 = Moderate satisfaction	19 (67.86)	1 (3.57)	
1 = Mild satisfaction	-	23 (82.14)	
0 = No change	-	4 (14.29)	
-1 = Worse	-	-	
Mean ± S.D.	0.82 ± 0.42	2.32 ± 0.48	
Median (Max-Min)	2 (2-3)	1 (0-2)	

Note. Values were represented as number (percent)

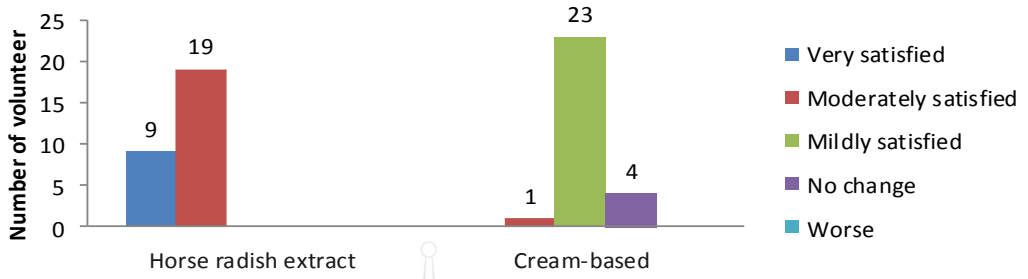


Figure 4.8 Bar chart reveals the frequencies of patient satisfaction scoring of 4% White radish root extract and standard cream base after treatment for 12 weeks

According to Table 4.12 and Figure 4.8 after 12th week, nine volunteers (32.14%) rated their satisfaction as very satisfied, 19 volunteers (67.86%) rated as moderate satisfied for the side that applied 4% White radish root extract cream. For standard cream base, one volunteer (3.57%) rated as moderately satisfied, 23 volunteers (82.14%) rated as mildly satisfied and four volunteers (14.29%) rated as not improved. The mean of satisfaction of face that applied 4% White radish root extract cream was 0.82 ± 0.42, while standard cream base was 2.32 ± 0.48. There was statistical significance with p< 0.001. The researcher did the following at significance levels of p-value < 0.05.

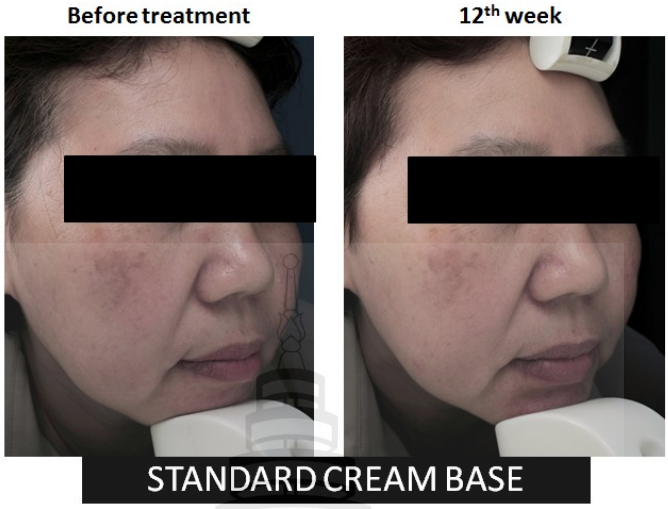


Figure 4.9 Volunteer photos compared before and after treatment with standard cream base

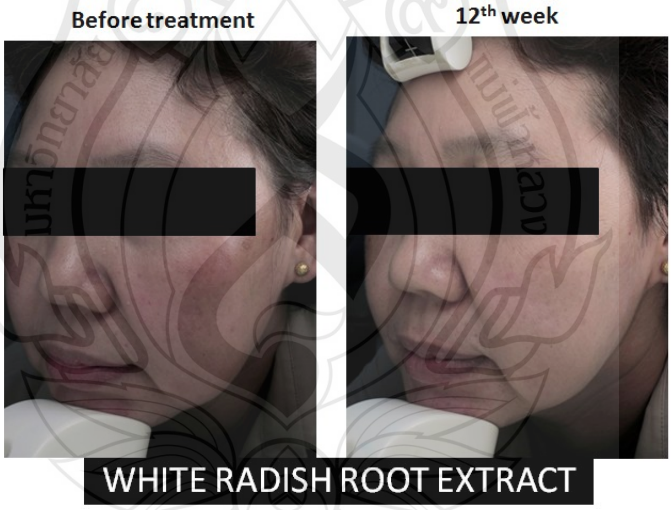


Figure 4.10 Volunteer photos compared before and after treatment with 4% White radish root extract cream

Figure 4.11 Volunteer photos compared before and after treatment with standard cream base

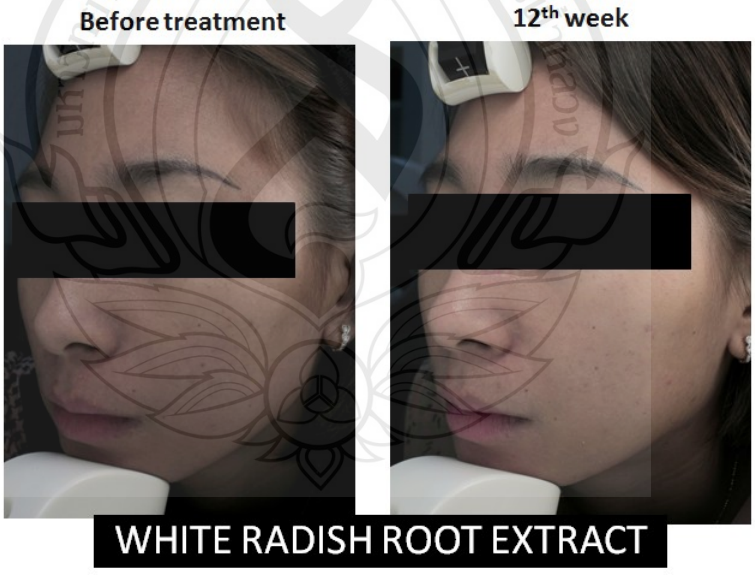


Figure 4.12 Volunteer photos compared before and after treatment with 4% White radish root extract cream

Figure 4.13 Volunteer photos compared before and after treatment with standard cream base

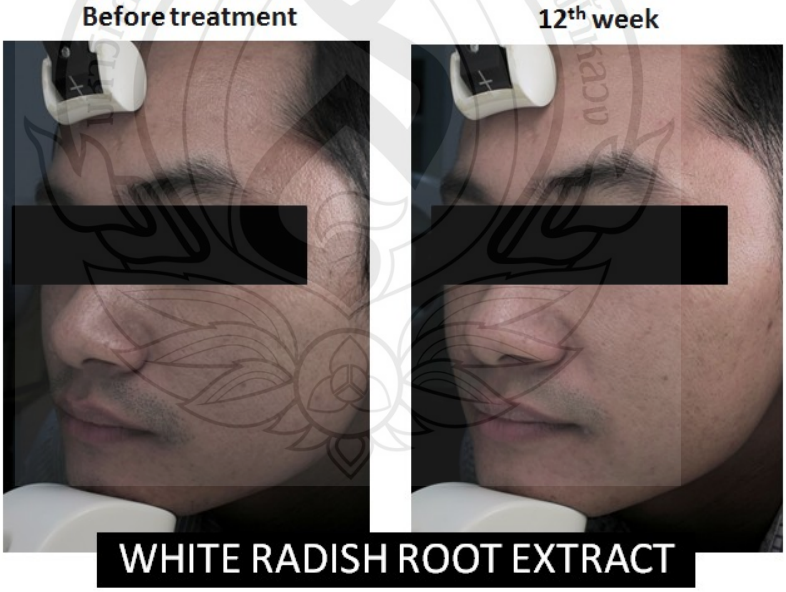


Figure 4.14 Volunteer photos compared before and after treatment with 4% White radish root extract cream

Figure 4.15 Volunteer photos compared before and after treatment with standard cream base

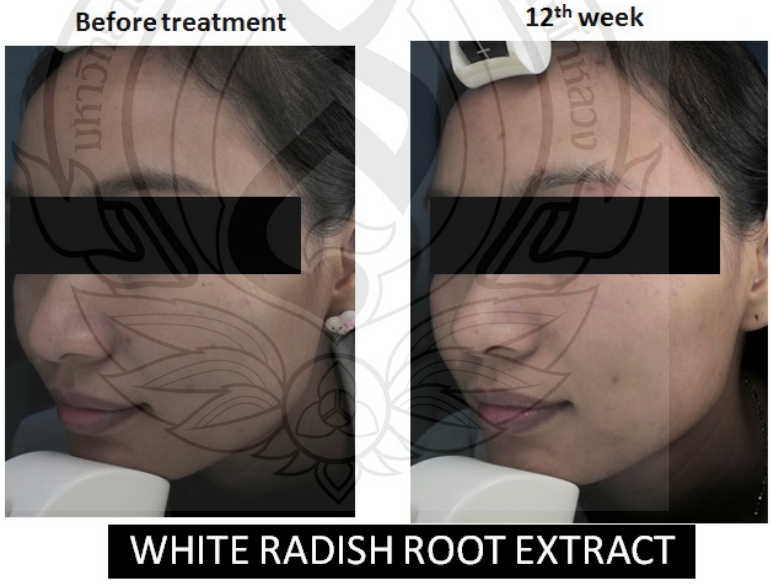


Figure 4.16 Volunteer photos compared before and after treatment with 4% White radish root extract cream

4.6 Complication

Table 4.13 Complication of standard cream base and 4% White radish root extract cream

Complication	Standard cream base		4% White radish root extract	
	n	%		n
Week 4				
NO	27	96.43	27	96.43
Acne	1(same case)	3.57	1(same case)	3.57
Week 8				
NO	28	100.00	28	100.00
Acne	-	-	-	-
Week 12				
NO	28	100.00	28	100.00
Acne	-	-	-	-

As shown in Table 4.14, after applied 4% White radish root extract cream and standard cream base for 4 week, one volunteer developed acne vulgaris on both side of face and clinical were subsided after 8 week without discontinuation of treatment. There was no statistical difference of complication between two groups.

Figure 4.17 Volunteer photo show mild acne vulgaris after applied standard cream base

Figure 4.18 Volunteer photo show mild acne vulgaris after applied 4% White radish root extract cream

CHAPTER 5

DISCUSSION AND CONCLUTION

5.1 Discussion

Pale skin creates a vaguely supernatural aura. It can make an appearance look elegant, pretty and seductive. Many countries consider that pale skin is beautiful [1]. Southeast Asia and also Thai women want to look white like Japanese and Korean [2]. Human skin color is the result of natural selection, comprised of genetic and extrinsic factors such as UV irradiation and hormones. UV irradiation and hormones stimulate melanin synthesis. The key enzyme of melanin synthesis is tyrosinase.

The main mechanism of skin whitening is interference with the pigmentary processes: tyrosinase activities, melanosome transportation, cytotoxic to melanocytes, and antioxidants. Synthetic products have been popular for several years. Prolonged using of these products may provide adverse reactions and drug dependence.

Over the past few years, increasing attention has been paid to herbal plants for development into modern medicine and cosmetic products. Because of suitable for long term application and mild side effects, skin whitening containing natural ingredients from plants has become very popular [6].

From the previous studies, antityrosinase activity of white radish root extract was revealed. Established to play a role in this activity without permanent damage, the main active components are phenolic, flavonoids and L-ascorbic acid [6, 7, 55]. Moreover, flavonoid and L-ascorbic acid give antioxidant properties, another mechanism of skin lightening under the hypothesis of reducing the direct photo-oxidation of pre-existing melanin. White radish is cheap and grows easily in Thailand. The purpose of this research is to study the effectiveness of white radish root extract for facial whitening in human volunteers.

To our knowledge, this is the double-blind, randomized, controlled, split-face clinical trial study to analyze the clinical effectiveness, side effect and satisfaction of 4% White radish root extract cream for facial whitening in Thais.

According to this study, all of volunteers were Thai with Fitzpatrick skin type 4, 3 and 5, respectively. This was consistent with Thai skin tone. There were female more than male volunteers. It is possible that women are concerned about their cosmetic and social implications more than men. Average age was 33.14±11.86 years. Main aggravating factors of melanin production was sunlight and average time exposure to the sunlight between 10am - 4pm was 18.57± 14.25 minutes. Most were officers who worked indoor. All of volunteers had history of using facial cosmetic products, at least. Two volunteers had mild hypertensive disorder and took antihypertensive drugs which were not effect to skin whitening. Nevertheless, some took supplements, so the researcher advised to temporarily discontinue taking supplements until the end of the study. To eliminate affecting external factors, this study was designated as split-face clinical trial, so the divergence of general characteristics of volunteers was insignificant.

When analyzing the reduction of mean melanin index of forehead and cheek after applied 4% White radish root extract cream and standard cream base for 4, 8, 12 weeks, statistical significance on both sides on independent area were exhibited. The decreasing of mean melanin index of radish sides may result from the antityrosinase properties, the key enzyme in melanogenesis and also antioxidant activities. This correlated with the studies of Pirodamornchai in 2005, Kamkaen in 2007 and Jakmatakul in 2009, observed of antityrosinase and antioxidant activities in laboratory test [6, 7, 55]. After stop applying for 4^{th} week, the reduction of mean melanin index was still statistically significant at $p<0.001$. No rebounding appeared. Nevertheless prolonged observation should be done. Without lightening properties, the reduction of mean melanin index on standard cream base side may arise from other factor such as moisturizing effect or broad spectrum sunscreen. Based on study of Chaudhuri in 2002, the using of broad spectrum sunscreen can protect skin from ultraviolet radiation and improve melasma [48].

Owing to statistically significant reduction of mean melanin index on both sides, paired different between 4%White radish root extract cream and standard cream base in the same period would be used. From 4^{th} week of application, in every area was considered to be statistically significant. When compared the paired different in each area

with same products, there were no statistically significant. From this correlation, it can be assumed that 4%White radish root extract cream can reduce the mean melanin index better than standard cream base without area dependence. Whitening effect also increased with prolonged application time.

Regarding the median of clinical evaluation score at 12^{th} week of 4%White radish root extract cream and standard cream base side was moderately and mildly satisfied, correspondingly. The clinical improvement compared different of mean change was statistical significance with $p<0.001$ after treatment for 4^{th} week. This was concordant with volunteers' satisfaction. It is possible that 4%White radish root extract cream can improve clinical pictures and give better result for facial whitening.

Concerning the side effects, no rash or serious skin disorders were observed. Correlated with the previous study of Jakmatakul in 2009, white radish root extract exhibited only mild cytotoxicity. Nevertheless, one volunteer developed mild acne vulgaris on both side of the face. This was statistically insignificant between two sides, so it is likely that 4% White radish root extract is not the cause. Intrinsic factors of the volunteer such as oily skin and hormonal disturbance or extrinsic factors such as chemical components of the cream base may be originator. Thus, the 4% White radish root extract appeared to be well tolerated and safe, however the extract should be further evaluated for a more prolonged usage.

5.2 Conclusion

The results of the study clearly demonstrated that white radish root extract was able to reduce melanin production in human volunteers. With significant lightening effect, requiring only 4 weeks of application, moderately satisfied by the clinical evaluation and volunteers satisfaction and also less side effects, white radish root extract have a very promising potential for use as a safe, effective and economical whitening agent. Nevertheless, the highest concentration with lowest side effects, the duration that white radish root extract cream will reach its maximum lightening effect and more prolonged usage complication should be find out.

REFERENCES

REFERENCES

[1] Smit, N., Vicanova, J. & Pavel,S. (2009). The hunt for natural skin whitening agents. *Int J Mol Sci., 10*(12), 5326-5349.

[2] Hodal, K. (2012). *Thailand's skin-whitening craze reaches woman's intimate areas.* Bangkok: The Guardian.

[3] Areeporn Ruksawong. (2011). *Behavior of purchasing whitening cream for maintaining face skin of women in Bangkok area.* Independent Study of Business Administration. Rajamangala University of Technology Thanyaburi, Pathum Thani.

[4] Mosher, D. B., Fitzpatrick, T. B., Ortonne, J. P. & Hori, Y. (1999). *Disorder of pigmentation*. In I. M. Freedberg & T. B. Fitzpatrick (Eds.), *Fitzpatrick's dermatology in general medicine* (5th ed.). New York: McGraw-Hill.

[5] Gillbro, J. M. & Olsson, M. J. (2011). The melanogenesis and mechanisms of skin-lightening agents--existing and new approaches. *Int J Cosmet Sci., 33*(3), 210-221.

[6] Rattanamanee Jakmatakul, Rutt Suttisri & Parkpoom Tengamnuay. (2009). Evaluation of antityrosinase and antioxidant activities of Raphanus sativus root: Comparison between freeze-dried juice and methanolic extract. *Thai J. Pharm. Sci., 33*, 22-30.

[7] Tidarat Pirodamornchai, Wisa Karnboon, Narisa kamkaen & Sinsupha Chuichulcherm. (2006). Preliminary screening of nine vegetables for anti-tyrosinase activity. *Thai Pharmaceutical and Health Science Journal, 11*(1), 99.

[8] Bolognia, J. L. & Pawelek, J. M. (1988). Biology of hypopigmentation. *J Am Acad Dermatol, 19*(2 Pt 1), 217-255.

[9] Szabo, G., Gerald, A. B., Pathak, M. A. & Fitzpatrick, T. B. (1969). Racial differences in the fate of melanosomes in human epidermis. *Nature, 222*(5198), 1081-1082.

[10] Yaar, M. (2012). Biology of melanocytes. In L. A. Goldsmith & T. B. Fitzpatrick (Eds.), *Fitzpatrick's dermatology in general medicine* (8th ed., pp. 765-780). New York: Mc Graw Hill.

[11] The Lone Ranger. (2007). *Intro to anatomy 7: The integumentary system.* Retrieved June 10, 2013, from http://www.freethought-forum.com/forum/showthread.php? t=11578 &garpg=2

[12] Hearing, V. J. (1993). Unraveling the melanocyte. *Am J Hum Genet, 52*(1), 1-7.

[13] Schallreuter, K. U. & Wood, J. M. (1999). The importance of L-phenylalanine transport and its autocrine turnover to L-tyrosine for melanogenesis in human epidermal melanocytes. *Biochem Biophys Res Commun, 262*(2), 423-428.

[14] Schallreuter, K. U., Lemke, K. R., Hill, H. Z. & Wood, J. M. (1994). Thioredoxin reductase induction coincides with melanin biosynthesis in brown and black guinea pigs and in murine melanoma cells. *J. Invest. Dermatol, 103*(6), 820-824

[15] Vance, K. W. & Goding, C. R. (2004). The transcription network regulating melanocyte development and melanoma. *Pigment Cell Res, 17*(4), 318-325.

[16] Goding, C. R. (2000). Mitf from neural crest to melanoma: Signal transduction and transcription in the melanocyte lineage. *Genes Dev, 14*(14), 1712-1728.

[17] Chiaverini, C., Beuret, L., Flori, E., Busca, R., Abbe, P., Bille, K., Bahadoran, P., Ortonne, J. P., Bertolotto, C. & Ballotti, R. (2008). Microphthalmia-associated transcription factor regulates RAB27A gene expression and controls melanosome transport. *J Biol Chem., 283*(18), 12635-12642.

[18] Schallreuter, K. U., Moore, J., Tobin, D. J., Gibbons, N. J., Marshall, H. S., Jenner, T., Beazley, W. D. & Wood, J. M. (1999). alpha-MSH can control the essential cofactor 6-tetrahydrobiopterin in melanogenesis. *Ann N Y Acad Sci, 885*, 329-341.

[19] Gillbro, J. M. & Olsson, M. J. (2011). The melanogenesis and mechanisms of skin-lightening agents existing and new approaches. I*nternational Journal of Cosmetic Science, 33*(3), 210–221.

[20] Gillbro, J. M., Marles, L. K., Hibberts, N. A. & Schallreuter, K. U. (2004). Autocrine catecholamine biosynthesis and the beta-adrenoceptor signal promote pigmentation in human epidermal melanocytes. *J Invest Dermatol, 123*(2), 346-353.

[21] Cardinali, G., Bolasco, G., Aspite, N., Lucania, G., Lotti, L. V., Torrisi, M. R. & Picardo, M. (2008). Melanosome transfer promoted by keratinocyte growth factor in light and dark skin-derived keratinocytes. *J Invest Dermatol, 128*(3), 558-567.

[22] Kameyama, K., Sakai, C., Kondoh, S., Yonemoto, K., Nishiyama, S., Tagawa, M., Murata, T., Ohnuma, T., Quigley, J., Dorsky, A., Bucks, D. & Blanock, K. (1996). Inhibitory effect of magnesium L-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. *J Am Acad Dermatol, 34*(1), 29-33.

[23] Barsh, G. & Attardi, L. D. (2007). A healthy tan?. *N Engl J Med, 356*(21), 2208-22010.

[24] Arndt, K. A. & Fitzpatrick, T. B. (1965). Topical use of hydroquinone as a depigmenting agent. *JAMA, 194*(9), 965-967.

[25] Lawrence, N., Bligard, C. A., Reed, R. & Perret, W. J. (1988). Exogenous ochronosis in the United States. *J Am Acad Dermatol, 18*(5 Pt 2), 1207-1211.

[26] Maeda, K. & Fukuda, M. (1996). Arbutin: Mechanism of its depigmenting action in human melanocyte culture. *J Pharmacol Exp Ther, 276*(2), 765-769.

[27] Hu, Z. M., Zhou, Q., Lei, T. C., Ding, S. F. & Xu, S. Z. (2009). Effects of hydroquinone and its glucoside derivatives on melanogenesis and antioxidation: Biosafety as skin whitening agents. *J Dermatol Sci, 55*(3), 179-184.

[28] Mukherjee, P., Fulton, D. B., Halder, M., Han, X., Armstrong, D. W., Petrich, J. W. & Lobban, C. S. (2006). Maristentorin, a novel pigment from the positively phototactic marine ciliate Maristentor dinoferus, is structurally related to hypericin and stentorin. *J Phys Chem B, 110*(12), 6359-6364.

[29] Picardo, M. & Carrera, M. (2007). New and experimental treatments of cloasma and other hypermelanoses. *Dermatol Clin, 25*(3), 353-62, ix.

[30] Badreshia-Bansal, S. & Draelos, Z. D. (2007). Insight into skin lightening cosmeceuticals for women of color. *J Drugs Dermatol, 6*(1), 32-39.

[31] Solano, F., Briganti, S., Picardo, M. & Ghanem, G. (2006). Hypopigmenting agents: An updated review on biological, chemical and clinical aspects. *Pigment Cell Res, 19*(6), 550-571.

[32] Jones, K., Hughes, J., Hong, M., Jia, Q. & Orndorff, S. (2002). Modulation of melanogenesis by aloesin: A competitive inhibitor of tyrosinase. *Pigment Cell Res, 15*(5), 335-340.

[33] Lin, C.B., Babiarz, L., Liebel, F., Roydon Price, E., Kizoulis, M., Gendimenico, G. J., Fisher, D. E. & Seiberg, M. (2002). Modulation of microphthalmia-associated transcription factor gene expression alters skin pigmentation. *J Invest Dermatol, 119*(6), 1330-1340.

[34] Fu, B., Li, H., Wang, X., Lee, F. S. & Cui, S. (2005). Isolation and identification of flavonoids in licorice and a study of their inhibitory effects on tyrosinase. *J Agric Food Chem, 53*(19), 7408-7414.

[35] Bissett, D. L., Oblong, J. E. & Berge, C. A. (2005). Niacinamide: A B vitamin that improves aging facial skin appearance. *Dermatol Surg, 31*(7 Pt 2), 860-865, discussion 865.

[36] Hakozaki, T., Minwalla, L., Zhuang, J., Chhoa, M., Matsubara, A., Miyamoto, K., Greatens, A., Hillebrand, G. G., Bissett, D. L. & Boissy, R. E. (2002). The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfe*r. Br J Dermatol, 147*(1), 20-31.

[37] Wallo, W., Nebus, J. & Leyden, J. J. (2007). Efficacy of a soy moisturizer in photoaging: A double-blind, vehicle-controlled, 12-week study. *J Drugs Dermatol, 6*(9), 917-922.

[38] Minwalla, L., Zhao, Y., Cornelius, J., Babcock, G. F., Wickett, R. R., Le Poole, I. C. & Boissy, R. E. (2001). Inhibition of melanosome transfer from melanocytes to keratinocytes by lectins and neoglycoproteins in an in vitro model system. *Pigment Cell Res, 14*(3), 185-194.

[39] Parvez, S., Kang, M., Chung, H. S. & Bae, H. (2007). Naturally occurring tyrosinase inhibitors: Mechanism and applications in skin health, cosmetics and agriculture industries. *Phytother Res, 21*(9), 805-816.

[40] Parvez, S., Kang, M., Chung, H. S., Cho, C., Hong, M. C., Shin, M. K. & Bae, H. (2006). Survey and mechanism of skin depigmenting and lightening agents. *Phytother Res, 20*(11), 921-934.

[41] Holmgren, A., (1981). Regulation of ribonucleotide reductase. *Curr Top Cell Regul, 19*, 47-76.

[42] Ebanks, J. P., Wickett, R. R. & Boissy, R. E. (2009). Mechanisms regulating skin pigmentation: The rise and fall of complexion coloration. *Int J Mol Sci, 10*(9), 4066-4087.

[43] Briganti, S., Camera, E. & Picardo, M. (2003). Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res, 16*(2), 101-110.

[44] Ando, H., Itoh, A., Mishima, Y. & Ichihashi, M. (1995). Correlation between the number of melanosomes, tyrosinase mRNA levels, and tyrosinase activity in cultured murine melanoma cells in response to various melanogenesis regulatory agents. *J Cell Physiol, 163*(3), 608-614.

[45] Usuki, A., Ohashi, A., Sato, H., Ochiai, Y., Ichihashi, M. & Funasaka, Y. (2003). The inhibitory effect of glycolic acid and lactic acid on melanin synthesis in melanoma cells. *Exp Dermatol, 12*(Suppl 2), 43-50.

[46] Noh, J.M., Kwak, S. Y., Seo, H. S., Seo, J. H., Kim, B. G. & Lee, Y. S. (2009). Kojic acid-amino acid conjugates as tyrosinase inhibitors. *Bioorg Med Chem Lett, 19*(19), 5586-5589.

[47] Kim, D. H., Hwang, J. S., Baek, H. S., Kim, K. J., Lee, B. G., Chang, I., Kang, H. H. & Lee, O. S. (2003). Development of 5-[(3-aminopropyl) phosphinooxy]-2-(hydroxymethyl)-4H-pyran-4-one as a novel whitening agent. *Chem Pharm Bull (Tokyo), 51*(2), 113-116.

[48] Leenutaphong, V., Nettakul, A. & Rattanasuwon, P. (1999). Topical isotretinoin for melasma in Thai patients: A vehicle-controlled clinical trial. *J Med Assoc Thai, 82*(9), 868-875.

[49] Ha, J. H., Jo, N. S., Lee, H. K., Kim, J. I., Lee, B. G. & Park, W. J. (1996). *The depigmentation effect of a new material extracted from paper mulberry and its comparison by three colorimetric instrument*. The IFSCC Congress Sydney, Australia.

[50] Chaudhuri, R. K., (2002). Emblica cascading antioxidant: A novel natural skin care ingredient. *Skin Pharmacol Appl Skin Physiol, 15*(5), 374-380.

[51] Rosa Martha Pérez Gutiérrez & Rosalinda Lule Perez. (2004). Raphanus sativus (Radish): Their chemistry and biology. *The Scientific World Journal, 4*, 811-837. doi:10.1100/tsw.2004.131.

[52] Harborne, J. & Baxter, Herbert. (1993). *Phytochemical dictionary: A handbook of bioactive compounds from plants.* London: Taylor & Francis.

[53] Nguyen, Q. L. & Chevalier, V. (2008). Process for preparation of a composition having antibacterial properties. *Fr Demande* 21.

[54] Perry, L. M. (1980). *Medicinal plants of east and southeast Asia*. Cambridge: MIT Press.

[55] Takaya, Y., Kondo, Y., Furukawa, T. & Niwa, M. (2003). Antioxidant constituents of radish sprout (kaiware-daikon), Raphanus sativus L. *J. Agric. Food Chem, 51*(27), 8061-8066.

[56] Jansen, F., Dickhof, S. & Emond, E. (2010). Antiwrinkle cosmetic preparations containing baicalin. *Ger Offen De*.

[57] Narisa Kamkaen, Narong Mulsri & Charoen Treesak. (2007). Screening of some tropical vegetables for anti-tyrosinase activity. *Thai Pharm Health Sci J, 2*(1), 15-19.

[58] Mitchell, J. C. & Jordan, W. P. (1974). Allergic contact dermatitis from the radish, Raphanus sativus. *Br J Dermatol, 91*(2), 183-189.

[59] Kim, O. K., Murakami, A., Nakamura, Y. & Ohigashi, H. (1998). Screening of edible Japanese plants for nitric oxide generation inhibitory activities in RAW 264.7 cells. *Cancer Lett, 125*(1-2), 199-207.

[60] Rojanapo, W. & Tepsuwan, A. (1993). Antimutagenic and mutagenic potentials of Chinese radish. *Environ Health Perspect, 101*(Suppl 3), 247-252.

[61] Kassie, F., Parzefall, W., Musk, S., Johnson, I., Lamprecht, G., Sontag, G. & Knasmüller, S. (1996). Genotoxic effects of crude juices from Brassica vegetables and juices and extracts from phytopharmaceutical preparations and spices of cruciferous plants origin in bacterial and mammalian cells. *Chem Biol Interact, 102*(1), 1-16.

[62] Nath, D., Sethi, N., Singh, R. K. & Jain, A. K. (1992). Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. *J Ethnopharmacol, 36*(2), 147-154.

[63] Alvin, G., Catambay, N., Vergara, A. & Jamora, M. J. (2011). A comparative study of the safety and efficacy of 75% mulberry (Morus alba) extract oil versus placebo as a topical treatment for melasma: A randomized, single-blind, placebo-controlled trial. *J Drugs Dermatol, 10*(9), 1025-1031.

[64] Dattalo, P. (2008). Determining sample size: Balancing power, precision, and practicality. New York: Oxford University Press.

APPENDICES

APPENDIX A

RESEARCH PROFILE

THE EFFICACY OF 4% WHITE RADISH ROOT EXTRACT CREAM FOR FACIAL WHITENING IN THAIS (Confidential)

ID.....

General information (Only official)

1. Date.....
2. Name.....
3. Hospital number.....
4. Address.....
5. Tel.....
6. Email.....
7. Sex O1 male O2 female: pregnancy or lactation? …..1.Yes.....2.No
8. Age.....year
9. Occupation

.....1. Government

.....2. Employer

.....3. Housewife

.....4. Student

.....5. Employee

.....6. Others

10. Underlyingdisease.....
11. Photosensitivity or drug induced photosensitivity.....
12. Personal medication and supplement.....

a. Chemo-radiotherapy

b. Active inflammatory skin disease, open wound in the treatment area

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

13. History of food or drug allergy.....

14. Current facial product use.....

15. History of following treatment within 4 weeks before the study

.....1. Yes (Identify.....).....2. No

O Ablative and non-ablative laser

O Intense pulse light

O Microdermabrasion

O Skin needling

O Chemical peeling

O Facial whitening treatment

O Facial whitening agent

16. Average time expose to the sunlight during 10 am – 4 pm..... min

17. Fitzpatrick Skin Phototypes ….I ….II ….III ….IV….V….VI

18. Aggravating factors

..... 1. Stress

..... 2. Hormone....Oral contraceptive pill, Hormone replacement therapy

....Thyroid hormone

....Other (Identify.....)

..... 3. Sun exposure

..... 4. Drug induced hypersensitivity

..... 5. Other.....

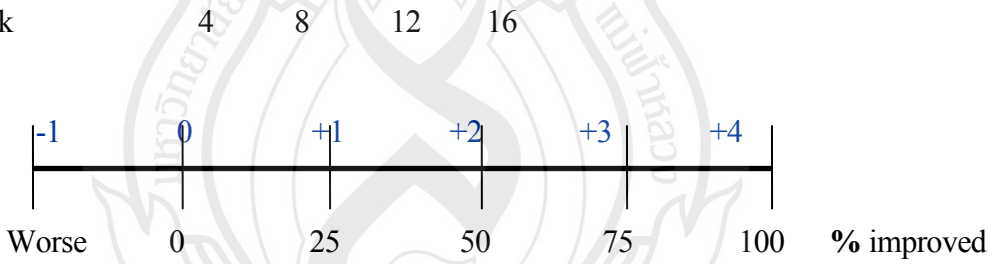
MEAN MELANIN INDEX

1. Melanin index (MI) by mexameter

Melanin index					
	Before Rx 0 wk	4 wk	8 wk	12 wk	After Rx 16wk
Rt Forehead					
Lt Forehead					
Rt Cheek					
Lt Cheek					

GLOBAL SATISFACTION

Global satisfaction by dermatologist: please draw the circle



*Score range from-1 to+4;

-1 = Worse

0. = Not improved

1. = Improved 1-25%

2. = Improved 26-50%

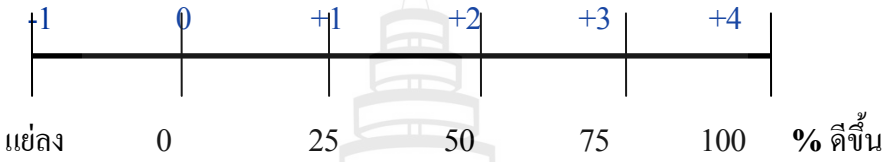
3. = Improved 51-75%

4. = Improved 76-100%

แบบประเมินความพึงพอใจ

คะแนนความพึงพอใจในการรักษาโดยผู้ป่วย: กรุณาวงกลมตัวเลขตามความเป็นจริง

ที่ 12 สัปดาห์



* คะแนนความพึงพอใจในการรักษา มีค่าตั้งแต่ -1 ถึง +4 โดยที่

-1	คือ	แย่ลง
0	คือ	ไม่ได้ผล
+1	คือ	ดีขึ้นน้อยมาก (1-25%)
+2	คือ	ดีขึ้นน้อย (26-50%)
+3	คือ	ดีขึ้นปานกลาง (51-75%)
+4	คือ	ดีขึ้นมาก (76-100%)

SIDE EFFECT RECORD
(แบบประเมินผลข้างเคียง)

การประเมินผลข้างเคียงโดยผู้ป่วย ประเมินในสัปดาห์ที่ 4, 8 และ 12

….. 1. มี ..... 2. ไม่มี

ผลข้างเคียง	ขวา				ซ้าย			
	ไม่มี	น้อย	ปานกลาง	มาก	ไม่มี	น้อย	ปานกลาง	มาก
แดง								
ลอก								
บวม								
คัน								
แห้ง								

อื่นๆ .....

โดยแพทย์ (Physician evaluation)

….. 1. Yes ..... 2. No

Adverse effects	Right				Left			
	No	Mild	Moderate	Severe	No	Mild		Severe
Erythema								
Scaling								
Edema								
Itchy								
Dry skin								

Other.....

APPENDIX B

ข้อมูลสำหรับผู้ป่วย

การวิจัยเรื่อง: การศึกษาประสิทธิผลของการทาครีมสารสกัดจากหัวผักกาดขาว 4% เพื่อการปรับผิวหน้าขาวในผู้ที่มารับบริการ ณ โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพฯ (ข้อมูลทั้งหมดจะถูกปิดเป็นความลับ)

เรียน อาสาสมัครทุกท่าน

ท่านเป็นผู้ได้รับเชิญจากแพทย์ให้เข้าร่วมการศึกษาทางคลินิกเพื่อศึกษาถึงประสิทธิผลของการทาครีมสารสกัดจากหัวผักกาดขาว 4% เพื่อการปรับผิวหน้าขาวในผู้ที่มารับบริการ ณ โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพฯก่อนที่ท่านตกลงเข้าร่วมการศึกษาดังกล่าว ขอเรียนให้ท่านทราบถึงเหตุผลและรายละเอียดของการศึกษาวิจัยครั้งนี้

ชื่อโครงการ

การศึกษาประสิทธิผลของการทาครีมสารสกัดจากหัวผักกาดขาว 4% เพื่อการปรับผิวหน้าขาวในผู้ที่มารับบริการ ณ โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพฯ

ผู้รับผิดชอบโครงการวิจัย

1. แพทย์หญิงอาภรณ์ คูสุวรรณ
2. อาจารย์นายแพทย์ไพศาล รัมณีย์ธร

สรุปย่อโครงการวิจัย

อาสาสมัครชายและหญิงไทยอายุ 20-60 ปี ที่มีสีผิวเกณฑ์การแบ่งมาตรฐานของ Fitzpatrick ลำดับที่ 3-6 จากการวินิจฉัยของแพทย์ผู้เชี่ยวชาญด้านผิวหนัง ที่ต้องการมีผิวหน้าขาว สามารถเข้ารับการติดตามผลที่โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร และผ่านเกณฑ์คัดเลือกตามที่กำหนด จำนวน 30 คน เข้าร่วมโครงการวิจัยโดยสมัครใจนาน 16 สัปดาห์ ระหว่างเดือนพฤศจิกายน พ.ศ. 2556 ถึงกุมภาพันธ์ พ.ศ. 2557 โดยอาสาสมัครแต่ละคนจะได้รับครีม 2 กระปุก โดยที่แต่ละกระปุกจะเขียนว่า “ทาหน้าซีกขวา” และ “ทาหน้าซีกซ้าย” ให้ทาที่หน้าซีกซ้ายและขวาเวลาเช้าและก่อนนอน นอกจากนี้จะได้รับสบู่อ่อนล้างหน้า และครีมกันแดดออกฤทธิ์ป้องกันกว้าง (Sunscreen SPF 60, PA+++) การกำหนดว่าครีมตัวใดทาที่หน้าฝั่งใดใช้วิธีการสุ่มแบบบล็อก (Block

Randomization) วิธีการทายา คือ หลังจากล้างหน้าด้วยสบู่ล้างหน้าที่จัดให้แล้ว ตวงยาตามช้อนตวงที่ได้ และปาดครีมให้เสมอช้อนโดยให้นิ้วด้านขวาทาครีมกระปุกที่เขียนว่าขวาบนหน้าซีกขวา และใช้นิ้วด้านซ้ายทาครีมกระปุกที่เขียนว่าด้านซ้ายบนหน้าซีกซ้าย โดยใช้เส้นสมมุติแบ่งกึ่งกลางใบหน้าเป็นด้านซ้ายและขวา ปริมาณที่ใช้ต่อข้างคือ 1.5 กรัม ทุกวันนาน 12 สัปดาห์ โดยตลอดระยะเวลาทั้ง 12 สัปดาห์แพทย์ผู้ประเมินผลกับผู้ป่วยต่างถูกปกปิดไม่รู้ว่าหน้าฝั่งใดทาครีมอะไร (Double blind) มีการติดตามผู้ป่วยเพื่อประเมินผลตั้งแต่ก่อนทาครีมและหลังจากทาครีมในสัปดาห์ที่ 4 , 8 , 12 และหลังหยุดทาครีมที่สัปดาห์ที่ 16 ตามลำดับ ประเมินความเข้มของเม็ดสีโดย เครื่องเมกซะเมเตอร์ 18 ได้เป็นค่า Mean melanin index ประเมินผลข้างเคียงจากการทาครีมโดยใช้แบบสอบถามจากผู้เข้าร่วมวิจัยและการตรวจร่างกายโดยแพทย์ผู้วิจัย ประเมินความพึงพอใจในการรักษาจากภาพถ่ายจากกล้อง VISIA® Complexion Analysis System ก่อนและหลังการรักษาโดยแพทย์ผู้เชี่ยวชาญด้านผิวหนังที่ไม่เกี่ยวข้องกับโครงการวิจัย 3 ท่าน ออกมาเป็นคะแนนความพึงพอใจในการรักษา และประเมินความพึงพอใจโดยรวมของผู้เข้าร่วมวิจัยโดยใช้แบบสอบถามที่ 12 สัปดาห์

วัตถุประสงค์การวิจัย

เพื่อศึกษาประสิทธิผลของการการทาครีมสารสกัดจากหัวผักกาดขาว 4% (White radish extract cream 4%) ในการปรับสภาพผิวขาวบนใบหน้า

สถานที่ทำการวิจัย

โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพฯ

ประโยชน์ของการวิจัย

1. เป็นข้อมูลในการใช้สารสกัดจากหัวผักกาดขาวเป็นทางเลือกหนึ่งในการปรับผิวขาว
2. เพื่อใช้เป็นข้อมูลพื้นฐานในการทำวิจัยต่อไปในอนาคต

เกณฑ์ในการคัดเลือกเข้าการศึกษา

1. ผู้ป่วยชายและหญิงชาวไทย อายุ 20-60 ปี ที่มีสีผิวตามเกณฑ์การแบ่งมาตรฐานของ Fitzpatrick ลำดับที่ 3-6 จากการวินิจฉัยของแพทย์ผู้เชี่ยวชาญด้านผิวหนังและสามารถเข้ารับการตรวจติดตามที่โรงพยาบาลมหาวิทยาลัย แม่ฟ้าหลวง กรุงเทพมหานคร
2. สุขภาพแข็งแรงดี
3. ยินยอมเข้าร่วมการศึกษาด้วยความสมัครใจ โดยยินยอมลงชื่อเป็นลายลักษณ์อักษร

เกณฑ์ในการคัดออกจากการศึกษา

1. ผู้ป่วยต้องเคยได้รับการรักษาผิวหนังโดยเครื่องมือต่างๆในช่วง 4 สัปดาห์
   การใช้แสงความเข้มสูง (Intense pulse light)
   การใช้เลเซอร์ทุกชนิด

การลอกหน้า (Chemical peeling)

การกรอผิว (Microdermabrasion)

การกระตุ้นผิวด้วยเข็ม (Skin needling)

การทำทรีทเมนท์ผิวหน้า

การทาผลิตภัณฑ์ที่ช่วยหน้าขาว

2. ได้รับการรักษาด้วยฮอร์โมน หรือยาที่มีผลต่อการเพิ่มขึ้นของเม็ดสี
3. ตั้งครรภ์และให้นมบุตร
4. มีประวัติแพ้ยาที่มีส่วนผสมในครีมเบสมาตรฐาน, หัวผักกาดขาว, สารกันแดด
5. มีโรคประจำตัวหรือโรคเรื้อรังที่ควบคุมไม่ได้ เช่นโรคหัวใจ, ลมชัก, โรคหัวใจ, มะเร็งทุกชนิด
6. ได้รับการฉายแสงหรือเคมีบำบัด
7. ไม่สามารถหลีกเลี่ยงแสงแดดมากๆได้
8. มีผิวบริเวณใบหน้าที่ผิดปกติเช่น ผิวหนังอักเสบ, มะเร็งผิวหนัง เป็นต้น
9. ไม่สามารถมาติดตามผลการรักษาได้
10. ได้รับการรักษาด้วยวิธีอื่นๆนอกเหนือจากที่กำหนดให้ ในขณะที่เข้าร่วมโครงการวิจัย

เกณฑ์การให้อาสาสมัครเลิกจากการวิจัย

1. ผู้ป่วยได้รับการปรับสภาพผิวขาวด้วยวิธีอื่น
2. มีอาการแพ้ยา เป็นผื่น หรือเกิดผลข้างเคียงที่ทนไม่ได้หรือเป็นอันตรายร้ายแรง
3. ตั้งครรภ์
4. ติดตามผลการรักษาไม่ได้ ไม่ให้ความร่วมมือในการรักษา
5. ผู้ป่วยต้องการออกจากการวิจัย

วิธีการศึกษา

การปรับผิวขาวเป็นการทำให้ผิวขาวขึ้น โดยการใช้สารจากธรรมชาติที่สามารถทำให้ความหนาแน่นของเม็ดสีเมลานินลดลง การรักษาในโครงการนี้อยู่ในรูปแบบการทาครีมสารสกัดจากหัวผักกาดขาว 4% เทียบกับครีมเบสมาตรฐาน

ในงานวิจัยนี้จะแบ่งผู้เข้าร่วมวิจัยทาครีมสารสกัดจากหัวผักกาดขาว 4% ครึ่งหน้า และทาครีมเบสมาตรฐานครึ่งหน้าอีกฝั่ง โดยผู้เข้าร่วมวิจัยไม่ทราบว่าทาตัวยาชนิดใดที่หน้าซีกไหน โดยก่อนรักษาจะมีการตรวจสภาพผิวโดยแพทย์ผิวหนัง ทำการทดสอบภูมิแพ้ต่อสารสกัดจากหัวผักกาดขาวด้วยการปิดบนผิวหนัง (Patch test) หากผลทดสอบปกติจะได้เข้าโครงการการ ทำการวัดเม็ดสีโดยเครื่องมือเมกซามิเตอร์ 18 วิเคราะห์สภาพผิวโดยเครื่อง VISIA ท่านจะได้รับยาทา 2 กระปุก โดยที่แต่

ละกระปุกจะเขียนว่า “ขวา” และ “ซ้าย” โดยให้ทาที่หน้าซีกซ้ายและขวาเช้าและก่อนนอน นอกจากนี้ท่านจะได้รับสบู่ล้างหน้าชนิดอ่อน และสารกันแดดชนิดออกฤทธิ์ป้องกันกว้าง

วิธีใช้สบู่ล้างหน้า

ใช้สบู่นวดทั่วใบหน้าที่เปียกพอหมาด จากนั้นล้างออกด้วยน้ำสะอาด

วิธีทาครีม

หลังจากล้างหน้าด้วยสบู่ล้างหน้าที่จัดให้แล้ว ตวงยาตามช้อนตวงที่ได้ และปาดครีมให้เสมอช้อนโดย

1. ใช้นิ้วด้านขวาทาครีมกระปุกที่เขียนว่า “ขวา”
2. ใช้นิ้วด้านซ้ายทาครีมกระปุกที่เขียนว่า “ซ้าย”

วิธีการใช้ครีมกันแดด

เฉพาะเวลาเช้าหรือก่อนออกแดด 30 นาที หลังจากทาครีม บีบครีมกันแดดลงบนนิ้วชี้ซ้ายให้ยาว 1 ข้อนิ้วมือทาลงบนใบหน้าครึ่งซีกซ้าย จากนั้นบีบครีมกันแดดลงบนนิ้วชี้ขวาให้ยาว 1 ข้อนิ้วมือทาลงบนใบหน้าครึ่งซีกขวา ให้ใช้ครีมต่อเนื่องเป็นระยะเวลา 12 สัปดาห์ ควรหลีกเลี่ยงแสงแดดช่วงที่ทำการวิจัยด้วย

หากเกิดการระคายเคืองให้หยุดยาทันที ติดต่อพญ.อาภรณ์ คูสุวรรณ ที่เบอร์โทร 0819360738 หรือมาพบที่รพ.มหาวิทยาลัยแม่ฟ้าหลวงโดยทันที หากท่านมีข้อสงสัยเกี่ยวกับวิธีการศึกษาวิจัย แพทย์จะแจ้งให้ท่านทราบและยินดีตอบคำถามต่าง ๆ ที่ท่านสงสัยโดยละเอียด

ผลข้างเคียงที่อาจเกิดขึ้น

1. จากการค้นคว้าหาข้อมูลเพิ่มเติมพบว่าส่วนผสมในครีมเบสมาตรฐาน สบู่ล้างหน้า และครีมกันแดด ผ่านการรับรองโดยองค์การอาหารและยา ผลข้างเคียงที่อาจเกิดขึ้น คือ มีการระคายเคืองต่อผิวหนัง หรือเกิดผื่นแพ้สัมผัสซึ่งพบได้น้อย จัดเป็นผลิตภัณฑ์ที่มีความปลอดภัยสูง
2. สำหรับครีมสารสกัดจากหัวผักกาดขาว มีการใช้อย่างแพร่หลาย ผ่านการรับรองโดยองค์การอาหารและยา จัดเป็นผลิตภัณฑ์ที่มีความปลอดภัยสูง อย่างไรก็ตามผลข้างเคียงที่อาจเกิดขึ้น คือ มีการระคายเคืองต่อผิวหนัง หรือเกิดผื่นแพ้สัมผัสได้ ดังนั้นผู้เข้าร่วมงานวิจัยทุกคนจะได้ทำการทดสอบภูมิแพ้แบบปิดบนผิวหนังก่อน เพื่อยืนยันว่าไม่แพ้ส่วนประกอบในครีมสารสกัดจากหัวผักกาดขาว จึงจะถูกคัดเลือกเข้าโครงการได้
3. หากเกิดผลข้างเคียงขึ้นกับผู้เข้าร่วมวิจัย แพทย์ผู้ทำวิจัยจะให้การรักษาผลข้างเคียงนั้นตามมาตรฐานวิชาชีพแพทย์ โดยไม่คิดค่าใช้จ่าย และผู้เข้าร่วมวิจัยสามารถออกจากโครงการวิจัยได้ตลอดเวลา

3.1 กรณีเกิดสิวขึ้นบนใบหน้า ให้หยุดใช้ยา ติดต่อแพทย์ผู้ทำการวิจัยตามรายละเอียดที่ให้ไว้ แพทย์แนะนำวิธีปฏิบัติตัวเบื้องต้น และนัดหมายเวลาเพื่อมาตรวจรักษา ให้การรักษาจนกว่าสิวและผลจากการเป็นสิวจะหายเป็นปกติโดยไม่คิดค่าใช้จ่าย และให้ค่าเดินทางแก่ผู้เข้าร่วมวิจัยครั้งละ 200 บาท

3.2 กรณีเกิดผื่นแพ้ ลอกแดง แห้ง ระคายเคืองให้หยุดใช้ยา ติดต่อแพทย์ผู้ทำการวิจัยตามรายละเอียดที่ให้ไว้ แพทย์แนะนำวิธีปฏิบัติตัวเบื้องต้น และนัดหมายเวลาเพื่อมาตรวจรักษา ให้การรักษาจนกว่าอาการจะหายเป็นปกติ โดยไม่คิดค่าใช้จ่าย และให้ค่าเดินทางแก่ผู้เข้าร่วมวิจัยครั้งละ 200 บาท

4. การศึกษานี้เป็นการศึกษาแบบแบ่งใบหน้าออกเป็นข้างซ้ายและข้างขวา โดยหน้าข้างหนึ่งทาครีมสารสกัดจากหัวผักกาดขาว หน้าอีกข้างหนึ่งทายาครีมเบสมาตรฐาน หากผลการศึกษาเป็นไปตามสมมติฐาน เมื่อทำการศึกษาไประยะเวลาหนึ่ง ครีมสารสกัดจากหัวผักกาดขาวอาจทำให้ใบหน้าขาวขึ้นได้ ทำให้ใบหน้าข้างหนึ่งดูขาวกว่าใบหน้าอีกข้างหนึ่งเล็กน้อย แพทย์ผู้ทำวิจัยให้แป้งอัดแข็งแก่ผู้เข้าร่วมวิจัยตกแต่งใบหน้า เพื่อให้ดูกลมกลืน

5. หากครีมสารสกัดจากหัวผักกาดขาว 4% สามารถปรับสีผิวหน้าให้ขาวขึ้นได้จริง หลังจากเสร็จสิ้นโครงการวิจัย แพทย์ผู้ทำวิจัยจะให้ครีมสารสกัดจากหัวผักกาดขาว 4% แก่ผู้เข้าร่วมวิจัยเพื่อนำไปทาใบหน้าอีกฝั่ง จนกว่าใบหน้าจะมีสีเท่ากัน โดยไม่คิดค่าใช้จ่าย

6. หากท่านตกลงที่จะเข้าร่วมการศึกษาวิจัยนี้ จะมีข้อปฏิบัติร่วมดังต่อไปนี้

1. ท่านจะได้รับสบู่ล้างหน้าอ่อน ครีมกันแดด และ ครีมทาปรับสีผิวขาวโดยไม่เสียค่าใช้จ่ายใดๆทั้งสิ้นตลอดการวิจัย

2. ท่านจะต้องเข้ามารับการรักษาและตรวจติดตาม ตามที่แพทย์นัดทุกครั้ง

3. หลังจากเสร็จสิ้นโครงการวิจัย ท่านจะได้รับครีมที่ได้ผลดีกับท่านมากกว่ากลับไปทาโดยไม่เสียค่าใช้จ่ายใดๆทั้งสิ้น

4. หากเกิดผลข้างเคียงขึ้นกับผู้เข้าร่วมวิจัย แพทย์ผู้ทำวิจัยจะให้การรักษาผลข้างเคียงนั้นตามมาตรฐานวิชาชีพแพทย์ โดยไม่คิดค่าใช้จ่าย และผู้เข้าร่วมวิจัยสามารถออกจากโครงการวิจัยได้ตลอดเวลา

4.1 กรณีเกิดสิวขึ้นบนใบหน้า ให้หยุดใช้ยา ติดต่อแพทย์ผู้ทำการวิจัยตามรายละเอียดที่ให้ไว้ แพทย์แนะนำวิธีปฏิบัติตัวเบื้องต้น และนัดหมายเวลาเพื่อมาตรวจรักษา โดยไม่คิดค่าใช้จ่าย และให้ค่าเดินทางแก่ผู้เข้าร่วมวิจัยครั้งละ 200 บาท

4.2 กรณีเกิดผื่นแพ้ ลอกแดง แห้ง ระคายเคืองให้หยุดใช้ยา ติดต่อแพทย์ผู้ทำการวิจัยตามรายละเอียดที่ให้ไว้ แพทย์แนะนำวิธีปฏิบัติตัวเบื้องต้น และนัดหมายเวลาเพื่อมาตรวจรักษา โดยไม่คิดค่าใช้จ่าย และให้ค่าเดินทางแก่ผู้เข้าร่วมวิจัยครั้งละ 200 บาท

5. การศึกษานี้เป็นการศึกษาแบบแบ่งใบหน้าออกเป็นข้างซ้ายและข้างขวา โดยหน้าข้างหนึ่งทาครีมสารสกัดจากหัวผักกาดขาว หน้าอีกข้างหนึ่งทายาครีมเบสมาตรฐาน หากผลการศึกษาเป็นไปตามสมมติฐาน เมื่อทำการศึกษาไประยะเวลาหนึ่ง ครีมสารสกัดจากหัวผักกาดขาวอาจทำให้ใบหน้าขาวขึ้นได้ ทำให้ใบหน้าข้างหนึ่งดูขาวกว่าใบหน้าอีกข้างหนึ่งเล็กน้อย แพทย์ผู้ทำวิจัยให้แป้งอัดแข็งแก่ผู้เข้าร่วมวิจัยตกแต่งใบหน้า เพื่อให้ดูกลมกลืน

6. หากครีมสารสกัดจากหัวผักกาดขาว 4% สามารถปรับสีผิวหน้าให้ขาวขึ้นได้จริง หลังจากเสร็จสิ้นโครงการวิจัย แพทย์ผู้ทำวิจัยจะให้ครีมสารสกัดจากหัวผักกาดขาว 4% แก่ผู้เข้าร่วมวิจัยเพื่อนำไปทาใบหน้าอีกฝั่ง จนกว่าใบหน้าจะมีสีเท่ากัน โดยไม่คิดค่าใช้จ่าย

7. หลังให้การรักษาแล้วแพทย์จะให้ใบประเมินผลการรักษากับท่าน กรุณากรอกใบประเมินตามความเป็นจริง เพื่อแพทย์ผู้ทำการวิจัยจะได้นำไปใช้ในการวิเคราะห์ข้อมูลต่อไป

8. ข้อมูลต่างๆของท่านจะถูกเก็บเป็นความลับ และจะเปิดเผยเฉพาะข้อมูลที่ได้สรุปผลหลังเสร็จสิ้นโครงวิจัยเท่านั้น

9. การเข้าร่วมการศึกษานี้เป็นไปโดยสมัครใจ ท่านอาจจะปฏิเสธที่จะเข้าร่วมหรือถอนตัวจากการศึกษานี้ได้ทุกเมื่อ โดยไม่กระทบต่อการดูแลรักษาที่ท่านจะได้รับจากแพทย์ ประการสำคัญที่ท่านควรทราบคือ ผลการศึกษานี้ใช้สำหรับวัตถุประสงค์ทางวิชาการเท่านั้น โดยข้อมูลส่วนบุคคลต่างๆจะถูกเก็บไว้ในคอมพิวเตอร์และไม่มีการเผยแพร่สู่สาธารณชน ขอรับรองว่าจะไม่มีการเปิดเผยชื่อของท่านตามกฎหมาย

ขอขอบคุณในความร่วมมือของท่านมา ณ ที่นี้

บัตรนัด

รายละเอียดการนัด

ชื่อ.....

ครั้งที่	ว/ด/ป	สัปดาห์	รายละเอียด	กิจกรรม
1		0	ก่อนการรักษา	เก็บข้อมูลVISIA MX18 รับครีม
2		4	รักษาครบ 4 สัปดาห์	VISIA MX18 รับครีม
3		8	รักษาครบ 8 สัปดาห์	VISIA MX18 รับครีม
4		12	รักษาครบ 12 สัปดาห์	แบบสอบถามVISIA MX18
5		16	หยุดรักษา 4 สัปดาห์	แบบสอบถามVISIA MX18 รับครีม

พญ. อาภรณ์ คูสุวรรณ

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โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพมหานคร

38/11-13 อาคาร อโศกเพลส ถ.อโศก สุขุมวิท 21

แขวงคลองเตยเหนือเขตวัฒนากรุงเทพฯ 10110

โทรศัพท์ : 02-664-2295

หนังสือยินยอมเข้าร่วมโครงการวิจัย (Informed Consent Form)

วันที่.....เดือน.....พ.ศ. .....

ข้าพเจ้า (นาย/นาง/นางสาว)..... อายุ.....ปี

อยู่บ้านเลขที่..... หมู่ที่.....ถนน.....ตำบล.....

อำเภอ.....จังหวัด..... รหัสไปรษณีย์.....

ขอทำหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

ข้อ 1. ข้าพเจ้าได้รับทราบโครงการวิจัยของแพทย์หญิงอาภรณ์ คูสุวรรณ และ อาจารย์ นพ. ไพศาล รัมณีย์ธรเรื่อง การศึกษาประสิทธิผลของการทาครีมสารสกัดจากหัวผักกาดขาว 4% เพื่อการปรับผิวหน้าขาวในผู้ที่มารับบริการ ณ โรงพยาบาลมหาวิทยาลัยแม่ฟ้าหลวง กรุงเทพฯ (THE EFFECTIVENESS OF 4% WHITE RADISH EXTRACT CREAM FOR FACIAL WHITENING AT MAE FAH LUANG UNIVERSITY HOSPITAL, BANGKOK)

ข้อ 2. ข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยนี้ ด้วยความสมัครใจ โดยมิได้มีการบังคับขู่เข็ญหลอกลวงแต่ประการใดและจะให้ความร่วมมือในการวิจัยทุกประการ

ข้อ 3. ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยเกี่ยวกับวัตถุประสงค์ของการวิจัย วิธีการวิจัย ประสิทธิผล ความปลอดภัย อาการหรืออันตรายที่อาจเกิดขึ้น รวมทั้งประโยชน์ที่จะได้รับจากการวิจัยโดยละเอียดแล้ว จากเอกสารคำอธิบาย โครงการวิจัย

ข้อ 4. ข้าพเจ้าได้รับการรับรองจากผู้วิจัยว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ จะเปิดเผยเฉพาะผลสรุปการวิจัยเท่านั้น

ข้อ 5. ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่า หากมีอันตรายใด ๆ อันเกิดขึ้นจากการวิจัยดังกล่าว ข้าพเจ้า จะได้รับการรักษาพยาบาลจากคณะผู้วิจัย โดยไม่คิดค่าใช้จ่ายและจะได้รับค่าชดเชยรายได้ที่สูญเสียไปในระหว่างการรักษาพยาบาลดังกล่าว ตลอดจน มีสิทธิ์ได้รับค่าทดแทนความพิการที่อาจเกิดขึ้นจากการวิจัยตามสมควร

ข้อ 6. ข้าพเจ้าได้รับทราบในการติดต่อกับ แพทย์หญิงอาภรณ์ คูสุวรรณ หัวหน้าโครงการวิจัยด้วยหมายเลขโทรศัพท์ 0819360738 แล้ว

ข้อ 7. ข้าพเจ้าได้รับทราบแล้วว่าข้าพเจ้ามีสิทธิ์จะบอกเลิกการร่วมโครงการวิจัยนี้ และการบอกเลิกการร่วมโครงการวิจัย จะไม่มีผลกระทบต่อการดูแลรักษาโรคที่ข้าพเจ้าจะพึงได้รับต่อไป

ข้อ 8. แพทย์หญิงอาภรณ์ คูสุวรรณ หัวหน้าโครงการวิจัย ได้อธิบายเกี่ยวกับรายละเอียดต่าง ๆ ของโครงการตลอดจนประโยชน์ของการวิจัยรวมทั้งความเสี่ยงและอันตรายต่าง ๆ ที่อาจจะเกิดขึ้นในการเข้าร่วม โครงการนี้ ให้ข้าพเจ้าทราบและตกลงรับผิดชอบตามคำรับรองในข้อ 5 ทุกประการ

ข้าพเจ้าได้อ่านและเข้าใจข้อความตามหนังสือนี้โดยตลอดแล้ว เห็นว่าถูกต้องตามเจตนาของข้าพเจ้า จึงได้ลงลายมือชื่อไว้เป็นสำคัญพร้อมกับหัวหน้าโครงการวิจัยและต่อหน้าพยาน

ลงชื่อ ..... ผู้ยินยอม
(.....)
ลงชื่อ ..... หัวหน้าโครงการวิจัย
(แพทย์หญิงอาภรณ์ คูสุวรรณ)
ลงชื่อ ..... พยาน
(.....)
ลงชื่อ ..... พยาน
(.....)

หมายเหตุ

กรณีผู้ยินยอมตนให้ทำวิจัยไม่สามารถอ่านหนังสือได้ให้ผู้วิจัยอ่านข้อความในหนังสือให้ความยินยอมนี้ให้แก่ผู้ยินยอมตนให้ทำวิจัยฟังจนเข้าใจแล้ว และให้ผู้ยินยอมตนให้ทำวิจัยลงนามหรือพิมพ์ลายนิ้วหัวแม่มือรับทราบในการให้ความยินยอมดังกล่าวด้วย

APPENDIX C

METERIAL

Figure C1 Packaging of standard cream base and 4% white radish root extract cream

Figure C2 Standard cream base and 4% white radish root extract cream (Similar consistency, color and smell)

CURRICULUM VITAE

CURRICULUM VITAE

NAME	Miss Arporn Koosuwan
DATE OF BIRTH	19 November 1985
ADDRESS	38/11-13 Asoke Place, Asoke Road, Klong toey, Wattana, Bangkok, Thailand, 10110

EDUCATIONAL BACKGROUND

2010	Bachelor of Doctor of Medicine Faculty of Medicine Mahidol University

WORK EXPERIENCE

2011-2012	Internship Kanthararom hospital, Srisaket, Thailand
2010-2011	Internship Angthong general hospital, Angthong, Thailand