



**THE EFFECTIVENESS OF ASTAXANTHIN CREAM
COMPARED WITH STANDARD CREAM BASE TO
IMPROVE SKIN MOISTURIZATION AND
REDUCTION OF SKIN WRINKLE**

WANVISA CHAROENWAT

**MASTER OF SCIENCE
IN
ANTI AGING AND REGENERATIVE MEDICINE**

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

2013

©COPYRIGHT BY MAE FAH LUANG UNIVERSITY

**THE EFFECTIVENESS OF ASTAXANTHIN CREAM
COMPARED WITH STANDARD CREAM BASE TO
IMPROVE SKIN MOISTURIZATION AND
REDUCTION OF SKIN WRINKLE**

WANVISA CHAROENWAT

**THIS THESIS IS A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ANTIAGING AND REGENERATIVE MEDICINE**

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

2013

©COPYRIGHT BY MAE FAH LUANG UNIVERSITY

**THE EFFECTIVENESS OF ASTAXANTHIN CREAM
COMPARED WITH STANDARD CREAM BASE TO
IMPROVE SKIN MOISTURIZATION AND
REDUCTION OF SKIN WRINKLE**

WANVISA CHAROENWAT

THIS THESIS HAS BEEN APPROVED
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
IN
ANTI AGING AND REGENERATIVE MEDICINE
2013

THESIS COMMITTEE

.....CHAIRPERSON
(Prof. Dr. Vijit Bunyahotara)

.....ADVISOR
(Lecturer Surapong Lookhanumanchao)

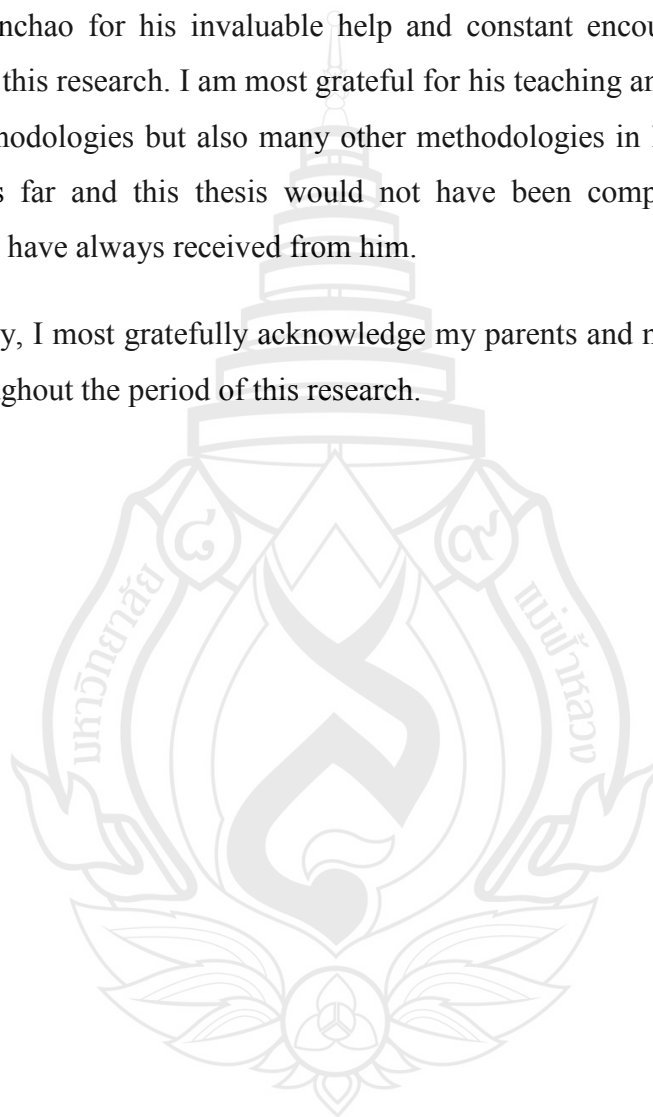
.....EXTERNAL EXAMINER
(Asst. Prof. Dr. Ekkarat Bumrungpert)

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my thesis advisor, Dr. Surapong Lookhanumanchao for his invaluable help and constant encouragement throughout the course of this research. I am most grateful for his teaching and advice, not only the research methodologies but also many other methodologies in life. I would not have achieved this far and this thesis would not have been completed without all the support that I have always received from him.

Finally, I most gratefully acknowledge my parents and my friends for all their support throughout the period of this research.

Wanvisa Charoenwat



Thesis Title	The Effectiveness of Astaxanthin Cream Compared with Standard Cream Base to Improve Skin Moisturization and Reduction of Skin Wrinkle
Author	Wanvisa Charoenwat
Degree	Master of Science (Anti-Aging and Regenerative Medicine)
Advisor	Lecturer Surapong Lookhanumanjao

ABSTRACT

Astaxanthin is potent antioxidant compared with vitamins and other antioxidants. Astaxanthin protects mitochondria from oxidation, anti-inflammation, reduce DNA damage and absorb UV light to prevent photo-oxidative damage that causing skin wrinkle and reduce skin moisturizer in aging population. A Prospective, Randomized, Double-blind, Experimental Clinical trial in 25 subjects, age greater than or equal 30 year olds in both male and female subjects. Apply astaxanthin cream in one side around eye and another side apply standard cream base. Then take a photo and measure skin moisturizer by Cutometer MPA 580, measure melanin pigment and skin erythema by Mexameter and measure skin wrinkle by The VISIA complexion Analysis System at week 0, 2, 4. Conclusion that Astaxanthin cream can increase skin moisturizer in 2 weeks and still increase skin moisturizer in 4 weeks ($p = 0.03$ and $p = 0.002$ respectively). Astaxanthin cream can decrease Melanin pigments in 2 weeks ($p = 0.02$) and decrease skin erythema in 4 weeks ($p = 0.01$). However Astaxanthin cream cannot decrease skin wrinkle in 2 weeks and 4 weeks ($p = 0.15$ and $p = 0.13$ respectively). The study found that Astaxanthin cream can reduce skin wrinkle but no statistically significant in 2 and 4 weeks ($p = 0.15$ and $p = 0.13$ respectively). The subjects reported satisfaction after study with statistically significant ($p = 0.0007$).

Keywords: Astaxanthin/Moisturization/Wrinkle/Cream

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	(3)
ABSTRACT	(4)
LIST OF TABLES	(8)
LIST OF FIGURES	(9)
CHAPTER	
1 INTRODUCTION	1
1.1 Significance and Sources of Research Problems	1
1.2 Research Question	2
1.3 Objective	2
1.4 Hypothesis	2
1.5 Benefit	2
1.6 Conceptual Framework	3
1.7 The Scope of the Research Project	3
2 REVIEW LITERATURES	4
2.1 Mechanism of Action in Antiaging	5
2.2 Skin Moisturization	5
2.3 Factors Effecting to Moisturize of Skin	5
2.4 Environment-Related Variables	6
2.5 Instrumental-Related Variables	6
2.6 Wrinkle	7

TABLE OF CONTENTS (continued)

	Page
CHAPTER	
3 RESEARCH METHODOLOGY AND EQUIPMENTS	9
3.1 Subjects and Sample Size	9
3.2 Inclusion Criteria	10
3.3 Exclusion Criteria	10
3.4 Discontinuation Criteria	10
3.5 Equipments	11
3.6 Step of Research	12
3.7 Data Collection	12
3.8 Statistic	13
4 RESULTS	14
4.1 General Characteristic	14
4.2 Result	17
4.3 The Side Effects	25
5 CONCLUSION, DISCUSSION AND RECOMMENDATION	26
5.1 Conclusion	26
5.2 Discussion	27
5.3 Recommendation	28

TABLE OF CONTENTS (continued)

	Page
REFERENCES	29
APPENDICES	33
APPENDIX A INFORMED CONSENT FORM	34
APPENDIX B RECORDING DATA	36
CURRICULUM VITAE	39

LIST OF TABLES

Table	Page
4.1 Sex of Subjects	14
4.2 Ages of Subjects	15
4.3 Skin Types	16
4.4 Result of Wrinkle	17
4.5 Skin Moisturization	19
4.6 Result in Dark Spot	21
4.7 Result in Erythematous	22
4.8 Result of Satisfaction	24
4.9 Side Effects	25

LIST OF FIGURES

Figure	Page
1.1 Conceptual Framework	3
2.1 Chemical Structure of Astaxanthin	4
2.2 Mechanism of Astaxanthin in Reduce Skin Wrinkle	7
3.1 Cutometer MPA 580	11
3.2 The VISIA complexion Analysis System	11
4.1 Sex of Subjects	15
4.2 Ages of Subjects	15
4.3 Skin Types	16
4.4 Result of Wrinkle	17
4.5 Y-axis: Change rate (Post/Pre)	18
4.6 Skin Moisturization	19
4.7 Y-axis: Change rate (Post/Pre)	20
4.8 Result in Dark Spot	21
4.9 Y-axis: Change rate (Post/Pre)	22
4.10 Result in Erythematous	23
4.11 Y-axis: Change rate (Post/Pre)	23
4.12 Result of Satisfaction	24
4.13 Side Effects	25

CHAPTER 1

INTRODUCTION

1.1 Significance and Sources of Research Problems

This research showed that the effectiveness and efficiency of wrinkle reduction and increasing skin moisturizing by using Astaxanthin cream. It found that Astaxanthin cream can reduce wrinkles and increase moisturizes by significant statistic, this will be the evidence base of important information and contribute to further research, particularly in Asia and Thai people.

In modern world has changed a lot in terms of health and technology. The longevity, more comfortable, more antiaging technology such as medical or alternative medicine has played the important roles. It is recognized that no one wants to old. But in reality, the physical condition deteriorated by age. Moreover, the current workload with more stress, lack of exercise, not enough sleep, eating bad food, no balancing diets and environment filled with pollution, dust, smoke, sunlight or living in air conditioning room for long time, causing decreased skin moisturization and increase wrinkle, which created anxiety that will become chronic problem in the further. Nowadays, there are many creams and many supplements are sale in the market, one of them is Astaxanthin.

Astaxanthin has high potent antioxidant level compared with other vitamins and other antioxidants. The mechanism of action in antiaging processes are protection of cell membrane and mitochondria from antioxidant, no pro-oxidant property, anti-inflammation, prevent NK-cell movement in many organs (such as gastrointestinal tract, vascular system, musculoskeletal, eye, kidney and brain), prevent DNA damage and prevent photo-oxidative damage from UV light that are the main cause of decrease skin moisturization and increase skin wrinkle in aging skin.

1.2 Research Question

Is Astaxanthin cream can improve skin moisturization and decrease wrinkle more than standard cream base?

1.3 Objective

1.3.1 For study the effectiveness of Astaxanthin cream to improve skin moisturization and decrease wrinkle.

1.3.2 For compare the effectiveness between Astaxanthin cream and Standard cream base to improve skin moisturization and decrease wrinkle.

1.4 Hypothesis

1.4.1 Astaxanthin cream can improve skin moisturization and decrease wrinkle.

1.4.2 Astaxanthin cream can improve skin moisturization and decrease wrinkle more than Standard cream base.

1.5 Benefit

1.5.1 As an alternative to reduce wrinkles and moisturize the skin.

1.5.2 As an alternative to anti-oxidants in the body.

1.5.3 To use as a basis data for future research.

1.6 Conceptual Framework

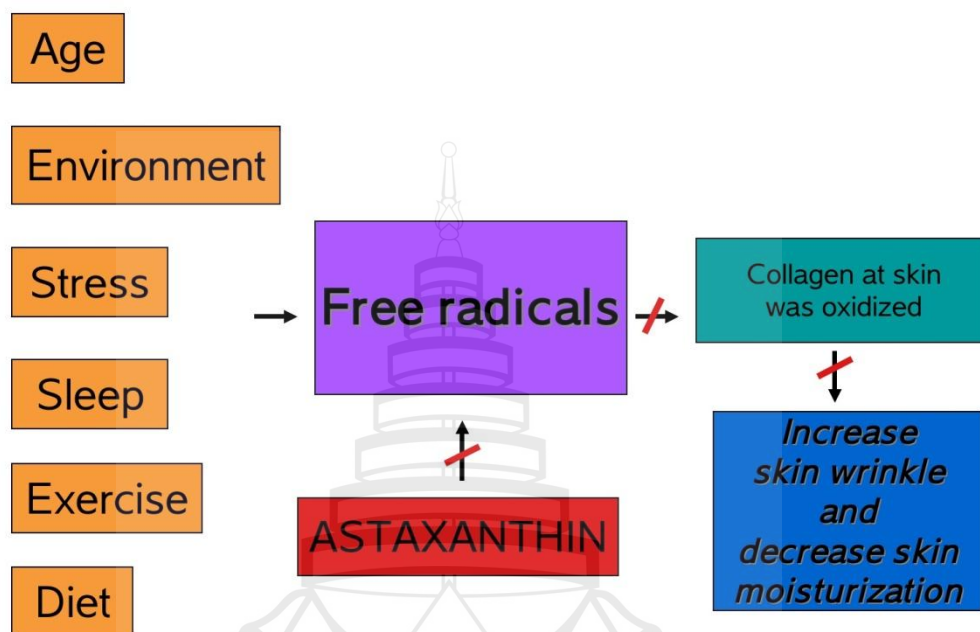


Figure 1.1 Conceptual Framework

1.7 The Scope of the Research Project

The sample size

Male and Female 25 subjects, age more than 30 years old.

CHAPTER 2

REVIEW LITERATURES

Astaxanthin is carotenoid group which both water-soluble and fat-soluble. They found in plants, algae, fungi, bacteria containing red, orange, yellow colors. (Cooper, Eldridge & Peters, 1999). Astaxanthin has antioxidant properties, catch with singlet oxygen (Edge, McGarvey & Truscott, 1997; Mortensen, Skibsted, Sampson, Rice-Evans & Everett, 1997) and also prevent light absorption by prevent photo-oxidation and light from ultraviolet.

Astaxanthin is most commonly found in Algae, Salmon, Lobster, Unicellular algae: *Haematococcus pluvialis* (*H.pluvialis*). (Odeberg, Lignell, Len Pattersson & Höglund, 2003; Refer, Moeseneder, Briviba, Rechkemmer & Bub, 2008; Barbosaa, Moraisa & Choubertb, 1999). Astaxanthin is similar structure as vitamin A, but the unique characteristic is a group of Hydroxyl group and keto-group that located at the end of the ring (Figure 1.1). These groups have potent anti-oxidant properties compared with vitamins and nutrients, such as the following lists below (Edge et al., 1997; Mortensen et al., 1997)

Antioxidant level 500 times more than Vitamin E 500

Antioxidant level 800 times more than Coenzyme Q10

Antioxidant level 3000 times more than Resveratrol

Antioxidant level 6000 times more than Vitamin C

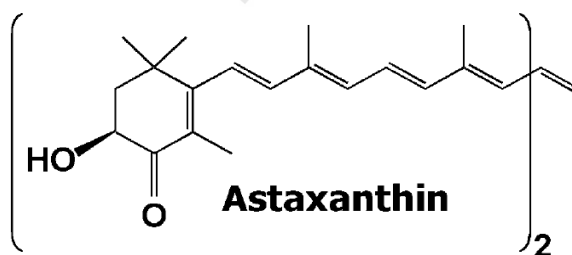


Figure 2.1 Chemical Structure of Astaxanthin

2.1 Mechanism of Action in Antiaging

2.1.1 Protect cell membrane by specific characteristic: hydrophilic and lipophilic, then Astaxanthin can catch both fat and water oxidants.

2.1.2 Protect mitochondria from damaged by oxidants.

2.1.3 Increase antioxidant level in blood, prevent decreasing of enzymes such as Catalase, Glutathione and Superoxide dismutase in body.

2.1.4 No pro-oxidant characteristic when compared with other Antioxidant.

2.1.5 Anti-inflammation by suppress movement of NF (Natural killer cell) that causing inflammation in many organs such as gastrointestinal tract, cardiovascular system, musculoskeletal system, eye, kidney and brain.

2.1.6 Reduces DNA damage.

2.2 Skin Moisturization

Skin Moisturization is measured by the water content in the stratum corneum. Based on the difference of the capacity in place to measure. Which is closely related to the amount of water in the stratum corneum by using Cutometer MPA 580.

Water is importance to physical properties of the stratum corneum. The relationship of the amount of water in stratum corneum and react to the skin cream, it is necessary to understand the role of the physical and the development of skin cream efficiency. Therefore, to measure the moisture of the skin, so it is commonly used in research on skin and cosmetics to assess the moisture and the skin response to skin cream.

2.3 Factors Effecting to Moisturize of Skin

Individual-related variables

2.3.1 Different skin regions have different moisturization, expectially forehead and palm that have more moisturization. Abdomen and extremities have less moisturization. But no different in both sides. (Blichmann & Serup, 1988; Rogiers, Derde, Verleye & Roseeuw, 1990; Bare1, Clarys, Wessels & de Romsee, 1991)

2.3.2 Sweat gland activity is effected to skin moisturization. Need to control temperature and humidity in research room. The subjects need to rest at least 10-20 minutes before measurement and open skin region. Choose the hairless area to be measured. Eliminate stress that caused sweat gland more production.

2.3.3 Characteristic of skin surface such as surfactant, cream, talc. Need to clean skin and avoid use skin surfactant at least 4 weeks

2.3.4 Sin cleanser has effect to skin moisturization then avoid clean skin at least 2 hours before measure. (Rogiers et al., 1990)

2.4 Environment-Related Variables

2.4.1 Room temperature and humidity have effected to skin moisturization in stratum corneum. Need to control room temperature and humidity when measurement. Keep 40 to 60 percent humidity and 20 to 22 degree Celsius. (Bare1 & Clarys, 1995; Clar, Her & Sturelle, 1975; Tagami, 1989)

2.4.2 Season and different time have effected to skin moisturization then need to measure in same time and same season. (Tagami, 1989; Prall, Theiler, Bowser & Walsh, 1986)

2.5 Instrumental-Related Variables

Probe measurement in perpendicular with skin. Meansurement in the same area need to rest at least 5 seconds or measurement nearby the same area. Because measurement in the same area decreasing skin hydration and impact probe and can causing abnormal test. (Beradesca, 1997)

2.6 Wrinkle

Wrinkle is aging skin condition causing by many intrinsic and extrinsic factors such as life style, foods and diets, exercise, health, stress, social, environment, home, pollution, toxin and heavy metal.

Nowaday, we have many technology to treatment skin wrinkle such as prevention, tropical drug use, oral drug, operative procedure (peeling, resurfacing, laser, filler, surgery). However no gold standard and best effective technology. (Yaar, M. & Gilchrest, 2003)

The VISIA complexion Analysis System: it photographs skin with different light lengths and analysis the skin condition with high effective and high accuracy. Report score and number of wrinkle.

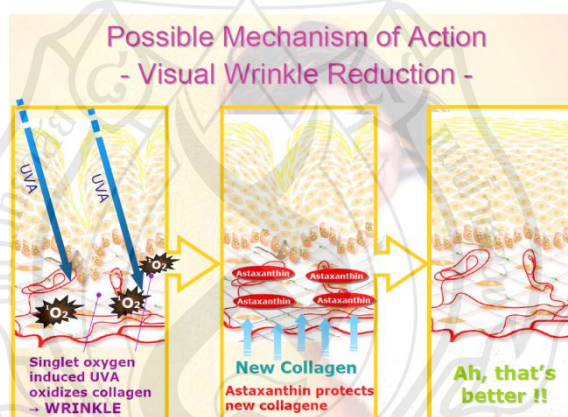


Figure 2.2 Mechanism of Astaxanthin in Reduce Skin Wrinkle

The pilot study Astaxanthin can reduce skin wrinkle (Seki, Sueki, Kono, Suganuma & Yamashita, 2001) by protect skin collagen and causing decrease skin wrinkle and increase skin moisturization. No side effect is found in this study. However this study is research in three subjects and young age group (26-30 years old). In 2002, The effectiveness of astaxanthin supplement and vitamin E (Yamashita, 2002) was researched in mean 47 years old subjects. After take 2 mg. astaxanthin supplement with 40 mg. vitamin E in 2 weeks, in dry skin group had increase skin moisturization and

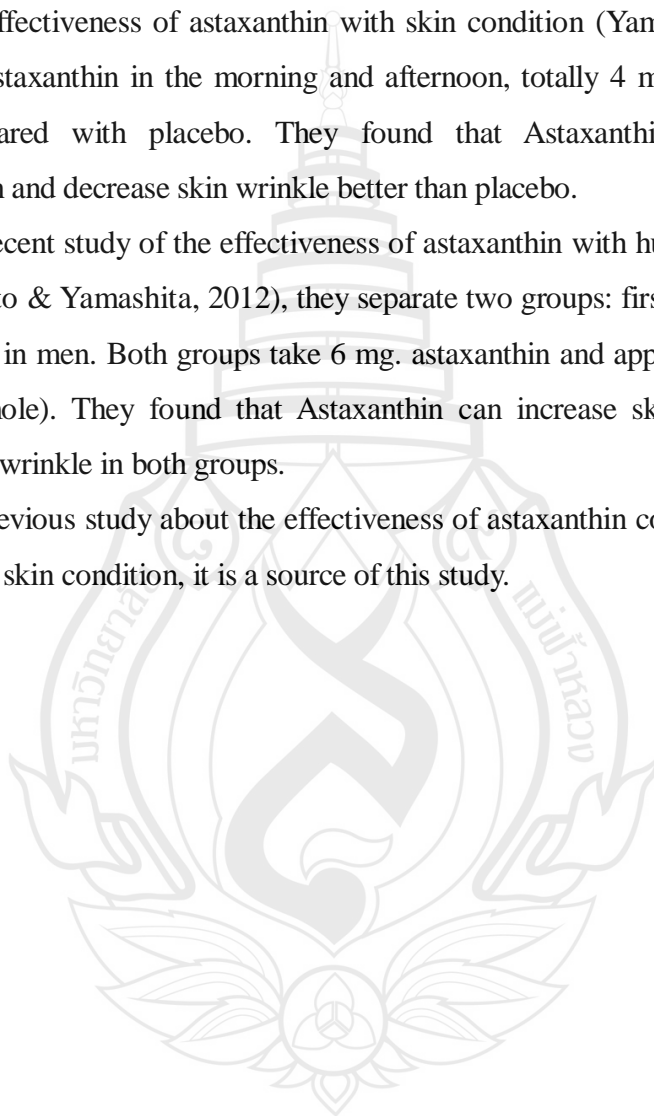
decrease skin wrinkle.

The study in USA in effectiveness of Astaxanthin supplement (Yamashita, 2005), used 4 mg. astacanthin combine with Canolar oil compared with placebo (Canolar oil). They found that astaxanthin combine with Canolar oil can increase skin moisturization and decrease skin wrinkle.

The effectiveness of astaxanthin with skin condition (Yamashita, 2006a; 2006b) used 2 mg. astaxanthin in the morning and afternoon, totally 4 mg. per day in 3 and 6 weeks compared with placebo. They found that Astaxanthin can increase skin moisturization and decrease skin wrinkle better than placebo.

The recent study of the effectiveness of astaxanthin with human skin (Tominaga, Hongo, Karato & Yamashita, 2012), they separate two groups: first group in women and second group in men. Both groups take 6 mg. astaxanthin and apply tropical astaxanthin (78.9 micromole). They found that Astaxanthin can increase skin moisturization and decrease skin wrinkle in both groups.

No previous study about the effectiveness of astaxanthin compared with standard cream base in skin condition, it is a source of this study.



CHAPTER 3

RESEARCH METHODOLOGY AND EQUIPMENTS

3.1 Subjects and Sample Size

3.1.1 Subjects

Male and female age at least 30 years old.

3.1.2 Sample size

25 subjects, follow up at Mea Fah Luang hospital, Bangkok.

Calculate sample size by Two mean independence

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \cdot (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

n = sample size

$Z_{\alpha/2}$ = Statistics under the curve when the level of statistical significance $\alpha/2 = 0.05$ is 1.96

Z_{β} = Statistics under the curve when the authority on the test 80%, $\beta = 0.2$ is 0.842

σ_1^2, σ_2^2 = The variance of population1 and 2 represented with SD_1^2, SD_2^2

(SD_1^2, SD_2^2 and μ_1, μ_2 come from Pawitra Aopaapragasit (2010) The effectiveness of 5 percent pomegranate peel in skin wrinkle, Thesis of Mae Fah Luang University)

$$n = \frac{(1.96 + 0.842)^2 \times (2.78 + 2.81)}{(41.468 - 37.671)^2}$$

$$n = 17.02$$

Calculate drop out 40% = 7

Total 17+7 = 24 subjects

Sample size in this study is 25 subjects, compared another sides between astaxanthin cream and standard cream base.

3.2 Inclusion Criteria

3.2.1 Male and Female, age at least 30 years old.

3.2.2 Accept to continuous use Astaxanthin cream and standard cream base in different side.

3.2.3 No change skin care during study.

3.2.4 No facial treatment or skin lazer during study.

3.2.5 No Botox and Filler used.

3.3 Exclusion Criteria

3.3.1 Allergy to Astaxanthin cream or standard cream base.

3.3.2 Medical condition

3.3.3 Pregnancy

3.4 Discontinuation Criteria

3.4.1 Severe side effect or allergy

3.4.2 Cannot continuous use Asthaxanthin

3.4.3 Pregnancy

3.4.4 Other medical condition

3.4.5 The sujet is deny

3.5 Equipments

3.5.1 Cutometer MPA 580 for measurement skin dehydration and skin moisturization



Figure 3.1 Cutometer MPA 580

3.5.2 The VISIA complexion Analysis System for report score and number of skin wrinkle



Figure 3.2 The VISIA complexion Analysis System

3.5.3 4.5% Astaxanthin cream (4.5 gram astaxanthin in 100 ml. standard cream base)

3.5.4 Standard cream base

3.6 Step of Research

Photograph skin before study: measure skin moisturization by Cutometer MPA 580 and measure skin wrinkle by The VISIA complexion Analysis System. Patch test before apply skin creams. Then apply 1 ml. astaxanthin cream in one side and apply 1 ml. standard cream base in another side in the morning and afternoon. Both subjects and Researcher do not know the side of astaxanthin cream or standard cream base.

Record data. Then 2 and 4 weeks later, follow up and take a photo as the first time: measure skin moisturization by Cutometer MPA 580 and measure skin wrinkle by The VISIA complexion Analysis System. Record data and analysis.

3.7 Data Collection

Record skin moisturization by Cutometer MPA 580 and skin wrinkle by The VISIA complexion Analysis System in record sheet and computer.

3.7.1 General data: age sex occupation address and previous medical history that related to skin moisturization and wrinkle.

3.7.2 Score from Cutometer MPA 580.

3.7.3 Score from The VISIA complexion Analysis System.

3.7.4 Assess satisfaction by subjects in 2 and 4 weeks.

Score 0 No satisfaction

Score 1 Little satisfaction

Score 2 Average satisfaction

Score 3 More satisfaction

Score 4 Most satisfaction

3.7.5 Side effect from the study.

3.8 Statistic

3.8.1 General data: Descriptive statistics

Percent Frequency Mean and SD

3.8.2 Compare before and after study in the same group: continuous data

3.8.2.1 Normal distribution: Pair T-test

3.8.2.2 No Normal distribution: Wilcoxon sign rank test

*CI 95% (p-value 0.05%)

3.8.3 Compare before and after study between two groups: continuous data

3.8.3.1 Normal distribution: Student t-test

3.8.3.2 No Normal distribution: mann-whitney u-test

*CI 95% (p-value 0.05%)

3.8.4 Treatment analysis between week 0, 2, 4 : continuous data

3.8.4.1 Normal distribution: one way ANOVA

3.8.4.2 No Normal distribution: friedman test

* CI 95% (p-value 0.05%)

3.8.5 Compare week 0, 2, 4 between groups: repeated measure ANOVA

3.8.6 Satisfaction analysis and side effect analysis by Descriptive statistics
between groups: Chisquare test

*CI 95% (p-value 0.05%)

CHAPTER 4

RESULTS

Results of Data Analysis

The aim of this research is study the effectiveness of astaxanthin cream compared with standard cream base for decrease wrinkle and increase skin moisturization in 25 subjects. Randomly, one side use astaxanthin cream and another side use standard cream base. Apply creams in the morning and afternoon for 28 days, then analysis the data and separate results in 3 steps as following:

1. General characteristic
2. Results
3. Assess satisfaction and side effects

4.1 General Characteristic

Demographic information

26 subjects (11 males and 15 females) 1 male subject was withdrawn due to political events, remaining 25 subjects (10 males and 15 females). All of them had complete follow up and data as following:

Table 4.1 Sex of Subjects

Sex	Total
Male	10
Female	15

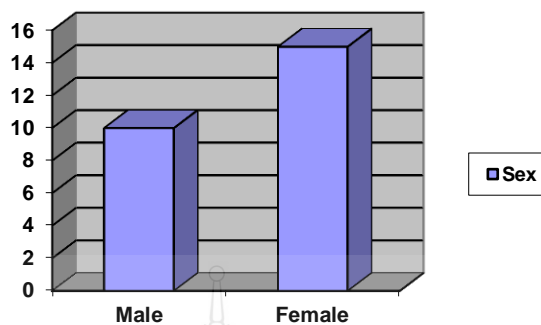


Figure 4.1 Sex of Subjects

From Table 4.1 and Figure 4.1 show 11 males and 15 females subjected and the ratio is 2:3.

Table 4.2 Ages of Subjects

Age	Total
30-39	12
40-49	8
50-59	4
60-69	1

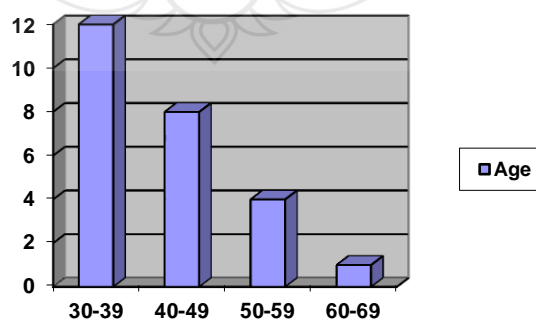


Figure 4.2 Ages of Subjects

From Table 4.2 and Figure 4.2 show 12 subjects with ages between 30-39 years old, 8 subjects with ages between 40-49 years old, 4 subjects with ages between 50-59 years old and 1 subject with ages between 60-69 years old. Total 25 subjects with maximal age is 67 years old and minimum age is 30 years old, mean age is 40.45 years old and SD = 10.99 .

Table 4.3 Skin Types

Skin type	Total
Dry	7
Oil	8
Combination	10

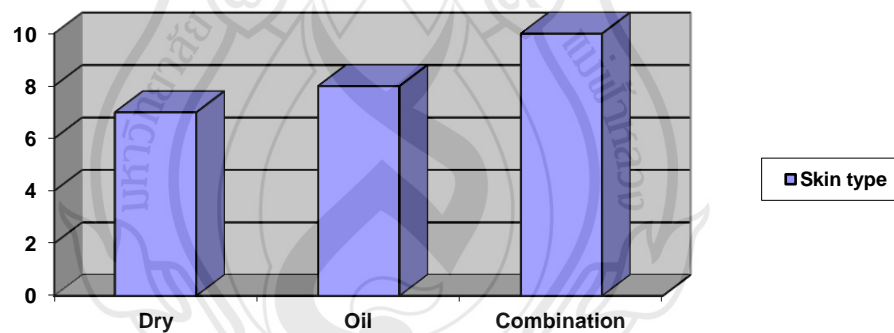


Figure 4.3 Skin Types

From Table 4.3 and Figure 4.3 shows 7 subjects had dry skin type, 8 subjects had oily skin type and 10 subjects had combination type.

4.2 Result

This research used The VISIA complexion Analysis System for study wrinkle and used Cutometer MPA 580 for measurement skin moisturization in week 0, 2 and 4. Randomly, one side used astaxanthin and another side used standard cream base in the morning and afternoon for 28 days. The results as following:

4.2.1 Result of wrinkle

Table 4.4 Result of Wrinkle

Wrinkle score	Treatment			
	Standard cream base		Astaxanthin cream	
	Mean \pm SD	p-value	Mean \pm SD	p-value
Week 0	5.65 \pm 5.27	Reference	4.94 \pm 4.26	Reference
Week 2	5.38 \pm 3.86	0.14	5.15 \pm 3.72	0.15
Week 4	5.48 \pm 4.37	0.43	6.07 \pm 4.85	0.13

Note. *Significant at $p < 0.05$, p-value from Student t-test

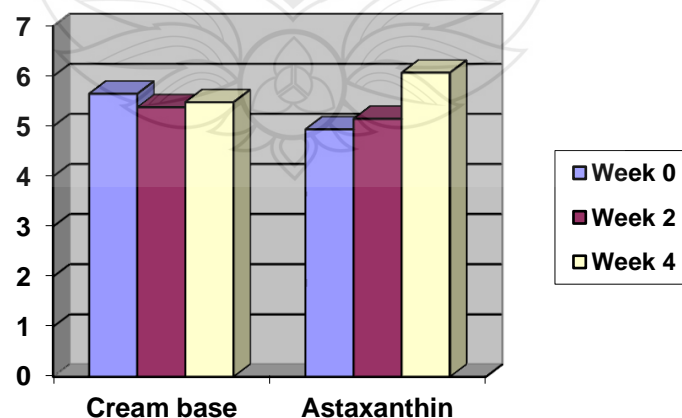


Figure 4.4 Result of Wrinkle

Figure 4.4 the result of astaxanthin cream compared with standard cream base on wrinkle at week 0, 2 and 4.

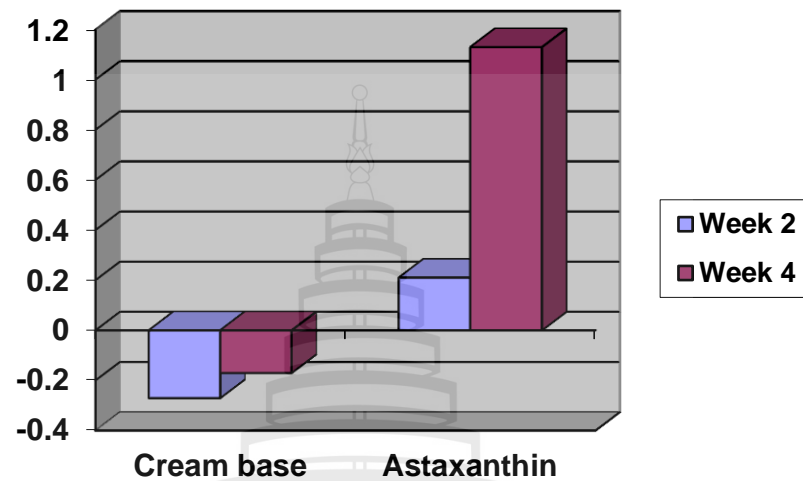


Figure 4.5 Y-axis: Change rate (Post/Pre)

From Table 4.4, Figure 4.4 and 4.5 show increasing mean wrinkle in standard cream base group, however no statistically significant when compared with week 2 and 4. ($p = 0.14$ and $p = 0.43$, respectively) Mean \pm SD in week 0 is 5.65 ± 5.27 , week 2 is 5.38 ± 3.86 and week 4 is 5.48 ± 4.37

The mean wrinkle in astaxanthin group is decreasing but no statistically significant in week 2 and 4 ($p = 0.15$ and $p = 0.13$ respectively) Mean \pm SD in week 0 is 4.94 ± 4.26 , week 2 is 5.15 ± 3.72 and week 4 is 6.07 ± 4.85

4.2.2 Result of skin moisturization

Table 4.5 Skin Moisturization

Corneometer score	Treatment			
	Standard cream		Astaxanthin cream	
	base Mean \pm SD	p-value	Mean \pm SD	p-value
Week 0	48.33 \pm 12.41	Reference	50.45 \pm 10.81	Reference
Week 2	52.56 \pm 10.53	0.18	53.70 \pm 12.00	0.03*
Week 4	49.13 \pm 111.24	0.49	54.34 \pm 13.62	0.002*

Note. *Significant at $p < 0.05$, p-value from Student t-test

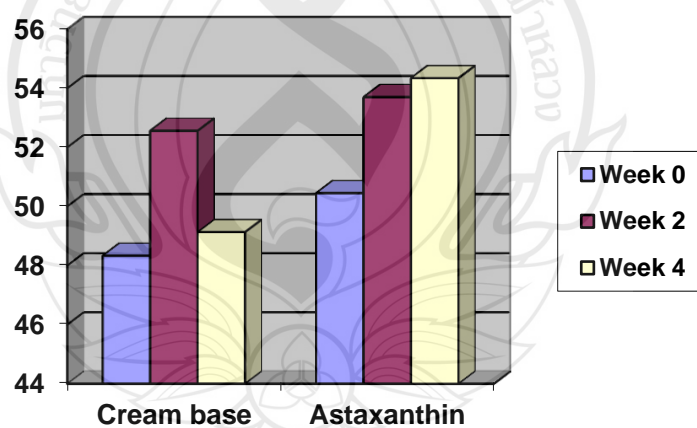


Figure 4.6 Skin Moisturization

Figure 4.6 The result of astaxanthin cream compared with standard cream base on skin moisturization at week 0, 2 and 4.

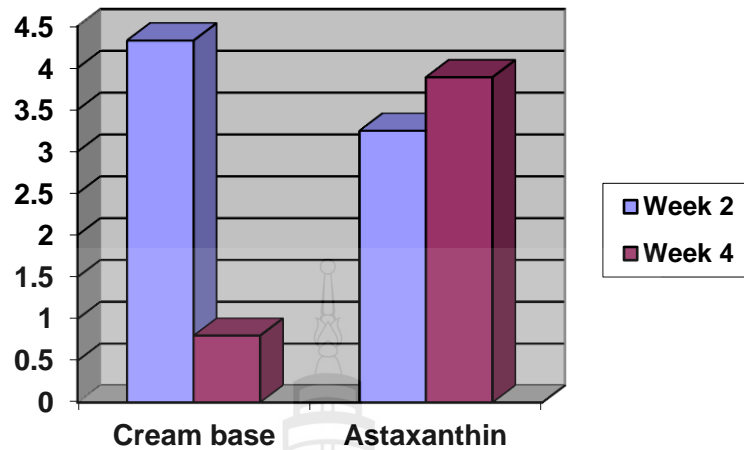


Figure 4.7 Y-axis: Change rate (Post/Pre)

From Table 4.5, Figure 4.6 and 4.7 show mean of skin moisturization in standard cream base group has no statistically significant when compared with week 2 and 4 ($p = 0.18$ and $p = 0.49$ respectively) Mean \pm SD in week 0 is 48.33 ± 12.41 , week 2 is 52.56 ± 10.53 and week 4 is 49.13 ± 11.24

The mean of skin moisturization in astaxanthin group has increasing and statistically significant when compared with week 2 and 4 ($p = 0.03$ and $p = 0.002$ respectively). Mean \pm SD in week 0 is 50.45 ± 10.81 , week 2 is 50.45 ± 10.81 and week 4 is 54.34 ± 13.62

4.2.3 Result in dark spot

Table 4.6 Result in Dark Spot

Mexameter score	Treatment			
	Standard cream		Astaxanthin	
	base Mean \pm SD	p-value	cream Mean \pm SD	p-value
Week 0	257.41 \pm 60.66	Reference	284.77 \pm 80.88	Reference
Week 2	237.05 \pm 61.18	0.11	259.82 \pm 83.27	0.02*
Week 4	286.53 \pm 56.30	0.06	245.14 \pm 64.38	0.18

Note. *Significant at $p < 0.05$, p-value from Student t-test

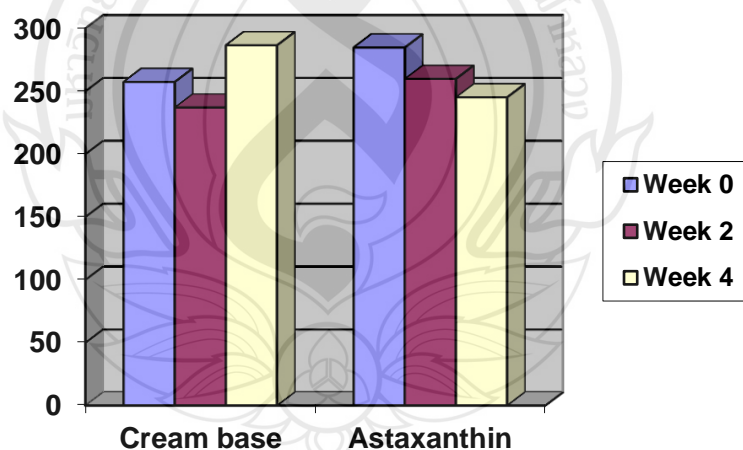


Figure 4.8 Result in Dark Spot

Figure 4.8 The result of astaxanthin cream compared with standard cream base on dark spot at week 0, 2 and 4.

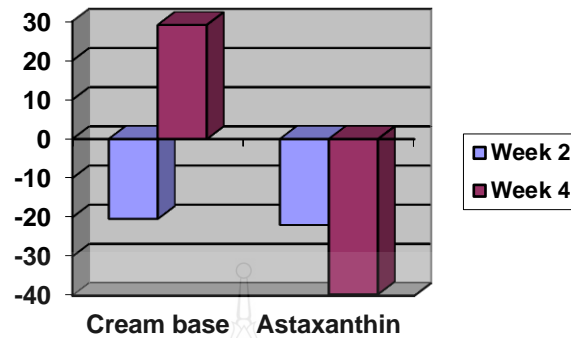


Figure 4.9 Y-axis: Change rate(Post/Pre)

From Table 4.6, Figure 4.8 and 4.9 show the mean dark spots in standard cream base group has no statistically significant when compared with week 2 and 4. ($p = 0.11$ and $p = 0.06$ respectively). Mean \pm SD in week 0 is 257.41 ± 60.66 , week 2 is 237.05 ± 61.18 and week 4 is 286.53 ± 56.30

But the mean dark spots in astaxanthin group has decreasing and statistically significant when compared with week 2 ($p = 0.02$). However in week 4 has no statistically significant ($p = 0.18$). Mean \pm SD in week 0 is 284.77 ± 80.88 , week 2 is 259.82 ± 83.27 and week 4 is 245.14 ± 64.38

4.2.3 Result in erythematous

Table 4.7 Result in Erythematous

Treatment				
Mexameter score	Standard cream base Mean \pm SD	p-value	Astaxanthin cream Mean \pm SD	p-value
Week 0	307.06 \pm 35.29	Reference	313.41 \pm 68.68	Reference
Week 2	306.27 \pm 58.74	0.48	301.79 \pm 79.81	0.32
Week 4	320.85 \pm 54.86	0.13	283.42 \pm 55.08	0.01*

Note. *Significant at $p < 0.05$, p-value from Student t-test

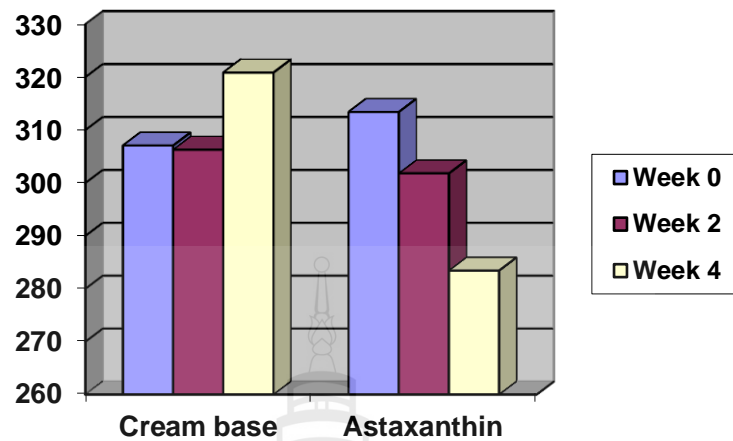


Figure 4.10 Result in Erythematous

Table 4.7 and Figure 4.10, the result of astaxanthin cream compared with standard cream base on erythematous at week 0, 2 and 4.

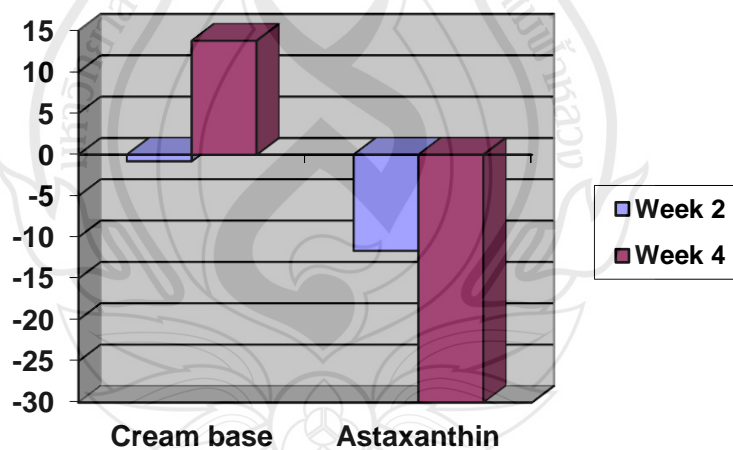


Figure 4.11 Y-axis: Change rate(Post/Pre)

From Table 4.7, Figure 4.10 and 4.11 show the mean of erythematous skin in standard cream base group has no statistically significant when compared with week 2 and 4 ($p = 0.48$ and $p = 0.13$ respectively). Mean \pm SD in week 0 is 307.06 ± 35.29 , week 2 is 306.27 ± 58.74 and week 4 is 320.85 ± 54.86

The mean dark spots in astaxanthin group has decreasing but no statistically significant when compared with week 2 ($p = 0.32$). However in week 4 has statistically significant ($p = 0.01$). Mean \pm SD in week 0 is 313.41 ± 68.68 , week 2 is 301.79 ± 79.81 and week 4 is 283.42 ± 55.08

4.2.4 Result of satisfaction

Table 4.8 Result of Satisfaction

	Treatment		p-value
	Standard cream base	Astaxanthin cream	
Satisfaction	1.55	4.73	0.0007*

Note. *Significant at $p < 0.05$, p-value from Student t-test

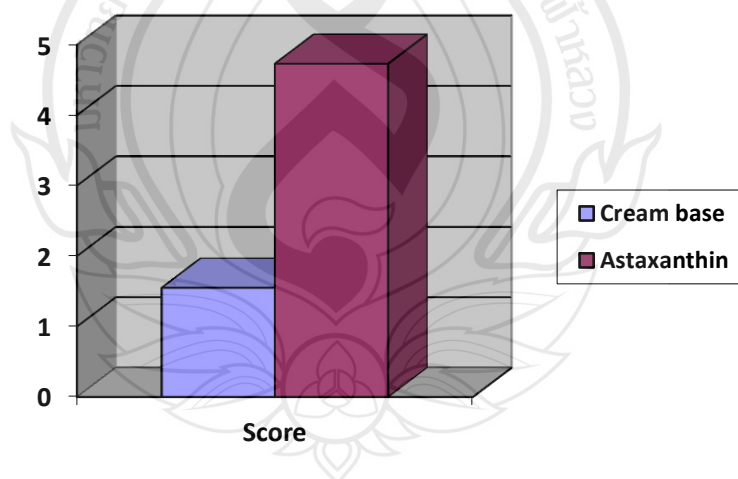


Figure 4.12 Result of Satisfaction

From Table 4.8 and Figure 4.12 show satisfaction in astaxanthin cream is more than standard cream base with statistically significant ($p = 0.0007$).

4.3 The Side Effects

Table 4.9 Side Effects

	Treatment				p-value
	Standard cream base		Astaxanthin cream		
	Total	Percent	Total	Percent	
Side effect	2	8	1	4	0.5

Note. *Significant at $p < 0.05$, p-value from Student t-test

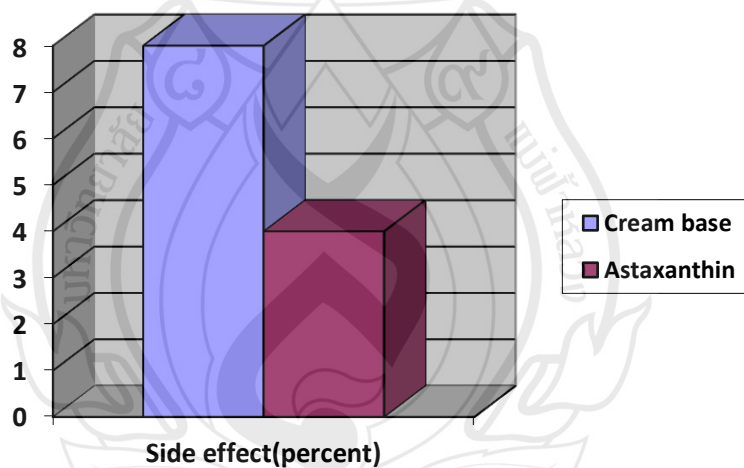


Figure 4.13 Side Effects

From Table 4.9 and Figure 4.13 show 2 subjects (8 percent) had mild side effect from standard cream base. And 1 subject (1 percent) in astaxanthin group had mild side effect. However no statistically significant ($p = 0.5$). All subjected has side effect in 1-2 hours after study and mild burning sensation for 2 hours. After use the cream, all of them had no further side effect.

CHAPTER 5

CONCLUSION, DISCUSSION AND RECOMMENDATION

5.1 Conclusion

This research is a Prospective, Randomized, Double-blind, Experimental Clinical trial for study for study the effectiveness of astaxanthin cream compared with standard cream base to improve skin moisturization and decrease wrinkle in 25 subjects.

There is increasing mean wrinkle in standard cream base group, however no statistically significant when compared with week 2 and 4. ($p = 0.14$ and $p = 0.43$, respectively). Mean \pm SD in week 0 is 5.65 ± 5.27 , week 2 is 5.38 ± 3.86 and week 4 is 5.48 ± 4.37 . The mean wrinkle in astaxanthin group is decreasing but no statistically significant in week 2 and 4 ($p = 0.15$ and $p = 0.13$ respectively) Mean \pm SD in week 0 is 4.94 ± 4.26 , week 2 is 5.15 ± 3.72 and week 4 is 6.07 ± 4.85 .

The mean of skin moisturization in standard cream base group has no statistically significant when compared with week 2 and 4 ($p = 0.18$ and $p = 0.49$ respectively). Mean \pm SD in week 0 is 48.33 ± 12.41 , week 2 is 52.56 ± 10.53 and week 4 is 49.13 ± 11.24 . The mean of skin moisturization in astaxanthin group has increasing and statistically significant when compared with week 2 and 4 ($p = 0.03$ and $p = 0.002$ respectively). Mean \pm SD in week 0 is 50.45 ± 10.81 , week 2 is 50.45 ± 10.81 and week 4 is 54.34 ± 13.62 .

The mean dark spots in standard cream base group has no statistically significant when compared with week 2 and 4. ($p = 0.11$ and $p = 0.06$ respectively). Mean \pm SD in week 0 is 257.41 ± 60.66 , week 2 is 237.05 ± 61.18 and week 4 is 286.53 ± 56.30 . But the mean dark spots in astaxanthin group has decreasing and statistically significant when compared with week 2 ($p = 0.02$). However in week 4 has no statistically significant ($p = 0.18$). Mean \pm SD in week 0 is 284.77 ± 80.88 , week 2 is 259.82 ± 83.27 and week 4 is 245.14 ± 64.38 .

The mean of erythematous skin in standard cream base group has no statistically significant when compared with week 2 and 4. ($p = 0.48$ and $p = 0.13$ respectively). Mean \pm SD in week 0 is 307.06 ± 35.29 , week 2 is 306.27 ± 58.74 and week 4 is 320.85 ± 54.86 . The mean dark spots in astaxanthin group has decreasing but no statistically significant when compared with week 2 ($p = 0.32$). However in week 4 has statistically significant ($p = 0.01$). Mean \pm SD in week 0 is 313.41 ± 68.68 , week 2 is 301.79 ± 79.81 and week 4 is 283.42 ± 55.08 .

The satisfaction in astaxanthin cream is more than standard cream base with statistically significant ($p = 0.0007$).

There are 2 subjects (8 percent) had mild side effect from standard cream base. And 1 subject (1 percent) in astaxanthin group had mild side effect. However no statistically significant ($p = 0.5$). All subjected has side effect in 1-2 hours after study and mild burning sensation for 2 hours. After use the cream, all of them had no further side effect.

5.2 Discussion

From this research found that mean of wrinkle in astaxanthin group had decreasing but no statistically significant when compared with week 2 and 4 ($p = 0.15$ and $p = 0.13$ respectively). The results were different from previous study (Yamashita, 2002; Tominaga et al., 2012).

The mean of skin moisturization in astaxanthin group had increasing and statistically significant when compared with week 2 and 4 ($p = 0.03$ and $p = 0.002$ respectively). Whereas standard cream base group had no statistically significant when compared with week 2 and 4 ($p = 0.18$ and $p = 0.49$ respectively). The results were related with the previous study (Seki et al., 2001; Yamashita, 2002; Tominaga et al., 2012).

The mean of dark spot in astaxanthin group had decreasing and statistically significant when compared with week 2 ($p = 0.02$) but no statistically significant when compared with week 4 ($p = 0.18$).

The mean of erythematous skin in astaxanthin group had decreasing but no statistically significant when compared with week 2 ($p = 0.32$). However in week 4 had statistically significant ($p = 0.01$). The results may be due to environment and temperature because there is long weekend during study. After take with subjects, all of them traveled with their family along the weekend and had cool temperature.

The mean of wrinkle, skin moisturization, dark spots and erythematous skin in standard cream base group had no statistically significant when compared with week 2 and 4. From the results can conclusion as the following:

5.2.1 Astaxanthin cream can increasing skin moisturization in week 2 and continuous increasing in week 4.

5.2.2 Astaxanthin cream can decreasing dark spots in week 2.

5.2.3 Astaxanthin cream can decreasing erythematous skin in week 4.

5.2.4 Astaxanthin cream cannot decreasing wrinkle in week 2 and 4.

5.2.5 Disadvantage of the research: temperature, environment, long weekend and difference in skin cream used in different subjects.

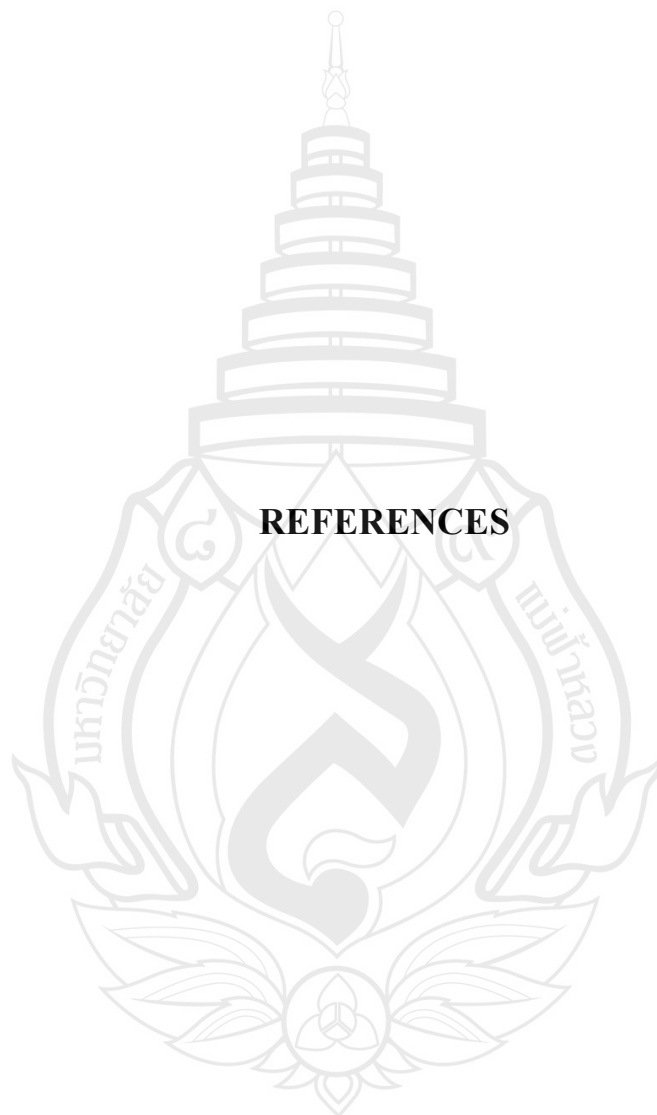
5.3 Recommendation

5.3.1 Can use research data to improve astaxanthin cream for increase effectiveness. For another option to increase skin moisturization, decrease dark spot and erythematous skin.

5.3.2 The research maybe used to be a database for further research about skin moisturization, dark spot and erythematous skin.

5.3.3 The temperature, environment and difference in skin cream used in different subjects are research variables and need to control in the next study.

5.3.4 For the next study maybe compared between cost-benefit between astaxanthin cream and other cream(s).



REFERENCES

- Barbosaa, M. J., Moraisa, R. & Choubertb, G. (1999). Effect of carotenoid source and dietary lipid content on blood astaxanthin concentration in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 176(3-4), 331-341.
- Barel, A. O., Clarys, P., Wessels, B. & de Romsee, A. (1991). Non-invasive electrical measurements for evaluating the water content of the horny layer: comparison between capacitance and conductance measurements. In R. C. Scott, R. H. Guy, J. Hadgraft & H. E. Bood (Eds.), *Prediction of percutaneous penetration: methods, measurements, modelling*. London: IBC Technical Services Ltd.
- Barel, A. O. & Clarys, P. (1995). Study of the stratum corneum barrier function by transepidermal water loss measurements: Comparison between two commercial instruments: Evaporimeter and TEWA meter. *Skin Pharmacol*, 8, 186-195.
- Beradesca, E. (1997). EEMCO guidance for the assessment of stratum corneum hydration: Electrical methods. *Skin Res Tech*, 3, 126-132.
- Blichmann, C. & Serup, L. (1988). Assessment of skin moisture. *Acta Derm Venereol*, 68, 284-290.
- Clar, E., Her, C. & Sturelle, C. (1975). Skin impedance and moisturization. *J SOC Cosm Chem*, 26, 337.
- Cooper, D. A., Eldridge, A. L. & Peters, J. C. (1999). Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: A review of recent research. *Nutr Rev*, 57(7), 201-214.
- Edge, R., McGarvey, D. J. & Truscott, T. G. (1997). The carotenoids as anti-oxidants - a review. *J Photochem Photobiol B*, 41(3), 189-200.

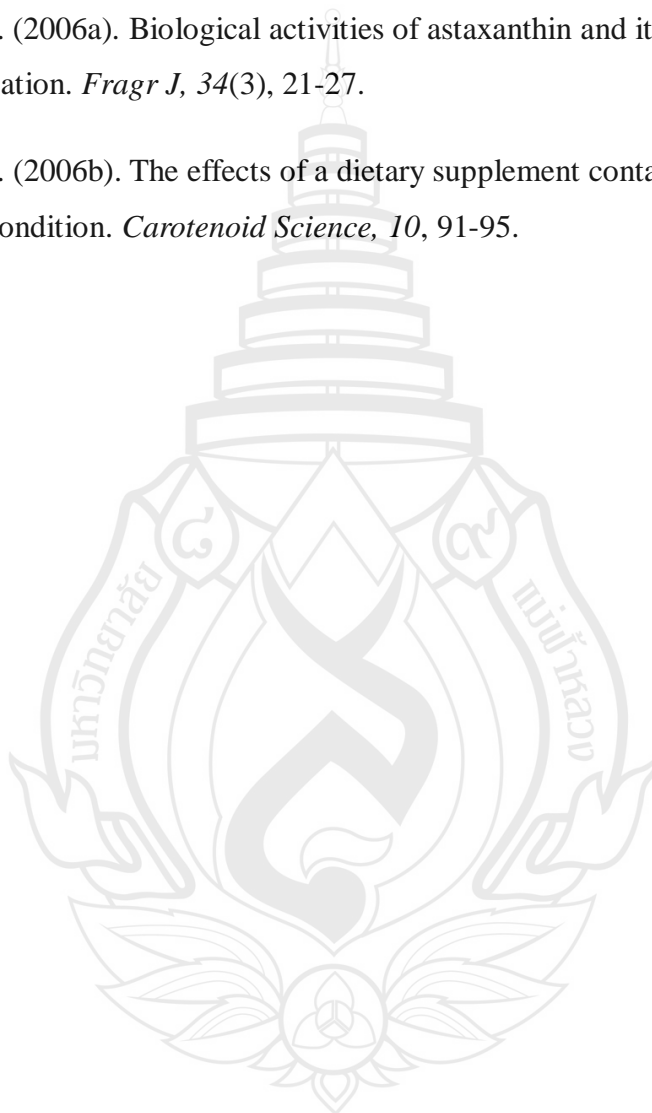
- Mortensen, A., Skibsted, L. H., Sampson, J., Rice-Evans, C. & Everett, S. A. (1997). Comparative mechanisms and rates of free radical scavenging by carotenoid antioxidants. *FEBS Letters*, 418, 91-97.
- Odeberg, J. M., Lignell, Å., Len Pattersson, A. & Höglund, P. (2003). Oral bioavailability of the antioxidant astaxanthin in humans is enhanced by incorporation of lipid based formulations. *E. J. Pharma. Sci*, 19, 299.
- Prall, J. K., Theiler, R. F., Bowser, P. A. & Walsh, M. (1986). The effectiveness of cosmetic products in alleviating a range of skin dryness conditions as determined by clinical and instrumental techniques. *J Cosm Sci*, 8, 159.
- Refer, C. E., Moeseneder, J., Briviba, K., Rechkemmer, G. & Bub, A. (2008). Bioavailability of astaxanthin stereoisomers from wild (*Oncorhynchus* spp.) and aquacultured (*Salmo salar*) salmon in healthy men: a randomised, double-blind study. *British Journal of Nutrition*, 99, 1048-1054.
- Rogiers, V., Derde, M. P., Verleye, G. & Roseeuw, D. (1990). Standardized conditions needed for skin surface hydration measurements. *Cosm Toil*, 105, 73-82.
- Seki, T., Sueki, H., Kono, H., Suganuma, K. & Yamashita, E. (2001). Effects of astaxanthin from *Haematococcus pluvialis* on human skin-patch test; skin repeated application test; effect on wrinkle reduction. *Fragrance J*, 12, 98-103.
- Tagami, H. (1989). Impedance measurements for evaluation of the hydration state of the skin surface. In J. L. LeV6que (Ed.), *Cutaneous investigation in health and disease*. New York: MarcelDekker.
- Tominaga, K., Hongo, N., Karato, M. & Yamashita, E. (2012). Cosmetic benefits of astaxanthin on humans subjects. *Biochimica Polinica*, 59(1), 43-47.
- Yaar, M. & Gilchrest, B. A. (2003). Topical retinoids. In S. Kang & J. J. Voorhees (Eds), *Fitzpatrick's dermatology in general medicine* (pp. 2106-2113, 7th ed.). New York: McGraw-Hill.

Yamashita, E. (2002). Cosmetic benefit of a dietary supplement containing astaxanthin & tocotrienol on human skin. *Food Style*, 21(6), 112-117.

Yamashita, E. (2005). The effects of astaxanthin on skin condition a UAS single-blind, randomized controlled study. *Food Style*, 21(9), 112-117.

Yamashita, E. (2006a). Biological activities of astaxanthin and its cosmeceutical application. *Fragr J*, 34(3), 21-27.

Yamashita, E. (2006b). The effects of a dietary supplement containing astaxanthin on skin condition. *Carotenoid Science*, 10, 91-95.





APPENDICES

APPENDIX A

INFORMED CONSENT FORM



หนังสือยินยอมเข้าร่วมโครงการวิจัย (Informed Consent Form)

วันที่.....เดือน..... พ.ศ.....

ข้าพเจ้า (นาย/นาง/นางสาว)..... อายุ.....ปี
 อยู่บ้านเลขที่..... หมู่ที่..... ถนน..... ตำบล.....
 อำเภอ..... จังหวัด..... รหัสไปรษณีย์.....

ขอทำหนังสือแสดงความยินยอมเข้าร่วมโครงการวิจัยเพื่อเป็นหลักฐานแสดงว่า

1. ข้าพเจ้ายินยอมเข้าร่วมโครงการวิจัยของแพทย์หญิงวันวิสา เจริญวัฒน์
2. เรื่องการศึกษาประสิทธิภาพของครีมแอสตาแซนทินเมื่อเปรียบเทียบกับครีมเบสมาตราฐาน เพื่อเพิ่มความชุ่มชื้นและลดเลือนริ้วรอย (The effectiveness of Astaxanthin cream compared with Standard cream base to improve skin moisturization and reduction of skin wrinkle) ด้วยความสมัครใจ โดยมีได้มีการบังคับ หลอกลวงแต่ประการใด และพร้อมจะให้ความร่วมมือในการวิจัย
3. ข้าพเจ้าได้รับการอธิบายและตอบข้อสงสัยจากผู้วิจัยเกี่ยวกับวัตถุประสงค์การวิจัย วิธีการวิจัย ความปลอดภัย อาการ หรืออันตรายที่อาจเกิดขึ้น รวมทั้งประโยชน์ที่จะได้รับการวิจัย โดยละเอียดแล้วตามเอกสารชี้แจงผู้เข้าร่วมการวิจัยแนบท้าย
4. ข้าพเจ้าได้รับการรับรองจากผู้วิจัยว่าจะเก็บข้อมูลส่วนตัวของข้าพเจ้าเป็นความลับ จะเปิดเผยได้เฉพาะในรูปแบบของการสรุปผลการวิจัยเท่านั้น
5. ข้าพเจ้าได้รับทราบจากผู้วิจัยแล้วว่า หากเกิดอันตรายใดๆ จากการวิจัย ผู้วิจัยจะรับผิดชอบค่ารักษาพยาบาลที่เป็นผลสืบเนื่องจากการวิจัยนี้
6. ข้าพเจ้าได้รับทราบว่า ข้าพเจ้ามีสิทธิที่จะถอนตัวออกจากการวิจัยครั้งนี้เมื่อใดก็ได้ โดยไม่มีผลกระทบใดๆ ต่อการรักษาพยาบาลตามสิทธิที่ข้าพเจ้าควรได้รับ

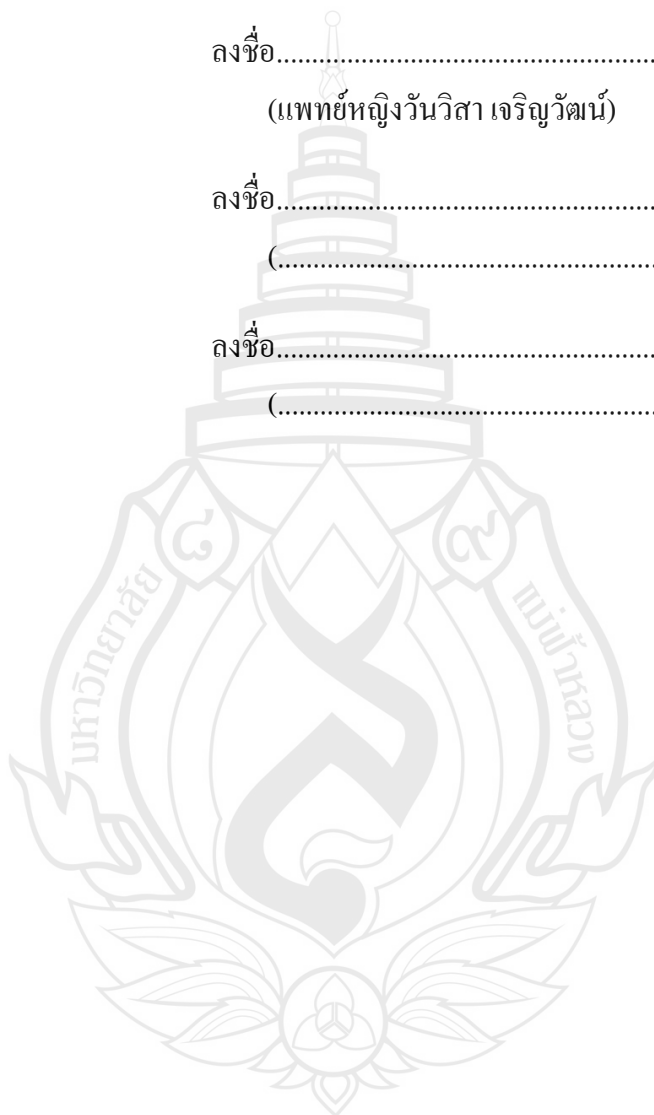
ข้าพเจ้าได้อ่านและเข้าใจข้อความตามหนังสือนี้แล้ว จึงได้ลงลายมือชื่อไว้เป็นสำคัญ พร้อม
กับหัวหน้าโครงการวิจัยและพยาน

ลงชื่อ..... ผู้ยินยอม/ผู้ปกครอง
(.....)

ลงชื่อ..... หัวหน้าโครงการ
(แพทย์หญิงวันวิสา เจริญวัฒน์)

ลงชื่อ..... พยาน
(.....)

ลงชื่อ..... พยาน
(.....)



APPENDIX B

RECORDING DATA

Case Number__

Name _____ Age _____

Adress _____ Tel. _____

1. Cutometer MPA 580: Corneometer

Corneometer	No.	Right	Left
Week 0	1		
	2		
	3		
	Total		
Week 2	1		
	2		
	3		
	Total		
Week 4	1		
	2		
	3		
	Total		

2. Mexameter
Melanin pigments

Melanin	No.	Right	Left
Week 0	1		
	2		
	3		
	Total		
Week 2	1		
	2		
	3		
	Total		
Week 4	1		
	2		
	3		
	Total		

Erythema

Erythema	No.	Right	Left
Week 0	1		
	2		
	3		
	Total		
Week 2	1		
	2		
	3		
	Total		
Week 4	1		
	2		
	3		
	Total		

3. The VISIA complexion Analysis System

Wrinkle: Right

Week	Feature count	Score	Percentile
0			
2			
4			

Wrinkle: Left

Week	Feature count	Score	Percentile
0			
2			
4			

4. Assess satisfaction (Subject Ranking)

Week	Right	Left
2		
4		

Score 0 No satisfaction

Score 1 Little satisfaction

Score 2 Average satisfaction

Score 3 More satisfaction

Score 4 Most satisfaction

5. Side effect(if has) _____ in week _____

Treatment _____

Result _____



CURRICULUM VITAE

CURRICULUM VITAE

NAME Miss Wanvisa Charoenwat

DATE OF BIRTH 30 May 1983

ADDRESS 1655/127 The address askoen, Petchaburi road,
Mukkason, Ratchathewi, Bangkok, 10400

EDUCATIONAL BACKGROUND

2002-2008 Doctor of Medicine
Chaing Mai University

2009-2012 General Radiology
Phamongkutklao Hospital, Mahidol University

WORK EXPERIENCE

October 2012- present General Radiologist at Mae Fah Luang Hospital

June 2009-May 2012 Resident at Phamongkutklao Hospital

May 2008-April 2009 General Practise at Chaiyaphum Hospital

October 2008-September 2008 General Practise at Klong Yai Hospital