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FULL REPORT

การพัฒนาสารสกัดจากสาหร่ายเกลียวทอง สาหร่ายไก และเทาน้ำ

เพื่อใช้เป็นสารเพิ่มความชุ่มชื้นแก่ผิว

Development of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. Extract for moisturizing agent

By

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PREFACE

Spirulina spp. is the common name for human and animal food supplements similar to Chlorella and Aphanizomenon flos-aquae (Blue Green Algae.). Spirulina contains an unusually high amount of nutrition which is wellknown as supplement in the market. *C. glomerata* and *Spirigyra* spp. have long been eaten and is highly nutritious which using as food for human being for a long time but rarely use in cosmetic product. The data obtained in this study could consequently be extended to the benefit from above algae especially for value adding to *Cladophora glomerata* and *Spirogyra spp.* which are the local algae in the north part of Thailand.

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Mayuramas Sang-ngern
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Executive Summary
(บทสรุปผู้บริหาร)

ในปัจจุบันมีการพัฒนาด้านวิทยาศาสตร์ และเทคโนโลยีอย่างต่อเนื่อง รวมถึงด้านเครื่องสำอาง ซึ่งนอกจากจะมีการพัฒนาเทคโนโลยีด้านการเตรียมวัตถุดิบ การตั้งตำรับ การผลิต และการนำส่งเครื่องสำอาง เพื่อเพิ่มประสิทธิภาพของเครื่องสำอาง โดยเฉพาะระบบการนำส่งด้วยนาโนเทคโนโลยี จากพัฒนาการด้านวิทยาศาสตร์เครื่องสำอาง ที่มีความก้าวหน้าอย่างต่อเนื่อง และไม่ได้จำกัดเฉพาะเพื่อการพัฒนาประสิทธิภาพและความปลอดภัย ของเครื่องสำอางเท่านั้น แต่ยังรวมถึงการผลิตเครื่องสำอางที่มีการใช้เทคโนโลยี ซึ่งไม่เป็นพิษต่อสิ่งแวดล้อม ประหยัดพลังงาน หรือที่เรียกกันว่า Green Technology การทดสอบประสิทธิภาพที่มีจริยธรรม และเป็นมิตรกับสัตว์ทดลอง หรือ Animal Friendly

เนื่องจากการที่อุตสาหกรรมเครื่องสำอางมีการขยายตัวอย่างต่อเนื่อง ประกอบกับมีความร่วมมือทางธุรกิจในกลุ่มประเทศอาเซียน รวมถึงการผลิตเครื่องสำอาง ซึ่งมีมาตรฐาน และศักยภาพในการผลิตแตกต่างกัน การวิจัยฉบับนี้ได้ตอบสนองด้านการพัฒนาด้านการนำวัตถุดิบที่มีอยู่ในธรรมชาติมาทำการทดสอบประสิทธิภาพด้านความปลอดภัย และประสิทธิผลด้านการนำสารสกัดจากวัตถุดิบทางธรรมชาติมาใช้ให้เกิดประโยชน์ในทางอุตสาหกรรมเครื่องสำอาง และยังเป็นการส่งเสริมด้านเศรษฐกิจโดยการสร้างมูลค่าเพิ่มให้กับพืชในท้องถิ่นต่อไป

การศึกษาวิจัยได้แบ่งออกเป็น 2 ส่วนตามวัตถุประสงค์ของโครงการคือ การศึกษาองค์ประกอบทางเคมี ซึ่งได้แก่ ปริมาณความชื้น โปรตีน ไขมัน แร่ธาตุ คาร์โบไฮเดรต และปริมาณสารสกัดหยาบโพ-ลีแซคคาไรด์ โดยผลการทดลองพบว่าพบว่าตำรับครีมให้ความคงตัวที่ดี และมีความปลอดภัย สามารถนำมาใช้ในผลิตภัณฑ์เครื่องสำอางได้ โดยประสิทธิภาพผลของครีมที่ผสมสารสกัดหยาบโพลีแซคคาไรด์จากสาหร่าย สไปรูลิน่า สาหร่ายไก และ เทาน้ำ 0.3, 0.5 โดยน้ำหนักในสูตรให้ความชุ่มชื้นได้มากกว่าผลิตภัณฑ์พื้นที่ไม่มีสารสกัดอย่างมีนัยสำคัญ ด้วยค่าความเชื่อมั่นร้อยละ 95

ดั้งนั้น จึงควรมีการส่งเสริมให้ทำวิจัยในขั้นสูง เช่น การทดสอบเชิงลึกถึงประเภท และโครงสร้างของสารสกัดหยาบโพลีแซคคาไรด์จากสาหร่าย สไปรูลิน่า สาหร่ายไก ต่อไป

บทคัดย่อ

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อสกัดและประเมินคุณภาพตำรับผลิตภัณฑ์ครีมที่มีส่วนผสมสารสกัดหยาบโพลีแซคคาไรด์จากสาหร่าย สไปรูลิน่า สาหร่ายไก และ เทาน้ำ เพื่อทดสอบการให้ความชุ่มชื้นเปรียบเทียบกับผลิตภัณฑ์พื้นที่ไม่มีสารสกัดเป็นองค์ประกอบ และทดสอบความปลอดภัยและประสิทธิภาพของสารสกัดหยาบโพลีแซคคาไรด์จากสาหร่าย สไปรูลิน่า สาหร่ายไก และ เทาน้ำ ได้ทดลอง ตั้งตำรับครีม 2 ตำรับ ผสมกับสารสกัดที่ความเข้มข้นร้อยละ 0.3, 0.5 โดยน้ำหนักในสูตรตำรับ และผลิตภัณฑ์พื้นที่ไม่มีสารสกัด แล้วทดลองความคงตัวที่อุณหภูมิห้อง (25± 0.5 องศาเซลเซียส) เป็นเวลา 1 เดือน พบว่าตำรับครีมให้ความคงตัวที่ดี จึงได้นำตำรับครีมมาทดสอบวามปลอดภัยและประสิทธิภาพผลของการให้ความชุ่มชื้นของครีมตัวอย่างในอาสาสมัคร 30 คน และประเมินผลโดยใช้สถิติ Paired samples test โดยวัดความชุ่มชื้นของผิวด้วยเครื่องคอรนีโอมีเตอร์ พบว่าครีมที่ผสมสารสกัดหยาบโพลีแซคคาไรด์จากสาหร่าย สไปรูลิน่า สาหร่ายไก และ เทาน้ำ 0.3, 0.5 โดยน้ำหนักในสูตรให้ความชุ่มชื้นได้มากกว่าผลิตภัณฑ์พื้นที่ไม่มีสารสกัดอย่างมีนัยสำคัญ ด้วยค่าความเชื่อมันร้อยละ 95

คำสำคัญ: สไปรูลิน่า, สาหร่ายไก, เทาน้ำ, ความชุ่มชื้น

ABSTRACT

The objective of this study was to extract and evaluate the safety and efficacy of base cream formulations containing crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. extract for moisturizing effect on skin comparing to the base cream formulation. Safety and efficacy activity of the extract in volunteers was also investigated. In the experiment two formulations of cream contained crude extract of 0.3, 0.5% w/w in the formula and base cream were prepared and kept under room temperature (25±0.5°C) for one month before tested. The result shown that the cream contained crude extract preparation showed the physical stability. So, further investigation was applied for safety and efficacy test. For safety test, the crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. extract were not presented any irritation on the skin. For performance test of the cream preparation for moisturization by Corneometer® CM 825 with 30 volunteers and evaluated by Paired samples test. The result shown that the cream preparation containing the 0.3% 0.5%w/w in the formula crude polysaccharide extract exhibited moisturizing effect significantly with respectively $p\text{-value} < 0.05$ within 95 % of the 100 confidence intervals when compared with base cream.

Key words: *Spirulina* spp., *Cladophora glomerata*, *Spirogyra* spp., Moisturizer

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CHAPTER 1

INTRODUCTION

1.1 Statement and significant of problem

Humectants are also found in many cosmetic products where moisturization is desired, including treatments such as moisturizing hair conditioners and also commonly used in body lotions. A wide variety of humectants are used in cosmetics including polyhydric alcohols like glycerin, propylene glycol, sorbitol, and including the main component of Natural Moisturizing Factor(NMF), pyrrolidonecarbonate and lactates. Humectants play as important role in cosmetics but at the same time they also work to maintain the moisture content and stabilize the cosmetic itself. In addition, they also have bacteriostatic and fixative activities.[1]

Hydrogels have been of great interest to biomaterial scientists for many years. Hydrogels are hydrophilic polymer networks which may absorb from 10–20% (an arbitrary lower limit) up to thousands of times their dry weight in water. Hydrogels have been of great interest to biomaterial scientists for many years. Among the numerous polymers that have been proposed for the preparation of hydrogels, polysaccharides have a number of advantages over the synthetic polymers which were initially employed in the field of pharmaceutics. Polysaccharides are usually non-toxic, biocompatible and show a number of peculiar physico-chemical properties that make them suitable for different applications in drug delivery systems.[2-4]

Spirulina is one of the most concentrated natural sources of nutrition known. It contains all the essential amino acids, is rich in chlorophyll, beta-carotene and its co-factors, and other natural phytochemicals. Spirulina is the only green food rich in GLA essential fatty acid. GLA stimulates growth in some animals and makes skin and hair shiny and soft yet more durable. GLA also acts as an anti-inflammatory, sometimes alleviating symptoms of arthritic conditions.

Spirulina acts as a functional food, feeding beneficial intestinal flora, especially Lactobacillus and Bifidus. Maintaining a healthy population of these bacteria in the intestine reduces potential problems from opportunistic pathogens like *E. coli* and *Candida albicans*.

Spirulina is gaining more attention from medical scientists as a nutraceutical and source of potential pharmaceuticals. There are several new peer reviewed scientific studies about Spirulina's ability to inhibit viral replication, strengthen both the cellular and humoral arms of the immune system and cause regression and inhibition of cancers.

In April 1996, scientists from the Laboratory of Viral Pathogenesis, Dana-Farber Cancer Institute and Harvard Medical School and Earthrise Farms, Calipatria, California, announced on-going research, saying, "Water extract of Spirulina platensis inhibits HIV-1 replication in human derived T-cell lines and in human peripheral blood mononuclear cells. A concentration of 5-10 mg/ml was found to reduce viral production." HIV-1 is the AIDS virus. Small amounts of Spirulina extract reduced viral replication while higher concentrations totally stopped its reproduction. Importantly, with the therapeutic index of >100, Spirulina extract was non-toxic to human cells at concentrations stopping viral replication.[5]

The study of Toshimitsu Hayashi and Kyoko Hayashit (1996) show that "bioactivity-directed fractionation of a hot H2O extract from a blue-green alga Spirulina platensis led to the isolation of a novel sulfated polysaccharide named calcium spirulan (Ca-SP) as an antiviral principle. This polysaccharide was composed of rhamnose, ribose, mannose, fructose, galactose, xylose, glucose, glucuronic acid, galacturonic acid, sulfate, and calcium. Ca-SP was found to inhibit the replication of several enveloped viruses, including Herpes simplex virus type 1, human cytomegalovirus, measles virus, mumps virus, influenza A virus, and HIV-1. It was revealed that Ca-SP selectively inhibited the penetration of virus into host cells. Retention of molecular conformation by chelation of calcium ion with sulfate groups was suggested to be indispensable to its antiviral effect.[6]

Spirulina spp. products are widely adopted as ingredients in making natural cosmetics, skin and hair care products for people with normal skin, dried skin, damaged skin and aging skin, and hair damaged, scalp protection in multiple applications such as astringents, elasticity, emollient, essential fatty acids, free radicals scavenging and humectants.

Cladophora glomerata is freshwater macro alga belonging to the division Chlorophyta, this specie naturally grown together in the Nan and Kong rivers. They are edible macro alga so called "Gai" by local people in Nan Province, Thailand. *C.glomerata* contains abundant proteins and fibers, and can be valuable sources of vitamins. This specie of alga are believed to offer many important health benefits; for instance, rejuvenating, promoting appetite, and remedying many common maladies.[7]

Spirogyra spp. is a genus of filamentous green alga of the order Zygnematales. The cell wall has two layers: the outer wall is composed of cellulose while the inner wall is of pectin. The cytoplasm forms a thin lining between the cell wall and the large vacuole it surrounds.[7]

The present work was focused on moisturizing capacity on the skin from crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata and Spirogyra* spp. which using as moisturizing substance in the cosmetic product.

1.2 Objectives

1) To extract crude polysaccharide from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.
2) To evaluate the moisture holding capacity on the skin of crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

1.3 Scope of study

1) Extraction crude polysaccharide from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

2) Safety and efficacy tests of crude polysaccharide extracted by using Corneometer®CM825.

1.4 Expected outcome of study

Spirulina spp., *Cladophora glomerata* and *Spirogyra* spp. contain a lot of nutrient which using as food for human being for a long time but rarely use in cosmetic product. The data obtained in this study could consequently be extended to the benefit from above algae especially for value adding to *Cladophora glomerata* and *Spirogyra* spp. which are the local algae in the north part of Thailand.

1.5 The main question of research

1. *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. have high quantity of polysaccharide.
2. The polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. have moisture holding capacity that effect to skin surface hydration and can be used for skin care products.
3. The polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. exhibit safety and has high efficacy when apply to be the moisturizing agent in cosmetic products.

1.6 Period of Study

Procedure	เดือน											
	1	2	3	4	5	6	7	8	9	10	11	12
1. Collected samples, dried and extracted with hot deionized water	←	—	→									
2. Safety and Efficacy testing				←	—	—	—	—	→			
3.Research summarization and report writing										←	—	→

1.7 Keywords: *Spirulina* spp., *Cladophora glomerata*, *Spirogyra* spp., Moisturizer

1.8 Researchers and Advisory Research

1) Head of research (80%)
   Miss Mayuramas Sang-ngern
2) The joint research project (20%)
   Mr. Jarupux Saansomchai

CHAPTER 2

LITERATURE REVIEW

A moisturizer is an agent designed to make the stratum corneum softer and more pliant by increasing its hydration. With the realization that the stratum corneum is a dynamic and interactive tissue, new emphasis has been placed on agents that are used to moisturize the skin; these are suitably termed moisturizers. Their multiplicity and potential effects, including barrier functions, trans epidermal water loss, and the exogenous or endogenous offenders that result in dry, scaly skin, parallel the increased understanding of the stratum corneum. Moisturizers are a group of cosmetic products designed for skin care and hygiene. Moisturizers have come under intense scrutiny in recent years regarding their therapeutic effects. They possibly are the most prescribed products in dermatology, and, until recently, dermatologists have received little or no training regarding these products, including their ingredients, pharmacokinetics, benefits, and toxicities.

Moisturizers, at times, are referred to as humectants, emollients, lubricants, oils, and greases; however, these terms are not interchangeable. Each term has a specific definition. The *Webster's Collegiate Dictionary of the English Language* defines these terms as follows:

- Moisturizer - A substance that imparts or restores moisture to (something); to supply moisture
- Humectant - A substance, such as glycerin, that absorbs or helps another substance retain moisture
- Emollient - A substance that makes something soft or supple; also, soothing especially to the skin or the mucous membrane
- Grease - Rendered animal fat; a thick lubricant; oily matter
- Lubricant - A substance, such as grease, that is capable of reducing friction, heat, and wear when introduced as a film between solid surfaces; something that lessens or prevents friction of difficulty

The extracellular membrane of the stratum corneum is composed of mainly ceramides, which constitute about 40% of lipid content, cholesterol (25%), and free fatty acids (10-15%), followed by smaller amounts of triglycerides, stearyl esters, and cholesterol sulfate. These lipids are synthesized throughout the epidermis where they are packaged in lamellar granules and subsequently undergo differentiation. In the lamellar granules, the lipids are stacked and flattened into lipid vesicles. After extrusion to the extracellular space, these flattened vesicles undergo rearrangement to form broad, multilamellar sheets.

The stratum corneum possesses approximately 30% water, which is mainly associated with its elasticity. Ten percent of the water is bound to lipids, and, the remaining 20%, which is resistant to solvent and water extraction, may be secondary to keratin components. The innermost layers of the stratum corneum contain a high level of water, while the outermost layers of the stratum corneum contain a water level largely dependent on the ambient relative humidity.

A healthy stratum corneum contains about 10% tightly held water. The tightly bound water is closely dependent on the presence of Natural Moisturizing Factor(NMF). NMF is a complex interaction of various substances, such as humectants, that are hygroscopic(ie, they attract water molecu). les from the environment). Data show that the water content of the stratum corneum increases with increasing relative humidity. The presence of NMF tends to increase the water content of the stratum corneum whenever the ambient relative humidity exceeds about 40-50%. The increased corneal thickness is about 10-15% when the stratum corneum is allowed to equilibrate in vitro from 0% relative humidity to 60%.[8]

2.1 Types of moisturizers

Moisturizers

Information on moisturizers has exponentially increased in recent years. Their structure and function are surprisingly complex and sophisticated; many are equidistant between cosmetics and drugs. Moisturizers of the new millennium include agents that mimic natural ingredients and function as botanicals, including vitamins,

hydroxy acids, and retinoids. Other common ingredients are collagen, elastin, DNA, ribonucleic acid (RNA), lecithin, sodium hyaluronate, sodium passive cutaneous anaphylaxis (PCA), and ceramides.

Disruption of intercellular lipid lamellae in the upper layers of the stratum corneum results in abnormal desquamation and an increase in TEWL. A simple explanation of a moisturizer's mechanism states that water, which otherwise would have been lost, is held by hygroscopic properties in the stratum corneum. Subsequently, this contributes to the smoothing of the skin surface due to swelling of the outer layers. Leveque [8] showed that occlusion of the skin by products, such as petrolatum, paraffin, waxes, or greases, restores and enhances the natural diffusion of moisture from the dermal capillary beds. Downing and colleagues indicated that properly functioning intercellular lipids trapped and redistributed water effectively throughout the epithelium.

Moisturizers impart a temporary barrier to damaged stratum corneum, which allows time for reparation of this layer. Two concepts have been proposed to explain water passage through the skin. First, the solubility-diffusion model postulates that water has a finite solubility in lipids; therefore, it can permeate through lipids in accordance with the accepted theory of P = KD/d. In this model, the water molecules are moving through the lipid barrier as individual entities or 1 molecule at a time. This model is probably the best description of transepidermal water loss through the stratum corneum. The second model postulates that water passes through lipids through transient pores or water-filled channels. The evidence for the existence of such channels or pores is inconclusive in the case of most biomembranes and especially for the stratum corneum.[8]

Humectants

Humectants are substances that attract water when applied to the skin. The source of the water is transepidermal, unless the relative humidity is very high (>80%). Humectants can also increase transepidermal water loss (TEWL). At times, this can lead to a perception of skin tightness or dryness. Examples of humectants include glycerin, sorbitol, urea, alpha hydroxy acids (AHAs), and sugars. Lactic acid,

particularly the salt form ammonium lactate, has demonstrated an ability to reduce the thickened stratum corneum of xerosis as well as to remove and clear the thick scales in ichthyosis and other hyperkeratotic conditions.

Occlusion

Lanolin was the first substance to be used in an occlusive system. Its use as a barrier has been known for thousands of years. Similar to other moisturizers, lanolin functionality was thought to be occlusive; therefore, lanolin prevents the loss of water. Today, other functions of lanolin are known as well. It has the reputation of being a contact sensitizer; however, this is controversial. Because of this reputation, petrolatum is now the principal ingredient used in occlusive formulations. Depending on the concentration, petrolatum physically blocks the surface of the stratum corneum and reduces transepidermal water loss. This increases the water content in the stratum corneum, thus producing a state of hydration.

Two reasons exist as to why occlusion is one of the best treatments of dry skin. First, transepidermal water is the most effective source of water (water added to the skin evaporates in 10-20 min). Second, these occlusive agents have an emollient effect.

Natural moisturizing factor

NMF is a combination of several low molecular weight substances. These substances include amino acids, pyrrolidone carboxylic acid, lactate, urea, ammonia, uric acid, glucosamine, creatinine, citrate, sodium, potassium, calcium, magnesium, phosphate, chlorine, sugar, organic acids, peptides, and other unidentified substances. Many of these substances are added to moisturizers to help its hygroscopic properties, too much of these substances cause irritation. For example, lactic acid and propylene glycol also act as exfoliants, and urea leads to dehiscence of corneocytes and contains broad-spectrum antibacterial properties. Thus, maintenance and hydration of the stratum corneum is the result of a multifaceted masterpiece.

Emollients

Emollients fill the spaces between the corneocytes, thus providing therapeutic improvement to defects in desquamation. Emollients function in smoothing the roughened skin, changing the skin's appearance, lubricating, replacing natural skin lipids, and providing occlusion. Emollients are composed of water in oil emulsions; thus, oil is the largest component, which ranges from 3-25%. The concentration of oil in emollients is important for easier spreading and for the degree of occlusion that is desired.

Emollients with low spreading value are most often used for night and facial creams, around eye wrinkles, and in cosmetics. They include castor oil, almond oil, and oleyl oleate. Emollients with medium spreading value are most often used in day and sun protection creams and oils. They include octyl dodecanol, hexyl decanol, oleyl alcohol, and decyl oleate. Emollients with high spreading value are most often used in body lotions, hand creams and lotions, and bath additives. They include isopropyl stearate, isopropyl palmitate, isopropyl myristate, hexyl laureate, and dioctyl cyclohexane.[8]

2.2 Action of Moisturizers on the Skin [9]

The urge to apply oils to the skin is almost intuitive and may be as old as mankind itself. The term "emollient" implies (from the Latin derivation) a material designed to soften the skin, ie, a material that "smooths" the surface to the touch and makes it look smoother to the eye. The term "moisturizer" is often used synonymously with emollient, but the term implies the addition of water to the skin. Therefore, moisturizers usually contain humectants to enhance the water-binding capacity of the SC. Moisturizers have multifunctional effects. Desired properties include reduction of clinical signs of dryness, like scaling and roughness, and decrease in perceived feelings of tightness and itching. Likewise improvement of skin barrier function is important. Sometimes moisturizers may appear to be ineffective because they are used in insufficient quantities or contain deleterious substances.

Humectant Effects

Moisturizers increase Stratum Corneum (SC) hydration by at least two different means: a) by occlusion of the skin surface and b) by introduction of humectants, which are able to maintain the moisture in the SC. Occlusion of the surface results in reduced water loss from the outside of the skin. Hydrophobic substances (eg, lipids) in moisturizers reduce water loss, but their effect may be diminished if they are combined with other ingredients. Water contained in the applied products causes a temporary increase in skin hydration, but most of the applied water soon evaporates from the surface. On the other hand, evaporation lowers skin temperature, which may relieve pruritus. The inclusion of humectants in moisturizers is believed to amplify their hydrating power. Humectants widely used are urea, pyrrolidone carboxylic acid (PCA), lactic acid, glycerin, panthenol, and sorbitol (**Table 2.1**). Which of these substances most efficiently increases skin hydration is not known. Besides differences in water-binding capacity, their absorption into the skin is important for their effect. The beneficial effect of humectants on dry skin has been demonstrated in several vehicle-controlled clinical studies. Another proposed effect of humectants is their influence on the crystalline arrangement of the bilayer lipids. In dry skin, the proportion of lipids in the solid state may be increased. Humectants may then help to maintain lipids in a liquid crystalline state at low relative humidity. For example, glycerin has been shown to maintain the liquid crystalline state of model lipids in low humidity conditions. Glycerin has also been proposed to aid the digestion of superficial desmosomes in subjects with dry skin and thereby ameliorate flakiness. Furthermore, α-hydroxy acids such as lactic acid might be useful in moisturizers because of their influence on SC elasticity.

Table 2.1 The moisture biding ability (%) different humectants

Humectants	Humidity 58-60%
Sodium-lactate	66
Sodium-PCA	61-63
Glycerin	35-38
Propylene glycol	32
Sorbitol	10
PCA	<1

2.3 *Spirulina* spp.

Spirulina spp. is the common name for human and animal food supplements similar to Chlorella and Aphanizomenon flos-aquae (Blue Green Algae.) Genus belongs to *Arthrospira* and Phormidiaceae (family). *Spirulina* spp. is specie naturally grown in generally. Spirulina contains an unusually high amount of protein, between 50%w/w and 70%w/w by dry weight and carbohydrate about 15.25%w/w and 25%w/w depend on the source as shown in **Table 2.2.** It is a complete protein, containing all essential amino acids, though with reduced amounts of methionine, cysteine, and lysine when compared to the proteins of meat, eggs, and milk. It is however, superior to typical plant protein, such as that from legumes. Spirulina has only one deficiency and that is in its carbohydrate content. The body could convert its high protein content to energy supplying carbohydrates, but harm would result and could not be recommended. While it is a complete food, in that it contains all the life sustaining nutrients it needs an added ingredient to make it a complete beneficial food, carbohydrates.[10] Spirulina extract inhibits HIV-1 replication in human lines, peripheral blood mononuclear cells (PBMC), and Langerhans cells (LC). A 2007 study found that 36 volunteers taking 4.5 grams of spirulina per day, over a six week period, exhibited significant changes in cholesterol and blood pressure: (1) lowered total cholesterol; (2) increased HDL cholesterol; (3) lowered triglycerides; and (4) lowered systolic and diastolic blood pressure. In cosmetic, spirulina is claimed in market that these used in hair care product and found that there cans antioxidant capacity.[11]

Table 2.2 List of nutrition from *Spirulina* spp.[10]

Nutrition	Amount (w/100 g dry weight)
Protein	50%-70%
Carbohydrate	15.25%-25%
Fat	5.6%-7%
Vitamin	Vitamin A, Vitamin B1, B2, B6, B12 and Vitamin E

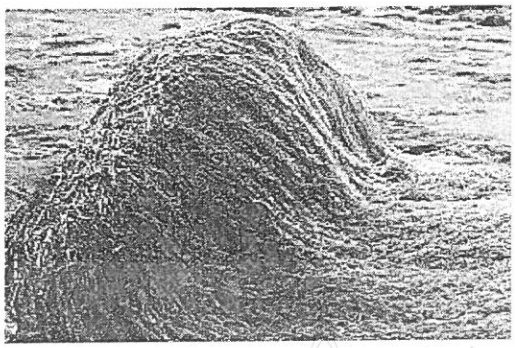

Figure 2.1 *Spirulina* spp. is blue green algae.[12]

2.4 *Cladophora glomerata*

Cladophora glomerata and *Microspora floccose* are freshwater macroalgae belonging to the Division Chlorophyta. These two species are naturally grown together in the Nan and Kong rivers. They are edible macroalgae so called "Gai" by local people in Nan Province, Thailand. Both of these algae have been used as a food source for centuries in local cultures, but only in the last few years they have become increasingly popular as food products marketed to tourists. *C.glomerata* and *M. floccosa* contain abundant proteins and fibers, and can be valuable sources of vitamins as shown in **Table 2.3**. Besides being one of the most popular food sources, these two species of algae are believed to offer many important health benefits; for instance, rejuvenating, promoting appetite, and remedying many common maladies. A small number of algae are highly toxic when consumed. Although *C. glomerata* has long been eaten and is highly nutritious, an evaluation of the safety of these algae, based on scientific studies, has not been published.[13] The antioxidant activity, marine macroalgae species (17 Chlorophyta, 8 Phaeophyta and 23 Rhodophyta) from the coasts of Yucatan and Quintana Roo (Mexico) were evaluated for antioxidant activity. The antioxidant activity was measured with the DPPH method.[14]

Table 2.3 List of nutrition from *Cladophora glomerata* [7]

Nutrition	Amount (w/100 g dry weight)
Protein	20.60
Carbohydrate	31.25
Fat	6.14
Moisture	6.61
Fiber	21.20
Ash	14.20

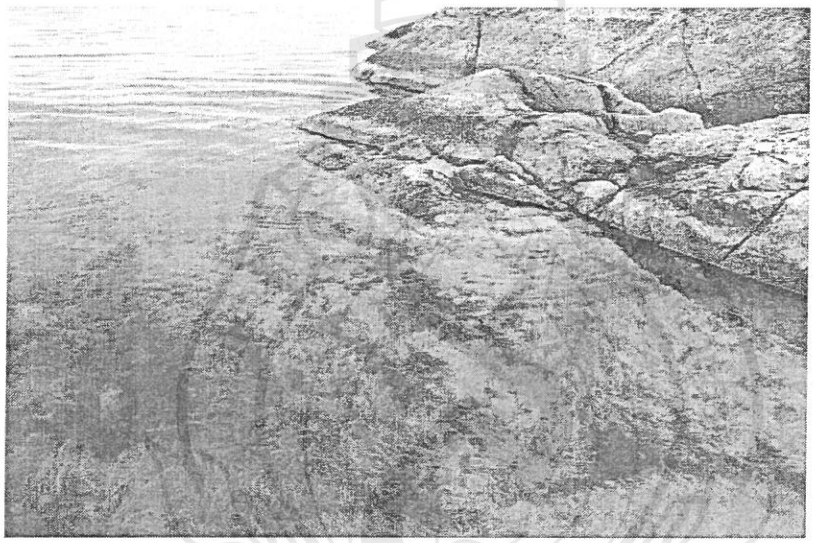

Figure 2.2 *Cladophora glomerata* is green algae.[15]

2.5 *Spirogyra* spp.

Spirogyra spp. is a genus of filamentous green algae of the order Zygnematales. *Spirogyra* spp. commonly found in northern Thailand especially in Mekong River, Chiang- Rai and Nan River. It is used by local people as favorite local food. *Spirogyra* spp. has highly nutritious as shown in **Table 2.4.** There are more than 400 species of *Spirogyra* spp. in the world. The cell wall has two layers: the outer wall is composed of cellulose while the inner wall is of pectin. The cytoplasm forms a thin lining between the cell wall and the large vacuole it surrounds. In this alga, it has research

about food ability of the inactive biomass to remove reactive dye from multi component textile wastewater. *Spirogyra* spp. was antioxidant property.[16]

Table 2.4 List of nutrition from *Spirogyra* spp.[16]

Nutrition	Amount (w/100 g dry weight)
Protein	18.65
Carbohydrate	56.31
Fat	5.21
Moisture	8.05
Fiber	7.66
Ash	11.75

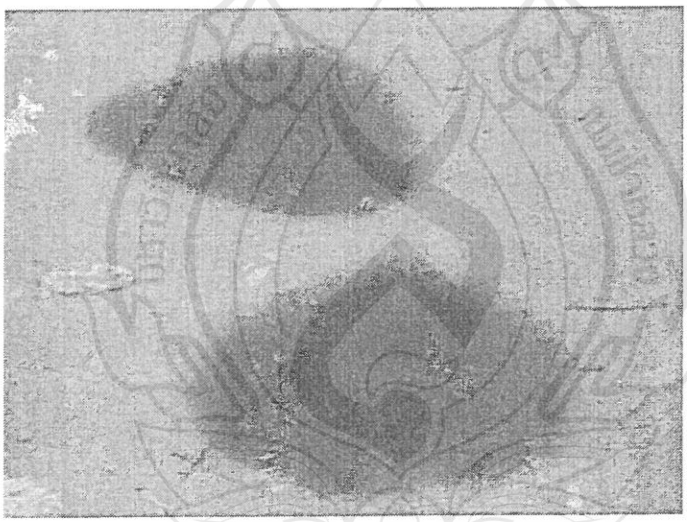

Figure 2.3 *Spirogyra* spp. is green algae.

2.6 Corneometer®CM 825

Since 1980 the Corneometer®CM 5825 has provided a well-established method to determine reproducibly and accurately the hydration level of the skin surface. This is documented by the numerous mentions in dermatology and cosmetology literature in which the terms "corneometry" and skin hydration measurements are inseparable.

The measuring principle of the Corneometer is based on capacitance measurement of a dielectric medium. Any change in the dielectric constant due to skin surface hydration variation alters the capacitance of a precision measuring capacitor. One of the greatest advantages of the capacitance measurement method, compared to other measurement methods, is the fact that products applied to the skin only have minimal influence on the measurements. The measurement can detect even slightest changes in the hydration level. The reproducibility of the measurement is very high and the measurement time is very short (1 s). Due to the construction of the measuring head, the measurement depth is very small (in the first 10-20 μm).

The modern, high quality electronics of the probe provide temperature stability and exclude interference of the base capacity and power supply fluctuations with the measurement. A spring in the probe head ensures constant pressure on the skin, enabling exact, reproducible measurements which do not influence the skin. The low weight of the probe and the small measuring surface allow easy handling, measurements on all body sites and simple cleaning after the measurement. Thus the probe is completely self contained and can be connected to different device types. It is also a significant advantage for quick and easy servicing of the probe.[17]

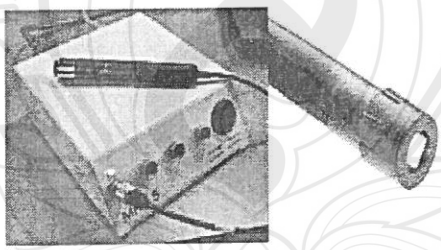

Figure 2.4 Corneometer®CM 825 use to determine reproducibly and accurately the hydration level of the skin surface.[17]

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and equipments

A. Equipments

- Centrifuge : Hettich/ Universal 32
- Corneometer : Corneometer®MPA 825
- Desiccators : C-3W Sanplatec
- Drying oven : Contherm/ Thermotech 2000 oven
- Extraction cups : Foss Tecator
- Extraction thimbles : Foss Tecator
- Freeze dry : Helo/ Drywinner8, FD8-55
- Homogenizer : IKA/T25D, Germany
- Hot plate : LMS HTS-1003-SP
- Kjeldahl digestion and distillation system : Foss Tecator
- Muffle furnace : Cabolite/ CWF 13/13/2416
- Refrigerator : SJ-W40J
- Soxhlet extractor : Foss Tecator, Sweden

B. Chemicals

- Benedict solution	: Merk, Germany
- Boric acid	:Carloerba/Reagents,Thailand
- Bromocresol green	: Fisher Scientific, India
- Distil deionized water	: Laboratory S4
	: Mallinckrodt Chemicals
- Ethanol, 95%	Baker, Malaysia
- Hydrochloric acid Methyl red	: RFCL limited, India
- Lugol's iodine solution	: Merk, Germany
- Methyl red	: RFCL limited, India
- n-octanol	: Sigma, Germany
- Petroleum ether or ethyl ether	: Food Laboratory
- Sodium hydroxide	: Merk, Germany
- Sulfuric acid	: Labsca, Thailand
- Tris(hydroxymethyl) aminomethane	: Ajax, Finechem, Australia

C. Natural material

- *Spirulina* spp. from Green Diamond Company, Chiang Mai, Thailand
- *Cladophora glomerata* from Khong River, Cheang Rai Province, Thailand
- *Spirogyra* spp. from Phrae Province, Thailand

3.2 Determination of chemical and physical composition from *Spirulona* spp., *Cladophora glomerata* and *Spirogyra* spp.[18]

3.2.1 Determination of moisture content

The each test was blended by the blender. The moisture can was weighed and recorded. One gram of those algae was placed in each moisture can and recorded the total weight. The can containing sample was placed in an oven at 103±2°C for 16-18 hrs (Open cover to allow water loss) and removed from an oven. Then, each can was stored in desiccators until cool down. The can containing sample was weighed and recorded. Each sample was done in triplicate.

3.2.2 Determination of ash content

Each fresh sample was blended by blender. The firstly, crucible was weighted and recorded. The secondly, one gram of each algae was placed in the crucible and recorded the total weight. The thirdly, crucible with sample was evaporated on hot plate. The fourthly, crucible with sample was transfer to muffle furnace, at 525°C for 4 hrs. Finally, crucible and sample was stored in desiccators until cool down. Sample with moisture can was weighed and recorded. Each sample was done in triplicate.

3.2.3 Determination of fat content

The test sample was blended by blender. One gram of those algae was placed in weight paper and placed in the thimble. The sample with thimble is placed in the Soxhlet extractor. Seventy milliliters of petroleum ether was put by using dispenser. The cup was heated in an oven at 105°C for 2 hrs. Cup and sample was stored in desiccator until cool down. Sample with cup was weighed and recorded. Each sample was done in triplicate.

3.2.4 Determination of protein content by Kjeldahl method

Digestion: Firstly, digestion block was heated to 420°C. One gram of test samples was placed in digestion tube and recorded the total weight. The secondly, placed 5.00 g of catalysts in digestion tube. The thirdly, 12 ml of concentrated H_2SO_4 were added into each tube. The fourthly, rack of digestion tubes were placed on digestion block. The system was turned on. Finally, those samples were digested until completion. The samples should be clear with no charred material remaining.

Distillation: Distillation system was start up. Receiving flasks were placed on distillation system. 25 milliliters of boric acid solution was dispensed in the flask. Each sample was done in triplicate.

Titration: 4-5 drops of methyl red indicator were added in to Erlenmeyer flasks. Each sample was titrated.

The Kjeldahl method and nitrogen combustion method for protein analysis are based on nitrogen determination. Both methods are official for the purposes of nutrition labeling of foods.

3.2.5 Determination of crude fiber

Each fresh sample was ground with mortar. 1 gram of each sample was placed in crucible. Firstly, crucibles were placed in the Fibertec cold extraction unit. 25 milliliters of acetone were filled in crucible. Then, leave for 10 min and filter. The secondly, crucibles were washed with water. Crucibles were placed in the Fibertec hot extraction unit. 105 milliliters of hot 1.25%w/v H_2SO_4 were added in to crucible. 4 drop of n-octanol were added and heated to boiling for 30 min. The thirdly, crucibles were flitted. The crucibles were washed three times with hot distilled water. 150 milliliters 1.25 % w/v hot sodium hydroxide was added and performs as above steps. Crucibles were placed in the Fibertec cold extraction unit. 25 milliliters acetones were added in to crucible for 10 min. Crucibles were filtrated and evaporated solvent. Finally, crucibles were dried at 130°C for 2 hrs. Crucibles and samples were stored in desiccators until cool down. Samples with crucibles were weighed and recorded. Each sample was done in triplicate.

3.2.6 Determination of carbohydrate content from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

The values of moisture, ash, fat, protein and crude fibers were calculated carbohydrate content. From the equation bellow:

Carbohydrate content (%w/w) = 100 - (% Moisture +% Ash+% Protein +% Fat +% Fiber)

3.3 Crude polysaccharide Extraction

The extraction method was modified by method of Marlène Godard et al.[19] The 150 g dried weigh of each sample *(Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.) was extracted by boiling with shacking with 500 ml of distilled water at 60°C for 6 hours. After cooling down at room temperature, each sample was filled through gauze and then centrifuged at 5000 rpm, 25°C for 60 min. The supernatant of each sample was extracted three times with ethanol (Ratio; Sample:EtOH, 1:3, v/v) and kept at 4°C for 24 hours. The precipitate was collected and dried.

3.4 Base cream and test sample preparation

Ingredients	%w/w
Active ingredients (*Spirulina spp., Cladophora glomerata* and *Spirogyra* spp.)	*
Polycrylamide and Laureth-7 (Emulbase®)	5.0
Hydrogenated polydecene (Cremaflow®)	5.0
Propylene glycol and diazolidinyl urea and iodopropynyl Butylcarbamate (Liquid Germall Plus®)	0.5
Perfume	q.s.
Deionized water	q.s. 100

* The concentrations of algae in the test sample were 0.1%w/w, 0.2%w/w and 0.3%w/w, respectively.

Procedure

1. The cremalflow® and Emulbase® was added together and then mixed until homogeneous.
2. Add the deionized water to the mixture and then mixed until homogeneous.
3. Add the extracted and stirred until the emulsion forming by using homogenizer.
4. The preservative (Liquid Germall Plus®) and perfume were added at the final step.
5. The formula of test samples was kept at room temperature for 1 month physical property observation.

3.5 Evaluate safety and efficacy test

The Permission for clinical trial was obtained from ethic committee of Mae Fah Luang University and complied with the Declaration of Helsinki before the evaluation of safety and efficacy testing. The safety testing was tested before the efficacy testing by observed the results which occurred at 72 to 96 hours after initial placement of the patch tests. For efficacy testing, a total of 30 healthy female subject's age between 20-60 years old having dry participated in this study after having given their informed

consent. They were instructed not to apply any topical products. Prior to all measurements, volunteers were left in the room for at least 30 min in order to allow full skin's adaptation to the room's temperature (20 ± 2°C) and humidity (45–60%). Volunteers were marked on forearms (As shown in **Figure 3.1**) then each sample was applied on standardized conditions 2 μl/cm^2. After that, volunteers were measured at T_0, T_1, T_3 and T_6 (measured at 0, 1, 3 and 6 hours after applied). The determination of moisture holding capacity on the skin was determined by using Corneometer® CM 825.

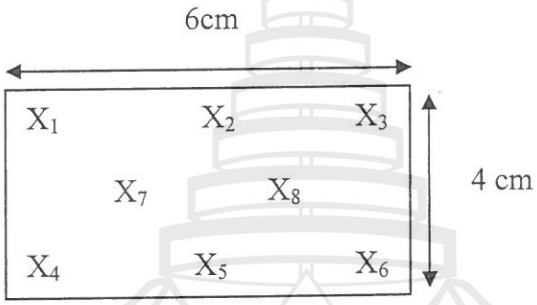

Figure 3.1 The marker for the efficacy testing.

X_1: The concentration of *Spirulina* spp.at 0.3% w/w
X_2: The concentration of *Spirulina* spp.at 0.5% w/w
X_3: The concentration of *Cladophora glomerata* at 0.3% w/w
X_4: The concentration of *Cladophora glomerata* at 0.5% w/w
X_5: The concentration of *Spirogyra* spp.at 0.3% w/w
X_6: The concentration of *Spirogyra* spp. at 0.5% w/w
X_7: Base cream
X_8: The position using for checking pH of the skin

3.6 Statistical analysis

The capacity to contain the moisture on the skin of the crude polysaccharide extracted was carried out using paired samples T test. The data was calculated the standard deviation and standard error of mean (SEM) from the equation bellow: [20]

Standard deviation (SD) by

$$S = \sqrt{\frac{\sum(X-\overline{X})^2}{n-1}}$$

Where: S is standard deviation,
$\sum$ is sigma, which told you to find the sum of what follows.
X is each individual score,
$\overline{X}$ is the mean of all the score,
n is the sample size

Standard error of mean (SEM) by

$$SE_{\bar{X}} = \frac{S}{\sqrt{n}}$$

Where: $SE_{\bar{x}}$ is standard error of mean,
S is the sample standard deviation,
n is the size (number of observations) of the sample.

After that, the data was calculated the %variation that it means a change within a population. It can calculate from the equation bellow:

$$\% \text{ variation} = \left(\frac{(Tn - T_0)}{Tn}\right) * 100$$

Where: Tn is the mean of time measurement (n= 0, 1, 3 and 6),
T_0 is the mean of initial time

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Chemical and physical composition of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

Table 4.1 Chemical composition of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

Sample	Ash (%w/w)	Fat (%w/w)	Protein (%w/w)	Fiber (%w/w)	Moisture (%w/w)	Carbohydrate (%w/w)
Spirulina spp.	10.25	1.27	76.98	1.19	1.51	**8.80**
Cladophora glomerata	27.45	2.58	22.92	14.28	3.44	**29.33**
Spirogyra spp.	16.20	1.32	24.85	6.94	2.30	**48.39**

4.2 Crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

Table 4.2 The percent yield of crude polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

Sample	Crude extract (g)	% yield
Spirulina spp.	14.37	9.58
Cladophora glomerata	13.10	8.7
Spirogyra spp.	4.23	2.82

From **Table 4.1 and 4.2,** Show the percentage yield of carbohydrate content and polysaccharide extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp., respectively. The carbohydrate content from *Spirulina* spp. was 8.80%

which is the smallness when compare to the carbohydrate content from *Cladophora glomerata* and *Spirogyra* spp. which were 29.33 and 48.39, respectively. The percentage yield of polysaccharide from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. were 9.58, 8.73, and 2.82, respectively. The percent yield of extracted depend on many factor such as method of extraction, source of the raw material or solvent, etc. The further study should be concern with the factor effecting percent yield of extraction for receiving the highest yield of the crude polysaccharide extraction.

4.3 Evaluate safety and efficacy test

From this research, O/W formula was selected as base cream because crude polysaccharides extract from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. were soluble in water. The efficacy of moisture capacity from those three algae was tested at concentration 0.3, 0.5%w/w in the formula. The samples were kept at room temperature for 1 month. It was suitable for using these formulas as base cream for the test. The resulting of polysaccharide is usually non-toxic, biocompatible and show a number of peculiar physico-chemical properties that make them suitable for different applications in drug delivery systems.

4.3.1. pH value of samples

The pH value of base cream for using in the formulation of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. were 6.24, 5.95 and 6.17, respectively. The pH value at concentration 0.3%w/w and 0.5%w/w of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. were observed at 5.5 to 6.5 as shown in **Table 4.3.**

Table 4.3 pH value of the formula at concentration 0.3 and 0.5%w/w of *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.

Concentration (%w/w)	pH value		
	Spirulina spp.	*Cladophora glomerata*	*Spirogyra* spp.
0.3	6.19	6.16	6.26
0.5	6.22	6.43	6.14

From **Table 4.3**, the pH value of test sample (*Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp.) at concentration 0.3 and 0.5%w/w in the formula. Overall of each concentration shown pH value about 6.14 to 6.29 which equivalence to the pH value of normal human skin which is slightly acid (pH 4.5-6.0)[49]. So, it was suitable for using these formulas as cream base for the test. Moreover, polysaccharides are usually non-toxic, biocompatible and show a number of peculiar physico-chemical properties that make them suitable for different applications in drug delivery systems.[2-4]

4.3.2. Efficacy test

4.3.2.1 *Spirulina spp.*

The test samples (concentration at 0.1, 0.2 and 0.3%w/w) and base cream A were applied on the skin of volunteer. The capacities of test samples to maintain the moisture on the skin were measured by using Corneometer®CM 825 as shown in **Table 4.4.**

Table 4.4 List of moisture on the skin of volunteer after applied the test samples 1A, 2A and Base cream A at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Samples	T_0 (Mean)	T_1 (Mean)	T_3 (Mean)	T_6 (Mean)
Sample 1A*	47.91	41.27	66.86	64.89
Sample 2A**	47.09	68.35	70.55	65.52
Base cream***	43.04	68.46	60.47	54.90

*Sample 1A: Base cream A with 0.3 %w/w of *Spirulina* spp.
**Sample 2A: Base cream A with 0.5 %w/w of *Spirulina* spp.
***Base cream A: Base cream A only

Table 4.5 The percent variation of moisture on the skin of volunteer after applied the test samples 1A, 2A and Base cream A at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Spirulina spp.	Time	Product (Mean ±SEM)	% Variation of *Spirulina* spp.	Base cream (Mean ±SEM)	% Variation of Base cream	***p*-value**
0.3% w/v	T_3-T_0	18.95±2.92	28.08	17.44±2.69	27.80	0.6628
	T_6-T_0	16.97±-2.78	25.89	11.86±-2.10	20.81	0.0009
0.5% w/v	T_3-T_0	23.46±3.62	32.21	17.44±2.69	27.80	0.6772
	T_6-T_0	18.44±3.09	27.36	11.86±-2.10	20.81	0.0261

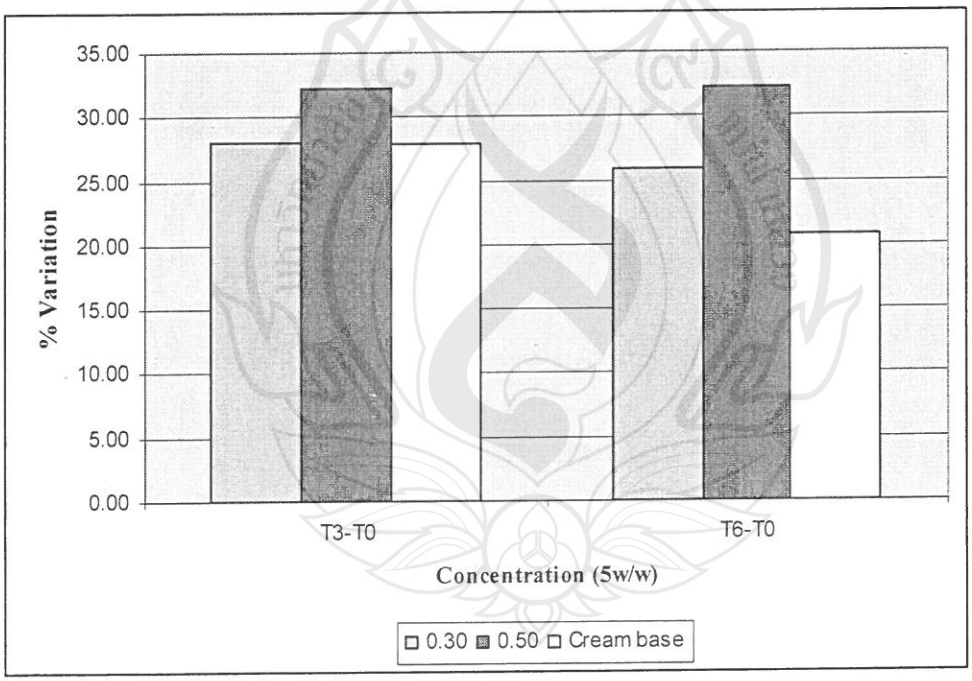

Figure 4.1 The comparison of moisture content on the skin between sample 1A and sample 2A with Base cream A after applied on skin of volunteer for 3 and 6 hours.

From **Table 4.4** show the mean value of skin moisture effect after applied on the skin of volunteers' skin for 3 and 6 hours. The comparison between base cream

and test samples after applied on the skin of volunteers for 3 hours shown that the test samples 1A and 2A exhibited increasing moisture content on the skin of volunteer with *p-value* 0.6628 and 0.6772, respectively (*p-value* < 0.05). However, the capacity to maintain the moisture on the skin of the test samples for 6 hours was decreased. Only the test sample 3A was maintained moisture on the skin at *p-value* 0.0389 as shown in **Table 4.5.**

4.3.2.2 *Cladophora glomerata*

The test samples 1B, 2B, and Base cream B were applied on the skin of volunteer. The capacities of test samples to maintain the moisture on the skin were measured by using Corneometer®CM 825 as shown in **Table 4.6.**

Table 4.6 The mean value of skin moisture of volunteers after applied the test samples 1B, 2B, and Base cream B at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Samples	T_0 (Mean)	T_1 (Mean)	T_3 (Mean)	T_6 (Mean)
Sample 1B*	44.31	68.84	65.84	66.51
Sample 2B**	41.46	74.12	64.24	60.43
Base cream***	43.04	68.46	60.47	54.90

*Sample 1B: Base cream B with 0.3 %w/w of *Cladophora glomerata*
**Sample 2B: Base cream B with 0.5 %w/w of *Cladophora glomerata*
***Base cream B: Base cream only

Table 4.7 The percent variation of moisture on the skin of volunteer after applied the test samples 1B, 2B and Base cream B at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Cladophora glomerata	Time	Product (Mean ±SEM)	% Variation of Cladophora	Base cream (Mean ±SEM)	% Variation of Base cream	***p*-value**
0.3% w/v	T_3-T_0	20.80±3.21	32.78	17.44±2.69	27.80	0.2300
	T_6-T_0	17.23±-2.94	33.15	11.86±-2.10	20.81	0.0388
0.5% w/v	T_3-T_0	28.06±4.33	35.78	17.44±2.69	27.80	0.0458
	T_6-T_0	23.54±4.00	31.28	11.86±-2.10	20.81	0.0408

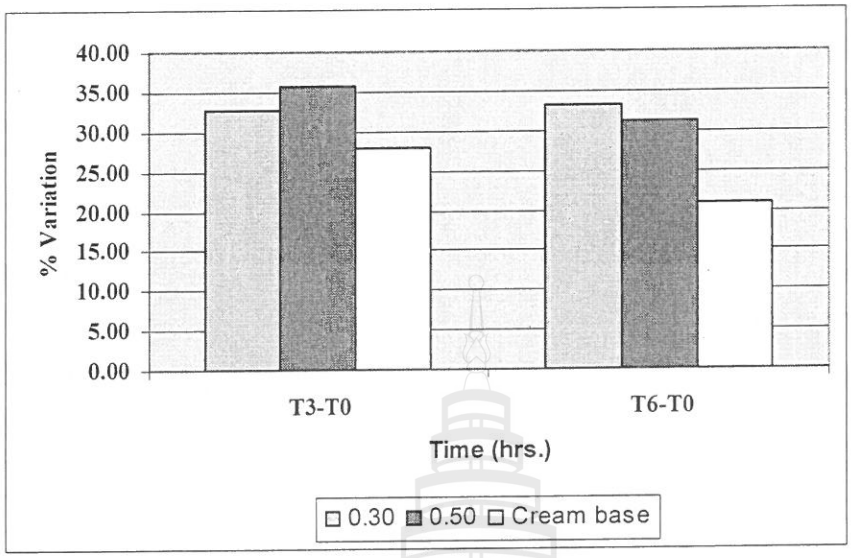

Figure 4.2 The comparison of moisture content on the skin between sample 1B, sample 2B with Base cream B after applied on skin of volunteer for 3 and 6 hours.

Table 4.6 shows the mean value of moisture content on the skin of volunteers. The comparison between base cream and test samples after applied on the skin of volunteers for 3 hours shown that the test sample 2A exhibited increasing moisture content on the skin of volunteer significantly with *p-value* 0.0458 (*p-value* < 0.05). However, the capacity to maintain the moisture on the skin of the test samples 1B and 2B after 6 hours with *p-value* 0.0388 and 0.0408, respectively. *Cladophora glomerata* at concentration 0.3%w/w exhibited increasing moisture capacity on the skin after apply more than 3 hours as shown in **Table 4.7.**

4.3.2.3 *Spirogyra* spp.

The test samples 1C, 2C and base cream C were applied on the skin of volunteer. The capacities of test samples to maintain the moisture on the skin were measured by using Corneometer®CM 825 as shown in **Table 4.8.**

Table 4.8 Moisture holding capacity on the skin of volunteer after applied the test samples 1C, 2C and base cream C at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Samples	T_0 (Mean)	T_1 (Mean)	T_3 (Mean)	T_6 (Mean)
Sample 1C*	43.75	70.41	64.55	60.98
Sample 2C**	41.25	72.64	69.31	64.79
Cream base****	43.04	68.46	60.47	54.90

*Sample 1C: Cream base C with 0.3 %w/w of *Spirogyra* spp.
**Sample 2C: Cream base C with 0.5 %w/w of *Spirogyra* spp.
***Cream base C: Cream base only

Table 4.9 The percent variation of moisture on the skin of volunteer after applied the test samples 1C, 2C and base cream C at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Spyrogyra spp	Time	Product (Mean ±SEM)	% Variation of *Spyrogyra spp*	Cream base (Mean ±SEM)	% Variation of Cream base	***p*-value**
0.3% w/v	T_3-T_0	20.80±3.21	31.38	17.44±2.69	27.80	0.9941
	T_6-T_0	17.23±-2.94	27.58	11.86±-2.10	20.81	0.4297
0.5% w/v	T_3-T_0	28.06±4.33	38.67	17.44±2.69	27.80	0.1059
	T_6-T_0	23.54±4.00	34.69	11.86±-2.10	20.81	0.0197

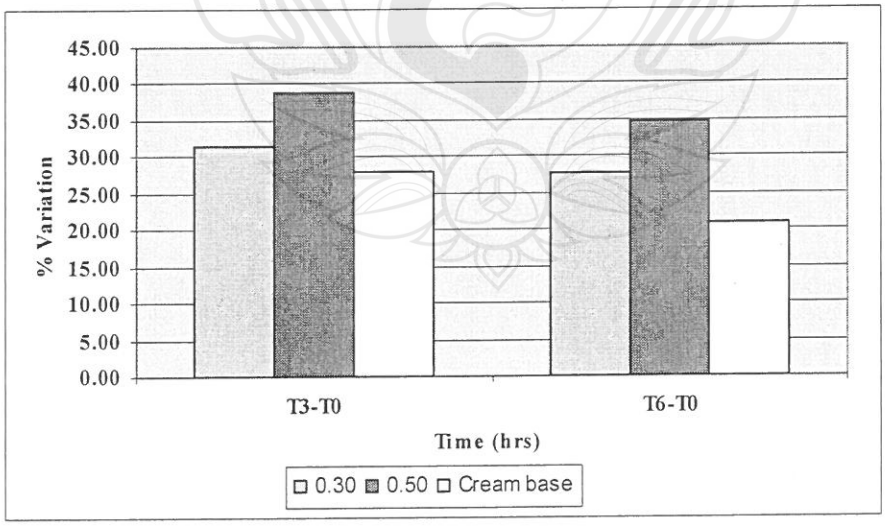

Figure 4.3 The comparison of moisture content on the skin between samples 1C, 2C with base cream C after applied on skin of volunteer for 3 and 6 hours.

. From **Table 4.9** The comparison between cream base and test samples after applied on the skin of volunteers for 3 and 6 hours shown that the test samples 2C exhibited increasing moisture on the skin of volunteer after apply for 6 hours with *p-value* 0.0197 (*p-value* < 0.05).

Table 4.10 The comparison of moisture holding capacity on the skin between test samples and base cream after applied at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825

Concentration (%w/v in the formula)	*Spirulina* spp.		*Cladophora glomerata*		*Spyrogyra* spp.	
	T_3-T_0	T_6-T_0	T_3-T_0	T_6-T_0	T_3-T_0	T_6-T_0
0.3	28.08	25.89	32.78	33.15	31.38	27.58
0.5	32.21	27.36	35.78	31.28	38.67	34.68
Base cream	27.80	20.81	27.80	20.81	27.80	20.81

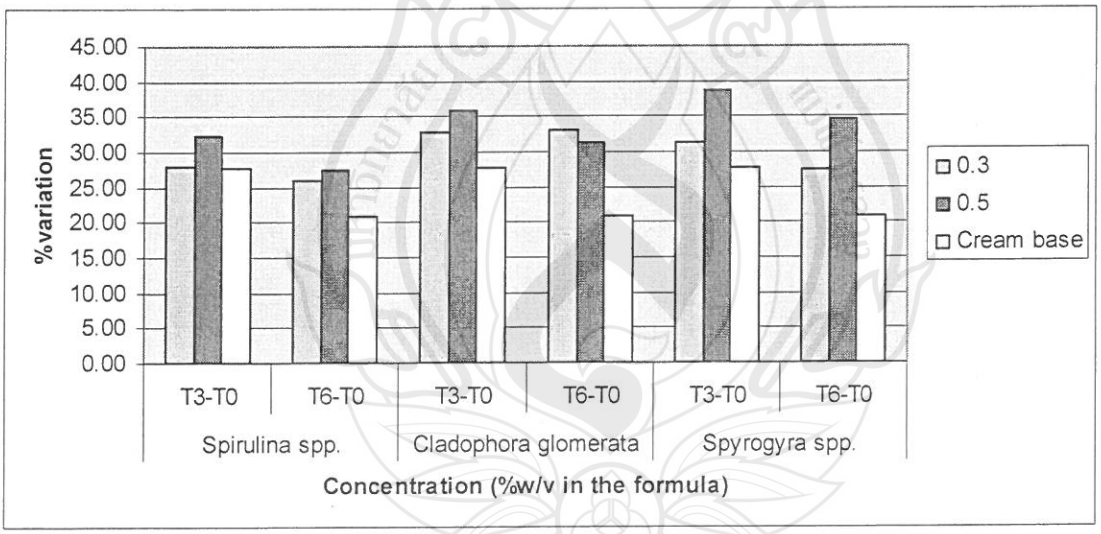

Figure 4.4 The comparison of moisture holding capacity on the skin between test samples and base cream after applied at T_0, T_1, T_3 and T_6 by using Corneometer®CM 825.

In this project, we were used 30 volunteers for measuring the moisture capacity test. The testing room was controlled the temperature at 24±2°C and humidity 40 - 60% for 15 mins then, the instrument should be calibrating before use. The reason of

temperature and humidity controlling is to decrease an error of moisture capacity. At the concentration 0.3%w/w, the crude polysaccharide extract from *Spirulina* spp. and *Cladophora glomerata* exhibit increasing moisture on the skin after apply for 6 hours while the crude polysaccharide extract from *Spirogyra* spp. did not show the capacity to increase the moisture on the skin. At the concentration 0.5%w/w, the crude polysaccharide extract from *Spirulina* spp. and *Cladophora glomerata* and *Spirulina* spp. exhibited increasing moisture on the skin after apply for 6 hours. Interestingly, the crude polysaccharide extract from *Cladophora glomerata* which show the potency of increasing moisture on the skin when apply for 3 hours with *p-value* 0.0388. The potency of this crude extract was slightly decrease after apply for 6 hours with *p-value* 0.0408. The reason supported to the above capacity from the extract due to the polysaccharide property.

Polysaccharides are relatively complex carbohydrates that consist of homopolysaccharide and heteropolysaccharide. Homopolysaccharide are polymer composed of a single type of sugar monomer. For example; cellulose is an unbranched homopolysaccharide comprised of glucose monomers connected via beta-glycosidic linkages (1, 4) and it combine by hydrogen bond that occur between hydroxyl group; Starch consist of a large number of glucose units joined together by glycosidic bonds. Starches have a different any molecule which affects to gel of starch. A large molecule of starch made up of simple gel. Starch is insoluble in cool water property but it can absorb water and swelling. A swelling of starch occur when increase temperature. Starches have 2 types; amylase and amylopectin. Amylopectin has high molecule and structure is any branch chain, so it is high viscose in a molecule. Homopolysaccharide are relatively complex carbohydrates. They are polymers made up of many monosaccharides joined together by glycosidic bonds. This project didn't compare moisture capacity on the skin of those three algae because volunteers were different group. The same volunteers were used to test for moisture capacity comparison and were resting period to skin about 1month for develop this test in the future. It was certainly value of moisture capacity and clearly. Finally, this project should continue to study mechanism of moisture capacity on the skin.

CHAPTER 5

CONCLUSIONS

In this research was studied and analyzed the carbohydrate content of *Spirulina spp., Cladophora glomerata* and *Spirogyra spp.* Then, polysaccharides were extracted from *Spirulina Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. The final, there were tested by clinical test.

6.1 Conclusion the result

6.1.1Determination of carbohydrate content found that *Spirulina* spp. *Cladophora glomerata* and *Spirogyra* spp. were 8.8%w/w, 29.33%w/w and 48.39%w/w, respectively.

6.1.2 Polysaccharides extracted from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. had percent yield 9.58%, 8.73%, and 2.82%, respectively.

6.1.3 Efficacy tests as shown that Sample 1A, 2A, 1B, 2B and Sample 2C were increased moisture capacity on the skin.

Therefore, the crude polysaccharide extract from *Spirulina* spp., *Cladophora glomerata* and *Spirogyra* spp. exhibit increasing the moisture capacity on the skin.

6.2 Suggestions

This research found that the crude polysaccharide extract exhibit increasing the moisture capacity on the skin which should be develop moisturizing agent in cosmetic product. Further study, the experiment should be concern with the comparison of moisture capacity between those three algae for selecting the best sample to develop into the cosmetic product. Moreover, the extraction process should be developing to obtain the highest percent yield of the extracts.

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Appendices

เอกสารเลขที่ 3/2552

มหาวิทยาลัยแม่ฟ้าหลวง
คณะกรรมการจริยธรรมการวิจัยในมนุษย์
ขอรับรองว่า

โครงการ : การพัฒนาสารสกัดจากสาหร่ายเกลียวทอง สาหร่ายไก และเทาน้ำ เพื่อใช้เป็นสารเพิ่มความชุ่มชื้นแก่ผิว (Development of Spirulina, *Cladophora glomerata* and *Spirogyra sp.* Extracts for moisturizing agent)

โครงการเลขที่ : REH-52003

ชื่อหัวหน้าโครงการ : อาจารย์มยุรมาศ แสงเงิน

สังกัด : สำนักวิชาวิทยาศาสตร์เครื่องสำอาง

เป็นโครงการวิจัยที่ไม่ขัดต่อหลักจริยธรรมสากลตามคำปฏิญญาเฮลซิงกิ (The Declaration of Helsinki) และแนวทางจริยธรรมการวิจัยในคนแห่งชาติ พ.ศ. 2545

จึงเห็นสมควรให้ดำเนินการวิจัยในขอบข่ายของโครงการที่เสนอต่อคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวงได้ ณ วันที่ 22 เดือน มกราคม พ.ศ. 2552

ลงชื่อ.....

(รองศาสตราจารย์ ดร.เทอด เทศประทีป)

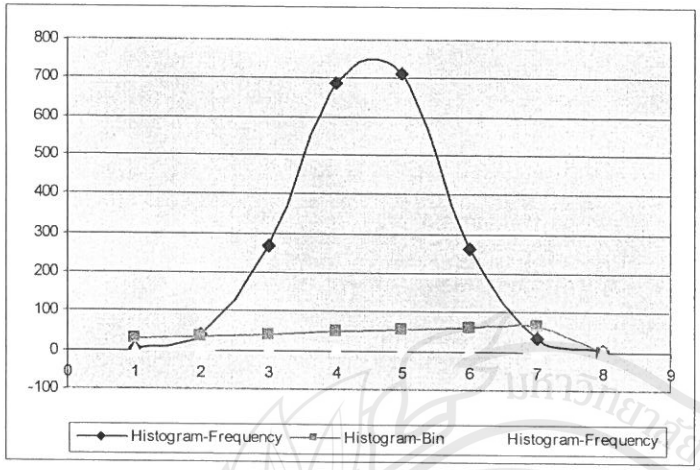
ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์

มหาวิทยาลัยแม่ฟ้าหลวง

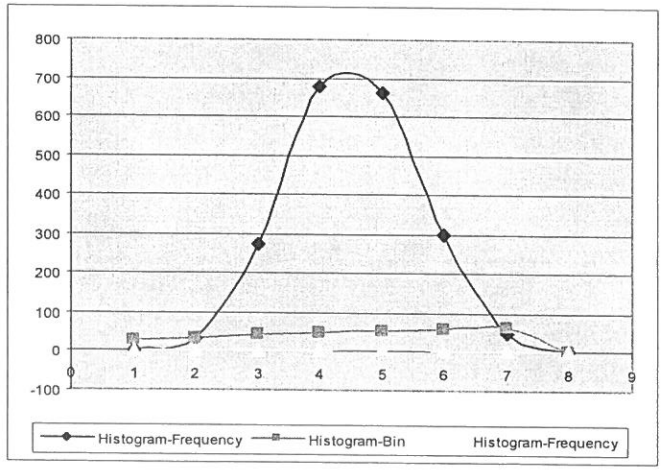
The moisture content on the skin from crude polysaccharide extract from *Spirulina* spp. and base cream after apply for 3 and 6 hours by using Corneometer®CM 825

Volunteers	T_0			T_1			T_3			T_6		
	Concentration of *Spirulina* spp. (%w/w)			Concentration of *Spirulina* spp. (%w/w)			Concentration of *Spirulina* spp. (%w/w)			Concentration of *Spirulina* spp. (%w/w)		
	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control
1	49.90	47.60	43.80	55.40	62.00	57.50	63.70	65.65	49.00	56.65	55.60	49.00
2	39.00	37.30	42.30	61.80	70.20	79.90	68.80	76.20	75.10	67.55	67.20	69.60
3	54.35	51.95	49.90	84.60	84.00	66.00	73.15	79.20	61.80	72.65	71.20	60.20
4	49.85	50.80	39.25	67.15	67.00	52.70	74.80	79.45	51.40	62.75	61.25	46.70
5	46.45	51.35	37.30	69.20	72.55	76.20	65.90	72.00	58.10	65.45	77.75	49.50
6	40.65	39.95	39.90	49.65	45.70	79.50	53.20	48.10	61.40	57.75	52.40	51.40
7	55.20	50.65	48.80	75.60	77.00	67.40	68.50	73.25	66.50	71.40	73.25	57.90
Mean	47.91	47.09	43.04	66.20	68.35	68.46	66.86	70.55	60.47	64.89	65.52	54.90
Minimum	39.00	37.30	37.30	49.65	45.70	52.70	53.20	48.10	49.00	56.65	52.40	46.70
Maximum	55.20	51.95	49.90	84.60	84.00	79.90	74.80	79.45	75.10	72.65	77.75	69.60
SEM	6.28	5.99	4.81	11.88	12.22	10.71	7.15	10.98	8.87	6.24	9.43	8.13

The sample distribution of *Spirulina* spp. at T_0



The Concentration 0.3%w/w



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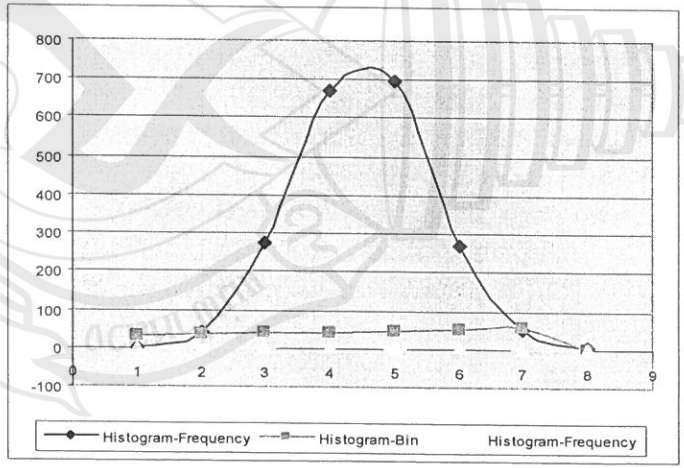
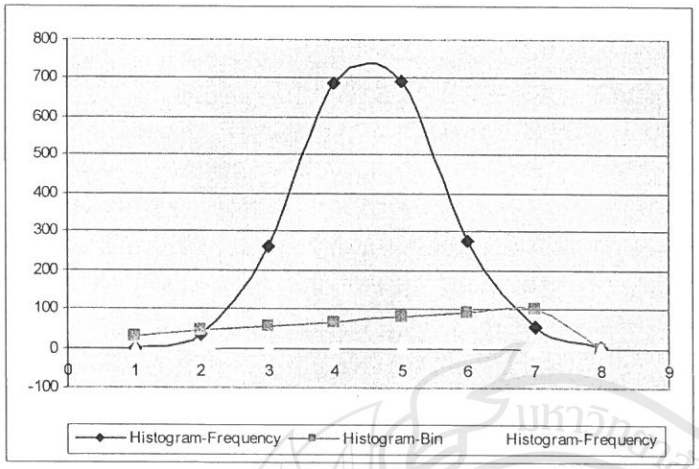
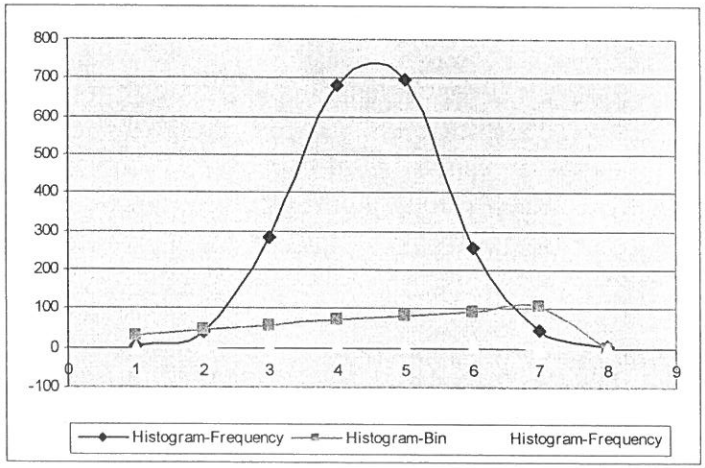


Base cream

The sample distribution of *Spirulina* spp. at T_1



The Concentration 0.3%w/w



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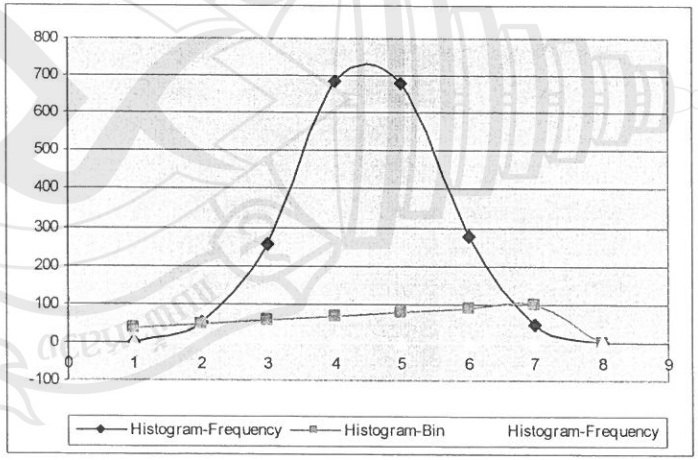
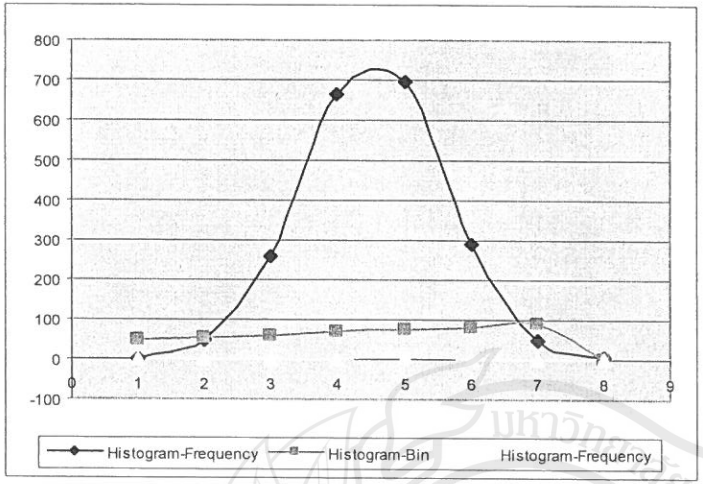
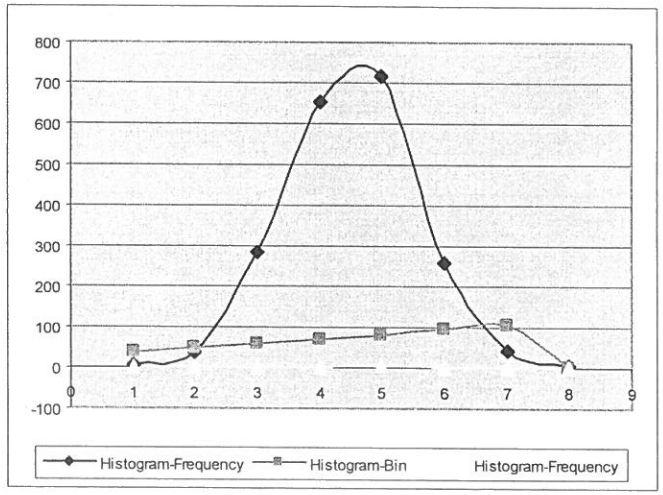


Base cream

The sample distribution of *Spirulina* spp. at T_3



The Concentration 0.3%w/w



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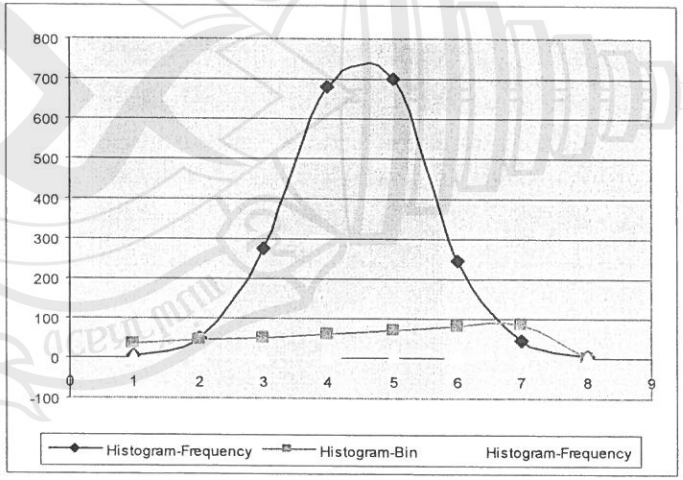
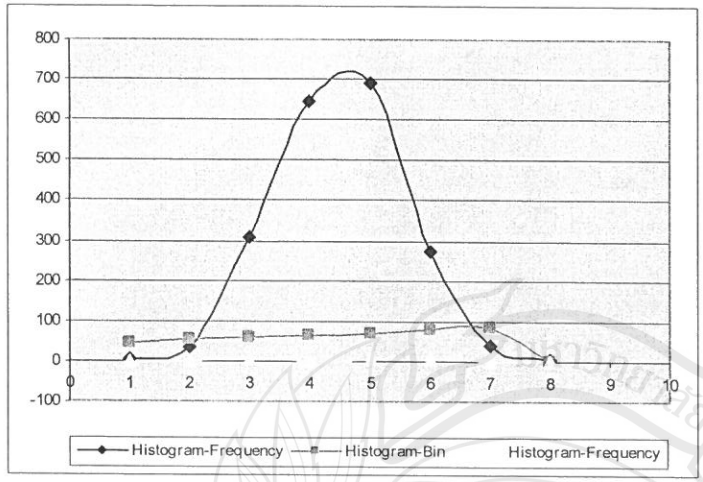


Base cream

The sample distribution of *Spirulina* spp. at T_6



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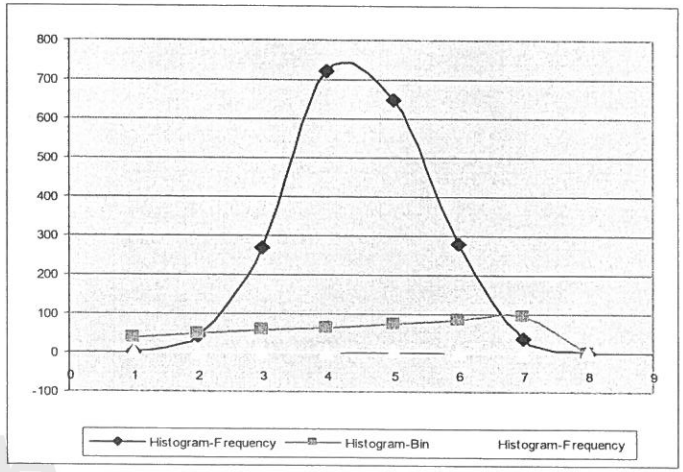

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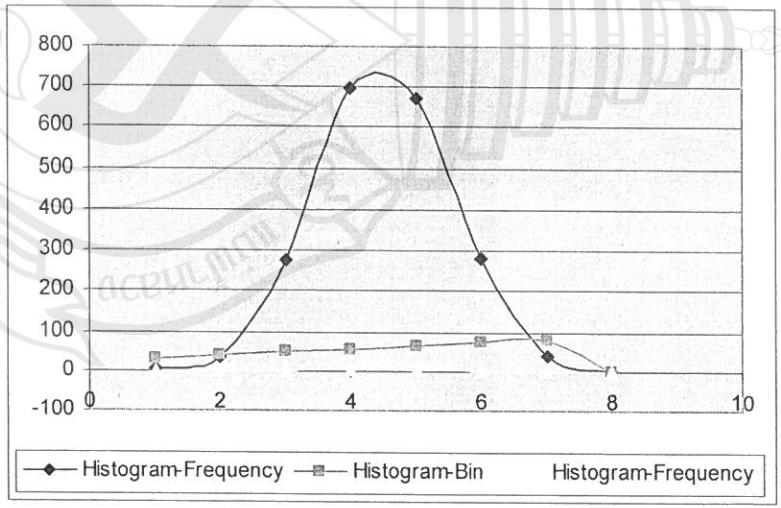
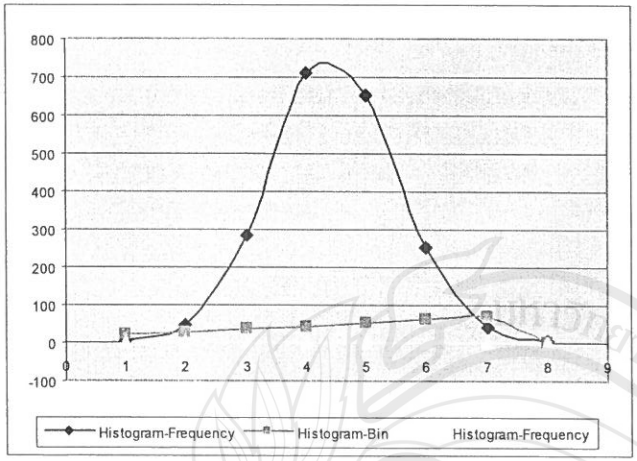


Base cream

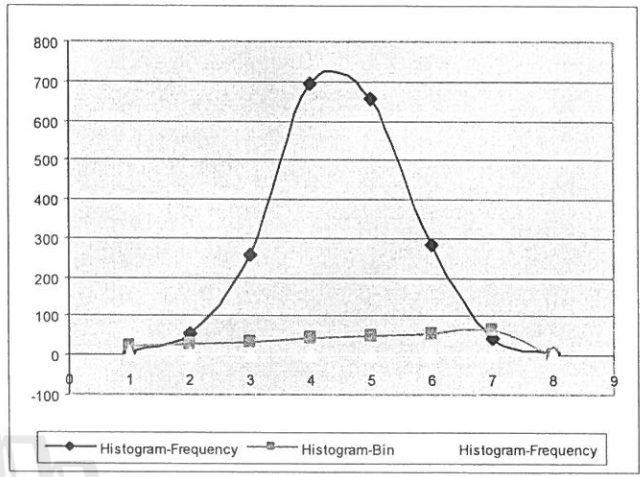
The moisture content on the skin from crude polysaccharide extract from *Cladophora glomerata* and base cream after apply for 3 and 6 hours by using Corneometer®CM 825

Volunteers	T_0			T_1			T_3			T_6		
	Concentration of *C.glomerata* (%w/w)			Concentration of *C.glomerata* (%w/w)			Concentration of *C.glomerata* (%w/w)			Concentration of *C.glomerata* (%w/w)		
	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control
1	44.40	42.60	43.80	61.30	97.80	57.50	61.10	68.30	49.00	55.60	54.29	49.00
2	39.80	36.05	42.30	60.10	62.35	79.90	66.85	61.10	75.10	64.30	60.30	69.60
3	49.90	46.85	49.90	72.15	76.75	66.00	70.25	65.05	61.80	72.75	60.58	60.20
4	53.40	49.70	39.25	82.40	77.45	52.70	77.55	70.60	51.40	63.90	64.35	46.70
5	37.05	37.45	37.30	63.20	70.40	76.20	58.50	64.80	58.10	67.85	65.80	49.50
6	31.95	30.35	39.90	56.20	65.45	79.50	47.55	53.75	61.40	63.70	57.75	51.40
7	53.70	47.20	48.80	86.55	68.65	67.40	79.05	66.05	66.50	77.50	59.95	57.90
Mean	44.31	41.46	43.04	68.84	74.12	68.46	65.84	64.24	60.47	66.51	60.43	54.90
Minimum	31.95	30.35	37.30	56.20	62.35	52.70	47.55	53.75	49.00	55.60	54.29	46.70
Maximum	53.70	49.70	49.90	86.55	97.80	79.90	79.05	70.60	75.10	77.50	65.80	69.60
SEM	8.44	7.07	4.81	11.78	11.81	10.71	11.13	5.49	8.87	7.07	3.86	8.13

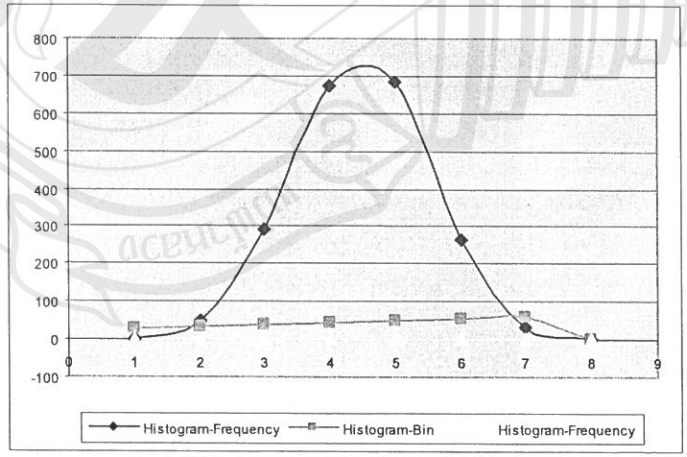
The sample distribution of *Cladophora glomerata* at T_0



The Concentration 0.3%w/w

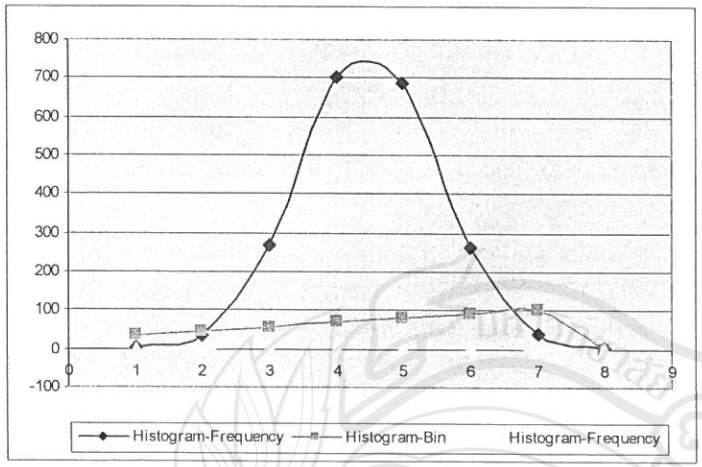


The Concentration 0.5%w/w

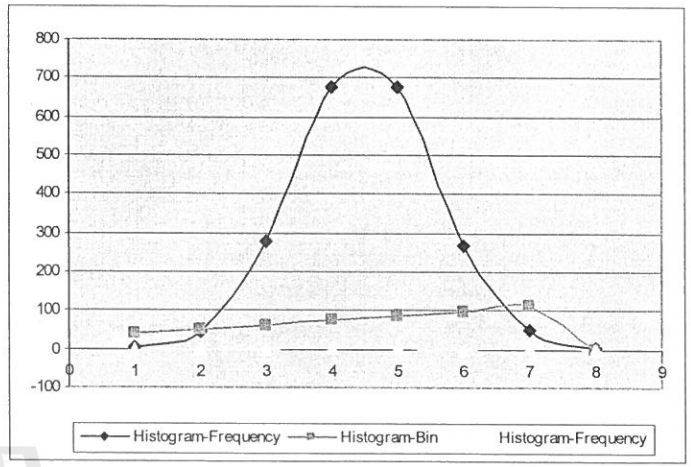


Base cream

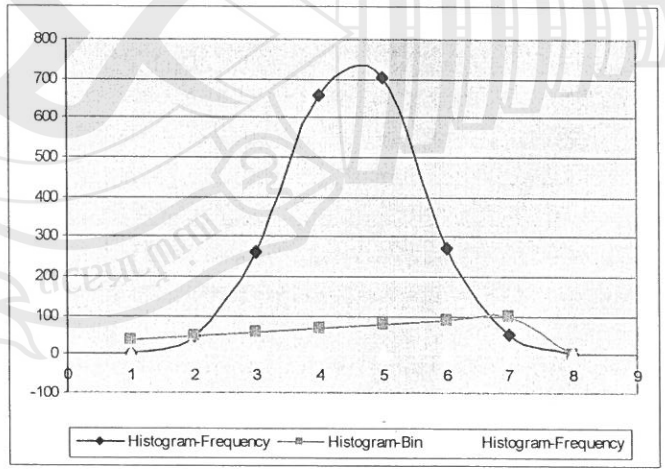
The sample distribution of *Cladophora glomerata* at T_1



The Concentration 0.3%w/w

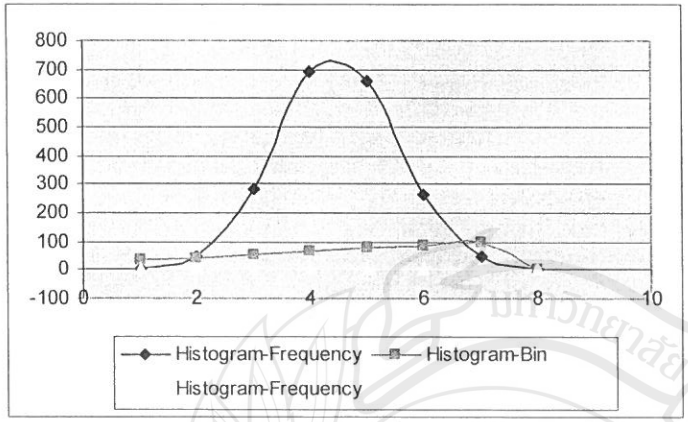


The Concentration 0.5%w/w

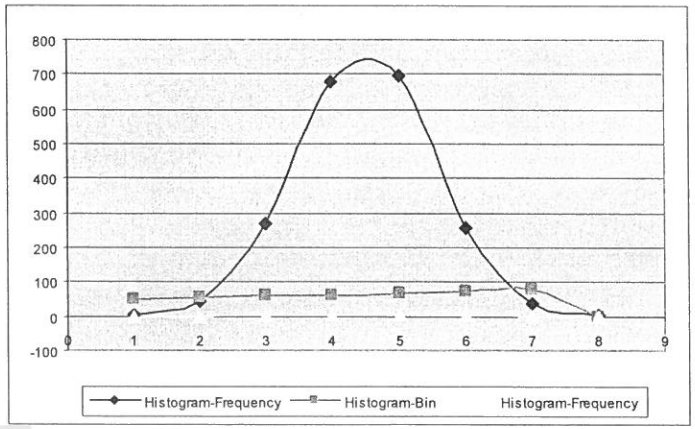


Base cream

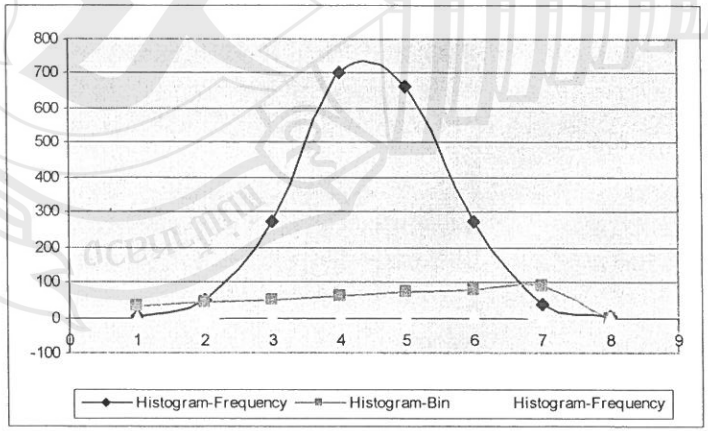
The sample distribution of *Cladophora glomerata* at T_3



The Concentration 0.3%w/w

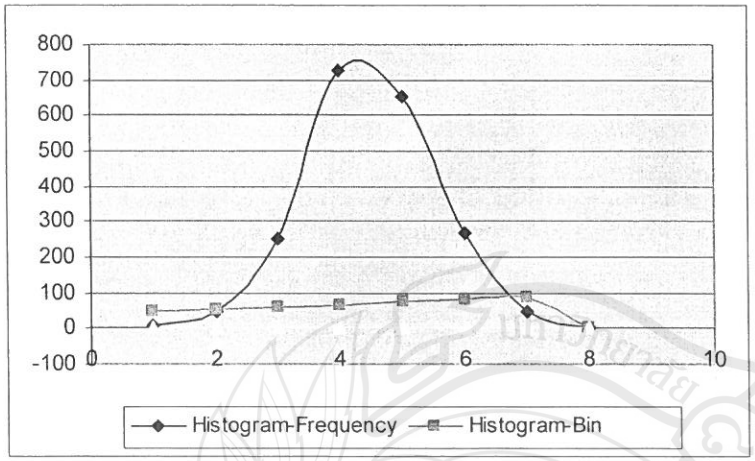


The Concentration 0.5%w/w

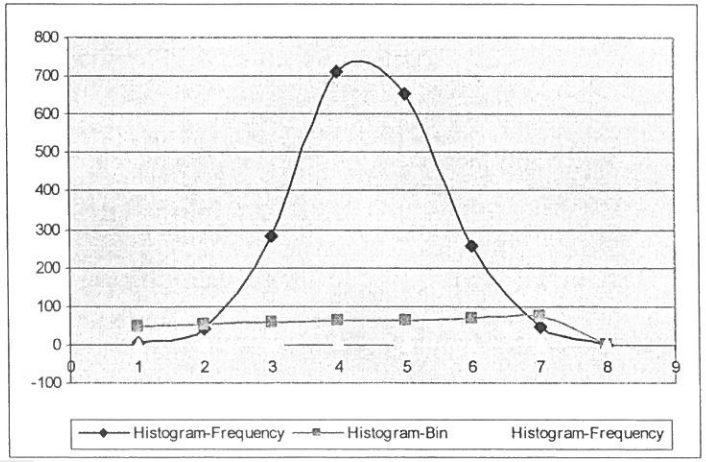


Base cream

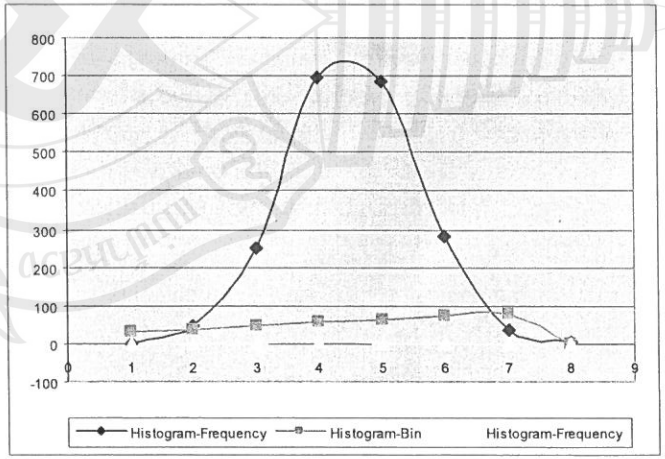
The sample distribution of *Cladophora glomerata* at T_6



The Concentration 0.3%w/w



The Concentration 0.5%w/w

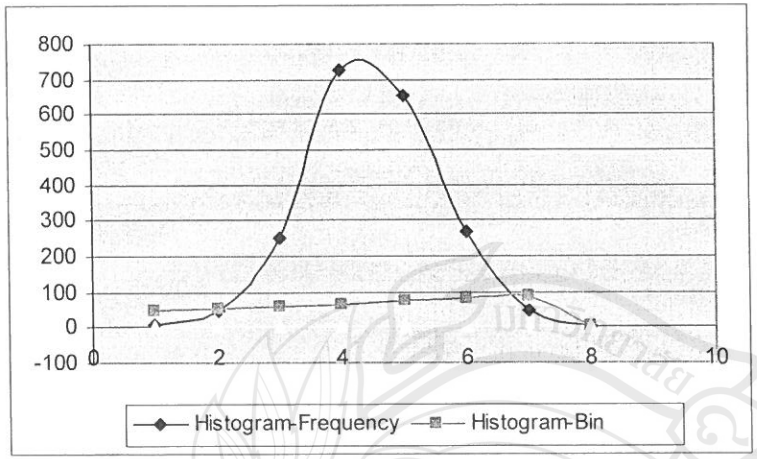


Base cream

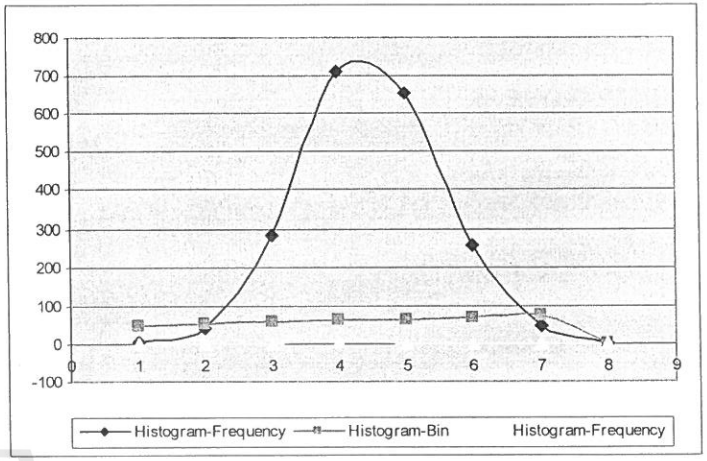
The moisture content on the skin from crude polysaccharide extract from *Spirogyra* spp. and base cream after apply for 3 and 6 hours by using Corneometer®CM 825

Volunteers	T_0			T_1			T_3			T_6		
	Concentration of *Spyrogyra* spp. (%w/w)			Concentration of *Spyrogyra* spp. (%w/w)			Concentration of *Spyrogyra* spp. (%w/w)			Concentration of *Spyrogyra* spp. (%w/w)		
	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control	0.3	0.5	Control
1	40.68	36.10	43.80	69.80	57.05	57.50	62.65	54.60	49.00	57.60	47.60	49.00
2	33.70	35.70	42.30	61.55	88.40	79.90	68.70	86.25	75.10	59.25	75.30	69.60
3	45.30	49.45	49.90	86.30	61.35	66.00	76.95	57.95	61.80	73.15	63.20	60.20
4	51.40	44.20	39.25	60.00	72.20	52.70	64.75	78.65	51.40	59.10	64.80	46.70
5	49.00	36.90	37.30	60.55	72.45	76.20	56.95	57.10	58.10	53.05	49.40	49.50
6	41.70	42.00	39.90	70.90	66.95	79.50	55.70	67.50	61.40	61.05	75.45	51.40
7	44.45	44.40	48.80	83.80	90.05	67.40	66.15	83.15	66.50	63.65	77.80	57.90
Mean	43.75	41.25	43.04	70.41	72.64	68.46	64.55	69.31	60.47	60.98	64.79	54.90
Minimum	33.70	35.70	37.30	60.00	57.05	52.70	55.70	54.60	49.00	53.05	47.60	46.70
Maximum	51.40	49.45	49.90	86.30	90.05	79.90	76.95	86.25	75.10	73.15	77.80	69.60
SEM	5.83	5.21	4.81	10.93	12.61	10.71	7.23	13.31	8.87	6.27	12.44	8.13

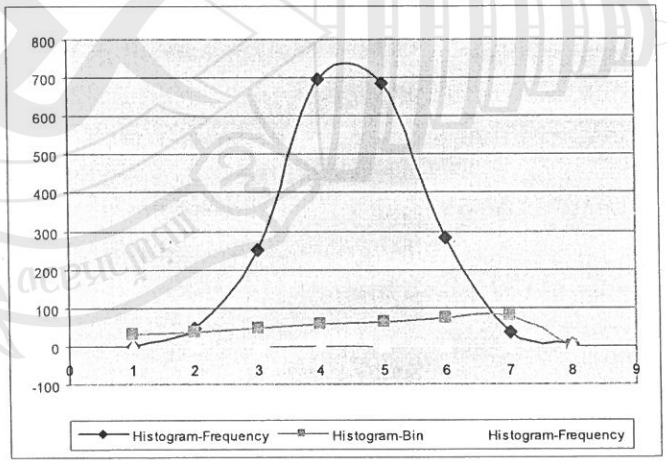
The sample distribution of *Spirogyra* spp. at T_0



The Concentration 0.3%w/w

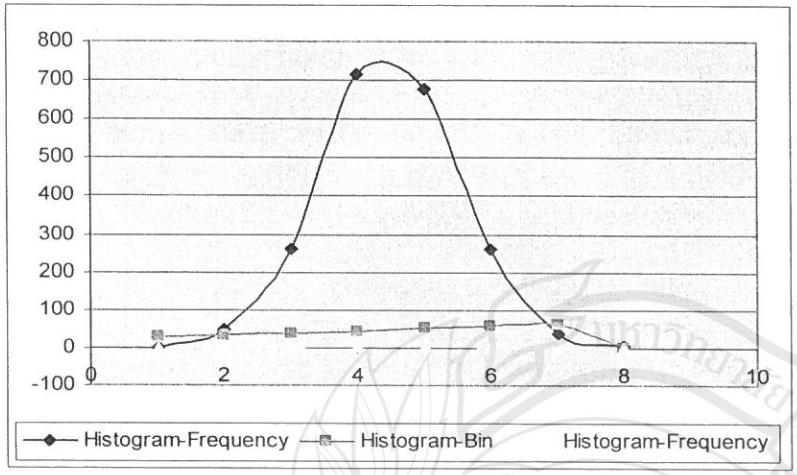


The Concentration 0.5%w/w

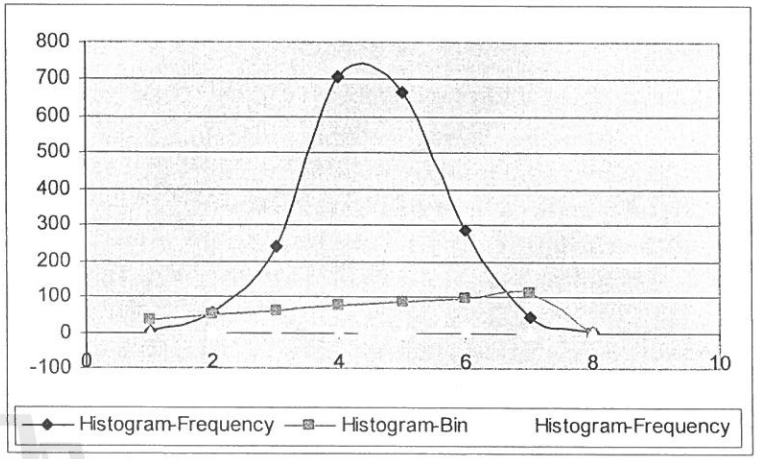


Base cream

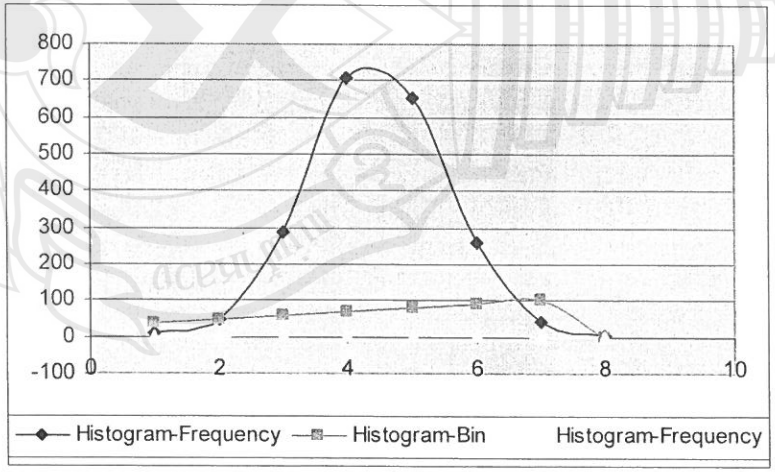
The sample distribution of *Spirogyra* spp. at T_1



The Concentration 0.3%w/w

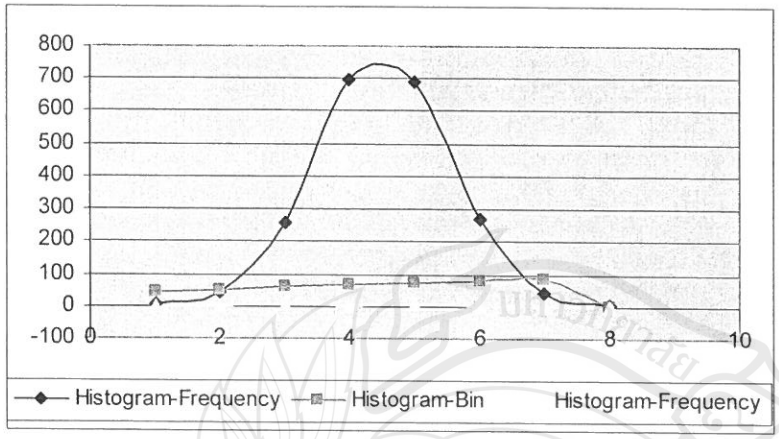


The Concentration 0.5%w/w

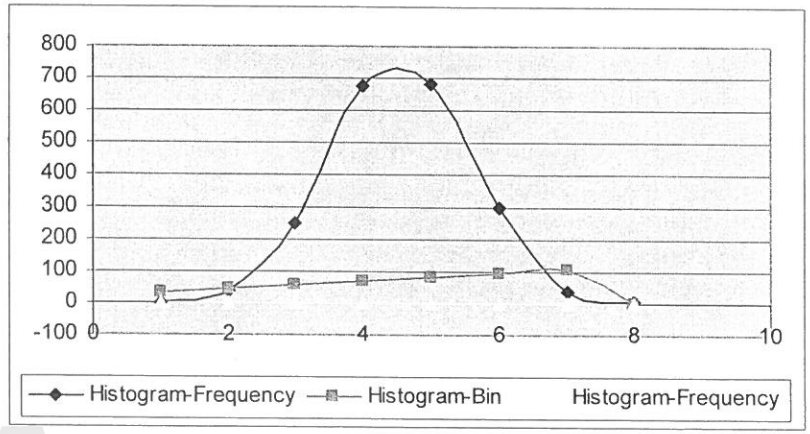


Base cream

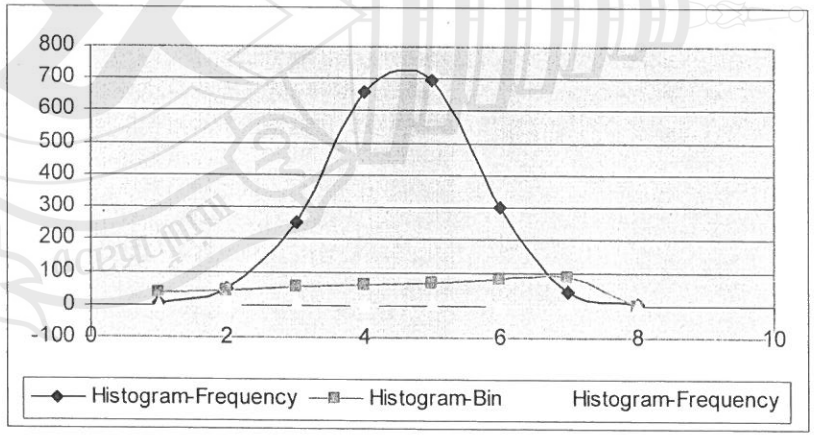
The sample distribution of *Spirogyra* spp. at T_3



The Concentration 0.3%w/w

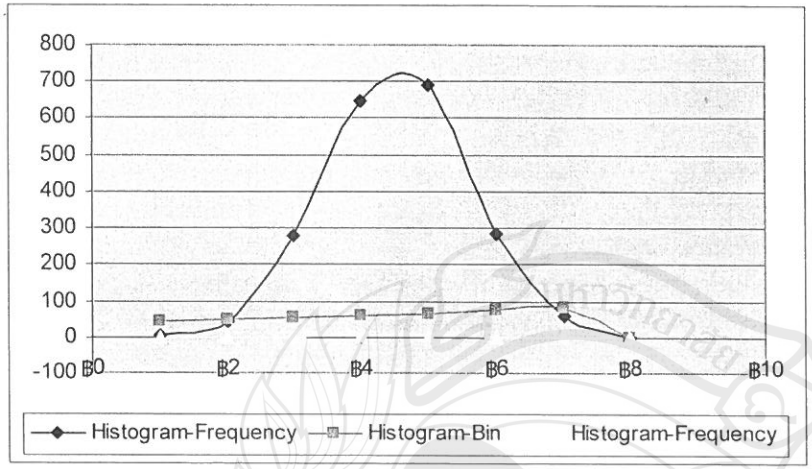


The Concentration 0.5%w/w

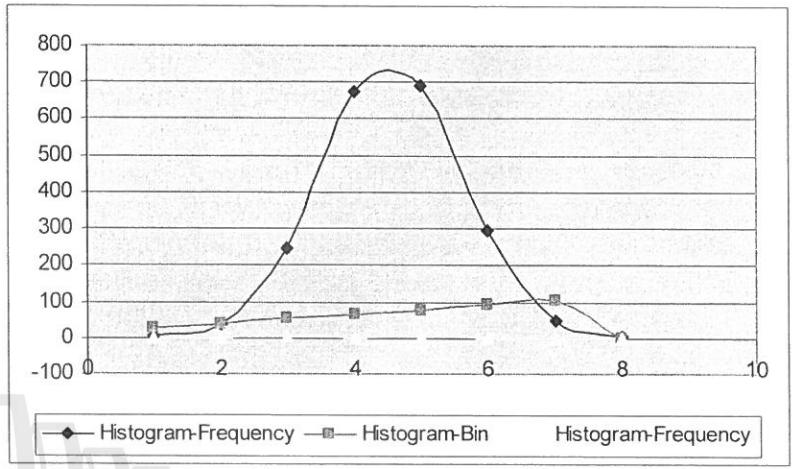


Base cream

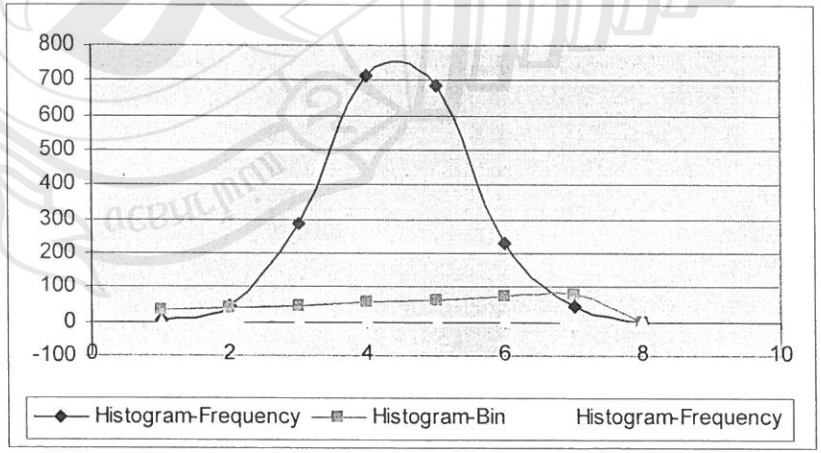
The sample distribution of *Spirogyra* spp. at T_6



The Concentration 0.3%w/w



The Concentration 0.5%w/w



Base cream

Biography

NAME	Mayuramas Sang-ngern
SEX	Female
BIRTHDATE	December 28, 1976
BIRTH PLACE	Phang-nga, Thailand
1995-1998	Bachelor of Science (Chemistry)
	Rajabhat Suratthani University (3.14 GPA)
2001-2004	Master of Science (Pharmaceutical Chemistry)
	Mahidol University (3.65 GPA)

NAME	Jarupux Saansomchai
SEX	Male
BIRTHDATE	January 04, 1979
BIRTH PLACE	Lumpoon, Thailand
EDUCATION	
1998 - 2001	Bachelor of Science (Biochemistry)
	Chiang Mai University
2002 -2005	Master of Science (Biochemistry)
	Chiang Mai University