



A Novel Moisturizer Extracted from Freshwater Macroalga [*Rhizoclonium hieroglyphicum* (C.Agardh) Kützing] for Skin Care Cosmetic

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ABSTRACT

Our previous study demonstrated that the aqueous extract from freshwater macroalga *Rhizoclonium hieroglyphicum* (C.Agardh) Kützing (RW) contained mostly polysaccharides and amino acids which might play an important role as a skin moisturizer. Therefore, its moisturizing effect in pig skin model and human skin was compared with some well-known commercial moisturizers such as glycerin (G), propylene glycol (PG) and hyaluronic acid (HA). The RW cream was then formulated and investigated its physical properties, pH and viscosity as well as stability under various storage conditions. The skin irritation and clinical evaluation for skin moisturizing effect in healthy volunteers were also evaluated. Skin patch test using Finn chamber[®] and skin moisturizing measurement using Corneometer[®] were conducted in 30 healthy volunteers. The skin hydration was measured at before and after applying the RW cream and control creams (cream base, G, PG and HA creams) for 15, 30 min, 1 hour for a short-term effect and 1 week (twice daily) for long-term usage. Satisfaction test using questionnaire was also performed. The results revealed that the moisturizing effect on human skin of RW is very similar to hyaluronic acid and glycerin. From clinical evaluation, the skin hydration increased after using RW cream in both short-term and long-term usage and no skin irritation occurred. Interestingly, the moisturizing effect of RW cream was comparable to HA cream ($p < 0.05$). More than 80% of the volunteers were satisfied with RW cream. In conclusion, the freshwater macroalga extract might be a promising natural skin moisturizer for skin care cosmetics.

Keywords: moisturizing effect, freshwater macroalga, *Rhizoclonium hieroglyphicum*, human skin, corneometer

1. INTRODUCTION

Moisturizers are a group of cosmetic products designed for skin care and hygiene. Besides daily use as a skin care, moisturizers

are also used for the treatment of dry skin. Recently, benefit of moisturizers when used on normal skin has been studied and found

to decrease skin susceptibility to irritants [1]. In addition, moisturizers are used to restore normal barrier functions of the skin and increase skin's hydration by reducing its evaporation [2]. Skin which is fully moisturized will appear to be healthy and good-looking [3].

Skin moisturizing products typically contain ingredients such as groups of emollients, humectants and natural moisturizing factor (NMF) [2]. Moisturization is accomplished by a combination of hydrating humectants, followed by the action of occlusive agents on the skin surfaces which are able to maintain the moisture in the stratum corneum [4]. A large number of preparations are available, many of which are marketed as cosmetic and therapeutic moisturizers. Moisturizing creams containing natural substances are currently popular since these are likely to be effective, safe and friendly to human skin.

Polysaccharides play a very important role in cosmetic formulation as humectants and moisturizers. They also act as a thickening agent, a gelling agent, a film former and an emulsifying agent [5-7]. Furthermore, they have been used as natural bioactive compounds in food and pharmaceutical industries [8].

Polysaccharides extracted from seaweed have been of great interest to natural resource researchers for many years. Red algae produce carrageenan and agar while brown algae produce alginates, fucoidan and laminaran [9-10]. These are used as ingredients in cosmetic and pharmaceutical skin care products [5, 11-12]. In addition, several researchers studied the freshwater algae on a topic of biodiversity, ecology and therapeutic (13-15). On the other hand, the properties of polysaccharides from freshwater algae such as *Cladophora* spp., *Nostochopsis* spp., *Spirogyra* spp., particularly, *Rhizoclonium hieroglyphicum*

and their potential for health and cosmetic applications have not been widely investigated.

R. hieroglyphicum, an edible alga which grows in the Nan River is rich in cell-wall polysaccharides. It exhibits maximum growth during dry season (November-March) when the temperature and water velocity are low [13]. Our previous study indicated that an aqueous extract from *R. hieroglyphicum* was mainly composed of polysaccharides (arabinose, rhamnose, xylose and galactose as a sugar unit) and amino acids such as tryptophan, tyrosine and phenylalanine [16]. Lautenschläger reported that the amino acids play an important role in the field of anti-aging skin care [17]. Furthermore, amino acids are substances found in the stratum corneum layer of the skin known as "natural moisturizing factor" (NMF), which regulate skin's moisture content [18]. The role of NMF is to maintain adequate skin hydration of the SC [19]. Therefore, *R. hieroglyphicum* is likely to be a new source of natural moisturizer to prevent and for the treatment of dry skin.

The objective of this research was to evaluate skin moisturizing effect of the aqueous extract from *R. hieroglyphicum* and its efficacy in cosmetic products, compared with some commercial skin moisturizers.

2. MATERIALS AND METHODS

2.1 Macroalga Sampling and Extract Preparation

R. hieroglyphicum was collected from the Nan River, Nan Province, Thailand (latitude 19°05'12.12"N and longitude 100°47'14.91"E) between November and March. Samples were identified using the morphological features of its macroscopic and microscopic structures [20-22]. The fresh alga was washed and dried at 60°C for 48 hours, then ground into powder. Extraction was performed on 50 g dry weight of the material in 1 liter of distilled water at

50°C for 1 hour. The solutions were separated from the residual alga by filtration using a No.1 Whatman filter, and were freeze-dried to obtain a dry extract (RW). The RW was stored in a vacuum desiccator at room temperature for further studies. These were done in triplicate.

2.2 Physicochemical Properties

2.2.1 The solubility test

The solubility of the RW extract in various solvents was tested. Each substance was used as a solvent; DI water, DI water:ethanol (ratio of 1:1, 1:2, 2:1), propylene glycol, tween 80 (1%), glycerin, mineral oil, jojoba oil and PEG-7 glyceryl cocoate. The RW extract was added in each solvent at the ratios of 1:10, 1:15, 1:20, 1:30 and 1:50. These were mixed with vortex mixer and kept at room temperature and 60°C for 10 min. Then, the tested solutions were observed for their solubility and compatibility.

2.2.2 Acid-base solubility and stability test

The solution of HCl (1N) or NaOH (10% w/v) was added to the RW extract solution (ratio 1:10 to 1:50) to adjust the pH to 2-9. Then, their physical changes were observed immediately and after storage in various conditions: at room temperature, 4°C and 45°C for 1 month and the 8 cycles of heating/cooling condition (45°C, 48 hrs alteration with 4°C, 48 hrs for 1 cycle).

2.3 Skin Moisturizing Test

2.3.1 Moisturizer testing on pig skin

The moisturizing capacity of the RW extract (0.1%) was examined on pig skin and compared with 5% glycerin (5G), 5% propylene glycol (5PG) and 0.1% of hyaluronic acid (0.1HA). The pig skins were prepared from abdomen of the pig; aged 6 months, and removed off the fat layer and cut into 1x1 inch. Each sample (0.2 ml) was

applied on the skin surface. The skin without any substances was used as a control. The moisture content had been measured before applying on sample and after application at 5, 15 and 30 min intervals using Corneometer®. Before applying the sample and recording the parameter, the pig skins were kept at room temperature for 30 min. This method had been adapted from O'Goshi *et al.* [23].

2.3.2 Moisturizer testing on human skin

The moisturizing capacity of the 0.1%RW extract (0.1RW) was examined on the skin of normal human volunteers and compared with 5% glycerin (5G), 5% propylene glycol (5PG), 0.1% hyaluronic acid (0.1HA), 0.1% carrageenan (0.1CA), 0.1% sodium alginate (0.1AL). The skin without any substances was used as a control. Before applying the sample and recording the parameter, the volunteers were rested for 30 min at room temperature. Each sample was applied on the forearm of the volunteers (aged 30-60, n = 30) in area of 1.5x1.5 inch. To area of testing, an amount of approximately 0.2 ml of each assigned test formulation was applied. The moisturizer content was measured before and after applying the sample at 10, 15 and 30 min. intervals using Corneometer®. This method had been adapted from Keng *et al.* [24].

2.4 Development of Freshwater Macroalga Extract Cream (RW cream)

2.4.1 Formulation of cream base

Five cream bases were developed from various compositions by conventional hot process. In the oil phase, glyceryl monostearate, stearic acid, cetyl alcohol, cyclomethicone, jojoba oil and cetareth-25 were heated at 70°C while triethanolamine (TEA), parabens and deionized water were heated together at 75°C as a water phase. These were then mixed and homogenized until the homogeneous emulsion was obtained and cooled down to

be a cream. The preparation was determined for their physical properties, pH, spreadability, viscosity (Pas) and feel on skin. In addition, the stability was tested in various conditions: room temperature, 4°C and 45°C for 6 month and 8 cycles of the heating/cooling condition (45°C, 48 hrs alteration with 4°C, 48 hrs for 1 cycle). The most stable cream base was then selected to incorporate with tested moisturizers.

2.4.2 Formulation of moisturizing cream

Each test substance: 5% glycerin (5G), 5% propylene glycol (5PG), 0.3%, 0.5% hyaluronic acid (0.3HA, 0.5HA) and 0.3%, 0.5% *R. hieroglyphicum* extract (0.3RW, 0.5RW) was incorporated into the selected cream base to obtain glycerin cream (CG), propylene glycol cream (CPG), 0.3%, 0.5% hyaluronic acid cream (CHA0.3, CHA0.5) and 0.3%, 0.5% *R. hieroglyphicum* extract cream (CRW0.3, CRW0.5), respectively. The stability of each cream was tested in various conditions as mentioned above in 4.1. Moreover, their pH and visually physical changing along with color, smoothness were also investigated.

2.5 Clinical Evaluation

Clinical evaluation on normal human volunteers for skin irritation and moisturizing efficacy were performed after it had been approved by the Ethical Review Committee of the Faculty of Pharmacy, Chiang Mai University.

The investigations were conducted on 30 healthy volunteers, both male and female (aged 30-60, n=30). They were enrolled in the study on the following criteria: had no history of cosmetic product allergy, no signs of skin disturbances, no hormonal alterations and not using any medications [25]. For 7 days interval before the beginning of the test, the subjects refrained from using cosmetic products. No

other products were used on each forearm during the trial period. The volunteers were recruited and asked to sign an informed consent statement.

2.5.1 Skin irritation test

Draize model has been modified from Bashir and Maibach [26] for skin irritation test using Finn chamber® with 30 normal healthy volunteers. Draize scoring system was used to calculate the primary dermal irritation index (PDII). The backs of the volunteers were covered with Finn chambers® which contained test substances for 48 hours. Then they were observed for any irritating reaction (erythema and edema) at 24 and 48 hours after removal of the patch. Sodium lauryl sulfate (SLS, 1 % w/v) was used as positive control and deionized water as a negative control.

2.5.2 Skin moisturizing test

Short-term moisturizing effect of each test cream was conducted with 30 healthy volunteers. They were instructed to apply each test cream on each site of their forearms. Before applying the test creams and recording the parameter, the volunteers were rested at room temperature ($21 \pm 2^\circ\text{C}$, 50-60% RH) for 30 min [27]. To each testing area (4cm x 4cm), 0.2 g of each assigned test cream was applied. Untreated area on one site of volunteer forearm was used as a control. The data was collected prior to applying the test cream and again after applying product for 15, 30 min, 1 hour using Corneometer®. In addition, the long-term use for 1 week (twice daily: morning-evening) was also evaluated [24]. At the end of the test, the volunteers finally filled out a questionnaire, presenting their satisfaction about the test creams after using them for 1 week.

2.6 Statistical Analysis

All data are expressed as means \pm S.D. Data were analyzed by an analysis of variance ($p < 0.05$) and the means compared between group by cluster analysis and ANOVA post hoc Tukey's b Test. Results were processed by computer programs: Excel and SPSS version 17.0

3. RESULTS AND DISCUSSION

3.1 Extraction and Physicochemical Characterization

R. hieroglyphicum was extracted with water and a pale yellowish dry hygroscopic powder was obtained (RW) with yield of $21.56 \pm 0.28\%$ w/w.

3.2 The Solubility Test

The RW extract was slightly soluble in water (1:30 to 1:50) and 1% Tween 80 solution (1:20 to 1:50) but swelling and formed gel in 1:10 and 1:15 water. The solution in 1:20 water and 1% Tween 80 (1:10 to 1:15) were slightly soluble with some swelling as shown in Table 1. It was sparingly soluble in H₂O: ethanol (1:10 to 1:50) with separation after 10 mins, but was insoluble in glycerin, propylene glycol, mineral oil, jojoba oil and PEG-7 glyceryl cocoate (data not shown). It can be explained that the water solubility of RW extract is due to the consisting sugar units and amino acids which are incompatible with alcohol and non polar solvents.

Table 1. The solubility of the *R. hieroglyphicum* extract (RW) in water and 1% Tween 80 solution.

Solvents	Ratio of RW:Solvent (w:v)	Dissolution	Appearances
H ₂ O	1:10	+	G, SW
	1:15	+	G, SW
	1:20	+	SW
	1:30	++	BS
	1:50	++	BS
Tween80 (1%)	1:10	+	SW
	1:15	+	SW
	1:20	+	BS
	1:30	+	BS
	1:50	++	BS

Dissolution: ++ Good, + Little, G: Gelling, SW: Swelling, BS: Brown solution

3.3 Acid-base Tolerance and Stability Test

The RW extract in water at concentration ratio of 1:10 (10% w/v) and 1:50 (2% w/v) were tested for acid-base tolerance. Initially, their pH was about 6.3. It was found that the 10% w/v RW formed gel at pH 4 and above in the same manner as agar and carrageenan [28-29], but was a low viscosity solution at pH 2 and 3 (data not shown). For the 2% w/v RW, the solution at pH 2 and 3 also showed lower viscosity whereas its viscosity increased when the pH of solution was 7 and above. At pH 4-6, the 2% w/v RW was stable at room temperature and 4°C except at 45°C

and heating/cooling condition which indicated that the viscosity was slightly increased as shown in Table 2. From these results, the RW extract at high concentration (10% w/v) presented the properties as other acidic polysaccharides such as agar and carrageenan, so it may be used as thickening and gelling agent in pharmaceutical as well as cosmetic products. Otherwise, at low concentration (ratio 1:50 or 2% w/v) was soluble in water and stable under tested conditions depending on pH and temperature. The RW was then further investigated for other properties such as moisturizer for cosmetic purpose.

Table 2. Acid-base stability of the RW extract (2% w/v) at 1 month.

pH	Condition									
	Observed-immediately		RT		4°C		45°C		H/C (8 cycles)	
	Appearances	Color	Appearances	Color	Appearances	Color	Appearances	Color	Appearances	Color
C	S	B	S	B	S	B	S	B	S	B
2	S	DB	S	DB	S	DB	V++ S	DB	S	DB
3	S	DB	S	DB	S	DB	S	DB	S	DB
4	S	DB	S	B	S	B	S	DB	S	DB
5	S	B	S	B	S	B	S	B	S	B
6	S	B	S	B	S	B	S V+	B	S	B
7	S	B	S	B	S	B	S V++	B	S	DB
8	V++ S	DB	V+ S	DB	V+ S	DB	V++ S	DB	V+ S	DB
9	V++ S	DB	V+ S	DB	V+ S	DB	V++ S	DB	V++ S	DB

V = Viscosity, S = Solution, +++ = Extremely, ++ = Very much, + = Medium, = Slightly
 B = Brown, DB = Dark brown
 C = control (pH 6.3)

3.3 Moisturizing Test

3.3.1 Moisturizing test on pig skin

The moisturizing effect of all tested substances showed significantly difference from untreated area ($p < 0.05$) whereas 5PG exhibited the highest increasing of moisture

content, followed by 0.1RW, 0.1HA and 5G (Figure 1). Interestingly, it was found that RW extract could keep the moisture on pig skin longer than HA when compared at 30 min, this may be due to the higher humectancy effect of the extract.

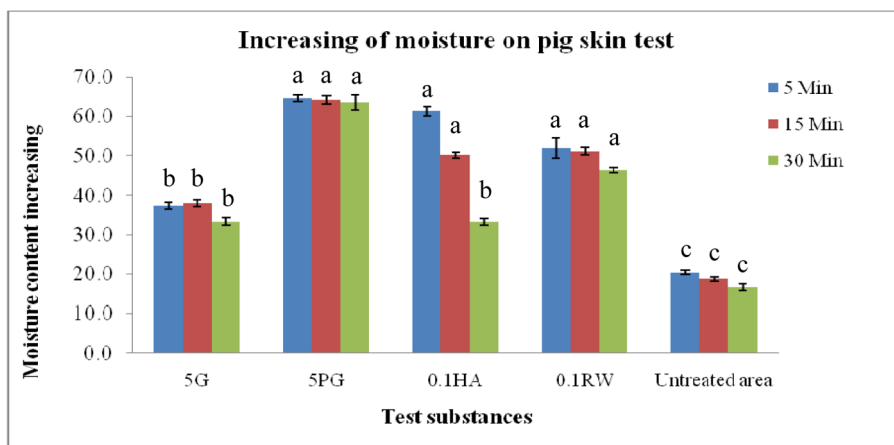


Figure 1. Moisture increasing on pig skin after application of the tested substances for 5, 15 and 30 min. (Data shown are mean \pm standard error (SD) of three replicates. Letters a, b and c are statistical comparison between groups in each time).

3.3.2 Moisturizing test on human skin

The results revealed that the moisture content increased in all test substances which was significantly different from untreated areas ($p < 0.05$) and showed the increasing of moisture content with times. Glycerin is more effective than propylene glycol due to the more swelling and hydrating to the stratum corneum [30] and higher water absorption capacity (Figure 2). For glycerin, the result was contrast to the test on pig skin showing the lowest moisture increasing capacity. This may

be due to the differences in pig skin structure from human skin that caused glycerin which is more viscous than others to less absorbed into pig skin leading to lower hydrating effect. In addition, at 30 min, the moisturizing effect on human skin of RW extract was not significantly different from carrageenan but less than glycerine and hyaluronic acid and also more than propylene glycol and sodium alginate. This indicated that RW extract could be an effective moisturizer which can be used in cosmetic products.

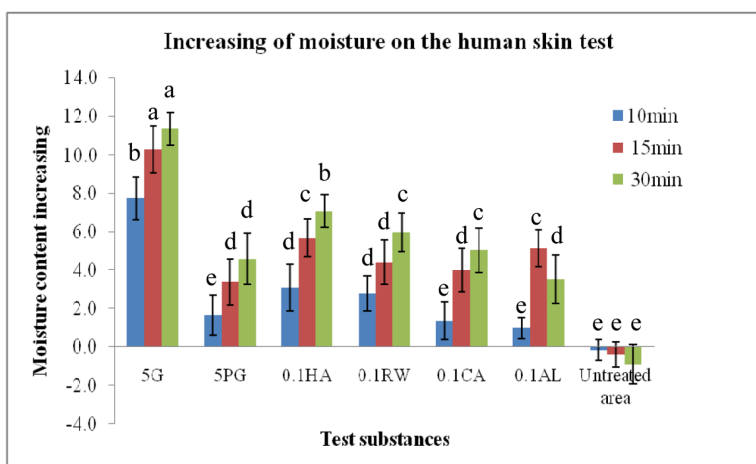


Figure 2. Moisture increasing on human skin after application of the test substances for 10, 15 and 30 min. (Data shown are mean \pm standard error (SD) of three replicates. Letters a, b, c, d and e are statistical comparison between groups in each time).

The results in both tests indicated that RW extract was an effective skin moisturizer comparable to HA, propylene glycol and glycerin which was then formulated into skin cream.

3.3.3 Formulation of moisturizing creams

The creams were determined for their physical properties, pH, spreadability, viscosity (Pas) and feel on skin. In addition, the stability was tested in various conditions as mentioned above in 4.2. In this result, the physical properties (color, smoothness and unstable conditions) of all tests creams did not change after test conditions (data not

shown). The pH of CHA increased whereas CRW decreased, which may be due to the effect of heat to the substances. The viscosity of all creams was almost unchanged except at 45°C and heating/cooling condition (Table 3), which may be due to heat effect. The heat affected to pH and viscosity but not the physical appearances, therefore, the CHA and CRW should not be stored at high temperature for long period. Regarding the pH and viscosity of the sample after heating/cooling condition, the CHA0.3 and CRW0.3 were likely to be more stable than the CHA0.5 and CRW0.5 creams. Therefore, CHA0.3 and CRW0.3 were selected for further study.

Table 3. pH and viscosity of test creams after various storage conditions for 6 months and the heating/cooling condition for 8 cycles.

Test creams	Start		RT		4°C		45°C		H/C	
	pH	Viscosity (Pas)	pH	Viscosity (Pas)	pH	Viscosity (Pas)	pH	Viscosity (Pas)	pH	Viscosity (Pas)
CB	5.5	3.15 ± 0.21 ^a	5.5	2.99 ± 0.07 ^a	5.5	2.77 ± 0.09 ^{ab}	5.5	2.16 ± 0.01 ^b	5.5	2.88 ± 0.10 ^{ab}
CPG	5.5	3.33 ± 0.14 ^a	5.5	3.29 ± 0.01 ^a	5.5	3.11 ± 0.01 ^a	5.5	1.81 ± 0.03 ^b	5.5	3.15 ± 0.04 ^b
CG	5.5	3.18 ± 0.28 ^a	5.5	3.45 ± 0.16 ^a	5.5	3.03 ± 0.03 ^a	5.5	1.81 ± 0.04 ^b	5.5	3.01 ± 0.06 ^b
CHA0.5	5.5	2.87 ± 0.02 ^a	5.5	1.51 ± 0.01 ^b	5.5	1.23 ± 0.01 ^d	6.5	1.44 ± 0.01 ^{bc}	6.5	1.33 ± 0.07 ^{cd}
CHA0.3	5.5	2.04 ± 0.07 ^a	5.5	2.04 ± 0.02 ^a	5.5	1.73 ± 0.04 ^b	6.5	1.71 ± 0.12 ^b	5.5	1.89 ± 0.14 ^{ab}
CRW0.5	5.5	2.26 ± 0.28 ^a	5.5	2.26 ± 0.05 ^a	5.5	2.22 ± 0.04 ^a	4.5	1.63 ± 0.12 ^b	4.0	2.21 ± 0.09 ^a
CRW0.3	5.5	3.27 ± 0.11 ^a	5.5	3.12 ± 0.09 ^a	5.5	2.42 ± 0.11 ^b	4.5	1.39 ± 0.01 ^c	5.5	2.57 ± 0.06 ^b

CB: Cream base, CPG: Cream base + PG, CG: Cream base + G, CHA: Cream base +HA CRW: Cream base +RW

Data shown are mean ± standard error (SD) of three replicates. Letters ^{a, b, c} and ^d are statistical comparison between groups in each conditions.

3.4 Clinical Evaluation

3.4.1 Skin irritation test

The skin irritation test was to determine the safety of the test creams. The dermal

irritancy potential of the test substances is shown in Table 4. All test substances were found to be non-irritating with low Primary Dermal Irritation Index value (PDII < 0.5).

Table 4. Primary Dermal Irritation Index (PDII) and skin irritation reaction in 30 volunteers.

Test substances	PDII value	Classification of skin
CB cream	0.00	reaction
CPG cream	0.00	No irritation
CG cream	0.00	No irritation
CHA cream	0.06	No irritation
CRW cream	0.16	No irritation
Positive (1 % w/v SLS)	1.18	No irritation
Negative (DI water)	0.00	Slight irritation

CB: Cream base, CPG: Cream base + PG, CG: Cream base + G, CHA: Cream base +HA CRW: Cream base +RW

3.4.2 Skin moisturizing test

The moisturizing effect of the test creams evaluated on 30 healthy volunteers. For the areas treated with test creams, the data showed an increased trend with the highest moisture content measured 15 min after the application. The moisture content in all tested areas was decreased after 30 min and

1 hour, which may be due to some moisture evaporated from skin in an air-conditioned room. However, the cream-treated areas still showed higher moisture content than the untreated area ($p < 0.05$). This indicated that the test creams had the desired moisturizing effect on skin with different capability.

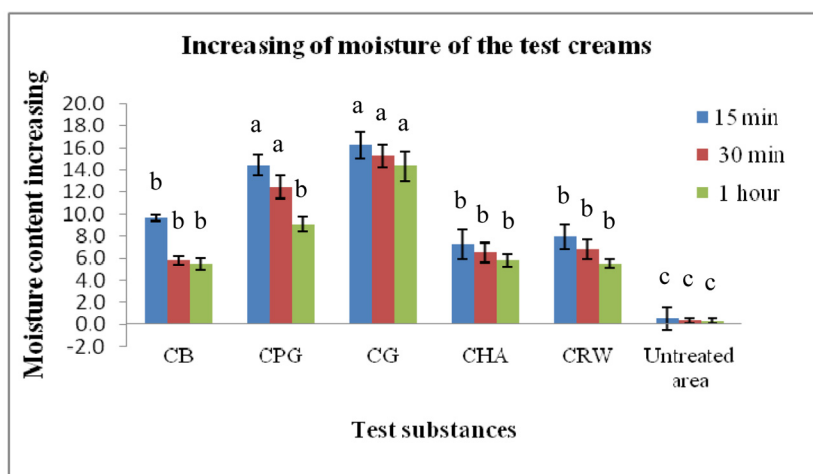


Figure 3. Moisture increasing after application of the test cream for 15, 30 min and 1 hour (Data shown are mean \pm standard error (SD) of three replicates. Letters a, b and c are statistical comparison between groups in each time).

Regarding a short-term moisturizing effect (15, 30 min and 1 hour) as shown in Fig.3, the CG showed the highest moisturizer capacity, and significantly different from CHA, CRW and CB. In addition, there was no statistically significant difference between CHA and CRW implying that RW was comparable to HA. The moisturizing effect

of cream base is due to occlusive effect of the containing oily material while the PG, G, HA and RW creams showed the additional moisturizing effect on the skin due to their humectancy capability. There were no significant changes on the untreated areas of the volunteers during the period of the experiment.

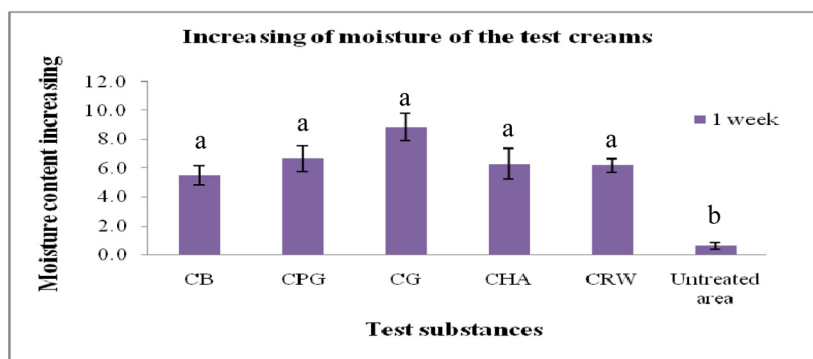


Figure 4. Moisture increasing after application of the test cream for 1 week (Data shown are mean \pm standard error (SD) of three replicates. Letters a and b are statistical comparison between groups in each time).

For a long-term moisturizing effect after one week application of the test creams by the volunteers as shown in Figure 4, CG showed the highest moisture content, but not significantly different from other test creams ($p < 0.05$). These results demonstrated that *R.hieroglyphicum* extract possessed a good moisturizing effect on human skin for long-term use similar to the effect of glycerin, propylene glycol and hyaluronic acid. The application of HA and RW creams could maintain good skin physiological function due to the humectancy effect.

Traditionally, humectants, occlusive agent and emollients have been and continue to be the mainstay of the medical and cosmetic treatments for xerotic skin and skin moisturizing products. The most widely-used and effective humectant is glycerin because of its excellent hygroscopicity. Application of glycerin leading to swelling and hydrating the stratum corneum will thus smooth down the scales. Benefits of the long-term application of glycerin can be seen and felt. The effect is the smoothening of the skin surface [30]. Therefore, the glycerin results in a higher moisturizer effect, probably from its humectant effect while the effect of propylene glycol is different. In this study, propylene glycol provides a lower moisturizer content than glycerin after 1 week of application.

Hyaluronic acid is a biopolymer naturally occurring in the skin and other tissues. It is an important component of the skin matrix. It is also a popular skin care ingredient often used topically. Hyaluronic acid is a highly effective humectant, it can hold thousands

of times its weight in water, and is used in moisturizing formulas and provides effective skin surface hydration [31]. The present findings indicate that the extract of *R.hieroglyphicum*, which is also biopolymer provides the moisturizing effect that resemble hyaluronic acid and glycerin. This result demonstrated that the extract of *R. hieroglyphicum* is a hygroscopic substance and probably a class of sugar units containing as in hyaluronic acid that provides the same function as a humectant on the skin's surface.

Moreover, the human primary skin irritation test exhibited that the RW caused no irritation to human skin, either immediately or during the entire course of the experiment. Therefore, it has a high potential for use in cosmetics and personal care products as well as topical pharmaceutical products, and is safe for human consumption by the topical route.

3.4.3 Satisfaction of RW cream and test creams by volunteers

The volunteers' feelings reflected in a questionnaire, the range of satisfaction with moisturizing creams from "like extremely to like moderately" exhibited more than 80% for all topics, which mean that the subjects thought the product application could add and retain moisture to the skin. Also, cream can control skin moisture for the whole day and even for one week under long-term application. Moreover, the range of satisfaction showed the subjects satisfaction with CRW was similar to CHA (Figure 5). Additionally, there was no report of skin irritation or allergic reaction during the period of application.

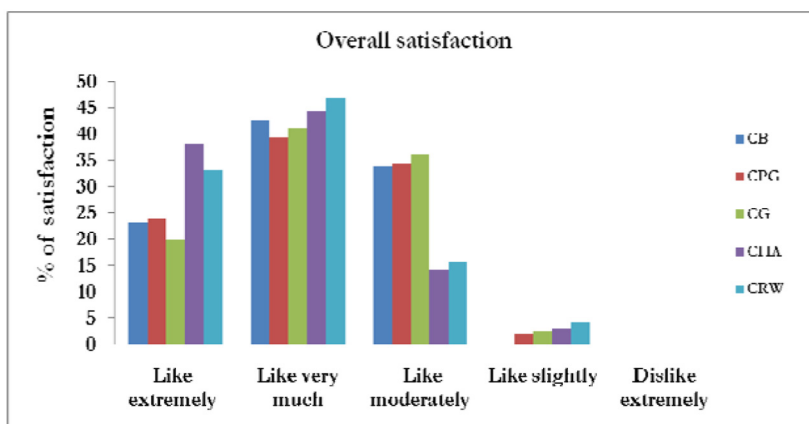


Figure 5. Satisfaction of volunteers on moisturizing effect of the test creams.

4. CONCLUSIONS

This work reveals that *R. hieroglyphicum* extract seems to be appropriated as a new skin moisturizing ingredient for cosmetic industry. The present data showed that when the moisturizer test creams were applied to the skin, the skin hydration had increased compared to untreated skin. This may be due to an increased penetration of the hydrophilic substances and occlusive barrier to prevent water loss from the skin, supporting results from a previous study on short- and long-term use of moisturizer [24,32]. For the aqueous extract from freshwater macroalga, it was safe and revealed the same moisturizing property as hyaluronic acid via humectancy effect, followed by occlusive effect which can retain longer effect for skin hydration. Therefore, the RW extract might be an alternative skin moisturizer to hyaluronic acid which is quite expensive.

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REFERENCES

- [1] Held E. and Agner T., Effect of moisturizers on skin susceptibility to irritants, *Acta Derm. Venereol.*, 2001; **81**: 104-107.
- [2] Robinson C., Hartman R.F. and Rose S.D., Emollient, humectant and fluorescent α , β -unsaturated thiol esters for long-acting skin application, *Bioorg. Chem.*, 2008; **36**: 265-270.
- [3] Schmitt W.H., Skin-care Products; in Williams D.F. and Schmitt W.H., eds., *Chemistry and Technology of the Cosmetics and Toiletries Industry*, 2nd Edn., Blackie Academic & Professional, London, 1996: 104-130.
- [4] Loden M., The skin barrier and use of moisturizers in atopic dermatitis, *Clin. Dermatol.*, 2003; **21**: 145-157.
- [5] Goddard E.D. and Gruber J.V., *Principles of Polymer Science and Technology in Cosmetics and Personal Care*, 1st Edn., CRC press, New York, 1999.
- [6] Ruperez P., Ahrazem O. and Leal J.A., Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*, *J. Agric. Food Chem.*, 2002; **50**(4): 840-845.

- [7] Lautenschläger H., (Poly) saccharides in cosmetic products – from alginate to xanthan gum, *Kosmetische Praxis*, 2009; 4: 12-15.
- [8] Rocha de Souza M.C., Marques C.T., Guerra Dore C.M., Ferreira da Silva FR., Oliveira Rocha H.A. and Leite E.L., Antioxidant activities of sulfated polysaccharides from brown and red seaweeds, *J. Appl. Phycol.*, 2007; 19: 153-160.
- [9] Percival E., The polysaccharides of green, red and brown seaweeds: Their basic structure, biosynthesis and function, *Brit. Phycol. J.*, 1979; 14(2): 103-117.
- [10] Pereira L., Amado A.M., Critchley A.T, van de Velde F. and Ribeiro-Claro P.J.A., Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman), *Food Hydrocolloids*, 2009; 23: 1903-1909.
- [11] Burlando B., Verotta L., Cornara L. and Bottini-Massa E., *Herbal Principles in Cosmetics Properties and Mechanisms of Action*, 1st Edn., Taylor & Francis Group, New York, 2010.
- [12] Dewick P.M., *Medicinal Natural Products A Biosynthetic Approach* 3rd Edn., John Wiley & Sons Ltd., United Kingdom, 2009.
- [13] Peerapornpisal Y., Pongsirkul I, Kanjanapothi D., *et al.*, Potential of Freshwater Macroalgae as Food and Medicine, *Final report submitted to Thailand Research Fund (TRF)*, 2005.
- [14] Thiamdao S., Boo G.H., Boo S.M. and Peerapornpisal Y., Diversity of edible *Cladophora* (Cladophorales, Chlorophyta) in northern and northeastern Thailand, based on morphology and nuclear ribosomal DNA sequences, *Chiang Mai J. Sci.*, 2012; 39(2): 300-310.
- [15] Peerapornpisal Y., Doungporn A., Chaiyong R., Khomson R. and Duangta K., Two endemic species of macroalgae in Nan river, Northern Thailand, as therapeutic agents, *ScienceAsia*, 2006; 32: 71-76.
- [16] Mungmai L., Jiranusornkul S., Peerapornpisal Y., Sirithunyalug B. and Leelapornpisid P., Extraction, characterization and biological activities of extracts from freshwater macroalga (*Rhizoclonium hieroglyphicum* (C.Agardh) Kützing) cultivated in northern Thailand, *Chiang Mai J. Sci.*, 2014; 41(1): 14-26.
- [17] Lautenschläger H., Peptides– more than transmitters and hormones, *Kosmetik International*, 2007; 9: 14-17.
- [18] Draelos Z.D., *Cosmetic Dermatology*, 2nd Edn., Churchill-Livingstone, New York, 1995.
- [19] Rawlings A.V., Canestrari D.A. and Dobkowski B., Moisturizer technology versus clinical performance, *Dermatol. Ther.*, 2004; 17: 49-56.
- [20] Whitford L.A. and Schumacher G.L., *A Manual of The Freshwater Algae in North Carolina*, The North Carolina Agricultural Experiment Station, North Carolina, 1969.
- [21] Prescott G.W., *How to Know The Freshwater Algae*, W.M.C. Brown Company Publisher, Iowa, 1970.
- [22] Jonh D.M., Whitton B.A. and Brook A.J., *The Freshwater Algae Flora of the British Isles*, Cambridge University Press, London, 2002.
- [23] O’Goshi K.I., Tabata N., Sato Y. and Tagami H., Comparative study of the efficacy of various moisturizers on the skin of the ASR miniature swine, *Skin Pharmacol. Appl.*, 2000; 13(2): 120-127.

- [24] Keng P.S., Basri M., Zakaria M.R.S., Abdul Rahman M.B., Ariff A.B., Abdul Rahman R.N.Z. and Salleh A.B., Newly synthesized palm esters for cosmetics industry, *Ind. Crop. Prod.*, 2009; **29**: 37-44.
- [25] Colipa, *Product Test Guidelines for the Assessment of Human Skin Compatibility*, Brussels, Belgium, 1997.
- [26] Bashir S.J. and Maibach H.I., *In Vivo Irritation*; in Paye M., Barel A.O. and Maibach H.I., eds, *Handbook of Cosmetic Science and Technology*, 2nd Edn., Taylor & Francis Group, New York, 2001: 905-916.
- [27] Gaspar L.R., Camargo F.B., Gianeti M.D. and Maia Campos P.M.B.G., Evaluation of dermatological effects of cosmetic formulations containing *Saccharomyces cerevisiae* extract and vitamins, *Food Chem. Toxicol.*, 2008; **46**: 3493-3500.
- [28] Armisen R., Agar; in Imeson A., ed., *Thickening and Gelling Agents for Food*, 2nd Edn., Blackie Academic and Professional, London, 1997: 1-21.
- [29] Thomas W.R., Carrageenan; in Imeson A., ed., *Thickening and Gelling Agents for Food*, 2nd Edn., Blackie Academic and Professional, London, 1997: 45-59.
- [30] Ramalho A., Silva C.L., Pais A.A.C.C. and Sousa J.J.S., *In vivo* friction study of human skin: Influence of moisturizers on different anatomical sites, *Wear.*, 2007; **263**: 1044-1049.
- [31] Baumann L., Saghari S. and Weisberg E., Moisturizing Agents; in Baumann L., ed., *Cosmetic Dermatology Principles and Practice* 2nd Edn., McGraw Hill Medical, New York, 2009: 273-291.
- [32] Elsner P. and Maibach H.I., Moisturizers; in Loden M., ed., *Cosmeceuticals Drugs vs. Cosmetics*, Marcel Dekker, New York, 2000: 73-93.