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Clinical Study on Skin Improvement Effect in Adult Women of Age 40 to 50 Using Cosmetics Containing Sea Cucumber Extract

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Abstract : The purpose of the study was to investigate the effects of sea cucumber extract on skin as a natural cosmetics functional material. Subjective evaluation of cosmetics before and after were conducted with questionnaires regarding moisture content, sebum content, melanin index, and erythema index. Experiments were conducted on improvement efficacy using skin clinical trials and questionnaires to evaluate changes in perception of skin condition and efficiency of products. With the aim of minimizing skin irritation, the efficiency of the solvent used for extraction was an important factor, and the sea cucumber extract was harvested with efficient extraction conditions at a ratio of 1:10 of 50% ethanol. The study aimed to identify the suitability of sea cucumber extract as a functional cosmetics material to improve the moisturizing ability of skin and its effect on the skin by adding marine natural animal sea cucumber extract. Clinical studies on cosmetics skin containing sea cucumber extract, excellent skin improvement effect from all items of clinical experiment in experimental and control groups. Sea cucumber extract was proved to be a stable, non-adverse physiologically active substance against abnormal symptoms or side effects of skin reactions and skin problems. In addition, the study found excellent results that can lead to its use as a cosmetics material. This is expected to contribute to the development of various cosmetics industries.

Keywords : Sea cucumber extract, Moisture, Sebum, Melanin index, Erythema index

1. Introduction

Sea cucumber is a collective term for the sea cucumbers belonging to phylum echinodermata class holothuroidea [1]. There are over 1,500 species of sea cucumbers in the world and about 40 kinds of edible species are known [2]. Sea cucumbers are called sea ginseng in Korean due to it being compared to wild ginseng or red ginseng [3]. There are three species of sea cucumbers in Korea including red sea cucumbers, blue sea cucumbers and black sea cucumbers[4]. The sea cucumber is rich in amino acids such as cysteine, histidine, lysine and iron (Fe), phosphorus (P) and bile components to prevent anemia and activate

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liver movement [4]. In a study on the composition of constituents of sea cucumber, the lyophilized sea cucumber powder consists of components of glycoprotein and chondroitin sulfate (ChS) [5]. The chemical composition is composed of sulfate esters of 0.09 to 1.21%, polysaccharide of 23.08 to 26.97%, and monosaccharide fucose constitution of more than 30% [6]. ChS of this sea cucumber is a mucopolysaccharide bound with D-galactosamine and sulfate group [7]. The mucolytic substance mucopolysaccharide helps to build up the mucous layer of the stomach. It functions to protect the epithelial cells of the gastrointestinal lining from chemical stimulation or mechanical damage [8]. Sea cucumber extract contains chondroitin sulfate that moisturizes and stores moisture in the skin. In addition, amino acid, protein, and iodine are stored in large quantities [9], and it is possible to alleviate the wrinkles of the skin, to prevent aging, to have whitening effect, to clean skin, and to exert an excellent effect on the skin [10]. Ingredients of sea cucumber extracts, Omega-9, oxaniloc acid lowers cholesterol in the body, promotes the immune system, and is involved in the growth and development of fetuses and children. It is a nutritional substance that prevents gastric acid secretion, improves constipation, prevents breast cancer, and activates nerve cells. [11, 12, 13]. Hydroxy-L-proline is an amino acid with a hydroxy group (-OH) attached to the 4th carbon atom of proline, and is a major constituent of collagen (11-14%), and it is known to be essential for the preservation of the triple helix structure of collagen [14]. Sea cucumber extract has antioxidant, antiviral, anti-cancer, anti-salt and immunoregulatory effects and wound healing effects on various causes of gastritis [15]. When sea cucumber extract was treated with melanin-producing M-3 cells, melanin synthesis was greatly suppressed, and it was reported that sea cucumber extract could be used as a natural whitening material for functional cosmetics [16]. Antioxidant activity and skin whitening effect of sea cucumber extract with physiological activity was also reported [17]. The method of manufacturing sea cucumber extract beauty soap was also studied [18]. The protein and chondroitin sulfate substance of sea cucumber extract is effective for moisturizing, anti-wrinkle of skin, aging prevention and skin whitening. It has been reported that there is no irritation or toxicity to the skin, and it is excellent in the function of stable skin moisturizing and wrinkle improvement [19]. Research has also been reported on chondroitin cosmetics that improve moisturizing and nutrients in the skin [20]. The physiologically active substance of sea cucumber extract inhibits the differentiation of lipogenic precursor cells (3T3LL). In this study, the physiological activity of cosmetics to improve skin aging was tested. The optimal extraction conditions were selected to obtain the ethanol extract of sea cucumber. Applying an effective extraction method of sea cucumber in accordance with the characteristics of ethanol solvent [21], a 50% ethanol solvent, which can effectively extract all of the ingredients contained [22] was used [23]. The effect of physiological activity of sea cucumber on skin was examined by cell experiment and clinical experiment to prove its efficacy and to provide it as a natural cosmetics material. As mentioned above, this study addresses sea cucumber extract's potential contribution to the manufacturing industry of functional cosmetics rather than cosmetics having a commercially available functional effect.

2. Experiment

2.1. Questionnaire

2.1.1. Subjects and period

The subjects of the study were 24 adult females of ages between 40 to 50 and the questionnaires were conducted after voluntary

written consent. After distributing 24 copies, 24 copies were collected and used as final analysis material.

2.1.2 Questionnaire contents

The contents of this study's questionnaire included general characteristics, lifestyle eating habits, and skincare and cosmetics use status pre-questionnaire, and the subjects' subjective skin condition for the clinical participants was investigated. The evaluation was done with the nominal scale of 3 items of general items, 7 items of skin sensation state, and 6 items of product evaluation. In order to analyze the degree of change of skin condition after the study period, the Likert 5-point scale was evaluated through questionnaires, and the items on the use of clinical manufacturing cosmetics on the skin during the clinical experiment and the satisfaction of the clinical experiment were evaluated.

2.2. Clinical experiment

2.2.1. Subjects and period

The 24 subjects participating in the study were female adult subjects between the ages of 40 to 50 residing in the greater Seoul metropolitan area. The experiment was conducted for 4 weeks divided into 2 groups of 12 subjects in the control group, and 12 subjects in the experimental group. The present study was conducted in accordance with the ethical regulations of the Institutional Review Board through preliminary deliberation and approval procedures. (Approval number : 7001355-201509-HR-085).

2.2.2. Clinical evaluation method

In this study, cosmetics for clinical use were used twice a day in the morning and evening. Moisture content, sebum content, erythema index, melanin content, and skin condition were measured before and after the clinical trial. The subjects of this study were selected except those who did not meet the criteria of the clinical subject according to the functional cosmetics guidelines of Korea Food and Drug Administration as follows.

(1) Those without chronic skin disease
(2) Those without sensitive and irritable skin
(3) Those who are taking medicines for skin allergy, photosensitivity, sunburn, serious illnesses
(4) Those who within the last 6 months, used steroid-containing external preparations for surgery and skin diseases
(5) Those with cancer, epilepsy, severe skin disease
(6) Those who have not passed a month after participating in the same experiment

2.2.3. Clinical measurement method

In this clinical trial, subjects used the same cleanser that was prepared after a certain period of time for accurate skin measurement. The indoor environmental conditions at the time of measurement were measured after a lapse of 30 minutes from the cleansing in the room temperature and humidity conditions where the room humidity was maintained at 40 to 60% and the room temperature was maintained at 22 to 23℃. For the measurement, the right and left cheeks of the face and the 3 cm side of the nose were repeatedly measured 5 times for accuracy. The moisture content, sebum content, erythema dose and melanin content for the sea cucumber extract were measured before and 4 weeks after use.

2.2.4. Clinical measurement tools

To measure the moisture of the face, a moisture meter Comeometer was used for the measurement. Comeometer® CM825 (Courage Khaazaka electronic GmbH, Germany) device measures the skin moisture content by measuring the capacitance, and it was repeated 5 times to get accurate data of the measurement value in the same part in 5

seconds by pressing on the measurement part to measure the facial skin. With sebum measurement Sebumeterⓡ SM815(Courage+ Khazaka electronic GmbHM Germany) a sebumeter cassette was used, and a probe having a translucent lipid-absorbing tape was gently adhered to the skin surface for 30 seconds at the measurement site. After the sebum was adsorbed, it was mounted on the measuring sensor part, and the amount of light transmitted was used to measure the sebum content of the facial skin by photometric reflection. The erythema index (EI) and melanin index (MI) of the skin were measured before and after the experiment using Mexameter (MX18, Courage Khazaka electronis, Germany). After the contact of the sensor to the skin surface of the selected site, the degree of erythema within 3 seconds was confirmed as the result. Below the left eye (2 ㎝ under the left pupil), below the right eye (2 ㎝ under the right pupil), left cheek (3cm adjacent to left nose cheek), right cheek(2cm adjacent to right nose cheek), left outer eye corner (3cm below left outer eye corner), right outer eye corner(3cm below right outer eye corner) were measured three times and the mean value was calculated and compared.

2.2.5. Statistical analysis

The collected data in the study were analyzed using SPSS Win 21.0. The $x2$ test was used to determine the general characteristics of the subjects. In addition, an independent t-test was performed to verify the homogeneity of the skin condition of the subjects, and a paired t-test (corresponding sample t-test) was performed to examine the pre- and post- skin condition change of the control and experiment groups. Statistical analysis was performed using an independent sample t-test to analyze the changes in the subjects' perception of skin condition and the effectiveness of the product after the end of the clinical trial.

3. Results and discussion

3.1. Verification of homogeneity of facial skin

The skin moisture and sebum content, melanin content, and erythema dose of the skin before the experiment were as shown in Table 1, and because there was no significant difference between the groups, the homogeneity of the subjects was secured(p〉. 05).

3.2. Measurement results of moisture content of facial skin

The results of analyzing moisture changes before and after according to the type of cream applied to the face of the subject were as shown in Table 2 below. In the control group, moisture decreased from 56.67 (M) to 56.20 (M), and in the experimental group, the

Table 1. Verification of identity for the state control and experimental group

Variable	Cont. (n=12) M±SD	Exp. (n=12) M±SD	t	p
Moisture (AU)	56.67±6.87	54.29±7.52	.810	.427
Sebum (㎍/㎠)	12.33±10.95	11.00±2.66	.410	.686
Melanin Index (MI)	140.50±35.63	156.66±34.79	-1.125	.273
Erythema Index (EI)	339.75±50.19	354.91±43.42	-.792	.437

Abbreviations: Cont, control ; Exp, It applied experimental *sea cucumber* extract-containing cosmetics

Table 2. Changes in facial skin moisture (Unit: AU)

Variable	Cont. (n=12)		E (n=12)	
	Before	After	Before	After
M±SD	56.67±6.87	56.20±5.53	54.29±7.52	66.77±5.74
t_1-t_2	0.47±2.81		−12.48±6.81	
t (p)	.584 (.571)		−6.344 (.000***)	

***$p<.001$

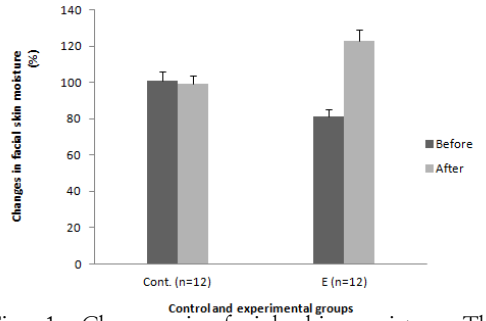

Fig. 1. Changes in facial skin moisture The results are presented as the mean ± standard deviation of three independent experiments.

moisture was significantly increased from 54.29 (M) to 66.77 (M)($p<.001$), and the high increase moisture is shown in Fig. 1. Sea cucumber extract obtained from sea cucumber contains physiologically active substance of mucopolysaccharide structure chondroitin. Chondroitin is involved in the aging of the skin, which declines with age as it performs the function of accumulating moisture and nutrients in the skin [24]. Thus, it has been confirmed that by supplying the chondroitin component of sea cucumber extract to skin, it stores moisture and nutrients of the skin, and it has been reported that the cosmetics exhibit skin improvement that is far superior to the efficacy of existing functional products [25].

3.3. Measurement results of sebum content of facial skin

The results of analyzing sebum changes before and after according to the type of cream applied to the face of the subject were as shown in Table 3 below. In the control group, sebum was significantly increased from 7.25 (M) to 8.58 (M)($p<.001$). There is a significant increase in sebum in both left and right. In the experimental group, sebum was significantly decreased from 10.58 (M) to 7.41 (M)($p<.001$). When looking at the results of a significant decrease in sebum, it can be interpreted that there is a reduction effect of sebum secretion, The subjects of this study were 40 ~ 50s and 58.3% of subjects in the control group answered that they had dry skin, and it is thought that the cosmetics used for the oily skin were recognized as dry skin and it is considered that they used products with excessive sebum content. Therefore, it can be said that it is cosmetics of appropriate function to appropriately adjust sebum of study subjects.(Fig. 2)

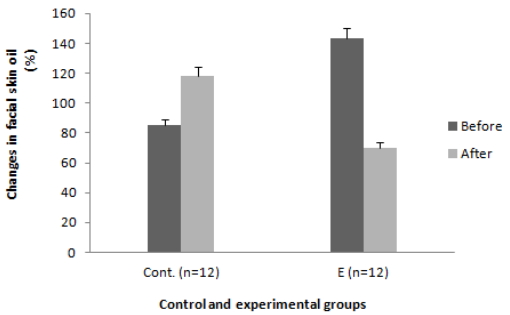

Fig. 2. Change in facial skin sebum. The results are presented as the mean ± standard deviation of three independent experiments.

3.4. Measurement results of melanin index of facial skin

The results of analyzing melanin changes before and after according to the type of cream applied to the face of the subject were as shown in Table 4 below. In the control group, melanin was significantly increased from 136.91 (M) to 146.91 (M)(p<.01), and in the experimental group, melanin was significantly decreased from 152.41 (M) to 128.75 (M)(p<.01). The 50% water soluble ethanol extract containing saponins derived from sea cucumbers has excellent whitening effect by inhibiting melanin production, and thus does not exhibit cytotoxicity as skin whitening cosmetics material of sea cucumber extract, and it has been proved that hydroquinone and Arbutin have a strong and high inhibitory effect on melanin formation at low concentration, and thus showed the result of improvement of spots and freckles and skin whitening efficacy through this clinical experiment. Therefore, as shown in Fig. 3, saponin, a component of the extract of sea cucumber, showed a significant decrease in melanin in the experimental group, and the efficacy of whitening compared to the results of the control group can be seen.

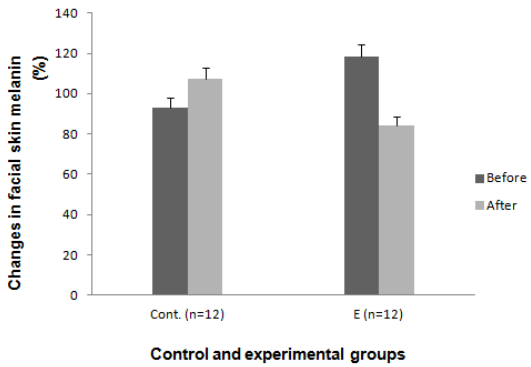

Fig. 3. Change in facial skin melanin. The results are presented as the mean ± standard deviation of three independent experiments.

3.5. Measurement results of erythema index of facial skin

The results of analyzing erythema changes before and after according to the type of cream applied to the face of the subject were as shown in Table 5 below. In the control

Table 3. Change in facial skin sebum. (unit: ㎍/㎠)

Variable	Cont. (n=12)		E (n=12)	
	Before	After	Before	After
M±SD	7.25±7.38	8.58±7.39	10.58±3.55	7.41±3.17
t_1-t_2	−1.33±0.65		3.16±1.74	
t (p)	−7.091 (.000***)		6.270 (.000***)	

***p<.001

Table 4. Change in facial skin melanin (Unit: MI)

Variable	Cont. (n=12)		E (n=12)	
	Before	After	Before	After
M±SD	136.91±38.38	146.91±33.80	152.41±29.72	128.75±31.92
t_1-t_2	−10.00±8.95		23.66±18.40	
t (p)	−3.869 (.003**)		4.454 (.001**)	

**p<.01, ***p<.001

group, erythema was significantly increased from 344.41 (M) to 353.91 (M)(p<.05). In the experimental group, erythema was significantly decreased from 376.50 (M) to 318.58 (M)(p<.001). Therefore, as shown in Fig. 4, there was a significant decrease in erythema in the experimental group. The experimental period was summer, which is considered to be the reason for the significant increase of melanin and erythema in the control group. Saponin, a hot-water extract of sea cucumber, increased collagen synthesis in a concentration-dependent manner and inhibited collagenase activity. The effect of wrinkle improvement and reduction of erythema content in the experimental group showed that 50% water soluble ethanol extract of sea cucumber extract had skin whitening and wrinkle improvement which is safe for skin and effective for improving spots and freckles.

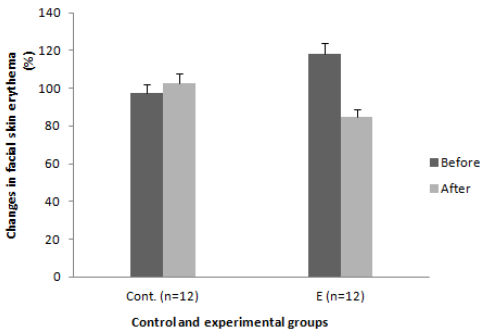

Fig. 4. Change in facial skin erythema. The results are presented as the mean ± standard deviation of three independent experiments.

3.6. Subjective evaluation results of cosmetics before and after use by subjects through questionnaire

3.6.1. Changes in feeling of skin condition

In this study, the subjects' subjective evaluation of changes in skin condition before and after the experiment was done after using the cream used in the experiment, and the results are shown in Table 6. The higher the score, the higher the improvement in skin condition after the experiment, according to 'strongly disagree' 1 point, 'disagree' 2 points, 'neutral' 3 points, 'agree' 4 points, and 'strongly agree' 5 points. Fig. 5 shows that the moisturizing ability of the control group was 3.41 (M) and that of the experimental group was 3.91 (M) showing higher experience of moisture improvement in the experiment group. In the case of sebum, it was 3.41 (M) in the control group and 4.08 (M) in the experimental group, indicating that the experimental group had significantly higher sebum improvement(p<.05). In the case of dead skin, 3.41 (M) in the control group and 4.08 (M) in the experimental group showed a significantly higher improvement of the dead skin in the experimental group than in the control group(p<.05), and in the case of whitening, 3.08 (M) in the control group and 3.66 (M) in the experimental group showed a higher whitening effect in the experimental group than in the control group. In the case of erythema, 2.83 (M) in the control group and 3.58 (M) in the experimental group showed a significantly higher erythema

Table 5. Change in facial skin erythema (Unit: EI)

Variable		Cont. (n=12)		E (n=12)	
		Before	After	Before	After
	M±SD	344.41±39.51	353.91±40.03	376.50±49.53	318.58±49.86
	t1-t2	-9.50±12.50		57.91±36.18	
	t (p)	-2.631 (.023*)		5.544 (.000***)	

*p<.05, ***p<.001

Table 6. Declining scale for the skin condition

Variable		N	M	SD	SE	t	p
Moisture power	Cont.	12	3.41	.668	.193	3.356	.081
	E	12	3.91	.668	.193		
Oil condition	Cont.	12	3.41	.668	.193	5.966	.023*
	E	12	4.08	.668	.193		
Keratinous condition	Cont.	12	3.41	.668	.193	4.958	.037*
	E	12	4.08	.792	.228		
Whitening effect	Cont.	12	3.08	.900	.259	2.882	.104
	E	12	3.66	.778	.224		
Erythema degree	Cont.	12	2.83	.717	.207	7.016	.015*
	E	12	3.58	.668	.193		
Pore size	Cont.	12	3.00	.426	.123	9.483	.005**
	E	12	3.83	.834	.241		
Wrinkles and elasticity	Cont.	12	3.58	.792	.228	1.774	.196
	E	12	4.00	.738	.213		

*p<.05, **p<.01

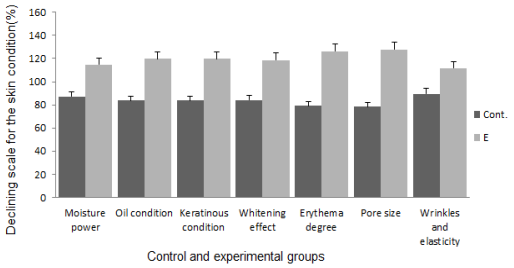

Fig. 5. After declining scale clinical trials for skin condition. The results are presented as the mean ± standard deviation of three independent experiments.

improvement in the experimental group than in the control group(p<.05), and pore size was 3.00 (M) in the control group and 3.83 (M) in the experimental group, indicating a significantly higher pore size improvement in the experimental group than in the control group(p<.01). The wrinkles and elasticity were 3.58 (M) in the control group and 4.00 (M) in the experimental group, indicating a higher improvement in wrinkles and elasticity in the experimental group than in the control group.

3.5.2. Evaluation of product efficiency

The results of the experiment subjects analyzing the effectiveness of the cream used in this study are shown in Table 7 below. The higher the score, the more positive evaluation of the effectiveness of the cream according to 'very bad' 1point, 'bad' 2points, 'average 3 points, 'good' 4 points, and 'very good' 5 points. In the case of fragrance, it was 3.50 (M) in the control group and 3.83 (M) in the experimental group which was higher than those of the control group, but there was no significant difference between groups. The color was 3.91 (M) in the control group and 4.00 (M) in the experimental group and the color satisfaction was higher in the experimental group than in the control group, but it did not show a significant difference between the groups. Spreadability was 3.33

Table 7. The efficacy of the product

Variable		N	M	SD	SE	t	p
Incense	Cot.	12	3.50	.522	.150	1.375	.253
	E	12	3.83	.834	.241		
Color	Cot.	12	3.91	.792	.228	.071	.792
	E	12	4.00	.738	.213		
Spreadability	Cot.	12	3.33	.651	.188	6.769	.016*
	E	12	4.00	.603	.174		
Satisfaction	Cot.	12	3.08	.514	.148	36.940	.000***
	E	12	4.33	.492	.142		
Trouble occurred	Cot.	12	4.50	.674	.194	.092	.764
	E	12	4.58	.668	.193		
Purchase physician	Cot.	12	3.16	.577	.166	27.077	.000***
	E	12	4.50	.674	.194		

*$p<.05$, ***$p<.001$

(M) in the control group and 4.00 (M) in the experimental group, and the satisfaction level of the spreadability was very high in the experimental group compared to the control group($p<.05$). The overall satisfaction with cream was 3.08 (M) in the control group and 4.33 (M) in the experimental group, which was significantly higher in the experimental group than in the control group($p<.001$). These results indicate that the experimental group experienced a skin improvement effect with the sea cucumber extract and thus the satisfaction level was significantly higher than the control group, and the results are synergistic effects and have a positive effect on the items such as fragrance, color, and spreadability of products that are not related to the functionality, and thus the satisfaction level of the experimental group was determined to be higher than that of the control group. In the case of the occurrence of trouble, it was 4.50 (M) in the control group and 4.58 (M) in the experimental group which showed higher satisfaction in the experimental group than in the control group and it did not show any significant difference between the groups. This indicates the level of 'disagree' in both the control and experimental groups, confirming the safety of the cream. The purchase intention was 3.16 (M) in the control group and 4.50 (M) in the experimental group, indicating a significantly higher purchase intention in the experimental group than in the control group($p<.001$). This showed the highest difference between the experimental group and the control group, and the control group showed the 'neutral' level, but the experimental group showed the level between 'agree' and 'strongly agree'. As described above (Fig. 6), it can be interpreted that the experiment group that experienced skin improvement effects showed high purchase intention.

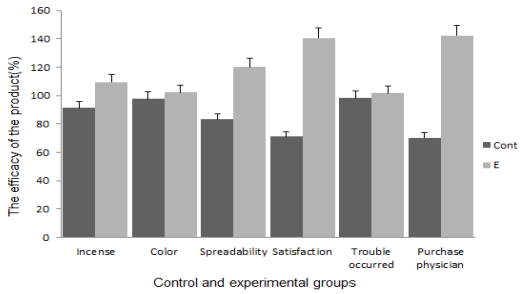

Fig. 6. After the clinical utility of the test product. The results are presented as the mean ± standard deviation of three independent experiments.

4. Conclusion

This study investigated the effect of sea cucumber extract on the skin as a natural cosmetics functional material with clinical trials and tested improvement efficacy. The purpose of this study was to investigate the suitability of natural marine animal sea cucumber extract on skin moisturizing ability as a functional cosmetics material. As of now, a variety of functions such as antiviral, anti-blood coagulation, immunity enhancement, antitumor, and regenerative efficacy have been reported as effects of the extract's excellent physiologically active substances with no adverse effects. In order to obtain ethanol extracts of superior physiologically active substances from sea cucumbers, efficient extraction conditions were selected for the purpose of maximizing the functional properties related to the quality of the extracted components and was applied to the experiment. Therefore, extracts were obtained by applying a 1:10 ratio of sample and solvent with 50% ethanol solvent. The experimental group of this study on skin cosmetics containing sea cucumber extract confirmed excellent skin improvement in all items of the clinical experiment compared to the control group. It was proved to be a stable, non-adverse physiologically active substance without abnormal symptoms or side effects of skin reactions and skin problems. In addition, the study found excellent results that can lead to the use of sea cucumber extract as a cosmetics material. It is believed that this will contribute to the development of various cosmetics industries.

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