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Effect of Continuous Ingestion of Pentadecyl-Containing Aurantiochytrium Oil on Skin Condition:

A Randomized, Double Blind, Placebo-Controlled Study

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● Abstract

Objective: The purpose of this study was to evaluate the effects of 12 weeks of consumption of pentadecyl-containing orlan oil for healthy adult women aged 35 to 59 years with dry skin and sagging faces.
Methods: The study design was a randomised, double-blind, placebo-controlled study. Fifty trial participants were randomly assigned to receive pentadecyl-containing orlane oil and placebo-controlled for 12 weeks. Skin elasticity was the primary outcome, and collagen score and stratum corneum water and water transpiration were secondary outcomes.
Results: In the test product group, there was a significant improvement in elasticity R2 (left, average), R5 (left), and R7 (left, right, mean) after 12 weeks compared to the control group. There was also a significant improvement in collagen score intensity (left, right, average), stratum corneum water content (left, right, average), and water evaporation (left).
Conclusion: These results suggest that the 12-week intake of pentadecyl-containing orlan oil improves skin elasticity by increasing collagen density, which in turn improves skin moisture.

Keywords: Aurantiochytrium Oil, Pentadecyl, collagen, endoplasmic reticulum stress, pentadecanoic acid

INTRODUCTION

The skin that covers the human body is the largest organ in the human body, accounting for about 16% of the body weight, and its structure is roughly divided into three layers: epidermis, dermis, and subcutaneous tissue [1)]. The skin functions as a barrier to protect the body from external influences such as temperature, humidity, ultraviolet rays, chemical substances, bacteria and viruses. Conversely, it also plays a role in preventing water evaporation from inside the body [2)]. The epidermis, the outermost layer of the skin, is divided into the stratum corneum, stratum granulosum, stratum spinosum, and stratum basale. The dermis, which is the lower layer of the epidermis, is composed of extracellular matrices such as collagen, elastin, and hyaluronic acid, and fibroblasts that produce them. The extracellular matrix is highly elastic and flexible, and is important as a supporting tissue that maintains the mechanical strength of the skin [1)]. That is, each layer of the skin must be in a healthy state in order for the skin, which serves as a barrier to the inside and outside of the body, to function normally. However, it has been reported that the extracellular matrix decreases with aging [4)] and also due to endoplasmic reticulum stress due to UV exposure [5)]. When the skin loses its flexibility and elasticity due to these factors, its function as a barrier against the inside and outside of the body is also impaired.

The microalgae Aurantiochytrium has the characteristic of accumulating lipids as oil droplets in cells under nutrient-rich conditions. Fatty acids accumulated at this time include not only even-numbered fatty acids such as DHA and EPA, but also odd-numbered fatty acids [6)7)]. Unlike even fatty acids, which are produced by condensation of acetyl-CoA and malonyl-CoA, odd fatty acids are synthesized by condensation of propionyl-CoA and malonyl-CoA. In recent years, odd-numbered fatty acids have been reported to have effects on hair growth, Alzheimer's disease, heart disease, cancer, and diabetes. It is believed that this replenishment reaction of propionyl-CoA is involved [8)9)].

Auran oil is produced by separate extraction from Aurantiochytrium and is mainly rich in odd-numbered fatty acids such as pentadecanoic acid. In particular, we identified a C42-C48 pentadecanoic acid lipid as pentadecyl and focused on its bioactivity [10)].

In a test in which pentadecyl was added to normal human epidermal keratinocytes, the expression levels of invocrine, transglutaminase 1, claudin 1, and filaggrin were significantly increased. In addition, in a test in

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2) Miyata Medical Clinic
3) Sea Act Co., LTD.

Table 1-1 Raw materials for test products

	Raw material name	amount
test article	Aurantiochytrium Oil* (Pentadecyl) edible corn oil	30 mg (6 mg) 120 mg
control	edible corn oil	150 mg
Coating part (common to test product and control product)	Gelatin (from pig) glycerin (vegetable) food additive glycerin Fatty acid ester	

* Contains 0.3% tocopherol (Emix D: Mitsubishi Chemical Foods) as an antioxidant.

Table 1-2 Fatty acid content of Aurantiochytrium Oil

Fatty acids	Content (g/100 g oil)
C13	1.1
C14	1.7
C15	30.5
C16	9.4
C17	4.3
EPA	0.5
DPA	4.1
DHA	31.8
Others	2.8
Total	86.2

Table 1-3 Fatty acid composition of Pentadecyle

Fatty acids	Fatty acids composition	
C42	C15, C14, C13	
C43	C15, C15, C13	C15, C14, C14
C44	C15, C15, C14	
C45	C15, C15, C15	
C46	C15, C15, C16	
C47	C15, C15, C17	C15, C16, C16
C48	C15, C16, C17	

C13 = Tridecanoic acid, C14 = Tetradecanoic acid, C15 = Pentadecanoic acid, C16 = Hexadecanoic acid, C17 = Heptadecanoic acid

which it was added to normal human dermal fibroblasts, it was confirmed that the production of type I collagen was significantly increased [11].

Furthermore, a test in which pentadecyl was added to human fibroblasts under endoplasmic reticulum stress confirmed an increase in collagen production due to the relaxation of endoplasmic reticulum stress [12]. From this, it is considered that one of the mechanisms of photoaging is that the endoplasmic reticulum in the dermal fibroblasts is abnormal and collagen production is reduced by UV exposure, so pentadecyl can be a strategy to suppress collagen reduction (Five).

Based on the above, pentadecyl intake is expected to have the function of keeping the skin in a healthy state, such as improving the skin's moisturizing function and maintaining elasticity by preventing a decrease in collagen production due to endoplasmic reticulum stress. A previous study in which pentadecyl-containing auran oil-containing food was continuously ingested for 6 weeks suggested an improvement in skin moisture content in a subgroup analysis based on skin moisture content. It is possible that differences in skin condition between subjects, due to whether or not they were aware of dryness, affected the results [13]. Therefore, in this study, we investigated the effects on skin functions of subjects who were aware of their skin dryness when they continuously ingested pentadecyl-containing auran oil-containing food for 12 weeks.

Table 1-4 Nutritional composition of test product (per capsule)

item	amount
energy	1.8 kcal
protein	0.1 g
lipid	0.15 g
carbohydrates	0 g
moisture	0 g
cholesterol	0 g
dietary fiber	0 g

1. SUBJECTS AND METHODS

1-1 Study design and ethics review committee

This study was a randomized, double-blind, placebo-controlled study with Akinobu Miyata (Director, Miyata Medical Clinic) as the principal investigator. This trial was previously registered in the UMIN Clinical Trials

Table 2　Subject Background of Valid Analysis Subjects

Item	unit	Test product group (n = 24)	Control group (n = 25)
age *	age	51.9 ± 4.0	50.1 ± 5.1
elasticityR2_left	index	0.645 ± 0.078	0.694 ± 0.052
elasticityR2_right *	index	0.658 ± 0.059	0.687 ± 0.054
elasticityR2_average	index	0.651 ± 0.061	0.691 ± 0.050
elasticityR5_left *	index	0.479 ± 0.088	0.508 ± 0.085
elasticityR5_right *	index	0.497 ± 0.103	0.506 ± 0.074
elasticityR5_average *	index	0.488 ± 0.092	0.507 ± 0.073
elasticityR7_left *	index	0.301 ± 0.054	0.329 ± 0.049
elasticityR7_right *	index	0.316 ± 0.053	0.336 ± 0.051
elasticityR7_average *	index	0.309 ± 0.051	0.333 ± 0.044
Intnsity_left *	index	43.78 ± 8.97	43.12 ± 8.54
Intnsity_right *	index	48.72 ± 10.08	46.12 ± 8.89
Intnsity_average *	index	46.25 ± 8.62	44.62 ± 8.05
LEB_left	μm	146.4 ± 109.7	75.3 ± 90.9
LEB_right *	μm	120.1 ± 116.2	66.3 ± 87.0
LEB_average	μm	133.3 ± 101.0	70.8 ± 71.6
Thickness_left *	μm	1157.0 ± 159.0	1248.7 ± 209.2
Thickness_right *	μm	1124.2 ± 177.1	1221.9 ± 220.9
Thickness_average *	μm	1140.6 ± 145.5	1235.3 ± 203.5
stratum corneum water content_left	index	25.65 ± 6.03	29.97 ± 7.06
stratum corneum water content_right	index	26.23 ± 6.42	30.36 ± 6.82
stratum corneum water content_average	index	25.94 ± 6.20	30.17 ± 6.63
water loss_left *	g/hm^2	11.51 ± 3.10	10.38 ± 3.54
water loss_right *	g/hm^2	10.81 ± 2.82	10.75 ± 3.22
water loss_average *	g/hm^2	11.16 ± 2.87	10.57 ± 3.30

mean ± standard deviation

* no significant difference between groups

Registry (UMIN000050031) and is compliant with the Declaration of Helsinki (Revised October 2013, Fortaleza) and the Ethical Guidelines for Life Science and Medical Research Involving Human Subjects (Effective March 2021), after obtaining approval from the Ethics Review Committee of the Pharmaceutical Affairs Law Advisory Council (Chairman: Toshio Houga, Attorney-at-Law) (January 4, 2023), the subject was given a written informed consent, and the purpose and method of this study were fully explained in writing and verbally. The study was conducted with written informed consent from the subject.

1-2 Target person

JACTA conducted general recruitment through Inchrome Co., Ltd. (Tokyo), and subjects were those who met the following selection criteria, who did not meet the exclusion criteria, and who voluntarily wished to use the test product.

1-2-1 Selection criteria

① Healthy women aged from 35 to 59
② Those with dry skin
③ Those who are concerned about facial sagging
④ Subjects with a wrinkle grade of 2 to 4 on the left and right corners of the eyes
⑤ Subjects who have given written consent of their own free will after fully understanding their participation in this research

1-2-2 Exclusion criteria

① Individuals with a history of food allergies
② Those who are pregnant, breastfeeding, or planning to do so during the test period
③ Those who are currently going to the hospital or receiving a doctor's prescription
④ Those who have experience in aesthetic medicine on the test site
⑤ Those who have inflammation or skin disease on the face
⑥ Those who are receiving hormone replacement therapy
⑦ Subjects taking supplements or medicines that affect the effects of the test product
⑧ Subjects who are likely to show allergic symptoms to drugs or food
⑨ Excessive smokers, regular alcohol users, and persons with extremely irregular eating habits

⑩ Subjects who have participated in other human clinical trials within one month prior to obtaining informed consent, and subjects who plan to participate in other clinical trials during the study period
⑪ Subjects deemed inappropriate by the study supervisor

1-2-3 Sample size
Based on the findings obtained from previous research, the significance level was set at 5%, the power was set at 80%, and the sample size was set at 48 ± 2 cases.

1-3 Test product
The test product was pentadecyl-containing auran oil provided by Sea Act Co., Ltd.

Subjects took 1 capsule each day for 12 weeks with water or lukewarm water after breakfast for test or control. The test product and the control product were provided with an identification code after making them indistinguishable in terms of shape, color and taste. **Table 1-1** shows the raw materials of the test product and control product, **Table 1-2** shows the Fatty acid content of auran oil, **Table 1-3** shows the fatty acid composition of pentadecyle, and **Table 1-4** shows the nutritional composition of the test product.

1-4 Randomization
Fifty applicants who met the inclusion criteria and did not meet the exclusion criteria were selected and randomly assigned by an allocator unrelated to the trial. In order to prevent bias, 25 people were divided into groups A and B, taking age into consideration (see **Table 2**). Allotment contents were kept strictly by the person in charge of allotment and disclosed to the study site after data fixation (key open). Group A took the test product and Group B took the control product for 12 weeks.

1-5 Test schedule
The study period will be from January to April 2023, with 4 observation days before the start, 4 weeks, 8 weeks, and 12 weeks. They were aligned and the times were almost the same. During the test period, instruct all subjects not to start using or taking any medicines or health foods that affect the test site, and to maintain a normal life, and submit a diary to confirm this point. (see 1-7). **Table 3** shows the test schedule.

1-6 Matters to be observed by subjects
All subjects were instructed to lead a normal life during the test period and to comply with the following items.

(1) To maintain the lifestyle habits such as eating, exercising, drinking alcohol, smoking, sleeping hours, etc. from before participating in the study without changing them.
(2) Avoid excessive exercise, sleep deprivation, excessive eating and drinking (banquets, all-you-can-eat, buffets, etc.) and diets that deviate from your daily routine.
(3) It is prohibited to use pharmaceuticals, quasi-drugs, health foods, etc. that claim or emphasize skin-related effects, and to receive esthetics and treatments.
(4) Do not take medicine unless it is unavoidable. When taking medicines, record the name of the medicine and the amount taken in the diary.
(5) Subjects who have been taking quasi-drugs and health foods before participating in the study should continue to take them without changing the intake amount, intake frequency, or intake method. Intake of new quasi-drugs and health foods is prohibited.
(6) Staying up late, staying up all night, and strenuous exercise (breathtaking running, swimming, mountain climbing, etc.) are prohibited for 3 days before the measurement.
(7) Do not drink alcohol the day before your visit, get enough sleep, and keep yourself in good physical condition.

2. EVALUATION ITEM

On the 4 observation days, the subjects washed their face with a commercially available cleansing agent to remove makeup and cleanse their face, and then rested for 20 minutes in a room maintained at a temperature of 21 ± 1℃ and a humidity of 50 ± 5RH% to acclimatize their skin. After that, each measurement was performed. Measurements were taken on the left and right sides of the face (once), and the values for each of the left and right sides and the average value for the left and right sides were used.

2-1 Elasticity
Cutometer® MPA580 (Courage+Khazaka electronic GmbH) was used to measure the intersection of a vertical line drawn from the outer corner of the eye and a horizontal line drawn from the nostril of each subject. R2 (total elasticity), R5 (net elasticity) and R7 (elasticity at retraction) were obtained. Measurements were taken once on each side. The unit is a percentage, and the closer to 1.0, the higher the elasticity.

2-2 Collagen score
DermaLab (Cortex Technology) was used to measure the left and right temple circumferences of the subjects. Intensity represents the collagen density of the dermis, and the higher the value, the better the condition. LEB represents the width of the portion with less collagen in μm, and the smaller the LEB, the better the condition. Thickness represents the thickness of the dermal collagen, and the narrower it is (the smaller the value), the better the condition.

Table 3　Test schedule

項目	consent	Main test				
		before start	start	4weeks later	8weeks later	12weeks later
Test explanation	●					
measurement		●		●	●	●
Ingestion of test and control products			←→	←→	←→	←→
Diary			←→	←→	←→	←→

● : Conducted on measurement day
←→: Every day during the period

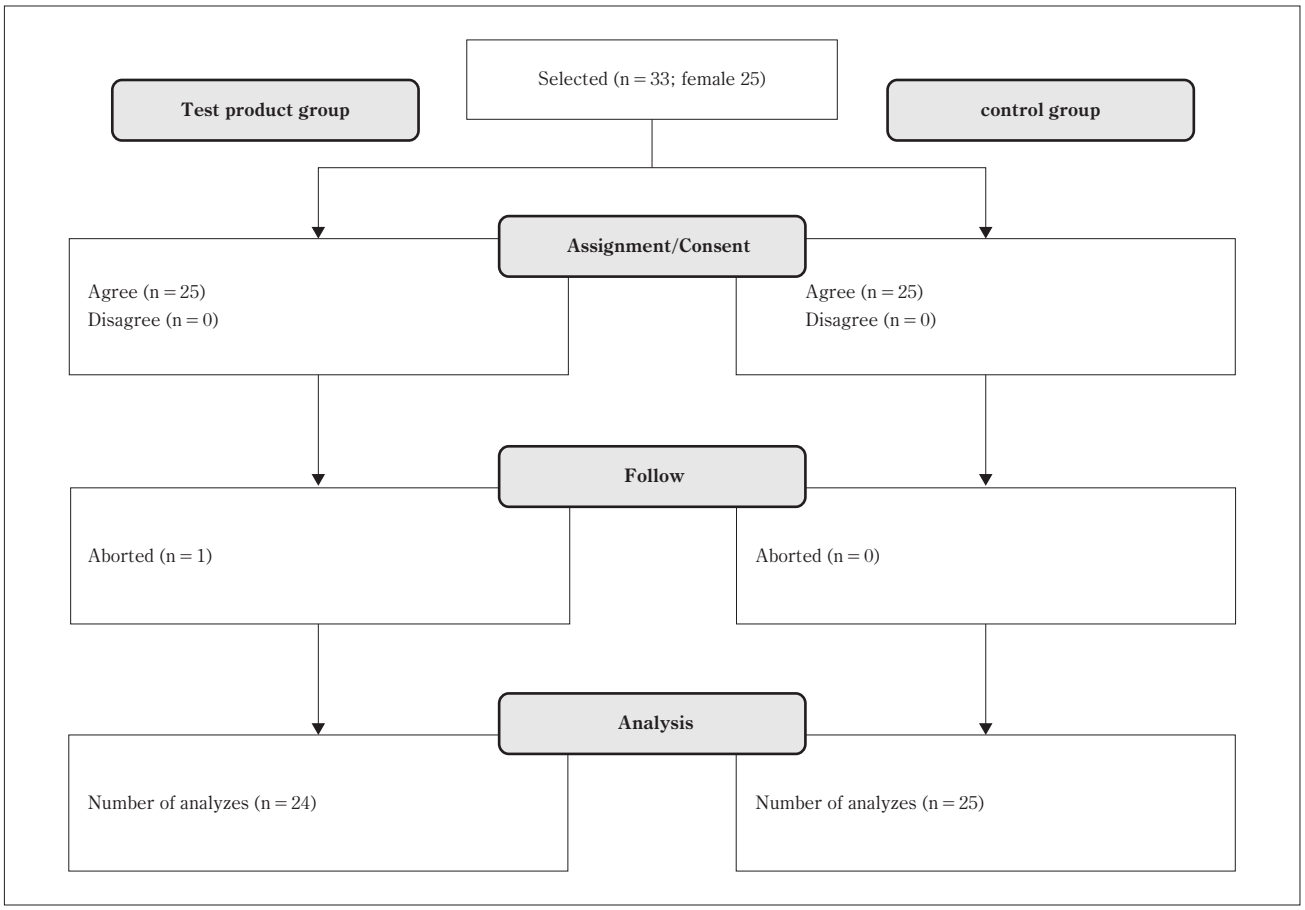

Figure 1　Analysis target decision process

2-3 Stratum corneum moisture content/moisture loss
The stratum corneum moisture content was measured using a Corneometer® CM825 (Courage+Khazaka electronic GmbH). Measurements were taken at the intersection of a line drawn vertically from the outer corner of each eye of the subject and a line drawn horizontally from the nostril. Measurements were taken once on each side. The unit is an index, and the higher the number, the higher the water content.

Water loss was measured using a Tewameter® TM300 (Courage+Khazaka electronic GmbH). A line drawn vertically from the outer corner of each eye of the subject and a line drawn horizontally from the nostril intersected, and the value was obtained. The unit is g/h m^2, and the smaller the number, the less water transpiration.

2-4 Safety
The safety of the study article was assessed based on diary surveys of lifestyle and adverse events during the study period. In addition, the principal investigator evaluated and judged the relationship between adverse events and the test product.

Table 4 Changes in elasticity (1)

Item	point in time	Index		p-value [2]
		Test product group (n=24) [1]	Control group (n=25) [1]	
elasticity R2_left	a before start	0.645 ± 0.078	0.694 ± 0.052	
	b 4weeks later	0.697 ± 0.066	0.672 ± 0.053	
	⊿ a − b	0.051 ± 0.093 *	− 0.022 ± 0.063 †	0.002 ##
	c 8weeks later	0.631 ± 0.076	0.687 ± 0.067	
	⊿ a − c	− 0.015 ± 0.093	− 0.008 ± 0.070	0.767
	d 12weeks later	0.666 ± 0.075	0.649 ± 0.077	
	⊿ a − d	0.021 ± 0.104	− 0.045 ± 0.078 **	0.015 #
elasticity R2_right	a before start	0.658 ± 0.059	0.687 ± 0.054	
	b 4weeks later	0.690 ± 0.061	0.669 ± 0.061	
	⊿ a − b	0.033 ± 0.072 *	− 0.018 ± 0.056	0.009 ##
	c 8weeks later	0.641 ± 0.057	0.668 ± 0.069	
	⊿ a − c	− 0.016 ± 0.069	− 0.019 ± 0.067	0.879
	d 12weeks later	0.654 ± 0.081	0.643 ± 0.076	
	⊿ a − d	− 0.003 ± 0.078	− 0.044 ± 0.064 **	0.052 ‡
elasticity R2_average	a before start	0.651 ± 0.061	0.691 ± 0.050	
	b 4weeks later	0.693 ± 0.058	0.670 ± 0.052	
	⊿ a − b	0.042 ± 0.072 **	− 0.020 ± 0.052 †	0.001 ##
	c 8weeks later	0.636 ± 0.060	0.677 ± 0.061	
	⊿ a − c	− 0.015 ± 0.075	− 0.013 ± 0.060	0.917
	d 12weeks later	0.660 ± 0.069	0.646 ± 0.072	
	⊿ a − d	0.009 ± 0.082	− 0.045 ± 0.066 **	0.016 #
elasticity R5_left	a before start	0.479 ± 0.088	0.508 ± 0.085	
	b 4weeks later	0.503 ± 0.092	0.517 ± 0.093	
	⊿ a − b	0.025 ± 0.029 **	0.009 ± 0.056	0.235
	c 8weeks later	0.472 ± 0.089	0.499 ± 0.095	
	⊿ a − c	− 0.007 ± 0.104	− 0.009 ± 0.093	0.941
	d 12weeks later	0.538 ± 0.073	0.501 ± 0.138	
	⊿ a − d	0.059 ± 0.072 **	− 0.007 ± 0.113	0.019 #
elasticity R5_right	a before start	0.497 ± 0.103	0.506 ± 0.074	
	b 4weeks later	0.528 ± 0.101	0.510 ± 0.090	
	⊿ a − b	0.031 ± 0.031 **	0.004 ± 0.062	0.060 ‡
	c 8weeks later	0.485 ± 0.075	0.506 ± 0.113	
	⊿ a − c	− 0.012 ± 0.096	− 0.000 ± 0.116	0.704
	d 12weeks later	0.530 ± 0.072	0.515 ± 0.118	
	⊿ a − d	0.033 ± 0.104	0.009 ± 0.099	0.416
elasticity R5_average	a before start	0.488 ± 0.092	0.507 ± 0.073	
	b 4weeks later	0.516 ± 0.088	0.513 ± 0.081	
	⊿ a − b	0.028 ± 0.023 **	0.006 ± 0.057	0.095 ‡
	c 8weeks later	0.479 ± 0.069	0.502 ± 0.097	
	⊿ a − c	− 0.009 ± 0.086	− 0.005 ± 0.090	0.851
	d 12weeks later	0.534 ± 0.060	0.508 ± 0.120	
	⊿ a − d	0.046 ± 0.081 *	0.001 ± 0.097	0.085 ‡

mean ± standard deviation

1) † :p<0.1, * :p<0.05, ** :p<0.01 vs. before start

2) ‡ :p<0.1, # :p<0.05, ## :p<0.01 vs. control group

Table 4　Changes in elasticity (2)

Item	point in time	Index		p-value [2]
		Test product group (n=24) [1]	Control group (n=25) [1]	
elasticityR7_left	a　before start	0.301 ± 0.054	0.329 ± 0.049	
	b　4weeks later	0.322 ± 0.056	0.328 ± 0.056	
	⊿ a − b	0.021 ± 0.014 **	− 0.001 ± 0.033	0.005 ##
	c　8weeks later	0.301 ± 0.054	0.324 ± 0.059	
	⊿ a − c	− 0.000 ± 0.061	− 0.005 ± 0.055	0.794
	d　12weeks later	0.332 ± 0.047	0.311 ± 0.072	
	⊿ a − d	0.030 ± 0.051 **	− 0.018 ± 0.053 †	0.002 ##
elasticityR7_right	a　before start	0.316 ± 0.053	0.336 ± 0.051	
	b　4weeks later	0.333 ± 0.062	0.324 ± 0.060	
	⊿ a − b	0.018 ± 0.021 **	− 0.012 ± 0.036 †	0.001 ##
	c　8weeks later	0.303 ± 0.044	0.330 ± 0.072	
	⊿ a − c	− 0.013 ± 0.051	− 0.006 ± 0.061	0.668
	d　12weeks later	0.335 ± 0.042	0.315 ± 0.063	
	⊿ a − d	0.019 ± 0.063	− 0.021 ± 0.046 *	0.015 #
elasticityR7_average	a　before start	0.309 ± 0.051	0.333 ± 0.044	
	b　4weeks later	0.328 ± 0.055	0.326 ± 0.052	
	⊿ a − b	0.019 ± 0.013 **	− 0.007 ± 0.032	0.001 ##
	c　8weeks later	0.302 ± 0.041	0.327 ± 0.061	
	⊿ a − c	− 0.007 ± 0.047	− 0.006 ± 0.046	0.924
	d　12weeks later	0.333 ± 0.038	0.313 ± 0.064	
	⊿ a − d	0.025 ± 0.051 *	− 0.020 ± 0.045 *	0.002 ##

mean ± standard deviation

1) † :p<0.1, * :p<0.05, ** :p<0.01 vs. before start

2) ‡ :p<0.1, # :p<0.05, ## :p<0.01 vs. control group

2-5 Statistical processing

Analysis adopted FAS. Measured values (scores) are shown as mean ± standard deviation. A paired t-test was performed for comparisons with before the start, and Student's t-test was performed for comparisons between groups and subject background bias. Data multiplicity was not considered and there were no missing values. In both cases, a significant difference was judged to be less than 5% (p<0.05) in a two-sided test. Statcel 4 (Hisae Yanai, 2015) was used as statistical analysis software.

3. RESULTS

3-1 Subject background

Fifty started the study, one discontinued for personal reasons, and 49 completed the study. Forty-nine subjects (41-58 years old, 51.0 ± 4.6 years old) were included in the analysis. **Figure 1** shows the flow up to the analysis, and **Table 3** shows the background of the subjects. The intake rate of the test product group was 100% for 20 people, 99% for 3 people, and 94% for 1 person. % had one and 89% had one.

3-2 Results of elasticity

Table 4 shows changes in measured values. In comparison with the control group, there was a significant difference in increase (improvement) in R2 (left, right, left-right average) and R7 (left, right, left-right average) after 4 weeks, and R2 (left, left-right average), R5 (left), and R7 (left, right, left-right average) after 12 weeks.

Compared to before start R2 (left, right, left-right average), R5 (left, right, left-right average) and R7 (left, right, left-right average) after 4 weeks, and R5 (left, left-right average) and R7 (left, left-right average) after 12 weeks showed a significant increase in test product group. In the control group, a significant decrease (exacerbation) was observed in R2 (left, right, left-right average) and R7 (right, left-right average) after 12 weeks.

3-3 Collagen score results

Table 5 shows changes in measured values. In comparison with the control group, there was a significant decrease (improvement) in LEB after 8 weeks,

Table 5 Changes in Collagen Score (1)

Item (unit)	point in time	Index		p-value [2]
		Test product group (n=24) [1]	Control group (n=25) [1]	
Intensity_left (index)	a before start	43.78 ± 8.97	43.1 ± 8.5	
	b 4weeks later	46.05 ± 10.71	45.5 ± 11.4	
	⊿ a − b	2.28 ± 7.80	2.4 ± 9.6	0.950
	c 8weeks later	45.12 ± 7.49	40.8 ± 10.7	
	⊿ a − c	1.34 ± 7.17	− 2.3 ± 10.9	0.174
	d 12weeks later	47.29 ± 8.83	38.6 ± 9.2	
	⊿ a − d	3.51 ± 7.32 *	− 4.5 ± 8.6 *	0.001 ##
Intensity_right (index)	a before start	48.72 ± 10.08	46.1 ± 8.9	
	b 4weeks later	45.44 ± 9.05	41.7 ± 9.1	
	⊿ a − b	− 3.28 ± 9.63	− 4.5 ± 9.2 *	0.663
	c 8weeks later	46.07 ± 7.35	38.4 ± 8.5	
	⊿ a − c	− 2.65 ± 9.36	− 7.7 ± 9.5 **	0.067 ‡
	d 12weeks later	47.60 ± 8.12	37.9 ± 8.0	
	⊿ a − d	− 1.12 ± 8.61	− 8.2 ± 7.8 **	0.004 ##
Intensity_average (index)	a before start	46.25 ± 8.62	44.6 ± 8.0	
	b 4weeks later	45.75 ± 8.93	43.6 ± 9.5	
	⊿ a − b	− 0.50 ± 5.89	− 1.0 ± 8.0	0.800
	c 8weeks later	45.60 ± 6.80	39.6 ± 9.2	
	⊿ a − c	− 0.65 ± 6.75	− 5.0 ± 9.1 *	0.064 ‡
	d 12weeks later	47.44 ± 7.92	38.3 ± 8.2	
	⊿ a − d	1.20 ± 5.57	− 6.4 ± 6.8 **	0.000 ##
LEB_left (μm)	a before start	146.4 ± 109.7	75.3 ± 90.9	
	b 4weeks later	108.5 ± 119.8	59.0 ± 93.8	
	⊿ a − b	− 37.9 ± 141.9	− 16.3 ± 101.7	0.543
	c 8weeks later	96.2 ± 145.2	95.8 ± 147.2	
	⊿ a − c	− 50.3 ± 109.8 *	20.5 ± 131.3	0.047 #
	d 12weeks later	136.6 ± 134.4	51.6 ± 81.6	
	⊿ a − d	− 9.8 ± 96.1	− 23.7 ± 89.1	0.601
LEB_right (μm)	a before start	120.1 ± 116.2	66.3 ± 87.0	
	b 4weeks later	171.8 ± 141.7	85.3 ± 125.5	
	⊿ a − b	51.7 ± 139.5 †	19.0 ± 147.9	0.430
	c 8weeks later	131.1 ± 145.9	37.9 ± 81.4	
	⊿ a − c	11.0 ± 153.2	− 28.4 ± 95.7	0.284
	d 12weeks later	125.8 ± 109.4	42.7 ± 79.1	
	⊿ a − d	5.6 ± 117.6	− 23.6 ± 96.7	0.346
LEB_average (μm)	a before start	133.3 ± 101.0	70.8 ± 71.6	
	b 4weeks later	140.2 ± 109.3	72.1 ± 91.2	
	⊿ a − b	6.9 ± 98.5	1.3 ± 88.1	0.835
	c 8weeks later	113.6 ± 120.2	66.9 ± 94.3	
	⊿ a − c	− 19.6 ± 78.3	− 3.9 ± 72.1	0.468
	d 12weeks later	131.2 ± 100.7	47.2 ± 68.1	
	⊿ a − d	− 2.1 ± 65.6	− 23.7 ± 63.2 †	0.247

mean ± standard deviation

1) † :p<0.1, * :p<0.05, ** :p<0.01 vs. before start

2) ‡ :p<0.1, # :p<0.05, ## :p<0.01 vs. control group

Table 5　Changes in Collagen Score (2)

Item (unit)	point in time	Index		p-value [2]
		Test product group (n=24) [1]	Control group (n=25) [1]	
Thickness_left (μm)	a　before start	1157.0 ± 159.0	1248.7 ± 209.2	
	b　4weeks later	1160.4 ± 202.4	1213.9 ± 195.2	
	⊿ a − b	3.4 ± 175.7	− 34.8 ± 179.1	0.455
	c　8weeks later	1146.0 ± 217.3	1258.6 ± 235.8	
	⊿ a − c	− 11.0 ± 202.5	9.9 ± 102.0	0.648
	d　12weeks later	1198.3 ± 231.3	1241.9 ± 194.1	
	⊿ a − d	41.3 ± 212.6	− 6.8 ± 149.5	0.363
Thickness_right (μm)	a　before start	1124.2 ± 177.1	1221.9 ± 220.9	
	b　4weeks later	1106.8 ± 195.7	1210.4 ± 218.9	
	⊿ a − b	− 17.4 ± 216.1	− 11.4 ± 149.1	0.910
	c　8weeks later	1155.4 ± 296.4	1165.5 ± 157.7	
	⊿ a − c	31.2 ± 310.1	− 56.4 ± 210.0	0.251
	d　12weeks later	1095.0 ± 223.9	1209.4 ± 184.0	
	⊿ a − d	− 29.2 ± 229.0	− 12.5 ± 203.1	0.789
Thickness_average (μm)	a　before start	1140.6 ± 145.5	1235.3 ± 203.5	
	b　4weeks later	1133.6 ± 177.1	1212.2 ± 171.2	
	⊿ a − b	− 7.0 ± 155.1	− 23.1 ± 114.1	0.680
	c　8weeks later	1150.7 ± 234.2	1212.0 ± 165.6	
	⊿ a − c	10.1 ± 220.7	− 23.3 ± 107.8	0.502
	d　12weeks later	1146.6 ± 213.9	1225.6 ± 164.2	
	⊿ a − d	6.0 ± 191.1	− 9.7 ± 133.8	0.739

mean ± standard deviation

1) † :p<0.1, * :p<0.05, ** :p<0.01 vs. before start

2) ‡ :p<0.1, # :p<0.05, ## :p<0.01 vs. control group

and a significant increase (improvement) in intensity (left, right, left-right average) after 12 weeks.

Compared to before the start of treatment, the test product group showed a significant decrease in LEB (left) after 8 weeks and a significant increase in intensity (left, right, average) after 12 weeks. In the control group, a significant decrease in intensity was observed after 4 weeks (left), 8 weeks (right, average), and 12 weeks (left, right, average).

3-4 Results of stratum corneum water content and water loss

Table 6 shows changes in measured values. In comparison with the control product group, the stratum corneum water content significantly increased (improved) after 4 weeks, 8 weeks, and 12 weeks (all left, right, average), and the amount of water loss was significantly increased after 12 weeks (left) showed a significant decrease (improvement) difference.

Compared to before the start, the test product group showed a significant increase in stratum corneum water content after 4 weeks, 8 weeks, and 12 weeks (all left, right, average). In the control product group, the stratum corneum moisture content (right) after 4 weeks significantly decreased (exacerbated), the moisture loss (left, right, average) significantly decreased (improved), and the stratum corneum moisture content after 8 weeks. (left, average), a significant decrease in stratum corneum water content (left, right, average) after 12 weeks, and a significant increase (exacerbation) in water loss (left, average).

3-5 Safety

As a result of the adverse event survey by measurement and diary, no adverse events occurred in this study, and no clinical side effects caused by the test product were observed.

4. DISCUSSION

A test was conducted to evaluate changes in skin elasticity when pentadecyl-containing auran oil was continuously ingested for 12 weeks. A randomized, double-blind, placebo-controlled study was designed in

Table 6 Changes in stratum corneum water content and water loss

Item (unit)	point in time	Index		p-value [2]
		Test product group (n=24)[1]	Control group (n=25) [1]	
stratum corneum water content_left (index)	a before start	25.65 ± 6.03	29.97 ± 7.06	
	b 4weeks later	31.42 ± 10.56	29.96 ± 7.70	
	⊿ a − b	5.77 ± 5.82 **	− 0.01 ± 1.70	0.000 ##
	c 8weeks later	36.22 ± 8.24	27.31 ± 8.04	
	⊿ a − c	10.57 ± 4.42 **	− 2.66 ± 4.50 **	0.000 ##
	d 12weeks later	47.48 ± 6.20	26.15 ± 6.22	
	⊿ a − d	21.83 ± 4.36 **	− 3.82 ± 3.98 **	0.000 ##
stratum corneum water content_right (index)	a before start	26.23 ± 6.42	30.36 ± 6.82	
	b 4weeks later	31.55 ± 8.73	29.34 ± 7.06	
	⊿ a − b	5.33 ± 4.50 **	− 1.03 ± 2.07 *	0.000 ##
	c 8weeks later	37.02 ± 8.91	28.06 ± 8.53	
	⊿ a − c	10.80 ± 5.92 **	− 2.30 ± 5.62 †	0.000 ##
	d 12weeks later	47.31 ± 5.99	26.54 ± 6.26	
	⊿ a − d	21.09 ± 4.90 **	− 3.83 ± 5.02 **	0.000 ##
stratum corneum water content_average (index)	a before start	25.94 ± 6.20	30.17 ± 6.63	
	b 4weeks later	31.49 ± 9.34	29.65 ± 6.92	
	⊿ a − b	5.55 ± 4.51 **	− 0.52 ± 1.37 †	0.000 ##
	c 8weeks later	36.62 ± 8.10	27.69 ± 7.70	
	⊿ a − c	10.68 ± 4.40 **	− 2.48 ± 4.31 **	0.000 ##
	d 12weeks later	47.40 ± 5.57	26.34 ± 5.72	
	⊿ a − d	21.46 ± 3.84 **	− 3.82 ± 3.54 **	0.000 ##
water loss_left (g/hm^2)	a before start	11.51 ± 3.10	10.38 ± 3.54	
	b 4weeks later	10.50 ± 4.51	9.22 ± 3.89	
	⊿ a − b	− 1.01 ± 2.94	− 1.16 ± 1.63 **	0.824
	c 8weeks later	10.23 ± 4.69	11.66 ± 5.65	
	⊿ a − c	− 1.28 ± 5.67	1.28 ± 4.41	0.083 ‡
	d 12weeks later	10.74 ± 4.04	12.86 ± 5.99	
	⊿ a − d	− 0.77 ± 4.63	2.47 ± 5.74 *	0.035 #
water loss_right (g/hm^2)	a before start	10.81 ± 2.82	10.75 ± 3.22	
	b 4weeks later	10.14 ± 3.10	9.99 ± 3.02	
	⊿ a − b	− 0.66 ± 2.24	− 0.76 ± 1.58 *	0.858
	c 8weeks later	10.23 ± 3.49	11.24 ± 4.50	
	⊿ a − c	− 0.58 ± 4.33	0.49 ± 3.12	0.324
	d 12weeks later	10.51 ± 2.37	11.81 ± 3.45	
	⊿ a − d	− 0.30 ± 3.49	1.05 ± 2.79 †	0.140
water loss_average (g/hm^2)	a before start	11.16 ± 2.87	10.57 ± 3.30	
	b 4weeks later	10.32 ± 3.72	9.60 ± 3.40	
	⊿ a − b	− 0.84 ± 2.42	− 0.96 ± 1.47 **	0.827
	c 8weeks later	10.23 ± 4.05	11.45 ± 5.04	
	⊿ a − c	− 0.93 ± 4.93	0.89 ± 3.67	0.149
	d 12weeks later	10.63 ± 3.10	12.33 ± 4.59	
	⊿ a − d	− 0.53 ± 3.86	1.76 ± 4.13 *	0.050 ‡

mean ± standard deviation

1) † :$p<0.1$, * :$p<0.05$, ** :$p<0.01$ vs. before start

2) ‡ :$p<0.1$, # :$p<0.05$, ## :$p<0.01$ vs. control group

women aged 35 to 59 who were concerned about dryness and sagging skin.

The primary outcome was skin elasticity, and the secondary outcomes were collagen score, stratum corneum water content, and water loss. There was a significant improvement difference in R2 (left, right, left-right average) and R7 (left, right, left-right average) after 4 weeks, and R2 (left, left-right average), R5 (left) and R7 (left, right, left-right average) after 12 weeks. As for secondary outcomes, there was a significant difference in improvement after 12 weeks of collagen score intensity (left, right, left-right average) and after 8 weeks of LEB. There was a significant improvement in the stratum corneum water content after 4 weeks, 8 weeks, and 12 weeks (all left, right, average), and for water loss on the left side after 12 weeks. Therefore, continuous intake of pentadecyl for 12 weeks can be expected to improve skin elasticity and moisturizing function.

It has been confirmed that pentadecyl increases collagen production in dermal fibroblasts [11], and the collagen score after 12 weeks was significantly increased in this study as well. Collagen is the main component of the dermis layer and is important as a supporting tissue that maintains the mechanical strength of the skin because it has strong resistance to tension acting along the running of collagen fibers [1]. It is considered that the skin elasticity was significantly improved due to the increase in the amount of collagen. Furthermore, since the dermis layer also plays a role in supplying moisture to the epidermis [14], it is thought that the moisturizing and barrier functions of the epidermis were improved by the dermal layer maintaining its elasticity and exerting sufficient functions. Apart from this, pentadecyl has also been reported to have the effect of activating epidermal keratinocytes [11], and it is speculated that it affected the moisturizing and barrier functions.

Exposure to various stresses such as photoaging can cause fibroblasts and keratinocytes to produce unfolded proteins in the endoplasmic reticulum. In particular, in fibroblasts, collagen proteins are produced in the endoplasmic reticulum, but accumulation of unfolded collagen proteins in the endoplasmic reticulum causes endoplasmic reticulum stress, resulting in decrease in normal collagen production in fibroblasts and exacerbation of the extracellular matrix of the skin. In human fibroblasts under endoplasmic reticulum stress, an increase in expression of XBP1S, an endoplasmic reticulum stress marker gene, was observed, and the addition of pentadecyl suppressed the increase in a dose-dependent manner. Similarly, the decrease in collagen production in human fibroblasts under endoplasmic reticulum stress was improved in a dose-dependent manner with the addition of pentadecyl [12]. Therefore, it is suggested that pentadecyl alleviates the endoplasmic reticulum stress of fibroblasts and promotes the production of normal collagen as a mechanism of action in improving skin condition.

Although this study was conducted with only women aged 35 to 59 as subjects, the mechanism of improving the structure of the skin such as the epidermis and dermis, moisturizing function, and elasticity is common to both men and women. For this reason, the effect of continuous pentadecyl intake that was clarified in this study can also be expected when targeting men.

We acknowledged that the results of previous studies may have been affected by seasonal variations, and that subgroup analysis suggested an improvement in moisturizing function for those with lower skin moisture content [13]. Since this test was conducted from January to April, it is thought that the influence of seasonal fluctuations was minimal. In addition, when the test subjects had dry skin, the numerical values of many items in the effect index were significantly improved, confirming the function of improving the elasticity and moisturizing function of the skin.

In addition, while the previous study involved continuous intake for 6 weeks, this study confirmed significant improvement in multiple effect indicators with continuous intake for 12 weeks. In this study, improvements were seen in some efficacy measures even at week 4, but more efficacy measures were improved at 12 weeks. These findings suggest that continuous intake over a certain period of time is desirable.

A limitation to mention in this study is that the test dose was only 6.0 mg/day. Therefore, it is unclear if similar efficacy can be observed even at lower doses, or if stronger effect can be obtained at higher doses. Further research is required to determine the optimal dose.

There were no adverse events or side effects in this study, and it was considered that there was no problem with the safety of the test product.

5. SUMMARY

To evaluate the effects of 12-week intake of pentadecyl-containing orlan oil on skin condition, the study was conducted in healthy adult women aged 35 to 59 years with dry skin and concern about facial sagging. In this study, the test food group showed significant improvement in elasticity R2 (left, average), R5 (left), and R7 (left, right, average) after 12 weeks compared to the control group. There were also significant differences in improvement of collagen score intensity (left, right, average), stratum corneum water content (left, right, average), and water loss (left). These results suggest that the 12-week intake of pentadecyl-containing orlan oil improves skin elasticity by increasing collagen density, which in turn improves skin moisture. During the study, no adverse events were observed that were attributed to the intake of test product.

Conflict of Interest

This research has received financial support and a manuscript request from Sea Act Co., Ltd.

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