



Topical Applications of *Caulerpa* spp. Extract Preventing Skin Aging through Improving Skin Moisture, Pigmentation and Decrease Laxity

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Abstract

Background: *Caulerpa* spp. extract contains neoxanthin, free astaxanthin, antheraxanthin, canthaxanthin, astaxanthin monoester, fucoxanthin, astaxanthin diester, and beta carotene, vitamins A, C, and E, minerals, amino acids and polyphenols which are photochemoprotective as to protect skin from damage caused by exposure to UV-B rays. This study aims to determine the effectiveness of topical 0.2% *Caulerpa* spp. extract in preventing premature skin aging in research subjects assessed by the parameters of skin moisture, pigmentation, and laxity of the skin. Method: This study was purely experimental research, using the randomized double-blind pretest-posttest control group design. A total of 40 research subjects participated in this study and were divided into two groups, namely the treatment group of 20 subjects, who were given interventions in the form of topical 0.2% *Caulerpa* spp. extract gel on the face, and the control group of 20 subjects who were given intervention in the form of application topical base cream on the face. All groups performed a pretest with a simple A-one scanner to assess skin aging with moisture, pigmentation, and laxity parameters on the skin. The two study groups were then treated for one month and post-tested. The research data was then collected for statistical analysis of using independent t-test and paired sample t-test. Results: Differences in skin assessments were analysed before and after therapy. Differences in assessment before therapy obtained $p < 0.05$ on laxity and pore variables. Differences in assessment after treatment were found to be $p < 0.05$ also in laxity and pores. Test differences in skin assessment in the two groups with Mann-Whitney using the difference in values after and before therapy obtained $p < 0.05$ in pigmentation, laxity, humidity, pores, sebum U zone, and sebum T zone. Conclusion: Topical administration of topical *Caulerpa* spp. gel on the face has a photo protective effect by reducing pigmentation and laxity and increasing skin moisture.

Keywords: *Caulerpa* spp., Skin aging, Pigmentation, Laxity.

Introduction

The aging process is a process of progressive accumulation of various pathological changes in cells and tissues that occur over time. One of the most widely adopted concepts of skin aging is the theory of free radicals [1]. Overall the effect of UV light on the skin results in collagen damage due to increased levels of the matrix metalloproteinase-1 (MMP-1), decreased collagen synthesis due to high levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), inflammation and oxidative stress, and reduced ability of damaged cells to be eliminated by the process of apoptosis [2, 3]. The body has a natural antioxidant defense system (AO) to neutralize free

radicals, but due to continuous exposure to UV light, this AO supply rapidly decreases [4]. Antioxidants used topically on the surface of the skin can reduce the effects of reactive oxygen species (ROS) [5]. Many skincare products use natural ingredients that contain antioxidants, both found in fruit, leaves, flowers, roots, and other parts of plants [6, 7]. In Bali, several types of seaweed are often consumed by the public, one of which is *Caulerpa* spp. Seaweed is often consumed as a vegetable or snack, but not yet commonly used topically to prevent or improve skin aging. Research conducted by Julyasih et al.

Found nine types of carotenoids contained in *Caulerpa spp.* extract namely neoxanthin, astaxanthin free, antheraxanthin, canthaxanthin, astaxanthin monoester, fucoxanthin, chlorophyll b, astaxanthin diester, and beta carotene [8]. *Caulerpa spp.* Also contain vitamin A, vitamin C, and vitamin E, 15 kinds of amino acids, total polyphenols of 567.06 mg/100 ml, and several minerals, and their antioxidant activity by 39.25% [9].

In studies on Wistar rats that were exposed to UV-B rays then smeared with astaxanthin gel, and *Caulerpa spp.* extract gel with doses of 0.01%, 0.02%, 0.05%, and 0.2% proved that *Caulerpa spp.* extract began with a dose of 0.02% to 0.2% have a protective effect on the skin of Wistar rats by increasing the expression of dermal collagen. The application of 0.2% *Caulerpa spp.* extract gel showed no significant difference with 0.02% astaxanthin gel in providing protection to the skin of Wistar rats exposed to UV-B rays [9].

Based on description above, it is necessary to conduct a research and develop sources of antioxidants derived from natural ingredients found in the oceans of Indonesia such as *Caulerpa spp.* This development is a strategy that can prevent and control skin damage from UV exposure.

Chemoprevention by natural compounds is a new concept in an effort to control the process of skin aging, specifically preventing premature skin aging, delaying the appearance of clinical symptoms or even reducing the clinical symptoms that already exist [10]. Active compounds contained in *Caulerpa spp.* are thought to affect one or more stages of the process skin aging.

Method

Study Design and Population

This research is a pure experimental study (true-experimental) with the randomized double-blind pretest-posttest control group design. This research was carried out in the Dermatology and Cosmetics Medical Sub-division in Sanglah Hospital Denpasar, while the production of *Caulerpa spp.* extract was carried out at the Agricultural Technology Laboratory of the Faculty of Agriculture, Universitas Udayana and the manufacturing of 0.2% *Caulerpa spp.* gel extract was done at the pharmaceutical company of PT United Farmaric Indonesia (UFI).

This research will be conducted for six months, which runs from June 2019 to December 2019. The samples in this study are people aged 18-65 years with skin aging, both men and women who are willing to participate in this study until completion. The exclusion criteria in this study were patients with skin aging therapy such as laser, filler or botox injection, oral or topical drugs, pregnant, suffering from chronic skin inflammatory diseases such as psoriasis and atopic dermatitis had severe systemic disease, consumed oral antioxidants like vitamins A, C, E, selenium, and zinc in the last four weeks. In this study 40 samples were selected based on inclusion and exclusion criteria which were divided into two groups randomly, namely the intervention group (receive *Caulerpa spp.* gel extract 0.2%) and the control group (placebo gel cream), the intervention was carried out by blinding and assisted by volunteers, then measurements were carried out at conditions before and after the intervention

Rating Parameters

The assessment parameters in this study are skin moisture, skin pigmentation, skin laxity, and the change in the protective effect of 0.2% *Caulerpa spp.* extract. Other variables measured in this study include elasticity, pores, sebum T zone and, sebum U zone.

Research Protocol

Freshly picked *Caulerpa spp.* is then aerated and extracted. *Caulerpa spp.* extract is processed and then made in the form of gel in a concentration of 0.2% and put in a tube. *Caulerpa spp.* extract gel was applied to the skin of the study subjects according to the group. The treatment was given for 1 month for each group and then observed. To assess the skin aging of all study subjects before and after treatment (pretest and post-test) was done with an A-one simple scanner using the parameters of moisture, pigmentation, and laxity on the skin.

Statistical Analysis

Statistical analysis in this study using SPSS 25.0 program (Armonk, NY, USA). Comparative analysis using independent t-test (if the data distribution is normal) or the Mann-Whitney test (if the data distribution is not normal) to determine the effect of the 0.2% *Caulerpa spp.* extract gel on moisture,

pigmentation, and laxity of the skin. All values are considered significant if $p < 0.05$.

Result

Sample Characteristics

In this study there were a total of 40 samples that met the inclusion criteria and were included in this study. Samples were divided into the intervention group ($n = 20$) given topically 0.2% *Caulerpa spp.* extract gel and the control group ($n = 20$) given topical base

cream on the face. The overall age of the sample ($n = 40$) was 45.25 ± 7.40 years with the youngest age being 31 years and the oldest age being 57 years. The sex of the entire sample ($n = 40$) was 25 women (62.5%) and 15 men (37.5%). Skin type based on Fitzpatrick in the whole sample ($n = 40$) was dominated by skin type IV by 32 people (80%), followed by type V by 7 people (17.5%) and type III by 1 person (2.5%). All samples ($n = 40$) had group III skin types based on Glogau (100%) (Table 1).

Table 1: Sample characteristics

Characteristics	Intervention Group (n=20)	Control Group (n=20)
Sex		
Male	8 (40.0%)	7 (35.0%)
Female	12 (60.0%)	13 (65.0%)
Fitzpatrick skin type		
Type III	0 (0)	1 (5.0%)
Type IV	15 (75.0%)	17 (85.0%)
Type V	5 (25.0%)	2 (10.0%)
Glogau skin type		
Group III	20 (100%)	20 (100%)
Age (years)		
Mean \pm SD	45.25 \pm 7.40	43.55 \pm 7.28

Normality Test

The normality test for all skin assessments by group can be seen in **Table 2**. Analysis of

the normality of the data at the assessment after administration of therapy in both groups was performed with the Shapiro-Wilk test.

Table 2: Normality test of skin variable

Skin variable	Group	p-value
Pigmentation	Control	0.008
	Intervention	0.237*
Laxity	Control	0.255*
	Intervention	0.187*
Moisture	Control	0.535*
	Intervention	0.040
Elasticity	Control	0.345*
	Intervention	0.195*
Pores	Control	0.010
	Intervention	0.006
Sebum T zone	Control	0.001
	Intervention	0.028
Sebum U zone	Control	0.001
	Intervention	0.008

*Normally distributed $p > 0.05$

Differences in Skin Assessment in the Intervention Group with the Control Group before and After Therapy

The distribution of skin assessments in the intervention group and control group can be seen in **Table 3**. The data shown are the median value (interquartile range), minimum value, and maximum value. Assessment tests after and before therapy with the Wilcoxon

test, found significant values ($p < 0.05$) in all skin assessments (pigmentation, laxity, humidity, elasticity, pores, sebum T zone and sebum zone U) in the intervention group. In the placebo group, significant values were found ($p < 0.05$) in humidity and sebum in the T zone (**Table 3**). Analysis of skin assessment after and before therapy in pigmentation and elasticity using paired t-

tests because the data were normally distributed (Table 2). Within 1 month of

therapy, no side effects were found in either group (100%).

Table 3: Distribution of skin assessment in both groups

Skin parameter	Intervention Group [Median (IQR) min – max]	p-value	Control Group [Median (IQR) min – max]	p-value
Pigmentation Before intervention	5.58 (2.92)	<0.001*	4.05 (2.15)	0.360
	2.72 – 9.02		2.23 – 8.00	
After intervention	5.55 (2.95)		4.04 (2.14)	
	2.69 – 8.98		2.24 – 8.04	
Laxity Before intervention	4.91 (1.51)	<0.001 ^a *	4.58 (0.66)	0.481 ^a
	3.85 – 6.78		3.81 – 5.92	
After intervention	4.90 (1.50)		4.59 (0.66)	
	3.83 – 6.77		3.84 – 5.93	
Moisture Before intervention	36.50 (18)	<0.001*	37.00 (13)	0.034*
	22 – 53		27 – 52	
After intervention	41.00 (11)		39.00 (2.17)	
	21 – 61		28 – 54	
Elasticity Before intervention	45.00 (22)	0.494 ^a *	41.50 (15)	0.846 ^a
	24 – 67		27 – 56	
After intervention	44.00 (23)		43.50 (12)	
	24 – 68		27 – 57	
Pores Before intervention	7.00 (0.88)	0.001*	5.80 (1.05)	0.132
	6.21 – 9.56		5.34 – 7.41	
After intervention	6.99 (0.87)		5.82 (1.07)	
	6.18 – 9.50		5.36 – 7.43	
Sebum U zone Before intervention	20.41 (28.30)	0.018*	14.55 (52.69)	0.086
	3.23 – 76.81		3.06 – 109.15	
After intervention	19.75 (28.03)		14.42 (52.33)	
	3.10 – 74.25		3.01 – 109.10	
Sebum T zone Before intervention	18.21 (30.23)	<0.001*	13.39 (15.32)	0.046*
	2.55 – 25.35		9.09 – 36.19	
After intervention	17.65 (29.89)		13.97 (15.34)	
	2.34 – 55.12		9.10 – 35.19	

*Significant (p<0.05)

^aPaired sample t-test

Test Differences in Skin Assessment in the Intervention Group with the Placebo Group

Differences in skin assessments were analysed before and after therapy. Differences in assessment before therapy obtained p values <0.05 on laxity and pore variables. Differences in assessment after treatment were found to be p <0.05 also in laxity and pores. Test differences in skin

assessment in the two groups with Mann-Whitney using differences in values after and before therapy obtained p <0.05 on pigmentation, laxity, humidity, pores, sebum U zone, and sebum T zone. Test the difference in the assessment of laxity and elasticity uses paired t test because the data are normally distributed (Table 2). The results of differences in skin assessment before and after therapy can be seen in Table 4.

Table 4: Test differences in skin assessment between the two groups

Skin parameter	p-value		
	Before intervention	After intervention	Δ Assessment
Pigmentation	0.130	0.168	<0.001*
Laxity	0.038 ^a *	0.043 ^a *	<0.001 ^a *
Moisture	0.654	0.135	<0.001*
Elasticity	0.787 ^a	0.873 ^a	0.795 ^a
Pores	<0.001*	<0.001*	<0.001*
Sebum U zone	0.607	0.626	0.006*
Sebum T zone	0.892	0.914	<0.001*

Δ Assessment = Assessment after intervention – before intervention

*Significant (p<0.05)

^aIndependent sample t-test

Discussion

Research on the effectiveness of topical 0.2% *Caulerpa spp.* extract gel on the face showed significant results decreased in pigmentation, decreased laxity, and increased skin moisture ($p < 0.05$).

Decreased pigmentation in the intervention group was associated with the content of *Caulerpa spp.* such as phenols hydroquinone, flavonoids, steroids, and saponins which have inhibitory effects on the melanin pigment [10, 11]. Research by Wiraguna et al. with Wistar rats exposed to UV-B rays and given *Caulerpa spp.* extract at a dose of 0.02% to 0.2% found to increase the expression of dermal collagen in Wistar mice [9].

The photoprotective effect on *Caulerpa spp.* is known to have the ability to reduce MMP-1 levels, an enzyme that degrades type I, II and III collagen in the skin. Decreased expression of 8-OHdG (increased at the time of DNA damage), decrease in MMP-1 ($p < 0.05$) and improvement in the structure of Wistar rat collagen was found after applying 0.2% and 0.4% *Caulerpa spp.* extract gel [10]. Findings decreased laxity ($p < 0.05$) and increased elasticity ($p < 0.05$) in this study are new findings that support the results of previous studies conducted in rats by Wiraguna et al. increased collagen expression in the dermis will increase skin elasticity.

In addition, phenolic extracts decrease the activity of proteinases which prevent the degradation of skin proteins such as collagen and elastin. Collagen plays a role in the strength of the skin and elastin fibers play a role in skin elasticity. Anticollagenase, antielastase, and antioxidant effects will reduce MMP-1 thus preventing skin protein degradation [12]. In addition, *Caulerpa spp.* contains vitamins A, C, and E. Vitamin A in the form of carotenoids is known to inhibit MMP-1 thereby inhibiting the degradation of

collagen, vitamins C decreases ROS activity and has an important role in collagen synthesis, and vitamin E prevents fat peroxidation, thus maintaining cell membrane structure [10, 13].

Skin moisture was found to increase significantly in the intervention group based on assessment after and before therapy was given ($p < 0.05$). In addition, in this study a significant effect was found to decrease pores, decrease in T zone sebum, and decrease in U zone sebum ($p < 0.05$). This shows that the use of topical *Caulerpa spp.* extract 0.2% on the face has a photoprotective effect compared to only using base cream in the control group. This is in accordance with the function of most algae which can moisturize the skin [11, 14].

The antioxidant content of *Caulerpa spp.* moisturise inhibits lipid peroxidase decreases the destruction of the skin barrier and decreases the performance of ROS, then controls oxidative stress [5, 11]. Which shows a decrease in sebum production in the T and U zones in this study. No side effects were found during the study. At present there are no studies that test the effectiveness of extract of *Caulerpa spp.* on human skin, so the research is a new finding and cannot be compared with similar studies using *Caulerpa spp.*

Conclusion

Application of topical *Caulerpa spp.* gel on the face has a photoprotective effect by reducing pigmentation and laxity and increasing skin moisture.

Publication Ethics

Current study has been approved by Ethical Committee Faculty of Medicine Universitas Udayana/Sanglah General Hospital, Bali-Indonesia.

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