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PHOTOCHEMOPROTECTION OF CAULERA SPP ACTIVE COMPONENT ON RAT MODEL SKIN

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ABSTRACT

Caulerpa spp. including seaweed and macro-algae can be found in a large number at Coastal Serangan, Bali-Indonesia. *Caulerpa spp* was historically used for food and vegetable. However, in our today life, this plant was applied as material for skin care products, especially for UV protection. The aim of this study is to know the active component of *caulerpa spp* extract and its inhibition of premature skin aging induced by UV-B radiation through collagen expression. This was descriptive and experimental research applying a randomized posttest only control group design. Active component contained in the *caulerpa spp* extract are carotenoid, vitamin A, C, and E, polyphenols, minerals and amino acids. Topical application of *caulerpa spp* can suppress the UV-B radiation induced collagen damage. Increased of collagen expression in all groups of *caulerpa spp.* just as good as astaxanthin group in improving collagen expression and 0.2% of *caulerpa spp.* extract is the most effective dose in improving collagen expression.

Keywords: *Caulerpa spp*, UV-B rays, collagen, expression

INTRODUCTION

The main environmental factor that may cause premature aging is ultraviolet (UV) radiation. Chronic exposure of ultraviolet radiation that comes from the sun such as UV-A (320–400nm) and UV-B (280–320 nm) known to cause destruction of structure and function of skin.[1] UV-B cause erythema, edema, hyperplasia, hyper-pigmentation, sun burn cells, immunesuppresor. Repeated exposure to UV radiation ultimately causes premature skin aging also called photo-aging and skin cancer.[2,3] UV irradiation also causes DNA damage, protein oxidation, and increasing matrix of metalloproteinases (MMPs).[4]

UV-B irradiation induces the production of reactive oxygen species (ROS) in skin cells, which is primarily responsible for photo-aging characterized by formation of fine and coarse wrinkles, increased skin thickness, dryness, laxity, and pigmenta-tion.[5,6] The wrinkles happened by photo-aging caused by disturbances of extracellular matrix of dermis such as collagen, elastin and glicosammino glican.

The Cellular and molecular mechanism which mediated the destruction of extracellular matrix in dermis involves cell surfaces receptors, signal tans-duction pathway of kinase protein, transcription factor and MMPs.[7] UV irradiation can activate cytokine receptors and growth factor in epidermal ceratinocyte surfaces and dermal fibroblasts.

The activation of this receptor induces intracellular signal such as mitogen-activated protein kinase (MAP kinase) and activates complex factor nuclear transcription AP-1. In dermis and epidermis, AP-1 will induce expression of MMP including collagenase (MMP-1), stromelysin 1 (MMP-3) and other MMPs that will destruct collagen and protein that build dermal extracellular matrix.[8.] In addition, AP-1 suppresses gene expression pro-collagen fibroblasts cells in dermis and cause decrease collagen synthesis. The overall effect of UV radiation on the dermis produces degradation of collagen, collagen synthesis obstacles, inflammatory and oxidative strees.[7]

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METHODS

Animal study

A number of 27 male wistar rat (*Rattus Novergicus Wistar Race*) used in this study obtained from animal unit laboratory department of pharmaco-logy Medical Faculty Udayana University. The age of rat around 2.5-3 months, with average weight of 200-250 g. Food and drinking water monitored twice a day, temperature and ventilation also moisture enclosure guarded properly. All rats are conditioned for 1 week

before the start of the study and a feather in all rats in the back shaved with a size 5 x 5 cm.

Administration of extract *caulerpa spp*, vitamin C and astaxanthin gel

The rats were randomly allocated to the non–irradiated control group (n=3), UV irradiated control group (n=3), vitamin C group (n=3), astaxanthin group (n=3) and *caulerpa spp* group, with each doses of 0.01% (n=3), 0.02% (n=3), 0.05% (n=3), 0.1% (n=3) and 0.2% (n=3). The vitamin C group applied with 0.5mg vitamin C gel 10%, astaxanthin group with astaxanthin gel 0.02% and *caulerpa spp* group each applied with *caulerpa spp* extract gel 0.01%, 0.02%, 0.05%, 0.1% and 0.2%, while the control group and UV-B group applied with 0.5mg vehicle. These topical materials are applied twice a day which is 20 minutes before irradiation and minimal 4 hours after irradiation.

UV-B Irradiation

The sources of UV-B rays obtained from UV irradiation treatment system made in China (KN-4003 A/B). The UV-B lamp placed 3 cm above the surface of the dorsal skin rats. The rats were exposed to UV-B radiation three times a week (10 AM) starting with 50 mJ/Cm2 for the first week, which was sequentially followed by 70 mJ/Cm2 (1week), then 80 mJ/cm2 (2 weeks) to afford a total dose of 840 mj/cm2 over the four weeks[9]. A non irradiated group of animals was induced as control. The animals were killed 48 hours after the final irradiation to allow the recovery from the acute UV effects.

Histology evaluation

Biopsies were obtained from the central dorsal skin, prependicular to the long axis of the trunk. The biopsies were fixed on 10% buffered formalin and prepared for optical microscopy. Following dehydration and inclusion in parafin wax, 5 mm thick sections were cut and stained with Sirius redto determine the collagen[10]. Sirius red were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and all other chemicals were of reagent grade and were used without further purification.Collagen expre-ssion was count used digital analysis method, each preparations was photographed using LV evolution camera, each preparations was photographed three times and saved in JPEG format.

Statistic analysis

Data were expressed as the mean ±SD and were analyzed by one way analysis of variance (ANOVA) followed by least significant difference (LSD) test and results considered significantly different when $p < 0.05$ was obtained.

RESULTS

Methanol extracts of condensed results of maceration gives the yield of as much as 25.43% blackish green. While the result of anti-oxidant activities to DPPH (1,1-difenil – 2 pikrilhidrazil) showed that *caulerpa spp* extract has percentage of the silencing of 39.25%. Further phtochemical screening done on extracts *caulerpa spp* to determine the compound contained in the extract of *caulerpa spp*. A positive test result indicates phytochemicals contains vitamin A, C, E, 15 amino acids and some types of minerals as well as total poly-phenol 567.06mg/100ml (Table 1).

Table 1
Active component of *caulerpa spp*

COMPOUND	TOTAL
Carotenoid	Rf value
Neoxanthin	0.09
Astaxanthin free	0.30
Antheraxanthin	0.36
Canthaxanthin	0.41
Astaxanthin Monoester	0.48
Fucoxanthin	
Klorofil B	0.50
Astaxanthin diester	0.59
Beta karoten	0.70
Vitamin	0.98
Vitamin A (µg/100mL)	
Vitamin C (mg/100g)	3438.3
Vitamin E (mg/L)	2.29
	55
Mineral (mg/L)	
Zn	1.596
Fe	15.453
Se	nd.
Cu	0.141
Mn	ttd
Na	45664.50
K	2287.85
Mg	562.71
Poliphenol Total (mg/100mL)	567.06
Amino acid (%)	
Aspartat acid	0.020
Glutamine	0.081
Serine	0.018
Histidine	0.017
Glycine	0.002
Threonine	0.021
Arginine	0.035
Alanine	0.030
Tyrosine	0.011
Methionine	0.014
Valine	0.001
Phenilalanine	0.017

nd = not detected

After the biopsy and histology preparations has been made, this continue with sirius red staining. In Figure-1 showed the destruction of collagen tissue after induced with UV-B, than after applied with vitamin C gel 10%, astaxanthin 0.02% *caulerpa spp* with various

doses strats 0.01% until 0.2%, the expression seemed to improve with the arrangement of the collagen network density better than the UV-B group ($p<0.05$). The profil of average dermal collagen expression in the rats skin both in the control group and experimental group showed in Figure 2. After the normality test and homogenicity result showed that all data have normal distribution and homogen with p value > 0.05

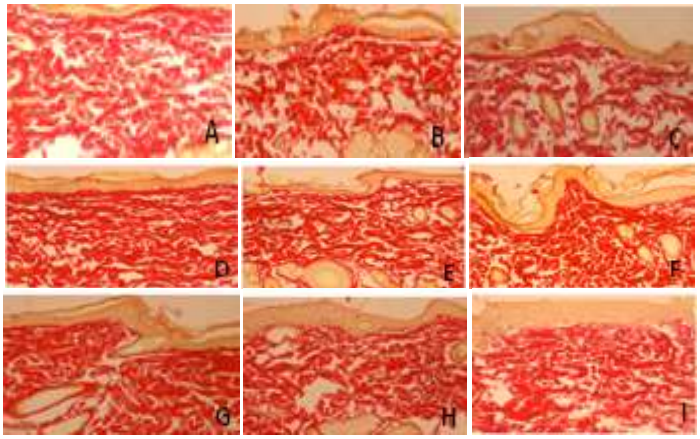

Figure 1
Histology expression of dermal tissue collagen of rats skin staining with sirius red
(A) Control group, showed collagen expression arranges regular and solid, (B) UV-Birradiation, showed damage of arrangement and collagen tissue structure. (C) Vitamin C group. (D) Astaxanthin group, collagen expression getting arranges closely the collagen tissue in the control group. (E) *Caulerpa spp* 0.01% group. (F) *Caulerpa spp* 0.02% group. (G) *Caulerpa spp* 0.05% group. (H) *Caulerpa spp* 0.1% group. (I) *Caulerpa spp* 0.2% group, collagen expression arrange closely the collagen tissue in the control group

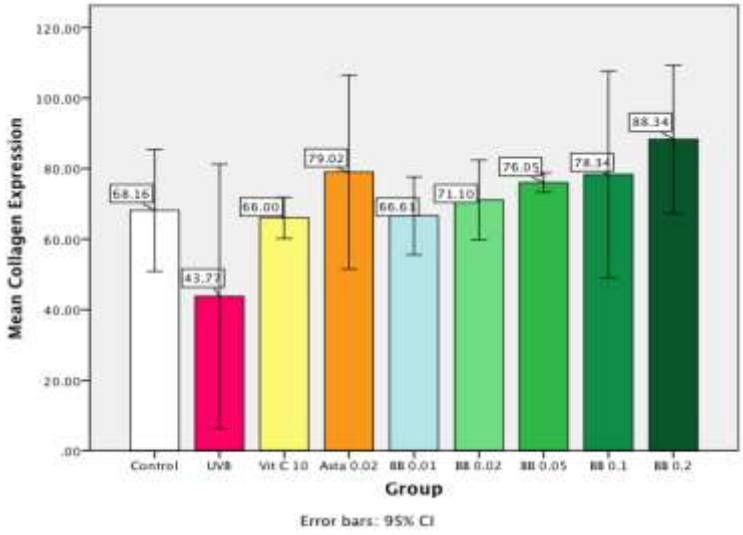

Figure 2
Collagen Expression Profile of Dermal Tissue in Wistar Rats Skin.

Control is non-irradiated group. UV-B group is group received UV-B irradiation. Vit C 10 group is group applied with vitamin C 10%. Asta 0.02 group is group applied with astaxanthin 0.02% gel. BB 0.01, 0.02, 0.05, 0.1 and 0.2 group is group applied with *caulerpa spp* gel extract 0.01%, 0.02%, 0.05%, 0.1% and 0.2%. All experimental grouped irradiated with UV-B.

The average result between group are: control vs UV-B, p < 0,005. The UV-B grouped vs all treated group p < 0,005, The BB 0, 2 group vs vit C 10, BB 0.01 and 0.02, p <0.05. The BB 0.1 groud is not siginificant different with vitamin C group, astaxanthin 0.02, BB 0.01, 0.02, 0, 05 and 0.2, p >0.05.

The analysis of the treatment group after being exposed to UV-Brays and administering vitamin C gel, astaxanthin and various dose*scaulerpa spp* tested based on average each collagen expression group by one way ANOVA. While to find out the differences in average each group performed with Post Hoc with least significant difference test (LSD).

Based on the one way ANOVA test found that the average decline in collagen expression of UV-B group different significantly with control group (p<0.05). While the average increase in the expression of collagen vitamin C group, astaxanthn and all *caulerpa spp* group differ significantly with UV-B group (p<0.05). The increasing collagen expression in astaxanthin group not differ significantly with control group, vitamin C and all doses of *caulerpa spp* (p>0.05). The increasing the average collage expression of *caulerpa spp* group 0.1% not differ significantly with vitamin C group, astaxanthin, *caulerpa spp*0.01%, 0.02%, 0.05% and 0.2%. While in *caulerpa spp*0.2% group, an average increase of collagen expression differ significantly with control group, vitamin C,*caulerpa spp*0.01% and 0.02% group.

DISCUSSION

The active compound in *caulerpa spp* extract can work synergisticallyto protect against UV-B induced-collagen damage. The combination between vitamin A, C, E and carotenoid also polyphenol is strong antioxidant that can bind singlet oxygen, superoxide anion, and hydroxil radical[11].The use of a combination of antioxidants sourced from plants have advantages in suppressing free radicals than with the used of a single antioxidant[12].

In this research there was a decrease of collagen expression significantly in UV-B group after induced by UV-B irradiation compare with control group (p <0.05). In rats grouped induced by UV-B irradiation and applied vitamin C, astaxanthin and all doses of *caulerpa spp* there was increase of collagen expression significantly compare to UV-Bgroup (p<0.05). The average of collagen expressio in *caulerpa spp* group 0.2% differ significantly with vitamin C group, *caulerpa spp*0.01% group and 0.02% (Figure 2).

The decrease of collagen expression in dermal tissue in rats group induced by UV-B irradiation is an alert of oxidative stress due to excessive formation of free radicals[3]. The oxygen molecules at the bottom of the epidermis is the main target of the UV-B radiation enter into the skin. UV irradiation through the skin may act as an electron donor molecule of oxygen that causes oxygen become unstable, being an aggressive free radicals. Superoxide anion will take randomly an electron from the nearest molecul and not only cause destruction of molecule but also changes it into free radicals and further cause chain rection[7.]

This bind free radicals will activate cytokine receptors and growth factor in the surface of keratinocyte in epidermis and dermal fibroblast[13]. The activation of these receptor will induced intracellular signal like MAP kinase that further will activate nuclear transcription factor AP-1 which a regulator production of MMP that may cause increase of MMP followed by collagen degradation[7,14]. The impact of increasing MMP1 enzymes in epidermal and dermal tissue will cause destruction of collagen tissue marked as decrease of collagen expression. In addition, AP-1 may suppres expression of procollagen gen of fibroblast dermis that cause decreasing of collagen synthesis[13].
In rats applied with vitamin C 10% gel, astaxanthin 0.02% gel and *caulerpa spp* extract topically may cause increasing of collagen expression significantly compare with UV-Bgroup (p<0.05). *Caulerpa spp* extract may reduce oxidative stress because these extract contain carotenoid[15]. vitamin A,C and E, polyphenol and mineral that act as an antioxidant. Astaxanthin also reduce oxidative stress because its a strong antioxidant that can muffle free radicals induced by UV-B irradiation[16]. As well as vitamin C, in addition bind free radicals also act in collagen synthesis.

CONCLUSION

ROS that come from inside and outside of the body, may destruct DNA, lipid membrane and protein structure also act in the accelerating of photoaging and skin cancer. Although the innate immune system may neutralized ROS, the protective agent may overwhelmed to excessive oxidative stress. The *caulerpa spp* topically with various doses potentially give extra-advantages in muffle free radical induced byUV-B irradiation and doses of *caulerpa spp*0.2% has the best effect of giving protection to collagen tissue damage in rats induced by UV-B irradiation.

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