PHOTOCHEMOPROTECTION OF CAULERA SPP ACTIVE COMPONENT ON RAT MODEL SKIN

1Wiraguna, A. A. G. P., 2Pangkahila, W., and 3Mantik-Astawa, N.

1Faculty of Medicine, Department of Dermato-Venereology, Udayana University, Bali-Indonesia
2Anti Aging Medicine Postgraduate Program, Udayana University, Bali-Indonesia
3Faculty of Veterinary Medicine, Udayana University, Bali-Indonesia

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 caulera 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 caulera spp extract are carotenoid, vitamin A, C, and E, polyphenols, minerals and amino acids. Topical application of caulera spp can suppress the UV-B radiation induced collagen damage. Increased of collagen expression in all groups of caulera spp. just as good as astaxanthin group in improving collagen expression and 0.2% of caulera 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, immunesupressor. 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 pigmen-tation.5,6 The wrinkles happened by photo-aging caused by disturbances of extracellular matrix of dermis such as collagen, elastin and glicosamminoglican.

The Cellular and molecular mechanism which mediated the destruction of extracellular matrix in dermis involves cell surfaces receptors, signal transduction 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 stress.7

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 pharmacology 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

Corresponding Author:
A. A. G. P. Wiraguna
Faculty of Medicine, Department of Dermato-Venereology, Udayana University, Bali-Indonesia
E-mail: wiraguna@yahoo.com

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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 weeks9. 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
derhydration and inclusion in parafin wax, 5 mm thick
sections were cut and stained with Sirius red to
determine the collagen10. 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 expression 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 pirkahlidrazil) showed
that caulerpa spp extract has percentage of the
silencing of 39.25%. Further phytochemical 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>
<thead>
<tr>
<th>Table 1</th>
<th>Active component of caulerpa spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMPOUND</td>
<td>TOTAL</td>
</tr>
<tr>
<td>Carotenoid</td>
<td>RF value</td>
</tr>
<tr>
<td>Neoxanthin</td>
<td>0.09</td>
</tr>
<tr>
<td>Astaxanthin free</td>
<td>0.30</td>
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<tr>
<td>Antheraxanthin</td>
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<tr>
<td>Canthaxanthin</td>
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<tr>
<td>Astaxanthin Monoester</td>
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<tr>
<td>Fucoxanthin</td>
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</tr>
<tr>
<td>Klorofil B</td>
<td>0.50</td>
</tr>
<tr>
<td>Astaxanthin diester</td>
<td>0.59</td>
</tr>
<tr>
<td>Beta karoten</td>
<td>0.70</td>
</tr>
<tr>
<td>Vitamin</td>
<td>0.98</td>
</tr>
<tr>
<td>Vitamin A (µg/100mL)</td>
<td>3438.3</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg/L)</td>
<td>2.29</td>
</tr>
<tr>
<td>Mineral (mg/L)</td>
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</tr>
<tr>
<td>Zn</td>
<td>1.596</td>
</tr>
<tr>
<td>Fe</td>
<td>15.453</td>
</tr>
<tr>
<td>Se</td>
<td>nd.</td>
</tr>
<tr>
<td>Cu</td>
<td>0.141</td>
</tr>
<tr>
<td>Mn</td>
<td>nd.</td>
</tr>
<tr>
<td>Na</td>
<td>45664.50</td>
</tr>
<tr>
<td>K</td>
<td>2287.85</td>
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<tr>
<td>Mg</td>
<td>562.71</td>
</tr>
<tr>
<td>Polipheno Total (mg/100mL)</td>
<td>567.06</td>
</tr>
<tr>
<td>Amino acid (%)</td>
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</tr>
<tr>
<td>Aspartat acid</td>
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<tr>
<td>Glutamine</td>
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<tr>
<td>Serine</td>
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<tr>
<td>Histidine</td>
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<tr>
<td>Glycine</td>
<td>0.002</td>
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<tr>
<td>Threonine</td>
<td>0.021</td>
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<tr>
<td>Arginine</td>
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<tr>
<td>Alanine</td>
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</tr>
<tr>
<td>Tyrosine</td>
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<tr>
<td>Methionine</td>
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</tr>
<tr>
<td>Valine</td>
<td>0.001</td>
</tr>
<tr>
<td>Phenilalanine</td>
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</table>

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

![Histology expression of dermal tissue collagen of rats skin staining with sirius red](image)

(A) Control group, showed collagen expression arranges regular and solid. (B) UV-B irradiation, 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.

![Collagen Expression Profile of Dermal Tissue in Wistar Rats Skin.](image)

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 group 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 group is not significant different with vitamin C group, astaxanthin 0.02, BB 0.01, 0.02, 0, 0.5 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 doses caulerpa 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, astaxanthin 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 expression of caulerpa spp group 0.1% not differ significantly with vitamin C group, astaxanthin, caulerpa spp0.01%, 0.02%, 0.05% and 0.2%. While in caulerpa spp0.2% group, an average increase of collagen expression differ significantly with control group, vitamin C, caulerpa spp0.01% and 0.02% group.

DISCUSSION

The active compound in caulerpa spp extract can work synergistically 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 radical11. The use of a combination of antioxidants sourced from plants have advantages in suppressing free radicals than with the used of a single antioxidant12.

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 group 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-B group (p<0.05). The average of collagen expressio in caulerpa spp group 0.2% differ significantly with vitamin C group, caulerpa spp0.01% 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 radicals3. 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 molecule and not only cause destruction of molecule but also changes it into free radicals and further cause chain reaction3.

This bind free radicals will activate cytokine receptors and growth factor in the surface of keratinocyte in epidermis and dermal fibroblast13. 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 degradation7,14. 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 synthesis13. 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 carotenoid15, 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 irradiation16. 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 spp0.2% has the best effect of giving protection to collagen tissue damage in rats induced by UV-B irradiation.

REFERENCES


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