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# The Administration of 35% Chocolate Seaweed (*Sargassum polycystum)* Extract Cream Does Not Inhibit TGF-β1 Decrease But Inhibit Collagen Decrease in Wistar Rats Exposed to Ultraviolet B

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**Abstract:** ***Background: Exposure to UV-B rays is one of the cause of the decrease in TGF-β1 and collagen in the skin. The mechanism of this decrease is caused by the formation of reactive oxygen species. One of the natural ingredients that can be used to inhibit collagen degradation is brown seaweed (Sargassum polycystum). The content of fucoxanthin, flavonoid, phenol and tannin in Sargassum polycystum is believed to be a high source of antioxidants, which can inhibit the decrease in TGF-β1 and collagen due to exposure to UV-B rays. The purpose of this study is to prove that the administration of brown seaweed (Sargassum polycystum) extract cream can inhibit the decrease of TGF-β1 and collagen in Wistar rats exposed to UV-B light. Methods: This research are an experimental study randomized post test only control group design. Samples were 45 male rats (Rattus novergicus) aged 10-12 weeks and weight 200-250 grams. The samples were divided into 3 groups, namely the untreated group, the control group which was only exposed to UV-B light and the treatment group which was given 35% chocolate seaweed (Sargassum polycystum) extract cream 20 minutes before UV-B exposure and 4 hours after. The control group and the treatment group were exposed to UV-B rays with the same total dose of 840 mJ/cm$^2$ for 4 weeks. The research data were then analyzed using the One way Anova test. Results: The results showed that there was no significant difference in the mean level of TGF-β1, where in the untreated group the average was 446.58±57.01 pg/mL, while in the control group the results were 458.11±49.63 pg/mL and in the treatment group it was 478.27±68.41 pg/mL (p=0.319). However, there was a significant difference in the amount of collagen, where in the untreated group was 72.15±4.28%, the control group was 49.16±3.22% and the treatment group was 64.29±4.02% (p<0.001). Conclusion: From the results of this study, it can be concluded that the administration of chocolate seaweed extract cream did not significantly inhibit the decrease in TGF-β1 levels, but it could significantly inhibit the decrease in the amount of collagen in Wistar rats exposed to UV-B light.***

**Keywords:** Chocolate Seaweed Extract Cream, TGF-β1, Collagen, UV-B Exposure

## 1. Introduction

The population of elderly people is reported to continue to increase. With this change, it is hoped that the community and especially health workers will change their perspective towards the elderly. We must focus more on improving the quality of life of the elderly. In aging all organs will experience its effects. One of the problems of aging that will inevitably occur is a decrease in collagen. This decline in collagen can also occur earlier, if there is chronic exposure to ultraviolet B light or what is known as photoaging. Radiation from UVB will increase the production of reactive oxygen species (ROS) and induce a decrease in enzymatic antioxidants. Then the ROS formed will play a role in increasing the transcription factor AP-1 which stimulates collagen breakdown through an increase in the matrix metalloproteinase (MMP) enzyme.[1] And on the other hand the accumulation of ROS will cause a decrease in the expression of TGF-β, which will cause a decrease in collagen production and increase in elastin production, causing changes in skin structure that are clinically manifested by deep wrinkles, rough texture, telangiectasia, and pigmentation.[2]

The cosmetic industry currently focuses on bioactive substances derived from natural ingredients such as plants, microbial metabolites, fungi, and seaweed.[3] Brown seaweed is known to contain active components such as flavonoids, tannins, phenols, saponins, pigments, proteins, polysaccharides and a source of several vitamins. The pigment in brown seaweed is called fucoxanthin.[4] The content of fucoxanthin has the effect of preventing skin aging by supporting the formation of collagen .[5] In previous studies it was also found that fucoxanthin is able to stimulate the expression of TGF-β expression which is important for tissue remodeling.[6] Other content, namely phenol is also known to be able to fight UV radiation.[7] Flavonoid compounds also accelerate the aging activation of NF-κB which acts on the skin .[8] And tannin compounds have high antioxidant properties that inhibit reactive oxygen species.[9] In previous studies the use of brown seaweed at a dose of 25% could not increase collagen density, but at dose of 50% it could increase collagen density.[10] So based on this, the researchers used doses between 25% and 50%, which is 35% in this study. Brown seaweed in Indonesia is very abundant and spread in almost all Indonesian waters.[11] However, the brown seaweed still neglected by the community as a wild seaweed that grows in the waters and many people still do


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not know the benefits of this brown seaweed.[12] So the researchers used this brown seaweed as a collagen inhibition modality whose sources are abundant in Indonesia but little is known about its benefits.

This study aimed to prove that the administration of 35% chocolate seaweed (*Sargassum polycystum*) extract cream can inhibit the decrease of TGF-β1 and collagen in Wistar rats exposed to UV-B light.

## 2. Methods

This research are an experimental study randomized post test only control group design. Study was conducted from December 2021 to March 2022 at Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University, Bali. Samples were 45 male rats (*Rattus novergicus*) aged 10-12 weeks and weight 200-250 grams. The samples were randomly divided into 3 groups, namely the untreated group, the control group which was only exposed to UV-B light and the treatment group which was given 35% chocolate seaweed (*Sargassum polycystum*) extract cream 20 minutes before UV-B exposure and 4 hours after. The control group and the treatment group were exposed to UV-B light using Philips PL-S9W/01/2P lamps three times a week (Monday, Wednesday, and Friday) with the same total dose of 840 mJ/cm$^2$ for 4 weeks. On days when the UV-B light exposure is not perfomed, the extract cream is still applied twice aday at the same time.

**Extraction of Brown seaweed**
Wash the seaweed using running water so that no dirt sticks to the seaweed. Then the sample was dried using an oven at 45˚C, until the sample was dry. After drying, the sample was mashed using a blender until it became a fine powder. Then the powder was macerated in 80% acetone at 30$^o$C for 24 hours, and the filtrate was taken for use. After that, the filtrate was evaporated with a rotatory evaporator at a temperature of 40$^o$C with a pressure of 100 mBar until a concentrated sample was obtained.

**Cream Preparation**
First prepare the ingredients of the cream consisting of stearic acid, anhydrous lanolin, triethanolamine, propylene glycol, and water. And 1%W/V ethylhexyl gilserin and 0.2%W/V phenoxyethanol were used as preservatives. Next, heat the oil phase and water phase to a temperature of 70˚C in a water bath. Then the oil phase was added to the water phase and stirred until homogeneous. The emulsion was obtained after cooling at room temperature. Then add 35% of brown seaweed extract to the emulsion base.

**Sample Prepration**
To avoid the acute effects from UV-B exposure, the biopsy was perfomed 24 hours after the final UV-B light exposure. The skin tissue was used for making histochemistry sample with Picro sirius red staining and for testing the levels of TGF-β1 by ELISA.

**Collagen Examination**
The percentage of collagen was determined by digital analysis method. In the analysis, an Olympus CX21 light microscope with an objective magnification of 4 times will be used. Each sample will be photographed with a magnification of 40 times using an Optilab Pro camera and then saved in JPEG format using the Optilab Viewer 2.2 software. Furthermore, the calculation of the amount of dermal collagen will be carried out using Adobe Photoshop CS3 and Image J software. The first step is to assess the amount of non-collagen by separating the red channels in the image using the "RGB stack" option in Image J. Then change it to black and white and block the dermis, so that the subcutaneous tissue and epidermis will be excluded which will appear with a yellow line. Next, adjust it by setting a threshold value for the black and white zone, where white is non-collagenous and black is collagen. Finally, it will be analyzed so that the pixel percentage value of the collagen area will be obtained automatically.[13]

**TGF-β1 Examination**
The examination of TGF-β1 levels was using ELISA kit (Enzyme-Linked Immunosorbent Assay) to obtain quantitative calculations of TGF-β1 levels in mice. TGF-β1 ELISA kit was by BT Lab. The yellow color results in the optical density was directly proportional to the level of TGF-β1.

**Data Analysis**
The research data were analyzed using SPSS software. The data was presented in descriptive analysis, Shapiro-Wilk test to determine data normality, Levene's test to determine data homogenity, and One way Anova test to compare data. The test is continued to the Post-Hoc test if there is a significant difference between groups.

## 3. Results

Descriptive analysis was perfomed as the basis for performing statistical analysis (hypothesis testing). The descriptive analysis results (Table 1) show that the mean of TGF-β1 level in the treatment groups was the highest. And the mean of total collagen in the untreated group was the highest, and the mean of total collagen in treatment group was higher than the control group.

**Table 1:** Descriptive Analysis Results of TGF-β1 Levels and Total Collagen

| Variable | Group | n | Mean±SD | Median | Min. | Max. |
|---|---|---|---|---|---|---|
| TGF-β1 (pg/mL) | Untreated | 9 | 446.58±57.01 | 455.84 | 343.34 | 508.4 |
| | Control | 18 | 458.11±49.63 | 474.15 | 364.24 | 562.55 |
| | Treatment | 18 | 478,27±68.41 | 475.32 | 377.46 | 669.81 |
| Collagen (%) | Untreated | 9 | 72.15±4.28 | 74.1 | 64.1 | 76.3 |
| | Control | 18 | 49.16±3.22 | 49.95 | 42 | 55.4 |
| | Treatment | 18 | 64.29±4.02 | 64.9 | 57.1 | 72.2 |

n : number of samples
SD : standard deviation


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The One-way Anova test results (Table 2) showed that the mean of TGF-β1 levels between group had no significant difference (p>0.05). The mean of TGF-β1 levels in the untreated group was 446.58±57.01 pg/mL. While in the control group was 458.11±49.63 pg/mL and in the treatment group was 478.27±68.41 pg/mL.

Meanwhile the One-way Anova test results showed that the mean of total collagen between group had a significant difference (p<0.05). The mean of total collagen in the untreated group was 72.15±4.28%. While in the control group was 49.16±3.22% and in the treatment groupwas 64.29±4.02%.

A follow up test with Post-Hoc test showed there was no difference between the TGF-β1 groups (p>0.05), but there was a significant difference in all collagen groups (p<0.05).

**Table 2:** Comparison of TGF-β1 Levels and Total Collagen in the No Treatment, Control, and Treatment Groups

| Variable | Group | n | Mean | SD | p |
|---|---|---|---|---|---|
| TGF- $\beta$ 1 (pg/mL) | No Treatment | 9 | 446.58 | 57.01 | 0.377 |
| | Control | 18 | 458.11 | 49.63 | |
| | Treatment | 18 | 478.27 | 68.41 | |
| Collagen (%) | No Treatment | 9 | 72.15 | 4.28 | 0.000 |
| | Control | 18 | 49.16 | 3.22 | |
| | Treatment | 18 | 64.29 | 4.02 | |

SD : standard deviation
p : level significance

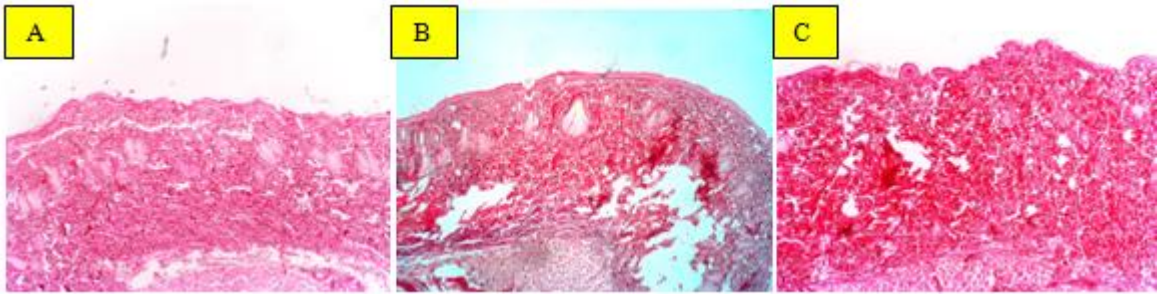


**Figure 1:** Histology of Total Collagen with Picro Sirius Red Staining (40x Magnification). The total collagen was assesed from the red collagen in the dermis by picrosirus red staining. Figure A, the untreated group, without UV-B light and cream: dense collagen fibers appear. Figure B, the control group, exsposed to UV-B rays without cream: there is a decrease in the density of collagen fibers and damage to the structure of collagen. Figure C, the treatment group, exposed to UV-B rays and treated with 35% brown seaweed extract cream: collagen fibers appear denser and more structured than the control group

## 4. Discussion

Brown seaweed is known to contain active components such as flavonoids, tannins, phenols, and fucoxanthin. Fucoxanthin was able to significantly reduce the intracellular ROS formation caused by exposure to UVB rays in fibroblast cells.[14] Fucoxanthin is also known to have the ability to prevent aging of the skin by supporting the formation of collagen.[15] It was reported in previous studies that fucoxanthin is able to inhibit the activity of the collagenase enzyme, so that the degraded collagen will decrease.[16] In previous studies it was also found that fucoxanthin is able to stimulate the expression of TGF-β which is important for tissue remodeling.[6]

Other ingredients such as flavonoids, and tannins also play a role in collagen by relying on components as antioxidants. [17] It was reported that polyphenolic compounds have UV protection ability.[7] Phenol is also able to regulate the pathway of mitogen active protein kinase (MAPK).[18] It was reported that some of the flavonoid compounds were able to inhibit the activation of NF-κB and related pathways including the kinase signaling pathway that plays a role in skin aging.[8] In addition, flavonoids also have effects such as estrogen-like. It is reported that estrogen is effective in treating aging, and improving elasticity and wrinkles in premenopausal women. Recent studies have also reported that estrogen can increase collagen production and increase TGF-β.[19]

The tannin compounds found in Sargassum polycystum have a very large number of hydroxyl groups in their structure, which causes the tannins to have a high antioxidant content. [9] Sargassum has the ability to inhibit reactive oxygen species by donating protons thereby accelerating the cessation of free radicals.[17]

In the results of this study, there were differences in the results of TGF-β1 and collagen. The results of the TGF-β1 examination did not find statistically significant differences, but in the amount of collagen there were statistically significant differences. This difference in results could be due to two possibilities. In the first possibility, it could be due to the presence of other pathways besides TGF-β/Smad in influencing the amount of collagen. This pathway can be seen when there is an increase in ROS due to UV exposure. ROS generated by UV exposure will react with cysteine at the catalytic site and thereby inhibit the enzymatic activity of receptor protein tyrosine phosphatases (RPTPs). Inhibition of protein tyrosine phosphatase by ROS reactions enhances receptor tyrosine kinase (RTK) phosphorylation and triggers a signaling cascade that includes phosphorylation of mitogen-activated protein kinase (MAPK) and transcription factor activation of activator protein-1 (AP-1).[20] AP-1 has an important role in regulating MMP-1, MMP-3, and MMP-9. Studies have shown that cAMP (cyclic adenosine monophosphate) regulates MMP expression by influencing the MAPK/AP-1 pathway, leading to the degradation of collagen. Apart from AP-1, nuclear


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factor-κB (NF-B) is another important transcription factor. ROS-induced activation of NF-B is associated with MMP-1 and MMP-3 in dermal fibroblasts.[21] The signaling pathway of MAPK can also be inhibited through compounds that are antioxidants. It was previously reported that antioxidant compounds such as polyphenols are able to regulate or inhibit MAPK pathways.[18] So offering Sargassum polycystum extract cream can also be affected through this pathway.

In the second possibility, the increase of TGF-β1 to be insignificant could be caused by an increase in the level of TGF-β1 in the control group. This increase can be explained by the presence of a wound healing response mechanism that is activated in the TGF-β/Smad pathway by thrombospondin. This is considered an acute response to UVB. While the decrease or low expression of collagen in the control group may indicate that the pathway activation is not good enough to resist UV radiation damage, or it could also be caused by cell death during the pathway activation process.[22]

In the amount of collagen, there was a significant difference in the mean results in the treatment group compared to the control group. This amount of collagen can be caused by an increase in the content of active substances found in brown seaweed (Sargassum polycystum), such as fucoxanthin, tannins, phenols, flavonoids, and tannins. The active substance content in Sargassum polycystum is able to act as an antioxidant that is able to neutralize ROS due to UVB exposure, so that it can inhibit the MAPK pathway. This may explain the inhibition of collagen reduction in this study.

## 5. Conclusion

It can be concluded that administration of 35% brown seaweed (*Sargassum polycystum*) extract cream twice a day for 4 weeks did not inhibit the decrease in TGF-β1, but can inhibit the decline of collagen in Wistar rats exposed to ultraviolet B light. Further research can be considered for the isolation of the active substances in brown seaweed (*Sargassum polycystum*) namely fucoxanthin, flavonoids, tannins, and phenols so that the role of collagen and TGF-β1 is known so that the results are not influenced by other ingredients contained in Sargassum polycystum. And it can be considered to examine 2 pathways, namely the MAPK pathway and the TGF-β/Smad pathway in influencing the amount of collagen, so that it can be known which pathway affects.

## References

[1] Rahmawati YD, Prasetyawan S. Effects of Oral and Topical Application of Centella Asiatica Extracts on The UVB-Induced Photoaging of Hairless Rats. *J Pure App Chem Res*. 2019;8(January):7-14. doi:10.21776/ub.jpacr.2019.008.01.430

[2] Mesa-arango AC, Florez-munoz SV, Sanclemente G. Mechanisms of skin aging. *IATREIA*. 2017;30(2):160-168. doi:10.17533/udea.iatreia.v30n2a05

[3] Berthon J, Nachat-kappes R, Bey M, Cadoret J, Renimel I, Filaire E. Marine algae as attractive source to skin care. 2017;5762(August). doi:10.1080/10715762.2017.1355550

[4] Pereira L. Seaweeds as Source of Bioactive Substances and Skin Care Therapy- Cosmeceuticals, Algotheraphy, and Thalassotheraphy. *Cosmetics*. 2018;5:68. doi:10.3390/cosmetics5040068

[5] Joshi S, Kumari R, Upasani VN. Applications of Algae in Cosmetics : An. Published online 2018:1269-1278. doi:10.15680/IJIRSET.2018.0702038

[6] Natsume C, Aoki N, Aoyama T, et al. Fucoxanthin ameliorates atopic dermatitis symptoms by regulating keratinocytes and regulatory innate lymphoid cells. *Int J Mol Sci*. 2020;21(6). doi:10.3390/ijms21062180

[7] Schagen SK, Zampeli VA, Makrantonaki E, Zouboulis CC. Discovering the link between nutrition and skin aging. *Dermatoendocrinol*. 2012;4(3):298-307. doi:10.4161/derm.22876

[8] Domaszewska-Szostek A, Puzianowska-Kuźnicka M, Kuryłowicz A. Flavonoids in skin senescence prevention and treatment. *Int J Mol Sci*. 2021;22(13):1-18. doi:10.3390/ijms22136814

[9] Fraga-Corral M, García-Oliveira P, Pereira AG, et al. Technological application of tannin-based extracts. *Molecules*. 2020;25(3):1-27. doi:10.3390/molecules25030614

[10] Karina A, Revianti S, Karsini I. Khasiat Ekstrak Sargassum sp. Terhadap Kepadatan Kolagen pada Proses Penyembuhan Ulkus Traumatikus. *J Kedokt Gigi*. 2014;8(1):34-42.

[11] Pakidi CS, Pada D, Manajemen J, Perairan S, Pertanian F, Musamus U. Potensi dan Pemanfaatan Bahan Aktif Alga Cokelat. *Octopus J Ilmu Perikan*. 2017;6(1):551-562.

[12] Santiyoga IKW, Suhendra L, Wartini NM. Karakteristik Ekstrak Alga Coklat (Sargassum polycystum) sebagai Antioksidan pada Perlakuan Perbandingan Pelarut Aseton dan Etilasetat. *J Rekayasa Dan Manaj Agroindustri*. 2020;8(1):91. doi:10.24843/jrma.2020.v08.i01.p10

[13] Miot HA, Brianezi G, Tamega A de A, Miot LDB. Techniques of digital image analysis for histological quantification of melanin. *An Bras Dermatol*. 2012;87(4):608-611. doi:10.1590/s0365-05962012000400014

[14] Peng J, Yuan J, Wu C, Wang J. Fucoxanthin , a Marine Carotenoid Present in Brown Seaweeds and Diatoms : Metabolism and Bioactivities Relevant to Human Health. Published online 2011:1806-1828. doi:10.3390/md9101806

[15] Joshi S, Kumari R, Upasani VN. Applications of Algae in Cosmetics : An. *Int J Innov Res Sci Eng Technol*. 2018;7(2):1269-1278. doi:10.15680/IJIRSET.2018.0702038

[16] Handayani T. Fukosantin: Karotenoid Berharga Dari Makroalga Coklat. *Oseana*. 2018;43(3):16-28. doi:10.14203/oseana.2018.vol.43no.3.60

[17] Lifie K, Mansauda R, Anwar E, Nurhayati T, Anwar E. Antioxidant and Anti-Collagenase Activity of Sargassum plagyophyllum Extract as an Anti-Wrinkle Cosmetic Ingredient. 2018;10(5):932-936.

[18] Behl T, Upadhyay T, Singh S, et al. Polyphenols Targeting MAPK Mediated Oxidative Stress and. *Molecules*. 2021;26(6570):1-24.


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[19] Liu T, Li N, Yan Y qi, et al. Recent advances in the anti-aging effects of phytoestrogens on collagen, water content, and oxidative stress. *Phyther Res*. 2020;34(3):435-447. doi:10.1002/ptr.6538
[20] Rittie L, Fisher GJ. Natural and Sun-Induced Aging of Human Skin. *Cold Spring Harb Perspect Med*. 2015;5:1-14.
[21] Geng R, Kang S-G, Huang K, Tong T. Boosting the Photoaged Skin : The Potential Role of Dietary Components. *Nutrients*. 2021;13(1691):1-27.
[22] Li Y, Xiong L, Tang J, Zhu G, Dai R, Li L. Mouse skin-derived precursors alleviates ultraviolet B irradiation damage via early activation of TGF-β/Smad pathway by thrombospondin1. *Cell Cycle*. 2020;19(4):492-503. doi:10.1080/15384101.2020.1717042


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