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# Rosella Flower 3% (*Hibiscus sabdariffa*) Extract Inhibited the Expression of Matrix Metalloproteinase-1 dan Collagen Reduction on Wistar Rat Exposed to Ultraviolet-B

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**Abstract:** ***Background: Rosella flower petals contain flavonols and anthocyanin pigments. Flavonols and Anthocyanins are compounds that have double bonds that play a role in preventing cell damage due to exposure to ultraviolet light. This study aims to see the effect of rosella flower extract in cream on preventing the expression of Matrix Metalloproteinase-1 (MMP-1) and prevent the reduction of collagen amount in male Wistar rats exposed to ultraviolet-B (UV-B). Method: The posttest only control group design was applied in 36 male Wistar rats aged 2 to 3 months. The subject was divided randomly into two groups evenly; the control and study groups. The control group was given a base cream in the exposure areas before and after UV-B exposure and continuously given even without irradiation. The study group was given a rosella extract cream with a similar procedure. A punch biopsy was conducted to assess the MMP-1 expression and the collagen amount after four intervention weeks. Comparative analysis was used to compare the mean of each variable. Results: The results showed the mean expression of MMP-1 in the treatment group was significantly lower than the control group (8,01±2,845 vs 30,17±4,68%;p<0,001). The mean collagen amount in the treatment group was higher than the control group (83,14±2,84% vs 56,80±3,65%;p<0,001). Conclusion: The study showed the effectiveness of rosella extract cream 3%inhibited the expression of MMP-1 and collagen reduction on male Wistar rats exposed to UV-B.***

**Keywords:** Rosella extract cream, *Hibiscus sabdariffa*, Matrix Metalloproteinase-1, Collagen, Anti-aging

## 1. Introduction

Matrix metalloproteinase (MMP) is a zinc-contained proteinase enzyme and plays a role in the degradation of extracellular matrix proteins. MMP is classified as collagenase, gelatinase, stromelysin, matrilysin, and membrane-type MMP. MMP-1 is an enzyme that is responsible for the breakdown of skin collagen that is photoaged.(1–3) Collagen is a human skin tissue derived from fibroblasts regulated by transforming Growth Factor-β (TGF β), which stimulates

collagen production.(3) Ultraviolet (UV) B leads to resulting damage on the skin and increases MMP-1. This is because UV radiation will induce MAP-kinase and will activate AP-1 (activator Protein-1). The protein-1 activator causes activation of MMP-1, which then degrades collagen. AP-1 also inhibits TGFβ signaling, resulting in suppressing TGFβ receptor 2 so that it inhibits collagen synthesis. Apart from MAP-kinase and AP-1, the increase in MMP-1 by free radicals was also triggered by the activation of NFkβ. (2, 4, 5)

Giving antioxidant supplements is one of the right solutions to reduce the impact of UV exposure on aging skin. Therefore, the effect of ultraviolet radiation is the formation of free radicals, and if the number of free radicals is high or exceeds the antioxidants in the body, it will cause oxidative stress. Some compounds work to prevent ROS formation through a mechanism of resistance to free radicals in some plant extracts.(6) Therefore, many studies use natural ingredients from plants to reduce the effects of free radicals caused by UVB rays.

Rosella (*Hibiscus Sabdariffa L.)* is a plant that lives in tropical, subtropical climates. Rosella belongs to the family of Malvaceae. Rosella plants are usually located in India to Malaysia. There are also vitamin A and amino acids needed by the body in the Rosella plant and vitamin C. Rosella flowers also contain calcium, protein, and other useful elements for the body. Rosella flower petals contain a mixture of citric acid and malic acid, and anthocyanins, namely gossipetin (hydroxy flavone) and hibiscin.(7,8). The antioxidants present in rosellas such as gossipetin, anthocyanin, and glucoside hibiscin can protect against various degenerative diseases such as coronary heart disease, cancer, diabetes mellitus, and cataracts (Fitriani, 2008).

Rosella flower petals contain flavonoids such as flavonols and anthocyanin pigments. Flavonols and anthocyanins are compounds that have double bonds that play a role in preventing cell damage due to exposure to ultraviolet light. Flavonoids can be used as sunscreens because of chromophore groups' presence (conjugated single, double bonds) that absorb ultraviolet A and ultraviolet B, reducing the skin's intensity. The results of phytochemical analysis of roselle petal extract carried out at the Faculty of Agricultural Technology, Agricultural Laboratory of Udayana University were IC 50 (236,2428ppm) Flavonoids (42938.72 mg / 100g), phenol (1758.68 mg / 100g GAE), tannin content (2865 , 25 mg / 100g TAE), vitamin C (1294,1176 mg / 100g), antioxidant capacity (2249.43 mg / L GAEAC), and saponins +. (7,9–11)These results show that the cream formula made can be developed as an anti-aging skin cream. The study was conducted to test the effect of topical rosellaflower extract cream to inhibit the increased expression of MMP-1 anddecrease of collagen amount in male Wistar rats skin (*Rattus norvegicus*) exposed to UV-B light.


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## 2. Method

This research was conducted at the Integrated Biomedical Laboratory Unit, Udayana University. Preparation of rosella extract cream conducted at the Department of Pharmacology and Therapeutics, University of Udayana. Preparation of Rosella extracts and phytochemical examination conducted at the Laboratory of Biochemistry and Nutrition Faculty of Agriculture, University of Udayana.The making of rosella flower extract cream starts by taking the flower extract by extraction using 96% ethanol then closed within one day at room temperature protected from light. Stirred, filtered with filter paper, and then obtained the filtrate pulp mass. The ethanol macerate is combined and evaporated using a vacuum evaporator at a temperature of 45 degrees until the ethanol extract is obtained from evaporation in a paste form. The base cream is made using Sepigel 305 as an emulsifier with a 3% concentration mixed into water, then add 2% lanolin, 2% dimethicone, and 0.5% phenoxyethanol mixing until it forms a cream. Rosella flower extract cream is a cream that contains 42938.72 mg/100g flavonoids, 1294,1176 mg/100g, vitamin C.

An experimental study using a randomized pre-posttest control group design was carried out on the male Wistar rats population aged 2-3 months with an approximate bodyweight of 180-200 grams. The determination of the sample size of 36 Wistar rats as research subjects followed the calculation using Federer's formula. Samples that meet the requirements are randomly divided into two groups: the control and study groups. The research subjects were adapted in advance for one week.
The control group was given a base cream as a placebo (n = 18 rats) exposed to UVB rays three times a week for four weeks. The shaved rats back (3x4 cm) were applied with a base cream (0.2 mg / cm2 for each application) twice a day, 20 minutes before irradiation, and 4 hours after irradiation. Topical applications have continuously been given every day, even without radiation. The study group (n = 18 rats) were exposed to UVB three times a week for four weeks. Before irradiation, the rats' backs were given 0.2 mg/cm2 rosella extract cream for each application, and the same procedure was carried out.

After four weeks of exposure, the mice were left for 24 hours to rule out acute irradiation effects. UVB irradiation was carried out using a UVB light simulator made by the KN-4003 brand, with a total dose of 840 mJ/cm$^2$ in the control and treatment groups, with details: 50 mJ/cm$^2$ at the first week, 70 mJ/cm$^2$ at week two and 80 mJ/cm$^2$ at week three and four. The irradiation is given three times a week for four weeks so that the total dose reaches 840 mJ/cm$^2$, 3 cm irradiation distance. The measurement of the exposure dose was carried out using a UV meter (Beltron, Germany).

Skin specimens of Wistar rats were taken from each group exposed to UVB light, from the skin on the backs of mice with a punch biopsy with a diameter of 4 mm and a depth of 0.2 mm (full thickness) the area exposed to UV light. Immunohistochemical staining using the LSAB technique with anti-mouse MMP-1 primary antibody (BIOS, USA). Observation of the results of the amount of MMP-1 expression was carried out histopathologically. The preparations were examined under a microscope at 400x magnification using a microscope and photographed with an Optilab Pro camera (Miconos, Indonesia). The expression of MMP-1 is the ratio of the number of brown fibroblast cells to total fibroblast cells in a specific field of view.

$$\text{MMP-1 expression (\%)} = \frac{\text{Fibroblast cells expressing MMP-1}}{\text{Total number of fibroblast cells}} \times 100\%$$

Analysis of the amount of collagen was performed using Picro-Sirius Red staining. Collagen is the percentage of collagen tissue pixels that are bright red with Sirius red staining compared to the entire tissue's pixels that appear on the histological preparation photo expressed in percent (%). The assessment was carried out on photo preparations in JPEG format taken with the LC Evolution camera and Olympus CX41 microscope with an objective magnification of 400 times in 3 fields of view and then obtained the mean percentage of the amount of dermal collagen from the three fields of view.

$$\text{Collagen Expression} = \frac{\textit{pixel}\ \text{collagen areas}}{\textit{pixel}\ \text{whole tissue areas}} \times 100\%$$

Data analysis in this study was conducted using SPSS ver 23. A comparative test using the independent t-test was conducted to compare MMP-1 and collagen expression in the study and control groups. The study's procedures have been approved by the Udayana University.

## 3. Results

MMP-1 and the amount of collagen were presented in descriptive analysis, including the mean, standard deviation (SB), median, minimum, and maximum values (Table 1). The mean of MMP-1 level was lower in the study group than the control group. Meanwhile, the amount of collagen in the study group was higher than the control group.

**Table 1:** Descriptive Data of MMP-1 Level and Collagen Amount

| Variable | Group | Freq | Mean±SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| MMP-1 levels | Control | 18 | 30,17±4,68 | 31,95 | 21,30 | 36,30 |
| | Study | 18 | 8,01±2,84 | 8,27 | 3,23 | 13,43 |
| Collagen amount | Control | 18 | 56,80±3,65 | 56,17 | 52,07 | 64,37 |
| | Study | 18 | 83,14±2,84 | 83,32 | 78,57 | 88,47 |

Comparability analysis aimed to determine the average ratio between the MMP-1 control group and the treatment group after treatment was given. The results of the analysis of significance with independent t-test were presented in Table 2. The study indicated a significant difference between the control and study groups for MMP-1 levels and the collagen amount. The average of MMP-1 levels was significantly lower, and the collagen amount was significantly higher in the study group than in the control group.

**Table 2:** Comparative Analysis of MMP-1 Levels and Collagen Amount Between The Study and Control Groups

| Variable | Group | Freq | Mean p<0,001SD | P |
|---|---|---|---|---|
| MMP-1 levels | Control | 18 | 30,17±4,68 | p<0,001 |
| | Study | 18 | 8,01±2,84 | |
| Collagen amount | Control | 18 | 56,80±3,65 | p<0,001 |
| | Study | 18 | 83,14±2,84 | |

Figure 1. shows the difference of MMP-1 expression using IHK staining in the study and the control group. The MMP-1


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expression was less visible in the study group than in the control group.Meanwhile, figure 2 shows the comparison of collagen expression in both groups, where the study group had more intact collagen tissue than the control group.

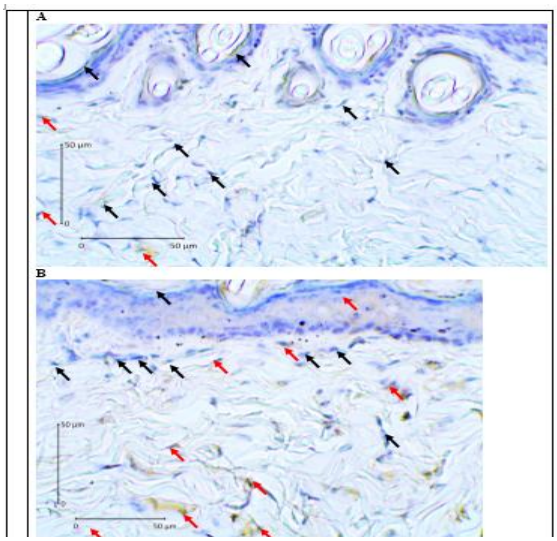


**Figure 1: Expression of MMP-1 in Dermis Tissue with CPI Stained.** A is the study group figure. Red arrows indicate fibroblast cells expressing MMP-1. B is the control group. The expression of MMP-1 (brown color) is increased compared to Figure A. Red arrows show fibroblast cells expressing MMP-1.

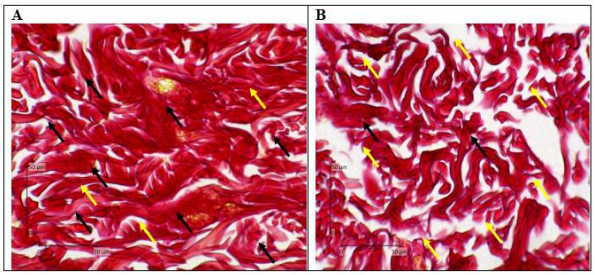


**Figure 2:** Expression of Collagen in Dermis Tissue with Picro-Sirius Red Staining. A is the study group imaging. The expression of collagen with red collagen fibers appears broader and thicker. B is the control group; damage to collagen pattern and structure with red collagen fibers appears thinner. Black arrows indicate intact collagen fibers. Yellow arrows indicate collagen fibers that are not intact.

## 4. Discussion

In this study, it has been shown that rosella flower extract cream can effectively prevent the increase in MMP-1 and prevent a decrease in the amount of collagen due to exposure to UVB rays. The protective effect of rosella flower extract cream on skin aging prevention due to exposure to UVB rays in this study was defined as the cream's ability to inhibit an increase in MMP-1 and decrease the amount of collagen due to exposure to UVB rays. This effect is obtained because the rosella flower extract contains bioactive compounds.

The phytochemical analysis results that have been carried out at the Agricultural Technology Laboratory, Udayana University, show high flavonoids and antioxidants. These are IC 50 (236,2428ppm) Flavonoids (42938.72 mg/100g), phenol (1758.68 mg /100g GAE), tannin levels (2865.25 mg/100g TAE), vitamin C (1294,1176 mg /100g ), antioxidant capacity (2249.43 mg/L GAEAC), and saponins +. Flavonoid compounds can be useful as photoprotective agents because of their ability to absorb UV rays into antioxidant agents both directly and indirectly. Flavonoids also are known as anti-inflammatory and immunomodulatory agents that mark photoprotection.(12) Rosella petal extract used in this study contains flavonoids that can suppress MMP-1 expression and induce type I procollagen protein expression in UV-induced cell culture.

## 5. Conclusion

Based on the results of this study, it can be concluded that the rosella flower extract cream inhibited the expression of matrix metalloproteinase MMP-1 inhibited the decrease in the amount of collagen in male Wistar rats exposed to UVB rays**.**Suggestions can be conveyed based on the research results that clinical trials need to be done on the role of rosella flower extract in humans, especially in blocking and improving skin aging signs due to UVB rays' exposure.

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