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Administration of Sugar Apple Leaf Extract Cream (*Annona squamosa* L.) Increased Superoxide Dismutase (SOD) Activity and Decreased Skin Matrix Metalloproteinase-1 (MMP-1) Activity in Male White Rats (*Rattus norvegicus*) Wistar Strain Exposed to Ultraviolet B Light

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ABSTRACT

Background: Ultraviolet B rays from sunlight causes increased levels of matrix metalloproteinase-1 (MMP-1). Superoxide dismutase (SOD) also will decrease to counteract these free radicals. Sugar apple (*Annona squamosa L.)* leaf extract showed significant antioxidant and anti-collagenase activity *in vitro*.

Methods: This is a posttest only control group design study. Thirty male white Wistar rats (*Rattus norvegicus*), 12 weeks old, 150-200 grams were divided into five groups, 1 group without UVB exposure and without cream, and 4 groups was exposed to UVB 3 times a week and by giving cream 2 times a day for 2 weeks, 20 minutes before exposure and 4 hours after exposure. Rats were fixated in a box. The UVB dose was 65 mJ/cm^2 for 65 seconds, with total 390 mJ/cm^2. Skin samples were tested using ELISA method.

Results: Negative control (P1) compared to Sugar apple (*Annona squamosa L.*) leaf extract cream 1%. (P2), 3% (P3), and 5% (P4) concentration for SOD levels were 2.3015 ± 0.32501 vs. 2.7817 ± 0.26899 vs. 4.7752 ± 0.65218 vs. 6.0890 ± 0.63946, respectively and for MMP-1 levels were 3.0753 ± 0.51113 vs. 2.3037 ± 0.15556 vs. 1.5840 ± 0.25473 vs. 0.9198 ± 0.16604, $p < 0.001$), respectively.

Conclusion: Sugar apple (*Annona squamosa L.*) leaf extract cream 3% and 5% can increase SOD levels and 1%, 3%, and 5% can reduce MMP-1 levels in the skin of male Wistar rats (*Rattus norvegicus*) exposed to UVB light. Concentration 3% is the most effective.

Keywords: sugar apple leaf extract, SOD, MMP-1, ultraviolet B rays

INTRODUCTION

Aging is natural and absolutely happen, but many people worry about it. One of the theories about the aging process is the free radical theory and is the most well-known theory as the cause of aging. The human body has a defense mechanism against oxidative stress through the production of endogenous antioxidants. However, exposure to ultraviolet (UV) rays and other sources of free radicals (such as smoking and pollution) causing endogenous antioxidant production unable to cope with them, resulting in oxidative damage.

The skin is the outermost and most extensive organ in the human body, so the aging process can be directly observed. The skin acts as an organ that is very important for survival because it functions to cover

and protect the organs underneath. The skin is also the organ most likely and most frequently exposed to the environment, such as UV rays, chemicals and air pollution.[1] The most important environmental factor is UV radiation which can damage telomeres and induce free radicals, causing cellular aging, so the term premature skin aging is often also referred to as photoaging. [2,3]

Free radicals are unpaired electrons, so they can damage molecules when these free radicals pull electrons from other molecules. If the levels of free radicals or oxidants exceed antioxidant levels, a condition known as oxidative stress will occur which causes the body to no longer be able to neutralize the free radicals that are formed. Antioxidants can help the body fight free radicals. Antioxidants will neutralize free radicals, causing free radicals to lose their reactivity. There are two types of antioxidants, namely endogenous antioxidants that can be produced by the body, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and exogenous antioxidants that are mainly found in foods such as flavonoids, phenols, vitamin C and vitamin E. [4]

UV rays are invisible rays whose wavelengths are shorter than visible rays, but longer than X-rays. The range of UV wavelengths is often divided into UV A with a wavelength of 320-400 nm, UV B with a wavelength of 290-320 nm, and UV C with a wavelength of 10-290 nm. The positive effect of UV rays is that they induce the production of vitamin D3 in the skin and can kill bacteria. However, if humans are exposed to UV rays for too long, acute and chronic health effects will appear, especially on the skin, which is the outermost organ of humans. Skin that is exposed to UV rays for too long will experience damage and thus accelerate aging. [5] The SOD enzyme is an enzymatic endogenous antioxidant produced by the body and works by neutralizing superoxide radicals. UV rays from the sun will activate a cascade of biochemical reactions in the skin. In short, UV light causes a decrease in skin enzymatic endogenous antioxidants such as SOD.[4] Matrix metalloproteinase-1 (MMP-1/collagenase-1) is an enzyme involved in UV-induced skin aging. ROS generated by UV light will increase the expression of MMP-1. The MMP-1 enzyme degrades type 1 collagen, the main extracellular matrix component that makes up skin structure. As a result, there is decomposition of the dermis and aging of the skin. Therefore, research on antiaging agents that inhibit the formation of ROS caused by UV rays and reduce MMP-1 activity is important to suppress the photoaging process.[6] There is no doubt about the contribution of ROS to the activation of mitogen activated protein kinase (MAPK), which then increases MMP and ultimately increases collagen degradation. Previous studies have shown that SOD can reduce MAPK activation caused by ROS and reduce MMP-1 levels. [7]

Sugar apple plant (*Annona squamosa L.*) is a fruit plant that grows in tropical and subtropical regions. The fruit can be consumed directly or processed into various dishes, and all parts of this plant have long been used traditionally for medicine. Various studies have found that Sugar apple (*Annona squamosa L.*) has antioxidant, anticollagenase, anticancer, antidiabetic, antihypertensive, hepatoprotective, antiparasitic, antimalarial, insecticide, microbicide, and molluscidal benefits. Phytochemical research found active substances from Sugar apple (*Annona squamosa L.*), namely flavonoids, diterpenes, alkaloids, acetogenins, and cyclopeptides. [8] Free radical test of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide with ethanol extract of Sugar apple leaves (*Annona squamosa L.*) showed significant antioxidant activity coupled with potential superoxide radical neutralizing activity. The antioxidant potency of Sugar apple leaf extract (*Annona squamosa L.*) was

determined by its activity against free radicals. The results obtained from an in vitro study regarding antioxidant activity clearly show that methanol extract, chloroform extract, and water extract from Sugar apple leaves (*Annona squamosa L.*) have antioxidant activity. [8,9] A study that compared 14 plant extracts, one of which was Sugar apple leaf extract (*Annona squamosa L.*) revealed that Sugar apple leaf extract (*Annona squamosa L.*) had the highest phenolic content, which correlated in free radical scavenging activity tests with ABTS and DPPH examinations. In the same study, it was found that only 4 of the 14 plant extracts had anticollagenase (anti-MMP-1) properties, and Sugar apple leaf extract (*Annona squamosa L.*) was one of them and had significant MMP-1 inhibitory properties. [10]

These studies were carried out in vitro, and in vivo studies in experimental animals, to the best of the researchers' knowledge, had never been carried out. Sugar apple leaves (*Annona squamosa L.*) which are antioxidants in vitro, contain flavonoids (polyphenol quercetin) and terpenoids (diterpenes) which are modulators of nuclear factor erythroid 2 related factor 2 (Nrf-2) for the skin's defense mechanism against oxidative stress by increasing this antioxidant in vivo via the Nrf-2 pathway. Activation of the Nrf-2 pathway activates cell defense mechanisms by increasing the expression of Nrf2 target genes, including the expression of the endogenous antioxidant gene SOD. [11–13] In addition, Sugar apple leaf extract (*Annona squamosa L.*) in vitro can reduce levels of MMP-1/collagenase-1. [10] This research was carried out on the skin because the skin is the outermost organ of the body that receives the most UV exposure. This became the background for researchers to conduct research on Sugar apple leaf extract cream (*Annona squamosa L.*) 1%, 3%, and 5% on the skin of male white rats exposed to UV B light. The 3% concentration was obtained from previous research on leaf extract. Sugar apple (*Annona squamosa L.*) for an anti-aging cream formula. Concentrations of 1% and 5% were studied to determine the effectiveness of creams at different concentrations.

MATERIALS AND METHODS

This research is an animal experimental study with a posttest only control group design. The independent variables were Sugar apple leaf extract cream (*Annona squamosa L.*) 1%, 3%, 5% and base cream. The dependent variables were skin SOD and MMP-1 levels. The precondition variable was UV B light. The control variable was the rat strain, age, sex, genetics, rat activity, rat health and rat body weight.

A total of 30 mice were adapted for 1 week. All rats were shaved on their backs with an area of 2 cm^2 before being adapted and every 1 day before being exposed to UV B light. Rats were randomly divided into 5 groups. The untreated group was not exposed to UVB light and was not given cream (P0), the group with base cream was exposed to UVB light and given base cream (P1), the first treatment group was exposed to UVB light and given Sugar apple leaf extract cream (*Annona squamosa L.*) 1% (P2), the second treatment group was exposed to UVB light and given Sugar apple leaf extract cream (*Annona squamosa L.*) 3% (P3), and the third treatment group was exposed to UVB light and given Sugar apple leaf extract cream (*Annona squamosa L.*) 5% (P4), each group consisted of 6 rats. Mice from group P0 were not exposed to UV B light and were not given cream. Mice from groups P1 to P4 were given cream according to the group, by means of which the cream was taken with a syringe, 0.2 gram and sprayed on the mice's skin and spread evenly with a cotton bud. The cream is applied 2 times a day, 20 minutes before exposure to UV B rays (to provide absorption time for topical ingredients into the skin) and 4 hours after exposure to UV B rays (ROS begins to form 4 hours after exposure to UV B rays), with a dose of 0, 2

mg/cm2 on the backs of rats that had been shaved for 2 cm^2. The application of topical materials is still carried out on days without irradiation by applying it 2 times a day at 08.00 and 16.00. Irradiation using Philips brand UV B rays was given 3 times per week (Mondays, Wednesdays and Fridays) at a dose of 65 mJ/cm^2 each exposure for 65 seconds each session, with a distance of 15 cm, so that the total UV B rays received by each mouse is 390 mJ/cm^2. The irradiation intensity was calculated by dividing the dose by the irradiation time (65 mJ/65 seconds), so that per cm^2 an intensity of 1 mW was obtained as measured by a UV light meter. To rule out the effects of acute irradiation, the skin samples of mice were taken 24 hours after irradiation. In the skin sampling process, experimental animals were euthanized by injecting Ketamine 20 mg/25 g IM rat weight and Xylazine 20 mg/25 g IM rat weight, by means of Ketamine 4 cc + Xylazine 6 cc mixed in a bottle, then taken 0.2 cc for rats weighing 200 grams, injected in the thigh intramuscularly (IM). Then a biopsy was performed using the punch biopsy method of 1 cm^2 area of the rat's dorsal skin in the central part of the shearing area. After that, the experimental animals were buried.

In this study, SOD and MMP-1 were examined using the Rat Superoxidase Dismutase Kit from BT Lab using the ELISA method with Cat. No. E2268Ra and Kit Rat Matrix metalloproteinase-1 by ELISA method with Cat. No. E0916Hu. Data analysis used SPSS Version 22 for Windows software.

RESULT

In the phytochemical analysis examination conducted at the Faculty of Agricultural Technology, Udayana University, it was found that the inhibitory concentration 50 (IC50) of Sugar apple leaf extract (*Annona squamosa L.*) was 16.8219 ppm, which means that to inhibit 50% of ROS determined in the laboratory, 16.8219 ppm Sugar apple leaf extract (*Annona squamosa L.*). In addition, an antioxidant capacity of 76519.82 mg/L gallic acid equivalent antioxidant capacity (GAEAC) was obtained which is a strong antioxidant, with phenol levels of 7036.52 mg/100 grams, flavonoids 20066.9486 mg/100 grams, and tannins 6823. 79 mg/100 grams.

Descriptive analysis includes mean, standard deviation (SD), median, minimum, and maximum on the variables SOD and MMP-1. The results of the analysis are presented in Table 1. From the descriptive analysis it was found that in the P1 group which was exposed to UVB light and only given basic cream, the SOD levels decreased and the MMP-1 increased compared to the P0 group which was the baseline value (without UVB light exposure and without given cream). Furthermore, by administering Sugar apple leaf extract cream (*Annona squamosa L.*) (groups P2, P3, P4), it can be seen that SOD levels increased and MMP-1 levels decreased compared to P1.

Table 1. Descriptive Analysis Variables SOD and MMP-1

Variable	Group	n	Mean	SD	Median	Min	Max
SOD	P0	6	3.2258	.08904	3.2015	3.11	3.34
	P1	6	2.3015	.32501	2.2740	1.89	2.85
	P2	6	2.7817	.26899	2.8190	2.42	3.05
	P3	6	4.7752	.65218	4.8955	3.52	5.33
	P4	6	6.0890	.63946	6.0130	5.35	7.22
MMP-1	P0	6	1.1785	.11134	1.2025	1.01	1.29
	P1	6	3.0753	.51113	2.9865	2.49	3.88
	P2	6	2.3037	.15556	2.3125	2.11	2.48
	P3	6	1.5840	.25473	1.5955	1.32	2.01
	P4	6	.9198	.16604	0.9950	.67	1.06

Analysis of the treatment effect was tested based on the average SOD between groups after being given treatment in the form of exposure to UVB rays and Sugar apple leaf

extract cream (*Annona squamosa L.*). SOD and MMP-1 data were tested for normality using the Shapiro-Wilk test. The results show that the data are normally distributed (p>0.05), and were tested for homogeneity using the Levene's test. The results show homogeneous data (p>0.05). Significance analysis using the one way ANOVA test shows that the value of p = 0.001. This means that the mean SOD in the five groups after being given treatment was significantly different (p <0.05). The test for differences in the mean SOD between groups was carried out using the Post Hoc Least Significance Different (LSD) test. The results of the Post Hoc SOD test between groups are presented in Figure 1.

Based on different tests on the mean SOD levels between groups, it can be concluded that there were statistically significant differences in SOD levels in all groups because they had a p value <0.05, except for groups P0 and P2 and P1 and P2 where no significant differences were found because they had p-value>0.05. The graph of the average SOD level is shown in Figure 1.

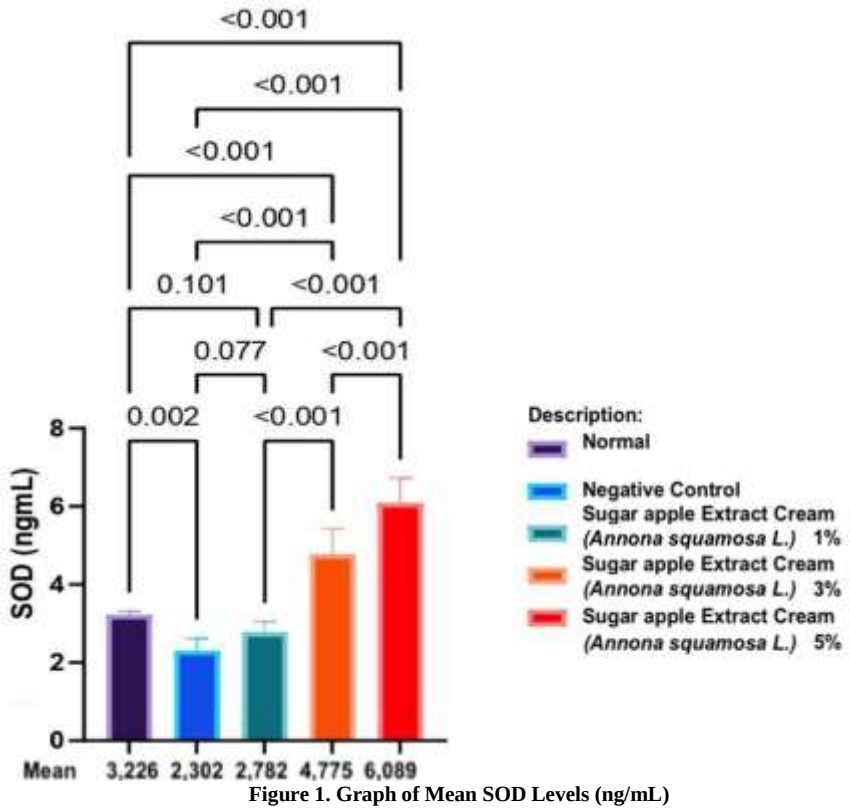

Figure 1. Graph of Mean SOD Levels (ng/mL)

MMP-1

Analysis of the treatment effect was tested based on the average MMP-1 between groups after being given treatment in the form of exposure to UVB rays and Sugar apple leaf extract cream (*Annona squamosa L.*). The results of the significance analysis with the One Way Anova test are presented in Table 6 below.

Table 2. Differences in Mean MMP-1 Between Groups After Exposure to Ultraviolet B Light and Sugar apple Leaf Extract Cream (Annona Squamosa L.)

Variable	Group	n	Mean MMP-1	SD	p
MMP-1	P0	6	1.179	.11134	0,001
	P1	6	3.075	.51113	
	P2	6	2.304	.15556	
	P3	6	1.584	.25473	
	P4	6	0.920	.16604	

Significance analysis using the One Way Anova test shows that the value of p = 0.001. This means that the mean MMP-1 in the five groups after being given treatment was significantly different (p <0.05).

The test for differences in the mean MMP-1 between groups was carried out using the Post Hoc Least Significance Different (LSD) test. The results of the MMP-1 Post Hoc test between groups are presented in Figure 2.

Based on the different test of the mean MMP-1 levels between groups, it can be concluded that there were statistically significant differences in MMP-1 levels in all groups because they had a p value <0.05, except for groups P0 and P4 where no significant differences were found because has a p-value> 0.05. The graph of the average MMP-1 levels is shown in Figure 2.

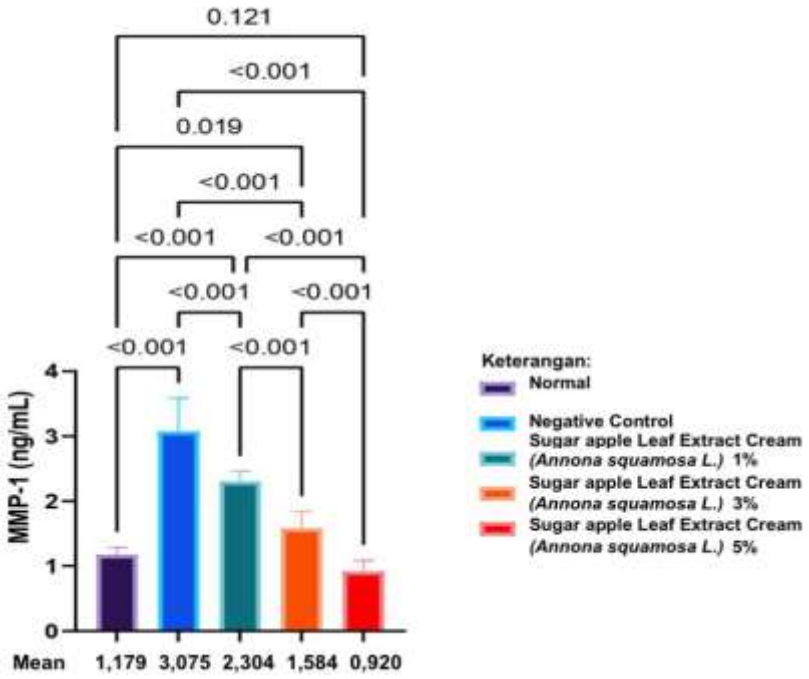

Figure 2. Graph of Average MMP-1 Levels (ng/mL)

DISCUSSION

The results of this study indicate that administration of Sugar apple leaf extract cream (Annona squamosa L.) with a concentration of 3% and 5% can increase the SOD levels of male Wistar rats exposed to UVB light. Likewise, administration of Sugar apple leaf extract cream (Annona squamosa L.) with a concentration of 1%, 3%, and 5% could reduce MMP-1 levels in the skin of male Wistar rats exposed to UVB light. This study used Sugar apple leaves which had been tested for phytochemicals containing an antioxidant capacity of 76519.82 mg/L gallic acid equivalent antioxidant capacity (GAEAC), phenol content of 7036.52 mg/100 gram, flavonoids 20066.9486 mg/100 gram, and tannins. 6823.79 mg/100 grams. The antioxidant capacity of Sugar apple leaves is a strong antioxidant. Inhibitory Concentration 50 (IC50) of Sugar apple leaf extract (Annona squamosa L.) is 16.8219 ppm, which belongs to a strong capacity. From these results, it was found that Sugar apple leaf extract had higher antioxidant capacity, phenol levels, flavonoid levels, and tannins than some plant extracts that had been previously studied in SOD and MMP-1 studies such as Bali Salak, Lemon Peel, Rice Bran. Black, and Moringa Fruit Seeds. The comparison can be seen in Table 8 below:

Table 3. Table of Comparison of Phytochemical Results of Several Extracts That Have Been Researched

Test	Sugar apple Leaf	Bali Snakefruit	Lemon Peel	Black Rice Bran	Moringa Seeds
IC50 (ppm)	16,8219	1119,5362	4100	1330	77750
Antioksidant Capacity (mg/L) GAEAC	76519,82	692,04	3976,79	1881	259,54
Flavonoid (mg/100 g)	20066,9486	2270,28	74,25	365,5	2,1
Fenol (mg/100 g)	7036,52	205,21	2788,79	1454	137,88
Tanin (mg/100 g)	6823,79	222,97	554,27	4174	1339,76

Exposure to UV B Light Reduces SOD Levels and Increases MMP-1 Levels in Rat Skin

UVB exposure to rat skin significantly reduced SOD levels in the P1 group compared to the P0 group. UVB exposure will produce free radicals, one of which is ROS which will activate the MAPK system and then increase MMP levels and ultimately increase collagen degradation, causing dermis decomposition and skin aging. The skin's endogenous SOD enzyme works to ward off free radicals caused by exposure to UVB rays, so SOD levels will decrease when the skin is exposed to UVB rays because it is used to ward off these free radicals [14]. From these results, the results of this study are in accordance with those obtained in previous literature, which said that exposure to at least one minimal dose of UV radiation erythema was shown to reduce levels of cellular antioxidants and antioxidant enzymes such as SOD. In addition, other studies examining SOD levels in the skin also found a decrease in SOD levels after exposure to UVB at certain doses and times. [15]

The results also showed that MMP-1 levels in group P1 increased significantly compared to group P0, after being exposed to ultraviolet B (UV B) light for 2 weeks, indicating that exposure to UV B light increased MMP-1 levels. This MMP-1 enzyme will further degrade collagen type 1, which is the main extracellular matrix component that makes up the structure of the skin resulting in decomposition of the dermis and skin aging. UVB exposure will cause an increase in ROS which will activate MAPK and then activate AP-1, which plays an important role in the regulation of MMP-1. [15] In addition, ROS will also activate NF-κB which in turn will also increase MMP-1. [16]

Effect of Sugar apple Leaf Extract Cream (Annona squamosa L.) on SOD Levels in Male Wistar Rats Exposed to UV B Light

The results of this study indicate that administration of Sugar apple leaf extract cream (*Annona squamosa L.*) at doses of 1%, 3%, and 5% can increase SOD levels in the skin of male Wistar rats exposed to UV B rays. However, at a dose of 1% (P2) levels SOD was not significantly different from normal conditions (P0). ROS. Likewise with the negative control (P1) with a dose of 1% (P2) where the average SOD was not significantly different, because in the negative control (P1), the SOD decreased greatly because it was used to counteract ROS, whereas at a dose of 1% the activation of Nrf2-ARE decreased. produced by a dose of 1% is not sufficient to replace the SOD used to counteract ROS. These results are in accordance with previous studies where Sugar apple leaf extract (*Annona squamosa L.*) can increase the activity of antioxidant enzymes such as SOD. [17,18] However, SOD levels should not be allowed to get too high, because high SOD levels are detrimental because excessive ROS counteracting causes increased lipid peroxidation. Removing ROS by SOD will produce Hydrogen Peroxide (H2O2), so that more SOD means more H2O2 is formed, which is actually toxic to cells and results in lipid peroxidation of the cell wall. From the literature it was found that SOD doses of up to 5 ng/ml are protective, but above that it loses its protective properties, and even at a dose of 50 ng/ml it causes cell injury due to lipid peroxidation. At a dose of 5% (P4) an average SOD of 6.0890 ng/ml was obtained,

so it might lose its protective properties. The dose of 3% (P3) is the most optimal dose with an average SOD of 4.7752 and this dose is in accordance with an in vitro study with DPPH which compared the ethanol extract cream of Sugar apple (Annona squamosa L.) leaves with Ascorbic Acid. [19,20]

Sugar apple leaf extract cream (*Annona squamosa L.*) in groups P3 and P4 can significantly increase SOD. This could be due to the fact that Sugar apple leaves (*Annona squamosa L.*) contain flavonoids (quercetin) and terpenoids (diterpenes) which are Nrf2 modulators so that they can increase SOD. Activate the Nrf2 pathway as well as by saving SOD (reducing SOD usage by reducing ROS production produced by UVB.[8,21–23] Under normal conditions, two Keap1 molecules, which bind Nrf2 and Cul3 ubiquitin ligase, bind Nrf2 at the high-affinity ETGE site and the low-affinity DLG site. If it is not activated, ubiquitination will occur in Nrf2 so that it is released from the bond with Keap1, and then Nrf2 is degraded by the proteasome. In the presence of ROS or Nrf2:Keap1 binding inhibitors (such as flavonoids or terpenoids), dissociation of the Nrf2:Keap1 bond will occur, possibly due to the interaction of the Nrf2:Keap1 inhibitor with the thiol chain on Keap1, Nrf2 phosphorylation, and then translocation of Nrf2 to the nucleus. Nrf2 translocation and accumulation in the nucleus will increase ARE promoter activity and further increase the expression of ARE target enzymes, including SOD.[8,10]

Effect of Sugar apple Leaf Extract Cream (*Annona squamosa L.*) on MMP-1 Levels in Male Wistar Rats Exposed to UV B Light

The results showed that administration of Sugar apple leaf extract cream (*Annona squamosa L.*) at doses of 1%, 3%, and 5% could reduce MMP-1 levels in the skin of male Wistar rats exposed to UV B light. These results were in accordance with previous studies where the extract Sugar apple leaf ethanol (Annona squamosa L.) is one of four plant ethanol extracts out of 14 plant extracts that have high MMP-1 inhibitory activity. [10]

Sugar apple leaf extract (*Annona squamosa L.*) has a high content of phenols and flavonoids which can inhibit MMP-1. From the research, Sugar apple leaf extract (*Annona squamosa L.*) contains flavonol type flavonoids which have the highest inhibitory activity compared to other types of flavonoids. Most of these flavonols are the flavonol quercetin which has the highest MMP-1 inhibitory properties because it has hydroxyl substitutions in the B-ring and it is strongly suspected that the hydroxylation pattern in this B-ring is an important determinant for the nature of MMP-1 inhibitory activity by flavonoids. [10]

In this study it was seen that the greater the dose of Sugar apple leaf extract cream (Annona squamosa L.), the greater the MMP-1 inhibition effect, but the administration of Sugar apple leaf extract cream (Annona squamosa L.) 3% (P2), the results were approaching the P0 group which was not given basic cream and was not exposed to UV B light. Meanwhile, in the P3 group with a concentration of 5%, the results were better than the P0 group and did not differ statistically significantly. These results indicate that although it is proven that Sugar apple leaf extract cream (Annona squamosa L.) has MMP-1 inhibitory properties, the optimal dose and duration of administration are still recommended for further research, because if there is excessive MMP-1 inhibition, ECM homeostasis will be disrupted. and instead will cause diseases such as keloids, neurodegenerative diseases, cancer, and others. [24]

Sugar apple Leaf Extract (*Annona squamosa L.*) in Anti-Aging Medicine

Sugar apple leaf extract (*Annona squamosa L.*) is an extract obtained from Sugar apple leaves (*Annona squamosa L.*) which is dried and macerated with 96% ethanol solvent.

Sugar apple leaf extract (*Annona squamosa L.*) contains various substances that are useful as antioxidants and anti-collagenase/anti-MMP-1, including flavonoids (quercetin) and terpenoids (diterpenes).[10]

In this study, administration of Sugar apple leaf extract cream (*Annona squamosa L.*) at a dose of 3% and 5% was shown to increase skin SOD levels and doses of 1%, 3% and 5% reduced skin MMP-1 levels compared to negative controls. A 1% dose has not been able to increase SOD levels, but it can reduce MMP-1. This may be due to the fact that 1% cream of Sugar apple leaf extract (*Annona squamosa L.*) contains flavonoids which are sufficient to reduce MMP-1 but have not been able to activate the Nrf2 pathway to increase SOD. Further research related to dosage, duration and use of the extract is needed to determine the optimal effect of the capacity of Sugar apple leaf extract (*Annona squamosa L.*) as an antioxidant and anti-collagenase to avoid the possible effect of quercetin as a pro-oxidant because in several journals it is stated that quercetin reduces ROS production in cells, but produce peroxide which is a pro-oxidant in the extracellular.

The weakness of this study is that it does not compare with the positive control (Ascorbic Acid), which is the standard drug for antioxidants. Consideration was taken not to compare with positive controls because there had been an in vitro study with DPPH that compared Sugar apple leaf extract cream (*Annona squamosa L.*) with Ascorbic Acid with similar results, namely Sugar apple leaf extract cream (*Annona squamosa L.*) 3% had effectiveness that exceeds Ascorbic Acid at a dose of 50 µg/ml, as a positive control.

CONCLUSION

Of the 3 doses studied, the concentration of 3% was the most effective in increasing SOD levels and reducing MMP-1 levels in the skin of male Wistar rats exposed to UVB light. Further research is needed to evaluate the optimal dose and duration of administration of Sugar apple leaf extract cream (*Annona squamosa L.*) in increasing skin SOD levels and decreasing skin MMP-1 levels. It is necessary to test the toxicity of Sugar apple leaf extract cream (*Annona squamosa L.*) on the skin, to find out potential short-term and long-term side effects when given at certain doses and to compare its effectiveness with vitamin C as the gold standard antioxidant and anti-collagenase.

Declaration by Authors

Ethical Approval: This research has been approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Universitas Udayana with number B/197/UN14.2.9/PT.01.04/2022.

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Authors' Contribution

All authors have the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis, to the interpretation of research data and presentation of the final report.

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