# Antioxidant And Anti Aging Activity Test Of Salak Skin Extract Cream (Salacca Zalacca)

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## ABSTRACT

This study aims to see the antiaging activity of snake fruit skin extract (Salacca Zalacca) through the difference in collagen density between treatment groups 1, 2 and 3 with a concentration of snake fruit skin cream extract (Salacca Zalacca) of 10%, 20% and 30% and compare it with the control group without extract. This study is a True experimental study, with the selection of the type of research design used is Post Test Only Control Group Design, the methods used include phytochemical tests, antioxidant activity tests with the DPPH method, and observations of collagen density in the skin of mice exposed to UVB light. This study used test animals in the form of male white rats (Rattus norvegicus) Wistar strain weighing 150-250 gr. The test animals were divided into 4 groups. The results showed that snake fruit skin extract has strong antioxidant activity and can increase collagen density, so it has the potential as an active ingredient in anti-aging cosmetic products.

***Keywords:*** *Salak Skin, Antioxidants, Anti-aging*

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## INTRODUCTION

Natural antioxidants are widely applied in the form of topical preparations. The purpose of these topical preparations is to reduce the damaging effects and prevent oxidative stress conditions that occur directly in the skin. Four different reaction mechanisms are used by the antioxidant mechanism to prevent lipid oxidation that occurs in the human skin layer. These include the release of antioxidant hydrogen, the release of antioxidant electrons, the addition of fatty acids to the antioxidant aromatic ring, and the formation of complex compounds between the antioxidant aromatic ring and fat.(Dipahayu, D., Soeratri, W., & Agil, 2014). It is possible that salak skin extract can be used as a source of antioxidants in topical preparations such as soap. Every day, people use soap to clean dirt and oil that sticks to the skin along with

water. The aesthetics of soap not only function as a skin cleanser, but also become a separate attraction for consumers in choosing various types of cosmetics. This new transparent scrub improves the appearance and has antioxidant content that can reduce premature aging of the skin. This study aims to determine the properties of solid and transparent soap from salak skin extract and its antioxidant activity. The description above shows that salak skin has great potential to be used as a source of antioxidants(Yousef H, Alhajj M, Sharma S. Anatomy, 2023) Skin aging generally begins approaching the age of 30. But a survey said, as many as 57% of women in Indonesia have realized the signs of aging at the age of 25. The survey conducted by a well-known skincare brand together with one of the online media, has studied 778 respondents. From the research results, it was also found that the most visible signs of premature aging were not fine lines or wrinkles, but dark skin with a percentage of 53.30%. Although aware of the emergence of premature aging signs, in fact many of them still postpone anti-aging treatments. Another survey conducted by independent research agency Taylor Nelson Sofres on 1,800 women aged 20-39 years in Asia (India, Korea, Philippines, Thailand) said, 1 in 3 women in Asia only use treatments for whitening, even though they also experience signs of aging(Irnawasti et al., 2022) An 8-year study published in the British Journal of Dermatology has shown that using skin care early can reduce signs of aging. The study also said that with the right treatment, the growth of fine lines and wrinkles can be reduced. Antioxidants are also one of the topics raised by the author related to snake fruit skin extract. This is based on the increasing understanding that some diseases begin with an excessive oxidation response in the body. Antioxidants outside the body can be obtained in synthetic and natural forms. Such as vitamin E and C supplements that can work in the body. Consuming antioxidants, both synthetic and natural, is very good at preventing the formation of oxidation in the body(Rusip, Ilyas, Lister, Ginting, & Mukti, 2022).

## LITERATURE REVIEW

Intrinsic skin aging is the natural skin aging process that occurs with age and begins in the late third decade. This process is slow and causes changes in the structure of skin tissue. Several mechanisms of change occur simultaneously during intrinsic skin aging.(Hwang KA, Yi BR, 2011). Changes mainly occur in the morphology or structure of the skin in the epidermis layer, while biochemical changes occur in the dermis layer. Changes also occur in the adnexal organs of the skin such as hair, sweat glands, and oil glands.(NoThakur R, Batheja P, Kaushik D, 2008)Intrinsic skin aging causes the skin surface to become paler, fine lines or fine wrinkles appear, and the epidermis and dermis layers become thinner, making the skin appear thinner, more transparent, and fragile. In addition, the skin becomes dry and itchy. A decrease in subcutaneous fat tissue, including facial fat, is followed by intrinsic skin aging, which causes

eye bags and deep, sunken cheeks. In addition to age, other intrinsic factors related to skin aging include race, anatomical variations in some areas of the skin, and hormonal changes.(Hwang KA, Yi BR, 2011). In Indonesia, there are three types of snake fruit plants that are generally different: Balinese snake fruit (Salacca amboinensis (Becc) Mogea) has 1-2 seeds, Javanese snake fruit (Salacca zalacca (Gaertner) Voss) has 2-3 seeds, and Padang Sidempuan snake fruit (Salacca sumatrana (Becc) Mogea) has red fruit flesh (Fransiskus 2010, in(Ismail, NA, & Abu Bakar, 2018). Seeing the actualization of the salak fruit or its age indicates when the salak fruit can be eaten. Salak fruit can be harvested after it is ripe at the age of six months after the flowers bloom.(Kusmana, C., Hikmat, A, 2015). The skin of the snake fruit becomes reddish black or dark yellow, the outer skin hairs are lost, the tip of the skin (the pointed part of the fruit) becomes soft when pressed, and the scales become denser. One of the signs of snake fruit is its shiny color, strong aroma, and easy to pick from the stalk. While harvesting snake fruit through appearance is usually done by experienced people, harvesting through age can be done by anyone. Snake fruit is a fruit that originates from Indonesia and grows well in tropical areas. This fruit has a sweet taste that is sometimes mixed with sourness and its shape resembles a cone. Snake fruit has various health benefits, such as improving heart health, improving the immune system, helping to improve memory, brightening the skin, and helping to destroy kidney stones. Despite its many benefits, more research is needed to reveal the full potential of snake fruit. This exotic fruit has diverse and potential pharmacological properties due to its high antioxidant content.

## METHODS

This study is a True experimental study, with the selection of the type of research design used is Post Test Only Control Group Design, which is a type of research that only observes the control and treatment groups after being given an action. The research sample in this study was a male white rat (Rattus norvegicus) Wistar strain weighing 150-250gr and aged 2-3 months. Researchers chose rats (Rattus norvegicus) as the subject of the research test because this animal has characteristics and physiology that are almost the same as humans and is also one of the most widely used animals in biomedical science research.

### Operational Definition

The following are operational definitions of each variable used in this study:

**Table 1 Operational Definitions**

| Variables | Operational Definition | Methods and Measuring Tools |
|---|---|---|
| Salak skin extract (Salacca zalacca) | Salak skin extract (Salacca zalacca) is a salak skin extracted using 70% ethanol. Ethanol extract of salak skin is given once a day for 14 days to test animals orally. | Measuring with the help of a needle and given by the remaceration method using a blunt probe |
| Antioxidants | The ability of a compound to inhibit oxidation reactions can be expressed as % inhibition (percentage of the sample's ability to capture DPPH radicals) which is measured using a spectrophotometer. | Spectrophotometer |
| Anti-aging | Antiaging activity was seen based on the collagen density of mouse skin exposed to UVB light | Identified using a microscope with 4x, 10x, 20x, and 40x magnification |
| Mice exposed to ultraviolet light B | Wistar strain rats (Rattus novergicus) were exposed to Philips brand PL-S9W/01/2P UVB lamps given 3 times a week, namely on Monday, Wednesday and Friday with a total dose of 840 mJ/cm2 for 2 weeks. | 200watt UV-B TL lamp |

The study was conducted by(Valentino et al., 2021)which has been changed by researchers, is the basis for making salak skin extract. To make salak skin extract, salak skin is collected from 20 kilograms of peeled salak fruit. To make salak skin extract, the blended salak skin is weighed about 150 grams and extracted with 900 milliliters of 70% ethanol solution. Exposure

to light was carried out with a frequency of 3 times a week (Monday, Wednesday and Friday) starting with 50 mJ/cm2 for 50 seconds in the first week, followed by 70 mJ/cm2 for 70 seconds in the second week and 80 mJ/cm2 for 80 seconds in the last week with a total UVB received of 840 mJ/cm2. Irradiation was carried out every day at 10.00 WIB using a Phillips UVB PLS9W/01/2P lamp (Haryanto et al., 2020) Salak Skin Extract Cream (Salacca zalacca) was applied after the mice were exposed to UVB light. During UVB light exposure, the extract was given twice a day for 2 weeks, 20 minutes after UVB light exposure at 09.40 WIB, 10.00 WIB and 4 hours later the administration was started again at 14.00 WIB. The application of Salak Skin Extract Cream (Salacca zalacca) was still given on days without irradiation. After 2 weeks, the mice were euthanized using an overdose of ketamine (125 mg/kg BW) intramuscularly in an anaerobic jar 48 hours after the last irradiation. The skin sampling process was carried out by biopsy in the back area where the skin would be taken, cleaned of fur, the skin was cut with a thickness of approximately 2 mm to the subcutaneous with a length of 2 cm and a width of 2 cm. After that, histopathological preparations were made and the amount of dermis collagen was calculated. The remaining unused mouse organs will be buried.

## RESULTS

### The results Antioxidant Activity Test

In determining the antioxidant activity test, it starts from determining the wavelength, determining the operating time and determining the antioxidant activity of the ethanol extract of snake fruit skin. The levels of antioxidant activity can be seen in Table 2:

**Table 2 Antioxidant Activity Levels**

| Mark | Levels |
|---|---|
| IC50 < 50 µg/ml | Very strong |
| IC50 50-100 µg/ml | Strong |
| IC50 101-150 µg/ml | Currently |
| IC50 > 150 µg/ml | Weak |

In making 0.5 mM DPPH solution (200 ppm), 10 mg of DPPH powder was weighed and dissolved in methanol up to 50 mL. Meanwhile, to measure the maximum absorption

wavelength of DPPH by pipetting 1 mL of DPPH standard solution. put into a 5 mL volumetric flask. then methanol was added to the mark so that a solution with a concentration of 40 ppm was obtained. The maximum wavelength was measured using a UV-Vis spectrophotometer (400 nm - 800 nm). The maximum wavelength was obtained at 515 nm. To make the extract test solution, 10 mg of thick extract was weighed and dissolved in methanol up to 10 mL. a solution with a concentration of 1000 ppm was obtained. Taken 0.1 mL; 0.2 mL; 0.3 mL; and 0.4 mL; from the 1000 ppm extract solution. then added 1 ml of DPPH solution (concentration 200 ppm) to each concentration and added with methanol to the mark limit (5 mL measuring flask). obtained concentrations of 20, 40, 60, and 80 ppm. Incubated for 30 minutes then measured the absorbance using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm. Determination of the free radical trapping process by the test sample using the DPPH free radical trapping method obtained different results. The results of the 20 ppm concentration were 33.6604%, the 40 ppm concentration was 52.6987%, the 60 ppm concentration was 70.0686%, the 80 ppm concentration was 74.5892%. For the calculation of the results of the DPPH trapping method is to calculate IC50. This value indicates that the plant extract can cause a reduction of 50% of DPPH activity. The results obtained were 44.0931 ppm, which means that based on the antioxidant activity level table, it is included in the very strong category. This can also be seen from the color change of the test sample which is dark purple when DPPH is added, it will turn yellowish if the extract has a reduction.

**Antiaging Testing**

Antiaging is a process of inhibiting premature aging of the skin. In this study, the number of wrinkles produced by exposure to ultraviolet light on the skin determines the anti-aging activity of the cream, if more wrinkles indicate that the cream does not work well on the skin, which means low anti-aging activity. To determine the effectiveness of the snake fruit skin extract cream (Salacca Zalacca.) which will be given to mice as a skin protector from exposure to ultraviolet type B (UVB) rays can be seen from the anti-aging activity test. The test animals were first exposed to ultraviolet type b (UVB) 311 nm in order to induce the formation of aging parameters, namely wrinkles, erythema, and exfoliation. After that, the mice were given treatment according to their respective groups. Experimental animals used were 24 mice divided into 4 groups including control group, treatment 1, 2 and 3. With the control group without any treatment, treatment group 1 (P1) was given snake fruit skin extract (Salacca Zalacca) with a concentration of 10%, treatment 2 (P2) 15%, and treatment 3 (P3) with a concentration of 20%. Furthermore, skin observations were carried out by looking at wrinkles and then the skin was observed through the density of collagenization after post-UVB irradiation. The process of taking skin samples was carried out by biopsy in the back area

where the skin would be taken, cleaned of fur, the skin was cut with a thickness of approximately 2 mm to the subcutaneous with a length of 2 cm and a width of 2 cm. after that histopathological preparations were made and the amount of collagen and melanin was calculated as post-test data. The remaining unused mouse organs will be buried. The following is a table of collagen density test results on mouse skin tissue:

**Table 3 Results of Collagen Density Test on Mouse Skin Tissue**

| **Repetition** | | | | |
|---|---|---|---|---|
| | **K** | **P1** | **P2** | **P3** |
| **1** | 37.41 | 43.23 | 52.58 | 56.85 |
| **2** | 35.30 | 40.28 | 48.87 | 55.78 |
| **3** | 36.42 | 41.64 | 52.63 | 56.63 |
| **4** | 34.30 | 42.65 | 44.79 | 53.19 |
| **5** | 35.60 | 42.32 | 50.32 | 55.21 |
| **6** | 37.40 | 44.71 | 52.23 | 58.43 |
| **Score** | **0** | **2** | **3** | **3** |
| **Mean** | **36.07** | **42.47** | **50.23** | **56.01** |
| **Std** | **1.235** | **1,492** | **3,057** | **1,765** |

## DISCUSSION

The percentage of collagen density in the control group of mice (K) without treatment only exposed to ultraviolet b light for 14 days had an average result with a standard deviation of 36.07 ± 1.235, for treatment group 1 (P1) mice exposed to ultraviolet b light and smeared with salak fruit skin extract cream with a concentration of 10% every day for 14 days had an

average result with a standard deviation of 42.47 ± 1.492, for treatment group 2 (P2) mice exposed to ultraviolet b light and smeared with salak fruit skin cream with a concentration of 15% every day for 14 days had an average result with a standard deviation of 51.23 ± 30.57, and for treatment group 3 (P3) mice exposed to ultraviolet b light and smeared with salak fruit skin extract cream with a concentration of 20% every day for 14 days had an average result with a standard deviation of 56.01 ± 1.765. Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. obtained a significance result of 0.200 in all groups that have been measured in the number of 6 mice in each treatment group. Data is said to be normally distributed if the p value> 0.05. Therefore, it can be concluded that the data is normally distributed. homogeneity results using Levene's Test. For decision making, the guideline is if the significance value <0.05 means the data is not homogeneous, conversely the significance value> 0.05 means the data is homogeneous. And the results of the t-test above can be seen the average value of 46.198 from each group and the sig value (2 tailed) is known to be 0.000 <0.05, then it is concluded that there is a significant difference (real) between each group.

## CONCLUSION

1. The active substance contained in the extract of snake fruit skin (Salacca Zalacca) which was tested through phytochemical testing is that the extract of snake fruit skin (Salacca Zalacca) contains secondary metabolites such as alkaloids, flavonoids, saponins, and tannins.
2. Based on the antioxidant activity level table, ethanol contained in the extract of snake fruit skin (Salacca Zalacca) is included in the very strong category. This can also be seen from the color change of the test sample which is dark purple when DPPH is added, it will change to yellowish if the extract has a dampening.
3. Snake fruit skin extract (Salacca Zalacca) contains secondary metabolite compounds, namely flavonoids, saponins, tannins, alkaloids, and glycosides which have antioxidant, antimicrobial, and anti-inflammatory effects and play a role in the healing process of cuts, collagenization, and weight loss in obese mice.

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