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# In Vitro: Sunscreen Activity of Red Lettuce Extract (*Lactuca sativa* var. *crispa* L.)

# In Vitro: Sunscreen Activity of Red Lettuce Extract (*Lactuca sativa* var. *crispa* L.)

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**Abstract.** This study aims to determine the activity of sunscreen with the SPF (Sun Protection Factor) value of red lettuce extract (*Lactuca sativa* var. *crispa* L.) in various solvents. Red lettuce samples used were obtained from Turirejo village, Lawang district, Malang. Simplicia red lettuce was extracted by maceration method for 24 hours using a various solvents which are methanol, ethyl acetate, ethanol, and n-hexane solvents. Sunscreen activity testing is done in vitro using a UV-Vis spectrophotometer. The results of the sunscreen activity test of red lettuce ethanol extract (*Lactuca sativa* var. *crispa* L.) had the highest SPF value with the ultra protection category (29.505 at a concentration of 800 ppm) compared to methanol, ethyl acetate, and n-hexane extracts. With the SPF value of each extract at concentrations of 200, 400, 600, 800, and 1000 ppm respectively 1.364; 1,941; 2,778; 4,089; and 7,675 (for methanol extracts); 1,449; 2,341; 3,868; 6,222; and 10,266 (for ethyl acetate extracts); 2,049; 2,381; 9,455; 29.505; and 35,698 (for ethanol extract); and 1,103; 1,294; 1,322; 1,379; and 1,983 (for n-hexane extract).

## 1. Introduction

Sunlight radiation consists of infrared light (wavelength >760 nm), visible light (400-760 nm), and UV (ultraviolet) rays consisting of UV-A (320-400 nm), UV-B (290-320 nm) and UV-C (200-290 nm). Sunlight that reaches the surface of the earth and has an impact on the skin is UV-A and UV-B rays [1]. Long term exposure of sun radiation could make degenerative changes in the skin cells which leads to premature ageing, sunburns and skin cancers [2]. Excessive sun exposure causes the skin's epidermal tissue to be inadequate to counteract the negative effects that can be caused by ultraviolet radiation on the skin such as the occurrence of epidermal damage commonly called sunburn, pigmentation, skin wrinkling, premature skin aging, and on long irradiation under blistering the sun can cause changes in the binding tissue in the stratum corneum layer to skin cancer, thus protection is needed both physically and chemically. Chemically using sunscreen cosmetics. Sunscreen is a preparation used on the surface of the skin that works to absorb, scatter or reflect ultraviolet light. Sunscreen can absorb at least 85% of the sun's rays at wavelengths from 290 to 320 nm for UVB but can continue rays at wavelengths more than 320 nm for UVA. Therefore we need sunscreen that can protect the skin from the dangers of sun radiation [3,4,5].

Research that has been done shows that n-hexane red lettuce extract has antioxidant activity with $IC_{50}$ of 52.27 ppm which is classified in the strong category while vitamin C has antioxidant activity which is classified as very strong at 3.21 ppm [6]. And $IC_{50}$ on the antioxidant activity test on red lettuce ethanol extract obtained very strong results in the amount of 4.7 ppm [7]. The amount of activity obtained shows red lettuce can eliminate free radicals that damage cells. Besides the high antioxidant activity obtained, red lettuce extract can also counteract ultraviolet radiation as a sunblock. The compounds that have sunscreen and antioxidant activity can prevent or minimize the negative effects of solar radiation on the skin. Therefore, we need further research on sunscreen red lettuce extract.

## 2. Method

The research design used in conducting this research was a descriptive quantitative on a laboratory scale. Where red lettuce (*Lactuca sativa* var. *crispa* L.) is the research subject.

Simplisia red lettuce was extracted by maceration method. A total of 50 grams of simplicia powder were macerated in 300 mL of methanol, ethanol, ethyl acetate, and n-hexane at room temperature for 24 hours. Maceration results are filtered. The residue obtained was re-macerated 5 times. Furthermore, the aqueous extract obtained was concentrated using a rotary vacuum evaporator at a heating temperature below 55℃ and a concentrated extract was obtained. Sunscreen activity is done by weighing 50 mg of each extract of ethanol, methanol, ethyl acetate, and n-hexane red lettuce and each dissolved with absolute ethanol, put into a measuring flask, homogenized, the final volume sufficient with ethanol up to 50 mL (1000 ppm). The stock solutions are 2.0 mL, 4.0 mL, 6.0 mL and 8.0 mL respectively. Then the volume is sufficient to 10.0 mL with absolute ethanol so as to obtain variations in solvent with concentrations of 200, 400, 600, and 800 ppm. Each concentration of each extract (200, 400, 600, 800, and 1000 ppm) was measured by absorption using a UV-Vis spectrophotometer at wavelengths that can cause erythema and pigmentation, ie 290-400 nm at 5 nm intervals.

Sunscreen activity data generated in this study SPF value calculation is performed. The Sun Protecting Factor (SPF) value is calculated in advance the area under the absorption curve (AUC) of the absorption value at wavelengths of 290-400 nm at intervals of 5 nm. AUC values are calculated using the following formula [8,9]:

$$\mathrm{AUC} = \frac{\mathrm{Aa+Ab}}{2} \times \mathrm{dPa-b} \quad (1)$$

where Aa is the absorbance at the wavelength a nm, Ab is the absorbance at the wavelength b nm, and dPa-b is the difference in wavelengths a and b. The total AUC value is calculated by adding up the AUC value for each wavelength segment. The SPF value of each concentration is determined using the following formula [8,9]:

$$\log \mathrm{SPF} = \frac{\mathrm{AUC}}{\lambda_n - \lambda_1} \times 2 \quad (2)$$

Where SPF is the sun protection factor, AUC is the amount of absorption at λn-1 divided by 2, λn is the wavelength that produces 0.05 absorption, and λ1 is 290 nm. To obtain the SPF value at the vulnerable wavelengths of UV A and UV B, it is first determined that the average value of A at the intervals of erythemogenic activity is the wavelength interval that can be absorbed by sunscreen which can cause erythemal which can be indicated by absorbance of 0.05 on the sample without dilution. The preparations used in determining the SPF value were 2 mg / cm2 which is equivalent to 2 mg/ml. Then erythema percent value is calculated. From observational data transmittance values at various wavelengths can be calculated percent of erythema transmission in the following formula [8,9]:

$$\%\ \text{erythema transmission} = \frac{\mathrm{Ee}}{\Sigma \mathrm{Fe}} \quad (3)$$

where T is the transmission value, Fe is the erythema flux, and Ee = ΣT. Fe is the amount of erythema flux which is passed on by wavelength extract 292.5-317.5 nm. And the percent transmission value of pigmentation is calculated as follows [8,9]:

$$\% \text{ pigmentation transmission} = \frac{Ep}{\Sigma Fp} \quad (4)$$

where T is the transmission value, Fp is the pigmentation flux, Ep = ΣT.Fp is the amount of pigmentation flux transmitted by the extract at a wavelength of 322.5-372.5 nm, and ΣFp is the total amount of UV light energy that causes pigmentation.

## 3. Results and Discussion

Based on the results of the identification of plants carried out at the Indonesian Institute of Sciences (LIPI) stated that the plants used in this study were red lettuce plants with species of *Lactuca sativa* var. *crispa* L. Sunscreen is a substance that can reduce the harmful effects of skin exposure to ultraviolet light by a mechanism of action that consists of a physical block (reflecting solar radiation) and a chemical absorbing mechanism (absorbing solar radiation) [4].

**Table 1.** SPF Value of Red Lettuce Ethanol, Methanol, Ethyl Acetate, and n-Hexane Extracts

| Extract | Concentration (ppm) | SPF Value | Rating Category |
|---|---|---|---|
| Ethanol | 200 | 2,049 | Minimum protection |
| | 400 | 2,381 | Minimum protection |
| | 600 | 9,455 | Minimum protection |
| | 800 | 29,505 | Ultra protection |
| | 1000 | 35,698 | Ultra protection |
| Methanol | 200 | 1,364 | Minimum protection |
| | 400 | 1,941 | Minimum protection |
| | 600 | 2,778 | Minimum protection |
| | 800 | 4,089 | Extra protection |
| | 1000 | 7,675 | Minimum protection |
| Ethyl Acetate | 200 | 1,449 | Minimum protection |
| | 400 | 2,341 | Minimum protection |
| | 600 | 3,868 | Minimum protection |
| | 800 | 6,222 | Extra protection |
| | 1000 | 10,266 | Maximum protection |
| n-Hexane | 200 | 1,103 | Minimum protection |
| | 400 | 1,294 | Minimum protection |
| | 600 | 1,322 | Minimum protection |
| | 800 | 1,379 | Minimum protection |
| | 1000 | 1,983 | Minimum protection |

Ultraviolet (UV) radiation is defined as that portion of the electromagnetic radiation lies between X-rays and visible light which is from 200 to 400 nm. This UV rays comprises into 3 categories as follows: UV-A, UV-B and UV-C which all have different wavelengths and radiation effects. UV-A rays (320-400 nm) have a radiation effect in the form of pigmentation which can cause the skin to turn brown and reddish, immediate tanning or dark- ening of the skin due to excess production of melanin in the epidermis, premature photo ageing, suppression of immunologic functions, and even necrosis of endothelial cells and damage of dermal blood vessels. UV-B radiation (290-320 nm) are known as burning rays as they are 1000 times more capable of causing sunburn than UV-A. UV-B rays act mainly on the epidermal basal cell layer of the skin but more genotoxic than UV-A radiations. Ultraviolet B (UVB) rays vary with time and season are major cause of sunburn. Radiation effect of UV-B that causes erythema (redness) that can cause skin cancer if exposed to excessive radiation. While UV-C (200-290 nm) is retained in the atmosphere so that it does not reach the earth due to absorption in the stratospheric ozone layer [4, 10, 11, 12]. So in this study, the potential for sunscreen is measured from wavelengths from 290 to 400 nm (UV-A and UV-B).

Determination of the sunscreen potential of red lettuce ethanol, methanol, ethyl acetate, and n-hexane extracts was carried out in vitro by the UV-Vis spectrophotometry method at wavelengths of 290-400 nm. The first test is done by calculating the SPF (Sun Protecting Factor) value of each extract. SPF is a

universal indicator that explains the effectiveness of a UV protective substance. The higher the SPF value, the more effective it is to protect the skin from the adverse effects of UV rays [3, 13].

Testing the SPF value begins with dissolving 50 mg each of ethanol extract, methanol, ethyl acetate, and n-hexane red lettuce. Each extract was dissolved in absolute ethanol, then put into a measuring flask, homogenized, the final volume sufficient with ethanol up to 50 mL (1000 ppm). The stock solutions are 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL and 10.0 mL respectively. Then the volume is sufficient to 10.0 mL with absolute ethanol so as to obtain variations in solvent with concentrations of 200, 400, 600, 800 and 1000 ppm. Each extract concentration was measured by absorption using a UV-Vis spectrophotometer at wavelengths that can cause erythema and pigmentation that is 290-400 nm at intervals of 5 nm.

After that the area of absorption curve (AUC) is calculated first, and the SPF value is calculated by adding up the AUC value in each wavelength segment then divided by the result of the largest wavelength (400 nm) minus the smallest wavelength (290 nm). SPF value test results can be seen in Table 1.

Based on Table 1, it can be seen that the SPF values of ethanol, methanol, ethyl acetate, and n-hexane extracts of red lettuce at concentrations of 200, 400, 600, 800, and 1000 ppm provide varying protection. The ethanol extract of the protection given shows the ultra protection at a concentration of 800 ppm with an SPF value of 29.505. Whereas in methanol extract, the protection provided is extra protection with an SPF value of 7.675 at a concentration of 1000 ppm. Then the ethyl acetate extract provides maximum protection with an SPF value of 10.266 at a concentration of 1000 ppm. Furthermore, the n-hexane extract provides minimal protection at all concentrations with the highest value at 1000 ppm with an SPF value of 1.983. Therefore, the sequence of best sunscreen based on the highest protection is ethanol extract, ethyl acetate, methanol, and n-hexane. This is caused by the content of secondary metabolites that are not completely attracted during the maceration process using non-polar (n-hexane) solvents. In the extraction with ethanol, methanol, and ethyl acetate solvents are solvents which are more polar in nature compared to n-hexane in the order of polarity of methanol>ethanol>ethyl acetate. Thus, many secondary metabolites will be extracted. Nevertheless, the extract that provided the highest protection was ethanol extract with an ultra-protection level (29.505). This is reinforced by the results of qualitative identification of phytochemicals and TLC, the existence of secondary metabolite compounds such as polyphenols (flavonoids and tannin) which have the potential as sunscreens are contained in red lettuce extracts [14]. Flavonoids, a group of natural occurring compounds, act as catalysts in the light phase of photosynthesis and as stress protectants in plant cells by scavenging reactive oxygen species (ROS). Natural flavonoids have the potential photoprotection because of their UV absorbing, their ability to act as direct and indirect antioxidants as well as anti-inflammatory and immunomodulatory agents which provide exciting platforms for the development of photoprotection [15]. Also strengthened by antioxidant activity of 4.7 ppm of red lettuce ethanol extract classified as very strong [7] compared to red lettuce n-hexane extract which has antioxidant activity ($IC_{50}$) of 52.27 ppm belonging to the strong category [6].

In addition, based on Table 1 can be seen the relationship between the SPF value and concentration. The higher the concentration of an extract, brings higher SPF value. This shows that the higher the concentration of a compound in solution, the more UV light is absorbed. Beside determining the SPF value, we also determined the value of percent transmission of erythema (% Te) and pigmentation (% Tp).Erythema is a sign of an inflammatory process due to exposure to UV-B rays which is characterized by the emergence of redness that can cause skin cancer if exposed to excessive radiation [3, 4]. Percentage in transmission of erythema (% Te) illustrates the amount of sunlight that continues after hitting the sunscreen, which can cause skin erythema. The smaller a Te% means the less UV-B rays are transmitted so that it can be said that the substance has great activity as a sunblock [4].Testing the erythema transmission value (% Te) is done by measuring the absorbance of 5 series of the concentration of the test solution using spectrophotometry at a wavelength of 292.5-317.5 nm at intervals of 5 nm. After the absorbance value is obtained then it is converted to percentage of transmittance. This conversion is done because what is measured is the absorbance value (the amount

of radiation beam absorbed by the substance) while the desired value is the transmittance (the amount of radiation beam that passes through the substance and is captured by the detector).

**Table 2.** Percentage Value of Erythema Transmission of Red Lettuce Ethanol, Methanol, Ethyl Acetate, and n-Hexane Extracts

| Extract | Concentration (ppm) | % Erythema Transmission | Rating Category |
|---|---|---|---|
| Ethanol | 200 | 33,131 | Fast tanning |
| | 400 | 22,689 | Fast tanning |
| | 600 | 4,171 | Extra protection |
| | 800 | 3,450 | Extra protection |
| | 1000 | 3,450 | Extra protection |
| Methanol | 200 | 64,244 | Fast tanning |
| | 400 | 53,197 | Fast tanning |
| | 600 | 36,604 | Fast tanning |
| | 800 | 18,430 | Fast tanning |
| | 1000 | 7,669 | Extra protection |
| Ethyl Acetate | 200 | 60,911 | Fast tanning |
| | 400 | 29,196 | Fast tanning |
| | 600 | 11,909 | Fast tanning |
| | 800 | 6,883 | Fast tanning |
| | 1000 | 4,120 | Fast tanning |
| n-Hexane | 200 | 93,084 | Fast tanning |
| | 400 | 55,151 | Fast tanning |
| | 600 | 62,323 | Fast tanning |
| | 800 | 55,412 | Regular suntan |
| | 1000 | 6,435 | Regular suntan |

After obtaining the percentage of transmittance value, it is then calculated to determine the percentage transmission value of erythema using the formula stated in the data analysis (equation 3). The results of the calculation of the percentage of erythema transmission can be seen in Table 2.

Based on Table 2, it can be seen (% Te) of each extract for 5 concentrations included in the category of fast tanning, regular injections, and extra protection, which means that in the category of fast tanning extract is only able to absorb a small amount of UV-B light, so that UV- B that is passed on is bigger or it can be said that the extract has not been able to prevent the occurrence of erythema on the skin. Whereas in the sun block category it means that the extract is able to absorb a lot of UV-B rays, so that the transmitted UV-B rays are smaller or it can be said that the extract is able to prevent the occurrence of erythema on the skin. This is thought to be caused by the content of secondary metabolites that are pulled by the solvent during the maceration process. In addition, the higher the concentration, the smaller the percent transmission rate of erythema. This can be seen at the concentration of 200 ppm which has the highest% Te value and the concentration of 1000 ppm has the smallest value of Te%. This is because the higher the concentration of a compound in solution, the more light is absorbed so that the light that is transmitted is getting smaller. In addition to calculating the SPF value and% Te, a percentage of pigmentation transmission (% Tp) was also determined.

Pigmentation is a change in darker skin color caused by exposure to UV-A rays with a wavelength of 320-400 nm. Percentage transmission of pigmentation is the amount of sunlight that is passed on after sunscreen, thus causing skin pigmentation. The smaller the percentage of pigmentation transmission means the less UV-A light is transmitted, so that it can be said that the substance has a great activity as a sunscreen [3,4,5].

Test solutions that have been made in 5 concentration series, absorbance values were measured using spectrophotometry at a wavelength of 322.5-372.5 nm at intervals of 5 nm. Similar to the determination of the erythema transmission value (% Te), determining the percentage of transmission value of pigmentation (% Tp) requires a transmittance value while the measured value is the

absorbance value, thus the absorbance value obtained is then converted to the percentage of transmittance value.

The percentage of transmittance value that has been obtained is then calculated the percentage of transmission pigmentation value (equation 4). The results of the calculation of the percent value of pigmentation transmission can be seen in Table 3.

**Table 3.** Percentage Value of Transmission Pigmentation of Red Lettuce Ethanol, Methanol, Ethyl Acetate, and n-Hexane Extracts

| Extract | Concentration (ppm) | % Pigmentation Transmission | Rating Category |
|---|---|---|---|
| Ethanol | 200 | 37,792 | Total block |
| | 400 | 27,979 | Total block |
| | 600 | 3,462 | Total block |
| | 800 | 0,706 | Total block |
| | 1000 | 0,454 | Total block |
| Methanol | 200 | 68,142 | Fast tanning |
| | 400 | 38,720 | Total block |
| | 600 | 23,431 | Total block |
| | 800 | 14,969 | Total block |
| | 1000 | 6,445 | Total block |
| Ethyl Acetate | 200 | 63,317 | Fast tanning |
| | 400 | 33,887 | Total block |
| | 600 | 18,357 | Total block |
| | 800 | 9,486 | Total block |
| | 1000 | 4,952 | Total block |
| n-Hexane | 200 | 95,562 | Fast tanning |
| | 400 | 74,696 | Fast tanning |
| | 600 | 23,681 | Fast tanning |
| | 800 | 26,348 | Fast tanning |
| | 1000 | 16,530 | Fast tanning |

As shown in Table 3, the percent transmission value of pigmentation (% Tp) for 5 concentrations in each extract of red lettuce belongs to the category of Fast tanning and total block. This means that in the category of fast tanning extract is only able to absorb a little UV-A rays, so that the UV-A rays that are transmitted are greater or it can be said that the extract has not been able to prevent the occurrence of pigmentation on the skin. Whereas in the regular suntan category it means that the extract is able to absorb a lot of UV-A rays, so that the UV-A rays that are transmitted are smaller or can be said to be able to prevent the occurrence of pigmentation on the skin. Based on this it can be concluded, the durability of sunscreen ethanol extract, methanol, ethyl acetate, and n-hexane red lettuce are in the category of fast tanning and total block. So that the highest in providing protection against UV-A and UV-B is ethanol extract. This is based on the SPF value in the ultra category, and the% Te value in the extra protection category, and the% Tp value in the sun block category.

The relationship between the value of percent transmission of pigmentation and concentration shows the greater the concentration, the smaller the value of percent transmission of pigmentation. This is because the higher the concentration of a compound in solution, the more light is absorbed and the less light is transmitted. Can be seen at the concentration of 200 ppm the highest% Tp value and the concentration of 1000 ppm has the smallest% Tp value which means at a concentration of 1000 ppm, the extract absorbs more UV-A rays than at a concentration of 200 ppm.

## 4. Conclution

The sunscreen activity of red lettuce ethanol extract (*Lactuca sativa* var. *crispa* L.) had the highest SPF value with the ultra protection category (29.505 at a concentration of 800 ppm) compared to methanol, ethyl acetate, and n-hexane extracts. With SPF values at concentrations of 200, 400, 600, 800, and 1000 ppm respectively for each extracts are:

a. 2,049; 2,381; 9,455; 29.505; and 35,698 for red lettuce ethanol extract.
b. 1,364; 1,941; 2,778; 4,089; and 7,675 for red lettuce methanol extracts.
c. 1,449; 2,341; 3,868; 6,222; and 10,266 for ethyl acetate red lettuce extracts.
d. 1,103; 1,294; 1,322; 1,379; and 1,983 for n-hexane red lettuce extract.

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