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## Phytochemical Screening Test and SPF Value of Ethanol Extract of Kundur Leaf (*Benincasa Hispida*) with In Vitro

Dwi Fitri Yani<sup>\*a</sup>, Lusiana<sup>a</sup>, Muntiya Permana<sup>a</sup>, Tirta Tri Valuvi<sup>a</sup>

**Abstract.** These bud leaves have been used in traditional medicine for a variety of health conditions. However, research on the potential SPF of *Benincasa hispida* has not been found. This study aims to conduct phytochemical screening tests and determine the Sun Protection Factor (SPF) value of kundur leaves (*Benincasa hispida*). The research methods used include extraction and determination of SPF values from *Benincasa hispida* extract at wavelengths of 290-320 nm using a UV-Vis spectrophotometer. The research results show that a concentration of 1000 ppm produces an SPF value of 25.89. And positive phytochemicals contain compounds such as flavonoids, terpenoids and saponins. The resulting SPF value shows that *Benincasa hispida* extract has the potential as a natural sun protection ingredient in the Ultra Protection category.

**Keywords:** Kundur leaves, Sunscreen, SPF, Ultra Protection.

<sup>a</sup>Chemistry, Faculty of Sains and Thecnology, Universitas Islam Negeri Raden Fatah, Palembang, Indonesia

Correspondence and requests for materials should be addressed to Dwi Fitri Yani (email: [dwifitriyani\\_uin@radenfatah.ac.id](mailto:dwifitriyani_uin@radenfatah.ac.id))

## Introduction

All living things use sunlight for their survival. As one example, sunlight is needed by humans as a source of energy and to maintain healthy skin and hair. Sunlight that reaches the surface has a negative effect on the skin, namely UV A and UV B rays. Because the two ultraviolet rays work together, some precautions or protection are needed to reduce the risk of skin cancer caused by UV A and UV B rays. UV A and UV B rays reach the earth and have a negative effect on the skin.

Side effects caused by ultraviolet radiation cause damage to the epidermis, commonly known as sunburn, skin shrinkage, premature aging, pigmentation etc. exposure to hot sunlight for a long time which causes changes in the connective tissue in the stratum corneum. Preventing harmful effects due to exposure to sunlight can be done by using sunscreen. Sunscreen is used as a preparation to protect skin health from the negative effects of solar radiation [1].

Sunscreen is a substance that contains ingredients that protect the skin from the sun so as to prevent UV rays from entering the skin (preventing skin diseases due to light radiation). Sunscreen contains chemical compounds that can absorb UV radiation and reflect sunlight by expelling or absorbing solar radiation energy that hits the skin, so that the radiation energy does not directly hit the skin. The effectiveness of sunscreen is based on determining the SPF (Sun Protected Factor) value. describes the ability of sunscreen products to protect the skin against erythema. To protect the skin from erythema, sunscreens are developed, but sunscreens are often developed with active chemicals that have a number of negative effects. One of the solutions that can be used is to use natural ingredients, namely from kundur leaf extract (*Benincasa hispida*).

Kundur leaf ethanol extract has antioxidant and flavanoid activity. Flanoids, saponins, triterpenoids and others have been reported to have the ability to protect against UV rays. Phenolic compounds, especially the flavanoid and saponin groups, have sunscreen potential because of the presence of chromophore groups (conjugated single double bonds) that are able to absorb UV rays, both UV A and UV B, so that they can reduce their intensity on the skin.

SPF values range from 0 to 100 and sun protection properties are considered good over 15. According to the FDA (Food Drug Administration). distribution of potential sunscreen products low (SPF between 2-4), Medium (SPF between 4-6), Extra (SPF between 6-8), maximum (SPF between 8-15) and ultra (SPF over 15). Testing of the antioxidant effectiveness of sunscreen of kundur leaf extract was carried out to determine the SPF value (Sun Protected Factor) using UV-vis spectrophotometry instruments [1].

## Experimental

**Sample Extraction.** Kundur leaf samples (*Benincasa hispida*) were taken in the KM. 12 area of the city of Palembang, South Sumatra. The kundur leaves are washed first with running water, slicing, slicing are placed in a container, the drying process is carried out in the open air under direct sunlight, then the dried kundur leaves are mashed using a blender to get it in powder form. after that it was sifted and obtained kundur leaf simplicia powder (*Benincasa hispida*).

In the extraction process of kundur leaf powder, it is carried out by the maceration method using 96% ethanol solvent. Dried kundur leaf simplicia is then weighed as much as 60 grams, then put into a 1000 ml beak, added 800 ml of 96% ethanol in a container until all samples are submerged by solvent and then the sample is macerated for 2 days. The extracts obtained are then evaporated by the solvent using a rotary evaporator to produce a viscous extract.

Phytochemical screening tests are carried out to determine the content of secondary metabolite compounds found in kundur leaves including flavonoids, alkaloids, tannins, saponins, steroids and triterpenoids using certain reagents [2].

### Test the content of secondary metabolite compounds of kundur leaves (*Benincasa hispida*).

#### Uji Flavonoid

A total of 0.1 grams of extract is added to 10 ml of hot water and boiled for 5 minutes. After that, the filtrate is filtered and tested. The filtrate is put into the test tube, 0.5 grams of Mg powder, 1 ml of concentrated HCl and 1 ml of alcohol, then shaken vigorously. The presence of flavonoids is indicated by the red/yellow/orange color of the alcohol layer.

**Alkaloid Test.** A total of 1 gram of sample extract was dissolved in 10 mL of  $\text{CHCl}_3$  with a few drops of  $\text{NH}_4\text{OH}$  and then filtered. The filtrate of  $\text{CHCl}_3$  extract is put into a closed test tube, 10 drops of  $\text{H}_2\text{SO}_4$  2 M are added while being beaten until 2 layers are formed, namely an organic layer and an acidic (colorless) layer, then Meyer, Wagner and Dragendorff reagents are added. The identification of alkaloid compounds was carried out by observing the results of reactions with phytochemical test reagents, namely the formation of white deposits after the addition of the Meyer reagent, brown deposits after the addition of the Wagner reagent, and the orange-red deposits after the addition of the Dragendorff reactant.

**Tannin Test.** The test solution was taken as much as 1 ml then a few drops of 10%  $\text{FeCl}_3$  solution were added and observed. A dark blue or greenish-black color appears that indicates positive results in the presence of tannins.

**Saponin Water.** 1 ml of sample extract (ethanol solvent) is put into the test tube. Then add 5 mL of hot water and 2 drops of  $\text{HCl}$  2 N and shake vigorously. The formation of a consistent foam for 15 minutes shows positive results against saponins.

**Steroid and Terpenoid Tests.** The sample of the test solution is put into the test tube, 3 drops of Liebermann Burchard reagent are added and observed. The formation of red or purple color indicates a positive result in the presence of terpenoids, the formation of brown rings, green or blue color indicates the presence of steroids [2].

**Determination of SPF Value.** Determination of SPF value of kundur leaf extract (*Benincasa hispida*) extract samples were taken as much as 0.0025 mg then diluted with ethanol and made in concentrations of 50 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm, and 1000 ppm. UV-vis spectrophotometry is calibrated in advance using ethanol. Its absorbance value was measured with a UV-vis spectrophotometer with an interval of 5 nm at a wavelength of 290-320 nm.

Analyze SPF value data using the Mansur method in Eq 1.

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times \text{I}(\lambda) \times \text{Abs}(\lambda) \quad (1)$$

Where, CF : Correction Factors (10); EE : Erythema Effect Spectrum; I : Light intensity spectrum; ABS : Absorbansi Sample.

The value of  $\text{EE} \times \text{I}$  is a constant whose value is already established as shown in the following Table 1.

Table.1  $\text{EE} \times \text{I}$  values

| Wavelength ( $\lambda$ nm) | $\text{EE} \times \text{I}$ |
|----------------------------|-----------------------------|
| 290                        | 0,0150                      |
| 295                        | 0,0817                      |
| 300                        | 0,2874                      |
| 305                        | 0,3278                      |
| 310                        | 0,1864                      |
| 315                        | 0,0839                      |
| 320                        | 0,0180                      |
| Total                      | 1                           |

**Data analysis.** To analyze the data, the maximum number of waves must be determined in advance by the UV-Vis spectrophotometry tool. After obtaining the absorbance of each concentration, the SPF of kundur leaf extract can be compared with the sunscreen protection category in the form of a table. The SPF level will be determined by the effectiveness of the sunscreen.

Table 2. The effectiveness of sunscreen products based on SPF values.

| SPF value | Categories of Protective Strength |
|-----------|-----------------------------------|
| 2-4       | Minimal protection                |
| 4-6       | Medium protection                 |
| 6-8       | Extra protection                  |
| 8-15      | Maximum protection                |
| >15       | Prosthetic ultrasound             |

## Result and Discussion

Kundur leaf samples (*Benincasa hispida* Thunb.Cogn) were used for this study. These kundur leaves are processed in the form of simplicia before being processed into extracts. The samples we used were taken in the KM 12 area of Palembang City, South Sumatra Province. Fresh, middle-aged kundur leaves are sorted wet, separated from unnecessary materials, and washed with running water to remove any dirt that sticks to the leaves. Drying in direct sunlight to avoid chemical damage. The purpose of drying is to reduce moisture to prevent microbial growth. Once the Simplicia is dry, it is sorted back to dry. The purpose of dry filtration is to pre-

vent *Simplisia* from being contaminated by unnecessary components that may affect the purity of the extract. Purification is carried out to increase the surface area of *Simplisia* and facilitate the adsorption of chemical components by the extract.

Kundur leaf extract is obtained by the maceration method. The maceration method is used because it is a low-temperature extraction method that contains *Simplisia* components that are not heat-resistant. The maceration is completed by drying it with the help of sunlight. Once dry, then ground into a powder. The active ingredient dissolves due to the difference in concentration. The maceration method was chosen because it does not require complicated and cheap equipment, and does not use heat so that it can prevent evaporation of compound components and avoid damage to compounds that are labile to heat, then soaking is carried out by adding 96% ethanol solvent. Ethanol was chosen as a solvent because of its volatility and because of its low boiling point. Concentrate and evaporate the sample using a rotary evaporator. Evaporation is carried out in such a way that no solvent remains and the validity of the test sample is not compromised [3]. Total yield from ethanol extract showed at Table 3.

Table 3 shows the yield of the extract produced during the extraction process. According to the literature, a good yield is >10%, and the ethanol extract of kunur leaves is considered

a good yield. This is also in line with previous research, which only produced an 8% yield. The concentrated extract color indicates that the sample contains many secondary metabolites with a compound structure that contains many aromatic groups and conjugated double bonds.

**Phytochemical Test of Kundur Leaves.** Phytochemical tests are carried out to find out the compounds present in the sample. Phytochemical screening is done to find out the group of compounds present in the sample [4]. The following phytochemical test results can be seen in Table 4.

Phytochemical screening results of kundur leaf extract (*Benincasa hispida*) The results obtained in this study show that kundur leaves contain alkaloids, terpenoids, flavonoids, and saponin compounds [5] which can be of interest to ethanol solvents. The concentrated extract of kundur leaves is produced then fractionalized, Fractionation aims to separate compounds based on polarity. Nonpolar compounds are soluble in nonpolar solvents, polar compounds are soluble in polar solvents, and semipolar compounds are soluble in semipolar solvents. The fractionation process is carried out using ethanol solvents. This is because ethanol has (-OH) and (-CH) groups<sup>3)</sup> which affects the polarity of ethanol solvents and can attract polar and non-polar compounds [6].

In other journal tests, and the samples used were taken in the Seberang Padang area, South Padang District, Padang City, West Sumatra Province.

**Table 3.** Yield of Kundur Leaf Ethanol Extract

| Sample          | Weight of Simplicia | Extract Weight | Rendemen | Extract Color |
|-----------------|---------------------|----------------|----------|---------------|
| Extract Ethanol | 60 grams            | 6.1304 grams   | 10,21%   | Deep Green    |

**Table 4.** Phytochemical Content of Kundur Leaf Extract (*Benincasa Hispida*)

| Compound Groups      | Ethanol Extract Test Results | Positive Test                     |
|----------------------|------------------------------|-----------------------------------|
| Flavonoid            | (+)                          | Red, yellow or orange color       |
| Terpenoid            | (+)                          | Red or purple color               |
| Tannins              | (-)                          | Dark blue or blackish-green color |
| Saponins             | (+)                          | Formation of foam or foam         |
| Steroids             | (-)                          | Brown, green or blue rings        |
| <b>Alkaloid</b>      |                              |                                   |
| Alkaloid Mayer       | (-)                          | White deposits                    |
| Alkaloid Wagner      | (+)                          | Brownish-orange sediment          |
| Alkaloid Dragendorff | (+)                          | Red-orange sediment               |

The results of the phytochemical test of kundur extract to identify the alkaloid group using the Mayer reagent and the Dragendorf reagent showed positive results, namely having white deposits and red deposits, and those with negative values when using Wagner reagents, the results were without brown deposits. Meanwhile, our phytochemical test data shows that the results for the Wagner alkaloids are negative and for the dragendrof are both positive, namely white and red deposits, and for the Wagner Alkaloids the results of our tests show positive results, in contrast to other journal tests. For this, the identification of saponin groups gives negative results because they do not form bubbles. Meanwhile, in our journal, the data showed the opposite result by marking the presence of bubbles or bubbles in the saponins. In addition, the identification of flavonoid groups using showed positive results with the formation of yellow deposits. In the flavonoid group, the results were the same as our data. Flavonoids have antioxidant activity, which is a type of reactive oxygen found in the body and is influenced by several factors. According to [7], antioxidants can protect the skin from UV radiation and prevent skin damage [8]. The tannin group also showed positive results with the formation of a bluish-green color, while in our data tannins showed negative results marked by a blackish-blue color not formed. There are similarities in the Dragendorf and Flavonoid Alkaloid tests while there are different results in the Mayer Alkaloids, Wagner Alkaloids, Saponins and Tannins tests, this is due to environmental factors during the kundur leaf growth process due to different leaf growth locations and other factors because each analyst is different in the process and produces different results.

**SPF Test.** SPF (Sun Protection Factor) is a general indicator that explains the effectiveness of a product or substance as protection against UV rays. The higher the SPF value of an active product or sunscreen, the more effective it is to

protect the skin from its effects. from UV radiation [7]. The effectiveness of sunscreen can be determined by using Sun Protection Factor (SPF). SPF shows how long sunscreen can protect your skin when exposed to the sun. This means that no more than 10 minutes per SPF value [3].

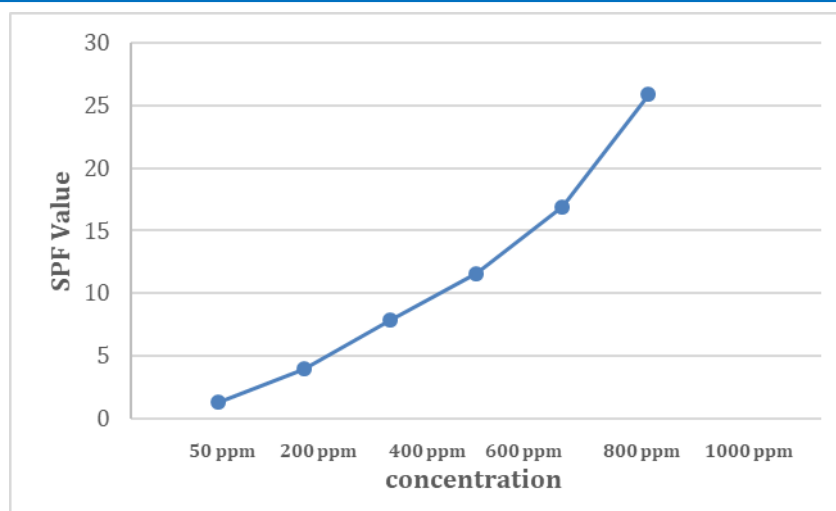
The SPF value is measured as an ingredient's ability to act as a sunscreen or sunscreen. The higher the SPF value, the better the sunscreen's UV protection. The SPF value is a ratio of how much UV is needed to burn the skin when protected versus not protected by sunscreen. So, the SPF value shows the usefulness of reducing erythema caused by UV radiation [9]. The purpose of the study was to prove the existence of sunscreen activity and SPF value from kundur leaf ethanol extract (*Benincasa hispida*) using a UV spectrophotometer used to analyze samples in vitro. Benefits of Using This method is more practical, cost-effective, and can be done in a short time [7]. Measurement of SPF value from kundur leaf ethanol extract (*Benincasa hispida*) measured using a UV spectrophotometer and its absorbance or absorption at wavelengths of 290-320 nm was calculated using the Mansyur equation [6][10].

In determining the SPF value of ethanol extract of kundur leaves (*Benincasa hispida*) by making a 1000 ppm master solution, it was then diluted into 5 concentrations, namely 50 ppm, 200 ppm, 400 ppm, 600 ppm and 800 ppm. The results of the measurement of sunscreen activity obtained a Sun Protection Factor (SPF) value, which can be seen in Table 5.

Based on the measurement of the SPF value of kundur leaves (*Benincasa Hispida*) In the table above, kundur leaf extract has excellent sunscreen activity because the results at concentrations of 800 ppm and 1000 ppm respectively are valued at SPF 16.89 and 25.89 which are classified as ultra protection types. With the SPF value, it can provide protection from UV A and UV B rays because the value range is quite high so it is included in the ultra protection type. According to [9] The Ultra Protection

**Table 5.** SPF Value of Kundur Leaf Extract

| No | Concentration | SPF value | Protection Type    |
|----|---------------|-----------|--------------------|
| 1  | 50 ppm        | 1.30      | -                  |
| 2  | 200 ppm       | 3.96      | Minimal Protection |
| 3  | 400 ppm       | 7.83      | Extra Protection   |
| 4  | 600 ppm       | 11.6      | Maximum Protection |
| 5  | 800 ppm       | 16.89     | Prosthesis Ultra   |
| 6  | 1000 ppm      | 25.89     | Prosthesis Ultra   |



**Figure 1.** Relationship Between SPF Value and Consentration of Kundur Leaf Ethanol Extract

category is able to protect the skin longer from sun exposure for 5 hours and 40 minutes. At a concentration of 50 ppm, kundur leaf extract does not have the category of sunscreen potential or does not provide effective protection against sunlight.

One of the factors that affect the determination of sunscreen factors is the concentration of the extract. This factor can increase or decrease the absorption of UV rays in sunscreen. This condition shows that the greater the concentration, the more protective effect against UV rays increases. Therefore, this corresponds to the results achieved as seen on Figure 1.

Sunscreen is said to protect if it has an SPF of at least 2 and is in good category if the test has a value of SPF 15 which is classified as an ultra-protective sunscreen. This is because SPF 15 provides better protection against long-term skin damage such as skin cancer. In addition, SPF 15 is able to protect the skin from harmful UV rays for up to 4-5 hours, while SPF 10 only lasts 1.5 hours [9].

Based on the graphic data, the results of the sunscreen research show that there is a comparison between 5 variations in the concentration of kundur leaf extract that has been carried out in this study. It can be seen that the highest SPF value on kundur leaves is 25.89 shown at a concentration of 1000 ppm with the category of ultra protection while the lowest is 1.30 shown at a concentration of 50 ppm. Based on the results of the highest SPF, it shows that kundur leaves have the potential to be a good sunscreen in protecting the skin.

## Conclusion

Based on the results of the research conducted on the phytochemical screening test of ethanol extract of kundur leaves (*Benincasa hispida*), it showed positive results of secondary metabolites of flavonoids, terpenoids, saponins, and alkaloids with a yield of 10.21%. The results of the test of the SPF value of kundur leaf extract obtained at the highest concentration at a concentration of 1000 ppm with an SPF value of 25.89 with the category of ultra protection, then based on the results of our research that kundur leaves have the best potential that is useful as a sunscreen and protects from UV A and UV B rays.

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