# Sofia Fatmawati-Antioxidant Activity and Sun Protection Factor (SPF) Graded Extract of Katuk Leaves (Sauropus androgynus (L.) Merr.)

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# Antioxidant Activity and Sun Protection Factor (SPF) Graded Extract of Katuk Leaves (*Sauropus androgynus* (L.) Merr.)

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**Abstract.** Katuk leaf (*Sauropus androgynus* (L.) Merr.) is believed to have medicinal properties, one of which is as an antioxidant. Its efficacy as an antioxidant cannot be separated from the phenolic and flavonoid compounds contained in katuk leaves. This study aims to determine the total phenolic and flavonoid levels as well as the antioxidant activity of graded extracts, namely *n*-hexane, ethyl acetate, and 70% ethanol extract of katuk leaves. The results showed that the total phenolic content and total flavonoid content of each extract were significantly different. Antioxidant activity by the DPPH method was calculated at $IC_{50}$ using quercetin as a comparison. The highest antioxidant potential was shown in the ethanol extract. Each extract has the potential as a sunscreen and ethanol extract provides the highest SPF value.

## 1. Introduction

Phenol compounds are characterized by the presence of an aromatic ring and one or two hydroxyl groups. Phenol compounds that have more than two hydroxyl groups are called polyphenols, for example the tannin, flavonoid, melanin, and lignin groups [1]. Flavonoid compounds have a characteristic structure of two aromatic rings connected by three C atoms, usually with O atomic bonds in the form of heterocyclic oxygen bonds [1]. Flavonoids are a group of secondary metabolites produced by plants which are included in the large group of polyphenols. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation [2].

Sunscreen contains compounds that can protect the skin by absorbing ultraviolet (UV) rays emitted by the sun [3]. Compounds contained in sunscreen can be used to prevent various skin diseases and to protect human skin health from the negative effects of UV rays. Sunscreens are divided into 2 groups, namely physical sunscreens and chemical sunscreens. Chemical sunscreens are sunscreens that absorb ultraviolet light, such as PABA, PABA esters, benzophenone, avobenzone, salicylate, cinnamate and camphor derivatives [4].

Katuk leaves (*Sauropus androgynus* (L.) Merr.) contain secondary metabolites such as tannins, saponins, alkaloids, flavonoids, glycosides, and phenols [5]. The 95% ethanol extract of katuk leaves has a high content of phenolic compounds with 1.49 mgGAE/g fresh weight and 8.71 mgGAE/g dry weight [6]. Antioxidant activity in previous studies stated that the $IC_{50}$ value of katuk leaf methanol extract had a value of 80.81 ppm [7]. Variations in ethanol concentration and extraction methods resulted in different phenolic and flavonoid content in katuk leaves [8], [9]. Multilevel extraction gives better results than individual extraction with each solvent [10].

The aim of the study was to determine the levels of phenolic, flavonoid, as well as antioxidant activity and SPF testing of the *n*-hexane, ethyl acetate, and 70% ethanol extract of katuk leaves from the extraction process with graded maceration, using UV-Vis spectrophotometry method.

## 2. Materials and Methods

### *2.1 Preparation Extract*

Katuk (*Sauropus androgynus* (L) Merr) leaves were collected and determined at the Institut Pertanian, Bogor, West Java. Simplicia processing begins with fresh katuk leaves taken and cleaned of impurities, then washed with water until clean, drained and finely chopped, and then dried again by aerating. After dried then powdered.

The graded extract was obtained by a stepwise method using solvents with different polarity levels, namely *n*-hexane, ethyl acetate, and 70% ethanol (1:10). The material is soaked for 24 hours, stirring occasionally for the first 6 hours. The residue is then separated from the filtrate. The process is repeated until the solvent is clear (3 repetitions). The residue was then extracted with ethyl acetate and 70% ethanol using the same procedure. Each maserate obtained was then concentrated with a vacuum rotary evaporator at a temperature of 40ºC - 50ºC to obtain a thick extract.

### *2.2 Characterization of Extract*

The organoleptic test was carried out by observing the shape, color, smell, and taste using the five senses. Each extract was weighed carefully as much as 2 grams, then put into a silicate crucible that had been sized and compared, incandescent in a kiln and the temperature was gradually increased to 600°C (temperature difference of approximately 25°C) until carbon-free. Next, it was cooled in a desiccator and then weighed. The total ash content is calculated against the weight of the test material and is expressed in % w/w. Each 1.0 gram extract was placed in a moisture content balance container which had been previously sized and the initial weight of the extract was seen. Then the extract was dried at a temperature of 105°C at the moisture content balance until a constant extract weight value was obtained, afterward the final weight of the extract was seen. Results are viewed as % MC values [11].

### *2.3 Phytochemical Screening of Extract*

The phytochemical compounds of kencur (aromatic ginger-*Kaempferia galanga*) ethanolic extract, such as phenolic, flavonoid, tannin, alkaloid, and terpene were qualitatively identified following standard procedures describing in the Harborne and Indonesian Herb Pharmacopoeia [12].

### *2.4 Determination of Total Phenolic Level*

From the base solution of 100 ppm gallic acid, several concentrations were made, namely 18 ppm, 30 ppm, 42 ppm, 54 ppm, and 66 ppm. From each concentration of the standard solution of gallic acid, 300 µl was pipetted and then 1.5 ml of *Folin-Ciocalteu* reagent (1:10) was added. After being allowed to stand for 3 minutes, each solution was added with 1.2 ml of 7.5% $Na_2CO_3$ solution, shaken homogeneously, and allowed to stand in the operating time range at room temperature. All solutions were measured for absorbance at the maximum absorbance wavelength obtained, which was 756.5 nm, then a calibration curve was made for the relationship between gallic acid concentrations. The extract solution obtained was pipette 300 l and added 1.5 ml of *Folin-Ciocalteu* reagent and shaken. It was allowed to stand for 3 minutes, added 1.2 ml of 7.5% $Na_2CO_3$ solution and allowed to stand again in the operating time range at room temperature. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at the maximum absorbance wavelength. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at a wavelength of 756.5 nm. Performed 3 repetitions [13].

*2.5 Determination of Total Flavonoid Level*

From 1000 ppm quercetin mother liquor, several concentrations were made, namely 33, 57, 81 and 105 ppm. A total of 0.5 ml of quercetin solution was made with several concentrations of pipette then added with 1.5 ml of methanol and added 0.1 ml of 10% $AlCl_3$ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water. Then the solution was shaken and left for an operating time of 60 minutes at room temperature. Measure the absorbance at a wavelength of 434 nm against the standard. Each concentration of 0.5 ml of the test solution was added with 1.5 ml of methanol and added 0.1 ml of 10% $AlCl_3$ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water, then allowed to react during the operating time at room temperature. Measure the absorption at the maximum wavelength against the standard [14].

*2.6 Antioxidant Activity Test*

Weigh 100.0 mg of ethanol extract of katuk leaves from each extraction time dissolved with methanol in a volumetric flask, then diluted to obtain concentrations of 20, 40, 60, 80 and 100 ppm. A total of 0.2 mL of sample solution of each concentration was added with 1 mL of 0.5 mM DPPH and 5 mL of methanol, allowed to stand according to the operating time for 30 minutes and then measured at a maximum wavelength of 515.5 nm. The percentage of radical scavenging activity was calculated by the formula [15].

*2.7 Sunscreen Potency Test*

The ethanol extract of Arabica coffee leaves was diluted with ethanol to a concentration of 100 ppm. The SPF value was determined by measuring the absorbance of the solution from the extract using a UV-Vis spectrophotometer at a wavelength of 290-320 nm. The sample was dissolved in 5 mL of ethanol p.a. The determination of the SPF value was carried out three times for each sample. The absorbance data obtained were processed by the equation of Mansur (1986) [16].

$SPF = CF \times EE \times I(\lambda) \times abs(\lambda)$

The SPF value can be calculated by multiplying the correction factor (CF), the erythema effect spectrum (EE), the intensity spectrum from the sun (I), and also the absorbance (Abs) of the cream sample of coffee leaf ethanol extract. The SPF assessment refers to the provisions of the FDA (Food and Drug Administration), namely minimal protection if SPF 2-4, moderate protection 4-6, extra protection 6-8, maximum protection 8-15, and ultra protection >15 [17].

*2.8 Data Analysis*

The observed data from the dispersion test and color stability test were then analyzed using one-way ANOVA. If there is a significant difference between the formulas, then it is continued with the Tukey HSD test with a 95% confidence level to see if the formula has a significant difference or not.

## 3. Results and Discussion

The purpose of using three solvents with different polarities is to obtain active compounds from katuk leaves based on their polarity [10]. In this study, repetition was carried out with the aim of providing sufficient extract weight and being able to attract residual compounds that were still left in the previous iteration. Extraction using solvents with different polarities will produce different polyphenol components so that the antioxidant properties or characteristics of each compound obtained from the extraction are also different [18].

**Table 1. Katuk Leaf Extraction Results**

| Extract type | Average extract weight (g) ± SD | Average extract yield (%) ± SD |
|---|---|---|
| *n*-Hexane | 18,05 ± 0,73 | 7,22 ± 0,29 |
| Ethyl acetate | 14,87 ± 1,15 | 5,95 ± 0,46 |
| Ethanol 70 % | 50,27 ± 1,90 | 20,11 ± 0,76 |

**Table 2. Characteristics of Katuk Leaf Extract**

| Parameters | Extract type | | |
|---|---|---|---|
| | *n*-Hexane | Ethyl acetate | Ethanol 70% |
| Organoleptic | | | |
| Form | Thick extract | Thick extract | Thick extract |
| Smell | Typical | Typical | Typical |
| Favor | Bitter | Bitter | Rather Bitter |
| Color | Green | Green | Chocolate |
| Ash Level | 2,59 % ± 0,22 | 0,55 % ± 0,05 | 6,88 % ± 0,93 |
| Drying Shrinkage | 1,08 % ± 0,48 | 3,04 ± 0,58 | 3,48 ± 2,41 |

The yield (Table 1) of the extract obtained from the multilevel extraction resulted in the lowest yield of the ethyl acetate extract, followed by the *n*-hexane extract. The yield obtained in the multistage extraction with 70% ethanol showed the highest yield. Based on the principle of mass transfer of solute into a solvent that is in accordance with characteristics *like dissolves like*, where there is a transfer at the interface layer (solvent and solute) then the solute diffuses into the solvent. The results are different because the ability to attract compounds in each solvent is different too. 70% ethanol extract has the highest yield because 70% ethanol solvent is universal. It can attract non-polar and polar compounds so that 70% ethanol extract is attracted to more secondary metabolites [8].

Organoleptic test on leaf extract was conducted to determine the characteristics of shape, smell, taste, and color. Of the three katuk leaf extracts the difference was only in the extract, the *n*-hexane, and ethyl acetate extracts had a green color while the 70% ethanol had a brown color.

The ash content obtained from this research is *n*-hexane 2.59%, ethyl acetate 0.55%, and ethanol 70% 6.88% as seen in (Table 2). The three extracts had simplicia criteria that were in accordance with the guidelines for a good extract ash content, namely the ash content of not more than 12%. The purpose of ash content is to show the content of organic and inorganic compounds from plants such as alkali metals, alkaline earth metals and heavy metals, as well as to provide an overview of internal and external mineral content until they become extracts.

Drying shrinkage has a goal to give the maximum amount of compounds lost in the drying process. In this study, a moisture balance was used at a temperature of 105ºC from the three extracts of *n*-hexane, ethyl acetate, and 70% ethanol. The three results (Table 2) fall into the drying shrinkage limit of no more than 10%.

**Table 3. Phytochemical Screening**

| Detected compounds | Extract type | | |
|---|---|---|---|
| | ***n*-Hexane** | **Ethyl acetate** | **Ethanol 70%** |
| Alkaloids | | | |
| - Dragendor | - | - | + |
| - Bouchardat | - | - | + |
| - Mayer | + | + | + |
| Phenolic | + | + | + |
| Flavonoids | + | + | + |
| Saponins | + | + | + |
| Tannins | + | + | + |
| Terpenoids | - | - | - |
| Steroids | + | + | + |

Note: (+) There is a compound (-) There is no compound

The results of phytochemical screening can be seen in (Table 3). Phytochemical screening was carried out to identify the compounds contained in *n*-hexane extract, ethyl acetate extract, and 70% ethanol extract of katuk leaves. Screening is an important step in an effort to reveal the potential of plant resources [1]. The 70% ethanol extract showed positive results for alkaloids, while the ethyl acetate and *n*-hexane extracts were negative.

Determination of total phenolic levels uses the *Folin-Ciocalteu* method because it is easy, cheap, fast to do, and can be done routinely in the laboratory. Polyphenols in plant extracts react with the reagent used, namely *Folin-Ciocalteu* (Blainski et al., 2013). Phenolic compounds react with *Folin-Ciocalteu* reagent only in an alkaline environment so that proton dissociation occurs in phenolic compounds into phenolic ions, hence 7.5% $Na_2CO_3$ is used to make an alkaline environment. The hydroxyl group in the phenolic compound reacts with the *Folin-Ciocalteu* reagent to form a blue *molybdenum-tungsten* complex which can be detected by a spectrophotometer [19]. Gallic acid (3,4,5-*Trihydroxybenzoic acid*) was used as standard. Gallic acid was chosen as the standard because it represents the general properties of phenolic compounds. In this case gallic acid is a natural phenolic compound that has a strong antioxidant effect [20].

Determination of the maximum wavelength of gallic acid aims to determine at what wavelength the compound reaches the highest absorbance value. The result of measuring the maximum wavelength is 765.50 nm with an absorbance of 0.3445 as the maximum absorbance value. The operating time of the results obtained is stable absorbance at 60 minutes so that it can be concluded that the purpose of determining the operating time is to get the measurement time when the reaction has been running optimally which is characterized by a stable absorbance, so as to maximize the measurement [21].

Various concentrations of gallic acid standards were made because the assay used a calibration curve equation (Table 4), so to make a calibration curve, several concentrations were made in order to obtain a linear equation. The results obtained were at concentrations of 18, 30, 42, 54, and 66 ppm, the data met the requirements of *Lambert Beer*'s law. The calibration curve obtained by the linear regression equation $y = 0.0093.x + 0.1824$ with a correlation coefficient $(r) = 0.9944$.

In the determination of the total phenolic content of katuk leaf extract, as seen in (Table 5) that the 70% ethanol extract had the highest concentration of 16.71 mgGAE/g. The extract was followed by ethyl acetate and *n*-hexane with a concentration of 2.95 mgGAE/g and 0.57 mgGAE/g extract, respectively. Phenolic compounds are extracted well in 70% ethanol because the phenolic content will increase in the extract as the polarity of the solvent increases. Phenolic also tends to be polar so it can dissolve in polar solvents well. These results are similar to other studies which stated polar extracts have the highest phenolic content followed by semi-polar and non-polar extracts. Methanol, a polar solvent, has a higher phenolic content than acetone which is a semi polar solvent [22].

**Table 4. Results of Total Phenolic Levels**

| Extract type | Average Phenolic content (mgGAE/g Extract ± SD) |
|---|---|
| *n*-Hexane | 0,57 ± 0,02 |
| Ethyl Acetate | 2,95 ± 0,07 |
| Ethanol 70% | 16,71 ± 0,43 |

**Table 5. Results of Total Flavonoid Levels**

| Extract type | Average levels of Flavonoids (mgQE/g Extract ± SD) |
|---|---|
| *n*-Hexane | 55,57 ± 0,11 |
| Ethyl Acetate | 88,79 ± 0,73 |
| Ethanol 70% | 6,23 ± 0,05 |

Determination of total flavonoid levels uses quercetin standards. Quercetin is a flavonoid class of the flavonol group [23]. Meanwhile, Quantitative Analysis is conducted by applying the UV-Vis spectrophotometric method (Hanani, 2015). The maximum wavelength resulted from this study was 434 nm. This was due to the reaction of the AlCl3 complex which caused the shift of the wave to become visible, which was indicated by the solution becoming more yellow, and the addition of sodium acetate to maintain the visible wavelength [24]. $AlCl_3$ reagents with flavonoids form complexes between neighboring hydroxyl groups and ketones or with neighboring hydroxyl groups. $AlCl_3$ will react with the ketone group at C4 and the OH group at C3 or C5 in flavone or flavonoid compounds to form a yellow stable complex compound.

The results of the absorbance measurement of the quercetin standard solution to obtain a linear calibration curve obtained results such as (Table 6). These results were entered into the regression equation which then produced linear y = 0.0024x+0.1897,r = 0.9931. The flavonoid content was expressed in mgQE/g Extract.

After reading the levels of flavonoids (Table 7) of the graded extract of katuk leaves, the results obtained were *n*-hexane 55.57 mgQE/g, ethyl acetate 88.79 mgQE/g, and 70% ethanol 6.23 mgQE/g. In this study, ethyl acetate had the largest concentration, this is because the content of flavonoid compounds contained in katuk leaves has a low polarity level, namely in the form of aglycones or commonly called aglycone flavonoids. Aglycone flavonoids have less polar properties and tend to be more soluble in chloroform and ether. Flavonoids generally bind to sugars to form glycosides which cause these compounds to be easily soluble in polar solvents, such as methanol, butanol, and ethyl acetate.

In this study, the free radical compound DPPH (2,2-*diphenyl*-1-*picrylhydrazyl*) was used. The results obtained on the measurement of the maximum wavelength is 515.5 nm, the wavelength can be used because the wavelength of the maximum absorbance for measurements with the DPPH method is 515-520 nm [25].

Quercetin was chosen as the standard for comparison because quercetin is a natural secondary antioxidant that has been shown to have free radical scavenging activity. Quercetin, which is a flavonoid group, has several biological activities. These activities can be attributed to the antioxidant properties of quercetin, including its ability to scavenge free radicals [23].

The results of antioxidant activity (Table 8) show that 70% ethanol extract has a lower $IC_{50}$ value than ethyl acetate and *n*-hexane, the smaller the % inhibition obtained, the stronger the antioxidant activity produced [7]. The compounds contained in the ethanol extract are an accumulation of polar, semi-polar, and non-polar compounds. When the extract is macerated in stages, the synergistic function

between the compounds will reduce because the components contained in the extract have been separated. The non-polar chemical components were extracted in the *n*-hexane solvent, the semi-polar chemical components were extracted in ethyl acetate, and chemical components that are polar were extracted in 70% ethanol solvent. This causes the antioxidant activity of 70% ethanol extract to be the strongest and at the same time has the least % inhibition, compared to others.

**Table 6. Antioxidant Activity Test Results and SPF**

| Sample Type | Average antioxidant activity ($IC_{50}$ ± SD) | SPF |
|---|---|---|
| Quercetin | 8,83 | - |
| *n*-Hexane | 88,43 ± 1,20 | 2,13 |
| Ethyl Acetate | 77,65 ± 1,78 | 5,23 |
| Ethanol 70% | 70,33 ± 1,64 | 2,13 |

Ethyl acetate extract gave the highest SPF results compared to ethanol and *n*-hexane extracts. The three extracts belonged to the minimum SPF capability (range 2 to 4). Phytochemical compounds in the extract that may play a role in the potential for absorption of ultraviolet light are flavonoids and phenolic compounds [26]. Several groups of active compounds derived from natural ingredients such as flavonoids, tannins, anthraquinones, cinnamates, and glycosides are reported to have the ability to protect against UV rays [27].

## 4. Conclusions

Based on the results obtained in this study, the determination of total phenolic content had the highest concentration in 70% ethanol extract, which was then followed by ethyl acetate extract and *n*-hexane extract. In the determination of total flavonoid content, the highest yield was in the ethyl acetate extract, then *n*-hexane content, while the 70% ethanol had the lowest content. The results of the antioxidant activity test used the DPPH method. $IC_{50}$ of *n*-hexane extract had the highest concentration in this activity test, followed by ethyl acetate and 70% ethanol extract. The smaller the $IC_{50}$ value, the higher the antioxidant activity produced. Multilevel extraction greatly affects the levels of each extract used. Besides, it will also look for different compounds in the solvent used.

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# Sofia Fatmawati-Antioxidant Activity and Sun Protection Factor (SPF) Graded Extract of Katuk Leaves (Sauropus androgynus (L.) Merr.)

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| 42% | 37% | 30% | 19% |
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