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Kacip Fatimah (*Labisia pumila*) as Natural Sunscreen: Analysis of Flavonoid Content and Ultraviolet Protection Ability

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Abstract: The cause of melanoma cancer is exposure to high-intensity ultraviolet rays from sunlight. Kacip Fatimah (Labisia pumila) utilizes the people of the interior of Kalimantan to protect their skin from sunlight by attaching leaves to the face or skin. Kacip Fatimah (Labisia pumila) contains flavonoid compounds, which are thought to impact these various properties. This study aimed to determine the ultraviolet protection ability and total flavonoid levels of Kacip Fatimah (Labisia pumila) leaves. Kacip Fatimah (Labisia pumila) leaves originating from Central Kalimantan were cleaned, dried in an oven, ground, and then extracted with air solvents, 70% ethanol, and 96% ethanol. Flavonoid identification was carried out with specific reagents, and total flavonoid levels were determined by colorimetric method using quercetin as a comparison. The ultraviolet protection ability of Kacip Fatimah (Labisia pumila) leaf extract was measured using a UV-Vis Spectrophotometer with the Sun Protection Factor parameter. The results showed the presence of flavonoids in the third extract. The total flavonoid content of air extract, ethanol 70%, and ethanol 96% from Kacip Fatimah (Labisia pumila) leaves were obtained respectively at 1.69%, 2.15%, and 2.38%. The most extracted flavonoids were in ethanol 96% solvent. The ultraviolet protection ability at a concentration of 100 - 500 ppm from air extract was in the range of 2.80 - 7.99, in ethanol 70% extract in the range of 5.69 - 38.39, while in ethanol 96% extract in the range of 8.76 - 49.07. The highest ultraviolet protection was in 96% ethanol extract from Kacip Fatimah (Labisia pumila) leaves. The results showed that Kacip Fatimah (Labisia pumila) leaves can provide ultraviolet protection with the content of active compounds of the flavonoid group. The research results can be scientific evidence for utilizing and developing products from Kacip Fatimah (Labisia pumila).

Keywords: Flavonoids, kacip fatimah, *Labisia pumila*, protection, ultraviolet.

INTRODUCTION

Skin problems often occur along with the thinning of the ozone layer. People living in equatorial regions receive the most significant exposure to sunlight¹. The equatorial region receives the strongest ultraviolet rays, so there is a high risk of causing skin damage, premature aging, sunburn, and melanoma cancer². Equatorial regions have high

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humidity, making them susceptible to skin infections. High temperatures also occur frequently in Indonesia, causing dry skin and making it susceptible to skin damage³. Based on World Health Organization data in 2022, the incidence of melanoma cancer in Indonesia reached 8,490 cases, which continues to increase with a death rate of 12 cases in every 1000 cases. The leading cause of melanoma cancer is exposure to ultraviolet rays from sunlight with high intensity⁴.

Kacip Fatimah (*Labisia pumila*) is a plant that grows in tropical forests. Kacip Fatimah is widely found in the Kalimantan region. Kacip Fatimah grows in valley areas protected from sunlight and high humidity⁵. Kacip Fatimah (*Labisia pumila*) is used by the community to facilitate the birth process and rejuvenate female organs. Rural communities also use Kacip Fatimah to protect the skin from sunlight by attaching the leaves to the face or skin⁶. Kacip Fatimah (*Labisia pumila*) contains flavonoid compounds that are strongly suspected of being responsible for these various properties⁷. Kacip Fatimah (*Labisia pumila*) is limited to traditional use, so scientific data is still limited. Kacip Fatimah (*Labisia pumila*) has excellent potential to be developed as a medicinal or cosmetic ingredient, so it has a high selling value for the community.

Flavonoid compounds such as quercetin, rutin, myricetin, and kaempferol have various abilities. Plant flavonoid compounds can be used as active ingredients in medicine and cosmetics⁸. Flavonoid compounds contain conjugated double bonds so that they can absorb ultraviolet radiation. Flavonoids are electron donors due to the presence of hydroxyl groups in their compounds' structure so that they can neutralize free radicals⁹. Free radicals can be produced from excessive exposure to sunlight, potentially triggering premature aging and even melanoma cancer¹⁰. Free radicals caused by exposure to ultraviolet light can be inhibited using skin protection mechanisms using natural ingredients, one of which is Kacip Fatimah (*Labisia pumila*).

The novelty in this study is that there is still limited research that measures the protective ability of Kacip Fatimah leaves (*Labisia pumila*) against ultraviolet light. Similar studies only measure antioxidant capacity and total phenolic content; this is different. The samples came from the Kalimantan forest, and variations of three different solvents were used to identify the most optimum solvent. In addition, the relationship between the protective ability of Labisa pumia (*Labisia pumila*) leaves and total flavonoid levels can be identified. This study aimed to determine the ultraviolet protection ability and total flavonoid levels of Kacip Fatimah (*Labisia pumila*) leaves.

MATERIALS AND METHODS

Research Design

This study used an experimental research design. The study was conducted qualitatively to determine the ultraviolet protection ability and total flavonoid content of Kacip Fatimah (*Labisia pumila*) leaves. Leaf extraction used three different solvent variations: distilled water, ethanol 70%, and ethanol 96%.

Materials

Kacip Fatimah (*Labisia pumila*) leaves from Dusun Tengah District, East Barito Regency, and Central Kalimantan are the main ingredients used. Other ingredients used are distilled water (Onelab), ethanol 70% (Merck), ethanol 96% (Merck), quercetin standard (Sigma Aldrich), aluminum chloride 10% (Merck), and pro-analysis ethanol (Merck).

Plant Determination

In this study, plant determination was carried out to ensure the authenticity of the plant. Determination was carried out at the Basic Laboratory of FMIPA, Lambung Mangkurat University, Banjarbaru City, South Kalimantan.

Sample Preparation

Kacip Fatimah (*Labisia pumila*) leaves are separated from other parts of the plant and washed using running water. The leaves are then cut into small pieces and put into a drying cabinet at 60°C for 2 x 24 hours. The dried leaves are then ground using a blender for 1 minute and then sieved with a sieve size of 117.

Extract Preparation

Kacip Fatimah (*Labisia pumila*) leaf powder was extracted using three different solvents: distilled water, ethanol 70%, and ethanol 96%. The extraction process using distilled water was carried out using the infusion method. The infusion method is a method commonly used in traditional communities. In contrast, ethanol 70% and ethanol 96% solvents were used using the maceration method for 3 x 24 hours. The maceration method is widely used in the traditional medicine industry. Each extraction process uses a ratio of powder to solvent of 1 to 10 parts. The extraction results were filtered with Whatman paper and then evaporated using a rotary evaporator (IKA® 60 rpm) until reduced to one-tenth. The extract was then dried using an oven at 60° C for 5 x 24 hours until the dry extract was obtained¹¹.

Flavonoid Identification

Flavonoid identification was carried out on the three Kacip Fatimah (*Labisia pumila*) leaf extracts. Identification was carried out using the tube method with the help of chemical reagents. Extract with a concentration of 1% was put into a test tube as much as 0.5 mL, then 1-2 mL of 50% hot methanol and five drops of magnesium powder (Mg) were added, then 4-5 drops of concentrated chloric acid (HCI) were added, the appearance of an orange color indicated positive results¹².

Total Flavonoid Content

The determination of total flavonoid levels was carried out using a colorimetric method with a UV-Vis spectrophotometer (PerkinElmer®, Lamda 365). The standard used was quercetin, which is included in the flavonoid group. Before determining the levels, the maximum wavelength, operating time, and standard curve equations were determined. Quercetin 10 mg was dissolved in 10 mL of ethanol pro analysis, then diluted to obtain a series of concentrations of 20, 40, 60, 80, and 100 ppm. Each concentration was taken at 0.5 mL, then put into a test tube, and 0.5 mL of 10% aluminum chloride was added. Next, 4 mL of 5% acetic acid was added and then mixed until homogeneous using a vortex mixer for 1 minute. One of the concentrations was read for its maximum wavelength, and then the operating time was determined. The five concentration series were also read for absorbance based on determining the maximum wavelength and operating time until a standard curve equation was obtained between concentration and absorbance¹³.

Determination of flavonoid levels from Kacip Fatimah (*Labisia pumila*) leaf extract by weighing 100 mg of extract, then dissolved in 10 mL of ethanol pro analysis. Dilution was carried out until a concentration of 4000 ppm was obtained, and then the standard compound was treated the same way. Absorbance readings were carried out using a UV-

Vis spectrophotometer. Flavonoid levels were calculated by integrating sample absorbance into the standard curve equation¹³.

Ultraviolet Protection

Distilled water extract, ethanol 70%, and ethanol 96% from Kacip Fatimah (*Labisia pumila*) leaves were weighed as much as 100 mg each, then dissolved with ethanol p.a in a 10 mL measuring flask to obtain a concentration of 10,000 ppm. The dilution process was carried out on each stock solution until five levels were obtained with concentrations of 50, 100, 200, 300, 400, and 500 ppm. The solution levels were read at a wavelength of 290-320 every 5 nm interval using a UV-Vis spectrophotometer using pro-analysis ethanol as a blank. The absorbance value obtained was used to calculate the ultraviolet protection power, which was determined in vitro using the Sun Protection Factor value¹⁴.

RESULTS AND DISCUSSION Results of Plant Determination

Determination of Kacip Fatimah (*Labisia pumila*) plants was carried out to establish the identity of the samples used in the study. The determination process of Kacip Fatimah (*Labisia pumila*) plants uses the roots, stems, and leaves. Determination was conducted at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru. The determination results are based on Number 232a/LB.LABDASAR/XII/2022. The results indicate that the plants used as samples in this study belong to the *Labisia pumila* species and the Labisia family.

Results of Extraction

The extracted Kacip Fatimah (*Labisia pumila*) leaf powder will then produce an extract. The extract is tested organoleptically, including odor, color, taste, and shape. The yield or amount of weight extracted is calculated. The results of the yield calculation are presented in Table 1.

Table 1. Percentage of Yield and Organoleptic of Kacip Fatimah (*Labisia pumila*) Leaf Extract

	LATIGOT	
Sampel	Persen Rendemen	Organoleptis
Water Extract	8.43%	Dry extract form, black in color, distinctive odor and bitter taste
Ethanol Extract 70%	15.21%	Dry extract form, black in color, distinctive odor and bitter taste
Ethanol Extract 96%	12.34%	Dry extract form, black in color, distinctive odor and bitter taste

The calculation of extract yield was carried out to determine the ratio of the amount of extract obtained from a sample to the initial weight of the herbal medicine and the amount of bioactive compounds contained in the extracted sample. The results of calculating the yield of herbal medicine extracts of water, ethanol 70%, and ethanol 96% from Kacip Fatimah (*Labisia pumila*) leaves were obtained, respectively, at 8.43%, 15.21%, and 12.34%. The highest yield was obtained in the ethanol 70% extract because ethanol 70% is semipolar, so it can attract various compounds with low to high polarity 15. The organoleptic results showed that the three extracts had the same characteristics; there was no difference in shape, color, odor, and taste. The shape of the extract is the

same because all three go through the same process, while the black color is a characteristic of natural ingredient extracts. The distinctive odor of an extract is also specific to plants that contain non-volatile compounds¹⁶. At the same time, the bitter taste is commonly found in natural extracts due to the influence of tannin compounds that are usually included in them¹⁷.

Results of Idenitifikasi Flavonoid

Chemical identification is the initial step to ensure the sample contains the compound group. Various methods can be used for identification, one of which is using specific reagents. The results of the identification of the flavonoid group are presented in Table 2.

Table 2. Identification of Flavonoid Groups of Kacip Fatimah	(Labisia pumila)
Loof Extract	

Leai Extract				
Compound	Reagents	Identification Results		
Group		Water Extract	Ethanol Extract	Ethanol Extract
			70%	96%
Flavonoid	Magnesium powder and chloric acid	Positive	Positive	Positive

The results obtained from the flavonoid test on the three extracts using magnesium and concentrated chloric acid (HCl) were positive for containing flavonoid compounds, indicated by the formation of an orange solution¹⁸. Magnesium (Mg) and chloric acid (HCl) react to form bubbles, which are H2 gas. Magnesium metal (Mg) and concentrated chloric acid (HCl) reduce the benzopyrone core in the flavonoid structure, which is indicated by the formation of a color change in the solution to brick red or orange. The results in red or orange are likely flavonoids belonging to the flavonol, flavanol, and flavanonol groups¹⁹. **Results of Total Flavonoid Content**

Total flavonoid levels were determined using a colorimetric method with an aluminum chloride reagent. Quercetin was used as a standard compound; quercetin was chosen because it belongs to the flavonoid group that can form a color complex with the reagent²⁰. The determination of the maximum wavelength and operating time was carried out at the beginning of the test. The scanning results obtained a maximum wavelength of 415 nm with an operating time starting at the 18th minute. The results of scanning the maximum wavelength and determining the operating time are presented in Figure 1.

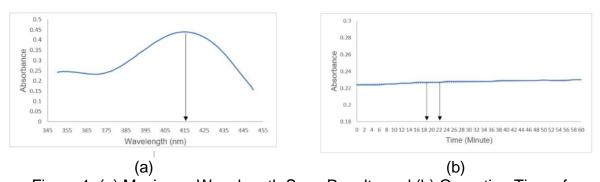


Figure 1. (a) Maximum Wavelength Scan Results and (b) Operating Time of Kacip Fatimah (*Labisia pumila*) Leaf Extract

The results of determining the maximum wavelength of quercetin using a UV-Vis spectrophotometer obtained a wavelength of 415 nm, with the highest absorbance value of 0.4827. The results are from research conducted by Ipandi (2016), which stated that the maximum wavelength of quercetin is 415 nm. The results of determining the operating time using a UV-Vis spectrophotometer, which was read at 0-60 minutes with an interval of every 2 minutes, obtained an operating time of 18-22 minutes²¹. The results obtained are from research conducted by Ipandi (2016) and Sari (2022), which stated that the stable time of quercetin is at the 22nd minute^{14,21}. Determination of operating time aims to determine the measurement time of a compound obtained when the absorbance is stable. Operating time is measured by measuring the difference between the measurement time and the absorbance of the solution. Determination of operating time needs to be done to minimize measurement errors²².

Total flavonoid levels are obtained from the results of the sample absorbance plot and entered into the standard curve equation. The standard curve equation obtained is $Y = 0.01328 \ X - 0.2104$. The total flavonoid content of the three Kacip Fatimah (*Labisia pumila*) leaf extracts is presented in Table 3.

Table 3. Total Flavonoid Content of Kacip Fatimah (Labisia pumila) Leaf Extract

Sample	Total Flavonoid Content (µ gEK /mg)	Flavonoid Content (% w/w)	Average Total Flavonoid Content (% w/w)
Extract	16,43	1,64	
Aquadest	17,62	1,76	1,69%
	16,82	1,68	
Extract	21,61	2,16	
Ethanol 70%	21,73	2,17	2,15%
	21,35	2,13	
Extract	23,96	2,39	
Ethanol 96%	23,83	2,38	2,38%
	23,73	2,37	

The results of determining total flavonoid levels from water extract, ethanol 70%, and ethanol 96% from Kacip Fatimah (*Labisia pumila*) leaves were obtained respectively at 1.69%, 2.15%, and 2.38%. The highest levels were found in samples extracted using ethanol 96%. Ethanol 96% tends to have the lowest polarity among these solvents. Ethanol contains polar hydroxyl groups and non-polar hydrocarbon groups²³. Flavonoid compounds tend to contain polar and non-polar properties so that they can be extracted in ethanol solvents. Flavonoid compounds above 1% indicate a reasonably high content in natural materials. Flavonoid compounds are secondary metabolites produced in small amounts in plants. However, this content is sufficient to produce pharmacological effects when used²⁴. Flavonoids are spread in many plants, and they have varying types of compounds with unique properties in each plant²⁵. According to the Indonesian Herbal Pharmacopoeia, flavonoid levels can be a specific parameter of an extract.

Result of Ultraviolet Protection

The protective power of the extract against ultraviolet light was determined in vitro using the Sun Protection Factor value using a UV-Vis spectrophotometer²⁶. SPF testing

using the in vitro method was carried out to characterize the UV absorption of the test solution using a UV-Vis spectrophotometer instrument. In vitro testing was used because it is simpler, faster, and less expensive than other methods. The results of the ultraviolet protection test are presented in Table 4.

Table 4. Results of Calculation of Ultraviolet Protection Ability of Kacip Fatimah

(Labisia pumila), Leaf Extract

(Labisia purilla) Lear Extract				
Sample	Sun Protection Factor Value			
Concentration (ppm)	Water Extract	Ethanol Extract 70%	Ethanol Extract 96%	
100	2,80	5,69	8,76	
200	3,56	12,12	17,75	
300	5,21	17,96	28,06	
400	7,99	25,13	40,39	
500	8,44	38,39	49,07	

The results of determining the ultraviolet protection ability of extracts with concentrations of 100-500 ppm showed varying results for each extract. In the water extract, the protection ability was in the range of 2.80 - 7.99; in the ethanol 70% extract, it was in the range of 5.69 - 38.39, while in the ethanol 96% extract, in the range of 8.76 - 49.07. A Sun Protection Factor value of less than 15 indicates low protection power, while high protection power is in the range of 30-49. The higher the Sun Protection Factor value, the stronger the protection ability of the sample²⁷. There has been no similar research that specifically tests the SPF value; however, there is research that tests Kacip Fatimah extract as an anti-aging agent for the skin. Kacip Fatimah can increase fibroblast collagen synthesis, which is disrupted by UVB exposure to the skin⁶. Based on these data, it is known that the 96% ethanol extract of Kacip Fatimah (Labisia pumila) leaves has the most potent ability to protect the skin from ultraviolet. This also correlates with the high levels of total flavonoids from the ethanol 96% extract compared to other extracts. These data show that the increase in total flavonoid levels is directly proportional to the ability of the Kacip Fatimah (Labisia pumila) leaf extract to protect the skin from ultraviolet. Kacip Fatimah (Labisia pumila) leaves have ultraviolet protection activity caused by the presence of flavonoid compounds7.

This is due to the results of identifying Kacip Fatimah (*Labisia pumila*) leaves, which are stated to contain flavonoids. Flavonoids are naturally found in plants, which also function as catalysts in the photosynthesis phase and protect plants from free radicals due to exposure to ultraviolet rays. Flavonoids are used in humans to add natural active ingredients that provide high protection to human skin²⁸. Flavonoids are efficacious in conjugated single and double bonds in aromatic compounds that absorb ultraviolet, thereby preventing ultraviolet²⁹. The ability of Kacip Fatimah (*Labisia pumila*) leaves to protect the skin from ultraviolet will avoid the risk of skin cancer. One of the risks of skin cancer is high exposure to ultraviolet substances, which can cause DNA damage, the formation of radical products, genetic mutations, oxidative stress, inflammation, and immunosuppression³⁰.

The limitations of this study include the fact that the plants used are clay plants originating from the Central Kalimantan region. Depending on where they grow, clay-

growing plants can have variations in active compound content in different areas. Cultivation of these plants and testing of cultivated plants is carried out. This testing is carried out in vitro, so further research is expected to be tested in vivo.

CONCLUSION

The total flavonoid content of water extract, ethanol 70%, and ethanol 96% from Kacip Fatimah (*Labisia pumila*) leaves was obtained respectively at 1.69%, 2.15%, and 2.38%. The most extracted flavonoids were in ethanol, 96% solvent. The ultraviolet protection ability at a concentration of 100-500 ppm from the water extract was in the range of 2.80 - 7.99, in ethanol 70% extract in the range of 5.69 - 38.39, while in ethanol 96% extract in the range of 8.76 - 49.07. The highest ultraviolet protection was in the ethanol 96% extract from Kacip Fatimah (*Labisia pumila*) leaves. The results showed that Kacip Fatimah (*Labisia pumila*) leaves can provide ultraviolet protection with the content of active compounds of the flavonoid group. The research results can be scientific evidence for utilizing and developing products from Kacip Fatimah (*Labisia pumila*). Further research is expected to be tested in vivo to obtain a picture of data on biological subjects. It is necessary to test melanoma cells in vitro and in vivo to see the potential of Kacip Fatimah in overcoming skin cancer.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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