
Determination Of Sun Protection Factor (SPF) Values Of Water, Ethyl Acetate, And N-Hexane Fractions Of Kepok Banana Flowers (*Musa acuminata* x *M. balbisiana*) Using The UV-Vis Spectrophotometry Method

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Abstract

Indonesia as a tropical country faces high UV exposure which causes premature skin aging and cancer risk, so it is necessary to have natural sunscreen from antioxidant-rich plants such as kepok banana flowers (*Musa acuminata* x *M. balbisiana*). This study aims to determine the Sun Protection Factor (SPF) value and protection category of water, ethyl acetate, and n-hexane fractions of kepok banana flowers. This type of research is descriptive experimental with ethanol maceration method, multilevel fractionation, phytochemical screening, TLC, and UV-Vis spectrophotometry (290-320 nm, Mansur formula). The results showed that the different types of solvents in each fraction influenced the resulting SPF value. The ethyl acetate fraction showed the highest protective activity compared to the other fractions. The SPF values of the kepok banana flower water fraction with concentrations of 60 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm were 4.662; 7.757; 9.055; 11.944; 15.264, respectively, with moderate-ultra protection. The ethyl acetate fraction obtained SPF values of 18.433; 18.968; 19.203; 19.672; 20.203 with ultra protection. The n-hexane fraction obtained SPF values of 1.190; 1.762; 2.278; 3.575; 4.598 with the category of no-moderate protection.. Concluded that kepok banana flowers have potential as a natural sunscreen agent, where the ethyl acetate and water fractions provide the most optimal sun protection effectiveness (SPF) compared to the n-hexane fraction.

Keywords: *Kepok Banana Flower, Ethyl Acetate, Water Fraction, N-Hexane, Sun Protection Factor.*

INTRODUCTION

Indonesia, as a tropical country located on the equator, exposes its population to high levels of ultraviolet (UV) light year-round. This exposure is necessary for vitamin D synthesis, but it also triggers free radicals that damage skin structure, causing erythema, pigmentation, dryness, dullness, and even the long-term risk of skin cancer (Yuni & Yani, 2021). Furthermore, the prevalence of premature skin aging is increasing, with 57% of Indonesians aged 25 showing signs of premature aging, faster than the normal age of 30 (Anggriani, 2023).

Using sunscreen is the main solution to prevent sunburn and skin cancer, assessed based on the Sun Protection Factor (SPF), which measures the ability to block UV rays, with a higher SPF indicating stronger protection (Yohanes, 2022). Phenolic compounds such as polyphenols and flavonoids from plants act as natural antioxidants, scavenging free radicals, inhibiting lipid oxidation, and exhibiting antibacterial, antiviral, and anticancer activity, with activity increasing with higher concentrations (Ngibad, 2023).

Kepok banana (*Musa acuminata* x *M. balbisiana*), especially the flower, heart, stem, and peel, are rich in antioxidants; kepok banana peel is superior to other varieties, while kepok banana flower extract showed an IC₅₀ of 26.00 µg/mL, a very strong category (<50 µg/mL) (Rahmi et al., 2021; Nurhaeni et al., 2025). Water, ethyl acetate, and n-hexane fractions of plant extracts are often extracted for the isolation of active compounds, with ethyl acetate usually being the most potent due to the solubility of moderately polar flavonoids.

The main problem is the lack of specific data on the potential of the water, ethyl acetate, and n-hexane fractions of kepok banana flowers as natural sunscreens in tropical Indonesia, where extreme UV exposure exacerbates skin damage. Previous research by Nurhaeni et al. (2019) focused on the antioxidant activity of the whole extract, but have not tested the SPF of the fraction in vitro using UV-Vis spectrophotometry for the protection category.

This study aims to determine the sunscreen activity and determine the SPF value and protection category of the water, ethyl acetate, and n-hexane fractions of Kepok banana flowers. The urgency lies in the need for alternative natural sunscreens amid the high prevalence of premature aging and UV-induced skin cancer in Indonesia, reducing dependence on potentially irritating synthetics. The novelty is the systematic testing of the SPF of specific fractions of Kepok banana flowers, complementing previous antioxidant studies for sustainable pharmaceutical and cosmetic applications (Ferdinan & Prasetya, 2018).

RESEARCH METHODS

This study used an experimental descriptive approach to explore the potential of water, ethyl acetate, and n-hexane fractions of kepok banana flowers (*Musa acuminata* x *M. balbisiana*) as a natural sunscreen by measuring the Sun Protection Factor (SPF) value in vitro. The independent variables included the fractions obtained from the separation of ethanol extracts based on the solvent polarity level (less polar n-hexane, semipolar ethyl acetate, and polar water), while the dependent variable was the SPF value calculated from the absorbance of the UV-B spectrum (290-320 nm) using the Mansur formula with a UV-Vis spectrophotometer (Yohanes, 2022). The experimental method involves manipulating the independent variable to observe the effect on the dependent variable, according to the operational definition where the fraction is measured through separating funnel partition and concentrated with a water bath, and the SPF through serial dilution (60-140 ppm) and calculation of $CF \times \Sigma(EE \times I \times Abs)$ with a correction factor of 10 (Mansur et al., 1986/2023; Shina et al., 2024). This approach ensures testing of causality and replicability of the data (Creswell & Creswell, 2023).

Research instruments include tools such as rotary evaporator, water bath, UV-Vis spectrophotometer (B-One), analytical balance, moisture analyzer, oven, TLC silica gel 60 F254, cuvette, micropipette, and separating funnel, as well as materials in the form of banana kepok flowers, 96% ethanol, n-hexane, ethyl acetate, distilled water, phytochemical reagents (Mg, HCl, FeCl₃, Liebermann-Burchard), and TLC solvents (chloroform: ethyl acetate, etc.). The data analysis technique is quantitative descriptive through the average absorbance table from three replications at λ 290-320 nm, SPF calculation, yield (simplicia, extract, fraction), standardization parameters (drying loss, water content <10%, ash content), as well as phytochemical screening and R_f TLC for flavonoids, saponins, phenolics, tannins (Hanifa et al., 2021; Veninda et al., 2023; Husnani et al., 2024). The data are presented in tables and flow charts for interpretation of SPF protection categories (minimum 2, moderate 4-<6, etc.), without statistical inference due to the descriptive focus (Juniasty, 2024).

The study population was all kepok banana flowers from Jalan Budi Utomo, Pontianak, West Kalimantan, with a purposive sample of 3,500 g of inner heart flowers (old fingers) which were sorted, chopped, dried (dry cabinet 40°C), and ground (blender, 40 mesh sieve), producing standardized simplicia. Species determination was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak to confirm *Musa acuminata* x *M. balbisiana*, while the test sample was 500 g of simplicia for extraction (96% ethanol maceration 1:10, 3 days, rotary 40°C), fractionation of 10 g of extract (3x replication per solvent), and SPF testing (0.1 g/100 mL ethanol) (Ferdinan & Prasetya, 2018; Shina et al., 2024).

The research procedure begins with sample collection, standardization of simplicia (drying shrinkage oven 105°C, moisture content moisture analyzer, ash content oven 600°C), ethanol maceration, concentration, extract standardization (water content, ethanol/H₂SO₄-CH₃COOH free, Pb/Cd free), stepwise fractionation (n-hexane → ethyl acetate → water), phytochemical screening (flavonoids Mg-HCl, saponins HCl, phenolics/tannins FeCl₃), TLC (specific eluent, UV 254 nm, reagent), to SPF measurement (dilution, absorbance 290-320 nm, EE×I table) (Yuni & Yani, 2021; Desriyani & Iskandar, 2021; Andini et al., 2023). The research took place from November 2025 to January 2026 at the Chemistry and Natural Materials Laboratory, Duta Bangsa University, Surakarta, with a systematic flow to minimize bias and ensure validity (Creswell & Creswell, 2023).

RESULTS AND DISCUSSION

Plant Determination

Plant identification was carried out by taking a few leaves, stems, fruits, hearts, and flowers of kepok bananas obtained from Jalan Budi Utomo, Pontianak City, West Kalimantan and tested at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. The results showed that the samples used were indeed kepok banana plants (*Musa acuminata* x *M. balbisiana*).

Collection of Raw Materials and Making of Simple Drugs

Table 1. Results of the yield of Kepok banana flower simplex

Wet Material Weight (g)	Dry Material Weight (g)	Yield (%)
3,500	619	17.69

Standardization of Simple Drugs

Table 2. Results of Standardization of Kepok Banana Flower Simplicia

Test Parameters	R1 (%)	R2 (%)	R3 (%)	Average (%)
Drying Loss	5.75	5.90	5.45	5.70
Water content	5.33	5.27	5.05	5.22
Ash Content	5.95	5.60	5.15	5.57

Extract Preparation

Table 3. Results of Kepok Banana Flower Extract Yield

Weight of simple substance (g)	Extract weight (g)	Yield (%)
500	63.90	12.78

Extract Standardization

Water content

Table 4. Water Content of Kepok Banana Flower Extract

Replication	Results Extract Water Content (%)	Average (%)
1	5.44	5.31
2	5.12	
3	5.37	

Ethanol Free and Metal Free

Table 5. Ethanol-Free and Metal-Free Results of Kepok Banana Flower Extract

Parameter	Reagent	Results
Ethanol free	H ₂ SO ₄ + CH ₃ COOH	No ester smell
Metal-free:		
Lead (Pb)	K ₂ CrO ₄	No yellow precipitate is formed
Cadmium (Cd)	NaOH	No white precipitate is formed

Extract Fractionation

Table 6. Fractionation Results of Kepok Banana Flower Extract

Solvent	Extract Weight (g)	Fraction Weight (g)	Yield (%)
<i>n</i> -Hexane	10	1,176	11.76
Ethyl acetate	10	5,514	55.14
Water	10	1,752	17.52

Phytochemical Screening of Kepok Banana Flower Extract

Table 7. Phytochemical Screening Results of Kepok Banana Flower Extracts and Fractions

Secondary metabolites	Reagent	Positive Results	Identification Results			
			Extract	Water fraction	Ethyl acetate fraction	n-hexane fraction
Flavonoid	Mg powder + concentrated HCl	Red, yellow or orange	(+) Orange	(+) Orange	(+) Orange	(-) Colorless
Polyphenols (phenols)	FeCl ₃ 1%	Green, purple, blue, black	(+) Blackish green	(+) Green	(+) Green	(-) White
Saponin	Aquadest	Foam or foam forms	(+) Foam formed	(+) Foam formed	(+) Foam formed	(+) Foam formed
Tannin	FeCl ₃ 1%	Blackish green	(+) Blackish green	(+) Blackish green	(+) Blackish green	(-) Green

Information:

(+) = Secondary metabolite compounds identified (positive)

(-) = No secondary metabolite compounds identified (negative)

TLC Examination of Extracts and Fractions

Table 8. TLC Examination of Kepok Banana Flower Extract and Fractions

Secondary metabolites	Motion Phase	Standard comparative value	Rf value			
			Extract	Water fraction	Ethyl acetate fraction	n-hexane fraction
Flavonoid	Chloroform: ethyl acetate (6 : 4)	0.58	0.64	0.50	0.58	-
Polyphenols	Chloroform : methanol (7 : 3)	0.40	0.40	0.40	0.40	-
Saponin	Chloroform : methanol : water (6 : 3 : 1)	--	0.86	0.86	0.84	0.64
Tannin	Chloroform : ethyl acetate : methanol (5 : 2 : 3)	0.90	0.90	0.90	0.90	-

Information:

(--) = No testing was done

(-) = No secondary metabolite compounds identified (negative)

SPF Value Testing with UV-Vis Spectrophotometry

Table 9. SPF Value of Ethanol Extract of Kepok Banana Flowers

Concentration (ppm)	SPF value	Sunscreen Protection Category
60 ppm	15,511	Ultra protection
80 ppm	16,978	Ultra protection
100 ppm	17,886	Ultra protection
120 ppm	18,796	Ultra protection
140 ppm	19,335	Ultra protection

Table 10. SPF Value of Kepok Banana Flower Fraction

Faction	Concentration (ppm)	SPF value	Sunscreen Protection Category
Water	60 ppm	4,662	Moderate protection
	80 ppm	7,757	Extra protection
	100 ppm	9,055	Maximum protection

	120 ppm	11,944	Maximum protection
	140 ppm	15,264	Ultra protection
Ethyl acetate	60 ppm	18,433	Ultra protection
	80 ppm	18,968	Ultra protection
	100 ppm	19,203	Ultra protection
	120 ppm	19,672	Ultra protection
	140 ppm	20,203	Ultra protection
<i>n</i> -hexane	60 ppm	1,190	Without protection
	80 ppm	1,762	Without protection
	100 ppm	2,278	Minimum protection
	120 ppm	3,575	Minimum protection
	140 ppm	4,598	Moderate protection

DISCUSSION

Plant Determination

Before plants are collected as research samples, a determination process is first carried out. Plant determination is carried out to determine the accuracy of the plants being studied and to avoid errors in selecting plant samples for use in the study.(Hanifa et al., 2021). Plant determination was carried out by taking a few leaves, stems, fruits, hearts, and flowers of kepok banana obtained in the area of Jalan Budi Utomo, Pontianak City, West Kalimantan and tested at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. The determination results showed that the samples used were indeed kepok banana plants (*Musa acuminata* x *M. balbisiana*).

Collection of Raw Materials and Making of Simple Drugs

The kepok banana flowers used are the inner part of the banana heart, which is shaped like a comb and is mature. The kepok banana heart is peeled and then wet sorted to remove dirt or damaged parts. 3,500 grams of kepok banana flowers are washed thoroughly with running water to remove dirt from the herbal medicine. Next, the washed and drained kepok banana flowers are chopped to reduce the size of the herbal medicine and facilitate the drying and grinding process. The samples are dried in a dry cabinet at 40°C. The purpose of drying is to reduce the water content and prevent the growth of microbes such as fungi and bacteria.(Maslahah, 2024). Drying is also done so that the simplicia is not easily damaged, so it can be stored for a long time. The dried simplicia is dry sorted to observe whether there are still impurities. Then the simplicia is ground by blending until it becomes powder and sieved with a No. 40 mesh sieve. The simplicia is ground so that the surface area of the sample increases and the contact with the solvent is higher, in this case it can increase the effectiveness during the extraction process.(Pane, 2023).

Based on Table 1, 619 grams of dried herbal medicine was obtained with a yield of 17.69% from 3,500 grams of kepok banana flowers. The yield calculation was carried out to determine the percentage of material remaining from the herbal medicine drying process and to determine the effectiveness of the resulting process. The higher the yield, the more efficient the treatment applied. A high yield value indicates the high amount of bioactive components contained in the herbal medicine.(John, 2022).

Standardization of Simple Drugs

Standardization of medicinal plants is carried out to ensure that the medicinal plants produced meet quality standards. The identified qualities include drying loss, water content, and ash content. Determining drying loss is one of the requirements for standardizing medicinal plants, with the aim of providing a maximum limit (range) for the amount of compounds lost during the drying process.(Sari et al., 2023). The drying loss parameter is a measurement of the remaining substance after drying at a temperature of 105°C for 30 minutes or until a constant weight is expressed as a percentage value. By knowing the drying loss, it can provide a maximum limit on the amount of compounds lost during the drying process. The drying loss of the kepok banana flower simplicia obtained was 5.70%. This indicates the amount of compounds lost during the drying process is 5.70%. A good requirement for

drying loss is <10%, because drying loss also represents the evaporated water content.(Maryam et al., 2020).

Determining water content is a parameter that indicates the minimum water content in a simple drug. Determining water content serves as the maximum limit for the simple drug.(Veninda et al., 2023)The water content of the Kepok banana flower simplicia is 5.22%. The water content test result is <10%, thus meeting the simplicia quality standards. The water content of >10% in simplicia is related to the requirements for the durability and quality of simplicia. If there is still a lot of water contained in the simplicia, it will cause the simplicia to rot easily and grow mold.(Shina et al., 2024).

Ash content determination is carried out to provide an overview of the mineral content originating from the initial process until the formation of the simplex. At this stage, the simplex is heated until the organic compounds and their derivatives are destroyed and evaporated, leaving only mineral and inorganic elements. The ash content obtained from the simplex of Kepok banana flowers was 5.57%, which meets the requirement of not being less than 10%. The ash content should have a low value because this parameter indicates the presence of heavy metal contamination that is resistant to high temperatures.(Juniasty, 2024).

Extract Preparation

Extraction aims to dissolve all compounds in the sample. Kepok banana flower powder was extracted using the maceration method (cold solid-liquid method) using 96% ethanol as a solvent. The maceration method was used because it can extract all secondary metabolites, including those that cannot withstand heat.(Amelia, 2021). The choice of 96% ethanol solvent, which is semipolar and tends to be polar, as a solvent extractor is based on the chemical properties of the compounds contained in Kepok banana flowers, namely flavonoids, saponins, and polyphenols, so that these compounds can be extracted well. The extraction process is carried out by changing the solvent three times every 24 hours with occasional stirring so that the ethanol can penetrate the walls of the Kepok banana flower pollen. The purpose of changing the solvent is to prevent the solvent from saturating, making it more effective in extracting the compounds.(Oktasila et al., 2019)The ratio between the simple drug and the solvent used was 1:10 (w/v). This is because a 1:10 (w/v) ratio produces good yields but does not reduce the absorption of the desired compound.(WAD Putri, 2021)The maceration results were then filtered with flannel and filter paper to separate the filtrate and residue. The maceration filtrate was evaporated using a rotary evaporator at 40°C, followed by a water bath. The evaporation process aims to separate the filtrate from the solvent.

Based on Table 3, the weight of the extract obtained was 63.90 grams with a yield of 12.78%, obtained from 500 grams of kepok banana flower simplicia. The yield is the ratio of the weight of the extract produced to the weight of the simplicia used as the raw material. A higher yield value indicates a greater extract yield.(John, 2022).

Extract Standardization

The thick extract obtained from the extraction results needs to be standardized to ensure that the extract quality meets the requirements. The identified extract qualities are water content, ethanol-free, and metal-free. Determining the water content in the extract aims to provide a minimum limit regarding the amount of water content in the extract. The higher the water content contained in the extract, the more easily the extract is grown by fungi, mold, or bacteria which results in a decrease in the quality and biological activity of the extract during storage. Determination of the water content of the kepok banana flower extract was carried out by weighing 2 grams of the extract. The water content was measured with a Moisture Analyzer at a temperature of 105°C. The resulting water content of the extract was 5.31%. The water content of the extract should not be >10%, if it is too high it can change the chemical composition of the extract, thereby reducing the quality of the herbal medicine, making it easy for bacteria to grow.(Shina et al., 2024).

The ethanol-free test was conducted to avoid the influence of ethanol solvent in the Kepok banana flower extract, which was feared to affect antibacterial activity because ethanol is a disinfectant. The results obtained from the ethanol-free test that had been conducted showed that the

sample was free of ethanol solvent because it did not detect an ester odor. The ethanol extract of Kepok banana flowers did not contain any residual solvent because it had undergone an evaporation process.(Achmad et al., 2024).

Metal-free testing was performed on Cadmium (Cd) and Lead (Pb). The purpose of the metal-free test is to ensure that the extract does not contain certain heavy metals exceeding the specified levels, as they are hazardous (toxic) to health. A positive lead content is indicated by the formation of a yellow precipitate, and a positive cadmium content is indicated by the formation of a white precipitate. The results in Table 5 show that the kepok banana flower extract did not contain any of the heavy metals cadmium or lead.

Extract Fractionation

Fractionation is a technique for separating and classifying the chemical content of an extract based on its polarity. The principle of liquid-liquid extraction is like dissolves like, where compounds with the same level of polarity will dissolve in solvents with the same level of polarity. The order of selection of fractionation solvents from nonpolar to polar is done so that nonpolar secondary metabolites can be extracted first with nonpolar solvents, because polar solvents have a wide range and are able to attract all compounds, whether nonpolar, semipolar, or polar.(Shina et al., 2024). A total of 10 grams of concentrated extract was dissolved in 100 mL of distilled water, placed in a separating funnel, and then fractionated with 100 mL of n-hexane. The separating funnel was shaken to release gas and left to stand until there were two layers (distilled water at the bottom and n-hexane at the top). The function of shaking was to maximize the extraction of secondary metabolite compounds by the solvent.(Herdiana and Aji, 2020)The two layers formed were then separated, and the n-hexane layer was taken. The process of adding the n-hexane solvent was repeated (replicated 3 times). The next fraction was repartitioned with ethyl acetate solvent in the same manner to extract semipolar compounds.

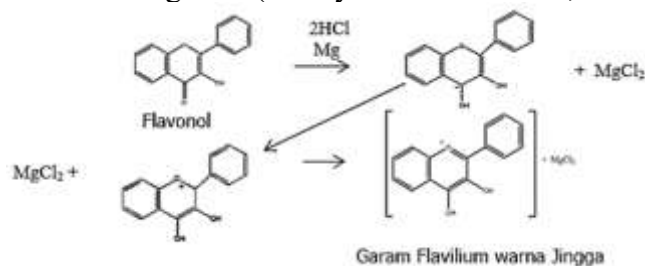
The yield of an extract fraction is the ratio of the fraction obtained to the amount of extract used. The higher the yield of an extract fraction, the greater the number of compounds extracted between solvents. Based on Table 6, the yield of the n-hexane fraction (nonpolar) was 11.76%, the ethyl acetate fraction (semipolar) was 55.14%, and the water fraction (polar) was 17.52%. The highest yield was in the ethyl acetate fraction and the smallest yield was in the n-hexane fraction. The semipolar nature of the ethyl acetate solvent causes the withdrawal of compounds from the extract to be large, where semipolar compounds are also attracted to the semipolar solvent. Meanwhile, the yield of the water fraction is small because the metabolites have been extracted in the ethyl acetate solvent phase, which has semipolar properties. The smallest yield is found in the n-hexane fraction, this is because the amount of nonpolar compounds in the kepok banana flower extract is very small.(Herdiana and Aji, 2020).

Phytochemical Screening of Extracts and Fractions

Phytochemical screening is a preliminary step in phytochemical testing that aims to provide an overview of the compound content of the plant being studied. Qualitatively, phytochemical screening is performed through color testing with the addition of reagents that will produce a color reaction to the chemical groups of the plant after being mixed.(Emilia et al., 2023)The examination aimed to confirm that the kepok banana flowers actually contain compounds with potential sunscreen properties, such as flavonoids and phenols, which are known to have antioxidant activity and can ward off free radicals.

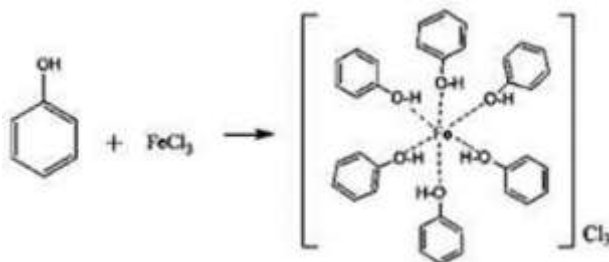
Based on the phytochemical screening data in Table 7, it shows that the extract, water fraction, and ethyl acetate fraction of Kepok banana flowers contain secondary metabolites in the form of flavonoids, polyphenols, saponins, and tannins. This is consistent with the results of research conducted by Nurhaeni et al. (2019)This showed that the ethanol extract of Kepok banana flowers contained flavonoids, polyphenols, and saponins. In contrast, only saponins were detected in the n-hexane fraction, while the other compounds showed negative results.

Flavonoid tests showed that the ethanol extract, water fraction, and ethyl acetate fraction of kepok banana flowers contained flavonoid compounds, which were characterized by the formation of an orange color. The addition of concentrated HCl to the sample solution aims to hydrolyze flavonoid glycosides, which are commonly found in plants, to form flavonoid aglycones. In an acidic environment, Mg reacts with HCl to form Mg²⁺ ions and H₂ gas. The formation of hydrogen gas is seen from the presence of bubbles in the mixture. Mg²⁺ ions will bind with flavonoids to form complex compounds and cause a color change to yellow, orange, or red. (Hanifa et al., 2021) The orange color produced by adding HCl and Mg to the sample is due to the formation of flavylum salts. The reaction that occurs can be seen in Figure 1. (Desriyana and Iskandar, 2021).



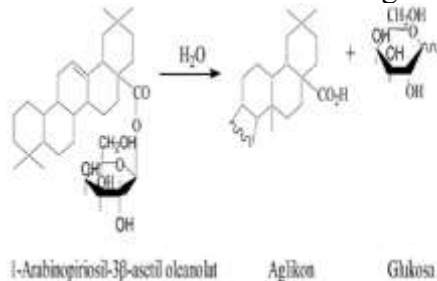
Picture 1. Reactions that Occur During Flavonoid Testing

The phenol test using FeCl₃ showed a positive result, indicated by the formation of a blackish green color in the ethanol extract, green in the water and ethyl acetate fractions. The addition of FeCl₃ to the phenol compound showed a blackish green color due to a complex reaction between the phenol compound and FeCl₃ with the transition of electrons from the central iron due to the presence of the Fe³⁺ ligand. (Ramayani et al., 2021).



Picture 2. Reactions that occur during the phenol test

In the saponin test, the results of the ethanol extract, water fraction, ethyl acetate fraction, and n-hexane fraction of kepok banana flowers were positive, indicated by the formation of foam. The formation of foam is caused by the structure of the saponin compound in the form of a glycoside that has a nonpolar sapogenin compound and a polar side chain that is soluble in water. When shaken with water, saponin can form micelles due to the presence of polar and nonpolar groups that are surface active. The polar group will face outward while the nonpolar group will face inward. This condition is what appears as foam. The reaction that occurs can be seen in Figure 3. (Hanifa et al., 2021).



Picture 3. Reactions that occur during the saponin test

In the tannin test, the sample reacted with FeCl₃ and produced a blackish-green color, indicating that the ethanol extract, water fraction, and ethyl acetate of Kepok banana flowers positively contained tannin compounds. The tannin compounds in the sample will bind with Fe³⁺ to form a

complex that causes a color change to blackish-green or blackish-blue depending on the type of tannin compound. The color change reaction that occurs in the identification of tannin compounds can be seen in Figure 4.

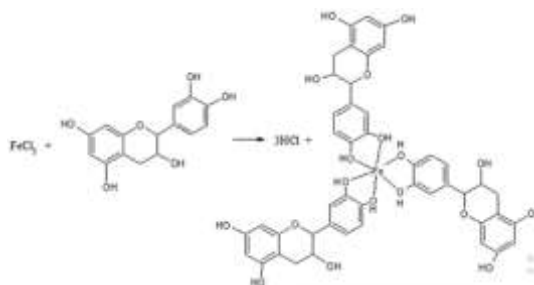


Figure 4. Reactions that Occur During Tannin Testing

The n-hexane fraction yielded negative results in the flavonoid, polyphenol, and tannin tests because these compounds are polar-semipolar, resulting in a negative result in the n-hexane fraction. In the multistage fractionation process, polar compounds will remain in the aqueous phase or be attracted to a more polar solvent, preventing them from dissolving in the n-hexane.(KA et al., 2022).

TLC Examination of Extracts and Fractions

The purpose of this study was to observe the separation of extracts and fractions of Kepok banana flowers based on the differences in polarity of compounds partitioned between the mobile and stationary phases. The TLC examination also provided an initial overview of the chemical composition based on the chromatogram pattern.(Peratiwi et al., 2023)The chromatogram results from TLC testing are generally colorless spots that can be measured chemically or physically. Physical observation of the chromatogram spots is carried out using 254 nm UV light.(Rahmi et al., 2021). The fluorescence of a compound under 254 nm UV light indicates the possible presence of a compound with conjugated double bonds. 254 nm UV light causes the plate to fluoresce (bright) and the spots appear dark.(Peratiwi et al., 2023).

Based on identification by thin layer chromatography (TLC), the results obtained from the extract, water fraction, and ethyl acetate fraction of kepok banana flowers positively contained secondary metabolite compounds in the form of flavonoids, polyphenols, saponins, and tannins in the range of 0.4-0.9. In contrast, in the n-hexane fraction only saponin compounds were detected with a value of 0.64, while other compounds were not detected.

Based on the results of identification by thin layer chromatography (TLC), the identification results obtained using yellow AlCl₃ detection were identified as flavonoids with R_f values of 0.58 in the quersertin reference standard, 0.64 in the ethanol extract, 0.50 in the water fraction, and 0.58 in the ethyl acetate fraction. These R_f values indicate that flavonoids were detected on the TLC plate spots. Research fromMaulana et al. (2018)stated that the yellow fluorescent spots were identified as flavones with an R_f of around 0.5-0.89.

The results of identification by thin layer chromatography (TLC) on polyphenols using FeCl₃ detection showed a blackish green color, so it was identified as polyphenols with an R_f value of 0.40 in the quercetin reference standard, ethanol extract, water fraction, and ethyl acetate fraction. This R_f value indicates that polyphenols were detected on the TLC plate spots. Research from(Sari et al., 2023)states that the green/blue-black spots are identified as polyphenols.

The next step was to identify saponins using the Liebermann-Burchard reagent. The R_f value of saponins ranged from 0.16 to 0.97.(Shina et al., 2024)The results of the study showed purple spots after being sprayed with Liebermann-Burchard reagent with an R_f value in the range of 0.64-0.86, thus being declared positive for saponins.

Tannin identification is performed using FeCl₃ spray reagent. This is because tannin is a phenolic compound that will react with FeCl₃. Tannin with FeCl₃ reagent will produce a blue-black color. The R_f value of tannin is between 0.19 and 0.96.(Shina et al., 2024). The research results were identified as blackish blue in the presence of FeCl₃ with an R_f value of 0.90 in the catechin comparison

standard, ethanol extract, water fraction, and ethyl acetate fraction so that it was declared positive for tannin.

SPF Value Testing with UV-Vis Spectrophotometry

Sun Protection Factor(SPF) is a universal indicator for sunscreen effectiveness. It is the ratio of UV energy (or time) required to cause redness (erythema) on skin with sunscreen compared to skin without. The higher the SPF, the more effective it is at preventing sunburn.(Ardiansyah and Ramayani, 2022).

The determination of the SPF value of Kepok banana flowers was carried out in vitro using the UV-Vis spectrophotometry method and was based on the Mansur equation. The steps to determine the SPF value were to first prepare a stock solution with a concentration of 1000 ppm. Then, dilutions were carried out by taking 0.6; 0.8; 1.0; 1.2; and 1.4 mL of the solution into a 10 mL volumetric flask, adding ethanol pa as a diluent solution to obtain concentrations of 60; 80; 100; 120; and 140 ppm. The sample was placed into a cuvette and the absorbance was measured using a UV-Vis spectrophotometer. A test absorption curve was made in the cuvette with a wavelength of 290-320 nm, which is the UV B wavelength that can cause sunburn. UV B rays are dangerous rays that can cause damage more quickly than UV A rays. Ethanol pa was used as a blank because most groups of compounds are soluble in this solvent, and ethanol relatively does not interfere with the absorption of the dissolved compounds.(Andini et al., 2023).

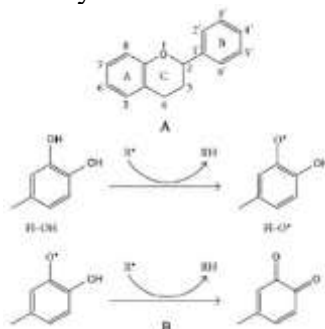
Based on Table 9, the SPF value of the ethanol extract of Kepok banana flowers ranges from 15.511 to 19.335. All concentrations fall into the ultra protection category, with values increasing with increasing concentration. In the ethanol extract of Kepok banana flowers, the compounds in it work together (synergistically) to absorb UV rays, indicating an increase in the effectiveness of these various compounds. Based on this, a concentration of 60-140 ppm, which already has ultra protection, can be used to test the SPF value of the kepok banana flower fraction.

The results of determining the SPF value of the kepok banana flower fraction can be seen in Table 10. The SPF value of the kepok banana flower water fraction ranged from 4.662 to 15.264. This concentration is in the moderate to ultra protection category, with the value increasing as the concentration increases. The SPF value of the ethyl acetate fraction of kepok banana flowers ranged from 18.433 to 20.203. All concentrations are included in the ultra protection category, with the value also increasing as the concentration increases. Meanwhile, the SPF value of the n-hexane fraction of kepok banana flowers ranged from 1.190 to 4.598. Based on Table 12, the SPF value of the n-hexane fraction at concentrations of 60 ppm and 80 ppm did not meet the minimum criteria for sunscreen protection because it was below the SPF value of 2, so it was categorized as having no protection. However, the SPF value continued to increase as the concentration increased until it reached the moderate protection category at 140 ppm.

The SPF value of the extract and fraction of the kepok banana flower can be useful as sunscreen protection. The secondary metabolites contained in the extract and fraction of the kepok banana flower contain flavonoids, polyphenols, and tannins, which are known to protect against free radical damage caused by UV rays.(Anjani et al., 2024)Ethanol and ethyl acetate are semipolar solvents that will attract compounds such as flavonoid aglycones, alkaloids, and polyphenols. Water is a polar solvent that will attract compounds such as flavonoid glycosides, carbohydrates, and tannins. Meanwhile, the n-hexane fraction is a nonpolar solvent that will attract fat, steroid, and terpenoid compounds. The SPF value in the water fraction is smaller than in the ethyl acetate fraction because flavonoid and phenolic compounds are more soluble in semipolar solvents such as ethanol and ethyl acetate compared to polar solvents that can only attract flavonoid and tannin compounds. Meanwhile, the SPF value in the n-hexane fraction is the smallest in accordance with the results of phytochemical screening of the n-hexane fraction of kepok banana flowers which do not contain flavonoid, polyphenol, and tannin compounds. The results of this study are in line with research conducted Astuti et al. (2024), the SPF values of ethanol extract, ethyl acetate fraction, water fraction and n-hexane fraction of kawista bark were 12.77 (maximum protection), 11.77 (maximum protection), 11.67 (maximum protection)

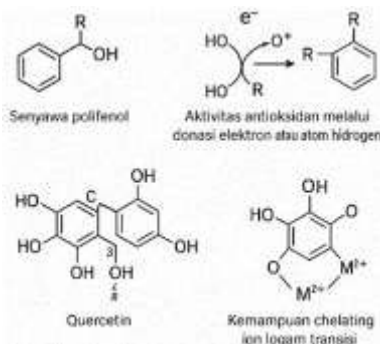
and 7.146 (extra protection), respectively. Based on the SPF value analysis, the order of solvent effectiveness in producing the highest SPF value was semipolar solvent, followed by polar solvent, and finally nonpolar solvent.

According to the research results of Nurhaeni et al. (2019) and Nurhaeni et al. (2025), the ethanol extract of kepok banana flowers has antioxidant activity with IC50 values of 13.21 $\mu\text{g/ml}$ and 26.00 $\mu\text{g/mL}$, respectively. The results of the study indicate that plant extracts with high antioxidant activity tend to have high SPF values as well. This is because the mechanism of free radical scavenging by antioxidants is in line with the mechanism of breaking the UV photon energy chain by the chromophore group. Based on the research John (2022), explained that there is a positive correlation between flavonoid content and the SPF value of the extract. Flavonoid compounds have high sunscreen potential due to the presence of chromophore groups (conjugated double bonds) that can absorb UV rays, thereby reducing their intensity on the skin.



Picture 4. Free Radical Scavenging Process by Flavonoids

Polyphenol compounds have the ability to act as antioxidants through several key mechanisms, which play a crucial role in protecting body cells from oxidative damage caused by reactive oxygen species (ROS). One key mechanism is that polyphenols provide assistance in the form of electrons or hydrogen atoms to free radicals. This converts aggressive free radicals into stable, harmless substances, thus preventing damage to DNA, proteins, and lipids in cells. Furthermore, polyphenol compounds are also able to bind metal ions such as iron (Fe^{2+}) and copper (Cu^{2+}). These metals typically trigger the formation of very dangerous free radicals through certain chemical reactions. By binding to these metals, polyphenols stop these dangerous reactions before they occur, thus protecting cells from oxidative stress.



Picture 5. Chemical Structure and Antioxidant Activity of Polyphenol Compounds

A sunscreen is considered to provide protection if it has an SPF of at least 2, and is considered good if the test sample has an SPF of over 15, which is considered ultra protection. This is because an SPF above 15 provides better protection against long-term skin damage, such as skin cancer. Furthermore, an SPF above 15 can protect the skin from sun exposure for approximately 4-5 hours. (Puspita et al., 2023).

Based on the results of this study, the water and ethyl acetate fractions of kepok banana flowers have potential as sunscreens because they show high SPF values (reaching the ultra protection category). Meanwhile, the n-hexane fraction has the lowest potential. The concentration of the solution

also affects the SPF value. The higher the concentration, the greater the number of active compounds (such as flavonoids and polyphenols) that can absorb or reflect UV radiation before it reaches the skin.

CONCLUSION

This study shows that the fraction Water, ethyl acetate fraction, and n-hexane fraction of kepok banana flowers have activity as sunscreens that can protect the skin from UV exposure. The SPF values of the kepok banana flower water fraction with concentrations of 60 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm were respectively 4.662; 7.757; 9.055; 11.944; 15.264 with moderate-ultra protection. The ethyl acetate fraction obtained SPF values of 18.433; 18.968; 19.203; 19.672; 20.203 with ultra protection. The n-hexane fraction obtained SPF values of 1.190; 1.762; 2.278; 3.575; 4.598 with the category of no-moderate protection. The research focused solely on in vitro testing without in vivo testing or long-term stability of the preparation, and lacked quantitative analysis of specific compounds, such as HPLC, for direct correlation with SPF. Suggestions for further research include sunscreen cream formulation, clinical trials on human skin, and isolation of key bioactive compounds for optimization. Practically, these results have implications for the development of natural sunscreens based on banana flowers as a safe alternative to synthetic ones to prevent premature aging and skin cancer in Indonesia's tropical climate.

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