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Flavonoid Compounds of the Catechin from Wungu (*Graptophyllum pictum* (L.) Griff) Leaves and the Sun Protecting Factor Value

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Abstract

The flavonoid content in wungu (Graptophyllum pictum (L.) Griff.) leaves has the potential as a sunscreen. The study aims to identify isolates of flavonoid compounds from wungu leaves and determine the SPF value. Steps are followed by extraction, fractionation, phytochemical test, separation by chromatography, identification, and SPF test. Extraction was carried out with methanol, followed by fractionation with n-hexane and dichloromethane. Dichloromethane fraction was chosen to proceed to the separation step because the results of the phytochemical test showed a vigorous color intensity for the content of flavonoids. The isolate (3.6 mg; dark green; amorphous; mp. 132-136 °C) was identified using a UV-Vis spectrophotometer using methanol as a solvent with a shift reagent NaOH, $AlCl_3$, and a mixture of concentrated $AlCl_3$ and HCl. Based on the UV-Vis spectra, the isolate was predicted to be flavonoid compounds belonging to the catechin group, which have a hydroxyl group at positions C-3, C-7, and do not have an ortho-hydroxy group in ring B. The SPF value of the isolate of 2.3244 at 100 ppm was determined in vitro and calculated by the Qian equation. Therefore isolate was categorized as sunscreens that provided minimal protection.

Keywords: Graptophylum, wungu leaves, catechin, flavonoid, SPF, UV-Vis

Introduction

Sunlight has many benefits for living things on earth. However, on the other hand, sunlight also has terrible effects on humans, one of which is the effect of the sun on the skin. On the earth's surface, sunlight consists of several very dangerous spectrums. They have very high energy and are carcinogenic, including infrared rays, visible rays, ultraviolet rays (UV-A), UV-B rays, and UV-C rays (Kaur & Saraf, 2010). Substances that can reduce the adverse effects of sunlight are known as sunscreens. The ability of sunscreen compounds to protect the skin from ultraviolet (UV) rays is defined as the Sun Protecting Factor (SPF) value (Widyastuti et al., 2016).

The performance of sunscreen through various mechanisms, one of which is photoprotective. Some plants contain natural substances that can be extracted and act as a potential source of sunscreen because they are photoprotective. The photoprotective properties of plants can protect the skin from UV radiation through bioactive compounds contained in plants, such as phenolic compounds and other compounds that have antioxidant activity (Prasiddha et al., 2016).

Flavonoid compounds have photoprotective properties to absorb ultraviolet light (Whenny et al., 2015). Flavonoid and phenolic compounds have the potential as sunscreens. The possibility of flavonoids as sunscreen is due to a chromophore group, a conjugated aromatic system that can absorb intense light in UV light wavelengths (Putri et al., 2019). In addition, substances that have antioxidant properties can also prevent various diseases caused by UV radiation, including flavonoids, tannins, anthraquinones, cinnamates, and others that can protect against UV rays (Hogade et al., 2010).

The SPF value of a compound extract will be different if various solvents are used. The SPF value of the methanolic extract of moringa leaves was higher than that of the ethanolic extract of the same leaf. This result is because methanol is more polar than ethanol (Ningsih & Oktadiana, 2019). Arya et al. (2021) reported that the higher the total phenolic and flavonoid content in the *Basella alba* leaf extract, the higher the SPF value. This test was carried out on *Basella alba* extract using various solvents. Wungu (*Graptophyllum pictum* (L.) Griff) leaves is a purple ornamental or medicinal plant that is easy to find. The leaves contain

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flavonoid compounds, tannins, alkaloids, glycosides, and saponins.

Flavonoid compounds have been successfully extracted based on the kinship of the same genus, namely Graptophyllum, from the Graptophyllum grandulosum plant. Purification of isolates from the butanol fraction *Graptophyllum grandulosum* plant produces five flavonoid glycosides (Kaur & Saraf, 2010). *Graptophyllum glandulosum Turrill (Acanthaceae*), a plant that grows on shrubs with green leaves, red-purple flowers and thrives on the African continent, contains several secondary metabolites polyphenols, flavonoids, and glycosides (Tagousop et al., 2018).

Antioxidant assay on the ethyl acetate fraction of wungu (*Graptophylum pictum* (Linn.) leaves Griff) obtained a relatively high value of 3.984×10^{-6} g QE/g. The flavonoid content in the ethyl acetate fraction of wungu leaves has been predicted to play a role in these results (Aminah et al., 2016).

Determination of the SPF value of the ethanol extract of jeruju leaves (*Acanthus Ilicifolius* L.) at various concentrations obtained an SPF value at 100 ppm of 1.3165 with the highest SPF value at 500 ppm of 3.8478 (Bahar et al., 2021). Jeruju leaves and Graptophylum is in the same family, Acanthaceae.

The published research results allow exploring other flavonoid compounds from other Graptophyllum plants, namely wungu (*Graptophyllum pictum* (L.) Griff) leaves from different solvent fractions. There has never been a characterization of flavonoid compounds from wungu leaves from Singkawang, Kalimantan Barat, and a sunscreen test throughout the literature search. In this study, the isolation of flavonoid compounds from wungu leaves and a sunscreen test on the isolates will be carried out. The characterization of wungu leaves isolates was carried out using a UV-Vis spectrophotometer.

Methods

The tools used in this study include laboratory glassware, separating funnels, Thin Layer Chromatography (TLC), Vacum Liquid Chromatography (VLC), Gravity Column Chromatography (GCC), analytical balance (BEL Engineering), melting point meter (Melting Point SMP10), UV lamp (UVGL-55 Handheld UV Lamp), rotary evaporator (Kika-Werke HB4 basic), and UV-Vis spectrophotometer (Genesys 10s UV-Vis Spectrophotometer).

The materials used in this study included samples of wungu (*Graptophyllum pictum* (L.) Griff) leaves obtained from Singkawang, Kalimantan Barat, hydrochloric acid pa (HCl), $FeCl_3$ solution, metal Mg, methanol (C_2H_5OH), ethyl acetate ($C_4H_8O_2$), *n*-hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), sodium hydroxide (NaOH), aluminum chloride ($AlCl_3$) and reagents used for phytochemical tests (Dragendorff, Wilstatter, and Lieberman-Burchard reagents).

Sample preparation

The wungu leaves are washed thoroughly with water and then dried without direct sunlight. The dried leaves are cut into small pieces, then mashed using a blender.

Extraction and fractionation

A total of 700 g of dried wungu leaves were macerated through immersion in methanol as solvent. The maceration process was left for 3 × 24 hours in a place protected from direct sunlight. After three days of immersion, the sample was filtered to obtain the filtrate and residue. The filtrate was concentrated with a rotary vacuum evaporator to get a thick methanol extract. The methanol extract of wungu leaves was fractionated using solvents with different polarity levels, namely methanol, *n*-hexane, and dichloromethane. The fractionation results were concentrated using a rotary evaporator. Three fractions of the viscous extract were obtained, namely the methanol fraction, the n-hexane fraction, and the dichloromethane fraction of wungu leaves.

Phytochemical test

The methanol extract, methanol fraction, *n*-hexane fraction, and dichloromethane fraction of wungu leaves were tested for phytochemicals. The tests carried out included alkaloids, flavonoids, terpenoids, steroids, and phenolics. A positive test for alkaloids was indicated by the appearance of an orange precipitate when Dragendorff's reagent was added to each extract and fraction. The Wilstatter test proved the flavonoid content by adding 2-4 drops of HCl and Mg metal powder to the section and a fraction of wungu leaves. A change in color to yellowish-green, brownish-yellow, blackish-green, yellowish-green, or light green indicates the presence of flavonoid compounds (Harborne, 1998). The phenolic compounds were identified by reacting the sample with a 1% $FeCl_3$ solution. A positive reaction occurred if there was a change in green, purple, blue, brown, and black color Triterpenoids and steroids were tested by adding Liebermann-Burchard reagent to the dissolved extract and fraction of wungu leaves. A positive result of terpenoids produces a red or purple color, or it can also be indicated by the formation of a brownish or violet ring on the boundary of the solution, indicating a positive terpenoid. In contrast, a greenish-blue circle indicates the presence of steroids (Harborne, 1998).

Compound separation and purification

The fraction that showed positive results containing the flavonoid compound group was continued to the separation and purification stage. The separation and purification process of compounds is carried out through TLC, VLC, GCC, preparative TLC, and one- and two-dimensional TLC (Destria et al., 2019).

Characterization using a UV-Vis spectrophotometer

Isolate as much as 0.9 mg dissolved in methanol. The isolate solution was added with several shift reagents, namely 2M NaOH solution, 5% $AlCl_3$ solution, and $AlCl_3$ and HCl solutions. The isolates were characterized using a UV-Vis spectrophotometer.

Melting point test

A total of ± 0.1 mg of the isolate was put into a capillary tube in a melting point apparatus, then measured at 132-133 °C. The temperature was increased gradually until the isolate melted completely.

Sunscreen activity test

In vitro test of sunscreen was carried out based on Dutra et al. (2004). 2.6 mg of the isolate was placed in a 10 mL volumetric flask, then adjusted to the 10 mL mark with methanol. The stock solution obtained has a concentration of 260 g/mL. The stock solution was diluted with methanol at 25, 50, 75, and 100 g/mL concentrations. The absorbance of the solution was measured at a wavelength of 200–400 nm using a UV-Vis spectrophotometer, and the maximum wavelength was measured at various concentrations of the test solution. The blank in this test used methanol as a solvent.

The SPF value was measured based on the absorption value of the compound following the equation of Qian et al. (2015):

$$\text{SPF} = \sum_{200}^{400} E\lambda.S\lambda \,/\, \sum_{200}^{400} E\lambda.S\lambda.T\lambda \qquad (1)$$

Where $E\lambda$ is the effectiveness of the CIE erythema spectra, $S\lambda$ is the solar irradiance spectra, and $T\lambda$ is the transmittance spectra of the test solution.

Results and Discussion

The dichloromethane fraction (FD) was continued to the separation and purification stage because the results of the phytochemical test on FD showed that it contained flavonoid compounds. The selection of the best solvent was carried out before VLC against FD, using various solvents with different polarity levels. Based on the number of spots and a good separation pattern on TLC, the best solvent was n-hexane: ethyl acetate (6.5:3.5).

The VLC fraction obtained was 38 fractions. TLC analysis was carried out on each fraction using the same solvent. The fractions with the same spot were combined so that 9 combined fractions were obtained, which are presented in **Table 1**. The FD_3 was continued to the GCC because it has a larger mass, and the fraction contains flavonoids, steroids, alkaloids, and phenolics based on the results of phytochemical tests.

Table 1. Combined fraction of VLC

Fraction	Combined fraction	Mass (g)
FD_1	1–2	0.0393
FD_2	3–4	0.0280
FD_3*	5–7	0.0565
FD_4	8–10	0.0140
FD_5	11–13	0.1563
FD_6	14–25	0.4435
FD_7	26–28	0.1563
FD_8	29–31	0.1185
FD_9	32–38	4.5283

*fraction continued to GCC

The subsequent separation stage is GCC. The best solvent was also selected for GCC using TLC to obtain the best eluent of *n*-hexane: ethyl acetate (7:3). The results of separation using GCC received 174 fractions. The fraction was analyzed by TLC using the best eluent. Fractions with the same spot are combined, resulting in 13 combined fractions. The results of the combined fractions of GCC are presented in **Table 2**. The $FD_{3.7}$ fraction was continued with separation using preparative TLC because this fraction contains flavonoid, steroid, and phenolic compounds with few spots and large mass.

Table 2. Combined fraction of GCC

Fraction	Combined Fraction	Mass (g)
$FD_{3.1}$	1-31	0.0068
$FD_{3.2}$	32-36	0.0067
$FD_{3.3}$	37-38	0.0016
$FD_{3.4}$	39-41	0.0039
$FD_{3.5}$	42–44	0.0027
$FD_{3.6}$	45	0.0026
$FD_{3.7}$*	46-48	0.0175
$FD_{3.8}$	49–52	0.0133
$FD_{3.9}$	53-56	0.0050
$FD_{3.10}$	57-68	0.0087
$FD_{3.11}$	69-74	0.0017
$FD_{3.12}$	75–171	0.0181
$FD_{3.13}$	172-174	0.0134

*fraction continued to preparative TLC

Preparative TLC was performed on fraction $FD_{3.7}$. The best eluent used for this step is *n*-hexane: ethyl acetate (5:5). The mass of the isolate after preparative TLC was 3.6 mg. The isolate purity test was carried out by one- and two-dimensional TLC, relatively pure if the TLC results show a single spot. A one-dimensional TLC test was performed using *n*-hexane: ethyl acetate (5:5). The results of one-dimensional TLC are shown in **Figure 1.**

Figure 1. One-dimensional TLC of FD_i isolate (UV at λ= 366 nm)

The purity test by two-dimensional TLC was carried out on the $FD_{3.7}$ fraction using *n*-hexane: ethyl acetate (5:5) followed by *n*-hexane: ethyl acetate (8:2) by rotating the TLC plate 5 × 5 cm by 90°. The results of the two-dimensional TLC are presented in **Figure 2**.

The TLC plate was observed under a UV lamp at emission wavelengths of 254 nm and 366 nm to reveal the compound as a dark or fluorescent spot (Gritter et al., 1991). In one- and two-dimensional TLC, the areas were observed under a UV lamp at a wavelength of 366 nm, showing a bright fluorescent spot, while it was not observed at a wavelength of 254 nm.

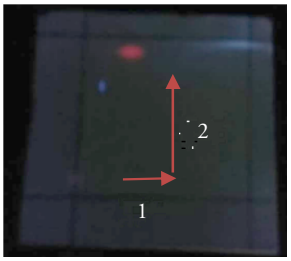

Figure 2. Two-dimensional TLC results of the FD_i isolate (UV at λ= 366 nm)

Based on the results of one- and two-dimensional TLC, it was shown that the isolate was relatively pure, which could be seen on a 366 nm UV lamp which indicated the formation of a single spot on the TLC plate. The FDi isolate was then characterized to identify the type of compound, and the SPF value was determined using a UV-Vis spectrophotometer.

Characterization of isolates using UV-Vis spectrophotometer and melting point test

Isolates were characterized using a UV-Vis spectrophotometer with a shift reagent NaOH solution, $AlCl_3$, and $AlCl_3$ solutions added with concentrated HCl. The UV spectrum of $FD_{3.7}$ isolated in methanol solvent with a concentration of 50 ppm without a shift reagent can be seen in **Figure 3** and **Table 3**.

Table 3. The absorbance of FD_i isolate without shift reagent

Wavelength (nm)	Absorbance
203	0.871
273	0.237

Based on the spectrum in **Figure 3** and **Table 3**, isolate FD_i has absorption at wavelengths of 203 nm (a band II) and 273 nm (a band I). The wavelength absorption range obtained indicates the catechin group of flavonoid compounds. The UV-Vis spectrum of catechin flavonoids gave a typical absorption in the maximum wavelength range of 270-274 nm (Nur et al., 2020).

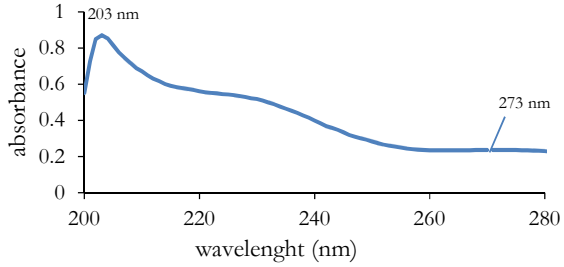

Figure 3. UV Spectrum of FD_i isolate without shift reagent

According to Tempesta (2007), catechin group flavonoid compounds have maximum absorption of band I at a wavelength of 275-280 nm and band II at 202-204 nm. It is also clarified by research from Rasidah et al. (2019), where the results of UV-Vis spectrophotometry give two absorption bands, namely at a wavelength of 272.6 nm (a band I) and 202.2 nm (a band II). Based on this data, the compounds are flavonoid compounds of the catechin group.

The spectrum of isolate FD_i did not show significant absorption at a wavelength of 300-550 nm because catechins did not have a carbonyl group (C=O) in their basic framework. Therefore, the absorption in this region shows the absence of an n → electron transition for the aromatic ring system substituted by a ketone group like most other types of flavonoids (Rasidah et al., 2019). Catechins have electron transitions $\pi \rightarrow \pi^*$ due to a chromophore group with a conjugated double bond. This group will absorb electromagnetic radiation in the UV and Visible wavelength regions (Husni & Puspitaningrum, 2017).

Based on **Table 4** and **Figure 4**, the addition of NaOH reagent causes a shift towards bathochromic in band II from 203 to 212 nm. A change in band II of 9 nm indicates a hydroxyl group at the C-7 position on ring A. There is no shift in the band I, so it is stated that there is no hydroxyl group at the C-4' work on ring B (Markham, 1988). Thus, the addition of NaOH reagent to isolate FDi resulted in a bathochromic shift in band II which indicated the presence of a hydroxyl group at the C-7 position on ring A.

Table 4. The maximum wavelength shift of isolate FD_i with shift reagent

Solvent	Wavelength maximum λ_{max} (nm)		Wavelength shift λ (nm)	
	Band I	Band II	Band I	Band II
Methanol	273	203	-	-
Methanol +NaOH	-	212	-	+9
Methanol + $AlCl_3$	275	203	+2	-
Methanol + $AlCl_3$ + HCl(conc.)	273	203	-	-

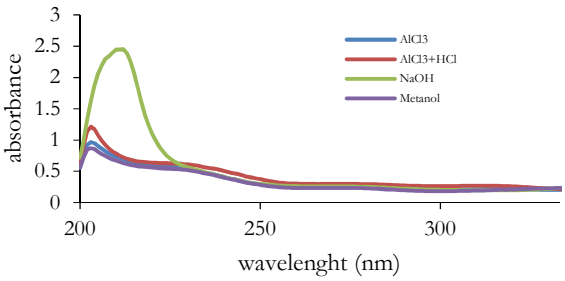

Figure 4. UV spectrum of isolate FD_i with shift reagent

The addition $AlCl_3$ reagent was carried out to determine the presence of a hydroxyl group at C-3 with or without C-5. The addition of the $AlCl_3$ reagent causes a bathochromic shift of 2 nm from 273 to 275 nm in the band I which indicates the presence of a hydroxyl group at the C-3 position on ring C. There is no shift in band II indicating the absence of a hydroxyl group at the C-5 position on ring A. Thus, the addition of the $AlCl_3$ reagent suggests the presence of a hydroxyl group at the C-3 place. A shift reagent ($AlCl_3$ + concentrated HCl) was added to determine the presence or absence of an ortho-hydroxy group in ring B. There was no shift in either band I or band II, indicating the absence of an ortho-hydroxy group in ring B (Markham, 1988).

Based on the analysis, isolate FD_i is predicted to be a flavonoid compound from the catechin group. Which has a hydroxyl group at positions C-3 and C-7, and there is no ortho hydroxy group in ring B. **Figure 5** shows the framework of flavonoid compounds and their numbering (Redha, 2010). **Figure 6** is a prediction of the framework of the structural formula for the isolate FD_i.

Catechins are flavonoid compounds in the natural phenolic group that have the potential as antioxidants and have bioactivity as drugs. Catechin compounds are natural and essential antioxidants that have a good level of safety in scavenging free radicals (Agustin et al., 2013). A Compound with antioxidant activity is due to the presence of flavonoid compounds. Maharini et al. (2019) determined the SPF value of dadap serep (*Erythrina Subumbrans* (Haks.) Merr) leaf extract due to the presence of flavonoid compounds. Therefore, it is estimated that catechins also have a photoprotective effect as sunscreen.

The melting point test results for isolate FDi were in the range of 132-136 °C. According to Taha et al. (2015), catechin compounds or 3-ol flavan derivatives have melting points of 131-132 °C. The melting point test results are classified as pure if the test results have

a maximum range of 2 °C. However, the effects on isolate FD_i were obtained in the field of 4 °C, presumably because it still contains impurities. **Figure 2** shows the presence of a spot from the impurity compound.

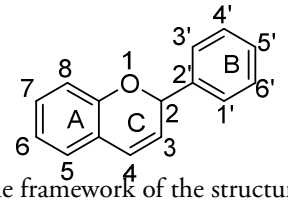

Figure 5. The framework of the structure of flavonoids

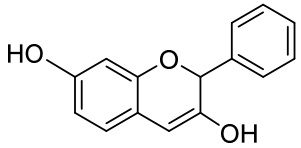

Figure 6. Prediction of the framework of the structure FD_i isolate (flavan 3,7-diol)

Determination of SPF value

Determination of the SPF value on isolates $FD_{3.7}$ was carried out in vitro using a UV-Vis spectrophotometer at a 200-400 nm wavelength range. A solution of FDi isolate was prepared in methanol solvent with various concentrations of 25, 50, 75, and 100 g/mL. The results of determining the SPF value can be seen in **Table 5** and **Figure 7**.

Table 5. Calculation of SPF value of FDi isolates based on the concentration

Concentration (ppm)	SPF value
25	1.296
50	1.626
75	1.943
100	2.253

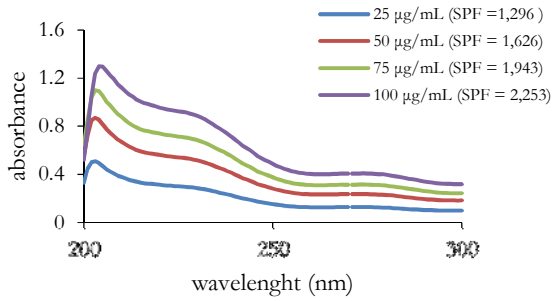

Figure 7. UV Spectrum of FD_i isolate and SPF value

The higher the concentration, the higher the SPF value. The higher the SPF value, the more influential the sunscreen activity (Daud et al., 2016). It is by the test results in **Table 5** and **Figure 7**. The SPF value of isolate FDi at a concentration of 100 ppm has a value of 2.25287, so it is categorized as a sunscreen with minimal protection.

Hashemi et al. (2019) have determined the content of flavonoid compounds, antioxidant activity, and sunscreen tests on leaf extracts of several plants. Leaf extracts with high content of flavonoid compounds tend to be directly proportional to antioxidant activity, but the SPF value is not always directly proportional to antioxidant activity.

The SPF test on extracts ethanol of jeruju leaves (*Acanthus Ilicifolius* L.) from the same family, Acanthaceae, showed a lower SPF value than the SPF value of isolating FD_i from wungu leaves at 100 ppm. The SPF value of extract ethanol of jeruju leaf is 1.31650 (Bahar et al., 2021), while the FD_i isolates from wungu leaves has an SPF value of 2.25287.

Based on the Least Significant Difference (LSD) test on statistics software against the SPF value of FDi isolate at various concentrations, it showed a significant increase in the SPF value with increasing concentrations of FDi isolate. It is indicated by the most negligible considerable difference between the SPF FDi value isolates at concentrations of 25, 50, 75, 100 ppm were smaller than 0.05, so there was a significant difference (Septiana, 2016). LSD test on statistics software against the SPF value of FDi isolate at various concentrations is presented in **Table 6.**

Human skin lasts for 10 minutes under sun exposure. Sunscreen material applied to the skin causes the skin's resistance to sun exposure to be ten times (Yulianti et al., 2015). The SPF value of isolate FD_i is 2.25287 at a concentration of 100 ppm, meaning that the isolate can protect the skin from sun exposure for 22,587 minutes.

Table 6. LSD test against the SPF value of FDi isolate at different concentrations

Concentration I (ppm)	Concentration II (ppm)	Significance
25	50	0.004
	75	0.000
	100	0.000
50	25	0.004
	75	0.004
	100	0.000
75	25	0.000
	50	0.004
	100	0.005
100	25	0.000
	50	0.000
	75	0.005

Conclusions

The UV-Vis spectrophotometer's characterization results predicted that FD_i isolate was a flavonoid compound from the catechin group, namely flavan 3,7-diol. The SPF value of isolate FDi at a concentration of 100 ppm was 2.25287. The isolated compound FDi was categorized as a sunscreen with minimal protection.

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