

# Phytochemical Test and Sunscreen Activities Etanol Extract Of Guava Tangkalak Leaves (*Bellucia pentamera*)

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**Abstract** : One of the most important sources of natural light for life is sunlight. The skin is the outermost and largest organ in the human body, which is easily exposed to sunlight. The aim is to determine the biochemistry of secondary metabolite compounds in the guava tangkalak leaves of the plant and their activities as an active ingredient of sunscreen using UV-Vis spectrophotometry. The results of this study indicate that the guava tangkalak leaves plant contains secondary metabolite compounds, such as flavonoids, alkaloids, tannins, saponins, and steroids. The SPF values produced at concentrations of 400, 500, 600, 700, 800, and 1000 ppm, SPF values of 2.96, 4.60, 7.70, 10.76, 15.13, and 27.41. The maximum concentration of 1000 ppm protects ultra-categories in SPF.

Keywords: Guava Tangkalak Leaves, Skin, SPF

### **INTRODUCTION**

Sunlight is a natural source of light that is essential for life. However, sunlight can also negatively affect the skin, especially if overexposed. Chronic exposure to sun ultraviolet (UV) light causes changes in the skin's structure, composition, and oxidative stress. The effects can include redness, hyperpigmentation, photosensitivity, and premature aging (Susanti, 2012).

Skin is the outermost and largest organ of the human body, which is very easily exposed to sunlight. Sunlight is one of the sources of energy that is beneficial for the lives of living things in all parts of the world, including Indonesia. Apart from being a source of energy, the sun also emits ultraviolet rays that can cause radiation (Isfardiyana & Safitri, 2014). Types of ultraviolet light can be divided into ultraviolet UV-A (320-400 nm), UV-B (290-320 nm), and UV-C (200-290 nm) (Pontoan, 2016). According to Hasanah et al. (2015), excessive exposure to ultraviolet light can cause various adverse effects on the skin, such as redness (erythema), black spots (pigmentation), dryness, dullness, and, over a long time, skin cancer (Hasanah et al., 2015).

Based on the analysis of Indonesia Cancer Care Community (ICCC) data 2018 in Indonesian there are around 1,392 cases of melanoma skin cancer. Melanoma is one of the more dangerous types of cancer and can cause death; in 2018 in Indonesia, there were 797 cases of death caused by melanoma skin cancer. Handling these problems can be done using sunscreen (Nirmala Sari, 2015).

Sunscreen is a product that can help protect the skin from the harmful effects of UV rays. Sunscreens contain chemical compounds that absorb, scatter, or reflect UV rays (Oktaviasari& Zulkarnain, 2017). The active ingredients of natural sunscreens can be obtained from natural ingredients, such as plants, which contain phenolic compounds and

flavonoids. These compounds protect plant tissues from damage from solar radiation (Tristantini et al., 2016). Guava tangkalak (*Bellucia pentamera*) leaves are one of the plants that can be used as a natural antioxidant. Antioxidants can help protect the skin from free radical damage, including free radicals produced by UV light (Windiyanti et al., 2023).

# **RESEARCH METHODS**

#### **Materials and Tools**

The tools used in the research are as follows: Standard glassware (pyrex), spatula, test tube, analytical balance, rotary evaporator (Buchi type R 300+V-300), glass funnel, drop pipette, hotplate, cuvette, and Shimadzu UV-1900 UV-Vis spectrophotometer instrument.

The materials used in this study are guava tangkalak (*Bellucia pentamera*) leaf powder obtained from Penukal Abab Lematang Ilir (PALI), South Sumatra Province. 96% ethanol solvent, distilled water, reagent of Mayer, Wagner, Dragendorff and Lieberman-Burchard, NaCl, Mg powder, hydrochloric acid (HCl), chloroform (CH<sub>3</sub>Cl), ammonium hydroxide (NH<sub>4</sub>OH), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), 1% gelatin, and 1% FeCl<sub>3</sub>.

#### Methods

#### Sample Preparation and Extraction

Guava tangkalak leaves were washed and dried, then mashed and filtered. Guava tangkalak (*Bellucia pentamera*) leaf powder was macerated with 96% ethanol solvent twice (1x24 hour). Next, the extract was concentrated using a rotary evaporator at 50°C.

# **Phytochemical Test**

Flavonoid Test

0,1 g sample extract was added to 10 mL of distilled water and boiled for 5 minutes. The filtrate was added 0.5 gr of mg powder, 1 mL of concentrated HCl, and 1 ml of alcohol, then shaken vigorously. Positive flavonoid reaction is indicated by red/yellow/orange color on the alcohol layer (Nurjannah et al., 2022).

#### Alkaloid Test

One gram of sample extract was dissolved in 10 mL CHCl<sub>3</sub>, added NH<sub>4</sub>OH, and filtered. The filtrate from the CHCl<sub>3</sub> extract was then put into a closed test tube, and 10 pieces of 2M H<sub>2</sub>SO<sub>4</sub> were added while shaking until two layers were formed, namely the acid layer (colorless). Meyer, wagner, and dragendorf reagents were dropped into each test tube to see the color changes. Alkaloid compounds react positively by forming a white precipitate when adding the reagent. Meyer's reagent, a brown precipitate in Wagner's reagent, and an orange-red precipitate in dragendorff's reagent (Membalik et al., 2020) (Fatahillah et al., 2022).

#### Tannin Test

One gram sample extract was added to 10 mL of water and shaken until cold. After that, four drops of 10% NaCl were added and filtered, and the filtrate was divided into two. The first filtrate was given five drops of 1% gelatin, and with a positive reaction, a white precipitate formed, indicating tannin content (Tri Wahyuono & Hidajati, 2020). The second filtrate was given five drops of FeCl<sub>3</sub> 1% with a positive reaction of color change to greenbluish, indicating tannin content (Arnida et al., 2021).

#### **SaponinTest**

One milliliter sample extract was put into a test tube. Five milliliter of water and two drops of HCL 2 N were added and shaken vigorously. The sample is positive for saponins if foam has a lot of intensity and consistency (Suleman et al., 2022).

### Steroid and Triterpenoid Test

Several samples were added to three drops of Lieberman-Burchard reagent. Changes in the sample were observed: a positive reaction of terpenoids if the red or purple color formed, positive steroid formation of brown rings, and green or blue color (Aritonang, 2022).

### **Preparation of Test Solution**

25 mg sample was dissolved with 25 mL of ethanol solvent in a volumetric flask. This solution has a concentration of 1000 ppm as a parent solution. The parent solution was then diluted to 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, and 1000 ppm.

# **Determination of SPF Value**

The SPF (Sun Protection Factor) value was determined by measuring the absorbance of the sunscreen solution using UV-Vis spectrophotometry. Measurements were taken at a wavelength of 290-320 nm, with an interval of 5 nm each time. The SPF value was then calculated based on the Mansur equation, which is as follows:

SPF = CF X 
$$\sum_{290}^{320} X EE(\lambda) X I(\lambda) X Abs(\lambda)$$

Explanation:

CF = Correction Factor (10)

I = Light Intensity Spectrum

EE = Erythema Effect Spectrum

Abs = Absorbance of Sunscreen Samples

While the value of EE X I is constant, where the value has been determined by Sayre in Dutra which can be shown in Table 1 as follows.

Table 1. EE X I Value				
Wavelength (λ)	EE x I			
290	0,0150			
295	0,0817			
300	0,2874			
305	0,3278			
310	0,1864			
315	0,0839			
320	0,0180			
Total	1			

The results of the analysis obtained to determine the level of ability of the sunscreen activity test of the Guavatangkalak leaf extract tested by classifying it based on the ultraviolet index value according to the Food and Drug (FDA) which can be seen in table 2 as follows.

Table 2.SPF Index Value			
SPF Sunscreen Protection Categ			
2-4	Minimal protection		
4-6	Medium protection		
6-8	Ekstra protection		
8-15	Maksimal protection		
≥15	Ultra protection		

## **RESULTS AND DISCUSSION**

Guava Tangkalak is a plant that contains secondary metabolites such as flavonoids, alkaloids, steroids, tannins, and saponins. These compounds have high antioxidant properties, which can help protect the skin from free radical damage. Thus, this tangkalak guava plant has the potential to be an ingredient in sunscreens to protect the skin from UV rays. The dried and ground tangkalak guava leaves were extracted using ethanol solvent after that, evaporated with a rotary evaporator and obtained a thick extract powder of tangkalak guava leaves weighing 2.4117 g with a yield of 8.03% as shown in table 3 as follows.

Table 3. Yield of guava leaf extract Guava Tangkalah Leaf

Simplisia Weight	Condensed Extract Weight	Yield (%)	Ekstract Color
30 gr	2,4117 gr	8,03%	Intense Green

According to the table above, the yield of guava tangkalak leaf extract is 8.039%. This yield falls short of the minimum standard of 10% established by the Indonesian Herbal Pharmacopoeia 2017. Several factors may contribute to this lower yield, including insufficient extraction time. In this study, the extraction time was set at 4 hours, which may not be long enough to fully extract all the beneficial compounds from the guava tangkalak leaves. Despite the yield being below the standard, this study demonstrates that the guava tangkalak leaf extract contains a diverse range of phytochemicals that are beneficial for health.

### Phytochemistry

This phytochemical test aims to determine the content of secondary metabolite compounds contained in guava tangkalak leaf extract. The results of phytochemical tests on guava tangkalak leaf extract show that there are secondary metabolite compounds in flavonoids, alkaloids, tannins, saponins, and steroids, as seen in Table 4.

Phytochemical results of ethanol extract of guava tangkalak have never been reported in research. Plants from the same family (Melastomataceae) as guava tangkalak one of them is water guava (Syzygium aqueum (Burm.f) Alston). The results of water guava phytochemistry have different compounds from the phytochemistry of guava tangkalak. According to Rusydi et al., 2022 water guava contains flavonoids, alkaloids, phenols, tannins, and saponins. These results differ from Table 4, where guava tangkalak is also positive for

steroids, while water guava, a family (Melastomataceae), is negative for the steroid test (Rusydi et al., 2022).

Table 4. Phytochemical content of Guava Tangkalak Leaf Extract test					
Compound Classes	Ethanol Extract Test Results	Phytochemical Results	Positive Reaction		
Flavonoid	(+)Brick red color		Yellow, red or orange		
Alkaloid Mayer	(+) White Precipitate	1. White Precipitate			
Wagner	(+) Brown precipitate	bitate 2. Brown Precipitate			
Dragendorf	(+) Red precipitate	-	3. Red Precipitate		
Tanin	(+) White precipitate (+) Green color		1. White Precipitate 2. Bluish Green		
Saponin	(+) Foaming		Bubble		
Steroid	(+) Brown color	Brown ring, gre or blue ring			
Triterpenoid	(-) Green color	-	Red or purple color		

The plants from the same family generally contain similar secondary metabolites (Yani et al., 2021). This can be seen from the phytochemical test results of the two plants (tangkalak guava and water guava), which gave positive phytochemical test results for flavonoid, alkaloid, tannin, and saponin compounds. Secondary metabolite compounds in plants are produced from the same biosynthetic pathway using primary metabolite precursor compounds, namely carbohydrates. Several groups of secondary metabolites, especially flavonoids, are thought to be found in abundance in this plant.

#### **Sunscreen Activity Test**

Testing the sunscreen activity of the guava tangkalak leaf extract results by screening using a UV-Vis spectrophotometer measured in the wavelength range of 290-320 nm, which is the wavelength of UV-B light which is in the erythmogenic region so that it can cause sunburn. UV-B is a series of harmful rays that can cause damage faster than UV-A, as seen in Figure 1. Absorbance graph of guava tangkalak leaf extract.

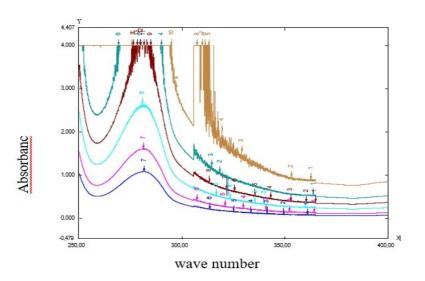


Figure 1. Absorbance Graph of Guava Tangkalak Leaf Extract

Figure 1 above shows the shape of the graph in the concentration of the solution used in the study, namely at a concentration of 400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, and 1000 ppm, which uses 96% ethanol solvent as a diluting solution and standard solution. Furthermore, the solution was measured for absorbance on a UV-Vis spectrophotometer with a 290-320 nm wavelength. The image shows a maximum absorption in the 2700 range and is almost uniform at each concentration variation. This maximum wavelength indicates that chromophores and auxochromes are absorbed in secondary metabolite compounds contained in the extract. Chromophores and auxochromes will provide absorption at UV-VIS light wavelengths. This indicates the compound's ability to absorb UV rays and act as a sunscreen (Sari et al., 2020), where the absorbance data obtained at a wavelength of 290-320 nm can be seen in Table 5 as follows.

Tabl	Table 5. Measurement of absorbance over wavelength 290-320 nm.				nm.		
Abs	Wavelength (nm)						
(ppm)	290	295	300	305	310	315	320
400	0,749	0,446	0,331	0,268	0,245	0,196	0,171
500	1,120	0,676	0,512	0,423	0,381	0,314	0,279
600	1,811	1,110	0,849	0,710	0,642	0,553	0,485
700	2,559	1,538	1,182	0,998	0,896	0,778	0,693
800	4,00	2,118	1,629	1,378	1,326	1,137	0,992
1000	4,00	3,52	2,587	2,172	4,00	1,909	1,774
Average	2,373	1,568	1,182	0,991	1,248	0,814	0,732

From table 5 above, it can be seen that the absorption value of a sample increases with each concentration of the guava leaf sample. Increased levels of flavonoid compounds cause this. Flavonoid compounds are secondary metabolite compounds with an aromatic 2ring structure and abundant chromor. Apart from that, the flavonoid structure is rich in

hydroxyl (OH) groups, causing flavonoids to also be able to absorb a lot of UV light due to the presence of auxochrome in the aromatic group. Tangkalak guava leaves contain various flavonoid compounds such as quercetin, kaempferol, and myricetin. This compound can absorb UV-B rays to protect the skin from damage due to sun exposure. Determination of SPF Value. The obtained absorbance value is then calculated to determine the SPF (Sun Protection Factor) value using the Mansur equation. So, the SPF value is obtained in Table 6 as follows.

Table 6. SPF Value				
Concentration (ppm)	SPF Value	SPF Category		
400	2,958	Minimal		
500	4,602	Minimal		
600	7,693	Ekstra		
700	10,756	Maksimal		
800	15,133	Ultra		
1000	27,408	Ultra		

The table above shows the relationship between the concentration of a substance, the SPF (Sun Protection Factor) value, and the SPF category. The SPF value is used to determine sunscreen activity (Sun Protection Factor) for a measure of a product's ability to protect the skin from ultraviolet B (UVB) rays, which cause sunburn (Hafshah, Rohmah, and Mardliyah 2022). Higher SPF values indicate better protection. The table also shows that higher concentrations of substances result in higher SPF values. This is because the substance absorbs more UVB rays, thus reducing the amount of rays reaching the skin.

#### CONCLUSIONS

Phytochemical tests on tangkalak guava leaf extract show secondary metabolite compounds in flavonoids, alkaloids, tannins, saponins, and steroids. In the Sun Protection Factor (SPF) value test, the SPF values produced at concentrations of 400, 500, 600, 700, 800, and 1000 ppm, SPF values were 2.96, 4.60, 7.70, 10.76, 15.13, 27.41. These values fall into the minimal, extra, maximum, and ultra SPF categories. The maximum concentration of 1000 ppm protects ultra-categories in SPF.

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