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PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY WITH SUN PROTECTION FACTOR VALUE OF RED BETEL LEAF ETHANOL EXTRACT (*Piper ornatum* N. E. Br)

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ABSTRACT

Red betel leaf is a plant that is rich in benefits, one of which is as an antioxidant. The presence of phenolic and flavonoid chemicals in plants provides antioxidant effects. Phenolic compounds also function as protection against UV-B rays; this ability can be seen from the SPF value. This study sought to ascertain the quantity of phenolic and flavonoid constituents as well as the antioxidant and sunscreen properties of red betel leaf ethanol extract. Phenolic content was determined using 10% Follin Ciocalteu reagent and 20% Na_2CO_3.On the other hand, DPPH was used to measure antioxidant activity, while $AlCl_3$ and CH_3COO_{Na} solutions were used to measure flavonoid concentration. In contrast, the ability to protect against sunlight (sunscreen) was determined by determining the sun protection factor (SPF) value. After measurements were carried out using a UV-visible spectrophotometer, the results obtained were a phenolic content of 257.56 mg GAE/g and flavonoids of 46.8008 mg QE/g. At concentrations of 100, 300, 500, 700, and 1000 ppm, respectively, they demonstrated sunscreen action with an SPF value of 5, 17, 28, 36, and 38, and extremely significant antioxidant activity with an IC50 value of 38.9338 ppm. Thus, it can be seen that the ethanol extract of red betel leaves can be used to prevent damage caused by free radicals with very strong capabilities and as an ultra category sunscreen (>15).

Keywords: *Red betel leaf, Piper ornatum, phenol, flavonoid, antioxidant, SPF*

ABSTRAK

Daun sirih merah merupakan tanaman yang kaya akan manfaat, salah satunya sebagai antioksidan. Keberadaan senyawa fenolik dan flavonoid dalam tanaman memberikan efek antioksidan. Senyawa fenolik juga berfungsi sebagai pelindung terhadap sinar UV-B; kemampuan ini dapat dilihat dari nilai SPF. Penelitian ini bertujuan untuk mengetahui kuantitas konstituen fenolik dan flavonoid serta sifat antioksidan dan tabir surya dari ekstrak etanol daun sirih merah. Kandungan fenolik ditentukan menggunakan reagen Follin Ciocalteu 10% dan Na_2CO_3 20%. Di sisi lain, DPPH digunakan untuk mengukur aktivitas antioksidan, sedangkan larutan $AlCl_3$ dan CH_3COONa digunakan untuk mengukur konsentrasi flavonoid. Sebaliknya, kemampuan untuk melindungi terhadap sinar matahari (tabir surya) ditentukan dengan menentukan nilai faktor perlindungan matahari (SPF). Setelah pengukuran dilakukan menggunakan spektrofotometer UV-Vis, diperoleh hasil kandungan fenolik sebesar 257,56 mg GAE/g dan flavonoid sebesar 46,8008 mg QE/g. Pada konsentrasi masing-masing 100, 300, 500, 700, dan 1000 ppm, senyawa ini menunjukkan aktivitas tabir surya dengan nilai SPF 5, 17, 28, 36, dan 38, serta aktivitas antioksidan yang sangat signifikan dengan nilai IC50 sebesar 38,9338 ppm. Dengan demikian dapat diketahui bahwa ekstrak etanol daun sirih merah dapat digunakan untuk mencegah kerusakan akibat radikal bebas dengan kemampuan sangat kuat dan sebagai pelindung sinar matahari kategori ultra (>15).

Kata kunci: Daun sirih merah, *Piper ornatum*, fenol, flavonoid, antioksidan, SPF

INTRODUCTION

One plant that has been extensively used in medicine is the red betel leaf, particularly in Southeast Asia, including Indonesia. The red betel leaf has a round stem, is greenish purple, and has no flowers (Suri et al., 2021). The leaves are heart-shaped with pointed tips, flat leaf edges, and a non-shiny surface. The length of the leaves can reach 15-20 cm, and the upper part is green with a grayish-white pattern, and bright red on the underside of the leaf (Chandran, 2023). Microscopically, red betel leaves have anomocytic stomata and essential oil glands. Red betel leaves have many benefits, including antibacterial for Propionibacterium acne, Staphylococcus aureus, and Staphylococcus epidermidis, antioxidant, antidiabetic, and vaginal discharge.

Red betel leaves contain triterpenoids/steroids, alkaloids, flavonoids, saponins, and tannins, among other chemical substances (Ginting et al., 2021). Flavonoids are substances that give flowers their color and fragrance. They also have antiviral, antibacterial, antiallergic, and anti-inflammatory

properties (Abotaleb et al., 2018). Anthocyanin, a flavonoid derivative molecule, is made up of two groups: anthocyanidin (aglycone), which is a non-sugar group, and glycone, which is a sugar group (Ali et al., 2018). Anthocyanins are the largest group of natural pigments in plants, responsible for giving color to fruits, flowers, and vegetables (Swami et al., 2020). Flavonoids are the largest group of polyphenols and are very effective as antioxidants. Antioxidant properties as free radical scavengers because they contain hydroxyl groups (Yuza et al., 2023). The adage "the higher the total flavonoid content of a material, the higher the antioxidant activity" describes the link between flavonoids and antioxidants (Muflihah et al., 2021).

Free radicals have unstable electrons that seek other molecules to join, causing damage to cells and DNA. One way to prevent the increase in free radicals is to use natural ingredients that contain antioxidants. Antioxidants are compounds that arise from phenolic compounds and have the function of neutralizing free radicals (Pulungan et al., 2022). Due to their molecular makeup, antioxidants can provide electrons to free radicals without interfering with their ability to do so, thereby halting the free radical chain reaction. Foods, plants, or supplements that contain substances like beta-carotene, vitamin C, vitamin E, polyphenol compounds, flavonoids, alkaloids, saponins, and tannins can all provide antioxidants that can help protect against free radical damage (Koomson et al., 2018; Nurmazela et al., 2022)

Flavonoid compounds are one of the antioxidants that can be used to counteract oxidative stress in the body because they have strong antioxidant properties (Adwas et al., 2019). The reaction between flavonoids and free radicals can occur through two main mechanisms, namely quenching and proton transfer. Quenching is a mechanism in which flavonoids can bind free radicals by forming stable compounds, which causes free radicals to be reduced and inactive. In proton transfer, a mechanism in which flavonoids can provide hydrogen from the hydroxyl group to free radicals, causing free radicals to be inactive (Panche et al., 2016).

One of the methods often used to determine antioxidant activity is by using DPPH. DPPH will demonstrate the presence of a bond reaction between the antioxidant compound's hydrogen atom and the free radical's electron compound (diphenyl picrylhydrazine), resulting in the formation of a non-radical compound (diphenyl picrylhydrazine). The reduction of the free radical complex by DPPH is indicated by the color changing from purple to yellow. Another sensitive technique for evaluating antioxidant activity is the DPPH method. The DPPH method is a method that is often chosen because it is simple, easy, sensitive, and only requires a small sample (Ridwanto et al., 2023). Phenolic substances called flavonoids protect DNA from dimerization and damage by preventing cell death and acting as a shield against UV-B radiation (Saewan & Jimtaisong, 2013).

If exposed to UV light in excess, it can have negative consequences on the skin. The adverse effects caused are homeostatic imbalances that cause sunburn and accelerate premature aging (Panich et al., 2016). Sun protection factor (SPF) or sun protection factor (FPM) can be interpreted as an index that is generally used as a measure of the level of effectiveness of protection from sunscreen products (Sultan & Salamah, 2024). The SPF value can be used to determine the activity of sunscreens because the higher the value, the more protection is offered. The length of time that a cosmetic preparation may shield the skin from sunlight is also indicated by the SPF number (Khunkitti et al., 2014). Previous research on the SPF Test of several plants showed a correlation between SPF values and antioxidant activity (Noviardi et al., 2020). Secondary metabolite compounds, including phenols and flavonoids, are also predicted to have a relationship with SPF values and antioxidant activity. The greater the total phenol and total flavonoid content, the IC_{50} value for inhibiting free radicals has a low concentration (very strong antioxidant), and has high protection against UV rays (Aryal et al., 2019). According to the preceding description, researchers are interested in studying the phenolic and

flavonoid content of red betel leaf ethanol extract, as well as its antioxidant activity and sun protection factor value (*Piper ornatum* N. E. Br).

METHOD

The materials utilized in this investigation included ethanol 96%, red betel leaf, gallic acid, Folin-Ciocalteau 10%, Na_2CO_3, quercetin, $AlCl_3$, CH_3COONa, methanol, and DPPH. The tools used in this study were a blender, a porcelain cup, a stir bar, a water bath, a heater, a thermometer, a volumetric flask, and a UV-visible spectrophotometer.

Collection of Red Betel Leaf and Determination

Central Simalanggang City, Payakumbuh District, Lima Puluh Kota Regency, West Sumatra Province, Indonesia, provided the Red betel leaves utilized as study samples. Plant identification was done at the University of North Sumatra's Herbarium Plant Systematics Laboratory (MEDA) with Number 823 / MEDA / 2023. According to the plant determination results, the species of plant that was utilized was *Piper ornatum* N.E.Br.

Making of Simplicia

After removing any debris that may have adhered to the fresh red betel leaves, they are rinsed under running water and drained. The sample is then placed in a drying cabinet coated with parchment paper to dry completely. Then the sample is ground using a crusher or blender. The simplicial powder is stored in a sealed container protected from direct sunlight (Syahputra et al., 2021).

Extract preparation

Red betel leaf powder is extracted using 96% ethanol solvent by maceration. This process uses 10% red betel leaf powder. 75 parts of solvent liquid are added to 10 parts of simplicial powder in a closed jar, and the mixture is left for 5 days with periodic stirring. The dregs are then cleaned with the solvent liquid till 100 parts of macerate are achieved. Store in a covered container away from direct sunlight for two days, and then pour or filter. The macerate obtained is then evaporated below its boiling point until a thick extract is obtained (Rafita et al., 2022).

Determination of phenolic compound levels

Assessment of the amounts of phenolic compounds is carried out in several stages:

Preparation of Gallic Acid Stock Solution

1000 µg/ml of gallic acid is added to methanol to create the stock solution.

Determination of the Maximum Wavelength of Gallic Acid

A gallic acid solution with a concentration of 300µg/mL is then created from the standard gallic acid stock solution, which has a concentration of 1000 µg/mL. After that, 7.9 mL of Aquadest, 0.5 mL of 10% Folin-Ciocalteau reagent, and 0.1 mL of 300µg/mL gallic acid solution are combined, and 1.5 mL of 20% Na_2CO_3 is added. A UV-visible spectrophotometer is used to measure the highest wavelength in the 400–800 nm region after the solution has been incubated for 90 minutes.

Operating Time (OT) Measurement

A test tube was filled with 0.1 mL of a 300µg/mL gallic acid solution. added 1.5 mL of 20% Na_2CO_3 after vortexing for one minute with 7.9 mL of distilled water and 0.5 mL of 10% Folin Ciocalteau reagent. The solution's absorbance was measured for 50 minutes at a wavelength between 400 and 800 nm for one minute. The operating duration was determined when the solution started to exhibit stable absorbance.

Determination of the Calibration Curve of Gallic Acid

Concentrations of 100, 200, 300, 400, and 500 µg/mL of gallic acid solution were utilized; 0.1 mL of each concentration was pipetted, combined with 7.9 mL of purified water, 0.5 mL of Folin-Ciocalteau

reagent, and then 1.5 mL of 20% Na_2CO_3. After 90 minutes of incubation, the maximum wavelength of the solution was measured with a UV-visible spectrophotometer in the 400–800 nm range. Gallic acid's linear regression line equation, y = ax + b, and calibration curve were acquired.

Determination of Phenolic Compound Content
A 10 mL flask was filled with methanol once the extracted sample had been weighed up to 10 mg. Following that, 0.3 mL was transferred to a test tube, which was then filled with 7.9 mL of distilled water, 0.5 mL of 10% Follin-Ciocalteu reagent, and 1.5 mL of 20% Na_2CO_3. Aluminum foil was then used to cover the tube, and it was incubated for 90 minutes. After that, each sample's absorbance was measured six times using a UV-Vis spectrophotometer during working (or operating) hours. The highest wavelength was then recorded.

Determination of flavonoid compound levels
Determination of flavonoid compound levels is carried out in several stages:
Preparation of Quercetin Standard Mother Solution
A concentration of 100 parts per million in ethanol is used to create the quercetin standard mother solution.

Quercetin Maximum Wavelength
Pipette Determination 0.1 mL $AlCl_3$, 0.1 mL CH_3COONa, 2.8 mL of aqua dest, 0.6 mL of 100 ppm quercetin standard mother solution, and adequate ethanol are added. The mixture is then incubated for 25 minutes. A UV-Vis spectrophotometer is used to measure the maximum wavelength, which falls between 400 and 800 nm.

Calculating Operating Time (Working Time)
A 10 mL measuring flask containing 0.6 mL of a 100 ppm quercetin standard solution, 0.1 mL $AlCl_3$, 0.1 mL CH_3COONa, 2.8 mL of distilled water, and ethanol is then incubated for 25 minutes. A spectrophotometer was used to measure the absorbance at a maximum wavelength of 429 nm.

Making a Calibration Curve
In each 10 mL measuring flask, add 0.5 mL of quercetin solution at concentrations of 2, 3, 4, 5, and 6 ppm. Then, add 0.1 mL of $AlCl_3$, 0.1 mL of CH_3COONa, 2.8 mL of distilled water, and ethanol. Incubate for 25 minutes. At a maximum wavelength of 429 nm, the absorbance of each concentration was measured with a UV-Vis spectrophotometer. The linear regression line equation, Y=ax+b, and the quercetin calibration curve were acquired.

Determination of Flavonoid Compound Levels
A 10 mL measuring flask was filled with ethanol once the extracted material had been weighed up to 10 mg. A 10 cc measuring flask was filled with 1 cc of the sample solution. Next, add 2.8 mL of Aquadest, 0.1 mL of $AlCl_3$, 0.1 mL of CH_3COONa, and enough ethanol, and mix until everything is uniform. For half an hour, the solution was exposed to the elements. After the operating period was reached, the absorbance of the sample solution was measured six times using a UV-Vis spectrophotometer set to its maximum wavelength.(Yuza et al., 2023).

Antioxidant activity measurement
Preparation of 0.5 mM DPPH solution (200 ppm):
DPPH solution with a concentration of 200 ppm in methanol was prepared.

Measurement of the Maximum Wavelength of DPPH
In a 10 mL volumetric flask, a 200 ppm DPPH solution was converted to a 40 ppm DPPH solution. A UV-Vis spectrophotometer was used to detect the maximum wavelength (400 nm to 800 nm).

Calculating the Operating Time

After that, the 40 ppm DPPH solution was monitored for 60 minutes at the Maximum Wavelength until a steady absorbance was achieved.

Preparation of Sample Solutions and measurement of absorbance

Vitamin C Reference Standard: A solution containing 100 parts per million of vitamin C in methanol was made. In a 10 mL volumetric flask, solutions with concentrations of 1, 2, 3, 4, and 5 ppm were made from this solution. Following this, methanol solvent was added to the mark line, and 2 mL of DPPH solution (concentration 200 ppm) was added to each measuring flask. At the selected maximum wavelength, the absorbance was measured.

Extract: To create a solution with a 1000 ppm concentration, 100 milligrams of thick extract was weighed and diluted in 100 mL of methanol. The concentrations of 10, 20, 30, 40, and 50 ppm were generated by taking 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL, adding 2 mL of DPPH solution (concentration 200 ppm), and then adding methanol to the mark line (10 mL measuring flask). After 30 minutes of incubation, the absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer.

Determination of DPPH Free Radical Trapping Process: The following formula is used to determine the free radical trapping process by test samples using the DPPH free radical trapping method:

$$\text{Absorption Activity (\%)} = \frac{Abs.\ control - Abs.\ sample}{Abs.\ control}\ x\ 100\ \%$$

Description:
Abs. control = Absorptive material is absent.
Abs. sample = sample absorbance

Determination of sunscreen activity

100 ppm, 300 ppm, 500 ppm, and 700 ppm ethanol extract solutions were prepared in 96% ethanol. A UV-visible spectrophotometer was used to detect the absorbance at a wavelength of 320-290 nm with a 5-nm interval, using ethanol as a blank. The calculation of the SPF value follows the Mansur equation (1986). The equation is

$$\text{SPF} = CF\ x \sum_{290}^{320} EE\ (\lambda) x\ I\ (\lambda) x\ A\ (\lambda)$$

The correction factor, or CF, has a value of 10, EE: The wavelength-dependent erythmogenic action of radiation (λ), I: Solar radiation simulation spectrum (λ), A: Wavelength-dependent absorbance value (λ)

RESULTS AND DISCUSSION

Extract preparation

The results of making the extract by maceration obtained a thick reddish brown extract with an extract yield of 10.16%. Maceration is one of the processes of extracting chemical compounds that can be used for leaf simplicia. In order to extract chemicals using the maceration method, the solvent liquid is first poured through the cell wall into the cavity of plant cells that contain active substances. Because of the difference in concentration between the active substance solution inside and outside the cell, the concentrated solution is then forced out of the cell cavity. This process is repeated until the concentrations of the solutions inside and outside the cell are balanced. (Rochani 2009 in Hujjatusnaini, N et al. 2021).

Results of phenolic content determination

Gallic acid standards were used to determine the phenolic concentration at the maximal wavelength and working time. Figure 1 displays the findings of calculating the maximum wavelength of gallic acid, and Table 1 displays the results of calculating the working time.

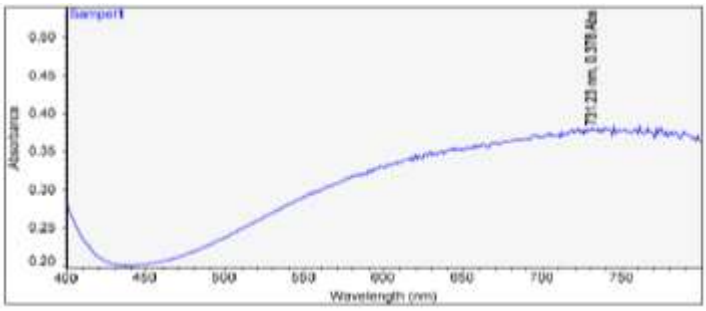

Figure 1. Maximum wavelength of gallic acid

The wavelength is the maximum wavelength, as shown in Figure 1 above, the highest absorption (0.378) is reached at 731.23 nm. The wavelength that offers the most absorption is known as the maximum wavelength. Table 1 and Figure 2 display the outcomes of the working time determination.

Table 1. Results of determining the working time (operating time) of gallic acid

Times (minute)	Absorbance	Times (minute)	Absorbance	Times (minute)	Absorbance
1	0.239	16	0.235	31	0.232
2	0.238	17	0.235	32	0.232
3	0.239	18	0.234	33	0.231
4	0.238	19	0.235	34	0.231
5	0.238	20	0.235	35	0.232
6	0.237	21	0.234	36	0.231
7	0.237	22	0.234	37	0.231
8	0.237	23	0.234	38	0.231
9	0.236	24	0.234	39	0.231
10	0.237	25	0.233	40	0.230
11	0.237	26	0.233	41	0.231
12	0.236	27	0.233	42	0.230
13	0.236	28	0.233	43	0.230
14	0.236	29	0.232	44	0.229
15	0.235	30	0.232	45	0.230

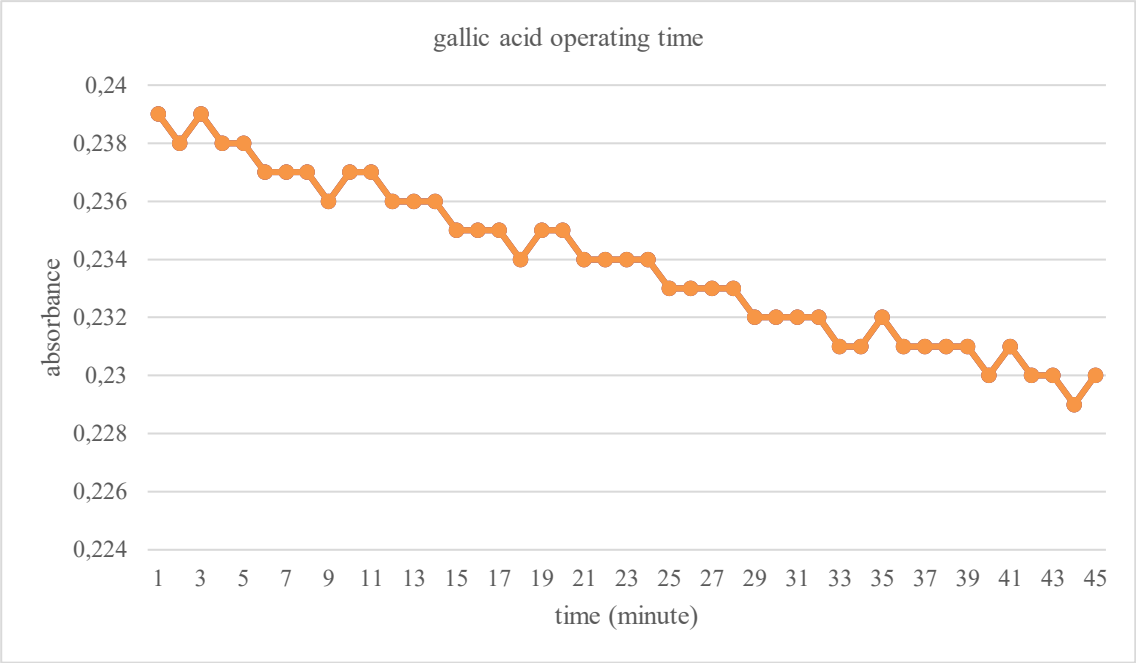

Figure 2. **Operating time graph of gallic acid**

It is evident from the data in Table 1 and Figure 2 that gallic acid's working period was measured between minutes 21 and 24. Working time is the best working time that provides the most stable absorption. The measurement time from minute 21 to minute 24 is the time that provides the most stable absorption, so this time is the best working time for gallic acid. The calibration curve was made at the Wavelength and working time of gallic acid. Table 2 displays the findings of the measurements of gallic acid absorption, and Figure 3 displays the calibration curve.

Table 2. Results of gallic acid absorbance measurements

Concentration (mcg/ml)	Absorbance
0	0.002
100	0.171
200	0.285
300	0.411
400	0.581
500	0.686

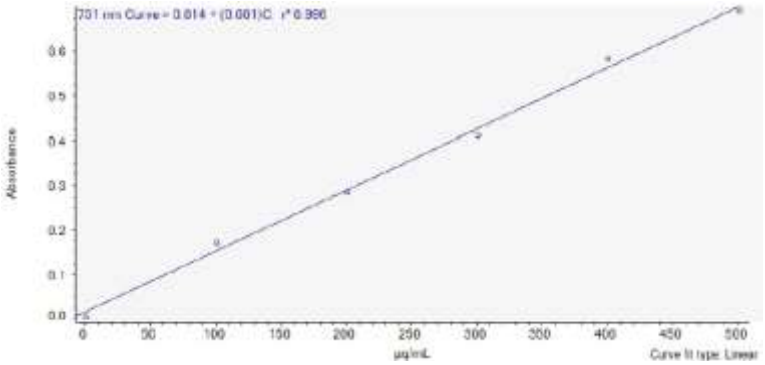

Figure 3. Calibration curve of gallic acid

The regression equation was derived from a study of the absorbance data. This yields the regression equation y = 0.001X + 0.014. The amounts of phenolic chemicals in the ethanol extract of red betel leaves are determined using this regression equation..

The total phenol content was then determined by entering the absorbance value of the red betel leaf ethanol extract into the regression equation, as indicated in Table 3.

Table 3. Results of calculating the phenol content of the ethanol extract of red betel leaves

Repetition	Absorbance	Concentration (x) (ppm)	Concentration (x) (mg/g)	Phenol content (mg GAE/g)
1	0.271	257.00	0.257	257.00
2	0.271	257.00	0.257	257.00
3	0.273	259.00	0.259	259.00
4	0.272	258.00	0.258	258.00
5	0.272	258.00	0.258	258.00
6	0.271	257.00	0.257	257.00
Average				257.56

After six repetitions, the average amount of phenolic components in the red betel leaf ethanol extract was 257.56 mg GAE/g, as shown in the above table.

Results of flavonoid content determination

Using quercetin standards, flavonoid levels were determined at the maximal wavelength and working time. Figure 6 displays the findings of the investigation on quercetin's maximum wavelength.

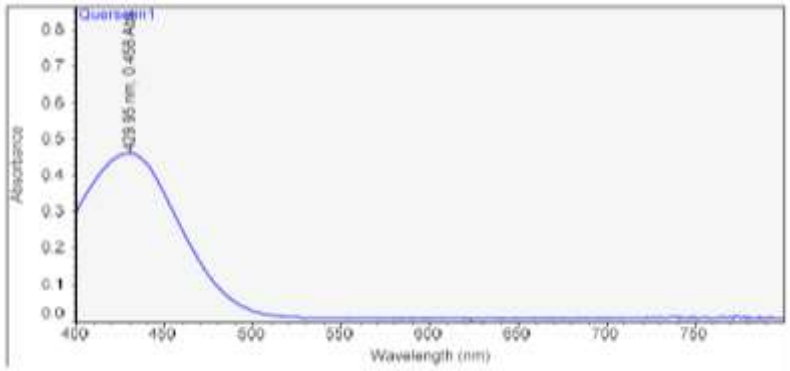

Figure 6 Maximum wavelength of quercetin standard

As can be observed from Figure 6 above, the wavelength is the highest wavelength since the maximum absorption (0.458) is attained at 429.95 nm. The wavelength that offers the most absorption is known as the maximum wavelength. The maximal wavelength is obtained, and then quercetin's operating time is calculated. Table 4 and Figure 7 display the operating time determination results.

Table 4. Results of determining the operating time of quercetin

Time (minutes)	Absorbance	Time (minutes)	Absorbance	Time (minutes)	Absorbance
1	0.458	16	0.461	31	0.475
2	0.458	17	0.462	32	0.475
3	0.458	18	0.463	33	0.475
4	0.458	19	0.464	34	0.475
5	0.458	20	0.465	35	0.475
6	0.459	21	0.466	36	0.476
7	0.459	22	0.467	37	0.476
8	0.459	23	0.468	38	0.477
9	0.459	24	0.469	39	0.477
10	0.459	25	0.47	40	0.478
11	0.459	26	0.471	41	0.478
12	0.461	27	0.472	42	0.478
13	0.461	28	0.472	43	0.479
14	0.46	29	0.473	44	0.48
15	0.461	30	0.474	45	0.48

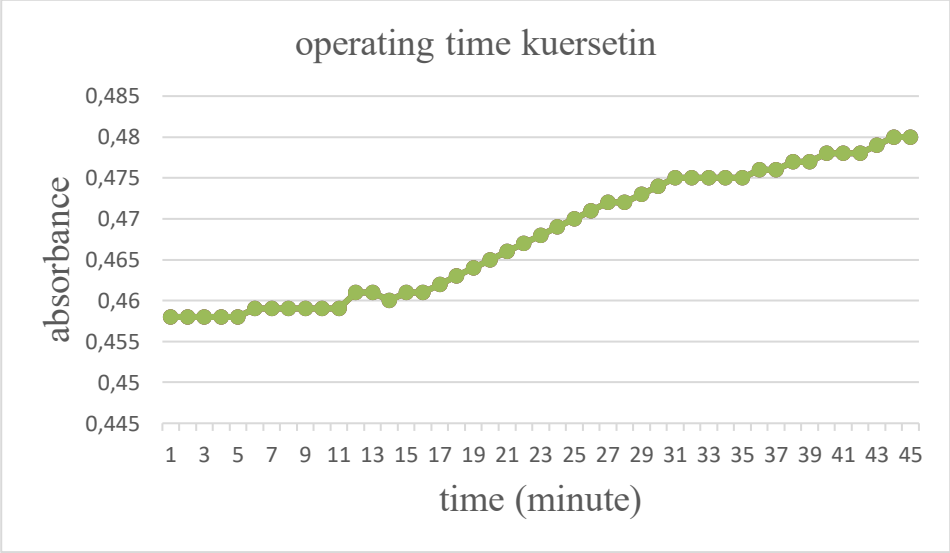

Figure 7. Operating time graph of quercetin

Because quercetin provided the most consistent absorption at those minutes, the working time of quercetin was determined to be between minutes 6 and 11, as shown by the results in Table 4 and Figure 7. The calibration curve was created using quercetin's wavelength and working time. Table 5 displays the quercetin absorption measurement findings, and Figure 8 displays the calibration curve.

Table 5. Results of quercetin absorbance measurements

Concentration (mcg/ml)	Absorbance
0	0.000
2	0.186
3	0.273
4	0.357
5	0.449

6	0.520

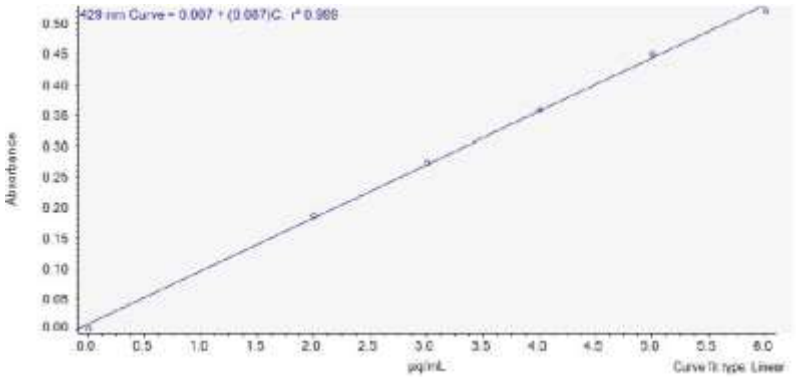

Figure 8. Quercetin calibration curve

From the absorbance data obtained, an analysis was carried out to obtain the regression equation. The regression equation obtained was y = 0.0872X + 0.007. The flavonoid content was determined using the regression equation that was derived. The flavonoid content was then determined by entering the absorbance value into the regression equation, as indicated in Table 6.

Table 6. Results of calculating the flavonoid content of the ethanol extract of red betel leaves

Repetition	Absorbance	Concentration (x) (ppm)	Concentration (x) (mg/g)	Flavonoid levels (mg QE/g)
1	0.0415	4.6897	0.00469	46.8966
2	0.0415	4.6897	0.00469	46.8966
3	0.0414	4.6782	0.0047	46.7816
4	0.0414	4.6782	0.0047	46.7816
5	0.0413	4.6667	0.00468	46.6667
6	0.0414	4.6782	0.0047	46.7816
Average				46.8008

The ethanol extract of red betel leaves after six repetitions had an average flavonoid content of 46.8008 mg QE/g, as shown in the above table. A quercetin solution is used to determine the total flavonoid content. Quercetin is included in the compounds with the highest potential in the phenolic group (Prakash et al., 2013).

Visible Spectrophotometry is used to determine the flavonoid concentration. The Visible Spectrophotometry method is used to measure the absorbance of a sample at a certain wavelength. This method is based on measuring light energy by a chemical substance at its maximum wavelength. UV light has a wavelength of between 200 and 400 nm, and visible light has a wavelength of 400-750 nm. This method refers to the quantitative determination of a substance with the Lambert-Beer law as the basis. This law states a directly proportional relationship between absorbance and the concentration of the analyte solution and is inversely proportional to transmittance.

Results of Antioxidant Activity Determination

As seen in Figure 9, the antioxidant activity of the red betel leaf ethanol extract was measured at the highest wavelength possible, 515 nm.

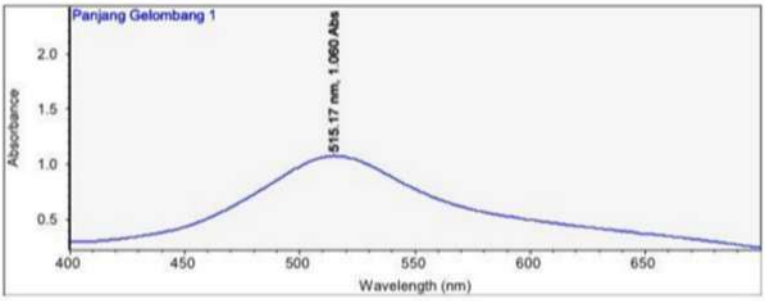

Figure 9. Results of determining the maximum DPPH wavelength

The wavelength of 515 nm is utilized as the maximal wavelength to assess antioxidant activity since Figure 9 shows that this wavelength has the highest absorption of DPPH (1,060). The DPPH working time is established when the maximum wavelength has been obtained. Table 7 displays the working time measurement findings, and Figure 10 displays the graph.

Table 7.Findings from calculating the DPPH's operating time

Time (minutes)	Absorbance	Time (minutes)	Absorbance	Time (minutes)	Absorbance
1	0.967	16	0.897	31	0.866
2	0.961	17	0.894	32	0.864
3	0.952	18	0.892	33	0.863
4	0.946	19	0.889	34	0.863
5	0.941	20	0.887	35	0.860
6	0.935	21	0.886	36	0.859
7	0.930	22	0.884	37	0.858
8	0.926	23	0.881	38	0.858
9	0.922	24	0.878	39	0.856
10	0.917	25	0.876	40	0.855
11	0.913	26	0.874	41	0.853
12	0.910	27	0.872	42	0.852
13	0.905	28	0.871	43	0.852
14	0.902	29	0.869	44	0.851
15	0.900	30	0.867	45	0.851

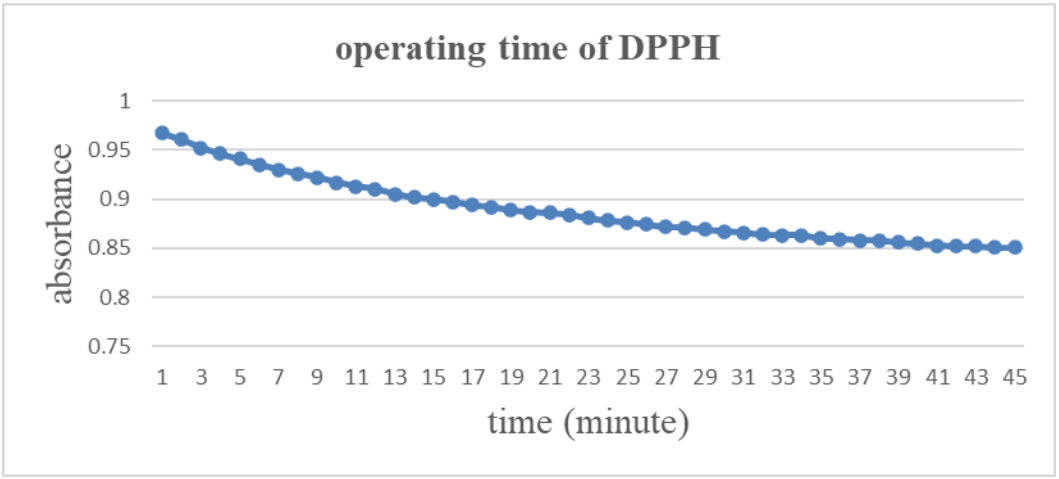

Figure 10. Operating time graph of DPPH

Absorbance measurements can be performed between 0 and 45 minutes after the sample reacted with DPPH, since Table 7 and Figure 10 show that there was no stable absorption from the first to the 45th minute.

Then the calibration curve was determined. The absorbance value obtained was used to determine the percentage of attenuation, which was then used to obtain a linear equation. Table 8 displays the findings of DPPH absorbance measurements following the addition of red betel leaf ethanol extract, as well as the results of determining the attenuation percentage.

Table 8. Data from absorbance measurements and % inhibition

Concentration (ppm)	Absorbance (Abs)			Average Abs	% Inhibition
	1	2	3		
DPPH	0.917	0.917	0.917	0.917	0
10	0.823	0.821	0.82	0.8213	10.4326
20	0.677	0.675	0.675	0.6757	26.3177
30	0.535	0.534	0.534	0.5343	41.7303
40	0.437	0.437	0.437	0.4370	52.3446
50	0.351	0.351	0.351	0.3510	61.7230

It is evident from Table 8 above that the inhibition percentage increases with decreasing absorbance. The ethanol extract of red betel leaves is an oxidizer, and the absorbance value measured here is the DPPH absorbance value after reacting with it as an antioxidant. The inhibition percentage is the ethanol extract's capacity to prevent the oxidation process that free radicals, in this case DPPH, carry out. From the absorbance measurement data and the inhibition percentage calculation above, a linear equation is obtained, namely Y = 1.2861x-0.0727.
The IC_{50} value (antioxidant activity) is calculated using the derived linear equation. The concentration of ethanol extract needed to 50% prevent oxidation by free radicals is known as the IC_{50} value. The ethanol extract of red betel leaves has an IC50 value of 38.9338 ppm, meaning that it may block the oxidation process by free radicals by 50% at this concentration, which falls into the category of extremely strong activity.

Results of Sunscreen Activity Determination

Red betel leaf ethanol extract was tested at concentrations of 100, 300, 500, 700, and 1000 ppm to determine its SPF value. To ascertain the sample's capacity and efficacy as a shield against free radicals, such as those produced by sun exposure, the SPF value test was conducted. The more successful the sample is at shielding the skin from the sun, the higher the SPF number it produces. Table 9 displays the findings of the absorbance measurement of the red betel leaf ethanol extract at a wavelength of 320–290 nm.

Table 9.Findings from absorbance tests using an ethanol extract of red betel leaves at 320–290 nm

Concentration (ppm)	Repetition	Wavelength (nm)/absorbance						
		290	295	300	305	310	315	320
100	1	0.680	0.623	0.585	0.568	0.570	0.570	0.568
	2	0.679	0.622	0.585	0.568	0.570	0.570	0.558
	3	0.680	0.622	0.585	0.568	0.570	0.570	0.558
	Average (A)	0.680	0.622	0.585	0.568	0.570	0.570	0.561
300	1	2.211	2.030	1.909	1.852	1.039	1.855	1.809
	2	2.214	2.032	1.013	1.853	1.040	1.857	1.812

	3	2.215	2.036	1.910	1.855	1.041	1.857	1.816
	Average (A)	2.213	2.033	1.911	1.853	1.040	1.856	1.812
500	1	3.259	3.041	2.872	2.768	2.774	2.791	2.704
	2	3.259	3.049	2.891	2.790	2.768	2.787	2.727
	3	3.223	3.051	2.884	2.767	2.797	2.795	2.685
	Average (A)	3.247	3.047	2.822	2.775	2.779	2.791	2.705
700	1	3.833	3.875	3.690	3.720	3.663	3.678	3.592
	2	3.832	4.224	3.900	3.710	3.586	3.699	3.501
	3	3.806	3.816	3.746	3.634	3.611	3.689	3.524
	Average (A)	3.824	3.935	3.779	3.688	3.620	3.355	3.539
1000	1	3.983	3.928	3.861	3.788	3.865	3.649	3.71
	2	3.808	3.869	3.867	3.752	3.738	3.743	3.761
	3	3.944	4.004	3.840	3.827	3.707	3.754	3.408
	Average (A)	3.912	3.934	3.856	3.789	3.770	3.715	3.627

The average absorbance at a wavelength of 320-290 nm is visible from the data in the above table. The Mansur equation is used to calculate the SPF value based on the absorbance values. Table 10 displays the findings of calculating the SPF value of the red betel leaf ethanol extract.

Table 10.Results of determining the SPF value of red betel ethanol extract

Concentration (ppm)	Value SPF	Category
100	5,795340333	Extra
300	17,381415	Ultra
500	28,36663267	Ultra
700	36,93742167	Ultra
1000	38,10022633	Ultra

The ethanol extract of red betel leaves at a concentration of 100 ppm has an SPF value of 5,79 with an additional category, according to Table 10. At concentrations of 300, 500, 700, and 1000 ppm, the SPF values are 17.38, 28.36, 36.93, and 38.1 with an ultra category (> 15). By preventing UV-B rays from reaching the skin, red betel leaf extract can shield the skin from sun exposure, as indicated by this SPF rating.

CONCLUSIONS AND SUGGESTIONS

From the data obtained, it can be concluded that red betel leaf extract contains phenol of 257.56 mg GAE/g, flavonoid of 46.8008 mg QE/g and has very strong antioxidant activity with an IC_{50} value of 38.9338 ppm, and has sunscreen activity with an SPF value of 5, 17, 28, 36 and 38 at concentrations of 100, 300, 500, 700 and 1000 ppm respectively. We hope that future researchers can formulate an ethanol extract of red betel leaves into a safe cosmetic product that can protect the skin from the harmful effects of sunlight.

ETHICAL CONSIDERATIONS

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There was no specific grant from any financial organization for this research.

Conflict of Interest Statement

About this article, we certify that there are no actual or possible conflicts of interest.

This research is expected to help the public in using cosmetics that are safe because they are derived from plant materials so that they can avoid the dangers of chemical/synthetic materials which, with long-term use, can damage the skin and cause cancer, and can protect the skin from the bad effects of sunlight due to the formation of free radicals which cause tissue damage.

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