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Research Article

Antioxidant Activity of Dechlorophyllation of Kejibeling Leaf (*Strobilanthes crispu* L.) Extract Using the Free Radical Reduction Method

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ABSTRACT: Kejibeling leaves *(Strobilanthes crispus* L.) from the *Acanthaceae* family is a medicinal plant that has medicinal properties. This research aims to determine and determine the antioxidant activity of the dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.*)* based on the IC_{50} value with the DPPH method. Kejibeling leaf extract *(Strobilanthes crispus* L.) extracted by soxhletation method, wherein the extraction process uses two types of solvents of different polarity, to attract chlorophyll compounds and antioxidant compounds, the percent yield of chlorophyll extract is obtained 0.0352% and percent yield of dechlorophyllation ethanol extract 9.604%. Then tested the antioxidant activity using the DPPH method. The results showed that there was antioxidant activity with an IC_{50} value of 106.375 µg/mL, that is, the result shows moderate antioxidant activity, while quercetin as a comparison has very strong antioxidant activity with a value of 1.391 µg/mL.

KEYWORDS: Antioxidants; dechlorophyllation; DPPH; kejibeling leaves; UV-Vis Spectrophotometry

1. INTRODUCTION

An unhealthy lifestyle and the impact of a bad environment can cause the immune system to become unbalanced, causing several diseases caused by free radicals. Free radicals can come from two sources, namely the rest of the body's metabolism and several factors from outside the body such as dietary factors, air pollution, cigarette smoke and ultraviolet light. This can trigger the formation of free radicals in the human body (Bherawi., 2017).

Free radicals are molecules that contain one or more unpaired electrons. The presence of an unpaired electron causes the compound to be very reactive looking for a partner by binding to the electrons of the molecules around it (Fawwaz et al., 2023). Free radicals can be reduced by giving antioxidants (Yuslianti, 2018)

Antioxidants are electron donor compounds that are used to prevent the formation of free radicals in the body by binding to free radical molecules. The presence of these antioxidant compounds can neutralize the formation of free radicals in the body so that they can inhibit cell damage (Salamah & Widyasari, 2015).

Many plants have the potential to have antioxidant activity, one of which is the kejibeling plant (*Strobilanthes crispus* L.). kejibeling (*Strobilanthes crispus* L.) is one of the tropical plants which can be found quite a lot in various places and has great potential as an antioxidant, because it contains chemical compounds, namely: potassium, sodium, calcium, silicic acid, alkaloids, saponins, flavonoids, and polyphenols. In traditional medicine systems, plant parts such as leaves, stems and roots can be used for different treatments. Kejibeling leaves (*Strobilanthes crispus* L.) are known to have pharmacological benefits for the treatment of kidney stones, gallstones, cholesterol, tumors, and it has a hypoglycemic effect so it is efficacious as an antidiabetic (Banjarnaho & Artanti, 2014).

To optimize the compound content in kejibeling leaves (*Strobilanthes crispus* L.) extraction can be carried out by soxhletation. Soxhletation extraction is a solvent extraction that is carried out with a special tool so that constant extraction occurs by back-cooling (condenser). The extracting liquid that is in the heated flask will evaporate and condense and then soak the solid so that the extraction process occurs, with the appropriate solvent, namely chloroform and 96% ethanol. It is very important to select the appropriate solvent for the extraction process in order to provide optimal results (Wewengkang & Rotinsulu, 2021).

Chloroform solvent was chosen because it is a good solvent for extracting non-polar compounds because it has advantages including because this solvent is relatively stable, easy to evaporate and selective in dissolving substances such as chlorophyll compounds which are known to dissolve in chloroform so that they can attract green dyes from kejibeling leaves to obtain chloroform extract, so that they are used in extraction by dechlorophyllation. Dechlorophyllation is the separation of chlorophyll in plants which contain pigments in the form of chlorophyll or green components found in the leaves and stems of plants (Pebriana, et al., 2017). The purpose of dechlorophyllation is to get pure natural compound components free from other chemical components that are not needed. chemical components in the extract that are not needed such as lipids, pigments (chlorophyll), tannins and carbohydrates. Another goal is to maintain some of the chemical constituents of the extract which have a synergistic effect so that they can maximize the treatment process because in some cases, the chemical components that have been isolated actually show a decreased effect (Malik et al, 2014). The 96% ethanol solvent was chosen because it has both polar and nonpolar groups so it can

dissolve polar, nonpolar and semipolar chemical compounds (Adibi, 2017) such as steroidal flavonoids, alkaloids and fatty acids. 96% ethanol solvent is known to be able to attract compounds from the ethanol extract to determine the presence of antioxidant compounds (Wardatun, 2011).

The most frequently used method for antioxidant testing is the 1,1 diphenyl -1-picrylhidrazil (DPPH) method. Where antioxidants react with DPPH which stabilizes free radicals and reduces DPPH. Then DPPH will react with hydrogen atoms to reduce free radicals to form DPPH due to the pairing of electrons and it becomes more stable. Antioxidant activity test using the DPPH method is one method to find out how much antioxidant activity is in kejibeling leaves (*Strobilanthes crispus* L.) (Utomo et. al., 2012). Based on the above, this research was carried out, testing the antioxidant activity of the dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) Using the DPPH method.

2. EXPERIMENTAL SECTION

2.1. Population and Sample

The population of this study is kejibeling leaves (*Strobilanthes crispus* L.). The sample used was the leaves of vile shard (*Strobilanthes crispus* L.) obtained from Bone District, Patimpang District, Masago Village, Indonesia.

2.2. Material and tools

The materials used in this study are aluminum foil, DPPH (*1,1 Diphenyl -2- Picrylhdrazil*), quercetin, 96% ethanol, 75% ethanol, methanol pa and dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) while the tools used were glassware (Pyrex, Japan), a set of soxhletation instruments, micropipette, UV-Vis spectrophotometer, analytical balance.

2.3. Dechlorophyllation and extraction

50 g of simplicia powder leaves (*Strobilanthes crispus* L.). extracted by means of soxhletation using chloroform solvent for 8 hours at 50 °C. The extract obtained was evaporated using a rotary evaporator. Furthermore, the extraction was continued using 96% ethanol for 8 hours at 500 °C, the extract obtained was evaporated using a rotary evaporator. The chloroform extract was analyzed using a UV-Vis spectrophotometer to see the absorbance of green pigment at wavelengths (665-666 nm) and yellow pigment (408-410 nm). The ethanol extract was then analyzed for its antioxidant activity using a UV-Vis spectrophotometer with the DPPH method (Kouar et al. 2018).

2.4. Determination of maximum wavelength of DPPH

1000 ppm DPPH solution was prepared by weighing 5 mg of DPPH powder, then dissolved with 5 mL of methanol solvent pain a pumpkin. A concentration of 50 ppm was made by pipetting 2.5 mL of 1000 ppm DPPH, then the volume was made up to 50 mL. Measurement of the maximum wavelength was carried out by measuring the DPPH solution which had been incubated for 30 minutes at room temperature in a dark place and measured on a UV-Vis spectrophotometer at a wavelength of 400-800 nm and a maximum wavelength of 514 nm was obtained (Fawwaz et al., 2020).

2.5. Determination of quercetin antioxidant activity

The stock solution was prepared at a concentration of 1000 ppm by weighing 5 mg of quercetin and dissolved in 5 mL of methanol pa and then homogenized. then a 1 mL pipette dilution was carried out and then diluted with methanol pa up to 10 mL (100 ppm). Furthermore, dilution was carried out by making 5 variations of concentration 1, 2, 3, 4, and 5 ppm. To determine the antioxidant activity of each concentration, 1 mL of sample solution was pipetted and 4 mL of 50 ppm DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in the dark, the absorption was measured with a UV-Vis spectrophotometer at a wavelength of 514 nm.

2.6. Determination of antioxidant activity of extract

The stock solution was prepared by weighing 50 mg of dechlorophyllation ethanol extract and then dissolving it with 10 mL of methanol pa in a volumetric flask. Next, dilution was carried out by making 5 concentration variations 50, 100,150, 200 and 250 ppm. To determine the antioxidant activity of each concentration, 1 mL of sample solution was pipetted and 4 mL of 50 ppm DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in the dark, the absorption was measured with a UV-Vis spectrophotometer at a wavelength of 514 nm.

2.7. Data analysis

The antioxidant activity of the samples was determined by the magnitude of the DPPH free radical uptake inhibition by calculating the percentage (%) of DPPH uptake inhibition using the formula (Melyneux, 2004).

$$\%\ Inhibition\ = \frac{Blank\ absorbance - Sample\ absorbance}{Blank\ absorbance}\ X\ 100$$

The IC_{50} value was calculated using the linear regression equation, sample concentration as the x-axis and % inhibition as the y-axis. From the equation: y = a + bx. Where y = inhibitor of 50% oxidation, x = IC_{50} number indicating the concentration of the extract capable of inhibiting the oxidation process by 50%, a = slope, and b = intercept. The IC_{50} value can be calculated using the formula:

$$IC_{50} = \frac{(50-b)}{a}$$

3. RESULTS AND DISCUSSION

Quantitative analysis of the absorption measurement of kejibeling leaf chloroform extract and determination of the antioxidant activity of the dechlorophyllation ethanol extract of kejibeling leaves using the DPPH method were analyzed using the UV-Vis spectrophotometer method. This method was chosen because it can be used for the analysis of a substance in small quantities, the process is easy, simple, quite sensitive and selective and also has the sensitivity to analyze a substance in small quantities.

In measuring the absorption of kejibeling leaf chloroform extract (*Strobilanthes crispus* L.) analysis was carried out using a UV-Vis spectrophotometer to see the absorbance of the green pigment at a wavelength of 665-666 nm and the yellow pigment at 408-410 nm. The measurement results can be seen in **Table 1**.

Table 1. The absorption of kejibeling leaf chloroform extract (*Strobilanthes crispus* L.)

Plant	Green pigment	Yellow pigment
Kejibeling leaves (*Strobilanthes crispus* L.)	0.114	1.048

Based on the results of measuring the absorption of kejibeling leaf chloroform extract (*Strobilanthes crispus* L.) **Table 1** shows a green pigment absorption of 0.114 and a yellow pigment absorption of 1.048. From these data indicate that the amount of chlorophyll compounds that can be extracted. According to research (Jihane Kouar, 2018) regarding the comparison of the electrocoagulation dechlorophyllation method and solvent extraction, it shows that the higher the absorbance value, the more chlorophyll content in the extract. Therefore, the electrocoagulation method is a suitable method for removing chlorophyll pigments because the absorbance measurement results are much lower compared to solvent extraction. According to research (Pebriana, 2017) the electrocoagulation process showed that the longer the electrocoagulation duration, the lower the value of the chlorophyll compounds obtained, but the lower the levels of phenolic compounds which are one of the antioxidant compounds. Solvent extraction in dechlorophyllation is an easy extraction to apply, the solvent is used in small quantities, although in solvent extraction the results obtained are less than optimal but can minimize damage to antioxidant compounds compared to other methods.

Measurement of antioxidant activity was first carried out to determine the maximum wavelength to determine λ which has the highest absorption of DPPH. Then it is measured using a DPPH concentration of 50 ppm, measured at a wavelength of 400-800 nm, the maximum wavelength is obtained at a wavelength of 514 nm. The sample measurement must be at the maximum wavelength so that the sensitivity is maximized and minimizes errors because at that wavelength the change in absorbance for each concentration unit is the greatest.

DPPH is a method that can be used to determine antioxidant activity in samples to be studied by looking at their ability to counteract free radicals. The DPPH method has advantages when compared to other methods, namely the method is simple, easy, and only requires a few samples and reagents in terms of antioxidant activity (Ngibad & Lestari, 2020).

The basic principle in antioxidant activity using the DPPH method is that there is a chemical reaction between antioxidant compounds and DPPH free radicals through a donation reaction mechanism or giving hydrogen atoms by antioxidant compounds to DPPH free radicals which results in a change in the color of the solution from purple to yellow. This color change causes a decrease in the absorbance value of the sample (Molyneux, 2004).

In measuring the antioxidant activity, the standard quercetin solution was measured using 5 concentration variations, namely 1, 2, 3, 4, and 5 ppm. To determine the antioxidant activity of each concentration, 1 mL of sample solution was pipetted and 4 mL of 50 ppm DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in the dark, the absorption was measured with a UV-Vis spectrophotometer at a wavelength of 514 nm. The measurement results can be seen in **Table 2** below.

Table 2. The results of measuring the absorption of quercetin.

Concentration (ppm)	Absorbance	% Inhibition
1	0.349	48.296
2	0.315	53.333
3	0.295	56.296
4	0.253	62.518
5	0.223	66.963

After measuring the absorbance, the percent inhibition value is calculated. Then do a regression between the quercetin concentration and the percent inhibition. Can be seen in **Figure 1**.

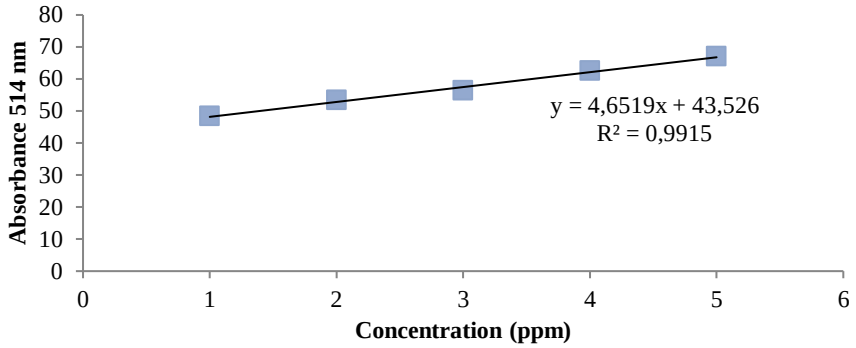

Figure 1. Calibration curve of quercetin series

Based on the quercetin standard curve obtained the standard curve of the equation y = bx + a where y = 4.6519x + 43.526 with R^2 = 0.9915 and r = 0.99574, then the value of IC_{50} is calculated that can be seen in **Table 3**.

Table 3. Measurement of quercetin comparator antioxidant activity

Concentration (ppm)	Absorbance	% Inhibition	IC_{50}	Antioxidant activity
1	0.349	48.296		
2	0.315	53.333		Very strong
3	0.295	56.296	1.391 µg/mL	(IC_{50} < 50 µg/mL
4	0.253	62.518		
5	0.223	66.963		

Furthermore, testing was carried out on samples of the dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) was measured at 5 concentration variations, namely 50, 100, 150, 200 and 250 ppm. To determine the antioxidant activity of each concentration, 1 mL of sample solution was pipetted and 4 mL of 50 ppm DPPH solution was added. The mixture was homogenized and incubated for 30 minutes in the dark, the absorption was measured with a UV-Vis spectrophotometer at a wavelength of 514 nm that can be seen in **Table 4**.

Table 4. The results of measuring the absorption of quercetin comparators.

Concentration (ppm)	Absorbance	% Inhibition
50	0.519	37.09
100	0.412	50.06
150	0.338	59.03
200	0.246	70.181
250	0.174	78.909

In **Tables 2** and **4** it can be seen that with increasing solution concentration, the absorbance value decreases and the percent inhibition value increases. The decrease in the absorbance value is caused by the capture or attenuation of free radicals by the test solution resulting in the transfer of electrons between antioxidant compounds and free radicals so that they neutralize free radicals characterized by a change in the color of the solution from purple to yellow (Molyneux, 2004). An increase in the percent inhibition value is related to a decrease in the absorbance value, this occurs because the lower the DPPH absorption value, the higher the inhibition percentage value because more DPPH is paired with hydrogen atoms from the extract so that DPPH absorption decreases. The DPPH free radical scavenging activity was based on the ability of the ethanol extract to dechlorophyllation kejibeling leaves (*Strobilanthes crispa* L.) in capturing free radicals which can be seen from the change in the purple color of the DPPH solution after being mixed with the test solution to a yellow color. Thus, it can be stated that the ethanol extract of kejibeling leaves dechlorophyllation (*Strobilanthes crispa* L.) has antioxidant activity. After measuring the absorbance, the percent inhibition value is calculated. Then do a regression between the quercetin concentration and the percent inhibition (**Figure 2**).

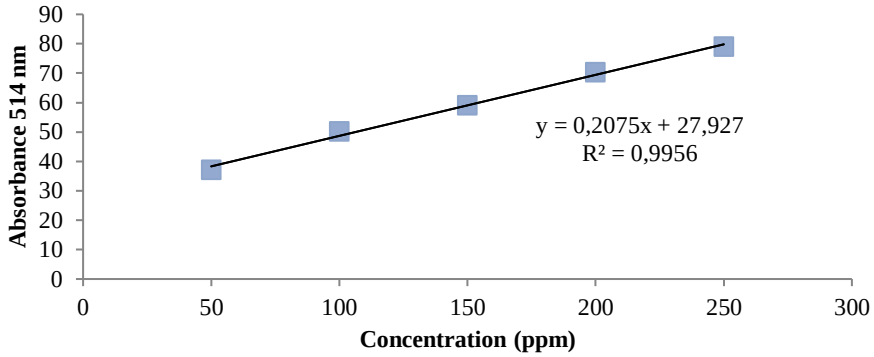

Figure 1. Calibration curve of antioxidant activity in dechlorophyllation ethanol extract of kejibeling leaves

Based on the quercetin standard curve obtained the standard curve of the equation y = bx + a where the equation y = bx + a where y = 0.2075x + 27.927 with R^2 = 0.9956 and r = 0.9977, then the value of IC_{50} is calculated that can be seen in **Table 5**.

Table 5. Antioxidant activity in dechlorophyllation ethanol extract of kejibeling leaves

Concentration (ppm)	Absorbance	% Inhibition	IC_{50}	Antioxidant activity
50	0.519	37.09		
100	0.412	50.06		Very strong
150	0.338	59.03	106.375 µg/mL	(IC_{50} < 50 µg/mL
200	0.246	70,181		
250	0.174	78,909		

Based on the results of value analysis IC_{50} in **Table 3** and **5** obtained based on the calculation of the linear regression equation can be seen in **Figure 1** and **2**. Where the concentration of the solution (ppm) is the abscissa (X axis) and the percent attenuation value is the coordinate (Y axis). Determination of free radical scavenging activity potential of dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) with value parameters IC_{50} is a number indicating the concentration of the test sample (µg/mL) which is able to reduce the free radical oxidation process by 50%. Measurement of antioxidant activity in the quercetin comparator obtained values IC_{50} of 1.391 µg/mL is a very strong antioxidant category while the ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) earned value IC_{50} 106.375 µg/mL is a medium antioxidant category. According to Kurniasih (2015), a compound is declared as a very strong antioxidant if the value IC_{50} less than 50 µg/mL, strong for IC_{50} worth 50-100 µg/mL, while if IC_{50} value of 100-150 µg/mL, whereas if IC_{50} value of 150-200 µg/mL is said to be low in antioxidants, and if IC_{50} is > 200 µg/mL, so its antioxidant activity is very low.

4. CONCLUSION

Based on the research results obtained, it can be concluded that the dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) has the potential as an antioxidant compound with moderate antioxidant activity so it has the potential to be developed as a natural antioxidant. The IC_{50} value of dechlorophyllation ethanol extract of kejibeling leaves (*Strobilanthes crispus* L.) was 106.375 µg/mL.

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Ethical Approval: Not applicable

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