PHYTOCHEMICALS SCREENING AND ANTIOXIDANT ACTIVITY TEST OF ETHANOLIC EXTRACT OF CHINESE BETEL LEAVES (Peperomia pellucida L.) AND ITS FRACTIONS

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ABSTRACT

Peperomia pellucida (Chinese betel) is considered as a plant with antioxidant activity for inhibiting oxidation reactions. This study aims to determine the yield of secondary metabolites, total flavonoid levels, and antioxidant activity. Dried leaves of Chinese betel were macerated using 70% ethanol as solvent. The extract was partitioned until the n-hexane and ethyl acetate fractions were obtained. A phytochemical screening test was followed by determining total flavonoid levels and antioxidant activity using UV-Vis spectrophotometry. Phytochemical screening results containing an ethanolic extract of Chinese betel leaves (ECBL) showed positive results on alkaloid, flavonoid, saponin, and tannin. Meanwhile, the ethyl acetate (EAF) and n-hexane (HF) fractions showed positive results only for flavonoid presence. The total flavonoid contents of ECBL, EAF, and HF were 3.30%, 2.54%, and 2.03%, respectively. The IC50 values of ECBL, EAF, and HF were 7.51 ppm, 8.02 ppm, and 8.04 ppm, respectively. The highest flavonoid content was found in ECBL. Antioxidant activity test of Peperomia pellucida ethanolic extract, ethyl acetate fraction, and n-hexane fraction showed potent antioxidant activity.

Keywords: Antioxidant, Peperomia pellucida, DPPH

INTRODUCTION

The metabolism that occurs within the body involves oxidation and reduction processes. The oxidation process can lead to the formation of harmful oxidants, or free radicals, for the body (Salamah & Lina, 2014). Free radicals can damage macromolecules such as lipids, cell membranes, DNA, and proteins, causing oxidative stress in cells. (Salamah & Lina, 2014). Antioxidants are compounds that can prevent oxidation reactions by providing their electrons to free radical molecules, thereby stopping the chain reactions caused by free radicals (Sitorus et al., 2013).

One type of plant that contains antioxidants is the Chinese betel leaves (Peperomia pellucida L.). The Chinese betel leaves have been traditionally used to treat several diseases, such as abscesses, boils, acne, skin inflammation, kidney disease, and stomach aches (Sitorus et al., 2013). According to research by Salamah and Lina (2014), ethanolic extract of Chinese betel leaves (ECBL) has antioxidant activity in inhibiting oxidation reactions. ECBL contains alkaloids, tannins, resins, flavonoids, steroids, phenols, and carbohydrates (A. Pratiwi et al., 2021). The secondary metabolite compound flavonoids in Chinese betel leaves act as antioxidants that are used to inhibit and stop free radicals and accelerate the wound healing process by increasing or accelerating the proliferation of fibroblast cells and collagen.
fiber production (Mulyani et al., 2018). Pratiwi et al. (2021) have also reported that ECBL has antioxidant activity.

This study aims to identify standard quality parameters of the extract based on specific parameters (organoleptic extract and phytochemical screening) and to determine the total flavonoid content and antioxidant activity of the extract and fraction of Chinese betel leaves (Peperomia pellucida L.) using DPPH (1,1-difenil-2-pikrilhidrazil). The mechanism of this method is a reaction between the antioxidant functional group present in the sample and DPPH. Antioxidants will donate their hydrogen atoms, inhibiting the activity of free radical compounds (Sitorus et al., 2013).

RESEARCH METHOD

This study is descriptive research to determine the content of secondary metabolites (alkaloids, flavonoids, triterpenoids, saponins, and tannins), total flavonoid levels, and antioxidant activity of the ECBL (Peperomia pellucida L.) using the DPPH method.

Tools

- Analytical balance (OHAUS PX224, 220 g/0.1 mg);
- Rotary evaporator (IKA®);
- Waterbath; measuring flask (Pyrex®);
- Measuring cylinder (Pyrex®);
- Beaker glass (Pyrex®);
- Kuvet; spectrophotometer UV-Vis (Shimadzu UV Mini-1240);
- Balance (OHAUS PX224, 220 g/0.1 mg);
- Measuring cylinder (Pyrex®);
- Measuring flask (Pyrex®);
- Beaker glass (Pyrex®);
- Kuvet; spectrophotometer UV-Vis (Shimadzu UV Mini-1240);

Materials

- Peperomia pellucida L. leaves; 70% ethanol (PT Brataco Indonesia);
- Concentrated sulfuric acid; 2 M sulfuric acid; Dragendorff reagent; Mayer reagent; acetic anhydride;
- Concentrated hydrochloric acid; chloroform (CV Sains Pratama) ; ferric chloride solution; aquadest (PT Brataco Indonesia); DPPH (Aldrich Chemistry); methanol (PT Brataco Indonesia); n-hexane (PT Brataco Indonesia); ethyl acetate (PT Brataco Indonesia); quercetin (Himedia Laboratory); vitamin C powder (CV Merck).

Research Procedure

1. Plant determination

   Plant determination was carried out at the Laboratory of Biology Study Program at IAIN Syekh Nur Jati Cirebon. This identification aims to examine and ensure the true identity and avoid errors in the plant samples used for study.

2. Dry powder preparation

   Chinese betel leaves (Peperomia pellucida L.) obtained from Kuningan Regency were collected and then sorted in fresh condition. Subsequently, the leaves were washed with flowing clean water. The clean sample were weighed and dried using an oven at 40°C. In the end, the dry sorting process is carried out prior to powdering process (Depkes RI, 1979).

3. Extraction procedure

   Approximately 300 grams of crude herbs were soaked in 3 L of 70% ethanol and left for 5 days. The stirring was conducted everyday to obtain effective contact between powder and solvent (Sani et al., 2014). After 5 days, the extract was filtrated using flannel to obtain macerate for further processing. The macerate was collected and subsequently concentrated in a rotary evaporator (Wungkana, 2013). The solvent was let to evaporate at 70°C in a water bath to get a thick extract.

4. Partition of ECBL

   Two grams of extract are dissolved in 10 mL of 70% ethanol and placed in a separating funnel. Thirty mL of distilled water was added and shaken prior to the addition of 30 mL of n-hexane for each process. The separating funnel is shaken and left to settle into two fractions. The n-hexane fraction is accordingly evaporated on a water bath until one-third remains, resulting in a non-polar fraction. The aqueous fraction was partitioned again using 2 x 30 mL of ethyl acetate solvent, shaken, and left to separate into two fractions. Then, the ethyl acetate fraction was evaporated in a water bath until one-third remains, resulting in a more polar fraction than the previous.
5. **Phytochemical Screening**
   a. Alkaloid identification (Harborne, 1987)
      
      Five hundred milligrams of ECBL were dissolved using 10 mL of chloroform, and three drops of ammonia were added. This was then acidified using 2 drops of 2 M sulfuric acid. The acidic fraction was divided into 2 tubes, and 3 drops of Dragendorff reagent were added to the first tube and 3 drops of Mayer reagent to the second tube. The presence of a white precipitate in Mayer’s reagent indicates that the sample is positive for alkaloids, while a positive result for alkaloids in Dragendorff's reagent is indicated by the formation of a red precipitate.
   b. Flavonoid identification
      
      Five hundred milligrams of ECBL was placed in a spot plate, and then a small amount of magnesium metal was added. To this, 1-2 drops of concentrated hydrochloric acid were added, stirring it well. The Appearance of pink color informs that the sample contains flavonoids.
   c. Triterpenoid
      
      Two grams of ECBL were dissolved in 25 mL of ethanol and heated. The sample was then filtered and evaporated. The remaining residue is added to ether and transferred to a test tube, then added to Liebermann Burchard reagent (3 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid). The appearance of a red or purple color indicates the presence of triterpenoids.
   d. Saponin identification
      
      To 500 milligrams of ECBL, 5 mL of distilled water were added, and this was heated for 5 minutes. The sample was then shaken for 5 minutes to let the foam appear. A stable foam is an indicator of the presence of saponins.
   e. Tanin identification
      
      One gram of ECBL was boiled in distilled water for 5 minutes. Then, the solution was filtered, and 5 drops of 1% (w/v) ferric(III) chloride were added. A positive test for tannins is indicated by the formation of a dark blue or black color.

6. **Evaluation of the extract**
   a. Organoleptic analysis of extracts
      
      The test was conducted to determine appearance, color, smell, and taste (Depkes, RI, 2000).
   b. Moisture content test
      
      One gram of ECBL was placed in an evaporating dish. This was weighed to obtain a constant mass. After that, the remains were put in an oven for drying at 105 °C for 5 hours and weighed again. The difference between two consecutive weighings is no more than 0.25%. This method is used to determine the moisture content of the extract (Depkes, RI, 2000).

7. **Total Flavonoid Test**
   a. Determination of quercetin's maximum wavelength
      
      The determination of quercetin’s maximum wavelength was carried out by measuring the absorbance of quercetin solution in the range of 400-450 nm. The absorbance reading showed that the maximum wavelength of the quercetin standard is 431 nm. The maximum wavelength was used to measure the absorption of the extract.
   b. Determination of operating time
      
      A solution of 50 ppm quercetin was taken in an amount of 1 ml and added to 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. The solution was measured for its absorbance at the maximum wavelength of 431 nm at 5-minute intervals until a stable absorbance was obtained. A curve was then created to show the relationship between absorbance, time, and the determined operating time.

*Phytochemicals Screening and Antioxidant Activity Test... (Naida Rahma Almira et al.)*
c. Preparation of quercetin standard solution
   Ten milligrams of quercetin were dissolved in 10 mL of ethanol p.a. to prepare a 1000 ppm quercetin standard solution. From the 1000 ppm quercetin standard solution, several concentrations were prepared, namely 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm. One milliliter of the quercetin standard solution was mixed with 0.1 mL of 10% aluminum chloride, 1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. The mixture was vortexed until homogeneous and allowed to stand for 30 minutes. Next, the absorbance of each concentration of the solution was measured using the UV-Vis spectrophotometry method at the maximum wavelength of 431 nm.

d. Measurement of blank solution absorbance
   One milliliter of ethanol was added to 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was then allowed to stand for 30 minutes. Next, the absorbance of each concentration of the solution was measured using the UV-Vis spectroscopy method at a maximum wavelength of 431 nm.

e. Determination of total flavonoid content in ECBL
   Twenty milligrams of the sample were weighed and dissolved in 10 mL of ethanol, resulting in a concentration of 2000 ppm. One milliliter of the test sample was added to 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was vortexed until homogeneous and then allowed to stand for 60 minutes. Then, the absorbance of each concentration of the solution was measured using the UV-Vis spectrophotometry method at the maximum wavelength of 431 nm.

f. Calculation of total flavonoid content
   \[
   \text{% concentration} = \frac{C \times V \times df \times 10^{-3}}{\text{weight of sample}} \times 100\% 
   \]
   where:
   \(C\) : concentration of sample (mg/L)
   \(V\) : Volume of sample (L)
   \(df\) : Dilution factor

8. Antioxidant activity test of ECBL
   a. Preparation of DPPH solution
      Two milligram of DPPH were dissolved in 25 mL of methanol to obtain a concentration of 80 ppm in the solution until it was homogeneous.

   b. Preparation of blank solutions
      One milliliter of methanol was placed within a vial and added to 2 mL of DPPH solution (80 ppm), shaken until homogeneous, and then incubated at 37˚C for 30 minutes.

   c. Preparation of ECBL, EAF, HF, and vitamin C stock solutions
      Five milligrams of ECBL were mixed with 50 mL of methanol (100 ppm) and vortexed until homogeneous.

   d. Preparation of concentrations of ethanolic ECBL, ethyl acetate fraction, n-hexane fraction, and vitamin C solutions
      Sample solutions with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were prepared by diluting each stock solution of ECBL with volumes of 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL, respectively, then transferred to 5 mL volumetric flasks, and added to 2 mL of DPPH solution and methanol up to the calibration mark.

   e. Determination of maximum wavelength and operating time
      The blank solution absorbance was determined using a UV-Vis spectrophotometer at a wavelength of 400-800 nm. The blank solution absorbance
was measured at 0 minutes (immediately after preparation) and at 10, 20, 30, 40, 50, and 60 minutes.

f. Antioxidant activity testing

Sample solutions with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were measured for their absorbance at a wavelength of 400-800 nm, and their absorbance was recorded. In each test, the blank absorbance was also determined.

g. Calculation of antioxidant activity

Antioxidant activity was calculated using the DPPH method, where the sample was reacted with a DPPH radical solution. The DPPH solution absorbance before and after the addition of the extract was calculated as the percentage inhibition (% inhibition) using the following formula:

\[
\% \text{ Inhibition} = \frac{(\text{blank absorbance} - \text{sample absorbance})}{\text{blank absorbance}} \times 100\%
\]

After obtaining the percentage inhibition from each concentration, the calculation was continued by linear regression using the equation \( y = bx + a \), where:

- \( x \) = concentration (ppm).
- \( y \) = percentage inhibition (%)

The antioxidant activity is expressed as inhibition concentration 50%, or IC50, which is the sample concentration that can scavenge 50% of the DPPH radical. The IC50 value is obtained from the X value by substituting Y with 50.

Data Analysis

The analysis of antioxidant activity data was calculated based on the percentage of inhibition (% inhibition) using the formula below:

\[
\% \text{ Inhibition} = \frac{(\text{blank absorbance} - \text{sample absorbance})}{\text{blank absorbance}} \times 100\%
\]

After obtaining the percentage of inhibition from each extract concentration, further analysis was conducted using a regression equation (simple linear regression) represented as \( y = bx + a \), where \( x \) represents the concentration (ppm) and \( y \) represents the percentage of inhibition (%). The antioxidant activity was evaluated by the inhibition concentration 50% (IC50), which refers to the concentration of the sample that can inhibit 50% of the DPPH radicals.

According to Puspita et al. (2021), the data analysis method used is descriptive and based on the determination of flavonoid content. The flavonoid content can be calculated using the following formula:

\[
\% = \frac{C \times V \times Fp \times 10^{-3}}{\text{sample (g)}}
\]
RESULTS AND DISCUSSION

The yield obtained from the Chinese betel leaf sample was 300 grams. The extract yield obtained was 17.75%. Based on these results, the extract yield meets the requirement as it is not less than 13.1% (Depkes RI, 2017). The concentrated ECBL was separated by partition using a separating funnel with n-hexane as a nonpolar solvent and ethyl acetate as a semipolar solvent. The partition separation of the ethanol extract yielded the n-hexane fraction and the ethyl acetate fraction.

Testing the characteristic parameters of the extract includes the identification of the extract, its organoleptic properties, and its moisture content. The results of testing the parameters of ECBL can be seen in Table I below:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity:</td>
<td></td>
</tr>
<tr>
<td>extract name</td>
<td>Ethanolic extract of Chinese betel leaves</td>
</tr>
<tr>
<td>Latin name</td>
<td>(Peperomia pellucida L.)</td>
</tr>
<tr>
<td>Plant part(s)</td>
<td>Leaves</td>
</tr>
<tr>
<td>Organoleptic:</td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Dark brown, almost black</td>
</tr>
<tr>
<td>Odor</td>
<td>Distinctive odor</td>
</tr>
<tr>
<td>Form</td>
<td>Viscous extract</td>
</tr>
<tr>
<td>Moisture Content</td>
<td>24 %</td>
</tr>
</tbody>
</table>

Organoleptic evaluation is one specific parameter determined by using the senses and aims to provide simple initial simple recognition, but it is subjective in nature (Cahyani, 2017). Based on the organoleptic evaluation results conducted, ECBL has a dark brown to almost black color, a distinctive odor, and a viscous consistency.

The moisture content test is conducted to determine the minimum limit or range of water content in an extract. Higher moisture content can promote the growth and proliferation of fungi and molds, thus reducing the biological activity and stability of the extract during storage. In the moisture content test, a result of 24% was obtained. Moisture content is considered to be at high risk if it exceeds 10% (Cahyani, 2017). This indicates that ECBL was considered to be at high risk due to the result exceeding the 10% limit. Such a high moisture content can affect the formulation and stability of the extract. In addition, the extract was susceptible to being a good medium for mold growth (Cahyani, 2017).

The partition of ECBL resulted in two fractions that should be observed to reveal information regarding with their chemical constituents. For that, phytochemical tests were conducted. The results of the test are shown in Table II:

<table>
<thead>
<tr>
<th>Identification</th>
<th>Results for</th>
<th>Ethanol extract</th>
<th>Ethyl acetate fraction</th>
<th>n-hexane fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>Flavonoid</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>(+)</td>
<td>(-)</td>
<td>(-)</td>
<td></td>
</tr>
</tbody>
</table>

(+): positive result  
(-): negative result
The phytochemical screening results of ECBL showed positive results for alkaloids, flavonoids, tannins, and saponins. However, the n-hexane fraction (HF) and ethyl acetate fraction (EAF) only showed positive results for flavonoids. The variation in phytochemical screening results among the different samples is due to the presence of different secondary metabolite groups with distinct properties. These results differ from the phytochemical screening results of the extract and fractions conducted by Jannah et al. (2020). Their study showed positive results for alkaloid testing in both the EAF and the HF, as well as positive results for triterpenoid testing in the n-hexane sample and ethanolic extract. On the other hand, the study conducted by Arifah et al. (2018) reported the presence of false positive results for flavonoid compounds, indicated by the formation of an orange-brown color, and for triterpenoid compounds, indicated by the formation of a dark purple color.

False negative results for steroid compounds are indicated by the formation of a dark green color, false positive results for tannin compounds are indicated by the formation of a black precipitate, and false positive results for alkaloid compounds are indicated by the formation of a white precipitate. False positive results occur when a sample shows positive results for a compound that should actually show negative results. False negative results, on the other hand, occur when a sample shows negative results for a compound that should actually show positive results. False positive results are errors made during the research process where a sample shows positive results for a compound that should be negative. False negative results are errors made when a sample shows negative results for a compound that should be positive.

From the phytochemical screening, flavonoid were found in ECBL. Subsequently, it was important to determine the quantity of flavonoid in ECBL. Figure 1 was the quercetin standard curve at a maximum wavelength of 431 nm:

![Figure 1. Quercetin Standard Curve](image)

The coefficient of correlation (r) indicates the linearity of the relationship between two variables. The obtained value of r for the quercetin standard curve was 0.999. This result is slightly different from the r value obtained in the quercetin standard curve performed by Pratiwi (2020), which was 0.9979. However, both results indicate a good linear relationship between the variables as the obtained r value approaches one. Linearity refers to the ability of an analytical method to produce proportional values to the analyte concentration in the sample within a certain concentration range (Pratiwi, 2020).

The data was collected by measuring the absorbance values of a series of standard solutions. According to Pratiwi (2020), there is a linear relationship between absorbance and flavonoid concentration, meaning that higher measured absorbance values indicate higher flavonoid concentrations. The absorbance data of the standard solutions will then be used to determine the flavonoid concentration using a standard curve, which is obtained from the
relationship between quercetin concentration (mg/L) and sample absorbance. The results of the calculation of total flavonoids are provided in Table III.

### Table III. Total Flavonoid Content

<table>
<thead>
<tr>
<th>Sample</th>
<th>Absorbance</th>
<th>Total Flavonoid Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic extract</td>
<td>1.472</td>
<td>3.30 %</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>1.090</td>
<td>2.54 %</td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>0.884</td>
<td>2.03 %</td>
</tr>
</tbody>
</table>

The results of the antioxidant activity test were based on a comparison with the reference, i.e., vitamin C. From the results given in Table IV, the best antioxidant could be identified based on the IC50 value.

### Table IV. The results of antioxidant activity measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ppm)</th>
<th>% inhibition</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin c</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67.87</td>
<td>1.97 ppm</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>91.64</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>95.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>96.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>95.74</td>
<td></td>
</tr>
<tr>
<td>ethanolic extract</td>
<td>0</td>
<td>0</td>
<td>7.51 ppm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>54.77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>48.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>47.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>48.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50.44</td>
<td></td>
</tr>
<tr>
<td>ethyl acetate fraction</td>
<td>0</td>
<td>0</td>
<td>8.02 ppm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>44.73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>49.87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>48.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>46.58</td>
<td></td>
</tr>
<tr>
<td>n-hexane fraction</td>
<td>0</td>
<td>0</td>
<td>8.04 ppm</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>46.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>48.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>48.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>46.42</td>
<td></td>
</tr>
</tbody>
</table>

After measuring the absorbance for the standard and sample solutions, the absorbance values of each tested sample can be obtained, allowing for the determination of the percentage of free radical inhibition (% inhibition). The percentage of inhibition for each sample and the standard at the same concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm.
ppm were as follows: ECBL sample: 54.77%, 48.83%, 47.06%, 48.83%, and 50.44%; EAF: 44.97%, 44.73%, 49.87%, 48.99%, and 46.58%; HF: 50.92%, 46.90%, 48.43%, 48.43%, and 46.42%; vitamin C (standard): 67.87%, 91.64%, 95.98%, 96.22%, and 95.74%. Vitamin C was used as a reference because it acts as an antioxidant by scavenging free radicals, preventing chain reactions. It exhibits high antioxidant activity, is readily available, and has a higher polarity compared to other vitamins. Vitamin C contains a free hydroxyl group that acts as a free radical scavenger (Damanis et al., 2020). The obtained % inhibition values can be used to determine the values of a, b, and r to obtain the IC50 value. The linear regression coefficient (r) obtained is 0.6043 for the ethanolic ECBL sample, 0.692 for EAF, 0.612 for HF, and 0.796 for vitamin C.

Based on the % inhibition values and IC50 values indicated in Table IV, it can be observed that ECBL had an IC50 value of 7.51 ppm, EAF had an IC50 value of 8.02 ppm, and HF had an IC50 value of 8.04 ppm. The sample showing the highest antioxidant activity was the ethanolic ECBL, followed by the EAF and the HF, respectively. However, based on the obtained IC50 values, ECBL was not stronger than vitamin C as an antioxidant.

The difference in antioxidant activity among the samples may be attributed to variations in the content of active compounds present in each sample, which affects their ability to scavenge free radicals (Mangela et al., 2016). Additionally, the use of different solvents with varying polarities can influence the types of compounds extracted (Mangela et al., 2016).

From the measurement of the antioxidant activity of ECBL, EAF, and HF in Chinese betel leaves, it can be concluded that they have the ability to scavenge free radicals and fall into the category of very strong antioxidants, as indicated by the calculated IC50 values being less than 50 ppm. According to Mangela et al. (2016), the smaller the IC50 value, the higher the antioxidant activity, and a compound is considered a very strong antioxidant if the IC50 value is less than 50 ppm, strong if it is in the range of 50–100 ppm, moderate if it is in the range of 100–150 ppm, weak if it is in the range of 150–200 ppm, and very weak if it is in the range of 200–1000 ppm.

CONCLUSION

Phytochemical screening of the ethanolic ECBL showed positive results for alkaloids, flavonoids, saponins, and tannins, while the ethyl acetate fraction and n-hexane fraction only showed positive results for flavonoids. The total flavonoid content obtained from the ethanolic ECBL was 3.30%, while EAF and HF had total flavonoid contents of 2.54% and 2.03%, respectively. The highest total flavonoid content was found in the ethanol extract.

The ethanolic ECBL, ethyl acetate fraction, and n-hexane fraction exhibited strong antioxidant activity. The IC50 values obtained were 7.51 ppm for the ethanol extract, 8.02 ppm for the ethyl acetate fraction, and 8.04 ppm for the n-hexane fraction. Vitamin C, used as the positive control, exhibited stronger antioxidant activity compared to the ethanolic extract, ethyl acetate fraction, and n-hexane fraction, with an IC50 value of 1.97 ppm.

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*Phytochemicals Screening and Antioxidant Activity Test... (Naida Rahma Almira et al.)*
