TOTAL PHENOLIC, FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACT OF BETEL LEAF (Piper betle L.)

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Abstract: Piper betle is an efficacious medicinal plants as anti-microbial, antiseptic and a bloody nose tackle and is known to have antioxidant activity. This study aims to determine the total phenolic, flavonoid content and antioxidant activity of the ethanol extract of green and red betel. The sample of leaves were macerated with 70 and 96% ethanol, the filtrate tested antioxidant activity using DPPH scavenging method, while total phenolic was measured by Folin-Ciocalteu assay and total flavonoids was measured using the aluminium chloride colorimetric assay. The result showed that total phenolic of 70 and 96% ethanolic extract 1.17% (of the green betel), 1.66% of the red betel. Total flavonoid content of 70 and 96% ethanolic extract were 0.061 and 0.050 % of the green betel and 0.071 and 0.064% of the red betel. The antioxidant activity with IC_{50} values were 8.72 and 10.74 μg/mL (70% ethanolic extract of red and green betel), 20.51 and 26.51 μg/mL (96% ethanolic extract of red and green betel). From the results showed that the total phenolic, total flavonoid content and antioxidant activity of red betel greater than green betel.

Keywords: red betel, green betel, antioxidant activity, total phenolic, flavonoids content

INTRODUCTION

Betel plant is one of the medicinal plants that have been used since ancient times as a medicine nose bleed, ulcer, cough, and eye pain. Besides that, it is also used in various religious rituals and customs. Along with the trend back to nature, different types of medicinal plants cultivated again and exploiting people, without exception betel plant. Betel contain saponins, flavonoids and polyphenols, as well as essential oils.

Betel plant is one the most important in human life which have a more significant medicinal value, benefits, a variety of applications from a variety of pharmacological activities (Shalini et al., 2011). The constituent of piper betle were hydroxychavicol, allylpyrocatechol, chavibetol, piperbetol, and others. There are other ingredients such as arecoline, charvacol, caryophyllene, piperitol, eugenol and others (Rimando et al., 1986).

Phenols comprise the largest group of plant secondary metabolites. Phenolic compounds commonly found in plants both edible and non-edible and they have been reported to have multiple biological effects, including antioxidant (Durgaprasa et al., 2011; Rathee et al. 2006; Manigauha et al. 2009; Pin et al. 2010; Arambewela et al. 2006). Antioxidants are compounds that emit potentially toxic oxygen species, suppress its formation, or against the effect.

This study aims to determine the total phenolic, flavonoid content and antioxidant activity of the ethanol 70 and 96% extract of green and red betel. The Folin-Ciocaltel method is a rapid, widely used assay to determine the total concentration of phenolic compounds. It is known that different phenolic compounds vary in their responses in the Folin-Ciocaltel method, and total flavonoids was measured using the aluminium chloride colorimetric assay, while the antioxidant activity using free radical scavenging DPPH method. The reaction is the formation of α,α-diphenyl - β – picryl hydrazine, through the ability of antioxidants to donate hydrogen. The more color fading (DPPH) after reacted with antioxidant showed that the greater of the antioxidant.

EXPERIMENTAL

Plant material and chemicals

Green and red betel leaf. ethanol, methanol, DPPH (1,1-diphenyl-2-picryl hydrazyl), sodium carbonate, hexamethylenetetramine, glacial acetic
acid, aluminium chloride, gallic acid, Folin-Ciocalteu’s, ascorbic acid.

**Total phenolic assay**
The total phenolic content of the dry leaf of green and red betel was determined with Folin-Ciocalteu assay. An aliquot (1 ml) of extracts or a standard solution of gallic acid (0.2, 0.4, 0.6, 0.8, and 1 mg/100ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of the Folin-Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% sodium carbonate solution was added to the mixture. The solution was diluted to 25 ml and mixed. After incubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with an UV-Vis spectrophotometer. The data for the total phenolic contents were expressed as milligrams of gallic acid equivalents (GAE) per 100 gram dry mass (mg GAE/100 g dw). All samples were analysis in duplicate.

**Total flavonoid assay**
*Test solution*
Unless otherwise stated, carefully weighed amount of 200 mg of crude extract, put in a round bottom flask, added consecutive 1 mL solution of HMT (hexamethylentetramine), 20 mL of acetone and 2 mL P hydrochloric acid solution P, refluxed for 30 minutes. Then the residue is filtered using cotton, enter the filtrate into a 100 mL volumetric flask. Refluxed again with 20 mL of acetone residue for 30 min, filtered and the filtrate was mixed into 100 mL volumetric flask. Pipetted 20 mL into a separating funnel, add 20 mL of water and extracted three times, each time using 15 mL of ethyl acetate P. Ethyl acetate phase included in the flask add 50 mL of ethyl acetate P up to the mark.

*Dilution test solution*

Pipetted 10 mL of test solution into 25 mL volumetric flask, add 1 mL solution of glacial acetic acid 5% v/v in methanol P up to the mark.

Pipetted 10 mL of test solution into 25 mL volumetric flask, add 1 mL solution of aluminium chloride and a solution of glacial acetic acid 5 % v/v in methanol P up to the mark.

*Measurement*
Measure 30 min after the addition of aluminium chloride solution using a spectrophotometer at a wavelength of 428 nm. Calculated as the total flavonoid content of flavonoids comparison.

**Antioxidant activity assay**
Test the antioxidant activity with the DPPH ethanol extract 70 and 96 % ethanol green betel and betel red leaf.  

a. Preparation of 0.4 mM DPPH
Approximately 16 mg DPPH (MW 394.32) dissolved in 100.0 mL of methanol pro analysis, and then placed in a dark bottle.

b. Preparation sample solution
Approximately 25 mg of sample extract, dissolved in 25.0 mL of methanol pro analysis (1000 mcg/mL), Pipetted 25 mL, 50 mL, 125 mL, 250 mL and 500 mL into 5.0 mL volumetric flask, methanol was added to 5.0 mL of pro analysis were homogenized

c. Preparation of vitamin C as a positive control
Approximately 10 mg, then put into 10 mL volumetric flask, add methanol to 10.0 mL pro analysis in order to obtain a concentration of 1000 µg/mL. Pipetted 5 mL, 10 mL, 15 mL, 20 mL and 25 mL into 5.0 mL volumetric flask to obtain a concentration of 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm.

d. Antioxidant activity test
Into each flask, test solution and reference solution (as a positive control) was added 1 mL of 0.4 mM DPPH solution and methanol to 5.0 mL pro analysis were homogenized . Flask covered with aluminum foil. Without inhibition of DPPH solution (blank solution), the test solution and reference solution (positive control) immediately incubated at 37 º C for 30 min and then absorbance was measured at a wavelength of 517 nm with a UV-Vis spectrophotometer.

**RESULT AND DISCUSSION**
The content of total phenols were determined spectrophotometrically using Folin-Ciocalteu reagent and gallic acid as a reference standard.
with various concentrations and absorption are shown in Table 1.
The relationship between the content of gallic acid and absorbance is expressed as the equation
\[ y = 1.0603x + (-0.0251) \]
with a correlation coefficient \( r = 0.9996 \).

Table 1. Concentration of gallic acid and absorbance at \( \lambda \) 645 nm.

<table>
<thead>
<tr>
<th>Concentration of Gallic Acid (mg/100ml)</th>
<th>Absorbance</th>
<th>Linear regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1984</td>
<td>0.1948</td>
<td>( y = 1.0603x )</td>
</tr>
<tr>
<td>0.3968</td>
<td>0.3873</td>
<td>( y = 1.0603x )</td>
</tr>
<tr>
<td>0.5952</td>
<td>0.6042</td>
<td>( y = 1.0603x )</td>
</tr>
<tr>
<td>0.7936</td>
<td>0.8063</td>
<td>( y = 1.0603x )</td>
</tr>
<tr>
<td>0.9920</td>
<td>1.0371</td>
<td>( y = 1.0603x )</td>
</tr>
</tbody>
</table>

Table 2. Total phenolic content of the extract and IC\(_{50}\) values betel extracts.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total phenolic content (%)</th>
<th>Total flavonoid content (%)</th>
<th>IC(_{50}) value (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green betel</td>
<td>Ethanol 70%</td>
<td>1.1700</td>
<td>0.0611</td>
</tr>
<tr>
<td></td>
<td>Ethanol 96%</td>
<td>1.1681</td>
<td>0.0504</td>
</tr>
<tr>
<td>Red betel</td>
<td>Ethanol 70%</td>
<td>1.6602</td>
<td>0.0714</td>
</tr>
<tr>
<td></td>
<td>Ethanol 96%</td>
<td>1.0060</td>
<td>0.0644</td>
</tr>
</tbody>
</table>

From Table 2, it can be seen that the red betel has a total phenol and total flavonoid content were greater than green betel. These results were in line with its antioxidant activity.

**CONCLUSION**

Total flavonoid content of 70 and 96% ethanolic extract were 0.061 and 0.050 % of the green betel and 0.071 and 0.064% of the red betel. The antioxidant activity with IC\(_{50}\) values were 8.72 and 10.74 µg/mL (70% ethanolic extract of red and green betel), 20.51 and 26.51 µg/mL (96% ethanolic extract of red and green betel). From the results showed that the total phenolic, total flavonoid content and antioxidant activity of red betel greater than green betel.

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