Antioxidant Activity of Pidada (*Sonneratia caseolaris* (L.) Engl.) Fruit Extract by DPPH Method

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Abstract

*Sonneratia caseolaris* (L.) Engl. or commonly called pidada fruit is one type of mangrove that is commonly found in the coastal areas of Muara Gembong, Bekasi, West Java. Pidada contains vitamins and secondary metabolite compounds that are beneficial for health. The purpose of this study was to determine the concentration of ethanolic extract, *n*-hexane fraction, ethyl acetate fraction, and butanol fraction of pidada fruit that could inhibit 50% absorbance of DPPH as free radical. Extraction was conducted by maceration method using 96% ethanol, then fractionated using *n*-hexane, ethyl acetate and butanol as the representatives of nonpolar, semipolar and polar solvent. This study used vitamin C and vitamin E as positive controls. The percentage of antioxidant activity was obtained from the data absorbance. IC₅₀ values obtained from linear regression between the concentration of tested series versus the percentage of antioxidant activity. The results showed that the ethanol extract of pidada fruit possessed very strong antioxidant activity while *n*-hexane fraction possessed weak antioxidant activity, ethyl acetate fraction possessed strong antioxidant activity, and butanol fraction possessed very weak antioxidant activity. IC₅₀ values of ethanol extract, *n*-hexane fraction, ethyl acetate fraction, and butanol fraction of pidada fruit were 32.58; 191.31; 96.02 and 371.16 ppm respectively.

Keywords: Pidada fruit, antioxidant activity, DPPH method

1. Introduction

As an archipelago, Indonesia is one of the countries which has the largest mangrove forests in the world. Mangrove forests have an important role and many benefits both directly and indirectly for the surrounding environment. One of its benefits is to resist abrasion of sea water¹. There are several types of mangrove plants that can be processed, such as *Nypa fruticans* which are used to repel insects, *Xylacarpus granatum* which is processed to become hair oil, and *Sonneratia caseolaris* which can be processed to become fruit syrup and dodol².

*Sonneratia caseolaris* or pidada fruit is one type of mangrove that is commonly found in the coastal areas of Muara Gembong, Bekasi-West Java. Pidada fruit is often used as raw material to produce syrup and dodol by the surrounding community. But widely the public has not known much about pidada fruit and its benefits.

Pidada fruit has a sour and fragrant taste that is typical like tamarin fruit. Processed pidada syrup, jam and pidada fruit itself contained 17.08%; 12.20%; and 56.74% ascorbic acid (vitamin C) compounds respectively³. Pidada fruit peel had a very strong antioxidant activity with IC₅₀ value of 25.27 ppm where the extraction was done by maceration using methanol as
a solvent. Antioxidant activity of *Sonneratia alba* which in one genus with *Sonneratia caseolaris* had an IC$_{50}$ value of 296.54 ppm where the extraction method was done by maceration using methanol as a solvent$^{[4]}$.

![Figure 1: Pidada fruit (Sonneratia caseolaris (L.) Engl.)](image)

The purposes of this study were to determine the antioxidant activity of ethanolic extract, $n$-hexane fraction, ethyl acetate fraction and butanol fraction from pidada fruit. Fractionation was done to see the tendency of the polarity of secondary metabolites which provide higher antioxidant activity.

2. Materials and Method

2.1 Materials

The sample of pidada fruit were taken at Bahagia Beach, Muara Gembong, Bekasi, West Java (Figure 2). The fruits were collected into vinylbags then carried out to laboratory. The leaves, trunks, fruit and flower were sent to Research Center for Biology, Indonesian Institute of Sciences (LIPI) for plant determination.

![Figure 2: Location of sampling](image)

2.2 Sample Preparation

The ripe fruit was washed with running water, peeled slowly to separate the flesh of the fruit and the skin, then the flesh crushed by hand and flattened on an aluminium sheet until full, dried at room temperature. Direct exposure to sunlight was avoided. The preparations
then monitored for 24 hours from the onset of fungus which would cause spoilage and affect the content in the fruit.

2.3 Extraction and Fractionation

The dried pidada fruit then mashed until it became powder. Furthermore it was stored in an airtight container at room temperature and protected from direct sunlight. 1 kg of powdered pidada fruit was put into macerator then added 96% ethanol (1:5). Result from maceration were filtered and evaporated at 40°C[4].

Fractionation of ethanol extract was carried out by liquid-liquid extraction method using separating funnel. 60 gram concentrated ethanol extract was diluted with 600 mL of distilled water, stirred until homogeneous, then put in a separating funnel and fractionated with 600 mL of \(n\)-hexane. The \(n\)-hexane fraction was separated, then the fraction of water was fractionated with 600 mL ethyl acetate. The ethyl acetate fraction was separated, then finally the fraction of water was fractionated with 600 mL of butanol. The fractionated extract was then concentrated with a rotary evaporator to produce extracts of \(n\)-hexane, ethyl acetate and butanol fractions[5,6].

2.4 Screening of Secondary Metabolite

The ethanol extract of pidada fruit, \(n\)-hexane fraction, ethyl acetate fraction and butanol fraction were obtained qualitatively by phytochemical screening. Alkaloid, flavonoid, saponin, steroid or terpenoid and tannin were tested in phytochemical screening.

1 mL of sample was added with 2 mL of chloroform and 2 mL of concentrated ammonia, shaken until homogeneous and filtered. The filtrate obtained was added with 3-5 drops of concentrated sulfuric acid and shaken to form two layers. The top layer was moved into 3 test tubes, then each tube was added with 5 drops of Mayer reagent. If a white precipitate was formed by Mayer reagent, it showed that the sample contained an alkaloid.

1 mL of sample was added magnesium powder and 3 drops of concentrated hydrochloric acid then shaked. A change in color from orange to red indicated the presence of flavonoids.

1 mL of sample was added with hot water. The mixture was cooled and shaken vigorously for 10 minutes. Positive samples contained saponins if there was a stable foam.

1 mL of sample was added 10 drops of glacial acetic acid, 2 drops of concentrated sulfuric acid and shaken. Samples were positive if there were red or orange color changes for terpenoids and blue or green color changes for steroids.

1 mL of sample was added with 2-3 drops of 1% iron (III) chloride. Positive samples containing tannins were indicated by the formation of a blackish green color.

2.5 DPPH Free Radical Scavenging Activity

DPPH is a free radical which can turn into a stable diamagnetic molecule by accepting hydrogen radicals. The prepared solution of DPPH turned deep violet color to become lighter with the presence of antioxidant. Antioxidant can quench DPPH free radicals by donating electron and converting them to colorless substances[7].

DPPH was prepared by dissolving 5 mg of DPPH powder in 50 mL ethanol. The DPPH solution was then stored in a dark place. To obtained maximum wavelength, 1 mL of 100 ppm DPPH solution was added by 2 mL of ethanol. The mixture was stirred with vortex mixer for 10 seconds and allowed to stand in the dark for 30 minutes. After 30 minutes, the solution was measured at a wavelength of 400-700 nm using UV-Vis spectrophotometer.
Sample was prepared by dissolving 2.5 mg of pidada fruit extract, fractions or standard (vitamin C and vitamin E) in 25 mL of ethanol. The sample solution was shaken then mixed with vortex until it was homogeneous. 2 mL of sample solution was put respectively into the test tube then each added 1 mL of 100 ppm DPPH solution. Subsequently incubated for 30 minutes in the dark then measured at maximum wavelength. Concentration series were prepared for each samples and standard.

2.6 Data Analysis

The formula for calculating the percentage of inhibition against DPPH from each concentration of sample and standard solution was as follows:

\[
\text{%Inhibition} = \frac{\text{Abs DPPH} \times \text{Abs Sample}}{\text{Abs DPPH}} \times 100\%
\]

Furthermore, statistical data analysis was performed using linear regression. Antioxidant activity was expressed by 50% Inhibition Concentration (IC50), which represented sample concentration that can reduce DPPH radical by 50%. IC50 value of pidada fruit extract was calculated from the equation of the regression curve between concentration and % inhibition of the extract (sample) solution while the IC50 values of vitamin C and vitamin E were calculated from the equation of the regression curve between concentration and % inhibition of vitamin C and vitamin E.

3. Results and Discussions

3.1 Sample Preparation

Pidada fruit was determined to find out its identity. Determination was carried out at Research Center for Biology, Indonesian Institute of Sciences (LIPI). The results of the determination showed that the sample used in the study came from species of *Sonneratia caseolaris* (L.) Engl. Pidada fruit was collected and cleaned from the skin, washed and separated between the seeds and flesh. After that the flesh was thinly sliced and air dried in an open space and protected from sunlight. This was purposed to reduce the water content in the sample, so that the growth of microorganisms and enzymatic activities that could cause decay was hampered or stopped. Proper drying could make simplicia had a long shelf life. Drying the sample was done by aeration without direct sunlight, because ultraviolet rays from the sun could cause damage to the chemical content of the dried sample\[^8,9\].

3.2 Extraction and Fractionation

Pidada fruit powder extraction was carried out by maceration method. The advantage of this method was that the tools and procedures used were very simple and could prevent damage to the active substance because of heating. Maceration process was done by immersing the sample with organic solvents at room temperature\[^10\]. The extracts with the maceration method had stronger antioxidant activity compared to other extraction methods\[^11\]. The organic solvent used for extraction in this study was 96% ethanol. 96% ethanol is a solvent that can extract secondary metabolites of unknown structure and for screening purposes. This solvent has extensive extraction power so that all secondary metabolites can be extracted.
A total of 1 kg of Pidada fruit powder was macerated with 96% ethanol as much as 10 L for 4x24 hours. Extraction was done 3 times or until the filtrate became colorless. The filtrate was then concentrated with rotary evaporator and concentrated ethanol extract was obtained. The principle of rotary evaporator was to reduce the vapor pressure of the solvent so that the solvent would evaporate below its normal boiling point. This principle made the solvent could be separated from the solute without high heating so that the phytochemical components contained in the sample did not undergo degradation due to high temperatures\(^{[11]}\).

The concentrated ethanol extract was then fractionated by liquid-liquid extraction technique. The purpose of fractionation was to separate the components of active compounds based on different levels of polarity from the extracts that had been produced. The principle of separation in the fractionation process was based on differences in the level of polarity and differences in specific gravity between the two fractions\(^{[12]}\). A total of 60 grams of concentrated ethanol extract was diluted with distilled water. Fractionation was conducted using \(n\)-hexane, ethyl acetate and butanol solvents which was purposed to separate nonpolar, semipolar and polar compounds. When fractionated with \(n\)-hexane solvent, two layers were formed, the top layer was \(n\)-hexane and the bottom layer was water. This was because the specific gravity of \(n\)-hexane (0.670-0.677 g/mL) was smaller than the specific gravity of water (1 g/mL). The same thing was done with the other solvent, ethyl acetate and the butanol. The results of the fractionation of each solvent were then evaporated using rotary evaporator at 40 °C to produce concentrated \(n\)-hexane, ethyl acetate and butanol fractions. Yield of extraction and fractionation process could be seen in Table 1.

Table 1: Yield of Extraction and Fractionation

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent</th>
<th>Weight (g)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>144,9260</td>
<td>14.49</td>
</tr>
<tr>
<td>2</td>
<td>(n)-heksan</td>
<td>2,4016</td>
<td>4.00</td>
</tr>
<tr>
<td>3</td>
<td>Etil asetat</td>
<td>4,4325</td>
<td>7.39</td>
</tr>
<tr>
<td>4</td>
<td>Butanol</td>
<td>5,3190</td>
<td>8.87</td>
</tr>
</tbody>
</table>

3.3 Screening of Secondary Metabolite

Phytochemical screening was carried out to find out or give an overview of secondary metabolite compounds contained in extract and fraction of pidada fruit. Phytochemical screening test results could be seen in Table 2.

Table 2: Results of Phytochemical Screening

<table>
<thead>
<tr>
<th>No.</th>
<th>Group of secondary metabolite</th>
<th>ethanol</th>
<th>(n)-hexane</th>
<th>Ethyl acetate</th>
<th>Butanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Terpenoid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tanin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The results showed that ethanol extract and fractions did not contain alkaloids but contain flavonoid, terpenoid, tannin and saponin. The principle of flavonoid test reaction was the redox reaction. Flavonoid compound would be reduced by hydrogen gas resulting from the reaction between Mg and HCl. Furthermore, the reduced compound will form a complex compound with \(Mg^{2+}\) which gave red color\(^{[13]}\). Terpenoid test was using Liebermann Buchard test. Acetic acid molecules and sulfuric acid will bind to molecules of terpenoids to produce a reaction that appeared to change color\(^{[14]}\). The tannin test was carried out by adding 1% iron...
(III) chloride to the sample which would later cause a blackish green or dark blue color because tannins will form complex compounds with Fe\(^{3+}\) ions.

3.4 DPPH Free Radical Scavenging Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay based on the ability of the antioxidant substance to neutralize free radicals\(^{[15]}\). Quantitative antioxidant activity tests were carried out using a UV-Vis spectrophotometer. First of all the determination of the maximum wavelength measured in the range 200-700 nm. The results showed that the maximum wavelength of 100 ppm DPPH solution was 515.93 nm.

The mechanism was through the donation of hydrogen atom so that the DPPH color changed from purple to faded purple or yellow. DPPH color changes occur due to the presence of compounds that could provide hydrogen radicals to DPPH radicals so that it was reduced to DPPH-H. The decrease in absorbance of DPPH was measured against the absorbance of control (DPPH in ethanol without the addition of sample). The decrease in absorbance was indicated by the change in color of the solution from purple to faded purple or yellow. The color degradation of the solution was directly proportional to the addition of the extract concentration\(^{[16]}\). When the absorbance value of DPPH obtained, it could be determined the percentage of radical inhibition (\% inhibition) of DPPH then plotted to regression curve against concentrations. The linear regression equation of ethanol extract and fractions of pidada fruit with vitamin C and vitamin E as a comparison represented as follows:

![Figure 3: Linear regression curves of ethanol extract (a), n-hexane fraction (b), ethyl acetate fraction (c), butanol fraction (d), standard of vitamin C (e) and standard of vitamin E (f)](image)
Based on Figure 3, it could be seen that the addition of concentration will affect the value of percent inhibition, where the higher the concentration the smaller the absorbance value due to the presence of antioxidant compounds.

**Table 3: IC\textsubscript{50} values**

<table>
<thead>
<tr>
<th>No</th>
<th>Ekstrak</th>
<th>IC\textsubscript{50} (ppm)</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Vitamin C</td>
<td>3.70</td>
<td>Very strong</td>
</tr>
<tr>
<td>2.</td>
<td>Vitamin E</td>
<td>3.99</td>
<td>Very strong</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanol extract</td>
<td>32.58</td>
<td>Very strong</td>
</tr>
<tr>
<td>4.</td>
<td>n-hexane fraction</td>
<td>191.31</td>
<td>Moderate</td>
</tr>
<tr>
<td>5.</td>
<td>Ethyl acetate fraction</td>
<td>96.02</td>
<td>Strong</td>
</tr>
<tr>
<td>6.</td>
<td>Butanol fraction</td>
<td>371.16</td>
<td>Weak</td>
</tr>
</tbody>
</table>

Based on data in Table 3, ethanol extract of pidada fruit had a very strong antioxidant activity with an IC\textsubscript{50} value of 32.58 ppm. According to Paputungan et al., (2017) in his study stated that the results of antioxidant activity tests on other mangrove species (*Sonneratia alba*) had an IC\textsubscript{50} value of 296.54 µg/mL which was stated to be very weak. This means that the fruit of pidada (*Sonneratia caseolaris* (L.) Engl.) has much stronger antioxidant power than *Sonneratia alba*. Therefore, the extract was fractionated to see the polarity of secondary metabolites which provide the most dominant antioxidant activity. Based on Table 3, it could be seen that ethyl acetate fraction had the highest antioxidant activity with an IC\textsubscript{50} value of 96.02 ppm compared to the n-hexane and butanol fraction. It could be concluded that the dominant polarity of secondary metabolite compound which provided antioxidant activity was semi-polar.

The difference of IC\textsubscript{50} values in each extract and fractions was caused by the distribution of types and amounts of secondary metabolite compounds which acted as antioxidants based on the polarity of the solvent used. From phytochemical screening data, ethanol extract contained flavonoids, terpenoids, tannins and saponins while ethyl acetate and butanol fractions also positively contained these compounds except saponins. The n-hexane fraction only contained terpenoids and tannins. Differences in compound content could affect antioxidant activity and IC\textsubscript{50} values. The ethyl acetate fraction extract gave high activity as an antioxidant against DPPH radical.

### 4. Conclusion

The IC\textsubscript{50} values of ethanol extract, n-hexane fraction, ethyl acetate fraction and butanol fraction of pidada fruit (*Sonneratia caseolaris* (L.) Engl.) were respectively 32.58; 191.31;
96.02 and 371.16 ppm, which are categorized as having very strong, moderate, strong and weak antioxidant activity.

Acknowledgement

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References


