DETERMINATION OF ANTIOXIDANT ACTIVITY IN OIL AND RED FRUIT JUICE (*Pandanus conoideus* Lamk) WITH DPPH METHOD

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**Abstract** Antioxidant compounds play an essential role in health because they can reduce the risk of chronic diseases such as cancer and coronary heart disease. This study analyzed the antioxidant activity of red fruit oil and juice. This study used an experimental method with oil and juice obtained through boiling for 4-5 hours. The stages of the study include flavonoid qualitative tests and antioxidant activity tests using the DPPH method using UV-Vis Spectrophotometers. Based on the research results, oil and red fruit juice contain flavonoids that have the potential to be antioxidants. Antioxidant activity test with the DPPH method showed IC50 in red fruit oil products on the market at 30.11 ppm and red fruit juice at 31.80 ppm, showing vigorous antioxidant activity compared to homemade red fruit oil with an IC50 of 85.98 ppm.

**Keywords:** Red Fruit • Antioxidants • DPPH • IC₅₀ • *Pandanus conoideus* Lamk.

**Introduction** Antioxidant compounds are increasingly used in the health sector. In the food sector, antioxidants can act as safe and natural preservatives. Antioxidant compounds have been scientifically proven to reduce the risk of chronic diseases such as cancer and coronary heart disease. The mechanism of action of antioxidant compounds in preventing chronic diseases is to ward off free radicals in the body (Purwanto et al., 2017).

Antioxidant compounds are found in many plants, including flowers, leaves, and fruits. Plants that contain bioactive compounds such as flavonoids, alkaloids, and terpenoids are potential raw materials that can be used as natural antioxidants. Red fruit is one type of plant that may contain antioxidant compounds. Flavonoids are a class of polyphenolic compounds with antioxidant properties. Antioxidants are chemical compounds that can reduce free radicals by providing one or more electrons so as not to damage body cells (Merdita, 2023).

People empirically use red fruit as traditional medicine. Red fruit contains various active ingredients that are important for health, including anticancer substances, energy enhancers, calcium, fiber, protein, vitamin B1, vitamin C, myristic acid, linoleic acid, acid, deconate, omega 3, omega 6, and omega 9. So far, red fruit contains many active ingredients that are good for health. The use of red fruit has focused on the fruit's flesh. In addition to the flesh, the remaining red fruit consists of seeds. The number of seeds in the red fruit is relatively high because the red fruit consists of thousands
of seeds that form the fruit shell. Red fruit seeds contain essential nutritional components such as carbohydrates, proteins, lipids, and secondary metabolites (Ayomi, 2015). Until now, the use of red fruit has only focused on the flesh. In addition to red meat, another part of the red fruit is the fruit seed. The number of red fruit seeds is relatively abundant because red fruit is composed of thousands of seeds that form the fruit’s skin. The seeds are thrown away after the fruit’s flesh is taken. Fruits and seeds are closely related because both have almost the same structural arrangement, and both function as storage of food reserves in plants (Sundari, 2010).

Red fruit is vital for the people of Papua for several reasons, namely because Red fruit oil is used as goring oil and as a primary ingredient for medicines. Related to its role as a primary medicinal ingredient, Red fruit oil has been studied for its chemical content and is known to contain fatty acids and derivatives (Husein et al., 2019). Soft Red fruit paste can be made into chili sauce and sauce, then applied to hipere (sweet potato), hom (taro), and rice to arouse the appetite (Husein et al., 2019).

Antioxidant activity can be proven by testing the DPPH (1,1-diphenyl-2-picrylhydrazil) method to reduce free radicals. Although there are several methods for testing antioxidant activity, the DPPH method was chosen because it requires a limited number of samples and is simple, easy, fast, and sensitive to evaluate the antioxidant activity of natural compounds. Determination of antioxidant activity with this DPPH using a UV-Vis spectrophotometer (Purgiyanti et al., 2022). Based on the explanation above, the research team conducted a study to test the antioxidant potential of red fruit oil and juice. This research is also expected to help make a meaningful contribution to science, especially pharmacy. In the next stage, it will also greatly benefit society. Therefore, the author is interested in using red fruit seeds to test antioxidant activity.

Materials and Methods

Materials and Objects of Research

The ingredients used include Red fruit and Red fruit oil purchased online with the brand Cahya, aquades, methanol, ethanol 96%, ethanol 70%, HCl 2N, HCl 32%, FeCl3 1%, H2SO4 98%, anhydrous acetic acid, Mg powder, Mayer reagent, Wagner reagent, Bouchardat reagent, DPPH powder (Sigma-Aldrich), and vitamin C powder. The object of the study was the determination of antioxidant activity in red oil and fruit juice (Pandanus conoideus Lamk.) with the DPPH method.

Samples and Sampling Techniques

The samples used in this study were self-made red fruit oil and the juice and red fruit oil sold online. In this study, the sampling technique was simple random sampling. Samples were obtained randomly regardless of the sample size studied (Salsabilla, 2023). The sample preparation used in this study was red fruit purchased directly from farmers in Manokwari, Papua. Fruits are selected at the optimal maturity level, with the harvest criterion being that the fruit grains are filled (pithy) and dark red. The fruit flesh becomes softer during shipping, making it easier to pick.

Macroscopic Identification Test

The purpose of macroscopic identification is to show the characteristics of Simplicia using direct observation of organoleptic Simplicia, ranging from shape, color, smell, and taste (Paramita et al., 2019).

Making Red Fruit Oil

According to Purgiyanti et al. (2022), the steps of making red fruit oil are (a) choosing a ripe fruit, (b) the fruit is split, and the pith is removed (c) the flesh of the fruit into pieces, and thoroughly washed; (d) the flesh of the fruit is steamed 1 hour to 1 hour 30 minutes until soft; (e) the fruit is removed and cooled; (f) add a little water and then knead and squeeze until it becomes a paste; (g) the paste is then filtered to separate the seed pulp from the paste; (h) pasta
is cooked 4 to 5 hours to boiling; (i) the paste is allowed to remain on the fire for 10 minutes until black oil appears on its surface; (j) remove the pasta decoction and let it sit for 1 hour; (k) gently draw the oil with a spoon into a transparent container; (l) Let stand for 2 hours until the oil separates from the water and paste. The steps for making red fruit oil are repeated several times until no more water is under the oil layer. Water can also be removed by heating the oil at 95 to 100 °C for 2 to 3 minutes until no more bubbles are visible. The final result is red fruit juice and oil, which is then cooled.

**Preparation of Vitamin C Stock Solution 1000 ppm**
This stage is the manufacture of a 1000 ppm vitamin C stock solution by weighing 10 mg of vitamin C and then put into a measuring flask plus 10 ml of whipped methanol until homogeneous (Atika, 2021).

**Operating Time**
Determination of operating time by taking 0.4 ml of vitamin C solution plus DPPH 40 ppm as much as 10 ml then homogenizing with a stirrer then measuring absorbance at minutes 0-60 every 5 minutes at the maximum length that has been obtained (Atika, 2021).

**Preparation and Readings of Vitamin C Solutions 10, 20, 40, 80 ppm**
Making a solution of the vitamin C is carried out by taking a stock solution of 1000 ppm vitamin C pipettes into a measuring flask as much as 0.1 ml, 0.2 ml, 0.4 ml, and 0.8 ml. Then, enough with 10 ml of methanol shake until homogeneous incubation with operating time (Purwaningsih et al., 2023).

**Preparation of Red Fruit Oil Solution 100, 150, 200, 250, 300 ppm**
Making a Red fruit oil solution with a stock solution of 1000 ppm Red fruit oil in a pipette into a 1 ml measuring flask; 1.5 ml; 2 ml; 2.5 ml; 3 ml. Suffice with 10 ml of methanol and beat until homogeneous (Atika, 2021).

**Preparation of Flavonoid**
Flavonoid compounds are carried out by taking a sample of 1 ml, adding 3 ml of 70% ethanol, then shaking, heating, and shaking again. Strain the filtrate. The filtrate obtained is added Mg 0.1 grams and two drops of concentrated HCl. A positive result shows a red color on the ethanol layer (Pardede et al., 2013; Febriyanti et al., 2022).

**Preparation of DPPH Solution 1000 ppm**
In this step, we made a 1000 ppm DPPH solution by weighing 10 mg of DPPH and then added 10 ml of methanol shake until homogeneous.

**DPPH Maximum Absorption Wave Length Setting**
Determination of the maximum absorption wavelength of DPPH by taking 4.0 ml of DPPH solution, inputting it into the cuvette, then measuring at a wavelength of 400-600 nm with UV-Vis spectrophotometry obtained absorbance to find the maximum wavelength (Atika, 2021).

**Preparation of DPPH Blanks 40 ppm**
Making a 40 ppm DPPH blank takes 0.4 ml of 1000 ppm DPPH solution input into a measuring flask, plus 10 ml of methanol shake until homogeneous, then read the absorption with the maximum wavelength that has been obtained by UV-Vis spectrophotometry (Atika, 2021).
Preparation of Red Fruit Juice Solution 100, 150, 200, 250, 300 ppm
Making a solution of the Red Fruit juice with a stock solution of 1000 ppm, Red Fruit juice in a pipette into a 1 ml measuring flask: 1.5 ml; 2 ml; 2.5 ml; 3 ml, suffice with 10 ml of methanol beat until homogeneous (Atika, 2021).

Analysis of Antioxidant Activity Data
Measurement of antioxidant activity with the DPPH method is expressed by DPPH reduction value (%inhibition), with higher absorption value showing higher antioxidant value. Percentage of DPPH inhibitory activity in each formulation (Pratiwi, 2021). The formula can express the percentage of DPPH inhibitory activity on the extract:

% inhibition = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100

Value Determination of IC_{50}
The value of IC_{50} is determined by linear regression of the relationship between the concentration of logarithms and probits. The lower the IC_{50} value, the lower the DPPH by 50%. IC_{50} is then calculated using a linear regression equation plotted with the logarithm of the sample concentration on the x-axis and probit on the y-axis. For good results in this study, use probit. The IC_{50} value is determined by probit, which is obtained by converting the % resistivity into a probit value and simultaneously converting the concentration value into a logarithmic concentration.

Results and Discussion
Red fruit (Pandanus conoideus Lamk.) is native to Papua Province, Indonesia. The fruit is 68-110 cm long and 10-15 cm in diameter, red in color, and contains a large amount of oil (Agnesa et al., 2017). This study aims to compare the content of flavonoids and antioxidant compounds in oil and red fruit juice (Pandanus conoideus Lamk.) obtained through the boiling process with red fruit oil products on the market. Flavonoid tests were carried out to determine the flavonoid content of red fruit.

Antioxidant analysis of the sample was performed using UV-Vis Spectrophotometry.

Macroscopic Test
Macroscopic tests aim to determine the characteristics of the fruit by direct observation, including shape, color, and size. The results of observations from macroscopic identification are presented in Table 1.

<table>
<thead>
<tr>
<th>Picture</th>
<th>Shape</th>
<th>Color</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triangular cylinder (tapering)</td>
<td>Red</td>
<td>52 - 74</td>
<td>2.8 – 6.1</td>
</tr>
</tbody>
</table>

The results of the physical character of the whole fruit from the red fruit in this study are tapered cylindrical, from the rounded base extending to the middle enlarged or reduced to the end shrinking. For the length and weight of red fruits vary from 2 (two) red fruits obtained, which on average ranges between 52-74 cm and 2.8-6.1 kg. Size variations between red fruit growing sites reported by Purgiyanti et al. (2022), that generally the highland area has a medium size (42 cm) to long (80.2 cm), while the lowland ranges from 59-66 cm, but in the lowland it varies more from short (25-29 cm) to long (70 cm). According to Agnesa et al. (2017), the red fruit weight of the 9 observed clones averaged 2.0-7.9 kg, which is relatively the same as report of Atika (2001) which ranges from 3.0-7.6 kg, while Hadad et al. (2006) grouped large fruits with a weight range of 5-10 kg and small fruits with a range of 4-7 kg.

Flavonoid Test
The flavonoid test showed that all three samples contained flavonoid compounds with the result that there was a red color on the ethanol layer. Identification of flavonoid entities is carried out using Mg powder and HCl concentrate. HCl solution aims to convert flavonoid glycosides into aglicon, which is then combined with...
magnesium to change color to yellowish, red, or orange. In addition, flavonoid compounds can be recognized by the reaction of NaOH which produces a color change that becomes reddish (Pardede et al., 2013; Salsabilla, 2023).

**Wave Length Determination**
The determination of the maximum wavelength aims to determine the wavelength characterized by maximum absorption, which is when the optimally formed colored compound to achieve maximum sensitivity (Pramiastuti et al., 2021). The wavelength used is between 400 nm to 600 nm. Based on the results, the maximum wavelength is 515 nm with an absorption of 0.374, is the maximum wavelength that has a high sensitivity to the most remarkable absorption change. To find out how much of the highest light energy is absorbed by the solution, the maximum wavelength is found (Pratiwi, 2021).

![Figure 1. Maximum wavelength measurement](image)

The maximum wavelength reading is determined using a standard control solution or a 40 ppm DPPH solution dissolved in methanol to obtain DPPH absorbance without interference with the absorbance of other compounds in the sample used (Atika, 2021).

**Operating Time**
The result of operating time at minute 0 with an absorbance value of 1.012 nm can be seen in Figure 2. This operating time indicates that the reaction between the test solution and DPPH is unstable, which can be indicated by a decrease in absorbance (Pramiastuti et al., 2021). This shows that the purpose of operating time is to determine the time needed for the comparison solution in this study, namely vitamin C, to react stably (Merdita, 2023).

![Figure 2. Operating Time Measurement](image)

**Figure 2. Operating Time Measurement**

**Determination of antioxidant activity**
Antioxidant activity tests were performed to determine the absorption value, % inhibition, probit value, and IC$_{50}$ value in Red oil and fruit juice and vitamin C comparison. Each sample was prepared from a 1000 ppm stock solution and dissolved in methanol (Purgiyanti et al., 2019). The stock solution is made into several concentrations, namely 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm. As for the comparison concentration, namely 10 ppm, 20 ppm, 40 ppm, 80 ppm. The comparison used as a positive control is vitamin C. Vitamin C serves as a positive control or comparison because it is a good antioxidant. Vitamin C has the molecular formula C$_6$H$_8$O$_6$ and is known for its powerful antioxidant effects as it acts as a reducing agent. This reducing property is caused by the release of hydrogen atoms in hydroxyl groups bonded to C2 and C3 (carbon atoms in double bonds) so that free radicals can easily trap and produce stable reduced free radicals (Purgiyanti et al., 2019).

The IC$_{50}$ value is determined by the linear regression equation of the sample and probit concentration log relationship curve using the equation $Y = ax + b$, where the sample concentration log is the (X) axis and the probit value is the (Y) axis. The IC$_{50}$ value is determined by entering the number 50 into the Y variable so that the X value will be known. X is the value of IC$_{50}$ (Arista & Siregar, 2023).
Table 2 Antioxidant Activity of Vitamin C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration Log</th>
<th>Probit % Inhibition</th>
<th>Linear Regression Equation</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>1.3</td>
<td>4.92</td>
<td>y = 2.46x + 1.723</td>
<td>19.6</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.6</td>
<td>5.64</td>
<td>R² = 0.9998</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.9</td>
<td>6.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that vitamin C has an IC<sub>50</sub> value of 19.62 ppm. Vitamin C has been shown to have highly active antioxidant activity. The lower the IC<sub>50</sub> value, the greater the antioxidant activity (Purgiyanti, 2019). Data on the results of probit inhibition, linear equations, and IC<sub>50</sub> values can be seen in Table 3 and Figure 3.

Table 3. Antioxidant Activity of Red Fruit Oil and Juice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
<th>Average Absorbance</th>
<th>% Inhibition</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>100</td>
<td>0.219</td>
<td>45.79 %</td>
<td></td>
</tr>
<tr>
<td>Sample A</td>
<td>150</td>
<td>0.208</td>
<td>48.51 %</td>
<td></td>
</tr>
<tr>
<td>Sample A</td>
<td>200</td>
<td>0.192</td>
<td>52.47 %</td>
<td>85.98</td>
</tr>
<tr>
<td>Sample A</td>
<td>250</td>
<td>0.185</td>
<td>54.20 %</td>
<td></td>
</tr>
<tr>
<td>Sample A</td>
<td>300</td>
<td>0.178</td>
<td>55.94 %</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>100</td>
<td>0.291</td>
<td>27.97 %</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>150</td>
<td>0.236</td>
<td>41.58 %</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>200</td>
<td>0.187</td>
<td>53.71 %</td>
<td>30.11</td>
</tr>
<tr>
<td>Sample B</td>
<td>250</td>
<td>0.182</td>
<td>54.95 %</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>300</td>
<td>0.162</td>
<td>59.90 %</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>100</td>
<td>0.273</td>
<td>32.42 %</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>150</td>
<td>0.216</td>
<td>46.53 %</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>200</td>
<td>0.209</td>
<td>48.26 %</td>
<td>31.80</td>
</tr>
<tr>
<td>Sample C</td>
<td>250</td>
<td>0.165</td>
<td>59.15 %</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>300</td>
<td>0.160</td>
<td>60.39 %</td>
<td></td>
</tr>
</tbody>
</table>

Antioxidant activity tests were performed using UV-Vis spectrophotometry at frequent concentrations of 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm with three repeats. This test is performed to determine the remaining DPPH absorbance after sample addition (Pramiastuti et al., 2021). The results of the antioxidant activity of oil and red fruit juice are presented in Table 3. Table 3 data shows that the greater the concentration of samples A, B, and C, the % inhibition value increases. The probit value is calculated from % inhibition data, then plotted into a graph between the concentration log (x) and probit (y), thus forming a linear equation = ax + b. The result of the curve between log concentration with probit % oil inhibition and red fruit juice is presented in Figure 4.
The value of $R^2$ (correlation coefficient) indicates a linear relationship between the probit and the concentration log. Based on the literature, the $R^2$ value that approaches 1 indicates that the data obtained is excellent (Pratiwi, 2021).

Based on the calculation results, IC$_{50}$ values of samples B and C have more significant antioxidant activity with IC$_{50}$ values of 30.11 ppm and 31.80 ppm compared to sample A with IC$_{50}$ values of 85.98 ppm, and it can be said that the activity in sample A is in the medium category. Vitamin C antioxidant activity is used as a reference substance in testing. The calculation results show the IC$_{50}$ value of vitamin C of 19.62 ppm. Compared to the IC$_{50}$ value in each sample, vitamin C still has highest antioxidant activity. According to Molyneux (Atika, 2021), the smaller the IC$_{50}$ value indicates, the higher the antioxidant activity. A compound is said to be a powerful antioxidant if the IC$_{50}$ value is < 50 ppm, a potent antioxidant for the IC$_{50}$ value ranging from 50-100 ppm, a medium antioxidant if the IC$_{50}$ value is 100-150 ppm, and a weak antioxidant if the IC$_{50}$ value is 151-200 ppm, while if the IC$_{50}$ value is above 200 ppm, then the antioxidant activity is very weak.

**Conclusion**

Based on the study's results, it can be concluded that Red fruit contains flavonoids. Red fruit oil from online stores has an IC$_{50}$ value of 30.11 ppm (very strong activity), self-processed Red fruit juice has an IC$_{50}$ value of 31.80 ppm (very strong activity), and self-made Red fruit oil has an IC$_{50}$ value of 85.98 ppm (strong activity).

**Compliance with ethical standards**

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**


