Antioxidant Capacity and Potential as an Alpha-Glucosidase Inhibitor in *Phaleria macrocarpa* (Scheff.) Boerl Fruit Peel Ultrasonic Extract

Candra Irawan¹, Maman Sukiman²-∗, Ismail³, Imalia Dwi Putri¹, Andita Utami², Andrean Nur Pratama⁴, M. Ilham Kumala Zalni²

**ABSTRACT**

*Phaleria macrocarpa* (Scheff.) Boerl is native to the tropical region of Papua Island, Indonesia, and has traditionally been used as a herbal drink, either alone or in combination with other medicinal plants, to treat diseases such as cancer, hypertension, and diabetes mellitus. This study aimed to obtain the fruit peel extract of *Phaleria macrocarpa* (Scheff.) Boerl. through the application of ultrasound-assisted extraction (UAE) with variations in time and amplitude to produce optimal extraction conditions. The potential of the extract as an antioxidant using the cupric ion reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) methods, and its potential as an antidiabetic through alpha glucosidase inhibition. The optimum extract selected was extract C (extraction time was 45 minutes and amplitude 77.37 ± 0.8 mg/L), while the inhibition of alpha glucosidase was 0.45 ± 0.007 mg/L. It can be concluded that the ethanol extract of *Phaleria macrocarpa* fruit peel has the potential as a source of antioxidants and anti-diabetic.

Key words: *Phaleria macrocarpa* (Scheff.) Boerl, Antioxidant, Antidiabetic, Alpha-glucosidase.

**INTRODUCTION**

Since 2000, a natural and healthy lifestyle has attracted the attention of people all over the world. The use of natural bioactive compounds in food or for medical applications is increasing. Because of its numerous antioxidant properties, the medicinal properties of this natural bioactive compound have been studied scientifically all over the world. Furthermore, the use of plants as traditional medicine is a topic of research because medicinal plants have good benefits such as low toxicity if used properly, low cost, and easy access. *Phaleria macrocarpa* (Scheff.) Boerl. is one of these plants. It is a native plant of Indonesia’s Papua Island’s tropical areas.

*P. macrocarpa* has traditionally been used as a herbal drink, either alone or in combination with other medicinal plants, to treat illnesses such as cancer, hypertension, and diabetes mellitus. Several studies have been conducted on the biological and pharmacological activities of the stem, leaves, fruit, and seed parts. Furthermore, studies show that secondary metabolites of this plant, such as tannins, saponins, phenolic compounds, flavonoids, terpenoids, and alkaloids, play an important role as antioxidants, anti-inflammatory, antimicrobial, and cytotoxic agents.

Antioxidants are important in life because they can neutralize or destroy free radicals such as reactive oxygen species (ROS). ROS can damage cell membranes, damage proteins, and cause DNA mutations. If this condition persists, it can cause oxidative stress, which can cause cancer, inflammation, arthritis, atherosclerosis, Alzheimer’s disease, Parkinson’s disease, neurodegenerative diseases, and diabetes mellitus.

According to the International Diabetes Federation (IDF), diabetes will affect 643 million people in 2030 and 783 million people in 2045. Type 2 Diabetes Mellitus (T2DM) accounts for approximately 90% of all diabetes cases, and it is primarily caused by obesity and a lack of physical activity. Agents with alpha-glucosidase inhibitory activity have been found to be effective as oral hypoglycemic agents in the treatment of hyperglycemia in T2DM patients. Acarbose, miglitol, voglibose, and emiglitate are currently the most commonly used drugs for the treatment of diabetes. Side effects of these medications include flatulence, stomach cramps, vomiting, and diarrhea. Because of these effects, several studies have been conducted to identify natural sources of alpha-glucosidase inhibitors.

The use of ultrasound-assisted extraction (UAE) to obtain an ethanolic extract of *Phaleria macrocarpa* fruit peel and assay of antioxidant activity using the FRAP and CUPRAC methods as well as alpha glucosidase inhibitory activity against ultrasonic extracts have not been previously reported. This extract will be prepared using an UAE method. In order to obtain optimum conditions, the extraction is carried out by varying the time and percent amplitude.

**METHODS**

**Simplicia setup**

The plants used were previously determined by the correctness of identity in the Herbarium Bogoriense, Botany field of the National Research and Innovation
The solution was incubated for 30 minutes at 37 °C, then the absorbance of the solution was measured using a visible spectrophotometer at a wavelength of 650 nm. The same steps were conducted on a standard solution of gallic acid with a concentration of 0.25, 0.5, and 0.75 mg/L. The IC<sub>50</sub> value can be calculated based on the equation as in the determination of the CUPRAC method.

Antioxidant activity test using Cupric Ion Reducing Antioxidant Power (FRAP) method

Antioxidant testing of the FRAP method using the procedure that has been used by Irawan et al. The work was done on the crude ethanol extracts of treatments A, B, C, and D that were obtained during the extraction process with the UAE. A solution of fruit peel ethanol extract with a concentration range of 40 to 160 mg/L was prepared from a mother liquor of 1000 mg/L fruit peel ethanol extract. Each solution was placed in five 5 mL volumetric flasks, then 0.4 mL of 0.001 M citric acid was added; 0.2 mL of 0.002 M Fe<sup>3+</sup> solution; and 0.4 mL of 0.2% o-phenanthroline, then filtered with distilled water and homogenized. The solution was incubated for 35 minutes at 37°C, then the absorbance of the solution was measured using a visible spectrophotometer at a wavelength of 510 nm. The same steps were conducted on a standard solution of gallic acid with a concentration of 0.25, 0.5, and 0.75 mg/L. The IC<sub>50</sub> value can be calculated based on the equation as in the determination of the CUPRAC method.

Total phenolic content

The total phenolic content of fruit peel Phaleria macrocarpa was determined using the Folin–Ciocalteu method. The crude extract solution with a concentration of 1000 ppm was pipetted as much as 400 µL and put into a measuring flask with a capacity of 10 mL. The solution was added with distilled water up to 5 mL, then shaken until homogenous, then 1 mL of Folin-Ciocalteu reagent was added and shaken again until homogenous, then allowed to stand for 3 minutes. Then 2.5 mL of sodium carbonate (10% w/v) was added and homogenized again. The solution was left in the dark for 60 minutes, and the absorbance was measured at a wavelength of 650 nm. Gallic acid calibration curves (concentrations of 0, 2, 4, 6, and 8 mg/L) were used to calculate the total phenol content. The result is given in milligrams of gallic acid equivalent per gram of dry mass. Use Figure 1: Curve for gallic acid used as a standard.

Alpha-Glucosidase inhibitor activity test

Testing of alpha-glucosidase inhibitory activity refers to the procedure used by Budiarsa. The work was done on the crude ethanol extracts of treatments A, B, C, and D that were obtained during the extraction process with the UAE. Acarbose as a standard and fruit peel ethanol extract samples were weighed and dissolved in phosphate buffer pH 6.8. Then, standard and sample solution were diluted into some concentrations. Thirty microliter standard and sample solution were added to 17 µL of para-Nitrophenyl-α-D-glucopyranoside substrate 4mM. The solutions were incubated at 37°C for 5 minutes, then 17 µL alpha-glucosidase solution was added. The solution was incubated again at 37°C for 15 minutes. After that, 100 µl sodium carbonate 200 mM was added, then the absorption of the solution was measured using a microplate reader at λ 405 nm. The same operation was carried out for sample control and blank control, but the addition of sodium carbonate was carried out before the addition of alpha glucosidase.

RESULT AND DISCUSSION

Ultrasound-assisted extraction

Ultrasound-assisted extraction of Phaleria macrocarpa fruit peel using 70% ethanol solvent with variations in time and amplitude resulted in a crude extract and yield of 1.5848 g and 21.83% for treatment A, 1.5494 g and 21.29% for treatment B, 1.5449 g and 21.33% for treatment C, and 1.3212 g and 18.47% for treatment D. Extracts A-C produced relatively
the same yield, which was about 21%, but in extract D (extraction time of 45 minutes and amplitude of 65%), the yield decreased to 18.47%. When compared with other extraction methods such as maceration, subcritical water, and microwave-assisted extraction (MAE), UAE was found to be more effective because it reduces the degradation of phenolic compounds. 19-21

Total phenolic content of ethanol extract

The total phenolic content of *Phaleria macrocarpa* fruit peel was determined using the Folin–Ciocalteu method, with gallic acid as a control compound. 22,23 The absorbance of the measured standard series of gallic acid can be seen in the standard curve as in Figure 1. The standard curve of gallic acid had a linear regression equation $y = 0.0961x - 0.1079$ with an $R^2$ value of 0.9781. The total phenol content in the ethanol extract of fruit peels with various times and amplitudes obtained from this equation can be seen in Table 1.

Table 1 shows that the highest total phenol content in the *Phaleria macrocarpa* fruit peel extract with treatment B was 2072.63 ± 0.6 mg GAE/g extract. For treatments A and C, the total phenolic content was relatively the same, while for treatment D it was lower. However, the total phenolic content identified from the 4 treatments was relatively high.

In general, phenolic compounds act as antioxidants. The greater the total phenolic content of natural ingredients, the greater their antioxidant activity. 24 Phenolic compounds donate a proton to free radicals, causing them to become stable radicals. 25,26

Antioxidant activity using CUPRAC method

The antioxidant activity test results with CUPRAC method are expressed as % reduction power (Table 2), which is then linked to a series of sample or standard concentrations to produce a curve, as shown in Figure 2. The second-order regression equation of BHT and ethanol extract of *Phaleria macrocarpa* fruit peel with treatments A, B, C, and D obtained were $y = 19.905x + 9.8499$; $y = 0.2612x + 38.913$; $y = 0.5285x + 39.99$; and $y = 0.4445 + 32.384$, respectively. From this equation, the IC50 values for BHT, treatment extracts A, B, C, and D were 2.02 ± 0.01, 42.45 ± 0.6, 39.99 ± 0.2, 39.63 ± 0.009, and 42.01 ± 0.03, respectively. From this equation, the IC50 values for BHT, treatment extracts A, B, C, and D were 2.02 ± 0.01, 42.45 ± 0.6, 39.99 ± 0.2, 39.63 ± 0.009, and 39.99 ± 0.2 mg/L, respectively. General, BHT has a stronger reducing power against CUPRAC reagent than the ethanol extract of *Phaleria macrocarpa* fruit peel. The antioxidant activity of *Phaleria macrocarpa* fruit peel extract with treatments A, B, C, and D was included in the very strong category because the IC50 value was less than 50 mg/L. 27

These findings suggest that an ethanol extract of *Phaleria macrocarpa* fruit peel could be used as an alternative source of natural antioxidants. The oxidation and reduction properties of phenolic compounds in natural materials can cause an antioxidative effect. This redox property is important in peroxide digestion, quenching singlet and triplet oxygen and trapping and neutralizing free radicals. 28,29

Antioxidant activity using FRAP method

The antioxidant activity test results with FRAP method are expressed as % reduction power (Table 3), which is then linked to a series of sample or standard concentrations to produce a curve, as shown in Figure 3.

Table 1: Total phenolic content of ethanolic extract of *Phaleria macrocarpa* fruit peel.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolic Content (mg GAE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (30 minutes - 60%)</td>
<td>1676.43 ± 0.2</td>
</tr>
<tr>
<td>B (35 minutes - 65%)</td>
<td>2072.63 ± 0.6</td>
</tr>
<tr>
<td>C (45 minutes - 60%)</td>
<td>1670.81 ± 0.3</td>
</tr>
<tr>
<td>D (45 minutes - 65%)</td>
<td>1633.71 ± 0.3</td>
</tr>
</tbody>
</table>

Table 2: The results of the CUPRAC method antioxidant activity test.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/L)</th>
<th>% Reduction Power</th>
<th>IC50 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT</td>
<td>1</td>
<td>27.42 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>54.34 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>67.23 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>44.05 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>40</td>
<td>49.54 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>54.50 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>38.14 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>51.95 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>70.50 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td>41.17 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>50.32 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>67.89 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>38.14 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>51.95 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>70.50 ± 0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The results of the FRAP method antioxidant activity test.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/L)</th>
<th>% Reduction Power</th>
<th>IC50 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic Acid</td>
<td>0.25</td>
<td>29.21 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>46.98 ± 0.1</td>
<td></td>
<td></td>
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<tr>
<td>0.75</td>
<td>56.90 ± 0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>19.71 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>80</td>
<td>42.01 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>160</td>
<td>63.62 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>40.14 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>60</td>
<td>47.98 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>56.28 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>26.43 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>37.92 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>52.15 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>33.20 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>44.70 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>52.56 ± 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: Graph of the relationship between concentration and % reduction power for IC\textsubscript{50} determination of BHT. (A) ethanolic extracts of Phaleria macrocarpa fruit peel in 30 minutes extraction time-60% of amplitude, (B) with 35 minutes extraction time-65% of amplitude, (C) with 45 minutes extraction time-60% of amplitude, and (D) with 45 minutes extraction time-65% of amplitude.

Figure 3: Graph of the relationship between concentration and % reduction power for IC\textsubscript{50} determination of gallic acid. (A) ethanolic extracts of Phaleria macrocarpa fruit peel in 30 minutes extraction time-60% of amplitude, (B) with 35 minutes extraction time-65% of amplitude, (C) with 45 minutes extraction time-60% of amplitude, and (D) with 45 minutes extraction time-65% of amplitude.
The regression equation of gallic acid and ethanol extract of *Phaleria macrocarpa* fruit peel with treatments A, B, C, and D obtained were $y = 55.37x + 16.68; y = 0.3522x + 8.9102; y = 0.4035x + 23.925; y = 0.6429x + 0.2571$ and $y = 0.4839x + 14.451$, respectively. From this equation, the $IC_{50}$ values for gallic acid, treatment extracts A, B, C, and D were 0.60 ± 0.006, 116.67 ± 1.4, 64.61 ± 0.9, 77.37 ± 0.8, and 73.46 ± 0.7 mg/L, respectively. In general, BHT has a stronger reducing power against CUPRAC reagent than the ethanol extract of *Phaleria macrocarpa* fruit peel. The antioxidant activity of *Phaleria macrocarpa* fruit peel extract with treatments B, C, and D was included in the because the $IC_{50}$ value was in the range of 50-100 mg/L. 27

The $IC_{50}$ value produced by the FRAP method is lower than the CUPRAC method because the FRAP method is limited to water-soluble antioxidants and cannot be used for compounds containing thiol or carotenoid groups. This is because carotenoids do not have the ability to reduce iron.30

**Potential inhibition of alpha-glucosidase activity of fruit peel ethanol extract**

The alpha glucosidase inhibitory activity assay results were expressed as a percentage of inhibition (Table 4), which was then linked to a series of samples or standard concentrations to produce a curve, as shown in Figure 4. The regression equation of acarbose and ethanol extract of *Phaleria macrocarpa* fruit peel with treatments A, B, C, and D obtained were $y = 0.4946x + 22.38; y = 0.4946x + 22.38; y = 0.4946x + 22.38; y = 0.4946x + 22.38$ and $y = 0.4946x + 22.38; y = 0.4946x + 22.38; y = 0.4946x + 22.38; y = 0.4946x + 22.38$, respectively. From this equation, the $IC_{50}$ values for acarbose, treatment extracts A, B, C, and D were 0.45 ± 0.007, 1.33 ± 0.03 mg/L, respectively.

The $IC_{50}$ value of *Phaleria macrocarpa* fruit peel ethanol extract was lower than the acarbose standard, meaning that the ethanol *Phaleria macrocarpa* fruit peel extract had higher alpha-glucosidase inhibiting activity than the acarbose standard. The high alpha-glucosidase inhibitory activity of *Phaleria macrocarpa* fruit peel extract was correlated with antioxidant activity. Based on the research, the most active result was obtained in extract C of 0.45 ± 0.007 followed by extract D of 1.33 ± 0.03. Therefore, the optimum condition chosen for this extraction was an extraction time of 45 minutes with an amplitude of 60% (C).

The antioxidant and anti-diabetic properties of natural material are related to the presence of phenolic compounds that can donate hydrogen atoms to free radicals to become less reactive. By acting as competitive inhibitors of carbohydrate-digesting enzymes, phenolic compounds can also inhibit alpha-glucosidase. As a result, it takes longer for carbohydrates to be hydrolyzed into glucose molecules.15

**CONCLUSION**

Based on the results of the study, it can be concluded that the *Phaleria macrocarpa* fruit peel extract in all treatments had strong antioxidant activity using either the FRAP or CUPRAC methods. In addition, it is very active as an antidiabetic by inhibiting alpha glucosidase. The optimum extract selected was extract C (extraction time was 45 minutes and amplitude 60%) with $IC_{50}$ values for antioxidant activity in the FRAP method of 77.37 ± 0.8 mg/L and the CUPRAC method of 39.63 ± 0.009 mg/L, while the inhibition of alpha glucosidase was 0.45 ± 0.007 mg/L. It can be concluded that the ethanol extract of *Phaleria macrocarpa* fruit peel has the potential as a source of antioxidants and anti-diabetic.
ACKNOWLEDGEMENT

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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