α-Glucosidase Inhibitory Activities of *Rhizophora mucronata* Fruit Powder

Hardoko¹*, Yunita Eka Puspitasari¹, Eddy Suprayitno¹

¹Fisheries Processing Technology, Fisheries and Marine Sciences Faculty, University of Brawijaya Jl. Veteran, Malang, East Java, Indonesia

**Abstract**: One of approaches methods used to control blood sugar level is inhibition of α-glucosidase enzyme activity. In this research, the α-glucosidase inhibitory activity of extracts of *Rhizophora mucronata* powder processed from immature and mature fruit were studied. *R. mucronata* fruit powder was extracted with hexane, ethyl acetate and ethanol. Phytochemical screening and inhibition of the α-glucosidase activity (IC₅₀) analyses were done to the ethanol extract of the fruit powder. Analysis of total phenolic content was conducted to the immature and mature fruit rind powder. The results showed that the extract of *R. mucronata* fruit powder contained flavonoids, saponins, tannins and steroids, while the *R. mucronata* rind contained flavonoids and tannins. Extract of unpeeled mature *R. mucronata* powder showed the highest inhibitory activity against α-glucosidase and can be considered as an antidiabetic candidate. The inhibition activity of α-glucosidase from unpeeled mature *R. mucronata* powder extract was assumed to be related to the presence of flavonoids and tannins, as well as high levels of total phenols of the rind.

**Keywords**: *R. mucronata* fruit, inhibitory activity, α-glucosidase, rind, phytochemical.

**Introduction**

Diabetes mellitus (DM) type 2 is more common in diabetic. Treatment of type 2 DM mostly is inhibition of α-glucosidase to delay glucose absorption postprandial. This enzyme is involved in carbohydrate metabolisms to form glucose as final product. The ability of the material to inhibit glucose absorption and reduce blood glucose level can be used as a guideline for antidiabetic type 2 drug. One of inhibitors developed as a drug is acarbose.

Mangrove is known as new bioactive source for drug and functional food development. *Rhizophora mucronata* is one of the flora found in mangrove in Indonesian coastlines. Hypocotyl of *R. mucronata* shows antibacterial activity. The bark of *R. mucronata* is astringent used to cure diabetic. Diabetic rat consuming *R. mucronata* leaf extract and glibenclamide was able to maintain body weight. In contrast, diabetic rat that did not consumed either *R. mucronata* leaf extract and glibenclamide experienced loss body weight. The antidiabetic activity of *R. mucronata* extract was detected by insulin presence in the extract. Numerous natural bioactives may function as antidiabetic and other therapeutic treatment, thus their exploration is important to provide safe products for human consumption.

The role *R. mucronata* extract made from bark, hypocotyl and root as hepatoprotective is related with the presence of phytochemical compounds namely flavonoids, alkaloids, coumarins and polyphenols. Furthermore, *R. mucronata* contained tannin as catechin and epigallocatechin gallate. Acetone (70%) extract of the bark of *Cynomorium songaricum* contained 18.3% tannin. The polyphenol flavan-3-ol oligomers showed inhibitory activity on α-glucosidase. It is reported that polyphenol-rich extracts of berries inhibited α-glucosidase activity in vitro. In addition to the bioactive compounds, both immature and mature fruit of *R. mucronata*...
mucronata contained more than 20% total dietary fiber. Dietary fiber can reduce blood glucose or insulin level postprandial. Fruit of R. mucronata can be harvested mature and immature, so the inhibitory activity by α-glucosidase of R. mucronata fruit flour prepared from mature and immature fruit should be studied.

Materials and Methods

Sample collection

R. mucronata fruit both mature and immature were collected from mangrove areas Penunggul village, Pasuruan, East Java, Indonesia in August 2013. The mature fruit is marked by a yellow line on the hypocotyl.

Preparation of R. mucronata fruit flour

R. mucronata fruit powder were made from peeled and unpeeled fruit. Size reduction began with cutting of fruit (10 cm length), peeling and soaking in distilled water. Furthermore, fruit was drained, soaked in citric acid solution (0.5%) for 10 minutes, and followed by immersion in water for 3 days. After that, the fruit was drained, cut to reduce its size and dried in oven at a temperature of 75°C for 3 hours or until the moisture content of the material below the 14.5%. The dried fruit pieces were milled by discmill and sieved (80 mesh) in order to obtain R. mucronata powder. The rind of fruit, byproduct of the process of stripping R. mucronata fruit, also prepared to support the analysis by reducing the size around 5 cm.

Preparation of ethanol extracts of R. mucronata fruit flour

R. mucronata fruit flour was first macerated in hexane for 3 days. Residue and filtrate were separated by Whatman filter paper. The residue was then macerated again in ethyl acetate for 3 days. On the fourth day, residue and filtrate were separated by Whatman filter paper. The residue of ethyl acetate was macerated again for the third time in ethanol for 3 days. On the fourth day, residue and filtrate were separated by Whatman filter paper. The filtrate of hexane, ethyl acetate and ethanol extracts were each concentrated by rotary evaporation. The extract obtained was properly labeled and stored in the freezer at 4°C until it was used.

Phytochemical screening

Phytochemical screening of R. mucronata fruit flour was done qualitatively to analyze the presence of natural compounds namely flavonoids, alkaloids, steroid, tannin and saponin.

In-vitro α-glucosidase inhibitor activity assay

α-glucosidase inhibitory activity assay was conducted by test tube method. One mg of α-glucosidase was dissolved in 100 ml of phosphate buffer (pH 7.0) containing bovine serum albumin. The enzyme mixture was diluted with the same buffer until 1/50 before being tested. Mixture containing 500 μL of 20 mM p-nitrophenyl α-D-glucopyranoside, 990 μL of 100 mM phosphate buffer and 10 mL of samplesolution or DMSO was made. After incubation (5 min, 37°C), the reaction was initiated by adding 500 μL of enzyme solution and incubated for another 15 minutes. Reaction was stopped by adding 2000 μL 200 mM Na₂CO₃. Absorbance of p-nitrophenol was measured at 400 nm wavelength by using a Hitachi U-2000 spectrophotometer. The inhibitory activity (%) was calculated with the formula [(CS) / C] x 100, where C is the absorbance of DMSO without sample and S is the absorbance of the sample. Quercetin solution of 1% was used as a positive control.

Total Phenolics Content

Total phenolics content was measured by spectrophotometry using Folin–Ciocalteau reagent method, with slight modification. Ethanol extract was diluted with distilled water until 10,000 ppm and then pipeted 0.1 mL. Ethanol extract was then reacted with Folin–Ciocalteau reagent (1mL reagent added with 2 mL distilled water). Reaction was stopped by homogenization with 7.5% (w/v) sodium carbonate and incubation at room temperature for 30 minutes. The absorbance of total phenol was measured with spectrophotometer at a wavelength of 760 nm. Gallic acid was used as standard and the content of phenol total was determined based on the regression gallic acid formula. Total phenol was measured as total gallic acid equivalent per 100 g extract (g GAE/100 g).
Results and Discussion

Phytochemical Screening

Phytochemical screening was conducted to prove an assumption that there was a correlation between phytochemical of the ethanol extract of *R. mucronata* fruit powder and antidiabetic activity. Both mature and immature fruit powder, peeled and unpeeled, contained saponin, flavonoid, steroid, and tannin (Table 1). The immature fruit rind contained flavonoid, steroid, and tannin. In addition, *Terminalia kaerbachii* contained alkaloid, flavonoid, and catechin tannin and had α-glucosidase inhibitory activity\(^{18}\). Flavonoid groups of tea leaves were used as antidiabetic\(^ {19}\).

### Table 1. Phytochemical Screening of Ethanol Extract of *R. mucronata* fruit powder

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peeled mature fruit</th>
<th>Unpeeled mature fruit</th>
<th>Rind of mature fruit</th>
<th>Peeled immature fruit</th>
<th>Unpeeled immature fruit</th>
<th>Immature fruit rind</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Saponin</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Steroid</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Tannin</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

α-Glucosidase Inhibitory Activity of *R. mucronata* fruit flour

Utilization of some terrestrial crude extract was used as antidiabetic drug. Parts of plant which have been studied are namely leaves, fruit, and bark. If the compound has α-glucosidase inhibitory activity, the compound is a potent drug for antidiabetic or antiobesity\(^ {20}\). Table 2 shows the α-glucosidase inhibitory activity of *R. mucronata* fruit flour.

### Table 2. α-Glucosidase Inhibitory Activity of *R. mucronata* fruit powder

<table>
<thead>
<tr>
<th>Ethanol Extract</th>
<th>IC(_{50}) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpeeled mature- <em>R. mucronata</em> powder</td>
<td>76.53</td>
</tr>
<tr>
<td>Unpeeled immature- <em>R. mucronata</em> powder</td>
<td>105.89</td>
</tr>
<tr>
<td>Peeled mature- <em>R. mucronata</em> powder</td>
<td>109.87</td>
</tr>
<tr>
<td>Peeled immature- <em>R. mucronata</em> powder</td>
<td>223.49</td>
</tr>
</tbody>
</table>

The highest inhibition activity against α-glucosidase was shown by unpeeled mature-*R. mucronata* powder with IC\(_{50}\) of 76.53 µg/mL. Extract of unpeeled *R. mucronata* fruit powder prepared from both mature and immature fruit showed higher α-glucosidase inhibition activity compared to the IC\(_{50}\) value of peeled fruit. It indicated the relationship between phytochemical compounds in fruit rind, such as the presence of flavonoid and tannin, with α-glucosidase inhibition activity. Tannin consists of hydrolyzed tannin and condensed tannin. Hydrolyzed tannin does not have a role in reducing blood glucose of diabetic rats\(^ {21}\). Condensed tannin, called as proanthocyanidin, consisted of catechin and epigallocatechin gallate.

Epicatechin-(4β,8)-epicatechin gallate (B2-3’-O-gallate), epicatechin gallate (ECG) and 2-(4-hydroxy phenyl) ethyl 3,4,5-trihydroxybenzoate (HETB) isolated from *Rhodia crenulata* root were potent drugs as α-glucosidase inhibitor. The inhibitory concentration of B2-3’-O-gallate, ECG and HETB were 0.30 ± 0.03, 0.21 ± 0.04 and 3.10 ± 0.09 µM\(^ {22}\). Catechin, called as proanthocyanidin, is one of flavonoid group\(^ {19}\). Catechin reduced blood glucose level diabetic rats over 50%\(^ {21}\). Tannins on *R. mucronata* fruit powder acted as antidiabetic by inhibiting the digestive enzymes in intestine and glucose transporter\(^ {23}\). Blackcurrant and rowan berry inhibited α-glucosidase IC\(_{50}\) 20 dan 30 µg GAE/ml\(^ {11}\).

One of standard drug widely consumed by diabetic patient is acarbose. Acarbose inhibits α-glucosidase
Enzyme, the digestive enzyme aids in glucose or carbohydrate absorption, and reduces blood glucose level. IC$_{50}$ acarbose value was 117.20 µg/mL. IC$_{50}$ value both of mature and immature fruit unpeeled werelower than IC$_{50}$ value of acarbose. It showed that inhibition of α-glucosidase enzyme of mangrove fruit flour was higher that acarbose. Thus, $R$. mucronata fruitpowder can be a candidate as functional food for antidiabetic.

**Total Phenolic Content**

Phenol has aromatic ring withone or two hydroxyl group. Flavonoid is the biggest of phenol group. Total phenol of $R$. mucronata fruit flour can be seen in Table 3.

**Table 3. Total Phenol of $R$. mucronata fruit flour**

<table>
<thead>
<tr>
<th>Ethanol Extract</th>
<th>Total Phenol (mgGAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$. mucronata mature fruit</td>
<td>37.35</td>
</tr>
<tr>
<td>$R$. mucronata mature rind</td>
<td>459.14</td>
</tr>
</tbody>
</table>

Generally, total phenol of ethanol extract of mature $R$.mucronata rind was higher than its fruit extract. Either the extract of fruit-rind or the extract of fruit tended to dilute in polar solution. Phenol compound tend to dilute in polar solution. Total phenol of mature $R$.mucronata fruit powder extract (37.5 mgGAE/g) was lower than mature $R$. mucronata fruit rind extract (459.14 mgGAE/g), both extracted by ethanol. It showed that there was a relation between total phenolic content of the fruit rind and the high α-glucosidase inhibitory activity. Linked with Table 1, total phenol could be flavonoid and tannin.

**Conclusion**

$R$.mucronata fruit flour contained flavonoid, saponin, tannin and steroid. Unpeeled $R$.mucronata fruit powder had the highest α-glucosidase inhibitory activity with IC$_{50}$/76.53 µg/mL, thus can be used as a candidate of antidiabetic.

**Acknowledgment**

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**References**